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Eloy Girela-López
Section of Forensic and Legal Medicine. Faculty of Medicine and Nursing.
University of Córdoba, Spain
E-mail: *eloygirela@uco.es*

Markers of alcoholism and ethanol consumption. A review

Ethanol is the most commonly used drug in the world and is of paramount importance in relation to medico-legal pathology. This is why ethanol analysis is the most frequently performed assay in forensic toxicology laboratories. Nevertheless, the diagnosis of alcohol consumption does not only rely on ethanol analysis, but it could also be improved by the use of laboratory tests, which provide information on recent and long-term drinking patterns. In this paper we carried out a review of both direct markers (ethanol, acetate, ethyl glucuronid, ethyl sulphate, fatty acid ethyl esters and phosphatidylethanol) and indirect markers (carbohydrate-deficient transferrin, gamma-glutamyl transferase, alanine amino transferase, etc).

Keywords: ethanol, acetate, ethyl glucuronid, ethyl sulphate, fatty acid ethyl esters, phosphatidylethanol, carbohydrate-deficient transferrin, gamma-glutamyl transferase, alanine amino transferase.

1. INTRODUCTION

Ethyl alcohol or ethanol is the most commonly used drug in the world and is of paramount importance in relation to medico-legal pathology. It is responsible of most drug-related deaths. Blood alcohol levels strengthen effects on Central Nervous System of most drugs of abuse, particularly those with depressant action (BZD, opiates…), combining to cause a fatal outcome. The chronic abuse of alcohol leads not only to definite pathological changes in a number of target organs, but also contributes to deaths from neglect, hypothermia, drowning and burns. Its abuse is a prime factor in many accidents – transport, domestic and industrial – and a lot of crimes and homicides are catalyzed by alcohol intake (Saukko and Knight, 2004).

Interpretation of ethanol levels in blood and other biological specimens is of medico-legal interest both in the living and in the dead. According to the «Global status report on alcohol and health 2014» (WHO), about 3.3 million deaths in 2012 are estimated to have been caused by alcohol consumption. This corresponds to 5.9% of all deaths, or one in every twenty deaths in the world (7.6% for men, 4.0% for women). This is why ethanol analysis is the most frequently performed assay in forensic toxicology laboratories. Nevertheless, the diagnosis of alcohol consumption does not only rely on ethanol analysis, but it could also be improved by the use of laboratory tests, which provide information on recent and long-term drinking patterns. There are indirect markers and direct markers, the latter of which derive from the ethanol molecule and still contain the two carbon atoms of ethanol.

2. INDIRECT MARKERS

The indirect state markers such as gamma-glutamyl-transpeptidase (GGT), aminotransferases, mean corpuscular volume (MCV), and carbohydrate deficient transferrin (CDT) are influenced by a number of factors like age, gender, and nonalcohol-related illnesses, and do not cover the entire time frame (acute, short-term, long-term) of alcohol use.

2.1. Carbohydrate-Deficient Transferrin (CDT)

Transferrin is a liver glycoprotein made up of a polypeptide chain and two glycan chains. CDT refers to transferrin isoforms lacking one or two complete or incomplete glycan chains, the most common of which are asialotransferrin, monoasialotransferrin and diasialotransferrin. CDT quantification generally refers to diasialotransferrin measurements. Chronic alcohol consumption interferes with the glycosylation of several glycoproteins, including transferrin. Risky to heavy drinking (50–80 g alcohol/day) for several days decreases the carbohydrate content of transferrin, thus giving rise to free sialic acid and sialic-acid deficient transferrin (Nanau and Neuman, 2015). CDT is a biomarker of risky to heavy alcohol consumption and CDT levels return to normal within approximately 2 weeks of drinking cessation. As such, CDT is a useful indirect marker for both initial screening as well as relapse (Allen et al, 2013).

CDT generally correlates well with an individual's drinking pattern, especially during the preceding 30 days. CDT has been studied mainly in serum and diagnostic performance reported is satisfactory, sensitivity ranging from 25–86% and specificitiy from 70–100%. CDT may be analysed by HPLC, radioimmunoassay, capillary electrophoresis isoelectric focusing and by nephelometry. CDT is generally expressed as the percentage of CDT divided by the amount of total transferrin. The diagnostic usefulness of CDT is the same when using absolute or relative values. Cut off point depend on the scenario, but in a clinical one use to be from 1.6% to 2.7% (Nanau and Neuman, 2015), while in post-mortem samples is higher.

