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New technologies and challenges in laboratory toxicology and pharmacogenetics

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Clinical significance of therapeutic drug monitoring

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Therapeutic drug monitoring (TDM) represents an important way to optimise dosing of a wide range of drugs. Optimal drug therapy includes attaining the desired pharmacological effect of the drug, while reaching the maximal effect in the shortest possible time and decreasing the risk of toxicity. Clinicians request TDM in order to monitor patient compliance, individualize therapy at the beginning of therapy or when the dose is changed, diagnose undertreatment, avoid drug toxicity and manage withdrawal of the therapy.

Individual drug and drug classes where the most clinical usefulness of TDM is gained include aminoglycoside antibiotics (e.g. gentamicin), vancomycin, immunosuppressants (e.g. tacrolimus, cyclosporine), digoxin, theophylline, methotrexate, tricyclic antidepressants, lithium and antiepileptic drugs (AED). Digoxin is less often used than before but it is essential to regularly monitor its concentration for it can be associated with fatal adverse drug reactions in case of toxic concentrations (e.g. severe arrhythmias), especially in patients with compromised renal function.

TDM of AEDs will most likely benefit the individual patient through assessing compliance (particularly in patients with uncontrolled outbreak through seizures), through detecting drug toxicity, guiding dosage adjustment in situations with increased pharmacokinetic variability (e.g., children, the elderly, drug formulation changes) and in situations when important pharmacokinetic change is anticipated (e.g., in pregnancy). Pregnancy is particularly challenging in patients receiving AEDs because those drugs are associated with increased risk of congenital malformations, while physiological changes during pregnancy result in a reduction in the serum concentrations of most AEDs. Increase in lamotrigine clearance is particularly marked, leading to significant fall in plasma concentrations and consequent breakthrough seizures in some women. Concentrations may rise after delivery, leading to lamotrigine toxicity. Regular monitoring of AEDs is advised in each trimester and shortly after delivery and more frequent monitoring has been recommended for lamotrigine.

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Clinical chemist's interpretative competence in toxicology

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The role of laboratory, and specially the role of clinical chemist, in detection of disease, based on action of toxins (intoxication), is not well defined. In reality, a clinician can (may) frequently detect the cause of intoxication with simple-direct observation of patient's clinical condition. Moreover, the clinical staff usually share the same opinion, that the laboratory analytics is performed only to confirm their judgment and to fill the patient's administrative documentation, but has no influence on course of treatment. Such thinking produces frequently the confusion between the laboratory personnel and represents an usual companion in clinical laboratories. An additional embarrassment is the lasting education and the lack of properly educated personnel for toxicological analytics. Such consideration is changed because the techniques and knowledge in (clinical) laboratories has developed. The determination of kind (type) and quantity of absorbed substances can relieve the course of treatment. The laboratory findings can be used for optimization of therapy, and additional treatment can be carefully performed (dialysis, haemoperfusion, antidote therapy). In some cases, even a negative result could be of crucial importance. However, the time is of crucial importance, and only the quick and effective analytics with proper results interpretation, has some value. There is a high agreement in the literature about the frequency of different intoxications. Far ahead are intoxications with medicaments and alcohol,

followed by drugs of abuse, mushrooms, food, chemicals from agriculture and gases. Clinical laboratories with their facilities perform number of analyses, which can be used as an aid in diagnosis of intoxication. Such analyses are complete blood count, concentration of sodium, potassium, chloride, urea, creatinine, glucose, calcium, magnesium, albumin, INR, liver tests, anion gap, serum osmolality and osmolality gap, blood gas analysis, some enzymes (pseudocholinesterase, creatine kinase) etc. With proper interpretation this data can be very helpful and of benefit for intoxicated patient.

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Toxicology in forensic medicine

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Forensic toxicology is a discipline that deals with the medical aspects of the harmful effects of xenobiotics on humans and animals, helps in determining death and determines the circumstances that results in death. It connects the natural sciences (biology and chemistry) and social science (law) as it deals with a trial testimony in legal procedures relating to various poisonings.

Often, only external examination and autopsy of the body cannot determine the cause of death. Therefore, the court medics (but also the court and the lawyers) require toxicological analysis at every sudden, unexplained death circumstances, traffic accidents, homicides or suicides.

The basic prerequisite for good toxicological analysis is certainly an appropriate sample, properly collected and stored. Samples in forensic processing dead bodies include body fluids, tissues and contents of the digestive organs. For reliable results of the toxicological analysis good laboratory equipment and trained personnel are also very important.

There are no "absolute" rules in interpretation of toxicology results. Results cannot be interpreted only on the basis of the so-called "normal" or "reference" values. In the interpretation of toxicological results except concentrations of xenobiotics in the blood it must be taken into account the sampling site of blood (blood samples from different sites may give different results), but also the rate of absorption of xenobiotics, bioavailability, volume of distribution, half-life and the rate of metabolism which are different from person to person and are rarely known for the deceased person. Also, it is very important to take into account all the information about the circumstances and events related to the case of poisoning, particularly just before death, because toxicological results should be interpreted in light of the police documents (investigation) and the findings of medical tests.

Finally, forensic toxicologist report can be used as evidence in court, and based on the results obtained, may seek its interpretation and opinion.

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Instrumental techniques in toxicology

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Huge chemical diversity of substances that may cause intoxication generates high demands for selectivity and sensitivity of analytical methods and instruments needed for determination of these substances in different biological matrices. Diversity of chemical properties of analytes and matrices is reflected in variety of different analytical instruments used in toxicology laboratory and in the reduced potential for analytical process automation.

