17th IATDMCT
2019 Congress
Conference Abstracts
MESSAGE FROM THE PRESIDENT

Dear delegates

The 17th International Congress of Therapeutic Drug Monitoring & Clinical Toxicology brings the main event of IATDMCT for Latin America and a Developing Country for the first time.

The Congress theme is TDM and CT for a globalized World, considering the perspective that knowledge and innovation in our field must benefit patients at the most diverse economic and social conditions, no matter where they live.

The state of the art in research and practices of TDM and CT is represented in the scientific program, which includes plenary sessions, symposiums, oral and poster sessions. Many networking possibilities will be available for delegates, a unique opportunity to expand partnerships with colleagues from all over the World.

Welcome to IATDMCT 2019!

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Symposium TDM in oncology: Exposure-response relationships of commonly used Mabs - TDM of biologics in oncology- what can we learn from biologics used in inflammatory disease and how to apply in oncology?
(Annick de Vries, The Netherlands)

Therapeutic drug monitoring (TDM) of biologics has been actively used for biologics as used in inflammatory disease. Gastroenterologists are among the early adaptors and followed by dermatologists and rheumatologists and neurologists. The use of TDM of biologics in oncology has been limited thus far.

Biologics bind specifically to their target and are prescribed as a one-dose-fits all. We observe a high inter-individual variability in PK. This can be caused by the variation in patient’s characteristics and is influenced by disease severity and immunogenicity. Hence, TDM for biologics seems useful.

Biologics in oncology target for instance blood vessel formation, cell division, the immune system (B-cells, PD-1, PD-L1) or carry therapeutics to the cancer cells. As biologics can be very highly dosed before causing any adverse effects this often leads to a prescribed dose that leads to over-exposure. Increasing use warrants efficient and cost-effective use of these effective and costly medication.

Here, I would like to extrapolate data as acquired on biologics in inflammatory disease to oncology. Highlight similarities and discrepancies; what can be learn from TDM of biologics that target a soluble protein versus a membrane bound? Concentration/effect data of several bio-therapeutics in oncology can be used to advocate personalized dosing. Largest hurdle here is acquiring good data. As most patients show an over-exposure, it is hard to acquire clear concentration/response data.
Morning session Anti-infective: Clinical use of PK/PD for individualization of antibiotic therapy at the ICU
(Birgit Koch, The Netherlands)

Mortality in critically ill patients with infection is a global health problem. Traditional antibiotic dosing is not designed for the complexity of critically ill intensive care unit (ICU) patients. Sub optimal dosing will lead to longer ICU and hospital stay and eventually higher health costs.
A ‘one-dose-fits-all’ approach seems inadequate to reach the pharmacodynamic target in ICU patients. Because of increasing antimicrobial resistance and the shortage of new antibiotics, there is a growing need to optimize the use of old and new antibiotics. Modeling of the pharmacokinetic/pharmacodynamic (PK/PD) characteristics of antibiotics can support the optimization of dosing regimens. Antimicrobial efficacy is determined by susceptibility of the drug to the microorganism and exposure to the drug, which relies on the PK and the dose. Population PK models describe relationships between patients characteristics and drug exposure.
Symposium Pharmacometrics - Implementing model-based dosing in clinical practice
(Birgit Koch, The Netherlands)

This presentation will highlight PK/PD and clinical applications of antibiotics in ICU patients, focussing on: 1) PK/PD and dosing evaluation of antibiotics, 2) setting and understanding clinical breakpoints and 3) dosing individualization using therapeutic drug monitoring (TDM). Therapeutic drug monitoring (TDM), determination of drug levels with the application of pharmacokinetic and pharmacodynamic principles to optimize dosage regimens, is likely to increase reachment of pharmacodynamic target. Information on several PK/PD and TDM studies of antibiotics in ICU patients are given. The results of the EXPAT and the status of the DOLPHIN study will be discussed.
Morning session Alternative sampling strategies - Dried blood sampling techniques distinct from dried blood samples: what’s available and what’s the status?
(Christophe Stove, Belgium)

Following up on the field of newborn screening, the field of therapeutic drug monitoring is becoming more and more acquainted with dried blood sampling as an alternative sampling strategy. However, to obtain a correct result from a dried blood microsample, several criteria need to be fulfilled. Amongst these are a correct sample collection and a correct (analytically and clinically validated) methodology to analyze the dried blood microsample. Conventionally, direct application of a drop of blood on dedicated filter paper is being used (similar as applied in newborn screening), to generate non-volumetrically applied dried blood spots (DBS). However, analysis of these is associated with several challenges, amongst which the unknown volume of blood that is deposited and the so-called hematocrit issue. The latter implies that, when taking a fixed-size partial punch from a DBS, more blood will be contained within this punch when it’s taken from a high-hematocrit-DBS than from a low-hematocrit-DBS. As a consequence, typically a positive, respectively negative bias will be obtained when the results from these partial punches are compared to those obtained from a DBS with intermediate hematocrit. Recently, new technologies have emerged, aiming at overcoming this hematocrit issue, simplifying the sampling, protecting the sample from contamination, facilitating transport, allowing volumetric collection of a non-volumetrically applied drop of blood, etc. This presentation aims at providing an overview of the distinct solutions that have been proposed and/or marketed to cope with the problems associated with conventional DBS sampling and analysis, thereby focusing on non-conventional dried blood spot sampling techniques.
Symposium New psychotropic drugs - Detection of NPS requires NPS
(New Performant Strategies)
(Christophe Stove, Belgium)

Novel Psychoactive Substances (NPS) pose a real challenge for the forensic and clinical lab. Although the last years the rate at which new substances enter the drug scene has decreased, the issue of potentially having to detect many hundreds of substances remains, as substances that were formerly used may re-enter the market at any time. Moreover, another challenge is the fact that the newest compounds (particularly synthetic opioids, cannabinoids and hallucinogens) are increasingly potent. This is not only accompanied by an increased risk of intoxication, but also by an increased challenge to detect these compounds, which may be active at subng/ml concentrations. In this presentation, an overview will be provided of the distinct technologies (analytical and other) that have been used for the detection of NPS in biofluids.

Special attention will be paid to a new strategy that was recently developed in our own lab, allowing universal, activity-based screening of biofluids for the presence of synthetic cannabinoids and synthetic opioids (but not of designer hallucinogens). The advantages and drawbacks of the different technologies will be discussed, using as a basis own published work and reports by others.
Morning session Toxicology & Environmental Health - Lead: The human risk from drinking water

(David Kinniburgh, Canada)

Lead has been a risk to human health since the time of Hippocrates (370 BC), and remains a risk today, particularly to vulnerable populations such as developing fetuses and young children. Blood lead concentrations less than 5 μg/dL in children are associated with adverse health effects, such as learning difficulties, reduced IQ scores, attention and behavior related issues. Indeed, major health organizations including Health Canada now recognize that there is no safe level of lead in blood especially in infants and children.

In North America lead exposure, as measured by blood lead levels, have decreased significantly since the removal of leaded gasoline in 1993. Today, one of the major sources of environmental exposure to lead is drinking water, which can contribute to 10-20% of lead exposure in children and adults and 40-60% in infants. Most lead in drinking water arises from plumbing systems and fixtures in buildings rather than source water. In March of this year the Maximum Acceptable Concentrations (MAC) for lead in drinking water was reduced to 5 μg/L in Canada and it was noted that every effort should be made to maintain lead levels in drinking water as low as reasonably achievable.

Following the recent growing concerns related to lead contamination of drinking water and the health concerns for infants and young children, a study was conducted to investigate the levels of lead at 150 daycares in Alberta, Canada. Samples (n=1431) were collected from all cold water taps and fountains that were regularly used for drinking or food preparation, using Random Daytime (RDT) sampling and without flushing.

Sample preparation followed the Environmental Protection Agency (EPA) 200.8 and analysis was performed using an Agilent 7700 Inductively Coupled Plasma - Mass Spectrometer. The limit of quantification was 0.1 μg/L.

The concentration of lead ranged from not detected (<0.1 μg/L) to 35.5 μg/L. Lead was detected in about 80% of the tested samples, however, the majority of these samples had lead concentration below the MAC (5 μg/L). Lead concentration was above 5 μg/L in 37 samples (2.6%) and above 10 μg/L in 9 samples (0.6%). In certain locations, it was observed that lead concentra- tion varied at different taps in the same building. This study demonstrates low levels of lead in drinking water at daycare centres in Alberta and shows that testing every tap in a building is important in order to evaluate lead exposure and to choose an appropriate mitigation strategy.
**Symposium Anti infective Not just tuberculosis: the therapeutic challenges of acid-fast organisms Nocardia, the ultimate opportunist**  
(Debbie Marriott, Australia)

Is there a role for therapeutic drug monitoring to optimise therapy and improve patient outcome?

Nocardia belong to a diverse group of bacteria known as aerobic actinomycetes. Some members of this group, including Nocardia and Mycobacteria, contain mycolic acid in the cell wall and share a common property of ‘acid-fast staining’ which is the formation of stable complexes between the mycolic acids and certain dyes such as fuschin that cannot be removed by exposure to acid-alcohol or strong mineral acids. About 100 species of Nocardia have been identified and they are commonly found in soil, dust, decaying vegetation, water and other environmental sources.

Clinical disease caused by Nocardia occurs primarily in immune-compromised hosts, in particular bone marrow and solid organ transplant recipients who represent about 1/3 of cases of nocardiosis overall. Inhalation is the most frequent source of infection so pulmonary infection is the most common but disseminated disease occurs in up to 40% with a significant mortality. Pre-disposing factors can include the degree of immunosuppression, high dose corticosteroid treatment, use of tacrolimus and concurrent cytomegalovirus infection. The protective role of cotrimoxazole prophylaxis against Pneumocystis jiroveci in the prevention of nocardiosis is unclear.

The treatment of nocardiosis depends on the susceptibility of the infecting organism. Identification of the Nocardia species helps predict antibiotic susceptibility patterns. The most commonly used antimicrobial agents include trimethoprim-sulphamethoxazole, amikacin, carbapenems (imipenem and meropenem), cefoxitin, ceftriaxone/cefotaxime, amoxicillin-clavulanic acid, quinolone antibiotics such as moxifloxacin, and linezolid. Treatment is often prolonged for many months.

There is almost no information in the literature on the use of therapeutic drug monitoring to optimise the treatment and outcome of Nocardia infection. A search of PubMed for ‘Nocardia’ and ‘therapeutic drug monitoring’ produced only 19 articles with only 3 of these actually describing the use of therapeutic drug monitoring in this setting (trimethoprim – sulphamethoxazole in two and linezolid in one). The need for prolonged treatment with potentially toxic agents such as amikacin, trimethoprim-sulphamethoxazole and linezolid would suggest an important role for therapeutic drug monitoring.

The presentation will review the general evidence for therapeutic drug monitoring of antimicrobial agents used to treat Nocardia infection, and provide new data from St. Vincent’s Hospital Sydney on the positive impact of therapeutic drug monitoring on the long-term safety and efficacy of linezolid in the setting of Nocardia therapy.
Laboratories are frequently required to perform comprehensive screening of complex samples to identify drugs and other toxicants. Within the field of forensic toxicology, there is high requirement for analytical certainty in the accuracy and completeness of the results. In recent years, owing to the high level of specificity and sensitivity obtained from full scan exact mass data acquisition, the utilization of Quadrupole Time-of-Flight (QTOF) technology for forensic toxicology screening has increased. Furthermore, the ability to retrospectively interrogate data for unexpected compounds, or emergent new “designer” drugs has significantly increased the uptake and adoption of this technology. We will present the application of QTOF technology for toxicological drug screening therefore offers forensic toxicologists a comprehensive solution for both targeted and nontargeted screening workflows. However, in order to realize this benefit, it is essential to have access to a high quality accurate mass reference database supported by powerful data processing software for cross referencing experimental observations.
Morning session Clinical Toxicology - Oxidative Stress and Inflammation in Kidney Transplantation. The effect of immunosuppression
(Eberhard Wieland, Germany)

Oxidative stress (OS) and its inseparable companion inflammation affect the kidney allograft but also contribute to the development of cardiovascular disease (CVD), cancer, and other disorders in kidney transplant patients (KTx) receiving lifelong immunosuppression. Particularly premature CVD is a major concern for the long term outcome of KTx. The higher incidence of CVD in KTx compared to the normal population has been suggested to be due in part to the high prevalence of metabolic disorders such as diabetes and metabolic syndrome (MetS) which are well known sources of OS and inflammation (1). Presence and severity of OS can be assessed by various biomarkers produced from interaction of reactive oxygen species with lipids, proteins, nucleic acids, nitric oxide, glutathione, etc. Biomarkers of inflammation such as high sensitivity CRP (hsCRP) and interleukin-6 (IL-6) have been shown to be predictive for future cardiovascular events and all-cause mortality in renal graft recipients (2). Oxidized low density lipoproteins (oxLDL) are known to play an important role in the development of CVD in patients with MetS (3) and circulating autoantibodies against oxLDL (ox-LDL-AB) have been considered to reflect the extent of LDL oxidation (4).

Calcineurin inhibitors (CNI) have been suggested to contribute to oxidative stress in kidney transplantation by triggering diabetes, hyperlipidemia and MetS (5). In addition, they have been shown to directly induce vascular inflammation (6).

In a single center study biomarkers of inflammation (IL-6, hsCRP) and oxidation (oxLDL and oxLDL-AB) have been assessed in healthy controls, non transplanted patients with MetS without immunosuppression and immunosuppressed KTx with and without MetS (>6 months after transplantation, triple immunosuppression with steroids, mycophenolic acid and CNI) to see whether inflammation and OS caused by MetS are enhanced in immunosuppressed KTx.
Symposium Clinical Toxicology: Emergencies in Toxicology - Acute Intoxications: taking clinical decisions before laboratory results
(Eduardo Mello de Capitani, Brazil)

The toxicology laboratory in the management of emergency acute poisoned patients can be very important in many ways: 1. identification and quantification of the substances involved; 2. confirmation or exclusion of a poisoning diagnosis (be aware of predictive positive and negative values of the tests); 3. grading and prognosis; 4. monitoring of therapies (elimination therapies; use of antidotes); 5. testing for drug-of-abuse (be aware of PPV and PNV of the tests); 6. proper documentation of the case; 7. further forensic and legal developments. However, the majority of acute poison cases at urgency departments can be managed, at first moment, using the history of exposure, syndromic manifestations (toxidromes), and rapid general laboratory results (blood gases and sugar, electrolytes, renal and hepatic function tests), without any information from toxicologic laboratory tests. The cornerstone of the management of a poisoned patient continues to be rapid and effective ventilatory, cardiovascular and metabolic support, independently of the substances implicated. In the majority of the cases the old saying “treat the patient, not the poison” is still the prevailed paradigm. Nevertheless, when a good exposure history is unreliable, or not available (patients with coma, unexplained seizures, or altered mental state, unexplained cardiotoxicity, unexplained acidosis, for instance), toxicology laboratory test results can be extremely useful in determining prognosis and redirecting first general treatment (adding serial activated charcoal, antidotes, hemodialysis, hemoperfusion, for instance).

A toxicology laboratory result will change clinical management of acute cases of paracetamol, salicylates, lithium, antidepressants, carbamazepine, phenobarbital, theophylline, methanol, ethylene glycol, digoxin, methemoglobinemia, lead, iron, arsenic. Many other poison acute cases will not need a change in clinical management regarding treatment, like in cocaine (in acute myocardial infarction), ethanol, and paraquat cases. No antidotal or other specific treatments will be necessary in those cases. Remember that a positive urine screen for drugs of abuse could not correlate with the clinical presentation, meaning sometimes only a consume or addiction pattern.

On the other hand, a small group of toxic substances will not need urgent laboratory confirmation before institution of antidotal treatment, like cyanide, CO, paracetamol, and opiates. These last examples will be discussed in detail in the talk.

Remember that a positive urine screen for drugs of abuse could not correlate with the clinical presentation, meaning sometimes only a consume or addiction pattern (false positive, in this sense) (1). Modified from Wennig, 2005.(2, 3)

Do not rely only on the availability of laboratory tests. Exposure information, and good clinical history and examination, are still of paramount importance during the management of a poisoned patient.


Symposium Biologicals: Proactive therapeutic drug monitoring of biologics in inflammatory bowel diseases: induction treatment and cost - Is there a place for therapeutic drug monitoring of drugs other than infliximab for inducing response in patients with inflammatory bowel diseases?

(Erwin Dreesen, Belgium)

For over a decade, the biologics armamentarium for treating patients with inflammatory bowel diseases (IBD) was confined to anti-tumor necrosis factor (anti-TNF)-alpha monoclonal antibodies. The lack of therapeutic alternatives made it imperative to develop strategies to restore and maintain the response to these therapies. With the understanding that the drug exposure rather than the administered dose is related to the response, therapeutic drug monitoring (TDM) of anti-TNFs became an accepted practice.

A broad range of drugs addressing targets other than TNF has recently entered the market. These include both monoclonal antibodies (e.g. integrin and interleukin targeted therapies) and small molecular drugs (e.g. Janus kinase targeted therapies). The opportunity to choose between drugs with different mechanisms of action offers in the first place the potential for improved patient care, but it might also open the door for ‘trial-and-error medicine’. Varying response rates to these novel drugs are boosting the scientific community to elucidate their dose-exposure-response relationships and to evaluate the potential for TDM to improve therapeutic outcomes.
Scorpion stings are frequent in South America, being the primary cause of envenomation in Brazil. Data from the Brazilian Ministry of Health (Notifiable Disease Information System, SINAN) indicate that nationwide there were 124,662 scorpion stings in 2017, with 87 deaths; this corresponds to an annual rate of 60 cases/100,000 inhabitants, with lethality of 0.07%. Only scorpions of the genus Tityus cause clinically relevant envenoming in South America, with the most important species being T. serrulatus and T. stigmurus (Brazil), T. trivittatus and T. confluens (Argentina), T. discrepans and T. zulianus (Venezuela), and T. obscurus (Amazon basin), which are responsible for most cases of severe envenoming, mainly in children <15 years old. Local manifestations are similar regardless of the severity of the sting, and involved pain in almost all cases. The mechanisms responsible for the systemic manifestations are complex and involve marked alterations in the function of sodium, potassium and calcium ion channels in excitable cells, associated with neurohormonal activation that triggers an “autonomic storm” characterized by transitory parasympathetic stimulation and prolonged sympathetic activation. Other mediators such as nitric oxide, kinins, circulating venom concentration and a direct cardiotoxic effect of the venom have also been implicated in the severity of envenoming. The rapid onset of the clinical manifestations in severe cases (in general <2 h post-sting) means that it is important to recognize risk groups in order to quickly refer them to reference intensive care units. Supportive treatment is essential and prognostic in severe cases, and includes the use of dobutamine in patients with myocardial depression and mechanical ventilation in individuals with respiratory failure. In addition, Brazilian and Argentinean Ministry of Health guidelines have recommended the use of scorpion antivenom for all patients with systemic envenoming.
Symposium Immunosuppressive Drugs TDM of Immunosuppressive Drugs-personalized therapy Tacrolimus intracellular concentrations: improving patient and graft care
(Florian Lemaitre, France)

Therapeutic drug monitoring (TDM) of immunosuppressive drugs (ISD) has revolutionized the management of solid organ transplant recipients. Hence, TDM of ISD participated decreasing the rate of graft rejections due to low exposure to the immunosuppressive therapy, limiting, then, graft immune lesions, graft loss and ultimately patients’ death. Moreover, as ISD and particularly calcineurin inhibitors (tacrolimus and cyclosporine), display an obvious concentration-toxicity relationship, TDM also allows decreasing drugs’ adverse events such as nephrotoxicity. However, in recent years and despite intensive TDM, graft rejection rate is no longer decreasing meaning that newer ways for ISD monitoring are needed. Among those new approaches, measuring intracellular ISD concentrations appears as a promising approach. Indeed, some patients exhibit graft rejections or adverse events while having whole blood concentrations within the therapeutic range. These observations suggest that whole blood concentrations are not completely related to the pharmacological drug effect. Measuring ISD concentrations inside its site of action (i.e the lymphocyte) might, therefore, be of better relevance than measuring drug in whole blood. Intracellular concentrations might also be a better surrogate of unbound drug concentrations that is the fraction available to exert the pharmacological effect of the drug. Most of the studies on that topic have been conducted on tacrolimus, which is the gold standard of actual immunosuppressive therapeutic regimens, probably because of its ability to interact with membrane transporters potentially influencing drug disposition into the intracellular compartment. The aim of this presentation is, then, to review the current evidences of the interest of intracellular TDM of tacrolimus as a tool aiming at helping better predicting clinical outcomes in various type of solid organ transplantations.
Symposium Toxicology & Environmental Health: Environmental pollutants and health outcomes: an overview of recent evidence - Pesticide environmental exposure: molecular and biochemical biomarkers and morphometric parameters in pregnant women, placenta and newborn

(Gladis Magnarelli, Argentina)

Background: In utero exposure is the first contact with environmental xenobiotics that may affect the maternal-placental-fetal balance. Effects may be evaluated by alterations in biomarkers reflecting underlying toxicological processes. Methods: prospective and transverse studies were conducted. Pregnant women and their newborns residing in communities surrounding agricultural areas (RG: rural group), where mainly organophosphate pesticides (OP) are applied, were studied. Samples were collected during both spraying (SS) and non-spraying seasons (NSS) and from urban residents (control group). Blood cholinesterases, biochemical and endocrine parameters and biomarkers of liver injury were analyzed during pregnancy. In at term placental villous carboxylesterase activity (CaE), oxidative status, nuclear and mitochondrial phospholipid composition, respiratory complexes and enzymatic antioxidant defense activities were assayed. Progesterone levels, endothelial nitric oxide synthase (e-NOS) and cytokine expression were studied. Biomarkers of oxidative stress/damage were analyzed in umbilical cord blood (UCB). Relationships between analytical variables and fetal and placental growth indicators were evaluated. Results: In SS, blood cholinesterases decreased while Cortisol increased in the first trimester. During the second trimester, alanine-aminotransferase activity was increased. Placental samples showed a decrease in CaE and no changes in the antioxidant/oxidant status or in Nrf2 levels. The expression frequency of IL-13 increased. Mitochondrial sncytiotrophoblast cytochrome c oxidase activity and cardiolipin content increased. Progesterone levels and e-NOS expression decreased in SS and RG. Arginase and ornithine decarboxylase (ODC) were induced. An inverse association between Catalase activity and placental index was found. UCB erythrocye osmotic fragility and superoxide dismutase activity changed and the DNA damage index increase. Conclusions: Studies showed maternal OP exposition, suggesting subtle hepatic injury and increase in the amount of maternal Cortisol reaching the fetus. Although OP reached the placenta, it was able to handle the prooxidant conditions that might have been generated. Interestingly placental Catalase activity may serve as a potential biomarker of susceptibility. Changes in IL-13 expression, Arginase and ODC activities, involved in tissue repair mechanisms were in agreement with increase cardiolipin content associated to mitochondrial biogenesis. They could represent a response to OP induced injury being mitochondria bioenergetics and steroidogenic function, a toxicity target. Reduction in placental Progesterone and e-NOS expression may account for low newborn weight in RG. Impact on the UCB antioxidant defense capacity may contribute to increased vulnerability to oxidative insults.
Plenary session: Pharmacology & Toxicology of New Psychoactive Substances
(Hans H. Maurer, Germany)

Background: New Psychoactive Substances (NPS) are a huge problem for the human health. Continuously, new substances or derivative appears on the drug market. They are all sold without any preclinical pharmacological and/or toxicological testing (EMCDDA, European Drug Report 2019; UNODC, World Drug Report, 2019). Methods: Thus, mostly academic institutions start with such investigations and collect clinical case date for risk assessment. Results: In the plenary, experimental and clinical data of the important classes of NPS such as designer benzodiazepines and opioids, cannabinoid receptors agonists, cathinones and other phenethylamine stimulants, arylocyclohexylamines, tryptamines, and other hallucinogens will be discussed. Conclusions: The presentation will show how complex and emerging the NPS problem is.

Scheduling of NPS in regular narcotic acts considering the pharmacological and clinical effects looks like a cat and mouse game. Therefore, many countries passed NPS laws scheduling a variety of possible chemical derivatives independent of their pharmacological effects in order to protect public health. Since that time the number of new substances per year decreased, but nevertheless, not yet scheduled structures appeared thereafter. For further reading: Maurer HH, Brandt SD. Pharmacology, Clinical, Forensic & Analytical Toxicology of NPS. Handb Exp Pharmacol., Heidelberg: Springer, 2018; ISBN 978-3030105600 (print), ISBN 978-303010561-7 (ebook)
Symposium Clinical Toxicology: Emergencies in Toxicology - Analytical Strategy for Effective 24/7 ClinTox Services - From Screening to Simplified Blood Level Assessment (Hans H. Maurer, Germany)

Background: Various analytical tools allow today a broad range of analysis in clinical toxicology, particularly in big centers. Of course, long distances may limit the usefulness (Maurer HH., Therap Drug Monitor, 2012). Current strategies for efficient analytical diagnostics in clinical toxicology are presented. Methods: GC-MS, LC-MSn, and LC-HR-MS/MS are used for comprehensive screening (Maurer HH, Therap Drug Monitor, 2018). For simplified blood level assessment, GC-MS (Meyer GM et al., Drug Test Anal, 2014), LC-MS/MS (Michely JA et al., Drug Test Anal, 2018), and LC-MSn (Caspar AT, Drug Test Anal, 2019) approaches were applied. Results: The screening methods were sufficient to detect the most relevant causes of poisonings. The quantification procedures fulfilled the acceptance criteria of the recommendations for quantification in emergency toxicology (GTFCh, Toxichem Krimtech, 2018) of the Clinical Toxicology Committee of the Society of Toxicological and Forensic Chemistry (GTFCh) as basis of a competent interpretation of the analytical result in correlation with the clinical signs presented by the patient. Conclusions: Such service must be available around the clock and reliable results should be provided in a short time frame relevant for treatment and at reasonable costs.
Symposium Clinical use of precision dosing software - Use of PBPK model simulator for the individualized therapy
(Ikuko Yano, Japan)

In living-donor liver transplant patients, postoperative days and CYP3A5 genotype of the donor and recipient are known to affect the tacrolimus pharmacokinetics. A physiologically based pharmacokinetic model (PBPK) adapted to the clinical data was constructed, and the contribution of liver regeneration as well as hepatic and intestine CYP3A5 genotypes on the tacrolimus pharmacokinetics were evaluated. As a result, the oral clearance could be classified into three patterns according to the CYP3A5 genotype combination of the donor and recipient. The genotype-guided initial dosage of tacrolimus is useful to maintain the therapeutic range quickly. The PBPK model simulator is a powerful tool to know the dosing design in the special population.
Symposium Anti-infective agents and therapeutic drug monitoring: the ultimate precision medicine when no two patients are alike - Renal replacement therapy and antimicrobial therapy: a knowledge vacuum (Jan-Willem Alffenaar, The Netherlands)

Antimicrobial drug dosing is of critical importance in severely ill patients. Rapid target attainment is required to cure patients. Renal replacement therapy does not make it easier to achieve the target concentrations. The variability in renal replacement therapy even aggravates the variability between patients and may influence whether appropriate antimicrobial drug exposure is achieved. This presentation will give an overview of the impact and variability of renal replacement therapy on frequently used antimicrobial drugs. TDM will be presented as practical application to improve target attainment and thereby treatment outcome.
Symposium Anti infective Not just tuberculosis: the therapeutic challenges of acid-fast organisms - Non-tuberculous mycobacteria: does TDM have a place?  
(Jan-Willem Alffenaar, The Netherlands)

Nontuberculous Mycobacteria cause a wide range of opportunistic infections ranging from asymptomatic to progressive inflammatory disease affecting the respiratory system, central nervous system, lymph nodes and joints and disseminated disease. Treatment of NTM is challenging because drug susceptibility is highly variable, multiple drugs have to be combined to build a regimen and duration is lengthy and often not well tolerated. I additional to the traditional indications for TDM for aminoglycosides and in patients with presumed malabsorption or drug–drug interactions. Although TDM has been recommended little information or practical guidance is available. The aim of this presentation is to provide an overview on pharmacokinetic/pharmacodynamic of the individual drugs used for the treatment of NTM disease and potential use of TDM.
Symposium Alternative sampling strategies: What’s new in microsampling - IATDMCT method validation guidelines for dried blood spot assays
(Jan-Willem Alffenaar, The Netherlands)

This guideline aims to define the elements necessary for the validation of quantitative dried blood spot-based methods. The main focus of this guideline is the analysis of dried blood spots (DBS) for the quantitative determination of small molecule drugs and drug metabolites using chromatographic techniques for therapeutic drug monitoring (TDM) purposes. In the presentation relevant aspects of method development and validation like sampling method, filter paper type, sample volume, drying, storage, internal standards will be discussed. The requirements for clinical validation will also be presented. In short presentation will give you a good overview of what your lab needs to do before implementation of dried blood spot in clinical practice.
Symposium Young scientists - Study design and reporting matters: why IATDMCT members work was not included in important Systematic Re-views of TDM
(Jana Stojanova, Chile)

In this brief presentation I will review classic study designs in clinical epidemiology, as well as their associated reporting guidelines and Risk of Bias tools. Various Systematic Reviews of TDM as an intervention will be presented and critiqued, and excluded studies reflected upon. The approach to use quasi-experimental designs as a means of providing quality evidence for system level clinical interventions will be discussed, with clinical examples where this has been successful. The feasibility of applying these ideas for TDM as an intervention will be discussed.
Symposium Pharmacometrics - Development and applications of pharmacometric tools for routine clinical practices (Jean Baptiste Woillard, France)

I will present the development of population pharmacokinetic models and bayesian estimators based on limited sampling strategies and how to validate them so that they can be implemented in routine practice. Then I will explain different applications of population pharmacokinetics models: 1) simulations and probability of target attainment in order to propose a first dose or more generally, to estimate proportions attaining a pre-defined target for different conditions 2) determination of exposure markers to investigate PK/PD relationships and 3) dose adjustment to individualize dosing schemes in patients with clinical examples for immunosuppressants and antibiotics. Finally, I will discuss some limitations in the generalized use of such tools in routine practice.
Symposium Pharmacogenetic: PGx in oncology - UGT1A1 and beyond. Can a validated molecular signature be used to guide the prescription of irinotecan with associated therapies in advanced colorectal or pancreatic cancers?

(Jean Christophe Boyer, France)

Over the past 10 years, combinations of chemotherapy (e.g., fluoropyrimidines, irinotecan, or oxaliplatin) and agents targeting vascular endothelial growth factor (VEGF) or EGFR (e.g., bevacizumab, cetuximab, or panitumumab) have become the standard treatment options for advanced colorectal or pancreatic cancers.

This active form of irinotecan is the SN-38 metabolite, which is cleared by the biliary route after glucuronidation by uridine diphosphate–glucuronosyltransferase 1A1 (UGT1A1). UGT1A1 activity exhibits a wide inter-subject variability, in part related to UGT1A1 gene polymorphisms in Caucasian as well as in non-Caucasian populations.

This talk aims to highlight the real impact of the allelic variations described in UGT1A1 gene on improvement in patient outcomes. After reviewing the main clinical studies seeking to confirm the impact of functional consequences of UGT1A1 gene polymorphisms (particularly that of the variants *28 and *6) on the toxicity and efficacy of irinotecan based chemotherapy, the current recommendations made by the international health agencies (EGFR, FDA, EMA, PMDA...) and the current guidelines by international scientific societies (eg, ASCO, ESMO, CPTIC, DPWG, RNPGx...) will be presented. Furthermore, as a linear relationship between exposure to SN-38 and UGT1A1 polymorphisms have been described, an attempt will be made to demonstrate a possible role for a PK approach, based on plasma SN-38 levels, to complete the PGx strategy. Then, the challenging issue of a molecular signature including well known pharmacogenes for conventional regimen (eg, UGT1A1, DPYD), less tested variants that may strengthen the potential of UGT1A1 genotype (eg, variants in STAT-3, VDR, NR112...) and a restricted subset of promising SNPs predictive for those patients who would benefit from bevacizumab-based chemotherapy will be addressed.

Best execution practices, cost-effectiveness, and result interpretation will be discussed with the aim of enabling a personalized irinotecan dosing based on UGT1A1 genotype.

In this effort to ensure an optimal implementation of UGT1A1 genotyping, a flow chart will be proposed for such testing, depending on initially intended irinotecan dose.

Finally, this talk aims to encourage clinical laboratories to offer the prospect of widespread UGT1A1-testing in routine management, thus guaranteeing equal access to safe treatment and optimized therapy for patients receiving irinotecan in advanced colorectal or pancreatic cancers.
Morning session Pharmacogenetic of tamoxifen - Prospective Studies of Tamoxifen Pharmacogenetics and Metabolism: what have we learned
(Jesse Swen, The Netherlands)

Tamoxifen is the cornerstone of the anti-estrogenic therapy in the early and metastatic breast cancer setting. Tamoxifen has a complex metabolism, being mainly metabolized by CYP2D6 into its 30–100 times more potent metabolite, endoxifen. A considerable variability in clinical response to tamoxifen treatment is observed with up to ~30% disease recurrence within 15 years of treatment in early breast cancer. Both non-gene- tic and genetic factors have been described to influence this high interpatient variability in response to tamoxifen. In the latter case the ef- fect of genetic variation in CYP2D6 has been extensively studied. Others postulated that endoxifen serum levels are a better predictor of tamoxifen efficacy than CYP2D6 genotype and an endoxifen threshold of 5.9 ng/ml has been suggested. I will present data from recent prospective studies investigating associations between endoxifen concentrations and CYP2D6 genotypes with clinical outcome data as well as results from our study investigating the added value of long-read sequencing combined with a neural network to improve our ability to predict CYP2D6 metabolic capacity.
New psychoactive substances (NPS) become a serious public health problem, highlighted mainly due to increased cases of acute poisoning. The detection of these substances has been a challenge since the great number of substances, in very different concentrations in biological fluids. The absence of epidemiological studies in Brazil makes the diagnostic/analytical field even more difficult and more than that restricts the advancement of the knowledge of health professionals involved in the care of the users, as well as the self-caring of these users in harm reduction. Using oral fluid samples in toxicological analyses has the advantages of a rapid and non-invasive collection. The aim of this presentation is to present the first epidemiological data on NPS consumption in Brazil, by collection and analysis of oral fluid samples of participant of electronic music parties.
Symposium Alternative sampling strategies: What’s new in microsampling - Adduct monitoring in blood microsamples
(Lisa Delahaye, Belgium)

Biomarkers of exposure can be looked at from a wide variety of perspectives, ranging from the actual agent (e.g. a heavy metal), to metabolites (pointing at exposure to e.g. a certain drug), to other short- or long-term indicators. They are typically used to evaluate to what extent there has been external or internal exposure to an (offending) agent. These biomarkers of exposure allow a more accurate determination of the internal dose of a substance than environmental measurements per se.

Furthermore, chemical carcinogens and other toxic substances are mostly converted to reactive electrophiles, which are short lived, rendering direct detection in a target tissue virtually impossible from a practical-point-of-view. Their reactivity often results in the formation of covalent adducts with nucleophilic groups of DNA and proteins, allowing long-term detection of the initial exposure (days to months). While in DNA the N7 position of the guanine residue is the most nucleophilic site, in proteins typically sulfhydryl groups of cysteine residues are targeted, as well as lysine, histidine and tyrosine residues. For protein adducts, the adduct that is chosen as a biomarker of exposure is greatly determined by the matrix from which a sample is being taken. Typically, the biomarker of choice will be an adduct with the most abundant protein in a given matrix, e.g. albumin or hemoglobin in blood or keratin in skin.

Throughout this presentation the focus will lie on the determination primarily quantitatively of protein adducts as biomarkers of exposure, and more specifically their determination in blood microsamples. The emergence of new-generation analytical instrumentation has made it possible to use microsamples such as dried blood spots, dried plasma spots and volumetric absorptive microsamples, instead of conventionally sampled blood or plasma for a wide variety of toxicological applications, amongst which the measurement of protein adducts. Several applications that were successful in determining protein adducts, starting from blood microsamples, will be discussed. Undoubtedly, many more applications will follow in the (near) future.
Morning session Clinical Toxicology - Immunotoxicity: drug-induced and herbal-induced hypersensitivity reactions
(Manuela Neuman, Canada)

This symposium will the role of TDMCT in monitoring safety and efficacy of therapies. Identification of drugs or biologics with potential to cause serious, life-threatening drug-induced injury requires causality assessment and comprehensive testing for alternative etiologies in each suspected case. Since idiosyncratic event is rare, prediction of the risk of serious drug-induced-injury post-marketing relies on identification of cases meeting criteria during phase II-III clinical trials. Subjects with pre-existing, chronic liver diseases (CLDs), with or without cirrhosis, are challenging because FDA guidance is based largely on criteria for studies that exclude subjects with abnormal liver tests. Chronic Liver. Diseases do not increase drug-induced liver injury (DILI), but multiple exceptions suggest that additional examples will be identified in future clinical trials. DILI in subjects with CLD is worrisome because subjects with reduced hepatic functional reserve are less likely to recover and more likely to die. The probability of subjects with CLDs enrolling in clinical trials is increasing, especially in therapeutic trials targeting components of the metabolic syndrome, antimicrobials, complications of cirrhosis and hepatocellular carcinoma. Monoclonal antibodies are used against inflammatory diseases. Definition of laboratory test and how it has been interpreted by clinician will be review. The reduce efficacy of the antibodies in time and the possible adverse reactions that they can produce due to immunogenecity will be explained. Moreover, drug monitoring of therapeutics used in cancer are link to immuno pharmaco-genetics. The role of interaction between genetic and epigenetic factors within this context will be exposed.
Symposium New psychotropic drugs - Techniques for Toxicokinetic Studies of NPS
(Markus Meyer, Germany)

This talk is part of the symposium entitled “New psychotropic drugs”. Knowledge of the absorption, distribution, metabolism, and elimination (ADME) of new psychoactive substances (NPS) in humans are of considerable importance for understanding their toxicokinetics, their potential for interaction, and possible pharmacogenetic risks. This also includes the enzymatic systems involved in these steps (e.g. cytochrome P450s, glucuronosyltransferases, sulfotransferases, glutathione-S-transferases, N-acetyltransferases, P-glycoprotein). The metabolism for instance is a prerequisite for developing toxicological analysis procedures, predicting drug-drug or drug-food interactions, and for understanding analytical pitfalls. In case of therapeutic drugs, ADME properties are usually examined as part of the drug authorization or risk assessment (see Directive 2001/83 / EC). However, NPS are offered for sale without such safety studies. Thus, this talk will provide an overview about strategies for elucidating the toxicokinetics of NPS including different in vitro or in vivo techniques as well as analytical solutions. After this presentation, the attendee will be able to describe some workflows to investigate the ADME properties of NPS for answering questions in the context of clinical and forensic toxicology.
Symposium Mass spectrometry in clinical and forensic toxicology - Qualitative and Quantitive Solutions in 24/7 Toxicological Analysis (Markus Meyer, Germany)

This talk is part of the symposium entitled “Mass spectrometry in clinical and forensic toxicology”. It will first be answering the question why measuring drug exposure can be of importance in 24/7 clinical toxicology. The talk will also start with some fundamental aspects of hyphenated mass spectral analysis. Afterwards, analytical techniques and prerequisites will be explained, which can be used to meet the demands in 24/7 clinical toxicology. Examples for analyzing blood samples, such as quick and easy quantification procedures based on hyphenated mass spectrometry will be included. Challenges such as metabolism studies, which are an important question to be answered prior to urinalysis, will also be discussed. In addition to these traditional matrices, the value of alternative samples will be highlighted. Selected applications will be presented and discussed. After this presentation, the attendee will be able to describe strategies and workflows to analyze drugs and drugs of abuse for answering questions in the context of 24/7 clinical toxicology.
Symposium Alternative sampling strategies: What’s new in microsampling - Drug monitoring via fingerprints (Melanie Bailey, UK)

The use of fingerprint samples as a drug testing matrix
Our group are exploring the chemical information that can be left behind in a fingerprint and detected using mass spectrometry imaging and profiling techniques (liquid chromatography, paper spray, matrix assisted laser desorption ionisation and secondary ion mass spectrometry). We are working with clinicians and forensic providers to use fingerprints to (a) improve the recovery of fingermarks at crime scenes and (b) explore the possibility of using fingerprints as a drug testing matrix. This talk will describe our work on exploring the significance of detecting cocaine and heroin in a fingerprint by comparing fingerprints produced by direct contact with a drug to those produced after ingestion of a substance. We will also present recent work which is exploring therapeutic drug testing from a fingerprint for testing adherence to antibiotic and antipsychotic medications.
Plenary session: Breaking the devotion to breakpoints and P-values for anti-infectives
(Michael Neely, USA)

Categories and breakpoints are convenient, but they obscure information and risk miscategorization. With the power of computers in our hands, clinicians can approach therapeutic decision making and optimization using quantitative and probabilistic models that permit far more informative risk-benefit analyses. In this plenary session, we will explore how drug and dose selection may be achieved in the future. We will use anti-infectives as an example, but the ideas are broadly applicable to any domain.
Mass Spectrometry for Fentanyl Analog Screening, Confirmation and Metabolite Discovery in Forensic Urine Casework (Michelle Wood UK)

Overdose deaths from opiates and synthetic opioids have risen substantially over the past few years, with the largest increases attributed to synthetic opioids such as fentanyl and its analogs. Identification of fentanyl and fentanyl analogs in forensic toxicology can be crucial in alerting public health authorities to potential overdose risks. However, due to their high potency concentrations are often in the sub-ng/mL range. Consequently, sensitive methods for detection and confirmation of these substances is essential for medical examiner as well as other court-related casework.

The potential of high-resolution mass spectrometry (HRMS) as both a screening and confirmatory method for urine is presented. In addition, forensic samples are often analyzed from blood, which can present challenges not seen in urine samples. A sample preparation method based on a novel mixed-mode SPE for fentanyl and analogs in blood has been developed. This validated method has also been applied, in combination with tandem mass spectrometry (UPLC-MS/MS), to analyse plasma samples collected in a recent study to better understand human metabolism of pharmaceutical grade fentanyl.
Symposium Biologicals - Proactive therapeutic drug monitoring of biologics in inflammatory bowel diseases: induction - Financial implications of anti-TNF therapeutic drug monitoring (Murray Barclay, New Zealand)

The anti-TNF drugs infliximab and adalimumab have been used in treatment of inflammatory bowel disease (IBD) for >20 years and about 15 years, respectively. For most of this history, dosing has been fixed for adalimumab or adjusted to patient weight for infliximab. In recent years however, research has shown that drug efficacy can be improved by using therapeutic drug monitoring and there are now a number of national guidelines recommending individualised dosing using TDM.

The cost of these monoclonal antibodies is high, representing a large proportion of national drug budgets and imposing a significant financial burden for many countries. Due to cost, most of the world's population does not have access to these drugs despite increasing incidence of the disease globally.

Even with the availability of biosimilars, with cost reduction for infliximab and also soon for adalimumab, cost is still too high for most of the world.

The introduction of therapeutic drug monitoring for infliximab and adalimumab in IBD naturally leads to a change in the average dose of these drugs after dose adjustment. Some patients require dose increase into the recommended therapeutic range to increase efficacy whilst other patients can have dose reduction into the range, which reduces cost for this group and the possible risk of toxicity. The results of several clinical studies of TDM in IBD show that average dose after TDM-dose adjustment increases from 5 mg/kg 8-weekly IV to 8-9 mg/kg 8-weekly for infliximab (final dose is less clear for adalimumab). Therefore if TDM is implemented across all IBD patients in a population, drug cost would be expected to increase by approximately 70%. On the other hand studies have shown substantially improved cost-efficacy with TDM that more than off-sets the increase in average drug cost.
Symposium Biologicals: Proactive therapeutic drug monitoring of biologics in inflammatory bowel diseases: induction treatment and cost - Induction anti-TNF concentrations and primary nonresponse in adult IBD patients
(Niels Vande Casteele, USA)

Although biologic agents are effective for the treatment of patients with inflammatory bowel disease (IBD), a substantial proportion of patients will experience either primary nonresponse or secondary loss of response to biologic therapy. The mechanisms of treatment failure are complex, including disease-related, drug-related, and patient-related factors. Given this complexity, treatment decisions directed by symptom assessment alone are unlikely to achieve optimal outcomes. Therapeutic drug monitoring (TDM) has emerged as a promising strategy to maximize treatment response in IBD. In patients treated with tumor necrosis factor-α (TNFα) antagonists, primary nonresponse has been attributed to “non-TNFα-driven disease” which is an overly simplified and potentially misleading approach to the problem. Recent studies in IBD suggest that TDM could help to better define primary nonresponse and guide patients to an optimal treatment strategy more efficiently. Moreover, a mode-ling approach including pharmacokinetic parameters including patient-related covariates could potentially be predictive for response to therapy. This lecture will cover the evolution in our thinking about this problem, underpinned by previous findings and we will discuss the potential role of early TDM in defining and managing primary nonresponse.
Symposium Toxicology & Environmental Health: Environmental pollutants and health outcomes: an overview of recent evidence - Effects of a mixture of persistent organic pollutants on the thyroid function of a Belgian population
(Patrice Dufour, Belgium)

The blood levels of 12 brominated flame retardants, 3 polychlorinated biphenyls, 16 organochlorine pesticides, 7 perfluoroalkyl substances and 16 phenolic organohalogens were measured in 35 hypothyroid and 44 hyper- thyroid volunteers, and in 160 healthy individuals. An original statistical approach (WQS- weighted quantile sum) was performed to assess the relation between the cocktail of pollutants and thyroid disorders, since the increasing incidence of thyroid diseases could be explained by the contamination by chemicals suspected to be thyroid disruptors. The WQS index was statistically significantly associated with an increased odds of hypothyroidism especially noticed with the combination of 5 chemicals (3-OH-CB 180 and 4-OH-CB 146, 4,4’-DDE, PCB 138 and PCB 180). The study did not support any evidence of association with increased odds of hyperthyroidism.
Symposium Pharmacometrics - R-shiny tools for pharmacometrics in drug development
(Patrick Nolain, France)

Shiny is an R package for turning script analyses into interactive web applications that has revolutionized how data scientists and analysts distribute their analytic results and research methods. It allows for the development of interactive tools using nothing else but R code and enjoys a growing popularity within the scope of drug development. In pharmacometrics, models are built to characterize and understand drug efficacy and safety. Shiny applications can empower pharmacometricians to develop their models and non-pharmacometricians to use and explore the insights generated by the models to implement a model-informed development strategy. I will present several examples of how Shiny applications can be used to support model development and usage and foster the use of modeling and simulation in drug development.
Symposium: Therapeutic Drug Monitoring in developing countries - How can we bridge the gap between developed and developing countries in TDM?
(Paula Schaiquevich, Argentina)

The clinical community has witnessed a sustained development in therapeutic drug monitoring (or more precisely therapeutic drug management, TDM) over the last decades. However, this strong development was mainly observed in high income in contrast to low and middle income countries that are still lagging behind.
Specifically, in Latin America there is a wide variation in the existence of TDM services among countries and even within the same country, with resource limited areas at disadvantages. It should also be considered that healthcare systems differ among these countries, an additional reason that impacts on the development and implementation of TDM.
Benefits of TDM have been extensively reviewed elsewhere but, special issues in low and middle income countries in America should still be addressed and are basic for contributing to the development in the region. In this sense, studies about the implications of TDM on the use of herbal medicines, existence of nutritional deficiencies and disease conditions prevalent in the region, ethnic differences, and the quality of pharmaceutical drug products will be discussed in the context of regional studies.
Undoubtedly, the correct measurement of drugs in biological samples is a critical aspect of TDM. In this sense, nationwide surveys of analytical facilities have been performed in an attempt to determine the state of the laboratory practices in relation to TDM in Latin America and results will be discussed.
We will also analyze potential barriers that hamper TDM development in Latin America including funds, facilities, qualified personnel and equipments. Also, we will propose strategies to encourage the introduction and sustain the advancement of TDM based on the benefits of progress in this area with a potential perspective of TDM in the region. We will also emphasize on education activities to promote TDM among health team members, building regional TDM networks and also training of decision-makers on the clinical value of TDM.
Altogether, this presentation will focus on the current situation of TDM in some Latin American countries, with emphasis on what has been done, benefits for local countries to develop TDM, current limitations and future perspectives.
Plenary presentation: Therapeutic Drug Monitoring and Clinical Toxicology for a Globalized World
(Pierre Wallemacq, Belgium)

Globalization is an inevitable phenomenon well known in the economic and business areas, but also in medical fields such as therapeutic drug monitoring or clinical toxicology (TDM/TOX). This phenomenon has undoubtedly advantages, such as a larger diffusion of informations and services, exchanges of expertises or guidelines, opening markets to other countries, etc… but may have major drawbacks due to important disparities in terms of socio-economic situation or GDP of countries, inequitable distribution of the benefits raised from globalization with as consequence sometimes, paradoxically, poor access to these medical and analytical activities. The FDA adverse events reporting system (FAERS) confirms that the use of drugs is still not as appropriate and safe as it should be, and that a progressive increase of serious adverse events is even observed. One of the drawbacks of globalization is to provide access to sometimes poorly controlled medicines produced abroad, with the risk of substandard medicines (production not responding to quality requirements or falsified medicines) both from branded or generic drugs. Availability through internet platforms makes this risk even higher. All these facts together with the exciting concept of precision medicine, largely contribute to make TDM/TOX expertise an essential tool to optimize drugs use.

Unfortunately, scientific literature and data obtained from a typical and large diagnostic industry emphasize important differences among continents and countries in terms of TDM/TOX practice, particularly in developing countries. TDM practice appears roughly limited to developed countries, whereas some sectors of toxicology seem better established world-wide. In all cases a lack of homogeneity in the practice is observed. Among the potential approaches to improve the practice of TDM/TOX world-wide, some may appear difficult to implement, whereas others not. Let’s consider the following: reduction of the number of unnecessary tests, reduction of the costs of the assays or selection of the more appropriate techniques, efforts to harmonize analytical methods, promotion of teaching and expertise in pharmacology and toxicology, development of softwares/artificial intelligence in all steps of the process (from prescription to final validation), promotion of clinical-trials focusing on cost-benefits of TDM/TOX practice, and put the question to political and healthcare decision-makers. IATDMCT, as well as other associations or institutions (e.g.WHO…), may play an important role in this improvement.
Symposium: Therapeutic Drug Monitoring in developing countries - Therapeutic monitoring of immunosuppressive drugs
(Renato Foresto, Brazil)

Kidney transplantation is the gold-standard treatment for end-stage renal disease. Immunosuppressive drugs prevents graft rejection and guarantees long-term graft survival. TDM plays an important role in the correct drug management in transplantation, ensuring maximum efficacy and safety and minimizing adverse events. Here, we will understand how are the major immunological mechanisms involved in graft rejection, as well as pharmacokinetics and pharmacodynamics of immunosuppressive drugs and the protocol used for their therapeutic monitoring.
Symposium Clinical use of precision dosing software - Current and Future Developments in Model-Informed Precision Dosing
(Ron Keizer, USA)

The past few years have seen a renewed interest in model-based precision dosing (MIPD), with a surge in publications and conference presentations. This presentation will focus on relevant current topics within the field of MIPD and speculate on what lies ahead in the next decade.

Current developments include a growing focus on the proper validation of the population models that commonly provide the basis of MIPD tools. Ensuring that such models translate across hospitals and patient populations and are “fit-for-purpose” is of key importance, and various approaches are available and being used. Optimization of user interface and workflows, as well as integration with hospital systems will be a key driver of adoption of MIPD tools, and current developments in this area will be presented. MIPD tools currently available can be divided in academic and commercial efforts, and it is essential to realize the distinctive features that each can provide. Strengths and weaknesses of both approaches should be recognized and where possible combined, to jointly support the interests of the field as a whole.

In the upcoming decade, we expect more interest from pharmaceutical industry in precision dosing. For example, while so far MIPD tools have only sparsely been released as regulatory approved companion applications, we expect this to change in the years to come. In addition, also due to the availability of developer tools such as R/Shiny that facilitate rapid development and deployment of MIPD tools, we expect an increase in such tools released by academia, while commercially, horizontal integration with other clinical software platforms is expected to increase. While other clinical fields have benefited greatly from machine / deep learning approaches in the past few years, the application to precision dosing are expected to be more limited, mainly due to the availability and nature of the required data. Finally, while so far, the integration of point-of-care tools has been mostly the domain of the clinician, the move towards value-based healthcare will increasingly involve payers. To justify adoption beyond academic and local use, more rigorous evidence is required to demonstrate actual health benefits and/or cost reductions for MIPD tools.
Symposium Young scientists - Alternative sampling strategies to support therapeutic drug monitoring of anti-TB drugs in TB endemic areas

(Samiksha Ghimire, The Netherlands)

It is well known that anti-TB drugs exhibit considerable pharmacokinetic variability. As a result, on the same standard dosages different individuals achieve a range of concentrations which can translate into different clinical outcomes. Therapeutic drug monitoring has the potential to address too low or too high exposure by measuring the concentrations of drugs in serum or plasma of individual patients. While TDM using venous sampling is a routine procedure in few treatment centers, it has yet to be adopted in the programmatic settings because of its perceived technical and cost constraints. At present, TDM using venous sampling do not seem feasible at all levels of care. However, alternative and limited sampling strategies can make TDM an attainable goal. The finger prick blood (dried blood spots method) and saliva could be suitable alternative sampling matrixes to predict drug levels in serum/plasma. Similarly, limited sampling strategies that utilizes two- to three- sampling time points for estimation of pharmacokinetic parameters, reduces sampling burden on both patients and clinicians. In this talk, Dr. Ghimire will review recent developments in the field of alternative and limited sampling strategies for anti-TB drugs and speculate about the changing landscape of TDM.
Symposium Clinical use of precision dosing software - Chances and challenges for implementation of precision dosing software from a pharmacometric perspective
(Sebastian Wicha, Germany)

Precision dosing software has the merit to streamline the TDM process by allowing for timely dosing decision making already before reaching steady state and even before the first dose through probabilistic simulation. Moreover less reliance on fixed sampling times, a lower required number of total TDM samples and potentially increased precision in the derived individual PK estimates and calculated doses make precision dosing software appealing for implementation. On the other hand, a number of scientific and implementation challenges remain to be addressed in order to benefit most from the merits of model-based TDM, which will be discussed in the presentation:

Variable data quality of TDM-related variables in clinical practice. Case studies with two pharmacokinetically different anti-infectives illustrating the impact of uncertain recording of dosing times, infusion duration and sampling times on the determined population and individual parameters will be shown. Moreover, the influence of the chosen sampling times itself on the accuracy of the determined PK parameters in Bayesian estimation will be presented. Handling of inter-occasion variability, which is regularly present in TDM data, but often neglected in pharmacometric analyses. A simulation-based analysis and a clinical example from TDM with an anti-infective drug will be presented and optimal approaches will be highlighted. Selection of a suitable pharmacometric model. Case studies with vancomycin, meropenem and coagulation factor VIII will be presented and strategies for a fit-for-purpose evaluation of pharmacometric models implemented in precision dosing software will be outlined.
Symposium Pharmacogenetic PGx in oncology - Tyrosine Kinase Inhibitors and PGx of ABCG2  
(Tomohiro Terada, Japan)

The human breast cancer resistance protein (BCRP/ABCG2) is an ATP-binding cassette efflux transporter that uses ATP hydrolysis to expel xenobiotics from cells, including tyrosine kinase inhibitors (TKIs). It is expressed in the gastrointestinal tract, liver, kidney, and brain endothelium. Thus, ABCG2 functions as a tissue barrier to drug transport that strongly influences the pharmacokinetics of substrate medications. It has been demonstrated that genetic polymorphisms of ABCG2 are closely related to inter-individual variations in therapeutic performance especially. The common single nucleotide polymorphism c.421C>A, p.Q141K reduces cell surface expression of ABCG2 protein, resulting in lower efflux of substrates. Interestingly, ethnic differences in the frequency of ABCG2 c.421C>A have been reported, with markedly higher frequency in East Asian (~30%–60%) than Caucasian and African-American populations (~5%–10%). In the symposium, we will talk about the overview of TKIs and PGx of ABCG2.
Immunosuppressive therapies are life-long treatments in transplant patients and patients with immune related diseases. Due to the large variability in drug response and narrow therapeutic index, there is a continuing unmet clinical need to better predict and control the dose-exposure-response and adverse events relationships of immunosuppressive drugs in pediatric patients. This presentation will provide a brief overview of the current practice and a summary of current applications of model-informed approaches to individualization and optimization of immunosuppressive therapy in pediatric patients as part of therapeutic drug management.
Symposium Young scientists - Challenges and opportunities for model informed precision dosing in very young children (Tomoyuki Mizuno, USA)

Evidence-based pharmacotherapy remains challenging in neonates and infants due to the paucity of PK/PD data for dosing, efficacy and safety for many medications. Effective and safe drug treatment in very young children should be based on integrated understanding of the rapid dynamic changes in growth and physiology over the course of early childhood. This presentation will describe ongoing research efforts to develop and implement model-informed precision dosing strategy especially focusing on very young children.
Oral Abstracts
FULLY AUTOMATED THERAPEUTIC DRUG MONITORING OF Anti-EPILEPTIC DRUGS MAKING USE OF DRIED BLOOD SPOTS
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Background: Fully automated dried blood spot (DBS) extraction systems, online coupled to standard liquid chromatography-tandem mass spectrometry (LC-MS/MS) configurations, decrease the hands-on time associated with conventional DBS analysis, resulting in a higher sample throughput, making the technique more compatible with a high-throughput bioanalytical workflow. The aim of this study was to develop and validate an LC-MS/MS method, using a DBS-MS 500 autosampler (CAMAG, Switzerland), for the determination and quantification of four anti-epileptic drugs (AEDs) (carbamazepine, valproic acid, phenobarbital and phenytoin) and one active metabolite (carbamazepine-10,11-epoxide) in DBS samples. Methods: Method development included thorough optimization of the fully automated extraction procedure (i.e. extraction solvent, extraction (loop) volume, internal standard application, internal standard drying time, etc.). Afterwards, the method was fully validated based on international guidelines and applied on capillary patient samples originating from African epilepsy patients. Results: Thorough optimization of the fully automated extraction method was of utmost importance, finally resulting in the exclusion of the built-in IS spray. Concerning method validation, accuracy (%bias) and precision (%RSD) (with a single exception) were below 13%, meeting the acceptance criteria. Neither carry-over nor unacceptable interferences were observed. Calibration data was found to be heteroscedastic. Using weighted linear regression, with 1/x² weighting, mean back-calculated concentrations did not differ more than ±15% for all analytes, which is in line with the acceptance criteria. All compounds were stable in DBS for at least 1 month when stored at room temperature, 4 °C and -20 °C and for at least 4 days when stored at 60 °C. Internal standard-corrected matrix effects were below 8%, with %RSDs below 9.1%. Reproducible relative recovery values (around 60% for all analytes) were obtained and the effect of the hematocrit on the relative recovery was overall limited. Finally, fifteen capillary DBS samples, originating from patients receiving AED therapy in remote areas within sub-Saharan Africa, were successfully analyzed, demonstrating the applicability of the developed procedure in a remote setting. Conclusions: An LC-MS/MS method for the determination and quantification of 4 AEDs and one active metabolite in DBS, making use of the DBS-MS 500 autosampler, was developed and validated. Thorough optimization during method development demonstrated that proposed, generic direct elution conditions, while of value for orientation, may require (substantial) adjustment, depending on the analytes of interest and the used instrumentation. Keywords: Therapeutic drug monitoring; dried blood spots; LC-MS/MS; anti-epileptic drugs; automated extraction.
EVALUATION OF THE HEMAPEN® AS A NEW HEMATOCRIT-INDEPENDENT DRIED BLOOD SPOT DEVICE
Deprez, S1; Paniagua-González, L2; Velghe, S1; Stove, C1
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2Toxicology Service, Institute of Forensic Sciences, University of Santiago de Compostela, Spain

Fig 1. HemaPEN® device
(with permission from Trajan Scientific and Medical, Australia.)