We studied CDT, among other post mortem markers of chronic alcoholism, in 32 alcoholics with 32 age-sex matched controls drawn from a forensic autopsy population. Comparison of receiver operating characteristic (ROC) curve, likelihood ratios and post-test odds showed CDT to be the best individual test, followed by ALD and GGT. Quantitation of CDT by IEF/laser densitometry performed slightly better than MAEC/RIA by CDTect™. CDT showed considerable promise as a post mortem marker of chronic alcoholism. In fact, CDT was the best individual test (sensitivity 72% and specificity 88%) with a cut-off point of 18% (Sadler et al, 1996).

Possible confounding factors are:

• Serum CDT can differentiate between heavy drinkers and non-drinkers, and between heavy drinkers and social drinkers ($p < 0.0005$ for both), but not between social drinkers and non-drinkers ($p = 0.063$) (Pirro et al, 2011).

• Little variation in CDT levels was seen for alcohol consumption below a threshold of 2 drinks/day (6–10 drinks/week), past which point a significant increase was observed (11–20 drinks/week) (Whitfield et al, 2013).

• CDT levels were significantly associated with the body mass index, female gender and smoking, but not with age (Whitfield et al, 2013).

• The usefulness of CDT is reduced in overweight or obese subjects, as CDT levels are lower in these compared to lean individuals consuming comparable amounts of alcohol. In contrast, CDT levels are higher in smokers compared to non-smokers consuming comparable levels of alcohol (Whitfield et al, 2008).

• Disialotransferrin, the CDT species most often analyzed, shows a high degree of increase during pregnancy (1.07% ± 0.17% baseline to 1.61% ± 0.23% before delivery). CDT levels return to normal in the post-partum stage (Kenan et al, 2011).

• CDT elevations can occur in sepsis, anorexia nervosa, and airway diseases (Anton et al, 2002).

• Although CDT is usually unaffected by the presence of liver disease, false positive results have been reported in patients with primary biliary cirrhosis and with severe non-alcohol-related hepatic failure (Bell et al, 1993).

2.2. Other indirect markers

Elevated levels of liver enzymes i.e. gamma-glutamyl transferase (GGT), alanine amino transferase (ALT) and aspartate amino transferase (AST) in blood are commonly used in clinical

practice as an indicator of alcohol-induced liver damage. However, these tests suffer from low sensitivity for early detection of risky drinking, and the specificity is only moderate. In most studies, GGT sensitivities exceed those of the other commonly used markers, in the range of 50–60% but it is significantly correlated to ethanol intake only in high alcohol consumers. Detection times of liver enzymes in serum depend on the amount and frequency of alcohol intake. AST, ALT and GGT have an elimination time of 2–3 weeks (Maenhout et al, 2013).

Furthermore, GGT is subject to significant post mortem changes. GGT levels in right heart blood may be 2–8 times greater than in the femoral vein due to post mortem diffusion of GGT from the nearby liver (Piette and De Schrijver, 1987).

We studied the diagnostic performance of the CAGE questionnaire, plasma levels of ethanol and acetate, Mean Corpuscular Volume (MCV), GGT, and Glycosylated Hemoglobin (Hb A1c) in a group of 50 healthy non-alcoholic controls and 31 patients with non-alcoholic liver disease, and in a second group of 44 alcoholic patients. Taken individually, the CAGE questionnaire was the most efficient (96% sensitive and 92% specific), followed by plasma levels of acetate (74% sensitive and 85% specific), MCV (64% sensitive and 91% specific) and GGT (72% sensitive and 80% specific). Hb A1c did not show any statistically significant difference between alcoholics and non-alcoholics and thus is of no use as a screening test for the diagnosis of alcoholism (Girela et al, 1994).

3. DIRECT MARKERS

3.1. Ethanol

Ethanol is rapidly absorbed across both the gastric mucosa and the small intestines, reaching a peak concentration in blood usually between 30–120 min after ingestion, largely controlled by the quantity of food in the gastrointestinal tract. The bloodstream transports ethanol to the body and after equilibration, most tissues are exposed to the same concentration as in the blood, though it depends on the volume of distribution and the water content of the tissue (Baselt and Cravey, 1989).

3.1.1. Ethanol in breath

Breath-analyzers provide a quick result and the levels correlate well with the Blood Alcohol Concentration (BAC). Breath alcohol is a representation of the equilibrium of alcohol concentration as the blood gasses pass from the blood into the lungs to be expired in the breath. However, the airway alcohol exchange process is subjected to interferences with some factors which may lead to variation in breath alcohol concentration measurements. A major limitation of this test is the short detection window (typically < 12 h), due to rapid ethanol elimination, with a mean hourly BrAC elimination rate of 0.082 mg/L h-1 (Pavlic et al, 2007).

