Satellitensymposium

VS-01 Biomarker des Alkoholkonsums – eine Übersicht

Torsten Arndt

Bioscientia GmbH, Ingelheim, Germany; torsten.arndt@bioscientia.de

Alcohol abuse and alcohol addiction cause considerable consequential damage for both the individual and society. In Germany, the follow-up costs of alcohol misuse are estimated to be several ten billions Euro. This is the reason why there has been an intense ongoing search for valid markers of alcohol abuse and alcohol dependency.

Such markers can be divided into so-called Trait- and State-markers. Trait-markers, e. g. platelet monoaminoxidase activity or specific alleles of the D2-dopamine receptor gene, are time-independent indicators, which (should) point to a genetic predisposition for the development of alcohol dependency. Until now, trait-markers have not gained much acceptance due to limited analytical robustness and/or diagnostic accuracy.

State-markers are time-dependent parameters which (should) indicate an (increased) alcohol consumption. Such markers are usually divided into those of acute or recent alcohol use and those of chronic alcohol abuse. The demands for being a valid State-marker are very high. In the final analysis, a maximum diagnostic specificity and sensitivity is needed, very often being mutually exclusive.

Valid markers of acute or recent alcohol intake are ethanol, ethylglycuronide and ethylsulfate in blood and urine. These parameters are well-established and are standard parameters of a clinical and toxicological laboratory. Congener analysis, e. g. methanol, is common in forensic labs and adds valuable information.

A wide range of parameters has been proposed for the detection of chronic alcohol abuse. Most of them have not proven analytical robustness and/or sufficient diagnostic accuracy, e. g. the “liver enzymes” ALT and AST and the mean corpuscular volume of erythrocytes. Both show low diagnostic accuracy and it is no longer acceptable to use these parameters for the diagnosis of chronic alcohol abuse.

Relatively new parameters like fatty acid ethyl esters (FAEE) in hair and phosphatidyl ethanol (PEth) in blood, showed in first reports an acceptable diagnostic accuracy. However, it is too early to make a final judgement about analytical robustness, diagnostic specificity and sensitivity and about the additional diagnostic information provided by these parameters in comparison with well established markers.

Those markers are carbohydrate-deficient transferrin (CDT) and serum activity of γ-glutamyl transferase (γ-GT). CDT is considered to be the most specific marker of chronic alcohol abuse so far. In fact, increased CDT values are almost specific for chronic alcohol abuse whereas, due to limited diagnostic sensitivity, normal CDT results do not rule out alcohol abuse. Parallel analysis of serum γ-GT and ethylglucuronide in hair can increase the diagnostic sensitivity.

In summary, laboratory markers of alcohol abuse provide valuable information regarding the individual alcohol consumption. They should, however, not be used alone but should always be interpreted within the context of the clinical (or case) background including (if possible) a structured questionnaire.

VS-02 Neuere Aspekte in Diagnose und Therapie alkoholbezogener Störungen

Friedrich M. Wurst

Salzburg, Österreich (Abstract lag bei Druck nicht vor / no abstract submitted).

VS-03 Presentation and methodological aspects on alcohol biomarkers GTOL, EtG and PEth

Olof Beck

Karolinska Institute, Stockholm, Sweden; olof.beck@karolinska.se

This contribution will review the development and methodology of alcohol biomarkers 5-hydroxytryptophol glucuronide (GTOL), ethyl glucuronide (EtG) and phosphatidyl ethanol (PEth).

GTOL. Upon exposure to ethanol the metabolism of serotonin shifts to produce more 5-hydroxytryptophol at the expense of 5-hydroxyindole-3-acetic acid (5HIAA). This shift is presented in urine as an increased ratio of GTOL/5HIAA and this ratio does not return to normal value until hours after exposure. This observation led to the proposal of using this as an alcohol exposure biomarker. This marker is sensitive for each single intake and can be used for several applications where this information is valuable. Methodology of GTOL comprises both chromatography and immunoassay.

EtG. EtG is a direct ethanol metabolite that was first discovered long ago but has been developed as an alcohol biomarker more recently. EtG has the same use as acute biomarker as GTOL, but compared to GTOL it is more sensitive and has a longer detection window. Methodology of EtG comprises both chromatography and immunoassay. The combined use of ethyl sulfate and EtG has been proposed to increase accuracy.

PEth. Following research on the effects of ethanol intake on membrane fluidity PEth was discovered in early 1980's. The formation of PEth is specific for ethanol exposure and has evidential value of ethanol exposure comparable with EtG. PEth is measured in the lipid membrane fraction from blood cells. So far only chromatography methods have been used. PEth is a biomarker integrating ethanol exposure during the preceeding two weeks. PEth is more sensitive than CDT, but experience from clinical applications is more limited.

Summary. In recent years several new biomarkers of ethanol exposure have been developed and made it possible to monitor ethanol intake longer than when using ethanol itself and also integrating ethanol exposure over time so that excessive alcohol drinking behaviour can be revealed at early stage. Early detection of alcohol abuse is of value for successful treatment.

