ABERRANT CYP2D6 METABOLIZER PHENOTYPES DO NOT SHOW INCREASED FREQUENCY IN PATIENTS UNDERGOING ECT AFTER ANTIDEPRESSANT THERAPY

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Introduction: Major depressive disorder (MDD) and bipolar disorder (BD) are common mental disorders. Antidepressant drugs (ADs) are important first-line treatment options in both unipolar and bipolar major depressive episodes. According to present guidelines, the effective AD for treatment of MDD and BD for each individual patient is identified through trial and error switching in sequential treatments. A substantial proportion of depressive patients do not benefit from treatment due to ineffectiveness of medication therapy or incurring serious side effects such that these patients are indicated for electroconvulsive therapy (ECT). CYP2D6 variants are associated with metabolic profile of ADs and have been investigated as a determinant contributor in treatment resistant depression (TRD). Hereby, we investigate the accumulation of aberrant CYP2D6 genotypes and predicted metabolizer phenotypes (UM, IM, and PM) potentially affecting the antidepressant treatment response in depressive patients indicated for ECT compared to patients with single episode of depression.

Method: 84 Dutch Caucasian subjects with unipolar or bipolar treatment resistant major depression who underwent ECT were genotyped using Amplichip® CYP450 Genotyping Test for CYP2D6 and its metabolizer phenotypes. 208 genotyped patients with single episode of unipolar or bipolar major depression were used as controls to examine differences in prevalence of CYP2D6 phenotypes.

Result: The mean age of ECT cases and subjects with single episode of depression was 62 ± 14 [range: 27-87, F/M: 46/29] and 49 ± 19 [range: 15-91, F/M: 91/117], respectively. Prevalence of CYP2D6 phenotypes (PM, IM, EM and UM) was "5.3%, 38.7%, 56% and 0.0%" for ECT patients, and "6.4%, 51%, 42.6% and 0.0%" for depressive patients. The type of depression (OR=0.33, p=0.018) and age (OR=1.55 for a 10 year increase, p=0.001), but not CYP2D6 phenotype were associated with the response to treatment.

Conclusion: The frequencies of genotype-predicted phenotypes (UM, IM and PM), potentially affecting the treatment response, did not show increased frequency in patients who received ECT for continuation of depression treatment as compared to patients with single episode of depression. Preemptive genotyping for CYP2D6 currently appears to have no clinical implications in treatment resistant depressive patients undergoing ECT. Further large-scale prospective clinical trials are warranted.

CYP3A5 AND ABCB1 POLYMORPHISMS AS PREDICTORS FOR SUNITINIB OUTCOME IN METASTATIC RENAL CELL CARCINOMA

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Background
In our exploratory studies we have associated single nucleotide polymorphisms (SNPs) in candidate genes with efficacy and toxicities of sunitinib in metastatic renal cell carcinoma (mRCC). The aim of the present study is to test these SNPs for association with sunitinib treatment outcome in the largest patient cohort to date.

Methods
mRCC patients treated with sunitinib and a DNA sample available were pooled from 3 exploratory studies conducted in the US, Spain and the Netherlands. A total of 22 SNPs and 6 haplotypes in 10 candidate genes related to pharmacokinetics and pharmacodynamics of sunitinib were tested for associations with toxicity, dose reductions, progression-free survival (PFS), overall survival (OS) and best objective response.

Results
Three-hundred and thirty-three patients were included. The presence of CYP3A5*1 was associated with dose reductions (OR=2.0, CI=1.0-4.0, P=0.039). Presence of CGT in the ABCB1 haplotype was associated with an increased PFS (HR=1.9, CI=1.3-2.6, P=0.000275) and remained significant after Bonferroni correction. These associations are consistent with prior observations. Similar size and direction of effect were observed for the
association of VEGFA rs1570360 with hypertension (OR=1.9, CI=0.8-4.5, P=0.173) and FLT3 rs1933437 with leukopenia (OR=3.6, CI=0.8-16.7, P=0.088).

**Conclusion**
The confirmation of previously reported associations between polymorphisms CYP3A5 and ABCB1 with sunitinib toxicity and efficacy respectively indicates that genotyping of these genetic variants may be useful for guiding sunitinib treatment.

003

**TACROLIMUS SUBSTITUTION OF COMMERCIAL FORMULATIONS IN PEDIATRIC TRANSPLANT PATIENTS**

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Background: Close patient monitoring during interchange of tacrolimus commercial formulations is essential to ensure similar exposure on substitution in pediatric transplant patients. Nonetheless, currently available reports in pediatrics undergoing innovator-to-generic interchange are extremely limited. The aim of this study was to compare the variability in tacrolimus blood levels and laboratory parameters in maintenance pediatric transplant patients undergoing switch and that of de novo patients receiving generic or innovator tacrolimus.

Methods: From April to August 2013, the Hospital Pharmacy dispensed a generic product of tacrolimus to the in-patient clinics according to the decision of the National Provision Program which provides treatment to all those patients without health insurance. Ten maintenance pediatric patients after kidney, liver, heart and hematopoietic stem cell transplantation were included if they were receiving the tacrolimus for at least 14 days after the switch. Tacrolimus dose-normalized trough levels (DNL) and laboratory parameters of renal and liver function were recorded before and after the switch. Furthermore, we analyzed DNL and the percent coefficient of variation (CV%) of the DNL in 22 de novo transplant patients receiving innovator (n=11) or generic product (n=11) during the first 4 post-transplant weeks. Both groups were chosen to be comparable in type of transplantation, donor type, age, co-morbidities and diagnosis.

Results: In the maintenance population, median (range) DNL on the innovator and generic tacrolimus was 75.1(ng/ml)/(mg/kg/day) (14.7-466.2) and 79.3 (ng/ml)/(mg/kg/day) (12.2-723.5), respectively (p>0.05). Routine laboratory values showed no differences in biochemical parameters after the switch (p>0.05). No significant difference was found in DNL and in CV% of DNL between products in de novo transplant patients when comparing the corresponding post-transplant weeks (p>0.05).

Conclusions: We report no significant differences in terms of pharmacokinetic or laboratory parameters in the studied pediatric patients subjected to switch between tacrolimus commercial products. We strengthen the need of further studies in bigger groups of patients undergoing substitution of tacrolimus formulations to ensure a safe interchange in this vulnerable population. Despite the small patient number of our study, there are no previous reports about substitution of tacrolimus in pediatric patients.

004

**AN OVERVIEW OF THE CURRENT STATE OF ANALYTICAL LABORATORY PRACTICES FOR THERAPEUTIC DRUG MONITORING OF IMMUNOSUPPRESSANTS IN ARGENTINA**

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Background: Despite the practice of transplant is well regulated in Argentina by the National Authorities of Health, only general requirements are stated for analytical laboratories involved in drug assays for clinical decisions. We decided to perform a nationwide survey on the analytical performance of current immunosuppressants assays in Argentina and identify areas of improvement as a result of the joint effort between the Unit of Clinical Pharmacokinetics (UFC)-Garrahan and the national authority INCUCAI.

Methods: Based on the list of transplant centers certified by INCUCAI, surveyed centers were those with greater percentage of transplant patients updated to January 2013. Private analytical laboratories were also included. The questionnaire was written in Spanish and previously approved by the IRB of our Hospital. The director of each clinical or analytical center agreed to participate. The survey was designed to obtain information about: facilities and demographics of the analytical laboratory; analytes and sample information; analytical assays and instruments; types and contents of reports; quality assurance.
Results
27 respondents were surveyed including 22 clinical centers and 5 private laboratories. Almost all clinical centers monitor calcineurin inhibitors while 86%, 64% and 14% request for sirolimus, everolimus and MPA, respectively. 15/22 clinical centers outsource at least one assay; everolimus was the most common outsource determination. Almost 60% of the samples were analyzed at the private practice. All bioassays were performed in the biochemistry department without the pharmacist participation. 4/24 laboratories provide the therapeutic range in the report and none gave other advice. 92% reported to use immunoassays as assay methodologies; 1 center used HPLC and 60% (3/5) of private practice employed LCMSMS. A quality assurance program was reported in 13/19 (68%) respondents from clinical centers but only 10 (53%) participated in an international QA program for external quality controls.

Conclusions
The present is the first survey carried out in a developing country and the first step of a national project in collaboration between UFC-INCUCAI to support and promote the development and conduct of bioanalytical assays for clinical decisions according to international consensus to improve TDM and also support the professional development on this field.

005
INSTRUCTIONS FOR CLINICAL AND BIOMARKER MONITORING IN THE SUMMARY OF PRODUCT CHARACTERISTICS (SMPC) OF PSYCHOTROPIC DRUGS: OVERVIEW AND APPLICABILITY IN CLINICAL PRACTICE
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Background
The Summary of Product Characteristics (SmPC) of psychotropic drugs includes instructions for clinical and biomarker monitoring intended to optimize effectiveness and minimize harm. The present study evaluated which monitoring instructions are given in SmPCs, determined the reasons for monitoring and assessed whether these monitoring instructions were informative enough to be applicable in clinical practice.

Methods
Instructions for monitoring were collected from complete SmPCs of psychotropic drugs (n=73). Reasons and requirements for monitoring were assessed. Monitoring of somatic markers was distinguished from non-somatic markers. The applicability was determined using the Systematic Information for Monitoring (SIM) score. Instructions were determined applicable when a SIM-score ≥3 was acquired.

Results
An average of 3.2 monitoring instructions per drug label was found comprising diverse topics. Monitoring was predominantly mandatory (71%). Somatic parameters were most often mentioned (in 80% of the psychotropic drugs). Only 34% of the instructions were determined applicable in clinical practice. Overall, an average SIM-score of 1.9 (SD:1.7) out of a maximum possible score of 6 was found.

Conclusions
Prescribing of psychotropic drugs is accompanied by diverse monitoring instructions aimed at improving safe use. However most instructions on monitoring do not provide sufficient information to be applicable in clinical practice.

006
ANALYSIS OF THE VARIABLE FACTORS IN THE BLOOD TACROLIMUS CONCENTRATION DURING THE SWITCH FROM CONTINUOUS INTRAVENOUS INFUSION TO ORAL ADMINISTRATION AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION
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Background
It is difficult to set dosage adjustment of tacrolimus, especially when switching from continuous intravenous infusion to oral administration, in the allogeneic hematopoietic stem cell transplant (allo-HSCT). In the present study, we examined to identify the variable factors affecting on the blood tacrolimus concentration during the switch from continuous intravenous infusion to twice-daily oral administration.

Methods
Seventy-three allo-HSCT patients receiving tacrolimus between December 2010 and December 2013
were reached in critically ill infants and children. Secondly, we aimed to determine variable factors associated with the variation of (C/Dpo) divided by (C/Div) ([C/Dpo]/[C/Div]). In addition, the associations between the (C/Dpo)/(C/Div) values and the occurrence of acute graft versus host disease (aGVHD) as well as kidney injury.

Results: The median (C/Dpo)/(C/Div) was 0.21 (range, 0.04 - 0.58). Multiple regression analysis showed that concomitant administration of oral itraconazole (n = 25) or voriconazole (n = 4) was significantly varied the (C/Dpo)/(C/Div) of tacrolimus (P = 0.002), probably due to the inhibition of enterohepatic CYP3A4/5. Five of 17 (29.4%) patients who had the lowest quartile (C/Dpo)/(C/Div) had developed a GVHD before the change in the route of tacrolimus administration, which was significantly higher than that in others [five of 56 (8.9%) patients, P = 0.032]. There was no statistically significant association between (C/Dpo)/(C/Div) and the occurrence of kidney injury.

Conclusions: Although the conversion from intravenous to oral administration of tacrolimus at a ratio of 1:4 seemed to be appropriate, a lower conversion ratio seems to be suitable in patients taking oral itraconazole or voriconazole. In the patients whom blood concentration decreased after the switch, GVHD should be monitored carefully, and dosage of tacrolimus should be adjusted again considering its blood level.

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POLYMORPHISMS OF CYP3A5/MDR-1 AND POSTTRANSPLANTATION DIABETES MELLITUS (PTDM)

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Objectives: Posttransplantation Diabetes Mellitus (PTDM) is a frequent complication in patients treated with the immunosuppressive calcineurin inhibitor tacrolimus (FK506). The oral bioavailability of tacrolimus varies greatly between individuals and depends largely on the activity of both the cytochrome P450 3A (CYP3A) subfamily and P-glycoprotein (P-gp). A member of the CYP3A subfamily, CYP3A5, is thought to participate in the oral clearance of tacrolimus. P-gp, an efflux transporter encoded by the MDR1/ABCB1 (adenosine triphosphate [ATP] binding cassette subfamily B, member 1) gene, is associated with multidrug resistance and to cause rejection episodes in transplant recipients. This study is to investigate whether the polymorphisms of CYP3A5/MDR-1 influence the pharmacokinetics of tacrolimus, thus result in PTDM.

Methods: Three SNPs: exon 12 (C1236T), exon 21 (G2677T, A) and exon 26 (C3435T) were investigated in 228 liver transplant recipients (PTDM vs. non-PTDM), by HRM analysis (high-resolution melting curve analysis).Tacrolimus blood concentration was determined by EMIT. Serum glucose and HbA1c were followed up for 16-96 months.

Results: We demonstrated that allele 3435-T (associated with low P-gp expression and high drug absorbance) had significantly higher percentage in PTDM patients than non-PTDM ones. PTDM patients have higher tacrolimus concentration than non-PTDM ones. C3435T polymorphism demonstrates a significant correlation with tacrolimus pharmacokinetics and PTDM in liver transplant patient.

Conclusions: FK506BP is not only expressed by T-cells but also pancreatic β-cells. By binding Tac, it has a significant impact on the intracellular calcium signaling pathway affecting the exocytosis of insulin-containing vesicles. Diabetogenic effect of Tac is predominantly caused by decreased insulin secretion by the pancreatic β-cells. MDR1/ABCB1 gene influence the pharmacokinetics of tacrolimus, 3435-TT is associated with low P-gp expression and high blood tacrolimus concentrations, this may be a molecular marker to predict the risk of developing PTDM, and is important to define individualized therapies.

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VANCOMYCIN THERAPY IN CRITICALLY ILL CHILDREN: AN URGENT PLEA FOR PHARMACOKINETIC-PHARMACODYNAMIC RESEARCH INTEGRATING PROTEIN BINDING

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Background and Objectives: The primary aim of this study was to investigate whether -with current vancomycin dosing regimens- PK/PD targets were reached in critically ill infants and children. Secondly, we aimed to document vancomycin plasma protein
ASSOCIATION ANALYSIS OF SINGLE NUCLEOTIDE POLYMORPHISMS RELATED TO RESPONSE AND TOXICITY OF SUNITINIB IN PATIENTS WITH MALIGNANT RENAL CELL CARCINOMA: A MULTICENTER STUDY.

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BACKGROUND:
The objective of this study is to identify novel single nucleotide polymorphisms (SNPs) and to investigate the association with progression free survival (PFS), overall survival(OS), clinical response, and toxicity in patients with clear-cell metastatic renal cell carcinoma (mRCC) treated with sunitinib.

METHODS:
A multicenter pharmacogenetic association study was performed in mRCC patients treated with sunitinib as first or second line treatment. A total of 10 polymorphisms in eight candidate genes, together with clinical characteristics were analyzed for a possible association with end points.

RESULTS:
We included 336 patients. Multivariate analysis showed that PFS was significantly improved when A allele was present in MET rs11762213 (P=0.041; HR: 0.59; 95%CI: 0.36-0.98), but the above significant association of MET disappeared (P=0.076; HR: 0.63; 95%CI: 0.38-1.05) when adjusted by CGT copy in ABCB1 haplotype. The presence of T allele in IL13 rs1800925 was related to a 6.5-fold increase in the risk of leukopenia (P=0.023; 95%CI: 1.3-32.6). Hypertension was increased when one or two T allele were present in IL8 rs1126647 compared to wild type AA (HR: 2.8 and 3.1; 95% CI: 1.2-6.7 and 1.1-9.0; P value: 0.023 and 0.036, respectively). The prevalence of any toxicity higher than grade 2 was 2.0-fold increased when T allele in IL13 rs1800925 was present (95%CI: 1.2-3.5; P=0.007).

CONCLUSIONS:
This exploratory study suggests that polymorphisms in IL8rs1126647 and IL13 rs1800925 are associated with sunitinib induced toxicities. Further validation in other cohort is needed. If confirmed, these results should help to optimize drug treatment in individual patients with such variants.
MICRO-SAMPLING APPLICATIONS IN PHARMACOKINETIC STUDIES
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Background: scientific research depends greatly on the availability of biological fluids. Collection of biological fluids from humans and/or testing animals can be invasive for the studied subject and might sometimes even cause conflicts with scientific ethical behavior or regulations. Collection methods which minimally interfere with the investigated individual are therefore highly desired. Readily compliance with scientific ethical regulations can be obtained by decrease of the invasive impact of the sample collection by improvement of simplicity, reduction of sample amount and when possible decrease of numbers of total samples to be collected. Modern micro-sampling techniques are in general compliance with mentioned requirements. Dried blood spots (DBS), capillary microsampling (CMS) and other recently introduced methods have seen a significant increase in popularity, not only in pharmaceutical- but also in clinical, medical and bioanalytical research, lately. This presentation will demonstrate the application of different micro-sampling methodologies used for sampling of micro-volumes of blood during a pharmacokinetic study using Acetaminophen as model drug.

Methods: different micro-sampling methods were used and compared applying a pharmacokinetic study of healthy volunteers applying Acetaminophen as model drug. Liquid chromatography high resolution accurate mass spectrometry (LC-HRAMS) was applied for quantitative analysis of Acetaminophen in blood and plasma samples collected post-dose.

Results: micro-blood samples on different time points were applied for the preparation of dry blood spots, dried matrix on paper disks and liquid plasma. Pharmacokinetic parameters determined were clearance (Cl), area under the curve (AUC), volume of distribution (Vd), peak concentration (Cmax), time of occurrence of peak concentration (tmax) and half-life time (t1/2). Observed pharmacokinetic values were not statistically (ANOVA) different compared to in literature reported values based on regular venous blood collection.

COMPARISON OF DIFFERENT VANCOMYCIN DOSE RATES IN PREMATURE NEONATES ACCORDING TO WEIGHT AND SERUM CREATININE VALUES USING A POPULATION PHARMACOKINETIC APPROACH.
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Background: Vancomycin is the first choice antibiotic for the treatment of methicillin resistant coagulase negative Staphylococci. The goal of this study was to develop a population pharmacokinetic model for vancomycin in premature neonates and to use this model to simulate steady-state vancomycin serum concentrations during continuous infusion at different dose rates and for different serum creatinine values.

Methods: Hundred and sixteen observations obtained from 87 premature neonates hospitalized in a neonatology reanimation department and given vancomycin were used. The model was developed using Pmetrics. After determination of the POP-PK parameters, 1000 time-concentration profiles were simulated over a range of pediatric weights and creatinine values for 4 different dose rates to predict the probability of maintaining serum vancomycin above different steady state concentration (Css) targets between 5 and 30 mg/L (i.e. AUC0-24h between 120 and 720 mg.h/L).

Results: The mean±SD vancomycin doses, postmenstrual ages, weights and serum creatinine were 17±5 mg/kg/24h, 28.5±3, 1.18±0.426 kg and 53 ± 21 µM respectively. The final structural model was a one-compartment model with linear elimination. Body weight (BW) and serum creatinine (SCR) significantly influenced vancomycin clearance. The mean relative bias of concentration was 1.5 % and the root mean squared error was 31.5 %.

Conclusions: The vancomycin dose currently used in premature neonates (20mg/kg/24h) does not allow reaching the Css target of 20mg/L usually targeted by pediatrician in Limoges University Hospital. Whatever the serum creatinine level, the vancomycin dose should be higher, up to 2-fold in neonates with a normal serum creatinine.
012

IMPACT OF CYP3A5 AND ABCB1 GENETIC VARIANTS ON BLOOD EXPOSURE OF AND CLINICAL RESPONSES TO TACROLIMUS IN PATIENTS WITH RHEUMATOID ARTHRITIS
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Background: Tacrolimus, a T cell-targeted immunosuppressant, is a good candidate for treatment of rheumatoid arthritis. Several studies have demonstrated its excellent efficacy and laboratory findings for rheumatoid arthritis. Tacrolimus is metabolized principally to 13-O-demethylate by CYP3A4/5. P-glycoprotein, ABCB1 acts as a membrane efflux pump, which affects tacrolimus absorption from the gut. The pharmacogenomic impact on tacrolimus therapy remains to be clarified in rheumatoid arthritis. The aim of this study was to evaluate the blood exposure of and clinical responses to tacrolimus based on genetic variants of CYP3A5 and ABCB1 in patients with rheumatoid arthritis.

Methods: Seventy rheumatoid arthritis patients treated with oral tacrolimus in the evening once daily were enrolled. The tacrolimus dose was increased from 0.5-1 to 3 mg in accordance with the clinical condition. Blood concentrations of tacrolimus and its major metabolite 13-O-demethylate at 12 hours after dosing were determined at the titration dose. Tacrolimus withdrawal was defined as discontinuation within 4 weeks of starting the medication because of drug resistance or intolerance. The relationships between the tacrolimus pharmacokinetics and efficacy, renal function, and genotypes (CYP3A5*3 and ABCB1 C3435T) were evaluated.

Results: Dose-normalized blood concentration of tacrolimus was significantly higher in the CYP3A5*3/*3 group than in the *1 allele carrier group. A lower metabolic ratio of 13-O-demethylate to tacrolimus was observed in the CYP3A5*3/*3 group. The ABCB1 3435TT group had higher dose-normalized blood concentrations of tacrolimus and 13-O-demethylate. The blood tacrolimus concentration was inversely correlated with the estimated glomerular filtration rate (eGFR). ABCB1 C3435T but not CYP3A5 genotype had decreased eGFR. The blood tacrolimus concentration and CYP3A5 and ABCB1 genotypes did not alter the serum CRP level. Patients lacking the CYP3A5*3 allele had a higher incidence of tacrolimus withdrawal.

Conclusions: CYP3A5*3 increased the blood exposure of tacrolimus through its metabolic reduction. ABCB1 C3435T led to a higher blood exposure of tacrolimus and its major metabolite. The ABCB1 genetic variant and its associated tacrolimus pharmacokinetics affected renal function in rheumatoid arthritis patients.

014

EFFECT OF CHANGES IN LEGAL SALES STATUS AND THERAPEUTIC RECOMMENDATIONS OF DOMPERIDONE AND METOCLOPRAMIDE ON THE NUMBER OF OVERDOSES
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Background: Recently, in the Netherlands therapeutic recommendations for the use of the antiemetic drugs domperidone and metoclopramide were adapted. Recommended doses were reduced, the number of indications was decreased and pediatric use was restricted. Furthermore, domperidone’s legal sales status was changed twice. In June 2009 it changed from “Pharmacy and Drugstore Only (PDO)” to “Pharmacy Only (PO)” and in July 2014 to “Prescription Only (PO)”. Metoclopramide has the PO-status. The changes were made to reduce side effects and for domperidone also to reduce life threatening interactions with other drugs. In this study, we describe the effects of these restrictions on the number of overdoses with these drugs as reported to the Dutch National Poisons Information Center (DPIC).

Methods: The DPIC’s database was searched retrospectively for overdoses with domperidone and metoclopramide in 2007-2014.

Results: The annual number of reported domperidone overdoses did not vary much in the period 2007-2009 (51, 54, 49), and peaked in 2010 with 65 overdoses. Over the following years, the number of overdose cases dropped to 45 in 2013, and 37 in 2014. On the contrary, the number of reported metoclopramide overdoses doubled from 25 in 2007 to 51 in 2014.

Conclusions: The annual number of reported overdoses with domperidone shows a descending trend. This decrease is not totally consistent with the timings of the changes in legal sales status. Although it is common that changes in drug overdoses run behind changes in prescription numbers, possibly also other factors are involved. In 2013 the European Medicines Agency’s Pharmacovigilance Risk Assessment Committee’s (PRAC)
recommedation to reconsider use of domperidone, was communicated to doctors and pharmacists. In addition, in 2013 domperidone received national media attention because of reported serious side effects and possible interactions. Together, this may have contributed to more cautious use of domperidone since 2013. However, the number of overdoses with metoclopramide seems to increase where domperidone decreases. When evaluating the effects of legal status changes, one should consider possible shifts to the use of similar drugs. As illustrated by this study, data from Poisons Centers make a valuable contribution to the pharmacovigilance of drugs.

015

'TO DETERMINE IF THE CONCENTRATION OF PHENOBARBITONE IN SERUM CAN BE ACCURATELY PREDICTED FROM THAT MEASURED IN DRIED BLOOD SPOT SPECIMENS'
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Background:
It is well accepted that phenobarbitone requires therapeutic drug monitoring (TDM). The conventional matrix of serum or plasma used for TDM of phenobarbitone is not easily, cheaply or safely transported long distances. An alternative is to measure phenobarbitone in dried blood spots (DBS). These are stable and easily transported. This provides an affordable and safe alternative for rural hospitals in India to send specimens for phenobarbitone monitoring.

Methods:
36 patients between the ages of 18 and 65, on phenobarbitone, were enrolled. The DBS specimens were prepared by spotting 20µl of whole blood on a standard Whatman filter paper using a calibrated micropipette. Separate validated HPLC assays were used to measure the concentration of phenobarbitone in serum and DBS. Normal distribution was assessed, precision and bias were calculated along with regression using the Spearman rank correlation test and the Wilcoxon signed rank test was performed.

Results:
The imprecision and the bias between the measured and the predicted serum concentrations were found to be higher (10% and 1% respectively), when the DBS concentrations were not normalised for haematocrit, than when corrected for haematocrit (8% and 0.49% respectively). Thus, corrected dried blood spot (CDBS) concentrations were used for analysis. The data were not normally distributed and on applying the Spearman rank correlation test, a correlation coefficient of 0.988 (p = 0.002) was obtained between the serum and CDBS concentrations. The Wilcoxon signed rank test showed that there was no statistically significant difference between the serum and the CDBS concentrations (p=0.3919). Thus, apart from normalizing the DBS for haematocrit no further correction equation is required to accurately predict the concentration of phenobarbitone in serum from that obtained from a DBS.

Conclusion:
The concentration of phenobarbitone in serum can be accurately predicted directly from that measured in a haematocrit corrected DBS. This means that a simple, cheap, easily accessible dried blood spot TDM service is available for rural hospitals in India which will give the patients all the benefits of therapeutic drug monitoring.

016

PRE-TRANSPLANT TACROLIMUS EXPOSURE PREDICTS POST-TRANSPLANT DOSE REQUIREMENT
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Background: The aim of this study was to investigate whether pre-transplant tacrolimus (Tac) dose requirement in patients scheduled to undergo kidney transplantation correlates with post-transplantation dose requirement.

Method: The predictive value of Tac dose requirement pre-transplantation on this same parameter post-transplantation was assessed retrospectively in a cohort of 57 AB0-incompatible kidney transplant recipients. These patients started with immunosuppressive therapy pre-emptively 14 days before surgery.

Results: Sixty-three percent of the Tac dose requirement on day 3 post-transplantation was explained by the Tac dose-corrected predose concentration immediately before transplantation. Serum albumin and hematocrit explained an additional 8.5% of the variance in Tac dose requirement on day 3 post-transplantation.

Conclusion: Steady-state Tac exposure before transplantation largely predicts post-transplantation Tac dose requirement.
Abstract: Background The increasing treatment failure of antimicrobials use arises in critical ill patients with infection partly as they display highly variable pharmacokinetics. By measuring antibiotics serum concentrations to the individuals can optimize treatment response and minimize side effects. Methods The methods in quantification of voriconazole (VOR), teicoplanin (Teic) and meropenem (Mero) in serum have been built and validated previously. A retrospective review of 91 patients in our Intensive Care Unit (ICU) who treated with the above antibiotics (n=46, 33 and 12, respectively) was conducted. Steady-state trough serum concentrations were measured at least 3 days after starting treatments. The dose adjustments were according to the reported therapeutic range of VOR (1.5 μg/ml), Teic (15-30 μg/ml) or by comparing the serum concentration of Mero with the corresponding MIC value. Results The most frequent underlying conditions in the study population were invasive pulmonary aspergillosis for Vor (67.4%), severe MRSA infection for Teic (75.6%) and klebsiella pneumoniae infection for Mero (71.4%). At the first analysis, the proportion of patients reached therapeutic trough levels were 78.3%, 57.6% and 83.3% for Vor, Teic and Mero, respectively. After receiving dose adjustment (by increased or decreased) for another 3 days, patients outside the therapeutic range experienced global improvement (n=11), few improvement (n=3) and 2 died despite medical treatment. Conclusions For critical ill patients, common empiric antimicrobials dosing regimens are not suitable all the time and routine use of therapeutic drug monitoring in these patients is strongly recommended.

Background: Cytomegalovirus (CMV) infection is a major issue in transplantation, being associated with a high morbidity. Prophylactic or preemptive therapy with ganciclovir (GCV) is used in this context, but it induces hematological adverse effects (mainly neutropenia) leading to premature treatment discontinuation or to the use of lower doses, favoring the emergence of resistance. This toxicity appears not to be associated with GCV concentration in plasma. Therefore, intracellular metabolites of GCV or intracellular GCV itself could be better biomarkers.

Methods: Twenty-two pharmacokinetic (PK) profiles (samples collected at pre-dose and 1, 2, 3, and 5 hours after the GCV dosing) for GCV in plasma and its intracellular metabolites were obtained renal transplant patients and were measured using a fully-validated LC-MS/MS method. A non-parametric model was developed using PMetrics® software to describe the concentration-time curves of the different forms. Then relationships between the exposure indices to GCV and the evolution of neutrophil counts were investigated using a multiple linear regression analysis.

Results: The developed model allowed good predictions of concentrations. R² between observed and predicted concentrations were: 0.83 for plasmatic GCV, 0.64 for intracellular GCV, 0.69 for GCV monophosphate and 0.70 for GCV triphosphate. Of the tested individual factors, only intracellular GCV triphosphate concentrations (expressed by its AUC_{0-24h}) was significantly associated with the decrease in neutrophil count over a 3-month period of treatment (β=-6.25.10^-4, sd=1.5.10^-4, p<5.10^-4). For every unit increase in the GCV TP AUC there was a corresponding decrease of 5.6% in the neutrophils count during the first 3 months of prophylaxis treatment.

Conclusion: We report on a 4-compartment open model able to accurately describe GCV and its metabolite concentrations. Decreased neutrophil count used as a surrogate marker of hematological toxicity could be associated to the accumulation of GCV triphosphate in blood cells. Further studies are needed to confirm that this biomarker is a predictive factor of toxicity.
Background:
Anti-citomegalovirus treatment of solid organ transplant (SOT) patients with ganciclovir (GCV) or valganciclovir (VGCV) following the manufacturer’s dosing recommendations may result in either over or underexposure to the drug. However, Bayesian prediction based on a population pharmacokinetics model has been suggested to more accurately optimize GCV/VGCV dosing thus, achieving more steadily desirable area under the curve (AUC) therapeutic target values (between 40-50 ug/mL).

Methods:
We conducted a two arm, randomized, open-label, clinical superiority trial (superiority margin = 40%) in adult SOT patients receiving GCV/VGCV either as prophylaxis or treatment of CMV infection. Group A received GCV/VGCV according to the manufacturer’s dosing recommendations and group B received adjusted doses of GCV/VGCV based on desirable exposure targets using a Bayesian prediction model (NONMEM). Drug exposure achieved was evaluated in both groups.

Results:
Fifty-three consecutive SOT patients were recruited in the study, 27 and 26 patients were included in group A and B, respectively. 88.4% (23/26) of patients with CGV/VGCV adjusted according to the Bayesian prediction model (group B) reached therapeutic target AUC values, whereas only 18.7% (5/27) of patients in Group A did so, achieving the desired 40% superiority margin of the study design (p<0.001, 95% CI for the difference: 54-86). The required time to reach target AUC values was significantly longer in Group A as compared to Group B (55.9±8.2 vs 15.8±2.3 days respectively, p<0.001). A numerically shorter time to viral clearance was observed in Group B as compared to Group A (12.5 vs. 17.6 days, p=0.125, respectively). The incidence of CMV relapse (Group A: 66.67% (8/12); Group B: 9.01% (1/11)) and late CMV disease (Group A: 36.7% (4/11); Group B: 7.7% (1/13)) were both higher in Group A than in Group B. No relevant differences in drug-related toxicity were observed between the two groups.

Conclusions:
GCV/VCGV dose adjustment based on a population pharmacokinetics Bayesian prediction model optimizes GCV/VGCV exposure and shortens the time to achieve a therapeutic AUC target in SOT patients.

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CO-ADMINISTRATION OF PROTON PUMP INHIBITOR DECREASES PLASMA CONCENTRATION OF ERLOTINIB IN PATIENTS WITH NON-SMALL CELL LUNG CANCER
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[Background] Erlotinib, a tyrosine kinase inhibitor targeting epidermal growth factor receptor, is used for treatment of advanced or metastatic non-small cell lung cancer (NSCLC) and pancreatic cancer in combination with gemcitabine. Since oral erlotinib shows lower dissolution at higher pH in the gastrointestinal tract, lower absorption of erlotinib may occur under co-administration anti-acid agents such as proton pump inhibitors (PPIs). The aim of this study is to investigate the drug interaction between erlotinib and PPIs in patients with NSCLC. [Methods] Seventy-eight blood samples were collected from 14 patients (male/female: 4/10, 67.1 ± 8.0 yrs) treated with erlotinib for NSCLC. Daily dose of erlotinib were 1.30 ± 0.69 mg/kg. Five patients received PPIs for their gastric symptoms. Steady state plasma concentrations and concentration per dose (C/D) ratio of erlotinib were compared between with and without co-administration of PPIs. Plasma erlotinib were determined by high-performance liquid chromatography. [Results] The plasma concentration and C/D ratio (median: range) of erlotinib was 0.687: 0.104-1.691 µg/mL and 0.480: 0.104-1.589 µg/mL/mg/kg, respectively. The C/D ratios for PPIs co-administration was significantly lower than those for without PPIs; 0.430: 0.104-0.696 vs. 0.548: 0.179-1.589 µg/mL/mg/kg (P=0.002). [Conclusions] It was confirmed that plasma erlotinib concentration was decreased under the co-administration of PPIs, which may induce lower gastrointestinal absorption of erlotinib via modifying pH-dependent aqueous solubility.
THE QUALITY OF DOSING RECOMMENDATIONS DURING THERAPEUTIC DRUG MONITORING OF AMINOGLYCOSIDES AT THE INTENSIVE CARE UNIT
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Background
Following recognition of preventable incorrect dosing recommendations due to lack of adequate information about dosing and patient details, an intervention aimed at improving the quality of Therapeutic Drug Monitoring (TDM) of aminoglycosides at the ICU was undertaken. Aim of this study was to measure the effect of the intervention on the quality of dosing recommendations during TDM of aminoglycosides, as assessed by the incidence of preventable incorrect dosing recommendations in a cohort of critically ill patients.

Methods
Data were collected prospectively in the periods May-June 2013 (before intervention) and 2014 (after intervention). The intervention consisted of clinical lessons for ICU nurses, recurrent evaluation of the TDM service with all hospital pharmacists, and participation of all pharmacists in the collaborative studies of the Association for Quality Assessment in TDM. Timing of dosing, as recorded in the Patient Data Monitoring System (PDMS) and on the TDM request on paper was obtained. Simultaneously, exact timing of administration was collected by the researchers by direct observation at the ICU.

Interpretation-related incorrect recommendations were defined as incorrect dosing recommendations due to errors made by the pharmacist interpreting the dosing information and drug levels. Registration-related incorrect recommendations were defined as incorrect dosing recommendations due to differences between dosing information as recorded vs. dosing information as observed by the researchers. Both types of incorrect recommendations are regarded as preventable.

The dosing recommendations by the pharmacist were recalculated by two researchers based on the routinely collected information to assess the percentage of interpretation-related incorrect recommendations, as well as with the exact dosing information to assess the percentage of registration-related incorrect recommendations.

Results
A total of 50 dosing recommendations were recalculated in 2013 and 91 in 2014. The percentage of registration-related incorrect recommendations decreased from 16% to 4% (p=0.11). The median difference between the in PDMS recorded and exact administration times decreased from 33 minutes in 2013 to 16 in 2014. The percentage of interpretation-related incorrect recommendations increased from 20% to 38% (p=0.04).

Conclusions
The quality of dosing recommendations during TDM of aminoglycosides has not improved in 2014, compared to 2013 where it concerns interpretation-related recommendations.

RAPID AND COMBINED MEASUREMENT OF TACROLIMUS, CYCLOSPORIN A, SIROLIMUS AND EVEROLIMUS IN WHOLE BLOOD AND DRIED BLOOD SPOT WITH LC-MS/MS
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Background
Therapeutic drug monitoring (TDM) of immunosuppressants is essential to avoid toxicity and rejection in organ transplant recipients. To date, whole blood sampling is ‘the gold standard’ for TDM of these vital drugs. Nevertheless, whole blood sampling in stable transplant recipients is limited to the outpatient clinic and is usually not carried out by the patient himself at home. Therefore, we have developed a rapid and practical method for the simultaneous measurement of tacrolimus, cyclosporin A, sirolimus and everolimus after sample preparation for dried blood spot (DBS) and whole blood.

Methods
The extraction method for DBS involved a 15 minutes extraction with ultrasonication. The whole blood sample preparation consisted of a protein precipitation with 0.1 M zinc sulphate and methanol. Extracts (25 µL) were injected onto a Thermo Scientific HyPurity C18 column, with methanolic mobile phase gradient elution. The analytes were detected with a Thermo Scientific triple quadrupole Quantum Access with positive ionization. The methods were analytically validated and clinically validated by comparing whole blood and DBS results. Venous whole blood samples of 30 patients on tacrolimus, cyclosporin A, sirolimus or everolimus were used to prepare DBS and whole blood.

Concentrations determined by DBS and whole blood methods were plotted and were statistically compared by Passing & Bablok regression.

Results
The methods were validated over a linear range of 2 - 75 µg/L for tacrolimus, 20 - 750 µg/L for cyclosporin A and 2 - 36 µg/L for sirolimus and everolimus. For the clinical validation all compounds demonstrated good correlation between the two methods yielding a linear regression of R²=0.99 for tacrolimus (Ht range 0.24 - 0.51), R²=0.98 for
cyclosporin A (Ht range 0.21 - 0.51), R²=0.99 for sirolimus (Ht range 0.25 - 0.51) and R²=0.96 for everolimus (Ht range 0.31 - 0.44). For all compounds the average concentrations of the population were within the 95% limits of agreement.

Conclusions
A rapid and combined analytical method of DBS and whole blood samples suitable for routine monitoring of immunosuppressants in organ transplant recipients was developed.

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QUANTIFICATION OF THE ANTIVIRAL DRUGS DOLUTEGRAVIR, ELVITEGRAVIR, RILPIVIRINE AND DACLATASVIR IN HUMAN PLASMA USING LC-MS/MS
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Background
HIV and hepatitis C are still abundant viral infections worldwide. For both viruses, therapy can be optimized using therapeutic drug monitoring (TDM). For the quantification of the novel antiviral drugs elvitegravir, dolutegravir (both HIV integrase inhibitors), rilpivirine (HIV non-nucleosidal reverse transcriptase inhibitor) and daclatasvir (hepatitis C NS5A inhibitor) in human heparinized plasma, we developed an online extraction LC-MS/MS method.

Methods
To 100 µL plasma, the internal standards elvitegravir-d6 and rilpivirine-d6 dissolved in methanol were added. After centrifugation, the supernatant was injected into the turbulent flow online extraction LC-MS/MS system. For the extraction, a Cyclone column (50x0.5mm, Thermo Fisher Scientific) was used. Analytical chromatography was performed under acidic conditions on an Uptisphere 5µ C18 column (125x2mm, Interchim): the mobile phases consisted of 10 mM ammonium acetate + 0.1% formic acid either in water or methanol/acetonitrile 50/50 v/v. A Quantum Access Max triple stage quadrupole mass spectrometer (Thermo Fisher Scientific), operated in positive heated-electrospray ionization mode, was used. Detection mode was selected reaction monitoring.

Results
The calibration range was either 10-500 µg/L for rilpivirine, or 0.1-5 mg/L for daclatasvir, dolutegravir and elvitegravir, respectively. Imprecision for both intra- and interday measurement was <6.03 % for all analytes. The accuracy was between 97.9 and 108% for all analytes. Sample stability was tested and was within the acceptance ranges (deviation <15%) for storage of one week at 4°C and -20°C. For rilpivirine, samples from an international proficiency testing scheme were re-analyzed and were within the acceptance range of the scheme.

Conclusions
We have developed and validated a robust online extraction LC-MS/MS method for the quantification of dolutegravir, elvitegravir, rilpivirine and daclatasvir.

025
A CASE REPORT OF CLONAZEPAM ABUSE - UTILIZATION OF THERAPEUTIC DRUG MONITORING DURING WITHDRAWAL PERIOD
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Background: Benzodiazepines are among the most commonly prescribed psychotropic medications worldwide. Associated with the use of benzodiazepines have been abuse, dependence, and withdrawal sequelae. Clonazepam is long-acting, high potency benzodiazepine agonist used in benzodiazepine withdrawal, however, recent observations suggest the existence of its abuse. Alprazolam is intermediate to short-acting benzodiazepine and its abusers are more likely to be male and often adolescent. We demonstrate a 40-year-old man with a 20-years history of psychiatric care with alcohol abuse in anamnesis and recently with benzodiazepine dependence (daily intake of about 60mg of clonazepam and 10mg of alprazolam).

Methods: Toxic levels of both drugs were analyzed three weeks before admission to hospitalization (clonazepam 543.9ng/mL, alprazolam 110ng/mL) and at the time of admission (clonazepam 286.2ng/mL, alprazolam 140ng/mL) without any signs of benzodiazepine intoxication. Subsequent trough plasma samples of clonazepam were collected consecutively almost every morning for 8 days. Quantitative analysis for clonazepam was performed by high performance liquid chromatography. All clonazepam contrantrations were interpreted by clinical pharmacologist and pharmacokinetic analysis we performed using software MWPharm 3.30.
Results: Alprazolam was discontinued almost promptly without severe withdrawal symptoms because the patient continued to use long-acting, high potency benzodiazepine agonist clonazepam. Gradual withdrawal of clonazepam and increase of gabapentin dose were used to minimize physical signs and symptoms of clonazepam withdrawal. The patient used clonazepam 4mg three times daily first eight days after admission and then the dosage was wind down using a rate of taper of by about 2mg/2-3 days with almost daily therapeutic monitoring of its level. Clinical consequences of the treatment were only controllable tension, intermittent headache and rarely insomnia. The patient was discharged 16th day of hospitalization without clinical signs of withdrawal and with clonazepam plasma level 81.3ng/mL, i.e. in upper limit of therapeutic range. At the time of discharge he used clonazepam 5mg daily (2mg in the morning, 1mg at noon and 2mg at evening). Patients clonazepam half-life achieved 32 hours which agree with general population.

Conclusions: It is, to our knowledge, the first case report showing strong benzodiazepine dependence with extreme toleration objectified via therapeutic drug monitoring.

026

THE ANALYSIS OF COMMON ANTIPILEPTIC DRUGS IN HUMAN URINE BY LC-MS/MS
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Background
The use of liquid chromatography coupled with mass spectrometry (LC-MS/MS) in therapeutic drug monitoring and toxicology labs has increased significantly over the years. LC-MS provides sensitivity, speed, and the ability to simplify sample preparation. The Raptor® Biphenyl column was developed to complement high-throughput LC-MS/MS analyses by combining the increased efficiency of superficially porous particles (SPP) with the resolution of Ultra Selective Liquid Chromatography® (USLC®) technology. In this example, a simple dilute and shoot method was developed for 14 common antiepileptic drugs in urine using a Raptor® Biphenyl column.

Methods
Human urine samples were diluted in 0.1% formic acid in water and injected into a Shimadzu Nexera UHPLC equipped with an AB SCIEX API 4500® MS/MS. Detection was performed using electrospray ionization in positive ion mode using scheduled multiple reaction monitoring (MRM). The separation was performed using water and methanol mobile phases modified with 0.1% formic acid under gradient conditions on a Restek Raptor® Biphenyl 2.7µm, 100 x 2.1mm column.

Results
Linearity and precision and accuracy experiments were performed during method development. Purchased human urine samples were fortified with 14 drug analytes and their deuterated internal standards. The calibration range for most analytes was from 10 to 1000 ng/mL; R values were all greater than 0.990. Accuracy and precision were determined by fortifying human urine at a concentration of 800 ng/mL prior to dilution. Mean values at this level ranged from 88% to 110% of nominal concentrations for all analytes. Coefficient of variation (CV) was calculated for the determination of precision and ranged from 6.2% to 10.5%.

Conclusions
An easy dilute-and-shoot method was developed for the quantitative measurement of 14 common antiepileptic drugs in urine using a Raptor® Biphenyl column.

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LONG TERM NON-COMPLIANCE OF DIGOXIN - A CASE REPORT
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Background: Digoxin is a positive inotropic drug approved for use in chronic congestive heart failure. Therapeutic and toxic effects of digoxin are related to its serum concentrations, recent evidence suggests that a lower therapeutic range 0.5 - 0.9 ng/mL is associated with reduced mortality. However, non-compliance with digoxin is considered as one of the problems in patients. Therefore, therapeutic monitoring of digoxin is essential in clinical practice. We report the case of almost 3-years persisting non-compliance of patient combined with non-acceptance of dose adjustment recommendation by clinician - ambulatory internist.

Methods: A 81-year-old male with chronic obstructive pulmonary disease was admitted to hospitalization and digoxin
0.125mg daily was added to losartan because of decompensated chronic heart failure. Trough serum levels of digoxin were estimated by immunoassay MEIA (AxSym Abbott) seven days later and than repeatedly by ambulatory internist. The long term serum concentration - time profile of digoxin has been predicted by the Bayesian analysis computer program MW-Pharm 3.30 MediWare.

Results: First measured digoxin level was in therapeutic range (0.7ng/mL) and the same dosage 0.125mg daily was recommended to continue. Five months later, digoxin level 0.14ng/mL was found, non-compliance was demonstrated and further analysis was advised. However, internist increased digoxin dosage to 0.125mg twice daily and digoxin level 1.16 ng/mL indicated patient’s non-compliance nine months later. Lower digoxin dosage 0.125mg daily was recommended once more but the recommendation of adjustment of the dose was not accepted by internist again and digoxin level 2.18 ng/mL was measured after one year. We suggested digoxin 0.125mg daily once again and level 0.9 ng/mL was achieved after another year.

Conclusions: There are three basic mistakes - non-compliance of patient, non-acceptance of digoxin dose adjustment recommendations by clinician and long intervals between analysis of digoxin levels. Therapeutic drug monitoring is very useful for improvement of digoxin compliance but good cooperation with clinicians is necessary.

028
USE OF GRAFT DERIVED, CELL-FREE DNA PERCENTAGES (GCFDNA) TO MONITOR EFFECTS OF IMMUNOSUPPRESSIVE DRUG (ISD) COMBINATIONS IN LIVER TRANSPLANT (LTX) PATIENTS INCLUDING DURING ISD SWITCHES
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Background: Individual LTx recipients require adaptation of ISD therapy, or even calcineurin/mTor inhibitor switches due to e.g. rejection, infections, or other signs of ISD toxicity, occurring even if ISDs in therapeutic ranges (TR) used as general guidance. Particularly in these situations, when therapeutic drug monitoring (TDM) alone is insufficient and TR values are unclear, other biomarkers are needed, such as GcfDNA, which directly monitors graft integrity in real-time.

Methods: Using a published digital PCR method, GcfDNA was analyzed (double-blind), while ISD concentrations were measured by LC-MS/MS in 6 adult LTx patients, who had clinically indicated Tacro/Evero ISD switches, all receiving concomitant MPA.

Results: In patient #1 (P#1) ISD doses were decreased because of neutropenia, resulting in initially subtherapeutic Tacro, Evero, MPA and clinical rejection, accompanied by high GcfDNA (~64%), which slowly normalized with sufficient Tacro (10µg/L). In P#2 GcfDNA also spiked (~55%) during a biopsy proven rejection (BPR), when Evero levels were low but MPA therapeutic and consecutively normalized after switching when Tacro (max.: 20µg/L) and MPA (max.: 4.7mg/L) were high.

In P#3 a clinical rejection with elevated GcfDNA (max.: ~99%) after Tacro to Evero switch required short-term steroids. Rejection was controlled and GcfDNA normalized with therapeutic Evero (~5.6µg/L) plus low Tacro and low MPA.

After ISD minimization for pancytopenia P#4 had slightly elevated GcfDNA (~15%) initially with decreasing Tacro and low MPA. GcfDNA increased episodically (max.: 62%) during a Tacro/Evero combination despite Tacro and Evero in TR, but with low MPA. In P#5 had low GcfDNA (<3%) both with high Tacro (max.: 12.6µg/L) and (post switch) with low Evero (<3.2µg/L) plus high MPA (max.: 5.2mg/L).

Finally, in P#6 GcfDNA remained low (<3%) when MPA ~1mg/L and low Tacro (<8µg/L, early post LTx); followed by low Evero (<3µg/L) levels after ISD switch, indicating lower ISD requirements than reflected by the TR alone.

Conclusions: These results indicate that GcfDNA, having a one-day turn-around, appears as useful biomarker to achieve personalized IS. Particularly if ISD related complications occur within TR, with ISD combinations, switching or minimization, GcfDNA is a valuable supplementation to TDM as aid for personalized IS management.

029
RELATIONSHIP BETWEEN THE OXYCODONE PHARMACOKINETICS AND SERUM INTERLEUKIN-6 IN CACHECTIC CANCER PATIENTS
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Background: Oxyccodone, a semi-synthetic opioid analgesic, is metabolized principally to noroxycodone by cytochrome P450 (CYP) 3A. Our previous studies demonstrated that cancer cachexia decreased the CYP3A metabolism of oxycodone. Proinflammatory cytokines such as interleukin-6 (IL-6) have been found to reduce hepatic CYP3A activity through the down-regulation of gene transcription. Few reports have been published on the relationship between the oxycodone pharmacokinetics and serum IL-6 in cachectic cancer patients. The aim of this study was to evaluate the plasma concentrations of oxycodone and its major metabolites and serum level of IL-6 based on the cachexia stage in cancer patients.

Methods: Forty-five patients treated with oral oxycodone for cancer pain were enrolled. Cachexia stage was evaluated by the Glasgow Prognostic Score (GPS), calculated using serum albumin and CRP. Predose plasma concentrations of oxycodone and its major metabolites were determined at the titration dose. IL-6 in human serum was measured by ELISA. The relationships between the oxycodone pharmacokinetics, serum IL-6, and GPS were evaluated.

Results: Seven patients had a GPS of 0, 18 a GPS of 1, and 20 a GPS of 2. Plasma concentration of oxycodone in patients with a GPS of 2 tended to be higher than that with a GPS of 0 or 1. The plasma concentration ratio of noroxycodone to oxycodone was significantly lower in patients with a GPS of 2 than with a GPS of 0 or 1. The median and interquartile range of serum IL-6 level were 12.5 and 9.4-17.7 pg/mL in patients with a GPS of 0 or 1, and 55.7 and 43.0-75.8 pg/mL in patients with a GPS of 2, respectively. Serum IL-6 level in patients with a GPS of 2 was significantly much higher than that with a GPS of 0 or 1. The plasma concentration ratio of noroxycodone to oxycodone was inversely correlated with serum level of IL-6.

Conclusions: Cancer cachexia raised the plasma exposure of oxycodone through the reduction of CYP3A. The reduction of CYP3A in cachectic cancer patients was associated with the elevation of serum IL-6.

SIRS DURATION INFLUENCES SERUM CONCENTRATIONS OF VANCOMYCIN IN SEPSIS PATIENTS
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[Background]
Vancomycin, which is used in the treatment of Methicillin-resistant Staphylococcus aureus infection, is mainly excreted directly in the urine. Renal function is the most important factor to determine vancomycin dosage. In sepsis patients, we often observe much lower or upper concentrations of vancomycin than predicted when vancomycin dosage is adjusted based on renal function. However, it remain unclear what factors affect vancomycin pharmacokinetics in patients with sepsis. The large individual variability in these patients is likely associated with the pathophysiology of sepsis and subsequent SIRS. In this study, we investigated factors influencing vancomycin concentrations in sepsis patients.

[Methods]
We performed a retrospective cohort study with patients administered vancomycin in Nihon University Itabashi Hospital between 2005 and 2012. Among 294 patients administrated vancomycin, 95 eligible patients were enrolled for therapeutic drug monitoring and were classified as sepsis and non-sepsis patients. Sepsis patients were further classified into three subgroups as no change, lower and upper groups referring to the mean absolute error (MAE) for non-sepsis patients. We detected factors that influence variation in vancomycin concentrations and performed multiple logistic regression analyses using candidates of explanatory variables with P values <0.2 on multiple comparisons among three subgroups.

[Results]
95 patients were classified 27 non-sepsis and 68 sepsis patients. Sepsis patients had a higher MAE (p=0.008) than non-sepsis patients. Multiple logistic regression analysis identified factors that influence variation in vancomycin concentrations in sepsis patients, showing that shorter (odds ratio (OR), 0.626, p=0.041) and longer SIRS durations (OR, 1.640, p=0.015) were associated with decreased and increased vancomycin concentrations, respectively. According to receiver operating characteristics analysis, the optimal cut-off value of SIRS duration for vancomycin concentrations lower than predicted was 2 days and that for concentrations higher than predicted was 6 days. The sensitivity and specificity of discriminating lower concentrations were 68.8% and 78.8%, respectively; those of discriminating upper concentrations were 63.0% and 92.7%, respectively.
Our study shows that SIRS duration influences vancomycin concentrations in patients with sepsis. The appropriate dosing of vancomycin needs to be modified with SIRS durations.

A NEW CHALLENGE: SUICIDE ATTEMPT USING NICOTINE FILLINGS FOR ELECTRONIC CIGARETTES

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Background
Electronic cigarettes are increasingly popular. The liquid fillings of the electronic cigarettes ('e-liquid') contain high concentrations of nicotine, which can cause potentially lethal poisoning when ingested.

Methods
We present a case of nicotine poisoning of a 27 year-old male who attempted suicide by ingestion of large amount of e-liquid.

Results
We encountered a 27 year-old man with a borderline personality disorder at our Emergency Department one hour after ingestion of five e-liquid fillings. He had also consumed five units of wine. The e-liquid contained a total of 420mg of nicotine and unknown amounts of propylene glycol. Before arrival at the ED, the patient had vomited three times. Initial evaluation showed a sinus tachycardia, a blood pressure of 144/99 mmHg and oxygen saturation of 98%. Apart from excessive salivation, his physical examination was unremarkable. Laboratory examination revealed an anion gap (16 mmol/L) and lactic acidosis (pH of 7.33 and lactic acid of 3.8 mmol/L). The osmol gap was 30 and ethanol 3.9. Activated charcoal was administered and repeated twice. During the actual observational period of 30 hours no adverse events occurred and the metabolic acidosis disappeared within 10 hours after admission. The serum concentration of nicotine was 50 micrograms/L at 2 hours and <50 micrograms/L at 4 hours after ingestion. The concentration of cotinine was 250 micrograms/L at 2 hours and 180 micrograms/L at 4 hours (toxicity concentration of nicotine + cotinine in smokers: > 1000 micrograms/L and in non-smokers: > 300 microgram/L).

Conclusions
The frequency of nicotine intoxications is likely to rise in the next years. Therefore, healthcare providers should become familiar with this intoxication.

TRENDS IN DRUG ABUSE CONSUMPTION AS ASSESSED BY DRUG SCREENING IN URINE AMONG EGYPTIANS: AN EXPERIENCE IN DRUG ABUSE LAB IN ASSIUT UNIVERSITY HOSPITAL DURING THE PERIOD 2007-2014.

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Background: Drug abuse represents a big medical and social problem for human health and welfare. By the end of 2006, the service of drug abuse testing (DAT) in urine has been applied to the therapeutic drug monitoring lab in Assiut University hospital.

Aim: The incidence of requests and possible changes in the trends of drug abuse consumption has been assessed in the light of results of DAT in urine during the period 2007-2014.

Methods: Retrospective analysis of the results of DAT in urine was done for the following drugs: tetrahydrocannabinoids (THC), benzodiazepines (BZD), tramadol, barbiturates (Barb), amphetamines (Amphet) and opiates. Tramadol testing was available in the lab from year 2011. The semi-quantitative analysis with cut-off value using the competitive immunoassay method on VIVA-system of Siemens was used in DAT and results were reported as positive or negative according to the manufacturer's cut-off.

Results: The total number of requests for DAT was 3908 increasing from 204 cases in year 2007 to 1411 cases in...
year 2014. In the period 2007-2010, positive results represented 53% of the total requests related to this period. The incidence of positive results was changeable in the period 2012-2014: 62.7% (2012), 81% (2013) and 48.8% (2014). The most frequently detected drugs were BZD (24.8%) followed by THC (22%) in 2007-2010, and tramadol (32.7%) followed by THC (23.5%) in 2012-2014. Poly-drug use was presented in 23% of all cases with highest incidence of BZD+THC in 2007-2009 (29 cases), BZD+Barb in 2010 (11 cases), and tramadol+THC in 2011-2014 (195 cases). Subjects within age range of 20-30 years old were the highest consumers for drugs particularly tramadol. Conclusion: The growing number of cases requested for DAT in urine along the period 2007-2014 indicates the requirement for this lab service in the hospital. Analysis of data showed a panoramic view about changeable trends in drug abuse consumption among Egyptians and tramadol now is coming on the top of abused drugs. The changeable incidence of positive results in DAT in the period 2012-2014 may be related to the societal events that happened in the Egyptian community in this period.

033

ANTIRETROVIRAL TESTING: DEVELOPMENT AND VALIDATION OF LC-MS/MS ASSAYS IN UNIQUE SPECIMEN SOURCES TO SUPPORT CLINICAL TRIALS
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Background: Infection with HIV/AIDS remains a significant public health concern, and a primary modality in disease management and treatment is the administration of antiretroviral (ARV) agents. In order to better understand the pharmacokinetic-pharmacodynamic (PK-PD) relationships of ARVs in disease prevention and management, compartmentalized PK studies are required to assess localized drug concentrations. This work focuses on the development and validation of liquid chromatographic-tandem mass spectrometric methods for the quantification of emtricitabine (FTC) and tenofovir (TFV) in cervicovaginal secretions (CVS) and rectal fluid (RF).

Methods: Blank CVS and RF were collected from human subjects who consented to an IRB-approved protocol for biological sample collection. Matrices were spiked with ARVs. To mimic clinical studies, drug-containing samples were applied to Dacron swabs. FTC and TFV were removed from swabs, and extracted materials were combined with internal standards and subjected to solid phase extraction. Post-reconstitution, samples were separated via reversed-phase liquid chromatography, and analytes were detected on an API 4000 mass spectrometer (SCIEX) operated in selective reaction monitoring mode. Assays were validated in accordance with the Food and Drug Administration (FDA) Guidance for Industry, Bioanalytical Method Validation guidelines.

Results: The analytical measuring ranges for FTC and TFV in both CVS and RF applied to polyester-based Dacron swabs were 2.5 - 640 ng/swab and 0.625 - 160 ng/swab, respectively. Quality control (QC) samples prepared at the lower limit of quantitation, as well as low, mid and high levels yielded intra- and inter-assay %CVs ≤12.1% for FTC and ≤16.0% for TFV for both matrices. Both analytes were stable in CVS and RF following three freeze thaw cycles, three days in sample matrix, and 3 days post-extraction from the Dacron swab, and demonstrated ≤15% deviation from immediately prepared samples. Matrix effects studies demonstrated ion suppression for FTC (71.3% of expected peak area signal) and TFV (63.9% of expected signal) in RF; however internal standards showed comparable suppression (72.2% and 61.7%, respectively), indicating negligible relative matrix effects. Minimal ion suppression was observed in CVS for both analytes.

Conclusions: The described work illustrates the workflow and considerations required for the development and validation of assays using CVS and RF.

034

INHIBITION OF PACLITAXEL METABOLISM BY LOSARTAN AND A LOSARTAN METABOLITE DEPENDS ON CYP2C8 AND CYP2C9 GENOTYPES OF HUMAN LIVER MICROSOMES
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Background
Our previous study (using human liver microsomes, HLms) suggested that differences in losartan-mediated inhibition of paclitaxel metabolism reflected distinct CYP2C8 genotypes (*1/*1 vs. *1/*3). The present study aimed to determine the effect of other CYP2C8 variants on the losartan-paclitaxel interaction. We noted that EXP-3174, an active metabolite of losartan, is generated primarily by CYP2C9, suggesting that CYP2C9 variants might affect inhibition of paclitaxel metabolism by losartan. We therefore evaluated the amount of EXP-3174 generated in the incubation mixture in relation to CYP2C9 genotypes.
Methods
We carried out an incubation study using seven HLMs with different variants of CYP2C8 (*1/*1, *1/*2, *1/*3, and *3/*3) and CYP2C9 (*1/*1, *1/*2, *2/*2, and *1/*3). These HLMs were prepared and genotyped from healthy organ donor livers after approval of the Ethics Review Board at the Karolinska Institutet. Losartan concentrations in the incubation mixture were set at 1, 5, 10, 25, and 50 µmol/L. After incubation at 37 ºC for 30 min, each incubation mixture was divided in two, and separate aliquots were used to measure the concentrations of paclitaxel metabolites (using HPLC-UV) or the concentration of EXP-3174 (using HPLC-fluorescence). All incubations were performed in triplicate.

Results
Losartan at 1 µmol/L significantly (p < 0.01) inhibited paclitaxel metabolism in HLMs harboring both CYP2C8*3/*3 and CYP2C9*2/*2 (7.1 ± 1.1% inhibition (mean ± SD)); no significant inhibition was observed in HLMs harboring other CYP2C8 and CYP2C9 variants. In the presence of 1 µmol/L of losartan, HLMs harboring CYP2C9*2/*2 produced EXP-3174 at levels 37.1-65.2% of those seen in HLMs harboring CYP2C9*1/*1; comparable decreases in EXP-3174 levels were seen in HLMs harboring CYP2C9*1/*3.

Conclusions
The present study showed that 1 µmol/L losartan inhibited paclitaxel metabolism in only HLMs harboring both CYP2C8*3/*3 and CYP2C9*2/*2; the effect appeared to be mainly influenced by CYP2C8 genotype. Considering the maximum plasma concentration of losartan and the accumulation of the compound in the liver in clinical settings, treatment with losartan could result in decreased paclitaxel metabolism in CYP2C8*3/*3 carriers dosed with both losartan and paclitaxel.

035
COULD DIFFERENCES IN POTASSIUM BASED HEMATOCRIT EXPLAIN DEVIATIONS BETWEEN DRIED BLOOD SPOT AND PLASMA ANALYSIS OF AMITRIPTYLINE, NORTRIPTYLINE, CLOMIPRAMINE, DESMETHYL-CLOMIPRAMINE, VENLAFAXINE, AND O-DESMEHYLVENLAFAXINE IN PATIENT SAMPLES?
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Background
Variations in hematocrit, can cause bias in dried blood spot (DBS) analysis. During prior validation in spiked samples, a negative bias up to ~30% was observed in DBS analysis of amitriptyline (ATP), nortriptyline (NTP), clomipramine (CMP), desmethyl-clomipramine (DCMP), venlafaxine (VEN), and O-desmethylvenlafaxine (ODV) when hematocrit was ≤ 0.30 L/L. Based on a potassium assay, we assessed if bias between DBS and plasma concentrations observed in paired patient samples of the mentioned antidepressants (AD) was related to hematocrit values. Methods DBS potassium concentrations were used to calculate the hematocrit of the DBS. Due to the study design, only for the minority of the patient samples, measured hematocrit was available. A DBS punch of 6 mm was extracted by two consecutive extractions with 2x 200µL KCL (2.5 mM) and potassium concentrations were analyzed by indirect potentiometry. Concentrations of the AD in DBS and plasma were analyzed by LC-MS/MS. Visual inspection of data plots and Pearson’s correlation coefficient were used to assess if there was a relation between the hematocrit and the bias between DBS and plasma analysis. Results In total, hematocrit (potassium derived) of 119 patients samples were analyzed (ATP:10; NTP:52 ; CMP: 43; DCMP:45; VEN: 16; ODV: 19). The hematocrit of these samples ranged from 0.30 - 0.56 (L/L). For the relation between potassium derived and measured hematocrit, 21 paired samples we available (r² = 0.67). Bland-Altman analysis indicated a mean difference of 0.01 (L/L). Except for ATP (r² = 0.59 ; p < 0.01 ), we observed no relationship in the bias observed between DBS and plasma concentrations of the AD and the hematocrit (potassium derived) found in the DBS (r² < 0.1). Although results were significant for ATP, they should be interpreted with caution, due to the small sample size. Conclusions We were able to asses hematocrit in patients DBS by potassium analysis. For ATP, more research is needed to assess if a hematocrit correction could improve analytical results. However, for the other AD the results revealed it is unlikely that a hematocrit correction would reduce the bias between DBS and plasma analysis.

036
A COMPARATIVE BLIND STUDY BETWEEN ELECTROCHEMILUMINESCENT IMMUNOASSAY ON 3 DIFFERENT ANALYZERS AND LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY FOR TACROLIMUS MONITORING.
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Background: Therapeutic drug monitoring of Tacrolimus is recommended in organ transplantation. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) is the gold standard method. In despite of the fact that immunosassay allows a faster time of results compared with LC-MS/MS, its main drawback is a lack of specificity, with an overestimation of tacrolimus blood concentration. Immunoassays include various methods (MEIA, EMIT, ACMIA, QMS) and Roche Diagnostics® have recently developed an electrochemiluminescent enzyme reagent (ECLIA).

Objectives: The purpose of our work is to compare tacrolimus blood concentrations between ECLIA in 3 different Cobas analyzers and LC-MS/MS method.

Methods: Three laboratories contribute in this study. In a first time samples are analyzed by a validated LC-MS/MS method (XEVO WATERS, "one minute kit ChromSystem®) in Timone Hospital Marseille. Then, aliquots are done and tacrolimus blood concentrations are determined on Cobas E411 (Marseille), MODULAR Evo 170 (Paris), and Cobas Module E602 (Grenoble) using ECLIA reagent. All dosages by ECLIA are performed at the same day. We compare both tacrolimus blood concentrations obtained on the different analyzers with LC-MS/MS and analyzers each other. Statistical analysis is performed by Analyse-it software.

Results: The study compared 89 Tacrolimus blood concentrations ranging from 1.1 ng/ml to 36.2 ng/ml (26 from 1 - 5 ng/ml; 36 from 5-10 ng/ml; and 27 >10 ng/ml). The comparison of ECLIA on the 3 automates with LC-MS/MS shows Spearman correlation coefficients higher than 0.95, bias (Bland Altman) lower than 20%. As expected, overestimation is noticed for all immunoassays and the highest bias is observed for LC-MS/MS versus CobasE411 (20.3%). Same results are observed comparing 62 concentrations picked up in the 89 samples ranging from 1 to 11 ng/ml.

There is no difference between the 3 analyzers compared each other.

Conclusion: Our results show a better correlation between LC-MS/MS and ECLIA on Modular Evo 170, or Cobas Module E602 than Cobas E411. The current trend calls for a minimization of doses. ECLIA method on both Modular Evo 170 and Cobas Module E602 are capable of measuring tacrolimus concentrations in the clinically relevant range between 1 and 11 ng/ml.

A LARGE CANDIDATE GENE APPROACH OF THE CALCINEURIN PATHWAY IDENTIFIES TWO VARIANTS ASSOCIATED WITH THE RISK OF SERIOUS INFECTIONS IN CALCINEURIN INHIBITORS-TREATED RENAL TRANSPLANTS

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Background: Interindividual variability in calcineurin inhibitors (CNI) efficacy and toxicity may be explained by genetic variations in proteins of the calcineurin pathway. Methods: We strategically selected candidate variants in a gene panel of the pathway and investigated, in a population of 381 renal transplant recipients, their influence on two major phenotypes in transplantation: biopsy proven acute rejection (BPAR) and serious infections within the first year post-transplantation. The effect of variants as well as of covariates (HLA mismatch score, age, gender of the recipient, cytomegalovirus (CMV) donor/recipient serology, CMV infections and immunsuppressant blood exposure) was investigated using time-dependent Cox proportional hazards regression models (R software version 3.1.1). The p-value threshold was set to 0.002 in order to take into account multiple testing (Bonferroni correction).

Results: Twenty-three genetic polymorphisms in 13 key proteins were studied. Those candidates were (i) Single Nucleotide Polymorphism (SNP) reported in the NCBI SNP database (dbSNP; as of August 2014) with a minor allele frequency (MAF) ≥ 10% in Caucasians; (ii) additional variants reported in the literature with convincing clinical or functional data and (iii) variants in the proximal promoters of genes coding for proteins directly interacting with
CNI (i.e. cyclophilin A, FKBP12 and calcineurine A and B subunits) identified through Sanger direct resequencing in 75 DNA from Caucasian healthy volunteers. None of the genetic variants studied significantly associated with acute kidney graft rejection or serious infections. CMV status combination (donor +/recipient -) was the major predictor of the serious infection risk.

Conclusions: We found no effect of a carefully selected panel of SNP in the calcineurin pathway on clinical outcomes in renal transplantation. Specifically, we found no impact of the IL2 rs2069762, which is in line with recent literature. Potential low size SNP effects might have been veiled by other clinical covariates with large significant effects but this large exploratory study cast doubts on the potential interest of genetic biomarkers related to CNI pharmacodynamics.

PRELIMINARY RESULTS ON THE PHARMACOEPIGENETICS OF CALCINEURIN INHIBITORS USING AN IN-VITRO MODEL: PITFALLS AND PERSPECTIVES

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Background: Transcriptional changes related to epigenetic modifications of the genome contribute to inter-individual variability in numerous phenotypes and increasing evidence suggests that pharmacological treatments with common drugs may affect patient epigenomes. This exploratory study aimed to investigate in vitro the influence of the calcineurin inhibitors (CNI), cyclosporine and tacrolimus, on epigenetic marks in the promoter of Interleukine 2 gene (IL2) constituting their pharmacodynamic effector. Methods: Experiments were conducted using immortalized T lymphocytes (JURKAT cells) exposed to the drugs (24hours; 5mM cyclosporine and 0.05mM tacrolimus) after stimulation with phorbol 12-myristate 13-acetate(PMA)/ionomycine. Two types of epigenetic regulation mechanisms were studied: (i) DNA-packaging histones (H3K4me3, H3K36me3 as a permissive state and H3K27me3 as a repressive state), and (ii) Methylation of the cytosine residues of DNA (5-MeC). These marks were detected using ChiP (Chromatin ImmunoPrecipitation) and MeDIP (Methylation DNA ImmunoPrecipitation) techniques, respectively. In each case, immunoprecipitation was followed by DNA purification and quantitative real-time PCR analyses (qPCR) to compare methylation profiles in treated versus control cells. Experiments were performed in triplicate. Results: Using this approach, no clear change in the methylation of IL2 promoter was observed. The results concerning the methylation of histones were highly variable but did not suggest that CNI have substantial effects either. Conclusions: The preliminary results obtained in vitro point out several limits and pitfalls related to the cellular model and the qPCR approach. The cellular model chosen has been adequate for the development of a method but it has several limitations (e.g. immortalized cancer cells can have intrinsic epigenetic modifications). In addition, the required duration of drug exposure to observe any epigenetic modifications caused by CNI needs to be further explored. Finally, qPCR seems too restrictive for the detection of epigenetics changes. A study on mice exposed to cyclosporine and tacrolimus (from 1 day to 3 months) with CD4+ T cells isolation and untargeted detection of epigenetic modifications using Next Generation Sequencing is on-going to solve these deficiencies. The limits and major pitfalls related to the cellular model, as well as preliminary results obtained in mice will be presented.

IMMUNOASSAY-BASED THERAPEUTIC DRUG MONITORING OF 5-FU IMPROVES TREATMENT OF METASTATIC COLORECTAL CANCER (MCRC) PATIENTS

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Background: Body surface area (BSA)-based dosing of 5-FU leads to significant underexposure (assessed by area under the concentration-time curve, AUC) in the majority of patients. Personalized TDM for dosing of 5-FU can optimize an individual’s exposure, resulting in a higher overall dose intensity with reduced toxicities and improved outcomes. This study was initiated to validate the use of TDM with an immunoassay to personalize 5-FU dosing in mCRC patients treated in routine clinical practice.

Methods: 75 mCRC patients from 8 different medical centers in Germany treated with a 5-FU infusional regimen (AIO, n=16; FOLFIRI6, n=26; FUFOX, n=33) were followed for up to 6 cycles of therapy. Initial 5-FU dosing for all patients was based on BSA. Individual 5-FU exposure (AUC) was determined from a single blood sample drawn during each infusion using a proprietary immunoassay. To achieve a target AUC of 20 to 30 mg·h/L, subsequent doses of infusional 5-FU were adjusted according to the AUC in the previous cycle. Primary objective - confirm that personalized TDM of 5-FU to optimize exposure resulted in an increased proportion of patients achieving target AUC range at cycle 4 versus cycle 1. Secondary objective - determine whether personalized TDM of 5-FU reduced treatment-related toxicities compared to historical observations.

Results: Median 5-FU AUC at cycle 1 was 16 mg·h/L (coefficient of variation, %CV=75%), with the majority of patients (62%) falling below, and only 32% falling within, the target exposure range. By cycle 4, median 5-FU AUC was 26 mg·h/L (%CV=27%), with significantly more patients having optimal exposure (55% within target AUC range, p=0.005). Overall, 53% of patients had their 5-FU dose increased, 21% were decreased, and 25% remained unchanged. Additionally, fewer 5-FU-related grade 3-4 toxicities of diarrhea (5%), nausea (3%), fatigue (0%) and mucositis (0%) were observed compared to historical data (12%, 9%, 12%, 15%, respectively).

Conclusion: Optimization of 5-FU dosing using an immunoassay for TDM in routine clinical practice resulted in significantly higher 5-FU exposure, more patients achieving optimal dosing, and less 5-FU-related toxicity. The increase in exposure with simultaneous reduction in toxicity demonstrates the potential for TDM in oncology.

040

TDM OF PACLITAXEL (PTX) REDUCES SEVERE TOXICITY AND MAINTAINS EFFICACY IN ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC): RESULTS OF A RANDOMIZED STUDY

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Background: Body surface area (BSA)-based dosing of PTX results in highly variable individual exposure. PTX exposure (time above threshold concentration, Tc>0.05) has been shown to predict toxicity. Neuropathy is a particular problem in PTX regimens: it cannot be resolved with medication and may become permanent or require suspension of PTX treatment. This study was initiated to validate use of TDM for PTX dosing to optimize exposure in advanced NSCLC patients.

Methods: 304 advanced NSCLC patients were randomly assigned to receive up to 6 cycles of first-line 3-weekly carboplatin AUC6 combined with PTX either at a standard dose of 200mg/m² (Arm A) or at a TDM-guided dose (Arm B). Initial PTX dose in Arm B was between 150 to 200mg/m² based on age and sex. PTX exposure was determined from a single blood sample drawn 18-30Hr after infusion initiation using LC-UV assay. PTX doses were adjusted according to the previous cycle PTX exposure to target a Tc>0.05 between 26 and 31 hours using our
Dosing algorithm. Primary objective of the study was to detect an 11% reduction of grade 4 neutropenia with TDM-guided PTX dosing. Secondary objectives included comparison of neuropathy rates, tumor response and progression-free survival (PFS) between the study arms.

**Results:** Compared to standard dosing, TDM-guided dosing of PTX reduced the incidence of grade 4 neutropenia (measured on day 15 of each cycle) (21% vs. 15%, P=0.029), grade ≥2 neutropathy (27% vs. 14%, P<0.001), and grade ≥3 neutropathy (8% vs. 1%, P<0.001). TDM-guided dosing of PTX lead to a dose reduction in 62% and a dose increase in 17% of the patients. The proportion above the target exposure was reduced from 41% in cycle 1 to 2% in cycle 6. Objective response rate between Arms A and B was similar (32% vs. 29%, P=0.70), as was the median PFS (5.2 vs. 4.7 months, hazard ratio 1.1, 95% CI 0.8-1.4, P=0.54).

**Conclusion:** TDM-guided dosing of PTX improved the risk-benefit profile in patients with advanced NSCLC. The dosing algorithm resulted in reduction of the worst toxicities, grade 4 neutropenia and neuropathy ≥ grade 3, without impacting efficacy.

**041**

**DRIED URINE SPOTS AN ALTERNATIVE FOR DRUG TESTING? COMPARISON OF DETECTABILITY USING AN LC-MSN SCREENING APPROACH AFTER URINE PRECIPITATION**

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**Background:** Sample storage and stability may be an issue for urine drug testing or adherence monitoring, especially for long time storage or long distances between sampling point and analysis. Dried urine spots (DUS) might be an alternative although the low sample volume could be challenging. The aim of this study was the comparison of DUS analysis after simple liquid extraction with urine analysis after urine precipitation (UP).

**Methods:** For determination of recoveries, matrix effects, and process efficiencies, blank urine or water were spiked with 1 mg/L each of twenty compounds covering a wide range of physico-chemical properties. For determination of detection limits, urine concentrations between 10,000 and 0.1 µg/L were tested. Sample preparations were as follows. Whatman Protein Saver Cards were spiked with 20 µL urine or neat samples and dried at room temperature. After cutting 12 mm diameter spots, microwave assisted enzymatic deconjugation followed by liquid extraction with dichloromethane-methanol mixture (1:1) containing 1% ammonia were performed. UP was done in accordance to Wissenbach et al. (ABC 2011). All extracts were reconstituted in 100 µL mobile phase and analyzed by LC-MS\(^\text{a}\) (Bruker AmaZon Speed). To test the applicability in routine clinical toxicology, over 50 authentic urine samples were prepared as described above using DUS and UP and the screening results compared.

**Results:** Recoveries were 38-76% (±13% mean), matrix effects 55-222% (±38% mean), and process efficiencies 21-135% (±21% mean). Limits of detection ranged from >10,000-100 µg/L for DUS and >10,000-10 µg/L for UP. In the authentic urine samples, 98 different drugs out of 36 categories could be detected in total. Altogether, 258 compounds could be identified, whereby 240 (93%) compounds were found using UP and 204 (79%) using DUS. In cases of missed compounds in DUS, the analytes were only presented in traces either due to low therapeutic ranges or long time difference between drug intake and urinalysis.

**Conclusion:** The developed sample preparation procedure for DUS showed higher detection limits compared to UP caused by the lower sample volume and dilution factors. Nevertheless, it was suitable for detection of various compounds comparable with the established screening approach.

**042**

**25B-NBOME: METABOLISM AND DETECTABILITY OF A NEW SYNTHETIC HALLUCINOGEN - STUDIED BY GC-MS, LC-MSN, AND LC-HR-MSN**

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**Background:** 25B-NBOMe belongs to a new class of highly potent 5-HT\(_{2A}\) receptor agonists, which are frequently abused due to their intense hallucinogenic potential. In the past, it was involved in several intoxication cases. Therefore, the aim of the presented work was to study its phase I and II metabolism and its detectability in standard screening approaches (SUSA) using GC-MS, LC-MSN, LC-HR-MS\(^n\) and LC-HR-MS/MS.

**Methods:** After application of 25B-NBOMe HCl to male Wistar rats for toxicological diagnostic reasons (10 and 0.1 mg/kg BW for metabolism and toxicological detection studies, respectively), urine was collected over 24h. The phase I metabolites were extracted and analyzed directly or after enzymatic cleavage by SPE (HCl) followed by GC-MS (TF ISQ) after acetylation and LC-HR-MS\(^n\) (TF Velos Orbitrap Pro) according to Welter et al (ABC 2013).

**Results:** Compared to standard dosing, TDM-guided dosing of PTX reduced the incidence of grade 4 neutropenia (measured on day 15 of each cycle) (21% vs. 15%, P=0.029), grade ≥2 neutropathy (27% vs. 14%, P<0.001), and grade ≥3 neutropathy (8% vs. 1%, P<0.001). TDM-guided dosing of PTX lead to a dose reduction in 62% and a dose increase in 17% of the patients. The proportion above the target exposure was reduced from 41% in cycle 1 to 2% in cycle 6. Objective response rate between Arms A and B was similar (32% vs. 29%, P=0.70), as was the median PFS (5.2 vs. 4.7 months, hazard ratio 1.1, 95% CI 0.8-1.4, P=0.54).

**Conclusion:** TDM-guided dosing of PTX improved the risk-benefit profile in patients with advanced NSCLC. The dosing algorithm resulted in reduction of the worst toxicities, grade 4 neutropenia and neuropathy ≥ grade 3, without impacting efficacy.
The phase II metabolites were analyzed after urine precipitation by LC-MS\textsuperscript{(2)} (TF LXQ) and LC-HR-MS\textsuperscript{(2)}. For the detectability studies, our four standard urine screening approaches (SUSA) by GC-MS, LC-MS\textsuperscript{(2)}, and LC-HR-MS/MS (TF O-Exacte) as well as the Bruker ToxTyper LC-MS\textsuperscript{(2)} system were applied to rat urine samples after low dose. Finally, an initial CYP activity screening was performed to identify CYP isoenzymes involved in the major steps according to Welter et al., 2013, 2014.

**Results:** 25B-NBOMe was mainly metabolized by O-demethylation, O,O-bis-demethylation, N-debenzylation, hydroxylation of the benzyl moiety, and combinations of them. The O-demethyl metabolites were additionally glucuronidated or sulfated. Intake of 25B-NBOMe was detectable, mainly via its metabolites, by the LC-MS\textsuperscript{(2)}, LC-HR-MS/MS, and by the ToxTyper system with the customized Maurer/Wissenbach/Weber library (Wiley-VCH, 2014). The GC-MS SUSA was not sensitive enough. Initial CYP activity screening revealed the involvement of CYP2C9 and CYP2C19 in O-demethylation, CYP2B6 and CYP3A4 in N-debenzylation, and CYP1A2 and CYP3A4 in hydroxylation.

**Conclusion:** The presented study demonstrated that 25B-NBOMe was extensively metabolized and could be detected by the three LC-MS screening approaches. Since CYP2C9 and CYP3A4 were involved in the main initial metabolic steps, interactions might occur in certain constellations.

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**043**

**POPULATION PHARMACOKINETICS AND EXPOSURE-RENAL TOXICITY STUDY OF CYCLOSPORINE A DURING THE FIRST WEEKS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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**Background**

Cyclosporine A (CsA) is an immunosuppressant used in prophylaxis and treatment of Graft versus Host Disease (GVHD) after allogenic hematopoietic stem cell transplantation (SCT). Our objectives were to describe pharmacokinetics of CsA and to study the association between CsA exposure and the occurrence of renal toxicity during the first weeks following transplantation in patients treated for various hematological malignancies.