Possible confounding factors with Breath alcohol test include the ratio BrAC/BAC used to estimate BAC (may range from 2000/1 to 2300/1 at 34 ºC). According to the Henry's Law an increase in 1 ºC theoretically may result in an overestimation of the BAC up to 6.5%.

Other possible factor is food, which has a strong influence on breath alcohol pharmacokinetics. BrAC maximum concentration was higher in fasting subjects and lower in subjects who consumed a light meal (Sadler and Fox, 2011). There are also inter-individual differences in ventilation, particularly in some pathologies such as Chronic Obstructive Pulmonary Disease… (Hlastala, 2010)

On the other hand, in my experience (unpublished results), the Widmark equation overestimate the maximum ethanol concentration peak (50.7% overestimation as an average), being the overestimation markedly higher in women (55.7%) than in men (42.2%).

3.1.2. Ethanol in Urine

Urine alcohol provides an indication of the BAC at the time when the urine was produced (generally in the previous 8–12 h), but it is of no significance in the interpretation as to the cause of death (Niemelä and Alatalo, 2010). A drug detected in the urine had an effect on the individual but one cannot say that it is having an effect at the time the patient died.

Nevertheless, it may help in the interpretation of post-mortem formation of ethanol as well as may assist to know the absorptive stage. Finding a ratio less than or close to unity suggests incomplete absorption of alcohol in all body fluids at time of death, which indicates fairly recent drinking and some of the ingested alcohol probably remains unabsorbed in the stomach, whereas finding a ratio of 1.25 or more suggests that absorption and distribution of ethanol was complete by the time of death (Jones and Holmgren, 2003).

3.1.3. Ethanol in Vitreous Humour

The main advantage of VH over blood, besides its watery nature, is that anatomically it is remote from the gut and therefore less prone to contamination by spread of bacteria. Owing to the remoteness of the eyes from the large blood vessels and the gut, VH provides a very useful specimen whenever the corpse has already undergone decomposition so that post-mortem synthesis is a real possibility (Kugelberg and Jones, 2007)

VH is considered a relatively sequestered fluid, and because of this, vitreous ethanol levels, when compared with blood ethanol levels, can help in the interpretation about whether the person was in the «absorptive» or «metabolic» phase of alcohol consumption. Generally, vitreous ethanol levels lag behind blood ethanol levels as the ethanol is being absorbed into the circulation and remain higher than the blood ethanol levels as the ethanol is metabolized from the blood. If the blood ethanol level is greater than the vitreous ethanol level, the person was likely in the absorptive stage of ethanol ingestion. It takes approximately 30 to 60 minutes for ethanol to equilibrate in the vitreous fluid. If the vitreous ethanol level is greater than the blood ethanol level, the individual had likely absorbed the majority of the ethanol into the blood, which had already diffused into the vitreous fluid (Dolinak, 2005).

3.1.4. Ethanol in blood

Peripheral blood is the gold standard sample for the detection of a recent ingestion of alcohol (Druid and Holmgren, 1997). In the living individual, the main limiting factor is how quickly ethanol disappears. The clearance rate for ethanol varies among individuals, but averages between 0.15 and 0.25 mg/ml/hour (Jones, 1993), consistent with the opinion of many other authors – including myself – nearly always over the commonly used in the Widmark equation of 0.15. This is why ethanol will be absent in blood no later than 15–20 h after ingestion, even after haven reached high levels.

The pathologist must offer interpretations of alcohol levels found at autopsy with caution, especially where retrospective calculations are requested. Less often, the pathologist may be asked what blood or urine levels might be expected at a certain time (for example, at the time of death) given a description and timetable of alcoholic drinks taken by the deceased. The same cautions against overprecise calculations must be offered here (Saukko and Knight, 2004).

Widmark, in 1932, produced his well-known formula for calculating the total amount of alcohol in the body, from which knowing the body weight and assuming equilibration throughout the water compartment, the blood alcohol level could be derived. The Widmark equation is: $A = R \times P \times C$, where A is the total body alcohol, C the blood concentration, P the body weight in kilograms and R a factor, which is 0.68 in men and 0.55 in women. The sex difference is due to the different fat: water ratios, men having about 54 per cent and women 44 per cent water partition by weight.

Interpretation of the results, especially for post-mortem specimens, is complex. Many factors must be taken into consideration, including ethanol distribution in the absorptive and postabsorptive phases, sample collection site, possible stomach trauma, aspirated vomitus, and post-mortem distribution and synthesis of ethanol (O'Neal and Poklis, 1996).