During the last few decades some analytical techniques proved to be the most versatile and became standard tools in analytical toxicology. These techniques include gas chromatography coupled to mass detectors, liquid chromatography coupled to the tandem mass detectors and atomic absorption spectroscopy. Basic principles and limitations

of these techniques are given in this presentation. Specific sample preparation requirements are also described. Besides standard techniques, the utility of some older, less reliable methods like immunoassays is discussed. Relevant properties of some emerging techniques like time-of-flight mass spectrometry or less frequently used techniques like enzyme assays and capillary electrophoresis are shortly described at the end of this presentation.

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Implementation of new techniques in toxicology

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Analytical toxicology deals with the detection, identification and measurement of xenobiotics in biological fluids, tissues and other specimens with the purpose of diagnosis, management and prevention of poisoning. Different techniques and methods are available for analysis of various compounds, but of the greatest interest is the field of pharmaceuticals and drugs of abuse analysis.

Great progress in drug discovery methodology is observed in the latter part of twentieth century with introduction of the modern instrumental techniques. Since then, a range of powerful novel technologies, especially techniques related to mass spectrometric methods are constantly evolving, bringing new and better solutions and making them attractive and more toxicologicaly informative. However, comprehensive analysis that can detect all drugs in the sample with adequate sensitivity does not exist yet.

The choice of method and instrument in analytical toxicology depends on the problems to be solved. Many factors must be considered like the nature of analyte supposed to be determined, sample matrix, expected concentrations, whether qualitative or quantitative analysis is required and the time available for the analysis. Hence, knowledge about desirable characteristics of the instrument is necessary to ensure required information. It is particularly important in the selection of detector and gases. The choice of instrument may influence the choice of sample preparation which is important for selection of appropriate equipment and space. Finally, such techniques pose requirements related to space, security, maintenance and servicing. Mentioned requirements will be described through examples of implementation of selected techniques in the University Clinical Centre Osijek.

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Pharmacogenomics in personalized laboratory medicine, a new opportunity for more effective and safer treatment

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Physicians, pharmacists and also laboratory medicine professionals are aware for a long time that theory "one fits all" is no longer valid. The tailoring of drug dosing or drug selection was recognized as crucial for higher drug efficacy and lower adverse drug effects as well as for cost-effectiveness of (biological) drug treatments. Genetic factors are one of many factors (along with for example kidney or liver dysfunction) influencing treatment efficacy and safety. Pharmacogenomics (PGx) is looking for the genetic changes of proteins involved in drug absorption, metabolism, elimination and therefore the genetic changes which could alter drug plasma levels (pharmacokinetics) or drug-target interactions (pharmacodynamics). PGx testing as DNA testing of an individual has therefore the potential for significant contribution to individualization of treatment. PGx is taking an important part in personalized medicine, which represent a new approach in patients manage-

ment and is based on molecular definition of diseases. The long time research in the field of pharmacogenomics (PGx) resulted in much important information on molecular basis of drug efficacy, individual drug response and especially of prediction of adverse drug effects. Our study of the molecular basis of imatinib resistance in patients with Ph-positive chronic myeloid leukaemia and role of drug transporters at raloxifen treatment will be presented in the talk. In general, pharmacogenomics shift the emphasis in medicine from the reaction to the prevention and from population to individual patient with the aim to avoid medical complications and to reduce the healthcare costs. However, the use of PGx testing is still not widely used in clinical practice. Only some of clinical chemistry labs are performing the PGx testing for thiopurines, clopidogrel, codeine, warfarin, abacavir, simvastatin and allopurinol treatments. Steadily increasing evidence for the benefits of PGx in several drug treatments schemes is likely to result in the wider entrance of PGx into laboratory medicine in the near future.

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Liquid chromatography- mass spectrometry and applications of therapeutic drug monitoring

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To the present time therapeutic drug monitoring (TDM) - the quantitative determination of drugs in biological fluids in clinical laboratories has been predominantly performed using automated immunoassays. But it is recognised that immunoassay methods can suffer with non-specific interference from related compounds, metabolite interference or matrix effects. In recent years, liquid chromatography (LC) coupled to mass spectrometry (MS) has also expanded in routine clinical laboratories. It provides higher analytical sensitivity and specificity and it has ability to develop methods in a more rapid time frame than immunoassays. LC-MS assays for most therapeutic drugs have been developed; however in clinical practice are just some of them, most often immunosuppressants and newer drugs.

Voriconazole and posaconazole are two triazole antifungals administered for the prevention and in the treatment of invasive fungal infections. Because of their broad inter- and intra-individual pharmacokinetic variability, TDM is suggested to be used for dose optimization.

We have developed a convenient assay for determination of voriconazole and posaconazole in serum by LC-MS. Sample preparation involved a simple one-step protein precipitation with acetonitrile. After centrifugation, the supernatant was injected directly into the HPLC with C18 reversed-phase column. Mass spectrometric detection was performed using electrospray ion source in positive ionization mode, with ion trap mass spectrometer we used the fragmentation transitions m/z 350.0 $\rightarrow$ m/z 126.9 and m/z 701.3 $\rightarrow$ m/z 683.3 for voriconazole and posaconazole, respectively. The retention time of voriconazole was 4.7 min and of posaconazole was 5.0 min. Standard curves were linear over the concentration range of 0.1 to 12 mg/L. Intra- and interday coefficients of variation (imprecision) were 6.4 and 12.7 for voriconazole and 5.3 and 12.9 for posaconazole. The recovery was 99.6 for voriconazole and 96.8 for posaconazole. This simple and rapid LC-MS assay offer an efficient method for TDM of antifungals voriconazole and posaconazole.

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