**Materials and methods**

Data from 66 adult patients treated by CsA for the prophylaxis of GVHD after allogenic SCT were studied retrospectively. 133 pharmacokinetic profiles of CsA with 3 to 10 blood samples were used to perform pharmacokinetic modeling of CsA with Monolix® software. Association between CsA exposure (based on trough levels (C\textsubscript{T}) and area under the curve (AUC\textsubscript{12h})) and renal toxicity (defined by a decrease in glomerular filtration rate of at least 30% from the initial value measured at the time of transplantation) was investigated using time dependent Cox models.

**Results**

A two compartment model with absorption following a gamma distribution and first order elimination was used to describe CsA concentrations (observed vs predicted concentrations: r\textsuperscript{2}=0.9781; relative bias=0.21%; RMSE=9.4%) and to predict AUC (trapezoidal vs modeled AUC: r\textsuperscript{2}=0.9940; bias=0.21%; RMSE=2.4%). AUC\textsubscript{12h} was significantly associated with renal toxicity (per unit increase: HR=1.096 [1.021-1.175]; p=0.011) while no association was shown with trough levels (per unit increase: HR=1 [0.999-1.001]; p=0.77). An AUC threshold of 4.4 mg.h.L\textsuperscript{-1} (sensibility=82%, specificity=41%) was determined by ROC analysis, patients over this threshold have a significantly increased risk of renal toxicity (high vs low: HR=1.735 [1.272-2.367]; p=5.10\textsuperscript{-4}).

**Conclusion**

These results are in favor of CsA dose adjustment in allogenic SCT based on AUC estimation: we determined that an AUC\textsubscript{0-12h} of 4.4 mg.h.L\textsuperscript{-1} was associated with renal toxicity in the first weeks following transplantation.

**Key words**

Cyclosporine, stem cell transplantation, population pharmacokinetics

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**044**

**IMPACT OF GENETIC POLYMORPHISMS OF P-GLYCOPROTEIN (ABCB1) ON INTRACELLULAR ACCUMULATION AND THERAPEUTIC ACTIVITY OF TYROSINE KINASE INHIBITORS.**

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**INTRODUCTION:** ABCB1 transporter, or P-glycoprotein, is an efflux protein implicated in the absorption and the distribution of various compounds, including anticancer drugs as tyrosine kinase inhibitors (TKIs). It has been
demonstrated that the overexpression of ABCB1 is one of the mechanisms responsible of resistance to TKIs treatments. Many single nucleotide polymorphisms (SNPs) have been reported for ABCB1. Some of these SNPs, alter the expression and/or the activity of ABCB1, and hence may affect the pharmacokinetics of its substrates. More particularly, the ABCB1 1199G>A SNP located in exon 11 appears quite promising, given its relatively high frequency in the Caucasian population and the location of the associated amino acid substitution within an important site of the protein (S400N).

**Aims:** The present study focuses on the impact of ABCB1 1199G>A SNP on the in vitro disposition of TKIs which are transported by ABCB1.

**Results:** Two recombinant cell lines, i.e. Human Embryonic Kidney (HEK293) and Human Myelogenous Leukemia (K562) cells were transfected with ABCB1 cDNA gene either wild-type (1199G) or mutated (1199A). Recombinant cell lines were shown to overexpress ABCB1. FACS also confirmed that ABCB1 was present at the cell membrane surface and rhodamine123 assays, in presence or absence of ABCB1 specific inhibitor (LY335979), further demonstrated that the protein was functional. Then, cellular proliferation assays were performed by measuring the incorporation of radioactive thymidine in the presence of TKIs in our K562 recombinant cell lines. We demonstrated that recombinant cell lines were more resistant to imatinib, nilotinib and dasatinib than controls cells, confirming that these TKIs are transported by ABCB1. Interestingly, cells expressing the 1199A variant were more resistant compared to those expressing the wild-type 1199G. Moreover, TKIs intracellular accumulation assays were performed by using radioactivity measurements after exposing cells to C14-TKIs. However, no difference was observed in 1199A variant compared to wild-type 1199G.

**Conclusions:** Our results demonstrated that the 1199A variant is associated with an increased resistance to imatinib, nilotinib and dasatinib, probably reflecting a higher ABCB1 activity towards these three drugs. However, our results do not indicate that this difference of resistance is related to a modification of TKIs intracellular concentrations.

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**T-EFFECTOR/T-REGULATORY BALANCE AS PREDICTIVE BIOMARKER OF THE RISK OF REJECTION AND GRAFT CLINICAL OUTCOME IN LIVER TRANSPLANT RECIPIENTS**

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**Background:** Alloreactive T cells are the key mediators of the alloimmune responses that lead to acute and chronic rejection of an allograft. Their monitoring after organ transplantation may allow us to understand the real immune status of the patient, and therefore, to personalize the immunosuppression to their real needs. However, allograft outcome is determined by the balance of effector and regulatory mechanisms. Both mechanisms are not independent and they are closely related.

**Methods:** Sixty-four liver transplant recipients were recruited from four Spanish centers. Intracellular expression of IL-2 and IFN-γ (T-effector response) as well as the frequency of circulating T-regulatory cells (Treg response: %CD4⁺CD25⁺highCD45RO⁻CD62L⁻) were evaluated both pre and post-transplantation (1st and 2nd week, 1st, 2nd, 3rd, 6th and 12th month).

**Results:** Fourteen liver recipients experienced AR before the end of 1st month post-transplantation. Pre- and post-transplantation intracellular expression of IL-2 and IFN-γ in CD4⁺ and CD8⁺ T cells identified transplant patients at high risk of AR. Furthermore, patients with a documented AR after the episode of AR was solved showed a persistent increased of IL-2 and IFN-γ in CD8⁺T-cells during the year of follow up in comparison with patients free of AR. At 1st month after transplantation, the frequencies of CD4⁺CD25highCD45RO⁻CD62L⁻ cells was significantly higher in AR patients than in NAR patients, and during the 1st year post-transplantation the frequency of this circulating Treg cells also was higher in AR than in NAR recipients, maybe as a response of the immune system to compensate this greater alloreactivity. When we evaluated the ratio T-effector/T-regulatory response, the results shown that this is ratio is also a good early predictive biomarker of AR in the early post-TX period (before 1st month) and also in Pre-TX, patients who rejected had higher ratio than those who did not rejected. Interestingly, one year follow up, no significant difference was observed between patients free of AR and patients with an AR event in their past history after AR was solved.

**Conclusions:** The balance between effector and regulatory T cell activity could be a more accurate tool to predict risk of rejection and identify patients candidates for a slightest immunosuppression.
CENTRAL MEMORY REGULATORY T CELLS (CD4+CD25hiCD45RO+CD62L+) PREDICTS ACUTE REJECTION IN ORTHOTOPIC LIVER TRANSPLANT RECIPIENTS ALONG FIRST MONTH POST-TRANSPLANTATION: A MULTICENTER STUDY
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Background: A number of single-center studies have analyzed the potential of T-regulatory cells (Tregs) as biomarkers of acute rejection (AR). Although their role in maintaining hyporesponsiveness status is being elucidated, the function of Tregs in AR processes is unresolved and remains controversial. Multi-center studies should be performed in order to achieve a better correlation.

Methods: Four Spanish centers included sixty-four liver transplant recipients. All participating centers used identical standard operating procedures. The percentages of memory regulatory T cells (cmTregs: CD4+CD25hiCD45RO+CD62L-) were monitored by flow cytometry at pre-transplant and at 1 week, 2 weeks, 1 month, 2 months, 3 months and 1 year after transplantation.

Results: Fifteen patients developed AR episodes (23.4%), all of them occurred within the first month post-transplantation. No significant differences on Tacrolimus or Mycophenolic acid concentrations between rejectors and non-rejectors were observed. All cmTregs populations markedly decreased during the first months post-transplantation. The kinetics of peripheral blood cmTreg cells was similar between AR and NAR: the number of peripheral blood cmTreg cells showed a sharp increase at 1st week after transplantation, decreased afterwards, and stabilized over the first year after transplantation. During the 1st year after OLT, the frequency of circulating cmTreg cells was higher in AR than in NAR recipients. Specifically, at 1 month after OLT, the frequencies of cmTreg cells was significantly higher in AR patients than in NAR patients (NAR=2.84±2.29; AR=4.53±2.70; P=0.028). Cut-off values for predicting AR were determined based on the area under the ROC curves for cmTreg cells, which showed significantly high levels in AR patients. ROC curve analysis showed that patients with a percentage of cmTregs higher than 3.7%, sensitivity of 72.73%, and specificity of 81.25% had a 1.3-fold increase in the risk of AR.

Conclusions: Based on this data, liver transplant patients who suffered AR during the first month after showed higher frequencies of peripheral CD4+CD25hiCD45RO+CD62L+ in this period, which could be useful for assessing the immune status of graft recipients. We developed a prediction model for assessing risk of AR that can provide clinicians with useful information for managing patients individually and customizing immunosuppressive therapies.

T CELL FUNCTION MONITORING AFTER ALLOGENEIC STEM CELL TRANSPLANTATION: A USEFUL TOOL FOR PERSONALIZED IMMUNOSUPPRESSION
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Background: Pharmacodynamic (PD) monitoring has been proposed as a useful tool for evaluating individual responses to immunosuppressive drugs and for predicting the risk of rejection in organ solid transplantation. Limited PD data related to allogeneic stem cell transplantation (allo-SCT) are available.

Methods: We have studied the relationship between a specific panel of PD biomarkers as indicators of the immunomodulatory effect of cyclosporine (CsA) and mycophenolate mofetil (MMF) and graft-versus-host disease
IS THERE A PLACE FOR ORAL FLUID TESTING IN THERAPEUTIC DRUG MONITORING OF ANTIPSYCHOTICS?

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Background: In a psychiatric population, the need for alternative ways of performing therapeutic drug monitoring (TDM) is high. Therefore, oral fluid (OF) is suggested as an interesting matrix.

Methods: Our UHPLC-MS/MS method for quantification of antipsychotics in serum was optimized and validated for OF testing [Patteet et al. 2014]. After liquid-liquid extraction of 500 µl of OF, using methyl tert-butyl ether at pH 9.5, the extract was reconstituted in 50 µl acetonitrile. Instrumentation specifications were identical to the serum method. Serum and OF samples (Quantisal® Immunalysis) were collected from schizophrenic or bipolar patients at 3 psychiatric hospitals. Patients had to be in 'steady-state' condition. All samples were taken at the same time (trough concentration). Scatter plots of serum and OF concentrations and median, range, and SD of the OF:serum ratios were calculated for all antipsychotics found in the patient samples.

Results: Linearity was evaluated on 8 calibration levels. Accuracy criteria were fulfilled for all compounds (bias ≤ 15%, for LLOQ ≤ 20%), except for norclozapine, paliperidone and quetiapine at LLOQ. Data from intra- and inter-day precision did not exceed the imposed CV (≤ 15%, for LLOQ ≤ 20%), except for norclozapine and quetiapine at LLOQ. Mean recovery was 45.5% (21.2-84.4%) when 1 ml of OF was collected and 46.9% (17.2-89.8%) for 0.5 ml of OF. When only 0.1 ml of OF was collected, result of recovery were unreliable. Mean absolute matrix effect (ME) was 101.1% (82.0-120.0%). IS corrected ME were all in accordance with the criteria (CV ≤ 15%). Ninety-eight OF patient samples containing 269 antipsychotics and metabolites, were acquired and the volume of collected OF was determined. The OF:serum ratios were > 1 for most antipsychotics (amisulpride: median (range) 5.09 (0.78-100.37), bromperidol: 10.53 (2.39-24.85), clozapine: 1.91 (0.72-35.05), haloperidol: 4.28 (2.72-16.21), olanzapine: 6.01 (0.16-37.67), paliperidone: 1.88 (0.19-21.90), quetiapine: 1.83 (0.38-9.07), risperidone: 2.67 (0.76-17.98), zuclopenthixol: 1.03 (0.22-2.65). Only for aripiprazole the ratio was 0.23 (0.09-0.66).

Conclusion: The UHPLC-MS/MS method can be used for quantification of antipsychotics in OF. However, the wide range of the ratios suggests that OF concentrations are not reliable enough for TDM of antipsychotics.

ARE CAPILLARY DRIED BLOOD SPOTS APPLICABLE FOR THERAPEUTIC DRUG MONITORING OF COMMON ANTIPSYCHOTICS? A PROOF OF CONCEPT

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Background: Dried blood spot (DBS) sampling has been proposed as an alternative for classical blood collection in therapeutic drug monitoring (TDM) of antipsychotics. A DBS method for quantification of 15 antipsychotics and 7 metabolites was developed and analytically validated [Patteet et al. DTA 2014]. A clinical validation, with comparison between capillary and venous blood concentrations, is mandatory for implementation in routine practice.

Methods: Serum, venous whole blood and fingerprick capillary blood was collected from schizophrenic or bipolar patients at 3 psychiatric hospitals. Patients had to be in 'steady-state' condition. All samples were taken at the same time (trough concentration). All samples were analyzed using validated UHPLC-MS/MS methods for the two matrices [Patteet et al. DTA 2014; Patteet et al. CCA 2014].
First, blood:serum ratios were calculated, compared with literature and whole blood therapeutic reference ranges were defined. Second, venous whole blood concentrations were compared with venous blood spotted on DBS (v-DBS) and capillary blood spotted on DBS (c-DBS) by calculating ratios and by Passing-Bablok regression analysis. Finally, the obtained blood levels were evaluated according to the clinical interpretation.

**Results:** Samples from 111 patients (75 male, 36 female; age 19-65 years) were included in the study. From 100 patients, serum, whole blood and v-DBS were collected. From 73/100 patients, also c-DBS was taken. Eleven antipsychotics were found: amisulpride, aripiprazole, bromperidol, clozapine, haloperidol, olanzapine, paliperidone, pipamperone, quetiapine, risperidone and zuclopenthixol. The calculated blood:serum ratio was in accordance with literature except for olanzapine. Whole blood therapeutic ranges can be calculated from serum therapeutic ranges by using this ratio. Calculation of DBS: blood ratios and Passing Bablok regression analysis, demonstrated that concentrations obtained by DBS analysis were highly comparable to those obtained by conventional whole blood analysis. Clinical interpretation of DBS concentrations will result in a reliable conclusion, which will be almost identical to the interpretation of venous and serum concentrations (sensitivity 91.6%-96.7%).

**Conclusion:** This is the first clinical study demonstrating the value of DBS sampling in TDM of commonly used antipsychotics. DBS is a promising alternative for classical blood sampling and can be used in routine practice.

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**051**

**THERAPEUTIC DRUG MONITORING (TDM) AND ADHERENCE EVALUATION: COMPLEMENTARY TOOLS FOR THE MANAGEMENT OF RENAL TRANSPLANT PATIENTS**

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In renal transplantation, TDM of immunosuppressants (IS) has been shown to improve patients outcome. Low adherence has been reported to lead to underexposure and poor outcome, while some authors suggest that it could be induced by adverse events, potentially caused by overexposure to IS. Therefore, our objective was to investigate the relationship between exposure to IS and adherence in renal transplantation. This study was conducted in 321 patients of the EPIGREN cohort, followed-up for up to 3 years post-transplantation. Patients benefited from TDM based on C0 for calcineurin inhibitors or on AUC for mycophenolate mofetil (MMF). Adherence to IS, estimated using the four-item Morisky medication scale (MMS-4), was considered low for MMS-4<0. Health quality of life (HQOL) was evaluated using the SF-36 scale. K-means for longitudinal data was used to identify clusters of patients according to adherence. Pearson chi-square test, one way Anova and t-test were used to study relationships between covariates.

In this cohort, 307 patients received tacrolimus and 74 received cyclosporine, and 280 patients were on MMF. The proportion of low-adherent patients increased from 7% at M1 to 18% at M36. Two clusters were identified: adherence over time was good in cluster A (n=267 patients) and low in cluster B (n=54). The IS regimen did not significantly differed between clusters. Exposure to cyclosporine was significantly different between clusters (C0=116±134 vs 68±23 µg/L in cluster A vs cluster B, p=0.01). No relationship was found between adherence and exposure for tacrolimus (C0=4.1±2.2 vs 4.2±2.5 µg/L) and mycophenolate (AUC=40.4±19.0 vs 39.0±17.2 h.mg/L). Low adherence over time was associated with young age (44 vs 52 year-old in cluster B vs cluster A, p=0.007), low adherence at M1 (66% vs 12% in cluster B vs cluster A, p<10^-6) and low mental HQOL score at M1 (OR=0.96, IC95%=0.93-0.99, p=0.010).

A significant relationship between exposure and adherence was found for cyclosporine but not for tacrolimus or MMF. Low adherence was associated with young age and low mental HQOL. These results suggest that the early evaluation of mental HQOL and adherence could be a complementary tool to TDM for the management of renal transplant patients.

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**052**

**FIRST EXPERIENCE WITH PROFICIENCY TESTING FOR THERAPEUTIC DRUG MONITORING OF RILPIVIRINE IN THE INTERNATIONAL QUALITY CONTROL PROGRAM FOR MEASUREMENT OF ANTIRETROVIRAL DRUGS IN SERUM**

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**Background:** Rilpivirine is the latest non-nucleoside reverse transcriptase inhibitor that has been licensed for treatment of HIV-infection. Its pharmacokinetic properties (food- and pH-dependent absorption, large interpatient variability in pharmacokinetics, small therapeutic range and established concentration-effect relationships) make rilpivirine an attractive candidate for therapeutic drug monitoring. Rilpivirine was added to The International Quality Control Program for Measurement of Antiretroviral Drugs in Serum (KKG) in 2014. The objective of this exploratory analysis is to present the first experience with rilpivirine in the Program.

**Materials and Methods:** Blank human serum samples spiked with either subtherapeutic (0.029 & 0.048 mg/L) or therapeutic (0.210 & 0.105 mg/L) concentrations of rilpivirine were shipped to participants in two rounds. Any reported concentration deviating more than 20% was considered to be inadequate. This was intended to be an exploratory analysis, so no statistical tests were performed.

**Results:** Twelve laboratories participated in both rounds of the Program (one in the 2nd round only) making a total of 46 results. Laboratories were located in The Netherlands (n=3), Germany (n=3), Canada, UK, USA, Italy, Switzerland and China (n=1 each). Eight laboratories used LC-MS/MS techniques, the other four HPLC/UPLC-UV. Out of the 46 results, 33 (72%) were within the 20% limits for acceptance. The 13 results outside this boundary varied from 26.3 - 795% and 5 results were reported as being below the lower limit of quantification (LLOQ) of the assay. Out of the 30 samples analysed with LC-MS/MS, 24 (80%) were reported adequately vs. 9 of the 16 samples (56%) with HPLC/UPLC-UV. There was a trend suggesting that the participants performed better in analyzing therapeutic rilpivirine concentrations (>0.100 mg/L) than subtherapeutic rilpivirine concentrations (< 0.050 mg/L): 78.3 vs. 65.2%.

**Conclusion:** Analogous to our previous experience with TDM of antiretroviral agents, there is a significant proportion of laboratories that have to improve their methods for analyzing rilpivirine. Participation in the program alerted laboratories to previously undetected problems, showing the utility of this ongoing quality control program.

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**DETERMINATION OF UNBOUND FRACTION OF TACROLIMUS IN PLASMA WITH LC-MS/MS**

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**Background**

Tacrolimus is an immunosuppressant mainly used for prophylaxis of solid transplant rejection. Therapeutic drug monitoring (TDM) of tacrolimus is essential to avoid toxicity related to overexposure and transplant rejection from underexposure. Previous studies suggest that the free serum concentration of tacrolimus may correlate better with tacrolimus-related nephrotoxicity than the whole blood concentration. Thus, the unbound fraction of tacrolimus might be of interest to predict and prevent tacrolimus related nephrotoxicity. Therefore, we aimed to develop a practical method for the measurement of unbound fraction of tacrolimus in plasma.

**Methods**

The sample preparation consisted of ultrafiltration to separate the unbound fraction followed by a solid phase extraction (SPE) method using an Oasis HLB cartridge (Waters, Milford, USA) for further clean-up and pre-concentration. Extracts (25 μL) were analyzed on a Waters Acquity BEH C18 column using an isocratic elution. The analytes were detected with a Thermo Scientific Quantiva triple quadrupole with positive ionization. Ions monitored in the selected reaction monitoring (SRM) mode were m/z 821.5 -> 768.5 for tacrolimus and m/z 825.5 -> 771.4 for tacrolimus [13C2,2H2] (internal standard for tacrolimus).

**Results**

The recovery for the analyte and internal standard was >92%. Matrix effect expressed as matrix factors ranged from 0.95 to 0.97. The correlation coefficient (R²) was 0.9995 over a linear range of 5 - 200 ng/L for tacrolimus. The results for accuracy and precision were within the maximum tolerated bias and coefficient of variation (CV) (20% for LLQ and 15% for LOW, MED and HIGH).

**Conclusion**

A highly sensitive and rapid method was developed to analyze the unbound fraction of tacrolimus in plasma. Monitoring of tacrolimus free concentrations may serve as an additional marker to assess the risk of developing tacrolimus related nephrotoxicity in individual patients.

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**THE ANALYSIS OF FENTANYL AND ITS ANALOGUES IN HUMAN URINE BY LC-MS/MS**

Frances Carroll, Paul Connolly, Sharon Lupo, Shun-Hsin Liang, Ty Kahler

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Background
Synthetic opioid drugs, such as fentanyl and sufentanil, have very high analgesic potency. Abuse of these prescription opioids and their illicit analogue, acetyl fentanyl, is a growing public health problem. In this study, a simple dilute and shoot method was developed with an analysis time of less than 3.5 minutes for fentanyl, norfentanyl, acetyl fentanyl, and sufentanil in human urine by LC-MS/MS using the Raptor™ Biphenyl column.

Methods
Pooled human urine was fortified with the analytes. The urine sample was diluted 5-fold in a water/methanol solution with the addition of internal standards (fentanyl-d5, norfentanyl-d5, sufentanil-d5, acetyl fentanyl-d5) prior to injection on the Raptor™ Biphenyl column (50x2.1mm, 5μm). The mobile phases used were 0.1% formic acid in water (aqueous phase) and 0.1% formic acid in methanol (organic phase) and the chromatographic separation was achieved with a gradient elution of 30% - 80% organic phase in 2 minutes. The analysis was performed on a Waters ACQUITY UPLC® I-Class System coupled with a Waters Xevo TQ-S mass spectrometer using electrospray ionization in positive ion mode.

Results
All four analytes were completely resolved on the Raptor™ Biphenyl column with a 2-minute gradient elution. No matrix interference was observed for quantitation. The calibration linearity ranged from 0.05 to 50 ng/mL for fentanyl, acetyl fentanyl, and sufentanil; and 0.25 to 50 ng/mL for norfentanyl with % deviation of less than 10.0% and the R² of ≥ 0.999. The LLOQ was 0.25 ng/mL for norfentanyl, and 0.05 ng/mL for fentanyl, acetyl fentanyl, and sufentanil in urine. Three levels of QC samples were analyzed for accuracy and precision. Based on three independent experiments conducted on multiple days, the mean accuracy values ranged from 94 to 110% of the nominal concentrations for all compounds and the %RSD ranged from 0.2 to 9.2%.

Conclusions
An easy dilute-and-shoot method was developed for the quantitative measurement of fentanyl, its metabolite, and analogues in human urine. The analytical method was demonstrated to be fast and sensitive with great accuracy and precision for urine sample analysis.
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METABOLIC AND KIDNEY DISORDERS CORRELATE WITH HIGH ATAZANAVIR CONCENTRATIONS IN HIV-INFECTED PATIENTS: IS IT TIME TO REVISE ATAZANAVIR DOSAGES?

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Ritonavir-boostered atazanavir (ATV/r) is a relatively well tolerated antiretroviral drug. However, side effects including hyperbilirubinemia, dyslipidemia, nephrolithiasis and cholelithiasis have been reported in the medium and long term. Unboosted ATV may be selected for some patients because it has fewer gastrointestinal adverse effects, less hyperbilirubinemia and less impact on lipid profiles. We investigated the distribution of ATV plasma trough concentrations according to drug dosage and the potential relationship between ATV plasma trough concentrations and drug-related adverse events in a consecutive series of 240 HIV-infected patients treated with ATV/r 300/100 mg (68%) or ATV 400 mg (32%) . Forty-three point nine percent of patients treated with ATV/r 300/100 mg had ATV concentrations exceeding the upper therapeutic threshold. A significant and direct association has been observed between the severity of hyperbilirubinemia and ATV plasma trough concentrations (ATV concentrations: 271 [77-555], 548 [206-902], 793 [440-1164], 768 [494-1527] and 1491 [1122-1798] ng/mL in patients with grade 0, 1, 2, 3 and 4 hyperbilirubinemia, respectively). In an exploratory analysis we found that patients with dislipidemia or nephrolithiasis had ATV concentrations significantly higher (582 [266-1148], and 1098 [631-1238] ng/mL, respectively) (p<0.001), as compared with patients with no ATV-related complications (218 [77-541] ng/mL).

In conclusion, a significant proportion of patients treated with the conventional dosage of ATV (300/100) had plasma concentrations exceeding the upper therapeutic threshold. These patients that are at high risk to experience ATV-related complications may benefit from TDM-driven adjustments in ATV dosage with potential advantages in terms of costs and toxicity.

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IN VITRO APPROACH FOR THE PLACENTAL DRUG TRANSPORT EVALUATION MODEL USING INDUCED PLURIPOTENT STEM CELLS

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Background: Although the evaluation of placental drug transport is the first step of chemotherapeutic safety evaluations during pregnancy, an in vitro model is not well established. We previously reported that a trophoblast layer model using differentiating choriocarcinoma JEG-3 cells (DJEGERs) can be used for placental drug transport studies (K. Ikeda, et al., Basic Clin. Pharmacol. Toxicol., Vol. 108, p138, 2011; Pharmazie, Vol. 70, in press, 2015). However, in some instances, the fetal drug concentration predicted in vivo was not reflect in DJEGs model. Therefore, it was necessary to improve the similarities between syncytiotrophoblast and the in vitro evaluation model for using as a marker of placental drug transport. We focused on the in vivo similarities of differentiating induced pluripotent stem cells (iPSCs). iPSCs can achieve a syncytiotrophoblast-like form and secrete the human chorionic gonadotropin (hCG) after treatment with high levels of bone morphogenetic protein 4 (BMP4) for 5 days. However, for the placental drug transport model, a dense cell layer is necessary. In this study, the conditions required to differentiate iPSCs to syncytiotrophoblasts were investigated using hCG secretion and transepithelial electric resistance of cell layers as markers of syncytiotrophoblast cell layers. Methods/Results: iPSCs secreted hCG after treatment with BMP4 (100 ng/mL) for 5 days, reaching a peak on day 7. After 7 days, the secretion of hCG decreased; however, it was maintained for at least 14 days. Discontinuation of BMP4 on day 7 inhibited hCG secretion. Moreover, replacement of BMP4 reestablished hCG secretion in the syncytiotrophoblast-like differentiating iPSCs. Conclusions: We demonstrated that iPSCs differentiate into syncytiotrophoblasts, characterized by marked hCG secretion after high level BMP4 treatment for 5 days. hCG secretion was reversible depending on the presence of BMP4. In the future, we will optimize the differentiation conditions for iPSC-derived syncytiotrophoblast cell layers and establish efficient maintenance culture conditions for placental drug transport evaluation studies.
ACUTE KIDNEY INJURY FOLLOWING ATRIPLA OVERDOSE: A CASE REPORT

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Background
A 50-year-old male was admitted to the hospital after reporting to have ingested 86 tablets Atripla® (efavirenz 600mg, emtricitabine 200mg, tenofovir disoproxil 245mg). Symptoms, 12 hours after the auto-intoxication, were nausea and diarrhea. MDRD prior to auto-intoxication, on admission and 48h after presentation were 76 mL/min, 61 mL/min and 10 mL/min respectively. This acute kidney injury (AKI) was most likely caused by tenofovir overdose. Hemodialysis was started 9 days after the auto-intoxication and could be stopped 6 weeks later. A year later the MDRD is stabilised at 35-40 mL/min.

Little is known about acute intoxication with cART. Efavirenz overdose can give transient CNS disturbances and neither emtricitabine nor tenofovir overdose have been reported.

Methods
Blood samples were collected to analyse efavirenz, emtricitabine and tenofovir plasma concentrations to study pharmacokinetics in acute overdose. Pharmacokinetic parameters were calculated with maximum a posteriori Bayesian estimation (MWPharm 3.60). Two-compartment open pharmacokinetic models, based on literature, were used. The clearance of tenofovir during hemodialysis was analysed and hemodialysis simulations were performed for tenofovir with MWPharm.

Results
A total of 12 blood samples were collected, ranging from 12 hours up to 14 days after ingestion. Efavirenz concentration at t= 12h was 11.2 mg/L and <4 mg/L in 3 days with an estimated t½ of 33h (normal range: 40-55h). Emtricitabine concentration at t= 30h was 5 mg/L (normal Cmax= 3.8 mg/L) and the elimination t½ was approximately 19h (normal: 10h).

Tenofovir concentration was 6.4 mg/L (normal Cmax= 0.3 mg/L) at t= 12h and was still >0.3 mg/L on day 14. The estimated terminal t½ of tenofovir was 129h (normal range: 12-18h). Measured tenofovir clearance during hemodialysis was consistent with the SmPC. A simulation of 4h of hemodialysis on 4 consecutive days starting on admission showed a decrease in tenofovir AUC- of 60%.

Conclusions
This case shows that efavirenz and emtricitabine are relatively safe in overdose. Tenofovir however can cause acute renal failure and irreversible kidney damage. We therefore advise to start hemodialysis as soon as possible in case of tenofovir overdose to minimise tenofovir exposure and hopefully prevent damage to the kidney.

CHANGES IN THE PHARMACOKINETICS OF TEICOPPLANIN IN PATIENTS WITH HYPERGLYCEMIC HYPOALBUMINEMIA: IMPACT OF ALBUMIN GLYCOSYLATION ON THE BINDING OF TEICOPPLANIN TO ALBUMIN

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Background
There is a large interindividual variability in serum teicoplanin (TEIC) concentration after administration of its loading dose, and the factors that influence the pharmacokinetics of TEIC are disputed. The aim of this study is to clarify the changes in the pharmacokinetics of TEIC that occur in patients with hyperglycemia, and the impact of glycosylation of albumin on the pharmacokinetics of TEIC.

Methods
This study consisted of retrospective and prospective investigations. The pharmacokinetic parameters of TEIC were retrospectively compared between patients with TEIC treatment. Ninety-four patients were divided into 4 groups according to their serum albumin and blood glucose concentrations (i) hyperglycemic hypoalbuminemia (albumin < 3.0 g/dL) (n = 16), (ii) non-hyperglycemic hypoalbuminemia (n = 29), (iii) hyperglycemic normoalbuminemia (albumin ≥ 3.0 g/dL) (n = 9), and (iv) non-hyperglycemic normoalbuminemia (n = 40)). In addition, the concentration of glycosylated albumin was prospectively determined in another 28 patients. The relationships between the percentage of glycosylated albumin in total albumin and each pharmacokinetic parameter (association constant (Ka) for albumin, distribution volume (Vd), and total clearance (CL)) of TEIC were analyzed.

Results
At 12 hours after the administration of loading dose, the patients with hyperglycemic hypoalbuminemia displayed significantly lower serum TEIC concentrations ($P < 0.05$) and higher Vd ($P < 0.05$) than the other three groups, whereas total CL did not differ significantly among the 4 groups. In addition, the percentage of glycosylated albumin was significantly correlated with Ka ($r = 0.53$, $P = 0.004$) and Vd ($r = 0.41$, $P = 0.031$) of TEIC.

Conclusions
Our results strongly suggest that hyperglycemic hypoalbuminemia lowers the serum TEIC concentration which is attributable to the decreased Ka and increased Vd of TEIC by glycosylation of albumin.

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STATISTICAL METHODOLOGY FOR THE UNTARGETED SCREENING OF URINE PEPTIDOMIC BIOMARKERS IN KIDNEY TRANSPLANT RECIPIENTS.
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Background: The untargeted screening of biomarkers is a topic of research in various domains; however, no statistical strategy has been consensually approved for this complex discovery step. Urinary biomarkers of graft lesions in renal transplantation could improve patient care and minimize the use of biopsies. The goal of our study was to set-up a statistical strategy using R software to help discover biomarker candidates of graft lesions in urine of renal transplant recipients.

Methods: After solid-phase extraction of 31 urine samples from kidney transplant recipients with a normal (n=18) or antibody mediated rejection (ABMR) (n=13) biopsy, native peptides were detected using nano-LC-MALDI-TOF/TOF mass spectrometry. After data alignment, normalization and log transformation, 1110 different peptides were identified using a combination of proteomics search engines, and their discriminatory power between patients groups was screened using several statistical strategies. A first univariate step was performed using a Mann-Whitney test for each candidate (p-value thresholds≤0.05). A second univariate step was performed using 4 other approaches in parallel: 1) volcano plot, 2) penalized logistic regressions, 3) random forest and 4) spls-da. The peptides which were significant with at least 3 methods were included in a multivariate procedure using logistic regression. The selection of the final model was performed using a stepwise process based on the BIC criteria bootstrapped 500 times. The sensitivity and specificity of the final combination was evaluated using ROC curve analysis.

Results: Using the Mann-Whitney test at the first univariate analysis step, we selected 151 peptides with a p-value<0.05 and included them in the second univariate step, where 58, 10, 31 and 20 peptides were selected by methods 1, 2, 3 and 4, respectively. Among them, 10 peptides were selected by at least 3/4 methods and were included for multivariate analysis. The final model after bootstrapping retained 2 peptides leading to an AUC ROC=0.966 associated with a sensitivity=92.3% and a specificity=94.4%.

Conclusions: The statistical approach used here with R software allowed the selection of a combination of two peptides among 1110, associated with excellent sensitivity and specificity. This approach may be generalized for the untargeted screening of biomarkers in other diseases.

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SIMULTANEOUS EMTRICABINE AND TENOFOVIR HAIR ASSAY FOR ADHERENCE STUDIES
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Background: Emtricabine and tenofovir are components of many co-formulated single-dose antiretroviral medications (ARVs) used in the treatment of HIV. Studies investigating adherence to ARVs have been limited by lack of an objective method that provides reliable information about drug ingestion, especially in low-resource countries. We therefore developed an LC-MSMS method for the simultaneous quantification of both drugs in hair samples.

Methods: A liquid extraction method for 50 mg of hair was followed by an isocratic Acquity UPLC method using water, acetonitrile, 0.1% formic acid and an Acquity UPLC HSS C18, 1.8 um, 2.1x150mm column, in combination with a Waters TQ tandem mass spectrometer. Compounds were detected with positive electrospray ionization in multiple reaction mode. Mass spectrometer settings were optimized for both compounds and their internal standards tenofovir-d6 and emtricitabine-13C,15N2, respectively. To test the assay, both drugs were measured simultaneously...
in n=12 hair samples from South African HIV+ patients who were participating in an IRB-approved study investigating ARV adherence.

Results: Runtime for the assay was 2 minutes per sample, intra-assay precision <10%, and the sensitivity of the assay was 1 pg/mg of hair for each compound. The method showed little matrix effect for emtricitabine but a substantial effect for tenofovir. Hair concentrations were similar to those recently reported by Baxi et al (J Acquir Immune Defic Syndr. 2015), with a mean emtricitabine concentration of 5229 pg/mg, ranging between 352 and 14549 pg/mg and a mean tenofovir concentration of 396 pg/mg, ranging between 78 and 678 pg/mg. There was a remarkably good correlation between the concentrations of both drugs (R2 = 0.77).

Discussion and Conclusion: Our data suggest that our method is able to simultaneously quantify tenofovir and emtricitabine in hair samples, and that the assay may be useful to determine adherence to these ARVs in low-resource settings. Ongoing studies are comparing this method with other adherence measures such as electronic monitoring and tenofovir triphosphate measurements in dried blood spots.

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PHARMACOKINETICS OF TOTAL AND FREE MYCOPHENOLIC ACID IN LUPUS NEPHRITIS: PRELIMINARY REPORT.
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Background: Mycophenolate mofetil (MMF) is recommended in induction, as well as in maintenance therapy of lupus nephritis. Therapeutic drug monitoring of mycophenolic acid (MPA) has been proven to decrease the frequency of acute allograft rejection incidences after kidney transplantation. Since the differences in pharmacokinetics between patients with autoimmune diseases and renal transplant recipients were reported, thus it is worth to investigate MPA pharmacokinetics in non-transplant patients. The aim of this preliminary study was to observe the total and free MPA pharmacokinetics in lupus nephritis patients.

Methods: Nine adult patients with lupus nephritis on MMF (daily dose range: 500-3000 mg) provided a total of twelve three-time point samples collected at pre-dose, 0.5 and 2 hours after MMF administration. Total and free MPA concentrations (C0, C0.5, C2) were measured by validated HPLC-UV and LC-MS/MS methods, respectively. For total MPA, concentrations at 6, 8 and 12 hours were estimated using an empirical algorithm.1 Abbreviated MPA AUC0-12h was calculated by the trapezoidal rule.

Results: The mean (±SD) total MPA C0, C0.5 and C2 were 1.74±1.02 µg/mL, 6.93±5.44 µg/mL and 5.02±4.07 µg/mL, respectively. The mean (±SD) free MPA C0, C0.5 and C2 were 17.43±20.69 ng/mL, 69.55±63.40 ng/mL and 46.69±38.33 ng/mL, respectively. The abbreviated MPA AUC0-12h for total MPA was 39.67±17.42 µg·h/mL. The total plasma MPA concentrations at trough and 2h after MMF dose were significantly correlated with abbreviated MPA AUC0-12h. (r=0.7375, p=0.006 and r=0.6482, p=0.023, respectively). The significant correlation was also observed between total and free MPA concentrations at trough and 0.5h after dose (C0: r= 0.9023, p= 0.0006; C0.5: r=0.7179, p=0.009).

Conclusions: In this preliminary study the significant correlation between total MPA trough concentration and MPA AUC0-12h was observed. The need for free MPA determination in autoimmune diseases should be further investigated as a strong correlation between total and free MPA through levels were noted. The influence of MPA concentrations on lupus nephritis treatment outcome in the studied population is of further evaluation.

Key Words: mycophenolic acid; mycophenolate mofetil; lupus nephritis; pharmacokinetics; free fraction

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COMPREHENSIVE TOXICOLOGICAL ANALYSIS OF CLINICAL URINE SAMPLES WITH THE TOXTYPERTM COMPARED TO A STANDARD GC/EI-MS-FULLSCAN METHOD
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Background: Screening for drugs in urine samples with GC/EI-MS-FullScan and EI-MS library search can be regarded as standard practice in clinical toxicology. The Toxtyper™ (TT, Bruker) uses LC-MS™ ion trap technology with MS™ library search probably allowing a simplified sample preparation. This study compares the performance of the TT with our standard GC/MS procedure on the reported result level.

Methods: EI-MS-FullScan (Shimadzu QP2010plus) with Maurer/Pfleger/Weber-library (4th Edition, Wiley-VCH) search was performed after enzymatic hydrolysis of 2 ml urine, alkaline liquid-liquid-extraction, acetylation and 16.5
min GC separation. For TT analysis 250 µL urine was protein-precipitated with 200 µL acetonitrile. The supernatant was evaporated to dryness and redissolved in 50 µL mobile phase A. All samples were analysed with TT method 1 (TT-M1, run time 11 min) and TT method 2 (TT-M2, run time 19 min) according to Bruker. TT-M1 includes continuous positive/negative switching and generates MS2/MS3 spectra according to a scheduled precursor list. Substances were automatically identified with the TT-library (900 entries) by Rt and MS, MS2 and MS3 information. TT-M2 data (MS, MS2/MS3) were acquired in positive mode and searched against TT-library and the Maurer/Wissenbach/Weber-library (TT-MWW; Wiley-VCH, 2014). TT-MWW contains >4500 entries including 3000 metabolites and conjugates. Routine urine samples from 150 patients mainly in substitution therapy were analysed. 

**Results:** For the 150 urine samples 482 results were reported which could be attributed to 61 different drugs and/or their metabolite(s). GC/MS found 2 substances which were not detected by the TT. With TT-M2 9 substances not identified by GC/MS were found. In 352 cases (73%) TT and GC/MS gave according results (51 substances; TT-M1+TT-M2+GC/MS: 258, TT-M1+GC/MS: 19, TT-M2+GC/MS: 75). In 53 cases (11.0%) 14 different substances were identified by GC/MS which were not found with both TT approaches. However, 77 reports (16%) were based on TT analysis only (25 substances; TT-M1+TT-M2: 42, TT-M2: 34, TT-M1: 1).

**Conclusions:** The presented TT screening analysis after simple sample preparation proved to be of equal value when compared to our standard GC/MS approach. Future improvements in TT sample preparation could lead to the use of TT-M2 only.

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**SAMPLING THE STRATUM CORNEUM TO QUANTIFY DRUG UPTAKE FROM TOPICAL PRODUCTS**

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**Background:** Approval of topical products requires expensive and time-consuming clinical trials. Particularly, bioequivalence tests are challenging as the systemic bioavailability is frequently low and unrelated to local bioavailability. This project addresses the development of an alternative method ('tape-stripping') to sample the skin for subsequent drug assay, and to assess thereby the bioequivalence of topical products. Adhesive tape-stripping is a non-invasive technique which removes successive layers of the stratum corneum (SC) the cornified, outermost layer of the skin. In this work, the concentration of diclofenac in the SC of healthy volunteers was determined at two sampling, uptake and clearance, times following application of the marketed formulations Voltaren, Solaraze and Pennsaid.

**Methods:** Healthy adults provided informed consent before participating in this study approved by REACH and RIHSC. 12 treatment sites (8 cm²), 2 sites per formulation per arm, were demarcated on the ventral forearm. The formulations were applied in clinically relevant amounts. After 6 hours, excess formulation was cleaned from the skin surface. Half the sites were tape-stripped (Book tape, 3M, US) immediately ('uptake'), and half were tape-stripped at a later time ('clearance'). Thirty tapes were taken from each site unless the rate of transepidermal water loss (AquaFlux, Biox, UK) reached a threshold value. The mass of SC removed (and thus the depth reached) with each tape was determined gravimetrically. Diclofenac was extracted from the tapes, and assayed by HPLC.

**Results:** The depth of SC sampled was 5.6±1.1 and 6.9±2.2 µm for the uptake and the clearance samples, respectively. The amount of diclofenac in the uptake samples and percentage of applied dose recovered were 26.6±14.0 µg / 4.9% (Voltaren), 22.7±17.1 µg / 0.73% (Solaraze) and 133.2±37.0 / 11.8% µg (Pennsaid). Diclofenac clearance from the SC was slow. As expected, inter-individual variation in diclofenac SC uptake was greater than the intra-individual variation.

**Conclusions:** Sampling the skin using the tape-stripping technique is a relatively straightforward, convenient and sensitive way to compare the uptake of a drug into the skin from different topical products. Definitive product comparisons will be done once statistical power is attained (14-20 subjects).

**066**

**FACTORS AFFECTING BONE DISEASES DEVELOPMENT IN HIV PATIENTS ON TENOFOVIR-BASED THERAPY**

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Epoxyeicosatrienoic acids (EETs), metabolites of arachidonic acid (AA) via CYP epoxygenases, have anti-inflammatory, vasodilatory, myocardial preconditioning effects. EETs are further metabolized by soluble epoxide hydrolase to corresponding dihydroxyepoxyeicosatrienoic acids (DHETs) with weaker activities of cardioprotective effects. We have previously demonstrated that some angiotensin II receptor blockers (ARBs) inhibit AA metabolism via CYP2C8, CYP2C9 and CYP2J2, and the degree of inhibition varied among the ARBs in vitro. However, it is unclear whether administration of ARBs affects serum concentrations of EETs and DHETs in patients taking ARBs. This study aimed to determine the serum concentrations of eicosatrienoic acids in patients treated with and without ARBs.

Methods
Seventy patients, 34 patients taking ARBs and 36 ARB-free patients, admitted to Teine Keijinkai Hospital were included in this study. The study protocol was approved by the ethics committee of Teine Keijinkai Hospital, and written consent was obtained from all participants. Serum residue (250 μL) from each patient after biochemical tests was used in this study. Deuterated eicosatrienoic acids were used as internal standards. Ethanol was added to each serum in order to precipitate serum proteins, and supernatant was applied to a solid phase extraction column. After extraction, all eicosatrienoic acids were determined by LC-MS/MS.

Results
No significant differences were observed for patients with and without ARB administration, but serum concentrations of eicosatrienoic acids in patients taking ARBs (median=0.458 ng/mL) were slightly lower than those in the ARB-free patients (0.692 ng/mL; p=0.211). In ARB-free patients with hypertension, eicosatrienoic acid concentration (0.539 ng/mL) tended to be lower than in the subjects without hypertension (0.819 ng/mL; p=0.108).

Conclusion
There were no significant differences in serum concentration of eicosatrienoic acids between patients treated with and without ARBs in this study. Most patients participating in this study took concomitant drugs that could have been metabolized by CYP enzymes. Further investigations to compare the effects of ARBs on metabolism of AA are necessary to clarify the possible differences in serum EETs and DHETs in relation to various clinical settings, including administration of ARBs and other concomitant drugs.
THE INHIBITORY EFFECT OF STATINS AND DICLOFENAC ON ARACHIDONIC ACID METABOLISM IN VITRO
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Background
Arachidonic acid (AA) is metabolized by cytochrome P450 (CYP) to four regioisomers of epoxyeicosatrienoic acid (EET), i.e. 5,6-EET, 8,9-EET, 11,12-EET, and 14,15-EET. EETs are known to show heart protective effects. We have already found that some angiotensin II receptor blockers (ARBs) inhibit CYP enzymes in AA metabolism to reduce EETs production. The aim of this study was to examine the inhibitory effect of statins and diclofenac on AA metabolism in vitro compared with that of telmisartan, which exhibits potent inhibitory effects on CYP enzymes among ARBs that we have studied.

Methods
An incubation study of AA metabolism was conducted using fluvastatin, atorvastatin, and diclofenac with human liver microsomes (HLMs). Concentrations of regioisomers of EETs and dihydroxyeicosatetraenoic acids (DHETs), metabolites of EETs, were determined by high performance liquid chromatography-tandem mass spectrometry (LC-MS/MS). The inhibitory effect was compared by calculating the half maximal inhibitory concentration (IC₅₀) value for the generation of the sum of EETs and DHETs.

Results
Fluvastatin and diclofenac significantly decreased production of EETs and DHETs at concentrations of 1 (p < 0.01) and 50 µmol/L (p < 0.05), and the IC₅₀ values were 12.6 and 53.0 µmol/L, respectively. Atorvastatin reduced production of EETs and DHETs by 20% at 100 µmol/L (not significant).

Fluvastatin and diclofenac exhibited stronger and weaker inhibitory effects compared to telmisartan (IC₅₀: 34.1 µmol/L) on production of EETs and DHETs from AA, respectively, while atorvastatin showed a negligible effect.

Conclusions
The concentrations of fluvastatin and diclofenac used in the present study were higher than the reported maximum plasma concentrations from clinical doses. As fluvastatin is a substrate of organic anion transporters, it accumulates in the liver rather than plasma. Diclofenac has been reported to be largely distributed to the liver in mice. These results suggest that fluvastatin and diclofenac may inhibit CYP enzymes in the liver, and plasma levels of EETs and DHETs may decrease in patients taking these drugs.

SEPARATION AND QUANTITATIVE DETERMINATION OF THE BCR-ABL TYROSINE KINASE INHIBITORS IN HUMAN PLASMA BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION
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Background: The BCR-Abl tyrosine-kinase inhibitor imatinib has been used as the first-line treatment for chronic myeloid leukemia (CML) in both adults and children. Recently, it has been reported that, in some cases, tumors develop resistance to imatinib; the second generation dasatinib and nilotinib are used for such patients. The determination of pharmacokinetic parameters such as plasma trough level, AUC, and CL is useful in setting the dose for patients experiencing intolerable adverse effects. This study is aimed at developing and validating a simple and reliable method for determining the plasma concentrations of imatinib, dasatinib, and nilotinib using reversed-phase high-performance liquid chromatography with ultraviolet detection (RP-HPLC-UV).

Methods: Imatinib, dasatinib, and nilotinib were extracted from plasma samples (0.5 mL) using Oasis MCX after adding erlotinib as the internal standard. HPLC separation was carried out on a Waters XTerra MS C18 analytical column (4.6 mm I.D. x 250 mm, 5 µm). The compounds were eluted with an acetonitrile-aqueous solution of ammonium acetate mobile phase at a flow-rate of 0.6 mL/min by increasing the acetonitrile ratio from 45% to 80% for 10 min. The detector wavelengths were set at 265 nm for imatinib and nilotinib, 322 nm for dasatinib, and 333 nm for erlotinib, respectively.

Results: The HPLC technique provided sharp and symmetrical peaks with retention times of 6.3, 8.0, and 14.4 min for imatinib, dasatinib, and nilotinib, respectively. Interference peaks derived from the plasma samples were not detected. Calibration curves were linear from 35 to 1409 ng for imatinib (r = 0.99), from 2.3 to 90.0 ng for dasatinib (r = 0.99), and from 35 to 1386 ng for nilotinib (r = 0.99). The intra-day reproducibilities in the amounts of three analytes determined were good agreement with the actual amounts added, the relative errors were ±1.2 to 2.1%. The inter-assay precisions were less than 2.8%.

Conclusions: This study describes a simple and reliable method for the determination of imatinib, dasatinib, and
nilotinib in plasma. The method can be applied for the determination of the pharmacokinetic parameters in routine clinical TDM for setting the dose with minimal side effects.

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CLINICAL PHARMACOKINETICS OF 3-WEEKLY DOCETAXEL IN CANCER PATIENTS
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[Background] Docetaxel, a semisynthetic member of the taxane family, has been used for the treatment of patients with breast cancer, gastric cancer, and non-small cell lung cancer. Docetaxel is generally given in body surface area (BSA) adjusted dose. The drug, however, has a large inter-patient variability in pharmacokinetics associated with toxicity such as neutropenia and diarrhea. Knowledge of factors that influence the pharmacokinetics of the drug should provide a way for individualized anti-cancer therapy. In this preliminary study, we evaluated the range of pharmacokinetic variability in order to predict the therapeutic efficacy and toxicity.

[Method] Fifteen patients aged 54-76 years were recruited: Eight patients had gastric cancer, 6 had esophageal cancer, and 1 had pharyngeal cancer. Blood samples were taken for pharmacokinetic analysis of docetaxel at 0, 1, 6, 9, and 24 h after the end of a 1-h infusion in the first course. Plasma concentrations of docetaxel were measured by HPLC-UV. Pharmacokinetic parameters were determined by two-compartment analysis using WinNonlin software. The study was approved by Kyorin University School of Medicine and Tokyo University of Pharmacy and Life Sciences Human Subjects Review Board and written informed consent was obtained.

[Results] The average dose administered was 103.7±12.2 mg (70 mg/m²) with a range from 90 to 130 mg. The peak plasma levels varied from 1.34-5.67 µg/mL. The docetaxel concentrations at 24 h after the end of infusion were 6.1-35.0 ng/mL in 15 patients. The values of AUC₂₄, total-body clearance (CL₂₄TX), and elimination half-life (t₁/₂) were 2.83-8.67 hr-µg/mL, 12.7-38.8 L/hr, and 8.9-29.2 hr, respectively.

[Conclusions] The predicted and observed concentrations were in close agreement in two-compartment model. The inter-patient variabilities in CL₂₄TX and t₁/₂ were approximately 3-fold. The variability may be due to the activity of CYP3A in patients, by which docetaxel is metabolized to an inactive metabolite, hydroxydocetaxel. Further studies investigating the relationship between CL₂₄TX and CYP3A activity should be needed to predict the optimal dosage range for the individualized cancer therapy of docetaxel.

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FLUOROMETRIC UHPLC METHOD USING NBD-F FOR QUANTITATION OF PREGABALIN IN HUMAN PLASMA
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Background: Pregabalin, a γ-aminobutyric acid analogue, is commonly used for the treatment of neuropathic pain. Pregabalin concentration in human plasma has been measured using several derivatization reagents or mass spectrometry, since pregabalin has no specific ultraviolet or visible absorption. Earlier chromatographic techniques using an HPLC coupled to fluorometric derivatization employed the time-consuming pretreatments or longer run times. The aim of this study is to develop the quantitation for pregabalin in human plasma using an ultra high-performance liquid chromatography (UHPLC) method coupled to fluorometric detection with 4-fluoro-7-nitrobenzofurazan (NBD-F) and to apply it to pharmacokinetic analyses in patients with neuropathic pain.

Methods: This analytical method was based on pre-column fluorescent derivatization with NBD-F. After the pretreatment involving protein precipitation, pregabalin and gabapentin as an internal standard (IS) were derivatized with NBD-F under basic conditions. After the derivatization, the reaction was terminated with acidic solution, and 0.5 µL of the supernatant was injected onto fluorometric UHPLC system. The UHPLC separation was performed using a 2.3 µm particle size ODS column with isocratic elution. Their derivatives were monitored at excitation and emission wavelengths of 470 and 530 nm, respectively. The method was validated according to the industry bioanalytical method guidance of US FDA.

Results: The derivatization of pregabalin in human plasma was optimized following the reaction conditions: time, 1 min; pH, 10; and temperature, 60°C. The pregabalin-NBD and IS-NBD derivatives were eluted at 0.71 and 0.85 min, respectively. The calibration curve was linear at a range of 0.05-10 µg/mL (r > 0.999). The lower limit of quantification was 0.05 µg/mL. The intra- and inter-assay precisions and accuracies were less than 15% and 85-
115% for all analytes. This validated method was applied to the measurement of pregabalin plasma concentration in 19 neuropathic pain patients and it ranged 0.275-6.54 μg/mL.

**Conclusions:** A validated fluorometric UHPLC method for the quantitation of pregabalin in human plasma using NBD-F derivatization has been established. This method presents acceptable analytical performance and can be helpful for evaluating the plasma exposure of pregabalin in clinical settings.

### 073

**POPULATION PHARMACOKINETICS OF AMIKACIN IN NON-CRITICAL CARE PEDIATRIC KUWAITI PATIENTS.**

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**Introduction:** Amikacin (AMK) is commonly used to treat gram negative infections in pediatric patients. AMK pharmacokinetic parameters such as volume of distribution (Vd) and clearance (Cl) in pediatrics displays an extensive interindividual variability’s. Age, body weight (bw), sex and creatinin clearance are the covariates of the interindividual variability in Vd and Cl. Published data on AMK pharmacokinetics for Arab pediatrics patients are very limited and in Kuwait are none. The aim of this study was to: (1) calculate the Vd and Cl in pediatric Kuwaiti patients; (2) investigate the effect the covariates on the individual Vd and Cl.

**Methods:** 62 non-critical care pediatric patients older than 0.3 years of age (29 male and 33 female) were identified for whom a confirmed steady-state AMK peak and trough levels were available. They have received a multiple daily dosing regimen of AMK (4 to 15 mg/kg intravenously during 0.1 hour, two to three times per day). Assuming one compartment linear model, the Vd and Cl were calculated using the method of Sawchuck and Zaske. Multiple regression analysis was performed to assess the effect of age, body weight, sex and creatinin clearance on Vd and Cl.

**Results:** The Mean±SD (median, range) of the AMK peak, trough, Cl and Vd were 18.14±6.95 (17.46, 6.20-37.72 mg/l), 1.56±1.18 (1.23, 0.30-5.85 mg/l), 1.77±1.38 (1.46, 0.19-5.03 l/h) and 6.52±5.67 (4.64, 0.73-21.07 l) respectively. The Vd and Cl were greater and smaller respectively, than those reported from western countries. The age and bw were found to have significant effects on both Vd and Cl as indicated by the following equations: Cl=0.431+0.128xage+0.031xwt, (R²=0.826); Vd=0.892+0.488xAge+0.142xbw, (R²= 0.86).

**Conclusion:** In this population of pediatric Kuwaiti patients we found a wide interindividual variability’s in AMK peak, trough, Cl and Vd, with age and bw exerting a strong effect upon Vd and Cl. Therefore, initial dose regimen based on age and bw models followed by measuring AMK concentration are recommended.

### 074

**DEVELOPMENT OF A LIBRARY OF HUMAN NATURAL URINARY PEPTIDES FOR BIOMARKER DISCOVERY IN KIDNEY TRANSPLANT RECIPIENTS.**

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**Background:** In renal transplantation, the discovery of early urine biomarkers of graft lesions would be useful to help physicians to improve patient care and minimize the use of invasive graft biopsies. For untargeted proteomic analysis, papers have recently reported using the SWATH™ MS (AB Scieix) technology (1), a data independent acquisition mode, with fixed or variable windows, allowing the simultaneous identification and quantitation of large numbers of compounds. To the best our knowledge, so far only tryptic peptides have been included in the spectra libraries required in the elaboration of these SWATH™ MS methods. However, the urine peptidome, made up of the peptides naturally present in urine, excreted or shed by the kidney, or resulting from natural enzymatic hydrolysis of urine proteins, may be another interesting source of such biomarkers. In this study, we aimed at building an MS library of human natural urinary peptides.

**Methods:** After solid-phase extraction of 52 urine samples from kidney transplant recipients (2), native peptides were detected using nano-LC-ESI-Q-TOF mass spectrometry. Identification of these peptides was performed using a combination of proteomics search engines. To check the robustness of the Q-TOF MS spectra obtained, we calculated for some characteristic peptides the inter-assay CV% (n=6) of the relative intensity of fragments with relative abundance>10%.

**Results:** Our MS library currently includes more than 900 Q-TOF spectra of naturally occurring peptides, belonging to 89 different proteins. Each sequence was checked against that of the respective MALDI-TOF/TOF spectrum. The inter-assay CV% (n=6) of peptide fragments with relative abundance>10% was always better than 15%.

**Conclusions:** We developed a library of humannatural urinary peptides using nano-LC-ESI-Q-TOF mass
spectrometry. This library is still evolving and will be incremented with other spectra after analysis of urine samples from different backgrounds.

(2) Sauvage FL, Gastinel LN, Marquet P. *J Chromatogr A.* 2012;1259:139-147.

075

**SCREENING FOR TARGETED AND NON-TARGETED NEW PSYCHOACTIVE SUBSTANCES AND DRUGS OF ABUSE BY ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TIME-OF-FLIGHT MASS SPECTROMETRY**

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**Background**

The established procedure in drug testing has been, until now, combining various types of immunological testing with mass spectrometry based confirmation analysis. The continuously increasing number of new psychoactive substances (NPS) has changed the scene dramatically during the last decade, as many of the NPS cannot be detected by immunoassay. Our laboratory has developed a comprehensive drug screening-confirmation approach covering NPS, drugs of abuse, and prescription drugs, based on ultra-high performance liquid chromatography time-of-flight mass spectrometry (UHPLC TOF-MS). This presentation will report the main findings from nearly 5000 clinical and post mortem cases during a one-year period (2014), with emphasis on NPS. In addition, authentic examples of retrospective data analysis for retrieving untargeted analytes will be presented.

**Methods**

The method combined mixed mode (cation exchange with C4) solid phase extraction (SPE) with UHPLC TOF-MS. The matrices included urine, blood, and serum for clinical cases, and urine, blood, vitreous humour, liver, and muscle for post mortem cases. Method details are available from the presenting author.

**Results**

The total number of findings in 2840 clinical cases analysed was 10217. The most common findings in the order of decreasing prevalence were buprenorphine and norbuprenorphine, naloxone, methadone and EDDP, tetrahydrocannabinolic acid, quetiapine, pregabalin, alpha-PVP and various benzodiazepines. A total of 189 NPS findings were made, representing 28 different NPS. The total number of findings in 1946 post mortem cases analysed was 3735. The most common findings in the order of decreasing prevalence were various benzodiazepines, buprenorphine and norbuprenorphine, codeine and morphine, tetrahydrocannabinolic acid, pregabalin, zopiclone, amphetamine, oxycodone, and tramadol. A total of 34 NPS findings were made, representing 11 different NPS. The complete statistics will be presented in table format.

**Conclusions**

The results showed, that mass spectrometry based screening methods for monitoring substance abuse are needed, as in both clinical and post mortem cases the most common findings included several drugs that are not detected by immunoassay. The high frequency of NPS in clinical cases further confirmed this phenomenon. The combined screening-confirmation approach proved to be especially feasible in optimizing laboratory resources.

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**LC-MS/MS QUANTIFICATION OF BIO-ACTIVE INFlixIMAB IN SERUM USING BIOTINYLATED-TNF-A AND STREPTAVIDIN MAGNETIC BEADS FOR A TARGET BASED PRE-ANALYTICAL SAMPLE PURIFICATION.**

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**Introduction**

Infliximab (IFX), a tumor necrosis factor alpha (TNF-α) blocking agent, has been approved for the treatment of a wide range of autoimmune diseases. Therapeutic Drug Monitoring (TDM) of IFX is increasingly applied in clinical practice, because there is a large inter-patient variability in treatment response. Traditionally, TDM of monoclonal antibodies such as IFX is performed by Enzyme linked immunosorbent assays (ELISA). However, ELISA may lack selectivity due to a higher risk of cross reactivity as was demonstrated in a recent publication where different ELISA assays for IFX determination were compared (Vande Casteele etal, 2012). Furthermore, in general LC-MS/MS has several advantages over ELISA such as wider linear dynamic range, shorter validation times and higher throughput and might be favorable for IFX quantification. Therefore, we aimed to develop a quantitative LC-MS/MS method with
imunoaffinity pre-analytical sample purification to determine the biological active IFX concentration in serum.

Methods
Biotinylated-TNF-α (0.25µg, excess based on stoichiometric calculation) was used as ‘bait’ protein coupled to 5µl streptavidin magnetic bead slurry to selectively extract the active IFX concentration (‘prey’ protein) from 5µl serum sample. Cetuximab, a monoclonal antibody that contains a near identical signature peptide sequence to that of infliximab, was used as an internal standard. Sample and standard extracts were treated with 5mM Dithiothreitol (DTT) followed by an overnight digestion with 0.25µg trypsin. The liberated peptides were separated and the signature peptide (a stable, non-endogenous peptide with high signal-to-noise ratio) was analyzed on a LC-MS/MS.

Results
The recovery of IFX in serum was 82% (RSD 5.2%, n=9). The concentration range of the assay (0.5 (LOQ)-15mg/L) covered the entire therapeutic range, with a lower LOQ compared to ELISA (1mg/L). The correlation of the polynomial calibration curve was $R^2 = 0.997$. Sensitivity can be increased even further with a larger sample volume. The method had a good correlation ($R^2 = 0.97, n=25$) compared with ELISA.

Conclusion
Target-based pre-analytical sample purification followed by LC-MS/MS quantification can be used to determine the biological active IFX concentration in serum and may serve as a valid option next to ELISA.

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STABILIZED SENSING OF HEPARIN IN BLOOD USING HEPARIN-IMPRINTED ELECTRODE BY SURFACE MODIFICATION OR WASHING
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Effect of coagulant efficiency of heparin during extracorporeal circulation is estimated by measurement of activated clotting time (ACT). However, ACT sometimes looses dependency on heparin concentration. A real-time heparin sensor is required to optimize dosage of heparin and its antidote protamine. Then, we are trying to develop a heparin sensor using a molecularly imprinted polymer (MIP). A MIP is a recognition polymer synthesized by simple template-polymerization in the presence of the target as a template. In the present work, we studied the method to stabilize measurement of heparin concentration in whole blood by using the MIP-grafted electrode. An initiator of radical polymerization (diethylthiocarbamic- benzyl group) was introduced on an indium-tin oxide (ITO) electrode. Heparin sodium, methacryloxyethyltrimethoxysilane, and acrylamide were dissolved in water. Methylenebisacrylamide was dissolved in dimethylformamide. Mixture of the both of the solutions were introduced into the gap (50 µm in thickness) between the surfaces of quartz crystal plate and the treated ITO electrode.

Ultraviolet was irradiated to the surface of the ITO to graft copolymer of the monomers. The ITO was washed with aqueous solution of 1 M sodium chloride to remove the template and to obtain the MIP-grafted electrode. Cyclic voltammetry was performed with the MIP-grafted electrode in physiological saline or bovine whole blood including 0-8 unit/mL heparin and 5 mM ferrocyanide as a redox marker. The relationship between the current intensity and the heparin concentration was analyzed.

The current intensity was decreased by the increase of heparin concentration either in the saline or the whole blood. However, the sensitivity to heparin in the blood was approximately 50% of that in the saline. The heparin-sensitivity of the electrode coated with SEC-1® (Toyobo) in the blood was 74% of that in saline. As another approach, uncoated-electrode was washed between each measurement in blood with protease-containing detergent (Sterizyme® S, Maruishi Pharmaceutical). The heparin-sensitivity in the blood with the washed electrode was 77% of that in the saline. The sensitivity to chondroitin sulfate C was not observed. Then, we can conclude that selective and stable sensing of heparin can be done with an electrode grafted with heparin-imprinted polymer.

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UPLC-MS/MS METHOD FOR THE SIMULTANEOUS QUANTIFICATION OF ANTI-HBV NUCLEOS(T)IDES ANALOGUES: ENTECAVIR, LAMIVUDINE, TELBIVUDINE AND TENOFOVIR IN PLASMA OF HBV INFECTED PATIENTS.
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Background: Hepatitis B infection affects 2 billion people worldwide and 350 million of these are chronically
infected (WHO, 2008). Chronic HBV is one of the most important cause of mortality and morbidity worldwide. 5 nucleos(t)ide analogs (NAs) are approved for the treatment of HBV infection: lamivudine (3'-TC), adefovir dipivoxil (ADV), telbivudine (TBV), entecavir (ETC) and tenofovir-disoproxil-fumarate (TDF). The most used NAs in high income countries are ETC, TBV and TDF: ETC and TDF are recommended as first-line treatment. Recently, ETC plasma concentration have been associated with outcome response. Moreover, therapeutic drug monitoring is necessary to evaluate inter- and intra-individual variability and to verify potential drug-drug interactions, in order to choose the most effective therapeutic strategy and avoid toxicity.

Methods: We developed and validated an UPLC-tandem mass spectrometry assay to quantify all these NAs, following FDA guidelines. Chromatographic separation was performed on an Acquity UPLC® HSS T3 1.8 µm (2.1x150 mm) column (Waters, Italy), heated at 40°C. The following mass transitions (m/z) were monitored: 242.3>116.1 for S’ amino-5’dexoy-thymidine (Internal Standard), 288.14>176.1 for tenofovir (TFV), 230.1>112.06 for 3-TC, 243.1>127.1 for TFV, 278.1>152.1 for ETC. The run was performed with a gradient of H₂O and ACN, both containing 0.05% formic acid. QCs at three different concentrations were prepared. Each standard, QC and patient sample was treated with a protein precipitation protocol with acetonitrile +0.1% formic acid.

Results: The calibration curves for all drugs were linear in the calibration range for all analytes (mean $r^2 > 0.998$). Accuracy, intra-day and inter-day precision fitted all FDA guidelines for all analytes. The extraction procedure showed a high and stable recovery for all analytes. LLOQ and LLOD values were: 15.6 ng/mL and 7.8 ng/mL for TFV, 9.8 ng/mL and 4.9 ng/mL for 3-TC, 19.5 ng/mL and 9.8 ng/mL for TBV and 0.039 ng/mL and 0.02 ng/mL for ETC. No significant matrix effect have been observed.

Conclusions: This simple analytical method could be applied in the near future to evaluate the optimal therapeutic range of these drugs in the real life and therefore, it could represent an useful tool for the correct management of anti-HBV treatment with NAs.

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FIRST RESULTS FROM STANDARDIZED ORAL FLUID CONTAMINATION EXPERIMENTS USING DIPHENHYDRAMINE AS A MODEL SUBSTANCE

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Background
Oral fluid (OF) is gaining increasing acceptance for drug of abuse testing. However, little is known about the probability and extent of OF contamination with drugs shortly after ingestion or after unintended/accidental oral contamination (e.g. kissing). We therefore performed an OF contamination simulating experiment with an uncoated tablet of the non-prescription drug Diphenhydramine.

Methods
20 individuals were divided into four groups (A-D) with five individuals each. All individuals placed a 50 mg Diphenhydramine tablet on their tongue for 5 seconds. OF samples were collected with the Greiner Bio-One SCS pH 4.2 device immediately after the tablet (time zero), followed by an hourly sampling interval. Group A stopped sampling after one, group B after two, group C after three and group D after eight hours. Each individual from group A to C drank 250 mL water right after their last sampling and subsequently took a final OF sample. Analysis was performed with a fully validated UPLC-MS/MS method on a Waters Acquity system connected to a Xevo TQ-S using Diphenhydramine-D₃ as an internal standard (LoD: 0.34 ng/mL; LoQ: 0.42 ng/mL).

Results
The Diphenhydramine concentration range fell from 5.6 - 679 µg/mL (n=20) at time zero to 23.1 - 22763 ng/mL (n=20) after the first hour and 2.7 - 59.9 ng/mL (n=10) after the third hour. The mean drug concentration decrease after the first hour was 99.0% (n=20; CV: 1%) from the initial concentration. After the second and the third hour the mean concentration decrease was 81.3% (n=15; CV: 9%) and 74.7% (n=10; CV: 14%) referring to the previous concentration. Drinking of 250 mL water led to an additional mean loss of concentration of 59.2 % (n=15; CV: 27%).

Conclusions
The unexpected high and variable OF Diphenhydramine contamination fell from a high µg concentration range to a low ng range within three hours. Even drinking of 250 mL water could not completely remove the remaining drug residues.
After four hours Diphenhydramine was still detectable in all individuals of group D.

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POPULATION PHARMACOKINETICS OF VANCOMYCIN IN JAPANESE PATIENTS WITH CONTINUOUS RENAL REPLACEMENT THERAPY
**Background:** Although the population pharmacokinetic (PPK) parameters of vancomycin during continuous renal replacement therapy (CRRT) for Caucasians are well-documented, those for Japanese subjects have yet to be determined. The aim of this study was to determine the PPK parameters of vancomycin for Japanese subjects using a nonlinear-mixed effects modeling (NONMEM) approach.

**Methods:** This retrospective study was approved by the ethical institutional review board of the Kumamoto university hospital. PPK analysis was performed using the software of NONMEM ver 7.3 (ICON, Dublin). Adult patients who received CRRT for acute kidney injury at the intensive care unit between September 2010 and August 2014 were included. The 2-Compartment model with first order elimination, and interindividual and residual variability as distributing exponentially were applied. To estimate significant covariance, we extracted patient’s data those were considered to affect the daily vancomycin pharmacokinetics such as body weight, total effluent rate (ER), creatinine clearance (CCR), calculated using the Cockcroft-Gault equation), and the others from the electronic medical records.

**Results:** 17 patients were enrolled in the study. The median (range) values for age, body weight, ER at the initiating CRRT, CCR and blood trough level were 64 (19-92, years), 58.8 (43.6-119, kg), 0.75 (0.60-3.00, L/hr), 1.38 (0.34-11.39, L/hr), and 11.3 (5.8-24.7, g/mL), respectively. Final PPK parameters were constructed as follows, total clearance (CL, L/hr) = 0.372*CCR^0.478+0.672*ER, volume of distribution (VSS, L/kg) = 1.25. The values for interindividual variability were 60.2% for CL, 43.2% for VSS, and the value for residual variability was 14.4%. These results suggest that the clearance of vancomycin for Japanese patients was approximately half to one third of the values reported value for Caucasians. In the case of a Japanese patient with the median values of body weight, ER, and CCR observed respectively, the blood trough level in a daily dosage of 0.5 g was calculated to be between 15 and 20 g/mL.

**Conclusions:** We successfully demonstrated the Japanese PPK parameters of vancomycin in special population with CRRT. 

**Key words:** vancomycin, renal replacement therapy, population pharmacokinetics, NONMEM, acute kidney injury

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**MELANOTAN II SKIN-TANNING DRUGS SOLD ILLEGALLY ON THE INTERNET: POOR MANUFACTURING AND CHALLENGES TO PUBLIC HEALTH**

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**Background**
Melanotan II is a synthetic heptapeptide hormone, sold on the illicit market and used to enhance the appearance of the skin by increasing skin pigmentation.

**Methods**
A new method was validated, based on liquid chromatography with ultraviolet detection (LC-UV) and tandem mass spectrometry (LC-UV-MS/MS), to determine the contents of injectable melanotan II products purchased from three Internet shops (n=26). Multiple reaction monitoring of the double charged [M+2H]^2+ precursor ion was used for identification of melanotan II in samples and LC-UV data (218 nm) were used to quantify melanotan II and estimate impurities.

**Results**
The amount of melanotan II in vials from shop A ranged from 4.70 to 5.12 mg, from shop B melanotan II ranged from 6.06 to 8.84 mg and from shop C melanotan II ranged from 4.32 to 5.56 mg in products purported to contain 10 mg. The level of unknown impurities in vials was in the range 4.1 to 4.8% for products from shop A and 4.6 to 5.9% for products from shop B. No impurities were detected in products from shop C. Vials from shop A were unlabelled. In contrast, products from shop C were labelled and arrived with a ‘tanning instruction leaflet’ similar to patient information leaflets included in licensed medicinal products.

The method was applied to determine the contents of a vial obtained from a user of enhancement drugs. Although the vial was purported to contain the synthetic peptide hormone GHRP-6 (and labelled accordingly), drug analysis revealed that the vial contained melanotan II as the only active substance.

**Conclusions**
A wide range of enhancement drugs are being sold on the illicit market which presents challenges to public health. From the analysis of melanotan II products, we conclude that products are mislabeled, vary considerably in amount of active substance and may contain high levels of impurities, potentially exposing users to a range of harms.
IN VITRO AND IN VIVO METABOLISM OF THE NPS METHOXYPIPERAMIDE (MEOP) STUDIED BY HYPHENATED MASS SPECTROMETRY
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Background
In 2013, the NPS methoxypiperamide (MeOP) - a piperazine-derived designer drug - was notified to the European Monitoring Drug Centre for Drug and Drug Addiction via the Early Warning System. The aims of this work were to identify its metabolites in rat urine for toxicological diagnostic reasons and the human cytochrome P450 (CYP) isoenzymes responsible for the initial metabolic steps to assess possible drug/drug or drug/food interactions.

Methods
After sample preparation using enzymatic cleavage followed by solid-phase extraction for elucidating phase I metabolites, the analytes were separated and identified by GC-MS (after additional derivatization) as well as LC-HR-MS/MS. For detection of phase II metabolites, the analytes were separated and identified after rat urine precipitation followed by LC-HR-MS/MS. [all according to Meyer et al., J.Mass Spectrom. 48 (2):243-249, 2013]

Results
The following metabolic steps could be observed in rat urine: cleavage of the carbonyl carbon-nitrogen bond, N-oxide formation, N- and/or O-demethylation, oxidation of the piperazine ring to the corresponding keto-piperazine, piperazine ring opening followed by oxidation of a methylene group to the corresponding imide or reduction of the carbonyl group to the corresponding alcohol, and hydroxylation of the phenyl group. Furthermore, N-acetylation, glucuronidation and sulfation were observed. Using recombinant human CYPs, CYP1A2, CYP2C19, CYP2D6, and/or CYP3A4 were found to catalyze N-oxide formation, N-, O-demethylation and/or oxidation. Mostly MeOP and N-oxide-MeOP, but to a minor degree also other metabolites could be detected in the GC-MS and LC-MSn standard urine screening approaches. [according to Maurer et al. Wiley-VCH, 2011 and Wissenbach et al. Anal.Bioanal.Chem. 400 (1):79-88, 2011.]

Conclusions
The NPS MeOP was excreted as metabolites but also unchanged in rat urine samples. Hence, MeOP and its metabolites could be detected in the authors SUSAs. Assuming similar metabolism and dosages in humans, detection should also be possible in human urine. Different CYPs were found to be involved in the initial metabolic steps, therefore, genetic polymorphisms and/or interactions should be of minor relevance.

Key Words
NPS, methoxypiperamide, metabolism, cytochrome
which corresponds to the minimum level in the blood, increased the intensity by 15%. However the most outstanding dynamic range existed between 100-1000 µg/mL, which exceeds the maximum safe level.

The electrode grafted with VCM-imprinted polymer including ferrocenyl group is feasible for reagentless sensing of TDM and might be useful for TDM of VCM after improvement of sensitivity.

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TRAMADOL PHARMACOKINETICS IN EXHALED BREATH, ORAL FLUID AND PLASMA

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Background: Exhaled breath is an interesting new matrix for bioanalysis. It has been routinely implemented for the analyses of drugs of abuse due to the simple collection procedure. So far, very little has been done regarding the time-concentration relationships of drugs and their metabolites in exhaled breath. Hitherto, nothing has been done regarding the pharmacokinetics of therapeutic drugs and their metabolites in exhaled breath. Therefore, we used tramadol as a model drug in the present study.

Methods: Twelve healthy volunteers (six men and six women) were recruited. Their mean age was 27 years (range 19-42). They received a single oral dose of tramadol (50 mg). Repeated sampling of exhaled breath, oral fluid and plasma was done for 48 hours after dosing. Samples were analyzed with LC-MS/MS and the pharmacokinetic correlations between different matrices were investigated.

Results: The clinical study was conducted in April 2015 and preliminary results indicate that the half-lives of tramadol in oral fluid and exhaled breath are roughly equivalent to those in plasma. One subject with a genotype suggesting poor metabolizer phenotype (CYP2D6*4/*4) had a delayed tramadol peak (3-4 hours compared to 1-1.5 hours) and prolonged half-life in plasma compared to the other subjects (9 hours compared to mean 5 (sd 0.6) hours). Tramadol levels in oral fluid (AUCinf 7474 µg*h/L; sd 4394) were considerably higher than in plasma (AUCinf 1240 µg*h/L; sd 929 ) but had otherwise similar pharmacokinetics. However, oral fluid levels of ODT (AUCinf 293 µg*h/L; sd 220) were much more variable and lower than the plasma levels (AUCinf 324 µg*h/L; sd 113). Preliminary data of tramadol and ODT concentrations in exhaled breath suggest peak levels are slightly delayed compared to plasma levels, but elimination kinetics are more variable.

Conclusion: This pilot study compares for the first time the pharmacokinetics in three different biomatrices. The true novelty of this study is to investigate the potential for exhaled breath as a matrix for TDM. In order to be useful for TDM, time-concentration relationships for therapeutic drugs in plasma and exhaled breath need to be clarified.

DETERMINATION OF ADALIMUMAB, INFlixIMAB AND ETANERCEPT TROUGH LEVELS: AN ASSAY COMPARISON

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Background: The currently available anti-TNFα biologics infliximab, adalimumab and etanercept are used to treat immune mediated inflammatory diseases, including psoriasis. Interest in the determination of through levels of these biologics is increasing to investigate dose-effect and concentration-effect relationships. To detect these biologics in plasma or serum, i.e. to determine through levels, ELISA-based test systems have been developed by different companies. Preferably, such assays should be cross-evaluated using the same reference samples to allow comparison of results obtained in different centres, using different assays. In the current study two available ELISA systems for the determination of the aforementioned anti-TNFα biologics are evaluated and compared.

Methods: Serum samples derived from infliximab-, adalimumab- and etanercept-treated patients, 40 each, were obtained and stored at -20°C until use. As control samples we obtained serum samples derived from ustekinumab-treated patients and healthy donors, 10 each. Furthermore, normal human serum was spiked with known amounts infliximab, adalimumab or etanercept. Two ELISA systems, biologic level ELISA produced by Sanquin (Amsterdam, The Netherlands) and Lisa Tracker produced by Theradiag (Marne La Vallee, France), were used. In addition, the ilite reporter gene-based bioassay produced by Biomonitor (Copenhagen, Denmark) was used. All assays were performed according to the manufacturer’s instructions.

Results: Both Sanquin and Theradiag ELISAs concordantly and sensitively detected infliximab, adalimumab and etanercept in the relevant patient groups showing only a single discrepancy in an infliximab-treated patient. The Sanquin ELISAs specifically detected the anti-TNFα biologic they were designed for, whereas the Theradiag ELISAs
showed cross-reactivity between the different anti-TNFα biologics. The anti-IL12/23 biologic ustekinumab was detected in neither ELISA. The Sanquin and Theradiag ELISAs showed a linear quantitative correlation in all respective assays (Pearson R²: 0.77 for infliximab level, 0.88 for adalimumab level and 0.68 for etanercept level). The comparative results for the ILite assay are currently in preparation.

**Conclusion:** Both Sanquin and Theradiag ELISAs appear suitable for therapeutic drug monitoring of anti-TNFα biologics. These ELISAs allow sensitive and comparable detection of infliximab, adalimumab and etanercept, however with clear differences in specificity.

**Key words:** adalimumab, etanercept, infliximab, ELISA, assay, biologic.

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**086**

**GREAT CLINICAL BENEFIT FROM MONITORING PROTEIN-UNBOUND VALPROIC ACID PLASMA CONCENTRATIONS IN SELECTED PATIENTS**

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**BACKGROUND**

Valproic acid (VPA) is an effective anti-epileptic drug, which is also used as mood stabilizer. A key characteristic of VPA is its high binding of approximately 90% to plasma proteins, predominantly albumin. While the 10% protein-unbound concentration of VPA is responsible for its pharmacological activity, total drug concentrations are monitored in routine clinical practice, mainly for feasibility reasons. In the local therapeutic drug monitoring (TDM) protocol of the Rijnstate Hospital, TDM of unbound VPA is recommended for specific clinical scenarios, such as decreased renal function or hypo-albuminemia. The goal of our study was to evaluate the use of TDM of unbound VPA.

**METHODS**

We evaluated all TDM requests for unbound VPA in 2014. In patients with potentially toxic concentrations, we evaluated whether toxicity was noted and whether the result was followed by a dose reduction of VPA. Unbound VPA concentrations were measured by means of a validated immune-assay at the laboratory of the Department of Clinical Pharmacy of the Radboudumc.

**RESULTS**

In 2014, we analyzed 82 unbound VPA plasma concentrations in 44 different patients. The median age of these patients was 65 years (range 12-86); 54% of them were men. The main reasons for requesting TDM of unbound VPA were decreased renal function (41%) and a low serum albumin (34%).

In the initial concentration measurement in these 44 patients, the median (range) unbound VPA concentration was 11.4 (3.5-34.7) mg/L (therapeutic concentrations 4-12 mg/L). The median (range) total VPA concentration was 57 (17-102) mg/L (therapeutic concentrations 50-100 mg/L). Thus, the median unbound fraction was 20%, which is a 2-fold elevation. In 20 of the 44 patients (45%), the unbound VPA concentration was above the threshold of 12 mg/L, potentially resulting in toxicity. In 14 of these 20 patients, clinical toxicity was noted, varying from drowsiness (n=14) to rigidity (n=1), lethargy (n=1) and even decreased consciousness (n=2). In 16 of the 20 patients with elevated unbound VPA concentrations, a dose reduction was applied (mean dose reduction 50%).

**CONCLUSION**

TDM of unbound VPA is an important tool to manage VPA therapy in selected, vulnerable patients.
Background: Sirolimus is an mTOR inhibitor which is increasingly being used in pediatrics. However, there is limited dosing experience in neonates and infants. Furthermore, there is a lack of mechanistic understanding of age-dependent changes in sirolimus disposition. The objective of this study was to analyze the developmental trajectory of sirolimus clearance (CL) in neonates and infants using a novel approach that combines population pharmacokinetic (PPK) analysis (Top-down) and physiologically-based pharmacokinetic (PBPK) modeling (Bottom-up).