In order to avoid problems related to post-mortem formation of ethanol and to interpret properly BAC, we must follow these requirements (O'Neal and Poklis, 1996; Kugelberg and Jones, 2007):

– Try to know the history of the deceased in the previous hours before death, though is not easy to have reliable information.

– Collect peripheral blood (femoral or jugular) with FNa (10 mg/ml) and fill completely the container, without head space.

– Compare the concentrations of ethanol in different body fluids such as femoral blood, urine and VH.

– Assess the degree of decay of the specimens… if clearly evident one must assume PM formation

– Pay attention to atypical distribution of ethanol: if is found in blood but not in urine and VH… think in PM formation

– If there are other volatiles: acetone, acetaldehyde, methanol, n-propanol, n-buthanol, isopropanol, isobuthanol... assume PM formation

– BAC very low. In most cases in which the ethanol is attributed to decomposition alone, the ethanol level will be less than 0.07% (0.7 g/L) though exceptionally may be as high as 1.5 g/l.

Because alcohol is rapidly eliminated from the circulation, there is a need for a biomarker that will bridge the gap in the time window, between one day and one week, left by the current biomarkers of alcohol consumption. In this respect, several markers have been proposed to extend the interval and sensitivities of detection, including ethyl glucuronide and ethyl sulfate in urine (up to 5 days), phosphatidylethanol in blood (over 2 weeks), and ethyl glucuronide and fatty acid ethyl esters in hair, among others (over months).

About 92–95% of the consumed ethanol undergoes biotransformation via oxidative metabolism. Non-metabolized alcohol is eliminated in small quantities by the kidneys (0.5–2%), lungs (1.6–6%) and the skin (<0.5%). A small proportion of the ingested ethanol, undergoes non-oxidative metabolism. The latter, i. e. ethyl glucuronide (EtG), ethyl sulfate (EtS), phosphatidylethanol (PEth) and fatty acid ethyl esters (FAEE), allow to detect a single alcohol intake several hours up to some days afterwards, the time window largely being dose-dependent. (Maenhout et al, 2013). Even the time window can extend to several months by analysing ethyl glucuronide and fatty acid ethyl esters in hair.

3.2. Acetate

Acetate, is the second metabolite resulting from ethanol oxidation, but it is also an ubiquous compound of intermediary metabolism. It had been established that during ethanol oxidation, blood acetate levels are higher both in alcoholics and heavy drinkers than in occasional drinkers; hence, it was proposed as a laboratory marker of alcoholism back in 1985 (Korri et al, 1985).

Though this proposal has hardly been examined, we investigated to explore the role and pharmacokinetics of acetate in two groups of rats (alcohol fed and nonalcohol fed) after administration of 1.5 ethanol/kg i. p. Results showed that acetate levels were higher in alcoholics rats from 0 up to 180 minutes after ethanol injection. (Girela et al, 1993)

In a second step (Girela et al, 2012), we studied ethanol and acetate concentration and distribution in several fluids (peripheral blood from femoral vein, vitreous humour, pericardial fluid and urine) and tissues (brain, lungs, liver and kidneys) in 98 human cadavers. Presence of ethanol ($n = 22$) was the most significant variable related to acetate increase in most specimens ($p < 0.0001$), except for urine and brain (Figure 1). Post-mortem interval tended to increase acetate concentrations in most specimens, although no statistically significant differences were observed. Presence of drugs of abuse in urine ($n = 21$) showed a significant acetate increase in blood, kidneys, and particularly in lungs and pericardial fluid ($p < 0.0001$).

Distribution of acetate, expressed as the ratio acetate in fluid or tissues/ acetate in blood showed a specific pattern in the cases with presence of ethanol -n = 22- (Figure 2), with statistically significant differences in brain/blood and kidney/blood acetate ratio ($p < 0.0001$). A Brain Acetate/ Blood Acetate ratio <2 and a Kidney Acetate/Blood Acetate ratio <2.5 were characteristic of ethanol consumption.

In spite of the interesting results, there were some relevant objections in our work with cadavers, which make acetate less valuable as a post-mortem marker of ethanol consumption:

– The greatest unanswered question was whether acetate concentrations would be increased in tissues in which ethanol is formed post-mortem by the action of microflora. In fact it may only be answered by in vitro experiments.