VS-04 Sucht und Sorgerecht

Claudia Zimmer-Odenwälder

Amtsgericht Schwetzingen, Germany; claudia.zimmer_odenwaelder@freenet.de

1. Feststellung von kindeswohlgefährdenden Situationen (z. B. Alkohol-, Opioidabhängigkeit oder Ähnliches) in der Familie

2. Eingriffmöglichkeiten des Jugendamts: Das Jugendamt hat verschiedene Möglichkeiten nach dem SGB VIII auf freiwilliger Basis Hilfen in der Familie zu installieren (Sozialpädagogische Familienhilfe, Einweisung der Kinder in stationäre oder teilstationäre Einrichtungen u.a.). Daneben hat das Jugendamt die Möglichkeit der Inobhutnahme der betroffenen Kinder, auch ohne Einwilligung der Eltern. Das bedeutet, dass die Kinder in einer Krisensituation vorübergehend (höchstens für 1 Tag) in einer Einrichtung untergebracht werden (§ 42 SGB VIII).

3. Eingriffsmöglichkeiten der Gerichte: Die Gerichte sind gehalten, bei kindeswohlgefährdender Sucht zunächst Maßnahmen im Einverständnis mit den Eltern herbeiführen. Gelingt dies nicht, kann das Gericht zwangsweise Maßnahmen anordnen (§ 1666 Abs. 3 BGB), z.B. kann den Eltern aufgegeben werden, Kinder- und Jugendhilfemaßnahmen in Anspruch zu nehmen, die Kinder einen Kindergarten/hort besuchen zu lassen, ärztliche Behandlung der Kinder wahrnehmen zu lassen, usw.

Das Gericht kann auch Erklärungen des Sorgerechtinhabers ersetzen, z B. eine Entbindung des Arztes von der Schweigepflicht oder Einwilligung in eine Operation § 1666 Abs. 3 Nr. 5 BGB. Verbessert sich die Situation nicht, können die Gerichte als ultima ratio das Sorgerecht entziehen und einen Vormund bestellen. Bei dieser Maßnahme ist stets die Hinzuziehung eines Sachverständigen erforderlich. Sämtliche Maßnahmen, die das Gericht ergreifen kann, beziehen sich immer auf das Kind, das heißt, dem Gericht ist es nicht möglich, den Eltern aufzugeben, sich einer Drogentherapie zu unterziehen oder Ähnliches.

VS 05 Testing for Alcohol and its Markers: Ethyl Glucuronide (EtG) and Ethyl Sulfate (EtS) on a Large Scale

Sumandeep Rana

Redwood Toxicology Laboratory, Santa Rosa, California, USA

OBJECTIVES: Since 2006, we have been testing for EtG/EtS, minor but important metabolites of ethanol, in urine and has analyzed tens of thousands of specimens. The purpose of this study was (1) evaluate trends in urine ethanol and urine EtG/EtS concentrations obtained from thousands of paired ethanol and EtG/EtS specimens and (2) examine the data from a statistically significant high number of EtG positive specimens to determine positivity rates based on various proposed EtG cut-offs. Since EtG/EtS testing is relatively new, the data is presented to the scientific community in an attempt to help establish guidelines for EtG/EtS testing in urine and to aid in understanding and interpreting urine ethanol and EtG/EtS results.

METHODS: Specimens submitted to the laboratory for urine ethanol and EtG/EtS were first screened for ethanol using an enzyme assay method with a 0.4 g/L screening cutoff. The screened positives were subjected to GC confirmation. The limit of quantitation was 0.2 g/L. Since the specimens were unpreserved, a microscopic examination for the presence of yeast (Candida sp.) was performed on all ethanol positives to rule out fermentation as a consideration. All specimens were also screened for EtG using a fully validated LC/MS/MS method with a screening cut off of 100 ng/mL. A separate aliquot of the screened EtG positives was extracted and subjected to separate LC/MS/MS analysis for confirmation. Both EtG and EtS were monitored in the confirmatory method to increase analytical specificity. A cutoff of 100 ng/mL for EtG and 25 ng/mL for EtS was used. Both EtG and EtS were required to be

present in the specimen above the laboratory cutoff levels in order to call a specimen positive for EtG/EtS.

RESULTS: 59,438 specimens were received in a six month period for ethanol and EtG/EtS testing. 7,488 (12.6%) were positive for ethanol, EtG/EtS or both. The specimens were divided into three categories:
1 – Ethanol and EtG/EtS positives. 547 specimens out of 560 were positive for ethanol, EtG and EtS with 83.5% specimens having EtG concentrations above 10,000 ng/mL, 11.9% had EtG concentrations between 1,000-10,000 ng/ mL and 4.4 % had EtG concentrations below 1,000 ng/mL. EtS was above 1,000 ng/mL in 93% of these specimens. 13 specimens (2.3%) were negative for EtS but positive for ethanol and EtG. 10% of the total specimens in this category (1) contained yeast.
2 – Ethanol negatives, EtG/EtS positives. 6781 specimens were negative for ethanol but positive for EtG and EtS (95.9%) or EtG only (4.1%). This is over 90% of the total positives in all three categories indicating the importance of EtG/EtS testing in ethanol abstinence programs. 62.9% of the specimens had EtG concentrations in excess of 1000 ng/mL, and 25.6% had EtG concentrations above 10,000 ng/mL in this category.
3 – Ethanol positives, EtG negatives. 147 specimens tested positive for ethanol and negative for EtG. Yeast was detected in 69% of these specimens indicating that ethanol may have been present due to fermentation. 4% of the specimens were positive for EtS only with concentrations in excess of 5000 ng/mL indicating EtG may have been subjected to bacterial decomposition.

