JOURNAL OF APPLIED BIOANALYSIS, July 2015, p. 76-79.
http://dx.doi.org/10.17145/jab.15.013 (ISSN 2405-710X)
Vol. 1, No. 3

OPEN ACCESS

EXPERT OPINION

Direct biomarkers to determine alcohol consumption during pregnancy, which one to use?

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(Received: 01 July 2015, Revised 07 July 2015, Accepted 08 July 2015).

Keywords: alcohol, pregnancy, meconium, FAEE, EtG, PEth.

Background

Alcohol consumption during pregnancy, even probably social alcohol consumption, can lead to (severe) fetal damage, such as fetal alcohol syndrome (FAS) or fetal alcohol spectrum disorder (FASD). In order to diagnose a child with FAS or FASD, maternal alcohol consumption during pregnancy needs to be proven. This makes the diagnosis of FAS and FASD a difficult one since self-reported questionnaires underreport the use of alcohol and are therefore biased [1]. Further, research regarding the harmful effects of alcohol during pregnancy is mostly performed with such questionnaires, so results of this kind of research is doubtful. Implementation of a reliable objective marker for alcohol detection would therefore be invaluable. Alcohol markers can be divided into two categories: direct and indirect biomarkers. Indirect biomarkers such as liver enzymes and carbohydrate deficient transferrin, are neither specific nor sensitive enough to be used for detection of alcohol consumption during pregnancy (beyond the scope of this article). Direct biomarkers are chemically derived from ethanol and are way more reliable to detect alcohol consumption during pregnancy. Three biomarkers can be used to detect alcohol in pregnant women: Fatty Acid Ethyl Esters (FAEE), ethyl glucuronide (EtG) / ethyl sulfate (EtS) and phosphatidylethanol (PEth) [2].

Fatty Acid Ethyl Esters (FAEE)

FAEE are produced by an enzymatic esterification of ethanol with free endogenous fatty acids, triglycerides, lipoproteins and phospholipids by means of two enzymes, FAEE synthase and acyl-CoA/ethanol O-acyl-transferase (AEAT). The FAEE group has more than 20 different compounds including ethyl laurate (E12), ethyl myristate (E14), ethyl palmitate (E16), ethyl palmitoleate (E16:1), ethyl stearate (E18), ethyl oleate (E18:1), ethyl linoleate (E18:3), ethyl arachidonate (E20:4) and ethyl docosahexanoate (E22:6). All have a lipophilic character. In addition, they are stable at neutral pH. FAEE do not cross the placenta into the fetal circulation, and because they can be detected in fetal matrices, must be produced in the fetus itself from the ethanol which crosses the placenta [2]. FAEE can be measured in blood for 24-44 hours, so it is still, such as ethanol in blood and breathing air, only a snapshot of the alcohol exposition to a child. For chronic exposition hair or meconium can be used. Newborn's hair will cover the last 16 weeks of pregnancy and meconium the last 20-24 weeks of pregnancy, the latter also having a greater ease of collection. Meconium comprises the neonates first several bowel movements, identified most commonly by its dark green-black color and lack of odor. Meconium formation begins at approximately 12 weeks of gestation (i.e. at the end of the first trimester), when fetal swallowing of amniotic fluid is initiated [2].

FAEE can be quantified in meconium by means of gas chromatography coupled with either flame ionization or mass spectrometry (GC-MS). MS allows measurement of lower levels, which makes MS in favor of FID when quantifying FAEE.

As meconium is a 'dirty' compound, several pre-analysis cleaning and extraction steps are necessary to have an interpretable chromatogram. To date, analytical methods used to quantify FAEE in meconium are based on Bern-

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hardt et al and involve liquid-liquid (hexane/acetone) and solid phase extraction [3]. Good extraction results have been seen using solid-phase microextraction (SPME), and headspace solid-phase microextraction (HS-SPME) [4,5]. Depending on the volume of meconium extracts being analyzed, simple liquid-liquid and solid phase or more sophisticated SPME or HS-SPME can be used.

A positive cut-off level of 2 nmol/gram (or 600 ng FAEE/gram) meconium using a cumulative sum of four FAEEs (ethyl palmitate, ethyl linoleate, ethyl oleate and ethyl stearate) has been shown to have 100% sensitivity and 98.,4% specificity as an objective marker for maternal alcohol use in pregnancy [6]. These analytical results are possible with GC-MS.

A drawback of analyzing meconium is that it can only be measured if the child is already born and the damage is already done. However, by identifying this woman and helping her to overcome the drinking habit, it can prevent a new child being born with damage due to alcohol consumption during pregnancyacy. Furthermore, in Ontario (Canada) the close follow-up of a baby identified as 'at risk' for alcohol-related disabilities facilitated early detection of developmental delays and initiation of interventions [7].

In a study comparing the prevalence of prenatal alcohol exposure obtained via maternal self-reports versus meconium testing, the pooled prevalence of alcohol exposure by meconium testing was 4.26 (95% Confidence Interval 1.34-13.57) times the pooled prevalence as measured by maternal self-reports [1].

A decision analytic model was developed to assess the cost-effectiveness of analyzing meconium. Meconium analysis costs, lifetime societal costs of disease, benefit of early intervention and quality of life and increased adult lifetime earnings were taken into account. Targeted and even universal screening by means of meconium were good value for money [8].

