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Research Article

Phosphatidylethanol, ethyl glucuronide and ethanol in blood as complementary biomarkers for alcohol consumption

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ABSTRACT

Alcohol biomarkers can monitor both recent and long-term drinking and provide information about drinking habits as a complement to self-reporting. Ethyl glucuronide (EtG) and phosphatidylethanol (PEth) are the most sensitive available biomarkers for this purpose. The present study aimed to collect data on both PEth and EtG in the same blood sample, in addition to ethanol, in order to evaluate the combined use of these biomarkers. Venous EDTA blood samples (n = 1149) sent to the laboratory as part of a clinical routine service for measuring PEth were investigated. PEth and EtG concentrations were analyzed using liquid chromatography–mass spectrometry methods and ethanol with an enzymatic method. Of the 1149 samples, 95 were positive for ethanol (range 0.11–3.12 g/L), 454 for EtG (1.0–9739 ng/mL), 635 for PEth (0.014–6.0 μmol/L), 534 for PEth ≥ 0.050 μmol/L, and 315 for PEth ≥ 0.30 μmol/L. EtG and PEth concentrations seemed largely independent as the coefficient of determination (r^2) between PEth and EtG concentrations was 0.15. However, when the EtG concentrations were evaluated for different subgroups depending on ethanol or PEth concentrations a statistically significant difference between successive higher concentrations was observed. EtG and PEth are independent measures of recent alcohol drinking reflecting different time windows. Their combined measurement in the same blood sample is possible and will provide valuable information regarding recent alcohol consumption as a complement to self-reporting.

Introduction

Measurement of ethanol in blood and breath is an established method for estimating the degree of intoxication when investigating a person suspected of being under the influence. This is the standard measure when acute intoxication is the focus of an investigation, e.g. in emergency care or traffic medicine [1]. However, when the purpose is more towards investigating long-term unhealthy and risk associated alcohol consumption, the measurement of ethanol is not a good choice as it is rapidly eliminated from the body, therefore alternative measures are needed.

Biomarkers aiming to detect excessive alcohol consumption have been used clinically for many years, but many lack sensitivity and specificity [2]. The evolution of ethylglucuronide (EtG) and phosphatidylethanol (PEth) analysis has provided biomarkers that are much more specific for alcohol consumption as compared to the others currently available [3,4].

PEth appears to be the best biomarker for investigating recent exposure to ethanol (i.e, within weeks) as it provides increased sensitivity and specificity versus all others [5–9]. PEth is formed by transesterification of phosphatidylcholines when ethanol is present in blood and is incorporated into blood cell membranes [5]. The main problem when using PEth as an alcohol consumption biomarker is arrival at a reliable conclusion regarding recent alcohol consumption based on a single blood concentration measurement in blood [9]. There is a consensus that PEth concentrations above 0.3 μmol/L indicate recent heavy drinking [7,9,10]. However, confounding factors include the inter-individual variability in formation and elimination rates of PEth, as well as both the person's drinking pattern and the fraction of cells in the blood [10].

EtG has become the most sensitive biomarker for detecting a single intake of ethanol [4]. The detection window compared to measuring for ethanol is extended by 1–4 days [11]. EtG is usually measured in urine, but recent analytical development has made it possible to use blood and

Abbreviations: EtG, ethyl glucuronide; PEtH, phosphatidylethanol; LC-MSMS, liquid chromatography tandem mass spectrometry; WB, whole blood.

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still achieve similar detection times to those for urine [12].

PEth, EtG and ethanol have different elimination characteristics resulting in varying detection windows. PEth is accumulated over repeated intake and is eliminated with a half-life of about 6 days [13]. Both EtG and ethanol are eliminated much faster, with a half-live for EtG of about 2.2 h [14] and an even lower half-life for ethanol due to its zero-order elimination kinetics.

The combined use of alcohol biomarkers for both recent and long-term drinking provides an opportunity to collect additional information about drinking habits to complement self-report [15]. However, the combined use of EtG and PEth, which are the best available biomarkers for monitoring recent and long-term alcohol consumption, has until recently required both a urine and blood sample. In this study, collected data on both PEth and EtG in the same blood sample, in addition to ethanol, were used to evaluate their combined use.