Background: Dried blood spots (DBS) are often used as a less invasive alternative to venous blood sampling. Despite its numerous advantages, the use of conventional DBS suffers from the hematocrit (Hct) effect when analyzing a sub-punch. This effect could be avoided by using Hct-independent sampling devices, of which the HemaPEN® is a recent example. This device collects the blood via 4 integrated 2.74µL microcapillaries, which each deposit the blood on a pre-punched paper disk. In this study we evaluated the technical performance of the HemaPEN® devices, using an extended bioanalytical validation and application on authentic patient samples. Methods: An LC-MS/MS method quantifying caffeine (CAF) and its metabolite paraxanthine (PRX) in dried whole blood (using the HemaPEN® device) was fully validated based upon the European Medicines Agency (EMA) guidelines on bioanalytical method validation. Additionally, DBS-specific parameters such as the impact of Hct were assessed. Results: All preset acceptance criteria were met. Using linear 1/x² calibration models, the method was found to be accurate (with a bias ±6% for both analytes) and precise (with a repeatability <8% and an intermediate precision <11% (CV%)). Selectivity and carry-over were within the preset limits (<20% of the LLOQ). CV’s on the internal standard compensated recovery were <9%. The recovery was Hct-independent (i.e. within ±15% of the recovery for DBS with a 0.41 Hct). A minimal absolute matrix effect was compensated for using 13C/15N-labeled internal standards, yielding CV’s <6%. Both CAF and PRX were stable for at least 2 months at room temperature and 4 days at 60°C when storing the HemaPEN® devices in their original packages. Analysis of 96 authentic patient samples (Hct range 0.17 to 0.53) confirmed the applicability of the validated method, with a very good concordance (≥0.976) between Hemapen® and blood concentrations. Only a slight Hct-based bias (≤10% concentration difference over a 0.20-0.50 Hct range) was observed for the Hemapen®, in contrast to DBS, showing concentration differences of ≥25% over this Hct range. Conclusions: We successfully validated a Hemapen®-based LC-MS/MS method for the quantitation of CAF and PRX in dried blood. All evaluated parameters met the pre-set acceptance criteria. A comparative analysis of Hemapen®, 3mm DBS sub-punches and whole blood revealed a good concordance with blood for the Hemapen® devices, with only a small Hct-dependence. Keywords: Alternative sampling strategies; Microsampling; DBS; HemaPEN®; method validation; LC-MS/MS
Theme: Alternative sampling strategies

**ADDECT-BASED MONITORING OF PARACETAMOL TOXICITY IN BLOOD VIA VOLUMETRIC ABSORPTIVE MICROSAMPLES**

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**Background:** Risk assessment in case of acute intoxications with paracetamol is currently based on the Rumack-Matthew nomogram. However, this has several drawbacks and limitations. Several studies have pointed out the potential value of an alternative marker, i.e. circulating proteins adducted with NAPQI, a reactive paracetamol metabolite. A 1 μM cut-off value in plasma of APAP-Cys (derived from the NAPQI-protein fraction after protein digestion) has been proposed for treatment with the antidote acetylcysteine. APAP-Cys could also be used to tailor paracetamol therapy in critically ill children and patients with liver disease, who have an increased risk for paracetamol-induced liver damage. We developed a method for the determination of these paracetamol-protein adducts, making use of a microsampling technique, namely VAMS (volumetric absorptive microsampling). Thereby, larger scale studies with a minimally invasive sample collection can be set up, to increase the knowledge about the presence and pharmacokinetics of this marker. **Methods:** We developed an LC-MS/MS method for the determination of APAP-Cys in blood making use of 10 μL VAMS devices. The method was validated with acceptance criteria based on international guidelines. Next, VAMS samples collected from patients receiving paracetamol treatment were analysed with the developed method. **Results:** The method was successfully validated with a maximum bias below 6% and the total imprecision lower than 9% for all QC levels. For both the low and the high QC level, there was no relevant difference in recovery over a hematocrit range of 0.20 to 0.60. Up till now, 39 samples, collected from 4 different patients, were analysed. In 59% (23/39) of the samples APAP-Cys was not detected. 25% (10/39) of the samples contained APAP-Cys, but in concentrations below the limit of quantification of 0.25 μM. 15% (6/39) of the samples had concentrations of APAP-Cys above the limit of quantification, ranging from 0.32 μM to 4.23 μM of APAP-Cys in blood. Thereby, the presence of paracetamol protein adducts in blood was demonstrated, even in patients receiving therapeutic concentrations of paracetamol. **Conclusions:** We have developed and validated a method for the quantitative determination of paracetamol-protein adducts in blood using VAMS. Applicability was demonstrated using a set of patient samples, demonstrating its feasibility. **Keywords:** alternative sampling, paracetamol, protein adduct
Background: PaperSpray is a rapid analysis technique which is particularly beneficial for the analysis of compounds in biological matrices, such as blood or urine. Because no sample preparation is required and analysis times are as short as 1-2 minutes, the technique offers a strong advantage over traditional methods which rely on chromatographic separation with their associated solvent use and time required for samples preparation and analysis. Methods: For the screening method, a total of 19 compounds (opiates, amphetamines, cocaine and PCP) were spiked into donor urine. For the quantitation method, EDDP was spiked into whole human blood at various concentrations. Samples were spotted onto the paper plates at a sample volume of 8 μL each and were oven dried for 30 minutes at a temperature of 50 °C. Sample plates were then loaded onto the PaperSpray mass spectrometry. Total mass spectrometer run time was one minute. Results: All 19 compounds met their respective screening cutoff levels (≤15% RSD of response ratio at the cutoff level, and the absolute AUC at least 4x higher than the AUC of the matrix blank). The lower limit of quantification (LLOQ) for EDDP was 3.5 ng/mL and was set to the lowest calibration standard analyzed that yielded < 20 % accuracy and < 15 % CV for 9 replicate injections. The labeled d3-EDDP present in all 240 samples was monitored for the entire run and had a peak area RSD of 32% spanning the entire run and a RSD of 1.5 % when comparing the peak area ratio of the labeled and unlabeled EDDP standard at 50 ng/mL level. Conclusions: PaperSpray technology can be used as a rapid screening method for drugs of abuse directly from dried urine spots. It can also be further applied for the confirmation and quantitation in whole blood. No sample preparation other than spiking an internal standard and spotting the urine or whole blood samples on the paper is required. A total method length (analysis time + automated sample handling time) of 1.5 minutes makes it a highly efficient and cost-effective screening and/or quantitation method. The method proved to be reproducible over 240 injections without any significant loss in signal, which makes it appropriate for a routine analytical method. Keywords: PaperSpray; Mass spectrometry; Drug of abuse; EDDP; Drug screening; Clinical research.
Background: Therapeutic drug monitoring of tacrolimus based on trough concentrations (C0) is a compromise for AUC-monitoring. New techniques may allow the patients themselves to collect a finger prick capillary blood sample. The aim of the present study was to validate a commercial device (Mitra tip®, Neoteryx, USA) based on volumetric absorptive microsampling (VAMS™) and compare this to simultaneously drawn venous samples in stable kidney transplant recipients. Methods: Blood samples were obtained by Mitra tip® finger prick sampling (10 µL). Tacrolimus was analyzed using a mass spectrometry assay, and the microsampling method was validated according to the guideline on bioanalytical method validation of the European Medicines Agency (EMA). Two 12-hour tacrolimus pharmacokinetic investigations (13 samples each), separated by at least one week, were performed in 27 renal transplant recipients included 3 ± 1 weeks after transplantation, receiving mycophenolate and prednisolone in addition to twice daily tacrolimus. At each sampling point 2 venous and 2 capillary samples (10 µL) were obtained; one pair (venous/capillary) went directly to the lab while the second pair was sent via ordinary mail. A non-parametric population model (Pmetrics) was used to calculate the reference AUC-12h ref based on all 13 measured concentrations. In addition, AUCO-12 LSS was predicted from 3 samples collected at 0-, 1- and 3-hours, and AUCO-12 C0 was estimated from the trough concentration with the same population model. The predictive performance of the 3-point LSS and C0 estimated AUCs were evaluated by comparison with the reference AUCO-12h ref. Results: All results of the method validation (tacrolimus concentration range 0.7 to 57 µg/L) were within EMA criteria. The Mitra tip® samples were stable for at least 30 days in room temperature and were not influenced by postal service shipment. In a total of 682 pairs the micro sample concentrations were on the average 4.2% (95% CI: -5.1% to -3.4%) lower than the venous samples. AUCO-12h ref was 148 ±41 µg*h/L and the 3-point prediction, AUCO-12 LSS, bias was 9 µg*h/L (RMSE=13). AUCO-12 C0 bias was 101 µg*h/L (RMSE=123). Conclusions: Measurement of whole blood tacrolimus concentrations in 10 µL capillary blood from renal transplant recipients can be reliably and safely performed using the VAMS™ technique, providing an option for self-collection by patients and the opportunity to simplify investigations of tacrolimus including repeated measurements within a dosing interval.

Keywords: tacrolimus; volume absorptive microsampling VAMS™; limited sampling strategy; Mitra tip®; renal transplantation.
RESULTS FROM A PROFICIENCY TESTING PILOT FOR IMMUNOSUPPRESSANT DRIED BLOOD SPOT ASSAYS

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Background: Therapeutic Drug Monitoring (TDM) of immunosuppressive drugs such as tacrolimus and cyclosporine A is important for prevention of allograft rejection in transplant patients. To facilitate TDM, several hospitals offer Dried Blood Spot (DBS) sampling, giving patients the opportunity to sample a drop of blood from a finger prick at home, which can be sent to the laboratory by mail. Currently, no external quality program exists for DBS methods. Methods: Seven laboratories (6 NL, 1 USA) participated in a pilot two-round proficiency test. Six participants report to use DBS sampling as part of routine care. In the first round, 6 DBS samples were prepared from spiked citrate whole blood (3 tacrolimus, 3 cyclosporine A) on Whatmann DMPK-C and 903 paper. In the second round, 4 DBS samples were prepared from spiked EDTA whole blood (2 tacrolimus, 2 cyclosporine A). The spiked whole blood used for sample preparation was also sent as a sample. In addition, a patient tacrolimus sample was sent (as whole blood and as DBS). Participants were asked to report values as in their regular practice. Results: In the first round the range of reported DBS values for tacrolimus and cyclosporine A were 74.0-123.3% and 57.4%-128.3%., compared to the spike values. In the second round this was 86.4-129.4% and 74.2-150.5%. The reported whole blood values for tacrolimus and cyclosporine A in the second round were 99.1-115.0% and 109.0-129.5%. For the patient sample, the range of reported DBS and whole blood values were 9.7-15.7 µg/L and 10.8-12.5 µg/L. Conclusions: Current DBS assays for tacrolimus and cyclosporin A that are used in transplant patient care show clinically relevant differences between participating laboratories. Standardization and harmonization are needed to improve accuracy and precision of DBS assays and decrease inter-laboratory variation in order to optimize immunosuppressant dosing in transplant patients. Proficiency testing of DBS assays can be of great value.

Keywords: Dried Blood Spots, Microsampling, Quality assessment, Proficiency testing, tacrolimus
ASSESSMENT OF THE DERMAL BIOAVAILABILITY OF TOPICAL GLUCOCORTICOIDS: STRATUM CORNEUM SAMPLING VERSUS THE VASOCONSTRICTION ASSAY.

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Background: The skin vasoconstriction assay is used as a surrogate marker of potency and as a metric for bio(in)equivalence for topical corticosteroid products. Here, stratum corneum (SC) sampling, a minimally invasive approach to assess dermal bioavailability, was used to quantify steroid delivery to the deeper skin. Methods: Betnovate® 0.1% Betamethasone valerate (BMV) cream was applied (2-10 mg/cm²) to the ventral forearms of 12 healthy volunteers (ethical approval EP 17/18 154). At separate skin sites, the mass of drug in the SC was measured following adhesive tape-stripping (a) after a 4-hour ‘uptake’ period, and (b) after removal, subsequent to a further 6-hour ‘clearance’. The BMV-induced vasoconstriction was assessed with a chromometer over a 22-hour period post-application of the cream for 4 hours. Results: BMV uptake by the SC was significantly higher (p < 0.05) when 5 mg/cm² of the cream was applied compared to the 2 and 10 mg/cm² doses. In all cases, ~30% of the BMV in the SC at the end of uptake period was cleared in the subsequent 6 hours. The BMV flux into the viable epidermis and the first-order elimination rate constant from the SC were approximately 4 ng.cm⁻².hr⁻¹ and 0.07 hr⁻¹, respectively. Vasoconstriction results were highly variable and insensitive to the applied BMV dose, even when non-responders were excluded from the data analysis. Conclusions: Evaluation of BMV dermatopharmacokinetics from SC sampling enabled quantitative assessment of drug delivery into the viable skin. In contrast, the pharmacodynamic data based on the vasoconstriction assay were dose-insensitive and poorly reproducible. Acknowledgements: This research was funded by the Leo-Foundation (Project LF16117).
Keywords: Betamethasone valerate; topical glucocorticoids, vasoconstriction; stratum corneum; tape stripping; skin.
PERFORMANCE OF AN APP MEASURING SPOT QUALITY IN DRIED BLOOD SPOT SAMPLING
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Background: The Dried Blood Spot (DBS) method allows patients and researchers to collect blood on a sampling card using a fingerprick. An important issue in the application of DBS is that samples are frequently rejected because of poor spot quality leading to delayed monitoring or missing data. We describe the development and performance of a web-based application (app) accessible on smartphone, tablet or desktop capable of assessing DBS quality at time of sampling by means of analyzing a picture of the DBS. Methods: The performance of the app was compared to the judgment of experienced lab technicians for samples obtained in a trained and untrained setting. A robustness- and user test were performed. Results: In a trained setting the app yielded an adequate decision in 90.0% of the cases with 4.1% false negatives and 5.9% false positives. In an untrained setting this was 87.7% with 5.5% false negatives and 6.7% false positives. A patient user test resulted in a system usability score of 74 out of 100 with an average time of 2 minutes and 11 seconds to use the app. Robustness testing showed a repeatability of 84%. Using the app in a trained and untrained setting improves the amount of sufficient quality samples from 80% to 95.9% and 42.2% to 87.9% respectively. Conclusions: The app can be used in trained and untrained setting to decrease the amount of insufficient quality DBS samples.
Keywords: Dried Blood Spots, Specimen Quality Evaluation, App, Therapeutic Drug Monitoring
Theme: Anti-infective drugs

VORICONAZOLE THERAPY OPTIMIZATION BASED ON TOTAL BILIRUBIN IN PATIENTS WITH LIVER DYSFUNCTION

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Background: Voriconazole, a first-line agent for the treatment of invasive fungal infections, has significant interpatient variability in exposure. This study aimed to investigate the population pharmacokinetics of voriconazole, identify the factors significantly associated with voriconazole pharmacokinetics and optimize voriconazole dosage regimen in patients with liver dysfunction.

Methods: The study prospectively included 51 patients with 272 voriconazole concentrations to develop the population pharmacokinetic model. The pharmacokinetic data were analysed using a nonlinear mixed-effects population approach with Phoenix NLME software. Dosing simulations based on the final model and voriconazole target attainment (1–5.5 mg/L) were performed. Results: A one-compartment pharmacokinetic model with first-order absorption and elimination was used to describe the data. Population estimates of clearance (CL), the volume of distribution (Vd) and oral bioavailability (F) were 0.88 L/h, 148.8 L and 88.4%, respectively. Voriconazole CL was significantly associated with total bilirubin (TBIL) and platelet count. The Vd increased with body weight. Considering that the elimination of voriconazole is markedly prolonged in patients with liver dysfunction, the dosing regimen was simulated for 7-days and 30-days, respectively. Dosing simulations stratified by TBIL (TBIL-1: TBIL <51 μmol/L; TBIL-2: 51 μmol/L≤TBIL<171 μmol/L; TBIL-3: 171 μmol/L≤TBIL<342 μmol/L; TBIL-4: TBIL>342 μmol/L) were performed. For 7-days, 100 mg every 12 h (q 12 h) orally or intravenous for patients with TBIL-1 or TBIL-2 and 100 mg every 24 h (q 24 h) orally or intravenous for patients with TBIL-3 or TBIL-4 were sufficient. For 30-days, TBIL-1, TBIL-2, TBIL-3 and TBIL-4 patients could be treated with 100 mg q 12 h, 150 mg q 24 h, 50 mg q 24 h, 50 mg q 24 h orally or intravenous, respectively. Conclusions: Lower doses and longer administration intervals should be considered for patients with liver dysfunction. TBIL-based dosing regimens provided a rational strategy for achieving voriconazole therapeutic target to maximize treatment outcomes.

Keywords: voriconazole; population pharmacokinetics; dosing regimen; liver dysfunction; therapeutic drug monitoring.
Theme: Anti-infective drugs

POPULATION PHARMACOKINETICS OF IMIPENEM IN CRITICALLY ILL PATIENTS: A COMPARISON BETWEEN PARAMETRIC AND NONPARAMETRIC METHODS

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Background: Population pharmacokinetic (popPK) models for antibiotics are used to improve dosing strategies for subpopulations and to individualize dosing by therapeutic drug monitoring. While both parametric and nonparametric popPK models are published, little is known about the differences in modelling and simulation results and consequences for dosing recommendations. Our objectives were to develop a parametric and nonparametric model using data of critically ill patients treated with imipenem and to compare the modelling results.

Methods: Twenty-six critically ill patients treated with imipenem/cilastatin were included. Median eGFR (CKD-EPI) was 116 ml/min (IQR 104-124) at inclusion. The usual dosing regimen was 500mg/500mg four times daily. Peak, intermediate and trough levels (n=138) were drawn on day 1, 2, 3, 4 and 6 of therapy. Imipenem concentrations were determined by an HPLC-UV method. Concentration-time profiles were analyzed using parametric (NONMEM 7.2) and nonparametric (Pmetrics 1.5.2) popPK software.

Results: For both methods, the data were best described by a model with 2 distribution compartments with CKD-EPI as a covariate on clearance. The parametric population parameter estimates were: first order elimination rate constant (Ke) 0.637 h⁻¹ (between-subject variability, BSV: 18.6% CV) and central distribution volume (Vc) 29.6 L (without BSV). The nonparametric values were: Ke 0.681 h⁻¹ (34.0% CV) and Vc 31.1 L (42.6% CV).

Conclusions: Both parametric and nonparametric models described imipenem popPK well and the popPK parameter estimates were comparable. However, the estimated BSV was remarkably different. The consequences of this finding for probability of target attainment simulations based on popPK models should be further studied.

Keywords: population pharmacokinetics; parametric; nonparametric; imipenem; critically ill.
Theme: Anti-infective drugs

VANCOMYCIN DOSING TARGETING TROUGH CONCENTRATIONS LEADS TO UNNECESSARILY HIGH DRUG EXPOSURE

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Background: A recommended target for guiding intravenous vancomycin dosing is the area under the concentration-time curve (24 hours, AUC24) of 400-700 mg/L.h. A convenient surrogate for this is a trough of 15-20 mg/L. However, in the setting of intermittent infusions, this may lead to an excessive AUC24. Further, as vancomycin clearance does not scale linearly with total body weight (TBW), ideal body weight (IBW) may be a better metric for estimating clearance, and hence initial maintenance dose. Using a real-world dataset, we aimed to evaluate the differences in vancomycin exposure using a) AUC24 vs trough targets, and b) IBW vs TBW. Methods: Data were extracted from local hospital databases of adult inpatients receiving intermittent vancomycin infusions (12-hourly) with at least one vancomycin serum concentration measured by immunoassay. Following feedback from measured vancomycin concentrations, dosing to achieve either a target AUC24 of 400-700 mg/L.h or a trough of 15-20 mg/L was predicted using a vancomycin pharmacokinetic model implemented in a Bayesian forecasting tool. Further, using the posterior model for each patient, dosing for the first 24 hours of the vancomycin course was simulated using IBW or TBW. Wilcoxon and McNemar’s tests were performed. Results: A total of 60 patients were evaluated with a median (range) age of 66 years (19-97), estimated glomerular filtration rate of 78 mL/min/1.73m2 (29-129), and total body weight of 86 kg (40-176). Following vancomycin concentrations, dosing according to AUC24 and trough concentration targets led to median (range) 12-hourly doses of 1000 mg (200-2500) and 1250 mg (200-3200), respectively. The corresponding median (range) AUC24 values were 449 mg/L.h (406-528) and 550 mg/L.h (438-711), respectively (P < 0.0001). Only one patient, dosed using the target trough concentration, would have exceeded an AUC24 of 700 mg/L.h. Use of IBW and TBW to guide initial dosing led to median (range) first 24 h AUC24 of 369 mg/L.h (210-610) and 424 mg/L.h (255-680). Significantly more patients dosed using IBW (63%) than TBW (35%) would have had an AUC24 below 400 mg/L.h (McNemar’s $\chi^2(1) = 14.1$, P = 0.0002). Conclusions: Dosing according to target trough concentrations of 15-20 mg/L resulted in higher AUC24 values than targeting 400-700 mg/L.h. Dosing using IBW was associated with an AUC24 below 400 mg/L.h.

Keywords: vancomycin; TDM; AUC; trough; Bayesian.
Theme: Anti-infective drugs

DEVELOPMENT OF A TOOL FOR OFLOXacin AND LEVOfLOXacin THERAPEUTIC DRUG MONITORING IN BONE AND JOINT INFECTIONS

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Background: Ofloxacin (OFLO) and levofloxacin (LEVO) are recommended treatments for bone and joint infections (BJI). Their efficacy is related to the area under the curve of their concentrations (AUC0-24h) over the minimum inhibitory concentration (MIC) for a given bacteria. Hence, the drug exposure appears as a crucial determinant of their efficacy and could improve the therapeutic drug monitoring (TDM) of these drugs. However, there is a paucity of data in gram + bacteria treatment which is the most common situation in BJI. The aim of the present study is to model the pharmacokinetics (PK) of oral OFLO and to establish a limited sampling strategy (LSS) to estimate AUC0-24h. The model will be externally validated on LEVO data to evaluate its replicability to this drug.

Methods: PK of OFLO was described using a compartmental modeling with population approach using Monolix. Three approaches were tested out to determine the LSS. Every 30 min between 0 and 4h were allowed as possible sample time. LSS performances were evaluate using the mean bias, precision, R2 adjusted and Bland-Altman graphics. Monte-Carlo simulations were conducted to evaluate the probability of attainment of AUC0-24h/MIC > 115 h, 230 h and 383 h targets (assuming a diffusion of 100%, 50% and 30% respectively into bones). The external validation consisted on the evaluation of predicted versus observed profiles obtained from 59 LEVO patients. Results: Thirty patients were included in the study. OFLO PK was best described using a bicompartamental model with a first order elimination, and a transit compartments model absorption. CKD-EPI and sex had a significant influence on apparent total clearance and allowed explaining half of the variability. The three different approaches for the LSS had similar results. The best performing LSS for predicting AUC0-24h consisted of sampling times: 0, 1h and 3h (R2 adjusted = 95.5%). The optimization results highlighted the difficulty in reaching PK-PD thresholds at the infectious site. The model performance for LEVO was good (R2 = 96.5 %, Bland Altman outliers = 5.7%). Conclusions: This study is the first description of OFLO PK in BJI. The model allows adequate PK estimation for OFLO and LEVO. We described a LSS which will allow OFLO/LEVO TDM based on AUC0-24h.

Keywords: Fluoroquinolone; Ofloxacin; Bone Joint Infection; Modeling.
Theme: Anti-infective drugs

ASSESSMENT OF MEROPENEM PHARMACOKINETICS AT THE EARLIER VERSUS LATE PERIOD OF SEPTIC SHOCK IN ADULT CRITICALLY BURN PATIENTS BASED ON DRUG SERUM MEASUREMENTS DONE IN A REAL TIME
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Background: Physiological changes due to sepsis and burning can alter the pharmacokinetics (PK) of antibiotics with impact on the desired outcome in critically ill septic patients from the Intensive Care Unit [1]. The aim of the study was to compare PK changes registered for meropenem at the earlier versus late period of septic shock in burn patients receiving the recommended dose. Methods: Ethical approval was obtained N. 069/09-2015. Thirteen adult burn patients, 3F/10M: medians, 25 yrs, 70 kg, 36% total burn surface area, inhalation injury (8/13), with renal function preserved undergoing meropenem treatment for septic shock received 1g q8h by 0.5hr infusion. Only two blood samples were collected (1.5 mL/each) at the steady state for drug serum measurement by liquid chromatography [2]. PK results from burn patients were compared with data previously described in healthy volunteers [3]. Furthermore, PK data were compared at the earlier with the late period of septic shock which was respectively at the 3rd and 10th day of septic shock. Results: Patients received 43 mg/kg (40-48 mg/kg) daily dose equivalent to 14 mg/kg (13-16 mg/kg) q8hr. PK data are shown in figure 1. Conclusion: Changes on plasma clearance and volume of distribution occurred at the earlier period of septic shock. Then drug plasma monitoring done in real time based on PK/PD approach can be an important tool to assess PK changes that can impact drug effectiveness and the desired clinical outcome in critically ill septic burn patients.


Keywords: Septic shock; Pharmacokinetics; Meropenem serum monitoring; Burn patients
Theme: Clinical Toxicology/drugs of abuse

NEW PSYCHOACTIVE SUBSTANCES (NPS) AND PSYCHOACTIVE PHARMACEUTICALS, A HARMFUL INTERACTION: REPORT OF TWO INTOXICATION CASES

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Background: Case 1: A 17 years-old female was admitted to emergency unit (EU) after a supposedly suicide attempt with unknown pesticide. The patient experienced seizure which was totally controlled with benzodiazepines. Urea and potassium levels were slightly decreased. According to her family, a possible LSD use was performed together. Case 2: A 16 years-old female was found in a party by her colleagues with visual hallucination, confusion, vacant stare and disconnected speech. Beside her body, empty packages of ibuprofen, fexofenadine associated to pseudoephedrine, venlafaxine and fluoxetine were encountered. At EU patient experienced hypertension, tachycardia, fever, visual hallucinations and delusions. Laboratory tests for magnesium, calcium, CK-MB, C-reactive protein and serum creatinine were altered. Patient was sedated and monitored. Methods: In both cases, urine and plasma samples were submitted to GC-MS (STA) and LC-MS/MS (target MRM screening) to determine the substances involved in both cases. Plasma from the first case was also analyzed for lithium (flame photometry) due to psychiatric treatment. Results: Case 1: Toxicological analysis detected in urine and plasma 2C-E (plasma level 2.5 ng/mL), an illicit phenethylamine drug used to obtain hallucinogenic effects. No LSD and pesticide were detected. Lithium level was 0.3 mEq/L (subtherapeutic level). Case 2: STA in urine detected all the pharmaceuticals described earlier (except of venlafaxine), methcathinone (synthetic cathanone with stimulant effects) and ephedrine. Plasma quantitative levels were: methcathinone (4 ng/mL), ephedrine (5 ng/mL), pseudoephedrine (1.1 µg/mL), fluoxetine/norfluoxetine (89 ng/mL, 84ng/mL). Conclusions: Lithium and 2C’s have a potential to lead to seizures when in high plasma levels separately. According to the report’s data, plasma levels aren’t in high concentration, thus, the signs and symptoms presented seen to been produced by the joint of action of this drugs. Methcathinone is metabolized into ephedrine and pseudoephedrine therefore dopaminergic/stimulant effects are waiting. Serotonin syndrome was the main effect of case 2 instead of adrenergic effects, therefore association between fluoxetine, pseudo-/ephedrine and methcathinone not in high levels it must to be considered. These two presented cases lead to infer that the association between psychoactive substances with recreational purpose such as NPS and pharmaceuticals even in therapeutic levels can be potentially dangerous. Keywords: NPS; 2C-E; lithium; methcathinone; fluoxetine; ephedrine.
SPECIALTY TOXICOLOGY INVESTIGATION: IDENTIFICATION OF AMB-FUBINACA FOLLOWING A HOSPITAL MEDICAL EMERGENCY

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Background: The accessibility and/or the type of novel psychoactive substances (NPS) consumed in an area at a given point in time is highly unpredictable. Advanced analytical tools are often needed to identify them, however, if not available, NPS exposure remains unknown to the health care providers. Following a hospital medical emergency we identified AMB-FUBINACA, an ultra-potent synthetic cannabinoid (previously linked to the 'Zombie' syndrome/outbreak), in the herbal product which the patient smoked. We combined data from experimental HR/MS, in-silico analysis and m/z cloud (an external mass spectral database), and further verified compound identity with commercial reference standard. Methods: 214.1 mg of product (light brown crushed leaves) was extracted in 1 mL methanol. 20 μL extract was drug-screened by high resolution mass spectrometry using: 1) Q-Exactive™ Orbitrap/HESI and Accela UHPLC quaternary-pump/autosampler (ThermoFisher); 2) Kinetex PFP/F5 column (Phenomenex) 2.5 μm/100 Å/100 x 2.1 mm; 3) in-house developed targeted Broad Drug Screening (tBSS) protocol (725 drugs/metabolites; mass accuracy < 5ppm; retention time (RT) ± 0.5 min; positive/negative switching-mode; spectral match to reference standard); 4) in-house validated suspect screen/confirmation algorithm for untargeted/unknown NPS. In-silico fragment analysis was with Mass Frontier (v.7.0.5.9 SR3). AMB-FUBINACA standard was from Cerilliant (S-111-1ML). Results: There was a high likelihood that a synthetic cannabinoid was the toxic ingredient. None of the synthetic cannabinoids in our drug library (n=24) were identified and nicotine was the only compound found with tBSS. Using exact (monoisotopic) molecular weights, the total ion chromatogram was manually queried against an expanded list of suspected synthetic cannabinoids not included in our library (n=50). A major peak was extracted at RT=11.98 with M+H=384.1718 corresponding to AMB-FUBINACA (mass accuracy of +1.3 ppm). The observed MS2 fragments (109.045; 253.077; 271.088; 285.104; 324.151 and 338.166) fully aligned with the in-silico fragmentation data, and returned a match of 90.5 % to AMB-FUBINACA in the m/z Cloud database. Its identity was further verified with reference analytical standard. Only the desmethyl AMB-FUBINACA metabolite (M+H= 370.1562) was identified in patient’s urine specimen (RT= 9.96 min) using the same retrospective query approach. Conclusions: To our knowledge this is the first report of a Toronto’s hospital medical emergency involving AMB-FUBINACA. This finding raised awareness of this ultra-potent NPS on hospital ground (and Toronto’s neighborhoods) and triggered extra security measures for patient safety. The Drug Analysis Service of Health Canada had only reported few instances of AMB-FUBINACA product seizures (n=3) across Canada prior to this finding.

Keywords: Novel Psychoactive Substance; Synthetic cannabinoid; AMB-FUBINACA; High Resolution Mass Spectrometry
PHOSPHATIDYLETHANOL AS A SCREENING TOOL OF ALCOHOL USE DISORDER IN TRAUMA PATIENTS
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Background: Trauma is the leading cause of death worldwide among those aged 5-49 years. Alcohol misuse is a known risk factor for injury and trauma recidivism. Since 2006, to prevent alcohol-related injury, all Level 1 trauma centers are mandated to provide alcohol screening in all patients admitted and an intervention on those who screen positive. The screening tool often used is the Alcohol Use Disorders Identification Test (AUDIT) or its shorter version: AUDIT-C. These self-reported questionnaires have limited diagnostic efficiency and studies show under-reporting of alcohol consumption on different populations. A novel approach to screening for alcohol-use disorder is the quantification of Phosphatidylethanol (PEth) in blood. PEth is a direct ethanol metabolite formed in the erythrocyte membrane exclusively in the presence of ethanol. The level of PEth in blood can characterize alcohol consumption patterns and it exhibits high diagnostic accuracy for detecting excessive alcohol misuse. The aim of this study was to compare the performance of PEth levels and the AUDIT-C score for alcohol screening in trauma patients. Methods: Prospective cohort study of 111 adult patients presenting in the emergency department with any type of trauma. PEth 16:01/18:01 homologue concentration was determined in whole blood by LC-MS/MS. Consent, AUDIT-C score and demographic data were obtained by the researchers. Results: The sample consisted of majority male (72%), single (44%) and employed (62%) patients. The most common type of trauma was traffic collision (60.4%). The mean age was 42 years (± 19 years). The severity of trauma was classified by the injury severity score (ISS) in: <8=minor (48.6%), 9-15=Moderate (32.4%) and >16=Serious (18.9%). PEth levels ranged from not detected (ND) to 2905 ng/dl. 61 subjects had ND PEth levels (LOQ=5 ng/ml). The patients were classified according to the AUDIT-C score into two (2) groups: 35 patients with excessive alcohol consumption (AUDIT-C ≥4 men; ≥3 women) and 76 patients with no-risk alcohol use (AUDIT-C <4 men; <3 women). We found a significant correlation (r=0.654; p<.0001) when compared the PEth levels with AUDIT-C score as continuous variables with Spearman’s rho. Also, the medians PEth levels were significant different between the two AUDIT-C groups (127.87 vs. 0.00; p<.0001 Mann-Whitney). We also observed that 4 patients that reported abstinence (AUDIT-C score =0) had quantifiable levels of PEth. Conclusions: We found a strong correlation between PEth levels and AUDIT-C score in our population. We identified 4 cases of clear under-reporting of alcohol consumption habits using PEth concentration. More research is needed to identify the best approach to screen for alcohol misuse in trauma patients.

Keywords: trauma; ethanol; phosphatidylethanol; emergency medicine; secondary prevention; screening
LARGE-SCALE STUDY TO EVALUATE THE VARIABILITY IN ELIMINATION RATE OF THE DIRECT ALCOHOL MARKER PHOSPHATIDYLETHANOL DURING ONE MONTH OF ABSTINENCE

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Background: Phosphatidylethanol (PEth) is a group of phospholipids that is formed in erythrocyte cell membranes in the presence of ethanol only. We set up a large-scale population study, with the aim to get more insight in the variability of the elimination rate of PEth during a month of abstinence and to evaluate our current PEth limits for social drinkers. Methods: Over 700 volunteers who voluntarily stopped drinking for one month took three fingerprick blood samples (early, mid and end of February 2019) via self-sampling at home using a volumetric absorptive microsampling (VAMS) device (Mitra®, Neoteryx). Self-sampling kits were sent from and back to the lab via regular mail, together with a short questionnaire (based on the alcohol use disorders identification test (AUDIT)). PEth 16:0/18:1 was quantified using a validated liquid chromatography – tandem mass spectrometric method. Results: 685 volunteers completed the study, the majority being female (63%) and European (99%), with an even spread in the age range 18 to 60 years. The reported alcohol use in January ranged from none to over 50 units per week. The majority reported drinking two or three times a week (40%), with a frequency of one or two drinks per day (44%). At the time of writing, PEth data are available for samples from 307 volunteers (analysis is still ongoing). PEth values ranged from <10 to 1142 ng/mL, 404 ng/mL and 189 ng/mL early, mid and end of February, respectively. A correlation was found between the initial PEth level and the AUDIT-C score ($r^2 = 0.36$). When PEth values were organized according to AUDIT-C scores (group 1: AUDIT-C score <4, group 2: AUDIT-C score 4-8, group 3: AUDIT-C 9-12), statistically significant differences were seen in the mean PEth values. The mean half-life was calculated as $7.9 ± 2.2$ days, with no correlation to the initial PEth value ($r^2 <0.01$). There were no significant effects of sex or AUDIT-C score on the half-life. The correlation between the average units per week and the PEth concentration can be described by a linear regression line (PEth = 6.4 average units a week + 5.7, $r^2 = 0.51$). Conclusion: A large-scale study was set up, in which PEth levels in an unprecedented number of alcohol drinkers (covering almost abstainers to possible heavy drinkers) were monitored during one month of abstinence. Analysis of the wide range of measured PEth 16:0/18:1 concentrations in the resulting microsamples revealed that (i) there is a correlation with the reported average alcohol intake in January, (ii) the decrease in PEth is not related to the initial PEth level and (iii) the half-life ($7.9 ± 2.2$ days ) is longer than currently assumed (3-5 days). Further analysis is ongoing, and the overall results of this study will be reported.
Theme: Clinical Toxicology/drugs of abuse

**SUSPECTED INTOXICATION WITH NITRITE: QUANTIFICATION OF NITRITE AND NITRATE IN POST-MORTEM HUMAN MATRICES**

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**Background:** Sodium nitrite has various applications, such as a food additive in processed meat and medically as a third line treatment of cyanide poisoning in combination with sodium thiosulfate. Nitrite and its metabolite nitrate are endogenous compounds as well. Endogenous nitrite and nitrate plasma concentrations are <0.01 – 0.8 and 0.6 – 6 mg/L respectively. Average nitrite and nitrate urine concentrations are 0.2 and 61 mg/L respectively. In 2018, oral ingestion of nitrite was suspected to be the cause of death in two forensic cases in the Netherlands. The toxicity mechanism of nitrite is twofold. First, nitrite is reduced in vivo to nitric oxide, causing vasodilation and subsequent hemodynamic instability. Second, nitrite induces methemoglobinemia and subsequent hypoxia. Ingested doses of more than 4 g are potentially lethal. An analytical method was developed to quantify nitrite and its metabolite nitrate in biological matrices (including postmortem whole blood and urine) and powder form. **Methods:** Sample preparation is based on the method from Yang et al (2013) with a few modifications (sample volume, temperature, evaporation protocol). Samples are precipitated (diluted for powder) with acetone and derivatised with pentafluorobenzylbromide (PFBBr) using tetraoctylammonium bromide as a phase transfer catalyst. The derivatised products of nitrite and nitrate are extracted with iso-octane and analysed with GC-MS in single ion monitoring (SIM) mode. Because nitrite and nitrate are endogenous compounds, the quantification is performed with calibrators prepared in deionized water, and the quality of the method is monitored using internal controls prepared in deionized water. Analog (15)N-labeled internal standards are used for the quantification of nitrite and nitrate. **Results:** The calibration curve is linear between 5.0 mg/L and 50 mg/L. The lower limit of quantification (LLOQ) is 5.0 mg/L. The bias of the method is within 15%, and the repeatability within 16%. The recovery of the quality controls lies within 90%-110%. The method has been applied to two forensic cases. In the first case, nitrite was detected in powder (646 g/kg) and in urine (lower than 50 mg/L) but not in blood, whereas nitrate was detected in blood (571 mg/L) and urine (85 mg/L), but was not detected in powder. In the second case, nitrite was detected in stomach content (4.1 g/L) and in heart blood (lower than 25 mg/L). Nitrate was detected in stomach content (141 mg/L) and in heart blood (273 mg/L). **Conclusions:** In case of a suspected death due to ingestion of nitrite powder, it is important to quantify both nitrite and nitrate in blood and urine. The analysis of stomach content is also a good indicator of a possible oral intake of nitrite. **Keywords:** toxicology; post-mortem; forensic; mass-spectrometry
Background: Previously we developed an activity-based screening assay for (synthetic) opioids/opiates in biological matrices, allowing the detection of all opiate/opioid substances regardless of their (un)known structure. This approach had one major limitation: because opioid receptor antagonists (e.g. naloxone) can prevent the in vitro opioid receptor activation induced by opioid agonists, some opioid positive samples were missed. As it is difficult to know if a person with a (potential) opioid overdose received opioid antagonists as treatment, it is important to be able to assess if an antagonist is present in the sample. Therefore, we adapted our protocol to distinguish between ‘real negative’ samples and ‘negative’ samples due to the presence of e.g. naloxone.

Methods: In the bioassay, 20 µL of blood extract is applied onto stable cell lines, in which activation of the µ-opioid receptor can be monitored via a split-luciferase system. By adding a small amount of agonist (0.5 ng/mL hydromorphone) after 30 min, we can determine if an antagonist is present in the extract. The applicability was assessed by blind-coded analysis of 136 authentic blood extracts and comparing the results to those of a GC-MS and LC-Q-TOF analysis. Results: In the bioassay, four distinct profiles can be distinguished: 1) the samples that show net opioid activity (indicating the presence of opioid agonists); 2) samples that do not contain any opioid agonists nor antagonists; 3) samples that contain naloxone at sufficient high concentrations to block receptor activation; 4) samples that contain a combination of opioid agonists and antagonists. Within the 136 blood extracts, there were 43 duplicates, which all gave the same result, showing the robustness of the assay. After subtracting the duplicates, we ended up with 93 unique samples of which 68 of the 69 opioid positive samples (agonist and/or antagonist) could be identified, resulting in a sensitivity of 98.6%. 23 of the 24 opioid negative samples were correctly scored negative (specificity = 95.8%).

Conclusions: This improved bioassay allows the simultaneous screening of opioid agonistic and antagonistic activity in biological matrices, thereby further supporting its application as a useful first-line screening tool to investigate potential opioid intoxications in clinical/forensic settings, complementing conventional analytical methods which are currently used.

Keywords: synthetic opioids; naloxone; activity-based; screening; NPS.
MEDICAL CHILD ABUSE THAT WENT UNDETECTED FOR YEARS BECAUSE OF INCOMPLETE TOXICOLOGICAL ANALYSIS

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Background: Medical child abuse refers to a child receiving unnecessary and (potentially) harmful medical care due to a caregiver’s overt actions like the exaggeration of symptoms, lying about the history, fabricating clinical findings, or intentionally inducing illness in their child. The annual incidence is approximately 0.4 to 1.2 per 100,000. Case presentation: A 3-year old boy was admitted to the emergency department of another hospital with obtundation and a tendency to fall. No other symptoms were reported. The medical antecedents included epilepsy, bronchial hyperreactivity, and eczema. Medicine intake consisted of desloratadine and levetiracetam. Clinical examination showed a normal heart rate and blood pressure, shallow breathing with intermittent apnoea. Neurologically, the patient was somnolent with short episodes of being well-oriented and responding adequately. The differential diagnosis included encephalitis/meningitis, acute toxic metabolic encephalitis, and intoxication. CT, lumbar puncture, and laboratory investigation, including urine toxicology screening by immunoassays, did not reveal a clear aetiology. Due to the persistent somnolence, shallow breathing and decline in oxygen saturation, the child was referred to our hospital where he was mechanically ventilated. In our hospital, all toxicological immunoassay screenings on serum and urine (by EMIT and CEDIA), including an opiate assay, were negative. A general unknown screening by GC-MS on urine revealed the presence of tramadol, nortramadol, O-desmethyltramadol, and bis-desmethyl-tramadol. Over the course of the next 12 hours, the patient recovered spontaneously and was extubated. A thorough investigation of the medical history showed seven previous visits to the emergency department of different hospitals with obtundation, supposedly after convulsions at home (never objectivated by medical staff). On 3 such occasions, a urine toxicology screening by immunoassays was performed and turned out negative. Social services were involved because of a potentially worrying social situation. Conclusions: Because the toxicological analysis was limited to immunoassays for drugs of abuse, the correct diagnosis was delayed. This case demonstrates the added value of a general unknown screening, e.g. by GC-MS, when an intoxication cannot be excluded and remains unexplained based on the negative immunoassay screening results. Toxicologists have a responsibility in educating clinicians in the limitations of immunoassays.

Keywords: Clinical toxicology, medical child abuse, Munchausen by proxy, tramadol, immunoassays.
Theme: Clinical Toxicology/drugs of abuse

METABOLIC INTERACTIONS BETWEEN OXYCODONE, BENZODIAZEPINES OR DESIGNER BENZODIAZEPINES PLAY AN IMPORTANT ROLE IN OXYCODONE INTOXICATIONS

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Background: Benzodiazepines (BZD) are commonly used/misused in combination with opioids, including oxycodone (OXC), to enhance the “high” and to reduce the descent phases. Designer benzodiazepines (DBZD) have become of particular importance in this context. BZD and DBZD are frequently implicated in cases of oxycodone overdoses. Slight modification in the structure of BZD has a heavy impact on the pharmacokinetic profile and could also induce important interactions, especially in chronic intoxications. Additionally, little is known about potential metabolic interactions between OXC, BZD or DBZD. Our study aims to explore metabolic interactions in acute and chronic patterns between OXC, one of the most abused opioid, and diazepam (DZ) or, its designer derivative, dclazepam (DCZ), one of the most popular DBZD. Methods: (i) acute toxicity was induced by concomitant single doses administrations to 2 different groups of Swiss mice: OXC (1.5 mg/kg) and DZ (5.0 mg/kg) in a first group and OXC (1.5 mg/kg) with DZC (0.5 mg/kg) in a second group. Blood concentrations of the drugs and their metabolites: nordazepam, temazepam, oxazepam for DZ; lorometazepam, lorazepam for DCZ; noroxycodone and oxymophore for OXC were measured at 0.5, 1.5 and 3h (LC-MSMS method). (ii) chronic intoxication was induced after continuous infusion of OXC for 15 days followed by injection of DCZ on day 10 to mice. Drugs and metabolites were measured in blood and urines after sacrifice on day 15. For both protocols motor coordination and pain tests were performed. Results: In acute condition, both BZD were able to inhibit OXC metabolism. Noroxycodone/OXC ratio significantly decreased at 3h from 19.29± 8.02 in presence of OXC alone to 11.07 ± 7.14 in presence of OXC + DZ and to 3.70± 1.80 in presence of OXC + DCZ (p<0.05, ANOVA). OXC has no impact on both DZ and DCZ metabolism. In the chronic condition, we observed that DCZ enhanced the accumulation of oxymophore. Oxymophore/OXC ratio in presence of DCZ was 0.13± 0.04 but non detected in absence of DCZ. Conclusions: DZ and DCZ interact clearly with the metabolism pathways of OXC. An acute dose of DZ or DCZ increases the accumulation of OXC in blood while in chronic condition; DCZ promotes the production of oxymophore, rather than noroxycodone. This could be due to a metabolic shunt leading to a more potent and toxic metabolite. These results indicate that oxycodone overdoses in the presence of a BZD or DBZD may be partly due to metabolic interactions. Designer benzodiazepines inhibition is more important than classic BZD and could be more dangerous when co-abused with opioids.

Keywords: metabolic interactions; oxycodone; benzodiazepines; designer benzodiazepines; overdose.
Background: The diagnosis of toxic cirrhosis in early stages remains difficult especially for alcoholic cirrhosis. It relays on clinico-biological tests of late apparition. In patients suffering form cirrhosis, the human serum albumin (HSA) presents chemical and structural modifications expressed by the apparition of several isoforms. For instance, it has been demonstrated that the increased percentage of the oxidized forms of HSA is related to the level of liver dysfunction and predicts patient morbidity/mortality. Our hypothesis is that HSA modifications occur at early stages of cellular hepatic injuries. The aim of this study is to demonstrate the precocity of apparition of the different HSA isoforms in an animal model. Methods: Animal experimentation- Hepatotoxicity was induced in albino male Wistar rats by the administration of high daily doses of ethanol in 5 different groups of rats for 1, 3, 7, 10 or 14 days (n=7 for each group). Rats were scarified at D+1 where blood was collected for isoforms determination and classic biochemistry analyses. Livers were used for the determination of the degree of histological injuries. The same experimentation was repeated by using another known hepatotoxic, CCl4 (n=7x5 groups). A control group (n=10) received NaCl 0.9% for 14 days. Isoforms determination- rats serums were diluted (1:100) and injected on a μLC-tripleTOF 5600 Plus system (Sciex). HSA was separated on a C4 Chrom XP column (100 mm x 0.3 mm ; 3 μm) (Sciex). HSA spectra were deconvoluted by using PeakView 2.1 software (Sciex) for the identification of the different isoforms. Results: in addition to the native HSA we identified 4 different isoforms in rats serums: acetylated, cysteinylated, glycosylated and glutathinoylated. All the isoforms were present at relative abundances significantly higher than the control group (3 to 5 folds). The apparition of HSA isoforms was observed from D3 indicating early liver injuries after hepatotoxic administration. The classical biochemistry test (ALT, AST, bilirubin) significantly increased starting D7 for CCl4 groups, and only a slight but significant increase was observed for ethanol groups from D10. Conclusions: The increase of HSA isoforms is an early biomarker of liver injuries induced by a hepatotoxic. It is now clear that not only the quantity, but also the quality of albumin could reflect liver dysfunction. It is also important to consider HSA modifications in free drug studies since such modifications alter the conformation and binding properties of HAS.

Keywords: Liver injuries, alcoholic cirrhosis, albumin structural modifications, hepatotoxicity, ethanol, CCl4.

NEW PSYCHOACTIVE SUBSTANCES: METABOLIC FATE, ISOZYME-MAPPING, AND PLASMA PROTEIN BINDING OF 5-APB-NBOME, 2C-B-FLY-NB2ETOSCI, AND 2C-B-FLY-NBOME

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Background: Studies on the toxicokinetics of new psychoactive substances (NPS) are important in clinical and forensic toxicology to understand drug-drug interactions, influence of individual polymorphisms, and elimination routes. In addition, they are essential for identification of targets for toxicological screening procedures aiming to detect NPS in human biosamples for example in intoxication cases. Stimulant and hallucinogenic substances with an N-methoxybenzyl moiety, the so-called NBOMes, are well known on the NPS market and recently novel derivatives appeared, namely 5-APB-NBOME, 2C-B-FLY-NB2EtOSCI, and 2C-B-FLY-NBOME (see Figure). The aim of the present study was to investigate their toxicokinetic features focusing on metabolic fate, enzymes involved, and plasma protein binding. Methods: Hyphenated high-resolution mass spectrometry (LC-HRMS/MS) was used to identify phase I and II metabolites in in vitro incubations with human liver cells (HepaRG cell line). Incubations with recombinant human enzymes were used for identification of isozymes involved in the major metabolic steps. Plasma protein binding for an NBOMe concentration of 5 µM was determined by ultracentrifugation and subsequent quantification. Results: The investigated compounds were extensively metabolized in HepaRG cells mainly via O-dealkylation, hydroxylation, glucuronidation, and combinations thereof. The isozymes CYP1A2, 2C8, 2C19, 2D6, and 3A4, were involved in the initial metabolic reactions of all investigated compounds. Glucuronidation of the phase I metabolites was mainly catalyzed by UGT1A9. Extensive plasma protein binding of ≥90% was determined for the three NBOME derivatives. Conclusions: The identified metabolites are essential as toxicological screening targets in human biosamples due to the expected extensive metabolism of the investigated NPS. Interindividual differences in the metabolism rates of the NBOME compounds due to polymorphisms (CYPs/UGT) or drug-drug interactions for example via metabolic enzyme inhibition or displacement from plasma proteins may lead to increased concentrations and toxicity. Keywords: NBOME phenethylamines; NBOME amphetamines; drugs of abuse; metabolism; plasma protein binding; LC-HRMS/MS.
Theme: Clinical Toxicology/drugs of abuse

DO WE NEED POINT-OF-CARE TESTING OF GAMMA-HYDROXYBUTYRIC ACID (GHB) AT THE EMERGENCY DEPARTMENT?

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Background: Gamma-Hydroxybutyric acid (GHB) is a recreational drug of abuse (DoA) with central depressing effects. Patients with a GHB intoxication presenting at the Emergency Department (ED) have typical symptoms including unconsciousness, coma with Glasgow Coma Scale (GCS) ≤ 3, bradypnea and bradycardia. A quick ‘GHB intoxication’ diagnosis may prevent unnecessary further diagnostic work-up, and may lead to better patient treatment. The objective of this prospective study is to compare the diagnostic performance of medical doctors (MD) with point-of-care testing in GHB-intoxicated patients. Methods: Patients presented at the ED with a GCS ≤ 14 and a potential intoxication with DoA were included. The MD performed an initial examination at the ED and registered the working diagnosis in the hospital information system. Urine of these patients was tested afterwards for the presence of GHB using an immunoassay method (Viva-E® System; Bühlmann Laboratories assay kit; LLQ=8 μg/mL). The test was considered positive if the GHB concentration was 10 μg/mL or higher. Results on the diagnosis of the MD and the immunoassay were compared for agreement with results generated with a validated gas chromatography method (LLQ=5 μg/mL). Besides, after initial examination the MD’s were asked if they would have performed a point-of-care GHB test, if this test would have been available. Results: A total of 506 patients were included, resulting in 100 patients who tested positive for GHB using gas chromatography. The specificity and sensitivity of the MD’s was 93% and 66%, respectively. The specificity and sensitivity of the immunoassay was 93% and 85%, respectively. MD’s were inferior to the immunoassay for diagnosing GHB intoxications, because of the substantial amount of false negative diagnoses by MD’s. In addition, in 16% of the true positive and true negative cases by the MD’s, they would have performed a point-of-care test, if available. They would also perform a point-of-care test in 27% of the false positive and false negative cases. Conclusions: Point-of-care testing of GHB in addition to clinical judgement is especially valuable for reducing false negative diagnosis.

Keywords: gamma-Hydroxybutyric acid (GHB); immunoassay; differential diagnosis, emergency department, point-of-care.
PERFORMANCE OF AN IMMUNOASSAY METHOD FOR GAMMA-HYDROXYBUTYRIC ACID (GHB) IN PATIENTS PRESENTED AT THE EMERGENCY DEPARTMENT, A PROSPECTIVE STUDY

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Background: Gamma-Hydroxybutyric acid (GHB) is a recreational drug of abuse (DoA) with central depressing effects. An immunoassay test for GHB in urine can be of great diagnostic value, since this test can be available 24/7 in most hospitals and results are known within one hour. The presence of GHB confirms the diagnosis ‘GHB intoxication’ and may prevent additional diagnostic work-up. The objective of this prospective study is to determine the analytical performance of a GHB immunoassay method in a real life clinical setting. Methods: Patients presented at the Emergency Department (ED) with a Glasgow Coma Scale ≤ 14 and a potential intoxication with DoA were included. Urine of these patients was tested for the presence of GHB using the immunoassay method (Viva-E® System; Bühlmann Laboratories assay kit; LLQ=8 µg/mL). The test was considered positive if the GHB concentration was 10 µg/mL or higher. Positive and negative results were compared for agreement with results generated with a validated gas chromatography method (LLQ=5 µg/mL). Possible cross-reactivity with ethanol was investigated by analyzing ethanol concentrations in patients’ urine and serum. Results: A total of 506 patients were included, and 100 of these patients tested positive for GHB using gas chromatography. The specificity and sensitivity of the immunoassay was 93% and 85%, respectively. Serum and urine ethanol levels in the false positive group were significantly higher (median 2,8 and 3,2 mg/mL, respectively) compared to the true negative group (median 0,9 and 1,0 mg/mL, respectively; p<0.05). High ethanol levels in GHB negative samples caused elevated GHB levels in the immunoassay, resulting in false positives. Excluding patients with serum ethanol levels > 2,0 g/L and using a GHB cut-off value of 50 µg/mL, resulted in a specificity of 99% and a sensitivity of 94%. No trend was observed in other possible interfering substances among the false positives after screening with a LC-MSn method. Conclusions: The performance of the immunoassay method to detect GHB in urine is adequate if a cutoff of 50 µg/mL is used and samples with an ethanol concentration > 2 g/L are excluded.

Keywords: gamma-Hydroxybutyric acid (GHB); immunoassay; ethanol; emergency department.
INTOXICATION OF GAMMA-BUTYROLACTONE (GBL) WITH SEVERE METABOLIC ACIDOSIS

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Background: Gamma-Butyrolactone (GBL) is a precursor of the recreational drug gamma-Hydroxybutyric acid (GHB). GBL is used as a recreational drug as well and has similar effects compared to GHB. After ingestion, GBL is rapidly absorbed and quickly transformed to GHB by in vivo lactonase in plasma and liver. The absorption of GBL is faster than the absorption of GHB, due to its lipophilicity. Moreover, the bioavailability of GBL is higher and effects tend to occur faster. We report an intoxication with GBL, resulting in a severe metabolic acidosis coinciding with an elevated anion gap after drinking an unknown fluid. Methods: We performed toxicological analysis on patient specimen and the unknown fluid. A validated gas chromatography method was used to measure volatile substances e.g. methanol, ethanol, ethylene glycol and GHB. FTIR spectroscopy was used to analyze the unknown fluid. Results: A 51 year-old patient was brought to the Emergency Department with a Glasgow Coma Scale (GCS) of 3 after drinking a fluid in a sauna, that the patient bought online. The patient suffered from a severe metabolic acidosis with an elevated anion gap (20 mmol/L) and an inadequate respiratory compensation due to respiratory depression, resulting in a pH of 6.96. The patient had myoclonia not provoked by stimuli. A CT scan of the brain showed no abnormalities or trauma. Subsequently, the patient was intubated for ventilator failure and transferred to the Intensive Care Department for respiratory support and treatment with intravenous bicarbonate. Overnight, the patient regained consciousness (E4M6Vtube) without residual myoclonia and was detubated in the morning. The toxicological analysis showed no presence of methanol, ethanol and ethylene glycol. GHB-levels were extremely high: 1400 µg/mL (plasma) and 12000 µg/mL (urine). The unknown substance was identified as GBL. After 15 hours the blood pH was fully recovered and the patient was discharged from the hospital in good condition. Conclusions: GBL intoxications can be life-threatening due to respiratory depression and metabolic acidosis. GBL intoxications should be detected early by toxicology screening and treated in an Intensive Care setting.

Keywords: gamma-Butyrolactone (GBL); gamma-Hydroxybutyric acid (GHB); metabolic acidosis; anion gap; coma.
Theme: Immunosuppressive drugs

NEW POPULATION PHARMACOKINETIC MODEL THAT PREDICTS THE STARTING DOSE OF TACROLIMUS FOLLOWING ADULT RENAL TRANSPLANTATION

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Background: Multiple clinical, demographic and genetic factors affect the pharmacokinetics (PK) of tacrolimus (Tac), yet in daily practice the starting dose is based solely on bodyweight. TDM limits the time a patient is exposed to concentrations outside the target range, but it can take two weeks to reach the target Tac concentration. The aim of this study was to describe the PK immediately after transplantation, and to develop a tool for selecting the best starting dose. Methods: Clinical, demographic, PK and genetic data were collected for the first three months after renal transplantation. A population PK analysis was conducted using NONMEM. Demographic, clinical and genetic parameters were evaluated as covariates for all PK parameters containing interpatient variability. Results: A total of 4527 tacrolimus blood samples collected from 337 kidney transplant recipients were available. Data were best described using a two-compartment model. The mean absorption rate was 3.6 h⁻¹, clearance was 23.0 l h⁻¹ (39% interindividual variability, IIV), central volume of distribution was 692 l (49% IIV) and the peripheral volume of distribution 5340 l (53% IIV). Interoccasion variability was added to clearance (14%). CYP3A5 expressers, higher BSA, lower creatinine, younger age, higher albumin and lower hematocrit levels were identified as covariates enhancing tac clearance (CL). CYP3A4*22 carriers had a significantly lower CL. Age, BSA, CYP3A4 and CYP3A5 genotype were incorporated in a second model to individualize the tac starting dose. Both models were successfully internally and externally validated. A clinical trial was simulated to demonstrate the added value of the starting dose model. Conclusions: For a good prediction of tacrolimus PK, age, BSA, CYP3A4 and CYP3A5 genotype are important covariates. The model proved effective in calculating the optimal tacrolimus dose based on these parameters and can be used to individualize the tac dose in the early period after transplantation.

Keywords: tacrolimus, pharmacokinetics, pharmacogenetics, renal transplantation
BASING THE STARTING DOSE OF TACROLIMUS ON A VALIDATED DOSING ALGORITHM IN PEDIATRIC RENAL TRANSPLANT RECIPIENTS: A MULTICENTER PILOT STUDY

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Background: Multiple clinical, demographic and genetic factors affect the pharmacokinetics of tacrolimus in children, yet in daily practice a uniform body-weight based starting dose is used. It can take weeks to reach the target tacrolimus predose concentration (C0). To investigate whether adaptation of the tacrolimus starting dose according to a validated dosing algorithm¹ increases the proportion of pediatric kidney transplant recipients being within the target tacrolimus predose concentration range (10-15 ng/mL) at first steady state. Methods: In this multi-center open-label hypothesis generating pilot study 28 children were planned to receive a tacrolimus starting dose based on a dosing algorithm. Bodyweight, CYP3A5 genotype and donor status were taken into consideration when calculating the dose. After five doses the tacrolimus predose concentration was determined and the dose adjusted accordingly. Results: This abstract describes the interim analysis after inclusion of 16 patients. At day 3 (the first steady state) following kidney transplantation 31% of the children had a tacrolimus predose concentration within the target range of 10-15 ng/mL. This is a sufficient proportion on target and supports continuation of the study. On day 7 this was 20% and on day 10 it was 36%. At day 3, 2 children had extreme subtherapeutic tacrolimus concentrations (<5 ng/mL) and 3 extreme supratherapeutic concentrations (>20 ng/mL). The incidence of biopsy proven acute rejection during follow-up was 12.5%. Conclusions: On day 3, 31% of the children had a tacrolimus concentration within the prespecified target range. So far this is comparable with the 30% in the historic bodyweight-based dosed control group.


Keywords: tacrolimus, pediatrics, starting dose
LONGITUDINAL FOLLOW-UP OF LIVER TRANSPLANT RECIPIENTS USING INTRACELLULAR TACROLIMUS CONCENTRATIONS AND PHARMACODYNAMICS MONITORING: PRELIMINARY RESULTS OF THE OPTILTH STUDY.

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Background: Despite intense therapeutic drug monitoring some liver transplant recipients exhibit rejection and/or adverse events while having tacrolimus (TAC) whole-blood (WB) concentration within the therapeutic range. Thus, new ways of treatment monitoring should be explored. Measuring TAC intracellular concentrations (IC) (i.e. measurement of TAC concentration in peripheral blood mononuclear cells) and calcineurin activity (CA) appears as promising methods but there is few data on their practical relevance. The aim of this study was to assess the value of TAC IC and of CA measurements as biomarkers of rejection during longitudinal follow-up in liver transplantation.