Ethyl glucuronide (EtG)/Ethyl Sulfate (EtS)

EtG and EtS are minor metabolites of ethanol. Since EtS is rarely used, this article will only focus on EtG. The alcohol marker EtG can be detected in different biological matrices, including hair and meconium. In urine it can be detected up to 3-4 days after alcohol use, in blood only 18 hours. Hair and meconium are useful to establish chronic alcohol consumption, but EtG has a small incorporation in hair because of its acidic profile. In meconium it is easier to detect. Recently, a cut-off of 0.5 nmol/gram meconium for a positive test on prenatal alcohol intake has been established [9]. Several methods are described in literature to detect EtG, ranging from an immunoassay to LC-MS/MS [9,10,11]. Extraction techniques have not varied much over the years, ranging from ultrasonification, SPE, microwave-assisted extraction (MAE) and HS -SPME [2].

When combining FAEE and EtG analysis in meconium, both biomarkers are very specific and together can be used for confirmation. Meconium can be analyzed for both biomarkers via GC-MS, MAE, and LC-MS/MS [2].

Phosphatidylethanol (PEth)

PEth is a non-oxidative and exclusive product of alcohol metabolism. It is an abnormal phospholipid formed in the cellular membrane only in the presence of alcohol. It is formed during a transphosphatidylation of alcohol and phosphatidylcholine (PC). Normally PC is degraded by phospholipase D (PLD) to phosphatidic acid (PA) and choline. However the 100 to 1000 fold higher affinity of PLD for alcohol promotes the transphosphatidylation of alcohol and PC, therefore PEth is always formed when alcohol is present [12,13].

Due to the half-life of 4 to 5 days, PEth however can be detected up to at least two weeks on average in blood [14]. This relatively long half-life compared to for instance EtG makes PEth a promising retrospective marker for alcohol consumption detectable in blood.

Of over 40 PEth homologues discovered, 16:0/18:1 (POPEth) is the most abundant one in alcoholics [15]. Further, homologues 16:0/18:2 (PLPEth) and 18:0/18:2 are found frequently as well in both alcoholics and social drinkers [16]. Analysis is best performed by LC-MS/MS methods and LOQs reported ranges start from 3,1 nmol/L to 30 ng/ml [14,16,17]. Research has focused on cut-off levels to differentiate between abstainers, social drinkers and alcoholics [14]. Unfortunately, no convincing results to set these levels have been published so far. However, due to the formation of PEth exclusively in the presence of ethanol, false positives are impossible. So any concentration above the limit of detection indicates the woman has consumed alcohol.

Up till now, PEth measurement will only give information on the amount of PEth present in the blood at the moment of sampling (in case the blood alcohol concentration is 0 mg/ml). How positive results should be interpreted remains a difficult issue. The ratio of different PEth homologues might indicate if consumption was very recent or not [16]. To answer this question it might be useful to combine PEth analysis with EtG testing. Although results of EtG testing should always be interpreted with caution, a positive PEth analysis combined with positive EtG testing could indicate that the positive PEth analysis is at least a result of the alcohol consumption in

the previous days. On the contrary, a positive PEth analysis combined with a negative EtG testing is indicative for alcohol consumption that took place less recently, so before the previous 3 to 4 days.

Opinion

When determining alcohol consumption during pregnanacy, focus can be on the mother (being pregnant and drinking ethanol). PEth and EtG can be used as markers in blood or urine. Focus can also be on the child (being born and having been exposed to alcohol during pregnancy). In this case, EtG or FAEE analysis in hair or meconium can be used.

Based on developmental stages, damage to the unborn child due to alcohol consumption is assumed to be the most evident in the first trimester. Therefore the focus on preventive measures, amongst all by using PEth analysis, should primary be on the early first trimester and ideally even the period before conception. Although the most harmful first trimester is almost over when most women have their first trimester laboratory checkup, in case a woman has a positive result for PEth analysis, further harm to the child from then on can be prevented. Further, research on alcohol consumption during pregnancy objectified by PEth analysis combined with FAEE measurement in meconium as a proxy of alcohol consumption in the second and third trimester, can give valuable information on characteristics of women that continue alcohol consumption while pregnant. Characteristics identified by such research could help improve preventive actions, which apparently are still too general and therewith less effective. At this moment PEth analysis is not yet part of standard antenatal or prenatal care. However, analysis sometimes is requested to get a global indication of the amount of alcohol consumed, or to see whether a woman known to have continued alcohol consumption indeed did stop consuming alcohol.

At this moment PEth is the best marker to get insight in retrospective alcohol consumption over a longer period while the woman is pregnant. However, a disadvantage of PEth as a sole indicator for alcohol consumption is the fact that it is not yet possible to reliable translate the measured concentrations into an amount or drinking pattern related to a specific time point. Though, we think that when more research is performed, there might be a basis to make this biomarker part of standard practice in prenatal care.

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Citation:
Wassenaar S, Koch BCP. Direct biomarkers to determine alcohol consumption during pregnancy, which one to use? J Appl Bioanal 1(3), 76-79 (2015).

Funding/Manuscript writing assistance:
The authors have no financial support or funding to report and they also declare that no writing assistance was utilized in the production of this article.

Competing interest:
The authors have declared that no competing interest exist.