Materials and methods

Clinical samples

Venous EDTA blood sent to the laboratory as part of a routine clinical service was used. Precisely 1149 samples were received from addiction clinics, unemployment agencies, occupational health units and forensic hospitals for analysis of PEth. The samples reached the laboratory by postal service between 1 and 3 days after collection. They were stored at 4 °C after arrival to the laboratory and the analytical investigations were performed within 24 h.

Analytical methods

The analytical methods used are described in detail in recent publications [12,16]. PEth (16:0/18:1) and EtG in whole blood (WB) were measured by liquid chromatography–tandem mass spectrometry (LC–MSMS). Ethanol in plasma was measured with an enzymatic method using the DRI™ Ethyl Alcohol Assay from Thermo Scientific (Waltham, MA, USA) applied on an AU680 Clinical Chemistry Analyzer (Beckman Coulter Inc., Brea, CA, USA). All three methods were used in clinical and forensic routine and operated under ISO 15189 and ISO 17025 accreditation. The measuring ranges were from 1 ng/mL to 5000 ng/mL for EtG in EDTA whole blood and EDTA plasma, and from 0.014 μmol/L to 7.112 μmol/L (10 ng/mL–5000 ng/mL) for PEth. For PEth, the lower limit for reporting it as present was 0.05 μmol/L; the higher limit of 0.30 μmol/L as used as an indicator for "heavy drinking" [17]. The measuring range for ethanol was 0.10 g/L–6.0 g/L. Concentrations above the measuring ranges were measured after dilution.

The imprecision in quantification was assessed using commercial quality controls from ACQ Science GmbH (Rottenburg, Germany), which in some cases were diluted with blank blood. For PEth, three concentration levels were used: 0.064, 0.44 and 2.84 μmol/L, with a CV and bias of <8%. For EtG, the concentration levels used were: 3.20, 32.0 and 4100 ng/mL, with a CV and bias of <9%. For ethanol, the concentration levels used were: 0.51, 1.30 and 3.03 g/L, with a CV and bias of <5%. Stability of PEth in ethanol-containing blood during storage at 4 °C was documented by reanalysis after 28 days, with a mean ratio for day28/day0 of 0.99 (n = 83).

Statistical calculations

Statistical calculations were done using Xact® chart software (Scilab, Hamburg, Germany) and MedCalc (MedCalc Software, Ostend, Belgium).

Results

Out of the total 1149 blood samples investigated, 95 were positive for ethanol (range 0.11–3.12 g/L), 454 for EtG (1.0–9739 ng/mL), 635 for PEth (0.014–6.0 μmol/L), 534 for PEth ≥ 0.050 μmol/L, and 315 for PEth ≥ 0.30 μmol/L. The distribution of qualitative results for the three analytes is shown in Fig. 1.

In 91 cases, all three biomarkers were positive (with PEth ≥ 0.05 μmol/L). Out of the 95 positive ethanol samples, 77 (81 %) had PEth values ≥ 0.30 μmol/L. In three cases where ethanol was present in the blood (0.13–0.20 g/L), PEth was <0.05 μmol/L, and in three cases where ethanol ranged from 0.13 to 0.64 g/L EtG was negative. In cases with PEth concentrations ≥ 0.050 μmol/L, 73% were positive for EtG, while in cases with PEth ≥ 0.30 μmol/L 86% were positive for EtG. In PEth negative cases (<0.05 μmol/L), 0.5% were positive for ethanol and 10% were positive for EtG.

Fig. 2 presents the data as a plot of PEth versus EtG concentrations with color-coded indications of ethanol concentrations. Ethanol-negative samples scatter over the graph, while ethanol-positive samples appear to distribute along a positively sloped curve. Cases with high ethanol concentrations (red dots) generally also had high EtG concentrations. The coefficient of determination (r^2) between PEth and EtG concentrations was 0.15.