Methods: We analyzed preliminary results (82 out of 110 patients) of the OPTILTH study (NCT02877628). TAC WB, IC and CA measurements were conducted at day-7, -14, -21 and -28, week-6, -8, 12 and -24 and suspected rejection was validated by an external committee. Measurements were compared between the group of patients with a suspected rejection and the group without suspicion of rejection. Relationship between WB, IC and CA were explored using Pearson’s correlation test. Results: The results of 82 patients were analyzed. Rejection was suspected in 42 patients (51%) mainly at day-7 or before. TAC IC were lower in the rejection group (median 20.5 pg/million cells [IQR: 15.2-33.8 pg/million cells] versus 27.5 pg/million cells [IQR: 21.2-43.0 pg/million cells], p=0.036) but TAC WB or CA were not different between the groups. Rejection was suspected only in 9 patients after day-7 (11%). Overall, a moderate correlation was found between TAC WB and IC (r=0.54; p<0.001) but not between TAC WB and CA (p=0.34) or between TAC IC and CA (p=0.16). Conclusions: Measuring TAC IC might be helpful for therapeutic drug monitoring of TAC in liver transplant recipients. Hence, it allows discriminating patients with or without rejection during the early post-operative period while TAC WB and CA measurement appears not to be sensitive biomarkers of rejection in the early post-operative days.

Keywords: Liver transplantation; Immunosuppressive drugs; Tacrolimus; Therapeutic drug monitoring; Pharmacokinetics; Pharmacodynamics.
Theme: Immunosuppressive drugs

**NOVEL INTERACTION BETWEEN TACROLIMUS AND MEROPENEM IN PEDIATRIC RENAL TRANSPLANT PATIENTS**

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**Background:** Tacrolimus is the cornerstone of immunosuppressive regimens in pediatric renal transplant patients. Urinary tract infections (UTI) in these patients may require antibiotic therapy including carbapenems. A drug–drug interaction was previously reported between the carbapenem ertapenem and tacrolimus with significant increases in tacrolimus levels in adult renal transplant patients. Meropenem is a widely used carbapenem in our clinical setting. The aim of this study was to evaluate a possible drug-drug interaction between meropenem and tacrolimus in pediatric renal transplant patients. **Methods:** An observational retrospective cohort study was conducted in pediatric renal transplant patients treated with tacrolimus and meropenem between December 2011 and March 2018. Patients receiving a stable tacrolimus dose (i.e. variation in dose <20%) were included. Data collected included demographic, biochemical, and clinical parameters and tacrolimus doses, trough concentrations (C0) and dose/kg-normalized C0 (C0norm) before, during and after concomitant treatment with meropenem. Pharmacological interactions with other concomitant medications were discarded. Differences in tacrolimus C0 and C0norm during and after administration of meropenem were analyzed using Wilcoxon matched paired T test. The Drug Interaction Probability Scale (DIPS) was performed. **Results:** In total 31 patients were identified in the study period. A total of 12 patients were excluded due to diarrhea (n=2), hemodynamic alterations such as plasmapheresis (n=1), liver dysfunction (n=1), unavailability of medical/nurse records (n=7), and co-administration of azoles, macrolides, nifedipine or other interacting drugs (n=1). Nineteen patients fulfilled inclusion criteria for the study. Median age was 13.4 years (range 4.9-17.2), and median post-transplant time at the infection was 17 days (range 6-820). The incidence observed of the drug-drug interaction was 42% (8/19 patients). Tacrolimus C0norm values increased by a median of 61.8% (range 14.1-556.3%) during the concomitant administration of meropenem compared to baseline (p=0.004). Peak effect of the interaction was seen after a median (range) of 10 days (6-17) post-initiation of the antibiotic and C0norm decreased when meropenem was discontinued in all these patients. The interaction was classified as probable (Score 6-7). **Conclusions:** We reported for the first time the interaction between meropenem and tacrolimus, confirmed by the increase in tacrolimus C0norm. A potential drug-drug interaction between meropenem and tacrolimus may be mediated by inhibition of CYP3A4 altering tacrolimus metabolism. Further studies are required to confirm the proposed mechanism. The identification of this novel interaction may help transplant physicians to avoid tacrolimus overexposure by close C0 monitoring when concomitant administration of meropenem is required. **Keywords:** drug interactions; tacrolimus; meropenem; pediatrics; therapeutic drug monitoring; kidney transplantation.
Theme: Immunosuppressive drugs

THE INHIBITION OF IMPDH AND PURINES IN LYMPHOCYTES BY MYCOPHENOLIC ACID AFTER TRANSPLANTATION

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Background: Proliferation of activated lymphocytes is a vital step in an adaptive immune response and the nucleotides of guanine and adenine are in this respect essential for the nucleic acid synthesis. Mycophenolic acid (MPA) is a reversible inhibitor of inosine monophosphate dehydrogenase (IMPDH), a rate-limiting enzyme in the de novo synthesis of guanine nucleotides. Thereby, MPA inhibits the proliferation of lymphocytes and is frequently used as an immunosuppressant to prevent rejection after solid organ transplantation and to treat some auto-immune disorders. Patients and methods: Adult patients (n=29) receiving a renal transplant from living donors were included. All patients received TAC and prednisolone. Data on concentration of tacrolimus in PBMC and NFAT-regulated gene expression have been reported previously. Blood samples were collected at 0-4 days before transplantation and at 6-9 days, 5-7 weeks and at 1 year after transplantation. On each occasion blood samples were collected immediately prior to (t0) and 1.5 hours (t1.5) after the morning dose. At each sampling, blood was collected EDTA-tubes for plasma MPA concentration and in heparinized tubes for measurement of IMPDH-capacity and guanine and adenine content in PBMC, using a previously published assay in split samples, unstimulated as well as ex vivo stimulated by the lymphocyte activating mitogens phorbol-12-myristate-13-acetate and ionomycin. Results: In patients not receiving MMF before the pre-transplant sample, mitogen stimulation increased the IMPDH capacity on average 22 fold. For all biomarkers, MPA showed a stronger inhibitory effect in stimulated PBMC compared to non-stimulated PBMC. Whilst IMPDH-capacity was only reduced 20-44 % in non-stimulated PBMC when patients were given MMF, the reduction in stimulated PBMC was 66-79 %. Conclusions: By measuring IMPDH inhibition as the pharmacodynamic response of MPA in renal transplant patient over a prolonged period, we have shown that MPA inhibits activated lymphocytes to a larger degree than resting lymphocytes. In resting lymphocytes, the nucleotide pool appeared unaffected by MMF treatment, probably because nucleotide levels could be maintained by the salvage pathway. Our study indicated that patients needing reduction of MMF dose may have lower IMPDH-capacity and levels of purines. These measurements may therefore be utilized to identify those patients who could benefit from lower MMF dose before clinical indications (diarrhea or leucopenia) are presented. Keywords: mycophenolate mofetil; inosine monophosphate dehydrogenase (IMPDH); renal transplantation.
MODEL-BASED REGIMEN ADJUSTMENT OF INFliximAB IN INFLAMMATORY BOWEL DISEASE: CONTRIBUTION TO CLINICAL RESPONSE.

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Background: Infliximab (ifx) is effective for inducing and maintain remission in inflammatory bowel disease (ibd) patients but 24-46% of patients have secondary loss of response in the first year of treatment. Aim: to determine the impact of Bayesian forecasting methodology on clinical response in ibd patients. Methods: Quasi-experimental study with one-group pretest-posttest design involving ibd adult patients treated with model-based personalized ifx between July 2013-February 2018. Intervention: dose adjustment based on clinical/biochemical response in combination with Bayesian forecasting. Primary end point: Harvey Bradshaw Index (hbi)≤4 for Crohn’s disease (cd) and partial Mayo score (pm)≤2 for ulcerative colitis (uc). Secondary end points: (a) decrease of symptoms after intensification if hbi or pm≥2 before adjustment; (b) ifx discontinuation rates one year after intervention. Results: 108 patients (72 cd/36 uc). Regimen adjustment was performed in 59 patients (34 intensifications/9 deintensifications/16 stops). 26/34 (76%) patients in the intensification group achieved ifx cₘᵢₙ>3 mg/L. Patients with hbi≤4 and pm≤2 in the intensification group increased from 8/21 (38%) to 13/21 (62%) and 6/13 (46%) to 9/13 (69%), respectively. Median hbi before and after intensification was 6 and 1, respectively. Median pm before and after intensification was 3 and 1, respectively. Treatment intensification resulted in a decrease of symptoms in 14/16 (88%) cd patients with hbi≥2 before intervention and 7/9 (78%) uc patients with pm≥2 before intervention. All patients in the de-escalation group maintained ifx cₘᵢₙ>5 mg/L and hbi 0-1 or pm 0 after intervention. Antibodies to ifx (ati) were detected in 16 patients before intervention: ifx was stopped/switched in 15 patients and treatment intensification was performed in 1 patient. No patients developed ati after regimen adjustment. Of the 92 patients receiving ifx after intervention, 85 patients were still on ifx therapy 1 year after intervention (32/34 (94%) after intensification; 9/9 (100%) after de-escalation, 44/50 (88%) of those patients maintaining the same regimen. Conclusions: Model-based intensification resulted in improved hbi and pm scores and a decrease of symptoms. All patients in the de-escalation group maintained hbi 0-1 or pm 0 after intervention. 88% of patients were still on ifx therapy one year after intervention.

Keywords: Infliximab; regimen adjustment; inflammatory bowel disease; clinical response.
Background: Anti-tumor necrosis factor (TNF) therapies, e.g. adalimumab, are effective but expensive treatments for rheumatoid arthritis (RA). Most anti-TNF dosing is fixed. Drug efficacy and cost savings could be made by altering dose or dose interval based on patient characteristics and drug concentration and/or presence of anti-drug antibodies (ADAs). The aim of this study was to determine the relationship between patient characteristics and adalimumab concentrations, and between adalimumab concentrations, ADAs and disease activity in people with RA. Methods: 156 people with RA receiving adalimumab were recruited. Detailed demographic and clinical variables collected. Disease activity was assessed using the DAS28 and good response defined as DAS28<3.2. Plasma drug concentration and ADAs were measured. Results: Of the 156 participants mean (SD) age was 57.4 (12.7) years and 69.2% were female. Multivariate analysis, revealed that CRP, weight and ethnicity were independently associated with adalimumab concentrations such that the higher the CRP and the higher the weight the lower the adalimumab concentrations. There was a significant negative correlation between adalimumab concentration and DAS28 (r=-0.37; p<0.001), CRP (r=-0.41; p<0.001), and swollen joint count (r=-0.22; p=0.002). Adalimumab concentrations were significantly higher in those with DAS28 ≤3.2 compared to those with DAS28 >3.2 (median (IQR) 10.8 (6.4-20.8)mg/l vs.7.1 (1.5-12.6) mg/l; p <0.001). Of those with DAS28≤3.2, 73% had adalimumab concentrations ≥7mg/dl. 8/48 with adalimumab concentration <5mg/l had neutralizing ADAs present. Mean adalimumab concentration was significantly lower in those with neutralizing ADA present (Figure). Conclusions: Neutralizing ADAs are associated with low drug concentrations and with poor disease control. Therapeutic drug monitoring of adalimumab and adalimumab ADA is likely to be useful in clinical practice.

Keywords: adalimumab, anti-drug antibodies, drug concentration, rheumatoid arthritis.
Theme: Other theme

CORRELATION AND INTERACTION BETWEEN CLOZAPINE CONCENTRATION AND THERAPEUTIC BIOMARKERS IN BLOOD OF SCHIZOPHRENIA PATIENTS IN A TWO-PERIOD PHARMACOKINETIC STUDY
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Background: A panel of biomarkers consisting of 15 compounds has been identified as the potential predictor of treatment response in patients with schizophrenia (SZ) by our group [Cai et al. Transl Psychiatry. 2017, 7: e1130; Cai et al., Psychoneuroendocrinology. 2018, 90: 43-51]. However, responses of these biomarkers to antipsychotic drug concentrations in blood of SZ remain to be elucidated. Methods: Twenty-eight stable patients with chronic SZ were enrolled and all of them were prescribed with clozapine 100 mg PO q12h during this two-period pharmacokinetic study. The effects of single-dose and multiple doses of clozapine were both taken into account. In the first period (Day 1-10), blood samples were collected at the steady-state (trough, 1 h pre-dose) from Day 7-9. On the morning of Day 10, blood samples were collected at 1 h pre-dose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8, 10 and 12 h post-dose. Blood samples were also collected in the same manner in the second period (Day 11-20). The plasma concentrations of clozapine as well as different categories of biomarkers were quantified using LC-MS/MS method. Results: There were no significant differences in the pharmacokinetic parameters of clozapine such as $C_{\text{max,ss}}$, $T_{\text{max,ss}}$ and AUC0-t between the two periods. The panel of biomarkers was divided into four categories for analysis: purinergic metabolites, neurosteroids, glucocorticoids and phospholipids. Firstly, as compared with period 1, baseline values of all four categories of biomarkers were increased after multiple doses in period 2, albeit that only the increment of glucocorticoids approached significance ($p=0.0015$). Secondly, clozapine showed an inverted plasma drug concentration–biomarker response relationship with purinergic metabolites and neurosteroids, with maximum suppression on biomarker levels at higher drug concentrations, and feedback stimulation when the drug concentrations declined. Plasma clozapine concentration and the sum of purinergic biomarkers were inversely correlated in period 1 ($p=0.0017$), period 2 ($p=0.0034$) and throughout the two periods ($p<0.0001$). Conclusion: The present study suggests that purinergic biomarkers of SZ is fast responsive to the changes of plasma clozapine levels and may have potential usefulness for individualized dosing of clozapine and other antipsychotic drugs.

Keywords: Antipsychotic drug; Clozapine; Schizophrenia; Plasma drug concentration; Therapeutic biomarker; Pharmacokinetics.
A CONSENSUAL PANEL FOR NEXT-GENERATION SEQUENCING IN PHARMACOGENETICS FROM THE FRENCH NATIONAL NETWORK OF PHARMACOGENETICS (RNPGx)

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Background: In France, 54 laboratories offer pharmacogenetic testing (2017 report of the biomedicine agency). Five tests are included in the list of complementary biological acts (i.e. CYP3A5, UGT1A1, DPYD, TPMT and CYP2D6 genotyping). Other tests are performed without dedicated nomenclature. The list of innovative nomenclature acts (RIHN) allows multiparametric analysis using Next Generation Sequencing (NGS). In this context, the French national network of pharmacogenetics (RNPGx) defined consensually a list of genes to be included in a pharmacogenetic panel, specifying for each gene the region to be sequenced. Methods: RNPGx constituted a group of 18 biologists with particular expertise in 7 pharmaco-therapeutic domains (i.e. oncology, immunosuppression, neuro-psychiatry, pain, cardiology, hypersensitivity and infectiology). A list of 68 candidate genes was proposed by the experts. Each gene was assigned a score incorporating 7 weighted criteria (1-classification as PharmGKB “Very Important Pharmacogene”; 2- RNPGx classification level (Therapie, 2017); 3-inclusion in the European UPGx gene panel; 4-availability of clinical pharmacogenetics implementation consortium guidelines; numbers of 5-relevant gene-drug pairs and 6- level 1 or 2 gene variants reported in PharmGKB, and 7-score given by RNPGx experts). The regions to be targeted (e.g. hotspots, coding regions, full gene sequence) were chosen consensually for each gene. Results: Of the 68 initial genes, 27 genes obtained a score > 10. This final list was completed by 17 genes considered relevant from recent literature despite having a score <10. In the final panel, 14 genes are drug metabolism enzymes; 8 are membrane transporters; 12 are drug targets. Others are miscellaneous relevant pharmacogenes. The regions to be sequenced varied from (i) isolated disruptive markers, (ii) combinations of markers that allow haplotypic inference (e.g. for cytochromes P450 or SLCO1B1), (iii) coding gene sequences (e.g. DPYD, VKORC1, CYP2D6, TPMT, NUDT15) or (iv) full gene sequences (e.g. CYP2D6, NAT2). Conclusions: RNPGx consensually defined a list of 44 pharmacogenes and specified relevant regions to be targeted in these genes. The proposed panel is suitable for pharmacogenetic analyses performed in the clinics and can be useful in research protocols. A diagnostic kit based on the panel and compatible with the two main NGS technologies (Ion Torrent and Illumina) is under development. Keywords: Pharmacogenetics; next-generation sequencing; recommendations; consensus.
**Background:** A large number of people treated with allopurinol for gout fail to reach recommended serum urate (SU). Given the variability in allopurinol dose required to reach target SU the ability to measure plasma oxypurinol to guide allopurinol dosing would be an advantage. The aim of this study was to determine factors that influence the serum urate lowering response to allopurinol dose escalation and the conversion of allopurinol to oxypurinol, and to determine if there is a minimum therapeutic oxypurinol concentration. **Methods:** Data from 129 participants in a 24-month open, randomized, controlled, parallel-group, clinical trial were analysed. Allopurinol dose, SU and plasma oxypurinol concentrations were available at multiple time points. A slope for the association between allopurinol dose and SU was calculated for each individual as a measure of *sensitivity to allopurinol*. A slope for the association between allopurinol dose and plasma oxypurinol was calculated for each individual as a measure of *allopurinol metabolism*. **Results:** There was a wide range of SU concentrations for each allopurinol dose. The relationship between sensitivity to allopurinol (change in SU) and allopurinol metabolism (change in oxypurinol) for each 100mg increase in allopurinol dose varied between individuals (Figure). BMI (p=0.023), CrCL (p=0.037), p.Gln141Lys variation in the ABCG2 urate transporter (p=0.019), and baseline SU (p=0.004) were all independently associated with sensitivity to allopurinol, such that those with higher BMI, better kidney function, or ABCG2 Lys/Lys had a smaller reduction in SU for a 100mg increase in allopurinol, while those with a higher baseline SU had a larger reduction in SU for a 100mg allopurinol dose increase. Those with worse kidney function required higher concentrations of oxypurinol to achieve target urate compared to those with better kidney function. **Conclusions:** Although there is a relationship between change in oxypurinol and change in SU, a minimum therapeutic oxypurinol concentration cannot be identified. Other variables including ABCG2 p.Gln141Lys genotype impact on sensitivity to allopurinol. **Keywords:** allopurinol, oxypurinol, gout, serum urate.
Background: TDM in conjunction with Bayesian forecasting can be used for individualised pharmacotherapy [1]. Some intra-individual variability in pharmacokinetics (PK) may be attributed to analytical error, which may differ between labs and methods, and is usually not explicitly separated from other intra-individual variability in PK models. Acceptable limits exist for lab precision, regularly based on analytical possibilities and often without regard for the impact on clinical outcome. Here, we aim to provide a model-based framework for the evaluation of acceptable levels of precision within TDM. Methods: Monte-Carlo simulations and re-estimations (N=1000) [1] of an established PK model for piperacillin-tazobactam [2] were used to determine the ability to estimate PK parameters (clearance, peripheral volume) depending on different levels of analytical error ranging from 0 to 500 CV% (originally: 25% [3]). After dose adjustments, probability of attaining the target of concentrations at least 50% of the time above the minimum inhibitory concentration (T>MIC, 16 mg/L) were calculated for each scenario. Results: Bayesian dosing adjustments typically resulted in an improvement of 9-15 percentage point of target attainment (up from circa 75%) compared to baseline. Improvement in target attainment was only significantly impacted once analytical error reached very high levels (CV > 200%), which suggest other factors to possibly be of higher importance, such as choice of sampling time and dosing adjustment algorithm. Conclusions: Our simulations show how a model-based approach may be integrated in the evaluation of acceptable precision of TDM where acceptability is taken within the context of clinical practice, i.e. based on pharmacodynamic principles rather than analytical possibilities. This approach can be extended towards other drugs and therapeutic areas.

Keywords: piperacillin-tazobactam, pharmacometrics, TDM, precision, individualized dosing, Bayesian forecasting

[2] De Cock et al., J. Antimicr. Chemother. 72(7);2017
[3] Carlier et al., Int. J. Antimicr. Agents 40(5);2012
Theme: Pharmacometrics

IMPACT OF INACCURATE DOCUMENTATION OF SAMPLING AND INFUSION TIME IN PHARMACOMETRIC ANALYSES OF TDM DATA

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Background: Routine clinical TDM data is often used to develop population pharmacokinetic (PK) models. The impact of uncertainty in documented sampling and infusion (inf.) times in population PK modelling has not yet been systematically evaluated. The aim of this study was to investigate uncertain documentation of (i) sampling times, (ii) inf. time and (iii) the combination of both exemplified with meropenem. Methods: A stochastic simulation and estimation study (1000 simulations for 100 patients, 10 samples per patient) was performed in NONMEM® 7.4.1 using a previously published population PK model of meropenem (inf. time 30 min). Uncertainties, i.e. deviation between real and planned sampling (standard deviation (SD) ± 5 min to ± 30 min) and inf. times (SD ±7.5 min to ±22.5 min) were added randomly in R (v 3.5.2) before carrying out the simulation step. The estimation step was then performed with the real or planned times (replacing real time points by scheduled study values). Moreover, the combination of uncertainties in both parameters was investigated. Relative bias (rbias) and coefficient of variation (CV) were calculated to determine accuracy and precision of the PK parameter on the population and individual level.

Results: On the population level, the estimates of the early distribution parameters (central volume of distribution (V1) and intercompartmental clearance (Q)) were most affected by erroneous records in the sampling time and inf. time. For example, the rbias for V1 at an uncertainty of ±5 min or ±30 min in sampling time was -4.7% or -65% vs. 3.9% or 6.2% (real) and at an uncertainty of ±7.5 min or ±22.5 min in inf. time was -5.2% or 34% vs. 3.7% or 3.8% (real), respectively. Already very small undocumented uncertainty in sampling time (5 min), increased the rbias of the estimated proportional residual error from 0.3% to 85%, whereas uncertainty of 30 min increased the rbias from 0.6% to 216%. On the individual level, uncertainty affected the rbias of the estimated individual PK parameters, e.g. for CL 6.9% or 17% vs. 1.2% or 1.9% in the sampling time (±5 min or ±30 min) and -0.03% or -5.4% vs. 1.3% or 1.6% in the inf. time (±7.5 min or ±22.5 min), planned vs. real, respectively. The combination of both uncertainties in incorrect documentation potentiated imprecision. Conclusions: Undocumented uncertainty caused bias and increased imprecision of population and individual parameters. Erroneous records with only 5 min uncertainty in documented time inflated the residual error model considerably. Our results underline the importance of accurate documentation of timing and call for extension of our work to further drug classes.

Keywords: meropenem; pharmacometrics; TDM; documentation
LIMITED SAMPLING STRATEGY FOR GANCICLOVIR AND VALGANCICLOVIR IN PEDIATRIC SOLID ORGAN AND HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENT

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Background: Cytomegalovirus (CMV) is a major cause of morbidity and mortality in children recipient of solid organ transplant (SOT) or hematopoietic stem cell transplant (HSCT). Ganciclovir (GCV) and its prodrug valganciclovir (VGCV) are recommended to prevent and treat CMV in this high-risk population. Given a high pharmacokinetic (PK) interindividual variability, therapeutic drug monitoring (TDM) is frequently performed. Determining the area under the curve from 0 to 24h (AUC0-24h; surrogate efficacy target) using the trapezoidal method is challenging in pediatrics because it requires many blood samples. We sought to develop a limited sampling strategy (LSS) for the estimation of GCV AUC for children with SOT or HSCT. Methods: Based on our previous population PK model developed in 50 children with SOT or HSCT on GCV and/or VGCV, we selected optimal sampling times (figure) among trough, maximal and elimination concentrations. The mean bias, imprecision (root mean square error: RMSE) and R² (between AUC obtained by Bayesian estimation based on the best LSS and trapezoidal method) were calculated. Results: Six LSS were tested for intravenous (IV) GCV and 1S for oral VGCV. For IV GCV, mean bias/RMSE/R² for 1h-2h-3h and 1h-3h LSS were -0.04/18.5%/94% and -0.07/21.2%/91%, respectively. For oral VGCV, mean bias/RMSE/R² for 1h-2h-6h and 2h-6h LSS were -0.01/16.6%/89% and -0.002/16.8%/90%, respectively. Conclusion: The best LSS for IV GCV required 3 samples (1h, 2h and 3h after the start of the 1h infusion) whereas the best LSS for oral VGCV required only 2 samples at 2h and 6h after the oral administration. This is the first LSS applicable for oral VGC and IV GCV, and to include both SOT and HSCT pediatric recipients. Keywords: Ganciclovir; Valganciclovir; Limited sampling strategy; pediatric; hematopoietic stem cell transplant; solid organ transplant.
Background: The antiepileptic drug lacosamide is approved in the USA and the European Union as monotherapy as well as adjunctive therapy for the treatment of focal seizures in children >4 years and adults. Here, we present a pharmacometric analysis for pediatric patients (≥1 month to <18 years) who received lacosamide as both mono and adjunctive therapy. Methods: Lacosamide serum concentrations obtained during therapeutic drug monitoring were collected from 315 patients with epilepsy treated over an 8-year period. Population pharmacokinetic modeling was performed using nonlinear mixed effects modeling (NONMEM) with a first order conditional estimation. A one-compartment structural model with linear elimination and allometrically scaled effect of body weight on clearance and volume of distribution best described the data. Interindividual variability (IIV) was estimated for clearance and residual error was modeled using a proportional (log-additive) error term. Additional covariates evaluated on clearance included age, sex, race, and coadministration of both hepatic enzyme-inducing & inhibiting antiepileptic drugs. Results: Body weight as a covariate on clearance and volume improved both population and individual predictions while decreasing the interindividual variability (-4%) and residual proportional error (CV: -25%). Coadministration of hepatic enzyme inducing antiepileptics such as phenobarbital and felbamate were identified as significantly increasing clearance (1.9-, 1.6-fold respectively) and reduced the overall interindividual variability (-15%). Population pharmacokinetic parameters obtained from this final model are shown in the Table. Conclusions: This pharmacometric analysis based on routine therapeutic drug monitoring data indicates that the pharmacokinetics of lacosamide in pediatric patients older than 1 month of age can be fully described by weight-based allometric modeling without the need for any further age-associated maturation function. In addition, co-administered antiepileptic drugs that are known hepatic enzyme inducers were identified as increasing lacosamide clearance and reducing exposure. Thus, dose adjustments of lacosamide may be necessary while prescribing lacosamide in adjunctive therapy.

Keywords: Lacosamide; Children; Pharmacometrics; Epilepsy; Nonlinear mixed effect modeling.
Theme: Pharmacometrics

OPTIMIZING TREATMENT OF RISPERIDONE IN CHILDREN BY CORRELATING POPULATION PHARMACOKINETICS TO SAFETY AND EFFECTIVENESS

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Background: Risperidone is the most frequently prescribed antipsychotic to children worldwide. However, concerns have been raised about the safety in children due to serious metabolic side-effects, increasing the risk for cardiovascular morbidity and mortality. This study aims to define therapeutic reference ranges for risperidone in children by correlating population pharmacokinetics to clinical outcomes in a pediatric population. Methods: We conducted a prospective observational multicenter trial (NTR 6050) with children diagnosed with Autism Spectrum Disorder (ASD) using risperidone. During the 6 month follow up, pharmacokinetic sampling of risperidone and 9-OH risperidone was performed by venepuncture and dried blood spots. Several indices for side-effects and therapeutic effectiveness were systematically collected including BMI, cholesterol, glucose, prolactin, Abnormal Involuntary Movement Scale (AIMS) and Aberrant Behavior Checklist (ABC). Genotypes of CYP2D6, CYP3A5, CYP3A5 and PGP were determined, and adherence was assessed. Population pharmacokinetics were modelled using NONMEM 7.4. The correlations between pharmacokinetic parameters and clinical effects were subsequently analyzed. Results: 404 concentrations in 42 children (median age 10.8 years range [6.0-17.5], 76.2% male, median body weight 41 kg [20.2-88.9]) were best described using a 2-compartment model for risperidone combined with a 1-compartment model for 9-OH risperidone. Allometric scaling was applied. The error model included corrections for differences in matrices; BMI was found a significant covariate for clearance (CL) of the metabolite, explaining 32% of the variability. The mean volume of distribution (V) of risperidone was 37.8 L/70 kg and the apparent clearance (CL/F) was 24.9 L/h/70 kg (interpatient variability, IPV 88.4%); for 9-OH risperidone V was 95.4 L/70kg and CL/F 5.1 L/h/70 kg (IPV 19%). The median weight gain during 6 month follow up was 4.05 kg and the median improvement in ABC-irritability score was 5 points (on a total maximum of 45 points) for children starting risperidone treatment. We are currently correlating the pharmacokinetic parameters to effectiveness and safety outcomes, which will be available at IATDMCT 2019. Conclusions: The pharmacokinetic parameters of risperidone in pediatric patients with ASD are comparable to other pediatric populations. This population pharmacokinetic model with clinical safety and effectiveness outcomes can guide Therapeutic Drug Monitoring for risperidone in this vulnerable population.

Keywords: risperidone, children, therapeutic drug monitoring, Dried Blood Spot, effectiveness, safety
Theme: TDM in Oncology

PRETREATMENT TPMT ACTIVITY AND ERYTHROCYTE 6-TGN LEVELS ARE PREDICTORS OF TOXICITY OF 6-MERCAPTOPURINE IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Background: More than half the patients with Acute Lymphoblastic Leukemia (ALL) do not tolerate standard doses of 6-Mercaptopurine (6MP) requiring frequent dose interruptions or modifications. TPMT polymorphisms account for approximately 10% population showing intolerance to 6MP. TPMT genotyping is not routinely done in the Indian sub-continent due to high cost and limited access to the test. The present study aimed at identifying other predictive markers of 6MP toxicity. Methods: Adult ALL patients (≥18 years) receiving 6MP (75mg/m² per oral) during interim maintenance (IM) phase of MCP841 protocol were enrolled. Pretreatment TPMT activity was measured using a HPLC method. Erythrocyte 6-methylmetcaptopurine (6-mMP) and 6-thioguanine nucleotides (6-TGN) levels were also measured using HPLC on day 8 and day 22 of IM. Treatment related toxicity, dose reductions, interruptions and cumulative duration of interruption were documented. Difference in means between groups was compared using Mann-Whitney U test. Non-linear regression was performed to assess relationship between markers and outcomes of toxicity. Results: 100 patients were enrolled (82M/18F). The average age was 26.6±7.9 years. Grade-4 cytopenias was the most cause of dose interruption or reduction. Day 22 erythrocyte 6-TGN levels were higher in patients who had dose interruption compared to patients who did not require dose interruption (0.7±0.27 vs. 0.2±0.05 ng/mL; P<0.05). 6MP treatment was better tolerated in patients who had higher pretreatment TPMT activity [14.25±2.51 vs. 10.36±2.17 Units/gHb in patients with ‘No dose reduction’ and ‘Dose reduction’ respectively and 13.24±2.5 vs. 9.98±2.1 Units/gHb in ‘No dose interruption’ and ‘Dose interruption’ groups respectively]. The relationship between TPMT activity and cumulative duration of interruption was best fitted using a hyperbole function (Figure). The minimum TPMT activity that is required to achieve the least cumulative duration of interruption was found to be 13.3 Units/gHb. Conclusions: Erythrocyte 6-TGN levels and pretreatment TPMT activity can predict toxicity. A threshold pretreatment TPMT activity level is defined which future studies should validate and suggest dose selection based on TPMT activity.

Keywords: 6-mercaptopurine; 6-TGN; TPMT activity; Toxicity; Dose reduction; Dose interruption.
Background: User-friendly therapeutic drug monitoring (TDM) software tools are needed for dose individualization strategies in clinical practice. The implementation of a population pharmacokinetic (PK) model into a TDM software tool is important to obtain accurate and precise estimates of individual PK parameters. The aim of this study was to assess the suitability of MwPharm++ for the 5-FU dose adjustment during continuous intravenous infusion. Methods: A validated one-compartment 5-FU population PK model developed with NONMEM® was integrated into MwPharm++ using the EDSIM++ platform. The drug exposure estimates, expressed as area under the concentration-time curve (AUC), obtained with both programs were compared using a dataset of 62 colorectal cancer patients treated with three 5-FU infusional treatment regimens. Results: The validated 5-FU population PK model was successfully integrated into MwPharm++ obtaining a total of 311 AUC values. The Spearman’s rank correlation coefficient ($r_s$) resulted in 0.77 (95% CI) indicating a high positive correlation between programs. The Bland-Altman plot (see Figure) revealed a good agreement between programs by resulting in a bias of 0.3 mg·h/L, which means that MwPharm++ AUC values tended to be slightly lower than those obtained with NONMEM®. Discrepancies in analytical imprecisions might be attributed to the different optimization algorithms. In 81% of the cases, NONMEM® and MwPharm++ resulted in the same dose recommendation. Interchangeability between programs was not evaluated because no criterion of precision was set before conducting the study. Conclusions: The implemented 5-FU population PK model in MwPharm++ represents a valuable tool to guide 5-FU dose adjustment in clinical practice and it is much easier to use than NONMEM®. Further studies should be addressed by establishing cut-off criteria to determine interchangeability between MwPharm++ and NONMEM® for its application in the oncology clinical setting. Keywords: Dose adjustment; fluorouracil (5-FU); pharmacokinetic software tool; therapeutic drug monitoring.
Poster Abstracts
Theme: Alternative sampling strategies

**LC-MS/MS DETERMINATION OF 3 IMMUNOSUPPRESSANTS FROM DRIED BLOOD SPOTS WITH AUTOMATED SAMPLE PREPARATION**

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**Background:** Dried blood spots (DBS) are an attractive specimen of choice for immunosuppressant therapeutic drug monitoring as DBS can be collected by the patient, require small amounts of blood and are very stable. Here, we describe the development of a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to simultaneously quantitate 3 immunosuppressants (tacrolimus, sirolimus and cyclosporine) from DBS. Sample preparation and extraction was also adapted for use on an automated liquid handler to improve laboratory workflow. **Methods:** DBS samples were spotted on Whatman 308 Filter paper and left to dry overnight. An 8 mm punch was removed from each spot, added to 200 μl water and vortexed for 3 min followed by a 15 min incubation at 21°C. Then, 200 μL of zinc sulfate in methanol and the internal standard mixture was added to each sample, centrifuged and the supernatant aliquoted for analysis. This process was automated using a Microlab® NIMBUS™ pipetting workstation (Hamilton). LC-MS/MS analysis was performed using a 1260 HPLC coupled to a 6410 triple quadruple mass spectrometer (Agilent Technologies). The three analytes and two internal standards (ascomycin and cyclosporine-d12) were analyzed using multiple reaction monitoring in positive electrospray ionization mode. Chromatographic separation was achieved using C18 column in a run time of 3 min. **Results:** The developed assay was linear between 2-50 ng/mL for tacrolimus and sirolimus and 25.3-1838 ng/mL for cyclosporin. Between run precision CVs ranged from 5-13% for sirolimus, 5-9% for tacrolimus and 5-10% for cyclosporine. When compared to our existing clinical LC-MS/MS assay for immunosuppressants, the developed DBS method had R² >0.98 for all three analytes but with a proportional positive bias. **Conclusions:** A method was successfully developed to analyze for three immunosuppressants from DBS with automated sample preparation. This allows for introduction of DBS as a specimen of choice for immunosuppressant monitoring, but also demonstrates that this workflow can be adapted for use with automated liquid handling systems. **Keywords:** dried blood spots, therapeutic drug monitoring, immunosuppressants, LC-MS/MS
Theme: Alternative sampling strategies

EVALUATION OF THE STABILITY OF ABIRATERONE, D4 ABIRATERNE AND 5α-ABIRATERONE IN DRIED PLASMA SPOTS BY LC-MS/MS

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Background: Abiraterone efficacy against prostate cancer has shown variability among patients. Two active metabolites have been associated with treatment efficacy: D4 abiraterone with potent anti-tumor activity and 5α-abiraterone (3-keto-5α-abiraterone), a tumor growth promoter. To personalize chemotherapy and improve efficacy, plasma levels of abiraterone and its active metabolites should be monitored. Therefore, this study evaluated the applicability of dried plasma spots (DPS) as an alternative sampling strategy to overcome the short stability of abiraterone in plasma. Methods: DPS were prepared applying 50 µL patient EDTA plasma onto Whatmann 903® paper. Stability was evaluated in triplicate after 7, 14, 21 and 28 days at 25, -20 and 45°C. Whole-spot extraction was performed with 1 mL freshly prepared 0.1% formic acid in methanol containing 2.5 ng/mL internal standard 3H4-abiraterone, incubated 15 min. at 1250 rpm and 30°C. Samples were evaporated to dryness at 45°C for 60 min, reconstituted with 100µL mobile phase and filtered to vials. LC-MS/MS analysis was performed on an Acquity UPLC Class system coupled to a Xevo TQ-D mass detector (Waters, Ireland) and Cortecs UPLC column (2.1 x 100 mm, 1.6 um d.p.) (Waters, Ireland), based on the method described by Alyamani et al. (2015). Results: At room temperature and -20°C, abiraterone and D4A were stable for 28 days considering the stability acceptance criteria of ±15% variation. 5α-abiraterone stability decayed after 14 days at 25°C (83.34-86.09%) and 28 days at -20°C (77.66-80.62%). At 45°C, only abiraterone was stable for 28 days, while D4A and 5α-abiraterone lost stability after 14 days (82.94-84.47% and 80.30-81.47%, respectively). Conclusions: Studies have reported short stability of abiraterone in plasma and whole blood, and there is few data about the stability of abiraterone metabolites. This study shows that accurate results for abiraterone and its active metabolites can be obtained after up to 7 days with DPS storage at room temperature, and 21 days with DPS storage at -20°C.

Keywords: abiraterone; dried plasma spots; LC-MS/MS; prostate cancer; therapeutic drug monitoring.
CLINICAL VALIDATION OF A DBS AND DPS SAMPLING STRATEGY FOR ESTIMATION OF LITHIUM IN PATIENTS WITH BIPOLAR DISORDER

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**Background:** Lithium (Li) is the main used drug for the treatment of bipolar disorder. Despite its clinical utility, it has a narrow therapeutic index (0.6-1.2 mEq/L) and several adverse effects, including acute and chronic toxicities. Therefore, Li is a suitable candidate for Therapeutic Drug Monitoring (TDM). Dried Blood Spots (DBS) and Dried Plasma Spots (DPS) sampling can be an attractive alternative due to conventional transport and collection in TDM. The clinically validate a Graphite Furnace Atomic Absorption Spectrometry (GFAAS) method for quantification of Li in DBS and DPS samples to estimate systemic Li exposure through a limited sampling strategy in patients with bipolar disorder. **Methods:** Paired serum, DPS, and DBS samples from 43 patients were collected after 12 hours after last Li administration. Li was extracted from 8 mm DBS and 6 mm DPS discs with nitric acid 4.5% and diluent solution. Both extracts were injected into GFAAS. Estimated serum concentrations (ESC) were obtained after adjustment of DBS levels (DBS_{conc}) by patients Hct and Li fraction in serum and DPS. Li concentrations obtained were correlated and compared with ESC using Passing-Bablok and Bland-Altman methods. **Results:** Mean ESC_{Hct} was 0.57 mEq/L, representing 82% of percentage fraction in serum (0.56 mEq/L, interval 0.18-1.1 mEq/L). On DBS and DPS samples averages were 0.42 (interval 0.17-0.92 mEq/L) and 0.47 (interval 0.15-0.99 mEq/L) respectively. Correlation with serum samples presented r values of 0.734, 0.866, 0.734 and 0.740 for DBS, DPS, ESC and ESC_{Hct} respectively. Passing-Bablok regression indicated no constant or proportional errors on the correlation samples. Serum vs DBS intercept 95% CI of -0.14 to 0.10; slope 95% CI of 0.5 to 1. Serum vs DPS; intercept 95% CI of -0.18 to 0.03; slope 95% CI of 0.77 to 1.15. Serum vs ESC intercept 95% CI -0.28 to 0.11; slope 95% CI 0.75 to 1.49. Serum vs ESC_{Hct}; intercept 95% CI of -0.27 to 0.10, slope 95% CI of 0.74 to 1.45. The mean residual standard deviation among methods was 0.12%. **Conclusions:** The DBS estimation arise feasible and with good correlation with serum levels and could be used to further Li measurements. Stem from dried samples could estimate Li erythrocyte concentrations and interpretation of serum levels stem from DPS samples. **Keywords:** Lithium, Serum concentrations, Dried Blood Spots, Dried Plasma Spots, Therapeutic Drug Monitoring, Validation.
Theme: Alternative sampling strategies

VALIDATION AND CLINICAL APPLICATION OF AN ULTRAHIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR THE QUANTIFICATION OF TACROLIMUS USING VOLUMETRIC ABSORPTIVE MICROSMPLING. Camille Tron¹,², Marie-José Ferrand-Sorre¹, Pauline Houssel-Debrý³, Marie-Clémence Verdier¹,², Florian Lemaitre¹,², Eric Bellissant¹,²

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Background: Volumetric absorptive microsampling (VAMS) is an innovative alternative sampling strategy to venepuncture which presents many advantages for therapeutic drug monitoring (TDM) of tacrolimus (TAC). Indeed, this approach is minimally invasive and the device is also suitable for self-sampling by the patient at home. The aim of this work to validate an ultrahigh performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of TAC in blood collected with VAMS. Methods: After sampling, VAMS tips were dried at room temperature (RT) for at least 1h30. To extract TAC, the tips was immersed in 200 µL of water containing the internal standard and mixed for 15 min followed by 10 min of sonication. Extraction was performed by protein precipitation then 10 µL of the supernatant were injected in the LC-MS/MS system. Elution was performed on a C₁₈ reversed column and a mobile phase composed of acetonitrile/ammonium acetate in water (95%/5%). The method was validated according to the European Medicines Agency guidelines. A clinical validation was performed by comparing TAC concentrations in whole blood from venepuncture, to concentrations in finger capillary blood measured in VAMS collected at the same time in 30 liver transplant recipients. Results: The method was linear within 1 to 45 ng/mL. Within-day and between-day precisions and overall bias were within ±15%. Relative matrix effect fulfilled the acceptance criteria. TAC was stable in VAMS for 1 week at RT, 48 h at 60°C and 4°C. No significant effect of the hematocrit was observed within the range of 0.25-0.50. In the clinical validation part, a good linear correlation was found between the two methods (Deming coefficient = 0.97, Passing-Bablok analysis: slope = 0.9 (IC95%: 0.82-0.99), intercept = 0.17 (IC95%: -0.42-0.56)). Conclusion: A LC-MS/MS method was successfully validated regarding analytical performances and clinical requirement. This assay using VAMS is reliable to be further implemented in routine TDM of TAC or to perform pharmacokinetics studies. This approach may represent a crucial step forward for tacrolimus TDM and for improvement of the quality of life of transplanted patients. Keywords: tacrolimus, volumetric absorptive microsampling, mass spectrometry, therapeutic drug monitoring, transplantation
Background: Valproic acid (VA) is a widely used antiepileptic drug, with a plasma therapeutic concentration range of 50-100 μg mL⁻¹. TDM of VA requires the measurement of the compound using immunochemical or chromatographic assays. Recently, a new sample preparation strategy was introduced, named biocompatible solid-phase microextraction (Biocompatible SPME). In this study, the first application of LC Tip SPME C18 to the determination of VA in biofluids is described.

Methods: The extraction procedure was simple, with four steps: fiber conditioning with a mixture of methanol: water (1:1, v/v), mixing of 50 μL of plasma and 150 μL of HCl 0.1 M in a vial containing the internal standard, extraction by adding the fiber in the mixture and homogenization for 30 min at 450 rpm, and extraction by transferring the fiber to another tube containing 150 μL of methanol, which was agitated at 450 rpm for 30 min. An aliquot of 1 μL of the extract was injected in a ZB-WAX column (30m x 0.32mm x 0.25μm). The GC oven temperature ranged from 80°C to 240°C, with a run time of 10 min. Monitored m/z were 102 (quan), 73 and 115 (qual) for VA and 116 (quan), 81 and 108 (qual). The assay was validated according to international guidelines and applied to 25 clinical samples. Results: The assay was linear in the range of 10 to 150 μg mL⁻¹. The performance was evaluated with QC samples with concentrations of 10, 15, 60 and 120 μg mL⁻¹. Intra-assay precision was 3.97-9.62% and inter-assay precision 5.57-10.01%. Accuracy was in the range of 92.57-104.12%. Extracts were stable at the GC autosampler for 10 h. VA was also stable after 3 freeze and thaw cycles. Extraction yield was 93.7-101.34%. Concentrations measured with biocompatible SPME assay were highly correlated with the routine liquid-liquid based GC-MS assay, with a correlation coefficient (r) of 0.9631. Conclusions: A new and simple assay for the measurement of VA levels in plasma using biocompatible SPME and GC-MS was developed and validated and its clinical performance is currently under evaluation. Biocompatible SPME is a sample preparation strategy with high potential for drug measurement in the field of TDM.

Keywords: Valproic acid, solid-phase microextraction, therapeutic drug monitoring, GC-MS.
DEVELOPMENT OF A BIOANALYTICAL METHOD FOR SIMULTANEOUS QUANTIFICATION OF ANTIRETROVIRALS USED IN PREGNANT WOMEN IN DRIED BLOOD SPOT BY LC-MS/MS

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Background: Universal antiretroviral therapy in pregnant women is an important element in the prophylaxis of vertical HIV transmission. Because of physiological changes occurring during pregnancy, therapeutic drug monitoring (TDM) can be considered to ensure adequate plasma concentrations of antiretrovirals and maintenance of undetectable levels of HIV RNA. Dried Blood Spot (DBS) sampling offers several advantages over traditional blood collection, such as greater convenience, smaller sample volumes, reduced risk of infection, and improved sample stability. The objective of this study was to develop a bioanalytical method for simultaneous quantification of raltegravir, atazanavir, ritonavir and efavirenz in DBS by LC-MS/MS for TDM application in pregnant women living with HIV. Methods: For this, tests were carried out using variations in the parameters of column chromatography, mobile phase, elution type, flow, injector wash solution and injection volume. Preliminary validation tests of selectivity, matrix effect, residual effect, cross-detection and linearity were conducted according to the RDC 27/2012 and FDA bioanalytical validation guidelines (2018). Results: The developed bioanalytical method showed symmetry of chromatographic peaks (asymmetry <1.5) as shown in the figure, analysis time of 6 min, using a C18 column of 150x4.6mm and particle size of 5μm, water and methanol as mobile phase, both with 0.1% formic acid, eluted in gradient mode at a flow rate of 1mL/min, and a sample injection volume of 7μL. In the preliminary validation tests, the method showed necessary linearity, selectivity and sensitivity for the analytes, absence of interferences of the matrix, residual effect and cross-detection. The methanol extraction procedure followed by 30min agitation showed adequate recovery and efficiency for atazanavir, ritonavir and efavirenz, presenting reproducible values for these drugs. Conclusions: The developed bioanalytical method presented promising potential for the simultaneous quantification of the target antiretrovirals.

Keywords: Antiretrovirals; Pregnancy; Dried Blood Spot; Therapeutic Drug Monitoring; LC-MS/MS.
THE FEASIBILITY OF DRIED BLOOD SPOTS IN CHILDREN WITH AUTISM SPECTRUM DISORDER AND SEVERE BEHAVIORAL PROBLEMS

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Background: Blood sampling in children with Autism Spectrum Disorder (ASD) can be challenging. Dried Blood Spot (DBS) sampling might be a less invasive sampling method for drug concentration measurement, as this involves only a fingerprick. The aim of this study was to evaluate the pain scores of DBS finger pricks in children with ASD and severe behavioral problems. Methods: Blood was collected by DBS fingerpricks between 2016 and 2019 during the Dutch multicenter SPACE trial (NTR 6050) in children with ASD and behavioral problems aged 6-18 years. The pain severity was scored using the Numeric Rating Scale (NRS) by the child, parent and/or researcher. The fingerprick could be performed by parent or caregiver, researcher, nurse or by the child itself. Descriptive statistics were used to analyze frequencies. Mixed effect linear modelling was used to analyze the influence of child sex, child age and type of performer of the fingerprick on the NRS pain score reported by the child. P<0.05 was considered significant. Results: A total of 247 fingerpricks was performed in 70 children (70% Male, median age 11 years). The median (min-max) pain scores rated by child, parent and researcher were 3 (0-10), 3 (0-7), 2 (0-7) respectively. In the majority of n=147 fingerpricks, the parent rated the same painscore as the child (60.5%), and in 26.5% the parent scored the pain lower. 17.4% (n=61) of the fingerpricks were rated ≥4 (distressing or higher) by the child, but nevertheless of these, 68.9% (n=42) agreed with a next fingerprick. Multivariate mixed effect analysis could be performed for n=167 children, and showed no significant covariates for the NRS painscore by the child; sex (b=0.37, p=0.36), age (b=-0.08, p=0.18), performer (parent b=-0.97, p=0.623, nurse b=-1.64, p=0.414, researcher b=-1.19, p=0.56 with self as reference category). Conclusions: The painscores of DBS fingerpricks in children with ASD and severe behavioral problems are generally low and were not dependent on age, sex of the child or performer of the fingerprick. DBS Fingerpricks seem well tolerated in this population.

Keywords: Dried Blood Spots, Psychiatry, Children, Pain
Background: Prednisolone is usually given under a fixed dosing regimen to prevent rejection following renal transplant. It displays significant pharmacokinetic variability and exposure-outcome relationships, suggesting a role for measuring drug exposure in certain clinical scenarios. Prednisolone is highly protein bound, with free prednisolone the pharmacologically active form. Measurement of free prednisolone in plasma is cumbersome. Saliva prednisolone concentrations may reflect the free form in plasma and may be an alternative for drug measurement. This study aims to examine the correlation between total and free plasma and saliva prednisolone concentrations in adult renal transplant recipients in the early post-transplant period.

Methods: Total and free plasma and saliva prednisolone concentrations were measured simultaneously in twenty patients receiving oral prednisolone one to two months’ post-transplant. Samples were taken pre-dose and 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 6, 9 and 12 hours post-dose. Prednisolone concentration was determined using high performance liquid chromatography. Pearson’s coefficient was used to assess the association between plasma and salivary prednisolone concentrations and the area-under-the-concentration-time-curve from 0 to 12 hours post-dose (AUC₀₋₁₂).

Results: When considering all time points there was a strong correlation between total and free plasma prednisolone concentrations (r²=0.81), but a poor correlation between both free and total plasma and saliva prednisolone concentrations (r²=0.003 and 0.01 respectively). There was a moderate correlation between free and total plasma prednisolone AUC₀₋₁₂ (r²=0.62), but a poor correlation between free and total plasma prednisolone AUC₀₋₁₂ and saliva AUC₀₋₁₂ (r²=0.07 and 0.17 respectively).

Conclusions: Total and free plasma prednisolone measurements correlated poorly with saliva prednisolone measurements. Measurement of prednisolone concentration in saliva is not currently a reliable option for estimation of free plasma concentrations in adult renal transplant patients.

Keywords: Prednisolone, saliva, therapeutic drug monitoring, renal transplant
Theme: Alternative sampling strategies

**NOVEL TDM OF INHALED BUDERSONIDE IN EXHALED BREATH FOR CONFIRMATION OF ADEQUATE INHALATION**

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**Background:** Although inhalation is a useful method for the treatment of asthma and chronic obstructive pulmonary disease, the proper use of inhalers is essential to achieve the best therapeutic response. However, there is no quantitative assessment method to confirm the patients’ inhalation are adequate or not. Our previous in vitro studies have reported that pulmonary drug deposition rates of dry powder inhalation, budesonide Turbuhaler®, were drastically depended on inhalation flow rate. Here, because a part of inhaled drugs is exhaled to breath, quantitative assessment of exhaled drugs could predict the pulmonary deposition rate of inhaled drugs. The aim of the present study is to investigate the possibility of TDM of inhaled budesonide in exhaled breath for confirmation of adequate inhalation.

**Methods:** Three healthy volunteers were enrolled in this study, and inhaled budesonide Turbuhaler® at inhalation flow rate of 30 L/min and >60 L/min, respectively. The nasally exhaled breath after the inhalation of budesonide were directly collected in plastic bag (volume >4 L). The collected drugs were dissolved in 10 mL of 20% ethanol, and measured by HPLC-UV method.

**Results:** Recovered budesonide amount at higher inhalation flow rate of >60 L/min (310 ± 58.0 ng) was significantly higher than that at 30 L/min (75.1 ± 29.6 ng, P<0.05). Our previous study described that the pulmonary deposition rate of budesonide at higher inhalation flow rate of >60 L/min (74.8 ± 4.1%) was also higher than that at 30 L/min (33.3 ± 4.7%). These results demonstrated the relationship between the amount of budesonide in exhaled breath and the pulmonary drug deposition rate.

**Conclusions:** TDM of budesonide in exhaled breath may be a promising noninvasive marker for pulmonary deposition rate of budesonide inhalation.


**Keywords:** inhalation; exhaled breath; budesonide; adherence.
Background: Amikacin (AMI) is a broad-spectrum antibiotic used in treatment of infections in critical ill patients. Critical patients present significant pharmacokinetics variability, making AMI a drug of interest for therapeutic drug monitoring (TDM). AMI clearance is highly correlated with creatinine (CRE) clearance, making CRE plasma levels an important data for applying PK models to AMI dose individualization. Dried plasma spots (DPS) can be used as an alternative for the transportation of specimens for AMI measurements, which are not readily accessible in Developing Countries. The aim of this study was to evaluate the applicability of DPS for TDM of AMI. Methods: Paired samples of DPS and plasma from 36 patients in treatment with AMI were analyzed applying UPLC-MS/MS. Method comparison was evaluated by Passing-Bablok (PB) regression and Bland-Altman (BA) plots. Results: AMI plasma concentrations varied between 0.56-71.0 mg L\(^{-1}\) for AMI and 3.65-86.4 mg L\(^{-1}\) for CRE. DPS concentrations showed high correlation with plasma levels (r=0.987 for AMI and r=0.991 for CRE, p<0.001), being in the range of 0.6-79.3 mg L\(^{-1}\) for AMI and 3.73-87.3 mg L\(^{-1}\) for CRE. PB analysis (Figure A1/B1) showed no systematic bias and small proportional bias for AMI, while CRE measurements were absent of both biases. BA plots (Figure A2/B2) showed mean differences between DPS and plasma determinations of -1.9% for AMI, with 2 samples outside the ±1.96 standard deviation range, and -0.5% for CRE, with 4 samples outside the deviation range. Conclusions: These findings suggest that DPS quantifications of AMI and CRE are representative of measurements obtained in plasma, justifying its use in TDM of AMI. Keywords: amikacin; creatinine; DPS; LC-MS/MS; therapeutic drug monitoring; vancomycin.
Background: Therapeutic drug monitoring (TDM) is routinely applied for amikacin (AMI) and vancomycin (VAN), once they are largely used in the empiric treatment of severe infections. TDM allows to maximize treatment efficacy and minimize the occurrence of adverse effects, especially for critical patients with considerable pharmacokinetics (PK) alterations. The clearances of AMI and VAN are highly correlated to creatinine (CRE) clearance, making CRE levels in plasma an important data in PK applications. Usually, the measurement of AMI and VAN levels is performed in plasma or serum specimens, however the use of dried matrices, such dried plasma spots (DPS), can be an important alternative because it simplifies the logistic to reference laboratories. Thus, the aim of the study was to determine simultaneously AMI, VAN and CRE in DPS using LC-MS/MS. Methods: An 14 mm diameter punch of Whatman 903 paper, containing 25 µL of plasma, was extracted with 450 µL of methanol:purified water:formic acid (49.5:49.5:1, v/v) containing internal standards (kanamycin B 0.5 µg mL⁻¹ for AMI, creatinine-D₃ at 0.25 µg mL⁻¹ for CRE), incubated at 30°C at 1,500 RPM for 30 min. An aliquot of 1 µL from the supernatant was injected in the UPLC-MS/MS. Separation was achieved on an Acquity HSS T3 column eluted with purified water and acetonitrile, containing 0.1% formic and 0.01% of HFBA. Calibration curves were in the range of 5-100 µg mL⁻¹ for CRE and 0.5-100 µg mL⁻¹ for AMI and VAN. QC samples were prepared at the concentrations of 1.5, 8 and 80 µg mL⁻¹ for AMI and VAN, and 6, 20 and 80 µg mL⁻¹ for CRE. The assay was validated according to international guidelines. Results: The total run time was 5.5 min with retention times of 0.92, 0.93, 2.12, 2.17 and 2.27 min for CRE, CRE-D₃, AMI, KAN and VAN, respectively. Intra-day imprecision was in the range of 3.8-4.9% for AMI, 2.4-6.5% for VAN and 1.6-4.2% for CRE. Inter-day imprecision varied between 3.6-6.6% for AMI, 3.2-6.9% to VAN and 4.5-6.5% for CRE. Accuracy was in the range of 95.9-100%, 89.0-102.2% and 95.9-100.4% for AMI, VAN and CRE, respectively. Conclusions: This study developed and validated the first method for simultaneous determination of AMI, VAN and CRE in DPS. This method provides a useful logistic strategy for allowing a more widespread access to dose individualization of AMI and CRE in limited resources settings. Keywords: amikacin; creatinine; DPS; LC-MS/MS; therapeutic drug monitoring; vancomycin.
A SIMPLE AND NON-DESTRUCTIVE MEASUREMENT OF HEMATOCRIT IN DRIED BLOOD SPOTS

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**Background:** The use of dried blood spots (DBS) as a sampling method has several benefits such as simple storage and transportation, which enable sampling in rural areas and self-sampling at home. During the last decade, the development of analytical methods using DBS has increased in the field of therapeutic drug monitoring (TDM). The main argument against using DBS with TDM however, is that different hematocrit (HCT) and indeterminable blood spot volumes can add to the bias of the measured concentration.

The aim was to develop a simple and non-destructive method to estimate the HCT and volume in a DBS sample. **Method:** Dried blood spots were scanned using a conventional flatbed scanner. The luminescence and the area of the spots were measured using ImageJ, a free image analysis software. DBS calibration curves were constructed for a range of HCT values (20-65 [%]) and blood spot volumes (10-60 µL). The HCT of 50 scanned patient samples was calculated from their respective luminescence and compared to HCT values obtained with fluorescence flow cytometry (FFC) on whole blood. Blood spot volumes were calculated from the spot area of 7 different volumes per patient and compared to nominal pipetted volume. **Results:** For HCT, the mean absolute difference ± SD between the scanning method and the FFC method was -2.0 ± 2.5 HCT [%]. For the blood spot volumes, the mean absolute difference ± SD for the scanning method calculated against nominal pipetted volume was 0.95 ± 1.43 µL. **Conclusions:** With the use of conventional filter paper and office equipment (i.e. a flatbed scanner), the method can be used to estimate the hematocrit and the volume of a DBS sample. If there is a robust and validated DBS-method for a specific analyte, this scanning method can be used to exclude deviating DBS samples in order to limit the bias from hematocrit and volume on the measured analyte concentration.

**Keywords:** HCT; DBS; TDM; sample volume; fluorescence flow cytometry; scanner
THE ROLE OF THE LABORATORY IN ACHIEVING PERSONALISED ANTI-INFECTIVE THERAPEUTIC DRUG MONITORING

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Background: For many drugs there is significant inter-individual variability in the pharmacokinetics and/or pharmacodynamics. This variability is of clinical concern particularly for drugs such as anti-infectives where therapeutic drug monitoring (TDM) is recommended to avoid subtherapeutic and toxic drug concentrations. In order to successfully deliver a TDM service to achieve the optimal dose in every patient every time, engagement of the laboratory is an integral step. Therefore, the aim of this presentation is to provide an overview of the advantages and disadvantages of current analytical methodologies and identify approaches which maximise the efficiency, quality and quantity of TDM results utilizing available resources.

Methods: There are numerous bioanalytical methods developed on several different platforms which are used to determine drug concentrations. The advantages and disadvantages of major platforms (immunoassays, High-Performance Liquid Chromatography (HPLC); HPLC coupled with Tandem Mass Spectrometry (MS/MS) technology) will be presented with the examples of potential clinical consequences of not undertaking TDM. In particular, the use of HPLC-MS/MS will be discussed together with a novel approach (Active Flow Technology) to enhance sensitivity and decrease analysis time by up to five-fold.

Results: Effective TDM ultimately ensures optimal patient outcomes including minimizing toxicity, reducing the number of expensive and often avoidable and invasive diagnostic tests and shorter hospital stay. Currently, the use of HPLC-MS/MS is considered a gold standard approach to determine concentrations of drugs requiring TDM including immunosuppressants, antifungals, antibiotics, antiretrovirals. However, the use of immunoassays and simple HPLC-UV assays can offer a more affordable approach with a significant impact on effective TDM, particularly in developing countries.

Conclusions: The TDM service should be more than just determining drug concentrations to inform dose adjustments to attain therapeutic targets. Ideally it is a patient centred service involving the multidisciplinary team of clinicians, clinical scientists, pharmacists, pathologists and nurses working towards delivering safe, effective and personalized drug treatment.