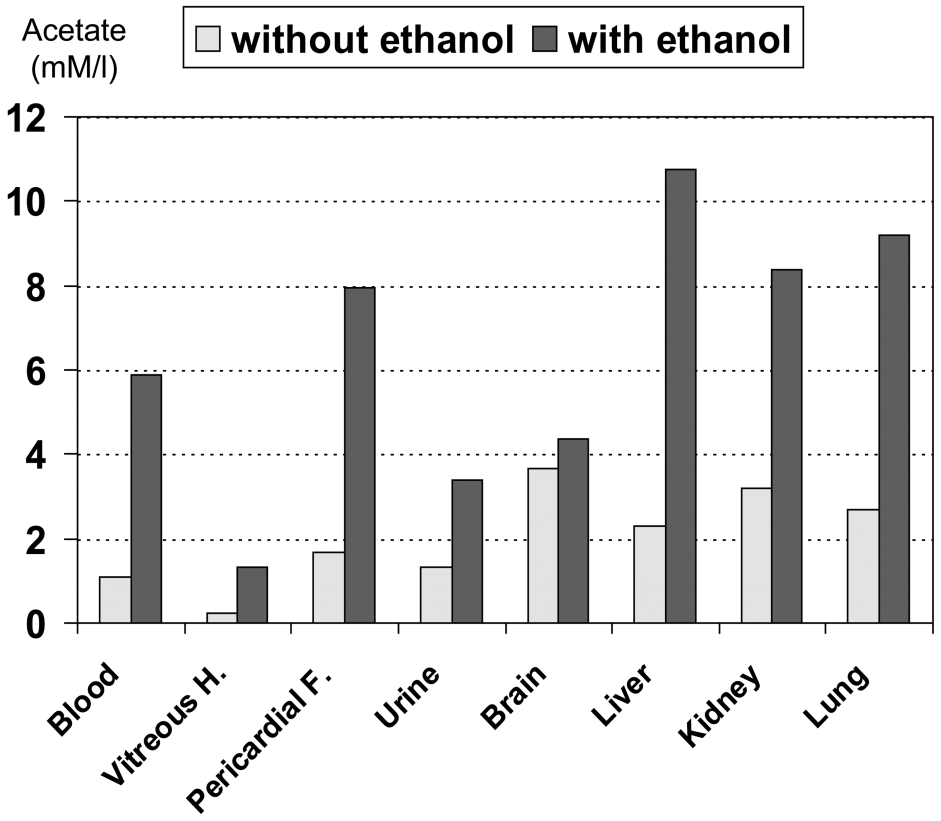

Figure 1 – Acetate concentrations (mM) in presence or absence of ethanol

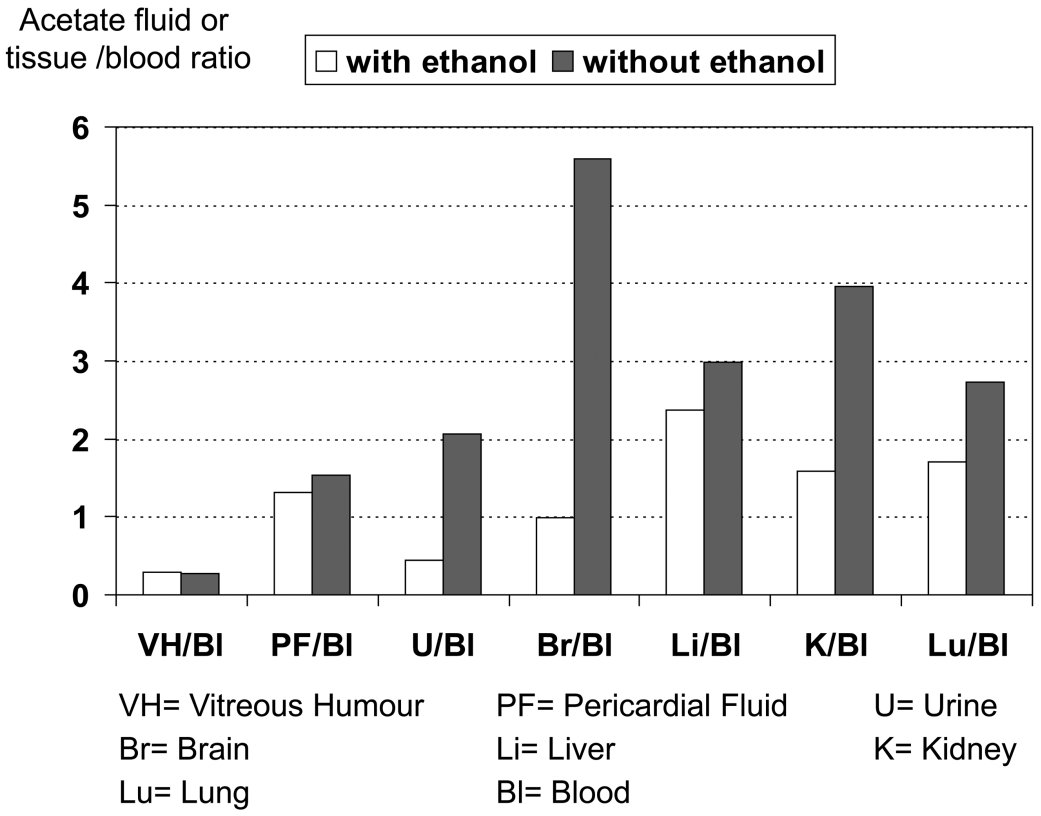

Figure 2 – Acetate fluids or tissues/blood ratios in presence or absence of ethanol

– The findings regarding increased acetate in alcohol and drug related deaths were also puzzling, and raises questions about the diagnostic value or conclusions regarding alcohol when drugs are present, because is difficult to assess the independent influence of the ethanol (in 13 out of 22 cases of drugs in urine we found also alcohol).

– The observation that acetate increases with post mortem interval also raises questions about the diagnostic value of the test, as a possible confounder, when coupled with decomposition – the very kinds of cases where this is an issue.

3.3. Ethyl glucuronid (EtG)

EtG is a non-oxidative ethanol metabolite nonvolatile, water-soluble, and stable in storage. The possible effect of nutritional components such as flavonoids on EtG formation is currently under investigation and seems to be a possible partial explanation of the variability of EtG formation in humans (Schwab and Skopp, 2014). Only a small proportion (0.6–1.5%) of ethanol is conjugated with glucuronic acid catalyzed by the UDP-glucuronosyltransferase (UGT) superfamily of enzymes. Approximately 0.02–0.06% of the total amount of ethanol consumed is eliminated as EtG in the urine (Maenhout et al, 2013). Depending on the amount of consumed alcohol and time spent for consumption, EtG is still detectable in the body long after completion of alcohol elimination: up to 36 h in blood, 4–5 days in urine and several months -6- in hair (Wurst et al, 2015).

The use of EtG as a possible marker for alcohol intake was first discovered in 1995 by Schmitt. In recent years, it was demonstrated that the consumption of alcohol can be indirectly determined by analyzing the hair for EtG. The solid and durable nature of hair ensures a substantially longer detection time for chemical substances. During the growth period, substances can enter the hair either via incorporation by diffusion from blood into growing cells or by deposition from sweat or sebum into the hair. The level of incorporation is affected by the amount of exposure to the hair from each of these sources, the pH of the surrounding mediums, physicochemical properties of the analyte, its opportunity and ability to penetrate the hair and its binding sites (Maenhout et al, 2013).