In Fig. 3, the EtG concentrations in four subgroups, based on ethanol concentrations, are presented. Subgroups with higher ethanol concentrations also had significantly higher EtG concentrations. In samples that were negative for ethanol (i.e., <0.1 g/L), mean and median EtG concentrations were 85.1 and 16 ng/mL. At ethanol concentrations between 0.1 and 0.5 g/L, mean and median EtG concentrations were 276 and 125 ng/mL. For samples with ethanol concentrations between 0.5 and 1 g/L, mean and median EtG concentrations were 671 and 456 ng/mL. For samples with >1 g/L ethanol, mean and median EtG concentrations were 2259 and 1815 ng/mL. In three cases where ethanol was present, the EtG concentration was below 1 ng/mL.

Fig. 4 shows the EtG concentrations in five subgroups based of PEth concentration ranges. At increasing PEth concentrations the EtG concentrations are significantly higher. In cases with PEth concentrations >0.3 but below 1.0 μmol/L the mean and median EtG concentrations were 189 and 78 ng/mL, respectively, for PEth concentrations between 1.0 and 3.0 μmol/L the the mean and median EtG concentrations were 651 and 137 ng/mL, and for PEth concentrations >3.0 μmol/L the mean and median EtG concentrations were 1275 and 784 ng/mL. In one case, with ethanol present, PEth was not detected (<0.014 μmol/L), nor was EtG. The EtG WB and plasma concentrations were highly correlated with a coefficient of correlation of $p < 0.00001$ and a slope of 0.66 with higher values in plasma.

Discussion

The present study measured ethanol and EtG concentrations in blood samples sent in for routine measurement of PEth concentration in order to evaluate the possibility and value of having a combined measurement of both short- and long-term biomarkers for consumption of alcohol. From an analytical point of view, the same whole blood specimen can be used for measurement of all three analytes, but at present it requires separate aliquots and methods. While PEth and ethanol are already measured using blood, routine EtG measurements are carried out using urine. Using blood for EtG was considered, which would result in shorter detection times [14,18]. However, we recently demonstrated that measurement in blood with improved sensitivity (lower limit of 1 ng/mL) provides the long detection time of ethanol intake offered by urine analysis [12].

More than half of the specimens analyzed had PEth concentrations above the detection limit and about half of those above the level established for diagnosing heavy alcohol consumption (0.30 μmol/L), thus demonstrating a relatively high prevalence of heavy drinking in the study population. From inspection of Fig. 2 it is evident that PEth and EtG concentrations are not correlated supporting that they should be considered as independent measures. This means that the two measurements provide complementary information, PEth reflecting long-