Keywords: Anti-infective; Therapeutic Drug Monitoring; Mass-Spectrometry; Personalized Medicine; Clinical Pharmacology & Toxicology; Active Flow Technology.
FULLY AUTOMATED THERAPEUTIC DRUG MONITORING OF ANTI-EPILEPTIC DRUGS MAKING USE OF DRIED BLOOD SPOTS
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Background: Fully automated dried blood spot (DBS) extraction systems, online coupled to standard liquid chromatography-tandem mass spectrometry (LC-MS/MS) configurations, decrease the hands-on time associated with conventional DBS analysis, resulting in a higher sample throughput, making the technique more compatible with a high-throughput bioanalytical workflow. The aim of this study was to develop and validate an LC-MS/MS method, using a DBS-MS 500 autosampler (CAMAG, Switzerland), for the determination and quantification of four anti-epileptic drugs (AEDs) (carbamazepine, valproic acid, phenobarbital and phenytoin) and one active metabolite (carbamazepine-10,11-epoxide) in DBS samples. Methods: Method development included thorough optimization of the fully automated extraction procedure (i.e. extraction solvent, extraction (loop) volume, internal standard application, internal standard drying time, etc.). Afterwards, the method was fully validated based on international guidelines and applied on capillary patient samples originating from African epilepsy patients. Results: Thorough optimization of the fully automated extraction method was of utmost importance, finally resulting in the exclusion of the built-in IS spray. Concerning method validation, accuracy (%bias) and precision (%RSD) (with a single exception) were below 13%, meeting the acceptance criteria. Neither carry-over nor unacceptable interferences were observed. Calibration data was found to be heteroscedastic. Using weighted linear regression, with 1/x² weighting, mean back-calculated concentrations did not differ more than ±15% for all analytes, which is in line with the acceptance criteria. All compounds were stable in DBS for at least 1 month when stored at room temperature, 4 °C and -20 °C and for at least 4 days when stored at 60 °C. Internal standard-corrected matrix effects were below 8%, with %RSDs below 9.1%. Reproducible relative recovery values (around 60% for all analytes) were obtained and the effect of the hematocrit on the relative recovery was overall limited. Finally, fifteen capillary DBS samples, originating from patients receiving AED therapy in remote areas within sub-Saharan Africa, were successfully analyzed, demonstrating the applicability of the developed procedure in a remote setting. Conclusions: An LC-MS/MS method for the determination and quantification of 4 AEDs and one active metabolite in DBS, making use of the DBS-MS 500 autosampler, was developed and validated. Thorough optimization during method development demonstrated that proposed, generic direct elution conditions, while of value for orientation, may require (substantial) adjustment, depending on the analytes of interest and the used instrumentation. Keywords: Therapeutic drug monitoring; dried blood spots; LC-MS/MS; anti-epileptic drugs; automated extraction.
Theme: Anti-infective drugs

THERAPEUTIC MONITORING OF AMIKACIN IN PATIENTS HOSPITALIZED IN THE SOUTH OF BRAZIL
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Background: Amikacin (AMK) is an aminoglycoside widely used for the treatment of severe infections caused primarily by gram-negative bacteria. It can be used in combination with other antimicrobial drugs, such as β-lactams and penicillins. It is a drug that presents a significant interindividual variability and the toxicological effects are nephrotoxicity and ototoxicity. Therapeutic drug monitoring (TDM) may help to optimize the results of AMK treatment. The aimed of this study was to quantify the plasma levels of AMK in patients hospitalized at the University Hospital of Santa Maria (HUSM), Brazil.

Methods: Participants in this study were those treated with AMK from May 2018 to May 2019. The collected samples were carried out at steady state, and plasma levels of AMK were determined by LC-MS/MS after liquid-liquid extraction. Severe infections require peak steady-state plasma concentrations of 25-30 µg/mL when using conventional dosing. Patient medical records were individually reviewed using a standardized data collection model at each study site to collect information demographics and clinical data on therapy outcomes and adverse events. This study was approved by the UFSM Ethics Committee (CAAE: 83200618.7.0000.5346).

Results: A total of 90 samples of AMK through concentrations of 24 patients were enrolled. The majority of patients were affected by a multiresistant microorganism, such as Klebsiella pneumoniae or Pseudomonas aeruginosa. The dose of AMK administered IV in these patients ranged from 500 to 1500mg once daily. The patients’ age range was 29 to 80 years old. The mean peak AMK concentration was 43.67 µg/mL (range 10.1 to 102.71 µg/mL). Among these patients, eight were from the nephrology department and one patient had the amikacin suspended due to the ototoxicity reported.

Conclusions: The results of this study provided useful information on AMK dosage adjustments in patients with infections caused by multiresistant bacteria. Also, these patients had elevated plasma AMK levels. Thus, routine TDM may be useful for dose optimization and individualization while maintaining appropriate drug concentrations, avoiding possible toxic effects.

Keywords: amikacin; aminoglycosides; drug safety; antibiotics
Background: Although recent data in vitro and some case reports indicate that the combination of daptomycin (DAP) with ampicillin (AMP) has synergistic effects against Enterococci, their combined in vivo efficacy in serious non-endocardial infections is unknown. Here, we present PD data with DAP plus AMP against two strains of enterococci, derived from our optimized mouse model that allows Enterococci to grow at least 2 log_{10} CFU/g in 24 h. Methods: For in vitro tests, the drugs were tested in time-kill curves (TKC) alone and combined in concentrations sub-MIC against E. faecalis ATCC 29212 or E. faecium ATCC 19434. For in vivo studies, the optimized neutropenic mouse thigh infection model was used. Two hours post-infection, groups of 2 mice were allocated to receive single or combined therapy in doses from 0 to 200 mg/kg/day for DAP divided every 6 hours and fixed doses of 125 and 250 or 100 and 150 mg/kg/day for AMP divided every 3 hours, for E. faecium and E. faecalis respectively. Hill’s model was fitted to estimate PD parameters E_{max} and ED_{50}. In vivo synergism was defined as an increase of the E_{max} ≥2 log CFU / g or a reduction ≥50% of the ED_{50}. Results: For TKC, the combination of DAP plus AMP increased the bacterial killing more than 2 log CFU/mL against both strains (i.e. it was synergistic). In vivo, against E. faecalis the potency of DAP increased when combined with AMP 100 mg/kg/day, without changes in efficacy (ED_{50,DAP} 15.4 vs. ED_{50,DAP + AMP} 0.09 mg/kg/day); and against E. faecium the potency of DAP also increased when combined with AMP 250 mg/kg/day, also without changes in efficacy (ED_{50,DAP} 31.9 vs. ED_{50,DAP + AMP} 0.006 mg/kg/day), indicating a strong synergistic effect against both strains, with a 171 to 5315-fold reduction of ED_{50}. Conclusions: In vivo combinations of daptomycin and ampicillin against enterococci showed strong synergism, with a huge impact on potency (hundreds to thousands-fold increase). These results suggest that this synergistic combination may be daptomycin dose-sparing. Keywords: daptomycin; ampicillin; synergism; antibiotic combination; enterococcus.
**Background:** Nifurtimox (NFX) is a drug commonly used for the treatment of Chagas disease, but its metabolism is not fully described in humans. It is necessary to search for reactive species that may be involved in the appearance of adverse effects observed in patients, as well as to identify metabolic pathways affected by treatment. This work aims to identify by hydrophilic interaction liquid chromatography (HILIC) drug metabolites and/or potential biomarkers in urine of NFX treated patients. **Methods:** Two sets of 24-hour urine samples were prepared, one from 8 controls and one from 12 patients. For each group a pool of urine was prepared as quality controls. 200 µL of urine were precipitated with 800 µL of a cold ACN/MeOH mixture (1:1), centrifuged at 13,200 rpm and the supernatants were placed in the autosampler of the HPLC equipment (Shimadzu Nexera X2) at 5 ºC. 2 µL were injected into a 2.7 µm Restek Raptor HILIC-Si column (100 x 2.1 mm). The mobile phases A and B contained ACN/Buffer acetic-ammonium acetate (10 mM, pH 4.75), in 50/50 and 95/5 proportions, respectively. Chromatography was performed at 0.6 mL/min in gradient: 85 %B (0.0-1.0 min), 85 to 0 %B (1.0-12.0 min), 0 to 85 %B (12.0-12.2 min), and 85 %B (12.2-15 min). The pharmacometabolomic screening was done with an AB-Sciex QTRAP 6500 with Turbo-IonSpray ionization source, in positive and negative modes, by means of Enhanced Mass Scan (EMS) and Enhanced Product Ion (EPI) experiments under a Data-Independent Acquisition (IDA) criterion, from 50 to 1000 Da. Data were analyzed with the LightSight (Sciex) software. **Results:** From 116 relevant signals in patients, 13 potential metabolites were found, among which the ions m/z(+) 362, 404, 379 and 421 were identified as GSH-derivatives, m/z 436 as a glucuronide, as well as other phase I NFX metabolites. **Conclusions:** The HILIC screening method allowed the identification of a large number of specific signs of NFX treatment. This research suggests that the main detoxification pathway of NFX is most likely glutathione conjugation. Additional characterization experiments such as search for precursor ions, neutral losses and MS³ fragmentation are needed to discern between drug metabolites and possible endogenous markers. Some of the metabolites found had been characterized by our group by C18 reverse-phase chromatography. Knowledge of these species could allow the development of targeted methods to study the pharmacokinetics of NFX and its metabolites in various biological matrices, and try to design better treatments. **Keywords:** Nifurtimox; HILIC; UHPLC-MS/MS; Chagas.
DETERMINATION OF THE ANTVIRAL POTENTIAL OF BENZIMIDAZOLIC COMPOUNDS IN FRONT OF ZIKA VIRUS

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<table>
<thead>
<tr>
<th>Concentration</th>
<th>% reduction NB2</th>
<th>% reduction NB5</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 μM</td>
<td>15,21</td>
<td>*</td>
</tr>
<tr>
<td>5 μM</td>
<td>13,36</td>
<td>*</td>
</tr>
<tr>
<td>2.5 μM</td>
<td>15,67</td>
<td>*</td>
</tr>
<tr>
<td>1.2 μM</td>
<td>33,64</td>
<td>39,17</td>
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<tr>
<td>0.6 μM</td>
<td>43,78</td>
<td>41,94</td>
</tr>
<tr>
<td>0.3 μM</td>
<td>38,88</td>
<td>16,59</td>
</tr>
</tbody>
</table>

* indicates cytotoxicity results.

Background: Benzimidazoles are aromatic heterocyclic organic compounds which consist of the melting of benzene and imidazole. These compounds have several applications in the pharmacological area with antineoplastic, anti-inflammatory, analgesic, antihelmintic, antiparasitic and antimicrobial action. Therefore, taking into account the great diversity of applications of benzimidazoles, we evaluated the antiviral potential of NB2 and NB5 compounds against the Zika virus (ZIKV). Methods: Vero cells, susceptible to ZIKV, were cultured in DMEM supplemented with 10% fetal bovine serum and maintained in a humid atmosphere at 37°C with 5% CO2. Subsequently, the least squares regression method was used to obtain the cytotoxic concentration for 50% of the culture (CC50) through the tetrazolium salt reduction assay MTT, which determines the mitochondrial functionality. These values were used to define the test concentrations for the antiviral analysis. For this, 2.5x10^5 cells/well were inoculated into 24-well plates, and the viral suspensions were adjusted to 100 PFU/well. Infected cells were exposed for 72 hours at concentrations of 0.3 to 10 μM of compounds NB2 and NB5. The tests were performed using the plaque assay method in medium with 2% CMC and 0.4% crystal violet staining. Results: The results obtained in the determination of CC50 indicated concentration-dependent toxicity profile for both compounds, with values 15.73 μM and 0.85 μM for NB2 and NB5, respectively. In the antiviral analysis the highest reduction was 43.8% for NB2 and 41.9% for NB5, both at concentration of 0.6 μM. However, a cytotoxic effect was observed in the plaque assay for NB5 at concentrations higher than 2.5 μM. Conclusions: It may be concluded that compounds NB2 and NB5 showed a weak antiviral potential against ZIKV. However, further trials using other methodologies are required to confirm these results. Financial support: FAPERGS, FEEVALE, Croatian Science Foundation (Project 4379). Keywords: Cell culture. Pharmacological activity. ZIKV.
Theme: Anti-infective drugs

PHARMACOKINETICS OF INTRATHECAL ADMINISTRATION OF GENTAMICIN IN PATIENTS WITH INFECTIOUS MENINGITIS

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Background: Gram-negative bacteria are common organisms in nosocomial meningitis in neurosurgery. The mortality and morbidity associated with Gram-negative meningitis have remained significant. Because of poor blood-brain barrier permeability of some antibiotics, intrathecal administration is common. We are referring a case report of a patient with Serratia marcescens meningitis who was treated with intravenous meropenem plus intrathecal gentamicin. The gentamicin cerebrospinal fluid (CSF) “peak” concentration 10-20 mg/l (10xMIC) have proven as good treatment success predictors. Usually recommended gentamicin intrathecal dose doesn’t exceed 5 mg per day. This case describes pharmacokinetics of intrathecal gentamicin in context of intraindividual variability, risk of neurotoxicity and reliability to achieve recommended target.

Patients and methods: CSF gentamicin concentration were determined by validated LC/MS method. The patient with fulminant Serratia marcescens meningitis (age 62 years, weight 95 kg and GFR 100 ml/min) received 10 mg of intrathecal gentamicin every 24h concomitantly with intravenous meropenem 2 g every 8 h. After the first 48 h, the dose was increased to 20 mg every 24h due to repeated blood culture and liquor positivity. The total treatment time was 10 days for gentamicin and 15 days for meropenem. CSF samples were obtained by lumbar drainage at 0, 0.25, 0.5, 1, 3, 5, 8, 12, 24h after the first gentamicin dose (10 mg) and before and after the third increased dose (20 mg). Gentamicin CSF concentration/time profile data were used to calculate PK/PD target and individual pharmacokinetic parameters. Results: The CSF peak concentration after the first dose of 10 mg gentamicin at 0.25h was 738.7 mg/l. Cmin before the third double dose of 20 mg gentamicin was 0.0 mg/l and Cmax was 2737.6 mg/l. MICs for Serratia marcescens was 1 mg/l. Conclusions: The results indicate probably unnecessarily high doses administered intrathecally. The initial failure in achievement of liquor sterility may have been related to the leakage of gentamicin outside the intrathecal space at the second day of therapy due to the lumbar drainage dislocation. Timing of new drainage replacement corresponds to the increase of gentamicin dose to 20 mg per day. CFS sterility was found within next 24 hours. Patient clinical status improved dramatically after these both changes. No symptoms of neurotoxicity were observed.

Keywords: meningitis, intrathecal gentamicin, pharmacokinetics. Supported by the projects IGNNH 151102, IGNNH 168601, IGNNH 168602.
Theme: Anti-infective drugs

GENTAMICIN AND PIPERACILLIN – TAZOBACTAM is there a CHEMICAL INTERACTION?
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Background: As part of the surgical prophylaxis protocol in liver transplantation 60 minutes of pre-surgical incision, the recipient receive a loading dose of gentamicin of 5mg/Kg diluted in 200 mL of saline solution 0.9% in 30 minutes associated with 4.5 g of piperacillin-tazobactam diluted in 250 mL of glucose solution 5% in short infusion. This is followed by continuous infusion of piperacillin-tazobactam 13.5g over 8h and then 4.5g every 8h for the next 2 days. If bleeding exceeds 1500 mL intra-procedure, a second drip of gentamicin 2mg/kg is administrated. The goal is to prevent surgical site infection due to Coccus Gram+ and Multidrug resistant Gram-negative bacteria t, due to synergistic bactericide effect and extended spectrum, avoiding carbapenems. As it has been postulated that the carboxylate group of the piperacillin is susceptible of nucleophilic attack by the amino group of the glycoside resulting in an inactive amide, with beta-lactam as the limiting substrate of the reaction we wanted to assess this potential interaction in-vivo. Concomitantly use of other antibiotics with gentamicin yield lower target peaks and through concentrations of gentamicin.

Methods: Our sampling strategy was to take peripheral blood samples 30 minutes, 3.5 and 6.5 hours after gentamicin administration was finished. Piperacillin –tazobactam begins once of gentamicin load finishes. Results were modelized by Bayesian estimation in Limoges. Gentamicin determinations were measured by Architect. 14 patients who underwent for liver transplantation in Uruguay during 2018 with this prophylactic regimen were compared with 10 patients of Limoges cardiac surgical services on similar doses of gentamicin.

Results: After Student’s test, significant p values were obtained for AUC, half-life elimination time and creatinine clearance. Gentamicin elimination is by glomerular filtration. In both groups of patients Cmax did not reach theoretical 30-40 mg/L levels. Despite they exhibit similar distribution volume, Cmax and Caverage, their AUCs are quite different, pointing toward a disparity in absorption regardless Vd and Cmax. similarities. None of our patients developed intra-abdominal infections.

Conclusions: Both groups achieved theoretical target peaks; besides our Cmin remains within the non-toxic levels. AUC, creatinine clearance and half-life elimination of gentamicin could be more reliable parameters to evaluate PK profile during liver transplant procedures.

Keywords: gentamicin, aminoglycoside interaction, beta-lactams, surgical prophylaxis, liver transplant procedure.
Theme: Anti-infective drugs

QUANTIFICATION OF EFAVIRENZ HYDROXY METABOLITES IN PLASMA USING LC-HRMS/MS
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Background: Efavirenz is a non-nucleoside reverse transcriptase inhibitor (NNRTI) used worldwide to treat HIV. Efavirenz undergoes major metabolic steps influenced by genetic characteristics and there are concerns about the present dosing schedules in adults and children. Central nervous system adverse events have been linked to efavirenz treatment. Several studies have been performed to investigate the relationship between these side effects and the plasma concentrations of efavirenz and its metabolites. Conflicting results have been reported, emphasizing the need for further investigations requiring quantification of efavirenz and its metabolites.

Methods: An LC-HRMS/MS method was developed for the quantification of efavirenz hydroxy metabolites in human plasma. Sample preparation consisted of protein precipitation with methanol containing internal standards (efavirenz-d4, 7-OH efavirenz-d4 and 8-OH efavirenz-d4) followed by centrifugation. One supernatant aliquot was analyzed for the non-conjugated efavirenz hydroxy metabolites. A second and third aliquot was evaporated and reconstituted with arylsulfatase/β-glucuronidase for selected hydrolysis of sulfates and glucuronides, respectively. Analysis was performed using a Thermo Dionex Ultimate 3000RS LC-system coupled to a Thermo Scientific Q Exactive Orbitrap mass spectrometer.

Results: The method can be applied for quantification of efavirenz, 7-OH efavirenz and 8-OH efavirenz in human plasma samples. Furthermore, the hydrolysis step enables indirect quantification of the corresponding glucuronides and sulfates by comparing the concentration in unhydrolyzed plasma samples with hydrolyzed samples. The addition of an inhibitory solution to one aliquot during the hydrolysis step allows for selected hydrolysis of sulfates, while a second aliquot is used to hydrolyze both sulfates and glucuronides, resulting in indirect quantification of both phase II metabolites.

Conclusions: This newly developed method may be used as a tool to further investigate the relationship between efavirenz metabolism, genetics and reported central nervous system adverse events.

Keywords: HIV; efavirenz; LC-HRMS/MS
HIV MAINTENANCE TREATMENT WITH DARUNAVIR MONOTHERAPY: EXPOSURE-RESPONSE RELATIONSHIP (MONDARREE STUDY)

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Background: Darunavir (DRV) monotherapy is an appealing option in virologically suppressed patients as it reduces drug burden, cost, and the risk of adverse events while maintaining high efficacy (85%). Moreover, there is no evidence of specific resistance substitutions selected in patients experiencing viral failure (VF) during monotherapy. Some risk factors for failure have already been reported but little is known about the impact of DRV exposure on VF. Moreover, to date, no concentration target has been validated for maintenance treatment with DRV. We aimed at exploring the relationship between DRV concentrations and treatment outcome in patients treated with DRV monotherapy.

Methods: De novo and on-treatment patients treated were included in the study. Demographic, biological and treatment data were collected with the NADIS national database. DRV concentrations, obtained with a validated LC-MS/MS method, were gathered. Trough DRV concentrations (Cmin DRV) were estimated by individually modeling DRV pharmacokinetics using a 1-compartment model and the MWPharm++ software¹. Median Cmin DRV were compared between patients with, or without viral suppression (defined as VL < 50 copies/mL). Results: Forty-six patients (22 de novo and 24 on-treatment) were included in the study from January 2016 to December 2018. Mean zenith VL was 276,925 copies/mL and mean CD4 nadir was 198 cells/mm³. During the study, 8 patients (16.6%) presented at least one episode of VF. The mean treatment duration before VF was 5.3 months. Median Cmin DRV were not different between patients with virological control (2.22 ± 1.19 μg/mL), and failure (1.82 ± 1.47 μg/mL), p=0.36. Most patients (75%) who maintained virological control throughout the study period had Cmin DRV > 1.43 μg/mL. Conclusion: In this study, we confirmed that loss of virological control is not a rare event on DRV monotherapy in a non-selected population of virologically controlled HIV-infected patients. Cmin DRV was not associated with VF. A Cmin DRV of 1.43 μg/mL may be sufficient to maintain drug efficacy during monotherapy.

References:

Keywords: HIV, Antiretroviral, Protease inhibitor, Simplification, Pharmacokinetics.
Theme: Anti-infective drugs

POPULATION PHARMACOKINETIC ANALYSIS OF AMIKACIN IN PATIENTS IN INTENSIVE CARE UNIT: PRELIMINARY STUDY

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4University Hospital of Santa Maria, Brazil.

Background: Amikacin is aminoglycoside antimicrobial widely prescribed to treat infections caused by Gram-negative microorganisms. However, the widespread use only limited data are available on amikacin disposition in different patients. By the concept, the drug disposition in target population allows clinicians to optimize the design of drug regimen both “a priori” and later in the course of therapy. In this sense, the objective of this study was to estimate the population pharmacokinetics parameters (popPK) of amikacin in patients in the intensive care unit. Methods: Adult patients (n=6) received amikacin dose 500 mg twice or 1000 mg once daily (slow infusion) at the University Hospital of Santa Maria (Santa Maria, Brazil) from 2018 and 2019 were enrolled in this study. The blood sample collection was designed in patients to access peak (one and a half hour after dose) and trough (within before 30 minutes of the next dose) at steady-state concentrations. The blood samples analyzed by LC-MS/MS after liquid-liquid extraction method previous validated. All human procedures were submitted and approved by the UFSM Ethics Committee in research (CAAE: 83200618.7.0000.5346). Population pharmacokinetic parameters were estimated by nonlinear mixed effect model using a software MONOLIX™ v.2018R1 (Liosoft, Antony, France). Patient characteristics such as biochemical parameters, body weight and age type were investigated as covariate. Results: A one compartment model was found to be appropriate to characterize concentrations in total plasma showing the best curve fitting, precision of parameter estimates and model stability. The following parameters were estimated by the popPK model: volume of the central compartment (V = 24.5 L); total plasma clearance (CL = 2.26 L/h); standard deviation of the random effects omega_V (0.507 %), omega_CL (0.501 %) error model parameters a (2.92), b(0.14). The interindividual variabilities were reasonably small for the parameters in the model. Conclusions: In the present study, Amikacin PopPK in patients in the intensive care unit was built and well-validated. Although a further study in a large number of patients should be conducted and covariate effect analyzed in this population, the obtained results would be useful information on the dosing optimization and individualization in patients in intensive care unit.

Keywords: Amikacin; POPPK analysis; MONOLIX.
A PRELIMINARY STUDY FOR THERAPEUTIC DRUG MONITORING OF VORICONAZOLE IN BRAZILIAN PATIENTS WITH HAEMATOLOGICAL MALIGNANCIES
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4University Hospital of Santa Maria, Brazil.

Background: Voriconazole (VCZ) is a triazole antifungal with broad-spectrum indicated for the treatment of invasive fungal diseases. In addition, this drug has nonlinear pharmacokinetics. With the increasing use of antifungal agents in immunocompromised patients, therapeutic drug monitoring (TDM) can help optimize treatment outcomes of VCZ. This preliminary study aimed to evaluate plasma concentrations of VCZ in Brazilian patients with haematological malignancies treated at the University Hospital of Santa Maria (HUSM) (Santa Maria, Brazil) in order to optimize the dosage. Methods: The participants of this study were those being treated with VCZ from March 2018 to March 2019 in HUSM. The collected samples were carried out at steady state, and plasma VCZ levels were determined by high-performance liquid chromatography and UV detection. Dose adjustment was conducted according to a target concentration range of 1.0-5.5 mg/L. Patient medical records were individually reviewed using a standardized data collection template at each study site to collect demographic information and clinical data on outcomes of therapy and adverse events. This study was approved by the UFSM Ethics Committee (CAEE: 82281518.3.0000.5346). Results: A total of 22 VCZ samples trough concentrations from 8 patients were enrolled. The majority of patients received VCZ for the treatment of a known or presumed fungal infection. The patients’ age range was 11 to 43 years old, and all patients had haematological malignancy diagnosis. Among these patients, acute myeloid leukemia was the most common condition (n=6). The mean VCZ concentration was 2.4 μg/mL (range, 0 to 4.5 mg/L). Voriconazole was administered orally by all patients, and an initial dose was determined by standard dosing regimen (200 mg) twice a day for 8-12 weeks. Three patients had at least one subtherapeutic level, and the dose was adjusted. No patients showed level in the toxic range (more than 5.5 mg/L). Conclusions: The results of this preliminary study provided useful information on the dosage settings of VCZ in patients with haematological malignancies. Besides that, these patients showed longer medication time, thus routine TDM could be helpful in keeping therapeutic drug concentrations.

Keywords: drug safety; voriconazole; LMA; LLA
Background: omeprazole is a proton pump inhibitor largely used on the treatment of peptic ulcers and gastroesophageal reflux disease. Despite being considered safe, evidences relate its use with cognitive decline. Thus, the goal of the present work was to evaluate the cognitive ability of patients undergoing treatment with omeprazole. Methods: a case-control study was developed with 24 volunteers not users of omeprazole and 37 volunteers using omeprazole for longer than 6 months. The cognitive function was evaluated through the application of the Rey Auditory Verbal Learning Test, the Verbal Fluency Subtest Analysis of the Montreal Cognitive Assessment, the Brief Neuropsychological Assessment Battery (NEUPSILIN), the Psychological Battery for Attention Assessment, the Five Digit Test and, finally, The Hayling Test. Results: from the 37 volunteers in the group using omeprazole, 30 (81.1 %) were female and 7 (18.9 %) were male, while in the group of 24 volunteers not users of omeprazole, 20 (83.3 %) were female and 4 (16.7 %) were male. The average age in both groups was also very similar, being 64 (± 8.7) in the group of users and 63 (± 8.5) in the group of not users of omeprazole. Until this moment, through the evaluations, the group using omeprazole presented inferior results in 4 of the 6 tests compared to the group of not users of omeprazole: in the sustained attention (p=0.000), divided attention (p=0.012) and alternating attention (p=0.042) subtests from the Psychological Battery for Attention Assessment, in the reading (p=0.005) and counting times (p=0.001) of the Five Digit Test, in the phonemic (p=0.021) and semantic (p=0.007) fluency tests of the Montreal Cognitive Assessment and in the retroactive interference evaluation of the Rey Auditory Verbal Learning Test (p=0.032). Those results represent impairments on the attention, automatic cognition and executive function (inhibitory control).

Conclusions: it was possible to identify a decline of the cognitive function among the omeprazole users in comparison to the performance of the not users, which suggest a possible connection between the long-term omeprazole use with the decline on the cognitive function of patients undergoing treatment for longer than 6 months. However, it is necessary to increase the sample studied in order to obtain more reliable results.

Keywords: omeprazole; ppi; long-term use; cognitive function; decline.
Theme: Anti-infective drugs

COMPARISON OF TWO CARBAPENEMS KINETIC DISPOSITION AT THE EARLIER PERIOD OF SEPTIC SHOCK IN CRITICALLY ILL BURN PATIENTS BASED ON DRUG PLASMA MEASUREMENTS
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Background: Carbapenem recommended dose usually cannot achieve the target in critically ill septic patients from the Intensive Care Unit (ICU) against the most common MIC 2 mg/L strains due to pharmacokinetics changes that can impact the desired outcome [1]. The aim of the study was to compare changes on the pharmacokinetics of meropenem versus imipenem at the earlier period of septic shock in adult ICU burn patients receiving the recommended daily dose. Methods: Ethical approval was obtained N. 069/09-2015. Thirty-seven burn patients, 13F/14M were 28 yrs, 70 kg, 33% total burn surface area (medians), inhalation injury (32/37), renal preserved function were included. Patients received meropenem 1g q8h or imipenem 0.5 g q6h, both by 0.5hr infusion. Only two blood samples were collected (1.5 mL/each) at the steady state for drug serum measurement by liquid chromatography [2]. Pharmacokinetics (PK) results from burn patients were compared between the groups and furthermore with data previously described in healthy volunteers [3]. Results: PK data are shown in figure 1. It was registered significant changes on PK parameters by comparison of burn patients with healthy subjects. Conclusion: Important changes on pharmacokinetics occurred in a different manner, at the earlier period of septic shock, once the volume of distribution of imipenem was increased by trice compared to meropenem. Drug plasma monitoring done in real time in septic burn patients can be important to assess PK changes that can impact drug effectiveness and the desired clinical outcome.


Keywords: Septic shock; Pharmacokinetics; Meropenem versus imipenem; Burn patients
**Theme:** Anti-infective drugs

**VANCOMYCIN PHARMACOKINETIC-PHARMACODYMANIC APPROACH PERMITS DOSE ADJUSTMENT IN A REAL TIME FOR THE CONTROL OF SEPTIC SHOCK CAUSED BY GRAM-POSITIVE PATHOGENS IN CRITICALLY ILL PAEDIATRIC BURNS**

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**Background:** Paediatric septic ICU burn patients present metabolic conditions that change pharmacokinetics with impact on pharmacodynamics during severe infections (1). Effective vancomycin dose regimen in ICU pediatrics is a challenge to clinic staff, once the initial dose recommended cannot reach the target against MIC 1mg/L strains; then therapy fails with impact on desired outcome (2). The rational of study was to apply vancomycin serum monitoring through pharmacokinetic-pharmacodynamic approach to perform dose adjustment in a real time. **Methods:** 20 septic pediatric burn patients 6F/14M were investigated: 5.0 (3.0-9.0) yrs, 22 (16-38) kg ideal body weight, (20-48) 30(20-39%) total burn surface area, CLcr 240 (201-295) ml/min, medians (quartiles). Therapy started with empirical dose regimen 10-15mg/kg q6h. Predictive index of drug effectiveness based on area under the curve:MIC ratio was AUC\(_{\text{0-24h}}\)/MIC>400. Only two blood samples were collected (2 mL/each). Serum levels were obtained by liquid chromatography. **Results:** Target was reached in 50% patients after the initial dose against MIC 1mg/L gram-positive strains. If therapy fails dose was adjusted; the target was reached against MIC 1mg/L in all patients, while it was attained in 65% (13/20) patients against MIC 2mg/L strains. **Conclusions:** Vancomycin serum levels and the predictive index of drug effectiveness AUC\(_{\text{0-24h}}\)/MIC>400 must be considered as important tools for dose adjustment in order to eradicate nosocomial gram-positive pathogens in ICU pediatric burns. **Reference:** (1) Gomez et al., 2013 (2). Santos et al., 2015. **Keywords:** PK/PD approach; Septic paediatric burns; Vancomycin serum monitoring; Dose adjustment in a real time.
SIMULTANEOUS QUANTIFICATION OF PLASMA LEVELS OF 12 ANTIMICROBIAL AGENTS USED IN INTENSIVE CARE UNIT USING UPLC-MS/MS METHOD

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Background: Critically ill patients in intensive care unit (ICU) are susceptible to infectious diseases. Empirical therapy for methicillin-resistant Staphylococcus aureus, Pseudomonas aeruginosa and candida spp., which are the major pathogens in ICU, is recommended. However, it is difficult to predict the therapeutic effect in ICU patients because of pharmacokinetic fluctuation due to various factors such as sepsis, organ failure, and continuous renal replacement therapy. Although therapeutic drug monitoring can overcome such problems, analyses of multiple drugs take much time and require many processes, because patients in ICU are often administrated more than one antimicrobial agent including antifungals. Therefore, we developed simultaneous quantification of 12 antimicrobial agents commonly used in ICU, including antibiotics, antifungals and metabolite of an antifungal agent, using UPLC-MS/MS method. Methods: Plasma protein was precipitated by adding acetonitrile and 50% MeOH containing standard and labeled internal standard. The analytes were separated with an ACQUITY UPLC® CSH C18 column, under a gradient mobile phase consisting of water and acetonitrile containing 0.1 % formic acid and 2 mM ammonium formate. The assay was validated according to the Food and Drug Administration guidance on bioanalytical method validation. Results: Using plasma samples from six healthy volunteers, no interfering peaks derived from plasma matrix were observed. The calibration curve had a good linearity (R² ≥ 0.9984) in the calibration range, which was sufficiently wide to cover the C_max and C_trough of all of the drugs. For all drug measurements, within batch and batch-to-batch precision of 3 QC samples were less than 15.0% and 12.4%, respectively. Within batch and batch-to-batch accuracy ranged from 90.4 to 113.7% and 94.6 to 117.9 %, respectively. Average recovery rates of all the drugs were higher than 84.8 %. Matrix effect of the drugs was 61.3-119.3%. Conclusions: We succeeded to develop a sensitive and selective method using UPLC-MS/MS for simultaneous quantitative measurement of 12 antimicrobial agents in plasma.

Keywords: Therapeutic drug monitoring; UPLC-MS/MS; Antibiotics; Antifungals; Intensive care unit.
THE EFFECT OF TIGECYCLINE ON COAGULATION FUNCTION IN PATIENTS WITH PULMONARY INFECTION BY ACINETOBACTER BAUMANNII

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ABSTRACT: OBJECTIVE To evaluate the effect of tigecycline on coagulation function in patients with pulmonary infection by acinetobacter baumannii and to guide the rational use of tigecycline in clinical. METHODS Clinical materials and coagulation parameters of 81 ICU patients with pulmonary infection by Acinetobacter baumannii were collected for retrospective analysis. Tigecycline concentrations in serum were quantified by using a validated HPLC methodology. RESULTS With tigecycline treatment, the mean values of prothrombin time (PT), activated partial thromboplastin time (APTT) and D-dimer (D-D) were increased significantly (p < 0.05), plasma fibrinogen (FIB) level decreased significantly (p < 0.05). No significant differences in the level of platelet count (PLT) and thrombin time (TT) were found. Nevertheless, female, patients with high tigecycline peak concentration or long-term use of tigecycline were more likely produce uncommon coagulation indexes. CONCLUSION Therapeutic drug monitoring of tigecycline can serve as a good tool for individualized medication and coagulation level should also be monitored routinely.

KEY WORDS: tigecycline; acinetobacter baumannii; pulmonary infection; coagulation function
Theme: Anti-infective drugs

SIMULTANEOUS QUANTIFICATION OF AMIKACIN, VANCOMYCIN AND CREATININE IN PLASMA EMPLOYING UPLC-MS/MS

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Background Vancomycin (VAN) and amikacin (AMI) are largely used in the treatment of severe infections in intensive care. Therapeutic drug monitoring is largely applied for AMI and VAN, especially because of their narrow therapeutic range and high intra and interindividual pharmacokinetic variability. AMI and VAN are mainly eliminated by glomerular filtration, presenting a body clearance highly correlated to creatinine (CRE) clearance, making plasma creatinine levels an important parameter for the clinical use of population PK models for these antibiotics. The aim of this study was to develop and validate a method for the simultaneous determination of AMI, CRE and VAN in human plasma, using UPLC-MS/MS. Methods: Aliquots of 50 µL of spiked plasma were added with 50 µL of acetonitrile containing internal standards (kanamycin B and creatinine-D3 at 10 µg mL−1), followed by vortex mixing. Supernatant (70 µL) was diluted 1:3 with 0.1% formic acid and 1 µL was injected into the UPLC system. Separation was achieved on an Acquity HSS T3 (100 x 2.1 mm, p. d. 1.8 µm) column, eluted with mobile phase composed of purified water and acetonitrile, both containing w 0.1% formic acid and 0.01% HFBA. Calibration curves were in the range of 5-100 µg mL−1 for CRE and 0.5-100 µg mL−1 for AMI and VAN. Quality control (QC) samples were prepared at the concentrations of 6, 20 and 80 µg mL−1 for CRE and 1.5, 7.5 and 75 µg mL−1 for AMI and VAN, and the assay was validated according to international guidelines. The method was applied to 22 patients in treatment with AMI. Results: Analytical run time was 5.5 min with retention times of 0.92, 0.93, 2.12, 2.17 and 2.27 min for CRE, CRE-D3, AMI, KAN and VAN, respectively. Intra-day imprecision was in the range of 4.7-6.0% for AMI, 5.0-5.8% to VAN and 2.3-3.0% for CRE. Inter-day imprecision varied between 3.8-5.6% for AMI, 4.9-6.9% to VAN and 3.8-4.7% for CRE. Accuracy was in the range of 94.2-99.7%, 98.3-102.2% and 101.4-107.7% for AMI, VAN and CRE, respectively. The assay was applied to 22 clinical specimens, with AMI concentrations in the range of 0.6 to 113.6 µg mL−1 and CRE concentrations in the range of 5.0 to 72.3 µg mL−1 for CRE. Four samples presented CRE concentration lower than 5 µg mL−1. Conclusions: An easy and rapid method for simultaneous determination of AMI, VAN and CRE in plasma was developed and validated. The concentrations of AMI and CRE varied widely in plasma samples, especially because of inadequate empirical dosage, confirming the importance of this method to AMI therapy optimization. Keywords: Amikacin; creatinine; UPLC-MS/MS; pharmacokinetics; therapeutic drug monitoring; vancomycin.
Background: The aminoglycoside antibiotic amikacin is commonly used in pediatric cystic fibrosis (CF) patients. Mostly, amikacin efficacy is related to maximum plasma concentration/minimum inhibitory concentration (Cmax/MIC) ratio >8. Nevertheless, pharmacokinetic data in pediatric CF patients are scarce and recommendations on the therapeutic target are empirical. The objective of this study was to develop a population pharmacokinetic model describing amikacin disposition in pediatric CF patients. Methods: CF patients less than 18 years old with pulmonary exacerbation who received IV amikacin were enrolled. Patients received different amikacin dosage regimens (30 mg/kg/day every 8h [Q8], 12h [Q12] or 24h [Q24]) depending on physicians’ clinical criteria and the hospital protocols. Amikacin serum levels were obtained for therapeutic drug monitoring. A population pharmacokinetic model was developed using MONOLIX Suite-2018R1 (Lixoft, France) with 114 amikacin concentrations obtained from 39 patients. Results: Population estimates for the elimination rate constant (k) and the volume of distribution (V) were 0.541 h⁻¹ and 0.451 L/kg, respectively. Between-subject and between-occasion variability were 53% and 16.5% for k and 31% and 22% for V, respectively. Body-weight remained a significant covariate associated with V. One third of the patients receiving 30 mg/kg/day amikacin once daily, (see figure: comparison of predicted distribution for amikacin concentration vs time in hours) would achieve a Cmax/MIC of 10, which is an appropriate therapeutic Cmax/MIC goal while no patient would achieve that objective in the other two groups (Q8 and Q12). Conclusions: 30 mg/kg Q24 regimen fulfilled more adequately with the therapeutic aim for amikacin. Although all our patients had good clinical results and a very good adverse events profile, further studies are necessary to redefine an optimal treatment strategy.

Keywords: amikacin; population pharmacokinetics; cystic fibrosis; pediatrics.
IDENTIFYING FACTORS AFFECTING THE PHARMACOKINETICS OF VORICONAZOLE IN PATIENTS WITH LIVER DYSFUNCTION: A POPULATION PHARMACOKINETIC APPROACH

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Background: Voriconazole is a broad-spectrum antifungal agent commonly used to treat invasive fungal infections. Voriconazole has significant intraindividual and interindividual pharmacokinetics variability in different patient populations. Pharmacokinetic data of voriconazole in patients with liver dysfunction is limited. The aims of this study were to evaluate the population pharmacokinetics of voriconazole in patients with liver dysfunction, and to identify the factors that affect voriconazole pharmacokinetics.

Methods: A population pharmacokinetic analysis was based on 166 samples taken from 57 patients with liver dysfunction. The population pharmacokinetic models were developed by NONMEM software to determine the relationships between the pharmacokinetic parameters of voriconazole and covariates.

Results: A one-compartment pharmacokinetic model with first-order absorption and elimination adequately described the data. Voriconazole clearance (CL) was 0.58 L/h, the volume of distribution (Vd) was 134 L, and oral bioavailability (F) was 80.8%. Platelet count was significantly associated with CL, while CYP2C19 polymorphisms had no effect on voriconazole pharmacokinetic parameters.

Conclusions: The study identified platelet count as being significantly associated with voriconazole pharmacokinetic parameters. The CL of voriconazole was significantly decreased in patients with liver dysfunction. This study provides useful pharmacokinetics information for patients with liver dysfunction while highlighting the value of therapeutic drug monitoring in adjusting doses.

Keywords: voriconazole; population pharmacokinetics; liver dysfunction; therapeutic drug monitoring; CYP2C19 polymorphisms
A COMMERCIAL SOLUTION (XSOLUTION®) FOR ULTRA-FAST SAMPLE PREPARATION OF HAIR, URINE AND ORAL FLUID FOR DRUG DETERMINATION USING UPLC/MS/MS

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Background: An easy and efficient sample preparation method was developed for the determination of amphetamines, methamphetamines, cocaine, opiates, opioids, benzodiazepines, cannabinoids, and z drugs in hair, oral fluid and urine. A diluted and shot sample was applied for urine (53 drugs analyzed) and oral fluid (47 drugs analyzed). 50 µL of urine or oral fluid was diluted with xsolution® and injected into Waters® UPLC/MS/MS, Xevo TQS micro. For hair drug screening and confirmatory analyses, 10 mg of hair (45 drugs analyzed) was weighted in a 2 mL tube and xsolution® extraction formula was add, after 4 minutes of rigorous agitation and centrifugation, an aliquot of 1.5 µL was injected and analyzed in a single chromatographic run with 80 seconds. For cannabis use confirmation, 30 mg of hair was used at the same protocol (LOD 0.15pg/mg). The chromatography run time was 2.2 min. Reverse phase separation was performed with the Acquity BEH C18 chromatographic column (50 and 100 × 2.1mm×1.7µm - Waters®). The xsolution® sample preparation method was fully validated, and the cutoff levels applied were based in EWDT and SOHT, the majority of LOQ was performed 60% below the recommended cutoff value. Proficiency sample tests as ARVECON, CONTROLLAB, ACQ-SCIENCE, SOHT and real samples were analyzed using xsolution® sample preparation. The xsHolution® recovery was compared with kinetic methanol extraction using different time of incubation (2h; 4h; 6h; 10h; 15h and 24h). Results: For hair analyses validated data, was in according of ISO17025/2017 guide and Brazilian Department of Transportation (DENATRAN) regulation. For urine and oral fluid, also was validated based on ISO17025/2017 guide and UNODOC. Volunteer samples were for THC, THCCOOH, cocaine, norcocaine, AEME, cocaethylene, codeine, amphetamine, MDMA and MDA, some isolated or in concomitant with more than one class of drug. Xsolution® presented better extraction efficiency and recovery when to compare the results to the incubation extraction methanol. Some drugs would be considered negative in methanol process but in Xsolution® are confirmed positive. Conclusions: Xsolution® protocol demonstrated to be more effective in the process of extracting real positive hair samples for the evaluated drugs when compared to methanol extraction in different incubation time. For proficiency results, the score, for all rounded, including urine, oral fluid and hair, was greater than 90%. Keywords: xsolution®, hair, drug of abuse.
USE OF TOXICOLOGICAL HAIR TEST TO EVALUATE CANNABINOID USE (MARIJUANA) IN JUDICIAL PROCEEDINGS - REPORT OF A CASE

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Background: Forensic toxicological analyzes involve the application of toxicology for legal purposes, where the results generated will represent an important tool for the final decision making of the legal process. The main advantage of hair is the ability to provide historical consumption data for months, increase or decrease profile or even the cessation of chronic use. The present work will present a case where a toxicological analysis of hair with judicial purpose was used to evaluate the possibility of obtaining custody of a minor. Methods: The method used is validated and accredited in the ABNT ISO/IEC 17025:2017 standard. It consists of washing hair for removal of external contamination, sample pulverization, subsequent solid phase extraction and LC-MS / MS analysis. 14 segments of hair of different lengths were analyzed in different periods, covering the period from 08/20/2018 to 01/28/2019. Results: The figure shows the segments analyzed in relation to the studied period. The report of cocaine use during the September holiday and before can be confirmed through the results of segments 1 to 5 of Figure, where the highest concentration of cocaine is in segment 4 and decreasing in segments 3, 2, 1 and 5. A concentration is in accordance with his report. The specific metabolite of crack use, the AEME, was found. The donor does not specifically report that he used crack, but confirms that the crack was present in the environment and that he may have put the crack along with the marijuana weed in the preparation of the cigarette. The pattern of consumption follows the same pattern of cocaine. He reported consistent but not daily use of marijuana, but confirms that he made use of larger and daily amounts in the Christmas and New Year period, which can be proven through segments 10, 11, 13, and 14. He decreased the use of marijuana on the dates close to the collection date, which can be proven by the negative or low results of segments 1, 6, 9 and 12. Conclusions: Segmental hair analysis may provide useful information regarding the history of drug administration. The results of the washing samples were negative, which confirms that the THC values are due to consumption and not to external contamination. The concentrations found, the pattern of use and the substances used could be confirmed in relation to the donor’s report and this analysis can thus be a very useful tool in the legal area, helping judges and lawyers to better handle cases like this.

Keywords: hair; toxicology; cocaine; thc; cannabinoid; hair test.
A HIGHLY SENSITIVE LC-MS/MS ASSAY FOR ANALYSIS OF AMPHOTERICIN B IN PLASMA, URINE AND CEREBROSPINAL FLUID: APPLICATION TO PHARMACOKINETICS IN A PATIENT WITH NEUROCRYPTOCOCCOSIS

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Background: Neurocryptococcosis is a subacute meningoencephalitis most commonly affecting patients diagnosed with HIV. Amphotericin B (AMB), the drug of choice for the treatment, presents low permeability into the blood-brain barrier. The assessment of the pharmacokinetics (PK) of AMB in these patients requires the development and validation of the methods of analysis of AMB in plasma, urine, and cerebrospinal fluid (CSF) with sensitivity compatible with the expected low concentrations in CSF. Methods: Plasma and urine samples (25 µL), and CSF samples (100 µL) were precipitated with 0.1% formic acid in acetonitrile after the addition of piroxicam (internal standard, IS) and injected into the chromatographic system XEVO TQ-S® (Waters Corp.). The separation was performed on a LiChrospher® 60 RP-Select B column (Merck) using a mixture of water:acetonitrile (40:60, v/v, for plasma and urine; 50:50, v/v, for CSF) with formic acid 0.1% as mobile phase. The monitored transitions were m/z 906 → 743 for AMB, and m/z 332 → 95 or 332 → 121 for the IS. The methods were linear in the ranges of 5–1000 ng/mL for plasma and urine, and 0.1–250 ng/mL for CSF. Inter- and intra-assay precision and accuracy values presented deviations below 10%, and the obtained limits of quantification were inferior to 12% for all the three matrices. The methods were applied to the investigation of the PK and renal excretion of AMB (Abelcet®, 100 mg, 6h-iv-infusion) in an HIV patient undergoing neurocryptococcosis treatment. Serial blood samples were collected up to 24 h (dose interval) after the start of the iv-infusion; urine was collected during 24 h. AMB PK parameters were calculated with Phoenix® WinNonlin® software (Certara USA, Inc.). Results: The obtained PK parameters were AUC0–24h = 5402.53 h-ng/mL, Clss = 327.62 mL/h·kg, Vss = 11.86 L/kg and Frel = 1.46. Conclusions: The methods were successfully applied to the study of PK and renal excretion of AMB in an HIV patient, and the amount recovered in the urine suggest there is accumulation in the 24 h-dose interval. The method for the quantification of AMB in CSF is the most sensitive to date. Keywords: amphotericin B; neurocryptococcosis; pharmacokinetics; LC-MS/MS.
PHARMACOKINETICS OF BETAMETHASONE IN TWIN PREGNANCY

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Background: The main complication of twinning is prematurity, which occurs in 30 to 50% of cases and may result in Acute Respiratory Distress Syndrome and neonatal death. In singleton and multiple pregnancies, when there is a risk of preterm delivery, antenatal administration of corticosteroids is recommended to stimulate fetal pulmonary maturation. There are controversies about the benefits of corticosteroids and evidences that their pharmacokinetics are different in twin pregnancies. This study aims to compare the pharmacokinetics of betamethasone between singleton and twin pregnancies at risk of preterm delivery. Methods: After antenatal administration of 12mg of betamethasone intramuscularly, serial samples of maternal blood were collected for pharmacokinetic analysis. Chromatographic analysis of betamethasone was performed by LC-MS/MS and pharmacokinetic parameters were evaluated using the Phoenix® WinNonLin® software. Results: Seventeen pregnant women were included in the control group (n=9, singleton pregnancy) and twinning group (n=8, dichoronic pregnancies). The test for statistical analysis was Mann-Whitney with significance level set at p <0.05. The pharmacokinetic parameters found for the control group were C<sub>max</sub> 45.31ng/mL, t<sub>max</sub> 1.79 h, AUC<sub>0-∞</sub> 533.40 ng.h/mL, t<sub>1/2</sub>0.44 h, Ka 1.59 h<sup>-1</sup>, t<sub>1/2</sub>el 6.68 h, K<sub>e</sub> 0.11 h<sup>-1</sup>, Cl/F 11.26 L/h, Vd/F 102.10 L; and for twin group were C<sub>max</sub> 40.22ng/mL, t<sub>max</sub> 1.94 h, AUC<sub>0-∞</sub> 337.80 ng.h/mL, t<sub>1/2</sub>0.60 h, Ka 1.16 h<sup>-1</sup>, t<sub>1/2</sub>el 4.86 h, Kel 0.14 h<sup>-1</sup>, Cl/F 17.76 L/h, Vd/F 115.90 L. Conclusions: The pharmacokinetic parameters obtained indicate that in twin pregnancies the elimination half-life was significantly lower and the elimination rate constant was significantly higher when compared to the control group (box plot). These findings demonstrate the need for further studies relating pharmacokinetics and the effects of corticosteroids in newborns and suggest the necessity of different doses of the drug when considering twinning.

Keywords: Pharmacokinetics; betamethasone; twin pregnancy; prematurity.
APPLICATION OF A MU OPIOID RECEPTOR BIOASSAY PLATFORM TO GAIN INSIGHT INTO THE
SIGNALING OF NOVEL SYNTHETIC OPIOIDS

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Background: Fentanyl and morphine are agonists of the Mu opioid receptor (MOR), which is a member of the G protein coupled receptor (GPCR) family. While, on a signalling level downstream from MOR, analgesia is mainly mediated through the G protein pathway, the undesirable effects of opioids have been linked to the β-arrestin pathway. Little is known about a potential ‘bias’ (i.e. the preferential activation of one pathway over the other) of the new synthetic opioids-including fentanyl analogs- that have emerged on the illegal drug market. We have therefore developed a novel, robust bioassay platform to study the activity of synthetic opioids through both the G protein and β-arrestin pathways, in order to evaluate to what extent these MOR agonists show biased signalling. Methods: The bioassays are based on functional complementation of two split fragments of Nanoluciferase (LgBiT and SmBiT), either fused to the receptor or to a cytosolic protein such as mini G protein (GTPase subunit of Gα subunit as a measure for the G protein pathway) or β-arrestin2, that is recruited to the receptor upon activation. These assays were used to evaluate a panel of 10 fentanyl analogs (fentanyl, acetylfentanyl, valerylfentanyl, tetramethylcyclopropylfentanyl, tetrahydrofuranylentanly, ocfentanil, methoxyacetylfentanyl, cyclopropylfentanyl, cyclopropylfentanyl and crotonylfentanyl) in a 96-well plate format. Concentration-response curves were generated to yield Emax and EC50 values that were finally used to calculate the ligand bias.

Results: In the assay set-ups, MOR fused to LgBiT is combined with either β-arrestin2 or mini Gi fused to SmBiT. All tested fentanyl analogs demonstrated a concentration-dependent response at MOR in both bioassays. Only two of the ten ligands, valerylfentanyl and crotonylfentanyl, showed a significant bias (of approximately 2.5-fold) towards the G-protein pathway when compared to the unbiased ligand, hydromorphone. On the other hand, fentanyl was found to be more biased towards the β-arrestin pathway, in line with literature. Other fentanyl analogues showed no preference towards either of the pathways and hence, can be considered unbiased ligands. Conclusions: The developed bioassays allow to gain insight into the signalling of synthetic opioids, which may eventually help to better understand why certain opioids are associated with higher toxicity. The knowledge that is gained via these bioassays could possibly also help to develop novel ligands devoid of side effects, with a better therapeutical profile.

Keywords: Mu opioid receptor; new psychoactive substances, mini G protein, β-arrestin; biased signaling; designer opioids
Theme: Clinical Toxicology/drugs of abuse

ASSESSMENT OF BIASED AGONISM AMONGST DISTINCT SYNTHETIC CANNABINOID RECEPTOR AGONISTS SCAFFOLDS

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Background: Cannabinoid receptors are considered as key drug targets for a number of diseases. Most of the typical orthosteric cannabinoid ligands still provoke adverse psychotropic side effects that impair their therapeutic utility. Although the research on biased signaling of synthetic cannabinoid receptor agonists (SCRAs) is still in its infancy, the outcome of these studies could contribute to a better understanding of the exact mechanism of action of these compounds. Therefore, we have developed and applied bio-assays based on the recruitment of different transducers (G\(_{α_i}\)-protein or β-arrestin2) to the activated cannabinoid receptor 1 (CB\(_1\)) to assess the occurrence of biased signaling among SCRAs. Methods: The complementation-based bio-assays require the fusion of both the transducer and the CB\(_1\) to one part of a split luminescent protein, the Nanoluciferase. The development of the stable CB\(_1\);β-arrestin2 cell system has been reported previously (Cannaert et al., Anal Chem, 2017). A similar approach, based on retroviral transduction, for the generation of a stable CB\(_1\);mini-G\(_{α_i}\) cell system was executed in this study. Both stable cell lines were used for the simultaneous screening of a panel of 21 SCRAs for their preference in provoking the recruitment of the mini-G\(_{α_i}\)-protein or β-arrestin2. For quantification of the ligand bias, data were normalized to the efficacy of CP55,940 (arbitrarily set as 100%), which served as the ‘balanced’ reference compound. Relative intrinsic activities were calculated from the efficacy (E\(_{max}\)) and potency (EC\(_{50}\)) values, to quantify biased agonism. Results: For most of the selected SCRs (e.g. 5F-APINACA, CUMYL-PEGACLONE, among others) a higher efficacy for the β-arrestin2 read-outs was observed in comparison to the signal provoked by mini-G\(_{α_i}\)-protein recruitment. In contrast, MMB-CHMICA showed no difference in the EC\(_{50}\) nor E\(_{max}\) values in both bio-assays, which means it could serve as potential ‘balanced’ agonist. Interestingly, EG-018 showed a pronounced (10-fold) preference towards G-protein over β-arrestin2 recruitment. Conclusions: The CB\(_1\) activation bio-assays allow the observation of biased agonism among SCRAs in the same cellular context and using the same functional assay. Therefore, these in vitro cell-based techniques, which are based on the recruitment of mini-G\(_{α_i}\)-protein or β-arrestin2, can be applied to screen for the occurrence of biased agonism of a broad panel of SCRs. This might allow a better insight into the structure-‘functional’ activity-relationship of these compounds, which may aid the development of new therapeutic compounds with less unwanted psychoactive effects. Keywords: synthetic cannabinoid receptor agonists; biased signaling; activity-based; bio-assay; NPS.
UNUSUAL BODY PACKING CASE: CANNABIS INTO THE PRISON SYSTEM
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Background: A 44 years-old man was admitted to emergency service, coming from the prisional system, with suspected ingestion of wrapped packets of unknown psychoactive substance. During the early medical service he presented agitation, violent behavior and convulsion, requiring administration of haloperidol and diazepam. Minutes later, he evolved with lowering of consciousness and needed endotracheal intubation. In endoscopy, it was possible to analyze the presence of plastic material loose, apparently packages' leakage, along with a package blocking the passage of the esophagus, and a laparotomy was performed to remove it. Patient had postoperative paralytic ileus, severe asthma, and bronchospasm. During hospitalization, he presented intercurrences as hypotension followed by hypertension and tachycardia, evolved to severe edema in the lower members and evisceration. Patient only started being able to expel the packages in the 19th day of hospitalization. Methods: Admission toxicological screening in the service performed by urine immunoassay the day he entered the emergency service and also twelve days later. Also plasma samples were analyzed on the day of operation and also twelve and eighteen days later, by gas chromatography-tandem mass spectrometry (GC-MS/MS) toxicological drug screening. Results: In the admission, immunoassay detected cannabinoids and cocaine, which made doctors suspect cocaine intoxication. In a new screening during the hospitalization, urine presented positive for cannabinoids. The amount of THC found in the samples was 2.3 ng/ml on day 1, 0.8 ng/ml on day 2 and 1.0 ng/ml on day 3. The amount of 11-hydroxy-THC (OH-THC) found in the samples was 6.5 ng/ml on day 1, 3.4 ng/ml on day 2 and 3.5 ng/ml on day 3. And the amount of THC-carboxylic (THC-COOH) found in the samples was 172.4 ng/ml on day 1, 10.9 ng/ml on day 2 and 11.8 ng/ml on day 3. The cocaine detected in the first urine screening was consequence of a previously use, not from the packages. Plasma THC concentrations rapidly decline as a result of tissue distribution and extensive metabolism, but in the case of the patient, even after 18 days of hospitalization, the levels found were constant (about 1.0 ng/ml), indicating absorption of the substance. The last quantifying analysis (of OH-THC) (about 3.4 ng/ml) also indicated absorption because it does not accumulate due to its rapid oxidation to THC-COOH. Conclusions: Here we presented an unusual cannabis body packing case.
Keywords: Body Packer; Cannabinoids; GC/MS.
DEVELOPMENT AND VALIDATION OF ANALYTICAL METHOD TO ANALYZE BENZODIAZEPINES IN ORAL FLUID SAMPLES BY LC-MS/MS

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<td>8.9 4.6</td>
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<tr>
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<td>8.6 9.8 8.5</td>
<td>2.0</td>
<td>7.0</td>
<td>13.3 5.5</td>
</tr>
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<td>15.2 12.3 8.2</td>
<td>4.2</td>
<td>14.8 4.7</td>
<td>8.9 4.6</td>
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<tr>
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<td>2.0</td>
<td>4.9</td>
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<td>9.6</td>
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<td>17.8</td>
<td>17.5</td>
<td>5.5 5.3</td>
</tr>
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</table>

Table 1. Values obtained from the method validation.

Background: Benzodiazepines are commonly used as sleeping pills, to treat anxiety disorders, convulsions as well as preoperative sedation for outpatient surgery. According to European Monitoring Centre for Drugs and Drug Addiction (EMCDDA), in Europe (2012) benzodiazepines were the most often detected medicine in drivers and also the second more common substance found in fatal accidents. These drugs can affect brain functioning impairing cognitive and motor functions causing higher incidence of motor vehicle accidents. Methods: The method validation was performed by liquid-liquid extraction using 500 µL of oral fluid sample collected with Quantisal® device, 500 µL of saturated sodium tetraborate aqueous solution and 1 mL of methyl tert-butyl ether. 700 µL of the organic supernatant was transferred to a conic plastic tube, evaporated to dryness under nitrogen stream and ressuspended with 100 µL of solution with (A) water and (B) methanol, both containing 0.1% formic acid and 2mmol ammonium formate (80:20). 5 µL were injected in LC-MS/MS system, equipped with a Raptor biphenyl column (100 x 2.1 mm, 2.7 µm, Restek, USA). The method analyzes 10 analytes (benzodiazepines and z-drugs) using diazepam-d5 and clonazepam-d4 as internal standards. Mobile phase A was water containing 0.1% formic acid and 2mmol ammonium formate and mobile phase B was acetonitrile with a flow rate of 0.4 mL/min and a 9.5 min run. Data acquisition was programmed in MRM mode. Results: The method was validated following the SWGTOX guideline and was linear between 0.1-20.0 ng/mL and 0.5-25.0 ng/mL (1/x², r > 0.99). The limit of quantification (LOQ) was 0.1 ng/mL and 0.5 ng/mL. Other results and parameters are shown on Table 1. Conclusions: A sensitive method was developed to quantify benzodiazepines in oral fluid samples. The method will be now applied to real samples collected in Brazilian roads.

