The Markers for Alcohol Abuse: The Good, the Bad and the Ugly

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Abstract

In the past decades' different biomarkers for alcohol abuse has been proposed, but, despite this, the research for newly ones is still actual. To overcome the lack of the "perfect" biomarker, at this time, different strategies has been proposed, but none of them has demonstrated the required sensibility and specificity for general population screening. As to date, the determination of carbohydrate deficient transferrin (CDT) alone is the standard serum analysis for forensic examination, despite its chance to be interfered by genetic variants (<3%) and by particular physio-pathological condition. It should be considered the evaluation of ethyl glucuronide on hair coupled with CDT on serum for forensic examination to be able to overcome the intrinsic limits of the laboratory tests and, the combination of the two together with an accurate anamnestic analysis, will give the ability to prevent misinterpretation due to the genetic and metabolic variability providing an accurate patients evaluation.

Short Communication

In the past decades' different biomarkers for alcohol abuse has been proposed [1-3] despite this, the research for newly ones is still actual [4]. We can summarize the alcohol biomarkers in three main categories: The direct ones ("the good"), the indirect ones correlated to alcohol abuse ("the ugly") and the indirect ones influenced by alcohol abuse, but unspecific ("the bad"). In the first category we can include fatty acids ethyl esters (FAEE) ethyl glucuronide (EtG), ethyl sulphate (EtS) and phosphathidylethanol (PEth) [5-7] that are metabolites related to alcohol consumption. EtG, EtS and PEth cannot be found without drinking [6,7] FAEE account for more than 20 substances and it is possible to find them in total abstainers in very small quantities due to normal metabolic production [5]. Carbohydrate-deficient transferrin (CDT) alone make the second category, that, exception made for its genetic variants and interindividual enzymatic related difference, it is used for forensic evaluation of alcohol abuse [8] and it is actually the biomarker used in general population analysis [9]. In the third category we have the "classical" indirect biomarkers that are represented by mean corpuscular volume (MCV), gamma glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) [2] which are influenced by alcohol intake, but are altered also in many other physio pathological conditions and they have demonstrated poor correlation with alcohol abuse [10].

The research for the optimal biomarker of alcohol abuse, which has theoretically 100% specificity and sensibility in the general population is still actual. To overcome the lack of the "perfect" biomarker, at this time, different strategies has been proposed both by researchers [11-13] and by scientific societies [9].

Two different research group [11,12] proposed the use in combination, integrated into a mathematical equation, of different biomarkers (MCV/GGT and CDT) to evaluate patients alcohol consumption. These combined biomarkers shown higher specificity (at 95% C.I.), when analysed with ROC curves, than the biomarkers alone. Despite the results, as concluded by the authors, the equations are not performing well enough for the general population screening, but they

are efficient in controlled settings (e.g. inpatients settings). A similar study [13] reported that the analysis of these biomarkers, even if combined, is not good enough for the evaluation of alcohol abuse or dependence without the correlation with clinical context.

As reported by Neels et al. [7], the determination of CDT alone is, so far, the standard serum analysis for forensic examination, despite its chance to be interfered by genetic variants (<3%) and particular physio-pathological condition, e.g. the carbohydrate-deficient glycoprotein syndrome (CDGS) or the use of oral contraceptive [3,7]. The other aspect in which a consensus is important is the cut-off definition for positivity [3]. Another important aspect about CDT determination is the kind of analysis performed. There are a few types of analytical methods for CDT determination [3], but two of them are the most commonly used: the capillary electrophoresis (CE) and the HPLC (high performance liquid chromatography) method [14]. As reported by Veronesi et al. [14], the CE is relatively cheap and fast analysis, that is good for screening propose; while the more expensive and time consuming HPLC analysis is well suited for confirmatory step.

To date, the best biomarkers that the laboratories are able to provide are the direct ones (FAEE, EtG, EtS and PEth). These biomarkers demonstrate a brief half-life in blood (up to 4 days) compared to the others blood biomarkers (MCV, AST, etc.). EtG, EtS and PEth are stable in collected blood, while FAEE are demonstrated to form in fresh samples in less than an hour when alcohol is present, thus requiring special storage conditions, like dry blood spots, as reported by Luginbuhl et al. [15]. The short half-life and storage requirements can be both overcome by the measurement in other matrix, i.e. the hair [6,16]. The hair has undoubtedly some advantages over the blood, for instance the virtually absence of half-life (it is limited to the hair length, which it can be considered approximately one month for each centimetre from scalp) due to the metabolites integration into the matrix. Another advantage is the non-invasive collection. FAEE and EtG has been demonstrated to be complementary when analysed in hair. Their different biochemical nature (FAEE are lipids and EtG is a polar molecule) affect the integration process into hair matrix and the

effect of exogenous substances (e.g. hair treatments) on their stability [5,16]. There are a few drawbacks too, first of all the costs to perform the analysis, due to the instrumentation required and the operators expertise [9] to reach the required LOD (limit of detection) and LOQ (limit of quantitation) and, secondly, the biological variation related to the process of integration into the hair matrix [5,7,16] to define the cut-offs.

It is our opinion that, to the best of our knowledge, it should be considered the evaluation of EtG on hair coupled with CDT on serum for forensic examination (e.g. for driver license assessment or abstinence from alcohol use in patients with liver transplantation) as well as, in a two steps process, for general population screening (CDT as first level, followed by EtG as confirm for positive results). The determination of alcohol itself and its metabolites in the subject blood, as well as in urine, are helpful for acute use/abuse evaluation (e.g. the direct determination of alcohol level in blood is used for forensic identification of drivers under alcohol influence). To be able to use these matrix (blood and urine) for chronic abusers' identification will require an extensive patients monitoring due to their short half-life. To overcome these limits, the use of a biomarker (or a matrix) with longer half-life is desirable. These aspects are important to consider because they will give the ability to prevent misinterpretation of patient's clinical condition as happened in a recent case that we reported [17]. The proposed solution would be able to overcome the intrinsic limits of the laboratory tests and, the combination of the two together with an accurate anamnestic analysis, will give the ability to prevent misinterpretation due to the genetic and metabolic variability providing an accurate patients evaluation, thus meeting the required sensibility and specificity for general population screening and forensic analysis of chronic alcohol abusers.

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