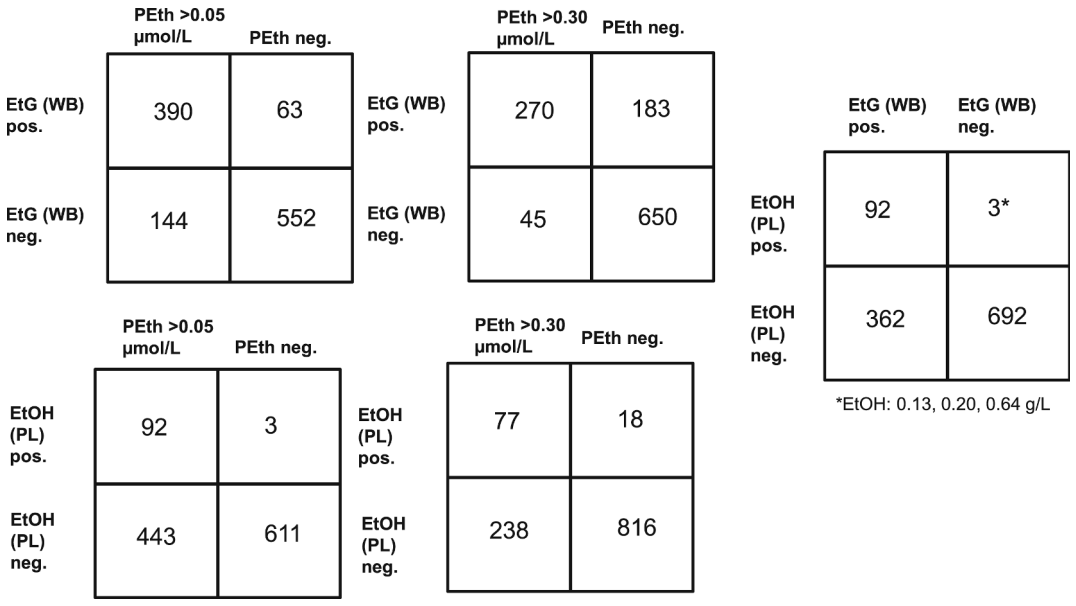

Fig. 1. Agreement of positive/negative outcome between PEth, EtG and ethanol.

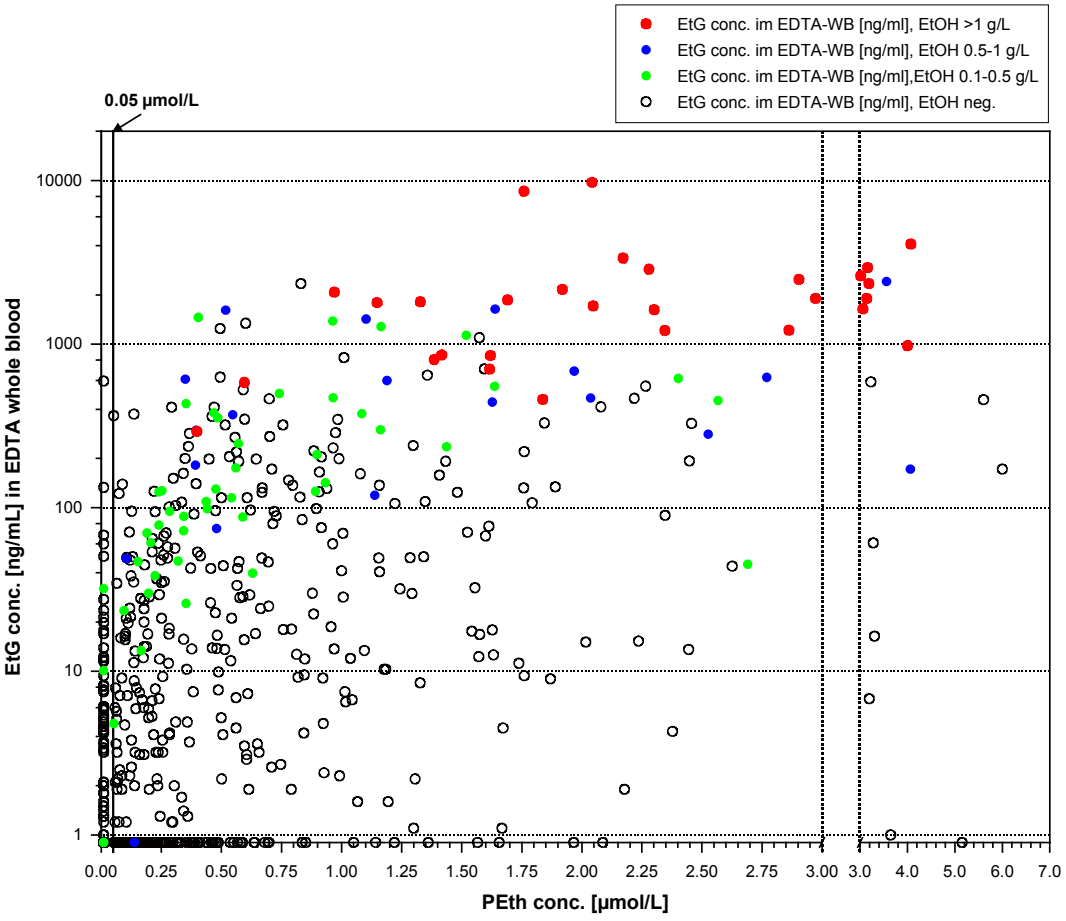

Fig. 2. Plot of PEth and EtG concentrations in whole blood (n = 1149). Ethanol concentrations in plasma indicated by color-coded dots. Coefficient of determination (r^2) of 0.15.