Keywords: benzodiazepines; validation; oral fluid; LC-MS/MS; SWGTOX.
VASCULAR ENDOTHELIAL GROWTH FACTOR A DIAGNOSTIC TEST FOR POEMS SYNDROME

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Background and aims:
POEMS syndrome is characterized by poly-neuropathy, osteo-sclerotic myeloma, organomegaly, endocrinopathy, monoclonal plasma cell disorder, and skin changes. We aim: (1) to demonstrate the utility of quantitative measurement of serum levels of VEGF in the diagnosis of POEMS and the monitoring of therapeutic interventions; (2) to demonstrate that overproduction of pro-inflammatory cytokines is a characteristic of POEMS. Methods: We studied 14 POEMS patients clinically presenting POEMS. We compare the serum levels of cytokines and chemokines between the POEMS patients with 80 patients with viral hepatitis C (HCV), 12 healthy controls, and 80 individuals with alcoholic liver disease (ALD). We quantified (ELISA pg/mL) the levels of VEGF, Interferon gamma (IFN-γ), Tumor Necrosis Factor alpha (TNF-α), Regulated-upon-Activation Normal-T-cell-Expressed and presumably-Secreted (RANTES), and Nuclear Factor kappa-B (NFκB).

Results: In POEMS patients, VEGF levels were 20 x elevated versus control, 16 x ALD, 14 x vs HCV. TNFα levels were 8x higher versus control, but significantly lower when compared with HCV or ALD patients. VEGF levels in POEMS patients decreased with therapeutic intervention. Interferon gamma (IFN-γ), RANTES levels were x10 vs, control, but not differed significantly from ALD and HCV. NFκB levels were not significantly different from HCV and ALD. The follow up of individual cytokine kinetics during the 4 years showed significant reduction in all parameters.

Conclusions: Extreme elevation of VEGF levels is diagnostic for POEMS syndrome, and should be followed to assess response to therapy.
**DEVELOPMENT AND VALIDATION OF A QUECHERS-UPLC-MS/MS METHOD FOR THE DETERMINATION OF 21 DRUGS OF TOXICOLOGICAL INTEREST**

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**Background:** The QuEChERS method (Quick, Easy, Cheap, Effective, Rugged, Safe) was initially developed for analysis of pesticide residues in plants. However, it was later adopted in analytical toxicology as an efficient alternative to overcome the disadvantages of conventional extraction methods, such as protein precipitation, liquid-liquid extraction and solid phase extraction. This study aims to develop and validate a quantification assay for 21 drugs relevant in the context of emergency toxicology (benzodiazepines, opioids, amphetamines, antidepressants, and cocaine metabolites), based on QuEChERS extraction from blood and UPLC-MS/MS analysis. **Method:** Aliquots of 250 µL whole blood were added with 25 µL of internal standard solution (D3-Trimipramine, 0.5 µg mL⁻¹ in methanol) and 0.5 mL of cold acetonitrile. To the same tube, 100 mg of a salt mixture composed of magnesium sulfate, sodium chloride and sodium citrate monohydrate (4:1:1, m/m/m) was added. After homogenization, followed by centrifugation at 13.000 rpm for 10 min, 300 µL of the upper organic layer was evaporated at 45 °C. The extract was recovered with 200 µL of mobile phase and 2 µL were injected into the UPLC-MS/MS system. Chromatographic separation occurred in an Acquity C18 column (2.1 x 150 mm, 1.8 µm) at 50 °C. The mobile phase was ammonium formate 5mM (A) and acetonitrile 0.1% formic acid (B), eluted in gradient mode at 0.4mL min⁻¹. Linearity, precision, accuracy and matrix effects were evaluated. The assay was applied to blood samples from 130 polytraumatized admitted at an emergency department. **Results:** method was linear from 25 to 1500 ng mL⁻¹, with accuracy of 92.2 to 104.6% and intra-assay precision within 1.3-10.2% and inter-assay precision within 2.0-11.9%, mean extraction yield was 60.6 to 97.7%. Matrix effects ranged from -2.7 to +4.4%, with exception of the early eluting compounds codeine (-35.4 to -28.2%) and EME (+15.9 to +17.9%). Among the 130 tested blood samples, 24 had positive results for one or more of the 21 evaluated compounds. The most frequently detected compounds were cocaine metabolites (11 samples) and benzodiazepines (9 samples). **Conclusions:** A QuEChERS-UPLC-MS/MS assay for the determination of multiple drugs was developed and validated, presenting acceptable characteristics for use in clinical and forensic toxicology.

**Keywords:** QUECHERS. Whole blood. Emergency toxicology. UPLC-MS/MS
OPTIMIZATION OF HAIR PULVERIZATION CONDITIONS USING NOVA 2200e TO ANALYSE THE SURFACE AREA IN THE VALIDATION OF ANALYTICAL METHODOLOGY FOR DRUGS DETECTION

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**Background:** Toxicological analyzes in hair allow a retrospective investigation of drug use, considering its wide window of detection. The pulverization step is critical in this process, because the analytes are extracted from the inside of the hair. Therefore, greater efficiency decreases the particle size distribution of the hair, and allows a higher rate of recovery of the analytes, associated with a high reproducibility. **Methods:** Initially 10 mg of hair, form the head, cut into small fragments were weighed into 2 mL Sarstedt microtubes. Three replicates were weighed, for each one of the different pulverization times: 6, 8 or 10 minutes, and, in additional, one test was frozen with liquid nitrogen before being pulverized for 8 minutes (8N), the use of liquid nitrogen aims to maintain the temperature controlled, during pulverization step. Then, 60 μL of methanol solvent was added into the eppendorfs, corresponding to 50 μL of the internal standard and 20 μL of the calibrator solution, foreseen in the drug extraction protocol, for further pulverization. The replicates 8N were frozen with liquid nitrogen and the other three were directly pulverized at their respective times. All pulverization tests were performed directly on the microtube tube, by adding two steel microspheres, with 5 mm of diameter, into it, using a RETSCH automatic mill, at a speed of 30 Hz, in their respective times. Posteriorly, the similar triplicates were combined to enable the analysis of the pulverized hair surface area by NOVA 2200e, with a heat treatment at 70 °C for 5 hours. **Results:** The surface area calculated at the time of 6 minutes was: 3,854 m\(^2\)/g; 8 minutes: 43,381 m\(^2\)/g; 8 minutes with nitrogen: 38,341 m\(^2\)/g; 10 minutes: 424,831 m\(^2\)/g. It is noted that the increase in pulverization time was directly proportional to the increase in capillary surface area, therefore a better pulverization efficiency. However, the pulverization process when comparing the similar time of 8 minutes, with and without prior freezing with nitrogen, showed no significant difference in surface area between these, showing that the freezing step did not improve pulverization efficiency. **Conclusions:** The increase in pulverization time positively affects the quality of the capillary particles distribution, increasing surface area between the particles. However, additional tests are required, such as analyte recovery in positive samples to obtain more accurate results. **Keywords:** hair, pulverization, toxicology, drugs, area surface.
Theme: Clinical Toxicology/drugs of abuse

SENSITIVE DETERMINATION OF THC-COOH IN HAIR BY ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY-TRIPLE QUADRUPOLE MASS SPECTROMETRY

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Background: Confirmatory analysis for the determination of Cannabis use in hair requires the quantification of its metabolite 11-nor-9-carboxy-tetrahydrocannabinol (THC-COOH). According to the recommendations of DENATRAN (Brazilian National Traffic Department), at least 0.2 ng g\textsuperscript{-1} of THC-COOH (the mandatory cut off level) must be present in hair sample to constitute a positive result in a drug test. We developed a sensitive assay for THC-COOH in hair using ultra-performance liquid chromatography coupled to a triple quadrupole mass spectrometer (UPLC-MS/MS), which fulfills the Brazilian regulatory requirements for confirmation of Cannabis use. Methods: Hair samples were cut in small pieces and 50 mg were transferred to a plastic micro tube. Then, 50 µL of internal standard solution (THC-COOH-D3 at 0.3 ng mL\textsuperscript{-1} in methanol) and 400 µL of the mixture methanol: ethyl acetate (1:1, v/v) were added, followed by incubation at room temperature for 4 hours, at 800 rpm. After incubation, an aliquot of 600 µL of water and 500 µL n-hexane were added to the micro tube, vortexed by 5 min and centrifuged. An aliquot of 500 µL of the extractive phase was separated to another micro tube and evaporated to dryness at 30 °C. The dried extract was recovered with 100 µL of acetonitrile: water (1:1, v/v), vortexed and then centrifuged at 14,000 g for 10 min. An aliquot of 10 µL of the supernatant was injected into the UPLC-MS/MS system. Separation was achieved on an Acquity UPLC BEH C18 column (100 x 2.1 mm, p.d. 1.7 µm), eluted at gradient mode with mobile phases composed of water containing 0.1 % formic acid and acetonitrile containing 0.1 % formic acid. The analytical system was composed of an Acquity I-Class chromatograph coupled to a Xevo TQ-S Micro Triple Quadrupole mass detector. Quality control (QC) samples were prepared at the concentrations of 0.25, 0.50 and 1.2 ng g\textsuperscript{-1} and the assay was validated according to international guidelines. Results: Chromatographic run time was 14 min. The assay was linear in the range 0.15-6.4 ng g\textsuperscript{-1}. Precision assays presented CV% of 5.27-10.19, and accuracy was in the range of 97.33-105.87%. The extracts had adequate autosampler stability, with variations of 1.87% for CQA and 10.86% after 15 hours. Matrix effect were observed in the range of 1.25-16.17%. The expanded measurement uncertainty at the cut off level was ± 5.15%. Conclusions: A sensitive method for determination of THC-COOH in hair using UPLC-MS/MS was developed and validated, with acceptable performance for forensic and clinical cases.

Keywords: COOH-THC; hair; UPLC-MS/MS; forensic toxicology; clinical toxicology.
Theme: Clinical Toxicology/drugs of abuse

**ASSAY PERFORMANCE OF THE NEW ARCHITECT® cSYSTEMS**

**6-ACETYL-MORPHINE, BUPRENORPHINE, EDDP, FENTANYL, OXYCOTIN, AND TRAMADOL URINE ASSAYS**

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¹Abbott Diagnostics, Immunoassay², USA

**Background:** Opioid use disorder and opioid addiction are reported to be reaching epidemic levels in the US and worldwide. Roughly, three million US citizens and 16 million citizens worldwide have had or currently suffer from opioid use disorder, and deaths attributed to or associated with opioid use disorders were estimated to be 118,000 in 2016. The drastic increase in opioid use disorder is at least partially due to overprescribing of opioid medications, yet these medications play an important role in pain management if prescribed and used as indicated to provide an effective modality for pain relief. In response to the risk of opioid misuse or abuse, more stringent recommendations for prescribing and monitoring patients have been made by medical professionals. These recommendations may include expansion of current urine drug screening panels to help meet guideline recommendations. This study characterizes 6 new opioid assays developed for use on the Abbott ARCHITECT cSystems. **Methods:** Precision, method comparison, linearity, specificity, and endogenous substance interferences were evaluated for each of the six assays on the ARCHITECT cSeries instruments. **Results:** All six assays evaluated met acceptance criteria for precision (<4% Qualitative; <10% Semi-Quantitative) using multiple target drug concentrations representing levels above, below and at the cutoff levels for each assay under investigation. Method comparison was performed against the AU400 which currently has 510(k) clearance for use or directly with LC-MS/MS. Results for each assay were 100% concordant (n ≥ 80). A range of known concentrations covering the dynamic range of each assay was tested to evaluate linearity. Acceptability criteria, < 15% difference from nominal values, were met with R² > 0.990 for each assay. Structurally similar compounds to the target analytes as well as other relevant compounds and endogenous substances were tested to evaluate specificity, resulting in little to no major interferences. **Conclusions:** Assay performance on the ARCHITECT cSystem instruments was found to be acceptable for accurately screening patients. Performance of all six assays met acceptability criteria for each analytical parameter investigated. Addition of these opioid specific assays to the Abbott ARCHITECT family of chemistry systems expands the current urine drug testing options available for testing and meets 92% of the recommended routine assays to be screened according the American Association of Clinical Chemistry (AACC). **Keywords:** Opioids, Urine Drug Screen, Architect, 6-AM, Buprenorphine, EDDP, Fentanyl, Oxycotin, Tramadol
Background: Non-adherence with antipsychotic treatment is a major health issue, and none of the current methods used to promote adherence work well. Recently, several authors have shown the possibility to detect drugs of abuse as well as pharmaceutical drugs from a fingerprint. The advantage of using a fingerprint as a sampling matrix is that it can be donated easily and painlessly. Furthermore, the fingerprint sample conveys the identity of the donor and therefore is not easily falsified. This gives an unexploited opportunity for unsupervised sampling. In this study, we are exploring the possibility to use fingerprints for the detection of four antipsychotics (risperidone, quetiapine, clozapine and olanzapine) and their metabolites.

Methods: Fingerprints from patients undergoing antipsychotic treatment (n=9 for quetiapine, n=7 for olanzapine, n=4 for risperidone and n=1 for clozapine) were collected on two different substrates (one porous, one hydrophobic) and were analysed for risperidone, olanzapine, quetiapine, clozapine and metabolites using liquid chromatography mass spectrometry (LC-MS).

Results: Separation of all four antipsychotics and their metabolites was successfully obtained using LC-MS. The linearity ($R^2$) of all calibration curves of analytes extracted from substrate were above 0.988. The limit of detection of the analytes were between 1 pg and 20 pg with an %RSD below 20%. From the patient samples, it was possible to obtain proof of concept data for the detection of all four antipsychotic drugs targeted and their metabolites.

Conclusions: We have demonstrated here the detection of antipsychotic and their metabolites in fingerprints for the first time using LC-MS.

Keywords: antipsychotic drug; fingerprint; therapeutic drug monitoring; LC-MS.
SCREENING OF NEW PSYCHOACTIVE SUBSTANCES, THC AND COCAINE IN URINE SAMPLES OBTAINED AT TWO MUSIC FESTIVALS IN THE METROPOLITAN AREA.

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Background: We present the results of two studies of effluent samples that were obtained in the context of two electronic music parties. The objective was to generate accurate information on the circulation in the Uruguayan territory of New Psychoactive Substances and other drugs of abuse that are consumed in these events. 

Methods: A total of 28 samples of the first party (2017) and 25 samples of the second party (2019) were analyzed by: immunological tests for the detection of: Opiates, Fentanyl, Synthetic Cannabinoids, Catinones, THC, Cocaine and its metabolites and 6-MAM and by GC/MS. The samples were processed by four different methods, in different physicochemical conditions to make possible, in each case, the extraction of drugs of diverse nature contemplating the neutral, basic, acid substances and those excreted as metabolites and/or conjugated. Each of the extractions were then analyzed by Gas Chromatography coupled to Mass Spectrometry (GC/MS).

Conclusions: As remarkable results in the first party was found: Synthetic Cannabinoids in 11% of the samples, Catinones in 52%, Cocaine/PBC/Cocaethylene and metabolites in 82%, Ketamine in 11%, Levamisol in 7%, LSD/LAMPA in 4%, MDMA in 71%, THC in 100%, and 2.5B-NBOMEs or bk-DMBDB were not detected. In the second party was found: 6-Acetylmorphine (Heroin) in 8% of the samples, indicating the appearance of consumption of this substance, Catinones in 52%, Cocaine/PBC/Cocaethylene and metabolites in 96%, LSD/LAMPA in 8%, MDMA in 64%, THC in 96%, 2.5B-NBOMEs in 4% and bk-DMBDB in 4% of the samples analyzed. Levamisole, Fentanyl and Ketamine were not detected. This work provides accurate and valuable information for Public Health of substances consumed in Uruguay, which are not normally seized, and cause intoxication.

Keywords: SAT; NPS; Drug Abuse
A NOVEL OBJECTIVE QUANTIFICATION OF ALCOHOL CONSUMPTION IN EARLY PREGNANCY: PHOSPHATIDYLETHANOL SCREENING IN A LARGE CROSS-SECTIONAL STUDY
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Background: Alcohol consumption during pregnancy is associated with major birth defects and developmental disabilities. The use of a reliable and objective biomarker for alcohol consumption enables more accurate screening for alcohol consumption during pregnancy. To determine the prevalence of alcohol consumption during pregnancy in a large urban population in a 40 university hospital in the Netherlands using phosphatidylethanol levels and self-reported alcohol consumption was conducted in a cross-sectional study from January 2016 till October 2017

Methods: A single center study in the obstetric outpatient clinic in the largest university hospital of the Netherlands. All pregnant women referred for pregnancy care to the outpatient obstetric clinic undergoing routine pregnancy laboratory testing, were eligible if below 15 weeks of gestational age (N=776). Women who did not consent to the use of residual material for research purposes and women under the age of 18 years were excluded (n=92). Convenience samples (from rest material) were used. Alcohol consumption during pregnancy was assessed by phosphatidylethanol in blood (FDA validated UPC2MS/MS method). The main outcome measure was Phosphatidylethanol above limit of detection in blood, indicating alcohol consumption in the previous two weeks.

Results: In total, 684 women were included. The mean age was 32 years (range 18-50), the median weeks of gestation was 10 weeks (range 5-15). Of these women, 5.3 percent (n=36) were tested positive for phosphatidylethanol, indicating alcohol consumption in the previous two weeks. Eleven percent (n=4) of women with a positive phosphatidylethanol test had reported alcohol consumption to their obstetric care provider.

Conclusions: A significant number of women consumed alcohol during early pregnancy, and the majority of these women reported no alcohol consumption to their obstetric care provider. Determining phosphatidylethanol in maternal blood can be of added value to identify women who consume alcohol during pregnancy.

Keywords: Pregnancy, Alcohol Drinking, Biomarkers, FASD, fetal alcohol spectrum disorder, PETH
SENSE DETERMINATION OF ETHYL GLUCURONIDE IN HAIR AS A BIOMARKER OF CHRONIC ETHANOL USE USING ULTRA-PERFORMANCE LIQUID CHROMATOGRAPHY COUPLED TO TANDEM MASS SPECTROMETRY (UPLC-MS/MS)

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Background: Ethyl Glucuronide (EtG) concentration in hair is an important biomarker of the chronic use of ethanol. EtG is a secondary metabolite formed by the conjugation of ethanol with glucuronic acid. EtG hair levels higher than 30 ng mg⁻¹ are considered indicative of chronic abusive use of ethanol (consumption of 60 g or more of pure ethanol per day, over several month). Methods: Hair samples were washed with dichloromethane and methanol. Washed hair was milled in a ball mill. EtG was extracted from milled hair (50 mg) with 1.5 mL of ultra-purified-water, with the addition of 25 μL of the internal standard solution (EtG-D5 at the concentration of 120 ng mL⁻¹). Water extraction was performed by ultra-sonication at 50 °C for 1.5 h, followed by incubation at room temperature for 24 h at 900 rpm. The mixture was centrifuged at 14.200 rpm for 10 min. The resulting supernatant was applied to Oasis® MAX SPE cartridges, previously conditioned with 2 mL of methanol and 2 mL of water. Cartridges were washed with 2 mL of 5% ammonia in water and 2 mL of methanol. After vacuum drying, EtG was eluted with 2 mL of 2% formic acid in methanol. The eluate was evaporated to dryness at 60 °C and re-suspended in 100 μL of initial mobile phase initial (95% A: 5% B). An aliquot of 10 μL was injected into the UPLC-MS/MS system. Separation was achieved on an Acquity UPLC® CSH™ Fluoro-Phenyl column, eluted with mobile phase composed of ultra-purified-water containing 0.1% formic acid (A) and acetonitrile containing 0.1 % formic acid (B). Linearity was evaluated in the range of 4-500 pg mg⁻¹. Quality control (QC) samples were prepared at the concentrations of 5.5, 80 and 450 pg mg⁻¹, and the assay was validated according to international guidelines. The assay was applied in hair samples from 46 patients. Results: Precision assays presented CV% of 3.02-10.42, and accuracy in the range of 100.30 - 103.16%. The extraction yield was satisfactory (96.93 - 101.06%). The assay was sensitive enough to detect the recommended cut off for the identification of ethanol abstinence. EtG was detected in all samples. Among tested samples, 20 (43.5%) were above the cut-off values established to characterize chronic consumption, 15 (32.6%) were within the concentration range established to characterize moderate alcohol consumption, and the remainder 11 (23.9%) were below the cut-off value for abstinence, of 7 pg mg⁻¹. Conclusions: A method for determination of EtG, using UPLC-MS/MS was developed and validated for use in forensic and clinical cases and was applied in 46 volunteer patients.

Keywords: Ethyl Glucuronide, Hair, Ethanol, Liquid Chromatography, Mass Spectrometry.
Theme: Clinical Toxicology/drugs of abuse

OPTIMIZED METHOD FOR THE DETERMINATION OF ETHYL PALMITATE IN HAIR USING AUTOMATED HEADSPACE SOLID-PHASE MICROEXTRACTION COUPLED TO GAS CHROMATOGRAPHY-MASS SPECTROMETRY (HS-SPME-GC-MS)

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Background: Abusive consumption of ethanol is a worldwide public health problem, and its characterization is relevant in forensic investigations. Fatty Acid Ethyl Esters (FAEE) are secondary metabolites formed from free fat acids, triglycerides, lipoproteins or phospholipids in the presence of ethanol, being important biomarkers of chronic use of ethanol. Recently, the determination of ethyl palmitate (EtP) hair levels instead of the combined concentration of FAEE as a marker of chronic ethanol consumption was proposed. EtP hair levels higher than 0.35 ng mg⁻¹ are considered indicative of chronic abusive use of ethanol (consumption of 60 g or more of pure ethanol per day, over several months). Methods: Hair samples were washed with a sodium dodecyl sulphate solution and with N-heptane, before milling. Acetone, methanol: dichloromethane (1:1, v/v), and dimethyl sulfoxide (DMSO): N-heptane (1:4, v/v) were evaluated as hair extraction solvent at extraction times of 0.5, 4 and 15 hours. After solvent evaporation, the dried extract was recovered with phosphate buffer 0.1M (pH 7.6), followed by HS-SPME-GC-MS. SPME was performed with a PDMS/DVB (65 µm) fiber. The optimal conditions for HS-SPME were selected by response surface analysis, after a Box-Behnken designed experiments. The following extraction variables were evaluated: pre-adsorption time of 5 to 15 minutes, adsorption time of 15 to 60 minutes and incubation temperature of 75 to 95 °C. Linearity was evaluated in the range of 0.05 to 3 ng mg⁻¹. The assay was validated according to international guidelines. Results: The optimized HS-SPME conditions were pre-adsorption time of 6 min, adsorption time of 60 min and incubation temperature of 94 °C, resulting in EtP peak areas approximately 33% higher than previously described. Retention time for EtP was 15.3 minutes with total run GC time of 26 minutes. The assay was linear in the evaluated range, with r higher than 0.99. The lower limit of quantification was 0.05 ng mg⁻¹, with acceptable precision and accuracy. Considering all QC levels, accuracy was 95.15-109.91%, between-assay and within-assay precision were 8.58-12.53% and 6.12-6.82%, respectively. The extraction yield was satisfactory. Samples were analyzed from 46 patients, all users of ethanol. EtP was detected in all samples, with 38 (82.6%) presenting concentrations higher than 0.35 ng mg⁻¹, characterizing chronic consumption of ethanol. Conclusions: A method for determination of EtP, using GC-MS-SPME-HS, was developed and validated for use in forensic and clinical cases. The optimization of extraction conditions allowed a response 33% higher than a reference method.

Keywords: Ethyl Palmitate, Hair, Ethanol, Gas Chromatography, Mass Spectrometry, SPME.
VALIDATION PROCESS OF ANALYTICAL METHODOLOGY TO QUANTIFY DRUGS OF ABUSE IN HAIR SAMPLES BY LC-MS/MS

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Background: Toxicological analyzes in capillary matrix allow a retrospective investigation of drugs use, because of its large window of detection. Abuse drugs and their metabolites, when present in hair, have very low concentrations, demanding methods of analysis with high sensitivity and specificity, consequently an extensive methodological validation. Methods: For the decontamination procedure, the hair was washed with ultrapure water for 2 minutes and then with methanol for another two minutes, both sonicated during the process. Posteriorly 20 mg of hair, from the head, without substances, were weighed into 2 mL Sarstedt microtubes, then 20 μL of the respective calibrators, 50 μL of the internal standard solution and 500 μL of the extraction solvent methanol were added, for further spraying. All pulverization tests were performed directly into the microtube, by adding two steel microspheres, with 5 mm of diameter, using a RETSCH automatic mill, at a speed of 30 Hz, for 5 minutes. The assays were incubated for 15 hours in ThermoMixer at a temperature of 50 °C and 1000 rpm, then the microspheres were removed with a magnet and the tubes were centrifuged. 1.5 μL of the extract was injected into LC-MS/MS. The validation process included tests of sensitivity, linearity, reproducibility, self-sampler stability, matrix effect and carry over. Results: Concentration ranges: 100 – 1200 ng/g for: morphine, 6-ACM, codeine, amphetamine, MDA, MDMA, femproporex, amfepramone; 250-3000 ng/g for: cocaine, mazindol; 25 – 300 ng/g for: tetrahydrocannabinol, AEME, norcocaine, cocaethylene, benzoylecgonine. The accuracy of the method was 86.63 to 105.87%, the intra-assay precision ranged from 3 to 13.5% and the inter-assay precision ranged from 1.65 to 12.02%. The stability of the extract inside the self-sampler ranged from -5.11 to 7.23% between time zero and 15 hours. The highest carry over effect observed after injection of a high control was 15.54%, relative to the concentration of the quantification limit, it was evaluated for the analytes and their analogous patterns, individually. The matrix effect ranged from -15.38 to 24.56%. Conclusions: A methodology for drug detection in hair by LC-MS/MS was validated, according to the international guidelines.

Keywords: hair; drugs; validation; quantify; LC-MS/MS.
Theme: Clinical Toxicology/drugs of abuse

CHARACTERIZATION OF PHARMACOKINETICS IN CAFFEINE OVERDOSE CASES
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Background: Cases of drug overdose are sometimes encountered in the emergency department. During treatment, prediction of the time-blood concentration profile of the causative drug may be crucial for treatment decisions. Pharmacokinetics (PKs) in cases of overdose are considered to be different from those in typical dose cases; however, the PK information in cases of overdose has not been determined sufficiently because of the impossibility of clinical trial and the infrequency of the incidence. Caffeine is one of the most common causative drugs; thus, we aimed to reveal its PK characteristics following massive ingestion. Methods: The population PK analysis was conducted by using dedicated software (NONMEM 7.4.3). In total, 22 points of blood concentrations from our seven and the reported five cases were included in the analysis. In most cases, one-point sampling was conducted within 5 h of ingestion, whereas four cases conducted multiple-point sampling at unprescribed timing during hospitalization. In our cases, blood caffeine level was measured by using gas chromatography-mass spectrometry. Results: The PK was described by a one-compartment model; and additive error was decided as preferred. The estimated typical values of clearance (CL) and volume of distribution (V) were smaller than those in typical dose cases (i.e., CL, 6.84 L/h; V, 41.5 L) reported by Seng et al.\textsuperscript{1} The elimination half-life calculated from our estimated parameters (i.e., approximately 16 h) was very close to that reported in a previous caffeine overdose case reported by Leson et al.\textsuperscript{2} Conclusions: Our PK parameters were suggested to reflect the PK characteristics in cases of caffeine overdose. These parameters may be applicable to the prediction of individual PK parameters or time-concentration prolife in a caffeine overdose case. [References: 1) Seng K.Y. et al., J Clin Pharm Ther. 2009;34(1):103-14; 2) Leson C.L. et al., J Toxicol Clin Toxicol. 1988;26(5-6):407-15.] Keywords: Population pharmacokinetics; Overdose; Clinical toxicology; Bayesian forecasting; Caffeine; Pharmaceutical care.
ANALYSIS OF DRUGS OF ABUSE IN ORAL FLUID BY LIQUID CHROMATOGRAPHY AND HIGH-RESOLUTION MASS SPECTROMETRY

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Background: Taking illicit drug is a serious public health concern that affects almost every society in the world. Use of these substances may lead to a severe mental and physical disorder, and life-threatening consequences. Most commonly used matrices are blood, urine as well as hair to analyze the drugs of abuse in clinical and toxicology laboratory. In addition, dried blood spot and urine spot also found an alternative methodology for the identification of illicit drugs. However, the sampling techniques of these methods sometimes create an inconvenient situation for the donor and often needs proper supervision to prevent any tampering of the sampling. In recent years, oral fluid has become an alternative and non-invasive sample matrix to determine the drugs of abuse. In this study, a liquid chromatography (LC) coupled to a high-resolution mass spectrometry (HRMS) method was developed to investigate multiple drugs of abuse in oral fluid. Methods: Total 34 drugs of abuse including 6-mam, 7-aminoflunitrazepam, 7-aminoclonazepam, 7-aminonitrazepam, alprazolam, amphetamine, benzoylecgonine, bromazepam, buprenorphine, clonazepam, diazepam, ephedrine, fentanyl, flunitrazepam, hydromorphone, ketamine, codeine, cocaine, MDA, MDMA, metamphetamine, methadone, methylphenidate, midazolam, morphine, nitrazepam, nordiazepam, oxazepam, oxycodone, phenaepam, ritalinic acid, temazepam, THC, tramadol and respective internal standards were included in the method. 200 μL oral fluid was extracted with solid phase extraction in a µelution plate (Oasis HLB, Waters Sweden) and injected directly to a LC-HRMS system without evaporation. The LC system was ultimate 3000 coupled to an orbitrap HRMS from Thermo Fisher Scientific. Acquisition was performed with full scan in electrospray positive ion mode. The chromatographic separation was achieved on an acquity UPLC BEH phenyl column (2.1 × 100 mm, 1.7 μm) with injection volume of 3 μL at a mobile phase flow rate 600 μL/min in a gradient mode with total run time 5 min. Column oven temperature was 60 °C. Mobile phase A consisting of water and a mobile phase B consisting of MeOH with 4 mM ammonium formate and 0.05% ammonia in both A and B. Results: The acquired detection limits were in the range of 0.1 to 2 ng/mL for all substances. According to a preliminary experiment, method linearity over a concentration range from 2 to 200 ng/mL with 6 data points was ≥ 0.99 (for example cocaine given in the figure). Method recoveries at 5 and 100 ng/mL were in the range of 80 to 110% for all substances. Conclusions: The LC-MS method developed in this study can reliably analyze 34 drugs of abuse in oral fluid. The present method is characterized by partial validation with satisfactory recovery, linearity, and full validation data will be presented at the conference.

Keywords: Drugs of abuse; LC-MS; HRMS; Oral fluid.
**IS N-ACETYLCYSTEINE ABLE TO REVERT NIMESULIDATE-INDUCED HEPATOTOXICITY?**

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**Background:** Anti-inflammatory agents and antibiotics are the most common causes of drug-induced liver damage. Nimesulide is a drug that is at high risk for hepatotoxicity, characterized by disorders ranging from mild elevation of aminotransferases to fulminant hepatitis with a high mortality rate. N-acetylcysteine (NAC) has been widely used in studies for being precursor of glutathione, raising antioxidant defenses. In this context, the objective of this study was to evaluate the effects of NAC as a hepatoprotective on the hepatotoxic effects induced by nimesulide. **Methods:** Twenty-four male Wistar rats were randomized into 4 groups of 6 animals per group: Control group, treated with saline solution, nimesulide group (100mg/kg/day) nimesulide+NAC group (100mg/kg/day of nimesulide and NAC) and NAC group (100mg/kg/day). Animals were treated for 15 days by gavage. Body mass gain ratio was daily followed and in the end of the study the relative weights of the organs (liver, kidneys, spleen and heart) were evaluated. This project was approved by CEUA (protocol n° 020/2018).

**Results:** Two rats from nimesulide group died in the day 5 and 6 of the study. There was no significant difference between the body mass gain between control and NAC groups. The nimesulide group had a significantly lower body mass gain than control and although NAC significantly improved the weight gain of the nimesulide + NAC group compared to the nimesulide alone group, this protection was not enough to restore the weight gain to similar levels to control groups. Liver and spleen of the animals treated with nimesulide (alone and wth NAC) had a relative weight higher than control. The heart of animals treated with nimesulide alone had a higher relative weight than the other groups. No significant changes were observed in kidneys. **Conclusions:** NAC showed partial hepatoprotective effect for the hepatotoxic effects of nimesulide. More detailed studies of animal organs may confirm this hepatoprotective effect.

**Keywords:** Nimesulide; Hepatoprotection; Clinical Toxicology; Hepatotoxicity.
SURVEILLANCE OF SPIDER BITE CASES REPORTED TO THE DRUG AND POISON RESEARCH AND INFORMATION CENTER (CIEMTO) IN MEDELLIN, COLOMBIA

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Figure 1. Some representative photographs obtained of the clinical cases attended at CIEMTO

Objective: to characterize the clinical and epidemiological variables related to the cases of spider bites attended by the Poison Control Center in Medellin between January 1st, 2016 and November 19th, 2018. Methods: descriptive, retrospective study, nested to the database where the clinical and epidemiological information of all the cases advised by the telephone line of the Poison Control Center at the University of Antioquia Medical School are registered. From this database we selected the clinical cases of spider bites diagnosed by the Emergency Department physician who requested the advice of a toxicologist of CIEMTO and the toxicologist who provided it regardless of the taxonomic identification. Dubious araneism cases or not confirmed by the clinical specialist were excluded. Results: 112 cases related to spider bites were attended in the period. The majority of cases were reported from Antioquia (66%), affecting mainly males (68%) with a median age of 37 years (range: 1 to 84) and a median time from accident to medical attention of 2 h ranging from 1 to 96 hours. Follow-up was achieved in 27% of cases without deaths nor clinically relevant complications. Conclusion: Araneism is an unattended disease that continues to be a cause for medical consultation in Colombia. Keywords: Spider Bites; Poisoning; Poison Control Centers; Colombia.
Theme: Clinical Toxicology/drugs of abuse

**BROAD SPECTRUM URINE DRUG SCREENING WITHIN CLINICAL TOXICOLOGY IN FINLAND**

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**Background:** In addition to conventional drugs of abuse, drug screening today must also encompass prescription drugs and newly emerging drugs. The emergence of these new psychoactive substances (NPS) widens the range of toxicologically relevant compounds making the limited scope immunoassays unsuitable for comprehensive drug screening. In Finland, two separate analyses are required if there are any sanctions to be applied based on the test result. Consequently, we rely on two mass spectrometry (MS) based techniques providing results suitable for forensic and clinical casework. **Methods:** The multi-analyte drug screening approach comprises a Bruker Daltonics Impact II quadrupole time-of-flight (QTOF) MS and an Agilent Technologies 6460 triple quadrupole tandem (QQQ) MS method. Both techniques were operated in the positive-ion mode and utilized electrospray ionization. QTOF data was acquired in full scan mode with high and low collision energies and QQQ data with selected reaction monitoring (SRM) mode with optimized MS parameters including collision energies. Each MS instrument was interfaced with an Agilent 1290 Infinity II ultra-high performance liquid chromatography system. The target database for QTOF screening included hundreds of compounds. The QQQ gave at least semiquantitative results for more than 200 compounds. For emerging NPS the QQQ identification remained qualitative, since only a partial revalidation was performed during the method update. The sample preparation for QTOF and QQQ consisted of solid-phase extraction and liquid-liquid extraction, respectively. **Results:** In 2018, the top ten most common findings in clinical urine samples were buprenorphine (43% of all samples), oxazepam (23%), cannabis (18%), quetiapine (17%), methadone (15%), 7-aminoctclonazepam (15%), amphetamine (13%), temazepam (12%), mirtazapine (10%), and olanzapine (9%). Buprenorphine is the most commonly abused opioid in Finland, although the high positivity rate here can partly be explained by the origin of the samples (rehabilitation clinics, hospital, and reformatories). Similarly, the frequently abused oxazepam is also a common relieve for anxiety, insomnia, and agitation. The abuse of cannabis has increased in Finland over the past years nowadays mimicking the European average. The most prevalent NPS was phenazepam probably due to the proximity of Russia, where it is in medicinal use. **Conclusions:** This two-dimensional MS-based multi-analyte drug screening enables high throughput identification and quantification of hundreds of toxicologically important substances yielding results applicable in court, if necessary. Such feature yields cost-effective screening results at a scope and sensitivity beyond ordinary drug testing.

**Keywords:** urine drug screening; drugs of abuse; time-of-flight mass spectrometry; triple quadrupole tandem mass spectrometry.
BE AWARE OF A BARBITURATE INTOXICATION
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\textbf{Background:} The last several years the number of suicide is increasing. The majority of suicide attempts involve the use of medication. Pentobarbital is one of the recommended drugs to perform self- euthanasia by diverse websites. Due to online access, pentobarbital can easily be obtained or ordered with the help of an organization Pentobarbital is labelled as a different substance to mislead custom control. In this abstract 4 cases on pentobarbital intoxications are presented, in which 3 resulted in death.

\textbf{Methods:} Patients (n=4, 2 male, 2 female) were admitted to the emergency room or found death in their home. Toxicology analysis was performed with a validated HPLC-UV method in the ISO15189 pharmacy laboratory of Erasmus MC.

\textbf{Results:} The first case concerns a successful tentamen suicidii (TS) where the laboratory pharmacist received two bottles of 100 mL of ‘natural skin cleanser’. After analysis these bottles contained pentobarbital in a concentration of 70 g/L. Two other postmortem investigations represented two cases with measured serum pentobarbital concentrations of 45 mg/L and 18 mg/L (therapeutic range 20-40 mg/L for coma). Antiemetics were also identified, i.e. domperidon and metoclopramide. These are recommended to be ingested during 24 hours prior to the pentobarbital liquid. Lastly, at the Emergency Unit a patient was admitted in a critical sedated condition after an attempted suicide without indication which substance was taken. Surprisingly the urine screening turned positive for barbiturates, consequently the serum pentobarbital concentration was 9 mg/L. After a time period on the ICU, the patient survived.

\textbf{Conclusions:} Health care providers should be aware of an increased risk of pentobarbital intoxications, since it can easily be accessed online and it is a recommended drug on diverse suicide websites.

\textbf{Keywords:} barbiturates; internet selling; forensic toxicology; suicide kits
FULLY AUTOMATED THERAPEUTIC DRUG MONITORING OF ANTI-EPILEPTIC DRUGS MAKING USE OF DRIED BLOOD SPOTS
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Background: Fully automated dried blood spot (DBS) extraction systems, online coupled to standard liquid chromatography-tandem mass spectrometry (LC-MS/MS) configurations, decrease the hands-on time associated with conventional DBS analysis, resulting in a higher sample throughput, making the technique more compatible with a high-throughput bioanalytical workflow. The aim of this study was to develop and validate an LC-MS/MS method, using a DBS-MS 500 autosampler (CAMAG, Switzerland), for the determination and quantification of four anti-epileptic drugs (AEDs) (carbamazepine, valproic acid, phenobarbital and phenytoin) and one active metabolite (carbamazepine-10,11-epoxide) in DBS samples. Methods: Method development included thorough optimization of the fully automated extraction procedure (i.e. extraction solvent, extraction (loop) volume, internal standard application, internal standard drying time, etc.). Afterwards, the method was fully validated based on international guidelines and applied on capillary patient samples originating from African epilepsy patients. Results: Thorough optimization of the fully automated extraction method was of utmost importance, finally resulting in the exclusion of the built-in IS spray. Concerning method validation, accuracy (%bias) and precision (%RSD) (with a single exception) were below 13%, meeting the acceptance criteria. Neither carry-over nor unacceptable interferences were observed. Calibration data was found to be heteroscedastic. Using weighted linear regression, with 1/x² weighting, mean back-calculated concentrations did not differ more than ±15% for all analytes, which is in line with the acceptance criteria. All compounds were stable in DBS for at least 1 month when stored at room temperature, 4 °C and -20 °C and for at least 4 days when stored at 60 °C. Internal standard-corrected matrix effects were below 8%, with %RSDs below 9.1%. Reproducible relative recovery values (around 60% for all analytes) were obtained and the effect of the hematocrit on the relative recovery was overall limited. Finally, fifteen capillary DBS samples, originating from patients receiving AED therapy in remote areas within sub-Saharan Africa, were successfully analyzed, demonstrating the applicability of the developed procedure in a remote setting. Conclusions: An LC-MS/MS method for the determination and quantification of 4 AEDs and one active metabolite in DBS, making use of the DBS-MS 500 autosampler, was developed and validated. Thorough optimization during method development demonstrated that proposed, generic direct elution conditions, while of value for orientation, may require (substantial) adjustment, depending on the analytes of interest and the used instrumentation.
Keywords: Therapeutic drug monitoring; dried blood spots; LC-MS/MS; anti-epileptic drugs; automated extraction.
CONCENTRATION-TOXICITY RELATIONSHIPS OF MEROPENEM IN PATIENTS WITH SEVERE INFECTION
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ABSTRACT
Objective: To determine the existence of concentration–toxicity relationships for meropenem adverse effects and define thresholds above which toxicity is more likely. Methods: Retrospective review of consecutive patients treated with meropenem who underwent therapeutic drug monitoring (TDM) at Nanjing Drum Tower Hospital between January 2017 and December 2018. Adverse events investigated included neurotoxicity, nephrotoxicity, hepatotoxicity and opportunistic Clostridium difficile infection. Toxicity was measured using observational grading criteria, clinical assessment and relevant serum biomarkers. These findings were correlated with trough TDM measurements at the time of toxicity presentation. Results: TDM results from 94 patients were investigated. A statistically significant elevation in mean meropenem serum trough concentrations (Cmin) was found in patients diagnosed with neurotoxicity (P=0.04) and those who developed nephrotoxicity whilst being treated with meropenem(P=0.01). Incidence of hepatotoxicity and C. difficilewas not related to Cmin. Threshold concentrations for which there is 50% risk of developing a neurotoxicity or nephrotoxicity were Cmin>64.2mg/L and Cmin>44.45mg/L respectively. Conclusion: Our data reveal an association between toxic concentrations and neurotoxic/nephrotoxic effects for meropenem. We have defined threshold concentrations above which these toxicities become more likely. Clinicians should balance concerns for therapeutic efficacy with potential toxicity when considering aggressive therapy. Regular drug concentration monitoring with TDM may be an effective method to reduce the occurrence of adverse reactions.

KEY WORDS: Meropenem; Concentration-toxicity Relationships; Neurotoxicity; Nephrotoxicity; Hepatotoxicity
EVALUATION OF VANCOMYCIN PLASMATIC CONCENTRATIONS IN PATIENTS ATTENDED IN INTENSIVE CARE UNITS OF A PORTO ALEGRE HOSPITAL
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Background: The plasmatic concentration monitoring of glycopeptide antibiotics are crucial for evaluation of the effectiveness associated with security therapy. In this scenario, vancomycin is one of the antimicrobial agents most widely used for treatment of gram-positive infections. The use is recommended for infections with methillin resistant Staphylococcus aureus. However, vancomycin intoxication is observed as a result a short therapeutic window. This report aimed to describe the therapeutics monitoring profile of the vancomycin patients attended by Hospital Pronto Socorro (HPS-Porto Alegre). Methods: A retrospective study was performed based on vancomycin therapeutic concentration determined by enzyme multiplied immunoassay technique (EMIT). Were analyzed data obtained in Madya Computerized System (local database) of adult, pediatric and burn patients attended in the period of January to December 2018 in the intensive care units. Results: Of the 138 patients included in the study, 83.3% were adults and 77.5% were male. Each patient was submitted at least two EMIT analysis resulting in 381 vancomycin plasma dosing. Only 39.4% results were in accordance with therapeutic dose. The therapeutic level was higher than the reference level in 30.9% of samples and below the recommended in 29.7% of samples. Considering the short therapeutic window, the patients that present a concentration above the therapeutic range can be susceptible an intoxication or a prognosis including nephrotoxicity and ototoxicity. Additionally, concentrations below the recommended level should be considered, once the therapeutic efficacy can be compromised. Different factors such as individual variability, epigenetics, nutritive pathways or lifestyle may influence the vancomycin pharmacokinetics. Furthermore, other variables including sample collecting and drug administration, should be also considered. Conclusion: This is an initial study to report the misuse vancomycin treatment alerting the need of multidisciplinary activities to promote a better healthcare quality and reducing costs. Keywords: Vancomycin; Therapeutic drug monitoring; Clinical toxicology.
Theme: Clinical Toxicology/drugs of abuse

ANALYTICAL DIAGNOSIS OF POISONING BY NAPHAZOLINE
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Background: Naphazoline is a sympathomimetic drug which acts as an agonist at α2-adrenergic receptors, promoting a vasoconstriction. The drug is indicated for nasal or ophthalmic use. Clinical effects are reported in a range of few minutes lasting six hours. Despite local action, the use of naphazoline can generate systemic effects in the human body in small amounts, with a toxic dose of 0.05 mg/kg body weight. The most common symptoms related to naphazoline poisoning are drowsiness, bradycardia, hypothermia, hypotension and sweating. The numbers of cases of naphazoline poisoning have been increased over the last years. However, there are few published case reports showing efficient diagnostic methodologies for naphazoline intoxication. Methods: Were evaluated all cases of naphazoline poisoning (n=115) attended by the Toxicological Information Center of Rio Grande do Sul (CIT/RS - Brazil) during 2018. The variables studied were age, sex, chemical substance, type of event, route of exposure, type of use and exposure, type of effect and case development. Urine and blood samples were analyzed by gas chromatography coupled to mass spectrometry (GC-MS) in cases where the toxicological analyses were requested. This report aimed to describe the specific case of a male patient, two years old, who had an individual accident, with suspected ingestion of naphazoline and paracetamol, presenting drowsiness in medical care. Results: The main clinical manifestations reported were drowsiness (n=48), bradycardia (n=23), hypothermia (n=26) and generalized sweating (n=19). About 90% of the cases occurred in children under six years and 80% was characterized as individual accident. The analysis of the two-year-old child showed a positive result in the urine, indicating the use of naphazoline. The method implemented in the CIT/RS presented the limit of detection (LOD) of 100 ng/mL. When performed with other less sensitive methodologies, such as thin-layer chromatography, the result was negative. Conclusions: GC-MS analysis showed to be sensitive and efficient, since it has a low LOD, necessary in the diagnosis of naphazoline intoxication, because, mostly, the amount ingested is small. The treatment of naphazoline poisoning is mostly supportive and symptomatic. However, the analytical confirmation, in patients where there is uncertainty about the drug involved, is useful to guide the medical team in the precise diagnosis and appropriate treatment, avoiding unnecessary therapeutic procedures. Moreover, the finding of intoxication provides the toxicovigilance system with a basis for the elaboration of educational policies.

Keywords: naphazoline; poisoning; GC-MS.
DEVELOPMENT AND VALIDATION OF A QUECHERS EXTRACTION METHOD FOR THE DETERMINATION OF DRUGS IN LIVER SPECIMENS BY UPLC-MS/MS

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Background: Liver is a specimen frequently used in forensic toxicology testing, particularly where urine and blood cannot be obtained. We applied a QuEChERS (Quick, Easy, Cheap, Effective, Rugged, Safe) extraction method to measure concentrations of several relevant drugs (citalopram, clomipramine, fluoxetine, nortriptyline, paroxetine, venlafaxine, clozapine, sulpiride piroxicam, clonazepam, diazepam, flunitrazepam, flurazepam, midazolam, nitrazepam, oxazepam, carbamazepine, atenolol, metoprolol, propranolol, verapamil) in liver. This extraction method consists essentially of two steps: extraction/partitioning, and dispersive solid phase extraction.

Methods: A homogenate of liver was prepared in ration of 2:8 (w/w) with water. An aliquot of 200 µL of the homogenate was fortified with working solutions of the analytes and internal standard (trimipramine-D3), in a polypropylene tube. To this tube, 0.4 mL of acetonitrile containing 0.4% formic acid, 80 mg of MgSO₄, 20 mg of NaCl and one stainless ball (5 mm of diameter) were added, followed by homogenization and centrifugation. After, 200 µL of the supernatant was transferred to a polypropylene tube containing 15 mg MgSO₄and 5 mg of C18, followed by vortex mixing and centrifugation. An aliquot of 100 µL of the supernatant was transferred to another tube, and evaporated. The dried extract was recovered with 100 µL of initial mobile phase (5 mM ammonium formate pH3 and 17% ACN with 0.1% formic acid (83:17, v/v)). Quality control samples were prepared at the concentrations of 25, 400 and 800 ng g⁻¹. During the assay validation, precision, accuracy, sensitivity, dilution Integrity, matrix effects and extraction yield were evaluated. Analysis were performed by UPLC-MS/MS in ESI positive mode. Separation was performed in an Acquity HSS C18 column in a flow rate of 0.4 mL min⁻¹, kept at 50 °C. Results: Matrix effects were in range -11.53% (verapamil) to +44.70% (atenolol). Excellent recoveries were achieved for all analytes, in the range of 54.21% (piroxicam) to 104.78% (sulpiride). The assay was linear from 20-1,000 ng g⁻¹ (r>0.99), with accuracy in the range of 92.29% (verapamil) to 114.67% (metoprolol). Precision presented intra-assay CV% in the range of 2.72% (fluoxetine) to 15.59% (oxazepam) and inter-assay CV% in the range of 0.78% (flunitrazepam) to 14.33% (atenolol). Additional validation parameters fulfilled acceptance criteria. Conclusions: The developed QuEChERS extraction method, associated to UPLC-MS/MS, presented acceptance performance for the quantitation of a range of drug in liver specimens. Further evaluation in case samples is warranted.

Keywords: Quechers; liver; validation; UPLC–MS/MS.
Background: We present the determination of benzodiazepines and tramadol, in blood samples and human urine from a male patient of 31 years, who is admitted to emergency on repeated occasions in a period of 4 months for confusional syndrome, cerebellar syndrome, depression of oscillating consciousness. The initial clinical approaches were oriented towards an infectious encephalitis and then to immune-mediated or genetic neurological pathology. Given the normality of the paraclinical studies and the poor response to pharmacological treatments, the possibility of intoxication arises. In the emergency, an immunological test was performed on his first admission with a positive result for benzodiazepines. Given that the patient reported receiving tramadol for pain in the gluteal region, the search for this opiate was also carried out. Methods: An enzymatic hydrolysis was performed with B-glucuronidase from Hélix pomatia, and subsequent liquid-liquid extraction, in basic medium, at pH 9-10, using dichloromethane as extraction solvent. They were then derivatized with a mixture of MSTFA/NH4I/Ethanethiol for analysis by GC/MSD: HP 5890 Gas Chromatograph coupled to HP 5972 Mass Detector. Conclusions: Tramadol (Concentration: 2ug/mL), Ketoprofen, OH-Ketoprofen, Paracetamol and Diclofenac were detected in the first urine sample, 72 hs after admission to the emergency, asymptomatic, and no relevant substance was found in the blood sample. Subsequently another urine sample is received to perform the same analysis, one week after the previous one, with symptoms, upon re-entry to Emergency. Tramadol (Concentration: 11.3ug/mL), Salicylic Acid, Ibuprofen, Paracetamol, Hydroxythrazole, Desmethyltramadol and Traces of Nordiazepam were detected. The results obtained in the analysis of these samples highlight the importance of using absolute methods such as Gas Chromatography/Mass Spectrometry, after obtaining presumptive positive results by immunological methods, since they present cross reactivity with other substances giving false positives. The presence of Tramadol was confirmed in the three urine samples, considering that the clinical picture presented by the patient was explained by the use of it. Keywords: Tramadol; Benzodiazepines; Intoxication; Drug Abuse.
Theme: Clinical Toxicology/drugs of abuse

TOXICOKINETIC STUDIES OF THE FENTANYL-HOMOLOGES CYCLOPROPANOYL-1-BENZYL-4’-FLUORO-4-ANILINOPIPERIDINE AND FURANOYL-1-BENZYL-4-ANILINOPIPERIDINE USING IN VITRO TOOLS

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Background: There has been an increasing emergence of new psychoactive substances (NPS) with opioid-like effects over the last decade. Unfortunately, little is known about their toxicokinetic profiles. Therefore, the most important in vitro toxicokinetic features of cyclopropanoyl-1-benzyl-4’-fluoro-4-anilinopiperidine (cyclopropanoyl-B-4F-AP; 4F-cyclopropylbenzylfentanyl) and furanoyl-1-benzyl-4-anilinopiperidine (furanoyl-BAP, furanylbenzylfentanyl) were determined such as metabolic stability, most abundant phase I and II metabolites, and involved isozymes. Methods: Pooled human liver S9 fraction was used to determine the metabolic stability and for metabolite identification. Substrates (2.5 µM) were incubated for 360 min and samples were taken at different time points. For isozyme mapping, the NPS were incubated with one out of ten heterogeneously expressed CYP-isozymes (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5) or flavin-containing monooxygenases 3 for 30 min. All samples were analyzed by hyphenated high resolution mass spectrometry (Dionex Ultimate 3000 + Thermo Fisher Q-Exactive plus). Results: Furanoyl-BAP showed a shorter half-life of approximately 60 min in comparison to cyclopropanoyl-B-4F-AP (>90 min). The main phase I steps of furanoyl-BAP and cyclopropanoyl-B-4F-AP were hydroxylation and N-dealkylation. However, no phase II metabolites could be detected. CYP3A4 and CYP2C19 were involved in the metabolism of both compounds and additionally CYP2D6 in case of furanoyl-BAP. Conclusions: The identified metabolites of furanoyl-BAP and cyclopropanoyl-B-4F-AP may facilitate the detection of both NPS in toxicological urine screenings. However, based on the in vitro metabolic stability a low metabolic rate and low intrinsic clearance in vivo is expected. Both compounds are substrates of polymorphically expressed CYP-isozymes. Therefore, potential drug-drug or drug-food interactions should be considered.

Keywords: synthetic opioids; pS9; metabolic stability; CYP-isoenzyme mapping; LC-HRMS/MS
Theme: Immunosuppressive drugs

EFFECTS, COSTS AND IMPLEMENTATION OF MONITORING KIDNEY TRANSPLANT PATIENTS’ TACROLIMUS LEVELS WITH DRIED BLOOD SPOT SAMPLING: A RANDOMIZED CONTROLLED TRIAL

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Background: Dried Blood Spot (DBS) home sampling allows monitoring creatinine levels and tacrolimus trough concentrations as an alternative for blood sampling in the hospital, which is important in kidney transplant patient follow-up. This study aims to assess whether DBS home sampling results in decreased patient burden and lower societal costs. Methods: In this single-center randomized controlled trial, adult kidney transplant patients were enrolled while still hospitalized after transplantation. The intervention group (n=25) used DBS home sampling on top of usual care in the first 6 months after transplantation. The control group (n=23) received usual care only. Primary endpoint was the number of outpatient visits. Secondary endpoints were: (1) costs per patient (2) patient satisfaction and (3) implementation. Results: There was no statistical significant difference in the average number of outpatient visits between the DBS group (11.2, SD: 1.7) and the control group (10.9, SD: 1.4) (p = 0.48). Costs per visit in the DBS group were not significantly different (€537) compared to the control group (€510) (p = 0.66). Most patients (82.6%) were willing to perform DBS home-sampling if this would reduce the number of hospital visits. Only 55.9% of the expected DBS samples were received and one-fifth analyzed on time. Conclusions: Adult kidney transplant patients are willing to perform DBS home sampling. However, to decrease patient burden and costs in post-transplant care, optimization of the logistical process concerning mailing and analysis of DBS samples is crucial.

Keywords: RCT, Dried Blood Spots, cost-effectiveness, implementation
Theme: Immunosuppressive drugs

**CHINESE CHARACTERISTICS ABOUT RENAL FUNCTION AND PLASMA METHOTREXATE CONCENTRATIONS PREDICT TOXICITIES IN CHINESE ADULTS RECEIVING HIGH-DOSE METHOTREXATE**

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**Background:** Methotrexate (MTX) is an effective drug for the treatment of Chinese adults with acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL), but toxicity remains a significant problem. Toxic reactions may occur when patients use high-dose MTX, but the correlation between its toxicity and concentration in Chinese adults is controversial. The purpose of this study was to discover the incidence of renal injury in China and the relationship between MTX concentration and renal function, as well as to assess toxic reactions of MTX in Chinese adults. **Methods:** We used a retrospective study and enrolled 97 patients who had been diagnosed with ALL or NHL at the Hemopathology Department of Shanghai Changhai Hospital from January 2015 to June 2016. A total of 149 courses of MTX infusion were enrolled in this study. All date assessments were double-tailed and p≤0.05 was considered statistically significant. SPSS19.0 statistical software was used for statistical analysis. **Results:** Our results suggest that the incidence rate of nephrotoxicity was 8.05% in our study (12/149 courses) which was higher than other countries, forty-one (27.5%) episodes of elimination delay were observed and may be related to the genetic polymorphism of the population. We found negative correlations between creatinine clearance rate before MTX infusion and the plasma concentrations of MTX at 36 h after MTX infusion(P=0.005). The serum creatinine at 48 h and plasma concentrations of MTX at 48 h and72 h were significantly positively correlated (both p=0.000). High blood concentration of MTX was positively associated with nephrotoxicity> grade 1 (P<0.01). Infection > grade 1 was more likely to occur if a patient had high MTX levels at 36 h, 48 h, and 72 h (P<0.01). **Conclusions:** Our results show that renal function is associated with MTX concentration, and high MTX concentration can predict the occurrence of renal toxicity and infection. It is unrealistic to monitor blood drug concentration of MTX in some institutions, but regular measurement of renal function is feasible. Our results also suggest that the blood concentration of MTX can predict whether MTX will induce infection, so clinicians could prophylactically administer antibiotics in advance based on MTX concentration. **Keywords:** Methotrexate, nephrotoxicity, MTX concentration.
REDEFINING THERAPEUTIC DRUG MONITORING OF TACROLIMUS IN LIVER TRANSPLANTATION: CAN WE TARGET TROUGH CONCENTRATIONS BELOW 7 NG/ML DURING THE FIRST MONTH?
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Background: Minimizing TAC drug exposure might lead to decrease adverse events in patients and could then be of utmost importance for long-term outcome but also has to maintain drug efficacy. This led to the current TAC trough blood concentration recommendation of 6-10 ng/mL in liver transplantation. However, most of the randomized controlled trials leading to this recommendation targeted low TAC exposure (up to 5-8 ng/mL during the first month) but hardly reached this objective with mean Cmin frequently above 7 ng/mL and/or a low number of patients included. Therefore, uncertainties remains regarding the outcome associated with a Cmin below 7 ng/mL during the first 4 weeks after liver transplantation. The aim of the present study was to evaluate the outcomes according to TAC exposure during the first 4 post-operative weeks in liver transplant recipients.

Methods: We retrospectively analyzed data from liver recipients transplanted from 2002 to 2017. Patients were classified to low (4-7 ng/mL), normal (7-10 ng/mL) or high (>10 ng/mL) exposure groups according to their median TAC trough concentration calculated during the first 4 post-operative weeks. A propensity score matching (including recipients and donors characteristics as well as incidence of acute rejection and TAC related toxicity) was performed in order to reduce biases. Clinical (patient’s and graft survival) and biological outcomes were then compared between groups.

Results: From 904 initial patients, 552 were eventually analysed after matching procedure (n=184 patients by group). The month-3, -6 and -12 patient’s and graft survival were not different between the groups while month-1 and -3 creatinin level were lower in the low and normal exposure groups compared to the high exposure group (p<0.001 and p=0.001 respectively).Liver function tests seems also improved by the minimization with month-1 ALT and INR, month-1 and -3 ALP and month-1, -3 and -6 bilirubin level significantly improved in the low exposure group. Conclusions: Targeting a TAC trough blood concentrations between 4 and 7 ng/mL during the first 4 post-operative weeks after liver transplantation is safe regarding grafts and patients survival and improves liver and renal biological parameters.

Keywords: Liver transplantation; Immunosuppressive drugs, Tacrolimus, Therapeutic drug monitoring, Outcome.
VALIDATION OF AN ULTRAHIGH PERFORMANCE LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR THE QUANTIFICATION OF CYCLOSPORINE IN ENDOMYOCARDIAL BIOPSIES
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Background: The pharmacological response of CSA is highly variable and partially managed by therapeutic drug monitoring in whole blood. However, some patients experience acute cellular rejection (ACR) while their CSA blood concentrations are within the therapeutic range. Thus, it appears crucial to develop newer pharmacological monitoring approaches to optimize immunosuppressive drug therapy. In renal and liver transplantation, several studies suggest that graft concentration would better reflect immunosuppressant efficacy. The interest to monitor intra-graft concentration of CSA in heart transplantation remains to be investigated provided that an analytical method allows quantifying the drug in this alternative matrix. The aim of this study was to develop and to validate an ultrahigh performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the quantification of CSA in endomyocardial biopsies (EMB).