Methods: 316 sirolimus concentrations were obtained as part of a prospective Phase II concentrations-controlled clinical trial of sirolimus in 24 patients with vascular anomalies (age: 1.1 months-4 years). Sirolimus and metabolite concentrations (24-, 25-, 46-hydroxy sirolimus, 16-O- and 39-O-demethyli sirolimus) were determined by LC-MS/MS. Individual sirolimus CL estimates at each sampling point over the course of treatment (1 month-1 year) were generated using Bayesian estimation (MW/Pharm, version 3.82). The relationship between individual sirolimus CL estimates and age at each sampling point was analyzed with NONMEM (version 7.2). In vitro incubation studies were conducted using recombinant P450s and results were used to develop the pediatric PBPK model with Simcyp Pediatric platform (version 14.1).

Results: Allometrically scaled sirolimus CL increased up to 2 years. Data were best described by the sigmoidal E\text{max} model. Estimates of TM\text{50} (postmenstrual age at which 50% of matured CL is reached) and matured CL were 60.1 weeks and 20.6 L/hr/70kg, respectively. An age-dependent increase was also observed in each individual patient over their treatment period. 25-OH-sirolimus and 16-O-demethyli sirolimus, normalized by sirolimus concentration, increased in a similar age-dependent manner not only in the population but also in individual patients over time. In vitro study results revealed that sirolimus metabolism is mainly catalyzed by CYP3A4 followed by CYP3A5>CYP3A7>CYP2C8. The PBPK model revealed a good age-dependent prediction of sirolimus CL.

Conclusion: This study suggests that CYP3A4 ontogeny is the most important factor responsible for age-dependent changes in sirolimus clearance in neonates and infants. Simulations with the PPK and PBPK models will facilitate the development of age-appropriate dosing guidelines for neonates and infants. Our study demonstrates the effective utilization of TDM data to explore the developmental trajectory of sirolimus disposition.

A MULTI-CLASS DRUG AND METABOLITE SCREEN OF 231 ANALYTES BY LC-MS/MS
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Background
The use of pain management drugs has been steadily increasing. As a result, hospital and reference labs are seeing an increase in patient samples that must be screened for a wide variety of drugs to prevent drug abuse and to ensure patient safety and adherence to their medication regimen. Therapeutic drug monitoring can be challenging due to the low cut-off levels, potential matrix interferences and isobaric drug compounds. To address these challenges, many drug testing facilities are turning to liquid chromatography coupled with mass spectrometry (LC-MS/MS) for its increased speed, sensitivity, and specificity. The Raptor[TRADEMARK] Biphenyl column was developed to complement high-throughput LC-MS/MS analyses by combining the increased efficiency of superficially porous particles with the resolution of Ultra Selective Liquid Chromatography[TRADEMARK] column technology. In this example, a method was developed for a 231 compound multi-class drug and metabolite screen.

Methods
There are many challenges one must consider when developing a large screening assay. Experiments performed included mobile phase considerations, sample diluent, isomer resolution, drug interferences, and instrumentation capabilities. Analytes were diluted in water and injected into a Shimadzu Nexera UHPLC equipped with an AB SCIEX API 4500[TRADEMARK] MS/MS. Detection was performed using electrospray ionization in positive and negative ion modes using scheduled multiple reaction monitoring (MRM).

Results
During mobile phase investigations, it was found that methanol provided the best retention of early eluting analytes, such as morphine, oxymorphone, nicotine and norcotinine. Sample diluent was optimized to improve the peak shape of the early eluting compounds. Scan rates and retention time windows were optimized to insure enough data points per peak were collected.

Conclusions
The final optimized separation was performed using water and methanol mobile phases modified with 0.1% formic acid and 2 mM Ammonium formate under gradient conditions on a Restek Raptor[TRADEMARK] Biphenyl 2.7µm, 100 x 2.1mm column equipped with a Raptor[TRADEMARK] Biphenyl EXP® 2.7µm, 5 x 2.1mm guard column. The gradient run time was 10 minutes, with a total cycle time of 12 minutes. Of the mixture of analytes, 209 were analyzed in positive ion mode, and 22 were analyzed in negative ion mode.
LC-MS/MS METHOD DEVELOPMENT CHALLENGES FOR THE SEPARATION OF 43 OPIOIDS AND METABOLITES
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Background
The use of liquid chromatography coupled with mass spectrometry (LC-MS/MS) has become a routine method of analysis in therapeutic drug monitoring and clinical toxicology labs. LC-MS/MS provides sensitivity, speed, and specificity when analyzing drugs in complex biological matrices, such as plasma, serum, and urine. Because of the similarity of many opioids and metabolites, chromatographic separation becomes increasingly difficult due to the number of structural isomers and the need to chromatographically resolve these isomers due to their identical mass spectral fragmentation patterns. In this example, a method was developed for the separation of 43 common opioids and their metabolites on a Raptor® Biphenyl column. The Raptor® Biphenyl column was developed to complement high-throughput LC-MS/MS analyses by combining the increased efficiency of superficially porous particles (SPP) with the resolution of Ultra Selective Liquid Chromatography (USLC®) technology.

Methods
During development, 3 mixtures, containing a total of 43 opioids, were diluted in water and injected into a Shimadzu Nexera UHPLC equipped with an AB SCIEX API 4500® MS/MS. Detection was performed using electrospray ionization in positive ion mode using scheduled multiple reaction monitoring (MRM). During initial method development, multiple mobile phase combinations and additives were investigated. Comparisons were made using scouting gradients to evaluate retention, resolution, and sensitivity.

Results
During the mobile phase investigation, it was found the use of a neutral pH mobile phase resulted in peak tailing. It was also noticed that when a buffered, acidic mobile phase was used, there was a distinct decrease in sensitivity for buprenorphine and norbuprenorphine. The use of methanol as the organic mobile phase had the benefit of retaining early eluting compounds, at the sacrifice of the resolution of noroxycodone and dihydrocodeine.

Conclusions
The final optimized separation was performed using water and acetonitrile mobile phases modified with 0.1% formic acid under gradient conditions on a Restek Raptor® Biphenyl 2.7µm, 100 x 2.1mm column equipped with a Raptor® Biphenyl EXP® 2.7µm, 5 x 2.1mm guard column. Resolution of all isobars was achieved under these mobile phase conditions, and gradient conditions were optimized to retain early eluting analytes.

AN EVALUATION OF DUTCH PHARMACISTS' KNOWLEDGE, EXPERIENCE, AND ATTITUDES TOWARDS PHARMACOGENETIC TESTING: RESULTS OF A NATIONWIDE SURVEY
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Background:
Pharmacists, as medication experts could play an essential role in the implementation of pharmacogenetics (PGx) guided drug therapy in routine patient care. The goal of this study was to assess knowledge, experience, and attitudes towards PGx among Dutch pharmacists.

Methods:
A web-based survey with 35 questions concerning PGx related topics was sent to Dutch pharmacists both in community and hospital setting using the website netq-enquete (1). Statistical analysis was performed with chi² analysis using SPSS software.

Results:
Out of the 3550 invited pharmacist pharmacists a total of 667 (18.8%) completed the survey. Virtually all respondents believed in the concept of Pharmacogenetics (99.7%) and had high expectations of PGx testing to improve efficacy, reduce toxicity and prevent erroneous drug therapy. Almost all respondents (89%) would like to receive additional training on the subject. Approximately 40% of the pharmacists had received any education on PGx in their curriculum. However, only 27.0% of the responding pharmacists consider themselves qualified to interpret a PGx test result. 14.7% of the respondents had recommended PGx testing in the previous 6 months. Significant differences in experience, attitudes and knowledge of PGx were observed between community and hospital based pharmacists.

Conclusion:
Pharmacists' attitude towards PGx is very positive and they express high expectations that PGx can improve efficacy, reduce toxicity and prevent erroneous drug choice or dosing. However, only a small number has recommended a PGx test and there is a clear demand for training on the subject.


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VANCOMYCIN CLEARANCE IN NEONATES AND INFANTS WITH SINGLE VENTRICLE: INFLUENCE OF SURGICAL PROCEDURE ON THE DEVELOPMENTAL TRAJECTORY WITHIN INDIVIDUALS
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BACKGROUND:
Vancomycin is eliminated mainly through the kidneys. Due to rapid developmental changes in renal function and body weight, renal elimination of vancomycin increases during the neonatal and infantile periods. The recommended doses in the Sanford Guide To Antimicrobial Therapy are 36-44 and 40-60 mg/kg/day for neonates and infants; however, from our experience, these doses appear to cause excessive exposures in patients with single ventricle who undergo early neonatal major surgeries. We hypothesized that a bidirectional Glenn procedure which reduces the volume overload of single ventricle alters vancomycin pharmacokinetics. The objective of this study was to examine the pharmacokinetics of vancomycin in patients undergoing graded procedure for single ventricle and analyze the changes after the procedure within individuals.

METHODS:
From January, 2012 to September, 2014, 21 infants were identified undergoing bidirectional Glenn procedure for single ventricle at the National Cerebral and Cardiovascular Center. All patients were treated with vancomycin pre- and post-bidirectional Glenn procedure by intravenous infusion for 2 hours. Serum vancomycin concentrations at trough were measured by enzyme immunoassay. Vancomycin clearance was calculated by the Bayesian method.

RESULTS:
Mean patient ages were 5.79 weeks and 41.9 weeks, and mean vancomycin doses required to maintain the trough concentrations of 10-15 mcg/mL were 20.5 mg/kg/day and 34.2 mg/kg/day pre- and post-procedure, respectively. The dose pre-procedure was lower than the recommended dose. Allometrically-scaled estimated vancomycin clearance post-procedure (3.06 ± 1.24 L/hr/70kg) was significantly elevated from that pre-procedure (1.29 ± 0.47 L/hr/70kg). Eighteen out of 21 patients demonstrated larger elevation in vancomycin clearance as a ratio than presumed elevation in glomerular filtration rates as a marker of kidney function based on the reported developmental trajectory. This elevation in clearance over time was probably due to maturation of the kidneys. In addition, improvement of systemic circulation by the bidirectional Glenn procedure could account for a better agreement between observed and reported doses and clearances after the bidirectional Glenn procedure.

CONCLUSION:
This study suggests that vancomycin clearance is decreased in neonates with single ventricle and is improved by bidirectional Glenn procedure in the infantile period. A careful dosage with drug monitoring is strongly recommended.

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GENOTYPE-PHENOTYPE RELATIONSHIP OF INOSINE TRIPHOSPHATE PYROPHOSPHATASE (ITPA) AMONG NORMAL AND ABNORMAL THIOPURINE S-METHYLTRANSFERASE (TPMT) ACTIVITY SAMPLES.
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Background - Azathioprine and 6-mercaptopurine (6-MP) are commonly used as anticancer and immunosuppressant agents. Thiopurine S-methyltransferase (TPMT) catalyzes the primary inactivation pathway in which ~90% of 6-MP is converted to an inactive metabolite, 6-methylmercaptopurine (6-MMP). In addition, inosine triphosphate pyrophosphatase (ITPA) is also involved in the inactivation of 6-MP. Life-threatening adverse effects have been documented when standard dosing is administered to individuals with TPMT deficiency, due to accumulation of toxic drug and metabolites. TPMT genotype and/or phenotype can be used to identify patients at risk for adverse effects prior to drug administration to minimize the incidence of toxicity and improve dose selection. The purpose of this study was to evaluate the relationship of ITPA mutations in a sample population of
normal and abnormal TPMT activity. Method - TPMT enzyme activity was determined from lysed red blood cells (RBCs). The concentration of 6-MMP produced from the enzyme reaction in the lysate was determined by LC-MS/MS and results were converted to units of activity per mL of lysed packed RBCs (U/mL). 191 blood samples were collected from patients with TPMT activity in the range of 15 - 35 U/mL. TPMT (r2,*3A-C) and ITPA (rs1127354, rs7270101) genotypes were determined using a custom TaqMan® OpenArray® on the QuantStudio® (TRADEMARK) 12K Flex system (Life Technologies). Results - No TPMT variants were detected in 69 of the total patients tested. However, 33% of these normal TPMT phenotype samples had at least one ITPA mutation. The most common variant TPMT alleles (‘*3A and ‘*3C) were found in the subpopulation of samples with abnormal TPMT activity, of which at least one ITPA mutation was detected in 33%. Lastly, there were a few patients identified as deficient TPMT from the detection of two mutant alleles and each of these patients had at least one ITPA mutation. Conclusions - Based on this small subset of patients, this data demonstrates a clustering of variant TPMT genotypes with phenotype, but a somewhat equal distribution of ITPA mutations among normal and abnormal TPMT phenotype and genotype.

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PHARMACOKINETIC AND PHARMACODYNAMIC (PK/PD) GUIDED ECULIZUMAB TREATMENT IN CHILDREN WITH HEMATOPOIETIC STEM CELL TRANSPLANT-ASSOCIATED THROMBOTIC MICROANGIOPATHY (HSCT-TMA)
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Introduction
Eculizumab is a humanized monoclonal antibody that binds to C5 complement protein and inhibits terminal complement-mediated activation. It has been used for Paroxysmal Nocturnal Hemoglobinuria (PNH) and Atypical Hemolytic Uremic Syndrome (aHUS). HSCT-TMA is a life-threatening post-transplant complication (with high mortality rates up to 90%). Our pilot study clearly indicates that eculizumab is a promising therapeutic option for children with severe HSCT-TMA (Jodele, BBMT 2014). The purpose of this analysis was to evaluate eculizumab pharmacokinetics in relation to the degree of complement blockade and clinical response using PK/PD modeling and to propose an optimal dosing strategy for children with HSCT-TMA.

Methods
Prospectively collected laboratory samples and clinical data of eighteen children with severe HSCT-TMA on eculizumab therapy were available for analysis. The first starting dose was based on recommendations for children with aHUS (Schmidtko, AJKD 2013). Subsequent dose adjustments were at physician’s discretion based on clinical symptom and laboratory biomarkers available. Eculizumab concentrations, total hemolytic complement activity (CH50), and soluble terminal complement complex (sC5b-9) were obtained daily. Population PK/PD analysis was performed to correlate eculizumab concentrations with the degree of complement blockade and to examine inter-individual differences in PK parameters.

Results
Twelve of 18 children (67%) achieved resolution of HSCT-TMA. Increased and/or more frequent doses (2-3 times per week) were needed for the first 2 weeks to reach complete complement blockade (CH50) and to achieve the suggested therapeutic pre-dose eculizumab target of >99 mg/L recommended for children with aHUS. Individual Eculizumab clearances calculated after the first dose were significantly higher than estimates at 3rd week (p<0.0003). Pre-treatment sC5b-9 values in addition to body weight significantly correlated with initial clearances (p<0.01). CH50 activity directly correlated with eculizumab concentration and clinical response.

Conclusions
Our analysis suggests that higher and/or more frequent dosing is required to achieve the therapeutic target more rapidly, especially in patients with highly elevated sC5b-9 values at start of treatment. Since clearance changes over time, real-time PK/PD(CH50) guided dosing would allow tailoring of dose to individual response. This has a high likelihood to further improve cure rates for children with HSCT-TMA and is currently being studied.

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CANNABIS USE IN PREGNANCY AND NEONATAL ABSTINENCE SYNDROME: A CASE REPORT.
Introduction: Cannabis is the most commonly used drug in pregnant women with a prevalence rate between 3 and 16% in Western countries. The active ingredient of cannabis, Δ9-tetrahydrocannabinol (THC) and its metabolites can cross the placental barrier and then accumulate in the fatty tissue fetal.

Case report: We report the clinical evolution of a patient born by caesarean section for difficult progression during labor at 39th gestational week. Thirteen hours after birth muscular hypertonia, hyperexcitability, tremors and high-pitched crying ensued. On the next day, symptoms were attenuated but persistence of tremors required treatment with diazepam (0.5 mg/kg/day). Other causes potentially responsible for the clinical picture were excluded. Routine laboratory tests were normal. Two days after the onset of symptoms the mother reported daily use of cannabis for over 10 years. Newborn's urine screening confirmed the presence of cannabinoids, while in blood samples the level of 11-nor-9-carboxy-delta-9-tetrahydrocannabinol (THC-COOH) was 17.2 ng/ml (cut-off 5 ng/ml) and THC level was below of 1 ng/ml (cut-off 2 ng/ml).

Discussion: In 16-29% of adults regular cannabis users, the abrupt termination may get a withdrawal syndrome and occasional case reports of withdrawal manifestations have also been reported in infants whose mothers routinely used cannabis during pregnancy. Late gestational exposure THC could be associated with a neonatal toxicity syndrome with immediate onset at birth or soon after birth and sometimes may be confused with neonatal withdrawal syndrome: the differential diagnosis between intoxication and neonatal abstinence is possible only through the plasma assay of cannabinoids and metabolites, as occurred in the present case.

Conclusion: Despite the large prevalence of cannabis use during pregnancy, the cases of neonatal abstinence reported in the literature are extremely scarce. This is probably due to the fact that the self-reporting of drug use is omitted by the mother, since the use of cannabis is illegal. The clinical case presented suggests that in case of neonatal neurological symptoms of unclear etiology, urinary screening of drugs of abuse in the newborn and a possible confirmation by determining plasma THC and its active metabolites may be useful for a correct diagnosis.
Background: Major depressive disorder (MDD) is a common mental disorder. The treatment response to antidepressants is still limited and varies for each patient. However, there have been a few studies about biomarkers to predict the antidepressant response in MDD and also no firm predictor to choose antidepressants. To discover peripheral biomarkers related with antidepressant response and pathophysiology of MDD, we profiled the RNA expressions in peripheral blood specimens from MDD and healthy individuals.

Methods: We performed gene expression profiling using GeneChip Human Gene 1.0 ST Array (Affymetrix, Santa Clara, CA, USA) on peripheral blood specimens at baseline and at 6 weeks after the initiation of either selective serotonin reuptake inhibitor (SSRI) or non-SSRI treatment from MDD patients and from healthy individuals. The gene expression patterns were compared between each comparison group; patients vs. controls, response vs. non-response, and pre-treatment vs. post-treatment.

Results: Sixteen genes, ACRC, APHTA, BBS10, GOLPH3L, HDGF, KCTD20, LRIF1, NDUFAT1, OMA1, PAPOLG, PPID, SNORD41, STK17A, TRIP11, VTA1, and ZSWIM3, showed different gene expressions between MDD patients and controls. Hierarchical clustering using above 16 genes demonstrated that patients at baseline were separated from controls, and this separation was not dependent on gender and antidepressants. In comparison of RNA expression at baseline to discover biomarkers to predict treatment response, the expression of FAM118A gene (corrected p-value = 0.018, fold change = 0.49) was shown a difference between response and nonresponse group treated with SSRI at baseline.

Conclusions: Our study shows a disruption of RNA expression in MDD patients, and gene expression differences as candidate markers for the prediction of antidepressant response in the pretreatment specimen between response and nonresponse MDD patients treated with SSRI. These findings may be helpful to provide a rationale for personalized medicine for the antidepressant treatment of MDD patients in the future.

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ANTIMICROBIAL UNDEREXPOSURE IN INTENSIVE CARE PATIENTS WITH 'NORMAL' RENAL FUNCTION

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Background
Adequate antimicrobial exposure is crucial during critical illness. Recent work has shown that antimicrobial exposure varies dramatically in intensive care patients, and that augmented renal clearance may contribute to under-exposure.

Methods
Retrospective review of piperacillin, ciprofloxacin and meropenem therapeutic drug monitoring (TDM) in a 20-bed intensive care unit (ICU) at a tertiary teaching hospital, using the first TDM order to reflect empiric dose selection. Piperacillin and meropenem were collected as troughs (Cmin) and ciprofloxacin was collected as a peak (Cmax). Concentrations were determined in EDTA plasma, using HPLC-MS/MS. Data were extracted from the patient record and the ICU admissions and laboratory databases. Minimum inhibitory concentrations (MICs) were determined by broth microdilution. If a pathogen was identified without an MIC, the relevant European Committee for Antimicrobial Susceptibility Testing (EUCAST) breakpoint was used. If cultures were negative the EUCAST breakpoints for Pseudomonas spp. were used. The PK/PD targets were Cmin/MIC>1 for piperacillin and meropenem and Cmax/MIC>10 for ciprofloxacin. Estimated glomerular filtration rate (eGFR) was estimated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula.

Results
250 tests (ciprofloxacin 55, piperacillin 118 and meropenem 77) were performed on 202 patients (216 admissions). Median age of patients was 56y (71% male). Median duration from prescription to collection of the TDM sample was 36h. A pathogen was identified in 39% of cases, 47% of these had an MIC determined. There was large variability in exposure to the study drugs. Patients with 'normal' renal function (eGFR>90) were underexposed (Cmin/MIC<1) to piperacillin and meropenem on 47% (p<0.0001) and 29% of occasions respectively, more often than those with lower renal function. The median age of these patients was less than those with renal impairment (44 vs. 59y p<0.0001). Higher doses of meropenem were prescribed in renal impairment, otherwise prescribing was consistent with recommendations.
Underexposure occurred from 46/56 ciprofloxacin, 29/118 piperacillin and 15/77 meropenem prescriptions respectively. Patients with adequate exposure were prescribed 22% more than the recommended dose (p<0.0001).

**Conclusion**

Underexposure to antimicrobials occurs frequently in the ICU. Clinicians should be wary of selecting manufacturer recommended doses especially when treating young patients with 'normal' renal function.

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**HONEY-PROPOLIS HEPATOPROTECTIVE EFFECT ON ALCOHOL-INDUCED HEPATOCYTOTOXICITY IN VITRO IN NORMAL HUMAN HEPATOCYTES**

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Background: Alcohol consumption induces liver injury.

Objective: To evaluate whether caspases are involved in ethanol (EtOH)-induced apoptosis and if honey-propolis affects EtOH-apoptosis, in vitro in normal human hepatocytes (NHH).

Methods: 100 mmol/L EtOH for 24 hrs and with 2 doses of 100 mmol/L EtOH (1/24 hrs) in the presence of absence of 1mg/mL of PPC or 50 mmol/L caspase 3 inhibitor (IDN). Cells were analyzed for apoptosis by transmission electron microscopy (TEM) 6000 cells/treatment, DNA fragmentation of cytokeratine CK-18 (M30). Cytotoxicity was determined using succinate dehydrogenase activity.

**Results:** 100 mmol/L dose of EtOH resulted in 22±2.5 % (p<0.001) apoptosis (vs. control). Two consecutive doses of 100 mmol/L EtOH for 24 hours each caused 36±3.0 % (p<0.001 vs. control and p<0.05 vs. one dose). Honey-propolis significantly reduced apoptosis (vs. non exposed to honey-propolis): 100 mmol/L -12±1.5 % (p<0.05) and 2x100 mmol/L -20±4.0 % (p<0.001). Pre-treatment with 50 mmol caspase inhibitor reduced EtOH-induced apoptosis in a similar proportion. By TEM ethanol-exposed cells present, ballooning, changed in mitochondrial cristae and apoptosis. Also Mallory bodies have been observed. Pre-treatment with 50 μmol caspase inhibitor reduced EtOH-induced apoptosis significantly (vs. non-exposed to caspase-inhibitor): Δ -14±0.5 % (p<0.05).

Conclusion: Honey-propolis (APISAN) downregulates EtOH-apoptosis by caspase-effector inhibition.

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**POPULATION PHARMACOKINETIC MODELING OF LEVETIRACETAM IN PEDIATRIC AND ADULT EPILEPTIC PATIENTS USING THERAPEUTIC DRUG MONITORING DATA**

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**Background:** Levetiracetam, a new antiepileptic drug of second generation, is frequently used as an adjunctive therapy for partial onset seizures. About 70% of administered dose is excreted in urine as an unchanged form, and dosage adjustment is recommended based on the individual renal function. In this study, we developed a population pharmacokinetic model of levetiracetam using routinely monitored plasma concentration data.

**Methods:** Patients whose plasma concentrations of levetiracetam at steady state were measured at Kyoto University Hospital from April 2012 to March 2013 were enrolled in this study. The influence of patient characteristics on levetiracetam pharmacokinetics was evaluated using the nonlinear-mixed effects modeling program NONMEM.

**Results:** A total of 583 steady-state concentrations from 225 patients were used for the analysis. Patient age and estimated glomerular filtration rate (eGFR) were from 1 to 89 years old, and from 15 to 189 mL/min/1.73m², respectively. Plasma concentration-time data of levetiracetam were well described by a one-compartment model with first-order absorption. Oral clearance was allometrically scaled to body weight and eGFR. An increase in the dosage significantly increased the oral clearance. No improvement in the model fit was observed by including any concomitant antiepileptic drugs. Population clearance for an adult with 70 kg with normal renal function was 4.8 and 5.9 L/h for 500 mg bid and 1500 mg bid, respectively.

**Conclusions:** Oral clearance allometrically scaled to body weight and eGFR can predict the routine therapeutic drug monitoring data from children to aged patients with several renal functions. Levetiracetam showed a non-linear pharmacokinetics. These findings will contribute to the management of individualized therapy of levetiracetam.
HEPATIC SINUSOIDAL OBSTRUCTION SYNDROME INDUCED BY PYRROLIZIDINE ALKALOIDS
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Background: Traditional herbal medicines containing pyrrolizidine alkaloids (PAs) induce hepatic sinusoidal obstructions syndrome or veno-occlusive disease (VOD). PAs are consumed as teas, or as infusions for medicinal purposes. PURPOSE: 1) to present two cases biopsy-confirmed VOD and one case of hypersensitivity (HSR), ultrasound confirmed VOD; 2) to encourage recognition and prevention of VOD encountered when using complementary and alternative medicine containing PAs. METHODS and RESULTS: The first case, a 71-year-old woman was hospitalized twice with the same clinical picture: cahectic with a palpable liver. Laboratory work revealed pancytopenia, low haemoglobin levels, hypoglycemia, elevated aminotransferases. The liver biopsy presented central areas with marked congestion and hepatocellular atrophy, as well as centrolobular necrosis consistent with VOD. Upon the discontinuation of the herbal remedies the patient improved clinically and all liver functions returned to normal. The second case was a 3.5 month old infant that had been given an infusion of herbal medicine daily for one week before admission. She developed hepatomegaly and ascites. Laboratory results presented 1.5-2.5 x normal aminotransferases, gamma glutamyl transferase x 4 normal and x 2 bilirubin. A post-mortem liver biopsy revealed hepatocellular necrosis, the collapse of reticulin structure in zone 3 and occlusion of the effenter hepatic venules and hepatic sinusoids consistent with VOD. The mechanism of PA-injury consist of depletion of glutathione leading to mitochondrial damage. Also the release of pro-inflammatory signals such as cytokines that promote cell damage and integrins that link the leukocytes to cell adhesion molecule and injure the venules. The PA-induced HSR, was a 65 old women that used a natural-herbal cosmetic product for several weeks before presenting an HSR. Doppler ultrasound was useful in confirming the VOD diagnosis. Upon discontinuation of the product the elevated transaminases and clinical picture improved. CONCLUSIONS: Our results confirm that herbal remedies containing PAs induce VOD. Stopping immediately the "remedy" prevents further harm to the liver. The active metabolite PA-induced cytotoxicity should be analyzed in remedies and cosmetics containing herbal ingredients.

ALCOHOL-ENHANCE HEPATOCYTOTOXICITY PROFILE OF PYRROLIZIDINE ALKALOID-CONTAINING MEDICINAL PLANTS IN VITRO NORMAL HUMAN HEPATOCYTES
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Pyrrolizidine alkaloids (PAs) are found in various plant genera worldwide. Poisoning by PA-containing plants is usually accidental, by the ingestion of grain inadvertently contaminated with seeds of pyrrolizidine-containing weeds, or the consumption of herbal or bush tea, or when taken as herbal infusions for medicinal purposes. The interaction between PA and alcohol is the purpose of the present work.

OBJECTIVES: 1- To determine the mechanism(s) of PA-induced toxicity in human normal hepatocytes (HNH) in vitro; 2- To evaluate whether caspases are involved in PA-induced apoptosis.

Design and Methods: 1- Cells were treated with aqueous extracts of Senecio (10 mg/mL) with/without 100 mM ethanol. Cytotoxicity was determined using succinate dehydrogenase activity. Total glutathione (GSH) was measured using the Tietze assay. 2- Cells were treated with aqueous extracts of PA in the presence or absence of 50 μmol/L caspase 3 inhibitor for 24 h. Apoptosis was determined by transmission electron microscopy (TEM) and CK-18 (M30).

Results: PA produced cytotoxicity and depleted GSH in a concentration- and time-dependent manner. A significant depletion in GSH was observed after 15 min (p < 0.001 vs. control), whereas significant cytotoxicity was only observed after 3 h (p < 0.001 vs. control). Treatment with alcohol enhanced PA-induced GSH depletion and resulted in a significant increase in PA-induced cytotoxicity (p < 0.001 vs. ethanol-un treated cells). Treatment with PA for 24 h resulted in 22±2.5 % (p<0.001) apoptosis (vs. control). Pre-treatment with 50 μmol caspase inhibitor reduced PA-induced apoptosis significantly (vs. non-exposed to IDN): Δ -12±1.5 % (p<0.05). Pre-treatment with 50 μmol caspase inhibitor in cells treated with PA + ethanol reduced apoptosis significantly (vs. non-exposed to caspase-inhibitor): Δ -22±3.0 % (p<0.05).

Conclusions: Our results suggest the mechanism of PA-induced cytotoxicity in HNH in vitro involves depletion of cellular GSH. Preventing GSH depletion by supplementation with antioxidants may abolishes PA-induced hepatocytotoxicity. Caspase activation is involved in the apoptosis induction by PA.
Elucidating the factors involved in herbal remedies-induced toxicity has medical significance. Currently, there is no antidote for natural-substances that induce liver damage.

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NFAT-REGULATED CYTOKINE EXPRESSION DURING TACROLIMUS THERAPY EARLY AFTER TRANSPLANTATION
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Background
Although calcineurin inhibitors (CNI) have contributed to significant improvements in short-term outcome after transplantation (Tx), long-term outcome is still hampered by the severe CNI side effects. Monitoring NFAT-regulated gene expression is a promising pharmacodynamic strategy for further improvement of CNI therapy. The aim of this study was to characterize NFAT-regulated gene expression in relation to tacrolimus (Tac) concentrations and clinical outcome in 29 renal allograft recipients.

Methods
Samples were drawn pre-Tx and pre-dose and 1.5 hours post-dose approximately 1, 5-7 and 52 weeks post-Tx. Tac concentrations were measured by immunoassay, while the expression of nuclear factor of activated T-cells (NFAT)-regulated cytokines (IL2, IFNG, CSF2) was determined by real-time PCR after ex vivo immune activation.

Results
Tac pre-dose concentrations (C0) were median 5.1 μg/L (2.5-7.8 μg/L, n=26), 6.15 μg/L (3.5-9.3 μg/L, n=26) and 5.85 μg/L (3.2-7.4 μg/L, n=16) at week 1, 5-7 and 52, respectively. Tac concentrations 1.5 hour post-dose (C1.5) were more variable, ranging 3.5-23.3 μg/L (n=26), 5.6-18.7 μg/L (n=26) and 4.2-12.7 μg/L (n=16) at 1, 5-7 and 52 weeks post-Tx.

The strongest inhibition of NFAT-regulated cytokines was observed 1 week post-Tx with median residual gene expression (RGE) of 18.5 % (1.5-68.7 %, n=26) versus 42.2 % (5.7-76.2 %, n=22, P=0.039) and 47.7 % (18.6-84.5 %, n=16, P=0.004) at 5-7 and 52 weeks, respectively. Tac C1.5 >15 μg/L was associated with strong cytokine inhibition (RGE ≤10 %), while lower Tac C1.5 resulted in highly variable inhibition (RGE 2.5-68.7 %).

Patients with ongoing subclinical acute rejection (n=5) demonstrated limited cytokine inhibition (RGE 39.7-72.6 %), while patients with persisting polyoma virus viremia (n=3) showed relatively strong inhibition (RGE 2.5-32.5 %). In contrast, there was no association between Tac exposure and clinical outcome.

Conclusions
Despite similar Tac exposure, the pharmacodynamic response varied considerable between patients. The difference in cytokine inhibition among patients with rejection versus viremia supports the potential of NFAT-regulated gene expression as a tool for further improvement of Tac therapy. However, further knowledge is needed considering different patient populations, interfering parameters, monitoring strategies and target ranges.

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QUALITATIVE COMPARISON OF LC-MS AND IMMUNOAASSAYS IN SERUM OF EMERGENCY TOXICOLOGICAL CASES
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Background
Usually drugs of abuse screening tests are performed in urine and not in blood as the commercially available tests are only intended for this sample material. However, the collection of urine may be difficult in acutely intoxicated patients. Furthermore, the substances detected in urine do not necessarily reflect the circulating substances that cause the acute toxicity. At the University Hospital Basel, drugs of abuse immunoassays in serum are performed in every patient presenting to the emergency department with a suspected intoxication. In the literature, cut-off concentrations in serum are only available for forensic applications, where all positive results are mandatory confirmed by a chromatographic method.

Methods
Serum samples of over 600 patients attending the emergency department with a suspected intoxication were analyzed using different DRI® and CEDIA® immunoassays intended to be used with urine and oral fluid (n = 300) using different pipetting protocols. For confirmation analysis a targeted LC-MS screening method using online extraction1-2 and detecting over 700 drugs (including novel designer drugs) was applied. The sensitivity and specificity of the different immunoassays in serum were established and cut-off concentrations defined for clinical
situations.

Results
An increased sample volume using the urine drugs of abuse immunoassays resulted in a decreased sensitivity of the assays without more false positive results. Despite a comparable metabolite pattern in oral fluid and serum for most drugs, the immunoassays intended to be used with oral fluid did not show any or only partial superiority over the standard immunoassays. After ROC-curve analyses, cut-off concentrations for the modified urine immunoassays could be defined for serum samples. Nevertheless, several cases were found with false negative results compared with LC-MS\(^3\), likely due to bad cross reactivity of the compound.

Conclusion
In a clinical laboratory immunoassays are an essential drug screening method in the clarification of an intoxicated patient where time is crucial and LC-MS analysis is often not available during 24 hours. With the newly defined cut-off concentrations for the DRI® and CEDIA® immunoassays, serum can be used as sample material without an increased risk for false positive or negative results compared to urine samples.

VALIDATION OF A RAPID LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY ASSAY FOR QUANTIFICATION OF IMATINIB IN HAIR SAMPLES.

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Background
Studies estimated imatinib (a tyrosine kinase used in chronic myeloid leukemia) treatment adherence rates at approximately 75 to 90%, with higher adherence associated with better treatment outcome. However, the assessment of compliance presents many challenges, usually associated to the reliability of patient and pharmacy reports. Therefore, as an additional tool to serum imatinib monitoring, selective and sensitive liquid chromatography tandem mass spectrometric (HPLC-MS/MS) assay was validated for measuring this tyrosine kinases levels in human hair samples.

Methods
Imatinib calibrators were prepared in drug-free hair sample covering concentration range from 0.5 to 25 ng/mg. A 5mg hair strand was decontaminated by washing it successively in water and acetone for 15 minutes at 37°C. Sample was then dried and finely cut with a scissors. After decontamination, imatinib-d8 used as internal standard, and 500 µL of ethyl acetate were added. After two hours of sonication at 60°C, and centrifugation, organic phase was evaporated under nitrogen, and reconstituted in 100 µL of acetonitrile. 20 µL was injected into HPLC-MS/MS system (Alliance 2795 from Waters\(^\circledR\)) coupled with a Quattro-micro from Micromas\(^\circledR\)). Separation was achieved using a Waters® XTerra® C8 (2.1 mm x 50 mm, 3.5 µm) column maintained at 50°C, in isocratic conditions, at a flow rate of 0.3 ml/min with a mixture consisting of 45%:55% solvents A (2mM ammonium acetate buffer; 0.1%formic acid) and B (0.1% formic acid in acetonitrile). The total run time was of 5.5 minutes. Imatinib and IS were identified in positive electro spray ionization mode using ion transitions of m/z 494.5>394.3 and 503.0>394.3 respectively.

Results
The assay was validated in the concentration range of 0.5-25 ng/mg, with intra- and inter-assay imprecisions of <13.1 % and <9.3%, respectively. The limits of quantification and detection were 0.5 and 0.125 ng/mg, respectively. The recovery and ion suppression ranged between 71.7% to 80.7% and -10% to -17.3%, respectively.

Conclusions
We propose a fast, sensitive, and selective LC-MS/MS method allowing quantification of imatinib in hair samples. This assay might be suitable to investigate the eventual correlation between hair and plasma drug concentration, with potential contribution to therapeutic drug monitoring.

COMPARISON OF ISONIAZID AND RIFAMPICIN EXPOSURE BETWEEN INTERMITTENT AND DAILY ANTI-TUBERCULOSIS REGIMEN, IN CHILDREN WITH TUBERCULOSIS-AN INTERIM ANALYSIS

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BACKGROUND:
The currently recommended doses of antitubercular (ATT) drugs in children are extrapolated from adult pharmacokinetic studies. The Revised National Tuberculosis Control Programme in India advocates treatment through intermittent drug regimen, whereas WHO recommends daily therapy during intensive phase of the ATT. Sub-therapeutic ATT concentrations could lead to failure of therapy and emergence of drug resistance. This study compared the exposure of isoniazid and rifampicin in children receiving either intermittent or daily ATT regimen.

METHODS:
Children aged 2 to 15 years initiated on either daily or intermittent (thrice weekly) ATT were recruited. Towards the end of intensive phase, blood specimens were collected predose, followed by 0.5hrs, 1hrs, 1.5hrs, 2hrs, 2.5hrs, 4hrs and 6hrs postdose. The concentrations of isoniazid and rifampicin were analysed using validated LC-MS/MS and HPLC assays, respectively. Results were analysed using Wilcoxon rank sum test with R-version 3.1.2.

RESULTS:
The median dose (mg/kg) for isoniazid was 8.48 versus 8.0; C0 (µg/ml) was 0 versus 0.16 (p=0.02) and Cmax (µg/ml), C0 (µg/ml), C0 (µg/ml) were 6.5, 4.2 and 1.6 versus 6.2, 5.7 and 2.1 respectively. Median AUC0-6hrs (mg.hr/L) was 20.54 versus 27.33.
The median dose (mg/kg) for rifampicin was 8.48 versus 11.25; C0 (µg/ml) was 0 (for both regimens) and the Cmax (µg/ml), C0 (µg/ml), C0 (µg/ml) were 5.96, 3.41, 0.54 and 1.6 versus 5.9, 4.4 and 2.12 respectively. Median AUC0-6hrs (mg.hr/L) was 12.06 versus 18.43.
Correlation between age and dose normalised AUC0-6hrs was poor for isoniazid and rifampicin ($r^2=0.01$ and 0.14 respectively). All patients (except one with poor compliance) had isoniazid $C_{\text{max}}$ above 3µg/ml (recommended range: 3-6µg/ml) $^\ast\ast$, of which 62% had $C_{\text{max}}$ above 6µg/ml. 82.3% of the patients had rifampicin $C_{\text{max}}$ less than recommended range (8-24 µg/ml)$^\ast\ast$
$C_{\text{max}}$ correlated well with AUC0-6hrs in both regimens ($r^2=0.81$ and 0.92 for isoniazid; $r^2=0.74$ and 0.92 for rifampicin; intermittent and daily regimen respectively). However, only 12% and 23% of all patients had $C_{\text{max}}$ at 2hrs for isoniazid and rifampicin respectively.

CONCLUSION:
82.3% of the patients had a $C_{\text{max}}$ rifampicin <8 µg/ml. The $C_{\text{max}}$ and AUC0-6 hrs was not different for both drugs between daily and intermittent regimen.

$^\ast$Intermittent versus daily ATT regimen; Median. 

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URINARY BENZOYLECGONINE AND LEVAMISOLE CONCENTRATIONS IN SAMPLES SENT FOR CLINICAL DRUG SCREENING: FAILURE TO DETECT AMINOREX

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Background:
Levamisole, an antihelmintic, is commonly-encountered as an adulterant of illicit cocaine. Only 2-5 % of levamisole is excreted as unchanged drug in man, with a major urinary metabolite being p-hydroxylevamisole. Aminorex has also been reported as a levamisole metabolite in man, although the route by which this compound could be formed is unclear. Data on urinary levamisole and metabolite concentrations in clinical samples are scant. We have thus developed a method to measure benzoylecgonine, levamisole, and aminorex in urine and have applied this method to clinical samples.

Methods:
Patient urine samples were screened for drugs of abuse using immunoassay (CEDIA, Microgenics). Samples (N = 100) that were benzoylecgonine positive (CEDIA, ‘cut-off’ 0.3 mg/L) were further analysed using LC-MS. Briefly, centrifuged urine (50 µL) was diluted with aqueous LC eluent [10 mmol/L ammonium formate containing 0.1 % (v/v) formic acid and amfetamine-D5 and benzoylecgonine-D3, 10 µg/L], and a portion (50 µL) of the resulting mixture was analysed using an Accucore Phenyl-Hexyl column (2.6 µm a.p.s., 100 x 2.1 mm i.d.) with gradient elution (flow-rate of 0.3 mL/min). Detection was by high-resolution MS in positive ion mode using heated electrospray ionisation, with all-ion fragmentation MS2 scans collected to confirm peak identity (ThermoFisher Q Exactive). Calibration (all analytes) was over the range 0.10-10.00 mg/L (lower limit of measurement for all analytes 0.01 mg/L).

Results:
The median (range) benzoylecgonine concentrations measured (LC-MS) were 18.02 (0.38-1092) mg/L. Leva mimosole was present in 67 samples. The median (range) benzoylecgonine and levamisole concentrations in these 67 samples were 24.3 (0.65-1092) and 0.49 (0.04-14.6) mg/L, respectively. Aminorex was not detected in any sample.

Conclusion:
adulteration of cocaine with levamisole is common. The absence of aminorex in all samples, even those that
containted high concentrations of levamisole, requires further investigation.

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URINARY MEPHEDRONE CONCENTRATIONS AND CROSS-REACTIVITY WITH AN AMFETAMINE CLONED ENZYME DONOR IMMUNOASSAY (CEDIA)
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Background:
Mephedrone, a β-ketoamfetamine, is a widely-used recreational drug. Detection in urine using an amfetamine-group immunoassay may be appropriate, but little is known as to the cross-reactivity of mephedrone in commercially-available immunoassays. We have thus investigated the cross-reactivity of mephedrone in an amfetamine CEDIA (Microgenics; ‘cut-off’ 0.50 mg/L), and report the mephedrone concentrations measured by LC-MS in urine samples submitted for clinical drug screening.

Methods:
To investigate CEDIA cross reactivity, mephedrone was added to analyte-free human urine over the range 1-100 mg/L. Patient urine samples were screened for drugs of abuse using CEDIA. Samples that were opiate or amfetamine-group positive were further analysed using LC-MS. Briefly, centrifuged urine (50 µL) was diluted with aqueous LC eluent [10 mmol/L ammonium formate containing 0.1 % (v/v) formic acid and 10 µg/L amfetamine-D3], and a portion (50 µL) of the resulting mixture was analysed using an Accucore Phenyl-Hexyl column (2.6 µm a.p.s., 100 x 2.1 mm i.d.) with gradient elution (total flow-rate 0.3 mL/min). Detection was by high-resolution MS in positive ion mode using HESI, with all-ion fragmentation MS2 scans collected to confirm peak identity (ThermoFisher Q Exactive). Retrospective data interrogation was performed for mephedrone ([M+H]+ 178.1226) and amfetamine-D3 ([M+H]+ 141.1435). Calibration was over the range 0.01-1.00 mg/L (lower limit of measurement 0.01 mg/L).

Results:
Mephedrone had a cross-reactivity of <1 % with the amfetamine CEDIA over the concentration range studied. As to the patient samples, mephedrone was detected in 24 (1.5 %) of 1,593 samples submitted for LC-MS (median concentration 3.9, range 0.08-62.0 mg/L). Mephedrone alone was detected in twelve of these samples (median concentration 3.82, range 0.13-62.0 mg/L). The corresponding median (range) amfetamine concentrations (CEDIA) were 0.15 (<0.01-1.03) mg/L. Of the twelve samples where only mephedrone was detected, seven contained mephedrone at concentrations below the amfetamine-group screening ‘cut-off’: median (range) mephedrone and amfetamine-group (CEDIA) concentrations 0.36 (0.13-5.11), and 0.10 (<0.01-0.24) mg/L, respectively.

Conclusion:
Theoretically, a urinary mephedrone concentration of 82.6 mg/L would be required to produce an amfetamine-group result of 0.50 mg/L using CEDIA. The amfetamine-group CEDIA is not suitable for detecting mephedrone routinely in urine.

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STRATUM CORNEUM UPTAKE REFLECTS THE AREA-CORRECTED TOPICAL DOSE OF HYDROCORTISONE
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Background: The uncertainty in dosing topical formulations containing hydrocortisone (HC) is worsened by the so-called “corticosteroid phobia”, leading to poor adherence. The suggested standard “fingertip unit” (FTU) corresponds to the amount of formulation dispensed by a standard nozzle of tube between the tip of the index finger and the distal crease of the finger and should cover an area of skin equivalent to two hands, providing an area-corrected dose of 1.6 mg/cm². Although brief counselling helped volunteers to apply one FTU of a HC cream (1.7±2.2 mg/cm²) and ointment (3.2±3.5 mg/cm²) the doses ranged from 0.1 to 14 mg/cm². This project investigated whether differences in the area-corrected dose modify the uptake of HC into the stratum corneum (SC) and potentially its skin absorption.

Methods: Five healthy adults provided informed consent before participating in this study approved by REACH. Four treatment sites (4.9 cm²) were demarcated on each ventral forearm. Two HC 1% w/w generic formulations, a cream and an ointment, were applied ‘thinly’ (2.2±0.17 mg/cm²) and ‘thickly’ (9.2±0.48 mg/cm²) on both forearms and removed after 6 hours. The application sites on one arm were tape-stripped (Book tape, 3M, US) immediately (‘uptake’ samples) and the remaining sites were tape-stripped after a further 18 hours (‘clearance’ samples). Thirty tapes were taken from each site unless the rate of transepidermal water loss (AquaFlux, Biox, UK) reached a
threshold value. The mass of SC removed with each tape was determined gravimetrically. HC was extracted from the tapes, and assayed by HPLC with UV detection.

**Results:** The mean amount (±SD) of HC recovered from the uptake samples (Table 1) was similar for both formulations but higher for thicker applications (p<0.05) thus reflecting the area-corrected dose applied.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Thin application (µg)</th>
<th>Thick application (µg)</th>
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<tbody>
<tr>
<td>Cream</td>
<td>4.1±3.4</td>
<td>5.3±5.2</td>
</tr>
<tr>
<td>Ointment</td>
<td>4.6±2.5</td>
<td>6.7±3.5</td>
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The amounts of HC recovered from uptake and clearance samples were very similar, an observation in agreement with the formation of a corticosteroid skin reservoir following topical administration previously described.

**Conclusions:** HC uptake was similar for two generic formulations and discriminated between high and low area-corrected doses.

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**SINGLE CELL PHOSPHO-PROTEIN ANALYSIS DEMONSTRATES THAT IMMUNOSUPPRESSIVE DRUGS INHIBIT MAPK AND MTOR SIGNALING MOLECULES IN PRIMARY MONOCYTES AFTER TRANSPLANTATION**

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**Background**

Monocytes have been identified as key players driving rejection processes. It is therefore surprising that there are almost no data available about the impact of immunosuppressive drugs on monocyte activation. Here, we studied the cell signaling state to obtain valuable information about signaling events and as a measure of how monocytes interact with immunosuppression.

**Methods**

By phospho-specific flowcytometry we measured the phosphorylation levels of the signaling molecules NF-κB, MAPK (p38, ERK) and mTOR (AKT) in peripheral blood samples of kidney transplant patients (N=14) taken in the first 6 months after transplantation. Both the in vivo phosphorylation levels and the phosphorylation capacity after PMA/ionomycin stimulation were determined in CD14+ monocytes. Patients received tacrolimus, mycophenolate mofetil and prednisone in combination with basiliximab induction therapy during the first 4 days.

**Results**

Before transplantation the in vivo phosphorylation levels of p38MAPK, ERK and AKT but not of NF-κB are highly expressed by monocytes (MFI: 2258, 486, 1176 and 255 respectively) compared to isotype controls (MFI: 683, 185, 602 and 248 respectively; p<0.001 for p38MAPK, ERK and AKT). In addition, these monocytes have significantly higher p38MAPK phosphorylation levels than cells of healthy controls (p<0.05). After transplantation lower levels of these phosphorylated signaling molecules were measured (p<0.05 for p38MAPK, ERK and AKT) and reached the baseline level of the negative controls. Whole-blood stimulation with PMA/ionomycin resulted in high phosphorylation levels of p38MAPK, ERK and AKT. In these stimulated samples, the phosphorylation levels inversely correlated with tacrolimus predose concentrations (p38MAPK: p<0.05; ERK: p<0.05; AKT: p<0.01) and with prednisolone dosages (p38MAPK: p<0.05; ERK: p=0.10; AKT: p<0.01). No correlation was found between phosphorylated p38MAPK, ERK and AKT levels and kidney function, i.e. serum creatinine or eGFR levels.

**Conclusions**

These data show that before transplantation, monocytes of patients are more activated than monocytes of healthy controls. The decreased phosphorylation levels of the MAPK (p38, ERK) and mTOR (AKT) after transplantation demonstrate that current immunosuppression protocols adequately suppress early monocyte activation.

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**SIMULTANEOUS QUANTIFICATION OF TAMOXIFEN AND METABOLITES IN DRY BLOOD SPOT BY LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY**

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**Background** Therapeutic drug monitoring of tamoxifen (TAM) and its main active metabolites, N-desmethytamoxifen (NDT), 4-hydroxytamoxifen (4HT) and endoxifen (END), during hormonal therapy of breast...
Background: Intoxications are a considerable burden to health care. In 2013, the Dutch Poisons Information Center (DPIC) received approximately 40,000 information requests by telephone from medical professionals, of which 11% originated from Emergency Departments (EDs). In this study, we describe characteristics of intoxicated patients presented at EDs, about whom the DPIC was contacted by telephone. Methods: The DPIC’s database was queried for information requests by telephone from EDs on all types of exposures (January 1st-March 31st, 2014). Online consultations by EDs for toxicological information (DPIC’s website: www.vergiftigingen.info) were not included. Data were collected on patient and exposure characteristics. For each patient, we estimated the severity of the intoxication taking into account the dose (mg/kg), and by using a comprehensive database containing toxicological information on the specific compound(s) involved. Results: From January-March 2014, the DPIC received 981 information requests from EDs, of which 19 were excluded, because these involved multiple patients. The remaining 962 information requests involved 898 patients (for some patients we received more than one information request). In 77% of the patients, adults were involved, whereas 13% concerned small children (0-4 yrs old). Females (56%) were more often involved than males (40%; 4%; gender unknown). The information requests involved 2019 exposures, of which most (73%) involved human pharmaceuticals. Pharmaceutical exposures most often involved central and peripheral nervous system agents (75%), most commonly hypnotics/sedatives/anxiolytics (33%), antidepressants (21%) and analgesics/antipyretics (18%). The most reported drug was acetaminophen (6% of all exposures reported). In 17% of the patients exposed to pharmaceuticals, we estimated the severity of the intoxication as "none" or "mild" and in 63% of the patients as "moderate" or "severe" (19%; unknown severity, e.g. exposure to unknown substance(s) or unknown dose). Conclusions: The DPIC receives a substantial amount of information requests from EDs. 17% of the pharmaceutical exposures was estimated as "none" or "mild" intoxication, indicating that EDs encounter many patients who most likely do not need urgent care. We have started a prospective study, focused on gathering more data on exposure, symptoms and treatment, to improve triage and treatment of poisoned patients, and to reduce associated costs and hospitalization.
Background: To optimize pharmacotherapy in psychiatry the expert group AGNP issued guidelines for TDM. For many psychoactive drugs therapeutic reference ranges (TR), dose related reference ranges (C/D) and expected drug metabolite to parent drug ratios (M/P) are provided to individualize drug therapy. The aim of our study was to assess the prevalence of patients within the recommended TR for clozapine, olanzapine, or risperidone and to evaluate whether the C/D and M/P can help to explain drug concentrations outside the TR.

Methods: Clozapine/norclozapine, olanzapine/desmethyl-olanzapine and risperidone/9-OH-risperidone were measured in 852 plasma samples of 204 patients (82 female, 122 male; 19-76 years) between 2010 and 2013 under steady state conditions by a validated LC-MS/MS method. C/D and M/P were calculated according to AGNP. Patients were divided in cohorts with and without co-medication and categorized according to median concentrations from at least 5 measurements.

Results: Patients outside TR were predominantly below the TR. With clozapine (n=66) only 50% of patients were within, 45% below and 5% above TR, respectively. With olanzapine (n=76) most patients (78%) were within, 18% below, and 4% above TR. With risperidone (n=62) the majority of patients (66%) was also within TR, 27% below, and 6% above. Co-medication had a significant effect. An aberrant C/D was noted in 41% of all patients below the TR. An increased M/P was noted in 23% of patients below TR and a decreased M/P in 40% of patients above TR.

Conclusions: These results show that 22% to 50% of psychotic patients are not within the TR of their drugs despite regular TDM. In patients below TR more than half of the cases could be explained by dosing errors or non-compliance (C/D) and/or increased drug metabolism (M/P). Patients above TR had in 40% an inhibited drug metabolism. Our study suggests that the calculation of C/D and M/P according to the AGNP guidelines can be a helpful tool to better individualize pharmacotherapy of antipsychotics.

APPLICATION OF DRIED BLOOD SPOTS COMBINED WITH ULTRA-HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY FOR THE IDENTIFICATION AND QUANTIFICATION OF THE ANTIPSYCHOTICS RISPERIDONE, ARIPIPRAZOLE, PIPAMPERONE AND THEIR MAJOR METABOLITES

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Background: Risperidone, aripiprazole and pipamperone are antipsychotic drugs frequently prescribed for the treatment of comorbid behavioral problems in children with autism spectrum disorders (ASD). It has been shown that therapeutic drug monitoring (TDM) is useful to decrease side effects and to improve patients outcomes. The aim of this work was to develop a dried blood spots (DBS) assay suitable for TDM and home sampling.

Methods: The method was adapted from a validated method quantifying these drugs in plasma. DBS were prepared by spotting spiked whole blood on a filter paper card. Six mm circles were punched out from DBS. Analytes were extracted with a mixture of acetonitrile/methanol containing the internal standard. The extract was analyzed by UPLC-MS/MS. Gradient elution was performed on a C18 reversed phase column with a mobile phase consisting of ammonium acetate/formic acid 0,1% in water (A) or methanol (B). The suitability of DBS for TDM was assessed by studying the influence of critical parameters: type of DBS cards, extraction solution, EDTA, punching carryover, hematocrit, punching location, spot volume, hemolysis and stability of DBS stored at room temperature (RT).

Results: Optimal results were obtained using Whatman DBS cards, spots dried 4 hours at room temperature and extracted with methanol/acetonitrile (1:1, v:v). EDTA did not skew the results and no punching carryover was observed. Risperidone, 9-OH-risperidone and pipamperone were influenced by the hematocrit. No significant influence of the spot volume neither the punch location (edge/center) was observed. DBS of hemolyzed blood was associated with a negative bias for pipamperone. The antipsychotics were stable in DBS stored 1 week at RT.

Conclusions: This UPLC-MS/MS method allows measurement of all five analytes in DBS. It is important to investigated impacts of specific parameters to optimize and to implement a DBS method. The challenge is to deal with variability sources linked to this specific matrix to avoid misinterpretation in the drug level result. Further development of the method to use DBS at patients’ homes as an easy, stressless and painless sample collection alternative in pediatric patients with ASD has started.

THERAPEUTIC DRUG MONITORING OF RIFAMPICIN : AN INDIAN EXPERIENCE.

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**Background:** Rifampicin (Rif), an important first-line drug in treatment of tuberculosis (TB), exhibits wide inter-individual variability. Factors like inadequate absorption, inaccurate dosing (as per mg/kg), altered metabolism, clinical conditions or drug-drug interactions etc may interfere with drug exposure thus decreasing the bioavailability & causing a slow response to therapy. In the present study, we have assessed plasma rifampicin levels & correlated them with the factors contributing to sub-optimal levels & response to therapy.

**Method:** A detailed clinical & drug history was obtained from 83 clinically proven susceptible TB patients on ongoing Rif therapy. An in-house validated HPLC method was used for plasma rifampicin estimation. Therapeutic range for peak rifampicin level is 8-24 mg/L. Clinical outcomes i.e bacteriological reports, reduced cough or sputum, weight gain, improvement in imaging reports were available in 80 patients which were then classified as clinically improved or partial responders to therapy.

**Results:** Among the study population, 46 patients (58%) had plasma Rif levels in the sub-therapeutic range while 1 patient (1%) had a toxic level. We observed that only 50% (n=40) clinically improved while the remaining 50% (n=40) were partial responders. About 75% of the population in the therapeutic range improved clinically while 70% of the population in the sub-therapeutic range were partial responders. Six patients (12%) among the partial responders had developed Rif resistance over the time period. Factors attributing to this variability may be food interaction (n=2), low body weight adjusted dose (n=19), extended treatment therapy i.e > 6 months (n=13), & factors unknown. Females attained higher therapeutic levels as compared to males (p=0.000).

**Conclusion:** The results of this study suggest that low levels of rifampicin are common in our patient population & monitoring drug levels is necessary to optimize drug doses & achieve therapeutic efficacy & desired patient outcome.

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**ANALYTICAL VALIDATION AND CROSS VALIDATION OF A COMMERCIALLY AVAILABLE NFAT-REGULATED GENE EXPRESSION ASSAY**

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**Background:** Quantitative analysis of NFAT-regulated genes (IL-2, INF-γ and GM-CSF) has been developed as a new method to monitor therapy with calcineurin inhibitors. The implementation of this assay in routine practice requires analytical validation and harmonization across laboratories. The availability of a commercial kit (Search LC, Heidelberg), the feasibility of overnight shipment of the samples and the convenience for routine use make this assay a promising candidate to be utilized clinically. However, data on assay performance between laboratories are lacking.

**Methods:** The residual expression of NFAT-regulated genes (RGE) after drug intake was measured using RT-PCR and following essentially a previously-published protocol (1). For the analytical validation of the assay in our laboratory, anonymized whole blood samples of healthy donors were incubated with tacrolimus in vitro. Linearity, precision, limit of quantification as well as sample stability were investigated. For inter-laboratory comparison, 10 samples of patients under cyclosporine therapy were analyzed at the University Hospital Heidelberg and then reanalyzed in our laboratory within 24 hours.

**Results:** Tacrolimus decreased the expression of NFAT-regulated genes in vitro in a concentration-dependent manner with RGE values of 15%, 47%, 71%, and 89% when the concentrations of tacrolimus were 50µg/L, 25µg/L, 12.5µg/L, and 6.25µg/L respectively. The within- and between-assay CVs at 3 different concentrations (n=6 each) were <20%. The limit of quantification was 100 cDNA copies for each of IL-2, INF-γ and GM-CSF genes. The difference between RGE of the same samples measured twice within 24 hours was below 19% (n=3). The inter-laboratory comparison showed a very good correlation (r=0.951). The difference of RGE between the 2 labs was <30% except for one sample with a RGE ≤ 6% in both labs.

**Conclusion:** We confirmed an appropriate analytical performance of the NFAT-regulated gene expression assay in our laboratory where it has shown to be sensitive and reproducible. We proved the potential to harmonize the results of NFAT-regulated gene expression assay across laboratories which facilitates the implementation this assay in routine diagnostics in many centers.

PERFORMANCE OF A PHOSPHOFLOW ASSAY TO DETERMINE PHOSPHYRINATION OF S6 RIBOSOMAL PROTEIN AS A READ OUT FOR MTOR INHIBITION

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Background: The S6 ribosomal protein (S6RP) is a well-characterized downstream target of mTOR; hence there is an interest to measure its phosphorylation as a biomarker of mTOR inhibition. Whether the technique of phosphoflow cytometry would fit this purpose still needs to be thoroughly addressed.

Methods: A commercial kit (PerFix-p, Beckman-Coulter) using a fluorescent anti p-S6RP antibody (Alexa Fluor® 488 conjugated rabbit anti-p-S6RP) was employed. Phosphorylation of S6RP (p-S6RP) was examined separately in CD3+/CD4+ and CD3+/CD8+ cells using whole blood stimulated with PMA (150 µg/L, 6 min, 37°C). Median S6RP phosphorylation of stimulated vs. non-stimulated cells was reported. Samples from healthy persons (n=8), dialysis patients (n=5), patients with inflammatory disease (CRP > 10 mg/dL, n=5) and pre-dose samples of transplant patients treated with mTOR inhibitors (n=32) or other immunosuppressants (n=24) were applied for the assay evaluation. We investigated the specificity, linearity and precision of the assay as well as the sample stability. In a separate set of experiments, we compared the results of phosphoflow cytometry with western blot technique (n=13) using an anti-phospho-p70S6 kinase (Thr389) antibody instead of anti-p-S6RP.

Results: Everolimus decreased the level of p-S6RP in vitro and the effect was linear in the concentration range 0 - 0.03 µmol/L. Assay imprecision was ≤16% and ≤18% for CD3+/CD4+ and CD3+/CD8+ cells respectively in samples of transplant patients. Using samples of healthy persons the imprecision was ≤27% for both cell subsets. Taking the healthy persons as a control group and comparing different patient groups showed that mTOR-treated patients had lower median p-S6RP in both CD3+/CD4+ and CD3+/CD8+ cells. However, only the difference between the sirolimus and the control group in CD3+/CD8+ cells reached significance (p=0.03). Phospho-S6RP was found to be of limited stability (<24h) in blood samples. Neither significant correlation between phosphoflow and western blot nor between mTOR concentrations in blood and both pharmacodynamic markers was found.

Conclusion: Although the phosphoflow assay of p-S6RP provides several advantages including the possibility to work with whole blood samples and its analytical performance is satisfactory, results from the experiments with clinical samples question its fitness for purpose.

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DETERMINATION OF THE SEPARATION METHOD OF AMINOALKANOL DERIVATIVES OF DICARBOXIMIDES AS A POTENTIAL ANTICANCER DRUGS BY CAPILLARY ELECTROPHORESIS.

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Abstract
Purpose in this study is direct separation of derivatives of dicarboximides: I (4-[2-hydroxy-3-(propan-2ylamino)propyl]-1,7-diyethyl-8,9-diphenyl-4-azatricycl[5,2.1.02,6]dec-8-ene-3,5,10-trione hydrochloride), II (4-[2-(dimethylamino)ethyl]-1,7,8,9-tetraphenyl-4-azatricycl[5,2.1.02,6]dec-8-ene-3,5-dione hydrochloride) and III (4-[2-(piperidin-1-yl)ethyl]-1,7-diyethyl-8,9-diphenyl-4-azatricycl[5,2.1.02,6]dec-8-ene-3,5,10-trione hydrochloride), which were found in earlier studies as a potential anticancer drugs. The separation was performed with the use of capillary electrophoresis which offers the possibility of fast, cheap and reproducible separations for compounds I, II and III.

Methods
The Capillary Electrophoresis Beckman Coulter P/ACE MDQ system is equipped with an autosampler and UV/Visible detector. All the parameters of CE were controlled by Karat software version 32. An eCAP fused silica capillary (37 cm length, 50 µm I.D.) was used. Electrophoretic separations were obtained using an eCAP fused silica capillary (37 cm length, 50 µm I.D.). The compounds I, II and III were determined using 25 mM phosphate buffer as a background electrolyte (BGE) adjusted to pH = 2.5 by the addition of phosphoric acid. Buffer before introduction into the capillary was filtered through a 0.45 µm pore size filter. Serum samples after extraction of n-hexane-ethyl acetate mixture in the ratio of 90:10 (v/v) and reconstitution in the 0.1 mL water solution were automatically injected using pressure injection.

Results
In this paper, simultaneous separation of I, II and III by capillary zone electrophoresis has been achieved within 16 min by use of 25 mM phosphate buffer of pH 2.5. Analysis of the four compounds in the serum plasma standards were conducted. Limits of detection of I, II and III by UV absorbance at 200 nm were achieved in the range of 10.0-83.0 µg/mL. The method was validated for linearity, accuracy, precision, limits of detection and quantification. The calibration equation revealed a good linear relationship (r² = 0.998-0.999). The sufficient recovery was observed in the range of
A UPLC-MS/MS CLINICAL RESEARCH METHOD FOR THE ANALYSIS OF PLASMA MYCOPHENOLIC ACID

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Background: Mycophenolic acid is an immunosuppressant drug used in treatment after organ transplantation. An analytically sensitive method was developed using protein precipitation extraction (PPE) followed by UPLC-MS/MS for the analysis of mycophenolic acid in human plasma for clinical research purposes.

Method: Calibrators, controls and samples were prepared for analysis by PPE with the addition of methanol and zinc sulphate. The percentage of aqueous methanol added allows for the supernatant to be directly injected without the need for evaporation and reconstitution, thus sample preparation time is minimal. Using an ACQUITY UPLC® I-Class system, samples were injected onto a column using a water/methanol/ammonium acetate gradient elution profile and quantified with a Xevo® TQD mass spectrometer.

Results: In accordance with CLSI EP6-A, the method was shown to be linear from 0.1 - 20 µg/mL and analytical sensitivity experiments indicate that this method would allow precise quantification (± 20%) at 0.075 µg/mL. Coefficients of variation (CV) for total precision and repeatability on 5 separate days for low (0.5 µg/mL), mid (2.4 µg/mL) and high (5.0 µg/mL) QC material were all < 10% (n = 25, days = 5). An initial assessment of method accuracy was derived by analysing external quality assessment samples from an international proficiency testing mycophenolate scheme (Bioanalytics, UK), the determined bias was ≤ 5.1% for all samples. Comparison with samples previously analyzed by an independent LC-MS/MS method demonstrated good agreement using Deming and linear regression (r = 0.998). No significant carryover was observed and quantitation of mycophenolic acid was free from interferences of metabolites, endogenous and exogenous compounds.

Conclusions: We have successfully quantified mycophenolic acid in plasma using PPE with UPLC-MS/MS analysis, for clinical research purposes. The method demonstrates good analytical sensitivity, accuracy and precision with minimal matrix effects. For Research Use Only, not for use in diagnostic procedures.

AN AUTOMATED MULTI-BIOANALYSIS METHOD, DETERMINATION OF HALOPERIDOL, ZUCLOPENTIXOL, RISPERIDONE, 9-HYDROXYRISPERIDONE, SERTRALINE AND ITS METABOLITE DESMETHYLSERTRALINE IN HUMAN PLASMA BY LC-MS/MS

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Background: Concentrations of the antipsychotic drugs haloperidol, zuclopentixol, risperidone and 9-hydroxyrisperidone and the antidepressant sertraline are commonly monitored on routine basis. At Karolinska University Hospital, approximately 1600 samples are analyzed a year. A multi-method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed and validated according to European Medicines Agency guidelines, replacing earlier manual liquid-liquid extraction methods. Verification of automated sample preparation was performed on a Hamilton STARlet pipetting robot.

Method: Patient samples, 100 µL aliquots, were prepared by protein precipitation with methanol containing haloperidol-d₄, zuclopentixol-d₄, risperidone-d₄, 9-hydroxyrisperidone-d₄, sertralin-d₄ and desmethylsertralin-d₁₅ as internal standards. After centrifugation, the supernatant was transferred and diluted with mobile phase A (2 mM ammonium formate 0.1% formic acid in Milli-Q water) and then injected on a LC-MS/MS. Separation of the analytes was achieved on a Hypersil Gold C18 column, 1.9 µm, 2.0 x 20 mm, using a gradient run with mobile phase A and 2 mM ammonium formate 0.1% formic acid in methanol as mobile phase B. The analytes were detected by electrospray ionization (ESI) in positive mode utilizing selected reaction monitoring (SRM) for the transitions 376→123 m/z for haloperidol, 401→221 m/z for zuclopentixol, 411→191 m/z for risperidone, 427→207 m/z for 9-hydroxyrisperidone, 306→159 m/z for sertraline and 292→159 m/z for desmethylsertraline. Runtime was 3.5 min.

Results: The calibration curves in plasma were linear over the range 1.6 - 240 nmol/L for haloperidol, 2.0 - 240 nmol/L for zuclopentixol, 10.4 - 191 nmol/L for risperidone, 12.7 - 207 nmol/L for 9-hydroxyrisperidone, 306 - 159 nmol/L for sertraline and 292 - 159 nmol/L for desmethylsertraline. The method showed good reproducibility with intra- and inter-day precision of 0.97% - 1.76 %, respectively. The quantification limits for the compounds were in the range of 32.0-240.0 µg/mL respectively.
nmol/L for zuclopenthixol, 1.8 - 320 nmol/L for risperidone, 1.4 - 640 nmol/L for 9-hydroxyrisperidone, 2.5 - 1000 nmol/L for sertraline and 20 - 1000 nmol/L for desmethylsertraline. Between-run precision of low-, medium- and high quality control samples were below 13% and between-run accuracy -11% to 5.7% over the quantification range for all analytes.

Conclusion: A specific, rapid and convenient multi-method was developed and validated on a LC-MS/MS instrument. The method is currently used in routine analysis for therapeutic drug monitoring.

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PHYSICIAN ADHERENCE TO THERAPEUTIC DRUG MONITORING RECOMMENDATIONS ON THIOPURINES
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Background Therapeutic drug monitoring (TDM) of thiopurines is frequently used to optimize efficacy and to minimize hepatic and bone marrow toxicity. Little is known on physician adherence to TDM recommendations for thiopurines. In other therapeutic areas, such as HIV, low physician adherence (30-35%) has been reported. We evaluated physician adherence to TDM recommendations in the context of thiopurine monitoring.

Methods At our TDM service, we evaluated all TDM data for thiopurines obtained from patients using azathioprine or mercaptopurine from November 2014 to January 2015. All results were accompanied by a dosing advice written by the hospital pharmacist. The medical charts were reviewed to determine physician adherence to the recommendations. Blood concentrations of 6-thioguanine nucleotides (6-TGN) and 6-methyl-mercaptopurine (6-MMP) were analyzed by a validated HPLC method (Dervieux) at the laboratory of the department of Clinical Pharmacy (Rijnstate, Arnhem, The Netherlands).

Results A total of 80 patients were included: 39 patients using azathioprine (48.8%) and 41 patients using mercaptopurine (51.3%). The median (range) 6-TGN and 6-MMP concentrations were 290 pmol/8x10⁸ red blood cell (RBC) (156-700 pmol/8x10⁸ RBC; therapeutic concentrations 600-1200 pmol/8x10⁸ RBC) and 1240 pmol/8x10⁸ RBC (300-18000 pmol/8x10⁸ RBC; toxic concentration >5700 pmol/8x10⁸ RBC), respectively. A total of 41 patients had subtherapeutic 6-TGN concentrations (<600 pmol/8x10⁸ RBC), not caused by skewed metabolism to 6-MMP. The advice in these patients was to increase the dose. This recommendation was adopted in 24 patients (58.5%). In 20 patients (25%), skewed metabolism was detected (i.e., 6-MMP/6-TGN ratio >10), potentially leading to high 6-MMP concentrations. In these patients, the recommendation was to add allopurinol and to decrease the thiopurine dosage by approximately 67%. The adherence to this advice was 50% (n=10). Overall, physician adherence to TDM recommendations was 53.3%. The main documented reasons for not following the advice were (fear of) toxicity (n=4, 14.3%) and sufficient efficacy with relatively low 6-TGN concentrations (n=4, 11.4%).

Conclusions Physician adherence to TDM recommendations was approximately 50%. Fear of toxicity by increasing the dose and sufficient efficacy despite low 6-TG exposure were the main documented reasons for not adopting an advice.

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HIGH ALBUTEROL PLASMA CONCENTRATIONS CAUSED BY FINGERPRICK BLOOD FOLLOWING INHALATION ON THE ICU
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Background Children suffering from severe status asthmatics are treated with continuous intravenous albuterol. In the context of a population pharmacokinetic study, we noticed high plasma concentrations for both R-albuterol and S-albuterol in 8 samples, obtained from 7 different patients, which we could not explain clinically. Six out of these samples concerned fingerprick blood samples collected after nebulization was restarted. Nebulization is not performed in a closed system, therefore contamination of skin of the hands is a possibility. In literature misleading high tobramycin plasma concentrations after nebulizing are described. [1] Our hypothesis is, that these high concentrations were caused by skin contamination of fingerprick blood samples following nebulizing of albuterol.

Methods A healthy adult volunteer, not treated with albuterol, sprayed albuterol on the hands using the ICU nebulising
This volunteer was not involved in connecting the nebulizer and did not touch the albuterol solution. After approximately 5 minutes a fingerprick blood sample was taken and albuterol plasma concentrations were measured using a validated LC/MS-MS method.

**Results**

The fingerprick blood sample taken after spraying albuterol over the hands of the volunteer contained concentration of >1000 µg/l R-albuterol and S-albuterol. This is much higher than therapeutic concentrations analysed during intravenous albuterol infusion in children included in the trial (R-albuterol: median 66 µg/l and S-albuterol: median 106 µg/l).

**Conclusions**

Due to skin contamination after nebulizing albuterol extreme high plasma concentrations can be found in fingerprick blood samples. Fingerprick blood sampling should be used carefully in pharmacokinetic studies of inhaled drugs. These results may also have implications for blood sampling procedures in other pharmacokinetic studies.


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**QUANTATIVE EFFECT OF CYP2C19 AND CYP2C9 GENOTYPE ON CONCENTRATION PREDICTION OF VALPROATE IN CHINESE EPILEPTIC PATIENTS**

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Background: Valproate (VPA) application is very difficult to handle because of the significant individual pharmacokinetic variability. Its serum levels have a close relationship with the single nucleotide polymorphism in CYP2C19 and CYP2C9 genes. VPA serum concentration in patients with mutant-type CYP2C19 gene is significantly higher than that in patients with wild-type gene. Different genotypes had different influence on the VPA serum concentration. However, the influence of genotype on the prediction of drug serum concentration is unknown.

Objective: To study the influences of genotype of CYP2C19 and CYP2C9 on prediction of the serum concentration of VPA.

Method: Three Population pharmacokinetic (PK) models which included different factors were investigated in a previous study. They are SNP model only included genotype factors as covariates, BIO model only biological factors, and SNP+BIO model simultaneously genotype and biological factors. The parameter values of the 3 models were fixed and based on them, VPA serum concentrations of 200 patients were predicted with NONMEM software. The accuracy and precision of prediction was evaluated by indexes, such as the mean prediction error (MPE), mean squared prediction error (MSPE), root mean squared prediction error (RMSPE).

Results:

The result of MPE was -8.16, -7.47, -6.91 µg/mL in SNP, BIO, SNP+BIO model, respectively. MSPE was 1535.23, 1049.95, 672.31 (µg/mL)², and RMSPE was 37.58, 25.03, 20.12 µg/mL in 3 models, respectively (Table 3), indicating the SNP+BIO model was superior to the SNP model and the BIO model in terms of accuracy and precision of prediction. Among 3 models, the prediction of the BIO model was more accurate than SNP model, and the SNP+BIO model was the most accurate. Based on the effect of biological factors, the genotype factors can increase accuracy of prediction once more.

Conclusion: Including the genotype factor into Population PK model, the accuracy and precision of concentration prediction for VPA can be enhanced. This finding could be valuable to design individualized dosage regimen.

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**ASSESSMENT OF CORRELATION BETWEEN HEPATIC TISSUE AND BLOOD TACROLIMUS CONCENTRATION DETERMINED BY LC-MS/MS METHOD IN LIVER TRANSPLANT RECIPIENTS.**

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Background: Tacrolimus (TAC) is an immunosuppressive drug widely used after liver transplantation. TAC was licensed for liver transplantation in the 1990s. It has become progressively more popular, to such extent that by 2010, it was a component of about 90% of immunosuppression regimens for liver transplant recipients. An increasing number of studies indicate that intra-tissue concentration of the drug may be better predictor with respect to acute rejection compared with the measurement of drug concentration in whole blood.
**Objectives:** The aim of this work was to determine hepatic tissue and blood TAC concentration using validated liquid chromatography-mass spectrometry (LC-MS/MS) method and evaluate correlation between them.

**Methods:** The compound was isolated from the liver tissue using tris buffer. After homogenization, the proteins precipitation by using acetonitrile with zinc sulfate addition was applied. Analytical technique that was used was of LC-MS/MS, due to the high selectivity and sensitivity of the method and short time of analysis. Tacrolimus-$^{13}$C$_2$d$_2$ was selected as the internal standard (I.S.) in the analysis. Multiple reaction monitoring of TAC was performed using electrospray in positive ion mode (TAC: m/z 821.5 $\rightarrow$764.4 ; I.S. m/z 824.6 $\rightarrow$ 771.5).

**Results:** The method for measuring TAC was assessed by determining the validation parameters: linearity, accuracy, precision, limit of detection, limit of quantification and stability of TAC in hepatic tissue. The analytical measurement range was 15 - 750 pg/mg tissue. The coefficient of determination ($r^2$) of calibration standards was 0.997 for a linear regression equation $y=1.07x+0.05$. Fifty six samples containing TAC in whole blood and liver biopsies taken at the same time were determined by LC-MS/MS. The range of therapeutic concentrations in blood and tissues of patients was 2.0 - 10.5 ng/mL and 35 - 200 pg/mg, respectively.

**Conclusions:** Validated performance evaluation in terms of analytical method showed that tacrolimus can be quantified in biological material. This assay met requirements as fast, specific and sensitive LC-MS/MS method. There was no correlation between same-day TAC C$_0$ and liver concentration.

**References:**

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**PERFORMANCE EVALUATION OF A NEW AUTOMATED TACROLIMUS ASSAY ON DIMENSION® SYSTEMS**
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**Background.** Tacrolimus (FK-506) TDM is a crucial requirement in post-transplant patient care. Therapeutic strategies are increasingly reliant on sensitive and reproducible assays, especially in the presence of low sample concentrations. HPLC-MS/MS methods are both sensitive and selective for FK-506 determination, however not all centers have the instrumentation and expertise to provide such a service. Thus immunoassays remain an important tools in the management of transplant patients.

A new automated immunoassay, the Dimension®TAC assay has been assessed in comparison with laboratory routine Dimension®TACR assay, ARCHITECT Tacrolimus assay and a HPLC-MS/MS validated assay.

**Methods.**
Assessment of precision was carried out according to the reduced (n=10) CLSI-EP5A2 protocol and three commercially available QC materials and five pooled EDTA blood samples (BS) from patients under FK-506 treatment were employed. Linearity of the method was performed according to the CLSI-EP6A2 protocol. To assess the functional sensitivity, fortified blank pooled EDTA whole blood samples with pure standard at decreasing concentrations (3.0-0.5mg/L) were used. Accuracy of the method was evaluated using EQAS samples. Method comparison was assessed on 60 samples covering the range of 1.3 to 19.2mg/L. To define the relationship and the agreement between Dimension®TAC assay and comparison methods, Passing-Bablok non-parametric linear regression and Bland-Altman plots were performed, respectively.

**Results.**
Within-assay reproducibility (CV%, n=40) of QCs at 3.9/9.1/18.6mg/L and BS at 0.7/3.5/6.5/11.1/24.9mg/L were <7.4% and <21.4%, respectively. Linearity was 1.1-28.2mg/L. Functional sensitivity was 0.8mg/L. The bias using EQAS samples with a target values of 2.0/8.0/10/20/30mg/L were <14%.

The comparison made with the established methods were FK-506[TAC assay] =1.54xFK-506[TACR assay] +2.13, and with the Bland-Altman plot showing a significant bias +1.9 (+1.3 to +2.4, 95%CI); FK-506[TAC assay] =0.97xFK-506[ARCHITECT Tacrolimus assay] -0.01, and with the Bland-Altman plot showing no significant bias -0.34 (-0.72 to +0.04, 95%CI); FK-506[TAC assay] =1.2xFK-506[HPLC-MS/MS] -0.71, and with the Bland-Altman plot showing a significant bias +0.71 (+0.2 to +1.2, 95%CI).

**Conclusions.** The Dimension® TAC assay appears to be particularly promising since it exhibits good sensitivity, reproducibility and a satisfactory correlation with comparison methods. The significant bias against HPLC-MS/MS is probably due to cross-reactivity with FK-506 metabolite.
THERE IS STRONG EVIDENCE THAT THERAPEUTIC MONITORING SHOULD BE MANDATORY FOR PATIENTS ON HIGH DOSE BUSULFAN REGARDLESS OF AGE
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Background: High dose busulfan is frequently used as component of conditioning regimens prior to haemopoietic stem cell transplantation (HSCT). It was suggested that body-weight based busulfan doses given every 6 hourly for four days may be fitting the required target. However, series of observations and further studies indicate unpredictable variability challenging the accuracy of the body-weight based busulfan dosing scheme. Aim: The purpose of this alalysis is to describe that body-weight based fixed busulfan dose prediction may not guarantee target AUC and safety demonstrating the importance of therapeutic drug monitoring (TDM) in this challenging task for both paediatric and adult patients. Methods: Data of 28 patients (20 children and 8 adults), who received High dose busulfan for myeloablative target before HSCT since 2014 to February 2015 is included. Limited sample strategy was applied so that trough concentration (immediately before the 5th dose) followed by samples immediately after the end of 2 hour lasting infusion (peak), 4hr, and 6hr from the start of the infusion, respectively. Busulfan plasma concentrations were determined by high performance liquid chromatography (HPLC) and the AUCAwas calculated using the trapezoidal rule Results: Wide range of busulfan trough levels, 138-1042µg/L and peak levels 679-2947µg/L in children versus through levels 132-674µg/L and peak 704-1583µg/L in adults resulting in AUC 2221-10042µg/L-hr following body weight-based busulfan dosing is observed. Dose adjustment was recommended in 14/20 (70%) paediatric and 4/8 (50%) adult cases. Conclusions: Our results demonstrate that intra-individual PK/PD variability known for oral busulfan is challenging despite intravenous formula administration both in children and adults. Although AUC does not necessarily correlate with outcomes due to co-factors, the fact that dose at least in half of cases doses needed change underlines the importance of TDM as mandatory care. The overall idea could be drawn from our published previous series of observations and the present results is to recommend therapeutic drug monitoring with careful interpretation of drug levels including all influential factors instead of relaying on initial body-weight based dose to guarantee aimed target while reducing toxicity risk in both adult and paediatric HSCT patients.