term alcohol consumption and EtG recent consumption. In cases with PEth < 0.05 µmol/L and no EtG detected, sobriety can be confidently concluded, while in cases with PEth < 0.05 µmol/L and positive EtG, sporadic alcohol drinking can be concluded. In cases with PEth values indicating heavy drinking, EtG can be used to discriminate between a period of active drinking or abstention. This can be highly valuable if the patient is undergoing repeat measurements in the context of treatment.

Blood PEth concentration has been reported to be best correlated to

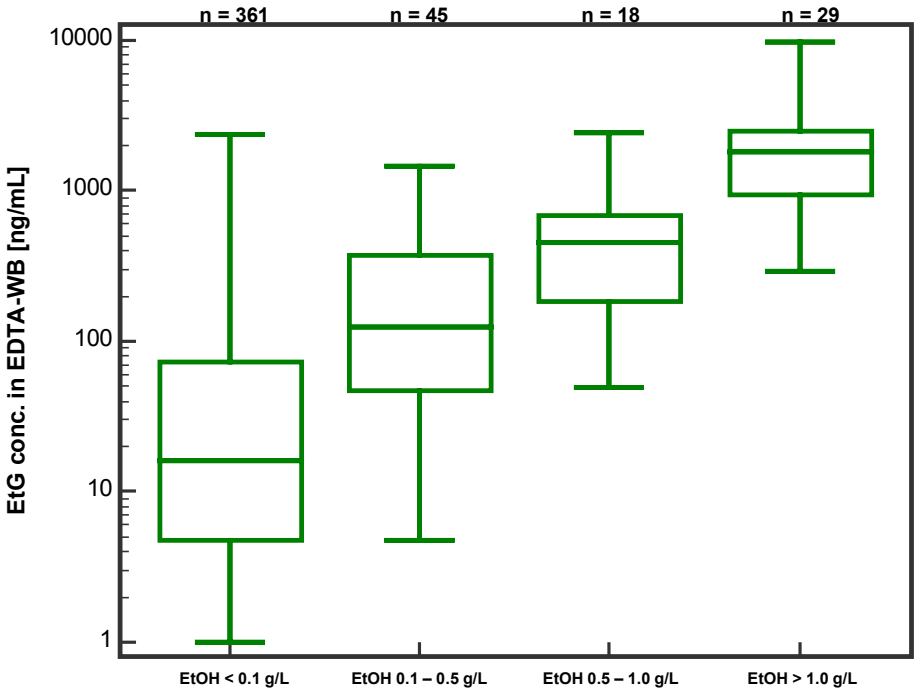

Fig. 3. Box-and-whisker diagrams showing whole blood EtG concentrations in subgroups based on ethanol concentration ranges. Mann-Whitney calculations of differences between group are shown in the figure together with median concentration values.