Methods: Calibrants and quality controls (QC) were prepared by spiking CSA in pork EMB homogenates. Sample purification was achieved using protein precipitation with a mixture of acetonitrile/zinc sulphate in water and a salting-out with ammonium acetate. Chromatography was performed in a 2.5 min run time using isocratic elution on a C18 reversed phase column with a mobile phase consisting of acetonitrile/ammonium acetate in water (95%/5%). Detection was performed in positive-ionization mode. The method was validated according to the European Medicines Agency guidelines. Results: The method was linear from 50-2500 pg/mg of biopsy. Within-day and between-day precisions as well as overall bias were within ±15%. Matrix factor assessed for 3 QC levels were lower than 1.1 meaning that matrix effect was negligible. The method was specific since there was no interference with CSA signal in samples spiked with a mixture of 35 drugs. No carry-over was observed.

Conclusion: A LC-MS/MS method was developed and allows measurement of CSA in EMB using a sensitive, easy and fast experimental procedure with good analytical performances even though stability of CSA in BEM remains to be evaluated. Then, this method could be applied to measure CSA in routine EMB from heart transplant recipients in order to assess the relationship between CSA intra-graft concentration and histological ACR score. This approach might help optimizing the management of immunosuppressive therapy in heart transplantation.

Keywords: cyclosporin, endomyocardial biopsy, mass spectrometry, alternative matrix, heart transplantation
Theme: Immunosuppressive drugs

**TACROLIMUS IS A P-GLYCOPROTEIN BUT NOT A MRP, ENT2 OR CNT3 SUBSTRATE**

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**Background:** Tacrolimus (TAC) is an immunosuppressive drug prescribed to prevent organ transplant rejection. Its target, the serine-threonine phosphatase calcineurine, is located inside the T-lymphocyte. The poor correlation between blood and intracellular concentrations is possibly related to membrane transporters. MDR1/P-glycoprotein (P-gp), MRPs, ENT2 and CNT3 are membrane transporters expressed at the T-cell membrane. For P-gp, MRPs and CNT3, genetic polymorphisms have been shown to influence TAC pharmacokinetics. This study aimed to determine if these transporters are involved in intracellular diffusion of TAC. **Methods:** TAC accumulation (for P-gp, ENT2 and CNT3) or retention (for MRPs) was studied, respectively, in P-gp-expressing MCF7R cells, ENT2- and CNT3-expressing BeWo cells, and MRPs-expressing HuH-7 cells, and compared to those of reference substrates (rhodamine 123 for P-gp, uridine for ENT2 and CNT3, and carboxy-2',7'-dichlorofluoresceine (CF) for MRPs), in the presence or absence of reference inhibitors (cyclosporin A (CsA) for P-gp, NBMPR for ENTs, inosine for CNT2 and CNT3, thymidine for CNT1 and CNT3, and probenecid (PBN) for MRPs). **Results:** As expected, TAC appeared to be a substrate of P-gp, as shown by the four-time increase in TAC cellular accumulation in presence of CsA. This result was confirmed by accumulation assay of rhodamine 123 (2.7-time increase). TAC accumulation was not significantly influenced by ENT2 and CNT3 inhibitors, suggesting that TAC is not substrate of these transporters. Regarding MRPs, cellular efflux of CF was inhibited by PBN but PBN failed to enhance TAC retention. Together, these data suggests that TAC is not a substrate of MRPs. **Conclusions:** Here we report an original *in-vitro* study to describe the interplay between TAC and T lymphocyte membrane transporters. TAC appears to be transported by P-gp but not by ENT2, CNT3 and MRPs. **Keywords:** Tacrolimus; transplantation; drug transporter; lymphocyte; individualization.
DECIPIERING PHARMACOGENETIC-WHOLE BLOOD/INTRACELLULAR PHARMACOKINETIC-PHARMACODYNAMIC (PG-PK-PD) RELATIONSHIP OF TACROLIMUS IN LIVER TRANSPLANT RECIPIENTS

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Background: Tacrolimus (TAC) pharmacological response exhibits substantial inter-individual variability and despite therapeutic drug monitoring in whole blood, some patients experience acute cellular rejection or toxicity while having blood concentration within the therapeutic range. These observations emphasize the need to look for alternative pharmacological biomarkers of TAC response. This study aimed at elucidating the interplay between pharmacogenetic determinants of TAC whole blood and intracellular exposures as well as the pharmacokinetic-pharmacodynamic relationship of TAC in both compartments. **Methods:** A non-parametric population pharmacokinetic model was developed using complete pharmacokinetic profiles of twice daily TAC in whole blood and peripheral blood mononuclear cells (PBMC) of 32 liver transplanted patients in the first ten days post transplantation. Concurrently, calcineurin activity was measured in PBMC. Influence of genetic polymorphisms of \textit{ABCB1}, \textit{CYP3A4} and \textit{CYP3A5} on TAC exposure was assessed. **Results:** The model lead to good prediction of TAC concentrations in both compartments. A moderate linear correlation was found between whole blood and intra-PBMC TAC exposures (\(r^2=0.51\), \(p<0.001\)). \textit{ABCB1} polymorphisms 1199G>A was shown to influenced whole blood and intracellular exposures (\(p=0.009\) and 0.006). In addition, intra-PBMC calcineurin activity appeared incompletely inhibited by TAC and a few patients are expected to achieve intracellular \(IC_{50}\) concentrations (100 pg/millions of cells) at therapeutic whole blood concentration. **Conclusion:** TAC intracellular concentration and \textit{ABCB1} genetic polymorphism could be promising alternative biomarkers to whole blood therapeutic drug monitoring, to widespread personalized medicine in the management of TAC therapy of liver transplanted patients. Finally, further studies are required to clarify the relationship between these biomarkers and clinical outcomes. **Keywords:** tacrolimus, pharmacogenetics, pharmacodynamics, modeling, liver transplantation
Theme: Immunosuppressive drugs

**IL-10 AND CYP3A5 GENE POLYMORPHISMS SIGNIFICANTLY INFLUENCE DOSE-ADJUSTED TROUGH BLOOD TACROLIMUS CONCENTRATIONS IN CHINESE EARLY POST-RENAL TRANSPLANT RECIPIENTS**

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**Background:** The highly inter-individual pharmacokinetic variability and narrow therapeutic index of tacrolimus (TAC) has hampered its clinical use. Genetic polymorphisms may contribute to the variable response, but the evidence is not compelling, and the explanation is unclear. In this study we attempted to find previously unclear genetic factors that may affect the TAC dose requirements in Chinese early post-renal transplant recipients. **Methods:** A total of 188 renal transplant recipients were enrolled in the analyses. Genetic polymorphisms of IL-10, CYP3A5, CYP2C8 and ABCB1 genes were determined. Tacrolimus doses and blood concentrations were determined at 5, 10, 15 days, and 1 month after transplantation. IL-10 polymorphisms at G-1082A, C-592A and C-819T; CYP3A5 polymorphisms at A-6986G; CYP2C8 polymorphisms at G-416A and A-1196G; and ABCB1 polymorphisms at A-61G were assessed by Kompetitive Allele Specific PCR. **Results:** In the first one month, the patients carrying the IL-10 rs1800896 AA genotype showed a significantly higher TAC logC/D than those with the GA genotype. Recipients with the capacity for low IL-10 production (-1082AA) engrafted with CYP3A5 nonexpressors had the highest logC/D ratios, whereas recipients with intermediate or high production of IL-10 (-1082GA or GG) carrying CYP3A5 nonexpressors were found to have the lowest ratios. We further validated the influence of CYP3A5 rs776746, CYP2C8 rs11572080 and rs10509681, ABCB1 rs9282564 on the TAC logC/D; no clinical significances of TAC logC/D difference of CYP2C8 and ABCB1 polymorphisms were found in our data. **Conclusions:** Genetic polymorphisms in the immune gene IL-10 rs1800896 may influence the TAC logC/D; it may together with CYP3A5 rs776746, contribute to the variation in TAC dose requirements during the early posttransplantation period. This finding provided a new interpretation for the variable immunosuppressive disposition after renal transplantation. **Keywords:** IL-10; CYP3A5; kidney transplantation; polymorphism; tacrolimus.
Theme: Immunosuppressive drugs

AN IN VITRO STUDY TO EXPLORE A DRUG-DRUG INTERACTION BETWEEN MYCOPHENOLIC ACID AND GANCICLOVIR INVOLVING MRP4 EFFLUX TRANSPORTER INHIBITION

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Background: Cytomegalovirus (CMV) infection is a major issue in immunodepressed patients, since it is associated with high morbidity. Ganciclovir (GCV) is frequently prescribed in these cases, but is characterized by an important hematological toxicity, mainly neutropenia. In kidney recipients, we previously demonstrated that (i) GCV is a substrate of MRP4 efflux transporter; (ii) that a genetic polymorphism causes intracellular accumulation of GCV; (iii) this accumulation leads to a diminution of neutrophil count (1). In transplant patients, multiple drug-drug interactions (DDI) can arise that could favor the toxicity of GCV. In the present work, we explored the existence of a DDI between GCV and mycophenolic acid, one of the most prescribed immunosuppressant in transplant patients.

Methods: In the first step, we developed a model of HEK293 cells that transiently overexpress MRP4. Then, we tested the impact of MPA on the transport of GCV in multiple conditions: GCV from 5 to 10 mg/L with/without MPA from 2.5 to 50 mg/L, for 30 minutes. Specific inhibition of MRP4 was performed using Ceefourin 1. Measurements of GCV and MPA were performed using LC-MS/MS methods. A classical Bradford method was used to quantitate proteins in each experiment. Results: Whatever the GCV concentration, the addition of MPA inhibited the efflux of GCV, and the accumulation of GCV into cells was at least as much as for ceefourin 1 (specific inhibitor). For example, for GCV concentration of 5 mg/L, concentrations of MPA of 2.5, 10 or 50 mg/L, increased intracellular of GCV by 137%, 239% and 267%, respectively. Conclusions: These results suggest that MPA could be a potent MRP4 inhibitor and could inhibit the efflux of GCV, which could favor its toxicity.


Keywords: Ganciclovir; MRP4; mycophenolic acid; transplantation; toxicity; drug-drug interaction
**SERUM IP-10 AS A POTENTIAL BIOMARKER TO COMPLEMENT DRUG CONCENTRATIONS IN THE MANAGEMENT OF KIDNEY GRAFT RECIPIENTS: RESULTS OF A PRELIMINARY SINGLE CENTER STUDY**

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### Background:
Interferon γ (INFγ) has been used as a biomarker to guide therapy with immunosuppressive drugs (ISD, 1), however limited stability of the molecule ex vivo is a critical preanalytical issue. IP-10 is produced in response to INFγ-release by activated T-cells and might be therefore a more robust alternative to the measurement of INFγ. Urinary concentrations have been shown to be predictive for acute rejection (AR) in kidney transplant recipients (KTR, 1). Biomarkers complementing TDM of ISD are required to guide more precisely immunosuppression. Therefore, the focus of this study was a possible association between serum concentrations of IP-10 and acute rejection (AR), impaired graft function (IGF) 12 months after transplantation (Tx) as well as pre-dose concentrations of ISD. **Methods:** IP-10 was retrospectively determined in KTR (116, 44 females; median 54 years, range 19-78) by ELISA (RayBiotech Life; Norcross, GA; USA) in 4 time-windows (week 2-4; months 2-3; 4-6 and 7-12). Concentrations of the ISD tacrolimus (Tac), mycophenolate sodium (MPA) were measured chromatographically. Statistical analysis included Mann-Whitney test, Kaplan-Meyer survival statistics, Spearman’s rho, and ROC analysis. **Results:** IP-10 concentrations over the entire observation period (12 months) were neither associated with Tac nor with MPA concentrations (p>0.05) but elevated at the time of AR (n=40, median 16 ng/mL (range 0-340) vs. 0 ng/mL, p<0.05). IP-10 concentrations at week 2-4 after Tx were predictive for IGF 12 months post Tx, particularly in KTR, who had ≥2 dialyses (DGF) in the first week post-Tx. In a subgroup of high-risk KTR (panel reactive antibodies >15%, ≥2 Tx) and without simultaneous AR, IP-10 at week 2-4 was predictive for AR in month 2-12 (Table). **Conclusions:** Our results suggest that serum IP-10 might be an appropriate biomarker to reflect immune activation in specific populations of KTR and complement ISD concentrations to better manage the therapy with these drugs.


**Keywords:** Immunosuppression; Immune Activation; Chemokines; IP-10
Background: Monitoring of mycophenolic acid (MPA) could be performed by measurement of trough concentrations with target therapeutic range of 1.3 to 3.5 mg/L, or by calculation of area under the drug concentration versus time curve (AUC) utilizing 3 or 4 samples within a dosing interval, with a target range of 30 to 60 mg*h/L. AUC is considered more reliable compared to trough levels, and MPA kinetics are dependent on the type of the co-administered calcineurine inhibitor. This study compares trough versus AUC MPA monitoring alone or in co-administration with Cyclosporine (CsA) or Tacrolimus (TAC). Methods: 52 transplanted patients receiving MPA, 25 of them on CsA, 27 on TAC, and 11 non-transplanted patients on MPA alone, were evaluated more than once, yielding 197 monitoring consults included for assessment. MPA, CsA, and TAC were measured by validated LC/MS/MS methods. Validated pharmacokinetic programs for the calculation of MPA AUC were used, adapted to the type of calcineurine inhibitor. Assessment was made by comparison of the distribution and coincidence between trough levels and AUC with respect to the above therapeutic ranges. Results: Transplanted patents: when combined with CsA, MPA AUC was in the target range in 60% of cases, under it - in 32%, and in 8% - over it, while trough levels were 49.5% under, 45% within, and 5.5% over the therapeutic range; agreement between trough MPA and MPA AUC was poor - there was coincidence in 40% of sub-therapeutic, 63% of therapeutic and 0% of supra-therapeutic ranges, 2 cases were identified with sub-therapeutic trough and supra-therapeutic AUC; when combined with TAC, MPA AUC was in the target range in 50% of cases, under it - in 15%, and in 35% - over it; trough levels being 22% under, 53% within and 25% over the therapeutic range; agreement between trough MPA and MPA AUC was much better, compared to the combination with CsA: there was coincidence in 61% of sub-therapeutic, 79% of therapeutic and 86% of supra-therapeutic ranges. Nevertheless, 1 case was found with sub-therapeutic trough and supra-therapeutic AUC. In non-transplanted patients MPA AUC was in the target range in 47% of cases, under it - in 9%, and in 44% - over it; trough levels being 12% under, 59% within and 29% over the therapeutic range; agreement between trough MPA and MPA AUC showed coincidence in all of sub-therapeutic, 87.5% of therapeutic and 60% of supra-therapeutic ranges. Conclusions. This study confirms that MPA AUC is more relevant than trough monitoring for dose individualization. It also shows that MPA trough levels are much more concordant with MPA AUC in non-transplanted patients and in transplanted patients on TAC, while in combination with CsA, discordance remains significant.

Keywords: MPA; trough monitoring; AUC monitoring; comparison.
FLUCLOXACILLIN-INDUCED LOW TACROLIMUS AND EVEROLIMUS TROUGH CONCENTRATIONS: A CASE REPORT

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Tacrolimus and everolimus are commonly used for prevention of allograft rejection and are both metabolized by the hepatic cytochrome P450 (CYP) enzyme CYP3A4. Drugs influencing the activity or expression of CYP enzymes can cause clinically relevant changes in immunosuppressant metabolism. We describe the case of an 11-year old kidney-liver transplant patient who received flucloxacillin treatment for pyelonephritis. Seven days after the onset of treatment, everolimus and tacrolimus trough concentrations dropped from 5.3 µg/L to 1.5 µg/L and from 2.9 µg/L to 2.3 µg/L, respectively, despite dose increase. After flucloxacillin discontinuation, trough concentrations returned to the target range within 3 days and increased further in the following days. We hypothesize that the changes in tacrolimus and everolimus trough concentrations were caused by a CYP3A4-inducing effect of flucloxacillin. An inducing effect of flucloxacillin has been reported for other drugs metabolized by CYP3A4 but not yet for tacrolimus and everolimus. We advise physicians to be aware of possible interactions of flucloxacillin with tacrolimus and everolimus. Extra monitoring of immunosuppressant levels during and up to at least 2 weeks after discontinuation of flucloxacillin is strongly recommended.

Keywords: Immunosuppressants, drug-drug interaction, tacrolimus, everolimus, TDM
Theme: Immunosuppressive drugs

VOLUMETRIC ABSORPTIVE MICROSPAMLING AND DRIED BLOOD SPOT MICROSPAMLING VERSUS CONVONITIONAL VENOUS SAMPLING FOR TACROLIMUS TROUGH CONCENTRATION MONITORING

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Background: Monitoring of immunosuppressive drugs such as tacrolimus is important for prevention of allograft rejection in transplant patients. To facilitate monitoring, our center offers Dried Blood Spot (DBS) sampling, giving patients the opportunity to sample a drop of blood from a fingerprick at home, which can be sent to the laboratory by mail. In this study, both a novel Volumetric Absorptive Microsampling (VAMS) device [Mitra©] and conventional DBS sampling are compared to venous wholeblood sampling. Methods: A total of 130 paired fingerprick VAMS, fingerprick DBS and venous whole blood samples were obtained from 108 different kidney transplant patients by trained phlebotomists for method comparison using Passing-Bablok regression. Bias was assessed using Bland-Altman. A multidisciplinary team pre-defined a validation limit requiring >80% of all paired samples within 15% of the mean of both samples. Sampling quality was evaluated for both DBS and VAMS samples. Results: Of the VAMS samples 32.3% and of the DBS samples 6.2% were of insufficient quality, leading to 88 paired samples fit for analysis. Passing-Bablok regression showed a significant difference between VAMS and whole blood, with a slope of 0.88 (95%CI slope, 0.81-0.97). After correction of VAMS results for this systematic difference, Bland-Altman analysis showed no significant bias. Passing-Bablok regression showed no differences between DBS and whole blood, with a slope of 1.00 (95%CI slope, 0.95-1.04). No significant bias was observed in Bland-Altman analysis. For VAMS (after correction) and DBS, validation limit was met for resp. 83.0% and 96.6% of the samples. Conclusions: VAMS sampling can replace whole blood sampling for tacrolimus trough concentration monitoring, but conventional DBS sampling is superior to VAMS sampling, both regarding sample quality and agreement with whole blood tacrolimus concentrations.

Keywords: Tacrolimus, Dried Blood Spots, Volumetric Absorptive Micro Sampling, immunosuppressants
THE NEMATODE *Caenorhabditis elegans* AS A TOXICOLOGICAL TOOL TO MONITORING WATER QUALITY

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**Background:** Anthropogenic activities, such as agriculture, have been increasing the concentration of toxic metals and pesticides in the environment. The use of pesticides in Brazil has increased alarmingly in recent years. Usually, metals are present in small quantities in aquatic environments, but they can be discarded in significant amounts due to human activities, including agricultural activities. Thus, the present study aimed at evaluating potential contaminants, such as metals, in the water samples collected in the South region of Brazil. Also, the toxic effects in the water were assessed using the nematode *Caenorhabditis elegans* (*C. elegans*). **Methods:** Water samples (*n* = 8, site 1 to 8) were collected in July 2017 from different points in Agudo-Rio Grande do Sul, Brazil. This period in which the application of agrochemicals does not occur in the region. This region has tobacco and rice farming. The metals levels were determined by inductively coupled plasma-mass spectrometry (ICP-MS) using a Nexion 300X spectrometer (PerkinElmer-Sciex, USA). The toxic effect in *C. elegans* was evaluated using the N2 wild type strain (Caenorhabditis Genetics Center - CGC) and maintained at 20°C in nematode growth medium (NGM) with *E. coli* OP50 as a food source. The development of worms as a toxic end point was evaluated in this model. The assays were performed after synchronization, in the L1 larval stage. One thousand L1 worms were treated with 2 mL of each water sample. In the adult phase, the worms (20/treatment) were photographed and their body lengths were measured using ImageJ software. Statistical analyses were performed using ANOVA, followed by the Tukey test. **Results:** The toxic metals most frequently detected were Cu, Cr, Mg, Fe, and Mn. Levels that exceed the ones permitted by the Brazilian law were found in at least two of the collected sample. The development of *C. elegans* was affected in 7 of the water samples, showing a significant reduction in the body length of worms, when compared to control group (*p*<0.001). Mean body length of 679.6 ± 35.4 µm for control group, 617.5 ± 78.9 µm for S1, 631.0 ± 44.6 µm for S2; 559.2 ± 56.1 µm for S3, 545.2 ± 60.1 µm for S4, 505.5 ± 50.4 µm for S5, 611.0 ± 75.2 µm for S6, 611.6 ± 64.3 µm for S7, 553.5 ± 65.4 µm for S8. **Conclusions:** This study demonstrated that the presence of metals in water may be represented as a source of environmental and human contamination. Besides, *C. elegans* could be used as an integrative tool to evaluate water quality.

**Keywords:** metals; water; *C. elegans*; pollution
COCHRANE SYSTEMATIC REVIEWS OF THERAPEUTIC DRUG MONITORING AS AN INTERVENTION
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Background: Clinical interventions that are complex and involve implementation of a service may be difficult to evaluate by Randomized Clinical Trials (RCTs). Therapeutic Drug Monitoring (TDM) might be considered a complex, service level intervention, and the evidence base that justifies its clinical application may be served by the inclusion of quasi-experimental designs. Methods: The present work evaluated published Cochrane Reviews of Therapeutic Drug Monitoring as an intervention, and considered a) the restrictions on the types of studies included, and b) evaluation of quality of the included primary studies. Results: The present work is descriptive. Three systematic reviews were identified. One of these considered the inclusion of controlled before/after and interrupted time series analyses, however no such studies were identified. A number of challenges in conducting RCT with TDM as an intervention were identified. Conclusions: RCTs are the pillars of evidence based medicine, however complex, service level interventions are difficult to evaluate through RCTs. Quasi-experimental designs such as controlled before/after and interrupted time series analyses may serve to support the evidence base that justifies TDM as a valuable clinical intervention.

Keywords: therapeutic drug monitoring; methods; randomized controlled trials; quasi-experimental; controlled before/after; interrupted time series analysis
**Background:** Evaluation of water quality in rivers is important to establish management policies. The use of organisms tests for the evaluation of water quality is an interesting tool, and *Caenorhabditis elegans* is described as a good bioindicator for this. The aim of this study is to apply the alternative model *C. elegans* to evaluate the water quality of Sinos River/Rio Grande do Sul/Brazil and verify the relationship with the physicochemical parameters. **Methods:** Water samples were collected at three sites in Paranhama and Ilha Rivers (S1, S2 and S3) in November 2018, January 2019, and April 2019 (n=9). Physicochemical parameters were measured according to standardized methods. The N2 wild type of *C. elegans* strain was obtained from the Caenorhabditis Genetics Center (CGC) and maintained in nematode growth medium (NGM) with *E. coli* OP50 as a food source at 20°C. Before the tests, the nematodes were synchronized. 1000 L1 nematodes were treated with 2 mL of each water sample. Twenty nematodes/treatment were photographed and the surface area of nematodes were measured using ImageJ software. **Results:** Aluminum presented higher values than the preconized by the Brazilian Agency for quality water assessment (0.1 mg.mL⁻¹) for sites S1 and S2 in November (0.92 and 1.08 mg.mL⁻¹, respectively), for sites S1 and S3 in January (0.84 and 1.90 mg.mL⁻¹), and for sites S1, S2 and S3 in April (1.50, 1.41 and 1.40 mg.mL⁻¹). Development of *C. elegans* was affected in all sites collected in all periods, with a significant reduction in surface area of nematodes when compared to control group (p<0.05). **Conclusions:** It was possible to observe a negative effect in development of *C. elegans*, showing that this alternative model could be an integrated tool to evaluate water quality. **Keywords:** water quality; *C. elegans*; bioindicator; alternative model.
A RAPID AND SIMPLIFIED LC-MS/MS WORKFLOW FOR THE ANALYSIS OF PAIN MANAGEMENT DRUGS FOR CLINICAL RESEARCH.
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Background: The objective of this study was to develop a comprehensive LC-MS/MS analysis strategy for a large drug panel (80 compounds from 22 drug classes) using a simplified solid phase extraction (SPE) protocol that incorporates in-well hydrolysis and pre-treatment of the urine sample. Methods: Calibration curves were prepared by spiking the compounds into urine covering the appropriate measurement range for each compound, from 2-200 ng/mL for 6-MAM and fentanyl, to 25-2,500 ng/mL for many opiates and amines. Urine samples (spiked calibrators, QCs and independent QC material) were extracted using mixed-mode cation exchange polymeric SPE plates. LC-MS/MS analysis was conducted using a Waters ACQUITY I-Class UPLC system coupled to a Xevo TQ-S micro mass spectrometer. Results: The method was evaluated for linearity, precision, accuracy (recovery), extraction efficiency and matrix effects. All analytes eluted within 3.1 minutes, maintaining baseline separation of all isobaric compounds. Calibrator linearity was assessed, and QC results were accurate and precise for all compounds over the measurement range. Precision performance over five days was acceptable both within and between runs (%CV<12%). Accuracy was assessed using commercially available control materials from UTAK (mean bias < 6.2 %) and all spiked QC sample-determined concentrations, ranged from -7.8% to 16.2% bias of the target values at the lowest QC level, with an overall mean bias of <3%. Extraction efficiencies were high and consistent for all compounds, averaging >80% with %RSDs under 15% for all analytes. Conclusions: This method enables the rapid extraction and analysis of a diverse panel of drugs for clinical research. A single, rapid SPE method is used to extract all 80 compounds. The combination of sample preparation, chromatography and tandem MS analysis results in a complete and comprehensive workflow. Keywords: toxicology; urine; LC-MS/MS; opioids; SPE; quantitative.
Theme: Other theme

**CAFFEINE MONITORING AND ECOTOXICOLOGICAL RISK ASSESSMENT IN SURFACE WATER BODIES OF THE SINOS RIVER BASIN, SOUTHERN BRAZIL**

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![Figure 1. Risk Quotient of caffeine for two years at the 12 different collecting sites. S: spring; I: intermediate point; M: mouth; LR: Luiz Rau; P: Pampa; S: Schmidt; PEV: Estância Velha/Portão.](image)

**Background:** Caffeine is a human-related chemical frequently found in surface waters. It has been suggested as an anthropogenic marker for fecal contamination of water, mainly because of its high human consumption and urinary excretion. The aims of this study were to measure caffeine levels in water samples collected in streams of the Sinos River Basin (SRB), as well as to evaluate the ecotoxicological risk for *Prochilodus lineatus*, fish species found in this basin, assuming the correlation between caffeine and domestic sewage. **Methods:** Surface water samples were collected in three different points (spring, intermediate point and mouth) from four SRHB streams: Schmidt, Estância Velha/Portão, Pampa and Luiz Rau. The sampling occurred bimonthly, from June 2016 to April 2018 (n=144). The caffeine was determined by high-performance liquid chromatography. The ecotoxicological risk was estimated base on the risk quotient (RQ); which was calculated through the ratio between measured environmental concentration of the chemical and the predicted no-effect concentration (PNEC). PNEC for caffeine was established as 30 ng/L, according to studies using *Prochilodus lineatus*. Different risk levels are determined by the resulting RQ value (minimal risk: RQ <0.1, median risk: 0.1 ≤ RQ<1 and high-risk: RQ ≥ 1). **Results:** Caffeine was detected in 90.97% (n=131) of the samples and high ecotoxicological risk was characterized in all the streams. As expected, the springs presented lower concentrations of caffeine, followed by the intermediated and mouth points, respectively, due to the interaction with anthropogenic activities. The RQ evaluation follows the pattern of the caffeine concentrations found in the three different collecting points, being the higher RQ values found in the mouth streams. This study report there is a high ecotoxicological risk to *Prochilodus lineatus* specie, due the presence and concentrations of caffeine detected in all sites throughout the two years research. **Keywords:** caffeine; ecotoxicological risk; risk quotient; surface water.
Background: Lithium carbonate remains the main drug for the treatment of bipolar disorder. Besides its high clinical efficacy, the drug has a narrow therapeutic range (0.5-1.2 mEq/L), and is associated to the development of several adverse events, which can reduce the adherence to the pharmacotherapy. Considering the interindividual variability on drug pharmacokinetics the objective of this study was to monitor lithium carbonate therapy as well as the adherence in patients with bipolar disorder in a public psychiatric health service in South Brazil. Methods: A total of 47 patients who provided blood samples for serum lithium by graphite furnace atomic absorption spectrometry (GFAAS) participated in the study. Adherence was assessed through the Mars and Morisky Green scales, patients' knowledge and attitudes regarding lithium therapy were assessed using lithium knowledge tests and lithium attitudes questionnaire. The study was approved by the research ethics committee (REC) by report number 2.231.794. Results: Most of the patients were female (68%), with age from 18 to 73 years. Lithium serum concentrations ranged from 0.18 to 1.1 mEq/L, being 42% below the therapeutic range. Within the group of patients with low lithium levels, 55% had low or no adherence to the treatment according to the Morisky Green scale, of these, 54.5% received less than two minimum wages monthly. There was a significant but poor correlation between the concentration/dose ratio of lithium and the adherence indicated by the Mars scale (r = 0.353, p<0.05). Conclusions: This study demonstrated a high prevalence of patients with lithium serum levels below the therapeutic range and with poor adherence. The serum lithium monitoring in addition to adherence scales demonstrate to be effective tools to identify the main problems related to non-adherence and inefficacy of treatment with lithium carbonate. Keywords: Lithium; bipolar disorders; therapeutic drug monitoring, adherence.
**ANTIDEPRESSANT-LIKE EFFECT OF LIPOSUMME AND NANOCAPSULES OF PAROXETINE IN MICE**

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**Background:** Depression is a mood disorder that affects 322 million people and is characterized by depressed mood and anhedonia. Patients with depression presents lower levels of serotonin availability in the brain. One of the most commonly drugs classes used for the treatment of depression are the Selective Serotonin Reuptake Inhibitors (SSRIs), such as paroxetine. Nanotechnology is a new approach to development of drugs with better pharmacokinetic profiles. The objective of the present study was to evaluate the antidepressant potential of a liposome and a nanocapsule containing paroxetine in order to optimize bioavailability of the drug. **Methods:** Liposomes and nanocapsules were prepared using reverse phase evaporation and nanoprecipitation methods, respectively. The nanostructures were characterized in terms of particle size, polydispersity index and zeta potential using NanoBrook 90Plus®. The paroxetine content in the formulations was assayed by liquid chromatography. In order to determine the association efficiency, the ultrafiltration-centrifugation was performed using Ultrafree® MC 10,000 MW (Millipore, USA) membrane for 15 min at 500 rpm. The amount of paroxetine associated to the nanostructures was calculated as the difference between the total and free paroxetine concentrations determined in the formulation and ultrafiltrate, respectively. BalbC mice, female, between 45 and 60 days old, nulliparous, obtained from in-house breeding colonies at the Feevale University were used for pharmacological evaluation. The animals were treated orally (1 hour before the behavioral tests) and were divided into the control groups, paroxetine (10 mg.kg$^{-1}$), paroxetine containing liposome (1 mg.ml$^{-1}$) and paroxetine (1 mg.ml$^{-1}$). To evaluate the effect of antidepressant-type, the animals were submitted to the tail suspension test (TST) and the immobility time was recorded for 6 minutes, divided into the initial 2 min, 4 min and total time times. The open field test (OFT) was used to evaluate the animals locomotor activity. The number of crossings, rearings, groomings and poops were recorded for 15 minutes. All experimental protocols were previously approved by The Animal Care Local Ethical Committee (CEUA Feevale - protocol n° 06/2016). **Results:** The formulations presented particle sizes in the range of 200.8 to 245.8 nm, polydispersity index between 0.087 and 0.283, zeta potential -13.76 to -17.9 mV, drug content in the range of 83.83 to 67.75% and association efficiency of 98.09 and 98.83%. Pharmacological tests in animals receiving paroxetine or nanocapsule containing paroxetine showed a significant decrease in the time of immobility in the TST when compared to the control group; and the paroxetine-containing liposome group reduced the immobility time when compared to free paroxetine, suggesting a potentiation of the antidepressant-like effect. However, the animals treated with paroxetine incorporated in both formulations significantly increased the number of crossings in the OFT when compared to the control group, without affecting the other parameters analyzed in the OFT. **Conclusion:** Since the liposome containing paroxetine potentiated the effect of the free drug, it is suggested that the liposome may be improving the bioavailability of the drug or, furthermore, this effect may be associated with the increased effect on locomotion. All these hypotheses are being investigated. **Keywords:** depression; paroxetine; liposome; nanocapsule; open field, tail suspension test.
Theme: Other theme

EVALUATION OF ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY OF OZONE IN ALTERNATIVE MODELS IN VIVO

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Background: Fungus infection is a crucial problem because there are few drugs with fungicidal properties compared to antibacterials and the complexity of the fungi nature, a molecule being tested recently is ozone, so this work tests ozone properties and toxicity using alternative methods in vivo, following the standards of the OECD, thus causing no harm to pain sentients animals. Methods: The tests began with HET-CAM testing ozone toxicity. Staphylococcus aureus and Candida albicans strains were used for the antibacterial and antifungal test, using the IET-CAM and CAM-TBS method with the chorioallantoic membrane. Afterwards, tests with the larvae of Tenebrio molitor and Zophobas morio infected by C. albicans and S. aureus, using ozone for treatment in therapeutic doses in a scenario of chronic infection. Results: Ozone was shown to be non-irritating to the chorioallantoic membrane through the HET-CAM (the irritant score was 2.61). The result of the CAM-TBS in the concentration of 50μg.ml⁻¹ was considered Non-irritant/Weak irritant (NI/WI) (1.73). In the IET-CAM it was possible to observe bacterial or fungal growth and death in the untreated infected eggs, whereas in the infected and treated eggs with ozone there was no death and no bacterial and fungal growth (100% efficiency in antifungal and antimicrobial activity in vivo). All mealworms survived the injection procedures (PBS) and the concentration of ozone tested showed an increase in mortality rates over time of exposure, demonstrating the efficiency of the method to evaluate the acute toxicity. In relation to the test of antimicrobial activity in larvae, the ozone presented an efficiency rate around 90% in the treated mealworms. Conclusions: The HET-CAM is a sensitive test to determine the toxicological parameters, in this way, the use of such methodology is shown to be acceptable, becoming an alternative to other in vivo. CAM-TBS has advantages such as its outcome quantitative and the most efficient classification criteria for products with low irritant potential, in this case ozone. Work with animals includes interventions or treatments for experimental purposes that may involve pain, suffering or harm to them. Therefore, the use of alternative models is necessary, based on the 4 Rs (“Reduce”, “Refine”, “Replace” and “Responsibility”). Thus, this research made it possible to evaluate the antifungal and antibacterial activity of ozone in alternative models in vivo.

Keywords: ozone; HET-CAM; antimicrobial; antifungal; IET-CAM; mealworms.
Theme: Other theme

ACUTE TOXICITY EVALUATION AND ANTIDEPRESSANT-LIKE EFFECT OF VORTIOXETINE LIPOSOME

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Background: Depression is a mood disorder that affects 322 million people in the world and is characterized by depressed mood and anhedonia. Patients with depression have decreased levels of serotonin in distinct brain regions. One of the most commonly used classes of drugs for the depressant treatment are the Selective Serotonin Reuptake Inhibitors (SSRIs), such as fluoxetine and paroxetine and, more recently, vortioxetine (Brintellix®) a drug with multimodal mode of action. A new strategy for the development of drugs with better pharmacokinetic profile is the use of nanotechnology. The aim of this study was to evaluate the acute toxicity of vortioxetine liposomes, as well as to evaluate the antidepressant potential, in order to evaluate the bioavailability of this drug.

Methods: Liposomes consisting of egg lecithin were prepared by the lipid film hydration method. Acute toxicity was performed using OECD 423 guideline. Pharmacological evaluation was conducted with BalbC mice, female, between 45 and 60 days old and nulliparous. The animals were divided into four groups: white liposome, Brintellix (10 mg/kg) and vortioxetine liposome (1 mg/ml) and received oral treatments 1 hour before the behavioral tests. To evaluate the antidepressant-like effect, the animals were submitted to the tail suspension test (TST) and the immobility time was recorded for 6 minutes, divided into the initial 2 min, 4 min and total time. The open field test (OFT) was used to evaluate the locomotor activity of the animals. The number of crossings, readings, grooming and poops were recorded for 15 minutes. All experimental protocols were previously approved by CEUA Feevale (protocol number 06/2016). Results: Non-drug formulations (white liposomes) and vortioxetine liposomes had particle sizes of 183.69 and 176.83 nm, polydispersion index 0.204 and 0.19, zeta potential -22.19 and 7.33 mV and pH 4.12 and 5.92, respectively. In the acute toxicity test, no signs of toxicity were observed. In addition, food intake and weight gain were not affected. Animals that received Brintellix® or vortioxetine liposome showed a significant decrease in immobility time at all times analyzed in the TST when compared to the control group and the liposome blank group. However, no differences were observed between the Brintellix® group and vortioxetine liposome in the TST. In the OFT, the animals that received Brintellix® presented a decrease in the number of grooming when compared to the animals of the control group, suggesting a reduction of the type-anxious behavior. No differences were observed in the other parameters analyzed in the TST. Conclusions: Vortioxetine liposome demonstrated safety at category 5 (maximum), according to the OECD classification; however, more studies are necessary to investigate its pharmacological effect.

Keywords: Depression; Vortioxetine; Liposome; Acute toxicity; Tail suspension test.
PT-31, A MOLECULE WITH ANTIPSYCHOTIC PROFILE, DOES NOT AFFECT THE NEMATODE C. ELEGANS SURVIVAL AND DEVELOPMENT

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Background: Schizophrenia is a disease that affects about 21 million people. The treatment is done with typical and atypical antipsychotics; however, they may cause serious side effects and toxic effects. PT-31, an imidazolidinone derivative, has demonstrated promising results in the pre-clinical evaluation. The aim of this study was to evaluate the PT-31 effects on survival rate and development in an alternative test of toxicity, C. elegans, when compared to typical (haloperidol) and atypical (clozapine) antipsychotics. Methods: The N2 wild type of C. elegans strain was obtained from the Caenorhabditis Genetics Center (CGC) and maintained in nematode growth medium (NGM) with E. coli OP50 as a food source at 20°C. Before the tests, the nematodes were synchronized. 1500 L1 nematodes were treated with 3 concentrations of PT-31, haloperidol and clozapine (80, 160 and 320 μM) for 30 minutes and then placed in NGM medium. 48 hours later, the number of live nematodes per treatment was verified. The development was evaluated after nematodes reached adulthood (3 days after synchronization) by measuring body length (20 nematodes/treatment). Three independent experiments were performed for each test. Results: Clozapine 320 μM affected survival rate, demonstrating significant death (about 40%), but not PT-31. The development showed a significant reduction in body length for clozapine and haloperidol at 320 μM (p<0.05), with means of 381.3±52.93 μm and 394.1±21.62 μm, respectively. PT-31 did not affect the nematodes survival and development in any of the tested doses. Conclusions: This study suggests that, at the concentrations tested, PT-31 does not affect the nematodes survival and/or development, suggesting a profile safer than the antipsychotics haloperidol and clozapine in this alternative model of toxicity. Keywords: PT-31; haloperidol; clozapine; C. elegans; antipsychotics; toxicity.
SUBCUTANEOUS LEVETIRACETAM IN THE MANAGEMENT OF SEIZURES IN THE PALLIATIVE CARE SETTING

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Background: Levetiracetam (LEV) formulations are only available for oral and intravenous administration. However, subcutaneous (SC) administration is commonly used in palliative care. There are some case reports and reviews of this off-label route of administration of LEV in humans with determination of serum concentration in some cases but pharmacokinetics studies taking into consideration this way of administration are lacking. Methods: Trough LEV plasma levels were determined by a previous validated high performance liquid chromatography technique. Adverse effects were registered. Data was analyzed using Monolix 2018R2 (Lixoft, France) in order to estimate the population apparent elimination clearance (CL/F) and its between-subject variability. Results: Seven patients (aged 53-86 years), five female and two male, took part in the study. In two cases, dose increase was required to control seizures. In one of these patients, midazolam was also added. Adverse effects were local irritation in the injection site in three patients, and somnolence in one patient receiving 3000 mg/day. Irritation was resolved rotating the injection site. The plasma level that caused somnolence (74.8 mg/L) was outside the therapeutic range reported in the literature for LEV: 12-46 mg/L. After reduction of dose to 2000 mg/day, plasma level decreased to 45.6 mg/L, and both somnolence and seizures disappeared. Mean (±SE) LEV levels after dose adjustment for the different doses (1000 and 2000 mg/day) are shown in the figure. A one-compartment linear model with instantaneous drug input adequately described LEV plasma concentrations. The apparent volume of distribution was fixed to a value of 33 L. The final model estimates (R.S.E) were: CL/F =2.51 L/h (21.6 %), between subject variability= 42.0% (40.3 %) and residual error=10.5 mg/L (22.1 %). The estimated elimination half- life was 10.4 h. Conclusions: SC use of LEV seems to be effective and well tolerated. Therapeutic drug monitoring was a useful tool in order to optimize individual dosage regimens. Doses of 1000-2000 mg/day yielded concentrations within the therapeutic range reported in the literature. The population CL/F and t1/2 were successfully estimated and they are in agreement with the expected values reported by other authors.

Keywords: Levetiracetam; off-label; drug monitoring; subcutaneous administration; pharmacokinetics.
Background: Cannabidiol (CBD) is a natural product found in *Cannabis sativa* L, with a chemical structure very similar to tetrahydrocannabinol (THC) but without the psychoactive effects or the potential for dependence associated to THC. Preclinical studies recognized CBD safety and potential pharmacological action in neurological conditions such as epilepsy, which led to its development as a promising new drug. We developed an ultra-fast UHPLC-MS/MS method to detect and quantify CBD in biological samples, especially in serum of patients under treatment. Methods: The [M+H]⁺ fragmentation pattern of a pure CBD standard (500 ppb in MeOH) was investigated by direct infusion in an ABSciex QTRAP 6500, and compound and source parameters were optimized for the MRM transitions m/z 315→259, 315→193 and 315→123. Standard curves with CBD (15 ppm) and internal standards (benznidazole and mfenamic acid, 3 ppm each in cold ACN) were prepared in serum. Samples were then vortexed, centrifuged at 13200 rpm, and the supernatant was diluted 1/10 in ultrapure water for injection (2 µL) into a 1.8 μm (100 x 2.1 mm) Restek Force C18 column on a Shimadzu Nexera X2 HPLC with autosampler at 8 °C. Chromatography was optimized using water (A) and ACN (B) as mobile phases (both with 0.1% formic acid), at a flow rate of 0.7 mL/min, with a gradient from 70 to 90% B in 2 min. Six-point calibration curves were prepared in water and serum (1 - 1000 ppb) following the same procedure. Results: The method was selective, with linearity throughout the range studied (R² > 0.993). The repeatability (RSD%, n=6) of peak areas were < 10%, and detection (LOD) and quantitation (LOQ) limits were 4.6 ppb and 15.3 ppb, respectively. Conclusions: A sensitive, precise and accurate method is presented to quantify CBD, capable of rapidly monitoring the drug in blood and in turn facilitating pharmacokinetic studies in patients. Keywords: CBD; Cannabidiol; UHPLC-MS/MS; Ultra-Fast; Serum.
DETERMINATION OF VITAMIN K\textsubscript{1} AND VITAMIN K\textsubscript{2} (MK-4, MK-7) IN SERUM OF PATIENTS WITH CYSTIC FIBROSIS BY LY-MS/MS

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Background: Vitamins K\textsubscript{1} and K\textsubscript{2} are the cofactors for the enzyme gamma-glutamylcarboxylase, which is involved in carboxylation of the vitamin K-dependent proteins. Vitamin K deficiency is frequently observed in patients with cystic fibrosis due to bowel resection, malabsorption of fat, liver disease, etc. Objective of this study was to determine the levels of vitamin K in the pediatric patients with cystic fibrosis by a new LC-MS/MS method.

Methods: A UPLC-tandem mass spectrometry method for the determination of vitamin K\textsubscript{1} and vitamin K\textsubscript{2} (MK-4 and MK-7) in human serum was developed. Mass spectrometric detection was performed on a triple-quadrupole in the positive electrospray ionization mode by multiple reaction monitoring. \textit{d}7-MK-7, \textit{d}7-MK-4 and \textit{d}7-K\textsubscript{2} were used as internal standards. Chromatographic separation was carried out on SB-C8 column (2.1x100 mm, 1.8 \textmu m, Agilent Technologies) using a binary gradient of mobile phases. We measured samples from 44 pediatric patients with cystic fibrosis and compared the results with healthy population.

Results: The LC-MS/MS method has been successfully validated. Lower limits of quantitation were 0.03 ng/mL for K\textsubscript{1} and MK-4, and 0.05 ng/mL for MK-7. The intra and interday accuracy and precision were evaluated on two QC samples by multiple analysis and coefficients of variation were 2.3-7.0% for intra-assay, 7.2-11.3% for inter-assay, and 0.5-6.6% for precision. Serum levels of vitamin K\textsubscript{1}, menaquinone-4 and menaquinone-7 in patients with cystic fibrosis were (median \pm SEM): 0.045 \pm 0.510 ng/mL; 0.003 \pm 0.021 ng/mL; and 0.140 \pm 0.198 ng/mL. Serum levels of vitamin K\textsubscript{1}, menaquinone-4 and menaquinone-7 in healthy population were (median \pm SEM): 0.412 \pm 0.150 ng/mL; 0.063 \pm 0.021 ng/mL; and 0.309 \pm 0.146 ng/mL.

Conclusions: We developed and fully validated a new LC-MS/MS method for determination of vitamin K\textsubscript{1}, MK-4 and MK-7. The comparison of vitamin K levels in patients with cystic fibrosis and healthy population showed statistically significant differences. Vitamin K supplementation in patients with cystic fibrosis seems to be necessary.

Supported by the project (Ministry of Health, Czech Republic) for conceptual development of research organization 00064203 (University Hospital Motol, Prague, Czech Republic).

Keywords: vitamin K\textsubscript{1}, vitamin K\textsubscript{2}, LC-MS/MS, cystic fibrosis
Background: The fluoride is an important agent against the dental caries, being present in the network of public supply through the water. However, excessive intake of fluoride can cause problems in both hard and soft tissues. Considering the influence of F intake on glucose homeostasis, our study investigated the effect of fluoridated water administration on the development of type-1 diabetes mellitus (T1D) in NOD mice. Methods: Seventy-two 35 day old female NOD mice (Protocol Ethics Committee: 013/2017) were randomly divided into two groups, according with the fluoride concentration (F) in the drinking water containing 0 (control group) and 10 ppm F (treated), administered for 14 weeks. This concentration of F administered to mice is the concentration found in water artificially fluoridated to humans. After the experiment period, the animals were euthanized. Plasma F was collected analyzed with the ion-specific, glucose was analyzed with test glucose - oxidase and insulin with ELISA and pancreas was performed morphometric analysis, staining with (hematoxylin and eosin). Results: The treated group had significantly higher concentrations of F in the plasma (Mann-Whitney, p <0.05) and a tendency to decrease (20%) in glycemic, (unpaired t, p> 0.05). Plasma insulin concentrations were similar in both groups (unpaired t, p> 0.05). In the morphological analysis, the % of the total area of the pancreas occupied by islets did not present significant difference between the groups (unpaired t, p> 0.05). Future studies are needed to help clarify the pancreatic changes caused by F, in addition to confirming the tendency to reduce glycemic, which is very relevant in terms of public health, since the fluoridation of public water supply is widely used for the control of dental caries.

Keywords: Sensitivity; Fluoreto; NOD mice; Diabetes.

Financial Support: CAPES and FAPESP Process:2016/20020-4
Background: Depression is a relatively common, chronic and recurrent disease. It is often associated with functional disability and impaired physical health. Its treatment is through psychotherapy and drug therapy, with fluoxetine, a Selective Serotonin Reuptake Inhibitor (SSRI), the most widely used. There are currently few studies on genotoxicity involving fluoxetine in humans and most of them show inconclusive results. Thus, the objective of this work was to evaluate the genotoxicity in fluoxetine users through the comet assay. Methods: A cross-sectional study was conducted to assess the socio-demographic characteristics, concomitant diseases, drug use and fluoxetine plasmatic concentration. Genotoxicity was evaluated by the comet assay in a heparinized blood drop. This assay detects different types of DNA damage in eukaryotic cells, forming a comet-like pattern (DNA concentration in the head and fragments in the tail). For each individual, 100 cells were analyzed, generating an index ranging from 0 to 400. Statistical analysis was performed using Mann-Whitney U test. Results: Were evaluated 31 users of fluoxetine [80.6% women, 52.9 ± 14 years, using fluoxetine for 4.0 (2.0 – 14.8) years] and 43 non-users of fluoxetine (79.1% women, 58.7 ± 11 years). The groups present a similar profile regarding the concomitant diseases and the use of drugs. The fluoxetine group presented higher DNA damage index when compared to non-users [174 (73-244) versus 115 (36-165), respectively; p = 0.036]. On the other hand, no correlation was found between the time of use and the plasma concentrations of fluoxetine. Conclusions: The continuous use of fluoxetine for more than 6 months causes increased DNA damage compared to non-fluoxetine users, regardless of other concurrent diseases, and use of other medication. Keywords: depression, fluoxetine, genotoxicity, DMA damage and comet assay.
Background: Micafungin is an antifungal medicine that is licensed by the European Medicine Agency (EMA) for the treatment of invasive Candidiasis in critically ill patients, children and adolescents. These patients often are in such conditions that may affect the distribution, metabolism and elimination of medicines whereby dose adjustments might be mandatory. [1]. Research for the therapeutic range of micafungin is still on going and therefore we present an assay for the detection of micafungin in plasma. Methods: A fast and efficient sample preparation was performed by plasma protein precipitation using 13C6-Micafungin as internal standard. Extracts (5 µL) were analyzed on a Waters Acquity BEH C18 column using a gradient elution with a mixture of aqueous ammonium formate 10 mM with a pH of 7 (eluent A) and acetonitrile (eluent B). The analytes were detected with a Thermo Scientific Quantiva triple quadrupole, in negative ionization mode. Results: The analytical method was validated according to European Medicine Agencies guidelines. The lower limit of quantification (LLOQ) was 0.01 mg/L and linearity was at least >R² 0.999 from 0.01 to 50 mg/L (figure 1). Variation coefficients of LLOQ were within 20% and for low, medium and high controls were all within 15%. Conclusions: A simple LC-MS/MS method was developed, which provides a high specificity, precision, and accuracy for the quantification of micafungin in plasma.

Keywords: Micafungin, LC-MS/MS, TDM, pharmacokinetics research

References:
Background: Benzene (BZ) is considered carcinogenic by International Agency for Research on Cancer. BZ is one of the constituents of gasoline, representing a hazard of occupational concern. Some studies have reported the capacity of BZ to induce epigenetic changes, which may be related to the carcinogenicity of BZ to humans. The aim of this study was to investigate the possible effects of occupational exposure to BZ on the global DNA methylation in gas station workers (GSW). Methods: A total of 80 individuals were divided in two groups: Non-occupationally exposed (NOE, n=30) and GSW (n=50). The environmental BZ levels were determined through SKC 575-002 passive samplers. Global DNA methylation in whole blood were assessed by HPLC-DAD. Results: All participants were male. The mean age were 34.9±5.8 and 32.2±7.8 for NOE and GSW groups, respectively. The levels of BZ measured in passive samplers were 0.04±0.02 mg.m⁻³ for NOE group and 0.57±0.10 mg.m⁻³ for GSW (p<0.001). The median global DNA methylation levels were 3.52% (3.05-3.80) for NOE and 3.83% (3.61-3.96) for GSW (p=0.010). It was observed a weak but significant correlation between BZ in passive samplers and global DNA methylation levels (rs=0.310, p=0.005). Conclusions: The GSW group was exposed to BZ levels higher than NOE group. It was possible to observe an increase of global DNA methylation in GSW group. Our results suggest that even low exposure to BZ could lead to epigenetic changes and might be related to the carcinogenicity of BZ. Keywords: Benzene; Epigenetic; Gasoline; DNA methylation; Occupational Exposure; Biomarkers of effect.
EVALUATION OF MITOCHONDRIAL VIABILITY IN VERO CELLS AFTER TREATMENT WITH TWO DIFFERENT IMIDAZOLIC SALTS

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Background: Imidazole (IS) salts are hydrophilic anion-associated cationic nuclei and amphiphilic long-chain hydrophobic N-alkyl chain molecules. Its molecular structure can be adjusted by modifying the structural arrangement of ions, including anion substitutions. These characteristics facilitate the electrostatic interaction with biological systems, evidencing the importance of these constituents for the pharmaceutical industry due to the great potential of their derivatives. Understanding the molecular mechanisms of cellular cytotoxicity is necessary to develop compounds with lower toxicity and greater selectivity and specificity for their therapeutic target. Therefore, we evaluated the cytotoxicity of two compounds: IS05 (PhC3-o-MimCl) and IS06 (PhC3MimMes), using a mitochondrial functionality assay based on the MTT reduction of the methyl tetrazolium salt. **Methods:** VERO cells were maintained at 37°C and 5% CO₂. Cultures with 80% confluence were exposed for 48 hours at serial dilutions at concentrations of 1250μM to 9.76μM. Cells maintained in standard condition were used as control group and were considered equivalent to 100% viable cells. After the incubation time, the MTT assay was performed and the cytotoxic concentration was calculated for 70% of the cells (CC₇₀). **Results:** It was observed that cultures exposed to both SI did not present a significant reduction of cell viability in the lowest concentration evaluated (IS05 95% and IS06 99%). However, they showed a 50% reduction in cell viability at the highest concentration tested. Preliminary in silico trials demonstrate the low toxicity of these molecules. Therefore, it was decided to estimate the CC₇₀ of these IS, finding them in the range of 888μM for IS05 and 775.92μM for IS06. **Conclusions:** We conclude that ISs have a concentration-dependent toxicity curve and exhibit CC₇₀ in the range of 775 to 888μM, confirming its low cytotoxicity and possible use of pharmacological prototypes. Financial support: CAPES, CNPq, FEEVALE.

**Keywords:** Cytotoxicity; cell culture; cell viability; MTT
EVALUATION OF SERUM CYTOKINES IN CHRONIC PATIENTS USERS OF MEDICINAL PLANTS
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Background: The immune system plays an important role in several processes that involve chronic diseases (CD), such as the activation of inflammatory cytokines. Medicinal plants (MP) have traditionally been used to treat diseases, commonly combined with the pharmacological treatment. Therefore, the aim of this study was to evaluate the profile of pro and anti-inflammatory cytokines in chronic patients users of MP. Methods: An interview comprising patient’s illness, use of MP and medications, and a venous blood collection were performed. Plasma levels of cytokines (IL-2, IL-4, IL-6, IL-10, TNF, IFNγ and IL-17A) were determined by flow cell cytometry using BD™ Cytometric Bead Array TH1/TH2/TH17. Statistical analysis was performed using Mann-Whitney U test. Results: The studied group included 49 volunteers, 89.8% women with average of 59.2 ± 9 years and typically non-smokers (90%). The prevalent CD were systemic arterial hypertension (55%), depression (43%), hypothyroidism (17%) and diabetes mellitus (11%). Most patients presented more than one illness. Based on patient reports, the most frequently used MP was lemongrass (C. citratus), followed by chamomile (M. chamomilla), pennyroyal (M. pulegium) and macela (A. satureioide). The concentrations of interleukin 4 (IL-4, an anti-inflammatory cytokine) were highest in diabetic patients users of “canela de velho” (M. albicans) and princess vine (C. sicyoides). TNF levels (a pro-inflammatory cytokine) were increased in users of princess vine (C. sicyoides) when compared to non-users of this plant. According to studies, these plants present anti-inflammatory activity through different mechanisms. There was no correlation between CD and cytokines. Conclusions: These primary results show increase of IL-4 in diabetic patients, which was suggested to be related with the use of MP, as well as the increase in TNF levels. Further studies are required to understand the increase of IL-4 and TNF levels. Financial support: FAPERGS, CNPq, PPSUS 03/2017, CAPES, FEEVALE. Keywords: cytokines; flow cytometry; inflammation; phytotherapy.
PARTICULATE MATTER INCREASE OXIDATIVE STRESS IN WISTAR RATS
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Background: Particulate matter (PM) is an air pollutant related to many deleterious health effects. Oxidative stress is among these effects, which can cause from a local inflammatory response to systemic damage to the organism. Therefore, the aim of this study was to evaluate the effects of the PM at different concentrations under oxidative stress parameters in Wistar rats. Methods: The study evaluated the effects of particulate matter (PM2.5 and PM10), collected in the metropolitan region of Porto Alegre-RS/Brazil, under oxidative stress parameters in male Wistar rats. The animals were randomly divided into 3 groups (n=8): G1 – control; G2 – exposed to PM2.5; G3 – exposed to PM10. The animals were submitted to a nasal instillation protocol of the PM for 5 weeks. Posteriorly, dosages of ferric reducing antioxidant power (FRAP); catalase (CAT); superoxide dismutase (SOD); glutathione peroxidase (GPx) were accomplished. Results: In relation to the oxidative stress parameters analyzed, CAT and GPx presented significant differences among G2 and G3, according to Table 1, indicating that the larger the particle size, the greater the activity of these enzymes. The initial and final weights presented significant differences in all groups. Besides, a correlation between the weight differences and the inhaled solution (r=0.296 and p=0.041) was observed, in which the control group (G1) had a smaller weight difference than the groups exposed to PM2.5 and PM10, thus denoting a negative correlation. Conclusions: From the results obtained, it is possible to infer that the particulate matter is related to oxidative stress and that the particle size interferes with the degree of injury to the organism.

Keywords: pollution; particulate matter; oxidative stress; health.
Theme: Other theme

THE COMBINED IN VITRO EFFECTS OF ARSENIC, CADMIUM AND MERCURY ON HEPATOCARCINOMA CELLS
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Background: Arsenic, cadmium and mercury are among the most toxic environmental heavy metals due to their distribution, longevity and ease of contamination. Few combinational studies are available to define their mechanism of cytotoxicity in organ systems, such as the liver. The study determined the individual and combinational (at the ratio of the maximum exposure levels set by the Environmental Protection Agency) effects of arsenic, cadmium and mercury in a cellular model of hepatotoxicity. Methods: Cytotoxicity was determined in the HepG2 hepatocarcinoma cell line after 24 h exposure by assessing the effects on cell density (sulforhodamine B), oxidative stress (dihydro-dichlorofluorescein diacetate and monochlorobimane), mitochondrial membrane depolarization (JC-1), adenosine triphosphate levels (chemiluminescence) and caspase-3/7 activity (Ac-DEVD-AMC). Results: Cadmium was most cytotoxic (IC50 = 0.43 mg/L), followed by mercury (IC50 = 26.23 mg/L) and arsenic (IC50 = 6.71 mg/L). Combinational treatment decreased cell density by 93% at a reference concentration of 4 mg/L As, suggesting additive cytotoxicity between the metals. The combination induced greater detriments to all cytotoxic parameters (mitochondrial membrane depolarization [78%]; reduced reactive oxygen species [90%], glutathione [99%] and adenosine triphosphate reduction [93%]; increased caspase-3/7 activity [505%] at 4 mg/L As reference) in comparison to the single metals, however, similar patterns were observed. Deviations were noted where individual arsenic and mercury did not depolarize the mitochondrial membrane (~12%) or decreased caspase-3/7 activity (~85%), respectively, at similar concentrations. Conclusions: Combinational treatment was more cytotoxic than individual metals, causing gradual mitochondrial membrane depolarization, depletion of glutathione and induction of caspase-3/7 activity; such effects are consistent with pro-apoptotic features, however, it lacked induction of reactive oxygen species. Slight differences in the mechanism of cytotoxicity was observed. The study highlights alterations to the cytotoxic potency of arsenic, cadmium and mercury when acting in addition or synergism.

Keywords: Arsenic; Cadmium; Mercury; Heavy metals; In vitro; Hepatotoxicity.
Background: Pentachlorophenol (PCP) is a ubiquitous pesticide and a persistent environmental pollutant. Knowledge regarding its neurotoxic mechanisms is limited, thus the aim of the study was to evaluate the effects of PCP and its metabolites, tetrachloro-1,4-benzoquinone (TCBQ) and tetrachlorohydroquinone (TCHQ) in human neuroblastoma SH-SY5Y cells. Methods: Flow cytometry was employed to investigate effects on cell cycle, mode of cell death, reactive oxygen species (ROS) generation, and mitochondrial membrane potential (Δϕm) at different time points. Caspase-3 activity and glutathione (GSH) was assessed using fluorospectrometry, and cell morphology visualized using fluorescence microscopy. Effects on acetylcholinesterase were assessed using the Ellman esterase assay. Results: TCBQ and TCHQ were found to induce changes more rapidly than PCP, with S phase and G2/M blocks observed for the compounds respectively, after 12 h exposure. A G1 block occurred after 24 h exposure to PCP. The predominant mode of cell death after PCP exposure was necrosis, whereas TCBQ induced apoptosis. The switch from apoptosis to necrosis after cells were exposed to TCHQ is attributed to an overwhelming ROS insult on apoptotic machinery. All compounds resulted in increased caspase-3 activity. Decreased Δϕm was an early event for all compounds, which ultimately increased ROS production. Oxidative stress was a result of PCP and TCBQ toxicity, while a switch from reductive to oxidative stress in cells exposed to TCHQ was indicated by increased GSH and transient Δϕm recovery. Acetylcholinesterase inhibitory activity was exhibited only by TCHQ. Conclusions: PCP, TCBQ and TCHQ exhibited different mechanisms of toxicity toward SH-SY5Y cells. This study provides new insight into the toxic effects of PCP and its metabolites in neuronal cells, and highlights the importance of assessing the action of metabolites in conjunction with the parent compound, as extrapolation of effects cannot be assumed from the parent compound alone.