Assuming the positivity rate to be 100% with an EtG cut off of 100 ng/mL, a cutoff of 250 ng/mL detects 91.4% of the positives, 500 ng/mL cut off detects 78.1%, 1,000 ng/ml cutoff detects 63.8% and a cutoff of 2,000 ng/mL detects 51.1% of the positives in a given population.

CONCLUSIONS: 1) The majority of ethanol positive urine specimens were positive for both EtG and EtS and had very high concentrations for both; EtG>10,000 ng/mL and EtS> 1,000 ng/mL. 2) EtS concentration in more than 90% of the specimens was lower than EtG; with EtS being 33% of EtG on an average. This should be useful when establishing cutoff values for EtG/EtS in urine; however care must be taken while trying to determine a cutoff for EtG. A higher cutoff value will result in a significant number of undetected cases and a lower cutoff may result in “innocent positives”. 3) The presence of yeast in more than 69% of the specimens testing positive for ethanol and negative for EtG indicate the probability of fermentation in these cases. The absence of EtG can also be attributed to decomposition by bacteria in some cases and hence, EtG/EtS combined test should be used since EtS is not known to be susceptible to bacterial decomposition. Urine preservative use should be recommended as well. 4) Majority of the EtG and EtS positives (90%) were negative for alcohol indicating the importance of EtG/EtS testing in abstinence programs. 5) There is little doubt that the analytical sensitivity and specificity of the LC/MS/MS methods for the detection of EtG/EtS have been firmly established. However, effective EtG testing cutoff levels to differentiate “innocent exposure” from ethanol consumption cannot be determined until diagnostic sensitivity and specificity studies have been performed.

VS-06 Model Calculation to Simulate Kinetics of Ethyl Glucuronide and Ethyl Sulfate

Detlef Thieme

Institute of Doping Analysis, Dresden, Kreischa, Germany; detlef.thieme@idas-kreischa.de

Objectives: A general benefit of examinations of longterm-metabolites is an extended temporal retrospection to substance abuse. On the other hand, the association with dosages, bioavailability of active compounds or biological effects is limited by individual variations of biotransformation reactions. Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are much longer detectable in biological specimens (e.g. blood, urine, hair) than ethanol and may hence provide valuable information on alcohol consumption long after its elimination.

Results and Discussion: A kinetic model utilizing the well established EtOH kinetics - followed by a first order invasion and elimination of EtG - could be analytically solved providing a robust kinetic model. A total of 24 EtG and EtS time courses from 16 subjects were tested under different conditions (e.g. single vs. multiple administrations) to verify the validity of the model and evaluate inter-individual variations of its parameters.
The invasion constant (0.558 ng/ml/h, RSD=0.28) and an elimination rate (0.181 h^{-1} , RSD=0.26) were fitted to the experimental data. The kinetics obey a two-phase mechanism which is very well reflected by the mathematical model. After total elimination of ethanol from the blood, a simple exponential elimination equation must be applied. This singularity impedes the potential of retrospective calculation from EtG (or EtS) concentration.

Conclusions: Based on this mathematical model, kinetic parameters and ethanol intake, the time course of EtG may be calculated and the validity of ethanol consumption statements in forensic cases may be challenged. This may be particularly interesting to evaluate potential ethanol consumption after total elimination of ethanol or in cases of post-offence drinking claims.

VS-07 Alcohol interlocks - contributing to greater road safety

Johannes Lagois

Dräger Safety AG & Co. KGaA, Lübeck, Germany; johannes.lagois@draeger.com

In view of the alarmingly high statistics relating to road traffic accidents and deaths caused by drink driving worldwide, new ways to reduce these figures have been sought in recent years. Starting from North America alcohol interlocks are used in more and more countries to prevent alcohol-impaired drivers from using their vehicles. An alcohol interlock is a breath-alcohol measuring instrument with a vehicle immobilizer. After taking a breath alcohol measurement, it prohibits a driver who has consumed alcohol from starting the motor. When the ignition is switched on, the alcohol interlock requests a breath sample from the driver. The result of the breath-alcohol concentration measurement determines whether the vehicle's starter is released and the engine can be started. Preventive installation of an alcohol interlock in vehicles used by the transport industry (hazardous goods carriers, haulage companies, buses and taxis) can reduce accidental damage and downtime, improve a company's image, and give customers a greater feeling of safety.

Bitte beachten Sie die Hinweise zu Einsendung und Abfassung der Beiträge zum Mosbach 2011 Proceedings Band auf Seite 161 dieses Heftes.