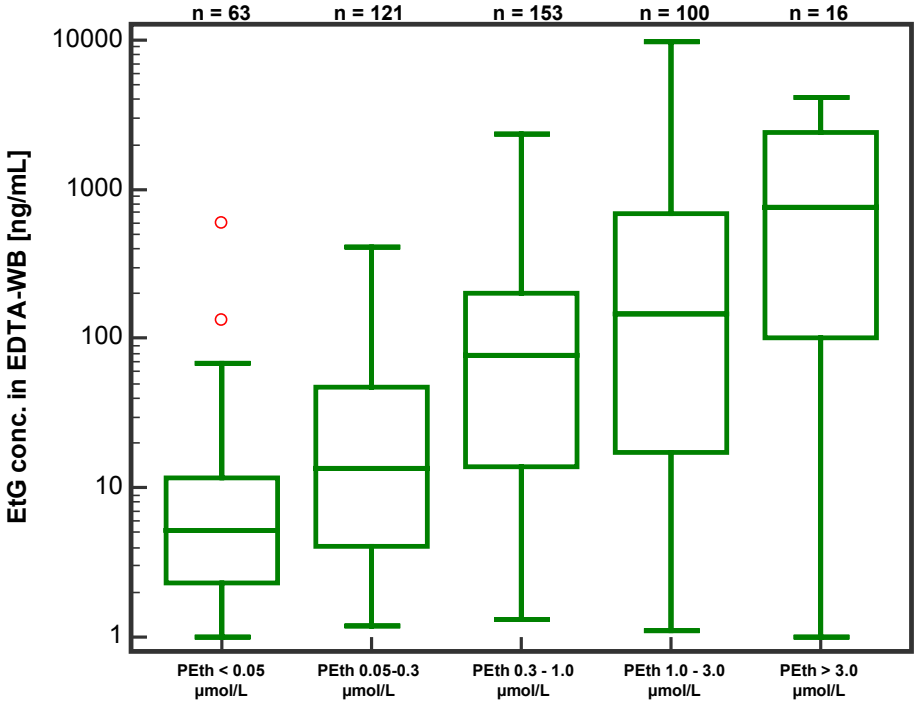

Fig. 4. Box-and-whisker diagrams showing whole blood EtG concentrations in subgroups based on PEth concentration ranges. Mann-Whitney calculations of differences between group are shown in the figure together with median concentration values. Outlier values shown as red circles. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the previous two weeks alcohol consumption, as determined from self-reported drinking from patients in outpatient treatment [10]. It is well documented that the response of PEth to a dose of ethanol is subject to inter-individual variation, as is true also for the elimination rate following abstention [10,13,19–21]. The decision limit for PEth indicating heavy drinking of 0.30 µmol/L seems well accepted in the literature [7,9,10,17], but is to be considered as safe or conservative in the sense that heavy drinking might also be the case at PEth concentrations lower than 0.30 µmol/L. In cases of PEth concentration between 0.05 and 0.30 µmol/L, the result for EtG might therefore be helpful in the clinical context.

Also, the level of EtG concentrations may be of value, especially if

ethanol is not being measured. In a controlled experiment, maximal EtG concentrations of 300–1000 ng/mL are reported after consumption of 1 g/kg ethanol and with blood ethanol concentrations of 0.5 to 1.2 g/kg [22]. Such EtG concentrations are in agreement with those seen in our study when plasma ethanol concentrations were >1 g/L. Based on the results presented in Figs. 2 and 3, an EtG concentration of >100 ng/mL might be taken to be associated with ongoing or very recent alcohol intoxication, while EtG concentrations of <10 ng/mL are indicatives of the hang-over phase.

As previously noted, a fraction of clinical blood specimens coming to the laboratory for PEth measurement contain ethanol, at 8% in this study and 12% in a previous study [16]. This raises concerns about the possibility of post-sampling formation of PEth in those specimens. To address this, the use of inhibitors of the phospholipase enzyme responsible for PEth formation have been proposed [23], and were recently confirmed [24]. If ethanol is present in blood, PEth formation will continue post sampling if the sample is not stored in proper conditions. This will potentially result in slightly elevated analytical results. Even if the formation of PEth from ethanol in whole blood at room temperature is relatively slow, it is recommended that one keep control over the presence of ethanol in the analyzed specimen when making interpretation of an analytical result. The use of dried blood spots as specimens for PEth has been proposed to eliminate this problem as any ethanol present would disappear in the drying process. By harnessing a strategy with a volumetric DBS device it was confirmed that post-sampling formation of PEth could be stopped. A future strategy for reliably monitoring both short- and long-term alcohol biomarkers could be to develop a method combining EtG and PEth measurement from the same dried blood spot.

In conclusion, EtG and PEth are independent measures of recent alcohol consumption, reflecting different time windows. In combination with self-reporting, their combined measurement from the same blood sample will provide valuable complementary information regarding recent alcohol consumption.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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