Keywords: Pentachlorophenol; Tetrachloro-1,4-benzoquinone; Tetrachlorohydroquinone; Neurotoxicity; In vitro
Theme: Other theme

QUANTIFICATION OF STATINS IN BLOOD PLASMA BY MEANS OF HYPHENATED MASS SPECTROMETRY TECHNIQUES – METHOD DEVELOPMENT AND APPLICATION FOR ADHERENCE TESTING AS WELL AS THERAPEUTIC DRUG MONITORING

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Background: Statins are international standard therapy to reduce total serum cholesterol concentrations. Unfortunately, dose-related side effects and sub-optimal response, attributed to non-adherence amongst others, were described. Therefore, a fast and sensitive liquid chromatography (LC)-based method for adherence testing as well as therapeutic drug monitoring of seven frequently prescribed statins and four active metabolites in human blood plasma should be developed, validated, and applied. Methods: Atorvastatin (+ two active metabolites), fluvastatin, lovastatin (+ one active metabolite), pitavastatin, pravastatin, rosuvastatin, and simvastatin (+ one active metabolite) were analyzed using two analytical instruments, a low-resolution ion trap (LRIT) mass spectrometry device (MS) and a high-resolution orbitrap MS (HRMS/MS) in comparison. Furthermore, several internal standards (IS) were tested. Validation was performed according to international guidelines including selectivity, carry-over, accuracy, precision, matrix effects, and analyte stability. Finally, applicability was tested using 14 authentic patient samples containing three different statins and three active metabolites. Results: As atorvastatin-d5 was shown to cause an analytical interference, diazepam-d5 and pentobarbital-d5 were chosen as IS. Using LC-HRMS/MS, all validation criteria could be fulfilled for all statins and the metabolites except for fluvastatin, which failed the acceptance criteria for stability and thus for accuracy, and precision. Due to the lower sensitivity of the LRITMS, the method could only be validated for atorvastatin and its two metabolites, the active lovastatin metabolite, pitavastatin, and rosuvastatin in the range of high plasma concentrations, partly above the therapeutic range. Authentic human plasma samples were analyzed by HRMS/MS and concentrations of statins + metabolites were in the therapeutic range for 11, above for one, and below for two samples. Conclusions: A LC-HRMS/MS method for identification and quantification of atorvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, and simvastatin and four active metabolites was successfully developed and its applicability demonstrated. Keywords: HMG-CoA reductase inhibitors, cardiovascular diseases, adherence monitoring, TDM.
Background: Valproic acid (VPA) is an anticonvulsant with an increasing use in the psychiatric setting. Hyperammonemia has been reported as a rare adverse effect in the treatment with VPA. Serum carnitine (CAR) depletion is postulated in the literature as a possible cause of higher ammonia levels detected in patients under VPA treatment. The aim of this study was to analyze if low carnitine levels could result in increased ammonia levels in patients treated with VPA.

Methods: Two groups of subjects were studied: Group A) epileptic patients under phenytoin treatment (n=31); Group B) patients under VPA treatment for bipolar disorder (n=28); Predose plasma VPA concentrations were measured using a validated liquid chromatographic method. For the two groups CAR and ammonia blood levels were quantified using liquid chromatography-tandem mass spectrometry and Cobas c311, Roche Laboratories, respectively.

Results: Patients in Group A presented lower levels of ammonia than patients in Group B (61.7±27.3 µg/dL vs 105.5±57.2 µg/dL, p<0.01), population range 25-94 µg/dL. All VPA concentrations in patients with hyperammonemia were below or within the population therapeutic range (50-100 mg/L) reported in the literature for VPA in plasma. There were significant correlations between plasma VPA levels and ammonia (p< 0.025) and plasma VPA levels and blood CAR levels (p<0.001) The incidence of hyperammonemia in patients treated with VPA was 28.6%.

Conclusions: Although sometimes asymptomatic, hyperammonemia is not negligible in patients treated with VPA, thus ammonia blood monitoring should be performed routinely. Trough plasma concentrations of VPA are not useful in dosage adjustments as this adverse drug reaction better correlates with peak plasma levels. One possible solution is to avoid the peak-trough oscillation using extended release formulations of VPA.

Keywords: Valproic acid, hyperammonemia, carnitine depletion
Background: Benzimidazoles are heterocyclic aromatic organic compounds, which consist of the fusion of benzene and imidazole. Various biological actions have been identified for this class of compounds, such as antineoplastic, antimicrobial and anti-inflammatory. The in vitro cellular toxicity of biologically active compounds is a preclinical approach that defines the therapeutic window of drugs. Therefore, we evaluated the in vitro cytotoxicity of six fluorescent benzimidazole core compounds (NB2, NB5, NB6, NB7, NB8, NB9), using a mitochondrial assay based on the reduction of MTT. Methods: VERO cells were cultured in DMEM supplemented with 10% fetal bovine serum (conventional medium) at 37°C in a humid atmosphere with 5% CO2. Cell cultures at 80% confluence were exposed for 72h to 0.001, 0.01, 0.1, 1.0, 10 and 100 μM of each of the six compounds. Untreated cells maintained in conventional medium were used as negative control (100% viable cells). After the incubation time, the MTT assay was performed and the cytotoxic concentration for 50% of the cells (CC50) was calculated from the polynomial equation of the dose-response pattern. Results: All concentration versus cell viability curves obtained exhibit a concentration dependent profile. Taking into consideration the maximum concentration evaluated (100 μM) and the CC50 of each compound, NB5 (97%), NB7 (100%) and NB8 (100%) showed the highest cytotoxicity’s. NB2 and NB9 showed 54% and 72% of cytotoxicity, respectively, while NB6 was the least toxic compound with around 75% of viable cells remaining. The CC50 concentrations of the NB compounds were between 20 μM (NB8) and 2.35μM (NB6). Interestingly, increased mitochondrial activities of up to 25% were determined below 1.0 μM. Conclusions: This study demonstrates that the benzimidazole compound NB6 is the least toxic one and may be tested in a broader therapeutic window to evaluate its biological action like antiviral, antibacterial or antifungal. Financial support: CNPq, CAPES, FEEVALE, Croatian Science Foundation (Project 4379).

Keywords: in vitro toxicity; cell viability; MTT, benzimidazole analogs.
LIMITS OF DETECTION FOR ULTRATRACE DETERMINATION: A NEED FOR HARMONIZATION?
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Background: To validate a bioanalytical method, determination of limits is essential. Limit of detection (LOD), defined as the lowest amount of analyte in a sample which can be detected but not necessarily quantified as an exact value, is one of them. Its determination is of particular importance when ultratrace compounds, such as endocrine disruptors, are assessed. There are numerous methods to calculate LOD but they may yield different results and have an impact on the determination of exposure to ED. The objective of this study was to determine quantitatively the impact of the method used for LOD on exposure frequency.

Methods: Detection limits were calculated using peak area (AUC), according to five methods recommended by international guidelines: signal to noise ratio (either calculated or directly given by the software), three times the standard deviation of the blanks and 2 methods based on the slope of the calibration curve. LODs were calculated for each day (n = 14) of analysis to account for daily variations of detector response. These LODs were applied to a set of 346 urine samples obtained from pregnant women in the EDDS cohort study and assayed for BPA and monochloro-BPA in view of determining and comparing exposure frequencies.

Table 1: Mean detection frequencies ± SD (%) (n=14)

<table>
<thead>
<tr>
<th>analyte</th>
<th>Calculated S/N</th>
<th>Software S/N</th>
<th>SD blanks</th>
<th>Calibration curve 1</th>
<th>Calibration curve 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BPA</td>
<td>22 ± 22</td>
<td>71 ± 22</td>
<td>59 ± 32</td>
<td>51 ± 29</td>
<td></td>
</tr>
<tr>
<td>CH-BPA</td>
<td>21 ± 31</td>
<td>61 ± 39</td>
<td>52 ± 38</td>
<td>25 ± 31</td>
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</table>

Results: The method based on software determination gave the lowest LODs while the one based on S/N calculated by the operator gave the highest. This situation led to different detection frequencies with a 3 to 4 fold range of variation depending on the method used (Table 1).

Conclusions: The method used to determine the LOD has an impact on frequency of ED exposure. These results highlight a need for harmonization. An extensive study, with controlled samples spiked with known amounts of analytes, is needed to confirm these results and identify the most reliable method.

Keywords: Limit of detection, ultratrace, endocrine disruptors, harmonization
EXPOSURE VERSUS NEUROTOXICITY SYMPTOMS: ARE THEY ASSOCIATED? RESULTS OF AN AMAZONIAN RIVERINE POPULATION CHRONICALLY EXPOSED TO MERCURY

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Background: Mercury (Hg) is highly toxic and harmful to human health. In the Amazon, many riverine populations are chronically exposed to this metal. Exposure parameters such as mercury levels are frequently used for the biomonitoring of these vulnerable populations. The International Academy of Oral Medicine and Toxicology (IAOMT) proposed a questionnaire of symptoms to evaluate mercury neurotoxicity in humans, being a useful tool especially for remote populations where the evaluation by a neurologist is not always possible. The possible association between neurotoxic symptoms and mercury levels in Amazonian populations has never been evaluated using this questionnaire.

Methods: The adapted questionnaire with sixteen symptoms was applied and hair samples were collected (ethical approval by CONEP/Brazil-CAAE nº 43927115.4.0000.0018). Total mercury and its species were analyzed by inductively coupled plasma mass spectrometry and GC-pyro-AFS, respectively. Median values and frequencies were analyzed by Kruskal-Wallis and Fisher’s tests, respectively. Results: Three hundred and eighty participants were enrolled in this study with a median mercury exposure 3.64 μg/g. A significant part of the participants (22%) presented levels of mercury above the safe limit (11 μg/g). A high prevalence of symptoms was observed with more than a half of participants reporting dizziness, blurred vision, paresthesia, memory loss and hair loss. Interestingly, there was no association between mercury levels and the prevalence of symptoms, pointing at three possibilities: (i) despite of the exposure, participants are not intoxicated (unlikely, considering previous studies with exposed Amazonian populations and the high prevalence of symptoms detected in this study); (ii) data collected through the IAOMT questionnaire do not reflect mercury intoxication in Amazonian populations; and (iii) in a chronic exposure, symptoms are not necessarily associated with the present exposure. Conclusions: Although further studies are necessary to confirm the origin of the high prevalence of symptoms observed in this work, our data already support that epidemiological monitoring based solely on exposure level may not be the best strategy for the detection of intoxicated people in the chronically exposed populations.

Keywords: Mercury; Neurological symptoms; Amazon; Riverine.
Background: Pharmacoepidemiological data report increasing prescription rates for antipsychotics in pregnant patients. Nevertheless, concerns of safety for the mother and the fetus/newborn introduce a major challenge for clinicians. Available literature has assessed antipsychotics’ concentrations in several matrices of pregnant patients. Methods: We reviewed pharmacokinetic data for antipsychotic concentrations in amniotic fluid, umbilical cord blood and breast milk to quantify the extent to which antipsychotics enter the above mentioned target matrices. Results: Despite variations between antipsychotics, concentrations in amniotic fluid and umbilical cord blood were lower than in maternal plasma for all antipsychotics. On the other hand, antipsychotic concentrations in breast milk were comparable to maternal plasma concentrations. Considerable differences between antipsychotics in their ability to enter fetal/newborn circulation were observed. Maternal blood concentrations provided a reliable estimate of the child’s exposure to antipsychotics. Conclusions: Given the purely pharmacokinetic nature of the reviewed data, it is not possible to draw conclusions on the clinical sequelae of the reported ranges for antipsychotic concentrations. Nevertheless, there is evidence of accumulation of some antipsychotics in breast milk, whereas data for amniotic fluid and umbilical cord imply only partial placental passage during delivery. The integration of therapeutic drug monitoring in the routine psychopharmacotherapy of antipsychotic-treated pregnant patients can guide clinicians in adjusting doses when required and minimize child exposure to antipsychotics.

Keywords: pregnancy; antipsychotics; umbilical cord; amniotic fluid; breast milk.
Theme: Other theme

A NOVEL DIRECT METHOD TO EVALUATE ADHERENCE TO ATORVASTATIN THERAPY IN CORONARY PREVENTION
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Background: Poor statin adherence is a prevalent challenge in coronary prevention associated with adverse outcomes. Objective methods to monitor statin adherence in clinical practice are lacking. We have previously established an LS-MS/MS assay for quantification of atorvastatin and its five major metabolites in blood. Cut-off values to discriminate non-adherent from adherent patients are yet to be developed. Purpose: To develop an objective drug exposure variable reflecting the given atorvastatin dose and to establish cut-off values to discriminate adherence, partial adherence and non-adherence to atorvastatin treatment. Methods: The study included 25 coronary patients aged 44-84 years treated with atorvastatin 10 mg (N=5), 20 mg (N=6), 40 mg (N=7) and 80 mg (N=7). All patients were instructed to administer atorvastatin between 7 and 10 AM for at least 7 days prior to study start to ensure steady-state drug concentrations. At the first study day, patients participated in a directly observed atorvastatin therapy (DOT) study without having taken their morning dose. Blood samples were collected 1 hour before DOT and immediately before DOT to detect any unscheduled morning dose, and then at one and 24 hours after atorvastatin dose. After the DOT, half of the patients on each dose level (N=12, test group) were instructed to stop taking atorvastatin and return for additional blood samples the subsequent 3 days. Results: No significant differences in drug exposure immediately before DOT and 24 hour after DOT were observed, confirming complete adherence. The sum of parent drug and metabolites correlated most strongly with the dose taken (Spearman rho= 0.71, 95% CI 0.44-0.87). Age correlated significantly with the drug plus metabolites exposure. The dose-normalized sum of atorvastatin plus metabolite concentrations were completely separated from the controls at 0.2 (nmol/L)/mg when the test group had omitted tablet intake for 3 days. To decrease the risk of identifying adherent patients as partially non-adherent we suggest a cut-off at 0.1 (nmol/L)/mg as a practical approach, providing 100% sensitivity and 91% specificity according to ROC-curve analysis. We suggest defining complete non-adherence as non-detectable concentrations of parent drug and metabolites. Conclusion: A dose-normalized cut-off value for the sum of atorvastatin and metabolites in spot blood samples allows discrimination between adherence and partial adherence to atorvastatin therapy in CHD patients. The present direct method to determine atorvastatin adherence emerges as a useful tool for clinical practice and future interventions.
Keywords: atorvastatin; non-adherence; coronary prevention; cholesterol lowering agents.
A CONTINUOUS INFUSION WAS MORE EFFECTIVE THAN A SINGLE INJECTION OF PALONOSETRON AT REDUCING PONV
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**Background:** Postoperative nausea and vomiting (PONV) is a common complication after anesthesia and surgery, and 5-hydroxytryptamine type 3 (5-HT3) receptor antagonists have been considered as a first-line therapy. Palonosetron is a recently developed drug and it has greater receptor affinity and longer elimination half life compared with older 5-HT3 receptor antagonists. We studied to figure out that a continuous infusion using PCA (patient-controlled analgesia) device is more effective than a single injection of palonosetron at reducing PONV in patients undergoing gynecological laparoscopic surgery. **Methods:** 139 patients undergoing gynecological laparoscopic surgery were enrolled. Subjects were divided into group S (single injection of palonosetron 0.075 mg immediately before the induction of anesthesia) and group C (palonosetron 0.075 mg before the induction of anesthesia and 0.075 mg added to PCA). The occurrence of nausea and vomiting, severity of nausea and rescue antiemetic drug use were monitored immediately after the end of surgery and at 0-2 h, 2-6 h, and 6-24 h post-surgery. **Results:** The incidence of PONV was significantly lower in group C than in group S during 24 h after surgery (% vs %, P = 0.005). The severity of nausea and use of rescue antiemetic were not statistically different between the two groups. **Conclusions:** In conclusion, a continuous infusion was more effective than a single injection of palonosetron at reducing PONV in patients undergoing gynecological laparoscopic surgery. **Keywords:** Palonosetron; Patient-controlled analgesia; Postoperative nausea and vomiting
Theme: Other theme

ANTHONY POTENTIAL OF 1,2,3 TRIAZOLES LINKED TO FTALIMIDA
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Background: Anti-inflammatories are widely used drugs, but they have several side effects. Aiming to produce less toxic drugs, compounds derived from phthalimides and 1,2,3-triazoles gain a foothold in current medical chemistry research because of its already proven anti-inflammatory effects, as well as other biological activities. In view of the above, the present work aims to determine the anti-inflammatory activity of compounds derived from the binding of 1,2,3-triazoles to phthalimide.

Methods: The methodology was formed by the hybridization between 1,2,3-triazoles and phthalimide and by the evaluation of anti-inflammatory activity of two heterocyclic compounds derived from this process, through the models of paw edema and peritonitis, applied to albino Swiss mice (Mus musculus). This research was approved by the Research Ethics Committee of the Health Sciences Center of the Federal University of Pernambuco (Nº. 23076.015273 / 2017-27). Peritonitis was induced from an intraperitoneal injection of carrageenan into the animals 1 hour after treatment with vehicle, acetylsalicylic acid - ASA, ibuprofen and triazole derivatives. After 4 hours, a solution of 0.9% saline containing EDTA (1mM) was administered for preservation of peritoneal fluid’s leukocytes, and then the animals were sacrificed for collection of the peritoneal fluid. The cells were counted by an automatic cell counter (ABX MICROSC 60), and Levy’s method determined anti-inflammatory activity. A solution of carrageenan 1% (0.1 mL) in 0.9% NaCl was injected through the plantar tissue of the right hind paw of each mouse to induce inflammation. For the test groups, the compounds synthesized in the same dosage of the standard drug were administrated orally 1 hour prior to the carrageenan. Paw edema was measured using a digital caliper at 1, 2, 3, 4, 24, and 48 h intervals. Results: Through the model of paw edema, when applying the positive control drugs (ibuprofen and ASA), the negative control (CMC 1%) and the test compounds (hetero-tri-fta 3 and hetero-tri-fta 4), it could be observed, after 2, 3, 4, 24 and 48 h, an effective anti-inflammatory activity from test drugs, with an increasing edema reduction at all time intervals when compared to the positive controls. Thus, the potency of test compounds was established, considering how many times their reduction percentage would be greater than the percentage of positive controls. At the time of 4 hours, at which time the majority of anti-inflammatories have peak concentrations, the reduction of hetero-tri-fta 3 was 254.9% when compared to ibuprofen and 153.8% with respect to ASA; the hetero-tri-fta 4 presented potency of 294.1% in relation to ibuprofen and 177.5% in relation to ASA in the same time interval. The peritonitis model evidenced the migration of leukocytes to peritoneal fluid after induction of inflammation. The animals treated with the negative control (1% CMC) demonstrated an exacerbated inflammatory response with records of 15 x 10⁵/ mm³ total leukocytes. Those who were treated with the positive control drugs showed a decrease in total leukocytes, but the test drugs contained the inflammatory process more effectively, a fact evidenced by the higher percentages of leukocyte reduction (46.66% for hetero-tri-fta 3, 33.3% for hetero-tri-fta 4, 13.3% for ibuprofen and 20% for ASA). Conclusion: Through the models and results presented, it was demonstrated, in this study, that the heterocyclic drugs derived from the phthalimide linked to 1,2,3-triazoles present efficient anti-inflammatory activity. These findings broaden the basis for research into less toxic alternative anti-inflammatory drugs, a breakthrough for medicine and for medicinal chemistry.

Keywords: Anti-inflammatory; alternative drugs; toxicity; phthalimide; triazoles.
OXIDATIVE STRESS EVALUATION ON PATIENTS UNDER LONG-TERM OMEPRAZOLE USE
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Background: omeprazole was one of the first proton pump inhibitors developed and was soon largely used worldwide. Despite being considered safe, controversial evidences relate its use with oxidative stress, factor directly involved in the aging process and in neurodegenerative disorders. Therefore, the goal of the present work was to evaluate the oxidative stress parameters among patients undergoing long-term treatment with omeprazole. Methods: a case-control study was developed with 24 volunteers not users of omeprazole and 37 volunteers using omeprazole for longer than 6 months, both groups with similar profile. For the oxidative stress analysis, the parameters measured were superoxide dismutase, catalase and ferric reducing ability of plasma. Statistical analyses were performed using SPSS 25.0. Normality of data was tested by Shapiro-Wilk test and comparisons among groups were checked with T-Student test (parametric) and Mann–Whitney U test (non-parametric). Results: in both groups more than 80% of the volunteers were female (81.1 % in the group using omeprazole and 83.3 % in the group of not users of omeprazole) and the average age was 64 (± 8.7) and 63 (± 8.5) years old in the group of users and not users of omeprazole, respectively. Until the present moment, it was possible to verify a significant increase of the ferric reducing ability of plasma on the group of omeprazole users (1598 ± 390 µM) in comparison to the group not using omeprazole (1044 ± 447 µM; p < 0.001). Conclusions: the results found suggest a possible connection between omeprazole administration and the oxidative stress. However, further investigation is necessary, which will proceed increasing the evaluated sample and analyzing also other parameters involved in the oxidative stress, in order to produce more consistent results, mainly because so far little is known regarding the connection between omeprazole and oxidative stress, with several studies presenting conflicting results. Keywords: omeprazole; ppi; long-term use; oxidative stress.
Background: *Cissus sicyoides* is a climbing plant (Vitaceae). It is commonly known in Brazil as “insulina vegetal”, “anil-trepador”, “cortina-japonesa”, “uva-brava” and widely used to reduce glycemia. Furthermore, studies also revealed other activities such as antitumor, anxiolytic, anticonvulsant, anti-lipemic, anti-inflammatory and anti-diarrheal. However, studies about its mutagenic, genotoxic and cytotoxic effects has not been elucidated. The aim of this study was to investigate cytotoxic and mutagenic effect of the aqueous extract of leaves from *C. sicyoides*. Methods: The aqueous extracts of 2,5 mg/mL, 5 mg/mL and 10 mg/ml were tested on root meristems of *Allium cepa* after stimulating the root growth during 48 h. The extract was prepared by infusion of the dried leaves of *C. sicyoides*. Results were analyzed statistically using Kruskal-Wallis test. The level of statistical significance was in all cases *p* ≤ 0.05. Acetaminophen (800 mg/ml) was used as positive control and distilled water was used as negative control. Results: There was no significant difference between treatments for root lenght (*p* > 0.05) during 24 h of exposure, considered a macroscopic parameter of cytotoxicity. Aqueous extract of *C. sicyoides* at 10 mg/mL caused a significant reduction (*p* < 0.01) in the Mitotic Index (MI) of the meristematic cells when compared to the negative control. The induction of micronucleous (MN) was not observed in all tested concentrations except in the positive control which is an evidence of mutagenicity. Conclusions: Results of this study revealed that aqueous extract of *C. sicyoides* exerted significant mitodepressive effects at 10 mg/ml on root meristems of *A. cepa*. Significant reduction of MI may be due to the mitodepressive effect of the phytochemical substances that decrease the number of cells in division. Therefore, further studies are important to complement this research. It is suggested to evaluate the cytotoxicity of the extract in cell culture. Keywords: *Cissus sicyoides*; cytotoxicity; mutagenicity; mitotic index; micronucleous.
MEASURING CT-P13 AT WEEK 14 USING A LATERAL FLOW ASSAY ALLOWS IMMEDIATE DOSE ADAPTATION
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Background: Therapeutic drug monitoring has become a popular and objective tool to aid the management of patients with inflammatory bowel diseases (IBD) treated with infliximab (IFX). It was shown that the IFX serum trough concentration (i.e. the serum concentration just before a next dose administration) relates to clinical response to treatment. Available data on serum trough concentrations are however largely based on IFX originator drug. Therefore, we aimed to explore the serum trough concentrations upon induction treatment with the IFX biosimilar CT-P13. Methods: Fifty IBD patients initiated treatment with CT-P13 between December 2016 and May 2018 at the University Hospitals Leuven, Belgium. Of these, forty-three patients were eligible for pharmacokinetic analyses. Six patients were re-initiating IFX treatment, while the others were IFX naïve. IFX trough concentrations and anti-drug antibodies (ADAs) were measured in serum samples collected right before infusion of CT-P13, and 2 (T1), 6 (T2) and 14 (T3) weeks after induction, using RIDASCREEN® IFX Monitoring (ELISA) and RIDA®QUICK IFX Monitoring (lateral flow assay, LFA). ADAs were measured using a drug-tolerant ELISA. Results: Thirty-seven patients received standard 5 mg/kg induction treatment and the others received an intensified dosing regimen. In total, 21/43 (49%) patients achieved endoscopic remission, defined as complete absence of ulcerations (Crohn’s disease) or Mayo endoscopic sub-score ≤1 (ulcerative colitis), without need for dose-escalation prior to endoscopic assessment. Serum IFX concentrations, measured with ELISA, were higher in patients with endoscopic remission than in those without (T1: p=0.0215; T2: p=0.001; T3: p=0.0059). Receiver operating characteristic analysis showed an IFX concentration threshold ≥4.1 µg/mL at T3 as the best predictor of endoscopic remission and a similar threshold was found using the LFA (≥4.2 µg/mL). In 5/50 (10%) patients, ADAs were identified on at least one time point. In two patients who re-initiated IFX therapy, ADAs were detectable from T1 onwards. In the other three patients, ADAs were detectable at T3. Three out of five ADA-positive patients were dose-escalated at 14 weeks after induction. Conclusions: This study shows that IFX serum concentrations and incidence of ADAs during induction with CT-P13 are comparable to induction with IFX originator. Furthermore, an IFX concentration threshold ≥4.1 µg/mL at 14 weeks after induction was the best predictor of endoscopic remission. Finally, we have shown that IFX biosimilar concentrations can be reliably measured with a rapid LFA, enabling faster dose adaptation during induction and thereby overcoming drug underexposure.

Keywords: infliximab; biosimilar; lateral flow assay; induction; therapeutic drug monitoring; inflammatory bowel diseases.
Background: Cytotoxicity assays are commonly used preclinically in a variety of conditions. The characteristics that support this use are the low cost and the ease of obtaining returns. The use of medicinal plants is millenarian and is part of basic health care in several underdeveloped countries. Ensuring safety and efficacy in the use of vegetable preparations promotes rational use of this practice. Lemongrass (C. citratus) is a plant species very appreciated for its palatability and medicinal properties, being used in large scale by the population of the south of Brazil. Methods: MTT assay was performed in confluent cultures of two types of cells, Vero (monkey renal epithelium) and HepG2 (human hepatic carcinoma) previously exposed to five concentrations (100%, 50%, 25%, 1%, 0,5%) of aqueous extract of C. citratus for 24 hours. The medium culture was prepared by using the aqueous extract as solvent to medium components and the other concentrations were obtained by mixed with control medium (prepared with reverse osmose water). All mediums were supplemented with 1% of fetal bovine serum. The cell culture was maintained at 37°C in humid atmosphere with 5% of CO₂. The results were expressed as % in relation to control group (culture maintained in control medium, used as 100% of viability). Results: The full concentration of infusion was very toxic for Vero cells, drastically reducing (> 90%) mitochondrial activity; this effect was dose dependent increasing toxicity with increased concentration. In HepG2 cells, the aqueous extract was non-toxic at all concentrations, suggesting a resistance profile of this cell type. This profile may be related to a natural resistance to xenobiotics, once HepG2 is a tumor and hepatic lineage, having a greater capacity of recovery, specially to oxidative effects. Conclusions: The C. citratus extract citotoxicity was higher for Vero cells than for HepG2 cells, suggesting that the renal epithelium is more affected by the extract then liver. In HepG2 cell culture the extract may have a proliferative effect because of the increasing of mitochondrial activity. Other biomarkers and animal trial needs to be performed in future to support these results.
Keywords: mitochondrial function, toxicity, pre-clinical trial, MTT assay.
MODELING OF GLOMERULAR FILTRATION RATE (GFR) BASED ON THE CLEARANCE OF IOHEXOL IN INTENSIVE CARE UNIT PATIENTS

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Background: Iohexol is a contrast product used in imagering, but it can also be used to determine the Glomerular filtration rate (GFR) of patients as during its elimination, it is only filtered. Its uses in intensive Care units (ICU) is of particular interest as the “classical” estimation formulas based on creatinine (Cockcroft & Gault, CKD-EPI, MDRD) cannot be used for these patients who receive a large quantity of solutes or remain bedridden for several days. The aim of this work was to develop a parametric population pharmacokinetics (popPK) model for iohexol and associated Bayesian estimators (BE) based on a limited sampling strategy (LSS). Methods: Unstable patients hospitalized in the ICU at University Hospital of Tours received 3235 mg of iohexol and had 9 blood samples collected at different times between 0.1 and 24 hours. PopPK model were developed using ITSIM and Monolix in a building dataset (n=64). Eleven covariates were investigated on PK parameters based on the Akaike criteria and final models developed were evaluated using Visual Predictive Check. The best 3-point limited sampling strategy (LSS) was used to estimate the iohexol clearance (=GFR) by Bayesian estimation in the validation datasets (n=15) and comparison with the GFR obtained using all available points was performed. Results The popPK profiles were best fitted using a two-compartment model with first order elimination. Inclusion of the covariates did not significantly improve the models. The individual predicted vs. the observed concentrations in the development dataset show a RMSE=11.8% and 13.8% and a mean relative bias=-0.58% and 2.4% for Monolix and ITSIM respectively. The best LSS included samples at 0.1, 1 and 9h giving relative bias in iohexol clearance (RMSE%)/number of estimated GFR out of the ± 20% interval= -6.8% (17.3%)/2 for Monolix and -2.1% (12.8%)/2 for ITSIM, respectively. Conclusions: The popPK models and Bayesian estimators developed allow an accurate estimation of GFR in unstable patients hospitalized in ICUs based on a LSS of 0.1,1 and 9h. The next step is to generalize this tool to other populations and conditions (ICU stable patients, renal transplant patients).

Keywords: Iohexol; GFR; ICU; PKPOP; LSS.
Theme: Other theme

EXPOSURE TO BISPHENOL A AND ITS CHLORINATED DERIVATIVES IN WOMEN UNDERGOING BREAST SURGERY FOR CANCEROUS AND NON-CANCEROUS LESIONS. RESULTS FROM THE BREDI-1 (Breast Endocrine Disruptors, part 1) PILOT STUDY.

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Background: Bisphenol A (BPA) and its chlorinated derivatives (Clx-BPA) are environmental pollutants exhibiting endocrine-disrupting (ED) properties suspected to be involved in the pathogenesis of hormone-dependent cancers, such as breast cancer. We aimed to determine exposure to these ED compounds in a cohort of women undergoing breast surgery for cancerous and non-cancerous lesions. Methods: BREDI-1 (Breast Endocrine Disruptors, part 1) was a monocentric pilot study. Women aged over 18 year-old, hospitalized for breast surgery and who had given their informed consent could be enrolled. Women receiving breast surgery for confirmed breast cancer, epithelial atypia and benign breast lesions were included. During hospitalization, two urinary samples were drawn in a glass recipient, before and after surgery. A sample of breast adipose tissue was also collected in glassware during surgery. Urine and breast samples were kept frozen in glass tubes (-20°C) before analysis. Target analytes were assayed in these samples using validated LC-MS/MS methods. Results: Thirty-four women were included in this study. BPA was detected in near all urine and breast tissue samples. Higher mean BPA concentrations were found both in urine and adipose tissue of patients exhibiting cancerous lesions (Table 1). Clx-BPA were less frequently detected than BPA with detection rates ranging from 41 to 54% and 36 to 64%, in urine and adipose tissue, respectively. Higher mean Clx-BPA concentrations were found in urine of patients exhibiting cancerous lesions but this trend was not confirmed in adipose tissue. Conclusions: This pilot study confirmed that ED compounds are present in biological specimen of women exhibiting breast lesions. The pattern of exposure found in this pilot study requires further investigations and confirmation in a well-powered study.

Keywords: Bisphenol A and its chlorinated derivatives (Clx-BPA), breast cancer, endocrine disruptors, urine, adipose tissue.
Theme: Other theme

OXIDATIVE DEMAGES IN TADPOLES OF THE SPECIE Scinax Fuscovarius (AMPHIBIA: ANURA) UNDER THE INFLUENCE OF PESTICIDES USED IN SOYBEAN CULTIVATION

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Background: In the state of Rio Grande do Sul, it is estimated that about 90% of the wetlands have already been lost due to their conservation into arable land, considering that this is the natural habitat of tadpoles, can be considered excellent bioindicators of the impacts caused by pesticides. The process of oxidative stress is one of the biological indicators that can be caused as an oxidation mechanism between the chemical agent and the organism, for example, with the increase of oxidants, such as malondialdehyde (MDA), which is the product of lipid peroxidation and carbonylation of proteins that are highly toxic to living organisms. The objective was to evaluate and compare the levels of MDA and protein carbonylation in tadpoles exposed and not exposed to the use of pesticides in soybean cultivation. Methods: The present study was accepted by the Ethics Committee in research on the use of animals CEUA-UNISSINOS through the number: PPE CEUA 02/2017. Tadpoles are collected in the northwest of the state of Rio Grande do Sul in two areas with the influence of soybean cultivation and two without. After being separated by specie, and Scinax Fuscovarius was chosen because it was the most abundant, then a rest of approximately one hour, anesthesia and euthanasia. The tadpoles were separated in two pools, contaminated and uncontaminated and macerated in gral and pistil until obtaining a homogenous extract. For the determination of MDA the extract was composed of 250mg tissue sample for 2mL of 150mM NaCl. After the MDA levels were determined according to the technique described by Buege et. al., (1978) adapted for anurans and read in a spectrophotometer at 535nm. For the determination of the levels of protein carbonylation the extract was composed of 60mg of tissue that was homogenized in 540µl of 10mM Tris-HCL pH=7.4 and centrifuged for 10 min after being diluted 1:10 in distilled water. Then the determination of the protein carbonylation was made following the technique of Yan et. al., (1995), adapted for anurans and read in a spectrophotometer at 370nm. Two-way ANOVA followed by Tukey’s test was used for both techniques, considering statistically significant values when p<0.05. Results: Tadpoles collected from dams located in soybean plantations have high levels of oxidative damage in lipids and protein when compared to those collected at neutral local. This suggests that tadpoles are under toxic effects. Keywords: Oxidative demages; Lipid peroxidation; Protein carbonylation; Scinax Fuscovarius; Pesticides.
Theme: Other theme

**SIGNIFICANT DECREASE IN PLASMA CONCENTRATIONS OF COPROPORPHYRIN-I, A SPECIFIC ENDOGENOUS OATP1B PROBE, AFTER LIVING KIDNEY TRANSPLANTATION IN PATIENTS WITH END-STAGE RENAL DISEASE**

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**Background:** Chronic kidney disease alters the elimination of many drugs mainly by decreasing their renal elimination. Recently, several studies have shown that renal failure also decreases the metabolic clearance of drugs and the transportation capability of some drug transporters. However, whether organic anion transporting polypeptide (OATP)1B activities decrease in renal failure remains unknown. Recently, coproporphyrin-I (CP-I) in plasma have attracted attention as sensitive and specific endogenous probes for phenotyping OATP1B. In this study, we measured plasma CP-I concentrations in patients with end stage renal disease before and after living kidney transplantation and evaluated the effect of renal function on OATP1B activity. **Methods:** This prospective study recruited 13 patients with end-stage renal disease. Morning blood samples were collected before and 7, 14, 30 and 90 days after living kidney transplantation, and plasma CP-I concentrations were measured using ultra-performance liquid chromatography with tandem mass spectrometry (UPLC-MS/MS). The UPLC-MS/MS method was fully validated according to the recommendations published by the US Food and Drug Administration (FDA). **Results:** A significant decrease in serum creatinine level was observed on day 7 and thereafter remained almost stable until day 90 after living kidney transplantation, indicating that living kidney transplantations in the patients were successful. No significant differences in ALT and total bilirubin were observed between before and after living kidney transplantation, suggesting that hepatic function and bile excretion capacity were stable during the study. Plasma CP-I concentrations decreased over time after living kidney transplantation and showed significant difference on day 90 compared with before living kidney transplantation [1.12 ± 0.59 vs 0.65 ± 0.27 ng/mL, p < 0.05 (95% CI of difference -0.927, -0.013)]. A significant negative correlation was observed between estimated glomerular filtration rate and plasma CP-I concentration \( r = -0.30, p < 0.05 \), suggesting recovery of OATP1B activity with improvement in renal function. **Conclusions:** OATP1B activity may decrease in renal failure and dose adjustment of OATP1B substrates may be needed in patients with renal failure. **Keywords:** OATP1B; renal failure; coproporphyrin-I; kidney transplantation; end stage renal disease.
Background: Laboratory testing for trace and toxic elements is important to diagnose metal toxicity and nutritional deficiencies. There are several essential elements that are necessary for biological function and there are non-essential elements that can pose risk from exposure. Both essential and nonessential elements can be toxic if concentrations exceed a certain threshold. Methods: An aliquot of serum is diluted in a diluent solution, which contains iridium (Ir) as the internal standard, gold (Au), 0.05% Triton X-100, and 1% nitric acid (HNO₃). The diluted specimen is aspirated into an inductively coupled plasma-mass spectrometer (ICP-MS) for quantitative elemental analysis of chromium, cobalt, copper, manganese, nickel, selenium and zinc. The sample is then introduced into the instrument spray chamber to form aerosol droplets and then atomized and ionized in argon plasma. The ions exit the plasma, pass through the interface of the instrument, and arrive at the entrance of the collision cell where helium gas is introduced to remove polyatomic interferences. After exiting the collision cell, the ions are filtered by a quadrupole mass spectrometer. Results: The analytical measurement range is element specific. The lowest limit of quantification is 0.3 mcg/L for chromium and the highest upper limit of quantification is 22,500 mcg/L for zinc. Imprecision was < 10% CV for the lowest limit of quantification for each element and accuracy was within +/- 15%. Conclusions: This method has been implemented for the identification of element exposure of seven elements in serum to access nutritional deficiency and toxicity. The multi-element panel by ICP-MS has met the validation criterial for biological monitoring of trace and toxic elements in serum patient specimens. Keywords: ICP-MS, serum, trace elements, method validation
ANTI-INFlixIMAB ANTIBODIES: HOW TO COMPARE OLD AND NEW DATA?

Imbrechts, M1; Van Stappen, T1; Compernolle, G1; Tops, S1; Gils, A1.

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Background: Anti-infliximab-antibodies (ATI) are frequently measured in patients receiving infliximab treatment with loss of response and undetectable infliximab concentrations. Different drug-sensitive ATI bridging assays (1st generation, 2nd generation and ready-to-use kit) have been developed successively and were applied over the last 10 years, making comparison between ATI concentrations very challenging. A cutoff of 8 µg/ml was established to discriminate low from high ATI concentrations using the 1st generation ATI bridging assay. The objective of this study was to enable comparison of ATI concentrations determined with the different assays that were developed over the years.

Methods: 166 serum samples were collected from patients with inflammatory bowel disease treated with infliximab. 98 samples were measured simultaneously with the 1st and 2nd generation ATI assay, 68 serum samples were measured with the 2nd generation assay and the ready-to-use kit.

Results: It was shown that the previously established cutoff of 8 µg/ml for ATI concentrations measured with the 1st generation ELISA corresponds to a cutoff of 374 ng/ml in the 2nd generation ELISA and a cutoff of 119 ng/ml in the ready-to-use ELISA kit (Figure A).

Conclusions: ATI concentrations measured with the different assays were compared and a cutoff concentration was determined for each of them to distinguish between low and high ATI concentrations. These cutoff concentrations may serve as a tool for clinicians to make treatment decisions for patients with a loss of response to infliximab and undetectable infliximab serum levels (Figure B).

Keywords: Anti-infliximab-antibodies, inflammatory bowel disease, clinical decision making, ELISA cutoff concentration, TAXIT.
EFFECT OF PLASMA CONCENTRATION MEASUREMENT ON THE CLINICAL INTERVENTION AND PROGNOSIS EVALUATION OF THE ACUTE PARAQUAT POISONING PATIENTS

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Background: Paraquat (PQ), as a rank toxic herbicide, has caused deaths of numerous people around the world because of its acute toxic hazard, and seriously organ damage, which makes the rapid plasma concentration monitoring and clinical intervention been mandatory. Therefore, this study aims to establish a simple, rapid, and valid HPLC method for determining the plasma paraquat concentration and to explore the clinical efficacy of hemoperfusion on the intervention and prognosis evaluation in acute paraquat poisoning patients. Methods: A simple protein precipitation with perchloric acid (15%) was used to extract paraquat from patients’ plasma. Satisfactory separation was achieved by a mobile phase consisting of acetonitrile and aqueous phase (10:90;v/v), containing sodium heptane sulfonate (0.02mol/L) and phosphoric acid (0.26mol/L) (pH adjusted to 3.0 by triethylamine) at a flow rate of 1.0 mL/min on Hypersil® ODS C18 (4.6×250mm, 5 μm) column with the UV detector wavelength at 258 nm. The plasma paraquat concentrations were monitored before and after intervention. Meanwhile, the general information, causes of acute intoxication, therapy information, the parameters functions of blood coagulation, liver, kidney, as well as the disease outcomes were analyzed to evaluate the effect of the therapeutic strategy by multiple linear regression and spearman’s correlation analysis. Results: The developed method was validated in plasma with a lower limit of quantitation of 0.02μg/mL (S/N=3) for paraquat. Linearity was demonstrated over the concentration range 0.02-40μg/mL (R=0.9999). The observed within- and between-day assay precision ranged from 1.14 to 8.27%; accuracy varied between 98.32 and 105.16%, which indicated that the method is suitable for plasma paraquat determination. According to the data analysis, statistical significance correlation existed between age, gender, education level and acute paraquat poisoning among patients (p<0.01). The plasma paraquat concentration had a strong positive relationship to poisoning rescue time, mean hospital days and cured or survival rate. The initial hemoperfusion intervention could significantly reduce the plasma concentration of paraquat (p<0.05), and multiple intervention had achieved a remarkable result (p<0.01). Spearman's correlation analysis exhibited that the paraquat concentration had a strong relationship to parameters of kidney (BUN and Scr), liver (ALT and AST), inflammation(WBC and NEUT) and coagulation function (PT and PTA) (p<0.01). Conclusions: The acute paraquat poisoning patients should be received timely treatment by hemoperfusion to reduce the plasma paraquat level. Plasma drug measurement of paraquat concentration provided important reference for evaluating the organ functions, which could be used as a imperative clinical index to judge the intervention and prognosis evaluation of paraquat poisoned patients.

Keywords: high performance liquid chromatography; acute paraquat poisoning; plasma concentration measurement; clinical intervention; hemoperfusion.
ASSOCIATION OF N-ACETYLTRANSFERASE-2 PROFILE AND ISONIAZID SERUM LEVELS IN TUBERCULOSIS PATIENTS FROM NORTHERN BRAZIL
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Background: Isoniazid is one of the main drugs used in the therapy of tuberculosis (TB) and several reports around the world have shown that genetic polymorphisms of NAT-2 and CYP2E1 may result in discrepant serum levels of this agent. On the other hand, there is no study dealing with patients from Northern Brazil, despite the high incidence rates of TB in this region. Therefore, this investigation tried to analyze the association between the NAT-2 and CYP2E1 polymorphisms with serum levels of isoniazid in patients from Northern Brazil. Methods: Two hundred and twenty-two patients who had been treated with anti-TB drugs were studied. Isoniazid (INH) serum levels were determined by a validated liquid chromatography-mass spectrometry assay. The allelic and genotypic frequency distributions of the NAT-2 (282C>T, 481C>T, 590G>A and 857G>A) and CYP2E1 (399G>A, 807C>T, 1027T>C and 1295G>C) enzymes were studied using real time polymerase chain reaction methodology. Data analysis was conducted using STATA software and the significance level was set at 0.05. The study protocol was analyzed and approved by the Research Ethics Committee of Universidade Federal do Amazonas. Results: The genotypic frequencies of NAT-2 and CYP2E1 similar to the values reported in other studies involving the Brazilian population. The frequencies of slow and fast acetylators were 31.82 and 68.18% (respectively). There were no differences among individual polymorphisms of NAT-2 and CYP2E1 and INH levels. However, the median two-hour INH concentrations in slow and fast acetylators were 3.28 and 2.86 µg/ml, respectively, and the differences in INH concentrations among the genotypes were significant (P<0.05). Conclusions: This study showed that the N-acetyltransferase profile is an important factor related to low levels of isoniazid in tuberculosis patients. Therefore, the previous investigation of acetylator profile may be helpful prior to the beginning of the treatment.

Keywords: tuberculosis; isoniazid; serum; pharmacogenetics.
THE ROLE OF FLAVIN-CONTAINING MONOOXYGENASE POLYMORPHISM ON VORICONAZOLE IN VITRO METABOLISM

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Background: Voriconazole (VCZ) is a triazole broad spectrum antifungal agent with large interindividual variability in metabolic clearance. It is primarily metabolized by cytochromes P450 (CYP) 2C19, 3A4, and CYP2C9 to a minor extent (1). In addition, Flavin-containing monooxygenases 3 (FMO3) catalyzes the N-oxidation of VCZ. The role of CYP2C19 and 3A4 polymorphisms was already explored but the influence of FMO3 polymorphisms on VCZ clearance is still unknown. We investigated in vitro the impact of FMO3 genetic polymorphisms on the metabolism of VCZ. Methods: VCZ (0.35 µg/ml) was incubated with pooled Human Liver Microsomes (HLMs; 1mg/mL) purchased from Biopredic International as well as with individual preparations genotyped for FMO3 rs2266780 (p.Glu308Gly) and rs2266782 (p.Glu158Lys). Individual HLMs were selected as non-carriers of CYP2C19*2, CYP2C19*17, CYP3A4*22 and CYP3A5*1 alleles to avoid any bias from their effect on VCZ metabolism. The evolution of VCZ concentration was analyzed using a validated liquid chromatography-tandem mass spectrometry method. The intrinsic clearance (Clint) of VCZ depletion was estimated using the in vitro half-life method. As a proof of concept, we compared the Clint of VCZ for CYP2C19*1/*2 (intermediate metabolizer) and wild-type microsomes CYP2C19*1/*1 (normal metabolizer). Results: A clear difference in Clint was found between CYP2C19*1/*2 and CYP2C19*1/*1 pooled HLMs (5 µL/mg/min vs 21 µL/mg/min, respectively). The Clint of homozygous HLMs for both rs2266780 and rs2266782 (25.5 µL/mg/min) were similar to that of commercial HLMs (23 µL/mg/min). Conclusions: The two FMO3 variants studied might not have a major impact on VCZ clearance as compared to CYP2C19*2. This is consistent with the limited contribution of FMO3 in VCZ metabolism as compared to CYP (approx. 25% vs. 75%)(2). Other FMO3 variants in linkage disequilibrium with those tested herein (rs37754491, rs2064080) will be investigated.


Keywords: voriconazole; metabolism; FMO3; pharmacogenetics; polymorphism.
**CYPASCAN: AN ONLINE TOOL FOR STAR ALLELE CALLING IN PHARMACOGENETICS**

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**Background:** The “star” nomenclature is a standard to describe the different allelic versions of pharmacogenes. It was implemented in the 1990s by an international consortium and has been widely adopted in the field of pharmacogenetics (PGx), in particular for cytochromes P450. Variant alleles (*2, *3, etc.) correspond to gene versions with one or more Short Nucleotide Variations (SNVs) as compared to the reference (*1) allele sequence. Currently, the most common way to determine the star allele of a given gene relies on targeted genotyping of tag SNVs. It is time-consuming and can only confirm or exclude the presence of specific alleles, leading to potential mistakes. Next Generation Sequencing (NGS) offers a promising alternative but requires specific bioinformatic tools to produce comprehensive reports integrating the star nomenclature.

**Methods:** The French National Pharmacogenetics Network (RNPGx) recently defined a list of 44 genes of particular interest including drug metabolizing enzymes, membrane transporters and pharmacodynamic targets. For each star allele of the list, we computed GRCh37 and GRCh38 positions of related SNVs in a database. We developed a PHP online software Cypascan dedicated to geneticists and pharmacologists. Cypascan allows users to select genes of interest among those of the RNPGx list. It analyses combination of relevant SNVs from NGS variant calling format (vcf) file provided, and eventually provides comprehensive reports based on star nomenclatures publicly available.

**Results:** Cypascan was tested using 192 vcf files generated from LifeTechnologies® Ampliseq® PGx panel (Ion Proton technology). Among the 13 RNPGx genes available in this commercial panel, the frequencies of 17 alleles related to 7 genes were found similar with those expected in the European population. For other genes, allele frequencies were unreported or essential hotspots to perform allele inference were missing from the vcf. Cypascan was also tested regarding a very complex gene (CYP2D6) using 12 vcf files generated using the Illumina® technology by Sophia Genetics® (gene capture). Divergences between Cypascan results and those expected concerned only 2 samples and came from large gene rearrangements (i.e. CYP2D6*36; gene fusion) or gene deletion (i.e. CYP2D6*5). Both can be addressed using complementary tools prior to variant calling.

**Conclusions:** Cypascan allow a quick determination of relevant variant alleles from NGS data which might be very useful for the clinical implementation of NGS technology in PGx labs.

**Keywords:** star allele nomenclature, Next-Generation-Sequencing, online tool
Theme: Pharmacogenetics

COMPARISON OF THE CLINICAL PHARMACOKINETICS OF THE TWO FORMULATIONS OF ONCE-DAILY PROLONGED-RELEASED TACROLIMUS (ADVGRAF® AND ENVARSUS®) IN KIDNEY TRANSPLANT PATIENTS AT VARIOUS POST-TRANSPLANT MOMENTS
Salvador-Garrido P, Outeida-Macias M, Pedreira-Vázquez I, Martín-Herranz I.

Background: A new once-daily prolonged-release tacrolimus formulation with a Meltdose® technology is available. Aim: To compare the pharmacokinetic profiles of the two available once-daily prolonged-release tacrolimus formulations (Envarsus® and Advagraf®) in kidney transplant patients at various post-transplant moments. Methods: A retrospective study of 20 Caucasian cadaveric renal transplant patients co-treated with mycophenolic acid and steroids. All patients received the same tacrolimus dosage for at least 3 days prior to each profile. Blood levels were measured by CMIA on an Architect-C8000™Analyzer. Pharmacokinetic profiles were obtained around 15 days (15D) following the start of tacrolimus treatment, and 6 (M6) and 12-24 (M12-24) months after transplantation. Blood samples were taken pre-dose and 1, 2, 3, 4, 6, 8 and 12 hours after the oral morning dose. The area under the concentration-time-curve from 0 to 24 h ($\text{AUC}_{0-24}$) was calculated using the linear trapezoidal rule. A statistical analysis was conducted using SPSS 19.0, with the t Student test to compare the pharmacokinetic parameters between the two formulations and the Spearman Rho correlation coefficient (r) to assess the correlation between the trough concentration and the $\text{AUC}_{0-24}$.

Results: 20 patients (11 with Envarsus® and 9 with Advagraf®) were included. At 15D post-transplant, trough concentrations were comparable for both formulations (Envarsus®-Advagraf®: 8.17±3.40 vs. 6.78±2.62 ng/mL) after administering a lower daily dose of Envarsus®, but not significantly different (9.44±4.85 vs. 11.44±3.43 mg/day), and a significantly higher drug exposure ($\text{AUC}_{0-24}$) in the case of those treated with Envarsus® (383.86±115.10 vs. 270.65±78.87 ng.h/mL, p=0.031). Envarsus® also showed a significantly higher maximum concentration (28.54±7.41 vs. 20.98±4.92 ng/mL, p=0.019). At the stable periods, M6 and M12-24, both formulations were comparable for the pharmacokinetic parameters assessed. At all three moments, the maximum concentration for Envarsus® was achieved approximately at 6h ($T_{\text{max}}$) and for Advagraf® at 2-3h. Both formulations displayed a good correlation between the trough concentration and the $\text{AUC}_{0-24}$ at the three moments studied (Envarsus®-Advagraf®: 15D: r=0.921, p=0.000; r=0.803, p=0.009; M6: r=0.670, p=0.024; r=0.854, p=0.003; M12-24: r=0.881, p=0.004, r=0.934, p=0.001). Conclusions: In the early post-transplant period, Envarsus® showed a significant higher drug exposure with an approximately 20% lower dose (not statistically significant); however, these differences were not found in the stable periods. The correlation between the tacrolimus $\text{AUC}_{0-24}$ and $C_{\text{trough}}$ is good, indicating that trough measurements may give a good indication of overall drug exposure.

Keywords: Envarsus®, Advagraf®, clinical pharmacokinetics, kidney transplantation.
Theme: Pharmacogenetics

STUDY OF THE ASSOCIATION OF UGT1A9 GENE -2152C>T POLYMORPHISM WITH MYCOPHENOLATE MOFETILE OR ENTERIC-COATED MYCOPHENOLATE SODIUM PHARMACOKINETICS IN TREATED KIDNEY TRANSPLANT PATIENTS
Salvador-Garrido P, Outea-Macías M, Pedreira-Vázquez I, Martín-Herranz I.

Background: The enzyme UGT1A9 is principally involved in the glucuronidation of mycophenolic acid (MPA). Expression levels and the activity of UGT1A9 may depend on the presence of the -2152C>T SNP located in the promoter region. Aim: To assess the effect of UGT1A9 -2152C>T polymorphisms on MPA pharmacokinetics in kidney transplant patients who are taking equivalent doses of mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS) at two different post-transplant moments (15 days -15D- and 6 months -6M-). Methods: A retrospective study of Caucasian cadaveric kidney transplant patients co-treated with tacrolimus and steroids. All patients received the same MMF (Cellcept®) or EC-MPS (Myfortic®) dosage for at least 1 week prior to each profile. Dose correction: 500 mg of MMF= 360 mg of EC-MPS was applied. Plasma levels were measured by MEIA on a VIVA® Analyzer. Blood samples were taken pre-dose and 1, 2, 3, 4, 6 and 8 hours following the oral morning dose. AUC0-12 was calculated using the linear trapezoidal rule. A genetic test was used for the genotyping of UGT1A9 -2152C>T polymorphism, which was correlated to MPA trough concentrations (Ctrough) and AUC0-12 at 15D (dosage of 1000 mg/12h or 720 mg/12h, respectively) and 6M (500 mg/12h or 360 mg/12h) after transplantation. Each patient gave written consent of their participation. A statistical analysis was performed using SPSS 19.0 with the Mann Whitney test. Results: 50 patients (24 with MMF and 26 with EC-MPS), age 51 years, weight 72 kg, were included. All the patients of the study group presented the same genotype for this polymorphism: -2152 CC. When patients were divided according to MMF (25) or EC-MPS (25) treatment, AUC0-12 was significantly higher in EC-MPS treated patients than in those with MMF at 6M post-transplant moment (AUC0-12: 72.76±47.36 vs 38.67±11.96 ng.h/mL, p=0.006), albeit not during the early period. Conclusions: As only individuals homozygous for the -2152C allele were found, this study was unable to establish an association between UGT1A9 -2152C>T polymorphism and MPA pharmacokinetics. Regarding the two formulations of MPA, during the stable period (6M post-transplant), the degree of exposure (AUC0-12) for EC-MPS increased significantly, indicating that other polymorphisms and/or other factors (co-morbidities, concomitant drugs, etc.) could affect MPA levels.

Keywords: -2152C>T polymorphism, mycophenolate mofetile, enteric-coated mycophenolate sodium
Theme: Pharmacogenetics

INFLUENCE OF UGT1A9 GENE -275T>A POLYMORPHISM ON MYCOPHENOLIC PHARMACOKINETICS IN KIDNEY TRANSPLANT PATIENTS TREATED WITH MYCOPHENOLATE MOFETILE OR ENTERIC-COATED MYCOPHENOLATE SODIUM

Salvador-Garrido P, Outeda-Macias M, Pedreira-Vázquez I, Martín-Herranz I.

Background: UGT1A9 is a major enzyme engaged in the metabolism of mycophenolic acid (MPA). Expression levels and the activity of the enzyme may depend on the presence of -275T>A SNP located in the promoter region. Aim: To assess the effect of allelic variants of the UGT1A9 -275T>A polymorphism on MPA pharmacokinetics in kidney transplant patients who are taking equivalent doses of mycophenolate mofetil (MMF) and enteric-coated mycophenolate sodium (EC-MPS) at two different post-transplant moments (15 days -15D- and 6 months -6M-). Methods: A retrospective study of Caucasian cadaveric kidney transplant patients who were co-treated with tacrolimus and steroids. All patients received the same MMF (Cellcept®) or EC-MPS (Myfortic®) dosage for at least 1 week prior to each profile. Dose correction: 500 mg of MMF= 360 mg of EC-MPS was applied. Plasma levels were measured by MEIA on a VIVA® Analyzer. Blood samples were taken pre-dose and 1, 2, 3, 4, 6 and 8 hours after the oral morning dose. AUC_{0\rightarrow 12} was calculated using the linear trapezoidal rule. A genetic test was applied for the genotyping of UGT1A9 -275T>A polymorphism, which was correlated to MPA trough concentrations (C_{trough}) and AUC_{0\rightarrow 12} at 15D (dosage of 1000 mg/12h or 720 mg/12h, respectively) and 6M (500 mg/12h or 360 mg/12h) post transplantation. Each patient gave written consent of their participation. A statistical analysis was performed using SPSS 19.0 with the Mann Whitney test. Results: 50 patients (24 with MMF and 26 with EC-MPS), age 51 years, weight 72 kg, were included. The genetic variants identified were -275TT in 42 patients (84%), -275TA in 7 (14%) and -275AA in 1 patient (2%). When patients were grouped according to UGT1A9 -275T>A genotype, no differences were found at any time for the pharmacokinetic parameters assessed (C_{trough} and AUC_{0\rightarrow 12}). However, in the case of -275TT carriers, when patients were divided according to MMF (19) or EC-MPS (23) treatment, AUC_{0\rightarrow 12} was significantly higher in EC-MPS treated patients than in those with MMF at 6M (AUC_{0\rightarrow 12}: 75.02±49.53 vs 36.73±12.22 ng.h/mL, p=0.008), albeit not during the early period. Conclusions: This study did not reveal any associations between UGT1A9 -275T>A polymorphisms and MPA C_{trough} and AUC_{0\rightarrow 12} at any time. Regarding the two formulations of MPA, for patients presenting the -275T genotype, in the stable period (at 6M post-transplant), the degree of exposure (AUC_{0\rightarrow 12}) for EC-MPS increased significantly, indicating that other polymorphisms and/or other factors (co-morbidities, concomitant drugs,...) could affect MPA levels.

Keywords: -275T>A polymorphism, mycophenolate mofetile, enteric-coated mycophenolate sodium
Theme: Pharmacogenetics

ASSOCIATION OF UGT1A9 GENE c.98T>C POLYMORPHISM ON MYCOPHENOLIC ACID PLASMA LEVELS IN KIDNEY TRANSPLANT PATIENTS TREATED WITH MYCOPHENOLATE MOFETILE OR ENTERIC-COATED MYCOPHENOLATE SODIUM
Salvador-Garrido P, Outea-Macias M, Pedreira-Vázquez I, Martín-Herranz I.