INFLUENCE OF CLINICAL AND GENETIC FACTORS ON EVEROLIMUS MAINTENANCE THERAPY IN HEART TRANSPLANT PATIENTS
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Background: The mammalian target of rapamycin (mTOR) inhibitor everolimus (ERL) is a newer immunosuppressive drug with antiproliferative properties attenuating development of cardiac allograft vasculopathy in de novo heart transplant (HTx) patients. ERL has become an alternative to calcineurin inhibitors due to its renal-sparing mode of action improving renal function in HTx recipients. However, ERL-based immunosuppression is associated with dose-related toxicities, increased rates of infections and low-grade allograft rejections owing to its narrow therapeutic window combined with substantial variability in response. Mechanisms underlying this inter-individual variability are poorly characterized. Thus, we aimed to evaluate the association of clinical factors and genetic variation in ERL pharmacokinetic pathway with the ERL maintenance dose requirement in HTx patients. Methods: This pilot study comprised of 37 patients recruited at the Bern University Hospital who were treated with ERL maintenance therapy for at least 3 months. Functional polymorphisms in CYP3A45, CYP3A4, CYP2C8, POR, NR12, and ABCB1 were genotyped and data on patient characteristics and medication were retrieved from the patient charts. Results: A 20-fold variability in the dose-adjusted ERL trough concentrations (C0) was observed, with 15/37 (41%) patients having a C0 below or above the targeted range. Linear regression analysis of clinical factors revealed a negative effect of kidney function (eGFR) on the dose-adjusted ERL C0 (P = 0.041; R2 = 0.114). A multivariate regression model including eGFR as a covariate showed a significant effect of the common splice-site variant CYP3A5*3 on the dose-adjusted ERL C0 (P = 0.027; R2 = 0.234 for the full model). CYP3A5 expressors (i.e. CYP3A5*1/*3 or CYP3A5*1/*1) showed significantly lower ERL C0 compared to CYP3A5 non-expressors (CYP3A5*3/*3) indicating faster ERL metabolism in the expressors. None of the other genetic variants or co-medications known to interact with CYP3A and CYP2C8 enzymes had a significant influence on the dose-adjusted ERL C0 in this patient cohort. Conclusion: Our preliminary data indicates that ERL pharmacokinetics in HTx patients is highly variable making the dose adjustment in these patients challenging. Kidney function and CYP3A5 genetic variation may account for part of the observed variability in ERL exposure in HTx patients.
RILPIVIRINE CONCENTRATIONS AND EFFECTS ON QTc INTERVAL IN PATIENTS TREATED WITH BOCEPREVIR FOR ACUTE HEPATITIS C VIRUS INFECTIONS

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Background
Hepatitis C virus genotype-1 infection (HCV) protease inhibitors are associated with substantial drug-drug interactions (DDI), especially within HIV-coinfected patients treated with combined antiretroviral therapy (cART).

Based on healthy volunteer DDI-data, rilpivirine, a second generation non-nucleoside reverse transcriptase inhibitor, can be comedicated with boceprevir, telaprevir or simeprevir. This study is a subanalysis within the Dutch Acute HCV in HIV Study (DAHHS) and will describe the pharmacokinetic interaction between boceprevir (CYP3A4 inhibitor) and rilpivirine (CYP3A4 substrate) in HIV/HCV positive patients and the effects on QTc-interval prolongation.

Methods
In the DAHHS study, 12 patients treated for HCV with weight based peginterferon-alfa, ribavirin and boceprevir 800mg tid in combination with antiretroviral therapy consisting out of rilpivirine 25mg once daily, emtricitabine and tenofovir were included in this subanalysis. Rilpivirine plasma concentrations were measured before the start of HCV therapy (T0) and at week 4 of therapy (T4). Both samples were taken during the elimination phase of the drug (t=4-24h after administration). Concurrently, with the blood draw, a single 12 lead ECG was recorded in all participants at T0 and T4. Heart rate corrected QT (QTc) was calculated using Bazett’s formula. Concentration-time data were analyzed using nonlinear mixed effect modeling (NONMEM). The effect of boceprevir on rilpivirine apparent clearance (CL/F) was evaluated. AUC was calculated using individual estimations for CL/F and dose. The effect of AUC on QTc prolongation was analyzed using linear regression.

Results
Pharmacokinetics of rilpivirine were described using a 1-compartment model with fixed values for the absorption rate (ka=0.7h⁻¹) and volume of distribution (Vd=152L) based on literature. CL/F decreased from 7.2 to 3.8 L/h when patients were cotreated with boceprevir (p<0.005). This DDI explained 34% of the variability in CL/F. Measured QTc-intervals were not correlated to the calculated rilpivirine AUC (median AUC without boceprevir was 3.8 mg·h/L and 6.5 mg·h/L with boceprevir).

Conclusions
Concurrent treatment of boceprevir resulted in 47% decrease in CL/F, which is equal to an increase in AUC from 3.8 mg·h/L to 6.5 mg·h/L. This DDI could explain 34% of the variability in CL/F seen in these patients, but did not result in increased QTc-intervals.

DETERMINATION OF IBANDRONIC ACID IN HUMAN PLASMA BY A VALIDATED LC-MS/MS METHOD

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Background: Development and validation of LC-MS/MS determination of Ibandronic Acid (IBA) was performed with the aim to be applied in the course of a bioequivalence study. Methods: IBA and d3-IBA (Ibandronic Acid-D3, internal standard) were extracted from human plasma with Ethyl Acetate and derivatized. Chromatographic separation was performed on C18 analytical column with mobile phase consisting of 37% aqueous methanol, 0.001% formic acid and 2mM Ammonium Acetate. Positive electrospray ionization and multiple reaction monitoring were used to follow the predominant transitions: collision energy 20, m/z 376→114 for IBA, and m/z 379→117 for d3-IBA. Raw data of mass chromatograms were collected and processed by specialized software, and weighted (1/X) linear regression was performed to determine the concentration of IBA. Validation strategy was strictly adhered to current industrial guidance. Results: Selectivity was assessed with 8 individual sources of human plasma and associated with absolute matrix effect (ME) averaging 100-140% for IBA and 100-136% for d3-IBA. Despite the observed ion enhancement for both the analyte and internal standard, calculated relative ME averaging 103 - 106% confirmed that isotope labelled internal standard fully compensated for the ion enhancement, thus leading to
negligible overall matrix effect for IBA. Accuracy ranged from -11.5 to 7.9 % within runs and from -14.7 to 11.3 % between runs. Precision was up to 6.7% within-runs, and up to 9.7% between-runs. Linearity was assured in the range 1.0 ÷ 201.2 µg/L, R²>0.99. Freeze-thaw stability was determined for three cycles each lasting 24 h, post-preparative stability was documented for 72 h at 4°C, short-term stability at room temperature was proven for 6 h at daylight and 4 h in the dark; stock solution stability and long term stability in plasma were documented for 86 days at -20°C. With run time of 4 min, a throughput of over 190 samples per working day was achieved. Conclusion: The method was validated according to current industrial requirements and allows the accurate and precise determination of IBA in human plasma.

TOO GOOD TO BE TRUE? SAMPLE PREPARATION OF PEMIROLAST IN PLASMA USING SOLID PHASE EXTRACTION WITH SELF-CORRECTING DILUTION.
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Background: In a method using solid phase extraction plates (SPE) and determination with liquid chromatography followed by tandem mass spectrometry for determination of pemirolast concentrations in plasma, it was discovered that samples with concentrations above upper limit of quantification (ULOQ), got correct results without previous dilution into the concentration range.
Methods: The sample preparation is using an automated solid phase extraction with Evolute ABN, 25 mg, Biotage in 96-well formate with a Hamilton STARlet robot and determination with LC-MS/MS (Aquity Ultra Performance LC-system and Quattro Premier XE, Waters). The method uses a stable isotopically labeled internal standard.
Results: Quality control samples up to 10 times above ULOQ (calibration range 4-4000 ng/mL) have been determined correctly without previous dilution into the calibration range. The analyte absolute peak areas of very high quality control samples (40000 ng/mL) were in the same size as the QC H level (4000 ng/mL). The peak areas of the internal standard were, however, about 10 times lower in the very high QC than in the QC H sample. The SPE material functions as a dilution filter when all binding sites are occupied.
Conclusions: It is possible to get correct pemirolast concentrations above ULOQ without dilution when using SPE during sample preparation if a stable labeled internal standard is used. The limiting factor is the diminishing peak area of the internal standard with higher analyte concentrations.

TOXICOLOGY SCREENING METHOD FOR HUMAN BRAIN SAMPLES
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Background: Postmortem human brain sample repositories, such as the Macedonian/New York State Psychiatric Institute Brain Collection, are of instrumental value to research into neurodegenerative and psychiatric diseases. The interpretation of molecular, biochemical and neuropathological data from such samples is improved if the presence or absence of drugs in these brain samples can be demonstrated. We therefore set up and validated an LC-MSMS method to screen for compounds in human brain tissues.
Methods: 100 mg of brain samples was disrupted in liquid containing n=5 deuterated internal standards, followed by centrifugation and solid phase extraction through a HybridSPE cartridge. The eluent was evaporated and reconstituted, followed by LCMSMS analysis, as described earlier (Rosano et al. J Anal Toxicol. 2014). All experiments were carried out on a Waters Xevo TQ MS ACQUITY UPLC system (Waters, Milford, MA). A gradient of Acetonitril, water and 0.1% formic acid was run using an ACQUITY UPLC HSS C18 Column (2.1 mm inner diameter × 150 mm with 1.8 µm particles). Total runtime was 15 minutes per sample. N= 179 compounds were detected with positive electrospray ionization in multiple reaction monitoring mode, which included a confirmatory ion transition for each compound. The assay was validated for amitriptyline, clomipramine, clozapine, fluoxetine, haloperidol, olanzapine, paroxetine, promethazine, risperidone and venlafaxine and subsequently pilot-tested in samples from n=32 deceased schizophrenic patients.
Results: There was good recovery and little matrix effect for the standards, and the sensitivity of the assay was <5 ng/mg tissue for most standards. A total number of n=32 drugs tested positive beyond doubt in the samples, predominantly antipsychotics, antidepressants and benzodiazepines. Another n=18 compounds tested possibly positive and await confirmation. The data were consistent with medication history data.
Discussion and Conclusion: This assay, which is a relatively simple adaptation from a commercially available LCMSMS screening method, can be used to screen human brains for the presence of drugs and their metabolites.
The assay is currently further validated and compared with other screening methods such as GC-MS.

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SIMULTANEOUS DETERMINATION OF EIGHT 'NEW GENERATION' ANTIEPILEPTIC DRUGS (AEDS) IN SERUM BY LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (LC-MS/MS)

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Background: Epilepsy is a group of neurological disorders characterized by epileptic seizures affecting approximately 65 million people worldwide, about 100 thousand of them are from the Czech Republic. The mainstay of treatment of epilepsy is anticonvulsant medication. Very important part of the management of the pharmacotherapy is therapeutic drug monitoring, requiring the determination of drug concentration. This work presents development and validation of a LC-MS/MS method for simultaneous determination of eight antiepileptic drugs in human serum, namely gabapentin (GBP), lacosamide (LCS), levetiracetam (LEV), pregabalin (PGB), rufinamide (RFN), topiramat (TPM), vigabatrin (VGB) and zonisamide (ZNS).

Methods: The samples were prepared using a simply protein precipitation by acetonitrile. The analytes were separated on a Kinetex PFP 50 x 3 mm, i. d. 2.6 μm (Phenomenex) column. The mobile phase was consisted of 0.1 % formic acid in water and acetonitrile. The flow rate was 0.3 ml/min and total run time was 6.5 min. Quantification was achieved using positive mode by applying multiple reaction monitoring monitoring (MRM).

Results: Method validation as well as determination of serum concentrations in patient samples were performed. The linear dynamic range over the calibration curve for individual AEDs was 0.363 - 23.0 μg/ml for GBP, 0.368 - 20.0 μg/ml for LCS, 0.908 - 51.2 μg/ml for LEVE, 0.179 - 9.97 μg/ml for PGB, 0.773 - 40.9 μg/ml for RFN, 0.273 - 16.5 μg/ml for TPM, 0.335 - 20.9 μg/ml for VGB and 0.758 - 41.1 μg/ml for ZNS. Analytical recoveries for serum control level I (Recipe) were 97 - 107% and for serum control level II (Recipe) were 95 - 106 % for all determining AEDs. Limit of quantification was 0.1 μg/ml (CV = 1.2 %) for GBP, 0.06 μg/ml (CV = 1.2 %) for LCS, 0.05 μg/ml (CV = 3.7 %) for LEVE, 0.08 μg/ml (CV = 1.1 %) for PGB, μg/ml (CV = 1.6 %) for RFN, 0.5 μg/ml (CV = 3.4 %) for TPM, 0.2 μg/ml (CV = 3.2 %) for VGB, 0.35 μg/ml (CV = 2.6 %) for ZNS.

Conclusions: The developed method was successfully validated to analyze human serum samples for therapeutic drugs monitoring.

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COMPARATIVE PHARMACOKINETICS OF ECHINOCANDINS IN INTENSIVE CARE UNIT PATIENTS

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Background: Pharmacokinetics (PK) of antimicrobial agents in Intensive care Unit (ICU) patients can be highly variable. We conducted a head-to-head study of caspofungin and micafungin in ICU patients to determine PK and explore possible covariates.

Methods: Patients (6 hospitals) receiving caspofungin (70 mg on day 1, followed by 50 mg/day or 70 mg/day if BW>80kg) or micafungin (100 mg/day) as antifungal treatment were eligible. Daily trough samples (C₀) and PK curves (day 3 and 7) were taken. PK analysis was performed using a standard two-stage approach. Multiple linear regression was used to determine covariates on PK.

Results: 21 patients (8 female, 13 male) receiving caspofungin and 20 patients (12 female, 8 male) receiving micafungin were evaluable. Median (range) age and bodyweight was 71 (45-80) yrs and 75 (50-99) kg for caspofungin and 68 (20-84) yrs and 77 (50-134) kg for micafungin.

The PK curve on day 3 resulted in caspofungin (median; IQR) AUC₀₃₄ of 88.7 mg*h/L⁻¹ (72.2-97.5), Cmax 7.51 mg/L (6.04-8.17), C₀ 2.15 mg/L (1.40-2.48), V₀ 7.72 L (6.12-9.01) and CL 0.57 L/h⁻¹ (0.54-0.77) and micafungin AUC₀₃₄ of 78.6 mg*h/L⁻¹ (65.3-94.1), Cmax 7.22 mg/L (5.40-9.22), C₀ 1.55 mg/L (1.39-3.14), V₀ 25.61 L (21.32-29.07) and CL 1.27 L/h⁻¹ (1.07-1.53). Caspofungin and micafungin PK in ICU patients showed limited intra-individual variability (14.0% and 12.9%, respectively) and moderate inter-individual variability (45.6% and 57.9%, respectively). Caspofungin AUC₀₃₄ was significantly higher than AUC₀₃₄ (n=11, p=0.0014) suggesting steady state was not achieved on day 3. Micafungin AUC₀₃₄ was comparable to AUC₀₃₄. No significant covariates were identified on caspofungin and micafungin PK (e.g. bodyweight, albumin, liver function, disease severity scores APACHE II and SOFA).

Conclusions: Caspofungin PK in ICU patients was comparable to PK in healthy volunteers and (non-)critically ill patients. Micafungin exposure in ICU patients was lower compared to healthy volunteers but comparable to other reference populations. The question arising from our results is not whether echinocandins are efficacious for the
average ICU patient in this cohort but if low exposure in an individual patient correlates with reduced efficacy. We may still gain in efficacy and further improve outcome targeting those individuals with low exposure in relation to the susceptibility.

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LIMITED SAMPLING STRATEGIES FOR THERAPEUTIC DRUG MONITORING OF CO-TRIMOXAZOLE IN THE TREATMENT OF MULTIDRUG-RESISTANT TUBERCULOSIS

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Objectives: Therapeutic possibilities in the treatment of multidrug resistant tuberculosis are limited; there is a need to re-evaluate other antibiotics for their potential anti-mycobacterial activity. Sulfamethoxazole, one of the components of co-trimoxazole, has in vitro activity against M. tuberculosis and may therefore be a promising candidate for further testing. A population pharmacokinetic model based limited sampling strategy can be used in a prospective study to evaluate the effect of sulfamethoxazole in TB treatment.

Methods: Twelve patients were included in this prospective clinical trial. They received 960 mg co-trimoxazole on 5 ± 1 consecutive days. On the last day, 9 blood samples were drawn in a time range of 0 - 24 hours after the drug intake. A one-compartment model with lag time was developed, optimized and cross-validated. Based on the results of this model, stepwise multiple linear regression was used to optimize a limited sampling strategy.

Results: The model was successfully cross validated with the following pharmacokinetic parameters (median): absorption lag time 0.77 h (IQR: 0.56 - 1.34), t1/2 7.93 h (IQR: 6.3 - 8.52), K2 1.94 (IQR: 1.39 - 2.54), Fmax 0.95 (IQR: 0.98 - 0.96), Vv 17.57 L (IQR: 16.41 - 19.25), Cl 1.57 L/min (IQR: 1.38 - 2.10), AUC0-24h 457.71 (IQR: 382.61 - 537.21), Cmax 41.26 mg/L (IQR: 34.60 - 44.91), Tmax 2.72 h (IQR: 2.19 - 3.03). The cross-validation resulted in a RMSE of the AUC0-24h estimation of 3.39 and a CV (RMSE) of 0.75% compared to the model, and a CV (RMSE) of 8.07% in comparison to the observed pharmacokinetic parameters. Sampling only at 6 hours after administration results in an adjusted R² of 0.96 in estimating the AUC with a standard error of the estimate of 25.06 h*mg/L (p < 0.01, AUC = 1.389 + 14.85 x [concentration at T = 6]).

Conclusion: We developed a pharmacokinetic model that is able to predict the pharmacokinetic parameters of sulfamethoxazole in a reliable and robust way. A reliable estimation of the AUC can be made with only one sample 6 hours after administration in further prospective clinical trials.

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QUANTIFICATION OF CO-TRIMOXAZOLE IN SERUM AND PLASMA USING TANDEM MASS SPECTROMETRY

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Background: Co-trimoxazole is an effective drug in the treatment and prophylaxis of pneumocystis carinii pneumonia (PCP) in HIV infected individuals. However, the pharmacokinetic profile of both trimethoprim and sulfamethoxazole show a significant interindividual variation, which could reflect in decreased efficacy or increased toxicity. We therefore developed a new liquid chromatography tandem mass spectrometry (LC-MS/MS) method to analyse sulfamethoxazole, its nephrotoxic metabolite N4-acetyl-sulfamethoxazole and trimethoprim in calf serum, human serum and human plasma.

Methods: Selectivity, accuracy and precision, recovery, matrix effect, autosampler stability and freeze-thaw stability tests were performed. A Finnigan TSQ Quantum Discovery Max was used for the analysis. Gradient elution with ammonium acetate, acetic acid, trifluoroacetic acid and acetonitrile was used to perform the liquid chromatography. Concentration ranges for trimethoprim, sulfamethoxazole and N4-acetyl sulfamethoxazole were 0.2 - 10.0 mg/L and 5.0 - 100.0 mg/L, respectively. Cyanomipramine was used to quantify trimethoprim; sulfamethoxazole and its metabolite were quantified using [2H4]-sulfamethoxazole as internal standard.

Results: The correlation coefficient of all three calibration lines was 0.99. The accuracy of this method varied from -2.4 - 14.7% for all compounds. Within-day and between-day precision ranged from 0.0 - 8.0%. The stability was calculated at -7.2 - 9.4% at room temperature and in the autosampler, while the freeze-thaw stability was determined at 0.4 - 12.8%. Matrix effects were determined and calculated at 95.8 - 106.4% for all components. Matrix comparison tests did not show any difference in Y-intercept or slope of the calibration lines in calf serum, human serum and human plasma.
Conclusions: All validation tests resulted in biases and variations less than 15%. This method is suitable for the determination and quantification of sulfamethoxazole, N-acetyl-sulfamethoxazole and trimethoprim in serum and plasma.

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LETHAL TOXICITY OF 5-FLUOROURACIL IN THE ERA OF PERSONALIZED THERAPY: DISCOVERY OF A NOVEL MUTATION DISRUPTING THE DIHYDROPYRIMIDINE DEHYDROGENASE ACTIVITY.

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Introduction: Five-fluorouracil (5-FU) based chemotherapies remain the gold standard for the treatment of various cancers. Despite the growing evidence that patients affected by dihydropyrimidine dehydrogenase deficiency (DPD) have a higher risk to develop a severe toxicity, the majority of patients that are candidate for fluoropyrimidine-based treatment are not screened for DPD deficiency. We report here the analysis of a 63 years-old patient who died after a first cycle of 5-FU. A comprehensive familial approach using both phenotype and genetic analyses was undertaken in 6 members of the patient’s family.

Methods: Genetic analyses were performed by direct sequencing of the 23 exons (including exon-intron junctions) of the DPYD gene, the promoter region flanking the -1590T>C polymorphism and a part of intron 10 in which a functional polymorphism c.1129-5923C>G has been described (Van Kuilenburg ABP et al. 2010). DPD enzyme activity was assessed by measuring the plasma dihydrouracil to uracil ratio using HPLC method.

Results: The patient was found to be a novel compound heterozygote for two DPYD different mutations: a novel 8-bp duplication in exon 3 (yielding to a truncated protein) and the known polymorphism c.1679T>G, leading to a complete DPD deficiency (determined with plasma dihydrouracil to uracil ratio). The study of the 6 family members showed a high correlation between genotype and phenotype and provides functional characterization of the different DPYD genotypes observed among the family members.

Conclusion: We showed that the phenotyping analysis of DPD activity is a reliable tool to identify deficient patients and avoid lethal toxicities with 5-FU.

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SPURIOUS IMMUNOASSAY-BASED SALICYLATE IDENTIFICATION IN A PATIENT WITH ELEVATED BENZOIC ACID LEVELS

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Background

According to the manufacturer, benzoic acid does not interfere with commercially available homogeneous enzyme salicylate immunoassays at concentrations up to 1 mg/mL. We report a case of falsely elevated serum salicylate concentrations due to elevated benzoic acid levels.

Case Report

A 35 old female with a history of nonketotic hyperglycinemia (NKH) presented to the Emergency Department with non-bilious emesis, cough, and increased seizure frequency. Laboratory evaluation was significant only for a slightly low bicarbonate (15 mmol/L), attributed to recent emesis. Due to worsening respiratory status, two seizures, and worsening metabolic acidosis (pH 7.22, bicarbonate 9 mmol/L) the patient was transferred to the pediatric intensive care unit for monitoring. The patient’s list of medications include: albuterol, diazepam, erythromycin, levetiracetam, leucovorin, sodium benzoate, topiramate, and acetaminophen. Given the anion gap metabolic acidosis, additional laboratory studies were performed. Based upon the acidemia and presence of gasping respirations in a critically ill child, the possibility of benzoic acid toxicity was considered. Additionally, the patient had unexplained persistently elevated salicylate concentrations of approximately 10 mg/dL.

Methods

An acid-basic-neutral GC-MS drug screen did not identify salicylate in urine or serum. However, a large peak was
METHODS

Background: It has been reported that 70 - 80% of nursing women are on medication. Though many are safe, there is accumulating evidence of toxicity in some breastfed infants. Information on drug excretion into milk is lacking for most drugs, and this uncertainty in the risk of drug exposure causes maternal non-adherence to therapy or avoidance of breastfeeding. This is a clinical problem in drug safety and an important women’s health issue. The objective of this study is to investigate the risk of drug exposure of three drugs (lithium, methotrexate and tacrolimus) in nursing infants. METHODS: We established a drug safety monitoring program, Drugs in Lactation Analysis Consortium (DLAC), to measure drugs commonly used by nursing women. Breast milk is a complex lipid- and protein-rich matrix; we worked to create a simplified drug extraction method using organic solvents. Methods were then developed to measure these drugs using LC-MS/MS. Breast milk samples were obtained from lactating women receiving lithium, methotrexate and tacrolimus; samples were obtained pre-dose and then at various time-points throughout the dosing interval. A unique feature of this study is that both fore-milk and hind-milk were collected.

RESULTS: Time-concentration profiling of methotrexate and its metabolite in breast milk were determined following a once-weekly subcutaneous dose of 25 mg of methotrexate. Foremilk and hindmilk samples were measured; peak drug concentration was found around 12 hours post-dose, with low but detectable levels from 48-96 hours post-dose. Tacrolimus breast milk pharmacokinetics was assessed following an 8 mg dose. Peak milk tacrolimus concentration was found around 18-20 hours post-dose, with higher concentrations present in the lipid-rich hindmilk samples. Lithium time-concentration profiling was established, with peak concentration found between 1-8 hours post-900 mg oral dose, and selectively accumulating in the aqueous phase of breast milk. Data showing the potential risk to the nursing infant will be presented. CONCLUSION: These results demonstrate that measurable levels of drugs are observed in breast milk. Combining these results with milk-volume consumption, metabolism and clearance, this data can be used to determine the relative infant dose and the risk of adverse effects to the nursing infant.
was calculated. 5-FU concentrations were obtained and pharmacokinetic parameters were estimated for individual 5-FU dose adjustment and to evaluate their relationship with biomarkers.

**Results:** 192 patients (52 females and 140 males) with a mean ± SD age of 62 ± 10.78 years (range 36 - 85 years) were included. Of these, the cancers were colorectal in 95 cases, pancreatic in 41 and gastric in 38. Pretreatment U and UH2 concentrations exhibited a high interindividual variability with values ranged from 5.7 to 70 mcg/mL and from 34 to 326 mcg/mL respectively. 24.48% of patients have concentration >15 mcg/mL. The mean UH Ratio was 9.34 ± 3.83. Baseline concentrations showed a positive relationship with 5-FU AUC (R spearman 0.59 p<0.01). No association has been found between UH Ratio and 5-FU AUC. The mean AUC observed in patients with U <15 mcg/mL were significantly lower than those who have U >15 mcg/mL (28.8 versus 36.3 P < 0.01).

**Conclusion:** Baseline U concentration is a useful predictor of decreased DPD activity and the cutoff value of 15 mcg/mL is associated with a high exposure to 5-FU (AUC>30mcg*h/mL) and clinical toxicity. We cannot demonstrate the utility of UH2/U ratio in predicting 5-FU toxicity.

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**PLASMA LEVELS OF POLYMYXIN B IN CLINICAL PRACTICE AND CORRELATION WITH CLINICAL OUTCOMES**

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**Background:** Polymyxin B (PB) is reserved for infections caused by multidrug-resistant gram-negative organisms. Optimal dosing has yet to be defined. The utility of monitoring PB levels remains unknown.

**Methods:** Patients with ≥2 PB levels obtained ≥3 days after initiation of PB from January 2012-April 2014 were evaluated. PB dosing regimens were determined by the treating medical team. PB concentrations (B1+B2) were measured in plasma using a previously described LC-MS assay. Pharmacokinetics (PK) of PB was optimally described by a 2-compartment model. Parameters were calculated using Phoenix WinNonlin software and compared to evaluate any relationships with clinical improvement (CI), acute kidney injury (AKI), and hospital mortality (M).

**Results:** Data from 39 patients (pts) were included in the analysis: 25(64%) men, median age 53 yrs(IQR 39,63), median weight 65 kg(IQR 54,80). PB was the only active antimicrobial used in 12(31%) and all pts received concomitant nephrotoxins. Median daily dose of PB was 2.6 mg/kg/day with the most common dosing frequency being q12h in 23 followed by q24h in 12 pts. At the time of PB levels, 8 pts were on renal replacement therapy (RRT; 7/8 continuous RRT), 5 receiving ECMO, and 1 on both CRRT and ECMO. Median t1/2(1) and (2) for all pts was 0.3 h and 12.1 h, respectively. Median PB total clearance was 4.1 L/h for non-CRRT pts (n=25) and 5.3 L/h for CRRT pts (p=0.04). Median AUC0-24h for all pts was 31 mg*h/L. Comparing pts with CI to those without, no differences in dose or AUC0-24h were noted. No differences were also observed in these parameters for mortality and AKI. Given MICs of the targeted pathogens in 32 pts, the median AUC/MIC ratio was 58.5(IQR 21.4, 120.4). In pts achieving an AUC/MIC ratio of ≥50 vs <50, CI was 88% vs. 60% (p=0.1) and M was 24% vs. 47% (p=0.27).

**Conclusions:** In this study, total systemic exposure to PB did not demonstrate a significant relationship with clinical outcomes. The impact of PB dosing regimens and corresponding plasma levels in the setting of combination therapy as well as alterations in PK by medical devices deserve further exploration.

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**IS THERE ANY INTEREST FOR INTRACELLULAR MYCOPHENOLIC ACID MEASUREMENTS IN KIDNEY TRANSPLANT PATIENTS ?**

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**Background:** Mycophenolic acid (MPA) is regularly monitored in transplant patients through plasma concentrations and preferably through area-under-time-concentrations curves (AUC). MPA TDM is recommended in some clinical situations but appears less consensual than for other immunosuppressive drugs. Peripheral Blood Mononuclear Cells (PBMC) have been reported a better biological matrix to measure calcineurin inhibitors, as compared to whole blood, to assess the pharmacological activity.

**Methods:** The first objectives of this work were to develop analytical methods to measure intracellular (PBMC) MPA concentrations by LC-MSMS, and the activity of the target enzyme IMPDH by HPLC. The ultimate objectives were to compare the relationships between MPA plasma and PBMC monitoring at different time points and the IMPDH inhibition at the same time points (T0, T15, T35, T120) on day 2, 4 and 10 post kidney transplantation in a population of...
40 kidney transplant patients under 500 mg bid mycophenolate mofetil.

Results: A LC-MSMS method has been validated to quantify MPA in PBMC isolated by Leucosep tubes. Total run time was 6 min and LLOQ was 0.1 ng/mL. The method has been fully validated for interferences and ion suppression effects. An IMPDH assay has been validated by HPLC, using a protein concentration normalization method. Intra and inter-assay imprecision was < 3%. Important inter-patient variability was observed both for the MPA and for the IMPDH measurements. Pre-dose IMPDH activity increased during the 10 days post-transplantation. A significant inverse relationship was found between IMPDH activities and MPA concentrations in both plasma and PBMC. A significant correlation was found between plasma and PBMC MPA values, but intracellular MPA kinetics appeared slower than in plasma. Maximum IMPDH inhibition was found at T1/2 and was better related to plasma AUC (p = 0.027) than with PBMC AUC (p = 0.323).

Conclusions: These findings suggest that PBMC MPA concentrations do not provide any better correlation with the IMPDH activity than plasma MPA values, most likely due to the correlation between plasma and PBMC MPA levels and to the important inter-patient variability both in MPA levels and enzyme activities.

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INHIBITION OF OCT-2, MATE1 AND MATE-2K AS A POSSIBLE MECHANISM OF DRUG INTERACTION BETWEEN PAZOPANIB AND CISPLATIN.

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Purpose: Pazopanib is a multi-targeted tyrosine kinase inhibitor (TKI) with anti-angiogenic activity approved for the treatment of advanced renal cell carcinoma and some sarcomas. Several studies have shown that some other TKI could inhibit the activity of OCT2, MATE1 and MATE2K carriers. The aim of this study was to evaluate the inhibitory potential of pazopanib on renal transporters OCT2, MATE1 and MATE2K and estimate its impact on the transport of cisplatin.

Methods: In vitro experiments on HEK 293 cells transfected to stably express OCT-2, MATE1 or MATE2K were conducted. A control experiment was transfected with an empty vector (EV). The inhibitory effect of pazopanib on these transporters was measured using the uptake of fluorescent substrate ASP+ together with pazopanib in concentrations ranging from 0 to 20μM. The effect of pazopanib on cisplatin uptake was evaluated by intracellular transport experiments in the different cell lines. Finally, the effect of pazopanib (used at 1μM and 5 μM) on cisplatin induced cytotoxicity (as a means of assessing the intracellular quantity of cisplatin) was evaluated on different cell lines.

Results: Pazopanib inhibits ASP+ uptake mediated by OCT2, MATE1 and MATE 2K transporters with IC50 values of 3.5; 3.1 and 4.5μM respectively. Regarding the transport of cisplatin, the addition of increasing concentrations of pazopanib resulted in a decrease of the intracellular concentration of cisplatin in OCT2-HEK cells, MATE1-HEK cells and MATE2K-HEK cells whereas it remained stable in the EV control line. These results were confirmed by cytotoxicity experiments in which the addition of pazopanib led to a decrease of the cisplatin-induced cytotoxicity in the transfected lines, whereas this addition had no consequences in the EV control line. Pazopanib inhibits the transport of cisplatin by OCT2, MATE 1 and MATE2K leading to a decrease of cisplatin-induced cytotoxicity in lines overexpressing these transporters.

Conclusion: Pazopanib inhibits OCT2, MATE 2 K and MATE 1 transporters which could be the cause of a drug-drug interaction with cisplatin which enters in the renal cells by OCT2 and is extruded by MATE1 and MATE 2K.

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BUSULFAN DOSING AND VENO-OCCCLUSIVE DISEASE FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION: A PEDIATRIC CASE SERIES

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Background: Hepatic veno-occlusive disease (VOD) is a rare but potentially fatal complication seen following hematopoietic stem cell transplantation (HSCT). Conditioning regimens with intravenous (IV) busulfan doses targeting a low area under the curve (AUC) may result in poor disease outcomes while higher doses may be associated with toxicities such as VOD.

Objectives: To assess VOD rates and outcomes in pediatric (age ≤ 21 years) HSCT patients treated with six hourly
Concentrations. Our model can be used as a practical alternative to measuring unbound vancomycin concentrations.

Design/Method: Twenty-year retrospective chart review was conducted. The AUCs and predicted doses were calculated following first dose for the Q6H group and from a test dose for the Q24H group.

Results: Forty-seven patients (female n=27) received IV busulfan. Median age at HSCT was 7.8 years (range: 0.3-19.8 years). Transplant indications included AML (n=23), CML (n=9), myelodysplastic/myeloproliferative disorders (n=2), ALL (n=1), and non-malignant conditions (n=12).

Twenty-one (44.7%) patients had AUC data. Median calculated AUC was 1089 (Q6H dosing n=9) and 934 µMol*min⁻¹ (Q24H dosing n=12). Median clearance was 3.35 (Q6H dosing n=8) and 3.5 mL/min/kg (Q24H dosing n=12). No difference in AUC and clearance was noted between the two groups (p=0.24 and 0.81, respectively).

Overall, VOD was observed in 5 (10.6%) patients, all with AML as the underlying disorder. Median time to VOD onset was 11 days (range: 6-15 days). No association of VOD occurrence with age, gender or busulfan dosing frequency was noted (p=0.37, 0.26 and 0.45, respectively).

Amongst the 23 patients with AML, three of 19 in Q6H dosing group and two of four in Q24H dosing group, developed VOD. Conditioning regimens included busulfan/cyclophosphamide (n=19) or busulfan/cyclophosphamide in combination with gemtuzumab (n=1), thiotepa (n=1), alemtuzumab (n=1), and samarium (n=1). Four patients with VOD had AUC data available and had a target or test dose AUC of ≤ 1168 µMol*min⁻¹.

Conclusion: Although there was no significant difference in VOD occurrence when busulfan dosing was switched to Q24H dosing, a higher VOD rate was noted in patients with AML. Notably, two out of four patients with Q24H dosing developed VOD. However, larger studies are required to confirm these findings.

146 UNBOUND VANCOMYCIN CONCENTRATIONS IN DIFFERENT PATIENT POPULATIONS: IDENTIFICATION OF FACTORS THAT INFLUENCE ITS PROTEIN BINDING.
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Background
Controversy exists about vancomycin protein binding and its covariates. We determined unbound vancomycin concentrations in four different patient groups and identified factors that modulate its binding. We further created and validated a prediction model to estimate the unbound vancomycin concentration.

Materials and Methods
Vancomycin (unbound and total) concentrations were measured in 90 patients (male: n=50; female, n=40; 146 samples) from 4 different patient wards (intensive care unit (ICU) (n=51 samples); hematologic (n=33); orthopedic (n=44) and pediatrics (≥6 months, n=18) using a validated liquid chromatography mass spectrometry method. Unbound vancomycin was obtained by ultrafiltration (Centrifree Filter Devices) at 37°C during 30 min. The unbound vancomycin fraction was calculated as ultrafiltrate concentration/total vancomycin concentration. Multiple linear mixed model analysis was performed to identify patient variables that were predictive for unbound vancomycin concentrations. Variables included in the model were age, patient ward, number of co-administered drugs with high protein binding, kidney function (estimated glomerular filtration rate (CDK-EPI formula)), alpha-1-acid-glycoprotein, albumin, total bilirubin, IgA, IgM, urea, and total vancomycin concentrations.

Results
In the pediatric cohort, median unbound vancomycin fraction was 81.3% (range: 61.9-95.9%), which was significantly higher (p<0.01, Wilcoxon test for paired samples) than the unbound fraction found in the three adult cohorts (ICU (61.7% (47.0-87.6%)), hematologic (60.6% (48.7-90.6%)) and orthopedic (56.4 (45.9-78.0)) patients). No significant differences between the adult cohorts were found. The strongest significant predictor for unbound vancomycin was the total concentration, completed by albumin in the pediatric cohort and albumin and IgA in the adult cohorts.

As IgA was not predictive for unbound vancomycin concentrations in the pediatric cohort, validation of our model was performed in 13 adult patients. A mean difference of 0.13 mg/L (±2SD: ±1.4 - 1.1 mg/L; R²=0.99 (±2SD 0.97-0.99)) between measured and calculated unbound vancomycin concentrations demonstrated the predictive performance of our model was favorable.

Conclusions
Unbound vancomycin fractions vary significantly between pediatric and adult patients. Our findings highlight the importance of considering unbound vancomycin concentrations in patients with hypoalbuminemia and low IgA concentrations. Our model can be used as a practical alternative to measuring unbound vancomycin concentrations.
DEVELOPMENT AND VALIDATION OF A LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY (LC-MS/MS) ASSAY FOR THE CONCOMITANT QUANTIFICATION OF DABIGATRAN, RIVAROXABAN AND APIXABAN IN HUMAN PLASMA

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Novel oral anticoagulants (NOACs) are characterized by a wide therapeutic window. However, determination of plasma drug concentrations may be useful in the case of a suspected excess effect due to overdose or bioaccumulation, in patients with deteriorating renal or hepatic function, or when a drug-to-drug interaction is suspected. Here we aimed to develop and validate a LC-MS/MS method for the quantification of dabigatran, rivaroxaban and apixaban in human plasma.

Solid phase extraction was applied on C18 Bond Elute Cartridges. Samples were analyzed using a Quattro Premier XE triple quadrupole system. Chromatographic separation was performed under gradient conditions with a mobile phase composed by NH₄COOCH₂ 2 mmol/L 0.05% formic acid and 0.1% formic acid in MeOH. A reversed-phase C18 column thermostated at 40°C was employed. Positive electron spray ionization (ESI) mode was applied with nitrogen serving as the desolvation gas and high-purity argon as the collision gas. The method validation procedure was based on the recommendations of EMA guidelines. The calibration curves were satisfactorily fitted by quadratic regression for all drugs with correlation coefficients for all calibration curves above 0.990. Deviations of the back calculated concentrations in QCs were below 15% of the nominal concentrations. The analysis of plasma samples from healthy volunteers and patients given other concomitant drugs revealed no significant interference with the MRM ion transitions of the analytes. No ion suppression was observed in correspondence of analytes and IS retention times. Post-infusion of analytes showed minimal influence of the matrix analyte retention time. The stability of drugs in plasma QCs samples left at room temperature (RT) and at +4 °C was ascertained up to 48 h with variations comprised within the ±10% of nominal concentrations. By sample reanalysis of samples from patients on anticoagulant therapy we found the percentage difference in results between the concentration of repeat and the original sample was below the threshold limit of 20% in 60 over 63 samples.

We have developed an accurate, rapid, and sensitive LC-MS/MS assay for quantification of NOAs in human plasma that can be easily applied in the clinical practice for the therapeutic monitoring of patients on anticoagulation therapy.

VITREOUS HUMOR AS AN ALTERNATIVE MATRIX FOR THE ANALYSIS OF ALCOHOL INTOXICATION IN POST MORTEM FORENSIC TOXICOLOGY

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Background: blood alcohol analysis and its evaluation in post mortem body fluids is a complex task also for experienced forensic toxicologists. Different alternative matrices have been studied for ethanol and drug of abuse determination. Our goal is to establish if ethanol concentration in vitreous humor could be used as a valid and reliable alternative to cardiac and peripheral blood alcohol concentration in defining alcohol intoxication.

Methods: twenty vitreous humor samples were screened for alcohol quantification in car crash deceased subjects. All samples were collected in NaF vials after puncture of anterior eye chamber, for every patient a venous central or peripheral blood sample in NaF was collected too. For Ethanol analysis we have chosen the following instrumental conditions: Head Space Gas Chromatography was performed using a DANI GC 1000 GC, with FID detector, with autosampler DANI HSS 86.50, with a column Variant CP Porabound Q (fused silica 25 m x 0.320 mm), injector temperature 210°C, detector temperature 250°C. The flow rate was of 1.8 ml/min with a split 1:5.

Results: the analysis of the matrices produced similar results and there was a strict correlation (r²=0.89) between the results of ethanol blood concentration values, both peripheral and central, and humor vitreous ethanol values (mean ±SD, blood vs humor vitreous: 125.2 ± 89.0 mg/dl vs 153.4 ± 103.9 mg/dl ).

Conclusions: vitreous humor has some advantages among the other matrices: first of all the stability and it is the relatively exempt from post mortem redistribution. Even if Literature is scarce on pharmacokinetics of alcohol and...
Background: In our hospital Intensive Care Unit (ICU) patients on mechanical ventilation are treated for selective decontamination of the digestive tract (SDD). Purpose is to eradicate potentially pathogen micro-organisms from the oropharynx and gastro-intestinal tract, thereby reducing mortality. In SDD tobramycin is given as mouth paste and oral suspension (through nasogastric tube), in a starting dose of 80 mg 4 times daily. If necessary, the dose can be increased. Given intravenously, high trough blood levels of the aminoglycoside tobramycin can lead to nephrotoxicity and ototoxicity. However, in SDD tobramycin is given orally and since aminoglycosides aren’t absorbed via the gut it usually won’t give systemic exposure. However, we describe a case in which a severe illness patient with Graft Versus Host Disease (GVHD) developed toxic tobramycin levels.

Method: In this case we describe a young male patient, submitted to the ICU due to respiratory insufficiency. Primarily, he was admitted with a recurrent AML. After allogenic stem cell transplantation he developed GVHD of his skin and intestines, leading to severe diarrhea. Previously, several ICU patients with SDD and GVHD developed systemic tobramycin exposure. Therefore the tobramycin level was measured.

Results: After consecutive use of 80 mg tobramycin 8 times daily for 30 days, the tobramycin trough level was 3.5 mg/L (reference < 0.5 mg/L). Because the patient was bedridden for a long time, renal function was difficult to assess, but creatinin was 102 umol/L and urea was 23.1 mmol/L (increase > 20% last 4 days). Tobramycin was stopped and levels dropped to 0.87 mg/L and 0.22 mg/L after 2 and 4 days respectively. Tobramycin was started again in a regimen of 4 times daily under daily monitoring. High tobramycin levels were contributed to intestinal leakage of tobramycin.

Conclusion: In SDD tobramycin is usually not absorbed. However, in severe intestinal GVHD systemic absorption can occur. In this patient toxic tobramycin levels were measured and impaired renal function was seen. After stopping tobramycin, levels dropped to normal. In ICU patients with intestinal GVHD and frequent administration of tobramycin-containing SDD close monitoring of systemic exposure of tobramycin is recommended.

Improved Determination of Uracil and Dihydrouracil in Plasma After a Loading Oral Dose of Uracil Using High-Performance Liquid Chromatography With Photodiode Array Detection and Porous Graphitic Carbon Stationary Phase

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Background: Despite its use in many chemotherapy treatments, fluoropyrimidine drugs are associated to a high rate of severe adverse effects. The main reason for fluoropyrimidine toxicity is a reduced activity of dihydropyrimidine dehydrogenase (DPD) enzyme. Approaches to identify patients with reduced DPD activity are usually based on the measurement of the concentrations of the endogenous compound uracil (U) and its DPD metabolic product, 5,6-dehydrouracil (UH2). Recently, the use of an oral loading dose of U was described as way to evaluate DPD activity. Considering the high hydrophilicity of U and UH2, the use of porous graphitic carbon (PGC) stationary phases for the HPLC separation of U and UH2 represents a valuable alternative due its unusual retention characteristics for polar compounds. The aim of this study was to develop and validate an improved HPLC method for determination of U and UH2 in human plasma after administration of an oral loading dose of U, based on PGC separation. Methods: 500 µL samples were added with internal standard (5-fluorouracil) and extracted with a mixture of ethyl acetate-isopropanol (85:15, v/v) after protein precipitation with ammonium sulfate. The extract was inject in the porous graphitic carbon stationary phase (Hypercarb® 150 x 4.6 mm, 5 µm) eluted with water and acetonitrile in gradient mode, at a flow rate of 1 mL min−1. Chromatograms were monitored at 210 and 260 nm. Results: Total chromatographic run time, including reequilibration, was 30 min. Retention times were 5.0, 12.5 and 15.7 min for UH2, U and IS (5-FU), respectively. The assay was linear in the concentration range of 0.2 to 20 µg mL−1. Accuracy was 98.4-105.3%, intra-assay precision was 5.1-12.1% and between-assay precision was of 5.3-10.1%. Analytes were stable in plasma at room temperature up to 6 h and for three freeze and thaw cycles. Processed samples are...
stable up to 12 h. Conclusions: The developed method was fully validated and has significantly reduced running time when compared to previous assay using PGC stationary phase, allowing complete resolution of U, UH2 and internal standard, with potential clinical application to identify DPD deficient patients.

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SIGNIFICANTLY DELAYED ELIMINATION OF METHOTREXATE IN ASSOCIATION WITH IMPAIRED RENAL FUNCTION IN A MAN WITH OSTEOSARCOMA NECESSITATING MORE EFFECTIVE INTERVENTION IN ADDITION TO LEUCOVORIN RESCUE THERAPY.

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BACKGROUND: Methotrexate is a widely used anti-metabolite anti-cancer agent. High-dose methotrexate (HDMTX) followed by leucovorin rescue therapy is an important component in the treatment of a variety of cancers. Unfortunately, acute renal failure and other adverse effects are unavoidable under certain circumstances. Despite advanced management care measures, HDMTX-induced renal dysfunction continues to occur in approximately 2% of patients with osteosarcoma. OBJECTIVES: The aim of this contribution is to describe the case of an adult Caucasian male patient with osteosarcoma receiving HDMTX treatment who demonstrated extremely delayed MTX clearance; necessitating intervention. CASE DESCRIPTION: A 37-year-old Caucasian male with osteosarcoma received HDMTX treatment according to the European and American Osteosarcoma Study Group (EURAMOS) joint protocol using an initial dose of 12 g/m² over a 4-hour infusion. The MXT plasma concentrations determined by fluorescent polarization immunoassay (FPIA) method 24 hours post-infusion and later were extremely high, indicating poor elimination and were confirmed by significantly elevated serum creatinine. High drug levels were accompanied by abnormal aminotransferases, namely ALT (up to 30 U/L). AST moderately increased (4 U/L), but was shortly restored. In contrast, the serum creatinine level remained abnormal over the course of a month. Leucopenia was present in the week after drug exposure; preceded by thrombocytopenia few days earlier. Both events of leucopenia and thrombocytopenia had several phases demonstrating the instability of the blood labs. Drug plasma level monitoring was completed daily until levels declined to 0.11 mmol/L. In total, this was >1 month +8 days since start of treatment. Leucovorin rescue treatment results were unsatisfactory; necessitating carboxypeptidase-G2 (CPDG2) use. This significantly and rapidly reduced the drug level by 80% of the previously recorded value. CONCLUSIONS: Although acute liver toxicity manifesting with transient aminotransferases elevation was reversible, methotrexate-induced nephrotoxicity may be challenging and warrant rescue measures, including use of CPDG2; especially for the treatment of patients with evidently delayed MTX clearance due to impaired kidney function. Prompt recognition of patients with poor elimination after administration of HDMTX is of vital importance to guide rescue therapy, including CPDG2, for effective elimination of MXT to avoid further deterioration of health and enhance outcomes.

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A FAST LC-MS/MS METHOD FOR ANALYSIS OF PHOSPHATIDYLETHANOL (PETH) TO DETERMINE ALCOHOL CONSUMPTION

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Background: Phosphatidylethanol (PETH) is a metabolite of ethanol that, compared to others, is formed only in presence of ethanol. It has a long half-life of about 4 days and remains detectable in blood for an average of at least 15 days. This offers potential for retrospective detection of alcohol over a longer period. Such analyses are desirable for, among others, pregnant women, since they underreport to questionnaires. When their alcohol consumption from even several weeks ago can be objectified, these women can be offered special counseling programs. This might prevent further alcohol consumption and will therewith reduce the number of children born with Fetal Alcohol Syndrome and Fetal Alcohol Spectrum Disorder, both conditions in which the child can suffer from behavioral, emotional or intellectual problems. That’s why we developed a quick method to measure PETH.

Methods: Among all different homologues of PETH, POPEth (16:0/18:1) is the most abundant in alcoholics and social drinkers. PLPEth (16:0/18:2) and DOPEth (18:1/18:1) are also reported in literature frequently. We developed a LC-MS/MS method to measure these three homologues with PETH 17:0/18:1 as the internal standard. A suitable fast
sample preparation was applied. Results: Freezing of blood samples is required for blood lysis. The most suitable sample preparation was isopropanol and acetonitrile. 400 µL sample prep was added after vortex-mixing 50 µL blood with 100 µL isopropanol. Again the sample was vortex-mixed and centrifuged, and 450 µL supernatant was transferred into glass tubes. 5 µL was injected into the LC-MS/MS, which comprised a Thermo TSQ-Vantage with (H)ESI-probe. Chromatographic separation was performed on a Waters Acquity BEH C18 2.1x50mm, 1.7µm column. We found LOD's and LOQ's of 91.5, 57.9 and 14.7 ng/ml and 150, 95 and 24.2 ng/ml for POPEth, PLPEth and DOPEth, respectively. Linearity (r²) was 0.98, 0.97 and 0.99 respectively. Based on results of a consumption-study, and in accordance with literature, the cut-off for heavy drinkers was set at 210 ng/ml for POPEth. Conclusion: We developed a fast LC-MS/MS method to determine three PEth-homologue levels. It can be used to objectify alcohol use. Currently, we are further optimizing our method.

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NONLINEAR ABSORPTION PHARMACOKINETICS OF AMOXICILLIN
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on behalf of COMBACTE consortium
Background
Amoxicillin is an aminopenicillin that has been in clinical use for decades. Yet, the pharmacokinetic profile has been poorly described. Some small pharmacokinetic studies have shown nonlinearity in the absorption. The aim of this study was to describe the population pharmacokinetics of oral amoxicillin with a focus on absorption and consequences for exposure.

Methods
28 healthy volunteers received on 2 separate occasions either 2 (b.i.d. 875/125 mg or 500/125 mg) or 3 (t.i.d. 500/125 mg or 250/125 mg) single oral doses of amoxicillin/clavulanic acid at the start of a meal. Blood samples were collected just before administration and after 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 8, 10 and 12 h (t.i.d. until 8 h). 140 amoxicillin concentration-time profiles with 1428 samples were available. The data were analyzed with nonlinear mixed-effect modeling (NONMEM, version 7.2). Different absorption models (first-order, zero-order, Michaelis-Menten) with and without lag-time were evaluated in combination with one- and two-compartment disposition models. Model selection criteria were decrease in objective function, diagnostic plots and visual predictive checks.

Results
The increase in mean AUC0–24h was proportional to the dose for 250/125 mg t.i.d., 500/125 mg b.i.d. and 500/125 mg t.i.d. (750, 1000 and 1500 mg/day amoxicillin, respectively). However, the mean AUC0–24h of 875/125 mg b.i.d. (1750 mg/day) was equal to 500/125 mg t.i.d. (1500 mg/day), thus nonlinear absorption was to be assumed. As expected, a first-order absorption model did not fit the data. Nonlinear (zero-order or Michaelis-Menten) absorption models showed a significant improvement in diagnostic plots. Pharmacokinetics of amoxicillin was best described by a two-compartment model with time-lagged nonlinear (zero-order or Michaelis-Menten) absorption and first-order elimination. Mean central volume of distribution was 28.1 L and mean clearance was 21.2 L/h. With each concentration-time profile analyzed separately, variability was included for central volume of distribution, clearance, lag-time and the model-specific absorption parameters (duration of the zero-order absorption process and the Michaelis-Menten parameters K_m and V_max).

Conclusions
Absorption pharmacokinetics of amoxicillin is dose-dependent and best described by a zero-order or Michaelis-Menten absorption model.

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ENVIRONMENTAL AND BIOLOGICAL MONITORING OF OCCUPATIONAL FORMALDEHYDE EXPOSURE RESULTING FROM THE USE OF PRODUCTS FOR HAIR STRAIGHTENING
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**Background:** Formaldehyde (FA) has been used for hair straightening in Brazilian beauty salons as a component of several cosmetic products, even being prohibited by Brazilian Health Authorities due to its toxicity. The aim of this study was to assess the exposure of beauty salons workers to FA through environmental monitoring and biological, also evaluating the presence genotoxic effects in exposed workers. Additionally, FA concentrations were measured in the employed cosmetic products. **Methods:** 50 subjects from 6 different Brazilian beauty salons (A to F) were recruited. Air concentrations of FA were determined in each salon using passive sampling. Urinary concentrations of formic acid were measured at the beginning and at the end of the working day by gas chromatography. DNA damage was evaluated by micronucleus test in oral mucosa cells and the comet assay with heparinized venous blood. **Results:** The environmental concentration of FA was 0.02-0.04 ppm in salons whose cosmetic products did not contain FA (D and E). Cosmetics used in salons A, B, C and F contained 5.7; 2.61; 5.9 and 5.79% of FA, respectively, associated with environmental concentrations of 0.07; 0.14; 0.16 and 0.14 ppm. There were significant differences in the urinary concentration of formic acid before exposure (p = 0.016) and after exposure (p = 0.004), variation of formic concentrations between the beginning and end of work shift (p = 0.018) and environmental concentrations of FA (p <0.001). There was no significant difference in the comet assay for both damage index (p <0.001) and frequency of damage (p <0.001). In the micronucleus test, only karyorrhexis presented significant difference among salons (p = 0.001). There was no significant difference between the groups classified by salons for binucleated cells, broken eggs and micronucleus. **Conclusions:** FA was detected in 2/3 of the tested hair straightening cosmetics. Urinary concentrations of formic after exposure and the variation of formic acid concentrations between the beginning and end of the working shift were correlated with the environmental concentrations of FA and can potentially be used as biomarkers of exposure. Environmental concentrations of FA were positively correlated with DNA damage found in the comet assay.

**TDM OF MIDAZOLAM AFTER IV OR INTRANASAL ADMINISTRATION USING A SIMULATED COMPUTER MODEL AND BISPECTRAL INDEX (BIS), A NEED FOR INDIVIDUALIZED THERAPY?**

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**Background**
Midazolam (MDZ) is used in intravenous anaesthesia for hypnosis induction. The level of sedation is often monitored using clinical scores. The Bispectral Index (BIS) is derived from changes in the electroencephalogram profile that may provide an objective measure of the level of sedation. A great inter-individual variability is observed in pharmacological response/dose requirements. MDZ monitoring could be useful to characterize response and optimize drug regimen in routine anaesthesia practice.

**Objective**
To evaluate MDZ drug monitoring and pharmacological response with aim to individualize drug regimens in intravenous anaesthesia

**Methods.**
The volunteers who previously signed an informed consent form, received 3 mg of MDZ IV (bolus dose). MDZ concentration was simulated with ‘RUGLOOP’ a TCI software using Greenblatt MDZ pharmacokinetic model. A blood sample was drawn when equilibrium between blood (Cp) and effect compartment (Ce) simulated concentration was reached. Simulated plasma and effect compartment concentrations were recorded. After seven days washout, an intranasal (IN) dose of 3 mg was administered by a specific device (Mucosal Atomization Device MAD, LMA, USA).

BIS, heart rate, oxygen saturation were registered in both experiments.

**Results:**
Different sedatives responses were observed after IV or IN administration according to BIS records: between 100-80 (n=1), 79-60 (n=3), lower than 59 (n=1). The same pattern was observed in both administrations, although BIS decreased was verified at longer times in IN administration than IV.

After IV administration MDZ equilibrium was reached at 10 minutes, no inter-individual differences were observed in Cp/Ce at equilibrium time and high correlation was observed between Cp measured and Cp simulated (median Cpm: 34,27ng/mL; Cps: 36,44ng/ml).

**Conclusion**
Midazolam is used to produce moderate sedation in patients undergoing minor therapeutic procedures. Previous studies showed wide variability to MDZ response that might determine individual MDZ dose regimen (0.1-0.2 mg/Kg) for anaesthesia.
We observed low/high sedation effects in the volunteers after the same IV or IN MDZ dose. Despite no differences were achieved in MDZ Cp levels, MDZ Cp/Ce or pharmacological effects, MDZ monitoring could be useful to characterize pharmacokinetics (CYP3A4 polymorphisms, CYP3A inhibitors or inducers), or pharmacodynamics in order to individualize dose regimens.

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HIGHER INTESTINAL PEPTIDE TRANSPORTER PEPT1 IN HUMAN NEONATES AND INFANTS THAN IN ADULTS

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Background: The influx oligopeptide transporter PEPT1 (SLC15A1) is expressed in small intestinal tissue. Its role in drug and prodrug transport is increasingly explored. For example, valacyclovir and β-lactam antibiotics appear PEPT1 substrates. As these drugs are used in children, developmental changes in its expression may impact drug absorption and hence systemic exposure. Absence or overexpression of a transporter in children might lead to respectively inadequate therapy or toxicity of PEPT1 substrates. We aim to study the developmental expression and localization of intestinal PEPT1 transporters.

Methods: Small intestine tissues were collected from surgical procedures (infants) or biopsies (adults), snap frozen and paraffin-embedded. PEPT1 gene expression was determined using real time RT-PCR, relative to villin mRNA expression. PEPT1 was stained immunohistochemically. Mann-Whitney test was used to compare among pediatric and adult groups.

Results: PEPT1 mRNA expression could be determined in all 18 infants (median [range] gestational age at birth 31.6 [24.7-40] weeks; median postnatal [range] age 2 [0-16.6] weeks) and 11 adult samples. Median relative PEPT1 gene expression was 30% higher in the infant samples compared to adults samples (p<0.05). Within the pediatric group PEPT1 expression was not related to gestational age at birth or postnatal age. For immunohistochemistry, 12 infant small intestine (median [range] gestational age at birth 33.3 [25,3-40] weeks; median postnatal [range] age 2.4 [0-16.6] weeks), and two adolescent, but no adult samples were available. The mRNA expression of PEPT1 was confirmed with immunohistochemical staining showing protein expression. Clear apical staining at the brush border of enterocytes was visible in all samples.

Conclusion: This study is the first to show intestinal PEPT1 in neonatal and young infants, with higher gene expression in infants compared to adults. This finding may have implications for oral dosing of PEPT1 substrates to infants.

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CHARACTERIZATION OF THE EXPOSURE-RESPONSE RELATIONSHIP OF LAMOTRIGINE IN CHILDREN WITH CHILDHOOD ABSENCE EPILEPSY

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Background: Lamotrigine is increasingly being used for the treatment of childhood absence epilepsy (CAE). The large variability in LTG pharmacokinetics (PK) makes it a good candidate for individualized dosing based on therapeutic drug monitoring (TDM). The target concentration range of lamotrigine has been proposed based on empirical data but has not been well established. Furthermore, a recent randomized clinical trial reported that 71% of CAE patients experienced treatment failure particularly due to lack of seizure control (Glauser et al. NEJM, 362:790-9, 2010). The purpose of this study was to characterize the lamotrigine exposure-response relationship using data collected as part of the largest prospective randomized trial in children with newly diagnosed CAE.

Methods: A total of 1054 PK observations in 187 CAE patients (3.1-12.8 years) at 4 week intervals over the first 16-20 week was available for analysis. Lamotrigine dose was titrated based on response (seizure control) and/or adverse events. Individual AUC estimates were predicted by Bayesian estimation using a population PK modeling approach (NONMEM 7.2). Patient demographics and UGT genotype were evaluated in the covariate analysis. Seizure status data (PD) were available in 106 patients at week 16-20. The probability of response was predicted by logistic regression analysis using pre-dose trough concentrations and AUC estimates (glm function, R 3.0.3).

Results: Large between patient variability in lamotrigine clearance (91 L/h/70kg, 44.8% CV) was observed. Body weight, UGA1A4 genotype and race were significant predictors of lamotrigine clearance. Thirty four patients (32%)
achieved seizure freedom at a wide range of lamotrigine exposures (AUC 26 to 243 mg∙h/L). Half of the patients did not respond to the treatment even at the pre-defined maximum dose (‘non-responders’). In the non-responder group, AUC was significantly lower than in responders at the same dose (p<0.05). The full exposure-response relationship could be described with a sigmoidal logistic regression model with a 50% of maximum effective AUC of 201 mg∙h/L and an EC_{50} of 14 mg/L.

**Conclusions** This study successfully identified for the first time the full lamotrigine exposure-response relationship in patients with CAE. These findings will facilitate lamotrigine dose individualization using TDM with the established evidence based PK/PD relationship.

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**EVEROLIMUS PHARMACOKINETICS IN PEDIATRIC PATIENTS WITH SUBEPENDYMAL GIANT-CELL ASTROCYTOMAS ASSOCIATED WITH TUBEROUS SCLEROSIS COMPLEX**

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**Background:** Everolimus is an inhibitor of the mammalian target of rapamycin (mTOR) and increasingly a cornerstone drug for the treatment of various debilitating conditions in both adults and children. Everolimus was approved for subependymal giant-cell astrocytomas (SEGA) in 2010 and several clinical trials have been conducted in pediatric neuro-oncology. However, knowledge on age specific everolimus pharmacokinetics (PK) in children is limited. The aim of this study was to characterize everolimus PK in pediatric patients with SEGA associated with tuberous sclerosis complex (TSC).

**Methods:** Prospectively collected PK data obtained as part of a phase II/II clinical trial of everolimus in 28 patients with SEGA associated with TSC were available for the analysis (Krueger DA et al., N Engl J Med. 2010;363:1801-1811). Everolimus clearance (CL) estimates were generated with Bayesian estimation using MW/Pharm ver. 3.82 (Mediware, The Netherlands). Allometrically scaling was used to account for differences in body size and the relationship between CL and size was evaluated with nonlinear modeling (GraphPad Prism ver. 6.05, GraphPad Software, USA).

**Results:** Apparent everolimus CL (L/h) increased with age in the range of 3 to 34 years (median was 11 years). The CL is positively correlated with body weight in the range of 16 to 133 kg (median was 51 kg). The power for allometric scaling was estimated 0.62 (95%CI: 0.40 to 0.83). The mean (SD) allometrically scaled everolimus CL was 22.2 (6.7) L/h/70 kg.

**Conclusions:** This is the first report on the PK analysis of everolimus in pediatric patients with SEGA associated with TSC to show CL increases with age as function of body size. This information will allow improved model based dose individualization of everolimus in this population.

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**ASSESSMENT OF AN HPLC-MS/MS METHOD FOR QUANTITATIVE DETERMINATION OF SEVEN BETA LACTAM ANTIBIOTICS, TAZOBACTAM, CIPROFLOXACIN AND LINEZOLID IN HUMAN PLASMA.**

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**Background:** The aim of this project was to develop simultaneous quantitation of seven beta lactam antibiotics (cefepeim, cefazidine, cefotaxime, cefazolin, meropenem, flucloxacillin, piperacillin), tazobactam, ciprofoxacin and linezolid.

**Methods:** EDTA plasma samples were subjected to a simple protein precipitation step with acetonitrile containing norfloxacin as internal standard, vortex-mixing, centrifugation and further diluted before being injected into a Shimadzu LCMS-8050 HPLC-MS/MS with dual ion source (ESI/APCI), operated in positive ion mode. Chromatography was achieved using a 2.1x50mm Waters Acquity C18 1.7µm diameter column with gradient elution using water and acetonitrile containing 0.1% formic acid over 3.5 min at a flow rate of 0.4ml/min for 5.5min. Data acquisition was via multiple reaction monitoring with Lab Solutions software. Calibration curves were 0.5-200mg/L for cefepime, cefazidine, cefotaxime, cefazolin, flucloxacillin, 0.05-25mg/L for ciprofoxacin and linezolid, 0.1-50mg/L for meropenem and tazobactam and 1-500mg/L for piperacillin. Blank plasma was spiked at 3 concentrations and used to evaluate the inaccuracy and imprecision of the assay over five days, in quadruplicate. A range of calibration curve fitting options were also employed and the fitting method that gave the lowest coefficients of variation (CVs), with adequate accuracy, was chosen as most appropriate for the analyte. Ion suppression was tested by comparing standard curves prepared identically in both water and plasma. The presence of ion
suppression/enhancement was considered if the slopes showed more than 10% difference.

**Results:** CVs were less than 10% at all three concentrations for all analytes, except ceftazidime being 12% at the lowest concentration. Lower limit of quantification for piperacillin was 10mg/L, cefotaxime, cefazolin, cefepime, ceftazidime, flucloxacillin were 5.0mg/L. Meropenem and tazobactam were 1.0 mg/L and linezolid and ciprofloxacin were 0.5mg/L. Ion suppression was observed for meropenem (40%) and ceftazidime (50%), suggesting the need for a stable isotope internal standard for these analytes. Norfloxacin (internal standard) showed no ion suppression, when comparing responses in standards prepared in water and plasma extracts.

**Conclusion:** The analytes were validated over wide concentration ranges making the assay suitable for clinical pharmacokinetic studies, routine monitoring, and quantitation of free drug.

**SOLANUM TORVUM INDUCED-IMMUNOCYTOTOXICITY**
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Background: Previously we presented a case series of clinical manifestation due to poisoning by susumber berries (Solanum torvum). In the present work we aim to study the mechanism by which this toxicity appears. The non toxic (control) and toxic berries have been analyzed by liquid chromatography-UV. The toxic berry presented steroidal alkaloids not described not present in the non-toxic berry. We isolated solasonine. Mass spectroscopy detected solamargine and two additional polar diacetyl and monoaconty glycoalkaloids which hydrolyze to an aglycone resembling hydroxy-solanidine. Each one of the fractions have been tested for immunotoxicity by using lymphocyte toxicity assay. Normal human lymphocytes have been exposed to each one of the fractions for 24 hours. The mitochondrial toxicity was measured by succinate dehydrogenase activity.

Results: Solasoline and solamargine did not show toxicity. Hydroxysolanidine presented 25% toxicity. The acetylated forms of these glycoalkaloids presented 65% toxicity. Proinflammatory cytokines (tumor necrosis factor alpha and interferon gamma) release in the media was 4 times higher in the lymphocytes exposed to hydroxysolanidine when compare to the levels of the same cytokines in media of lymphocytes exposed to solamargine only.

Conclusion: Elucidating the factors involved in natural substances -induced toxicity has medical significance. It is important to understand the need for monitoring the use of plant remedies. It is also necessary to enhance communication between scientists and physicians of all disciplines involved in complementary alternative medicine and clinical toxicology.

**CYP2B6*2 AND CYP2B6*9 ALLELE SIGNIFICANTLY ELEVATE NEVIRAPINE PLASMA CONCENTRATIONS IN MALAYSIAN HIV PATIENTS.**
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**Background:** Drug metabolizing enzyme CYP2B6 has been identified as a contributing factor in the variability of the pharmacokinetics of nevirapine, therefore may lead to variation in nevirapine efficacy and toxicity. This study was conducted to explore the possible association of CYP2B6 allelic variant with nevirapine plasma concentrations amongst Malaysian HIV patients.

**Methods:** In total, 112 patients treated with 200 mg twice daily nevirapine-based antiretroviral therapy were included in the study. Blood samples were drawn at pre-dose, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 and 8.0 hours after morning dose. Plasma nevirapine concentrations were determined by high performance liquid chromatography with UV detector. Identical pre-dose level and 12 hours post-dose were assumed. The area under the curve plot for 12-hour dosing (AUC₁₂) was obtained by the trapezoid rule with linear interpolation with PK-Solver. The minimum (Cₘᵟᵣₜ) and the maximum (Cₘₐₓ) plasma concentration of nevirapine were obtained from visual inspection of the concentration-time curves. Patients were genotyped for CYP2B6*1/*1, elevated nevirapine plasma concentrations (Cₘᵟᵣₜ) were found to be associated with CYP2B6*1/*1 (p= 0.023) and CYP2B6*9 (p= 0.03). Higher Cₘᵟᵣₜ was also observed in both alleles but the differences were not significant. No association was observed in the AUC₁₂ between the CYP2B6*1 alleles.
Conclusion: Results of this study suggest that knowledge of the CYP2B6 alleles may be useful in identifying HIV-infected patients at risk for higher nevirapine concentrations.

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CYP3A5 POLYMORPHISM AND ACCUMULATION OF INDOXYL SULFATE AFFECT THE RATE OF INCREASE IN CYP3A ACTIVITY AFTER LIVING KIDNEY TRANSPLANTATION IN PATIENTS WITH END STAGE RENAL DISEASE

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Background
Several previous studies have shown that renal failure decreases not only renal elimination but also metabolic clearance of drugs, particularly those metabolized by cytochrome P450 (CYP)3A, via transcriptional or translational modifications of CYP3A enzymes and direct inhibition of CYP3A-mediated metabolism by uremic toxins. We have previously reported that CYP3A activity increases in patients with end stage renal disease (ESRD) after kidney transplantation, with wide interindividual variability in the degree of increase. The aim of this study was to evaluate the influence of CYP3A5 polymorphism and plasma concentration of indoxyl sulfate on the increase in CYP3A activity after living kidney transplantation, by measuring plasma concentration of 4β-hydroxycholesterol.

Methods
This prospective study recruited 22 patients with ESRD who underwent the first living kidney allograft transplantation, comprising 12 patients with CYP3A5*1 allele (CYP3A5*1/*1 or *1/*3) and 10 patients without CYP3A5*1 allele (CYP3A5*3/*3). Morning blood samples were collected before and 7, 14, 30, 90 and 180 days after living kidney transplantation and plasma concentrations of 4β-hydroxycholesterol and indoxyl sulfate were measured.

Results
No significant difference in creatinine clearance over time was observed between patients with CYP3A5*1 allele and patients without CYP3A5*1 allele, suggesting that the degrees of recovery in renal function after living kidney transplantation are similar in the two groups. Plasma concentrations of indoxyl sulfate decreased significantly on or after day 7 after living kidney transplantation, with wide interindividual variability in the degree of decrease. Plasma concentrations of 4β-hydroxycholesterol on days 90 and 180 after living kidney transplantation were significantly higher in the presence of CYP3A5*1 allele than in the absence of CYP3A5*1 allele. A negative correlation was observed between areas under the plasma concentration-time curves of 4β-hydroxycholesterol and indoxyl sulfate.

Conclusions
These findings suggest that CYP3A activity may increase markedly associated with recovery of renal function in patients with CYP3A5*1 allele, and accumulation of indoxyl sulfate after living kidney transplantation may inhibit the increase in CYP3A activity.

Key Words
CYP3A activity; 4β-hydroxycholesterol; CYP3A5 polymorphism; indoxyl sulfate; kidney transplantation; end stage renal disease

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INVESTIGATING A CASE OF SUSPECTED POISONING WITH THE SYNTHETIC CANNABINOID AB-CHMINACA.

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Background
Identifying specific compounds among a wide range of emerging synthetic cannabinoids presents laboratory challenges, compounded by identifying uncharacterised metabolites in urine. This case reports on the hospitalisation of a prisoner, the seizure and the analysis of ‘green vegetable matter’ thought to be synthetic cannabis, and the analysis of urine specimens collected over three days.

Methods
The green vegetable matter was analysed by GCMS in full scan mode after ethanol extraction. Without GCMS library data covering emerging synthetic cannabinoids, a general Internet search of the main fragment ions indicated
AB-CHMINACA and reference material was purchased from Cayman Chemicals. Potential or ‘expected’ metabolites proposed by Cayman Chemicals were purchased and tuned on LCMSMS to determine optimum Multiple Reaction Monitoring (MRM) transitions which were used to analyse the urine specimens, after β-glucuronidase hydrolysis of possible glucuronide-conjugated metabolites.

Results
Analysis of the seized vegetable matter and the reference standard AB-CHMINACA demonstrated consistent retention time and consistent GCMS full-scan fragmentation, with abundant ions at m/z 312, 241 and 145. LCMSMS-tuned MRM transitions for the AB-CHMINACA ‘expected’ metabolites (nominated M4 and M2 methyl ester on the Cayman Chemicals website) were not seen in the analysis of the urine specimens. Tuned MRM transitions for M1A and M1B, which are hydroxycyclohexyl metabolites at position 4 and 3 respectively, were used for urine analysis and chromatographic retention time of MRM peaks were found to correspond between the M1B metabolite and one of the urine specimens. M3A, a dihydroxylated metabolite, was purchased and tuned by LCMSMS for urine analysis but the retention time did not correspond, suggesting that there is another metabolite similar to M3A but with a hydroxyl group in a different position. Suspected theoretical MRM transitions for other potential metabolites of AB-CHMINACA, nominated M2 and M5A by Cayman Chemicals, are present in the analysis of the urine, but the laboratory is awaiting supply of the material for confirmation of retention times and other qualifying identification criteria.

Conclusions
Elucidating metabolites of emerging drugs of abuse in a timely manner remains a challenge for pharmacology and toxicology laboratories responding to clinical emergencies arising out of abuse of novel drugs.

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STUDY ON THE ORIGIN OF METHADONE IN EXHALED BREATH PARTICLES
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Background: Non-volatile compounds present in aerosol particles in exhaled breath may be collected and used for detecting exposure of exogenous compounds, e.g., drugs of abuse. This new method has already been successfully implemented for drug testing. When collecting a breath sample via the mouth it is of concern if any contribution from saliva occurs. In the present study, we used measurement of methadone and phosholipids to study the origin of collected specimen.

Methods: Thirteen patients on methadone maintenance treatment were recruited for sampling of breath. A liquid chromatography-tandem mass spectrometry method was developed for measurement of methadone and phospholipids in the breath samples. Breath samples were collected in two different ways; using the SensAbues® device based in filtration and the PEXa® instrument based on impactor technology (collecting the 0.3-7 µm fraction). Results: The analysis of methadone in samples collected with the SensAbues device confirmed that methadone is present in exhaled breath (85-1610 pg/filter). The extracts prepared from SensAbues device also contained a phospholipid pattern known to be typical for the surfactant phase, with phosphatidylcholine 16:0/16:0 being dominant. Collection of particles with PEXa confirmed the presence of methadone in the particle fraction known to originate from terminal part of the lung.

Conclusion: This study confirmed that samples collected from exhaled breath mainly originates from lung as the phospholipid pattern is the same as for surfactant and different from that in oral fluid. Although contamination from saliva cannot be excluded it can be concluded that methadone detected in exhaled breath from methadone patients mainly is coming from particles originating from lung.

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RAPID QUANTIFICATION OF DESFUROYLCEFTIOFUR IN DIFFERENT BIOLOGICAL MATRICES AFTER TREATMENT OF PIGS WITH CEFTIOfUR
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Background: Every usage of antimicrobials in animal production causes the risk of an entry and distribution of metabolites into the environment which promotes antibiotic resistance and the formation of multi-resistant microbes. In several studies high amounts of antimicrobial residues were detected in sedimentation dust and aerosols after treatment of animals with the drugs. Ceftiofur, a cephalosporin β-lactam antibiotic is being increasingly used in veterinary medicine for treating bacterial infections. Thus, in the present study the environmental contamination after an intramuscularly or oral application of ceftiofur in swine was investigated.

Methods: Six pigs were treated intramuscularly (i.m.) with the therapeutic dosage of 3 mg/kg and subtherapeutic dosages of ceftiofur (0.3 and 1 mg/kg b.w. i.m.). Additionally an oral administration of 3 mg/kg b.w. was investigated. The concentrations of desfuroylceftiofur (DFC) were examined by UPLC MS/MS in samples of plasma, urine, feces, and sedimentation dust after extraction, derivatization and solid-phase sample purification. The quantification of DFC based on a targeted method using multiple reaction monitoring (MRM) within 3 min per sample.

Results: The presented UPLC-MS/MS method was established and major analytical variables were validated. The method showed linearity within a concentration range of 0.1 - 500 ng/ml. The LOD for quantification of DFC was determined for each matrix and revealed values between 80 - 340 pg/ml. Accuracies for quality controls in low and high concentrations showed variabilities below 10%. Method precision was also determined below 10% for different sample materials.

After the application of all treatment varieties significant concentrations of DFC were detectable in all investigated biological matrices. As expected, the treatment with highest amounts of DFC revealed the highest concentrations measurable with values up to 20 µg/ml in urine and plasma and up to 60 ng/ml DFC per mg sedimentation dust. However, even at low dose treatment DFC was detectable in different biological matrices.

Conclusions: The renal and biliary excretion of antimicrobials leads to an entry of microbiological active substances into the environment. The presented data underline the necessity of a careful use of antimicrobials in a combination with good hygienic conditions to minimize the risk environmental drug exposure.
AN EXTERNAL EVALUATION OF PUBLISHED TACROLIMUS POPULATION PHARMACOKINETIC MODELS IN ADULT RENAL TRANSPLANT RECIPIENTS
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Background
Tacrolimus (TAC) is an immunosuppressant with narrow therapeutic indices and large pharmacokinetic variability, which is widely used in renal transplant patients. Population pharmacokinetics (PPK) is used for individualizing medication therapy. However, their ability to predict medication concentrations is not clear when models are extrapolated to other clinical centers. This study aimed to (1) evaluate their external predictive performance and (2) identify the potential influencing factors.

Methods
Trough concentrations (C0, n = 620) from 52 adult renal transplant recipients were collected in post-operative (POT) 90 days from our center, and used to evaluate 13 relevant PPK models (published up to 31 December 2014). Predictive performance was assessed by (1) prediction-based diagnostics including median prediction error (MDPE), median absolute prediction error (MDAE), and the percentage of prediction error falling in ± 20% (F30) and ± 30% (F30); (2) simulation-based diagnostics as normalized prediction distribution error (NPDE) tests; and (3) Bayesian-based diagnostics as Bayesian forecasting in simulated clinical settings. Potential influencing factors as covariates, analytical methods and modeling strategies were investigated.

Results
Prediction-based diagnostics showed an empirical steady-state infusion model with MDPE 1.3%, MDAE 32.1%, F30 31.9% and F30 47.1% and a theory-based two-compartmental model (i.e. relationships between concentrations and covariates were theoretically expected) with MDPE -19.3%, MDAE 33.0%, F30 32.4% and F30 46.0% performed best. While in simulation-based diagnostics, no model met the criteria of NPDE tests. Bayesian feedback with at least 3 prior observations could stably and significantly improve predictive performance. CYP3A5*3, POT and hematocrit were the 3 most recognized covariates. The importance of analytical method standardization was confirmed. Compared to other no-covariate-involved base models, including steady-state infusion and one- or two-compartmental models, empirical non-compartmental model following nonlinear Michaelis-Menten kinetics performed much better with MDPE -6.45%, MDAE 32.34%, F30 32.43% and F30 46.13%, which almost equaled to the covariate-involved models aforementioned.

Conclusions
Published PPK models predicted unsatisfactorily when extrapolated to our clinical data. Nevertheless, the study showed to incorporate nonlinearity of tacrolimus may be beneficial to PPK studies and clinical practice in the future.

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DRIED BLOOD SPOT (DBS) TECHNIQUES FOR THE MONITORING OF AMBRISENTAN, BOSENTAN, SILDENAFIL, AND TADALAFIL IN PATIENTS WITH PULMONARY ARTERIAL HYPERTENSION
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Background:
Patients with pulmonary arterial hypertension (PAH) commonly receive an endothelin receptor antagonist (ambrisentan, bosentan, macitentan), a phosphodiesterase-5 (PDE 5) inhibitor (sildenafil, tadalafil), or a combination of both [1]. Because inter-individual variability of drug exposure is large and drug interactions may cause substantial exposure changes, therapeutic drug monitoring (TDM) might offer a way to tailor therapies appropriately. The quantification of oral PAH drugs in plasma using LC/MS/MS techniques is well established [2, 3, 4] but cost-intensive and difficult to realize standardized sampling times which is essential for TDM. Therefore we developed dried-blood-spot (DBS) sampling techniques for the quantification of PAH drugs in capillary whole blood.

Methods:
Within a clinical study patient plasma and contemporaneously taken DBS containing at least one of the substances were processed by adding stable isotopically labeled internal standards and subsequent liquid/liquid extraction. The extracts were analyzed by LC/MS/MS [2, 3, 4], in multiple reactions monitoring mode. All methods were validated according to EMA and FDA guidelines [5, 6]. For further validation purposes plasma and corresponding DBS data of the respective drugs were correlated to achieve plasma/DBS ratios.

Results:
The lower limits of quantification for the DBS methods were 2.5 - 10 ng/mL for the analyzed drugs within a hematocrit range of 30% to 50%. The analytical methods fulfilled all validation criteria defined by the
VOR treatment: mean EVE

Results: statistically significant. 15 during concurrent treatment (at 3 different time points: 1, 7, and 14 days after the first VOR dose). The EVE D, C

Methods: Patients who have cardiovascular diseases and Diabetes were included. The screening of SCC was performed at the during hospital visit at Nawanakhon’s hospital under the consent form of the patients. The SCC which less than 20000 IU indicated that abnormal oxidative status. The association between SCC and the diseases progression such as Blood pressure and Fasting blood sugar was determined by using relative risk ratio. The statistical significant was defined as 0.05 (α= 0.05).

Results: From 65 Chronic diseases (Cardiovascular diseases and Diabetes) with average age = 53.46 + 10.28 years produced mean SCC equal to 18057.14 + 7336.22 IU. (range 4000-34000 IU). The association assessment found that patients who have low SCC have increased risk of high blood pressure, significantly (RR = 2.28, p = 0.027). While there was no significant association between SCC and FBG however, some patients who had low SCC also had high FBS. In addition, patients who had history of smoking, exposed to smoke or chemical reagents tend to have low SCC.

Conclusion: This study shows the association between SCC and disease progression. Further study should be conducted to confirm the association of low SCC and Blood pressure control in cardiovascular diseases patients.

THE SCREENING OF SKIN CAROTENOID CONCENTRATIONS (SCC) IN PATIENTS WITH CARDIOVASCULAR DISEASES AND DIABETES MELITUS: AN ASSOCIATION ASSESSMENT IN SINGLE STUDY.

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Background: There are several evidences support about oxidative stress theory in chronic diseases patients such as Cardiovascular diseases, Diabetes Mellitus. Skin carotenoids concentrations (SCC) have been proposed to be the marker for oxidative status. Therefore, the objectives of this research is aim to determine an association of SCC and risk of diseases progression in cardiovascular diseases and Diabetes patients.

Method: Patients who have cardiovascular diseases and Diabetes were included. The screening of SCC was performed at the during hospital visit at Nawanakhon’s hospital under the consent form of the patients. The SCC which less than 20000 IU indicated that abnormal oxidative status. The association between SCC and the diseases progression such as Blood pressure and Fasting blood sugar was determined by using relative risk ratio. The statistical significant was defined as 0.05 (α= 0.05).