The analytical methods used for EtG are LC/MS-MS and EIA. The LC/MS–MS method has the advantage that no derivatization is being required. This enabled determination of EtG in urine, serum, post-mortem body fluids and tissues and hair. However, since LC–MS/MS methods are relatively costly and only available in specialized laboratories, a widespread use of EtG has been hampering. In 2006, a commercially available enzyme immunoassay (EIA) method based on a monoclonal antibody (DRI Ethyl Glucuronide Enzyme Immunoassay) was developed for the analysis of EtG in urine. The EIA showed a good correlation with a well-established LC–MS method ($r^2 = 0.93$), indicating a high level of accuracy and selectivity of the EtG antibody. Despite its relative high specificity, EIAs are currently used as screening tests and should be confirmed using a robust LC–MS method (Maenhout, 2013).

EtG is also detectable in post-mortem body fluids and tissues like gluteal and abdominal fat, liver, brain, and cerebrospinal fluid, in bone marrow, muscle tissue, and finger nails (Wurst et al, 2015).

3.4. Ethyl Sulfate (EtS)

Ethanol is conjugated with sulfate by a superfamily of cytosolic sulfotransferases. Only a very small fraction (~0.1%) of the ethanol ingested undergoes sulfate conjugation in humans. About 0.010–0.016% of the ethanol dose is excreted as EtS in urine. EtS is detectable for up to 1.5 days in urine after intake of moderate amounts of ethanol (Maenhout et al, 2013).

EtS is detectable in the same body tissues as EtG. Urinary EtG correlates well with EtS. Both EtG and EtS were below the cut-off value among subjects who denied alcohol consumption, and were generally undetectable (<0.1 μg/mL) in subjects who reported only one drink on the day before sampling. A cutoff of 0.05 mg/L in urine for repeated alcohol intake was suggested (Albermann et al, 2012). As for EtG, there is evidence of prolonged elimination in reduced renal function. An immunochemical detection test is currently not commercially available for EtS. For combined detection of EtS and EtG, the most commonly used method is LC-MS/MS (Maenhout et al, 2013).