Background: The enzyme UGT1A9 is principally involved in the glucuronidation of mycophenolic acid (MPA). Expression levels and the activity of UGT1A9 may depend on the presence of the c.98T>C SNP located in the coding region. Aim: To assess the effect of allelic variants of UGT1A9 c.98T>C polymorphism on MPA metabolism in kidney transplant patients who are taking equivalent doses of mycophenolate mofetile (MMF) and enteric-coated mycophenolate sodium (EC-MPS) at two different post-transplant moments (15 days -15D- and 6 months -6M-). Methods: A retrospective study of Caucasian cadaveric kidney transplant patients co-treated with tacrolimus and steroids. All patients received the same MMF (Cellcept®) or EC-MPS (Myfortic®) dosage for at least 1 week prior to each profile. Dose correction: 500 mg of MMF= 360 mg of EC-MPS was applied. Plasma levels were measured by MEIA on a VIVA® Analyser. Blood samples were taken pre-dose and 1, 2, 3, 4, 6 and 8 hours after the morning oral dose. AUC₀₋₁₂ was calculated using the linear trapezoidal rule. A genetic test was used for the genotyping of UGT1A9 c.98T>C polymorphism, which was correlated to MPA trough concentrations (Cₜrough) and AUC₀₋₁₂ at 15D (dosage of 1000 mg/12h or 720 mg/12h, respectively) and 6M (500 mg/12h or 360 mg/12h) after transplantation. Each patient gave written consent of their participation. A statistical analysis was conducted using SPSS 19.0 with the Mann Whitney test. Results: 50 patients (24 with MMF and 26 with EC-MPS), age 51 years, weight 72 kg, were included. The genetic variants identified were c.98TT in 48 patients (96%) and c.98TC in 2 (4%). When patients were grouped according to UGT1A9 c.98T>C genotype, no difference was found at any time for the pharmacokinetic parameters evaluated (Cₜrough and AUC₀₋₁₂). However, in the case of c.98TT carriers, when patients were divided according to MMF (23) or EC-MPS (25) treatment, AUC₀₋₁₂ was significantly higher in EC-MPS treated patients than in those with MMF at the 6M post-transplant moment (AUC₀₋₁₂: 74.68±46.59 vs 38.86±12.52 ng.h/mL, p=0.005) but not in the early period. Conclusions: In this study, as no patient homozygous for the allelic variant (c.98CC) was found, it was not possible to determine any association between the UGT1A9 c.98T>C polymorphism and MPA plasma levels. Regarding MPA formulations, for patients presenting the TT genotype, in the stable period (6M post-transplant), the degree of exposure (AUC₀₋₁₂) for EC-MPS increased significantly, revealing that other polymorphisms and/or other factors (co-morbidities, concomitant drugs,...) could affect MPA levels.

Keywords: c.98T>C polymorphism, mycophenolate mofetile, enteric-coated mycophenolate sodium
Background: Human exposure to mercury is a worldwide concern because of its serious consequences. Long-term deleterious cardiovascular effects have been associated with chronic exposure. Still, no data are presently available about the cardiovascular risk (lipid profile and apolipoproteins A-1 (ApoA-1) and B (ApoB) ratio -ApoB/ApoA-1-) of exposed Amazonian riverine populations. Methods: After approved by CONEP/Brasil (CAAE N.° 43927115.4.0000.0018), the total mercury (Hg) and methylmercury (MeHg) content in hair was quantified by inductively coupled plasma mass spectrometry and gas chromatography system, respectively. The lipid profile (LDL, triglycerides, HDL, total and non-HDL cholesterol), glucose and ApoB and APOA-I levels were evaluated by spectrophotometry. The APOB gene (APOB, rs693) was genotyped by real-time PCR using the TaqMan system. Mann-Whitney, Spearman and Fisher’s exact tests were used to analyze differences between groups (high and low Hg), correlations and frequencies, respectively. Results: Median values of total cholesterol, non-HDL cholesterol and LDL of the 415 participants enrolled in this study were above the recommended limits, as well as HDL value was below, showing the dyslipidemic profile of the population. Total mercury content in hair was 8.30 μg/g (median), mainly as MeHg (87%). High mercury group (≥ 10 μg/g) showed higher total cholesterol, LDL, non-HDL cholesterol, ApoB, ApoA-1 and an increased ApoB/ApoA-1 ratio. All these parameters were significantly correlated with hair Hg (r-values: 0.1122, 0.1751, 0.1189, 0.1970, 0.0107 and 0.2052, for each parameter, respectively). The most frequent genotypes and alleles of APOB were A/G and G, respectively. No difference was detected in the APOB genotypic distribution between groups pointing to similar genetic susceptibility. Conclusions: We demonstrated that the Amazonian riverine population, chronically exposed to Hg, has a dyslipidemic profile associated with the Hg exposure without a higher genetic susceptibility. These data contribute to the early identification of lipid abnormality and to the development of appropriate measures for the prevention of cardiovascular diseases probably caused by the chronic exposure to mercury. Keywords: Mercury; Apolipoproteins; Dyslipidemias; Amazon.
Correlation between renal clearance of vancomycin and fluid input, weight, postmenstrual age and TDM in critically ill neonates and infants infected with *Staphylococcus* coagulase-negative bacteria

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**Background:** The immaturity of the kidney function, the low muscle mass and high water body content make difficult to predict the drug elimination in neonates. It is particularly important for the elimination of vancomycin to avoid nephrotoxicity. Therefore, our aim was to investigate which clinical parameters may influence the vancomycin clearance in septic neonates patients, infected with *Staphylococcus* coagulase-negative, using a linear mixed model analysis and its impact in the pharmacokinetic and pharmacodynamic (PKPD) target attainment. **Methods:** Preterm and term patients who underwent first (between 3-5th dose) and second (after dose adjustment) therapeutic drug monitoring (TDM) of vancomycin at doses of 10 and 15 mg/kg, between Jan 2015-June 2018, were included in the study. Linear mixed model analysis was applied using as reference the vancomycin clearance (CLvan) described by Tseng et al. (2017). **Results:** The postmenstrual age (PMA), weight (W), creatinine (sCr) and fluid input were significant. The resulting equation was \(\text{CLvan} = (0.0348 + 0.0022 \text{PMA} - 1.445 \text{Fluid} + 0.009 \text{MTV2} + 0.0614 \text{Weight} - 0.0946 \text{sCr} + 0.0356 \text{PMA}) \times \text{Fluid.} \) A good correlation between the calculated CLvan with the equations proposed by Schwartz creatinine clearance (CLcr) \((r = 0.83)\) and Leger CLcr \((r = 0.85)\) was met, but these equations are not suitable for PKPD target attainment. **Conclusions:** PMA, sCr, weight, fluid and dose adjustment correlated with CL in neonates and infant. The trough concentration between 10 – 15 mg/L and AUC/MIC ratio between 250-400 were suitable for treatment against *Staphylococcus* coagulase-negative infection.

**Keywords:** neonates, infants, sepsis, vancomycin, renal clearance, fluid input.
Theme: Pharmacometrics

POPULATION PHARMACOKINETIC MODELS OF ANTI-TUBERCULOSIS DRUGS IN PATIENTS
SYSTEMATIC CRITICAL REVIEW AND SIMULATIONS COMPARING RESULTS OBTAINED BY
PARAMETRIC AND NONPARAMETRIC APPROACHES
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Table 1. Results of simulation using the pop-PK parameter of 3 anti-TB drugs summarized during the systematic review

<table>
<thead>
<tr>
<th>Drug</th>
<th>Approach</th>
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Table 1. Results of simulation using the pop-PK parameter of 3 anti-TB drugs summarized during the systematic review

Background: Tuberculosis (TB) remains as one of the most important infectious diseases. Population pharmacokinetic (pop-PK) models are widely used to individualize dosing regimens of several antibiotics, but its application on anti-TB drugs is scant. Our aim was to provide an insight about the status of pop-PK for these drugs, and to compare the results obtained by parametric and nonparametric approaches in order to design precise dosage regimens with these drugs. Methods: First, a systematic approach was implemented searching in PubMed and Google Scholar. Articles that did not include human patients, without an explicit structural model, analyzing drugs inactive against M. tuberculosis, or without full-text access, were excluded. Second, the PK parameters were summarized and separated in two groups (parametric versus nonparametric results). Third, a Monte Carlo simulation was done under Pmetrics using the results of both groups and an error term (ET) was built to describe the imprecision obtained with each PK modelling approach. ET include the comparison of AUC reported in literature versus the AUC simulated (simAUC), a value of 0 means ideal approach and 100 the worst. Results: Thirty articles reporting at least one pop-PK model of 15 anti-TB drug were found, and 37 different models were reported. The PK parameter estimates, including relevant covariates, population and error estimates were summarized and it will be presented in the meeting. Only 8 models were performed using non-parametric approaches. Rifampin was the drug most studied, but only under parametric approaches. Table 1 shows that the non-parametric approaches minimize the ET. Conclusion. More and better models, ideally using non-parametric approaches and linking PK/PD data, are required to optimize anti-TB drug dosing as recommended by the WHO End TB strategy. Keywords: Pharmacokinetics; Statistical Models; Anti-Bacterial Agents; Tuberculosis; Precision Medicine.
PHARMACOKINETIC ASSESSMENT FOR A CASE OF ALPRAZOLAM-INDUCED NEONATAL ABSTINENCE SYNDROME USING PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

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Background: Use of benzodiazepines during pregnancy is related with a risk of neonatal abstinence syndrome characterized by apnea, vomiting, and other digestive events. The association of abstinence symptoms with plasma drug concentration in mother and neonate is not well characterized. In this case study, we performed pharmacokinetic assessment in a case of alprazolam-induced neonatal abstinence syndrome and conducted physiologically based pharmacokinetic (PBPK) simulation.

Methods: Blood samples of the neonate were collected as routine care immediately after birth, and once a day thereafter. Plasma concentration of alprazolam was measured by liquid chromatography–mass spectrometry (LC-MS/MS). Plasma alprazolam concentrations were simulated by Simcyp Population-based ADME Simulator. The compound file of alprazolam and the population physiological data of pregnant women, pediatric and Japanese patients were provided by the Simulator.

Results: A neonate born from the mother with chronic intake of alprazolam showed abstinence symptoms such as apnea and vomiting from 9 h after birth with a Finnegan score of 7. Apnea improved by 24 h after birth and the digestive symptoms disappeared by day 4. The plasma alprazolam concentration in the neonate was 15.2 ng/mL immediately after birth and showed a gradual decrease up to day 3. Plasma alprazolam concentrations in a neonate and a mother were simulated using the PBPK model. The estimated pregnant plasma concentration by the mother and fetus concentration ratio, observed neonate plasma concentration transition, and plasma concentration of patients after delivery were within the range of 5th–95th percentiles of the PBPK population of pregnant women, neonates, and Japanese non-pregnant patients, respectively.

Conclusion: Pharmacokinetic analyses and simulations by the PBPK model are useful for the toxicological assessment in special populations such as pregnant women or neonates.

Keywords: alprazolam; pregnancy; neonate; PBPK.
PIPAMPERONE IN CHILDREN: THE FIRST POPULATION PHARMACOKINETICS AND CLINICAL IMPROVEMENT

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**Background:** Although pipamperone is one of the most frequently prescribed antipsychotics in children in the Netherlands, Belgium and Germany, no pharmacokinetic data is known and information about efficacy is lacking in this population. The aim of this study was to describe the population pharmacokinetics of pipamperone in relation to clinical improvement in children.

**Methods:** We performed a population pharmacokinetic analysis based on 70 pipamperone concentrations from 30 children (median age 13.0 years, range [5.6-17.7], and bodyweight 50.4 kg [24.8-100.4]), using NONMEM 7.4. The sample consisted of Dutch patients from a prospective naturalistic trial (n=8), and German patients from a TDM service (n=22). Additionally, a random sample of 21 German patients with 33 pipamperone concentrations from the same TDM service was used for external validation (median age 14.9 years [7.2-20.6], bodyweight 47.4 kg [24.0-118]). Pipamperone samples were collected by venepuncture and Dried Blood Spot (DBS) and analyzed by previously validated LCMS and HPLC methods. The interquartile ranges (IQR) of through levels and AUCs for responders (n=34) and non-responders (n=15), based on Clinical Global Impression Improvement (CGI) scores were analyzed. **Results:** Pharmacokinetics of pipamperone in children and adolescents were described using a 1-compartment model. The mean volume of distribution (V) was 420 L/70kg and the apparent clearance (CL/F) was 22.3 L/h/70 kg (interpatient variability 20.6%). The error model included corrections for differences in matrix and analytical method and no significant covariates were identified. The pharmacokinetic model was successfully externally validated. The simulated trough concentrations were similar for responders and non-responders (median 93.1 µg/l, IQR [67.9-129.4] vs 92.1 µg/l, [47.2-129.8]), as were the simulated AUCs (2521.5 µg/l vs 1208.8 µg/l). In a limited subset of 12 responders without psychiatric comedication (n=14 measurements), the simulated trough levels in responders were higher (70.9 µg/l, [26.0-123.8] than in non-responders 37.7µg/l, [21.9-57.8]). **Conclusions:** This is the first study that describes pharmacokinetic parameters of pipamperone in children, which correspond to adult values found in the literature. Although more research is needed to support routine therapeutic drug monitoring in this population, the IQR found for responders are lower than recommended therapeutic reference ranges in adults.

**Keywords:** children, antipsychotic, pipamperone, population pharmacokinetics, efficacy, Dried Blood Spots
PHARMACOKINETIC AND PHARMACODYNAMIC STUDY OF PROPOFOL IN PATIENTS WITH RENAL IMPAIRMENT

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Background: Propofol is the most widely used intravenous anaesthetic agent. Though the kidney eliminates only a minor fraction, the physicians use of propofol sparingly in renal impairment (RI) due to anticipated change in the pharmacokinetics (PK) and pharmacodynamics (PD). The currently available literature is sparse and it is unable to provide guidance for dose modification in various class of RI. There is an evidence of alteration in PD, which has not been linked with PK. Methods: This study was conducted to evaluate the simultaneous PK and PD behavior of propofol in patients with various stages of RI where it was used for both for induction and maintenance of anaesthesia. The participants were categorized as per the NKF-KDOQI classifications of eGFR. A total of 34 patients (stage 1=10 [control], stage 2 = 11 patients, stage 3 = 10 patients, stage 4 = 3 patients) were included in the study. The participants underwent urological procedures under propofol, fentanyl and N2O anaesthesia. Propofol infusion was regulated for a target BIS of 40-60. Blood samples were collected at 12 time points continuing till the eye opening. Concentration of propofol in the plasma was estimated by using HPLC-florescence method. The data was analysed using NONMEM (version VII release III). Results: The log transformed propofol concentrations data was found suitable for a three-compartment model in NONMEM and fitted reasonably well. The Vss as well as V1 appeared to be unaffected between the stages of RI. However, the average total body clearance of propofol was significantly decreased in stage 4 (172.97±48.50), stage 3 (181.67±30.78 L/hr) and stage 2 (196.25±49.34 L/hr) compared to stage-1 (272.92±86.29 L/hr). We found that average time required for eye opening after stoppage of anaesthesia was significantly higher in stage 4 patients (12.5±2.12 minutes), stage 3 (12.87±4.70 minutes) patients as compared to stage 1 (7±3.17 minutes) and stage 2 (7.9±2.38 minutes) patients. The time to loss of consciousness (LOC) and BIS at LOC were similar in all the groups. There was no significant difference observed for the concentration of propofol or the average BIS at the time of loss of eye opening. Conclusions: Propofol may have a significantly decreased clearance and higher terminal elimination half-life in the patients with renal impairment that seem to increase progressively with decreasing creatinine clearance. Keywords: propofol; renal failure; pharmacokinetics; pharmacodynamics; PK/PD modelling; renal impairment.
Background: Traditional antibiotic dosing is not designed for intensive care unit (ICU) patients, as the extreme pharmacokinetic behaviour of drugs in critically ill patients threatens the achievement of optimal antibiotic treatment outcomes. For severe infections, the pharmacodynamic target (PDT) of 100% $\text{ft}>\text{MIC}$ and 100% $\text{ft}>4\times\text{MIC}$ are proposed for β-lactam antibiotics in ICU patients. The main objective of this study is to assess the PDT attainment of commonly used β-lactam antibiotics in critically ill ICU patients. Methods: A prospective observational PK/PD study was performed at two ICUs in the Netherlands. We enrolled patients ≥18 years old administered frequently used β-lactam antibiotics, over an 18-month period. Based on optimal sampling, five separate samples were taken at various time points. Plasma concentrations were determined by a validated multi-analyte UPLC-MS/MS assay. Non-compartmental PK analysis was performed and the percentage $\text{ft}>\text{MIC}$ were determined by calculating the intercept of the MIC values (0.0625 to 64 mg/L) with the concentration-time curve. For the primary endpoints, we used the epidemiological cut-off (ECOFF) values to calculate the percentage PDT attainment (100% $\text{ft}>\text{MIC}_{\text{ECOFF}}$ and 100% $\text{ft}>4\times\text{MIC}_{\text{ECOFF}}$).

Results: A total of 145 patients were included. The median age was 63 years, 61% of the patients were male. Other baseline characteristics of the study population were: serum creatinine 105 μmol/L, albumin 25 g/L, C-reactive protein 112 mg/L, the APACHE-II-score 23, and SOFA-score 11. The proportion of all patients achieving the PDT of 100% $\text{ft}>\text{MIC}_{\text{ECOFF}}$ and 100% $\text{ft}>4\times\text{MIC}_{\text{ECOFF}}$ were 63% and 30%, respectively. Using multivariate analysis, high serum creatinine and low albumin were found to be predictive for achieving the PDT. Conclusions: Empirical approach of β-lactam antibiotics dosing results in poor target attainment in the majority of the ICU patients. At present, TDM of β-lactams is not used as a routine intervention. We believe that should change, because the high variability of interindividual PK parameter emphasize the need of TDM with the intent of optimizing efficacy β-lactam antibiotics in critically ill patients.

Keywords: beta-lactam; ICU; target attainment.
Theme: Standards of practice

**CLINICAL PHARMACOKINETICS OF EVEROLIMUS COMBINED WITH ONCE-DAILY PROLONGED-RELEASED TACROLIMUS (ENVARSUS®) IN KIDNEY TRANSPLANT PATIENTS**

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**Background:** A strong recommendation for therapeutic drug monitoring of everolimus exists in the transplantation setting, with a proposed target range of 3-8 ng/mL for the trough blood concentration (C\text{trough}). The area under the concentration-time curve (AUC\text{0-12}) is the best strategy for pharmacokinetic study because it reflects overall drug exposure, however in the clinical practice the trough level is generally used. **Aim:** To evaluate the pharmacokinetics of everolimus in de novo kidney transplant patients who received everolimus in combination with once-daily prolonged-released tacrolimus (Envarsus®). **Methods:** Retrospective study of 16 Caucasian cadaveric renal transplant patients who were co-treated with once-daily prolonged-released tacrolimus (Envarsus®) and steroids. After a minimum of 7 days of receiving the same dose of everolimus, eight blood samples were collected at predose, and at 1, 2, 3, 4, 6, 8 and 12 h after oral morning dose. Pharmacokinetic assessments were performed around 1 month after transplantation. Blood concentrations were measured by QMS® everolimus immunoassay on the Architect-C8000™ analyser. The AUC\text{0-12} was calculated using the linear trapezoidal rule. Statistical analysis was performed using SPSS 19.0, with the Spearman Rho correlation coefficient (r) to evaluate the correlation between the C\text{trough} with the daily dose and with the AUC\text{0-12}. **Results:** 16 patients were included. Starting dose at 3 mg/day (1.5 mg twice a day), the mean dose after 1 month post-transplantation was 3.13±1.41 mg/day (range: 1-6). Everolimus mean trough concentration was 4.73±1.07 ng/mL (range:2.96-6.69), C\text{12h} concentration was 4.06±0.92 ng/mL (range: 2.91-5.89), and mean peak concentration was 11.87±3.98 ng/mL (range: 6.17-20.67) which was generally reached (T\text{max}) at 2 hours post-dose (range 1-6). The AUC\text{0-12} was 80.01±14.41 ng.h/mL (range: 51.26-104.59). The correlation between the C\text{trough} and AUC\text{0-12} was: r= 0.762 (p=0.001), and between the C\text{trough} and daily dose was: r=0.217 (p=0.419). **Conclusions:** There is a good relationship between the trough concentration and AUC\text{0-12} at steady state, but not between the trough concentration and the total daily dose. Trough level demonstrates a good relationship with overall exposure (AUC\text{0-12}), providing a simple and reliable index for therapeutic drug monitoring. **Keywords:** Everolimus, clinical pharmacokinetics, tacrolimus (Envarsus®)
Background: Sodium thiopental is a barbiturate that is administered at low doses for anesthesia and at higher doses to protect the central nervous system after a traumatic event. The main indication for analysis at the Karolinska TDM laboratory is to distinguish between brain death and thiopental induced coma after drug withdrawal where rapid test results are desirable. By the development of a new method to replace a HPLC-UV method, shortened turnaround times and better workflow was achieved. Methods: An LC-MS/MS method for the quantification of sodium thiopental and its metabolite pentobarbital in plasma samples was developed and validated. The analytical instrument was a Waters Acquity Ultra Performance LC-system with a Xevo TQ-S micro mass spectrometer operated in negative electrospray ionization mode. Sample preparation was performed by protein precipitation, using methanol containing isotopically labeled internal standards. Separation was achieved on an Acquity UPLC BEH C18 column (2.1 x 50 mm, 1.8 µm, Waters Co.) and the total chromatographic time was 2.5 min. Results: The method was validated according to European Medicines Agency guidelines. The acceptance criteria were fulfilled for accuracy and precision, robustness as well as qualitative and quantitative matrix effects within the quantification range of 1-250 µmol/L for both analytes. Sodium thiopental has poor stability in plasma but can be stored in stock solutions of methanol. The working solutions used for calibration and internal controls were prepared in plasma and can be stored at -80°C for one month. Conclusions: A rapid quantitative method was developed and validated to reduce the turnaround time for the analysis of sodium thiopental and pentobarbital “from 6 hours to 3 hours” and in this way providing a better service for the health service. The new method also provides a better workflow when these few, but very urgent patient samples arrive at the laboratory.

Keywords: thiopental, pentobarbital, LC-MS/MS, TDM
**COMPARATIVE PHARMACOKINETIC STUDY OF AN INDIAN GENERIC BRAND OF ERLOTINIB WITH TARCEVA**

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**Background:** The quality of generic drugs available in India is very often a matter of grave concern. Erlotinib (Tarceva, Roche) was approved for use in EGFR mutation positive non small cell lung carcinoma (NSCLC) in 2004. Following expiry of its patent in 2016, many Indian generic brands became available for clinical use at far cheaper price. A comparative pharmacokinetic study was conducted in real world situation in adult patients with stage IV NSCLC to demonstrate the equivalence of an Indian generic brand (Tyrokinin, Dr. Reddy’s Laboratories Ltd.) with respect to the innovator. **Methods:** The study was conducted in a single tertiary care cancer hospital in Western India. Patients were treated with erlotinib brand of their choice. The choice of brand depended on affordability since cancer treatment is an out-of-pocket expenditure for a majority in India. Standard dose of 150 mg erlotinib was administered to all patients. Blood was collected at predose, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 12.0, 24.0, 48.0, 72.0 hours after the first dose for the measurement of erlotinib levels using HPLC. The pharmacokinetic parameters were determined using WinNonlin version 7.0 (Certara, USA) and compared between brands using Mann-Whitney test. **Results:** 29 patients were enrolled in the study, 22 in test arm and 7 in the reference arm. The two groups were comparable for demographic variables such as age, sex, BMI and ECOG performance status. Pharmacokinetic parameters were also found to be comparable in the two groups as shown in Table 1. **Conclusions:** In this real world pharmacokinetic study, Tyrokinin was found to be pharmacokinetically equivalent to Tarceva. Thus, the two brands can be used interchangeably. Generic substitution offers significant cost saving to patients. **Keywords:** Erlotinib, Tarceva, Tyrokinin, Generic, Pharmacokinetics, NSCLC
PROTOCOLS OF THERAPEUTIC MONITORING OF VANCOMYCIN: IMPACT ON CLINICAL OUTCOMES

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Background: Therapeutic monitoring of vancomycin is considered as an important part of the therapeutic management of patients treated with this antibiotic. We analysed the impact of the vancomycin therapeutic monitoring protocols implemented in a Brazilian teaching hospital on patients' clinical outcomes. Methods: Retrospective observational study. The clinical impact of the protocols applied was investigated by evaluating the following clinical endpoints: therapeutic failure, nephrotoxicity, and death related to infection. Results: 743 patients were enrolled in the study, according to the defined inclusion criteria. Only 61.9% of patients had their plasmatic concentrations of vancomycin (PCVAN) determined. Of the monitored patients, only 36.1% achieved PCVAN within the extended therapeutic range of 10-20 mg/L, on the first determination. In the group of elderly patients, 56.7% (p<0.001) had PCVAN values greater than 20 mg/L. In the other hand, the percentage of patients with plasmatic concentrations over 20 mg/L was significantly lower in the newborns group than the other population subgroups. We also observed that plasma concentrations of vancomycin were higher [34.30±2.03 mg/L (IC95 30.32 - 38.28 mg/L), p=0.004] in patients with nephrotoxicity, comparatively to patients without nephrotoxicity [20.07±0.81 mg/L (IC95 18.48 - 21.67 mg/L)]. Therapeutic failure was assessed considering PCVAN. PCVAN was lower in adult patients with therapeutic failure [20.66±1.45 mg/L (IC95 17.82 - 23.50 mg/L)] compared to values determined in adult patients without therapeutic failure [26.72±1.59 mg/L (IC95 21.642 - 31.80 mg/L)], and this was the only situation statistically significant for this clinical endpoint (P = 0.037). Moreover, the study revealed that PCVAN was higher in adult patients with death related to infection [38.07±2.75 mg / L (IC95 32.67 - 43.46 mg / L)] compared to adult patients without death related to infection [18.79±1.15 mg / L (IC95 16.54 - 21.03 mg / L)] (P <0.001). Conclusions: This study allowed to define the minimum conditions for the efficient implementation of a vancomycin therapeutic monitoring protocol and revealed the importance of continuous and advanced training in the area of clinical pharmacokinetics for health professionals who work in hospitals.

Keywords: Clinical pharmacokinetics; Therapeutic Drug Monitoring; Vancomycin
Theme: Standards of practice

EVALUATION OF SALADAX MYPACLITAXEL™ IMMUNOASSAY ON THE ABBOTT ARCHITECT™ ANALYZER

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Background: Therapeutic drug monitoring of Paclitaxel is necessary to ensure appropriate therapy. The maximum concentration (C_max) and amount of time that the concentration remains above 0.05 mmol/L (Tc>0.05, 42.7 ng/mL) has been associated with peripheral neuropathy. Aim: To assess the Saladax MyPaclitaxel™ immunoassay on the Architect™-C8000 analyzer for measuring human plasma Paclitaxel concentrations. Methods: The study was conducted in accordance with the CLSI protocol (EP5-A3, EP9-A3, EP17-A2). Within-day imprecision: 20 replicated analyses of three patient samples and of MyPaclitaxel™ low (60 ng/mL), medium (120 ng/mL) and high (200 ng/mL) controls. Between-day 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 analyte-free sample (zero-calibrator) and low concentration calibrator (40 ng/mL). LoD= LoB+ 1.645 (SD_low concentration calibrator). Lower limit of quantification (LoQ): a low concentration plasma sample was diluted with paclitaxel-free sample to ten different concentrations in 5 different analytical runs. Dilution linearity: five high paclitaxel concentration plasma patients' pools were serially diluted with calibrator A. Analytical recovery: adding concentrated paclitaxel into paclitaxel-negative samples. Calibration curve stability tested on days 1, 7, 14 and 21 using the calibrators A-F (A:0, B:40, C:80, D:160, E:240, F:320 ng/mL) and controls (low, medium, high) in duplicate as were patient samples. Results: Within-assay coefficient of variation (CV) was 5.2 % for low (mean: 62.1 ng/mL), 4.9% for medium (mean: 123.5 ng/mL) and 5.4% for high control (mean: 198.6 ng/mL). The respective total CV for patients' pool was 5,8% (mean: 22.8 ng/mL), 5,4 % (mean: 50 ng/mL) and 6.2% (mean: 230ng/mL), respectively. Between-day imprecision was 6.3%, 5.7% and 6.6% for low, medium and high controls, respectively. LoB and LoD were 15 and 18 ng/mL, respectively. LoQ was 18 ng/mL. Dilution linearity displayed a high degree in the range studied (19-320 ng/mL, r= 0.98). Recovery was 95%. The calibration curve remained stable for 3 weeks. Conclusions: This study proves that MyPaclitaxel™ II Immunoassay, adapted to the Architect™-C8000 analyser, displays excellent calibration curve stability, precision, reproducibility, sensitivity, specificity. This technology could therefore be suitable for monitoring paclitaxel in routine clinical practice.

Keywords: Paclitaxel, Saladax MyPaclitaxel™ immunoassay, Architect™-C8000 Analyzer
Theme: Standards of practice

ASSESSMENT OF ARK™ OXCARBAZEPINE METABOLITE IMMUNOASSAY ON THE ABBOTT ARCHITECT™ ANALYZER
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Background: Therapeutic drug monitoring of Oxcarbazepine (OXC) and eslicarbazapine active metabolite is necessary to determine appropriate therapy. Aim. To assess ARK™ OXC Metabolite II immunoassay on the Architect™-C8000 analyzer for measuring human plasma OXC metabolite concentrations. Methods: The study was conducted in line with the CLSI protocol (EPS-A3, EP6-A, EP9-A3, EP17-A2). Within-day imprecision: 20 replicated analyses of 3 patient samples and of ARK™ OXC Metabolite low, medium and high controls. Between-day imprecision: over a 20-day period using the 3 controls and 3 patient samples; each sample was tested using 2 reagent lots and 2 runs per day. Limit of blank (LoB) and limit of detection (LoD): 10 replicates of an analyte-free sample (zero-calibrator) and low concentration calibrator (2 mcg/mL). LoD= LoB+ 1.645 (SDlow concentration calibrator). Lower limit of quantification (LoQ): a low concentration plasma sample was diluted with an OXC-free sample to 10 different concentrations in 5 different analytical runs. Dilution linearity: 5 high OXC metabolite concentration plasma patients’ pools were serially diluted with calibrator A. Analytical recovery: addition of concentrated OXC metabolite into OXC metabolite-negative samples. Calibration curve stability was tested on days 1, 7, 14 and 21 using calibrators A-F and the three controls in duplicate, as were patient samples. Therapeutic range: 3-35 mcg/mL. A statistical analysis was carried out on SPSS 19.0. The plasma levels obtained with the ARK™ OXC Metabolite were compared with LC-MS/MS from 50 patients ranging from 6 to 42 mcg/mL. Concordance between these concentrations was assessed using the intraclass correlation coefficient (CCI: 95% limit of agreement) and graphically with the Bland-Altman method. Results: Within-assay coefficient of variation (CV) was 4.2%, 3.1% and 3.8% for all 3 controls. The total CV for patients’ pool was 4.2%, 4.9% and 5.4% respectively. Between-day imprecision for the controls was 6.4%, 6.7% and 5.9% respectively. LoB and LoD were 0.1 and 0.3 mcg/mL respectively. LoQ was 0.5 mcg/mL. Dilution linearity displayed a high degree in the range studied (1-46 mcg/mL, r= 0.99). Recovery was 95%. The calibration curve remained stable for 3 weeks. CCI was 0.96 (0.93; 0.98) and concordance was confirmed using the Bland-Altman analysis. Conclusions: The ARK™ OXC Metabolite II Immunoassay adapted to the Architect™-C8000 analyzer displays excellent calibration curve stability, precision, reproducibility, sensitivity, specificity and a high degree of accuracy with the LC-MS/MS method. This technology could be suitable for monitoring OXC metabolite in routine clinical practice.

Keywords: Oxcarbazepine, ARK™ Oxcarbazepine metabolite immunoassay, Abbott Architect™ Analyzer
ASSESSMENT OF SALADAX MYLMATINIB™ II IMMUNOASSAY ON THE ABBOTT ARCHITECT™ ANALYZER

Background: Therapeutic drug monitoring of imatinib is a key aspect of therapy management. Aim: To assess Saladax Mylmatinib™ II immunoassay on the Architect™-C8000 analyzer for measuring human plasma imatinib concentrations. Methods: The study was conducted in accordance with the CLSI protocol (EP5-A3, EP6-A, EP9-A3, EP17-A2). Within-day imprecision: 20 replicated analyses of three patient samples and of Mylmatinib™ low (750 ng/mL), medium (1500 ng/mL) and high (2500 ng/mL) controls. Between-day 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 analyte-free sample (zero-calibrator) and low concentration calibrator (300 ng/mL). LoD= LoB+ 1.645 (SD_{low concentration calibrator}). Lower limit of quantification (LLoQ): a low concentration plasma sample was diluted with an imatinib-free sample to ten different concentrations in five different analytical runs. Dilution linearity: five high imatinib concentration plasma patients’ pools were serially diluted with calibrator A. Analytical recovery: adding concentrated imatinib into imatinib-negative samples. Calibration curve stability was tested on days 1, 7, 14 and 21 using calibrators A-F and controls (low, medium, and high) in duplicate, as were patient samples. Therapeutic range: 1000-2500 ng/mL. A statistical analysis was carried out on SPSS 19.0. Results: Within-assay coefficient of variation (CV) was 7.1% for low (mean: 765 ng/mL), 6.9% for medium (mean: 1486 ng/mL) and 6.6% for high control (mean: 2560 ng/mL). The respective total CV for the patients’ pool was 7.4 % (mean: 426 ng/mL), 7.2% (mean: 1460 ng/mL) and 6.8% (mean: 2190 ng/mL), respectively. Between-day imprecision was 7.1%, 6.7% and 7.2% for low, medium and high controls respectively. LoB and LoD were 50 and 123 ng/mL respectively. LLoQ was 240 ng/mL. Dilution linearity displayed a high degree in the range studied (300-2800 ng/mL, r= 0.99). Recovery was 90%. The calibration curve remained stable for 3 weeks. Conclusions: This study proves that Saladax Mylmatinib™ II immunoassay, adapted to the Architect™-C8000 analyzer, displays excellent calibration curve stability, precision, reproducibility, sensitivity and specificity. This technology could therefore be suitable for monitoring imatinib in routine clinical practice.

Keywords: imatinib, Saladax Mylmatinib™ immunoassay
**therapeutic drug monitoring of psychotropic drugs in children and adolescents**

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**Background:** Although Therapeutic Drug Monitoring (TDM) is well-established in adult psychiatry, it is far from routine practice in child- and adolescent psychiatry. We conducted a systematic review to review and assess therapeutic drug monitoring studies with psychotropic drugs carried out in children and adolescents. **Methods:** Studies assessing drug blood concentrations and efficacy or toxicity of antipsychotics, psychostimulants, alpha-agonists, antidepressants or mood-stabilizers in children and adolescents aged up to 18 years were eligible. Three databases were systematically searched according to PRISMA guidelines and a critical appraisal of the selected studies was performed (PROSPERO CRD42018084159). **Results:** A total of 67 eligible studies were identified from 9,298 records. Quality assessment identified 21 well-described sampling studies; within these, for 6 psychotropic drugs a concentration-efficacy relationship was found (citalopram, fluoxetine, nortriptyline, buproprion, quetiapine, lithium), for 2 psychotropic drugs a concentration-side-effects relationship (desipramine, ziprasidone), and for 2 psychotropic drugs a relationship with both efficacy and side-effects (methylphenidate, imipramine). **Conclusions:** For 10 psychotropic drugs sufficient evidence was found for either a concentration-efficacy or concentration-side-effect relationship. Interpretation of the retrieved studies was complicated as often patient samples were not collected according to a standardized protocol, such as sampling under steady state circumstances. In order to better support routine therapeutic drug monitoring in child- and adolescent psychiatry, more effort should be done to demonstrate a concentration-effect relationship in well-designed prospective studies.

**Keywords:** therapeutic drug monitoring, psychopharmacology, children, adolescents
Theme: Standards of practice

INTERACTION OF PSYCHOLOGICAL PROFILE, BLOOD PRESSURE LEVEL AND DRUG ADHERENCE IN HYPERTENSIVE PATIENTS

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Background. Poor drug adherence is considered as a major cause of uncontrolled and drug-resistant hypertension (HTN). Besides influencing drug adherence, specific psychological traits may confer an increased risk of high blood pressure (BP). In a cohort of patients with resistant HTN, we have recently identified distinct psychological profiles associated with drug adherence and drug resistance. The aim of the current study was to see whether these findings can be extrapolated to a broader range of hypertensive patients.

Methods. All patients with essential HTN seen at the hypertension consultation of the Cliniques Universitaires Saint-Luc were eligible, provided both office and 24-hour ambulatory BP were available. General demographic characteristics and other relevant variables previously associated with BP level and drug adherence were prospectively collected. Psychological profile was assessed using a broad array of validated questionnaires. Drug adherence was assessed by drug and metabolites detection in urine using LC-MS/MS.

Results. The analysis included 88 hypertensive patients (age: 59±14 years, 52% females, BMI: 28.0±4.7kg/m², office BP: 145/79±21/14mmHg, 24-hour ambulatory BP: 134/81±16/10mmHg, number of antihypertensive drugs/day: 3.0±1.5). The proportion of fully adherent, partly adherent and totally non-adherent patients was 69, 25 and 6%, respectively. In regression analysis, independent predictors of poor drug adherence were alexithymia (a personality trait characterized by difficulties in identifying and expressing feelings), smoking status and younger age. Independent predictors of severity of hypertension (defined as 24-hour ambulatory BP adjusted for the number of antihypertensive drugs and for drug adherence) were alexithymia and the number of previous traumatic events. The corresponding models accounted for 18 and 15% of the variance in drug adherence and severity of hypertension, respectively.

Conclusions. Poor drug adherence and severity of hypertension appear to be associated with distinct psychological characteristics, not only in patients with resistant hypertension, but also in patients with essential hypertension at large. As previously suggested, previous trauma and alexithymia are associated with the severity of hypertension. The association of alexithymia with poor drug adherence is a novel finding. Current plans include confirmation of our results in a larger sample, and replication in a cohort of patients followed by their general practitioner and a third cohort recruited in a specialized hypertension clinic in Southern Europe.

Keywords: hypertension, drug adherence, psychological profile
CHARACTERIZATION OF PHARMACOKINETIC PARAMETERS OF 5-FLUORURACILO IN CHILEAN PATIENTS WITH GASTROINTESTINAL CANCER WITH FOLFOX/FOLFIRI REGIMEN

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Background: 5-fluorouracil (5-FU) is the basis of chemotherapy for the treatment of cancers of digestive origin. Its dosage is based on the body surface area (BSA), which generates a wide interindividual variability in the systemic exposure to the drug, expressed as the area under the curve (AUC) and a high percentage of patients do not reach optimal therapeutic ranges. The aim of the present study is to evaluate the pharmacokinetic behavior of 5-FU in Chilean patients with digestive cancers with FOLFOX/FOLFIRI regimen. Methods: The research protocol was approved by the Scientific Ethics Committee of the Concepción Health Service. For the estimation of the pharmacokinetic parameters (PK), a linear monocompartmental model implemented in the Abbott base PKS System® pharmacokinetic software was used. Plasma concentrations of 5-FU were measured with the commercial immunoassay My5-FU®. By applying population analysis with conventional Bayesian estimation and with override, the AUC was estimated, considering as therapeutic objective, a range of AUC between 20 - 30 mcg x h/mL. Results: Seventeen patients were recruited; ten with colorectal cancer, six with gastric cancer and one with esophageal cancer. The PK parameters of 5-FU estimated were: volume of distribution (Vd) = 24.5 ± 2.7 L; clearance (Cl) = 155.2 ± 10.8 L/h and half-life (t1/2) = 0.110 ± 0.019 h. The interindividual variability in the PK parameters was 10.9 - 28.1% and the intraindividual variability was 11.6 - 24.1%, reaching differences in the AUC between patients up to 4 times. Only 16.6% of the patients managed to reach the therapeutic range and stay within it during the monitored cycles, using the ASC-based dosage. Conclusions: The high variability in the AUC, together with the low percentage of patients who manage to reach and stay within the therapeutic range, using the traditional dosage method, evidences the importance of the implementation of a therapeutic monitoring program within this group of patients, and a monitoring and follow-up during all the cycles of its chemotherapy treatment.

Keywords: 5-Fluoruracil; digestive cancers; pharmacokinetic; therapeutic drug monitoring; FOLFOX/FOLFIRI.
Theme: TDM in Oncology

THERAPEUTIC DRUG MONITORING OF IMATINIB IN CLINICAL PRACTICE
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Background: Therapeutic drug monitoring of imatinib (IMA) is useful for individualizing treatment and improving clinical response by dose adjustment. However, suboptimal response and failure of this therapy continue to be in patients with Chronic Myeloid Leukaemia, and the majority experience non-tolerable adverse reactions. Aim. To assess the clinical utility of therapeutic drug monitoring of IMA in our practice. Methods: A retrospective observational study of 100% of patients treated with IMA between July 2018 and January 2019. The variables analyzed were age, weight, height, disease and evolution, IMA dose and trough blood levels (C\text{trough}), BCR-ABL1/ABL1 transcripts (determined by an integrated real-time PCR system with specific Taqman probes, where values of $\leq 0.01\%$ are considered undetectable and in response); tolerance (classification of adverse reactions: 1: mild, 2: moderate or 3: severe) and clinical decision (1: the same dose, 2: increased dose, 3: decreased dose and 4: discontinued treatment due to ineffectiveness or adverse effects). Two measurements of the C\text{trough} were carried out for each patient. These plasma C\text{trough}, were determined by spectrophotometry on an ARCHITECT C800 analyzer using the ARK\textsuperscript{TM} Imatinib Assay. IMA standard dose ranged between 400 and 800 mg/day; therapeutic range (TR): 1000-2500 ng/mL. A statistical analysis was carried out on SPSS 19.0. A descriptive analysis of the variables studied was conducted. Results: 37 patients were included, 64% men, 66.8 ± 13.4 years, 74± 22 kg. The mean dose was 375.7± 68.3 mg/day and the C\text{trough} obtained was 1156 ng/mL (range: 425-3044.5); 66.4 % of our patients were within the TR, 29.7% were below and 5.4% above. 54.1% BCR-ABL1/ABL1 was undetectable. 40.5% of the patients showed adverse reactions (46.7% mild, 46.7% moderate and 6.6% severe). After monitoring, 62.2% of them continued to be treated with the same dose, 13.5% required a reduction and 8.1% an increase. IMA treatment was discontinued in 16.2% (3: ineffectiveness, 2: adverse effects). With this first monitoring, 62.5% of patients presented undetectable BCR-ABL1/ABL1, C\text{trough} = 1316.5 ng/mL (range: 623-2888) and 81.3% fell within the TR, 12.5% below and 6.3% above. 20% with mild adverse reactions reported no adverse reactions after dose adjustment. Conclusions: The therapeutic drug monitoring of IMA proved extremely useful for maintaining patients within the therapeutic range and with an adequate response, allowing for the control of adverse reactions and therefore enhanced patient management and treatment individualization.

Keywords: imatinib, therapeutic drug monitoring, clinical practice.
Background: Imatinib, the first tyrosine kinase inhibitor, has offered successful treatment not only in Chronic Myeloid Leukemia but also in cases with Gastro Intestinal Stromal Tumor. Many patients treated with imatinib are using other medicines that potentially could interact with the turnover and elimination of imatinib. Methods: We performed a retrospective study including all patients who had been treated with imatinib based on data from the Swedish Prescribed Drug Register, from July 2005 until September 2018. Our aim was to investigate the frequency of clinically relevant potential drug-drug interactions (DDI) between imatinib and concurrent medications. Clinically relevant interactions that should be avoided (defined as type D) and clinically relevant interactions that may be handled by for instance dose adjustments (Type C) were included in the study. Results: We found that among total 3379 patients, 1119 (33.1%) were prescribed at least one interacting medicine together with imatinib. Three hundred eighty-seven (11.4 %) were prescribed at least one drug interacting with imatinib that could either increase (10%) or reduce (1.4%) the plasma concentration of imatinib. The frequency of co-administration of drugs interacting with imatinib among men (n=1907) and women (n=1472) were comparable (33.4% vs 32.8%, respectively). The frequency of prescribed drugs that interact with imatinib during an imatinib treatment period varied from 20% to 50% in different regions of Sweden. Conclusions: We conclude that a significant number of patients are exposed to clinically relevant DDIs that may influence the imatinib plasma concentration. The consequence of DDIs could be suboptimal treatment with higher risk for toxicity or lack of effect. Consequentially, exposure to a DDI constitute an important indication for therapeutic monitoring of imatinib plasma concentrations, as a guide for adequate dose adjustments. Keywords: imatinib; drug-drug interaction; therapeutic drug monitoring.


SIMULTANEOUS MEASUREMENT OF REGORAFENIB AND ITS METABOLITES IN HUMAN PLASMA USING HPLC AND PHARMACOKINETICS IN JAPANESE PATIENTS

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**Background:** Regorafenib is an oral multikinase inhibitor predominantly metabolized to active metabolites of M-2 and M-5 by CYP3A. It is administered to colorectal cancer (CRC), gastrointestinal stromal tumors, and hepatocellular carcinoma patients for the first 21 days of each 28-day cycle. Many Japanese patients must stop its use or reduce dosage due to side effects including hand–foot skin syndrome. Understanding the regorafenib pharmacokinetics could facilitate individualized anticancer therapy. We developed a sensitive and specific HPLC method with UV detection for simultaneously determining regorafenib, M-2, and M-5 concentrations in human plasma and applied it in measuring regorafenib and its metabolite concentrations in the patients’ plasma levels.

**Methods:** HPLC separations were performed on XTerra RP18 (250 mm × 4.6 mm i.d.) operated at a flow rate of 0.8 mL/min by gradient elution of 20 mM CH3COONH4 and acetonitrile. Erlotinib and sorafenib were used as internal standards. Twelve patients with CRC hospitalized at the Kyorin University Hospital were enrolled in this study. Blood samples were obtained 1, 2, 4, 6, 8, and 11 h after the first administration of 160 mg of regorafenib on day 1, and at 10 am on day 2, 3, 4, 5, 7, 14, and 21 over a 21-day administration period, and at 10 am on day 28.

**Results:** All the target analytes were within 16 min without interference. The inter-assay precisions were <11%. Eight patients had to reduce the dosage or discontinue treatment. Seven patients experienced hand–foot skin syndrome. The area under the plasma concentration–time curve of regorafenib, M-2, and M-5, and their sum 24 h after administration were 52.5 ± 8.1, 23.0 ± 14.5, 4.1 ± 3.6, and 79.3 ± 22.4 nmol·h/mL for patients who could continue the 21-days treatment, and were 78.9 ± 25.5, 35.4 ± 14.7, 4.8 ± 3.2, and 119.2 ± 35.6 nmol·h/mL for patients who had to reduce the dose or discontinue treatment, respectively.

**Conclusions:** We developed a simple and specific HPLC analysis method. Patients who discontinued treatment were exposed to higher regorafenib and its metabolite concentrations. The proportions of regorafenib, M-2, and M-5 were different in each patient. The parent drug and its metabolite concentration can be used to predict its side effects and optimal dosages in individuals.

**Keywords:** regorafenib; M-2; M-5; HPLC; plasma concentration; pharmacokinetics
A MECHANISM-BASED POPULATION K-PD MODEL FOR LONG-TERM TESTOSTERONE INHIBITION IN PROSTATE CANCER PATIENTS UNDER INTERMITTENT ANDROGEN DEPRIVATION THERAPY

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Background: Intermittent androgen deprivation therapy (iADT) is a treatment option for selected populations of prostate cancer (PCa) patients that can prevent or delay disease progression and development of castration resistant prostate cancer (CRPCa). The aim of this investigation was to describe testosterone in PCa patients undergoing long term iADT during active treatment and recovery phases, to predict PSA response in stable and relapsing patients. Methods: PCa patients showing PSA relapse after previous radiation therapy started iADT treatment under a pre-defined titration scheme, with a 4 + 32 week treatment cycle followed by an off-drug period [1]. Repeated PSA recurrence above a threshold re-initiated active treatment, until PSA returned to below the target level. 72 patients underwent one to five active treatment cycles during a period of up to six years, in which testosterone and PSA levels were monitored continuously every four weeks. A mechanism-based K-PD model for long-term testosterone inhibition and recovery via GNRH-receptor downregulation was developed, based on a recently reported testosterone-PSA model for Leuprolone [2], under Monte-Carlo importance sampling (IMPMAP) in NONMEM. Results: The model could successfully describe testosterone inhibition during and after each active treatment cycle on population and individual patient level. Precision of model predicted testosterone response decreased approximately three-fold after the first active cycle, but remained constant during subsequent cycles. The median testosterone nadir during active treatment remained constant (0.13 nmol/L) for patients remaining in the trial. A non-normal distribution of individual estimates (EBE’s) for testosterone production decrease could be observed. The return rate of testosterone concentrations after the end of active treatment cycles was approximately 60% lower after cycle 2-5, compared to the wash-out phase after cycle 1. For a small sub-population of 12 patients designated as PSA relapers, slightly higher testosterone levels at the nadir of cycle 1 and 2 were observed compared to stable patients, but no explanatory covariate or parameter could be identified. From model-derived parameters, obtained by fitting to the data reported from cycle 1 and 2, the response during subsequent active cycles 3-5 could be adequately predicted. Conclusions: The developed K-PD model is able to describe and predict the long-term testosterone response under iADT in this patient population, including some typical aspects of receptor down-regulation and post-recovery decay in testosterone production. In the future, this model may be used to explain and predict the long-term disease progression as reflected by PSA response in the same PCa patient population.


Keywords: Testosterone, Intermittent androgen deprivation therapy; Oncology; K-PD population modeling
CARBOPLATIN: A DECADE OF THERAPEUTIC DRUG MONITORING IN A CHILDHOOD CANCER SETTING
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Background: Therapeutic drug monitoring (TDM) can be used as a personalized treatment approach when using anticancer drugs in challenging patient populations such as neonates, infants and high dose patients. Carboplatin is an example of a chemotherapeutic where the plasma exposure (AUC) has been shown to correlate with clinical efficacy and toxicity. Consequently carboplatin treatment is commonly targeted to cumulative AUC values of 5.2-7.8 mg/mL*min (standard) or up to 21 mg/mL*min (high dose) over three consecutive days, with exposures below or above these ranges considered sub-therapeutic or toxic, respectively. The current study aimed to assess our experiences with carboplatin TDM in a childhood cancer setting at centres across the UK over the past decade.

Methods: Pharmacokinetic (PK) data were collected over a 10 year period from challenging chemotherapy patients who received carboplatin TDM as part of their treatment. In total PK data were collated from 18 neonates (<12 weeks old on their first cycle) and 43 patients receiving high dose carboplatin. For selected neonates TDM was carried out on multiple carboplatin cycles, subsequently the number of TDM events within the neonate group increased to 43. Unbound carboplatin plasma concentrations were measured by atomic absorption spectroscopy analysis of ultra-filtrate samples at the Newcastle Cancer Centre Pharmacology Group, UK. A limited sampling approach was applied and a Bayesian model used to estimate PK parameters. Results: Using a TDM approach >80 % (36/43) of neonate analyses required dose adjustment on the third day of treatment to obtain the desired target AUC. Without dose adjustment, predicted cumulative AUC values ranged between 44 - 122 % of the target exposure. Of these 36 analyses, 33 required a dose increase and 3 required a dose decrease. An analogous approach was performed for children receiving high dose carboplatin chemotherapy, with dose adjustments recommended in >65 % (28/43) of patients. Similar to the neonate patient population, a trend was observed towards the under dosing of patients, with 20/28 requiring an increase in carboplatin dose. However, the predicted AUC range without dose intervention was greater (52 - 233 %) in the high dose patients group. Conclusions: Carboplatin TDM in neonates and high dose patients suggest that standard dosing for these patients is not optimal and a more personalized approach is required to achieve target plasma exposures. Given the success of carboplatin TDM, such approaches can potentially be utilized for a wide range of chemotherapeutics. Future studies should focus on collecting toxicity and response outcomes alongside PK data to inform dosing guidelines for neonates and infants, patients receiving high dose chemotherapy and other challenging groups.

Keywords: Oncology; TDM; Pharmacokinetics; Carboplatin; Neonates; High Dose Chemotherapy.
Theme: TDM in Oncology

DEVELOPMENT OF A SIMULTANEOUS QUANTITATION METHOD OF 19 ANTI-CANCER DRUGS IN PLASMA USING LC-MS/MS
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Background: The Newcastle Cancer Centre Pharmacology Group (NCCPG) is involved in the routine quantification of anticancer drug levels as part of clinical trials and therapeutic drug monitoring (TDM) approaches to patient treatment. A sensitive liquid chromatography mass spectrometry method for the analysis of 19 widely used anti-cancer drugs (Actinomycin D, Cyclophosphamide, Dexamethasone, Beclomethasone, Doxorubicin, Idarubicin, Etoposide, Ifosfamide, Imatinib, Sunitinib, Selumetinib, Teniposide, Vinblastine, Vincristine, Docetaxel, Paclitaxel, Nilotinib, Bosutinib, Dasatinib) from a minimal volume of plasma has been developed. This assay permits simultaneous pharmacokinetic profiling of patients undergoing combination chemotherapy with fewer samples collected. This is of particular benefit in a paediatric setting where obtaining sufficient blood volumes for analysis of multiple drugs using individual assays can be problematic.

Methods: Analytical samples were prepared in spiked blank plasma and extracted using the following method: protein precipitation using acetonitrile, evaporation to residue of supernatant, reconstitution in 50:50 acetonitrile:water. Compounds were resolved on an Agilent Eclipse plus C18 RRHD column (2.1mm x 50mm 1.8µm) maintained at 60°C using a gradient elution of 0.1% (vol/vol) formic acid (Phase A) and acetonitrile (Phase B) at a flow rate of 500µl/min. Detection was by dynamic MRM method on an Agilent 6460 Triple-Quadrupole mass spectrometer with JetStream source in positive mode coupled to an Agilent 1260 HPLC system.

Results: All compounds were eluted by 4.2 minutes, with an on-column lower limit of quantitation of 1ng or better, and linearity over 3 orders of magnitude. Depending on compound concentration to avoid overloading, chromatography was robust with injection volumes from 0.5µl to 20µl, permitting easy adjustment of analysis. No cross signal was observed between mass channels.

Conclusions: A robust, quick and simple method to simultaneously quantify a variety of frequently used chemotherapy drugs has been developed which permits faster analysis of samples from patients receiving combination chemotherapies and can reduce the sample volume burden for pharmacokinetic studies and TDM monitoring of patients. The developed method is now undergoing assay validation according to EMA and FDA Bioanalytical method validation guidelines within the NCCPG laboratories.

Keywords: Oncology; LC-MS/MS; therapeutic drug monitoring; multiplexed assays
EFFECT OF APREPITANT ADMINISTRATION ON CINV CAUSED BY CISPLATIN MULTI-DAY CHEMOTHERAPY AND PHARMACOKINETICS OF DOCETAXEL

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Background: To compare efficacy and safety of postponing administration of aprepitant and routine triple antiemetic treatment for chemotherapy-induced nausea and vomiting (CINV) in patients received docetaxel and cisplatin multi-day chemotherapy treatment, and to evaluate the effect of aprepitant on docetaxel pharmacokinetics in the Chinese population. Methods: A total of 24 cancer patients (22-74 years old) received two cycles of high-emetic DP (docetaxel 75 mg/m² d1 + cisplatin 25 mg/m² d 1-3) regimen. A randomized, two-period and cross-over study was applied for prevention of CINV. The patients in group A took aprepitant 125 mg d1, 80 mg d2-3 (administered aprepitant 1 hour before chemotherapy). In group B, the patients took aprepitant 125 mg d2, 80 mg d 3–4, which was delayed one day than group A. Efficacy and safety in overall phase were evaluated within 5 days after initiation of chemotherapy. Simultaneously comparing the differences in the pharmacokinetic parameters of docetaxel between two different antiemetic treatments. Results: The CR rate of delayed-phase nausea was compared between the routine triple antiemetic treatment (group A) and the aprepitant delayed one-day administration treatment (group B), and the difference was statistically significant (16.7% vs 45.8% P < 0.05), despite there were similar for two group in the CR rate of acute-phase nausea and vomiting and delayed-phase vomiting. In two group, the area under the docetaxel curve (mean ± SD) of docetaxel were 1134.21 ± 732.55 and 1080.94 ± 585.09 (ng-h/mL), respectively. There was no significant difference in AUC values between the two antiemetic treatments (P>0.05), as well as Cmax, CLv, T1/2 v, MRT and Tmax. But the AUC difference was as high as 20 times in docetaxel between individuals. After the preliminary statistical analysis, the docetaxel AUC value was not associated with CYP3A4*3. Conclusions: Delayed administration of aprepitant provided superior delayed-phase nausea protection for patients who received cisplatin-based chemotherapy in comparison with the routine triple antiemetic treatment. In addition, in the routine triple antiemetic treatment, aprepitant didn’t significantly affect the main pharmacokinetic parameters of docetaxel. But the AUC0-t values of most patients in the two treatments were not in the therapeutic window, which needs therapeutic drug monitoring to provide a reference for individualized therapy.

Keywords: Docetaxel; Aprepitant; DP chemotherapy; CINV; Pharmacokinetic; DDIs
USING HIGH-SENSITIVITY TROPONIN T TO PREDICT CARDIOTOXICITY OF RADIATION THERAPY IN NON-SMALL-CELL LUNG CANCER PATIENTS
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Background: Cardiac troponin is a sensitive and specific biomarker for myocardial injury. In this study, we investigated the impact of radiation dose to heart on level of serum high-sensitivity troponin T (hsTnT) in NSCLC patients receiving definitive radio(chemo)therapy (CRT). Methods: Serum hsTnT levels were measured in 26 NSCLC patients treated with definitive CRT (radiation dose ≥ 60Gy) on a prospective clinical trial. All samples were assayed using hs-TnT (Roche Diagnostics). Results: There is no difference in clinical characteristics between the two groups except treatment modality. All patients in high dose group were treated by IMRT, and 4 patients (44%) in low dose group were treated by Proton therapy. No difference was found in hsTnT levels of the two groups before CRT. During CRT, hsTnT levels increased in 15 (88%) patients in high dose group and stayed stable in 2 patients. hsTnT levels did not increase during CRT in majority of patients of low dose group, except for two patients who had preexistent cardiac problem (one had cardiomyopathy, the other one had LBBB). Radiation dose to heart was the only significant factor that impact the hsTnT dynamic change during CRT (coefficient: 0.41, 95% CI: 0.12-0.69, \(P=0.007\)). Furthermore, the ratio of hsTnT during CRT (TnTduring/TnTpre-CRT) was highly correlated with MHD at the time of hsTnT measurement (spearman’s \(r=0.39\), \(P=0.004\)). In addition, induction chemotherapy maybe a potential factor for TnT increasing during CRT (coefficient: 0.24, 95% CI: -0.04-0.52, \(P=0.085\)). Conclusions: Elevation of hsTnT during CRT is related to MHD. hsTnT is strongly predictive of cardiotoxicity and mortality at any time point. Early monitoring of hsTnT could identify patients who are more sensitive to cardiac damage from radiation. Keywords: high sensitivity troponin T; cardiotoxicity; radiation therapy; Non-small-cell lung cancer.
FULLY AUTOMATED THERAPEUTIC DRUG MONITORING OF ANTI-EPILEPTIC DRUGS MAKING USE OF DRIED BLOOD SPOTS
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Background: Fully automated dried blood spot (DBS) extraction systems, online coupled to standard liquid chromatography-tandem mass spectrometry (LC-MS/MS) configurations, decrease the hands-on time associated with conventional DBS analysis, resulting in a higher sample throughput, making the technique more compatible with a high-throughput bioanalytical workflow. The aim of this study was to develop and validate an LC-MS/MS method, using a DBS-MS 500 autosampler (CAMAG, Switzerland), for the determination and quantification of four anti-epileptic drugs (AEDs) (carbamazepine, valproic acid, phenobarbital and phenytoin) and one active metabolite (carbamazepine-10,11-epoxide) in DBS samples. Methods: Method development included thorough optimization of the fully automated extraction procedure (i.e. extraction solvent, extraction (loop) volume, internal standard application, internal standard drying time, etc.). Afterwards, the method was fully validated based on international guidelines and applied on capillary patient samples originating from African epilepsy patients. Results: Thorough optimization of the fully automated extraction method was of utmost importance, finally resulting in the exclusion of the built-in IS spray. Concerning method validation, accuracy (%bias) and precision (%RSD) (with a single exception) were below 13%, meeting the acceptance criteria. Neither carry-over nor unacceptable interferences were observed. Calibration data was found to be heteroscedastic. Using weighted linear regression, with 1/x² weighting, mean back-calculated concentrations did not differ more than ±15% for all analytes, which is in line with the acceptance criteria. All compounds were stable in DBS for at least 1 month when stored at room temperature, 4 °C and -20 °C and for at least 4 days when stored at 60 °C. Internal standard-corrected matrix effects were below 8%, with %RSDs below 9.1%. Reproducible relative recovery values (around 60% for all analytes) were obtained and the effect of the hematocrit on the relative recovery was overall limited. Finally, fifteen capillary DBS samples, originating from patients receiving AED therapy in remote areas within sub-Saharan Africa, were successfully analyzed, demonstrating the applicability of the developed procedure in a remote setting. Conclusions: An LC-MS/MS method for the determination and quantification of 4 AEDs and one active metabolite in DBS, making use of the DBS-MS 500 autosampler, was developed and validated. Thorough optimization during method development demonstrated that proposed, generic direct elution conditions, while of value for orientation, may require (substantial) adjustment, depending on the analytes of interest and the used instrumentation. Keywords: Therapeutic drug monitoring; dried blood spots; LC-MS/MS; anti-epileptic drugs; automated extraction.