Results: From 65 Chronic diseases (Cardiovascular diseases and Diabetes) with average age = 53.46 + 10.28 years produced mean SCC equal to 18057.14 + 7336.22 IU. (range 4000-34000 IU). The association assessment found that patients who have low SCC have increased risk of high blood pressure, significantly (RR = 2.28, p = 0.027). While there was no significant association between SCC and FBG however, some patients who had low SCC also had high FBS. In addition, patients who had history of smoking, exposed to smoke or chemical reagents tend to have low SCC.

Conclusion: This study shows the association between SCC and disease progression. Further study should be conducted to confirm the association of low SCC and Blood pressure control in cardiovascular diseases patients.

EVEROLIMUS AND VORICONAZOLE INTERACTION IN LUNG TRANSPLANT PATIENTS

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Background: Voriconazole (VOR) is the therapy of choice for invasive fungal infections in lung transplant patients (LTPs). Aim: to analyze the impact of VOR administration on everolimus (EVE) dosage requirements (daily dose-D), trough blood concentrations (C_{trough}) and the concentration/dose(C_{trough}/D) ratio in LTPs.

Methods: Retrospective study of 100% of consecutive LTPs on a stable EVE-based regimen to which oral VOR was added, from January 2013 to February 2014. EVE blood levels (therapeutic range: 3-8 ng/mL) were measured using the Thermo Scientific QMS® EVE Immunoassay on an ARCHITECT-C8000 analyzer. The Wilcoxon Signed-Rank Test was used to assess EVE D, {C_{trough}} and {C_{trough}/D} ratio variations before commencing VOR treatment, during concurrent treatment (at 3 different time points: 1-3 days after the first VOR dose-V1-, after 4-6 days-V2- and 15-20 days-V3-) and after azole withdrawal. Statistical analysis was performed using SPSS version 19.0. p<0.05: statistically significant.

Results: 16 patients (9 men/7 women) were included, age: 55 years, weight: 65 kg. Before commencing (baseline) VOR treatment: mean EVE D, {C_{trough}} and {C_{trough}/D} were: 2.66±0.51mg/day, 6.26±0.94ng/mL and 2.47±0.71ng/mL.
Methods: In this study, the effects of a bolus injection of 5-FU were examined from viewpoints of patient management, including exchange of long-term infusions of 5-FU. Recently, the options have been considered from different perspectives, including the use of computer software to optimize tacrolimus exposure in renal transplantation. The computer software is applicable as a clinical dosing tool to optimize tacrolimus exposure and may potentially improve long-term outcome.

Results: Seventy-eight of the 80 included patients were randomized and included in the analysis. Computerized dosing (n=39): 32 standard risk/7 high-risk, Conventional dosing (n=39): 35 standard risk/4 high-risk. A total of 1711 tacrolimus whole blood concentrations were evaluated. The proportion of concentrations per patient within the target range was significantly higher with computerized dosing compared with conventional dosing performed by experienced transplant physicians. In standard risk patients a median of 90% [95% CI: 71-79%] was achieved compared to 81% [95% CI: 69-87%] in the Conventional group (P<0.001). In high-risk patients the respective values were 77% [95% CI: 71-80%] vs. 59% [95% CI: 40-74%] (P=0.04).

Conclusions: Computerized dose individualization improves target concentration achievement of tacrolimus after renal transplantation. The computer software is applicable as a clinical dosing tool to optimize tacrolimus exposure and may potentially improve long-term outcome.

Background: The FOLFIRI and FOLFOX regimens have been understood as standard treatments for colorectal cancers. A bolus injection of 5-FU via the VIA THE SUSPENSION OF DIHYDROPYRIMIDINE DEHYDROGENASE ACTIVITY BY A BOLUS INJECTION OF 5-FU IN ADVANCE

INCREASE IN STEADY-STATE PLASMA CONCENTRATIONS OF 5-FU/VIA THE SUSPENSION OF DIHYDROPYRIMIDINE DEHYDROGENASE ACTIVITY BY A BOLUS INJECTION OF 5-FU IN ADVANCE

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Background: The FOLFIRI and FOLFOX regimens have been understood as standard treatments for colorectal cancers, in which 5-fluorouracil (5-FU) is administered by long-term infusion and a bolus injection in advance. Recently, the options have been examined from viewpoints of patient management, including exchange of long-term infusion of 5-FU with repetitive oral administration of 5-FU derivatives and omission of a bolus injection of 5-FU. In this study, the effects of a bolus injection of 5-FU were examined by clinical and preclinical studies.

Methods: Japanese patients with advanced colorectal cancer were divided into 2 subgroups. Group 1 (n=5) was treated with the CPT-11 + 5-FU/LV + UFT/LV chemotherapy, consisting of 100 mg/m² CPT-11, 15 mg/m² I-LV and
500 mg/m² bolus 5-FU on day 1, followed by 300 mg/m²/day UFT (as tegafur) and 75 mg/day LV on days 1-5. Group 2 (n = 5) was also treated with this regimen, but bolus injection of 5-FU on day 1 was omitted. This study was conducted with the authorization of the institutional review board and followed the medical research council guidelines of Kobe University. Written informed consent was obtained from all participants prior to enrollment. To clarify the effects of a bolus injection of 5-FU, the activity levels of dihydropyrimidine dehydrogenase (DPD), the rate-limiting enzyme of 5-FU catabolism, and the plasma concentration of uracil, dihydrouracil, and 5-FU were determined after infusion of 5-FU (200 mg/m²/4 hr) with or without a bolus injection of 5-FU (470 mg/m²) in rats.

**Results:** In group 1, the plasma concentration of 5-FU at 48 hr after the start of treatment was 31.3 ± 11.5 ng/mL (±SD) significantly higher than that in group 2. 10.4 ± 8.4 ng/mL. Preclinical studies in rats suggested that a bolus injection of 5-FU resulted in higher plasma concentrations of 5-FU via suppression of DPD activity.

**Conclusions:** The bolus injection of 5-FU suppresses DPD activity, resulting in an increase in plasma concentration of 5-FU.

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**EVALUATION OF THE EVEROLIMUS QMS® IMMUNOASSAY ON THE ABBOTT ARCHITECTTM ANALYSER**

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**Background:** Recently, a new everolimus particle-enhanced turbidimetric immunoassay was introduced by Thermofisher Scientific. The aim of this study was to evaluate this QMS® everolimus immunoassay on the Architect®-C8000 analyser for measuring whole blood everolimus concentrations.

**Methods:** The study was performed following the CLSI protocol (EP5-A2, EP17-A). Within-day imprecision: 20 replicated analyses of three patient blood samples and of QMS® Everolimus low (4 ng/mL), medium (8 ng/mL) and high (16 ng/mL) controls. Between-days imprecision: over a 20-day period using the three controls (low, medium, high) and patient samples; each sample was tested using two reagent lots and two runs per day. Limit of blank (LoB) and limit of detection (LoD): ten replicates of calibrator blank (zero-calibrator) and low concentration calibrator (1.5 ng/mL). LoD= LoB+ 1.645 (SD<sub>sub</sub>)<sup>-1</sup>. Lower limit of quantification (LoQ): a low concentration blood sample was diluted with an everolimus-free sample to ten different concentrations in 5 different analytical runs. Dilution linearity: five high everolimus concentration patients' blood pools were serially diluted with calibrator A. Analytical recovery: adding concentrated everolimus into everolimus-negative samples. Calibration curve stability tested on days 1, 7, 14 and 21 using the calibrators A-F and controls (low, medium, high) in duplicate, as were the patient samples. Therapeutic range: 3.0 - 8 ng/mL.

**Results:** Within-assay coefficient of variation (CV) was 6.3% for low (mean: 4.2 ng/mL), 4.1% for medium (mean: 8.2 ng/mL) and 9.2% for high control (mean: 16.0 ng/mL). The respective total CV for the patients' pool was 5.2% (mean: 3.2 ng/mL), 4.8% (mean: 6.5 ng/mL) and 6.2% (mean: 8.3 ng/mL), respectively. Between-days imprecision was 6.7%, 5.4% and 7.5% for low, medium and high controls, respectively. LoB and LoD were 0.3 and 0.8 ng/mL, respectively. LoQ was 1.2 ng/mL. Dilution linearity exhibited a high degree in the range studied (1.7-20 ng/mL, r= 0.99). Recovery was 94%. The calibration curve was stable for 3 weeks.

**Conclusions:** This study demonstrates that Everolimus QMS® immunoassay adapted to the Architect<sup>TM</sup>-C8000 analyser presents a very good calibration curve stability, precision, reproducibility, sensitivity and specificity. Therefore, this technology could be suitable for monitoring everolimus in routine clinical practice.

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**ESTABLISHMENT OF SIMULTANEOUS QUANTIFICATION OF ESTRONE AND 16a-HYDROXYESTRONE IN HUMAN URINE USING ULTRA PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY.**

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**Background** 16α-Hydroxyestrone (16α-OHE<sub>1</sub>) is an endogenous estrone (E<sub>1</sub>) metabolite which is present in urine with large inter-individual variation. It is reported that E<sub>1</sub> is metabolized to 16α-OHE<sub>1</sub> by CYP2C19 and CYP3A in vitro, suggested that the ratio of 16α-OHE<sub>1</sub> to E<sub>1</sub> (16α-OHE<sub>1</sub>/E<sub>1</sub>) may potentially be used as an endogenous biomarker for the activity of CYP2C19 and CYP3A in vivo. The aim of this study is to develop and validate an UPLC-MS/MS method for the determination of urine concentrations of 16α-OHE<sub>1</sub> and E<sub>1</sub> for CYP2C19 and CYP3A.
Methods To 500 µL of urine in a glass, β-glucuronidase/sulfatase, 500 µL of sodium acetate buffer (pH 4.6) and 50 µL of internal standard (estrone-d4) were added and kept at 37 °C for 16 h. After samples were extracted with 7 mL dichloromethane, organic solvent portion were evaporated. The residue was re-dissolved in 100 µL of sodium bicarbonate buffer (pH 9) and 100 µL of dansyl chloride in acetone (1 mg mL⁻¹). After vortex mixing for 45 s, sample were incubated at 60 °C for 15 min to form dansyl derivatives for UPLC-MS/MS. Separation was achieved on a reversed phase C18 column at 60 °C. A gradient mobile phase system was used and MS/MS analyses were performed in positive ion mode under constant electrospray ionization conditions. Urine concentrations of 16α-OHE₁ and E₁ were measured in 4 men and 3 women.

Results The calibration curves were linear (r > 0.99) in the range of 10-500 and 0.5-25 ng mL⁻¹, with lower limit of detection of 0.5 and 0.2 ng mL⁻¹ in 16α-OHE₁ and E₁, respectively. The intra- and inter-assay precisions were within ±15%. Urine concentrations of 16α-OHE₁ and E₁ (mean ± S.D.) were 59.1 ± 21.9 (men) and 105.4 ± 57.0 (women) and 2.7 ± 1.2 (men) and 6.9 ± 5.2 (women) ng mg creatinine⁻¹, respectively. Urine 16α-OHE₁/E₁ was 24.6 ± 13.0 (men) and 18.0 ± 5.3 (women).

Conclusion UPLC-MS/MS method for simultaneous quantitation of E₁ and its metabolite 16α-OHE₁ in human urine was developed. This method was successfully applied to determine 16α-OHE₁/E₁ in human urine sample.

[Keywords] UPLC-MS/MS, 16α-hydroxyestrone, CYP2C19, phenotype

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PHARMACOKINETIC INTERACTION BETWEEN TACROLIMUS AND VORICONAZOLE IN LUNG TRANSPLANT PATIENTS
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Background: Invasive fungal infections are an important complication in lung transplant patients (LTPs). Our aim was to analyze the impact of voriconazole (VOR) administration on tacrolimus (TAC) dosage requirements, exposure (trough blood concentrations -C₉₀₀₀-, and the concentration/dose (C₉₀₀₀₀/D) ratio in LTPs.

Methods: Retrospective study of 100% of LTPs with a stable dose of a TAC based immunosuppressive regimen to which oral VOR was added, from January 2013 to February 2014. TAC trough blood levels were measured using CMIA on an Architect[TRADEMARK]-C8000 analyzer, adjusting the dose to reach the desired trough levels (therapeutic range: 5-15 ng/mL). The TAC dosage requirements (daily dose -D- and weight-adjusted daily dose - D/kg-). C₉₀₀₀₀ and C₉₀₀₀₀/D ratio were compared before commencing VOR treatment, during concomitant treatment and after azole withdrawal using the Wilcoxon Signed-Rank Test. The statistical analysis was performed using SPSS version 19.0. P<0.05 was considered statistically significant.

Results: The study included 39 patients: mean age of 52 years and mean weight of 70 kg. Before commencing (baseline) VOR treatment, the mean TAC D, D/kg, C₉₀₀₀₀, and C₉₀₀₀₀/D were: 6.15±2.71 mg/day, 0.09±0.05 mg/kg/day, 9.49±2.40 ng/mL and 1.73±0.63 ng/mL per mg/day, respectively. During VOR treatment, the TAC total daily dose was significantly reduced by 78.17±10.74%, obtaining a mean D of 1.45±1.05 mg/day and a D/kg of 0.023±0.022 mg/kg/day, in order to achieve therapeutic trough levels (10.20±3.45 ng/mL) and a significant increase in the C₉₀₀₀₀/D ratio (11.63±9.51 ng/mL per mg/day, p=0.000). After VOR withdrawal, the C₉₀₀₀₀/D ratio returned to similar values to the baseline situation (1.67±0.75 ng/mL per mg/day) and the mean D and D/kg were significantly increased: 5.46±1.33 mg/day, 0.083±0.043 mg/kg/day, p=0.000, respectively. On comparing the pharmacokinetic parameters studied (D, D/kg, C₉₀₀₀₀, C₉₀₀₀₀/D) at stable moments, before and after the discontinuation of azole therapy with the cotreatment period, these were found to have undergone significant changes (p=0.000).

Conclusions: This study performed in LTPs suggested that oral VOR is a strong inhibitor of TAC, and therefore the required dose had to be reduced around 78% to obtain therapeutic levels. In clinical practice, close monitoring of TAC blood concentrations is required both on commencing and discontinuing VOR therapy.

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DOES VOLUMETRIC ABSORPTIVE MICROSAMPLING ELIMINATE THE HEMATOCRIT BIAS FOR CAFFEINE AND PARAXANTHINE IN DRIED BLOOD SAMPLES? A COMPARATIVE STUDY
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Background. Volumetric absorptive microsampling (VAMS) is a novel sampling technique that allows the straightforward collection of an accurate volume of blood (approximately 10 µL) from a drop or pool of blood by dipping an absorbent polymeric tip, attached to a plastic handle, into it. The resulting blood microsample is dried and
analyzed as a whole. The aim of this study was to evaluate the potential of VAMS to overcome the hematocrit bias, an important issue in the analysis of dried blood microsamples.

**Methods.** An LC-MS/MS method for analysis of the model compounds caffeine and paraxanthine in VAMS samples was fully validated based on international guidelines. In conjunction with previously validated procedures for these analytes in dried blood spots (DBS) and liquid whole blood (LWB), this allowed us to set up a comparative study in which both compounds were determined in over 80 corresponding VAMS, DBS and LWB samples. These originated from authentic human patient samples, covering a wide hematocrit range (0.21 - 0.50).

**Results.** All evaluated validation parameters for caffeine and paraxanthine met the pre-established criteria. By calculating the differences with reference LWB concentrations, we found that analyte concentrations in human VAMS samples were not affected by a bias that changed over the evaluated hematocrit range, in contrast to DBS results. However, VAMS concentrations tended to overestimate LWB concentrations, as a consistent positive bias was observed. A different behavior of VAMS samples prepared from incurred and spiked blood, combined with a somewhat reduced recovery of caffeine and paraxanthine from VAMS tips at high hematocrit values -an effect that was not observed for DBS using a very similar extraction procedure- was found to be at the basis of the observed VAMS-LWB deviations.

**Conclusion.** Based on this study, being the first in which the validity and robustness of VAMS is evaluated by analyzing incurred human samples, it can be concluded that VAMS effectively assists in eliminating the effect of hematocrit, although care should be taken when protocols developed for DBS analysis are transferred to VAMS samples.

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**INFLUENCE OF CYP3A4, CYP3A5 AND MDR1-C3435T GENETIC POLYMORPHISMS ON ITRACONAZOLE-TACROLIMUS INTERACTION IN ADULT RENAL TRANSPLANT PATIENTS**

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**Background:** Tacrolimus-azole antifungal interactions are characterized by their clinical relevance. Aim: to evaluate the influence of CYP3A4, CYP3A5 and MDR1-C3435T polymorphisms on changes in tacrolimus dosage requirements, trough concentrations($C_{\text{trough}}$) and the concentration/Dose($C_{\text{trough}}$/D) ratio during and after itraconazole administration in adult renal transplant patients (RTPs).

**Methods:** Retrospective study of 49 Caucasian RTPs receiving tacrolimus and cotreated with prophylactic itraconazole (200 mg daily) for the first three months post-transplant. At stable moments, closest in time to azole discontinuation and after its withdrawal, tacrolimus blood concentrations were measured using CMIA on an Architect®C8000 analyzer and the dose was adjusted to reach target levels($C_{\text{trough}}$:10-12 ng/mL; $C_{\text{trough}}$/D:2nd. 3rd post-transplant month, 8-10 ng/mL: between months 4-12).

CYP3A4, CYP3A5 and MDR1-C3435T polymorphisms were associated with dose requirements (daily dose -D- and weight-adjusted daily dose-D/kg-), $C_{\text{trough}}$ and $C_{\text{trough}}$/D ratio during cotreatment and after azole withdrawal using the Mann-Whitney Test. Statistical analysis: SPSS 19.0. P<0.05: statistically significant.

All patients provided their informed written consent.

**Results:** 49 patients (30 men/19 women) were included, age: 51 years, weight: 73 kg. Genotype distribution was in Hardy-Weinberg equilibrium.

A significant increase in dosage requirements occurred after itraconazole exposure (D:from 3.28±2.70 to 4.91±2.97mg/day; D/kg:from 0.05±0.04 to 0.07±0.05mg/kg/day, respectively), by a mean of 80±67%, in order to achieve levels of 11.09±2.75 and 8.66±2.64 ng/mL, respectively. These requirements were always higher in CYP3A5*1/*3+CYP3A5*1/*1 (expressers) carriers but showed a trend, which was not significant, towards lower increase than CYP3A5*3/3(non-expressers) carriers (66±58% vs. 86±93%).

Mean $C_{\text{trough}}$/D decreased significantly (from 5.23±3.01 to 2.35±1.42ng/mL per mg/day) after coadministration. Although this parameter did not differ significantly between non-expressers/expressers, CYP3A5*3/3 carriers experienced a 1.5-fold higher decrease ($p=0.082$).

No significant differences in the pharmacokinetic parameters evaluated were observed for CYP3A4, CYP3A5 and MDR1-C3435T polymorphisms during and after cotreatment.

**Conclusions:** This study in RTPs revealed that mean tacrolimus dose requirements significantly increased by around 80% after itraconazole withdrawal.

Although the studied polymorphisms did not significantly influence the pharmacokinetic parameters evaluated, CYP3A5 non-expressers seem to require a higher increase in doses and experience a higher decrease in $C_{\text{trough}}$/D after its withdrawal.

In clinical practice, close monitoring of tacrolimus levels is required during and after itraconazole coadministration.
ETHANOL SCREENING FOR EMERGENCY DEPARTMENT: THE ROLE OF THE REFERENCE LABORATORY

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Background
Italian recommendations suggest whole blood (WB) as correct biological sample to use either for clinical or legal purpose. In fact people could be at Emergency Department (ED) for intoxication/drunkenness or for accident having drive under influence of alcohol. It is well known that alcohol in plasma (P) is higher than 20% when compared to WB. In Italy there are not commercially available kits validated for alcohol measurement in whole blood.
Aim of this paper is compared the results obtained by a kit in complete automation on P to those obtained with HS-GC / MS (reference method, RM) on WB.

Methods
161 couples of P and WB samples (grey top) have been collected with chain of custody at ED. P has been measured by kit ETOH_2 Advia Chemistry on Advia 2400 (Siemens Healthcare Diagnostics), whereas WB has been analyzed in head-space GC-MS QP2010 ultra EI (Shimatsu) by a validated method. For insurance and legal reasons four intervals are important: less than 0.5 mg/L, 0.51-0.8 mg/L, 0.81-1.50 mg/L and greater than 1.51 mg/L.

Results
Linearity on patients samples 0.1 - 4.05 mg/L, CV% 0.9% either low or high alcohol concentration
Range 0.05-0.50 mg/L: Comparison between kit (Y) and RM (X): Y=1.08X+0.06 R² =0.87
Range 0.51-0.80 mg/L: Comparison between kit and RM + 26% Y=0.76X+0.32 R² =0.61
Range 0.81-1.50 mg/L: Comparison between kit and RM + 21% Y=1.26X+0.06 R² =0.95
Range over 1.51: Comparison between kit and RM + 17% Y=0.99X+0.37 R² =0.91
Range 0.05 - 4.05 mg/L Comparison between kit and RM + 20% Y=1.08X+0.15 R² =0.97

Discussion
The kit evaluated has a good linearity and a small inaccuracy. Results on P are always higher than those obtained on WB by HS-GC-MS (17% to 33%) and only few patients are misclassified (4%). It is a useful kit for diagnostic purpose, but HS-GC-MS is strongly recommended when the result has a legal aim.

IS IT POSSIBLE TO PREDICT THE REJECTION RISK AFTER KIDNEY TRANSPLANTATION WITH A PRE-TRANSPLANT IMMUNOSUPPRESSANT SENSITIVITY ASSAY?

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Background
The first-year incidence of rejection is generally 10-15 % despite strong immunosuppressive regimens following kidney transplantation. Early prediction of the rejection risk is of great importance to allow individual preventive intervention. We assessed whether the sensitivity to immunosuppressants before kidney transplantation could be a prognostic marker for the development of rejection episodes.

Methods
Twenty-nine adult, living-donor kidney transplant recipients were included. Blood samples were collected pre-transplant, and at week 1, week 5-7 and year 1 after transplantation. Protocol biopsies were examined at week 6 and year 1. Peripheral blood mononuclear cells (PBMC) were isolated and stimulated with phorbol 12-myristate 13-acetate and ionomycin, and metabolic activity was quantified with water-soluble tetrazolium-1.

Results
Twenty pre-transplant response curves were eligible for the preliminary data analysis with clinical data available for one year (n=14) or six weeks (n=6). Five subclinical and one clinical rejection were detected in five patients. In regression analysis, the maximum inhibition of metabolic activity with MPA was estimated to median (range) 88.9% (72.7-93.8) and 93.6% (89.1-97.8, one outlier excluded) in patients with and without subclinical rejection, respectively (P=0.035). The actual observed inhibition at the highest MPA concentration was median 89.9% (72.7-95.9) and 95.0% (91.8-100, one outlier excluded) in patients with and without subclinical rejection (P=0.013). ROC curve analysis with estimated values identified maximum inhibition <91.8% as a discriminator with 80.0% sensitivity and 71.4% specificity (area 0.82, P=0.037). Similar analysis with actual observed single-point values identified maximum inhibition <93.0% as a discriminator with 80.0% sensitivity and 92.9% specificity (area 0.87, P=0.016). No significant relationships were observed for the tacrolimus response curves.
Conclusions
Interpreted with caution due to few observations, the pre-transplant sensitivity to MPA was a significant prognostic marker for subclinical rejection. The test may allow early selection and follow-up of patients at risk.

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BIOAVAILABILITY OF VORICONAZOLE IN HOSPITALIZED PATIENTS
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Background: Voriconazole is generally accepted as the first line antifungal agent in the treatment of invasive aspergillosis. Efficacy and effectiveness of voriconazole is associated with the voriconazole plasma trough concentration, which is highly variable in clinical practice. Altered bioavailability could contribute to this variability. In healthy volunteers the bioavailability is estimated to be over 90%. Recently, two studies have shown that in patients the bioavailability of voriconazole is substantially lower. However, for both studies some factors that could influence the voriconazole plasma concentration like inflammation, concomitant intake of food with oral voriconazole, or gastrointestinal complications, were not included in the evaluation. Therefore, we performed a retrospective study with strict inclusion criteria to compare bioavailability in hospitalized patients with the bioavailability in healthy volunteers.

Methods: A retrospective chart review was performed in adult patients, treated with both oral and intravenous voriconazole in the same dose and within a limited (≤5 days) time interval. These strict inclusion criteria were chosen because of the non-linear pharmacokinetics of voriconazole and the large variability of voriconazole plasma concentrations over time. Statistical analyses were performed with a paired sample t-test for normally distributed data and a Wilcoxon signed-rank test for non-normally distributed data.

Results: In total thirteen patients were included. The mean voriconazole trough concentration for intravenous administration of voriconazole was 2.28 mg/L (95% CI: 1.29 - 3.26 mg/L) and for oral administration of voriconazole 2.04 mg/L (0.78 - 3.30 mg/L). The mean bioavailability was 82.2% (95% CI: 59.0 - 105.3%). No significant difference was found in the oral and intravenous trough concentrations of voriconazole (p = 0.390) and the bioavailability between hospitalized patients and healthy volunteers is comparable.

Conclusion: We found a comparable mean bioavailability of voriconazole in hospitalized patients and healthy volunteers. Presumably, other factors than bioavailability may cause the observed difference in voriconazole trough concentrations between oral and intravenous administration in the earlier studies. A prospective bioavailability study in critically ill patients could confirm the results of this retrospective study.

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FLUCONAZOLE EXPOSURE IN PATIENTS ON THE ICU WITH INVASIVE CANDIDIASIS: A PROSPECTIVE STUDY
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Fluconazole (FLZ) has been used for the treatment of Invasive candidiasis (IC) for more than two decades. Higher FLZ exposure is associated with improved treatment outcome. However, routine drug monitoring is not advised for FLZ due to its low toxicity and linear dosage/exposure relationship. The dosage is considered to be proportional to the exposure but several studies have shown considerable variability in exposure in different patient populations. As critically ill patients may be at risk for underexposure the objective of this study was to evaluate the dosage - exposure relationship of FLZ in ICU patients with IC.

Patients admitted to the ICU and (empirically) treated for IC with FLZ were included in this study. Demographics and medical data were collected. Multiple leftovers per drug dosage from routinely obtained blood samples were analyzed with LC/MS/MS to determine FLZ concentrations. MW/Pharm 3.82 pharmacokinetic software equipped with a single compartment population PK model for FLZ was used to calculate AUC24h values. The primary endpoint was the association between FLZ dosage and FLZ exposure. A total of 10 ICU patients were treated with FLZ for their possible or proven IC from November 2014 to March 2015. Isolated Candida species were C. albicans (6), C. glabrata (1), C. parapsilosis (1) and C. tropicalis (2), all isolates were FLZ susceptible. FLZ dosing ranged from 200 - 800 mg/day for a period of 4 to 30 days. Plasma concentrations ranged between 7.2 - 40.4 mg/L. AUC24h values calculated using MW/Pharm ranged from 117 to 862 mg*h/L. Regression analysis showed that the AUC24h could be estimated based on the dosage; AUC24h =
0.97788*dosage - 61.195 (R²=0.89).

A significant correlation was found between the FLZ dosage and FLZ AUC₂₄₈. However, the dosage did not fully predict the AUC₂₄₈. Since 5 patients had lower AUC₂₄₈ values than the dosage would predict. Multiple studies showed that FLZ exposure may differ in different patient population due to patient related factors (renal failure, BMI, malignancy, etc.). Optimal drug exposure improves outcome in severely ill patients and more aggressive dosing along with therapeutic drug monitoring may help to achieve this goal.

A RANDOMISED-CONTROLLED TRIAL TO STUDY THE ADDITIVE VALUE OF CYP3A5 GENOTYPE-BASED TACROLIMUS DOSING IN LIVING-DONOR KIDNEY TRANSPLANTATION

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Background: The exposure to tacrolimus (Tac) is correlated with the expression and activity of the Tac-metabolizing enzyme CYP3A5. This isozyme is polymorphically expressed. Patients expressing CYP3A5 require a higher Tac dose to achieve therapeutic predose concentrations (C₀). The aim of this study was to evaluate whether dosing of Tac according to CYP3A5 genotype leads to earlier achievement of target Tac C₀ and consequently to a better clinical outcome.

Method: Recipients of a living-donor renal transplant (n = 240) were 1:1 randomly assigned to receive Tac at either a standard, fixed-dose (0.20 mg/kg per day) or based on the individuals’ CYP3A5 genotype (0.15 mg/kg per day for non-expressers and 0.30 mg/kg per day for expressers). The primary endpoint was the proportion of patients within the target C₀ (10.0-15.0 ng/mL) at first steady-state (day 3 after transplantation). Secondary endpoints included the time required to reach the target C₀ range, the number of dose modifications to reach the target C₀ and clinical outcomes during the first 3 months after transplantation.

Results: Three days after transplantation, 37.4% (95% CI: 28.5-47.0%) of the patients in the standard-dose (SD) group and 35.6% (95% CI: 27.0-45.0%) in the genotype-based (GB) group were within the target Tac C₀ range (P = 0.79). There was no significant between-group difference in the number of dose modifications needed to reach the target C₀ (P = 0.30). The time to achieve the targeted C₀ was also not significantly different: SD group 6 days (3-17 days) vs. GB group 6 days (3-28 days); P = 0.36. The clinical outcomes were similar in both groups.

Conclusion: Pharmacogenetic adaptation of the daily dose of Tac is not associated with earlier achievement of the Tac target exposure range and does not lead to improved clinical outcome.

IDENTIFICATION AND QUANTIFICATION OF AMPICILLIN, CEFAZOLINE, CEFTRIAXONE, CIPROFLOXACIN, DAPTOMYCIN, LEVOFLOXACIN, LINEZOLID, MEROPENEM, MOXIFLOXACIN, PIPERACILLIN AND RIFAMPICIN IN PLASMA FOR THERAPEUTIC DRUG MONITORING PURPOSES-DEVELOPMENT AND VALIDATION OF A LC-MS/MS PROCEDURE

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Introduction: Antibiotics are indispensable in the treatment of infections. TDM of these drugs helps ensuring sufficient plasma concentrations and minimizing side effects. Different liquid chromatography (LC) methods employing ultraviolet, fluorescence, or mass spectrometric detection have been published for the analyses of particular groups of antibiotics or a few antibiotics from different groups. The aim of the present study was to develop and validate a multi-analyte LC-tandem mass spectrometry (MS/MS) method simultaneously covering eleven antibiotics from five different classes to enhance effectiveness in TDM.

Method: A Shimadzu LC system coupled to a QTrap 4000 MS (ABSciex) instrument operated in positive electrospray ionization scheduled multiple reaction monitoring (MRM) mode was used in this study. Elution was performed on a Nucleodur-HILIC column (150 x 4.6 mm, 5 μm) with a mobile phase gradient of 50 mM ammonium formate buffer and acetonitril. Plasma samples (100 μL plasma) were prepared by protein precipitation with 800 μL of methanol/acetonitril (50:50 v/v). The supernatant was dried under nitrogen and 5 μl were injected after reconstitution in 200 μL mobile phase. The method was fully validated with respect to selectivity, matrix effects, linearity, accuracy and precision, limit of quantification (LOQ), and stability.

Results: All analytes were separated and could be unambiguously identified. No interfering peaks were detected in the monitored MRM traces. Due to instability during sample preparation, ceftriaxone and daptomycin were not
included in the validation study. Extensive but reproducible (RSD with 20%) matrix effects (> 50%) were observed for ampicillin, ciprofloxacin, meropenem, and rifampicin. Linearity was demonstrated over ranges broadly covering the published plasma concentrations. All analytes showed acceptable (< 15%) bias and precision. The lowest calibrator concentrations corresponding to at least half of the lowest published concentration of the respective drugs were used as LOQs. Instabilities over a 20 h period were observed for moxifloxacin and linezolid on the autosampler tray. Since meropenem was unstable in solution the working solution was prepared freshly every third day.

**Conclusion:** The developed method allows unambiguous identification of eleven antibiotics from different classes and reliable quantification of nine of them. It can be used for TDM and pharmacokinetic studies.

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**HIGH LEVETIRACETAM CLEARANCE IN A PATIENT WITH SUBARACHNOID HAEMORRHAGE.**

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**Introduction:** The glomerular filtration rate (GFR) is an important tool for drug dosing of really excreted drugs. At decreasing GFR, the doses of therapies must be reduced to avoid overdose. At clearly elevated GFR, it would be reasonable to increase the doses to avoid treatment failure. Increased GFR can be seen as a result of neurosurgical conditions, e.g. subarachnoid haemorrhage (SAH), SIADH or salt-wasting syndrome. The anti-Ep-drug levetiracetam (LEV) is an important drug to prevent seizures in SAH-patients. Approximately 60-70% of LEV is primarily excreted by the kidneys with a renal clearance of 0.6 mL/min/kg. SAH patients at neurosurgical wards may present with high urinary output, very low serum creatinine levels and subtherapeutic serum concentrations of LEV, despite full dose. We hypothesised that SAH patients have a high clearance of both creatinine and LEV.

**Aim:** To perform a pharmacokinetic study in serum and urine of LEV in a SAH patient with subtherapeutic serum LEV and low creatinine concentrations and to measure creatinine clearance (mCrCl) in order to better understand the mechanism of low LEV concentration in neuro-intensive patients.

**Materials and Methods:** A 50-year-old woman, weight 75 kg, with SAH and meningitis treated with I.V infusion of LEV 1,500 mg x 2 daily. Blood samples were taken continuously throughout a 12 hours dosing interval of LEV. Pharmacokinetic parameters such as LEV half-life (t1/2), time to maximum concentration (Tmax), maximum concentration (Cmax), exposure (AUC area under the curve), volume of distribution (Vd) and clearance (ClLEV) were calculated as well as mCrCl.

**Results:** Urine volume was 2.78 L/12h and mCrCl was 208 ml/min. Vd was 71 L (40-50L), AUC was 1059 h * µmol/L (50% of normal), t1/2 6.5 h (4-8 h). Total ClLEV was 135 ml/min (normally 75 ml/min) and renal ClLEV 70 mL/min (normally 45 mL/min).

**Conclusion:** Consistent with our hypothesis we could show that our patient had an increased urine volume, an increased mCrCl and an increased total and renal clearance of LEV compared to the general population. Neurosurgical patients are at risk of developing subtherapeutic drug concentrations unless doses are adjusted accordingly to a high clearance.

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**GENETIC POLYMORPHISMS ARE PREDICTIVE FOR SURVIVAL OF PATIENTS WITH ADVANCED GASTROINTESTINAL STROMAL TUMORS TREATED WITH IMATINIB**

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**Background** Imatinib is first line therapy for patients with advanced gastrointestinal stromal tumors (GIST). Clinical response is high, but primary and secondary resistance occur frequently. This study explores the effects of single nucleotide polymorphisms (SNPs) in genes related to the pharmacokinetics and pharmacodynamics of imatinib on its efficacy in patients with advanced GIST.

**Methods** In 255 patients with advanced GIST, a retrospective pharmacogenetic pathway analysis was performed. Genotype data from 36 SNPs in 18 genes, as well as traditional clinicopathological factors were tested in univariate analyses to investigate their effects on progression free survival (PFS) and overall survival (OS). Variables which showed a trend (p <0.1) were included in the multivariate model. SNP selection was based upon the minor allele
frequency and a predicted functional change. Missing baseline clinical data were imputed.

Results In the multivariate model significant associations with shorter PFS were found for the presence of metastases at diagnosis (HR 1.74, p=0.005), KIT exon 9 mutation (HR 1.94, p=0.040), and for the SNPs KDR rs2305948 (TT genotype, HR 4.57, p=0.041) and VEGFA rs1570360 (AA genotype, HR 1.77, p=0.025). Significant associations with shorter OS were found for the presence of metastases at diagnosis (HR 2.07, p=0.004), and for the SNPs KDR rs1870377 (AA genotype, HR 2.33, p=0.017), VEGFA rs1570360 (AA genotype, HR 2.01, p=0.026), whereas a T allele in SLCO1B3 rs4149117 was associated with longer OS (HR 0.475, p=0.017). A genetic profile was made, consisting of an unfavorable genotype in either KDR rs1870377, KDR rs2305948, or VEGFA rs1570360. Patients with the unfavorable profile had shorter PFS (p= 0.002) and OS (p=0.0003), as shown in multivariate analysis.

Conclusion In addition to clinicopathological factors such as mutational status and metastasis at diagnosis, SNPs in VEGFA, KDR and SLC01B3 appear to be associated with PFS and/or OS in imatinib-treated advanced GIST patients. If validated, these SNPs may serve as biomarkers to identify patients with an increased chance of progressive disease.

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DISTRIBUTION OF ALLELIC AND GENOTYPIC FREQUENCIES OF N-ACETYLTANSFERASE-2 (NAT2) AND CYTOCHROME P450 2E1 (CYP2E1) VARIANTS IN AN TUNISIAN TUBERCULOSIS POPULATION

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Introduction: N-Acetyltransferase-2 (NAT2) and cytochrome P450 2E1 (CYP2E1) are two key enzymes implicated in hepatic metabolism of isoniazid. To date, NAT2 acetyl profile is known to be strongly associated to Isoniazid Induced Hepatotoxicity (IIH). Regarding CYP2E1 enzyme, several studies suggest that CYP2E1 RsaI/PstI/DraI polymorphism could increase the risk of IIH. Objective: we aimed to identify polymorphisms in both NAT2 and CYP2E1 genes in a sample of Tunisian tuberculosis patients. Materials and method: Genotyping analysis for NAT2 and CYP2E1 was performed using polymerase chain reaction (PCR) with restriction fragment length polymorphism (RFLP). Three restriction enzymes, Rsal(-1053 C>T), PstI(-1293 G>C), and DraI (T 7632 A) were used to detect CYP2E1 polymorphism. NAT-2 polymorphism was performed using four different restriction enzymes BamH (G857A), KpnI(C481T), TaqI(G590A) and DdeI(A803G). NAT2 RFLP allowed the determination of three NAT2 slow allelic variant (NAT2*5,NAT2*6,NAT2*7) and two rapid NAT2 alleles (NAT2*4, NAT2*12). We considered RA, IA or SA acetyl phenotype if a patient exhibits a combination of two rapid alleles, a combination of a rapid and slow alleles or a combination of two slow ones, respectively. Results: 71 tuberculosis patients who received antituberculosis treatment were included in this study. The allelic frequencies of NAT2 variants *4,*5,*6,*7 and *12 were 16.1%, 44.3%, 30.2%, 3.5% and 5.6%, respectively. With regard to acetyl phenotype, our results show that 56.3% of the study population was SA, 40.8% was IA and 2.8% was RA. Genotyping of CYP2E1 gene revealed a frequency of 95% and 5% for Rsal(C1/C1) and Rsal(C1/C2) genotype, respectively. CYP2E1 Drai(D/D) and DraI(D/C) were as frequent as 71% and 29%, respectively. Regarding CYP2E1 PstI polymorphism, the frequency of wild homozygous (C1/C1) and heterozygous for the C1 allele were 97.2% and 2.8%, respectively. Conclusion: This study shows that more than 75% of the studied population are carriers of NAT2*5, NAT2*6 and NAT2*7 genotypes compatible with a SA status. Moreover, we have shown the predominance of the wild homozygous for the C1 allele of both CYP2E1 PstI and CYP2E1 Rsal, which have been associated with an increased risk of IIH in previous studies. Let's suppose that most Tunisian tuberculosis patients require low doses of isoniazid in order to prevent IIH.

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THE USEFULNESS OF TACROLIMUS TROUGH CONCENTRATION MONITORING IN RENAL TRANSPLANT RECIPIENTS

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Background: Tacrolimus is a potent immunosuppressive agent prescribed for the control of rejection in renal transplant patients. Because of the large between and within individual pharmacokinetic variability and the narrow therapeutic index of tacrolimus, its therapeutic drug monitoring (TDM) is mandatory. Trough concentration (C0) is presently a widely used parameter for TDM of tacrolimus. The aim of our study was to evaluate the usefulness of C0 in TDM of tacrolimus.
Methods:
We performed a retrospective study including Tunisian kidney recipients. Their tacrolimus blood samples were obtained between March 2009 and December 2014. All patients were treated with a combined immunosuppressive therapy based on tacrolimus, mycophenolic acid and prednisone. The initial tacrolimus dose was 0.15 mg/kg per day administered in two divided doses. Blood samples were performed just before the morning dose (C₀). Subsequent tacrolimus doses were adjusted to maintain the whole-blood concentration within the recommended target range, defined in the study protocol as 10-15 ng ml⁻¹ during the first 3 months after transplantation and 5-10 ng ml⁻¹ thereafter. The dosage of tacrolimus was performed by EMIT Technique with a “V-Twin Siemens” system.

Results:
One hundred and eighty six Tunisian patients (125 men and 61 women) undergoing renal transplantation were included in the study. Their mean age and weight were 32.1 ± 11 years and 61 ± 14 kg, respectively. The mean tacrolimus daily dose was 0.1 mg/kg.

A total of 1009 blood samples for the determination of tacrolimus C₀ were obtained during the period of the study. The mean number of samples per patient was 6, ranging from 1 to 18. C₀ was within therapeutic ranges in 439 (43%), under the lower limit in 229 (23%) and over the upper limit in 341 (34%). The mean C₀ was 10.6 ng ml⁻¹. The rate of C₀ within therapeutic ranges has increased from 34.4 % in the first sample to 60.7 % at the 9th sample and thereafter.

Conclusion:
We conclude that pharmacokinetic and pharmacogenetic parameters of tacrolimus should be considered to increase the probability of obtaining C₀ within therapeutic ranges.

FIRST REPORT OF A SUCCESSFUL PREGNANCY IN AN EVEROLIMUS TREATED HEART TRANSPLANTED PATIENT: NEONATAL DISAPPEARANCE OF IMMUNOSUPPRESSIVE DRUGS
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Purpose: The use of Everolimus (EVL) as primary immunosuppression is increasing in heart transplant (HTx) patients. Due to the unknown risk of fetal damage, its administration is contraindicated during pregnancy. No data are available regarding pregnancy outcome in HTx women treated with EVL and no information about EVL pharmacokinetics in newborns. We report a successful maternal and fetal outcome in a HTx patient administered EVL throughout pregnancy.

Methods: A 40 years old female underwent HTx in 2011. The patient was initially immunosuppressed with cyclosporine (CyA) and azathioprine. Due to a decreased renal function, EVL was begun three months after HTx and CyA dosages reduced. Two years after HTx, an unplanned pregnancy at 20 weeks gestation was discovered. Since pregnancy was advanced, it was decided to keep immunosuppression unchanged. Because of reduced renal function and cholestasis, a cesarean section was planned at 36 weeks gestation. A healthy male infant, 2745 grams, normal Apgar score was delivered. No congenital malformations were identified. CyA and EVL levels were measured in the mother’s blood, amniotic fluid, umbilical venous and arterial blood, colostrum and in the newborn.

Results: At birth, no differences were found between EVL concentrations in maternal venous blood and neonatal umbilical arterial or venous blood (1.4 ng/ml); whereas CyA was 31 in the mother and 12 in the umbilical blood. Amniotic fluid concentration was undetectable for both drugs. Two days later, colostrum and mother concentrations were: CyA 16 and 32 ng/ml, EVL at the lower limit of detection and 1.8 ng/ml. The neonate’s CyA was at the lower limit and EVL was 2.2 ng/ml. In the newborn, CyA blood levels became undetectable at 48 hours, whereas EVL persisted for more than 5 days. Finally, cord blood displayed a normal count of B and T cells, as well as CD4, CD8 and NK population.

Conclusion: At birth, the mother and the neonate showed the same blood levels of EVL, suggesting no drug placental filter and a similar exposure to EVL throughout pregnancy. This unique experience suggests that newborns may not be able to eliminate EVL for a considerable period, whereas CyA disappeared earlier.

RISK FACTORS OF ISONIAZID-INDUCED HEPATOTOXICITY IN TUNISIAN TUBERCULOSIS PATIENTS
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Introduction: Previous studies have shown controversial results on whether acetylator status causes isoniazid-induced hepatotoxicity (IIH). Moreover, the contribution of CYP2E1, a hepatic enzyme implicated in the formation of hepatotoxins, to the risk of developing IIH remains unclear. The objectives of this study was 1) to assess the quantitative relationship between the level of isoniazid serum concentration and the incidence of IIH and 2) to evaluate the extent of implication of the N-Acetyltransferase-2 (NAT2) and CYP2E1 polymorphisms genes to induce this disorder. Material and methods: 71 patients with tuberculosis receiving a conventional antituberculosis regimen were included. NAT2 and CYP2E1 genotypes were determined using polymerase chain reaction. Three restriction enzymes, RsaI, PstI, and DraI were used to detect CYP2E1 RFLP and four different restriction enzymes, KpnI, TaqI, and BamHI, Ddel were used to determine NAT2 acetylator status. Therapeutic drug monitoring (TDM) of isoniazid (Serum concentration performed 3 hours after morning dose: C3) was performed. Cases of isoniazid-induced hepatotoxicity were diagnosed according to Benichou et al. Receiver Operating Characteristics curve analysis was used to evaluate the relationship between risk factors and the incidence of IIH. Results: Eleven (15.4%) patients have developed IIH. Demographic factors, including age, weight, gender were not associated with the incidence of hepatotoxicity. High serum concentration of isoniazid (C3) was found to be a risk factor of IIH (AUC: 0.74, p: 0.007), with a cut-off value at 3.69 mg/l (OR: 13.2, p=0.0007). Multivariate analysis showed that only a C3 over 3.69 mg/l remains a risk factor of IIH. NAT2 and CYP2E1 variants were not found to increase the risk of IIH when analyzed separately. However, combined analysis of the NAT2/CYP2E1 gene polymorphisms showed that patients with both DraI C/D and slow acetylator have an increased risk of IIH compared with other combined NAT2/CYP2E1 genotype profiles (OR: 10.8, p=0.001). Conclusion: Our results suggest that a serum concentration of isoniazid over 3.69 mg/l and a combined genotype CYP2E1 DraI/C/D slow-acetylator are major risk factors for IIH. Therefore, TDM of isoniazid and the determination of both NAT2 and CYP2E1 genotypes could be useful for the prediction and prevention of IIH in Tunisian tuberculosis patients.

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WHAT IS YOUR POLICY IF PROFICIENCY TESTING IS NOT AVAILABLE IMMUNO-PHARMACOGENETIC TESTING - LYMPHOCYTE TOXICITY ASSAY

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Background: Cardiovascular diseases are the leading cause of death in the world. The pathophysiology of cardiovascular diseases is linked to atheromatous coronary plaque formation, inflammation and fibrogenesis. Deregulation of hemostasis leads to the formation of thrombi and blood vessels obstruction. However these therapeutics may produce adverse reactions (ADR). In order to prevent or treat ADR to platelet inhibitors we use an lymphocyte toxicity assay (LTA) which is not an available proficiency test.

Our objectives are to characterize the cellular and immunologic mechanism of platelet-inhibitor-induced ADRs and describe a immunogenetic assay for the laboratory confirmation of clinical diagnosis in affected individuals. We also aim to understand the immune and pathophysiological mechanism(s) of platelet inhibitors-ADRs.

Blood samples from 20 patients with a clinically confirmed diagnosis of anti-platelet-HSR and 6 tolerant controls were analyzed for toxicologic and an immunologic response after the HSR. LTA was performed after a minimum of 12 months post discontinuation. Pro-inflammatory cytokine and anti-inflammatory cytokine profiles and apoptotic and necrotic assays were performed in all patients. Immunologic analysis has been employed to establish the nature and mechanism of action that led to the pathological lesions.

Results: The mean percentage LTA values were significantly higher for ADR compared to control patients (20±6 vs. 7±4%, p=0.0004). Tumor necrosis factor-α levels were significantly higher for the HSR group compared to controls (116±49 vs. 29±9 pg/mL). ROC curves for LTA demonstrated excellent utility for the diagnosis of ADR (AUC=0.97, p=0.003). LTA >16% showed a sensitivity of 86% and a specificity of 100% for the diagnosis of HSR.

Conclusion: Anti-platelet-ADR is an immune-mediated phenomenon. This ADR may be diagnosed and prevented by the use of the laboratory immuno-pharmacocgenetic. The LTA provides in vitro cell assay that is useful for patient diagnosis and drug monitoring, leading to personalize treating of the patients, and preventing ADR thus improving human health.

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SAFETY AND PHARMACOKINETICS OF DAPTOMYCIN IN JAPANESE PATIENTS

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Background: Daptomycin is a new cyclic lipopeptide antibiotic that exhibits concentration-dependent bactericidal activity against Gram-positive pathogens including methicillin-resistant *Staphylococcus aureus*. It is predominantly eliminated through the kidneys, and dosage adjustments are recommended in patients with renal impairment. Monitoring creatine phosphokinase (CPK) is also recommended since the symptoms of muscle pain or weakness associated with CPK elevations have been reported as adverse drug reactions. The objective of this study was to evaluate the safety of daptomycin treatment in relation to the pharmacokinetics in Japanese patients.

Methods: Fifty patients (range, 19-88, median, 67 years old) were identified who received daptomycin for >4 days according to recommended dosage in our hospital. Laboratory data before and after the treatment were reviewed to evaluate changes of liver, kidney and muscle functions. Trough (C trough) and peak (Cpeak) concentrations of daptomycin were measured by HPLC-UV and individual clearances were calculated by the Sawchuk-Zaske method. Creatinine clearance was estimated using the Cockcroft-Gault formula.

Results: No irreversible serious adverse reaction occurred during the course of treatment (median duration 16 days, range, 5-71 days) in our study cohort while 4 out of 50 patients discontinued daptomycin treatment due to adverse events. One of 4 patients was due to moderate elevation in CPK which was restored to normal after discontinuation. Daptomycin concentrations were available in 27 patients and demonstrated large inter-individual variability and dose-dependent increase in C trough with 12.4±7.6, 14.0±6.2, and 22.1±12.9 µg/mL in patients on 4 (n=10), 6 (n=35), and >7 mg/kg (n=5), respectively. C trough values in 2 patients discontinued were available and were both within the therapeutic range. Three out of 27 patients had C trough higher than 24.3 µg/mL which is an indicative level reported to put patients at risk for CPK elevations. Covariate analysis for clearance revealed that individual clearance correlated with body temperature in addition to creatinine clearance in non-hemodialyzed patients over 55 years of age.

Conclusions: Our pilot study suggested that daptomycin is relatively safe. Since there is large inter-individual variability in PK, patients, especially complicated and/or on high-dose daptomycin, would benefit from therapeutic drug monitoring and following dose adjustment to assure the target range attainment.

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**POPULATION PHARMACOKINETIC ANALYSIS OF PHENYTOIN IN EGYPTIANS WITH EPILEPSY**

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Background: Phenytoin (PHT) is still used in Epilepsy clinic to control fits among Egyptians. The complicated pharmacokinetic (PK) behavior of the drug can affect its plasma concentration, therapeutic and/or toxic effects. Therefore, a population PK analysis (POP-PK) was performed to help dose optimization of PHT in epileptic patients maintained on the drug.

Methods: A group of Egyptian epileptic patients (n=121; 86 males & 35 females) treated with PHT either alone or combined with carbamazepine (CBZ) or Valproic acid (VPA) were included in the study. The mean age (yr) and weight (kg) were 33.4±16.5yr and 66.1±14.1 kg respectively. The drug was given orally with a mean dose of 300.24±104 mg/day (range: 50-750 mg/day). One single trough steady state (SS) concentration was available per patient with a mean value of 12.5±9.5 µg/mL (range: 1-52.04 µg/mL). Since PHT exhibits capacity limited (zero-order) elimination, its clearance was estimated in terms of Vmax (mg/kg/day) and Km (mg/L). Due to the nature of data, Km and volume of distribution (Vd) values could not be estimated separately, hence they were fixed to population values of 4 mg/L and 0.65 L/Kg respectively based on the published literature.

Results: The estimated overall population (n=121) mean value for Vmax was 7.22 ± 3.5 mg/kg/day with individual values ranging from 1.5 to 29.3 mg/kg/day. A subgroup analysis based on age showed Vmax mean values of 6.37 ± 2 mg/kg/day (range: 1.5 - 12.6 mg/kg/day) in adults (n=97) and 10.5 ± 5.7 mg/kg/day (range: 4.5 to 29.3 mg/kg/day) in children (n=24). The effect of covariates on the variability in Vmax couldn’t be estimated due to single concentration data per subject.

Conclusions: This is the first population PK analysis study in Egyptians with epilepsy to estimate PK parameters of phenytoin using single steady-state plasma concentration per subject. Body weight adjusted mean Vmax in children was found to be slightly higher than in adults. The variability in Vmax was also higher in children than in adults. The model could be further refined in future with more subjects per dose group and two or more concentrations per subject in each dose group.
EFFECT OF CYP3A4*22, CYP3A5*3 AND CYP3A COMBINED GENOTYPES ON ENDOXIFEN SERUM CONCENTRATIONS IN BREAST CANCER PATIENTS USING TAMOXIFEN

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Background: Tamoxifen is the cornerstone of long-term adjuvant therapy for breast cancer. It is metabolized to Endoxifen that is 30-100 times more potent. We previously reported that CYP2D6 genotype is significantly associated with Endoxifen serum concentration in the CYPTAM study. However, CYP2D6 genotype only partially explained the observed variability in Endoxifen concentration. Recently, the alleles CYP3A4*22 and CYP3A5*3 have been described as potential predictive markers for Endoxifen formation. The aim of this study is to investigate the effect of CYP3A4*22, CYP3A5*3 and CYP3A phenotype on Tamoxifen and Endoxifen metabolism in addition to CYP2D6.

Methods:
DNA and clinical data from 671 patients participating in the CYPTAM study [NTR1509], a large prospective study investigating the effect of CYP2D6 genotype on pharmacokinetics and clinical outcome in Tamoxifen treated breast cancer patients were available. Steady state Tamoxifen and Endoxifen trough concentrations were measured with LC-MS/MS. CYP2D6 genotyping was performed with the Amplichip. Genotyping for CYP3A4*22 was performed with TaqMan and CYP3A5*3 with Pyrosequencing. To study the combined effect of CYP3A4*22 and CYP3A5*3, genotype clusters were made: Slow metabolizers (C1), Intermediate metabolizers group 1 (C2), Intermediate metabolizers group 2 (C3) and Extensive metabolizers (C4).

Results:
The mean (SD, range) Endoxifen serum concentration was: 29.4 nM (15.8, 2.1-121.9 nM), Tamoxifen and Endoxifen serum concentrations were significantly higher in patients with the CYP3A4*22 allele with 371.1 nM (148.2, 48.2-805.1) vs 304.4 nM (111.6, 95.2-1141.6) and 32.4 nM (18.0, 4.5-121.3) vs 28.8 nM (15.3, 2.1-87.3) respectively (Tamoxifen, p=<0.05; Endoxifen, p=<0.05). CYP3A4*3 showed no significant association with Tamoxifen (R²=0.007, p=0.1) or Endoxifen (R²=0.42, p=<0.05) concentration. The combined effect of CYP3A genotypes were not associated with Tamoxifen (R²=0.015, p=0.011) or Endoxifen (R²=0.43, p=<0.05) metabolism in our cohort.

Conclusions: CYP3A4*22 but not CYP3A5*3 contributes to explain variance in Endoxifen serum concentrations in addition to CYP2D6 genotype.

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EVALUATION OF THE PHARMACOMETABOLOMIC APPROACH FOR DEVELOPING TDM

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Evaluation of the pharmacometabolomic approach for developing TDM

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Background
TDM is an important and established tool for drug dose individualization. Modern bioanalytical techniques offer new possibilities to gain more information from laboratory investigation than just a drug concentration. We aim to explore the use of high resolution mass spectrometry in combination with liquid chromatography for applying a metabolomic approach to develop TDM further.

Methods
Plasma samples were collected from 18 patients treated with risperidone or paliperidone. Control plasma samples were from 4 groups of healthy volunteers. Plasma (100 µl) was mixed with 300µl of methanol in a 96 well format and centrifuged. The supernatants (100 µl) were transferred to glass vials. Samples (5 µl) were analyzed on UPLC-Q-Exactive (ThermoFisher). The column used was a Hypersil Gold 100x2.1 mm, 1.9 µm particles. A gradient (2-99% MeOH, pH 4.8, ammonium formate, 10mM) was applied. Data acquisition time was 0.8-12 minutes. The Q-Exactive was set to positive ion electrospray mode and a scan range of 100-1000.

Results
Depending on the parameter settings in the SIEVE software about 700 features (unique combination of M/Z and Rt) were detected. Many of these could be tentatively assigned based on the exact mass and the ChemSpider data base. A discrepancy between the available recorded patient medication and the drug screening result was observed. In a number of cases patients had taken other therapeutic drugs than being prescribed. In one case
amphetamine was detected. Based on the quantification of risperidone metabolites patient could be categorized as fast or slow metabolizers. One previously reported metabolite of risperidone was not detected in any sample.

Conclusions
The Q-Exacte combined with UPLC is a powerful tool for untargeted screening of drugs and their metabolites as well as endogenous metabolites. The simultaneous profiling of drug, drug metabolites and endogenous metabolites is a valuable tool for discovery and monitoring of biomarkers and patient stratification.

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DETERMINATION OF TIBOLONE IN HUMAN PLASMA BY A VALIDATED LC-MS/MS METHOD
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Background: Development and validation of LC-MS/MS determination of tibolone (TIB) was performed with the aim to be applied in the course of a bioequivalence study. Methods: TIB and d2-TIB (Tibolone-13C2, d2, internal standard) were extracted from human plasma with tert-Butylmethyl ether and derivatized. Chromatographic separation was performed on C18 analytical column with mobile phase consisting of 65% aqueous methanol and 0.2% formic acid. Positive electrospray ionization and multiple reaction monitoring were used to follow the predominant transitions: collision energy 20, m/z 427→368 for TIB, and m/z 431→372 for d2-TIB. Raw data of mass chromatograms were collected and processed by specialized software, and weighted (1/X) linear regression was performed to determine the concentration of TIB. Validation strategy was strictly adhered to current industrial guidance. Results: Selectivity was assessed with 6 individual sources of human plasma and confirmed with matrix effect (ME) averaging 98-103% for TIB, 76-101% for d2-TIB, and relative ME of 101-102% for TIB. Accuracy ranged from -0.2 to 13.8% within runs and from -9.5 to 13.3% between runs. Precision was up to 6.1% within-runs, and up to 8.8% between-runs. Extraction recoveries averaged 62-82% for TIB and 74-97% for d2-TIB. Linearity was assured in the range 20.4 ÷ 10200.0 ng/L, R2>0.99. Freeze-thaw stability was determined for three cycles each lasting 24 h, post-preparative stability was documented for 24 h at 4°C, short-term stability at room temperature was proven for 2.5 h at daylight; stock solution stability and long term stability in plasma were documented for 141 days at -80°C. With run time of 3.5 min, a throughput of over 200 samples per working day was achieved. Conclusion: The method was validated according to current industrial requirements and allows the accurate and precise determination of Tibolone in human plasma.

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HPLC METHOD FOR VALSARTAN DETERMINATION IN HUMAN PLASMA
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A considerable percentage of ineffective treatment in resistant hypertension may be related to subtherapeutic dosage or non-compliance. The aim of the study was to develop a simple analytical method suitable for monitoring selected sartanes in patient plasma.

Valsartan (VAL) was determined using simple HPLC system consisted of an isocratic pump, an injector with 20 μL loop, a fluorimetric detector and an integrator. The separation was performed at ambient temperature on LC-CN column (150x4.6 mm, 5μm), using mobile phase: CH3CN : H2O : KH2PO4 : H3PO4 (717 : 280 : 3 : 0.4 v/v, pH 2.5), with a flow rate of 1.8 mL/min. Fluorescence detection was performed at 234 / 378 nm. The 200 μL aliquot of plasma after addition of losartan (LOS) as internal standard and 100 μL 0.5 M KH2PO4 was extracted with 4 mL of a mixture: methyl-t-butyl ether - dichloromethan (2:1). After centrifugation and freezing the organic layer was quantitatively transferred into a conical glass tube and evaporated at 37°C, then the dried extract was reconstituted in a mixture: mobile phase - methanol (4:1) and injected onto HPLC column. The validation tests including precision and accuracy were performed using seven levels of calibration standards as well as three levels of QC samples. No significant interference with biological matrix was observed in described chromatographic conditions. VAL, LOS and LOS carboxylic acid (EXP 3174) were eluted at retention times of 5.6, 7.3 and 4.6 min, respectively. The method was calibrated in a range of 10-5000 ng/mL. The precision was satisfactory in whole range tested with RSD of 0.99-6.15% for intra- and 0.91-6.60% for inter-assay, respectively. The intra-assay accuracy was between -1.02% and +2.05% and the inter-assay accuracy was between -12.01 and +4.37%. Simple extraction resulted in mean recoveries of 81.9% and 78.7% for VAL and LOS, respectively. The procedure may be easily adapted for determination of LOS and its metabolite EXP 3174 using VAL as internal standard. The method has been successfully applied for samples obtained from patients treated with sartanes.
Presented method may be recommended as an analytical tool improving the efficacy of pharmacotherapy in resistant hypertension.

DETERMINATION OF DAPSONE AND MONOACETYLDAPSONE USING HPLC/UV
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Determination of Dapsone and monoacetyldapsone using HPLC/UV

Background: Dapsone (DAP) is a synthetic sulfone antimicrobial drug used for a number of disorders as brown recluse spider bites, leprosy, vasculotitis, pneumocystis carinii, and acne vulgaris. Its use is limited by its adverse effects such as methemoglobinemia resulting in hypoxia which incidence is correlated to evaluated drug serum concentration. DAP is metabolised though acetylation to acetyldapsone (MAD). MAD demonstrates differences in tissue accumulation and protein binding than its parent compound and could reduce the occurrence of methemoglobinemia. In this study we investigated the relation of DAP and MAD plasma concentrations to MetHb in an overdose case of a patient receiving DAP for the treatment of acne vulgaris.

Methods: The determination of DAP and MAD was performed by HPLC-UV. The method was fully validated has been successful applied to the overdosed case study. The ratio of MAD to DAP has been used to determine acetylator phenotype which shows interindividual differences resulting in clinically important plasma concentration.

Results: The patient was a 19 year old women treated with DAP for acne vulgaris. In an attempt of self-medication the patient took an unknown amount of pills in a single dose. She was immediately admitted to the ED of the local hospital. Treatment consisted in methylene blue administration. Blood samples were taken over a 5 day period to monitor methemoglobinemia, DAP and MAD levels. Plasma concentration of DAP and MAD declined from 26.5 µg/ml to 2,5 µg/ml for DAP and 7,4 µg/ml to 0,6 µg/ml for MAD respectively within 5 days. Average MAD/DAP ratio was found to be 0,24 +/- 0.05 independent of plasma concentration. MetHb levels declined discontinuously possibly related to treatment occasions with antidote. Maximum MetHb % was 32,8% at admission to the ED, it was 0.5 % after 5 days. The patient was shown to be a slow acetylator.

Conclusions: The patient fully recovered after 5 days and was able to leave the hospital without sequelas.

WITHIN-INDIVIDUAL BIOLOGICAL VARIATION DATA FOR THERAPEUTIC DRUG MEASUREMENTS
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Background. The difference between sequential drug concentration measurements in a patient on a stable dose represents a combination of the variability in the absorption, distribution and metabolism of the drug on each measurement, in addition to any effect of disease on these processes. This variation is described as the total result variability (CVtot) which is made up of the within-individual (CVi) and the analytical imprecision (CVa). CVi is commonly used for endogenous substances at a tool for setting assay quality specifications and determining the significance of differences between serial results. A variation on this concept has been proposed for use with therapeutic drug measurements (1,2). In this paper I refine this process and apply the concept to drugs not previously assessed.

Methods. Pairs of sequential results from patients in our pathology database were collected. The distribution of the ratios of the results (2nd result/1st result) were analysed and the Gaussian distribution of best fit for the CVrr (CV result ratio) was determined by Bhattacharya analysis. CVtot=CVrr/1.414 and CVi=Ó(CVtot² - CVa²), Analysis was restricted to sample pairs where the first result was within the therapeutic interval as an indication that the dose of the drug was likely to remain unchanged. Identifying the Gaussian distribution excludes outliers and bases the results on the majority of the data. Measurements were performed using HPLC or LSMSMS.

Results. CVtot were calculated as follows: posaconazole(CVtot=24%), itraconazole(20%), voriconazole(33%), clozapine(15%), cyclosporine(15%), tacrolimus(16%), everolimus(17%) and mycophenolate (26%) using between 460 and 8700 pairs of results. For all drugs the CVa from internal QC met the minimal precision standard (CVa<0.75xCVi) or better with the exception of a high control for cyclosporine.

Conclusions: The method described allows estimation of CVtot and CVi for therapeutic drugs from data already
held in pathology databases. CVtot can be used to assess changes in results and the CVi was used to determine that analytical precision was likely to be acceptable.


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CD86-EXPRESSİON ON MONOCYTES AS A TOOL FOR THERAPEUTİC DRUG MONİTÖRING OF BELATACEPT

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The selective inhibitor of T cell co-stimulation, belatacept, blocks CD28-mediated T cell activation by binding CD86 on antigen presenting cells such as monocytes. In contrast to several other immunosuppressive drugs, for belatacept no therapeutic drug monitoring of serum levels is recommended, because of low inter-patient variability in pharmacokinetic parameters. We questioned whether the CD86 competition flow cytometry assay can be used as a tool for pharmacodynamic monitoring of belatacept. The hypothesis was that the degree of blockade of CD86 by belatacept and saturation levels might be associated with acute rejection after kidney transplantation. CD86-expression was assessed on monocytes of patients treated with belatacept or tacrolimus in the stable situation, during rejection and after conversion of belatacept to tacrolimus. Patients were randomized to the Less-Intensive regimen for belatacept or with tacrolimus.

Before transplantation, flow cytometric analysis of whole blood samples showed that CD86 was abundantly expressed on monocytes: median 2029 molecules/cell [1179-4102]. After one dose of belatacept the numbers of unbound CD86-molecules per monocyte dropped by >85% in all patients (n=17) to 265 molecules/cell [54-696 mol/cell], p=0.0003. Also in tacrolimus-treated patients (n=19) the expression levels of this co-stimulatory molecule decreased by 33% (p=0.003), but significantly less than in belatacept treated patients (p<0.0001). For the entire one year study period the numbers of unbound CD86-molecules per monocyte remained stable in patients without rejection treated with either belatacept or tacrolimus. Surprisingly, also during rejection the CD86-expression on monocytes was blocked by belatacept, 269 unbound molecules/cell [192-380]. After conversion to tacrolimus, after 5 months the expression of CD86-molecules returned to the same levels as those found in tacrolimus-treated patients.

In conclusion, the degree of belatacept-mediated blockade of peripheral CD86 expression is not different between rejecters and non-rejecters. Despite CD86-blockade above 85%, patients can still develop acute rejection. We hypothesize that rejection is the consequence of insufficient inhibition of the CD28-CD80/86 pathway at the tissue level (lymph nodes and kidney) or that alloreactivity is initiated through other co-stimulatory pathways. Flow cytometric assessment of free CD86 molecules on monocytes in peripheral blood does not seem to be a promising tool to monitor belatacept treatment.

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IMPDH-MONITORİNG IN RENAL TRANSPLANT PATİENTS IMPROVED USING EX-VİVO ACTİVATED PBMC

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Background: Several studies have investigated the potential for monitoring mycophenolic acid (MPA) by assessing the activity of its target enzyme, IMPDH, in peripheral blood mononuclear cells (PBMC). The present study explores the potential for improvement by measurement of IMPDH activity following ex-vivo activation.

Methods: Kidney recipients receiving tacrolimus, corticosteroids with (n=24) or without MPA (n=5) were included. At 6-9 days, 5-7 weeks and 1 year after transplantation blood samples were collected predose (t₀) and 1.5 hours after (t₁) administration of immunosuppressants. PBMCs were isolated and incubated for 72 hours with and without phorbol myristate acetate and ionomycin. IMPDH-capacity in PBMCs was measured by determining enzyme reaction rate using HPLC-MS/MS. MPA was measured in plasma using HPLC.
Results: Compared to non-activated, the IMPDH-capacity in ex vivo activated PBMCs was median 21 times higher before transplantation (median 5.7 pmol/10⁶ cells/min, range 2.2-21 vs. 134, range 21-208, respectively). In patients receiving MPA, IMPDH-capacity in ex vivo activated PBMCs after transplantation increased 10-12 fold predose and 3-5 fold at t1.5 compared to non-activated. In patients not receiving MPA the IMPDH-capacity in ex vivo activated cells was 17-25 fold increased compared to non-activated cells predose and 20-26 fold at t1.5. As a percentage of the IMPDH-capacity pre-transplant, the predose capacity was higher at one year than at 6-9 days posttransplant, as shown in Table 1. In the same interval predose plasma MPA increased 37% (median 2.0 µg/L, quartiles; 1.7-4.1 vs. 1.5-9.3 µg/L, 1.2-2.4, respectively). IMPDH-capacity in ex vivo activated PBMC correlated with plasma MPA (R² = 0.468, p<0.0001) while IMPDH-capacity in non-activated did not (p=0.296).

Conclusion: MPA reduced the IMPDH-activity more consistently in ex vivo activated than in non-activated PBMCs. There may be a potential for IMPDH activity measurement in ex vivo activated PBMCs as a pharmacodynamic marker of MPA in transplant recipients.

Picture 1: https://www.eventure-online.com/parthen-uploads/7/15017/img1_265645_oRLLmE9jS7.jpg
Caption 1: Table 1

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NEONATAL SEROTONIN SYNDROME AFTER IN UTERO EXPOSURE TO SERTRALINE AND PERPHENAZINE
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Introduction: Neonatal serotonin toxicity following in utero exposure to serotonin reuptake inhibitors (SSRI) as sertraline is a well-known and usually mildly severity consequence due to an hyperserotoninergic state. To our knowledge, this is the first case report of a neonatal SS after in utero exposure to sertraline and perphenazine, meeting all major diagnostic criteria.

Case report: A 32-year-old primigravida was admitted at 36 gestation weeks with complaint of decreased fetal movements of 2 days duration. The patient was in therapy with sertraline 75 mg and perphenazine 4 mg once daily since 25 weeks of gestation for major depressive syndrome. At 4 hours after birth, generalized hypertonia and hyperthermia (temperature greater than 38.5 degrees C) resistant to paracetamol treatment ensued and the patient was intubated. Diazepam 0.4 mg/kg three times daily and cyproheptadine 0.25 mg/kg once daily were started for possible serotonin syndrome. Sertraline serum levels at 72 h of age were 39 ng/ml while perphenazine was absent in all samples. Four days later, the patient was extubated and underwent to continuous positive airway pressure ventilation; the infant was awake but hyporeactive, hugging and holding reflexes were incomplete and sucking and swallowing reflexes were all absent. A nuclear magnetic resonance showed antepartum cortical hypoxia/ischemic brain injury. Two months later the patient was still neurologically compromised with an high suspicion of permanent damage.

Discussion:
Neonatal toxicity after in utero exposure has been previously reported and it can be confirmed by high drug serum levels at birth, although into a normal adult range. To the best of our knowledge, SSRI treatment has never been related to hypoxic-ischemic encephalopathy (HIE) as well as HIE has never been associated to SS. The unusual severity observed in our case may rely on to different, coexisting aggravating factors: since the disturbance in the balance between dopaminergic and serotonergic systems leads to or may worsen serotonin syndrome, it is possible that the concomitant in utero exposure to perphenazine (acting on dopaminergic receptors) and sertraline (acting on serotoninergic system) led a more vulnerable (because of HIE) fetus to develop a serotonin syndrome of such severity.

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A NOVEL UPLC-MS/MS METHOD FOR CLINICAL RESEARCH OF METHOTREXATE PHARMACOKINETICS
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Background
Dosing with glucarpidase promotes extra-renal clearance of methotrexate. Understanding this alternative route of
elimination is confounded by over-estimation of methotrexate by immunoassay. Glucarpidase converts methotrexate to 4-deoxy-4-amino-N10-methylpteroyl acid (DAMPA), an immunoassay interference. An analytically sensitive UPLC-MS/MS method for clinical research of methotrexate pharmacokinetics using the Waters ACQUTY UPLC® I-Class and XEVO® TQD mass spectrometer has been developed. Applying the method to samples post-glucarpidase administration shows a methotrexate 'rebound' phenomenon, not detected by immunoassay.

Methods
Samples (50µL) were deproteinized with methotrexate-D3 internal standard in methanol. Isocratic separation was achieved within 5 minutes using a HSS-SB C18 UPLC column (2.1x30mm, 1.8µm). Matrix-matched calibrators (0.025-10µmol/L) and quality control samples were prepared by gravimetric weighings of independent stocks of methotrexate.

Results
Analytical sensitivity was calculated to be 0.0025µmol/L (n=10 extractions, 3 occasions, <17.7% CV). Linearity was confirmed by the absence of non-linear terms (p<0.01) between 0.0175-13µmol/L (CLSI EP6-A). Extended LC column-washing eliminated carryover in samples <100µmol/L. Recovery of 0.1 and 1.0µmol/L methotrexate (n=3) from pooled plasma was unaffected (mean 98.6%, range 90.4-102.9%) by co-spiking with high concentrations of endogenous compounds, implying measurement would not be affected by icterus or hyperlipidaemia (CLSI EP7-A2). Similarly, recovery was unaffected (mean 101.4, range 98.8-103.3%) when methotrexate pools were supplemented with 5 and 50µmol/L 7-OH methotrexate (n=3) and DAMPA (n=3), showing absence of interference from these metabolites (CLSI EP7-A2). Negligible qualitative and quantitative matrix effects were mitigated by use of deuterated internal standard. Methotrexate measurements were made on a plasma series 27 hours pre- to 147 hours post-glucarpidase administration. Methotrexate measurements by immunoassay differed to UPLC-MS/MS values by 36-99% <45 hours post-glucarpidase; the magnitude of discordance proportional to DAMPA chromatogram peak area (correlation coefficient = -0.87, n=16, one subject). Between 34-57 hours post-glucarpidase, the UPLC-MS/MS method detected an increase in methotrexate concentration which was not manifest by immunoassay. This increase may represent redistribution of methotrexate from tissue polyglutamates into plasma, an effect previously described in the literature.

Conclusions
The developed method provides analytical selectivity for clinical research of methotrexate clearance following glucarpidase administration. For Research Use Only, not for use in diagnostic procedures.

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TDM-GUIDED MANAGEMENT IN PATIENTS WITH DAPTOMYCIN
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【Aim】Daptomycin (DAP) is a recently developed cyclic lipopeptide antibiotic against most gram-positive pathogens including methicillin-resistant staphylococcus aureus (MRSA). Approved dosage of DAP is 4-6 mg/kg once daily, and some reports recommend higher doses (>8mg/kg/day) in patients with severe infection because of better clinical effects, however, therapeutic drug monitoring (TDM) required for vancomycin is not recommended for DAP. The purpose of this clinical investigation is to conduct TDM-guided management in patients DAP to avoid adverse effects.

【Method】We evaluated the plasma concentrations (Cmin and Cmax) and monitored adverse effects of DAP. Pharmacokinetics/pharmacodynamics parameters were referenced from current literature (CPK elevation risk: Cmin≧24.3mg/L, worse clinical outcome: AUC0-24 / MIC (minimum inhibitory concentration) <666). Pharmacokinetics data in our patients were calculated and Monte Carlo simulation was used to estimate the probabilities of achieving Cmin≧24.3 mg/L, and AUC0-24/MIC≦666. MIC was referenced European Committee on Antimicrobial Susceptibility Testing (EUCAST) rationale document and was chosen 0.5 mg/L, 1 mg/L, and 2 mg/L respectively. DAP concentration was measured using a HPLC technique.

【Results】Eighteen patients were enrolled in this study. CPK elevation was observed in 3 patients with high Cmin concentration, and other adverse effects were not associated with DAP concentration. The probability of achieving Cmin≧24.3 mg/L was increased with higher doses (more than 10%). While the probability of achieving Cmin≧24.3 mg/L was less than 5% in standard doses, the probability of achieving AUC0-24/MIC>666 in case of MIC=1 was less than 40%.

【Discussion】In consistent with previous reports, DAP Cmin was associated with CPK elevation in our study. Standard DAP dose could be inadequate to achieve AUC0-24/MIC>666 in the case of MIC more than 1 mg/L and
higher doses (more than 8mg/kg) would be needed to achieve AUC_{0-24}/MIC>666. However, higher doses of DAP increase the risk of CPK elevation, so TDM should be performed for the patients with more than 8mg/kg of DAP doses.

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DETERMINATION OF PLASMA CONCENTRATION OF DABIGATRAN BY LIQUID CHROMATOGRAPHY COUPLED WITH TIME OF FLIGHT TYPE SINGLE MASS SPECTROMETRY

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[Background] Dabigatran (DAB), an oral direct thrombin inhibitor, has widely used for anticoagulation therapy in the patients with nonvalvular atrial fibrillation (NVAF). Measurement of the activated partial thromboplastin time (aPTT) has been considered to be useful in managing DAB safety, however aPTT could not predict the effects of DAB sufficiently in some patients. Thus, development of more useful predictive marker for DAB effects is required. In this study, we developed a quantification method for plasma concentration of DAB (PCD) as a predictive factor for DAB efficacy, and applied to clinical samples.

[Methods] The system was equipped with ACQUITY UPLC and LCT premier XE TOF-MS system (Waters). An ODS column and gradient elution with 10 mM ammonium bicarbonate and acetonitrile was used. SPE using ABN column (Biotage) was performed to treat plasma samples. Clinical samples were obtained from 98 NVAF patients, and PCD at trough and peak (90 min after administration) were analyzed.

[Results] The lower limit of quantification was 5 ng/mL and good linearity were obtained over the range of 5-500 ng/mL. Accuracy and precision, extraction ratio was 85.6% 14.6% and 12.5%, respectively. The PCDs at trough and peak with patients receiving 220 mg/day DAB were 90.0 ± 46.4 and 124.3 ± 82.5 ng/mL, and that with 300 mg/day of DAB was 107.6 ± 53.6 and 129.5 ± 100.3 ng/mL, respectively. The correlation between PCD and aPTT were very weak (trough; R² = 0.2019, peak; R² = 0.1042).

[Conclusion] We developed highly-sensitive quantification method for DAB using LC/TOF-MS, and confirmed that aPTT cannot reflect PCD sufficiently. The assessment of correlation between PCD and the efficacy or ADR of DAB are now on going. To make more useful predictive marker for DAB, the reason of discrepancy between aPTT and PCD also should be studied well in next study.

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THE ROLE OF CLINICAL PHARMACOLOGICAL ADVICES BASED ON THERAPEUTIC DRUG MONITORING IN TAILORING ANTIMICROBIAL THERAPY IN CRITICALLY ILL CHILDREN

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Background. Pharmacokinetics of drugs in children is different from that observed in adults as a consequence of physiological differences. Attainment of therapeutic concentrations with most antimicrobials is frequently challenging for clinicians, especially in critically ill children. Therapeutic drug monitoring (TDM) is gaining relevance in personalizing antimicrobial therapy. To maximize the usefulness of TDM, results have to be interpreted in light of both the patient pathophysiological characteristics (i.e. clearance variability, site of infection, drug interactions) and the microbiological susceptibility of the clinical isolates. Clinical pharmacological advices (CPA) should be provided suggesting how to optimize dose adjustments.

Methods. The role of CPA based on TDM results in tailoring therapies in critically ill children with meropenem, linezolid and voriconazole was retrospectively assessed. Forty-seven pediatric patients who underwent real-time TDM during treatment with meropenem (n=16 patients; male/female: 5/11; mean age: 10.7±5.0 yrs; mean weight: 37.7±21.6 kg), linezolid (n=16 patients; male/female: 11/5; mean age: 9.6±5.4 yrs; mean weight: 37.8±24.2 kg) and voriconazole (n=15 patients; male/female: 6/9; mean age: 10.2±4.8 yrs; mean weight: 36.6±17.8 kg) were involved.

Results. At first TDM 68.7%, 37.5% and 66.6% of patients treated with meropenem, linezolid and voriconazole, respectively, had sub-therapeutic concentrations. Median (IQ range) of trough levels (C_{trough}) were 3.93 [2.5-13.5]
mg/L for meropenem (desired range: 8-12 mg/L), 3.29 [0.83-5.89] mg/L for linezolid (desired range: 2-7 mg/L) and 0.74 [0.37-1.48] mg/L for voriconazole (desired range: 1-5.5 mg/L) respectively. At start of treatment, dosing regimens were 126.7 [78.6-161.9] mg/kg/daily for meropenem, 25.63 [20.4-30.0] mg/kg/daily for linezolid and 8.9 [7.9-10.1] mg/kg/daily for voriconazole. The median (IQ range) of CPAs per patient was 1 [1-2] for meropenem, 2 [1-2] for linezolid, and 2 [1-4.5] for voriconazole. After dose adjustments, median Cmin improved at 9.34 [6.2-13.4] mg/L for meropenem, 3.7 [2.0-4.9] mg/L for linezolid and 1.99 [1.2-2.2] mg/L for voriconazole.

Conclusions. CPA based on TDM results may allow a real-time personalization of antimicrobial drug exposure in pediatric critically ill patients and can fill the gap between the paucity of pharmacokinetic studies devoted to identify the most appropriate antibiotic dosages and the needs of clinical practice.

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POLYMORPHISMS IN MIR27A ASSOCIATED WITH EARLY-ONSET TOXICITY IN FLUOROPYRIMIDINE-BASED CHEMOTHERAPY

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Background: The microRNA miR-27a has previously been implicated in chemotherapy resistance and was recently shown to directly regulate dihydropyrimidine dehydrogenase (DPD), the key enzyme in fluoropyrimidine catabolism. A common polymorphism (rs895819A>G) in the miR-27a genomic region (MIR27A) was associated with reduced DPD activity in healthy volunteers, but the clinical relevance of this effect has not been investigated. Here, we assessed the association of MIR27A germline variants with early-onset fluoropyrimidine toxicity in cancer patients.

Methods: The complete MIR27A region was sequenced in 514 cancer patients receiving fluoropyrimidine-based chemotherapy. Associations of MIR27A polymorphisms with fluoropyrimidine toxicity in the first two chemotherapy cycles were assessed in the context of known risk variants in the DPD gene (DPYD) and other covariates associated with toxicity.

Results: Overall, rs895819 was associated with a reduced risk of early-onset fluoropyrimidine toxicity (p=0.042). However, this association was strongly dependent on DPYD risk variant carrier status (interaction p=0.0025). In patients carrying DPYD risk variants, rs895819G was associated with a strongly increased toxicity risk (OR: 7.6; 95% CI: 1.7-34.7; p=0.0085) with 71% (12 of 17) of patients who carried both rs895819G and a DPYD risk variant experiencing severe toxicity. Conversely, an opposite effect of rs895819G was observed in patients without DPYD risk variants (OR: 0.62; 95% CI: 0.43-0.9; p=0.012).