Baranowski et al (2008) showed the stability of EtS for up to 11 days. Further studies with standardized test procedures for biodegradation showed that EtS in closed bottle test (OECD 301 D) remained stable for even longer periods whereas in the context of a higher bacterial density such as in the manometric respiratory test a reduction after 6 days was detected.

3.5. Fatty Acid Ethyl Esters (FAEE)

FAEE are minor metabolites of ethanol, which are formed after alcohol consumption in almost all tissues from free fatty acids as well from triglycerides, lipoproteins, and phospholipids (there are about 20 different FAEE, but the 4 more representative are ethyl myristate, ethyl palmitate, ethyl oleate and ethyl stearate). This pathway is an enzyme-mediated esterification of fatty acid or fatty acyl-CoA and ethanol (Maenhout et al, 2013). The determination of FAEE in blood was regarded as a useful marker for both acute and chronic ethanol intake for the first time by Doyle (1996). The results from this study indicate that the concentration of FAEE in the blood closely parallels the concentration of blood ethanol and that FAEEs are still detectable 24 h after ethanol ingestion. In heavy drinkers, FAEE could be detected up to 99 h after drinking cessation (Borucki et al, 2007). FAEEs have a half-life of about 16 h and the ability to accumulate in, among other organs, the brain, the pancreas, and the myocardium. Furthermore, FAEE accumulate in the adipose tissue and in hair (Kulaga et al, 2009). In 2001, Pragst and colleagues suggested the use of FAEE in hair as marker for chronically elevated alcohol intake.

The analysis of FAEEs alone is not recommended to prove alcohol abstinence but may be used in cases of suspected false negative EtG results (Wurst et al, 2015).

The SoHT (Society of Hair Testing) has defined the cut-off value as the sum of four FAEE (ethyl myristate, palmitate, stearate, and oleate) to differentiate between abusive consumption and moderate consumption of alcohol. The last consensus of this organization, in June 2012, established that if the sum of the concentrations of these four esters is ≥0.2 ng/mg in hair measuring 0–3 cm or ≥0.4 ng/mg in hair measuring 0–6 cm, it is an indication of heavy drinking. However, other authors, established a value of 0.5 ng/mg if the length of the hair was ≤3 cm, and a value of 1 ng/ mg for lengths of capillary hair in the range 3.1–6 cm (Cabarcos et al, 2015).

Depending of the study and the cut-off level, FAEEs in hair have shown a sensitivity between 59.3–90% and a specificity between 90–96% for heavy drinking (Nanau and Neumann, 2015).

The FAEE hair test measures cumulative levels of 4 FAEEs: ethyl myristate, palmitate, oleate, and stearate. Extraction can be conducted by liquid-liquid extraction with a mixture of heptane and dimethyl sulfoxide followed by automated headspace solid-phase micro-extraction and GC–MS/EI analysis with deuterated internal standards (Cabarcos et al, 2015).

Possible confounding factors are:

• Regular use of products with ethanol content as low as 10% can potentially elevate FAEE results above the recommended cut-off to determine heavy drinking behaviour by the Society of Hair Testing – SoHT – (Gareri et al, 2011).

• FAEE are mainly incorporated into hair through the sebaceous glands, making the incorporation rate depend on sex and age. Furthermore, capillary hair treatments can affect the sebaceous layer by eliminating it, or false positives can be given because of capillary treatments containing this substance (Nanau and Neumann, 2015).

• In serum and plasma, males have a peak serum FAEE concentration approximately two fold higher than that for females. It could be that women have a decreased activity of enzymes required for the synthesis of FAEE or an increased activity of enzymes involved in the degradation of FAEE (Soderberg et al, 1999).

• There is artifactual FAEE formation in vitro when serum samples are stored at room temperature for at least 1 day, due to synthetic activity from white blood cells or platelets, known to have FAEE synthase activity. Storage of the sample at 4 °C or −80 °C for up to 2 days do not alter the FAEE concentration (Soderberg et al, 1999).