Conclusions: These results indicate a clinically relevant role of miR-27a for further fluoropyrimidine toxicity risk stratification in carriers of DPYD risk variants. The non-additive effect between rs895819 and DPYD risk variants suggests that direct suppression of DPD by miR-27a may predominate in DPYD risk variant carriers with reduced DPD activity. In patients with normal DPD activity, on the other hand, this effect may be outweighed by miR-27a regulation of additional targets involved in drug transport or apoptosis regulation, explaining the negative association with fluoropyrimidine toxicity.

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OPTIMIZING BUSULFAN EXPOSURE IN PEDIATRIC HEMATOPOIETIC CELL TRANSPLANTATION USING A WEIGHT-BASED DOSSING NOMOGRAM AND THERAPEUTIC DRUG MONITORING

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Busulfan (Bu) is an alkylating drug used in conditioning regimens for allogeneic hematopoietic cell transplantation (allo-HCT). Its narrow therapeutic range in combination with large inter-individual variability in exposure, even after intravenous administration, necessitates dose individualization. Recently a weight-based dosing nomogram based on a pharmacokinetic (PK) model was developed and introduced at our pediatric HCT unit that is expected to result in more predictive Bu exposure with the aim of improving allo-HCT outcomes. In this study we prospectively assessed target attainment of Bu exposure using the weight-based dosing nomogram and therapeutic drug monitoring (TDM).

All pediatric patients who underwent allo-HCT in 2011 or 2012 receiving Bu-based conditioning were included. Bu was administered once daily in a 3-hour infusion on four consecutive days. TDM based dose correction was
standard protocol. Drug levels were measured on day 1 and 4. Bu exposure was expressed as cumulative area-under-the-concentration-time-curve (cAUC) and estimated using a PK model and bayesian-based PK software (MwPharm 3.60). Bu target exposure was 80-100 mg*h/L. For each patient a ‘hypothetical’ cAUC without TDM was determined by extrapolating the AUC day 1. Secondly the ‘true’ cAUC was estimated based on PK data obtained on day 1-4 including TDM-based dose adjustment. Means and ranges were compared between cAUCs determined with and without TDM-based dose corrections. Also target attainment rates (cAUC 80-100 mg*h/L) were compared between ‘hypothetical’ nomogram-based dosing simulation and the ‘true’ nomogram with TDM-based dosing situation.

Fifty patients were included with a mean age of 9 years (3 months-18 years). Without TDM mean cAUC was 85.3 mg*h/L versus 96.2 mg*h/L with TDM. The range in individual cAUCs was significantly larger without TDM (45-156 mg*h/L) than with TDM (74-117 mg*h/L) (p=0.001). Without TDM 34% of patients reached target cAUC 80-100 mg*h/L and with TDM this significantly increased to 70% of patients (p=0.001). The weight-based dosing nomogram overall led to a mean busulfan exposure within the target range 80-100 mg*h/L in pediatric patients, yet the inter-individual range was substantial. Therefore, TDM of intravenous busulfan remains recommended and is of utmost importance to reach optimal target exposure in pediatric patients in order to optimize HCT outcomes.

Picture 1: https://www.eventure-online.com/parthen-uploads/7/15017/img1_265660_nQz9uopmNg.jpg
Caption 1: Figure 1: busulfan exposure (cAUC in mg*h/L). The lines connect cAUC without and with TDM for each individual patient.

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CLOSER TO THE SITE OF ACTION: EVEROLIMUS CONCENTRATIONS IN PERIPHERAL BLOOD MONONUCLEAR CELLS CORRELATE WELL WITH WHOLE BLOOD CONCENTRATIONS USING A NONPARAMETRIC POPULATION MODEL

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Background: Everolimus is an immunosuppressive drug dosed according to therapeutic drug monitoring in renal transplant recipients. The primary site of action is within activated lymphocytes. Everolimus is a substrate of the efflux transporter ABCB1 also known as P-glycoprotein. Limited data exists regarding a possible association between whole blood and intralymphocyte concentrations of everolimus and the potential influence of ABCB1.

Methods: A nonparametric population model was developed for everolimus in renal transplant recipients. Twelve renal transplant recipients (5 men, 7 female) treated with everolimus underwent two pharmacokinetic investigations where everolimus concentrations in whole blood and in peripheral blood mononuclear cells (PBMC) were determined within a dosing interval. In addition, the activity of ABCB1 in PBMC was determined using the Rhodamine123 efflux assay and the patients’ genotypes of ABCB1 were determined.

Results: There was a significant correlation between everolimus AUC0-12 in whole blood and in PBMC (r = 0.90, P<0.01) and no association was demonstrated between the ABCB1 activity and everolimus PBMC/whole blood ratio of trough concentrations (r = 0.23, P=0.76). Furthermore, ABCB1 1236C>T, 3435C>T, 2677G>T/A polymorphism did not influence everolimus AUC0-12 PBMC/whole blood ratio.

Conclusions: The results revealed a significant association between everolimus whole blood and PBMC concentrations, suggesting ABCB1 mediated efflux from PBMC to be of minor importance for the distribution of everolimus.

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TACROLIMUS PHARMACODYNAMICS ALONG THE CALCINEURIN PATHWAY IN LIVER TRANSPLANT PATIENTS

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BACKGROUND: We investigated the pharmacodynamic behavior of tacrolimus along the calcineurin pathway in liver transplant recipients (LTR).

METHODS: 66 LTR grafted for 60±62 months and receiving tacrolimus were enrolled. PBMC were isolated from pre-dose whole blood. Inhibition of NFAT1 translocation to the nucleus of PBMC, intracellular expression of IL-2 in CD4+ and CD8+ T cells, and expression of the surface activation marker CD25 on CD3+, CD4+ and CD8+ cells were measured by flow cytometry before (NS) and after stimulation (stim) ex vivo.

RESULTS: Table 1 shows expression and inter-individual coefficients of variation of the PD biomarkers (in %) in NS and stim conditions. All selected markers except IL-2 in CD4+ T cells could be measured in NS conditions. None of the PD biomarker was influenced by TAC dose or trough level. IL-2 in CD4+stim was significantly influenced by patient age (p=0.003). IL-2<sup>+</sup>CD8+ and CD25<sup>+</sup>CD8<sup>+</sup>NS revealed the highest CV%, probably due to levels close to or below the limit of detection of the technique.

Table 1: Pharmacodynamic biomarkers along the calcineurin pathway (expression in %)

CONCLUSIONS: For the first time, the whole calcineurin signaling pathway was assessed in LTR on tacrolimus and that the corresponding pharmacodynamic biomarkers were measured in non-stimulated conditions, meaning that a "physiological reading" is possible without the introduction of mitogenic or polyclonal stimulant. These biomarkers were not influenced by TAC dose or trough blood levels and displayed a very large inter-individual variability in both NS and stim conditions. Whether individual values are predictive of drug responses is the object of an ongoing study.

Picture 1: https://www.eventure-online.com/parthen-uploads/7/15017/img1_265665_J68DakNcfk.jpg

IS CEFAZOLIN 30 MG/KG INSUFFICIENT FOR PERIOPERATIVE PROPHYLAXIS FOR NEONATES AND INFANTS UNDERGOING CARDIAC SURGERY?

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Background: Cefazolin (CFZ) is a first-generation intravenous cefalosporin used as perioperative prophylaxis against predominantly gram-positive skin flora. Recent data have suggested that conventional dosing (30 mg/kg/dose) may be insufficient and some have increased to 50 mg/kg/dose in adult patient populations. We investigated if the conventional CFZ dose regimen resulted in inadequate serum levels in neonates and infants.

Methods: Five neonates ≤30 days of age and five infants >30 days of age undergoing cardiac surgery received perioperative prophylaxis with CFZ 30mg/kg q8h for 48 hours. Subjects received intra-operative redosing for surgical duration > 4 hours. Blood samples were drawn 0.25, 2 and 3 hours after completion of the pre-operative dose and immediately prior to the next dose. Samples were also collected using the same schedule for the intra-operative dose (when applicable) and the final dose. Total CFZ serum concentration was determined by LC-MSMS. Data were analyzed with both compartmental and non-compartmental techniques.

Results: No subjects developed a surgical site infection. Samples were collected after the first dose in 10 and after the last dose in 6 patients; samples were also collected in 5 subjects requiring intra-operative doses. The data were well described by a 2-compartment pharmacokinetic model: $V_1=0.17±0.1L/kg$; $\kappa_1=0.74±0.2h^{-1}$; $k_{21}=4.2±2.0h^{-1}$, $k_{32}=2.5±1.0h^{-1}$. The level of Cmin remained higher than 16 mg/L in most subjects, but 4 had a lower Cmin.

Conclusions: Our data suggest adequate CFZ levels in most subjects treated with 30 mg/kg q8h. However, 30% of patients had CFZ levels <16 mg/L suggesting the potential for subtherapeutic levels for Staphylococcus aureus (susceptible breakpoint <4 mg/L).

PHARMACOKINETICS OF MORPHINE AND THE PREDICTABILITY OF METABOLITE CLEARANCE THROUGH CREATININE AND ALBUMIN LEVELS IN TERMINALLY ILL PATIENTS.

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Background: Despite its wide-spread use, little is known about the pharmacokinetics of morphine in terminally ill patients. Having clinically relevant covariates for individualised dosing would be helpful since dosing can be challenging in this population due to severe co-morbidity, including hepatic and renal impairment. We therefore performed a pharmacokinetic study on morphine, morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) in terminally ill patients.

Methods: A population pharmacokinetic analysis was conducted with 199 samples obtained from 47 terminally ill patients, using non-linear mixed effects modelling (NONMEM 7.2). The covariates analysed were blood chemistry levels (e.g. albumin and creatinine), patient characteristics (e.g. age, diagnosis) and time to death.

Results: The data were best described by a two-compartment model for morphine with two one-compartment models for M3G and M6G and proportional residual error models. Between-subject variability (BSV) was shown for the bioavailability of morphine, morphine clearance, metabolite clearance and the volumes of distribution of the metabolites. The population mean estimates for morphine, M3G and M6G clearance were 49.4 L/h (BSV 53%), 1.56 L/h (BSV 31%) and 1.94 L/h (BSV 31%) respectively. Serum creatinine was negatively correlated with metabolite clearance and serum albumin was positively correlated, together explaining 78% of the between-subject variability in metabolite clearance. Morphine clearance decreased when time to death decreased. This was a first order process with a decrease in morphine clearance of 18L/h in the last three weeks before death (rate constant 0.118 day⁻¹).

Conclusion: The population pharmacokinetics of morphine, M3G and M6G in terminally ill patients were accurately quantified. Serum creatinine and albumin levels together were a better predictor of metabolite clearance than creatinine alone. This may be caused by the presence of cachexia and loss of muscle mass in terminally ill patients, in whom serum creatinine may overestimate renal function. Lower albumin levels may be an indicator of cachexia, and for overestimation of GFR based on creatinine levels. Our results show that morphine clearance declines as death approached and that M3G and M6G can accumulate in patients with decreased renal function. Dose adjustment might therefore be required in these patients, however the clinical effect of this requires further study.

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C-REACTIVE PROTEIN AND SERUM CONCENTRATIONS OF THE ANTOPSYCHOTIC DRUGS CLOZAPINE, QUETIAPINE AND RISPERIDONE - IS THERE A RELATIONSHIP?
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Background: Inflammation may increase drug concentrations in blood. In psychiatric clinical practice, there is a need to identify psychotropic drugs whose metabolisms are prone to be altered with increased inflammatory activity in an individual patient. The aim of this study was to find out whether elevated serum levels (≥5mg/l) of C-reactive protein (CRP), an established laboratory marker of infection and inflammation, are associated with increased serum concentrations of the atypical antipsychotic drugs clozapine (CLZ), quetiapine (QUE) and risperidone (RIS).

Methods: 319 therapeutic drug monitoring (TDM) request forms of 106 patients whose antipsychotic drug concentrations had been measured under conditions of normal (<5mg/l) and pathological (>5mg/l) levels of CRP were retrospectively screened. The serum concentrations in relation to the daily doses [concentration per dose (C/D) (ng/mL/mg)] and the metabolic ratios [ratio of concentrations (metabolite/drug)] were compared intraindividually by the Wilcoxon signed-rank test. To evaluate the threshold CRP value above which patients are expected to obtain a 100% increase in drug serum level, a receiver operator characteristic (ROC) curve was constructed.

Results: Elevated levels of CRP significantly were associated with elevated values (P<0.01) in C/D for CLZ (n=116), QUE (n=102), and RIS (n=101). Median increases were 31.1% (CLZ), 11.9% (QUE) and 26.1% (RIS-OH-RIS), respectively.

Conclusions: In patients who exhibit signs of inflammation or infection with increased CRP values during psychopharmacological treatment with any of the three studied drugs, TDM is recommendable in order to minimize the risk of intoxications due to elevated drug concentrations.

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PREDICTION OF ACUTE REJECTION AND INFECTIONS AFTER ADULT LIVER TRANSPLANTATION:
The recommended immunosuppressant drugs (ISD) dosing targets in most of clinical centers after liver transplantation were originally assessed in clinical trials that replicated the doses employed in kidney transplantation, where overall immunosuppression requirements are higher than in liver. The optimal target exposure of most use IS drugs such as calcineurin inhibitors (CNI) and mycophenolic acid (MPA) have therefore not be rigourously characterised for liver transplantation. Pharmacokinetics (PK) of IS including CNI and mycophenolate mofetil (MMF), the most used ISD, have been extensively been described in solid organ transplantation over the past decades, mostly using a data-driven approach. In parallel, several studies have focused on the evaluation of the ability of biomarkers to early predict the risk of rejection and infections. In the great majority of these studies, the analyses were also performed using a data-driven approach.

Pharmacokinetic/pharmacodynamic (PK/PD) modelling and simulations (M&S) methods are very powerful to quantitatively describe the time course of biomarker levels given the disease progression and the drug effects. The value of PK/PD modelling to assess clinical outcomes in acute rejection risk after liver transplantation (LT) has not been explored so far.

The present study is a pilot and a feasibility study aiming to propose a library of PK/PD models that can serve as aids to evaluate the prognostic and predictive performances of different previously described biomarkers of acute rejection and/or infections after adult liver transplantation. The PK/PD models have been developed using a mechanism-based approach that have allowed determination of the optimal levels (and dose requirement) for each of IS when used either as monotherapy or in combination with other drugs given drug-drug interactions.

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THERAPEUTIC DRUG MONITORING OF ACETAMINOPHEN AND ITS GLUCURONIDE IN ACETAMINOPHEN INDUCED LIVER INJURY

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Background: Acetaminophen (APAP) is primarily conjugated by glucuronidation (UGT1A1, 1A6) and partially (10%) metabolized by cytochrome P450 (CYP) 2E1. In the toxic doses, conjugative pathways are overwhelmed and increased metabolism by CYP 2E1 results in significant formation of N-acetyl-p-benzoquinoneimine (NAPQI). The aim of the study was to investigate the relationship between the plasma concentration ratio of APAP glucuronide conjugate (AP-gluc)/APAP and the severity of APAP induced liver injury.

Methods: In vivo study: Using Wistar/ST rats (5 or 10 weeks) with APAP treatment (900 mg/kg, po), plasma concentration of APAP and AP-gluc were determined using HPLC. In vitro study: Human hepatocytes FLC-4 cells (2.5×10⁶/mL) were placed with siRNA of UGT1A1 or 1A6 for 72hr. The cells were mixed with growth factor-reduced Matrigel in equal amount and placed on a 96-well multplate with APAP (1mM) for 1 week. APAP and AP-gluc concentration in the medium was measured using HPLC and the cell viability was measured using WST-8 reagent.

Results: In vivo study: The toxic reaction with centrilobular hepatocyte necrosis was only shown in 10weeks APAP-treated rats. The plasma AP-gluc/APAP ratio of 5 weeks rats was higher than that of 10 weeks. In vitro study: Cellular viability was significantly decreased treated with APAP or APAP and UGT1A1 siRNA, compared to control group. The viability more decreased treated with APAP and UGT1A6 siRNA. In CYP2E1 siRNA treatment, there was no difference of cellular viability, compared with control group. The medium AP-gluc/APAP ratio of APAP and siRNA of UGT1A6 treatment was also significantly decreased compared with that of APAP treatment and APAP and siRNA of UGT1A1 treatment.

Conclusions: The pathway of APAP glucuronidation is associated with UGT1A6 rather than UGT1A1. Since companion diagnosis for APAP induced liver injury by Invader® UGT1A1 Molecular Assay cannot be performed, the monitoring of plasma AP-gluc/APAP concentration ratio makes the prediction of APAP induced liver injury possible.

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ATAZANAVIR AND CRISTALLURIA: ALL PATIENTS ARE CONCERNED.
Background: Recent studies have found that exposure to the protease inhibitor atazanavir (ATZ) is associated with an increased incidence of renal stones, which may be enhanced by treatment duration and high concentrations.

Objective: Our objective was to explore ATZ crystalluria in our HIV population and compare patients with a once-daily dose of 300mg ATZ boosted with 100mg of ritonavir (ATZ300/r) and unboosted ATZ 400mg daily (ATZ400).

Method: Blood and urine samples were collected simultaneously during a routine follow-up evaluation of HIV infection from 50 patients. Blood samples were used to measure ATZ concentrations, renal, hepatic, lipidic and ionic parameters. Urine samples were used for ATZ, renal parameters and pH measurement. A urine aliquot (20mL) was centrifuged at 1300g for 10 min; the supernatant was removed and the sediment was washed in triplicate with water. The sediment was then resuspended and sonicated with methanol (1mL) and ATZ concentrations were determined. ATZ levels were measured in all matrixes by LC-MS/MS. Clinical data including demographic data, history of HIV, previous history of urolithiasis and current antiretroviral regimen were collected.

Result: In all urine samples, ATZ was found in sediments. The concentrations were trend to be higher in patients with ATZ300/r than ATZ400 (42 ±38 vs 32 ±43 ng/mL of methanol; not statistically different). Although ATZ concentrations in plasma and urine were significantly higher in patients with ATZ300/r than ATZ400 (Plasma: 1310 ±179 vs 713.0 ±124ng/mL, Urine: 18720 ±2711 vs 11540 ±2062 ng/mL), a poor correlation was found between ATZ concentrations in urine sediment and plasma or urine. ATZ duration therapy was lower in patients with ATZ300/r than ATZ400 (2.6 ±2.4 vs 4.7 ±2.3 ng/mL; p=0.0043) and did not influence the sediment concentrations. The presence of another nephrotoxic drug tenofovir (n=28) did not modify ATZ concentrations in sediment. The evaluated biological parameters did not show statistically significant influence.

Conclusion: This study confirms that the ATZ therapy induces ATZ crystal formation in urine in all the patients. Although no renal function impairment was found among all of the patients, a follow-up study should be investigated to confirm the absence of ATZ nephrolithiasis.

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PHARMACOKINETICS OF PARACETAMOL AND GLUCURONIDE, SULPHATE AND CYP2E1 MEDIATED METABOLITES IN MORBIDLY OBESE AND NON-OBSESE PATIENTS

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Background
Paracetamol is mainly metabolized via glucuronidation, sulphation and to a minor extent by CYP2E1, with the latter being responsible for hepatotoxicity. In obese patients, CYP2E1 activity is reported to be induced, thereby potentially changing the safety profile of paracetamol. The aim of this study was to determine the pharmacokinetics of paracetamol and its metabolites (glucuronide, sulphate, cysteine and mercapturate) in morbidly obese versus non-obese patients.

Methods
Twenty morbidly obese patients (mean total body weight (TBW) of 142 kg (106-193.1 kg) and mean BMI of 46.2 kg/m² (40.5-55.2 kg/m²)) and 8 non-obese patients (mean TBW of 70.4 kg (53.4-91.7 kg) and mean BMI of 22.3 (19.4-27.4 kg/m²)) participated in the study. All patients received 2 gram of intravenous paracetamol. Fifteen blood samples were collected per patient until 8 hours postdose. For the metabolites, the relation between the area under the curve from 0-8 hours (AUC0-8), Cmax and Tmax and TBW was studied using SPPS. Population pharmacokinetic modeling was performed using NONMEM.

Results
For paracetamol cysteine, there was a trend towards a higher AUC0-8 (1564 vs 1243, p>0.05) and Cmax (5.0 vs 4.3, p=0.05) in morbidly obese versus non-obese patients while Tmax decreased with TBW (r=−0.518, p=0.005). For paracetamol glucuronide, there was a decrease in both the AUC0-8 (r=−0.44, p=0.021) and Cmax (r=−0.42 p=0.025) with TBW, while Tmax was unchanged (r=0.089, p=0.05). There was no influence of TBW on the AUC0-8, Cmax or Tmax of sulphate and mercapturate metabolite (p>0.05). Total clearance of paracetamol (population mean (RSE%)) 0.331 L/min (3.9%) increased linearly with lean body weight (LBW) (p<0.001), while peripheral volume of distribution (22.6 L (18.6%)) increased with TBW (p<0.001) with a power exponent of 1.94 (22.4%).

Conclusions
In morbidly obese patients, a substantial influence of LBW and TBW was found for total clearance of paracetamol and peripheral volume of distribution, respectively. More specifically, for the CYP2E1-mediated cysteine metabolite,
there was a significantly lower $T_{max}$ and a trend towards a higher exposure and $C_{max}$. While exposure and $C_{max}$ of paracetamol glucuronide was significantly lower. These findings may potentially preclude an increase of the dose of paracetamol in morbidly obese patients.

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CASPASE-3, -8, AND -9 ACTIVITIES IN CARBON TETRACHLORIDE, HALOTHANE, AND SEVOFLURANE-INDUCED LIVER INJURY
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Background: It is difficult to predict DILI in the pre-clinical stage. The aim of this study was to clarify the changes of caspase (C)-3, -8, and -9 activities in carbon tetrachloride (CCl₄), halothane (HALO), and sevoflurane (SEVO) treated rats and in CCl₄, HALO, and SEVO treated-cells using the 3-dimensional culture of human hepatocarcinoma functional liver cell (FLC)-4 and co-culture of FLC-4 and THP-1 cells.

Methods: Wistar/ST rats were treated with CCl₄, HALO, and SEVO (1, 2, 4 mL/kg, ip). The liver was examined by light microscopy and laboratory data (AST, ALT) were measured in plasma. C-3, -8, and -9 activities in the liver and cells, cellular mitochondria membrane potential (MMP), and cellular viability were determined.

Results: There was no change of laboratory data and no histopathological changes of liver tissues were observed in SEVO (1, 2, 4 mL/kg, ip) treated rats. The increased plasma AST-ALT, intracellular C-3-C-9 activities, and malondiadehyde (MDA) were observed and the dose-dependent toxic reaction with centrilobular hepatocyte necrosis was shown in CCl₄-treated rats, while the increased plasma AST-ALT, intracellular C-3-C-8 activities, and no change of MDA were observed and the formed hepatitis with idiosyncratic hepatotoxicity was shown in HALO treated rats. In single culture of FLC-4, the increased C-9 and the decreased MPP and cellular viability were observed in CCl₄-treated cells. In co-culture of FLC-4 and THP-1, the increased C-8 and the decreased MPP and cellular viability were observed in HALO group.

Conclusions: The increased C-8 in the hepatocyte indicates idiosyncratic liver injury. While, the increased C-9 in the hepatocyte indicates toxic liver injury with mitochondria dysfunction. Drugs in pre-clinical stage should be evaluated using C-3, -8, and -9 activities.

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THE EFFECT OF INFLAMMATION ON VORICONAZOLE TROUGH CONCENTRATIONS IN CHILDREN
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Background: Voriconazole is a first line antifungal agent for the treatment of invasive aspergillosis. Voriconazole is extensively metabolized by cytochrome P450 isoenzymes and there is a large inter and intra individual pharmacokinetic variability, which has not been fully explained to date. Inflammatory stimuli can provoke changes in expression and activity of cytochrome P450 enzymes. In adults higher voriconazole concentrations were observed during severe inflammation. We wanted to investigate whether inflammation, as reflected by C-reactive protein (CRP) concentration, has a relationship with voriconazole trough concentrations in children.

Methods: A retrospective chart review was performed for patients younger than 18 years, treated with voriconazole (7.50 - 12.38 mg/kg/day) and for whom a steady state voriconazole trough concentration and a corresponding CRP value was measured. Subsequently we determined whether the voriconazole trough concentration is correlated with inflammation in different age groups and to what extent. Children with CRP values between 0 and 160 mg/L were considered to have no to moderate inflammation and children with CRP values higher than 160 mg/L were considered to have severe inflammation.

Results: thirty-three patients were included in this study, of which 14 children < 12 years and 19 children ≥ 12 years. For children < 12 years, no significant difference ($P=0.571$) in voriconazole trough concentration was observed between children with no to moderate inflammation (1.05 mg/L, interquartile range (IQR): 0.86-4.78 mg/L n=10) and severe inflammation (3.00 mg/L, IQR: 1.43-3.90 mg/L n=4). For children ≥ 12 years, a significantly higher ($P=0.035$) voriconazole trough concentration was found in children with severe inflammation (5.80, IQR: 4.48-8.48 mg/L n=6) compared to children with no to moderate inflammation (3.30 mg/L, IQR: 0.90-5.15 mg/L n=13). All groups received similar voriconazole doses based on mg per kg body weight ($P=0.157$ and 0.539 respectively).

Conclusions: Inflammation, reflected by CRP concentration, is probably associated with increased voriconazole trough concentrations in older children and might be age-dependent. The effect of inflammation is less substantial.
compared to adults, presumably because children younger than 12 years have linear pharmacokinetics and their cytochrome P450 capacity may be higher than in adults.

Key words: Voriconazole, therapeutic drug monitoring, inflammation, children.

ACCIDENTAL DIGOXIN OVERDOSE DUE TO MEDICATION ERROR IN A CHILD
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Background
Accidental poisonings or overdoses occur often in children. Recognition of poisonings in children is difficult because a small child cannot communicate what his symptoms are. This means specific symptoms can be missed, which can delay the diagnosis of the poisoning.

Methods
A 5 month old boy was started on heart failure treatment. After transfer to another hospital, accidently a tenfold dose of digoxin was given for 5 days. He developed feeding problems, vomiting, weight loss, elevated urea and creatinine, hypotremia, hyperkalemia and ECG abnormalities. After five days the patient was transported to our hospital and admitted to the PICU. At that point the digoxin plasma concentration was 7,6 µg/L. The patient met multiple criteria for the administration of digoxin antibodies (ECG abnormalities, hyperkalemia and ingestion of >0,3 mg/kg by a child). The patient was administered 30 mg digoxin antibodies, resulting in digoxin concentration of <0,3 µg/L. Twelve hours later the digoxin plasma concentration was 3,1 µg/L due to redistribution. Two days after the administration of digoxin antibodies the plasma concentration was within the therapeutic range.

Results
A failure analysis was performed which showed six issues:
Medication reconciliation. The discharge letter was correct, but unfortunately it was incorrectly entered in the electronic prescribing system.
Little expertise with digoxin given to small children in the general hospital.
Studies have shown that medication errors occur in children in 13,2% of the medication orders, versus 2% in adults. The reason for this is that doses for children generally have to be calculated on body weight.
More than three, call the pharmacy. The patient received 3 ml instead of 0,3 ml digoxin, which is within the volume limit of <5 ml advised by the EMA for children younger than 5 years of age.
Electronic prescribing system with clinical decision support was inadequate.
Recognition of poisoning in small children is difficult, partly because children can’t communicate their symptoms.

Conclusion
Medication overdoses due to medication errors can and should be prevented. Hospitals should implement different strategies to prevent them. The (hospital) pharmacist plays an important role in pediatric medication error reduction.

A THERAPEUTIC DRUG MONITORING PROTOCOL FOR IMATINIB IN CHRONIC MYELOID LEUKEMIA PATIENTS: FEASIBILITY AND INTEGRATION WITH PHARMACOGENETICS
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Background
Adoption of therapeutic drug monitoring (TDM) protocols in onco-hematology is scarce, mainly because a well-defined therapeutic range is often lacking. Imatinib inhibits BCR-Abl tyrosine kinase activity and the greatest therapeutic benefit seems to be achieved when minimum plasma concentrations (Cmin) are higher than 1 mg/L. The TIKlet study (clinicaltrials.gov code: NCT01860456) was aimed at investigating the feasibility of a) a TDM approach in chronic myeloid leukemia (CML) patients receiving imatinib, b) a complete pharmacokinetic analysis and c) the identification of factors responsible for interindividual pharmacokinetic variability.

Methods
A validated HPLC-UV method was used to measure drug plasma concentrations in peripheral blood samples obtained from a sparse sampling strategy. A population pharmacokinetic analysis using NONMEM was adopted to obtain predicted Cmin values and to identify those factors that may significantly influence drug pharmacokinetics.
Polymorphisms in transmembrane drug transporters were investigated by RT-PCR assays and considered as potential covariates in population pharmacokinetic modelling. Finally, patients’ adherence was evaluated by a 17-item questionnaire.

**Results**
Although the sparse sampling strategy, pharmacokinetic model was able to fit harvested data and describe pharmacokinetic characteristics of imatinib in 62 patients (35 males and 27 females). Mean±SD predicted Cmin values accounted for 1.068±0.513 mg/L (range 0.749-1.289 mg/L), but only 45% of subjects had predicted Cmin values equal to or higher than 1 mg/L. However, all of the patients attained a deep molecular response. The high adherence of the present patients to the prescribed therapy could partially explain the discrepancy. In fact, 92% of patients did not forget drug intake for more than 3 consecutive days while none of them changed the daily dose by themselves. The pharmacokinetic model identified serum alfa1-acid glycoprotein as a significant covariate for both apparent clearance (CL/F) and volume of distribution. Interestingly, the c.480C>G polymorphism of the human organic cation transporter 1 was found to influence in a significant manner imatinib CL/F and predicted Cmin values.

**Conclusions**
The present results demonstrate that an imatinib TDM protocol based on a sparse sampling strategy may be adopted in CML patients, and it may be useful to investigate the role of pharmacogenetic factors in drug pharmacokinetics.

**INTOXICATION OF A YOUNG GIRL REVEALED THE PITFALLS OF GHB RAPID SCREENING**

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**Background**
We discuss the case of a 14 year old girl who was transferred to the ICU with an ethanol intoxication (3,3 g/L), loss of consciousness (E5M3V1) and severe amnesia upon recovery. Because of this latter and the circumstances in which she was found a GHB screening was requested. The diagnosis of GHB toxicity is made clinically, based on the patient history and exclusion of other etiologies. Confirmation of the diagnosis requires analysis using gas chromatography/mass spectrometry (GC-MS). This analysis requires 2-3 hours and therefore usually does not contribute to the diagnosis. A timely diagnosis is important to initiate further diagnostics and treatment when sexual assault is suspected.

**Methods**
To facilitate timely GHB analysis, we implemented an immuno-assay analysis designed for the direct and quantitative determination in urine and serum. We chose the only available kit by Bühlmann laboratories AG. Within 30 minutes this rapid screen determines the concentration of GHB in the sample. The assay was performed on a urine sample taken immediately at presentation.

**Results**
The immuno-assay reported a GHB concentration of 26 mg/L which is above the cutoff value of 10 mg/L for urine samples. This cutoff value is to differentiate endogenous and exogenous levels since low levels of GHB occur naturally in the body. However confirmation of these results with a GC-MS method, gave a negative result. We contribute this discrepant to interference of ethanol with the assay. This is very important to realize when interpreting the analysis results, seeing as most patients who ingest GHB, have also ingested alcohol. The manufacturer of the GHB kit, states that 1 g/L ethanol, raises the GHB value by 3 mg/L. However in our case an ethanol concentration of 3,3 g/L gave an even higher GHB result.

**Conclusion**
This substantial downside of the GHB rapid screen, necessitates caution when interpreting a positive GHB. Targeted analysis using GC-MS remains the gold standard. We advise everybody using the rapid screen, to also confirm all positive GHB results which are lower than 50 mg/L with the GC-MS. This way the chance of a false positive GHB test result is reduced significantly.

**THERAPEUTIC DRUG MONITORING OF DAPTOMYCIN IN SEVERELY-ILL PATIENTS**

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Background
Daptomycin represents an antibacterial of choice for those bloodstream and skin and soft tissues infections sustained by MRSA strains. Although its efficacy, clinical use may be associated with subtherapeutic plasma concentrations especially in the presence of sepsis when standard doses (4-6 mg/kg once a day) are used. Highest daily dosages (up to 10 mg/kg) may overcome that issue, but this strategy could expose the patient to an increased risk of toxicity. Because of the variability in drug pharmacokinetics among patients, a therapeutic drug monitoring (TDM) protocol should be included in daily routine. Therefore, the aim of this study was to set up TDM of daptomycin in severely-ill patients and to investigate drug pharmacokinetics.

Methods
Plasma daptomycin concentrations at the end of drug i.v. infusion, 30 min later and immediately before the administration of the next dose were measured by validated HPLC-UV and LC-MS methods in patients who received the drug because of severe infections (i.e., cardiovascular, bone, skin infection and septicemia). A population pharmacokinetic approach (NONMEM) was adopted to investigate drug disposition and the probability to attain the target (that is, drug bactericidal effect is expected when the area under the time-curve [AUC]/MIC ratio values are >800) with standard and modified regimens, and cumulative response fraction (CRF) values were calculated.

Results
In 60 patients who received daptomycin 6-10 mg/kg/day, minimum plasma concentration (Cmin) accounted for 11.3±2.9 mg/L (mean±SD) and were always <24.3 mg/L, which is the threshold for toxicity. Moreover, pharmacokinetic modelling identified creatinine clearance as a significant covariate for drug clearance, which was 0.80±0.14 L/h. Interestingly, median (range) AUC/MIC value was 1122 (250-5658), being lower than 800 in 29% of patients. Simulated regimens with highest daily doses (i.e., up to 14 mg/kg/day or 1000 mg/day) were characterized by high CRF values (99.9) but the probability of Cmin>24.3 mg/L was 40%.

Conclusions
TDM protocols and population pharmacokinetics are useful to identify the most appropriate chemotherapeutic regimens. The present results suggest the use of daptomycin in combination with other drugs rather than adopting highest daily doses in mono-chemotherapy, because patients could be exposed to an increased risk of severe toxicities.

USE OF PK/PD/PGX SOFTWARE FOR MODELING, TDM AND CLINICAL PRACTICE TO DEMONSTRATE THE TOLBUTAMIDE STIMULATION OF INSULIN RELEASE.
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Background Applying PK/PD models in a clinical setting proved to be a difficult task in the past because of the complexity of the required PK/PD modeling software. Recently were upgraded two existing pharmacokinetic software applications. Edsim++ is used for developing and visually designing PK/PD models, while MwPharm++ is aimed at routine clinical practice for dose calculations and therapeutic drug monitoring.

Objective The aim of the study was to process tolbutamide case study with the software and demonstrate the option to use the software for modeling case studies.

Methods Insulin compartments represented by the insulin minimal model and glucose compartments represented by the glucose minimal model were prepared. These compartments are always associated with each other. Insuline has two effects - it stimulates cellular glucose update and it stimulates glycogen synthesis. Tolbutamide in this model has effects - it stimulates the insulin response and increases glucose sensitivity of the β-cells.

Results Performing the simulations, the insulin response based on tolbutamide level for the individual patient was obtained.

Conclusions The visual modeling approach of Edsim++ greatly simplifies the development of complex models which are easily transferrable to MwPharm++. These models can be employed in a clinical environment providing the dosage regimen and optimization based on large database of pharmacokinetic/pharmacodynamic/pharmacogenetic drug parameters and individual patient physiological parameters. The previous version of MwPharm application obtained the highest score in the 'Benchmarking Therapeutic Drug Monitoring Software: A Review of Available Computer Tools' carried out by Fuchs et al. (Clin Pharmacokinet 2013; 52(1):9-22).
MONITORING OF PIPERACILLIN AND MEROPENEM IN SEVERE SEPSIS
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BACKGROUND: Despite overall progress in the treatment of critically ill patients within the intensive care unit (ICU), mortality in severe sepsis is still very high. Early optimization of antibiotic treatment is essential but complicated by highly variable and unpredictable pharmacokinetics in the individual case. In addition, pathogens in the ICU might often exhibit reduced susceptibility to leading antibiotics. In this context, there is a growing interest among clinical colleagues to explore the potential of TDM for essential ICU betalactams like meropenem and piperacillin.

METHODS: This was a single-center observational study on ICU-patients (n=39) treated with either meropenem (1g q8h) or piperacillin (4g q8h) against severe sepsis. 63 pairs of plasma samples (corresponding to half- and end of the dosing interval) taken at day 1-2 (and sometimes repeated later days) were analyzed. Additional ICU-patients (n>50) on the same dose but requiring continuous renal replacement therapy, are currently under investigation. The plasma concentration of each betalactam was determined by LC-MS. Protein binding for meropenem is negligible but piperacillin concentrations were adjusted for an assumed protein binding of 20%, and then concentrations were compared to the MIC breakpoint for Pseudomonas aeruginosa, a critical ICU pathogen. Modeling of individual betalactam AUC and clearance was based on NONMEM. Individual plasma levels and clearance of the antibiotics were correlated to renal function as assessed by analysis of creatinine clearance into urine collections, or standard estimates based on plasma creatinine or cystatin C.

RESULTS: At the same dose, a remarkably high inter- and intra-individual variability in betalactam plasma levels was evident. The correlation to standard renal function tests was weak, indicating low predictive utility in dose selection. Many patients appear to be at potential risk of sub-therapeutic betalactam exposure, with 10-20% of patients exhibiting sub-MIC concentrations already at the mid-dose interval and as much as 50-60% before the next dose.

CONCLUSIONS: Even though clinical outcome data is lacking, it appears that many critically ill patients are at risk of potentially sub-therapeutic exposure to ICU-betalactam antibiotics. Betalactam TDM in the ICU should help to identify patients in need of more frequent dosing to improve time over MIC (ft>MIC).

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MICROWAVE-ASSISTED ON-SPOT DERIVATIZATION FOR THE GC-MS BASED DETERMINATION OF POLAR LOW MOLECULAR WEIGHT MOLECULES -AMONGST WHICH GABAPENTIN- IN DRIED BLOOD SPOTS
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Background: Gabapentin is an anticonvulsant drug used for the treatment of epilepsy and neuropathic pain. Monitoring gabapentin blood concentrations may be relevant in assessing compliance and in avoiding potential toxicity. Furthermore, the illicit use of gabapentin as street drug also increases. To this end, we have developed a method for the determination of gabapentin in dried blood spots (DBS) using GC-MS. Besides gabapentin, other low molecular weight molecules of interest in clinical and/or forensic toxicology, including vigabatrin, beta-hydroxybutyric acid, gamma-hydroxybutyric acid, propyleneglycol, diethyleneglycol, 1,4-butanediol, and 1,2-butanediol can be detected as well. We also evaluated the applicability of this method on capillary DBS obtained from healthy volunteers who had ingested gabapentin.

Methods: After punching a 6-mm disc from a DBS and adding the internal standard gabapentin-d10, the punches are subjected to microwave-assisted "on-spot"derivatization by direct application of 25 µL acetic anhydride and 25 µL pyridine and heating (90s, 800W), followed by a second derivatization (90s, 800W) with 25 µL heptafluorobutanol. Following evaporation under nitrogen, the extract is reconstituted in 100 µL ethylacetate. One µL of the derivatized extract is injected into an Agilent 6890 GC system coupled to a 5973 mass spectrometer. Chromatographic separation is achieved on a 30 m x 0.25 mm i.d. x 0.25 µm Agilent HP-5MS column. Quantification of gabapentin and gabapentin-d10 is performed in SIM mode using m/z 153, 167 and 195 for derivatized gabapentin and 163, 177 and 205 for derivatized gabapentin-d10.

Results: The method was linear between 1 and 30 µg/mL. Imprecision and bias values at 4 different QC levels lay below 12%. Stability studies revealed no significant decrease of gabapentin. In our real-life samples, a Passing-Bablok scatter plot demonstrated a good overall correlation between the serum concentrations obtained using the above-mentioned method and those measured by an independent method. Preliminary results revealed that our DBS concentrations are somewhat lower than serum concentrations despite a reported equal blood:serum distribution.

Conclusions: We have developed and validated a GC-MS method for the determination of low molecular weight molecules, amongst which gabapentin, in DBS using microwave-assisted "on-spot"derivatization.
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**ARK™ PREGABALIN ASSAY ON BECKMAN AU480 ANALYZER**

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In Europe, pregabalin is approved for the treatment of epilepsy, neuropathic pain and generalized anxiety disorder. In the United States, pregabalin is approved for the treatment of epilepsy, diabetic neuropathy, postherpetic neuropathy, and fibromyalgia. In the United States pregabalin is classified as a schedule V drug by the Drug Enforcement Administration. In the European Union, pregabalin is not a controlled substance, but a warning related to its abuse potential was added to the Summary of Product Characteristics in June 2010. Reports suggest that pregabalin is a recreational drug and abuse potential may exist. Successful pain management demands consistent monitoring to ensure compliance as prescribed. Based upon illicit use of pregabalin and the need to verify treatment compliance, a urinary drug test applicable to general chemistry analyzers would be a useful for identifying pregabalin in urine. ARK[TRADEMARK] Pregabalin Assay is a liquid stable homogeneous enzyme immunoassay, consisting of two reagents, 5 calibrators (0, 100, 500, 1000, and 2000 ng/mL) and 2 controls (250, and 750 ng/mL). In addition to a semi-quantitative mode, the 500 ng/mL Calibrator can be used as a Cutoff reference in a qualitative mode for distinguishing “positive” from “negative” samples. Performance of this assay was evaluated using a Beckman AU480 analyzer. Precision, spike recovery, specificity and Histogram Overlap Analysis of Controls and Cutoff concentrations were evaluated. In the semi-quantitative mode, total precision ranged from 7.3% to 9.8% CV. Accuracy was determined by spiking pregabalin into pooled pregabalin-free urine. Using the semi-quantitative mode, spiked recovery of pregabalin ranged from 94.4% (1500 ng/mL) to 104.4% (200 ng/mL). Qualitative mode tests for gabapentin and 20 L-amino acids, which are structurally similar to pregabalin, did not result in false positive responses. Histogram overlap analysis showed no overlap between Cutoff and Control levels. ARK Pregabalin Assay measures pregabalin in human urine with acceptable performance in either the semi-quantitative or qualitative modes. The ability to measure urine levels of pregabalin with high accuracy and fast turn-around time makes this method ideal for identifying the presence of pregabalin in urine to detect misuse use or abuse and verify treatment compliance.

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**AN INNOVATIVE METHOD TO ESTIMATE THE HEMATOCRIT OF DRIED BLOOD SPOTS.**

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**BACKGROUND:** The hematocrit (Hct) effect is generally considered as one of the most crucial issues in dried blood spot (DBS) based quantitation, as it is known to impact the accuracy of the results and hence, correct interpretation. Recently, our group demonstrated that the Hct of a DBS can be predicted based on the potassium content of a DBS extract1. Moreover, a correction algorithm was set up that allows to correct for the hematocrit bias in the quantitative analysis of caffeine and paraxanthine, based on the potassium content of the DBS2. Unfortunately, this potassium-based method suffered from some practical drawbacks, as it was destructive and required an additional analysis. Therefore, we now developed a non-contact method that enables Hct prediction in mere seconds. This way the entire sample is preserved and the regular sample work-up is barely disturbed.

**METHODS:** This Hct estimation method, which is based on an optical technique, was thoroughly validated and applied to patient samples with varying hematocrit (n = 288, hematocrit = 0.20 - 0.50). The true Hct was determined using a Sysmex XE 5000 hematology analyzer and compared with the estimated Hct using Passing & Bablock regression analysis.

**RESULTS:** Accuracy (% bias) and precision (% RSD) were within the limits of 15% and the LLOQ and ULOQ were 0.20 and 0.64, respectively. The estimated Hct was independent of the age of the DBS (at least up to 5 months), and DBS homogeneity and volume effect were within acceptable limits. Although Passing & Bablock regression analysis (95% confidence intervals of slope = [1.1035 - 1.1813] and intercept = [-0.08543; -0.05886]) revealed a statistically significant difference between the estimated and true Hct, the non-contact Hct estimation method proved to be fit for purpose, as 234 out of 288 samples (or 81%) were within ± 15% of their true Hct value, whilst 270 out of 288 samples (or 94%) were within ± 20%.
CONCLUSION: A non-contact method was developed that allows accurate prediction of a DBS’ Hct in a straightforward and quick manner, without interfering with the regular sample preparation and analysis of the DBS.  


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 DARUNAVIR UNBOUND FRACTION: ASSOCIATION WITH VIRAL LOAD IN HIV PATIENTS.  

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Background: In previous study [1], virologic failure was not always related to Darunavir (DRV) total plasma concentration (Cτ). It may be useful to associate the unbound plasma concentrations (Cu) representing the pharmacological active form, to the Cτ to improve DRV therapeutic drug monitoring (TDM). Our objectives were (i) to develop a model to estimate trough Cu and (ii) to investigate relationships between pharmacokinetic exposure and viral load.  

Methods: Sixty-eight DRV Cτ and Cu were collected in 57 patients during a drug interdose and measured by liquid chromatography. Unbound fraction (f_u) was calculated as f_u=Cu/Cτ. A one compartment pharmacokinetic population model (POPPK) allowing C_u estimation was developed using the non-parametric NPAG algorithm in Pmetrics®. Relationships between dichotomized viral load (>or< 20 copies/mL) and pharmacodynamics/pharmacokinetics were investigated using logistic regression.  

Results: Samples were collected at pharmacokinetic steady state, at (mean ± SD) 18.9 ±13.6 months after drug initiation. Trough Cτ and Cu were (median [Inter Quartile Range IQR]) 89.9 [42.3-125.5] µg/L and 2435.6 [1414.0-3730.0] µg/L respectively and f_u was 3.4 [2.7-4.3] %. The POPPK model estimates adequately trough Cu (relative bias= 2.33%, RMSE= 17%). f_u was associated with an increased risk of positive viral load (per unit increase OR[Confidence Interval 95%]=1.91[1.07-3.4]; p = 0.0282), while no significant association was found between Cτ and virologic failure (OR = 1.04 [0.82;1.31]; p=0.751). Cu adjusted on both Cτ and distribution volume was associated with increased viral load (per unit increase OR=1.02[1.01-1.03]; p= 0.0309). A statistical interaction between Cu and DRV clearance (Cl), explaining the relationship between Cu and viral load, was investigated and was significant (p = 0.0325). Indeed, linear regression showed an inverse relationship between Cu and the Cl (Cu = 4.7 - 46.53*Ln(Cl); r² = 0.61 ; p= 1.7 10-11).  

Conclusion: Our model allowed accurate DRV Cu estimation. However, a prospective study on a larger population is required to use f_u, and Cu in DRV TDM as a complement of trough Cτ during virologic failure.  

content) by high performance liquid chromatography with fluorescence detection.

**Results**

Strong correlations ($r > 0.7, p = 0.001$) between plasma concentrations of WAR and WAROHs and INR were observed over time for about two third of the enrolled patients. For the remaining patients, the concentrations of WAR and WAROHs were not correlated with the INR values, suggesting the existence of other variables, not investigated in this work (e.g. vitamin K), which may play an important role in the anticoagulation process. The correlation between OF and INR are not as good for all the patients, however we observed that these correlations increase if the OF concentrations are calculated in terms of ng/min.

**Conclusions**

Cross-sectional studies in literature report the existence of weak correlations between INR and plasma concentrations of WAR (total and unbound fraction). We showed here that correlations increase if single patients are monitored over time and sampling is performed under pH and OF flow rate control. Even if statistics are insufficient for firm conclusions, these results suggest that potentially useful clinical information can be obtained from these measurements, with minimum invasiveness and easy sampling.

**Key words**

Oral fluid sampling, plasma, INR, Warfarin, Warfarin alcohols

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**TDM-VIGIL: A MULTICENTRE GERMAN-AUSTRIAN-SWISS PHARMA COVIGILANCE STUDY IN CHILD AND ADOLESCENT PSYCHIATRY**

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The pharmacological treatment of children and adolescents with psychiatric disorders is a challenging task. Young patients show particular pharmacokinetic and pharmacodynamic conditions and a higher vulnerability for adverse drug reactions. In addition, age specific safety- and efficacy-studies are scarce for most psychotropic drugs, which often are not approved for these age groups.

A close observation including the assessment of positive and negative drug effects of these patients treated with psychotropics seems mandatory to continuously evaluate the benefit-risk-balance. Therapeutic Drug Monitoring (TDM) is a tool to optimize the therapeutic management as it can assist the modern clinician to individualize dosing in order to achieve therapeutic targets. However, whereas TDM is well established for many antipsychotics and antidepressants in adults, systematic investigations/studies about the practical use of TDM in children and adolescents are lacking, e.g. it is unknown for many substances whether the recommended ranges of serum concentrations derived from studies with adults are appropriate for minors as well.

Pilot studies from health services research revealed first hints on possible age-specific particularities in the relationship between daily dose, blood concentrations and therapeutic effects of some psychotropics in children and adolescents. A large multicenter patient registry ("TDM-VIGIL"), funded by the German Federal Institute for Drugs and Medical Devices, in collaboration with the German-Swiss-Austrian Network on TDM in child and adolescent psychiatry (www.tdm-kp.com), is presented, which appeals to collect prescription, serum concentrations, and effectiveness and safety data of psychotropic drugs in children and adolescents using a modern internet-based data infrastructure. With this approach of standardization drug/patient monitoring of antipsychotic and antidepressant drug use as well as psychostimulants, knowledge about drug safety and effectiveness in daily clinical practice will be increased with the overall goal to optimize psychopharmacotherapy.

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**NEW METHOD FOR DETERMINATION OF VITAMIN K1 AND TWO FORMS OF VITAMIN K2 (MK-4, MK-7) IN HUMAN SERUM BY HPLC**

Eva Klapkova, Jana Cepova, Richard Prusa

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**Background:** Vitamins K₁ and K₂ play an essential role as cofactors for the enzyme gamma-glutamylcarboxylase, which is involved in carboxylation of the vitamin K-dependent proteins. Objective of this study was to evaluate a new HPLC method for determination of vitamin K₁ and two forms of vitamin K₂ (MK-4, MK-7) in patient serum.

**Methods:** We developed a new HPLC method for the determination of vitamin K₁ and vitamin K₂ (MK-4 and MK-7) in human serum with fluorescence detection after post-column zinc reduction. The internal standard was obtained
from Immundiagnostik AG, Germany. 20 μl of internal standard were added to 500 μl of serum and 2 ml of ethanol were added to precipitate the proteins. The mixture was extracted with 4.0 ml of hexane for 10 min and then centrifuged at 3000 rpm for 5 min. The organic layers were than evaporated at 50 °C under a stream of nitrogen. The dry residue was reconstituted with 2 ml of hexane and solid phase extraction was than used (Sep-Pak, 500 mg, Waters). The separation was accomplished on a Supersphere 100 RP-18 (Merck) column at 22 °C. The detection was performed at 246 nm (excitation) and 430 nm (emission). The flow rate of mobile phase was 1.0 ml/min. We measured 204 patient samples from postmenopausal women and 30 patients before and after treatment with vitamin K.

**Results:** A linear relationship between serum concentration and peak area was obtained for all three substances with correlation coefficient \( r^2 = 0.9959 \) for vitamin \( K_1 \); and \( r^2 = 0.9971 \) for vitamin MK-7 and \( r^2 = 0.9939 \) for MK-4. The measured serum levels were \( 0.220 \pm 0.228 \) ng/mL for vitamin \( K_1 \), \( 0.728 \pm 0.128 \) ng/mL for MK-4 and \( 1.923 \pm 1.256 \) ng/mL for MK-7.

**Conclusions:** We evaluated and validated a new method for determination of vitamin \( K_1 \), MK-4 and MK-7 and determined the serum levels in postmenopausal women.

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**EVALUATION OF A DOSE-RELATED REFERENCE RANGE OF BUPRENORPHINE IN OPIATE SUBSTITUTION TREATMENT**

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**Background:** Compliance-monitoring of patients receiving buprenorphine is one of the major pitfalls in substitution treatment. An objective measure of compliance is the dose-related reference range. From data reported in the literature, a dose related reference range of 0.29 to 0.78 ng/ml/mg was proposed for buprenorphine. We have studied patients substituted with buprenorphine under well-controlled conditions to find out whether this reference range can be confirmed and used to identify compliance problems.

**Methods:** Patients with opiate dependence and substituted with buprenorphine underwent blood withdrawal twice under steady state conditions. Buprenorphine and its major metabolite were measured by a validated gas chromatography-mass spectrometry method.

**Result:** The study included 54 patients (64.8% males) aged between 19 and 52 years. The mean±SD dose of buprenorphine was 11.3 ± 7.7 mg/day. Mean plasma concentrations of buprenorphine and its metabolite norbuprenorphine were 1.6 ± 0.9 (range 0.1 to 9.7 ng/ml) and 5.6 ± 7.3 ng/ml, respectively. They correlated well with the dose (r=0.719 and 0.577, respectively). The dose related plasma concentration (ng/ml/mg) was by mean±SD 0.16 ± 0.17 (range 0.01 to 1.25) for buprenorphine and 0.52 ± 0.48 (range 0.02 to 3.25) for norbuprenorphine. Applying the dose-related reference range of 0.29 to 0.78 ng/ml/mg of the literature most patients, i.e. 91%, had higher concentrations. They were, however, in accordance with the range of 0.07 to 0.22 ng/ml/mg computed from pharmacokinetic data of a recently published pharmacokinetic study by Masson and co-workers (2014). The new range was further confirmed when comparing data from buprenorphine 184 samples that had been analyzed in clinical routine. Of these samples 46% were within, 21% below and 33% above this range.

**Conclusions:** For objective evaluation of compliance in opiate dependent patients substituted with buprenorphine, the dose related reference range recommended in the literature could not be confirmed in a well-controlled patient sample. However, they were consistent with newer data reported in a pharmacokinetic study on 49 patients using a modern analytical method (liquid chromatography-electrospray ionization-tandem mass spectrometry). Therefore we recommend a dose related reference range of 0.07 to 0.22 ng/ml/mg for patients receiving buprenorphine for substitution treatment.

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**A NOVEL AND RAPID HOMOGENEOUS IMMUNOASSAY FOR QUANTITATION OF GEMCITABINE IN PLASMA AS A TOOL FOR THERAPEUTIC DOSE MANAGEMENT (TDM)**
Conclusions: Our results suggest that vancomycin concentrations could fail to reach the therapeutic level or exceed the toxic level. TDMs considered the sub-therapeutic level (3-11 µg/mL) and toxic level (≥20 µg/mL) in approximately half of the cases (n=1811, 51.2%), while 23.4% and 20.4% of TDMs were obtained at steady state and non-steady state, respectively.

Background: Vancomycin is a glycopeptide antibiotic used to treat gram-positive infections, including methicillin-resistant Staphylococcus aureus (MRSA). The objectives of the present study were to evaluate the appropriateness of the first dosing regimen for vancomycin using therapeutic drug monitoring (TDM), and to identify factors that should be considered when choosing it.

Methods: Vancomycin TDMs performed at Seoul National University Hospital, Seoul, Korea, from 2011 to 2013 were included in this analysis, for which the dosage regimen, plasma concentrations, and demographic data were collected. Plasma concentrations were determined using samples taken 30 minutes before vancomycin administration and trough concentrations at steady state were estimated using the Abbott’s PKS software program. Estimated vancomycin trough concentrations were categorized into three levels: sub-therapeutic, therapeutic, and toxic. The concentration ranges of 10-15 µg/mL and 15-20 µg/mL were considered the therapeutic level depending on the vancomycin indications.

Results: A total of 15,527 TDMs were retrospectively reviewed, 3,986 of which were included in the analysis: 2,635 (74.5%) and 902 (25.5%) samples were obtained at steady state and non-steady state, respectively. The therapeutic level was achieved in approximately half of the cases (n=1811, 51.2%), while 23.4% and 20.4% of TDMs were considered sub-therapeutic and toxic, respectively, which was not different between males and females. The proportion of those who reached the toxic level increased as their age increased, e.g., 40% TDMs of those older than 80 were considered the toxic level. Furthermore, those with a body mass index (BMI) > 30 kg/m² had more TDMs considered the sub-therapeutic level (30%), which indicates they might have been insufficiently treated. On the other hand, those with renal impairment (i.e., creatinine clearance <60 ml/min/kg) had a higher proportion of the toxic level.

Conclusions: Our results suggest that vancomycin concentrations could fail to reach the therapeutic level or exceed the toxic level.
the safe upper margin of the therapeutic level depending upon several factors such as age, BMI, and creatinine clearance. Therefore, vancomycin dosing regimen should be carefully chosen after taking into account all those factors, particularly in the setting where vancomycin TDM is not routinely provided.

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INTEROCCASION VARIABILITY OF GENTAMICIN PEAK CONCENTRATIONS IN CRITICALLY ILL PATIENTS, DO WE NEED MULTIPLE MEASUREMENTS?

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Background: The clinical condition of critically ill patients can vary substantially, influencing the pharmacokinetics of gentamicin within a patient over time. This may hamper the efficacy of gentamicin treatment. Hence, the aim of this study was to determine the extent of variability in gentamicin peak concentrations (C_{peak}) from one dosing interval to another within a patient (interoccasion variability, IOV) in order to determine how often C_{peak} should be measured during a course of gentamicin in critically ill patients.

Methods: Data were collected prospectively from patients receiving gentamicin (initial dose 5 mg/kg) at the ICU of the Academic Medical Center (AMC). Samples were collected randomly. A population pharmacokinetic model was developed using nonlinear mixed-effects modelling (NONMEM version 7.2). Using this model, gentamicin C_{peak} were estimated 0.5 hours after the end of infusion. IOV for C_{peak} and how often therapeutic concentrations (range 15-20 mg/L) became non-therapeutic, between subsequent administrations without dose alterations, and vice versa were calculated.

Results: The concentration data from 58 patients were described using a 2-compartment model with IIV and IOV on clearance (CL) and volume of distribution (V1). Typical values for CL and V1 were 2.4 L/h and 22.0 L. IIV and IOV for CL were 67.5% and 23.9%, respective values for V1 were 24% and 24.6%. A total of 98 C_{peak} were estimated, IOV for C_{peak} was 18.3%. 52% of all C_{peak} were not within the therapeutic range and 29% of the C_{peak} were subtherapeutic after the first administration. In 20 cases with subsequent C_{peak} values, the gentamicin dose was not adjusted. In 6 (30%) of these cases the latter C_{peak} was in the non-therapeutic range while the former C_{peak} was in the therapeutic range. In 3 (15%) of these cases, C_{peak} became therapeutic while the former C_{peak} was non-therapeutic.

Conclusion: A substantial IOV of 18.3% for C_{peak} was observed. C_{peak} should therefore be measured repeatedly when critically ill patients receive gentamicin treatment for longer than 2 days. Also, the initial gentamicin dose should be at least 6 mg/kg in critically ill patients as 29% of C_{peak} were below the therapeutic range after the first administration.

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TDM OF TACROLIMUS IN KIDNEY TRANSPLANT PATIENTS - EFFICACY OF DOSE ADJUSTMENTS

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Background
Tacrolimus is a substrate of the enzyme family CYP3A4/5 and as such is subject to a number of drug-drug interactions. Due to its narrow therapeutic index, TDM is used routinely to maintain an optimal therapeutic range in order to limit toxicity while ensuring effectiveness. We analysed retrospective data on drug level measurements in our transplant centre to evaluate success of dose adjustments in keeping the patient in therapeutic range.

Methods
The population included 181 renal graft recipients in a single transplant centre who were treated with a calcineurin inhibitor for more than 3 months and had data available for their plasma levels. Risk of levels outside of target range following prescription of an interacting drug and time spent outside of range were analysed.

Results
At 641 of the 3331 patient visits where levels were measured, the dose of tacrolimus was adjusted (1.2 dose changes per patient/year). Of these dose adjustments, 66% were done when the patient was outside of target range and 34% when the level was within therapeutic range. Of those adjustments done for levels outside of range, 54% corrected the successive level measured and 46% did not (of these 39% were insufficient and 61% actually overshot the range).

Conclusions
While drug level measurements helps maintain optimal target levels of tacrolimus, dose adjustments done without
pharmacokinetic modelling based only on physician’s experience can fail to quickly and effectively correct level outside of the intended range.

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**ABCG2 AND CYP3A4 POLYMORPHISMS AS A RISK FACTORS FOR DEVELOPING ADVERSE DRUG REACTIONS IN PATIENTS TAKING ATORVASTATIN: A CASE-CONTROL STUDY**

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**Background:** Statins are among the most prescribed drugs, with atorvastatin being the most frequently used statin. Although statins have been confirmed to be well tolerated agents, there is significant number of people who are intolerant of statin therapy or who suffer side effects. ABCG2 is efflux transporter of a wide variety of xenobiotics, which are among some classes of statins. Among other functions, ABCG2 limits the absorption of its substrates from the gut and increases the excretion into the bile and urine. Its polymorphism, c.421C>A, has been associated with reduced ABCG2 transport activity. CYP3A4*22 variant could have influence on atorvastatin metabolism. Objective of the study was to explore the association between most commonly reported ADRs (adverse drug reactions) of atorvastatin (myotoxicity and hepatotoxicity) and polymorphism of ABCG2 and CYP3A4 gene.

**Myotoxicity and hepatotoxicity** were investigated together as these ADRs are related to plasma concentration and influenced by changes in the pharmacokinetics of statins. **Patients and Methods:** Sixty patients who experienced atorvastatin-related myotoxicity or hepatotoxicity and sixty matched patients without ADRs were enrolled in the study. Data regarding age, sex, atorvastatin dose, concomitant drugs, comorbidities, data regarding risk factors for atorvastatin ADRs (hepatic or renal dysfunction, perioperative periods, multisystem diseases, small body size and untreated hypo-roidism) were collected. Genotyping for ABCG2 421 CYP3A4*22 variants was performed by real-time PCR methods. **Results:** The results showed that those having ABCG2 421CA genotype had 3.8-times greater odds of experiencing atorvastatin-related ADRs (χ²=7.222; df=2; p=0.015; Cramer’s φ=0.245, odds ratio [OR]=3.75; CI: 1.27-11.12) than those with ABCG2 421CC genotype. 3 patients who developed ADRs were patient from control group were CYP3A4*22 carriers. After adjustments for clinical risk factors, influence of ABCG2 421C>A remained a statistically significant predictor of adverse effects (OR=3.79; Wald c²=5.39; df=1; p=0.020; 95% CI: 1.23-11.69). **Conclusion:** Our results demonstrate an association between atorvastatin-induced ADRs and genetic variants in the ABCG2. Influence of CYP3A4*22 variants should be further investigated in bigger studies.

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**THE BENEFITS OF HIGH RESOLUTION LCMS FOR MONITORING BACLOFEN AND ITS METABOLITES**

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**Background:** Baclofen is a muscle relaxant and a number of studies also confirm its efficiency for the treatment of alcohol addiction. At the Talwar centre (GH Cochin) alcohol-dependency is treated using high and variable doses of baclofen. It is therefore of interest to explain this variability by monitoring baclofen and its major metabolites. We describe a method to determine plasma and urinary concentrations of baclofen and a semi-quantitative evaluation of the metabolic ratio of its major metabolites by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS).

**Methods:** Plasma and urine samples from patients treated with Baclofen were prepared by addition of baclofen-d4 followed by protein precipitation with acetonitrile. The organic phase was evaporated and re-suspended with 100 µL 80/20, water/acetonitrile. Chromatographic separation was achieved using an Accucore PFP column (100 x 2.1 mm , 2.6 µm, Thermo Scientific™) by gradient elution. Mass spectrometric analysis was performed on an Exactive Plus system (Thermo Scientific™) using electrospray ionization. Data was acquired in full MS, scanning in polarity switching up to 4.9 minutes at a resolution of 17500 (m/z=200) and negative mode only between 5 and 8 minutes at a resolution of 700000 (m/z=200). Quantitation was performed using the extracted masses of baclofen and its
metabolites. Results: The method was successfully validated for the quantitation of baclofen in urine and plasma with linearity between 10 ng/mL and 2000 ng/mL (LLOQ = 3 ng/mL, CV = 5.7%). Precision and accuracy were evaluated using QC samples (n=3) and was measured at <10%. The total extraction recovery was > 90%. For patients receiving doses around 100 mg for at least 1 month concentrations of baclofen in plasma varied from 24 to 1039 ng/mL. Baclofen-glucuronide, deaminated and hydroxylated metabolites were detected in both urine and plasma. The ratio of detected metabolites was then studied. Conclusion: A validated LC-HRMS method was developed not only to quantify baclofen but also simultaneously monitor its phase I and II metabolites in urine and plasma for purposes of determining variability in dose observed in patients receiving treatment in alcohol addiction.

249 DEVELOPMENT AND IMPLEMENTATION OF A RAPID HPLC-HRAMS SCREENING METHOD FOR DETECTION OF TWENTY ANTIRETROVIRAL (ARV) COMPOUNDS IN HUMAN SERUM
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Background: The goal of this work is to rapidly screen for the presence of a panel of ARV drugs using a multiplexed approach, which is amenable to a large-scale production setting. We describe this analytical method here and the improvements we have made to this assay over several generations of the method.

Methods: The analytical method was developed to detect the following ARV agents: abacavir, amprenavir, atazanavir, darunavir, efavirenz, entecavir, indinavir, lamivudine, lopinavir, maraviroc, nelfinavir, nevirapine, raltegravir, ripivirine, ritonavir, saquinavir, stavudine, tenofovir, tipranavir, and zidovudine. After solid phase extraction (SPE) was performed, 30 μL of SPE eluent was injected onto a four-channel HPLC system equipped with Dionex pumps (Thermo Fisher Scientific). Analytes were eluted from a Hypersil Gold PFP 50 x 2.1 mm; 5 μm particle size column (Thermo Fisher Scientific) over 3.1 minutes. We achieved a run time of 1.5 minutes per sample using a QExactive mass analyzer (Thermo Fisher Scientific) and the multiplexing capability of the HPLC system. The mass spectrometer was operated in positive ionization mode. The quadrupole was used for isolation of the precursor mass and the product masses were determined after fragmentation, in the ion trap. Validation studies were performed, including limits of identification, precision, carryover, selectivity, and method comparison. During multiple generations of this assay we have developed methods to eliminate false positive reporting, a common pitfall in qualitative screening.

Results: The analytical method was found to have a limit of detection of ≤10 ng/ml for all ARV’s, acceptable performance with respect to carryover, selectivity, and precision, and acceptable agreement with a reference method.

Conclusions: This rapid HPLC-HRAMS method allows for the multiplexed qualitative detection of ARVs in human serum.

250 DEVELOPMENT AND VALIDATION OF AN UHPLC-HRAMS CREATININE ASSAY USING AN ALTERNATIVE CALIBRATION STRATEGY
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Background: The measurement of creatinine in testing for drugs of abuse or pain management compliance provides a metric by which toxicologists and physicians can assess the quality of a sample. Here we propose a method by which urine and serum creatinine can be quantified using a response factor-based approach, without the use of a full calibration curve.

Methods: The analytical method was developed to detect creatinine and creatinine d3, a deuterated internal standard. After sample preparation, during which a defined concentration of internal standards was added to the sample, a volume of sample was injected onto a four-channel HPLC system equipped with Dionex pumps (Thermo Fisher Scientific). We were able to achieve an effective run time of one minute per sample using a QExactive mass analyzer, (Thermo Fisher Scientific) and the multiplexing capability of the UHPLC system. The mass spectrometer was operated in positive ionization mode. The quadrupole was used for isolation of the precursor mass and the product masses were determined after fragmentation, in the ion trap. Validation studies were performed, including limits of quantification, precision, and carryover. As part of the validation studies, we analyzed creatinine standard reference material from the US National Institute of Standards and Technology (NIST). We analyzed all validation
results using a response factor (RF) based approach, in which an equimolar mixture of creatinine and creatinine d3 were established as reference, and all samples were quantified relative to an established RF. We compared the RF-based validation data to data we quantified using a calibration curve.

**Results:** The analytical method was found to have acceptable performance with respect to precision, carryover, limit of quantification, and accuracy. The RF-based calibration data was comparable to data quantified using a traditional calibration curve.

**Conclusions:** As creatinine quantification is used in toxicology laboratories to assess sample quality, we offer a simple UHPLC-HRAMS approach to creatinine quantification, which obviates the need to analyze a full calibration curve to achieve results.

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**ESTIMATION OF PRECISION AND ACCURACY OF TWO POPULATION PHARMACOKINETICS MODELS OF INFILIXIMAB IN PATIENTS WITH INFLAMMATORY BOWEL DISEASES.**

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**BACKGROUND**

Infliximab is a monoclonal antibody approved for the treatment of inflammatory disease. High interindividual variability in serum infliximab concentrations has been reported during treatment. Despite the common mechanism of action, the difference of dosing requirements for optimal efficacy imply that patients and/or disease characteristics may influence the PK of infliximab differently among patient populations.

Our aim was to estimate the precision and accuracy of two pharmacokinetic models in patients with inflammatory bowel disease.

**METHODS**

An observational retrospective study was designed. Patients with ulcerative colitis or Crohn’s disease treated with infliximab during 2014 were included. Trough blood samples for determining infliximab were drawn. Two population pharmacokinetics (PopPK) models were implemented in NONMEM: PopPK in ulcerative colitis (Fasanmade et al, 2009; Model 1) and PopPK in Crohn’s disease (Fasanmade et al, 2011; Model 2). The Infliximab concentrations were estimated from both models at the samples times, through the empirical bayesian of estimates (EBEs) of the pharmacokinetic parameters. To validate these models, bias of estimated concentrations was calculated as the mean residual predictive error (MRPE) and the accuracy was calculated as the root mean square predictive error (RMSPE) for the two models in our population.

**RESULTS**

45 serum infliximab concentrations from 40 patients (19 males and 21 females) was included. The mean age was 36 years (IC95%: 31 - 41), weight 72.82 kg (IC95%: 68.2 - 77.44) and 4.26 mg/dL (IC95%: 4.17 - 4.35) baseline serum albumin concentration. The mean trough serum concentration of infliximab was 3.84 mg/L (IC95%: 2.76 - 4.93). Three patients developed antibodies to infliximab. Bias of estimated concentrations (MRPE) were 1.56 (IC95%: 0.93 - 2.19; p<0.0001) and 0.269 (IC95%: 0.017 - 0.521; p=0.037) in Model 1 and 2, respectively. Accuracy (MRPE) were 259% and 87.3% for Model 1 and 2, respectively.

**CONCLUSIONS**

In our study, both pharmacokinetics models overestimate infliximab concentrations in the population, although Model 2 bias was statistically better, (i.e. closer to zero) than Model 1 (p<0.001). In terms of accuracy, Model 2 performed also better, with a difference or more than 150 units in percentage.

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**URINARY ETHYL GLUCURONIDE VS. URINARY ETHANOL AS A SCREENING TOOL IN PATIENTS UNDERGOING DETOXIFICATION PROGRAMMES**


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**BACKGROUND**

Excessive use and abuse of alcohol represent a major health problem. In addition to interviews, and the routine state markers, many centers utilize alcohol markers. Ethyl glucuronide (EtG), a direct ethanol metabolite, seems to meet the need for a sensitive and specific marker for monitoring alcohol consumption. Although accounting for only <0.1% of the ingested ethanol dose, EtG is detectable in urine for up to 80 hours from the complete elimination of alcohol from the body.
Our aim was to test the applicability of the EtG test for identifying alcohol consumption in patients under monitoring abstinence as compared to ethanol (EtOH) test.

METHODS
It was designed as a prospective field study. An aleatory sample of urine specimens was obtained from patients under monitoring abstinence of alcohol consumption from September 2014 and December 2014. Urine concentration of EtOH and EtG was analyzed with an DRI enzyme immunoassay (EIA) method based on a monoclonal antibody (ThermoFisher Scientific®) using Indiko Plus® analyzer. Urine samples were stored at 4-8 degrees and taken for analysis no more than 24 hours after reception. The EtOH test was considered positive with an urinary concentration (Cu) \( >10 \text{ mg/dL} \) and the EtG test was positive with Cu \( >100 \text{ ng/mL} \). Concordance between EtG test and EtOH test was assessed by kappa coefficient (SPSS 17.0)

RESULTS
A total of 147 urine specimens were analyzed from 125 patients (102 males and 23 females); mean age of 37 years (IC95\%: 35 - 39). EtG test was positive for 79 (53.7\%) cases and EtOH test was positive for 38 (25.8\%) cases; these 38 cases were also identified by EtG test. Thus, in 41 cases (27.9\%), the EtG test revealed alcohol consumption that could not be proven by the EtOH test. The measurement agreement between both techniques was from fair to moderate (Kappa coefficient 0.46).

CONCLUSIONS
In our study, the use of EtG substantially improves the detection of alcohol consumption in patients undergoing detoxification programmes in comparison with EtOH (53.7 vs. 25.8\%). We suggest using EtG as routine test for screening alcohol consumption.

KEY WORDS
Ethyl glucuronide, ethanol, alcohol abstinence, toxicology.

PHARMACOKINETIC EXPERIMENT DESIGN BY MINIMIZING A WEIGHTED BAYES RISK OVERBOUND
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This paper describes a multiple model optimal design method (MMopt), based on minimizing a Bayes Risk upper bound. Bayes Risk can be used as a design criterion for PK models having discrete Bayesian priors, as with nonparametric population pharmacokinetic models, as in the Pmetrics software. MMopt minimizes a Bayes risk upper bound [1], to minimize support point misclassification, and is useful when Bayes Risk itself is not practical to compute. A simple two-model problem with additive noise is described here to motivate the methods. Both MMopt and the optimal Bayes Risk design place a single sample when the response trajectories of the two models are most separated. Intuitively, this best discriminates between the two models. Interestingly, Fisher-based designs fail to maximize response separation in this simplest of all problems, questioning their performance in more complicated problems having multiple support points.

A new weighting technique is now introduced into the Bayes risk, giving a cost function that can be interpreted as the expected weighted cost with respect to the Bayesian posterior. This weighted form of MMopt is especially useful for clinical problems, as the experiment design (TDM protocol) can be tailored to optimize performance of specific clinical tasks such as future patient doses to hit specific desired target goals, or to achieve a desired area under the curve (AUC). Results are compared to ED optimal design, a standard method that also incorporates a Bayesian prior. MMopt can improve on EDopt for problems of practical clinical interest. MMopt helps provide a TDM protocol for learning about patients optimally while treating them at the same time, tailored to optimize specific clinical tasks with optimally precise subsequent dosing.

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TENOFOVIR DISOPROXIL TREATMENT FOR A HIV-HBV CO-INFECTED PATIENT UNDERGOING PERITONEAL DIALYSIS.
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Tenofor disoprosil fumarate is the drug of choice for the treatment of HIV-HBV co-infected patients. Tenofor disoprosil is eliminated primarily by the kidneys through glomerular filtration and active tubular secretion. Therefore, patients with a reduced kidney function are treated with a less frequent dose regimen. Based on several findings a dosing regimen of 245 mg tenofor disoprosil once weekly is recommended in patients on haemodialysis (as compared to 245 mg daily in patients with adequate renal function). For patients undergoing peritoneal dialysis no adequate dosing regimen has yet been established.

We treated a 46 year old man co-infected with HIV-1 and hepatitis B with end-stage renal disease, undergoing peritoneal dialysis, with 245 mg tenofor disoprosil once weekly. We measured steady state tenofor concentrations in serum and dialysis fluid. The trough concentration in serum was 0.51 mg/l, which is well above the therapeutic window of 0.05-0.30 mg/l. We decreased the dose frequency to 245 mg tenofor disoprosil every two weeks and remeasured the trough serum concentration, being 0.20 mg/l and in the therapeutic range. The HBV load in our patient decreased from 9.9 x 10^8 copies/ml to 7.8 x 10^4 copies/ml 6 months after the start of tenofor disoprosil, indicating the tenofor exposure had been adequate. The HIV load was undetectable, both before and during treatment. Our patient did not experience adverse events.

We conclude that patients on peritoneal dialysis, may be adequately treated with tenofor disoprosil 245 mg on a 2-weekly regimen.

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NONPARAMETRIC POPULATION PHARMACOKINETIC MODELING OF MYCOPHENOLIC ACID IN HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS.

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Introduction: Mycophenolate mofetil (MMF) is an immunosuppressive drug used in the prophylaxis and treatment of acute and chronic graft-versus-host disease after haematopoietic stem cell transplantation (HCT). Currently, a bayesian estimator (BE) using 3 time-points, which was built using a one-compartment model with first order elimination and 3 gamma functions for the absorption phase is used on our dose individualization ISBA website. The objective of this study was to develop: (1) a simpler population pharmacokinetic model for mycophenolic acid (MPA) in HCT using a nonparametric method; and (2) a BE based on a limited sampling strategy (LSS).

Methods: 154 pharmacokinetic profiles made of 3 to 9 points sampled between 0 and 8h or were collected in 75 HCT patients given MMF corresponding. Data were split into: a building dataset (129 PK full or sparse profiles) and a validation dataset (25 full PK profiles). The MMF pharmacokinetic model was developed using the nonparametric adaptive grid algorithm in the Pmetrics R package. The model was internally validated using the visual predictive check (VPC) with 1000 simulations based on the median dose. The best sampling times were determined using the MMopt algorithm in Pmetrics.

Results: The PK profiles were best fitted using a one-compartment model with first-order elimination combined with two gamma functions for the absorption phase. A first order elimination parameter was added to the baseline to take into account the decrease in concentration between T0 and Tlast (the model with corrected baseline = cbl was better than no cbl: AIC = 2666 and BIC = 2686 vs. AIC = 2710 and BIC=2728).

The model developed yielded good individual predicted concentrations (r² = 0.963, mean ± SD relative bias = 4.13% ± 58.24%, RMSE = 58.38%). The derived BEs based on the 4 best sampling times (C20min-C40min-C1h-C2h) accurately estimated the inter-dose AUC (relative bias = -8.20% ± 28.36%, RMSE = 28.99%).

Conclusion: We developed a population PK model for MMF in HCT and a BE based on 4 points LSS. The next step will be to compare the performance of this model to that of the model used in routine practice.

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THE DIFFERENT REGULATION OF TREGS AND TH17/TH1 CELLS BY SIROLIMUS-BASED REGIMEN MIGHT BE DEPENDENT ON STAT-SIGNALING IN RENAL TRANSPLANT RECIPIENTS

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Background: mTOR inhibitors (Sirolimus) has been used as de novo base therapy with steroids and mycophenolate mofetil in an effort to completely avoid use of CNI. Our aim was to determine whether signatures of
immunoregulation are promoted by conversion from tacrolimus to sirolimus.

**Methods**: We included 24 renal transplantation recipients (converted from tacrolimus to sirolimus therapy for clinical indications) and 24 healthy controls. Of all these subjects, Th cells and the STAT proteins frequencies in the peripheral blood were analyzed by flow cytometry (FCM) before conversion, three months and six months after conversion. Plasma levels of IL-1β, IFN-γ, IL-17, IL-6 and IL-10 were also analyzed by Bio-Plex® suspension array system before and three months after conversion.

**Results**: The recipients who switched to sirolimus presented a significant increase in Treg frequencies when compared with the preconversion and healthy people (P<0.05). The plasma concentrations of Th17 related cytokines (IL-1β, IL-6, IL-17) and Th1 related cytokine (IFN-γ) were significant decreased after conversion to sirolimus. In the mean conversion, recipients who switched to sirolimus showed increased STAT5 activation and decreased STAT3 activation compared with tacrolimus group. Circulating p-STAT3 are positively correlated with the frequencies of Th17 cells (r = 0.435, P =0.003), while the circulating p-STAT5 are positively correlated with the frequencies of Treg cells (r =0.419, P =0.015).

**Conclusion**: Our results indicated that conversion to SRL may both minimize CNI toxicity and promote immune tolerance.

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**THE REMISSION OF RENAL INJURY IN TACROLIMUS TO SIROLIMUS CONVERSION RENAL TRANSPLANT RECIPIENTS IS DEPENDENT ON THE INHIBITION OF CYTOKINE- CHEMOKINES SIGNALING**

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**Background**: Conversion from tacrolimus to sirolimus-based immunosuppression is associated with improved renal function and stabilization of renal-allograft lesions compared with recipients maintained on tacrolimus. Cytokine and chemokines play an important role in renal-allograft lesions. When stimulated with proinflammatory cytokines, endothelial and parenchymal cells in the kidney produce chemokines that are necessary for the initial migration of leukocytes into the renal allograft. Subsequently, the renal injury after transplantation occurred.

**Methods**: We included 48 recipients who received a first renal graft in West China Hospital. Among all the renal transplant recipients, 24 recipients received a tacrolimus (TAC) based regimen, the other 24 recipients received a sirolimus (SRL) based regimen which conversion from tacrolimus. Plasma cytokine IL-1β, IFN-γ, IL-17, IL-6, IL-10 and ChemokinesIP-10, MCP-1, MIP-1b, IL-8 were measured using the Bio-Plex® suspension array system (Bio-Rad Laboratories Inc, Hercules, CA,USA) which utilizes Luminex® xMAP[TRADEMARK] multiplex technology.

**Results**: After conversion to SRL for more than one month, the renal function has turned better for those recipients. For the cytokine and chemokines analysis, IL-6, IL-1β and IL-17 were positive correlation with MIP-1b and IL-8 (P<0.05), we found that Th1 related cytokine (IL-1β and IFN-γ) and Th17 related cytokine (IL-17, IL-6) decreased significantly in the recipients used SRL based regimen compared with tacrolimus group (P<0.05). On the other hand, we found the plasma concentrations of MCP-1 and MIP-1b in SRL group decreased significantly when compared with TAC group (P<0.05).

**Conclusions**: In summary, we conclude that conversion from CNIs to sirolimus in kidney-transplant recipients is associated with improved renal function. The proinflammatory cytokines IL-6, IL-1β and IL-17 are important inducer of the production of chemokines MIP-1b and IL-8. Those proinflammatory Cytokine and chemokines play an important role for tacrolimus treated recipients in mediating the inflammatory process of renal nephrotoxicity formation.

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**LC/MS/MS QUANTITATIVE ANALYSIS OF ANTICONVULSANTS AND ANTIPELLEPTICS IN URINE, ORAL FLUIDS AND SERUM, AND EVALUATION AND COMPARISON OF SAMPLE PREPARATION TECHNIQUES**

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Anticonvulsants and antiepileptic drugs are a diverse group of pharmacological agents. A highly sensitive, simple, accurate and specific LC/MS/MS analytical method has been developed for the quantitation of these analytes by QQQ. Various sample preparation techniques that includes dilute and shoot in urine and oral fluid, protein crash (PPT), liquid-liquid extraction (LLE), supported-liquid extraction (SLE) and solid phase extraction (SPE) and one (1D)
and two (2D) dimensional chromatographic configurations are evaluated and compared based on their ease of use, analyte recovery and post-extraction cleanliness as well as different mass spectrometer platforms. The described analytical method achieves the required sensitivity and is capable of quantitating the analytes over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed to quantitatively measure Anticonvulsants/Antiepileptic using sample preparation techniques that are quick and easily applied for high throughput analysis.

An Agilent 6460 tandem mass spectrometer with Jet Stream technology in negative Electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 ul of urine, oral fluid and serum were used for the analysis of Anticonvulsants/Antiepileptic’s. Various columns were evaluated and an Agilent Poroshell 120 SB-C18 100 x 2.1 mm, 2.7 um with a water:methanol mixture containing 0.01% formic acid and 5 mM Ammonium Formate gradient achieved baseline chromatographic separation in less than 6 minute run time for all dimensions. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from NIST, UTAK and Recipe Controls and urine, oral fluid and serum samples. Good linearity and reproducibility were obtained with the concentration range from 1 ng/ml to 1000 ng/ml for the respective Anticonvulsants/Antiepileptic’s with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.1 to 0.5 ng/ml. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations.

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LC/MSMS QUANTITATIVE ANALYSIS OF ANTI-DEPRESSANTS AND TRICYCLIC ANTI-DEPRESSANTS IN URINE, ORAL FLUIDS AND SERUM AND EVALUATION AND COMPARISON OF SAMPLE PREPARATION TECHNIQUES
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Antidepressants and Tricyclic Anti-Depressants are pharmacological agents. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantification of these analytes by QQQ. Various sample preparation techniques that includes dilute and shoot in urine and oral fluid, protein crash (PPT), liquid-liquid extraction (LLE), supported-liquid extraction (SLE) and solid phase extraction (SPE) and one (1D) and two (2D) dimensional chromatographic configurations are evaluated and compared based on their ease of use, analyte recovery and post-extraction cleanliness as well as different mass spectrometer platforms. The described analytical method achieves the required sensitivity and is capable of quantitating the analytes over their dynamic range. Therefore, a simple and accurate quantitative analytical method was developed to quantitatively measure Anti-Depressants.

An Agilent 6460 tandem mass spectrometer with Jet Stream technology in negative Electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 ul of urine, oral fluid and serum were used for the analysis of Anti-Depressants. Various columns were evaluated and an Agilent Poroshell 120 SB-C18 100 x 2.1 mm, 2.7 um with a water:methanol mixture containing 0.01% formic acid and 5 mM Ammonium Formate gradient achieved baseline chromatographic separation in less than 6 minute run time for all dimensions. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from NIST, UTAK and Recipe Controls and serum samples. Good linearity and reproducibility were obtained with the concentration range from 1 ng/ml to 1000 ng/ml for the respective Anti-Depressants with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 0.1 to 0.5 ng/ml. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations.

A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry analytical method was developed and verified for the simultaneous measurement of Anti-Depressants. The sample preparation techniques are quick and easily applied for high throughput analysis.

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LC/MS ANALYSIS OF PHYTOCANNABINOIDS AND THEIR METABOLITES IN URINE, ORAL FLUID AND BLOOD
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Liquid chromatography triple quadrupole (QQQ) mass spectrometry (LC/MS/MS) is suited for rapid analysis of multiple analytes. A highly sensitive and specific LC/MS/MS analytical method has been developed for the quantitation of phytoannabinoids and their metabolites that include cannabidiol, cannabiol, cannabidivarin, cannabigerol, cannabichromene and tetrahydrocannabinol by QQQ. Simple sample preparation techniques such as dilute and shoot for urine and oral fluid, and protein crash for blood, and one dimensional (1D) chromatographic configurations achieved the required sensitivity and is capable of quantitating the analytes over their relevant dynamic range.

An Agilent 6460 QQQ with Jet Stream technology in positive electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 100 mL of human urine, oral fluid and blood were used for the analysis of the various drug classes. Various columns were evaluated and an Agilent Poroshell 120 was used with a water:acetonitrile mixture containing 5 mM ammonium acetate gradient achieved baseline chromatographic separation in an approximately 5 minute run time for all matrices. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from UTAK and human samples.

Good linearity and reproducibility were obtained across the dynamic range of the drugs with a coefficient of determination R²=0.995 for all drugs in the various matrices. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined and excellent reproducibility was observed for all compounds (CV < 15%) in all matrices. A sensitive, simple, specific and accurate liquid chromatography QQQ mass spectrometry method was developed and verified for the simultaneous measurement of phytoannabinoids and their metabolites in urine, oral fluids and blood.

POLYMORPHISMS IN GENES OF THE CALCINEURIN RESPONSE PATHWAY AND RISK OF POST TRANSPLANT LYMPHOPROLIFERATIVE DISEASE: A CASE-CONTROL STUDY

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Background: Post-transplant lymphoproliferative disorders (PTLDs) represent a serious complication in solid organ transplantation and are the first cause of cancer related mortality in this population. Given a viral aetiology in majority of cases, and a key role of the immune system, we hypothesise that a genetic susceptibility to iatrogenic immunosuppression might favour lymphomagenesis. We therefore tested candidate polymorphisms with purported functional consequences in genes of the calcineurin inhibitor response pathway (IL2, CD25, cyclophilin PPIA, calcineurin subunit A alpha PPP3R1, calcineurin subunit B alpha PPP3R1). We also included loci in cytokine and receptor genes with previous associations to PTLD (IL10, TNF, TGFB, TNFRSF1A). A total of 15 loci were included.

Methods: PTLD cases were identified through the French PTLD Registry, and were limited to adults >18 years, kidney-only transplant and no HIV infection. Fifteen French transplant centres agreed to participate. Cases and controls were matched one-to-one on: transplant centre; sex; age at transplant (± 5 years); year of transplant (± 1 year); graft order (ie. 1st, 2nd or 3rd transplant); and pre-transplant EBV status. Controls were selected to have the same time-length of immunosuppression as the period transplant-to-PTLD in the case. Longitudinal data were collected retrospectively and included the following: immunosuppression regimen (dose and blood level), acute rejection episodes and treatment, CMV infections and treatment. 143 case-control pairs were included. Genotyping was performed using TaqMan® SNP genotyping assays. Given limited knowledge about the mode of inheritance of the selected polymorphisms, the most probable genetic model was determined using the SNPassoc R package.

Multivariate conditional logistic regression was performed using the R package ‘epicalc’.

Results: TGFB-rs1800470, IL2-rs2069762, PPP3R1-rs1868402 and azathioprine use exhibited significant associations with PTLD in univariate analyses. Only azathioprine associated with increased PTLD risk in the multivariate model (ever vs never taken, OR=3.57, 95% CI 1.54-8.26, p= 0.001).

Conclusions: We did not find any association between polymorphisms in genes of the calcineurin inhibitor response pathway and PTLD. PTLD risk was associated with azathioprine, whose use as an immunosuppressant in transplantation has fortunately largely been replaced.
VORICONAZOLE CLEARANCE ESTIMATION USING PHARMACOKINETIC POPULATION MODELING.

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Background
Voriconazole (VOR) is a triazole antifungal agent indicated for the treatment of fungal infections and which requires Therapeutic Drug Monitoring (TDM). VOR is mainly metabolized by cytochrome CYP 2C19, which is characterized by a polymorphism which may increase VOR activity and apparent clearance (Clᵢₑ), leading to trough concentrations (Cᵢ) below the recommended threshold (1mg/L)¹. The aim of the study was to develop a simple population pharmacokinetic (PKPOP) model to estimate VOR pharmacokinetic (PK) parameters (Clᵢₑ and Vdᵢₑ).

Methods
VOR was assayed by HPLC for routine TDM in 77 patients (3 from the Toulouse University Hospitals sampled before and 2, 4, 6, 8 and 12 hours after dosing and 74 from the Rouen University Hospital sampled before and 2h after dosing). A one-compartment PKPOP model was developed using a non-parametric NPAG algorithm in the Pmetrics R Package². Clᵢₑ, Vdᵢₑ and Area Under Curve were estimated from the modeled profiles using non-compartmental analysis. The median and Interquartile range (IQR) were calculated for Clᵢₑ.

Results
Individual Predicted concentrations (IPRED) as a function of observed concentrations, normalized residues as a function of IPRED and individual fits showed that the model fitted adequately the data, with a bias of 1.5% and Root Mean Square Error of 12.3%. Clᵢₑ values followed a trimodal distribution: high Clᵢₑ (n = 4, median = 21.8 L / h; IQR (21.4; 22.2)), middle Clᵢₑ (n = 37, median = 8.3 L / h; IQR (7.0; 10.5)) and low Clᵢₑ (n = 36, median = 1.7 L / h; IQR (0.9; 2.5)).

Conclusion
The developed model allowed estimation of PK parameters and will be used in further studies to investigate the influence of CYP2C19 polymorphisms on VOR PK.

(1) 2013 Update of the ECIL Guidelines for Antifungal Therapy (ECIL-5)

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IDENTIFYING RISK FACTORS FOR EVEROLIMUS SERIOUS SIDE EFFECTS AND DISCONTINUATION IN RENAL TRANSPLANT RECIPIENTS ON EVEROLIMUS AND PREDNISOLONE DUAL THERAPY.

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Objectives:
Everolimus is an emergent non-nephrotoxic alternative for calcineurin inhibitors with substantial potential non-renal benefits in renal transplantation. Despite its proven efficacy and close therapeutic drug monitoring, everolimus is also known for some serious side effects and relatively high discontinuation rates. The primary objective of this study was to develop time-to-event models for the time to drug discontinuation and the key side effect (i.e pneumonitis, infection and new onset diabetes mellitus) to identify risk factors that may determine therapy outcome.

Methods:
An extensive dataset consisting of demographic, transplant related and pharmacogenetic data of 99 stable adult renal transplant recipients on a regimen of everolimus and prednisone dual therapy was used for a systematic analysis using a parametric survival model for each different endpoint to describe the time to discontinuation and the most hazardous side effects including pneumonitis, infection and new onset diabetes mellitus. Modelling was performed using NONMEM v7.3.0 and R statistics was used for summary statistics and plotting.

Results:
The baseline hazard of discontinuation, pneumonitis and infection data, respectively, was best described by a Gompertz function and an exponential hazard function was used to describe the baseline hazard of new onset
diabetes mellitus. Risk factors for everolimus discontinuation were excess everolimus exposure and increasing age. Furthermore, risk factors for the hazardous side-effect non-infectious interstitial pneumonitis were excess everolimus exposure and PXR(NR1|2)(-24113G>A):AA genotype. For infection and new onset diabetes mellitus no significant risk factors could be identified.

Conclusions
The current findings indicate that discontinuation rates and non-infectious pneumonitis in renal transplant recipients on everolimus can be prevented by avoiding excess initial and/or excess maintenance everolimus exposure.

METABOLIC RATIOS OF VENLAFAXINE AND RISPERIDONE STRONGLY PREDICTS CYP2D6 GENOTYPE

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Background
The clinical response and side effect risk of many commonly used drugs rely on individual CYP2D6 activity. Genotyping can identify patients with absent, reduced or increased activity, but this is not offered - or reimbursed - in all pharmacological laboratories/ countries. Potentially, metabolic ratios of CYP2D6 substrates, calculated from therapeutic drug monitoring (TDM) measurements, could be an alternative to identify patients with a poor metabolizer (PM) or ultra rapid metabolizer (UM) genotype. The aim of this study was to evaluate the ability of metabolic ratios of risperidone venlafaxine to predict CYP2D6 PM and UM genotypes of CYP2D6 in a large patient TDM population.

Methods
The study was based on retrospectively analysed serum concentrations available from the TDM database at Center for Psychopharmacology, Diakonhjemmet Hospital, Oslo, Norway, in CYP2D6 genotyped patients. Areas under the receiver operating characteristic (ROC) curves were used to calculate sensitivity and specificity of risperidone and venlafaxine metabolic ratios to predict CYP2D6 a PM and UM genotype.

Results
A total of 425 patients treated with risperidone and 498 patients treated with venlafaxine were included. The sensitivity and specificity (%, 95% C.I.) of risperidone/9-OH-risperidone metabolic ratios >1 to predict CYP2D6 PM was 91% (76-97 %) and 86% (83-89 %), respectively. The sensitivity and specificity of N-desmethylvenlafaxine/O-desmethylvenlafaxine >1 to predict CYP2D6 PM was 98% (88-100%) and 93% (90-95%), respectively. The sensitivity and specificity of corresponding metabolic ratios <0.1 to predict CYP2D6 UM was 75% (51-90 %) and 68% (64-73 %) for risperidone, 71% (45-88%) and 51% (46-55%) for venlafaxine.

Conclusions
Metabolic ratios derived from TDM analyses of risperidone and venlafaxine are suitable for detecting CYP2D6 PMs, and to a lesser extent CYP2D6 UM. When accessible from TDM analyses, this information should guide the patient’s treatment to prevent adverse events or suboptimal response of CYP2D6 substrate drugs.

EVALUATION OF THE PLASMA UNBOUND FRACTION OF VORICONAZOLE

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Background
Voriconazole is a triazole antifungal used in the curative treatment of invasive fungal infections (IFI) and the prophylactic treatment of opportunistic fungal infections in immunocompromised patients. It is a drug for which therapeutic drug monitoring (TDM) is highly recommended.

Methods
In order to determine the best TDM marker, the pharmacological active form of the drug, represented by the unbound plasmaconcentration (Cu) and fraction (fu), has been studied using a method based on ultrafiltration and ultrasensitive liquid chromatography. As albumin is a likely factor inducing fluctuations in fu, the correlation between albumin levels and fu was carried out. Similarly, correlations between trough plasma concentrations (total concentration (Ct) and Cu) and both efficacy and safety markers were determined. Efficacy evaluation was based on
monitoring fungal antigens and cultures, while safety was monitored by measuring bilirubin levels.

Results
In vitro, using blank human plasma, the mean fu was determined at 32.3 ± 5.5% while in patients’ plasmas treated with Voriconazole, the median (5th-95th percentiles) of the unbound Voriconazole fraction was 22.95% (14.95 - 38.42). A high correlation was found (rho = 0.956, p < 0.001) between Ct and Cu, though there was no correlation between serum albumin levels and fu, except for some patients with severe hypoalbuminemia (< 25 g/L). Based on efficacy/safety correlations, a therapeutic window has been defined ranging from 4.5 to 6.5 mg/L and 1.5 and 2.0 mg/L for trough Ct and Cu, respectively.

Conclusion
For the first time, the relevance of new pharmacokinetic parameters such as Cu and fu have been explored and discussed, and our results support the current TDM protocol for Voriconazole.

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POPULATION PHARMACOKINETICS AND PHARMACOGENETICS OF ONCE DAILY TACROLIMUS FORMULATION IN STABLE LIVER TRANSPLANT RECIPIENTS.
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Background:
The once daily formulation of tacrolimus is an important immunosuppressive drug. Inter-patient variability in tacrolimus metabolism has been related to genetic variation in CYP3A4 and CYP3A5. However, in liver transplantation, both donor and recipient genotypes may affect tacrolimus pharmacokinetics. The primary objective of this study was to investigate the effect of CYP3A4*22 and CYP3A5*3 of both donor and recipient on once daily tacrolimus pharmacokinetics. The secondary objective was to develop a limited sampling model able to accurately predict exposure.

Methods:
Stable liver transplant patients receiving once daily tacrolimus (N=66) were included. Blood concentrations were determined with liquid chromatography tandem mass spectrometry (LCMS/MS). Population pharmacokinetic analysis was performed with patients of whom DNA was available (N=49) and demographic factors, CYP3A4*22 and CYP3A5*3 were tested as covariates. Moreover, a limited sampling model was developed using data of 66 patients. Modelling was performed using NONMEM 7.2 and R statistics was used for summary statistics and plotting.

Results:
Pharmacokinetics was best described by a two compartment model with delayed absorption. CYP3A5*1 carrying recipients engrafted with a CYP3A5*1 carrying liver had an average 1.65 fold higher clearance compared to non-carriers. CYP3A5*1 carrying recipients engrafted with a CYP3A5*1 non-carrying liver or vice versa showed a average 1.13 fold higher clearance compared with non-carriers. CYP3A4*22 was not significantly associated with once daily tacrolimus pharmacokinetics. Using 0, 1 and 3 hours postdose as limited sampling model resulted in significantly improved prediction of tacrolimus exposure compared with trough concentration.

Conclusions
Dose adjustments based on donor and recipient CYP3A5 genotype are indicated. In contrast, CYP3A4*22 appears not suitable as biomarker. The developed limited sampling model can be used to accurately estimate tacrolimus once daily exposure in liver transplantation.

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VALIDATED UHPLC-ORBITRAP MS ANALYSIS FOR DETERMINATION OF METOPROLOL AND A-HYDROXYMETOPROLOL IN SERUM FOR APPLICATION IN PHARMACOKINETICS
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Background

To determine metoprolol and its metabolite α-hydroxy metoprolol in serum for application in pharmacokinetics a convenient and accurate method of analysis is necessary. So far UHPLC with high resolution and accurate mass detection has rarely been used for therapeutic drug monitoring or pharmacokinetic studies.

Methods

We developed and validated a UHPLC method with a combination of a Hypersil Gold PFP column (3 µm, 150x2,1 mm), a gradient elution using mobile phase A: 2 mM aqueous ammonium formate with 0.1% formic acid and mobile phase B: methanol with 0.1% formic acid, a flow-rate of 0.4 mL/min, an Exactive® Orbitrap mass spectrometer (Thermo Scientific) as detector and metoprolol-d7 as internal standard. A simple sample preparation was developed by using water:acetonitrile (15:85) as precipitation reagent. The method was validated according to the EMA guideline on bioanalytical method validation [1].

Results

This method has a chromatographic run time of 15 min and linear calibration curves over the concentration range of 5.0-250 µg/L for both metoprolol and α-hydroxy metoprolol. Validation showed the method to be accurate, with a good precision, selective and with a lower limit of quantification of 2.0 µg/L for metoprolol and 1.0 µg/L for α-hydroxy metoprolol respectively.

Conclusion

This validated UHPLC-Orbitrap MS analysis can be used for application in pharmacokinetics.

References


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EVALUATION OF THE CHROMSYSTEMS MASSTOX ASSAY FOR ANTIFUNGAL THERAPEUTIC DRUG MONITORING (TDM) USING LC-TMS.

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Background: Triazoles and 5-flucytosine (5-Flu) display clinically relevant concentration dependent effect and toxicity relationships. TDM for voriconazole (VRZ), posaconazole (PSZ) and itraconazole (ITZ) and its metabolite hydroxitraconazole (HITZ) is recommended particularly for patients within critical care, haematology, neurology and renal settings. The Chromsystems MassTox assay for antifungal drugs allows for a relatively short development of a rapid and reliable LC-TMS method using deuterated internal standards. We describe the results of our verification of the manufacturers’ method.

Methods: Chromsystems MassTox reagents were used as described in the instructions. Sample preparation was performed according to the MassTox procedure using 50µL of calibrator/control/sample. Separation of the antifungal drugs was performed on a Waters Acquity/TQD system using a Chromsystems column at a flow rate of 0.6mL/min with a run time of 4min. Analytes were detected by positive ion electrospray ionisation using multiple reaction monitoring. Chromatographic, mass-spectrometer and TargetLynx parameters were adjusted to minimise known interference from PSZ glucuronide, emtricitabine, metformin, vigabatrin, atazanavir and ritonavir. Certified reference materials (Cerilliant) were used to assess linearity and recovery. Imprecision was assessed according to CLSI EP5-A2 and UKEQAS samples were used for method bias. Uncertainty of measurement was evaluated using IQC. The significance of bias, (vs ALTMs) was determined from UKEQAS reports. Uncertainty of bias was greater than bias for all the antifungal drugs measured and was not significant in the assessment of uncertainty.

Results: The assay was linear ($r^2$ >0.99) from 4.0 - 250mg/L (5-Flu), 0.05 - 10.0mg/L (ITZ and PSZ), 0.08 - 10.0mg/L (HITZ) and 0.05 - 20.0mg/L (VRZ). Total imprecision was <10% for all analytes at nominal IQC values. Mean recovery was within ±15% of target value for 98% samples (n=48/analyte. Relative expanded uncertainty was (%CV [range mg/L]) 12.7% [19-90] (5-Flu), 9.4% [0.37 - 1.38] (ITZ), 10.2% [0.48 - 1.73] (HITZ), 12.1% [0.62 - 3.27] (PSZ), and 12.9% [0.86 - 4.23] (VRZ).

Conclusions: The Chromsystems MassTox reagents kit for monitoring antifungal drugs is simple and precise with a relatively high throughput (15 samples/hour) and is an effective method for the routine monitoring of antifungal drugs.

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PEGASYS, RIBAVIRIN OR LEUCOGEN CAN INDUCE ANTI-RR ANTIBODY FOR HEPATITIS C PATIENT?

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A 38 female patient, complaining of weakness was admitted to the infectious disease department of the West China Hospital affiliated Sichuan University 8 years ago. She was diagnosis as infection with hepatitis C virus (HCV gene type was 1b) and treated with Pegasys (PEG-interferon) and ribavirin. Blood tests including HCV viral load, routine blood test (RBC), antinuclear antibody (ANA), liver function tests and renal function tests etc. were performed every half a year. Half year ago the results of Liver function tests and renal function tests was normal. HCV viral load was positive as 8.31×10^3 IU/mL, white blood cell (WBC) was 2.36×10^9/L significantly lower than reference range and ANA was negative. Then the leucogen tablets were administrated to raise the level of white blood cells. Now the patient was still very weak. WBC was still lower as 2.52×10^9/L. HCV viral load was negative. But the ANA was positive as anti-rings and rods (anti-RR) pattern. This pattern is characterized by 3-10 μm rods or rings with 2-5 μm in diameter scattered throughout the cytoplasm of the cell. The antigenic target of this reaction was identified as inosine-5'-monophosphate dehydrogenase type 2 (IMPDH2) which is a key enzyme in the synthesis of purine nucleotides. We speculated that it is possible that the drugs such as interferon, ribavirin and /or leucogen stimulates the occurrence of anti-RR. So far it is unknown why the anti-RR in HEP-2 cells occurs only in a fraction of patients with HCV. The relationship between this pattern and the clinical feature of the patient and the role of the anti-RR should be clarified in future study.

Picture 1: https://www.eventure-online.com/parthen-uploads/7/15017/img1_266252_AO021cPgL.jpg  
Caption 1: anti-RR antibody fluorescence pattern

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**PREDICTION OF MAXIMUM TOLERATED DOSE (MTD) FOR HYDROXYUREA TREATMENT USING BAYESIAN ADAPTIVE CONTROL IN CHILDREN WITH SICKLE CELL ANEMIA**

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**BACKGROUND:** Hydroxyurea (HU), a once-daily oral medication, has emerged as the primary disease-modifying therapy for patients with sickle cell anemia (SCA). Currently, HU is initiated at a relatively low dose (15-20 mg/kg/day) and then carefully escalated based upon the degree of bone marrow suppression to the maximum tolerated dose (MTD, ~25-35 mg/kg/day) over 6-12 months. The clinical and laboratory benefits of HU are maximized at MTD, but the prolonged escalation process delays the full benefits of HU therapy. The purpose of this study was to develop a Bayesian adaptive dosing strategy to reduce the time required to reach HU MTD in children with SCA.

**METHODS:** Pharmacokinetic (PK) data from a large prospective study¹ (807 serum concentration measurements in 96 children with SCA at baseline and MTD) were used to develop a population PK model using nonlinear mixed effects modeling (NONMEM 7.2). A D-optimal sampling strategy (PopED for R, version 0.1.2) was developed to estimate individual PK and HU exposure (area under the concentration-time curve (AUC)). The predictive performance of candidate sparse sampling schemes was evaluated by bias and precision estimation (MW/Pharm version 3.82). AUC targets were derived from the clinical trial data and defined as the mean AUC and 95% confidence intervals at MTD.

**RESULTS:** A one-compartment model with first order absorption and lag time appropriately described the PK of HU. Accounting for feasibility of PK sample collection for pediatric patients, the following sampling times were included in a new prospective protocol: pre-dose (baseline), 15-20 minutes, 50-60 minutes, and 3 hours after an initial 20 mg/kg oral dose. The predictive performance of the proposed sampling strategy was reasonable with a bias of 5.2% and precision of 12.4%. The mean target AUC_{0-1} at MTD was 115 mg-h/L.

**CONCLUSIONS:** We developed a PK model-based individualized dosing strategy for the prospective Therapeutic Response Evaluation and Adherence Trial (TREAT, ClinicalTrials.gov:NCT02286154). We will present preliminary data on Bayesian dose optimization versus traditional stepwise dose escalation of HU to MTD. This Bayesian approach has the potential to optimize the initial dosing of HU therapy such that the benefits of the MTD are achieved more quickly.

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**PLASMA CONCENTRATION MONITORING OF TERIFLUONMIDE USING DRIED BLOOD SPOT METHODOLOGY WITH LIQUID CHROMATOGRAPHY/TANDEM MASS SPECTROMETRY**
Background
Measurement of plasma concentrations of teriflunomide, a once-daily oral immunomodulator approved for the treatment of relapsing-remitting multiple sclerosis, may be warranted, particularly in female patients who are planning to become pregnant soon after discontinuing treatment or who become pregnant while receiving teriflunomide; based on animal data, a teriflunomide plasma level of <0.02 µg/mL is believed to be a non-teratogenic level. Teriflunomide is eliminated slowly from plasma, and an accelerated elimination procedure, using cholestyramine or activated charcoal, may be used to rapidly reduce plasma teriflunomide concentrations. The current bioanalytical assay for determination of teriflunomide concentration in plasma requires access to laboratory facilities for blood centrifugation and storage of plasma. We have developed a more convenient method, based on dried blood spot (DBS) methodology.

Methods
As analytical and clinical validations are required in order to switch from plasma to DBS (finger-prick sampling) methodology, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay method was developed and validated using a deuterium-labeled test compound as an internal standard for quantitation of teriflunomide in DBS over a range of 0.01 to 10 µg/mL.

Results
The method was specific and selective relative to endogenous compounds, with a process efficiency of ~88%, and without any matrix effect. Accuracy and precision values for intra-day and inter-day analyses were <20% at the lower limit of quantitation and <15% at all other concentrations tested. The quantitation of teriflunomide in DBS assay was not affected by blood deposit volume and punch position within spot, and the hematocrit level had a limited but acceptable effect on accuracy of the measurements. Teriflunomide was shown to be stable for at least 4 months at room temperature, and for at least 24 h at 37 °C with and without 95% relative humidity, to cover sampling, drying, and shipment conditions in the field.

Conclusions
Using samples from healthy subjects in a clinical study of efficacy of colesevelam hydrochloride (Cholestage®) to accelerate elimination of teriflunomide, the correlation between plasma and DBS concentrations was confirmed. Thus, DBS may provide an easier, more practical alternative sampling method for measuring plasma concentrations of teriflunomide.

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TOXICOLOGICAL SAFETY ASSESSMENT OF NOVEL CARBOHYDRAZIDES: AN ANTITUBERCULAR AGENT
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Nowadays multidrug-resistant tuberculosis strain which is unaffected by the major anti-tuberculosis drugs currently on the market. That’s why there is an urgency to develop new drugs and strategies to fight against tuberculosis or a tragedy may occur. A novel series of 5,6-dihydropyridazine-1 (4H)-carbohydrazides and its analogs was synthesized and characterized spectroscopically. All the compounds were characterized and screened for in vitro anti-tuberculosis (anti-TB) activity against Mycobacterium tuberculosis H37Rv strains by using resazurin assay utilizing microtiter-plate method. These compounds also showed good antibacterial. Thus, the high level of activity shown by the compounds (7a, 7e) suggests that these compounds could serve as leads for development of novel synthetic compounds with enhanced anti-TB activity. After that, a toxicological safety assessment was conducted on 5,6-dihydropyridazine-1(4H)-carbohydrazides, to predict safety with oral consumption by rats. Two genotoxicity studies were conducted and no evidence of mutagenicity or genotoxicity was observed in the presence or absence of a rat liver 59 metabolic activation system at concentrations up to 5,000 µg of compound/plate in a chromosomal aberration assay. Studies conducted in Wistar rats included a 14-day acute oral toxicity study, and a 90-day repeated oral toxicity study. A 6-month repeated oral toxicity study was conducted in rats. In the 14-day study, the NOAEL was determined to be 5 g/kg bw. While a few statistically significant (p<0.05) findings were observed in the 90-day Wistar rat study, it was considered to be a sound basis for conducting a 6-month study. In the 6-month rat study, the no observed effect level was concluded as 1,000 mg/kg bw/d, the highest dose group tested. Finally, in a developmental toxicity study in rats no fetal abnormalities related to administration of the test article were observed.
Background: Dried blood spot (DBS) immunosuppressant assay has a great promise as an alternative to routine TDM. The standard assay methods add internal standards to the extraction solution and then proceed for estimation. There may be differential recovery of analytes from the spots may affecting the assay result. With an aim to have standardized recovery, we pre-impregnated the cards with Deuterated internal standards before it was spotted and validated the assay.

Methods: We used Whatman-903 protein saver snap apart card and spotted 10 ul of isotope-labeled internal standard solution (4H2-Cyclosporine-A1000ng/ml; 13C3, 14H2-Tacrolimus100ng/ml; 14H2-Sirolimus and 14H2-Everolimus500ng/ml) and allowed to dry. 40 ul of calibration standards (6) and quality controls (3) were spotted to make a 13 mm diameter spot. After drying overnight at room temperature, the whole spot was punched and transferred into a microtube containing 300 ul 100% Methanol and 15 ul 0.4M ZnSO4, sonicated for 15 mins and left in the bath for more 15 mins. The tubes were centrifuged at 15000 rpm for 10 minutes and 20 ul of the extract was injected to the HPLC system with a short C18 column. The analytes were detected in ESI+ mode on Waters LC-MS/MS system with a run-time of 3 mins. We analyzed 38 clinical venous blood samples from patients using this method.

Results: Data analysis was performed on the Masslynx, MS-Excel, and EP Evaluator software. The validation showed linearity for tacrolimus (2.63-41.57ng/ml), sirolimus (6.43-41.52ng/ml), everolimus (2.58-44.85ng/ml) and cyclosporine (28.20-973.95ng/ml). The overall mean recovery (over the linear range) from 100% was 11.7%, 13.04%, 13.44% and 8.5% for tacrolimus, sirolimus, everolimus and cyclosporine respectively. The overall mean intra-day CV were 10.2%, 15.6%, 12.1% and 8.8% and inter-day CV were 6.2%, 10.1%, 11.4% and 10.3% respectively. The LLOQ was 0.5ng/ml for tacrolimus, sirolimus and everolimus and 8ng/ml for cyclosporine. There was no significant ion suppression observed. The patient samples were carefully chosen over the whole range of concentration and our initial results show that it is comparable to the results obtained by routine TDM within less than 10% variation. Stable recovery from the spots was observed after 7 days of storage at 25°C and 37°C.

Conclusion: This multiplex dried blood spot assay is promising and larger correlation studies are required to explore its utility as a routine TDM method.

A MODEL FOR THE DESCRIPTION OF DRUG ACCUMULATION AND AUC ESTIMATION OF CYCLOSPORIN A USING THE MEAN RESIDENCE TIME APPROACH

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Background: Several models have been developed to describe the pharmacokinetics of cyclosporin A (CsA), especially for Area Under the Curve (AUC) estimation for Therapeutic Drug Monitoring (TDM). So far, none of them enables the assessment of the variance in the estimation of the AUC. Experience has shown that CsA elimination could be approximated using a power function (linear function with a log/log scale), illustrating drug accumulation. The mean residence time (MRT) approach allows the interpretation of the time-concentration curve as a probability density function (PDF) and Burr distribution would likely be a candidate capable of both describing CsA MRT and of directly computing the AUC from the distribution parameters. Our objective was to evaluate the ability of the Burr probability distribution to describe CsA pharmacokinetics in allogeneic stem cell transplant (ASCT) patients and to develop a Bayesian estimator for AUC estimation using a limited sampling strategy (LSS).

Methods: We obtained 178 CsA PK profiles from 77 patients treated after ASCT at Limoges University Hospital. We used 147 profiles for model building and 31 for validation. Modelling was performed using Monolix® with a model describing the time-concentration curve with Burr distribution PDF. The predictive performance of the model was evaluated by goodness of fit and Visual Predictive Checks. Bayesian estimation of individual parameters was performed in the validation dataset using 3. AUC was computed from Burr distribution cumulative distribution function and compared to the trapezoidal AUC.

Results: The model accurately fitted the data with a mean relative bias of 0.96% (RMSE: 12.1%). Mean MRT, Cl and Vss were respectively 4.1h, 48L.h⁻¹ and 195L. VPC showed that simulated data matched observations adequately.
Mean SD in the AUC estimation directly computed from model parameters was 16% of the AUC. In the validation group, Bayesian posterior estimates of model parameters with LSS yielded good AUC prediction in comparison to the trapezoidal AUC (relative bias: 0.6%; RMSE: 9.8%).

Conclusion: We developed an innovative model which allows accurate description of drug accumulation and AUC estimation together with a confidence interval. This model could be of particular interest in TDM by giving relevant information for clinical practice.

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LIMITED SAMPLING STRATEGIES FOR MONITORING OF ERTAPENEM IN MDR-TB PATIENTS


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Ertapenem is widely used against various infectious diseases. No pharmacokinetic data for ertapenem have been reported for patients infected with multi drug resistant strains of M. tuberculosis (MDR-TB). The aim of this study is to develop a model to predict the area under the concentration-time curve, measured over the first 24h after ertapenem drug administration ($\text{AUC}_{0-24}$) during steady state, using a limited sampling strategy in patients with MDR-TB. The $\text{AUC}_{0-24}$ can subsequently be translated in $\%T>\text{MIC}$ and can therefore conceivably be used to predict antimicrobial activity in prospective studies. Twelve patients TB patients were included in this population pharmacokinetic model. Plasma concentrations of ertapenem were collected at steady state before administration at 1, 2, 3, 4, 5, 6, 8 and 12 hours and were stored at -80°C before analysis. Ertapenem plasma concentrations were analysed by a validated liquid chromatography-tandem mass-spectrometry (LC-MS/MS) method (14). Mean $\text{AUC}_{0-24}$ value for ertapenem was 621.3 (403.4 - 1101) mg*h/L. A population one-compartment model was developed from an ertapenem dose of 1000 mg once daily using the ‘KinPOP’ module using a two-stage Bayesian procedure (MW/Pharm 3.82, Mediware, The Netherlands). $\text{AUC}_{0-24}$ values of the population model were underestimated by mean -14 (range: -25.4 - 5.6) %. Assuming a MIC of 0.25 mg/L, 11 out of 12 patients exceeded a minimum of 40% time above MIC. A Monte Carlo simulation model was used to calculate Limited sampling strategies. This simulation model consisted of 1000 random patients drawn from the population pharmacokinetic model. For each patient sequential and robust limited sampling strategies were calculated at 4 sampling time points. We favour the time sampling points at 1 and 4 h, since these time points are applicable for other TB drugs. This study shows that $\text{AUC}_{0-24}$ of ertapenem can be estimated in patients with MDR-TB at only 2 sampling time points using a limited sampling in a population model and in a linear regression model.

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THE ASSOCIATION OF CYP3A5, CYP2C8 AND ABCB1 POLYMORPHISMS WITH EARLY RENAL INJURY IN CHINESE LIVER TRANSPLANT RECIPIENTS RECEIVING TACROLIMUS

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Objective: The purpose of this study is to explore the association of CYP3A5, ABCB1 and CYP2C8 polymorphisms with the risk of developing early kidney impairment in Chinese liver transplant recipients receiving tacrolimus.

Methods: CYP3A5, ABCB1 and CYP2C8 polymorphisms were genotyped in the Chinese all liver transplant recipients in the study receiving Tac for at least two years by polymerase chain reaction and high-resolution melting method. Serum cystatin C and urine microprotein (αM, MA, TRU and IgU) of liver transplant recipients were used to determine not only if the recipients had the early renal injury, but also the site of injury.

Results: We documented three genotypes of CYP3A5 and ABCB1 and only two genotypes of CYP2C8 in our cohort. The levels of cystatin C and as well as all the four indicators of the urine microprotein in the recipient group were significantly higher than those in the control group ($P<0.05$). The concentrations of TRU differed significantly in each CYP3A5 genotype group ($P<0.05$). Based on diverse CYP2C8 genotypes, we divided all the recipients into two groups: CYP2C8*1*1 group and CYP2C8*3*1 group. The concentrations of αM and Cys-C differed significantly between the two groups ($P<0.05$).

Conclusions: CYP2C8*3 and CYP3A5*3 appear to be predictive of risk of Tac-induced early renal impairment. CYP3A5*3 was associated with the risk of early renal glomerular lesion, while CYP2C8*3 was associated with the risk of the tubulointerstitial injury. ABCB1 polymorphisms (both C3435T and C1236T) were not associated with the
early renal injury in liver transplant recipients.

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CLINICAL TOXICOLOGY OF POTENTIALLY SEVERE OLANZAPINE INTOXICATION
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Olanzapine is widely used as an atypical antipsychotic drug. Reported toxicological effects include somnolence, mydriasis, blurred vision, respiratory depression, hypotension, extrapyramidal and anticholinergic reactions. Only few cases of suicidal overdose of olanzapine have been described, even less reports include measurement of serum blood levels. We report a case of suicidal nonlethal overdose requiring unexpected minimal medical intervention.

A 26-year-old male was found unconscious and was last seen on the internet at approximately 10pm the previous night. Primarily, several empty tablet blister packs of olanzapine 5mg (120 tablets, total of 600mg) were found. The estimated time that he was found, was 24-36 h after ingestion. Upon arrival, we saw a less conscious male with a free airway, respiratory rate 18 breaths/minute, bilateral normal breath sounds, pulls rate 95 beats/minute, blood pressure 115/60 mmHg, normal heart sounds and capillary refill less than 2 seconds. Patient was somnolent, Glasgow Coma Scale upon arrival was 4-6-4 and further neurological examination revealed no abnormalities, especially normal tendon reflexes and no rigidity. Body temperature was 37.4 degrees Celsius. Laboratory results showed an elevated CRP, 363 mg/L, and elevated liver enzymes were detected three days after admission (ASAT 90 UI, ALAT 114 UI, LD 276 UI, GGT 106 UI). Focus was on the drug olanzapine because excessive ingestion was expected on account of the empty blisters that were retrieved. The diagnosis of olanzapine intoxication was confirmed by the analysis of two sequential whole blood samples. The initial concentration of olanzapine was 1170 
ug/L; a second measurement after 6 days showed a concentration of 52 ug/L, resulting in an calculated elimination halftime of approximately 30 hours. This case describes a markedly mild clinical course after severe overdose of olanzapine resulting in drug levels tenfold higher than the potential toxicity level of 100 mg/L at time of hospital arrival, with no apparent pharmacological or clinical sequelae. Supportive care, including invasive ventilation, is common in patients facing a massive olanzapine overdosing. This mild clinical course is not what is known from literature or to be expected from pharmacological profile and offers valuable information for clinical decision-making in similar cases.

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EXTERNAL EVALUATION OF PUBLISHED POPULATION PHARMACOKINETIC MODELS OF CYCLOSPORINE IN ADULT RENAL TRANSPLANT RECIPIENTS: WHAT DO WE LEARN?
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Background
Cyclosporine (CsA) is an immunosuppressant widely used after renal transplantation. Due to its large pharmacokinetic variability and narrow therapeutic range, therapeutic drug monitoring was recommended and population pharmacokinetic (PPK) models have been developed to optimize dosage regimen. However, no research was conducted to evaluate the predictability and transferability of these models. This study aimed to (1) evaluate their predictive performance and (2) identify the potential influencing factors.

Methods
Published PPK models of CsA in adult renal transplant recipients were screened. The predictive performance was evaluated using an independent dataset including 506 through concentrations (C0) and 633 2-hour post-dose concentrations (C2) collected from 48 patients. (1) Prediction-based diagnostics including median prediction error (MDPE), median absolute prediction error (MAPE), the percentage of prediction error within ±20% (F20) and ±30% (F30), (2) simulation-based diagnostic method normalized prediction distribution error (NPDE) were applied to evaluate the model predictability. Bayesian forecasting was also conducted to assess the influence of prior information on model predictive performance.

Results
14 PPK models were obtained, including 11 compartmental models (CMs) and 3 non-compartmental models using Michaelis-Menten formula (NCM-MMs) to describe the nonlinearity of CsA dose-concentration relationship. Body-weight, postoperative day and total bilirubin were the three most frequently identified covariates. For C0, NCM-MM developed by Wang et al. performed best with MDPE -10.4%, MAPE 13.5%, F20 67.2%, F30 77.8%; for C2, CM
developed by Wu et al. performed best with MDPE 3.9%, MAPE 26.5%, F20 37.5%, F30 56.5%, which both
developed base on Chinese population. No models met the criteria of NPDE tests. Then, the predictability of these
two modeling strategies was compared using base model without covariates, NCM-MM performed better than CM
(C0: MDPE 1.1% vs 9.9%, MAPE 19.7% vs 34.4%, F20 50.9% vs 30.1%, F30 67.0% vs 44.4%; C2: MDPE -0.5% vs
2.2%, MAPE 20.8% vs 30.6%, F20 48.2% vs 33.3%, F30 62.7% vs 49.6%). Moreover, Prior knowledge improved
the model predictive performance significantly.

Conclusions
Besides the conventional CMs, data-driven NCM-MM is also a potential strategy to describe CsA dose-
concentration relationship. Involved covariates, race, assay methods and modeling strategies were the factors
influencing model transferability.

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SIMULTANEOUS DETERMINATION OF NILOTINIB, IMATINIB, DASATINIB AND ERLOTINIB BY ULTRA-
PERFORMANCE LIQUID CHROMATOGRAPHY WITH MASS SPECTROMETRIC DETECTION
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Abstract

BACKGROUND: Nilotinib, imatinib, dasatinib and erlotinib are important anti-cancer agents for the treatment of
chronic myelogenous leukemia and non-small cell lung cancer, respectively. Interindividual differences in absorption
and metabolism exist and pharmacokinetic interaction with other drugs are described. In case of poor efficacy,
suspicion of nonadherence or excessive side effects therapeutic drug monitoring may be useful. Unlike venipuncture
blood, dry blood spot sampling is relatively painless and non-invasive.

OBJECTIVES: To develop and validate an ultra-performance liquid chromatography mass spectrometric detection
method for the quantification of nilotinib, imatinib, dasatinib and erlotinib in dry blood spot samples.

METHODS: The dry blood spot sample was obtained by punching a 7.5 mm diameter paper disk (DMPK-C) from
the center of the dried blood spot. To the disc 200 µL of methanolic internal standard solution was added. The
samples were shaken for 15 minutes and thereafter centrifuged at 18.500 g during 5 min. An aliquot of 7.5 µL of the
supernatant was injected into the system. Chromatographic separation was achieved on a Phenomenex Synergi
Fusion® RP18 column (2.5 µm, 50 x 4.6mm) using deionized water and methanol, both containing 0.1% of formic
acid and 2 mM ammonium acetate at flow rate of 0.400 mL/min. The chromatographic run time was 3.0 minutes.
Mass spectrometric detection was achieved with an electro spray ionization source, operated in the positive mode.

RESULTS: The method is found to be selective and specific for the quantification of these tyrosine kinase inhibitors.
The calibration curves were found to be linear from 17-4100 µg/L for nilotinib, imatinib, erlotinib and 0.9-210 µg/L for
dasatinib. Between- and within run accuracy and precision were ranged between 85-115% for all compounds.
Parameters such as haematocrit and blood spot volume showed no effect on the performance of the method.

CONCLUSIONS: A simple pre-treatment and sensitive ultra-performance liquid chromatography-tandem mass
spectrometric detection method was developed and validated for the simultaneous quantification of nilotinib,
imatinib, dasatinib and erlotinib in dry blood spot samples.

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INPATIENT POLYPHARMACY AMONG CHILDREN WITH LEUKEMIA OR LYMPHOMA RECEIVING IV
ANTIBIOTIC THERAPY WITHIN THE FIRST YEAR FROM DIAGNOSIS
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Background: Polypharmacy is often regarded as an important healthcare issue among the elderly and is well
recognized as the key risk factor for adverse drug reactions. While children with cancer who are being treated for
infection are considered at a high risk for adverse drug reactions, little is known on the degree of polypharmacy in
pediatric cancer patients. This study evaluated polypharmacy in children receiving the antibiotic vancomycin within
the first year after diagnosis with leukemia or lymphoma.

Methods: A multicenter retrospective study of patients from age 2 to 18 years of age who received ≥2 doses of IV
vancomycin between 01/2006 and 12/2012 was performed using an EMR database. Cancer diagnoses were
identified via a validated hospital registry. Generic drug names (International nonproprietary name) were aligned with
hospital pharmacy administration records using a drug dictionary. Statistical analyses were performed using Prism 6
(GraphPad®) and R. Heparin, artificial tears, IV fluids, fat emulsions, solutions for parenteral nutrition were excluded.

**Results:** During the study period, 100 patients received vancomycin (171 total hospitalizations) within the first year of cancer diagnosis. The median hospital stay was 23 days (IQR 6-31) during which 95% of patients received 5 or more unique drugs. The overall median number of unique medications per hospitalization was 18 (IQR 12-30).

However, adolescents (12-18 years of age) experienced the highest drug exposure rate per hospitalization with a median of 25 (IQR 16-38) compared to children 2-5 years of age (11 [IQR 7-21], p<0.01) or those 6-11 years of age (17 [16-38], p<0.05) but not to those between 28 days and 1 year of age. The most common medications used were acetaminophen (94%), diphenhydramine (81%), sulfamethoxazole and trimethoprim (75%), ondansetron (74%), oxycodone (73%), ceftazidime (66%), lorazepam (58%), voriconazole (48%), morphine (45%), and gentamicin (44%).

**Conclusions:** Medication exposure was high among children with leukemia or lymphoma receiving IV vancomycin within the first year after cancer diagnosis, particularly for adolescents. The issue of polypharmacy has received significant attention among older adult patients, however the management of polypharmacy in a complex and high risk pediatric population clearly warrants more investigation.

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**MULTIPLEX TARGETED LONG AMPLICON SEQUENCING METHOD FOR CYP2D6 GENOTYPING USING THE PACBIO RSII**

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Cytochrome P450 2D6, is among the most important enzymes involved in drug metabolism. Specific variants in the CYP2D6 gene result in different CYP2D6 enzyme amount and activity. Different technologies exist to determine these variants. However, information on which variants reside on the separate alleles cannot be determined with these assays.

Targeted long amplicon sequencing using the PacBio RSII third generation sequencing platform is able to sequence multi-kb amplicons without the need for fragmentation steps, obtain high accuracy consensus sequences (QV50) and delivering fully phased variant information for separate alleles.

We developed a new PCR based multiplexing strategy for long amplicon variant profiling using the PacBio RSII platform. When applied for Cyp2d6 profiling, for each individual, the ~6.6 kb gene is first amplified with a pair of gene-specific primers with forward and reverse M13 sequence tails. A sample barcode is subsequently introduced in a second PCR using a set of re-usable M13-tailed barcode primers. Barcoded samples are then pooled in equimolar amounts and processed for PacBio sequencing. Using this setup we sequenced the Cyp2d6 gene for 12 individuals with different Cyp2d6 activity. Full length Cyp2d6 sequences were obtained after sequencing, barcode demultiplexing and processing the data with the Long-Amplicon-Analysis software. Moreover, for four individuals, two different allele sequences were evident from the data, indicating the exact distribution of multiple heterozygous SNPs over the two separate Cyp2d6 alleles. Predicted genotypes were concordant with those obtained with the AmpliChip.

In conclusion, we have setup a new, versatile and easy to use method for targeted long amplicon sequencing on the PacBio RSII and successfully applied it to obtain fully phased allele sequences for Cyp2d6. Moreover, with minor modifications this method can in principle be applied for targeted long amplicon sequencing of other gene panels.

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**RELATIVE INFLUENCE OF DONOR AND RECIPIENT CYP3A5*3 VARIANT GENOTYPE IN PK-TACROLIMUS OF LIVER TRANSPLANT RECIPIENTS**

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**Abstract**

**Background:** Relative influence of donor and recipient CYP3A5*3 variant genotype in PK- Tacrolimus (TAC) of liver transplant recipients is inconclusive. The objective of this study was to investigate the influence of CYP3A5 polymorphism of recipients as well as donors on the (TAC) pharmacokinetics in Argentinean population.

**Methods:** We evaluated the influence of CYP3A5 polymorphism of recipients as well as donors on the Tacrolimus (TAC) pharmacokinetics in 24 stable liver transplant patient and 77 pediatric patients for two years of follow-up.
**Results:** The percentage of CYP3A5 expressers (*1/*1 and *1/*3 genotypes) were between 17% and 37%. In the immediate posttransplant period, recipient expression of a CYP3A5*1 allele seemed to have the greatest influence on TAC pharmacokinetics with donor expression of a CYP3A5*1 allele becoming more important with increasing time after transplant. In donor no expressors the Co/dose ratio is influenced by age, fluconazole, and time after transplant, while in donor expressors the Co/dose is only influenced by donor CYP3A5 genotype.

**Conclusions:** The frequency of CYP3A5 expressers in Argentinean population seemed to be higher than reported in Caucasians. The donor expression of a CYP3A5*1 has a differential and dynamic effect in the time after transplant, with serious implications for the clinical monitoring of patients.
The developed method allows the determination of the four DOACs in serum samples from 5 nM up to 800 nM, which covers concentration both beneath and above the therapeutic range. The method development was quite straightforward, with only a few minor problems on the way, which mostly concerns dabigatran. The problem was related to solubility/stability problems of dabigatran in standard/working solutions. The matrix effect was evaluated and an ion enhancement was observed for dabigatran (180-190%). However, when corrected with internal standard the observed matrix effect was reduced significantly (105-112%).

**Conclusion**

Since the developed and validated method is quite fast, the turnover time from sample arrival to sample report is possible within one day.

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**PHARMACOKINETICS AND QT PROLONGATION OF SINGLE DOSE ESCITALOPRAM IN THE HEALTHY ELDERLY COMPARED WITH THE YOUNG**

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**BACKGROUND:** Escitalopram is widely used selective serotonin reuptake inhibitor for major depressive disorder and generalized anxiety disorder. The objective of this study was to evaluate the pharmacokinetic characteristics and QT prolongation after single oral administration of escitalopram in the elderly compared with the young.

**METHODS:** Twelve healthy Korean elderly subjects (> 65 years) and 35 healthy younger adults (20-40 years) received single oral dose of escitalopram 20 mg tablet. For pharmacokinetic analysis, serial blood samples were collected up to 48 hours post dose. Liquid chromatography coupled with tandem mass spectrometry was used to determine the plasma concentrations of escitalopram. The pharmacokinetic parameters were derived using the non-compartmental method. Electrocardiogram was done for QT measurement until 48 hours post dose. The measured QT was corrected using Bazett (QTcB) and Fridericia method (QTcF) and the difference of QT from baseline (dQT) were calculated.

**RESULTS:** The mean ages of elderly and younger subjects were 68 and 26 years, respectively. The C\text{max} and the AUC\text{0-}\text{t} for escitalopram (mean ± standard deviation) in elderly subjects were 23.4 ± 4.7 ng/mL and 564.9 ± 124.4 h·ng/mL; in younger adults, 23.0 ± 4.6 ng/mL and 563.5 ± 142.5 h·ng/mL. The geometric mean ratios (90% confidence interval) of the elderly to the young were 1.02 (0.90 - 1.17) and 1.02 (0.88 - 1.18) for C\text{max} and AUC\text{0-}\text{t} of escitalopram, respectively. Mean apparent volume of distribution was slightly greater (1175 L versus 1070 L) and apparent clearance was lower (21.4 L/h versus 26.5 L/h) in the elderly than in the young, but the differences were not statistically significant (p=0.280 and p=0.083, respectively). The mean dQT-time profiles were similar between elderly and younger adults. Mean values of maximum dQT were slightly lower in elderly than in younger adults, but not statistically different (p=0.059 for QTcB, and p=0.677 for QTcF).

**CONCLUSION:** Elderly and younger adults showed similar pharmacokinetic characteristics of escitalopram after single oral administration. This study supports previous reports that escitalopram could be used without dose adjustment, and suggests no additional concern on QT prolongation compared to younger adults in elderly without comorbidities.

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**IDENTIFICATION OF DIFFERENT PATTERNS OF DABIGATRAN IN VIVO BIOACTIVATION IN PATIENTS ON MAINTENANCE ANTICOAGULATION THERAPY**

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Dabigatran (DAB) is a novel, synthetic, competitive, rapidly acting and reversible inhibitor of thrombin. Since thrombin enables the conversion of fibrinogen into fibrin during the coagulation cascade, its inhibition prevents the development of thromboses. DAB is poorly absorbed following oral dosing thus it is orally administered in the form of the pro-drug dabigatran etexilate which is rapidly absorbed and converted in the two intermediate pro-drugs BIBR1087SE and BIBR951CL (pharmacologically active) that subsequently are converted into DAB. Due to their intermediate nature, BIBR 1087 SE and BIBR 951 CL have been detected only in trace amounts in human plasma samples from healthy volunteers. Here we investigated the bioconversion of dabigatran etexilate to DAB in patients...
to support therapeutic drug monitoring we developed a population pharmacokinetic model of rilpivirine.

**Background**

Eveline Roelofsen

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VALIDATION OF A RILPIVIRINE POPULATION PHARMACOKINETIC (PK) MODEL

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The aim of the study was to assess the usefulness of mycophenolic acid (MPA) trough concentration monitoring for mycophenolate mofetil (MMF) dose optimization in heart transplant patients.

Study group consisted of 234 patients (187M, 47F; age 55 y. (17-89)) after OHT treated with MMF + tacrolimus (TAC, n=655), MMF + cyclosporine (CSA, n=141), MMF + everolimus (EVE, n=110) or MMF alone (n=25). MPA concentration was measured using specific HPLC-UV method in 931 steady-state trough plasma samples.

MPA trough concentration presented high interpatient variability within usually administered doses. Analyzing data by sample, mean MMF (CellCept®) dose was 1685 ± 577 mg/d (500-3000) resulting in mean MPA trough concentration of 2.03 ± 1.13 µg/mL (0.18-7.80). MPA concentration was not correlated to the dose at all (r²=0.0009, p>0.05) yielding 2.00 ± 1.00 (0.18-5.51), 2.17 ± 1.18 (0.33-5.47), 2.01 ± 1.19 (0.21-7.80) and 2.12 ± 1.15 (0.57-6.04) µg/mL for MMF doses: 1000, 1500, 2000 and 3000 mg/d, respectively (NS). That correlation was still weak when dividing samples by co-administered drug: r²=0.0098, r²=0.0339, r²=0.0129 for TAC, CSA, EVE, respectively, as well as by sex: r²=0.0012 (men) and r²<0.0001 (women). In samples taken from women (n=168) MMF dose was lower (1552 vs. 1715 mg/d, p=0.0008) than in those obtained from men (n=763), but higher when corrected for body weight (24.81 vs. 22.39 mg/d/kg, p=0.0071), however, the differences were not reflected in MPA concentration. Similar findings were noted analyzing data by patient: mean MMF dose was 1728 ± 481 mg/d (500-3000) resulting in mean MPA concentration of 1.95 ± 0.82 µg/mL (0.21-7.80) and the correlation remained weak (r²=0.0379, p=0.0028). The results were similar when dividing samples by co-administered drug: r²=0.0016, r²=0.0815, r²=0.0002 for TAC, CSA, EVE, respectively, and by sex: r²=0.0461 (men) and r²=0.0114 (women). Again, in women (n=47) MMF dose was lower (1613 vs. 1756 mg/d, p=0.0349) than in men (n=187), but higher when corrected for body weight (25.45 vs. 22.30 mg/d/kg, p=0.0392), however, the differences were not reflected in MPA concentration. MPA trough concentration was not correlated to MMF dose in a group of heart transplant patients suggesting the need for TDM guided individualized therapy.

**MONITORING MPA TROUGH CONCENTRATION IN HEART TRANSPLANT PATIENTS.**

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Here we characterize the pharmacokinetics of DAB in patients on oral anticoagulation therapy. Different patterns of drug activation were identified, and some cases of suboptimal DAB bioconversion were identified. The clinical evidence of these findings warrants future investigations.
Aim
To validate the population PK model of rilpivirine by means of external data and to establish the percentage of patient with fitted concentration for Cmax and Cmin outside the therapeutic range.

Methods
The original model population was derived from a single HIV-treatment centre in The Hague. The population used to validate the model consisted of a combination of different patients from the same centre and the Dutch national ATHENA cohort.
Predicted concentrations before and after Bayesian fitting with MWPharm 3.70 (Mediware, NL) were compared to measured concentrations using linear regression.
The percentage of patients with a predicted Cmin > 0.044mg/L or Cmax < 0.600mg/L was calculated.

Results
The original model, created on 53 plasma concentration from 50 patients, was validated by an external population of 99 patients (129 plasma concentrations). The correlation coefficient R for the original population, the validation population and the combined population was 0.453, 0.096 and 0.184 before and 0.892, 0.9998 and 0.974 respectively after fitting.
Of all 149 patients 126 (84.6%) had a predicted Cmin > 0.044mg/L and 147 (98.7%) a predicted Cmax < 0.600mg/L. In the original model population and the validation population these numbers were 47 (94.0%) and 79 (79.2%) for Cmin and 50 (100.0%) and 97 (98.9%) for Cmax, respectively.

Conclusion
The pharmacokinetic model of rilpivirine poorly predicts rilpivirine plasma concentrations of patients without a measured plasma concentration. This is most likely due to multiple covariates like food and co-medication and lack of data in the absorption phase. The model performed better in the original population, possibly because of less selection bias in this population due to routine testing of rilpivirine. A good correlation between prediction and plasma concentration was seen after Bayesian fitting.
Although the vast majority of patients had plasma concentrations within the therapeutic range, approximately 15% of patients had a subtherapeutic Cmin, placing them at risk for virological failure. Further analysis is required to identify patients at risk for suboptimal rilpivirine concentrations to facilitate targeted TDM.

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A CASE REPORT: SUSPECTED XTC INTOXICATION WITH UNSUSPECTED GUEST!
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Context
We present a case of multiple organ dysfunction syndrome and death of a patient within 36 hours after ingestion of contaminated MDMA tablets and powder.

Case details
A 27-year old, well-educated man, was found somewhere in open field during a big dance event. The patient was unconscious, had a body temperature of 42 degrees Celsius, tachycardia of 150 beats/min and a blood pressure of 80/40 (53) mm Hg. The friends of the patient estimated he had only taken 1.5 or 2 tablets of 3,4-methylenedioxyamphetamine (MDMA). In the hours following initial presentation the patient developed progressive multiple organ dysfunction syndrome with a maximum CK 82.000 U/L, LDH 10.380 IU/L, ASAT 7.812 IU/L, ALAT 7.493 IU/L, Lactate 17 mmol/L, creatinine 250 umol/L, BUN 13.6 mmol/L and aPTT 82.4 s. Despite extensive treatment with fluids, blood, plasma, thrombocytes, circulatory- and ventilatory support and central-veno-venous-hemofiltration on an ICU in a teaching hospital, the patient died within 36 hours. A routine immunoassay toxicology screen on Drugs of Abuse (EMITII, VIVA-E, Siemens, USA) of his urine sample showed the presence of amphetamines and MDMA in particular. A blood sample, and white powder found in the patient's pocket was sent in for further analysis. The serum MDMA level exceeded 600ug/L, well above reference values (110 - 350ug/L). The white powder was identified as MDMA. Further toxicology screening using LC-MSn (Toxtyper®, Bruker, Germany) revealed the presence of 3.4-methylenedioxyamphetamine (MDA, metabolite of MDMA) and paramethoxymethamphetamine (PMMA) in the blood and urine of the patient. PMMA is a contaminant of MDMA tablets and powders.

Discussion
PMMA has a delayed onset of action of the stimulant effect as compared to that of MDMA. This may have caused an accidentally overdosing of the MDMA as was confirmed by the toxic MDMA serum level in the patient. Furthermore, PMMA has a very small therapeutic window. In cases of overdose, PMMA and MDMA, initially induce tachycardia, hyperthermia and hypertension. These clinical symptoms can ultimately lead to shock, facilitate MDMA overdosing.
The pharmacokinetics of mycophenolic acid (MPA) depends on co-administered calcineurin inhibitor. The aim of the study was to compare MPA plasma concentration in heart transplant patients treated with mycophenolate mofetil (MMF) in co-therapy either with cyclosporine (CSA), tacrolimus (TAC) or everolimus (EVE).

A number of 906 steady-state trough plasma samples for routine MPA monitoring were obtained from 232 patients (186M, 46F; age 54 y. (17-88)) after OHT treated with MMF + TAC (n=655), MMF + CSA (n=141) or MMF + EVE (n=110). MPA concentration in plasma was determined by a specific HPLC-UV method. Mean MMF (CellCept®) dose was 1715 ± 562, 1846 ± 656 and 1334 ± 417 mg/d in TAC, CSA and EVE groups, respectively (p<0.05 for each pair; Mann-Whitney U test). MPA concentrations were lower in patients co-receiving CSA: 1.50 ± 0.88 µg/mL vs. 2.13 ± 1.18 (TAC, p<0.0001) or vs. 2.12 ± 0.93 (EVE, p<0.0001) also if corrected for daily dose: 1.00 ± 0.87 L^1 x 10^3 (CSA) vs. 1.38 ± 0.89 (TAC, p<0.0001) or vs. 1.72 ± 0.87 (EVE, p<0.0001); dose-corrected MPA concentration was significantly higher (p<0.0001) in EVE vs. TAC group as well.

The group of 100 patients having at least four MPA measures was selected from this database and consisted of: 72 patients (60M, 12F) with 486 samples (TAC); 14 patients (11M, 3F) with 66 samples (CSA) and 14 patients (12M, 2F) presenting 95 samples (EVE). MPA values averaged in individual patient confirmed the results presented above for total number of samples i.e. MMF dose of: 1658 ± 468, 1774 ± 747 and 1328 ± 398 mg/d (p<0.05 only for TAC-EVE) resulted in MPA concentration of: 2.13 ± 0.68, 1.52 ± 0.59, and 2.22 ± 0.53 µg/mL for TAC, CSA and EVE treatment groups, respectively (p<0.01 for CSA-EVE and CSA-TAC). Dose corrected MPA concentration was: 1.14 ± 0.89, 1.43 ± 0.73 and 1.79 ± 0.62 L^1 x 10^3 in TAC, CSA and EVE groups, respectively (p<0.05 for each pair).

Our observations supported the hypothesis on lack of pharmacokinetic interaction between MPA and EVE (similarly to MPA-TAC but contrary to MPA-CSA) suggesting lower MMF doses when combined with EVE.

We developed a method for the analysis of five immunosuppressants and creatinine in dried blood spot (DBS) samples to facilitate therapeutic drug monitoring for transplant patients outside the hospital. The analysis method was based on a previous study (Talanta 115 (2013) 47-54) with the following changes. The card type was changed to Whatman DMPK-C. The linear range of cyclosporin was shortened from 2000 ng/mL to 1000 ng/mL in order to change the quadratic curve fit to a linear curve fit. Since the hematocrit (HT) and concentration of the substance affected spot formation and recovery, the HT effects were now evaluated at a HT range of 0.23 to 0.53 L/L and at 3.0 and 10.0 ng/mL for tacrolimus, sirolimus and everolimus and at 60 and 200 ng/mL for cyclosporin A. Within this range, the HT effects were all within 15% bias (normalized at HT 0.38 L/L), except for Cyclosporin A at HT 0.53 L/L (bias -17.8%).

Validation of the adjusted linear range and HT effects at concentrations that are expected for the patient population (outpatients and trough levels) showed far less extreme HT effects and now provided acceptable biases (without HT correction).

The possibility of measuring of mycophenolic acid with the current LC-MS/MS method was shown earlier (Ther Drug Monit 2009;31:116-125). The simultaneous extraction of mycophenolic acid, the other immunosuppressants and creatinine showed to be possible with the previously developed extraction procedure. Since creatinine free blood and thus DBS is not available and can not be prepared without changing the matrix, this issue had to be overcome. Therefore, our analytical method was validated using three different calibration strategies: a 7-point calibration curve, one point calibration curve and the use of a creatinine[^1][H_3] calibration curve. For the analysis of creatinine a re-injection of 0.1 µL was performed on the same LC-MS/MS system with different chromatography settings and the use of a column switch (Anal Bioanal Chem (2015) 407:1585-1594).

The DBS extraction and LC-MS/MS analysis method was fully validated for all substances based on the FDA guidelines including additional DBS parameters like HT, concentration and spot volume effects.
THE 4-HOUR-AFTER-DOSE MPA CONCENTRATION IS USEFUL FOR INFECTION DIAGNOSIS IN ALLORENAL RECIPIENTS
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Objects: To explore whether immunosuppressive agent (FK506 and MPA) concentration measurements are useful in the diagnosis of infection in allo-renal recipients. Method: 146 allo-renal recipients from West China Hospital (Sichuan University, PRC) were included in this research, 76 of whom were with clinical-diagnosed infection (infectious group) and 70 were stable allo-renal recipient without infection nor adverse reaction (noninfectious group). All the patients were treated with FK506-MMF-prednisolone triple immunosuppressive regimen. The pre-dose blood sample was taken for measuring FK506 concentration (C₀). The pre-dose, 0.5h-after-dose, 2h-after-dose and 4h-after-dose blood sample were taken for measuring MPA concentrations and calculating the AUC₀₄ of MPA (AUC=14.81+0.8C₀₂+1.56C₂+4.8C₄). The concentration of FK506 or MPA was measured by EMT method (the automatic analyzer V-TWIN, SIEMENS). Results: The average pre-dose whole blood concentrations of FK506 were comparable between the infectious group (Male/Female: 56/20, average age: 35.24±10.81) and the non-infectious group (Male/Female: 50/20, average age: 33.21±12.32) (p>0.05). Similarly, the average MPA-C₀, MPA-C₂, and MPA-AUC₀₄ were all comparable between the infectious group and the non-infectious group. Interestingly, the average MPA-C₂ was significantly higher in the infectious group than that in the non-infectious group (p=0.009). Conclusion: Our data indicates that the MPA-C₂ is useful for infection diagnosis in allo-renal recipients. Further large ample size study is needed to confirm its clinical application value.

Picture 1: https://www.eventure-online.com/parten-uploads/7/15017/img1_266619_8t9XH6OJb.png
Caption 1: Box plot for drug concentration

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LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR SIMULTANEOUS QUANTIFICATION OF ECHINOCANDINS IN HUMAN PLASMA: APPLICATION TO PEDIATRIC PATIENTS
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The opportunistic fungal infections are an important cause of morbidity and mortality in the pediatric oncohematologic patients, both subjected to intensive chemotherapy and hematopoietic stem cell transplantation (HSCT). The echinocandins, micafungin (MCF), caspofungin (CSF) and anidulafungin (ANF) are a new class of antifungal drugs with a potentially fungicidal activity against Candida spp and fungistatic activity against Aspergillus spp. They display concentration-dependent fungicidal activity and seems that efficacy correlates best with Cmax or AUC/MIC ratio. The information on the pharmacokinetics of the echinocandins in infants and children are scarce and mainly derived from adult patients.

We developed and validated an HPLC-MS/MS assay for simultaneous quantification of plasma concentrations of echinocandins. The detection was performed by a TSQ Quantum Mass Spectrometer fitted with an ESI (+) and SRM mode. The simple sample preparation requires 100µL of plasma, followed by protein precipitation with acetonitrile and formic acid (0.5%). Correlation coefficients were 0.99 or better.

Clinical study is ongoing for the assessment of the relationships between efficacy, safety and exposure to echinocandins in pediatric population: we started analyzing MCF plasma level in a pediatric (13ys, 41kg) patient with AML in relapse, who was treated with 50 mg/day 1 hr e.v. for oral candidemia during reinduction therapy, and subsequently treated prophylactically during the second allogeneic HSCT procedure. The plasma concentration obtained at the end of the first MCF cycle (Cmax) was 3.0 µg/mL; the Cₘₑₙ on day +4 of HSCT prophylaxis was 2.2 µg/mL, and C₀ₘₑₙ and Cₘₐₓ on day +5 dose were 6.0 and 8.7 µg/mL respectively. We measured ANF in a pediatric liver transplant recipient (16 ys) who received a combined voriconazole and ANF therapy for probable invasive lung aspergillosis. Anidulafungin was administered as 1 hr infusion (single dose) at 3 mg/kg/day for the first day (loading dose) and at 1.5 mg/kg/day for subsequent days. The Cₘₐₓ plasma concentration obtained at day +3 from start was 5 µg/mL.

This analytical method is suitable to measure concentrations of the echinocandins in pediatric population, both for research and clinical practice, especially when altered pharmacokinetics can be expected and dosing guidelines
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ORAL RIBAVIRIN FOR (LUNG TRANSPLANTED) PATIENTS WITH A PARAMYXOVIRUS INFECTION: A COMPARISON OF RIBAVIRIN PLASMA LEVELS WITH THE IC50

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Background:
Lung transplant recipients are prone for a complicated course of paramyxovirus infection due to their immunosuppressive medication. Ribavirin treatment is associated with faster recovery. However there is no consensus about the dosage. Since steady-state concentrations are reached 4-8 weeks after treatment, it is questionable if therapeutic ribavirin concentrations are achieved during a 10 day treatment course for paramyxovirus infection. Prospectively ribavirin plasma concentrations were determined of patients receiving oral ribavirin for paramyxovirus infections in relation to the concentration that inhibits 50% of the virus replication (IC50).

Methods:
Patients presenting in the UMCG with a paramyxovirus infection requiring treatment were studied. Ribavirin plasma levels were measured on day 1, 2, 4, 7 and 10 with a validated analytical method using LC-MS/MS. Sample preparation consisted of dilution, followed by ultrafiltration. The calibration curves were linear in the range of 0.2-10 mg/L with within-run and between-run precisions (CVs) in the range of 0-10%. A nasal swab was tested by PCR on day 0 and 7. In vitro the IC50 of ribavirin for RSV was evaluated using Hep-2 cells.

Results:
During the study period (Okt 2014 - Feb 2015) six immunocompromised patients presenting with paramyxovirus infection required antiviral treatment. Five males and one female, aged 57 (34-73) years were included in this study. They received a dose of 200-400mg twice daily. In these patients ribavirin plasma levels ranged between <0.2-0.6 mg/L (D2), 0.2-0.6 mg/L (D4), 0.3-1.1 mg/L (D7) and 0.7-1.6 mg/L (D10). During the first four days of treatment concentrations did hardly exceed the IC50 of RSV of 0.5 mg/L. Three of the six patients showed sustained viral response at day 7.

Conclusion:
Oral ribavirin leads to subtherapeutic levels in plasma during the first days of treatment, if compared to the IC50. Potentially ribavirin concentrations exceed the IC50 in lung tissue. Further research is needed to investigate whether a loading regimen to boost plasma levels during first days of Ribavirin treatment for paramyxovirus infection could further optimize the antiviral treatment and subsequently improve the clinical outcome.

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CYP3A PHENOTYPE ASSOCIATED TO USE OF BIOLOGICS AND INFLAMMATORY STATE IN PATIENTS WITH RHEUMATOID ARTHRITIS

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Background: CYP3A enzymes are involved in metabolism of many drugs. Individual variability in CYP3A phenotype is extensive, and the endogenous agent 4β-hydroxycholesterol (4β-OHC) has the potential to serve as a therapeutic drug monitoring (TDM) biomarker for dose optimization of CYP3A substrate drugs. Inflammation has been suggested as a mechanism that downregulates CYP3A activity. The aim of this study was to investigate the association between CYP3A phenotype, as measured by circulating 4β-OHC levels, and inflammatory state in patients with rheumatoid arthritis (RA) before and after initiation of biological disease-modifying drugs (bDMARDs).

Methods: The study included 56 RA patients who were monitored before and 3 months after initiation of bDMARD treatment. The monitoring comprised measurements of CRP, sedimentation rate and 4β-OHC concentration (retrospectively analyzed). In addition, 52 TDM samples of randomly selected levetiracetam-treated patients with CRP <5 mg/L were included as ‘healthy’ controls. Concentrations of 4β-OHC were compared in RA patients before
and after bDMARD treatment, and also compared with ‘healthy’ controls. Moreover, correlation analyses between CRP/sedimentation rate and 4β-OHC levels were performed at both time points in RA patients.

**Results:** The median CRP level was reduced from 6 (range 1-69) to 2 (1-100) following bDMARD treatment (p=0.06), while median sedimentation rate declined from 22 (2-84) to 14 (2-69) in the same period (p<0.01). 4β-OHC levels in RA patients did not significantly differ following bDMARD treatment (median 52.5 vs. 50.9 nM, p=0.8), but median levels of 4β-OHC were significantly lower in ‘healthy’ controls (67.7 nM) at both time points (p=0.022 before, p<0.01 after). While there was no correlation between CRP or sedimentation rate and 4β-OHC levels before treatment (p=0.5), a highly significant correlation between CYP3A phenotype and inflammatory biomarkers was observed after 3 months of bDMARD treatment (p<0.01; Spearman r = -0.41 [CRP] and -0.35 [sedimentation rate]).

**Conclusion:** CYP3A phenotype is generally reduced in RA patients by approximately 20-30% compared to individuals without inflammatory disease. Anti-inflammatory treatment with bDMARD does not improve CYP3A phenotype in RA patients over a time span of 3 months, but CYP3A phenotype is significantly correlated to inflammatory biomarkers during bDMARD treatment.

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**IMPACT OF CYP3A4*22, CYP3A5*1 AND POR*28 POLYMORPHISMS ON TACROLIMUS DOSE OPTIMIZATION AND THE OUTCOME OF KIDNEY TRANSPLANTATION.**

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Due to the narrow therapeutic window and the large between-subject pharmacokinetic (PK) and pharmacodynamic (PD) variability of tacrolimus, clinical outcomes in transplantation exhibit substantial inter-patient variability. Indeed, over-immunosuppression causing toxicity and under-immunosuppression leading to graft rejection episodes are frequently encountered. Unraveling the impact of genetic polymorphisms on the PK and PD of Tac may help to refine therapy and improve clinical outcomes.

**Objectives.** Confirm the effect of single-nucleotide polymorphisms (SNPs) in drug-metabolizing enzymes of renal transplant recipients on tacrolimus PK/PD. In addition, the predictive value of donor genotype was assessed to observed the variables.

**Methods.** Recipients (N=332) and donors (N=180) were genotyped for CYP3A5*, CYP3A4*22 and POR*28. Associations between SNPs and dose-adjusted predose concentrations (C0) (ng/ml per mg/Kg/d) and daily doses (mg/kg) of tacrolimus at 1, 3, 6, and 12 months after transplantation were evaluated. Clinical outcomes evaluated: incidence of delayed graft function (DGF) and graft loss.

**Results.** Univariate analysis demonstrated that CYP3A4*22 carriers (from month 1 to 6) and CYP3A5*3/*3 homozygotes (from month 1 to 12) had significantly higher dose-adjusted C0 and received lower daily doses (p<0.05). Clustering of recipients combined CYP3A4*22 and CYP3A5*3 allelic status showed that extensive metabolizers (CYP3A4*1/*1 homozygotes with at least one active CYP3A5*1 allele) had lower dose-adjusted C0 although receiving higher doses when compared with poor (CYP3A4*22 carriers with CYP3A5*3/*3) and intermediate metabolizers (CYP3A4*22 non-carriers with CYP3A5*3/*3 or CYP3A4*22 carriers with CYP3A5*1/*1) (p<0.05). In the CYP3A5 expresser group, POR*28 carriers had 26% increased tacrolimus dose-adjusted C0 when compared to POR*1/*1 (p=0.014). Patients receiving a kidney from a donor CYP3A4*22 allele and homozygous for POR*28*28 had an increased risk of DGF (OR=3.8, CI95%=1.5-9.9, p=0.010; OR=4.7, CI95%=2.2-11.4, p<0.001 respectively). Donor carriers of CYP3A4*22 were identified as risk factor for graft loss (OR=5.27, CI95%=1.6-16.9, p=0.011).

**Conclusions.** The CYP3A5*, CYP3A4*22 and POR*28 polymorphisms have a major influence on the tacrolimus dose required to reach the target exposure in renal transplant recipients. The kidney donor CYP3A4*22 and POR*28 genotype was correlated with more DGF (POR*28 and CYP3A4*22) and graft loss (only CYP3A4*22). These results support the importance of CYP gene polymorphisms in tacrolimus dose optimization and the outcome of kidney transplantation.
Background: Vitamin A (retinol) is one of the most important micronutrients affecting the health of children. In the developing world, vitamin A supplementation programmes significantly reduce infant mortality as well as the incidence of xerophthalmia, respiratory infection and morbidity from gastrointestinal disease. Term infants are well supplied with vitamin A in utero, at the expense of maternal stores and human milk provides adequate amounts of vitamin A for normal growth and health in the first six months. Preterm infants, particularly those of low birth weight, have low plasma concentrations of both retinol and retinol binding protein at birth compared with term infants. We have developed and validated a sensitive HPLC assay that requires a small plasma volume (100µL) and can be a valuable tool for measuring low concentrations of retinol in preterm infants. Methods: Sample preparation was performed in one step and involved precipitation of protein and extraction of lipid with two volumes of an ethanol-chloroform mixture (3:1) without internal standard addition. 20µL aliquots of the supernatant were injected into the HPLC system equipped with a Water Spherisorb ODS 2 column coupled to a guard column RP C18 and the fluorescence detection at excitation and emission wavelengths of 325nm and 470nm. The mobile phase consisted of methanol (100%). The flow rate was 1mL/min. Validation of analytical method was performed according to the EMA Guideline (July, 2011). Results: A linear correlation was found in the range from 75 to 1200 ng/mL. The non-zero standards were within ± 15% of their nominal value. The intra-interday accuracy of the assay was within 85%-115% for each group of samples (QC, LLOQ), while precision data showed a coefficient of variation ≤ 10.2 % for both intra- interday runs. The method was applied to plasma samples from 18 preterm infants receiving oral supplementation of vitamin A from day 2-4, when enteral feeds were tolerated. Retinol mean plasma concentration at birth, on days 14 and 28 were 196.84±92.17; 201.29±106.7, 174.43±75.91ng/mL, respectively. Conclusions: We developed a very simple and rapid method for measuring low concentrations of vitamin A in preterm infants with sensitivity, precision and accuracy.

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AN ALTERNATIVE METHOD FOR QUANTIFICATION OF TIGECYCLINE, A RESERVE ANTIBIOTIC GLYCICYCLINE, BY MEANS OF LIQUID CHROMATOGRAPHY WITH ELECTROCHEMICAL DETECTION.

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Tigecycline is a quite novel anti-infective drug which shows antimicrobial effects against gram positive and gram negative bacteria including multiresistant enterococci, staphylococci and many species from the enterobacteriaceae family - making this substance a valuable reserve antibiotic in different clinical disciplines including intensive care. Although tigecycline TDM is not mandatory during therapy, exact knowledge of tigecycline concentration in different tissues and in serum might be valuable for optimization of anti-infective therapy and antibiotic stewardship in special clinical situations (especially critical patients). From chemical view, tigecycline represents a t-butyl-glycinated minocycline derivative which like minocycline and unlike doxycycline shows a p-aminophenol substructure. Standard methods used to quantify tigecycline in vitro or in biological matrices are liquid chromatography with UV- or MS/MS-detection. In comparison, UV-methods lack high detection sensitivity while LC-MS/MS-methods are rather expensive - at least for smaller TDM-performing hospitals. These method characteristics have been our motivation to test an alternative quantification method. Against the background, that substances containing a p-aminophenol substructure are highly susceptible for redox reactions (oxidation to quinoneimines), we decided to test the response of tigecycline by means of an electrochemical detector. In our study, we quantified tigecycline either by our standard TDM-setup (Shimadzu HPLC-DAD) or by the experimental HPLC-ECD apparatus. In both methods, the stationary phase was represented by a reversed phase C18-column (Waters Xbridge BEH C18 2.5µm column). The DAD method contained a spectrum range from 190-400 nm by means of a SPD-M10A vp detector (Shimadzu); 244nm and 350nm were chosen for peak analysis. The electrochemical detection was performed by means of a Coulochem II detector (Esac) using the ESA 5011A analytical cell. Tigecycline was reproducibly measured in both HPLC methods, the quantification limit was comparable or lower when using the electrochemical detector. Thus, from our point of view, the HPLC-ECD method represents a promising alternative method for quantification of tigecycline for TDM from tissues where low tigecycline concentrations are expected and for verification of peak identity. Further experiments are currently running to test the clinical implementation of this method.
OVER-ANTICOAGULATION WITH PHENPROCOUMON ASSOCIATED WITH CYP2C9 POLYMORPHISM: A CASE EXAMPLE OF INTEGRATED THERAPEUTIC DRUG MONITORING AND PHARMACOGENETICS
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Background
A 80+ years old female patient was initially treated with the oral anticoagulant acenocoumarol upon mitral valve replacement. INR was variable, which resulted in a thromboembolic event. Therapy was switched to phenprocoumon. One week after initiation of phenprocoumon treatment, INR was supratherapeutic. INR continued to increase, despite therapy discontinuation. Failure to maintain INR values within the target range, motivated pharmacokinetic and pharmacogenetic monitoring.

Methods
On treatment days 1-6, phenprocoumon doses of 6, 9, 9, 9 and 6 mg were administered and was discontinued thereafter. On treatment day 8, the phenprocoumon plasma concentration was assessed. The observed plasma concentration was interpreted relative to a typical concentration-versus-time profile, based on a two-compartmental model implemented in Excel using pharmacokinetic parameters reported by Haustein. [1]

Results
INR values were 1.1 on treatment day 1 and 7.7 on day 8. The phenprocoumon plasma concentration on day 8 was 4.6 mg/L (therapeutic range 1-3 mg/L). In a typical patient, the administered phenprocoumon doses would have resulted in an approximately 2-fold lower plasma concentration (Figure 1). CYP2C9 genotype was CYP2C9*1/*2 (intermediate metabolizer). VKORC1 (vitamin K epoxide reductase) genotype was 1639GG(1173CC) (normal sensitivity for oral anticoagulants). Temporary discontinuation of phenprocoumon treatment was advised (2 weeks). Thereafter phenprocouman dosed at 3 mg twice weekly was recommended to maintain therapeutic drug levels and to achieve adequate INR management.

Discussion
The observed elevated and increasing INR can be explained by a composite of 1) supratherapeutic phenprocoumon plasma concentration, 2) slow elimination of phenprocoumon (typical half-life 5-7 days) and 3) delayed pharmacodynamic effect (hysteresis, turnover of coagulation factors 1-2 days). The variant CYP2C9*1/*2 genotype, as well as concomitant treatment with the CYP2C9 inhibitor irbesartan, might have contributed to an increased half-life of the CYP2C9 substrate phenprocoumon, resulting in supratherapeutic drug levels. Reduced CYP2C9 activity may also explain the uncontrolled INR values during earlier treatment with acenocoumarol, which is also a substrate of the CYP2C9 enzyme.

Conclusions
Therapeutic drug monitoring and genotyping were used to explain an unforeseen INR elevation and to optimize anticoagulation therapy in a patient treated with phenprocoumon.

References

Picture 1: https://www.eventure-online.com/parthen-uploads/7/15017/img1_266511_RABsT0wMtd.jpg
Caption 1: Figure 1. Time profile of phenprocoumon plasma concentration.