3.6. Phosphatidylethanol (Peth)

This is the more recent marker and for sure the more promising for the future, but Peth still has not found its way into routine diagnostics. Phosphatidylethanol (PEth) is not a single molecular species but a group of phospholipids with a common non-polar phosphoethanol head group onto which 2 fatty acid moieties, which is formed in cell membranes only in the presence of ethanol and was first described by Gustavsson in 1994. The reaction is catalyzed by phospholipase D, an enzyme that normally catalyzes the hydrolysis of phospholipids leading to the formation of phosphatidic acid. However, short-chain alcohols compete with phospholipids as a substrate for phospholipase D resulting in the formation of the corresponding phosphatidylalcohol in tissues. Phospholipase D prefers ethanol over water as a substrate by a factor of several hundred (Maenhout et al, 2013).

In blood, PEth resides mainly in erythrocytes having a half-life of approximately 4 days (Aradottir et al, 2006), though in chronic alcohol-dependent patients it is possible to detect PEth even after 28 days of sobriety. There is a significant correlation between reported ethanol intake and measured PEth level. PEth is not detected after a single high alcohol intake. The threshold of total ethanol intake yielding detectable PEth is around 1000 g, with a mean daily intake of about 50 g during two weeks (Wurst et al, 2010). PEtH levels are not affected by sex or age, nor by liver disease.

PEth was described to bear higher sensitivity than CDT, GGT, or MCV (sensitivity of 99% vs 40% to 77%). Another study found an excellent diagnostic accuracy (sensitivity 94.5%, specificity 100%) superior to that of CDT, GGT, or MCV in patients with excessive alcohol consumption taking part in a detoxification program (Staufer and Yegles, 2016).

Analytical aspects

EDTA-anticoagulated or heparinized blood samples, even containing ethanol, can be stored refrigerated for up to 3 weeks or frozen without affecting PEth levels. Nevertheless false positive or high test results can occur if samples are stored at room temperature.

Nowadays the analytical technique of Peth is LC–MS/MS. Since PEth is a group of molecules, this could hamper its implementation in routine use. In blood collected from heavy drinkers PEth 16:0/18:1 and 16:0/18:2 have been demonstrated to be the predominant molecular species accounting on average for 37%–46% and 26%–28%, respectively, of total PEth in blood (Cabarcos et al, 2015).

A considerable limitation of the above-mentioned methods, however, is the absence of commercially available reference substances for PEth analogs (at the moment only PEth 16:0/16:0, 16:0/18:1 and 18:1/18:1 are available), complicating the validation process, and hindering the diffusion of these methods in clinical and forensic toxicology laboratories (Viel et al, 2012).

PEth is not detected after a single high alcohol intake. The threshold of total ethanol intake yielding detectable PEth is thought to be around 1000 g (≈ 50 g/d 2w). Nevertheless with LC-MS/MS PEth was detectable after drinking 1 g/Kg for 5 consecutive days in 90% of the sample (Gnann et al, 2012).

At the present time, the international scientific community has not yet established a cut-off value for PEth concentration in blood to be used for differentiating an acceptable social ethanol intake (<40 g for males and <20 g for females, according to the World Health Organization parameters), from an at-risk-alcohol-use (40–60 g/day) and chronic excessive drinking behavior (>60 g/day).

Possible confounding factors

• Samples collected when the blood ethanol concentration (BAC) is higher than 0.1 g/L can generate false positive results due to the neo-formation of PEth in vitro (in the post-sampling period), which can occur at room temperature (16 °C–20 °C), but also at −20 °C, being slower at +4 °C. Only at −80°C can the formation process be considered drastically inhibited (Aradottir et al, 2004).

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Элой Гирела-Лопез
профессор кафедры судебной медицины медицинского факультета
Университета Кордобы, Испания

Маркеры алкоголизма и употребления алкоголя. Обзор

Этанол – наиболее часто используемое психоактивное средство во всем мире. Он имеет огромное значение в судебно-медицинской практике. Именно поэтому анализ этанола является самым частым лабораторным исследованием, проводимым лабораториями судебно-токсикологического профиля. Вместе с тем диагноз употребления алкоголя основан не только на анализе самого этанола. Этот вопрос может быть решен на основании ряда лабораторных тестов, которые позволяют установить сведения о недавнем или длительном употреблении алкоголя. В данной статье мы представляем обзор как прямых маркеров (этанол, ацетат, этилглюкуронид, этилсульфат, этиловые эфиры жирных кислот и фосфатидилэтанол), так и непрямых маркеров (углеводдефицитный трансферрин, гамма-глутамилтрансфераза, аланинаминотрансфераза и т. д.).

Ключевые слова: этанол, ацетат, этилглюкуронид, этилсульфат, этиловые эфиры жирных кислот, фосфатидилэтанол, углеводдефицитный трансферрин, гамма-глутамилтрансфераза, аланинаминотрансфераза.