EFFECT OF CARBAMAZEPINE ON THE CYP3A BIOMARKER 4β-HYDROXYCHOLESTEROL DEPENDS ON DOSE RATHER THAN STEADY-STATE CONCENTRATION
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Background: 4β-Hydroxycholesterol (4βOHC) has recently attracted great interest as a potential endogenous biomarker for the activity of CYP3A enzymes. Studies have previously showed that patients treated with carbamazepine or other CYP3A inducers have elevated serum concentrations of 4βOHC. In this study, we compared to which extent 4βOHC levels were correlated to dose and steady-state concentrations of carbamazepine using a therapeutic drug monitoring (TDM) material.
Methods: The study comprised TDM material from 47 randomly selected carbamazepine-treated patients (cases) and 54 levetiracetam-treated patients (negative controls). Information about daily doses and serum concentrations of antiepileptic drugs (AEDs) measured in the serum samples was withdrawn from the TDM database. The UPLC-MS/MS assay for determination of AEDs included all clinically used agents, which enabled control for potential use of other CYP3A-inducing AEDs than carbamazepine (i.e., phenytoin and phenobarbital). Remaining serum samples from TDM were reanalyzed for determination of 4βOHC concentrations by UPLC-APCI-MS/MS after alkaline hydrolysis (de-esterification) and hexane-based liquid-liquid extraction. In the data analysis, 4βOHC levels in carbamazepine-treated patients were first compared with negative controls (levetiracetam samples) to confirm the CYP3A-inductive effect (Mann-Whitney test). Then, correlations between carbamazepine doses and steady-state concentrations of carbamazepine with individual 4βOHC levels were performed (Spearman's test).

Results: The median serum concentration of 4βOHC was 10-fold higher in carbamazepine- vs. levetiracetam-treated patients (241 vs. 22 ng/mL, P<0.0001). There was a highly significant correlation between carbamazepine dose and 4βOHC level (Spearman r=0.56, P=0.0001). In contrast, no significant correlation was observed between steady-state serum concentration of carbamazepine and 4βOHC level (Spearman r=0.23, P=0.1163).

Conclusion: This present study shows that the inductive effect of carbamazepine on synthesis of the endogenous CYP3A biomarker 4β-hydroxycholesterol depends on dose rather than steady-state concentration. Thus, as the presystemic exposure of carbamazepine is the strongest determinant of CYP3A induction, these findings may indicate that intestinal CYP3A is involved in formation of 4β-hydroxycholesterol.

FAST AND CONFIDENT IDENTIFICATION OF DRUGS AND THEIR METABOLITES USING ION TRAP LC-MSN ANALYSIS AND A LIBRARY OF 4,500 COMPOUNDS
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Comprehensive screening of urine samples in forensic toxicology and clinical research is focused on the unambiguous identification of parent drugs and their corresponding metabolites. GC-MS is currently the gold standard technology in toxicology screening due to the availability of large and well annotated spectral libraries. Here, we present the evaluation of a comprehensive and robust forensic toxicology ion trap based LC-MSN spectral library screening. Eleven urine samples were worked-up by acid hydrolysis, liquid-liquid extraction, acetylation, and analyzed after GC separation by full scan EI-MS according to the GC-MS standard urine screening approach (SUSA) as published by Maurer et al. For the LC-MSn analysis, the urine samples were prepared by protein precipitation according to the LC-MSn standard urine screening approach (SUSA). Acquired data (full scan MS, MS2 and MS3) were searched against the Toxtyper library (900 compounds) and the recently published Maurer/Wissenbach/Weber (MWW, Wiley-VCH, Weinheim, Germany, 2014) LC-MSn library which contains > 4500 compound entries including 3000 metabolites. A combined library search approach using Toxtyper and MWW library was evaluated. In the first round spectra were searched against the Toxtyper library resulting in highly reliable identification of mainly the parent drugs. In the second step non-identified compound spectra were searched against the MWW library providing additional detection of metabolites and thereby increased confidence for drug identification. Most compounds could be identified with both approaches, GC-EI-MS and LC-MSn. The GC-MS approach identified 50 different drugs in the 11 urine samples, whereas the LC-MSn approach revealed 60 drug identifications. Several hypertension drugs, antibiotics and neuroleptics such as pipamperone could be identified solely by LC-MSn. Identification of drugs with fast metabolic rate such as propranolol (4-5 hours half-life period) is only possible through detection of their metabolites. Three metabolites of propranolol could be identified via the MWW library whereas the parent compound had been already completely metabolized. The presented LC-MSn screening workflow using combined spectral library searching of both, the Toxtyper and Maurer/Wissenbach/Weber library represents a valuable tool for comprehensive and reliable identification of toxicologically relevant compounds and their metabolites in urine, blood and other body fluid samples.

DESIGN OF AN LC-MS/MS METHOD FOR MEASURING CONCENTRATIONS OF CYCLOSPORINE A AND TACROLIMUS FROM DRIED BLOOD SPOTS SUITABLE FOR AUTOMATION
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Background: Patients on immunosuppressive treatment after organ transplantation is a patient group subjected to long-term therapeutic drug monitoring that would benefit from the possibility of home sampling with dried blood spots.
Determinations of cyclosporine and tacrolimus concentrations in whole blood are high-volume analyses in many clinical laboratories and are good candidates for development of automated dried blood spot sample work-up. The purpose for this method development was to design a method with rapid and easy preparation, no sample transfer steps and short runtimes.

Methods: Fixed-size punches of 5 mm from Whatman 903 collection cards were extracted with 150 µL MeOH/water 80/20 (v/v) and internal standards in a 96-well filter plate (Millipore) by gentle mixing for 10 minutes. The extracts were filtered down into a Greiner 96-well plate during 5 minutes by centrifugation. A 20 µL aliquot of the filtrate was injected on a LC-MS/MS using a 20 mm reversed phase column (TSQ Quantum Ultra, and Hypersil Gold, both Thermo Scientific). The gradient chromatographic system consisted of mobile phases of 2 mM ammoniumformate with 0.1 % formic acid with water (A) or methanol (B) Total run time was 2.3 minutes.

Results: Calibration curves were linear with $r^2$-values of $< 0.98$ in the range 2-25 ng/mL for tacrolimus and 17.5-1000 ng/mL for cyclosporine with precision and accuracy fulfilling European Medicines Agency criteria. The use of a filter plate in the extraction step makes it easy to separate the spots from the liquid and increases security by reducing sample transfer steps.

Conclusion: A bioanalytical dried blood spot method for the most common immunosuppressive drugs was designed and developed with easy application to the routine therapeutic drug monitoring laboratory. The method will be used in a clinical study with patients from Department of Transplantation Surgery, Karolinska University Hospital, Huddinge. The goal is to use the method evaluating samples that were taken in a home-sampling situation.

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DEVELOPMENT OF AN OPTIMAL DOERING REGIMEN FOR ORAL METHADONE TREATMENT IN NEONATAL ABSTINENCE SYNDROME: APPLICATION OF POPULATION PHARMACOKINETIC MODELING AND SIMULATION

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Background: Neonatal abstinence syndrome (NAS) is a drug withdrawal syndrome as a result of chronic intrauterine exposure to a variety of substances including opioids. Methadone is used as one of the first-line drugs for opioid-induced NAS. The current treatment protocols of oral methadone for NAS have not been validated and good pharmacokinetic (PK) data for oral methadone in neonates are lacking. The aim of this study was to characterize the PK of oral methadone in neonates and to develop a population PK model to design a new dosing strategy based on target exposure simulations.

Methods: A total of 66 dried blood spot concentrations collected from 20 neonates enrolled in a prospective PK/PD study were available for analysis. Samples were analyzed by validated LC-MS/MS assay. Population PK modeling was performed by nonlinear mixed effect modeling with NONMEM ver. 7.2. Dosing regimens to accelerate target attainment were evaluated by simulating methadone blood concentration-time profiles with the developed population PK model using Monte Carlo Simulation.

Results: Methadone blood concentrations exhibited large inter-individual variability. A one-compartment model with first-order absorption was found to best describe the data. With the original dosing protocol, target exposure was on average achieved after 48 h with cumulative AUC estimates (mean ± SD) simulated as 468 ± 446 on day 1 (0-24h) and 793 ± 802 ng·h/mL on day 2 (24-48h), respectively. In order to achieve the desired drug exposure on day 1 of treatment with accelerated tapering, new dosing regimens with a loading dose were designed to achieve target AUCs more expeditiously. The simulated AUC for 0-24h and 24-48h with the newly-proposed regimen were 985 ± 1020 and 1046± 1199 ng·h/mL, respectively.

Conclusion: We developed a population PK model for oral methadone in neonates with NAS. An improved dosing regimen with a loading dose and an accelerated tapering phase was developed based on the PK simulations. The optimized dosing regimen is being evaluated in an ongoing prospective clinical study, and preliminary data suggest that the new protocol successfully reduces the duration of opioid treatment, the total methadone dose being administered and the length of hospital stay.

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ONLINE ANALYSIS OF IMMUNOSUPPRESSANTS IN WHOLE BLOOD WITH THE EVOQ TRIPLE QUAD

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Immunosuppressants are used after organ transplantation to prevent allograft rejection by inhibition of the body’s immune system. The drugs have a narrow active concentration range and highly variable pharmacokinetics. Therefore it is necessary to continue research on these drugs to improve the scientific understanding of dosage requirements.

This study demonstrates a robust and reliable research method to quantitate the immunosuppressants cyclosporine A, tacrolimus, sirolimus and everolimus in whole blood samples using the Bruker Advance™ UHPLC with OLE coupled to the EVOQ Elite™ triple quad. Reagents and columns were provided by the ClinMass® LC-MS/MS complete kit MS1100 (Recipe, Munich). Sample preparation was fast and easy. After protein precipitation samples were further cleaned using the integrated online extraction option of the UHPLC. Interlacing the online extraction and chromatographic separation reduces the overall run time to 3 minutes per sample. ESI in positive mode was used for ionization.

Calibration curves including six calibrators showed very good linearity \((r^2 \geq 0.997)\). Replicate injections \((n = 4)\) of the calibrators and three whole blood quality controls (QC) demonstrated good intraday precision with RSD <5% for all analytes. Interday precision on three days showed high robustness with RSD of means <5.5%. Accuracy of calibrators and QCs was excellent with bias < ±6.5%.

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ONLINE ANALYSIS OF 25-OH-VITAMIN D2/D3 IN PLASMA AND SERUM WITH THE EVOQ TRIPLE QUAD

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Vitamin D continues to generate significant interest and research in a number of fields. The form typically analyzed in biological samples is its metabolite 25-OH-vitamin D.

This study demonstrates a robust and reliable research method to quantitate 25-OH-vitamin D2 and D3 in plasma and serum samples using the Bruker Advance™ UHPLC with OLE coupled to the EVOQ Elite™ triple quad. Reagents and columns were provided by the ClinMass® LC-MS/MS complete kit MS7000 (Recipe, Munich). Sample preparation was fast and easy. After protein precipitation samples were further cleaned using the integrated online extraction option of the UHPLC. Interlacing the online extraction and chromatographic separation reduces the overall run time to 3 minutes per sample. APCI in positive mode was used for ionization.

Calibration curves including four calibrators (25-OH-vitamin D2) and three calibrators (25-OH-vitamin D3) showed excellent linearity \((r^2 \geq 0.999)\). Replicate injections \((n = 4)\) of the calibrators, two serum quality controls (QC) and a pooled serum sample demonstrated good intraday precision with RSD <5% for both analytes. Interday precision on three days showed high robustness with RSD of means <6%. Accuracy of calibrators and QCs was excellent with bias < ±6%.

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CONFIDENT IDENTIFICATION OF DRUGS IN URINE AND SERUM USING HIGH RESOLUTION QTOF MASS SPECTROMETRY AND NEW SOFTWARE TOOLS

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High-resolution time-of-flight mass spectrometry has become an excellent tool in forensic toxicology and clinical research. The accurate mass based inherent characteristics like sensitive wide scope screening together with retrospective and general unknown analysis capabilities make it an ideal tool for this work. We describe the development and evaluation of a new software tool allowing screening and quantification using ultra-high-resolution LC-QTOF, accurate mass analysis. The new software allows applying the diagnostic ion concept, using enhanced confirmation criteria such as isotopes, adducts, fragments and qualifier ratios for reducing false positive detection rates. 61 compounds covering several compound classes based on their relevance in post-mortem and routine drug screening were used in this study. After acetonitrile precipitation, urine and serum samples were spiked with the toxicological compound mixes at four levels (between 10-500 ng/ml) and analyzed in ESI(+) mode by LC-QToF MS (Impact II, Bruker Daltonics, USA), in full scan and bbCID modes using a 14 minute gradient, reverse phase UHPLC separation. The data acquired in bbCID mode were processed with the new software package including a database with about compounds of forensic relevance. In the full scan ToF-MS channel, all compounds were detected at all
concentration levels, no false negatives were encountered. In urine, the high matrix load produced a higher number of false positives Only seven compounds were causing ~75% of all false positives. These false positives typically arose due to a very low detection threshold. In total 35 different compounds appeared as false positives. After applying the enhanced diagnostic ion detection criteria the false positives were completely removed. A challenge was tramadol in the presence of o-desmethylvenlafaxine as these compounds have the same retention time, same sum-formula and the same main fragment ion. However, two smaller characteristic fragments allowed the unambiguous identification of o-desmethylvenlafaxine.

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IMPACT OF CYP3A GENETIC VARIANTS ON THE CYP3A ACTIVITY MARKER 4β-HYDROXYCHOLESTEROL
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Background: Cytochrome 450 (CYP) 3A is one of the most important enzyme groups in drug metabolism. The CYP3A phenotype has great inter-individual variation, and 4β-hydroxycholesterol (4β-HC) is a candidate biomarker for determining individual CYP3A activity. Studies have shown that the metabolism of cholesterol to 4β-HC is catalyzed by both CYP3A4 and CYP3A5. Our aim was to evaluate the influence of CYP3A4*22 and CYP3A5*3 alleles on serum levels of 4β-HC.

Methods: Serum and blood samples from 751 patients were collected from a routine therapeutic drug monitoring (TDM) service at Center for Psychopharmacology, Diakonhjemmet hospital, and were available for reanalysis of 4β-HC. CYP3A4*22 and CYP3A5*3. Serum concentration of 4β-HC was determined by UPLC-MS/MS using atmospheric pressure chemical ionization (APCI), while the selected variant alleles were detected by real-time polymerase chain reaction (PCR) methods. So far, CYP3A genotypes and 4β-HC levels have been determined in 400 of the patients.

Results: Seventy-eight of the hitherto analysed patients were homozygous or heterozygous carriers of the functional CYP3A5*1 allele (19.5 %), whereas 312 were non-carriers. Mann-Whitney statistical analysis showed that CYP3A5*1 carriers had significantly higher median concentration of 4β-HC than homozygous carriers of the non-functional CYP3A5*3 allele, i.e. 62.8 vs. 53.2 nmol/L, respectively (p < 0.01). Twenty-eight patients were heterozygous carriers of CYP3A4*22 (7.0 %), whereas 362 were non-carriers. CYP3A4*22 carriers had significantly lower median concentration of 4β-HC compared to non-carriers (Mann-Whitney, p = 0.01), i.e. 43.4 vs. 58.3, respectively.

Conclusion: The preliminary results indicate that CYP3A4*22 contribute to decreased levels of the CYP3A activity marker 4β-HC, and that CYP3A5*1 contribute to increased levels of 4β-HC. This supports the theory that both CYP3A4 and CYP3A5 catalyze the metabolism of cholesterol to 4β-HC.

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INFLUENCE OF CYP3A5 POLYMORPHISMS ON TACROLIMUS PHARMACOKINETICS IN RENAL TRANSPLANT RECIPIENTS AMONG CHINESE POPULATION
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Background: The calcineurin inhibitor tacrolimus is the backbone of immunosuppressive drug therapy after solid organ transplantation. Little evidence was found in randomized controlled clinical trials among Chinese population that the initial dosage of tacrolimus based on pharmacogenetics will improve the clinical outcome of transplant recipients.

Methods: A total of 120 Chinese patients who underwent renal transplantation in West China Hospital from July to December 2014 were studied retrospectively. All the recipients were treated with tacrolimus-based triple immunosuppression regimen and were genotyped for CYP3A5 (6986A>C) SNPs by HRM analysis (high-resolution melting curve analysis). The percentage of recipients who reached the tacrolimus target concentration range (C0) (5-10 ng/mL) after 4 dosages were calculated.

Results: 33.3% (40/120) of these renal recipients were within the tacrolimus target concentration range, while 66.7% (80/120) of these recipients were out of the range, including 74 recipients tacrolimus C0 lower than 5ng/ml and 6 recipients tacrolimus C0 higher than 10ng/ml. The percentage of recipients achieving the tacrolimus target C0 were 33.3% in CYP3A5*1/*1 group, 38.3% in CYP3A5*1/*3 group and 29.3% in CYP3A5*1/*3 group, respectively.
Within non-expressors group, 60.3% of tacrolimus $C_0$ were lower than target range, while 29.3% of tacrolimus $C_0$ were higher than target range. The expressors (CYP3A5*1 allele) had a lower trough concentration than non-expressors (CYP3A5*3/*3) (4.16 (0.70-9.10) vs. 5.25 (0.4-12.5) ng/ml; $P<0.05$). The dose-corrected tacrolimus $C_0$ was approximately 30% higher in CYP3A5 expressors compared to non-expressors (117.58 (8.00-366.95) ng/ml per mg/kg vs. 90.76 (10.75-234) ng/ml per mg/kg); $p =0.012$.

**Conclusion**: More recipients had a lower tacrolimus $C_0$ within non-expressors in Chinese population. This may be result from low initial dose (0.04-0.06mg/kg/d) in China. As CYP3A5 6986A>G genetic polymorphism affected tacrolimus concentration, optimization of tacrolimus initial dose based on the CYP3A5 gene polymorphisms might be helpful to increase the percentage of patients reaching the tacrolimus target $C_0$ range. And the affect of the proposal on clinical outcomes needs further evaluation.

**Picture 1**: https://www.eventure-online.com/parthen-uploads/7/15017/img1_266705_OomgCo5C9F.jpg

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**EVALUATION OF THE NEW METHOTREXATE CMIA ASSAY ON THE ARCHITECT i2000SR**

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**Background**

Methotrexate (MTX) is an antifolate drug used in high dose in the treatment of various malignant tumours. High dose methotrexate is monitored to avoid toxic drug concentrations and for timing of leucovorin therapy. Hence, plasma concentrations may vary from zero to 1 mmol/L. We evaluated the novel Architect Methotrexate assay (Abbott Diagnostics), a one-step competitive microparticle immunoassay, on the Architect i2000SR (Abbott Diagnostics).

**Methods**

Accuracy (n=11), within-run (n=11) and between-run (n=11) imprecision were assessed with MTX Architect Control material (L1, L2, L3 and LX with target concentrations of 0.07, 0.45, 1 and 10 µmol/L respectively). Functional sensitivity was checked using a sample with low MTX concentration. Linearity, within the measurement range 0.04-1.50 µmol/L, was evaluated according to CLSI EP6-A2. Method comparison was performed using 52 anonymized fresh leftover serum samples measured against the routinely used ARK MTX immunoassay (Ark Diagnostics). Samples with high MTX concentration were 1/10 or 1/100 diluted as needed, using assay-specific diluent. 9 external QC samples (EQAS, Biorad) were also evaluated.

**Results**

Imprecision was acceptable with within-run CV's being 2.8 to 3.1% and between-run CV's being 7.6, 5.4, 5.7 and 5.4% for L1, L2, L3 and LX respectively. Accuracy evaluation yielded a minimal negative bias for the medium, high and X control, but a positive bias of 8% for the low control. A functional sensitivity of 0.040 mmol/L (CV 25%) was confirmed and the method was found to be linear. For all results (diluted or undiluted) within the measurement range the calculated Pearson correlation coefficient was 0.983 ($p<0.0001$). Passing-Bablok regression generated a slope of 0.943 (95% CI 0.875-1.018) and an intercept of -0.011 (95% CI -0.027-0.006). Bland-Altman analysis showed that results on Architect are on average 0.04 µmol/L lower (1.96 SD -0.18-0.10). EQC samples resulted in a recovery of 94-110%.

**Conclusions**

We conclude that the Architect Methotrexate assay demonstrates an acceptable performance. The assay shows advantages of a slightly extended measurement range (compared with the ARK assay) and a good on-board reagent and calibration stability (>1month).

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**IGM INTERFERENCE IN THE ABBOTT IVANCO IMMUNOASSAY: A CASE REPORT**

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**Background**

Therapeutic drug monitoring of vancomycin using immunoassays is a common practice. Here, we describe a case
of IgM interference in the Abbott iVanco immunoassay.

Methods
An 82-year-old patient, with Waldenström’s disease, was admitted to our hospital with MRSA sepsis. Vancomycin treatment was started and total vancomycin serum concentrations were routinely measured on the Architect i2000SR (Abbott Diagnostics), using a competitive chemiluminescent microparticle immunoassay (iVanco assay, Abbott Diagnostics). Re-testing occurred using a validated LC-MSMS method and VANC2 assay (Cobas c502 Roche Diagnostics). Unbound vancomycin concentrations were determined using ultrafiltration as described previously. 

Results
Measured vancomycin concentration on day 1 was extremely high (>100 mg/L), requiring further dilution. Surprisingly, measurements on 1/4 and 1/5 diluted serum suggested a lower initial concentration (21.8 mg/L and 17.2 mg/L, respectively). Analysis on a sample, drawn prior to vancomycin treatment, resulted in a vancomycin concentration of 70.3 mg/L, which further confirmed the suspicion of paraprotein (IgM=74g/L) interference. Unbound vancomycin concentration was 14.8 mg/L and, based on a previously reported equation, a total vancomycin concentration of 20.0 mg/L was estimated, which was confirmed by re-analyzing the native sera using the VANC2 immunoassay, which was not affected by IgM interference, and LC-MSMS. Samples from two other patients with a high IgM paraprotein concentration, but not on vancomycin, were analyzed using the iVanco immunoassay. Results were below detection limit, indicating that interference is not common.

Conclusions
Laboratory staff needs to be aware of possible paraprotein interference in the iVanco immunoassay, even if this is not mentioned in the assay leaflet. In contrast with the falsely low vancomycin results, reported for another immunoassay, we obtained with our assay spuriously high results.

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A NEW CYP3A5*3 AND CYP3A4*22 CLUSTER INFLUENCING TACROLIMUS TARGET DOSE-CONCENTRATIONS: A POPULATION APPROACH.
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Background: The single-nucleotide polymorphisms CYP3A5*3 and CYP3A4*22 have been associated with the pharmacokinetic variability of tacrolimus in renal transplant patients. The aim of this study was to develop a population PK model considering the influence of these two pharmacogenetic polymorphisms on tacrolimus exposure.

Methods: Tacrolimus exposure data from n = 382 renal transplant recipients (n = 372 predose concentrations [C0] only and n = 10 extensive sampling) were collected during the first year after transplant and were simultaneously analyzed with a population pharmacokinetic approach using NONMEM 7.2

Results: A two-open-compartment model with inter-occasion variability in clearance (CL) best described the pharmacokinetics of tacrolimus. A new cluster variable combining the CYP3A5 and CYP3A4 genotype explained the substantial variability in CL. A significant difference in CL was observed among the three different genotype groups (extensive, intermediate and poor metabolizers) (p<0.001 and reduction of interindividual variability of 19.2%). The inclusion of hematocrit predicting a standardized tacrolimus concentration significantly improved the model fit (P<0.001). An external validation confirmed the prediction ability of the model with median bias and precision values of -0.39 ng/mL (95% CI: -15.86 ng/mL - 4.53 ng/mL) and 2.41 ng/mL (95% CI: 0.24 ng/mL - 16.62 ng/mL) respectively. Simulations showed that patients with a low hematocrit (22%) had lower tacrolimus C0 concentrations when compared to those with normal hematocrit (33%), treated with the same doses. High CYP3A metabolizer patients had lower levels at the same fixed-dose when compared to intermediate metabolizer patients and, both had lower C0 with respect to the poor metabolizer subpopulation. The table below presents the median simulated (n = 500) C0 concentrations in ng/mL at different fixed doses:

Conclusions: A new CYP3A cluster, including CYP3A5 and CYP3A4 genotypes, as well as standardized hematocrit were identified as predictors of tacrolimus exposure and should be considered as tools for dose individualization during therapeutic drug monitoring.
ANALYTICAL VALIDATION OF POINT OF CARE ANALYSIS OF LITHIUM

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Background:
Lithium is widely used to treat manic or depressive episodes in bipolar disorders. It has a small therapeutic window (0.6 - 1.2 mmol/l) range) and above 1.5 mmol/l lithium in generally becomes toxic. Since the difference between therapeutic window and a toxic concentration is small, close therapeutic drug monitoring of the lithium concentrations in patients, especially during the initial stages of the treatment, is necessary.

Lithium is usually measured within the routine clinical laboratory, using ion selective electrodes or flame photometric detection. The objective of this investigation was to test the analytical performance of a novel Point-of-Care lithium instrument (Medimate Multireader®), introduced as the first electrophoresis based POC-test, which makes it possible to quantify ad-hoc lithium concentrations of patients specifically in finger-stick whole blood, e.g. in the psychiatry office or at the emergency department.

Methods:
The Clinical Laboratory Standards Institute (CLSI) protocols EP-5, EP-6, EP-7, EP-9, EP-17A and EP-25A were applied to investigate repeatability, reproducibility (EP-5), linearity (EP-6), Limit of Quantitation (EP-17A) and stability (EP-25A). The method comparison (EP-9) was performed against the reference standard which is flame photometric detection. All protocols were performed using capillary blood (finger stick), whole blood (venous K-EDTA) and serum samples, to assess the possibility to use different matrices. Interference study was carried out according to EP-7. The study was performed with the approval of the Medical Ethical Committee Twente (METC) and all patients signed an informed consent prior to the start of the study.

Results:
Measurement results indicate for serum a performance within +/- 0.10 mmol/l or 10% and for capillary and whole blood measurements a performance within +/- 0.15 mmol/l or 15%. Hemolysis and sodium influence on lithium outcome was found but is found acceptable. A measurement range from 0.25 to 10 mmol/l is proven and validated.

Conclusions:
For whole blood and serum Li+ measurements the POC Lithium instrument showed good analytical performance. The Medimate Multireader is very well capable to measure the Lithium concentration within the general acceptance criteria. The results show that this POC meter can be used to obtain a reliable result usable in daily clinical practice.

PERFORMANCE STUDY USING MEDIMATE MINI-LAB: PROFESSIONAL VERSUS PATIENT-USER OUTCOMES IN DRUG MONITORING OF LITHIUM.

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Background:
Lithium is globally used to treat and prevent manic or depressive episodes in bipolar disorder. The drug has a small therapeutic window and is in potential a toxic substance. Since the difference between therapeutic window and a toxic concentration is small, close monitoring of the lithium concentration is necessary. The Medimate Minilab is
intended to assess the lithium concentration in serum as well as fingerstick whole blood. The objective of the study is to investigate performance differences between experienced professionals and patient users.

Methods:
Method comparison experiments are carried out according to CLSI EP-9A2 protocol. 46 Patients on lithium therapy were included in this study.

Each lithium measurement was performed 3 times, each set from one fingerstick. The measurements were conducted by the patient, followed by the physician officer and the specialist (psychiatrist). An extra fingerstick sample was obtained for reference measurements by the physician officer. A venous sample was obtained for serum analysis and was analysed as the reference standard which is flame photometric detection. Prior to the measurements the patient and the physician officer were instructed on site according to the training protocol.

Each measurement was conducted by performing a fingerstick, acquiring a blood droplet, placing the blood droplet on a lab-chip, placing the lab-chip in the Multireader followed by a readout of the result from the Multireader.

Results:
Sample evaluation showed that errors are included due to instability of the measurement sample. This is caused by time differences in sampling from the subject as well as differences in preparing the reference sample. One person’s sample was found instable and was rejected from the dataset. The medication intake took place three hours prior to performing the measurement instead of the required minimal time of 10 hours.

The following clinical relevant parameters were found that 95% of all fingerstick measurements are within 0.15 mmol/l and 95% of all serum measurements are within 0.07 mmol/l

Conclusion
One-Way Anova analysis indicated that there was no difference in measurements results when performed by patients, physician officer or specialist.

Picture 1: https://www.eventure-online.com/parthen-uploads/7/15017/img1_266724_IS9a7HD7bL.png
Caption 1: Measurement results

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POSSIBLE CONTRIBUTION OF INOSINE TRIPHOSPHATE PYROPHOSPHATASE GENE POLYMORPHISMS TO AZATHIOPRINE THERAPY IN CHILDREN WITH CROHN’S DISEASE
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Background: Azathiopurine (AZA) is widely used to induce and maintain remission in children with Crohn’s disease. Its use is limited by a large inter-individual difference of the drug response. Inosine triphosphate pyrophosphatase (ITPA) is an enzyme participating in AZA metabolisms. Its SNP is reported to predict efficacy of AZA in patients with systemic Lupus erythematosus. Then, we tried to find if ITPA polymorphism has any association to the clinical response of AZA therapy in pediatric Crohn’s disease.

Method: Among a cohort of unrelated Japanese patients with inflammatory bowel disease in the multi-centers, children with Crohn’s disease (CD) were investigated as a subgroup study. Patients were managed by pediatrician.

Disease activity was classified by global assessment, International Organization for Inflammatory Bowel Disease (IOIBD) score. The primary outcome was relationship between ITPA gene variation and the mean change of IOIBD score after six months of AZA treatment.

Results: A reduction in IOIBD score was observed in patients carrying the ITPA 94CC genotype and the ITPA 94CA genotypes. The mean change in IOIBD score during six months of AZA therapy was different between the two groups (p<0.05). A multiple analysis showed the gene variation as a factor on the response. Plasma concentration of 6-thioguanin and adverse reactions during the therapy did not show any relationship between gene polymorphisms studied, such as TPMT and ITPA, statistically.

Conclusions: Our results show the correlation of ITPA 94C>A (rs1127354) polymorphism with the change in IOIBD score during AZA therapy, which might be a genetic biomarker in the treatment of children with CD.

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A COMPARISON THE PHARMACOKINETICS OF VANCOMYCIN ADMINISTERED TO PATIENTS WITH SEPSIS AS A BOLUS AND CONTINUOUS INFUSION
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Introduction: Pathologic condition alters the kinetics of many drugs, which significantly affects the efficacy and safety of treatment, especially substances with a narrow therapeutic range. Knowledge of these differences can improve the patient's chances of survival.

Aim of the study: The aim of this study was to compare the pharmacokinetics of vancomycin after administration in the form of bolus and continuous infusion.

Material and methods: The study involved 50 adults hospitalized because of sepsis or septic shock. 30 patients received vancomycin as a continuous infusion, and 20 in the form of a bolus. Patients were treated in the Department of Anesthesiology and Intensive Therapy Wrocław Medical University. Antibiotic serum concentrations were determined immediately after Cmax and 30 min, 24h, 48h, 96h (a control measurement to determine the adequacy of the dose) for bolus injection. While for continuous infusion test was performed 30 minutes after loading dose (LD). Vancomycin concentrations were measured in the Department of Clinical Pharmacology Wrocław Medical University with using enzyme multiplied immunoassay technique (EMIT) applying a Viva E Siemens analyzer. Antibiotic used based on antibiogram and clinical signs in a starting dose in accordance with the SPC, then they be modified based on measurements of the concentrations in the blood. The protocol for the study was approved by the Ethics Committee of Wrocław Medical University.

Results: There is a statistically significant difference between the AUC24 parameter for continuous infusions and bolus administration (p < 0.015). There are also statistically significant difference for MRT parameter (p < 0.001). There is no statistically significant difference for AUC24 / MIC parameter, which means that the effectiveness of these two forms of drug in the aspect of bioavailability is similar. There is also no statistically significant difference between the duration of treatment in both forms of administration of vancomycin.

Conclusions: The form of administration of the drug does not affect significantly the effectiveness measured by the AUC24 / MIC parameter. Calculation of pharmacokinetic parameters of vancomycin in patients with multiple organ failure is an important factor in increasing the efficacy and safety of therapy.

THERAPEUTIC DRUG MONITORING: LITHIUM PATIENT’S SELF-TESTS AT HOME
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Background: Lithium is globally used to treat and prevent manic or depressive episodes in bipolar disorder. The drug has a small therapeutic window and is in potential a toxic substance. Since the difference between therapeutic window and a toxic concentration is small, close monitoring of the lithium concentration is necessary. The Medimate Minilab is intended to assess the lithium concentration in serum as well as fingerstick whole blood. The objective of the study was to investigate the performance of the Medimate Minilab when used at a patient’s home.

Methods: In total 5 patients were requested to perform at 5 different days three measurements within an hour. The test is performed according to EP15-A2 protocol. After receiving a training conform procedure the patient obtains a Medimate Minilab package consisting of a Multireader and 20 lab-chips. Then the patient is requested to perform measurements at home. The measurement results are independently stored in the Minilab. The measurement are performed 10 hours after medication intake to minimize the influence on the measurement results by a changing lithium reference level in the patient. The patient is allowed to perform multiple measurements to get familiar with the system prior to start the official test. To minimize stress and external influence it is chosen solely to focus on precision and not to perform reference measurement for accuracy. This study was performed with the approval of the Medical Ethical Committee Twente (METC) and all patients signed an informed consent prior to the start of the study.

Results: The individual measurements result were all within the precision limits. Outliers are detected based on a difference of lithium outcome larger than 0.20 mmol/l or 20% from the mean of the two other outcomes. Test results showed no outliers. 100% of the measurement results are within the 95% specification boundary, indicating that the acceptance criteria are met.

Conclusions:
Measurements show excellent results for home testing by patients were the analytical acceptance criteria are met. Patients were highly satisfied with the ease of use of the Medimate Minilab.

Picture 1: https://www.eventure-online.com/parthen-uploads/7/15017/img1_266727_j1c3RfIXcJ.jpg
Caption 1: Measurements results for patient at home precision test

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EFFECT OF CYP2B6 516 GT POLYMORPHISM AND PUBERTAL MATURATION ON THE METABOLISM OF EFAVIRENZ IN PEDIATRIC HIV-INFECTED PATIENTS.

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Background: Functional polymorphisms in CYP2B6 result and developmental changes during childhood result in large variability in Efavirenz (EFV) exposure. This study was aimed to evaluate differences in EFV metabolism and CYP2B6 activity measured through 8-hydroxylation of EFV in HIV-infected pre-pubertal children and adolescents.

Methods: A cross-sectional study of HIV-infected children and adolescents on EFV-based treatment. CNS toxicity was graded based on adapted ACTG questionnaire on the scale 0-70. CYP2B6 genotyping was performed using the ABI TaqMan assay. Twenty-four hour PK of EFV, 8-hydroxy-EVF (E8F) and 8-hydroxy-EVF glucuronide (E8G) were quantitated by validated HPLC-MS/MS method using the Sciex APT-2000. PK analyses were performed using non-compartmental methods. One-way analysis of variance methods with post-hoc adjustment for multiple testing were used to compare mean exposures among CYP2B6 516 genotypes. Normality of each exposure variable was confirmed and log-transformation used where necessary. All results report raw values.

Results: Twenty-one patients (20 Black; 11 Females; median age=13.5 years) were enrolled. CYP2B6 516 genotype distribution was GG=10, GT=7, TT=4 (HWE p-value=0.21). Median EFV AUC was 59.7mcg*h/mL (17.7-421.4) mcg*h/kg and CL/F was 0.196 L/h/kg (0.027-0.539). Patients with the CYP2B6 516 GT genotype had a significantly greater mean CL/F (13.8±6.9) than patients with the TT genotype (2.9±0.9) (p=0.045), but neither showed a significant difference to patients having the GG genotype. Patients with the GT genotype showed a greater mean EFV AUC than patients with the TT genotype, although not statistically significant (α=0.05; p=0.07). No significant differences were seen in E8F AUC, (E8F+E8G) AUC, E8F/EVF or (E8F+E8G)/EFV ratios. Median CNS toxicity score was 12.5 (1-23). There was no association between EFV AUC and CY2B6 genotype with CNS toxicity. No differences in EFV AUC, CL/F and (E8F+E8G)/EFV were observed in relationship to Tanner Stages of pubertal maturation.

Conclusions: CYP2B6 genotype was directly related to the CL/F of EFV in pediatric patients and showed a potential relationship with EFV AUC. EFV plasma exposure was not associated with CNS toxicity. Pubertal maturation measured though Tanner stage did not influence the PK of EFV and CYP2B6 activity.

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RISK OF GENTAMICIN-INDUCED NEPHROTOXICITY IN THE CONTEXT OF NEW RECOMMENDATIONS FOR ITS THERAPEUTIC DRUG MONITORING: A 4-YEAR RETROSPECTIVE STUDY.

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Introduction

Patients on gentamicin usually benefit from therapeutic drug monitoring (TDM) in order to maximize efficacy and minimize the risk of nephrotoxicity. In 2010, the French Medical Agency (ANSM) edited new recommendations for the TDM of gentamicin, resulting in an increase of proposed doses and patients’ exposure to gentamicin. In this context, the aim of this study was to investigate the relationship between drug exposure and renal toxicity in patients who benefitted from TDM.

Material and Methods

This retrospective study included 305 patients on gentamicin who benefited from TDM based on pharmacokinetic modeling (546 TDM requests), followed in Limoges University Hospital between 2010 and 2014. Data collected were age, sex, serum creatinine before, during and until the seventh day after the end of treatment, and gentamicin
exposure parameters: maximal concentration (Cmax), minimal concentration (Cmin), average concentration (Cavg) and area under the curve (AUC) estimated by Bayesian estimation using our Internet-based PK-Just software (https://www.pharmaco-limoges.fr). Nephrotoxicity was defined according to KDIGO criterion for acute renal failure (3 levels of nephrotoxicity: K1, K2 and K3). The influence of covariates on nephrotoxicity was investigated using a time dependent Cox proportional hazard model using R software. A threshold for nephrotoxicity of each significant exposure markers was then investigated using ROC curve analysis.

**Results**

Median [25-75\textsuperscript{th} percentile] of serum creatinine was 87 \textmu mol/L [65-124]. Nephrotoxicity was observed in 25% of the patients (16.8% K1, 6.3% K2, 1.9% K1). No association between exposure and K1 was evidenced. Multivariate analyses showed that Cavg (HR=1.263[1.071-1.363]) and Cmin (HR=1.161[1.101-1.222]) were associated with K2 while AUC (HR=1.00[0.983-1.018]), Cmin (HR=1.142[0.923-1.406]) and Cavg (HR=1.268[0.865-1.858]) were associated with K3A. Significant ROC AUC was found only for Cmin on the risk of K2 (AUC=0.7). A Cmin threshold of 0.36 mg/L provided a specificity of 76.6% and a sensitivity of 60.2%. After dichotomization, Cmin>0.36mg/L was associated with an increased risk of K2 nephrotoxicity (HR=3.7[1.87-6.80]; p=0.00016).

**Conclusion**

The results of this study are consistent with the recommended Cmin maximal target of 0.5 mg/L, to limit the risk of nephrotoxicity. Interestingly, Cavg and AUC seem also to be important predictive markers of the risk of nephrotoxicity.

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**THERAPEUTIC DRUG MONITORING OF PSYCHOTROPIC DRUGS AND MILD OPIOID ANALGESICS IN ELDERLY HIP FRACTURE PATIENTS AT HOSPITAL ADMISSION**

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**Background:** Hip fractures are highly prevalent in older persons, with great implications for morbidity and mortality. Psychotropic drugs and mild opioid analgesics are widely used and may cause injurious falls. We aimed to analyse concentrations of psychotropic drugs and mild opioid analgesics in blood taken at hospital admission of older hip fracture patients to get information on the use of these drugs at the time of fracture.

**Methods:** Routine blood tests of hip fracture patients ≥65 years were sampled on admission to Diakonhjemmet Hospital, Oslo, Norway. Plasma analyses of psychotropic drugs and the mild opioid analgesics codeine and tramadol were performed by validated and certified ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS) methods developed for routine therapeutic drug monitoring analyses. The occurrence of detected drugs among the included hip fracture patients was compared with prescription data of the same drugs in an age-, region-, and time-matched population from the Norwegian Prescription Database (reference group).

**Results:** 250 hip fracture patients admitted to hospital were included in the study. The mean age was 83 years, and 76 % of the patients were female. Psychotropic drugs and/or mild opioid analgesics were detected in plasma samples in 63% of the patients. Occurrence of detected drug in hip fracture patients versus occurrence of prescribed drug in the reference group, respectively: antidepressants 16.4% vs 10.5%; diazepam 11.6% vs 7.5%; oxazepam 11.2% vs 6.8%; nitrazepam 4.8% vs 2.2%; clonazepam 0.8% vs 0.6%; zopiclone 22.8% vs 20.7%; zolpidem 4.8% vs 3.5%; codeine 18.0% vs 16.6%; tramadol 8.8% vs 6.1%.

**Conclusions:** Psychotropic drugs and/or mild opioid analgesics were detected in plasma samples of nearly two thirds of elderly hip fracture patients at hospital admission. Our findings support that use of these drugs is associated with increased risk of falling.

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**A COMPARISON OF THE PHARMACOKINETIC PARAMETERS OF MYCOPHENOLIC ACID (MPA) OF THE CONCENTRATIONS OF THE TOTAL AND A FREE FRACTION IN SERUM OF PATIENTS WITH RENAL DISEASES**

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Introduction. Modern immunosuppressive drug therapy is used to treat many immune-mediated diseases. These include renal impairments, the state of the post-transplantation. One of the modern drugs used in these cases is mycophenolic acid and it's derivatives (mycophenolate mofetil, mycophenolate sodium). These drugs exhibit varied pharmacokinetic depending on many factors which are not fully explored. In recent years, attention is drawn to the possibility of increasing the safety and efficacy of mycophenolic acid therapy by measuring a drug's free fraction and the designation of the parameter - area under the curve.

Objective: To compare the pharmacokinetic parameters of total and free fraction of the drug based on steady-state measurements in patients with various renal diseases and in patients after renal transplantation.

Material and Methods: The study included 100 patients with glomerulopathy and patients after renal transplant treated on the Department of Nephrology and Transplantation Medicine, Wroclaw Medical University. The MPA concentrations was measured before next dose, 30 min and 2 hours after dosing. Measurement was performed in the Therapeutic Drug Monitoring Laboratory of the Department of Clinical Pharmacology Wroclaw Medical University by EMIT method, and measuring of the free fraction of MPA designation was performed in the Department of Medicinal Chemistry, Medical University of Warsaw, using HPLC/MS method.

Results: There were no statistically significant differences between the values of the pharmacokinetic parameters of MPA for various pathological conditions. The average AUC for the total MPA concentration was 16 mg/l/ml, while for the MPA free fraction of the average AUC (AUCfree) is 167.08 ng / h / ml. The results showed no correlation between the AUC and the AUCfree.

Conclusions: Administration of MPA in patients with glomerulopathy requires the determination of AUCfree by measuring the concentration of free fraction, especially in patients who frequently observed adverse reactions pharmacotherapy. Monitoring of the MPA can contribute to increasing the effectiveness and safety of treatment.

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BEYOND ACETAMINOPHEN: CHARACTERIZATION OF N-ACETYL CYSTEINE (NAC) INTERFERENCE ON MULTIPLE ANALYTES AND IMPLICATIONS FOR ASSESSING LIVER FUNCTION.
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BACKGROUND: N-acetyl cysteine (NAC) improves the outcome of acetaminophen poisoning by a number of suggested mechanisms including prevention of hepatic injury and reduction of the severity of hepatotoxicity. However, because of its additional utility as an antioxidant and free radical scavenger, the role of NAC has expanded to other uses in other poisonings as well as in patients with liver failure. The negative interference of NAC in acetaminophen assays has been previously noted, but not fully characterized. The objective of this study was to define the concentrations and timeframe at which NAC interference is observed, not only for acetaminophen, but also for other analytes including those of liver function and those dependent on redox-sensitive chemistry.

METHODS/RESULTS: Excess patient samples with high acetaminophen levels or blank patient pools were incubated with increasing concentrations of NAC (100 to 800 mg/L) prepared in blank serum. Samples were assayed for acetaminophen (Abbot Architect) and other analytes (Ortho Vitros and Abbott Architect) within one hour of NAC addition as well as up to three hours after NAC addition. NAC negative interference on acetaminophen measurement greater than 10% differences from baseline was observed at 100 mg/L NAC and increased in a dose-dependent manner to >60% difference when measurement was carried out within one hour. However, when NAC was incubated with the samples for increasing time, the assay interference was attenuated to <10% difference at 800mg/L NAC at 3 hours. Analysis of other analytes revealed significant (>10% difference) interference due to NAC for ALP, AST, glucose and cholesterol (negative) and for ALT (positive), but not for total protein, albumin, GGT, or total bilirubin.

CONCLUSIONS: Characterization of NAC interference in the Abbott Architect acetaminophen assay revealed the concentrations and time-dependence of this well-known interference and this study offers guidelines for most accurate acetaminophen measurement. In addition, novel identification of NAC interferences on other analytes revealed which liver markers are most and least affected ex vivo by NAC treatment, the redox sensitivity of some assays, and the need for communication to clinicians regarding the possible changes in analyte measurement due to NAC treatment.

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BROAD SPECTRUM URINE DRUG SCREENING: THE CHALLENGES OF ASSESSING QUALITATIVE CUT-OFF PERFORMANCE CHARACTERISTICS IN AN LC/MSMS SYSTEM.
BACKGROUND: LCMS/MS methods are valuable for front-line or confirmatory testing of urine samples for a broad range of drugs. The cut-offs adopted by most laboratories for positive screening results are derived from the Substance Abuse and Mental Health Services Administration (SAMHSA) regulations. The objective of this study was to determine how best to assess the linearity and qualitative cut-off performance characteristics in an LCMS/MS system used for screening.

METHODS/RESULTS: Forty-five drugs or drug metabolites were assessed for linearity below the SAMHSA-derived positive cut-offs in use at the Hospital for Sick Children in Toronto, Ontario. Additionally, the performance of our method at the cut-off of 50 ng/mL for 3,4-MDA and 3,4-MDMA was assessed by application of the CLSI Guideline EP12-A2. In the linearity study, analyte pools, ranging from 1.56 ng/mL to 100 ng/mL of each analyte, were analyzed in duplicate on an LCMSMS QTrap system. The linearity of the peak area-under-the-curve of each analyte in response to concentration was evaluated by application of CLSI Guideline EP6. Differences in linear performance were observed specific to drug, drug metabolite, and between mass-transitions. Additional analysis assessed normalization to the internal standard and the success rate of software peak identification without user intervention. In the qualitative cut-off study, the capacity of our broad spectrum urine drug screen to correctly partition samples into a qualitative screen Positive or screen Negative designation was evaluated. Replicates of 3,4-MDA or 3,4-MDMA samples at concentrations at and bracketing a 50 ng/mL cut-off were analyzed. Previously established area under the curve count cut-offs failed to correctly partition samples as Negative at the -20% bracket. However, normalization of the data to the internal standard and establishing a new normalized area cut-off at 50 ng/mL improved the method partitioning performance.

CONCLUSION: As a result of this evaluation, there is confidence that our method correctly partitions Positive versus Negative samples with concentrations ±20% from the SAMHSA cut-off of 50 ng/mL for 3,4-MDA and 3,4-MDMA. This work highlights the challenges with determining performance characteristics in an LCMSMS system used for screening.

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LC/MS/MS QUANTITATIVE ANALYSIS OF STEROIDS IN SERUM AND URINE, AND EVALUATION AND COMPARISON OF SAMPLE PREPARATION TECHNIQUES
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The steroids analyzed include androstenedione, DHEA-S, testosterone, cortisol, DHEA, 11-deoxycorticisol, corticosterone, 17-OH-pregnenolone, progesterone, 18-OH-corticosterone, deoxycorticosterone, etc. A highly sensitive, accurate and specific LC/MS/MS analytical method has been developed for the quantitation of steroids by QQQ in serum and urine. Various sample preparation techniques that include ultracentrifugation (UF), protein crash (PPT), liquid-liquid extraction (LLE), supported-liquid extraction (SLE) and solid phase extraction (SPE) and one (1D) and two (2D) dimensional chromatographic configurations are evaluated and compared based on their ease of use, analyte recovery and post-extraction cleanliness. The derivatization of these compounds was also evaluated as well as different mass spectrometer platforms. The described analytical method achieves the required sensitivity and is capable of quantitating the analytes over their dynamic range.

An Agilent 6495 tandem mass spectrometer with Agilent JetStream technology in positive and negative Electrospray mode and an Agilent Infinity 1290 HPLC system were utilized for this analysis. 200 µL of serum and urine were used for the analysis of steroids. Various columns were evaluated and an Agilent Poroshell 120 EC-C18 100 x 2.1 mm, 2.7 µm with a water:acetonitrile mixture containing 0.1% formic acid and 5mM ammonium formate gradient achieved baseline chromatographic separation in less than 9 minute run time for all dimensions. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from NIST, UTAK and Recipe Controls and serum and urine samples.

Good linearity and reproducibility were obtained with the concentration range from 5 pg/mL to 1000 ng/mL for the respective steroids with a coefficient of determination >0.995 for all sample preparation and chromatographic techniques. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were determined to be range from 1 to 5 pg/mL. Excellent reproducibility was observed for both compounds (CV < 10%) for all techniques and configurations. The sample preparation techniques are quick and easily applied for high throughput analysis.
LC/MS QUANTITATIVE ANALYSIS OF FAT SOLUBLE VITAMINS IN BLOOD
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A highly sensitive and specific LC/MS analytical method has been developed for the quantitation of the relevant fat soluble vitamins- vitamin A (retinol, retinal and retinoic acid), vitamin D (25-hydroxy-vitamin D3 and D2), vitamin E (alpha-, beta-, gamma- and delta- tocopherol and tocotrienols) and vitamin K (phyllloquinone). These compounds are essential nutrients required for normal physiological functioning that either cannot be synthesized at all or in necessary amounts but can be toxic at high levels. Therefore, a simple and accurate quantitative analytical method was developed to measure these fat soluble vitamins in human blood using a simple offline sample preparation.

An Agilent 6460 QQQ LC/MS with Agilent Jet Stream (AJS) technology in positive electrospray mode and an Agilent Infinity 1260 HPLC system were utilized for this analysis. 500 mL of serum was used for the analysis of the fat soluble vitamins and the sample preparation involved a simple protein crash followed by a simple liquid-liquid extraction for the fat Soluble vitamins in buffer. Various columns were evaluated and an Agilent Poroshell 120 PFP, 100 x 2 mm, 2.7 µm with water:methanol containing 0.1% formic acid gradient achieved baseline chromatographic separation of the fat soluble vitamins. Quantitative analysis was performed using multiple reaction monitoring (MRM) transition pairs for each analyte and internal standard in positive mode and accuracy of the method was verified using reference materials from Recipe and UTAK controls and serum and blood adult samples.

Good linearity and reproducibility were obtained for all the fat soluble vitamins across their respective ranges. The lower limits of detection (LLOD) and lower limit of quantitation (LLOQ) were achieved at well below their respective ranges. The intra- and inter-day CVs were < 15% and the calibration curves displayed linearity with an R² > 0.998 respectively for all the vitamins.

A sensitive, simple, specific and accurate liquid chromatography- tandem mass spectrometry analytical method was developed and verified for the measurement of fat soluble vitamins in blood. The sample preparation is quick and easily applied for high throughput analysis.

OPTIMIZATION OF DATA ANALYSIS PARAMETERS FOR CLINICAL TOXICOLOGY TESTING USING HIGH RESOLUTION MASS SPECTROMETRY
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Background: The use of high resolution mass spectrometry (HRMS) in toxicology laboratories is becoming increasingly common. While HRMS offers the ability to simultaneously identify common drugs and unknowns, the optimal data analysis parameters have not been explored. The objective of this study was to optimize data analysis for HRMS testing by; determining the optimum allowable error and weighting for mass (ME), retention time (RT), isotope pattern (IP) and library score (LS); comparing different library search algorithms, and assessing the performance of an in-house HRMS spectral library compared to a commercially library.

Methods: Data was acquired with a validated method using an ABSciex TripleTOF®6600 operating in positive mode, collecting full scan data with IDA triggered acquisition of product ion spectra. Data was analyzed with MasterView® software. To determine the optimum parameters (ME,RT,IP,LS) a set of training samples were prepared by spiking known compounds (N=210) into drug-free urine. Different library search algorithms (MasterView®, PROLS, NIST) were compared. An in-house HRMS library was compared to a commercial nominal mass library.

After optimization, performance of targeted, suspect and untargeted data analysis was compared using patient samples.

Results: Data analysis parameters (ME,RT,IP,LS) were recorded for compounds found in the training set. Considering the true positives (N=587), the average RT error was 0.11+0.5mins, mass error 3+4ppm, IP difference 10-20%, and library score 90-20. ROC analysis was used to determine the optimal cut-off values for each parameter. Additionally each parameter was combined to produce a combined score and statistical modeling was used to determine the optimal weighting for each parameter (10%-ME, 10%-RT, 10%-IP, 70%-LS). The library search algorithms performed similarly with slight variations in the match scores generated. The spectra from the in-house library matched with a higher score to the acquired spectra compared to the nominal mass library resulting in a slight increase in detection sensitivity. The overall sensitivity and specificity was greater for targeted analysis (97%,99%) compared to suspect (54%,99%) and untargeted analysis (35%,99%).

Conclusions: Data analysis parameters should be optimized and validated prior to implementation of a HRMS method in a toxicology laboratory as the results can vary depending on the parameters used.
BACKGROUND: Vancomycin efficacy is associated with area-under-the-curve (AUC) and bacterial minimum inhibitory concentration (MIC), with a suggested 24-h steady-state AUC:MIC for S. aureus of ≥400 mg*h/L. AUC<800 may reduce nephrotoxicity, but prolonged concentrations <10 mg/L can facilitate resistance. We wished to update vancomycin dosing guidelines to include these pharmacodynamic considerations in premature and term neonates likely to receive vancomycin for late-onset sepsis after 72 hours of life.

METHODS: We recorded post-menstrual age (PMA), post-natal age (PNA), length, weight, sex, serum creatinine (SCr), concomitant medications and other covariates, and vancomycin doses/concentrations from two groups: 1) 75 preterm infants and 2) 110 preterm/60 term infants. Using Pmetrics, we fitted allometric 1-compartment PK models with vancomycin elimination proportional to differing descriptors of renal function: modified Cockcroft-Gault (using PMA or PNA), Schwartz, PMA/SCr, or PNA/SCr. We modeled each population and cross-validated with the other. Simulating from the final model, we developed initial vancomycin dosages for neonates with PNA 3 to 7 or 7 to 30 days, normal (0.1 to <1.0 mg/dL) or high (1.0 to 2.5 mg/dL) SCr, and weights 0.25-1.2 kg, >1.2-2.0 kg, or >2.0-6.0 kg, all to achieve ~90% probability of AUC>MIC≥400. We fixed MIC=1, the S. aureus vancomycin MIC90. For each simulation, we calculated the probability of AUC >800 and % dosing interval >10 mg/L.

RESULTS: PNE, weight, and SCr ranges in the modeled population were 1-691 days, 0.5-12 kg, and 0.1-4.7 mg/dL. The model based on PNA/SCr, developed in the larger population, had the lowest prediction bias and imprecision. Simulations using this model are summarized in the table below.

CONCLUSION: While not intended to extend glomerular filtration, PNA/SCr best predicted vancomycin elimination and is unambiguous and easy to calculate. Our proposed initial doses will be validated prospectively, and TDM is recommended for subsequent dose adjustment.

Picture 1: https://www.eventure-online.com/parten-uploads/7/15017/1g1_266765_2xi0aHCSfZ.jpg
Caption 1: Recommended Dose Regimen

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IMMUNOREGULATION OF TACROLIMUS ON BTLA EXPRESSION ON T LYMPHOCYTE SUBSETS IN ALLO-LIVER RECIPIENTS

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Background and objectives: B and T lymphocyte attenuator (BTLA) is a negative costimulators expressed on lymphocytes, which binds to its receptor on T cells and delivers a coinhibitory signal to T-cell activation. Tacrolimus, as one of calcineurin inhibitors, plays a critical role in immunosuppression in transplantation. Here we explored the effects of tacrolimus on BTLA expression on T cells to further know the immunosuppressive mechanism of tacrolimus in liver transplantation.

Methods: 30 healthy controls and 48 allo-liver recipients were included. All the recipients were treated with tacrolimus-based regimen. And their transplant periods all were over 3 years. The range of whole blood concentration of tacrolimus was 1.90 ng/ml to 12.90 ng/ml. Apply flow cytometry to detect the expression of inhibitory costimulators BTLA on peripheral T lymphocyte subsets.

Results: 1. Imbalance of peripheral T lymphocyte subsets in recipients: The expression of peripheral CD3+ T cells and CD3+CD4+ T cells were inhibited in recipients compared with healthy control (P<0.001). 2. Inhibitory effects of Tacrolimus on negative costimulator BTLA: compared with healthy control, BTLA expression on T cell subsets obviously decreased in recipients (P<0.001). 3. BTLA expression positively correlated with tacrolimus levels. In recipients with high Tacrolimus level (Tac≥5.3 ng/ml) the percentage of peripheral BTLA+CD4+CD3+ T cells remarkably increased, compared with recipients with low Tacrolimus level (Tac<5.3 ng/ml) (P=0.035). (Fig.1) The good correlations between whole blood Tacrolimus levels and the percentage of peripheral BTLA+CD4+CD3+ T cells or BTLA+CD8+CD3+ T cells were shown (rs=0.424 (P=0.022) and 0.389 (P=0.037), respectively).
Conclusions: Tacrolimus could prompt BTLA expression on T lymphocytes, especially on CD3+CD4+ T cell subsets, which was another way for tacrolimus to exert its immunosuppressive effects and strengthen its inhibitory role in allo-liver recipients.

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Background. Tacrolimus and cyclosporine are routinely analyzed by 3200 QTRAP ® LCMSMS System - AB Sciex. The backup method has been Abbott Architect at another laboratory. At the beginning of the year Roche launched new immunoassay methods for tacrolimus and cyclosporine on cobas immunoassay series e411, e601/602. The purpose of this evaluation was to assess a change in our backup method to the Roche platform and perform the analyses in house.

Methods. Thirty samples for tacrolimus and cyclosporine were analyzed by both Abbott Architect i2000 and Roche cobas e411. Twenty samples for tacrolimus and 20 samples for cyclosporine were analyzed by LCMSMS, Architect i2000 and cobas e411. Quality control samples were analyzed with each batch of samples. Samples are pretreated for analysis similar to LCMSMS.

Results. Between-run precision was under 10% for all 3 methods. Comparison data between the 3 methods are shown below:

Discussion. Both Architect and cobas methods have a positive bias versus LCMSMS and a negative intercept that is more noticeable for the cobas for cyclosporine. The comparison between Architect and cobas is linear with a slight negative intercept for the cobas cyclosporine. Tacrolimus for both Architect and cobas compare very well to LCMSMS with a slope close to 1.0 and a small positive bias versus Architect and small negative bias versus cobas. Comparison of tacrolimus between Architect and cobas has a slope above 1.0 and a slight negative bias for cobas.

Conclusion. The Roche cyclosporine and tacrolimus immunoassays on the cobas e411 are acceptable as backup methods for our routine LCMSMS method. Monitoring and performance of external quality control is warranted to ensure accuracy of results.

Key words. Tacrolimus, cyclosporine, immunoassays, LCMSMS, comparison.

LC-MS/MS METHOD DEVELOPMENT - FOCUS ON BIOANALYSIS OF NON-VITAMIN K ORAL ANTAGONIST ANTICOAGULANTS (NOACS)
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Background: Conventional liquid chromatography-tandem mass spectrometry (LC-MS/MS) remains a powerful tool for small molecule bioanalysis. In terms of suitability, it facilitates quantification of multiple agents in one analysis. Consequently, LC-MS/MS methods can be tailored towards monitoring of agents of the same class of drugs prescribed in common clinical indications; such as the non-vitamin K oral antagonist anticoagulants (NOACs) apixaban, dabigatran, edoxaban, and rivaroxaban which are being used for the treatment and prevention of thromboembolic disease. However, as emphasized in current guidelines for bioanalytical method validation, these approaches require rigorous method validation.

Methods: NOACs calibrators and quality controls were prepared in plasma matrix of normal individuals. Stable-isotope-labeled analogues for each NOAC were obtained for internal standardization and quantitative analysis.
Plasma specimen were prepared by 2 independent methods: protein precipitation with acetonitrile containing internal standards and sample preparation using a mixture of magnetic beads (MagSi-TDMPREP, MagnaMedics Diagnostics), buffer, acetonitrile and internal standards. Chromatographic separation was achieved using a C8 column and a gradient elution. LC-MS/MS analyses were performed on a triple quadrupole mass spectrometer using positive electrospray ionization in selected reaction monitoring (SRM) mode. Ion transitions monitored for quantitation were m/z 460 → 443 for apixaban, m/z 472 → 289 for dabigatran, m/z 548 → 366 for edoxaban, and m/z 436 → 145 for rivaroxaban.

**Results:** All analytes were eluted in less than 2 minutes. Extraction with both acetonitrile and magnetic beads yielded clear supernatants that were subjected to LC-MS/MS analysis. For both extraction methods, linearity was demonstrated from 2 to 500 ng/ml for all analytes.

**Conclusions:** NOACs can be quantified by means of a LC-MS/MS multi-analyte method suitable for therapeutic drug monitoring (TDM) settings. Conventional LC-MS/MS methods may exhibit drawbacks, for instance, the limitation of sample throughput due to laborious clean-up procedures. In this respect, extraction with magnetic beads may offer advantages as sample preparation does not require centrifugation and can be easily automated. Based on these findings, the performance of both extraction methods is going to be further assessed.

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**DRUG INTERACTIONS: UPDATE FOR THERAPEUTIC DRUG MONITORING (TDM) ON NEONATAL INTENSIVE CARE UNITS (NICU)**
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**Background:** Therapeutic drug monitoring (TDM) is one of the most challenging areas in pharmacotherapy on ICU, on neonatal-pediatric intensive-care units (NICU) in particular. With respect to the given number of drugs the patients in the NICU routinely receive more therapies than on general medical ward or surgical ward. As a consequence set up of TDM is needed and useful with regard of potential drug-drug interactions and impact of altered (pathophysiological and age-dependent) pharmacokinetics (PK). Therefore the goal of the present work is to identify and assess the risk of relevant drug-drug interactions (DDIs) of commonly used drugs on NICU.

**Methods:** A retrospective web-based literature review study to identify and assess the risk relevant drug interactions of n=48 frequently used drugs on NICU (University Hospital of Cologne) was carried out. All identified DDI's were presented as a visual interaction triangle (VIT) and recommendation on the management of clinically significant DDI's were provided.

**Results:** DDIs were subdivided into three classes: DD-major, DD-moderate/minor and intravenous admixture drug interaction (IA). A total of n= 115 (10,4 %) possible interactions (DDI, IA) were found.

n=35 (3,17 %) cases were categorized as serious interaction (DD-major). n=43 (3,89 %) were less severe (DD-minor/moderate) and in n=35 (3,35 %) cases an intravenous admixture drug interaction (IA) were found clinically relevant. One drug-drug combination was contraindicated.

The presented visual interaction triangle (VIT) serves an effective tool as unique clinical reference to uncover possible drug-interaction potential on NICU.

**Conclusions:** A relevant number of DDIs were identified in this web-based study, but only a small number is clinically significant. Nevertheless practitioners can use the presented visual interaction triangle (VIT) to monitor for possible predictable adverse drug effects.

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**TOXICOLOGICAL SCREENING OF POST MORTEM BLOOD SAMPLES FROM UNEXPLAINED DEATHS IN THE AMSTERDAM REGION FROM 2011-2014**
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The Dutch system of deciding whether a death is of natural- non-natural or of criminal cause, is sometimes criticised. The Amsterdam Public-Health-Authority (GGD) decided some years ago to perform further investigations (including toxicological) on all death-cases of which the cause was not clearly natural. Earlier only urine was screened for alcohol and important classes of drugs by immuno assays. Results were published [1]. Now we report on a more comprehensive but still very efficient way of toxicological screening. Femoral blood was taken by forensic physicians of the GGD. In about 25% of all cases toxicological investigation was performed.
Solid phase extraction on the samples resulted in three fractions: acid/neutrals, lipophilic bases and polar bases ([2]). The fractions were analysed qualitatively by the Waters Toxscreen system consisting of liquid chromatography followed by single quadrupole mass-spectrometry at 6 different ionisation voltages with 6 corresponding mass-spectral libraries. Also a quantitative GC screen for ethanol and some other volatiles was part of the protocol. This toxicological analysis was performed by the toxicology department of Atalmedial at that time located in Amsterdam. From March 2011 up to June 2014, over 600 cases were investigated. The results was used to advise whether to start a full criminal investigation.

In an unexpected high number of cases, substances potentially relevant for the cause of death were found. This relevance being dependant of the concentration in the body. At the start of the project we intended to determine concentrations in all positive cases. But this soon became infeasible because of the financial implications of this extra work.

In this presentation we will report on the possibility of performing a quite extensive qualitative toxicological investigation in an efficient and cost effective manner.

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James Dear, United Kingdom

ANTIDOTES
Antoinette van Riel, The Netherlands

SERIOUS GAMING IN LIFE SUPPORT
Stephanie Klein Nagelvoort Schuit, The Netherlands

CLINICAL IMPACT OF LCMS AND DRUG LIBRARIES
Daniela Remane
Institute of Forensic Medicine, University Hospital Jena, Germany, JENA, Germany

QUALITY CONTROL IN CLINICAL AND FORENSIC TOXICOLOGY
Denise McKeown, United Kingdom

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, Nederland

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, Nederland

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Ron Mathot
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Kana Mizuno
Clinical Pharmacology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States of America
PK/PD GUIDED DOSING OF VORICONAZOLE, FLUCONAZOLE, AND POSACONAZOLE IN IMMUNOCOMPROMISED AND CRITICALLY ILL PATIENTS
Jan Willem Alffenaar
UMCG, GRONINGEN, The Netherlands

Evaluation of the Performance of an A Priori Population Pharmacokinetic Tool for Vancomycin in Premature Neonates, as Compared to Clinician Empirical Dosing
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Background
We previously developed a pharmacokinetic model and Bayesian estimator for premature neonates that help to calculate a priori the individual vancomycin starting dose to reach a concentration target based on demographic parameters (weight, height, creatinine). The goal of this study was to evaluate the performance of this model as compared to the clinicians’ routine dosing practice.

Methods
Forty one concentrations at steady-state (ss) obtained from 36 premature neonates treated with continuous vancomycin infusion were studied. Following empirical dosing by the clinicians, the proportions of patients below (<20 mg/L; BT), within (20-25 mg/L; WT) and above the target (>25 mg/L; AT) vancomycin ss concentration range were calculated. For all patients, the dose needed to reach the target was calculated using the a priori Bayesian estimator and compared to that prescribed by the clinician. A reference dose was then calculated using the priors and the observed dose/concentration and was compared to the a priori and to the clinician doses using ANOVA and Bonferroni correction for multiple comparisons.

Results
The median [25-75th percentiles] of age, weight, height, creatinine, empirical vancomycin dose and first vancomycin concentration were 11[8-18] years, 1.44[1.08-1.82] Kg, 37[33-42] cm, 48[35-71] µM, 35[25-52] mg and 17.2[15.7-22.15] mg/L respectively. After the first vancomycin measurement at ss following empirical dosing, 61% (25/41), 24% (10/41) and 15% (6/41) were in the BT, WT and AT range respectively. Among the 25 patients below target, 64% (16) of the doses proposed using the a priori model were higher than the empirical doses, while among the 6 patients above target, they were all lower. For the 10 patients in the WT, 4 patients had higher and 6 patients lower a priori calculated than empirical doses, with a mean[CI95%] difference of 4.5[-37 to +46]mg. ANOVA showed no significant difference between the 3 types of doses (p=0.501).

Conclusions
Although not statistically significant, the present results are in favor of the use of our a priori dose calculator for determining the starting dose of vancomycin in individual premature neonates, but they have to be confirmed in further prospective studies.

CYP3A4*22 Allele is Related to Increased Plasma Levels of 4-Hydroxytamoxifen and Partially Compensates for the Inefficient CYP2D6 Metabolic Activation of Tamoxifen
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3Université Catholique de Louvain, BRUSSELS, Belgium

Background: The therapeutic antiestrogenic effect of tamoxifen (TAM) requires metabolic activation to endoxifin (EDF) and 4-hydroxytamoxifen (HTF). Adequate therapeutic outcome seems to be dependent on the achievement of a threshold of EDF concentration (>5.9 ng mL⁻¹). EDF plasma levels are highly variable among patients, which
could be partly explained by polymorphisms in the CYP2D6 gene and the use of enzymeinhibitor drugs. From a genetic point of view, a recently described CYP3A4*22 polymorphism has been associated with reduced enzyme activity. However, there is little knowledge about the impact of CYP3A4 polymorphisms on TAM metabolic activation. Thus, this study aimed to evaluate the impact of CYP3A4*22 in the formation of EDF and HTF, under different CYP2D6 genotypic backgrounds. **Methods:** Trough blood samples were collected from 178 patients. CYP2D6 genotyping was performed using the Luminex xTAG® CYP2D6 Kit v3 (Luminex Corporation) and CYP3A4 using TaqMan® (Applied Biosystems) genotyping assay. TAM, HTF and EDF were measured in plasma by LC-MS/MS. **Results:** CYP3A4*22 allele was present in 9.5% of patients. Regarding CYP2D6, 5.6% of patients were poor metabolizers (PM), 7.8% intermediate metabolizers (IM), 36.5% extensive metabolizer, with activity scores of 1 or 1.5, related to moderate/rapid activity (EM-S). 42.1% were extensive metabolizer with activity scores of 2, corresponding to rapid activity (EM-F), and 4.5% were ultra-rapid metabolizer (UM). CYP2D6 gene score was significantly correlated to EDF (r=0.343, p<0.01), while CYP3A4 gene score was inversely correlated to TAM (r=-0.289, p<0.01) and HTF concentrations (r=-0.269, p<0.01), but not associated to EDF plasma concentrations (r=-0.064, p=0.43). EDF concentrations were lower in CYP2D6 PM (2.77 ng mL⁻¹) and IM (5.84 ng mL⁻¹), compared to functional group (EM-F) (10.67 ng mL⁻¹, p<0.001). HTF and TAM levels were respectively 47% and 53% higher in CYP3A4*22 carriers compared to *1/*1 patients in the whole group. Patients with impaired CYP2D6 metabolism and carriers of CYP3A4*22 had EDF levels comparable to CYP2D6 EM-F group (9.06 and 10.67 ng mL⁻¹, p=0.247). **Conclusions:** The presence of CYP3A4*22 might compensate the reduction of EDF concentrations related to CYP2D6 inactivity, especially due to increased HTF concentrations.

**DEVELOPMENT AND VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRIC METHOD FOR THE DETERMINATION OF IMATINIB IN DRIED BLOOD SPOTS**

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**Background:** The tyrosine kinase imatinib (IM) is a first-line drug for the pharmacological treatment of chronic myeloid leukemia (CML). A minimum plasma concentration threshold of 1.000 ng mL⁻¹ is currently the target for TDM. The measurement of IM concentrations in plasma is not widely accessible, especially in Developing Countries where sample logistics could be a difficulty. Due to its intrinsic stability and handling safety, the determination of IM in dried blood spots (DBS) can be a good alternative to facilitate sampling and transportation of the samples to reference laboratories. The aim of this study was to develop a method for the determination of IM in dried blood spots by LC-MS/MS. **Method:** One 6 mm DBS was punched and added with 300 µL of methanol containing IS (Imatinib-D8, 75 ng mL⁻¹), followed by incubation in a ThermoMixer for 45 min at 1,500 rpm and 25 °C. An aliquot of 5 µL of the supernatant was injected on a LC-MS/MS system. Separation was performed in a Kinetex C18 column (150 x 4.6 mm, 2.6 µm), maintained at 40 °C. Mobile phase was a mixture of formic acid 0.1% and acetonitrile (gradient from 65:35 to 30:70, v/v) at a flow rate of 0.8 ml min⁻¹. Monitored transitions for quantitation were 494/378 for IM and 502/378 for IS. **Results:** Retention time was 1.9 for IM and IS. The method was linear from 50 to 4,000 ng mL⁻¹ (r>0.99). Mean IM recovery from DBS was 91%. Accuracy was 101-107%, intra-assay precision was 3.98-10.96% and inter-assay precision was 8.32-8.38%. IM was stable in DBS at -20 °C, 25 °C and 40 °C up to 36 days (CV% <12%) and processed samples are stable up to 12 h. The Hct effect was in the range of 25 to 50%, presenting no significant effect on measurements. The method was successfully applied to 50 paired clinical DBS and plasma samples, with a high correlation between matrices (r=0.96, p<0.01). **Conclusions:** A LC-MS/MS method for the determination of IM in DBS was developed and validated. The procedure has adequate analytical performance and can be used for TDM of IM in CML treatment.

**INFLUENCE OF CYP2D6 AND CYP3A4 PHENOTYPES, DRUG INTERACTIONS AND VITAMIN D STATUS ON TAMOXIFEN BIOTRANSFORMATION**

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Background: The therapeutic antiestrogenic effect of tamoxifen (TAM) requires metabolic activation to endoxifen (EDF) and 4-hydroxytamoxifen (HTF). Adequate therapeutic outcome seems to be dependent on the achievement of a threshold of EDF concentration (>5.9 ng mL\(^{-1}\)). EDF plasma levels are highly variable among patients, mainly as a result of polymorphisms in the CYP2D6 gene and the use of enzymes inhibitor drugs. In a lesser extent, CYP3A4 also contributes to EDF formation and can be influenced by drug interactions and sun exposure. In view of the large variability on therapeutic response and the multiple factors associated to TAM metabolic activation, the present study aimed to evaluate the effect of CYP2D6 and CYP3A4 phenotypes, drug interactions and vitamin D exposure on TAM metabolism in a group of breast cancer patients. Methods: Trough blood samples were collected from 116 patients. TAM, EDF and HTF were measured in plasma by LC-MS/MS. CYP2D6 and CYP3A4 phenotyping were obtained according to [Dextromethorphan]/[Dextrophan] and [Omeprazole]/[Omeprazole Sulphone] metabolic ratios, measured by HPLC in plasma collected 3 hours after oral administration of dextromethorphan and omeprazole. 25-OH-vitamin D\(_3\) was measured in plasma by HPLC-UV. Results: about 20% of patients had reduced CYP2D6 metabolic activity and 7% CYP3A4 impaired metabolism. EDF levels diminished proportionally to the reduction of CYP2D6 metabolic activity (PM 2.79 ng mL\(^{-1}\), IM 5.36 ng mL\(^{-1}\) and EM 10.65 ng mL\(^{-1}\), P<0.01). Median plasma levels of TAM (161.50 ng mL\(^{-1}\)) and HTF (1.32 ng mL\(^{-1}\)) in CYP2D6 IM/CYP3A4 PM patients were higher (P<0.05) than those from CYP2D6 IM/CYP3A4 EM patients (122.07 ng mL\(^{-1}\)) and 0.61 ng mL\(^{-1}\), respectively). Seasons contributed to the interpatient variability of EDF and HTF levels, with Summer concentrations being 24% and 42% higher compared to Winter. Vitamin D\(_3\) status was not associated to CYP3A4 metabolic activity, indicating that other mechanisms might be involved in the relation between TAM metabolism and vitamin D exposure. Conclusions: CYP3A4 contributes to the bioactivation of TAM through formation of HTF and becomes increasingly important in case of reduced CYP2D6 activity. EDF and HTF exposure were associated to seasonal variations, with considerable higher plasma concentrations during summer.

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**EVALUATION OF DIHYDROPYRIMIDINE DEHYDROGENASE ACTIVITY IN PLASMA AND SALIVA SAMPLES OF COLORECTAL CANCER PATIENTS AND ITS RELATION WITH THE OCCURRENCE OF TOXICITY**

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Background: Fluoropyrimidine toxicity has been related to a deficiency on its metabolic clearance mediated by dihydropyrimidine dehydrogenase (DPD) enzyme. The identification of patients with reduced DPD activity is mostly based on the measurement of the concentrations of the endogenous compound uracil (U) and its DPD metabolic product, 5,6-dihydrouracil (UH\(_2\)) in plasma samples. Recently, saliva has been suggested as an alternative sample for DPD evaluation. The aim of this study was to evaluate DPD activity in colorectal cancer patients trough the metabolic ratio [UH\(_2\)]/[U] both in plasma and saliva samples and its performance to identify patients presenting grade III/IV toxicity after fluoropyrimidine chemotherapy. Methods: Paired plasma and saliva samples were obtained from 23 patients scheduled for 5-FU treatment for colorectal cancer, before starting of treatment. U and UH\(_2\) concentrations were measured in both matrices by HPLC-DAD. Adverse effects after the first chemotherapy cycle were classified according to NCI-CTCAE. Results: 30.4% of the patients presented grade III/IV toxicity. [UH\(_2\)]/[U] metabolic ratios presented a large amplitude of differences among patients, ranging from 3.2 to 17.8 in plasma and 0.3 to 29.1 in saliva. The [UH\(_2\)]/[U] metabolic ratio in saliva was significantly correlated to toxicity grade (rs=-0.469, P=0.028). Metabolic ratios in both matrices were correlated (rs=0.443; P=0.039), as were metabolic ratios in plasma and toxicity grade (rs=-0.427 P=0.042). Patients with toxicity grade III/IV (n=7) presented higher median metabolic in plasma compared to patients with grade I/II (n=8) or absent of toxicity (n=8) (median 2.2 vs 5.32 and 4.5, respectively, P=0.05). No significantly difference was found in plasma metabolic ratios among toxicity groups. A cutoff of 2.97 for metabolic ratio in saliva was set after ROC curve (area under the curve of 0.829), with 86% of sensitivity and 87% specificity to identify patients presenting grade III/IV toxicity. Similar evaluation was done with plasma, but with worse specificity (area under the curve of 0.750), with a 8.6 cutoff presenting 71.4% sensitivity and 75% specificity. Conclusions: The evaluation of the metabolic ratio[UH\(_2\)]/[U] is saliva is a promising alternative to identify patients with reduced DPD activity, presenting better performance than plasma testing in this preliminary study.

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**CLINICAL EVALUATION OF A DRIED BLOOD SPOT METHOD FOR DETERMINATION OF IMATINIB IN CHRONIC MYELOID LEUKEMIA PATIENTS**
Background: The tyrosine kinase imatinib (IM) is a first-line drug for the pharmacological treatment of chronic myeloid leukemia (CML). A minimum plasma concentration threshold of 1.000 ng mL⁻¹ is currently the target for TDM. The determination of IM in dried blood spots (DBS) can be a good alternative to facilitate sampling and transportation of the samples to reference laboratories, especially in Developing Countries. However, the clinical use of DBS requires the previous knowledge of the correlation between concentrations measured in DBS and those measured in the standard biological matrix used for TDM. The aim of this study was to evaluate the clinical application of DBS sampling in CML patients under IM treatment.

Methods: Paired DBS and plasma samples were obtained from 50 CML patients. IM was measured in both matrices by UHPLC-MS/MS. Estimated plasma concentrations (EPC) were calculated from DBS concentrations (DC) using correction factors with or without the inclusion of the individual hematocrit (Hct).

Results: IM concentrations in DBS were in mean 76 % of those measured in plasma. Mean IM concentrations in plasma and DBS were 1605.8 ng mL⁻¹ (62.5-4169.2) and 1216.2 ng mL⁻¹ (50.3-3074.1), respectively. DBS concentrations and EPC calculated using patient’s individual Hct or a multiplying factor of 1.28 were highly correlated to plasma concentrations (r > 0.96). EPC using either Hct or not presented comparable values to plasma concentrations, being in mean 99% and 101%, respectively, of plasma concentrations. From Passing Bablock equation (DBS= 4.488182 + 0.760915 x Plasma), a DBS concentration of 765 ng mL⁻¹ is equivalent to the plasma threshold of 1,000 ng mL⁻¹. 93% of the individuals with plasma levels below 1,000 ng mL⁻¹ were accurately identified by EPC and using the DBS threshold of 765 ng mL⁻¹. Conclusions: The DBS method was able to identify with high accuracy patients with plasma IM levels below the clinical threshold related to better prognosis, indicating that the DBS sampling can be used for TDM of IM in a clinical setting. The use of individual Hct on estimation of plasma concentrations from DBS showed no benefit over the use of a multiplying factor of 1.28.
FULLY AUTOMATED DIRECT EXTRACTION AND ANALYSIS OF DRIED BLOOD SPOTS FOR THE DETERMINATION OF FOUR ANTI-EPILEPTIC DRUGS AND TWO ACTIVE METABOLITES

Sofie Velghe, Christophe P. Stove
Ghent University, GENT, Belgium

Introduction: Dosage adjustment of anti-epileptic drugs by therapeutic drug monitoring is very useful, especially for children. Considering the benefits of dried blood spots (DBS), this matrix could be an alternative to conventional venous sampling for this purpose. Since manual punching and off-line extraction slow down DBS analysis, an automated direct extraction and analysis of DBS can be advantageous.

Methods: A method for quantifying four AEDs and two active metabolites was developed, including carbamazepine, valproic acid, phenytoin, phenobarbital, carbamazepine-10,11-epoxide and oxcarbazepine. To that end, we used a prototype on-line DBS-SPE device (Spark Holland) coupled to liquid chromatography (Shimadzu) and tandem mass spectrometry (AB SCIEX QTRAP® 5500) (LC-MS/MS).

Results: For the LC-MS/MS method, a Kinetex 2.6 µm phenyl-hexyl 50x2.10 mm column equipped with a SecurityGuard ULTRA UHPLC Phenyl cartridge was chosen as it gave the best results in terms of compound separation. A mobile phase consisting of 5 mM ammoniumacetate (A) and 5 mM ammoniumacetate in water/acetonitrile (5/95 v/v) (B) at a flow rate of 0.4ml/min turned out to be the best option. The MS detected all compounds, using an optimized scheduled multiple reaction monitoring algorithm, operating in negative mode for valproic acid and phenobarbital and in positive mode for the other four compounds.

For the on-line DBS-SPE system, a HySphere C18HD, 7µm, 2x10 mm internal diameter cartridge (Spark Holland) was best suited for sample clean-up. The following SPE conditions turned out to be the best option: (1) preconditioning with 1 mL of methanol (MeOH); (2) equilibration with 1 mL of water; (3) elution of the DBS sample from the paper card directly to the SPE cartridge using 1 mL of water; (4) washing the cartridge with 1 mL of 5% MeOH in water and (5) elution of the sample from the cartridge using the LC pump gradient. In a next step, the eluent is directed to the phenyl-hexyl column for LC-MS/MS analysis.

Preliminary experiments on real-life patient samples readily demonstrated the applicability of the method. Validation based on international guidelines is ongoing.

Conclusion: Automated direct extraction and analysis of DBS can open up new ways for TDM of AEDs, certainly in clinical routine.