

Phosphatidylethanol as a sensitive and specific biomarker—comparison with gamma-glutamyl transpeptidase, mean corpuscular volume and carbohydrate-deficient transferrin

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ABSTRACT

Phosphatidylethanol (PEth), a direct ethanol metabolite, is detectable in blood for more than 2 weeks after sustained ethanol intake. Our aim was to assess the usefulness of PEth [comparing sensitivity, specificity and the area under the curve (AUC)] as compared with carbohydrate-deficient transferrin (CDT), gamma-glutamyl transpeptidase (GGT) and mean corpuscular volume (MCV), calculating the results from sober patients against those from alcohol-dependent patients during withdrawal. Fifty-six alcohol-dependent patients (ICD-10 F 10.25) in detoxification, age 43 years, GGT 81 U/l, MCV 96.4 fl, %CDT 4.2, 1400 g ethanol intake in the last 7 days (median), were included in the study. Over the time of 1 year, 52 samples from 35 sober forensic psychiatric addicted in-patients [age 34 years, GGT 16 U/l, MCV 91 fl, CDT 0.5 (median)] in a closed ward were drawn and used for comparison. PEth was measured in heparinized whole blood with a high-performance liquid chromatography method. GGT, MCV and %CDT were measured using routine methods. A receiver operating characteristic curve analysis was carried out, with 'current drinking status' (sober/drinking) as the state variable and PEth, MCV, GGT and CDT as test variables. The resulting AUC was 0.974 ($P < 0.0001$, confidence interval 0.932–1.016) for PEth. At a cut-off of 0.36 $\mu\text{mol/l}$, the sensitivity was 94.5% and specificity 100%. The AUC for CDT, GGT and MCV were 0.931, 0.894 and 0.883, respectively. A significant Spearman's rank correlation was found between PEth and GGT ($r = 0.739$), CDT ($r = 0.643$), MCV ($r = 0.639$) and grams of ethanol consumed in the last 7 days ($r = 0.802$). Our data suggest that PEth has potential to be a sensitive and specific biomarker, having been found in previous studies to indicate longer lasting intake of higher amounts of alcohol.

Keywords Gamma-glutamyl transpeptidase, harmful ethanol intake, mean corpuscular volume, phosphatidylethanol, sensitivity, specificity.

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INTRODUCTION

Phosphatidylethanol (PEth) is one of the direct ethanol metabolites that, especially during the last decade, have attracted attention as a biomarker of ethanol intake (Alling, Gustavsson & Änggård 1983; Alling *et al.* 1984; Hansson *et al.* 1997; Gunnarsson *et al.* 1998; Varga *et al.* 1998, 2000; Wurst *et al.* 2003, 2004; Aradottir, Moller & Alling 2004a; Aradottir *et al.* 2004b). PEth is an abnormal phospholipid formed only in the presence of ethanol via the action of phospholipase D (Gustavsson & Alling

1987; Kobayashi & Kanfer 1987). Recent studies have supported the use of PEth in blood as a marker of alcohol abuse (Hansson *et al.* 1997; Varga *et al.* 1998).

One study found detectable PEth in the blood of chronic alcoholics admitted for detoxification (mean PEth levels of 13.2 $\mu\text{mol/l}$ on the first day), up to 14 days (Hansson *et al.* 1997). By using electrospray mass spectrometry, the molecular identity of PEth has been proven in extracts of blood from alcoholics (Gunnarsson *et al.* 1998). These patients had PEth levels of 5–13 $\mu\text{mol/l}$, detectable up to 3 weeks after the beginning of an

alcohol-free period. A third study on chronic alcoholics showed mean PEth levels of 5.1 $\mu\text{mol/l}$ (range 1–18) the day after admission for detoxification (Varga *et al.* 2000). A study on healthy volunteers revealed that a single dose of ethanol (32–47 g) does not produce measurable amounts of PEth (Varga *et al.* 1998). However, out of 12 volunteers who consumed between 624 and 2134 g of ethanol during 3 weeks, eight persons had detectable levels of PEth (1.0–2.1 $\mu\text{mol/l}$). A threshold of total ethanol intake yielding detectable PEth seems to be around 1000 g, with a mean daily intake of about 50 g. So far analysis of PEth has been performed by the use of whole blood. A recent study on blood from chronic alcoholics showed that almost all PEth was found in the erythrocyte fraction (Varga *et al.* 2000). The mean half-life of PEth in blood from alcoholics was found to be 4 days, and PEth was still measurable after up to 2 weeks of sobriety (Varga *et al.* 2000). Two recent studies found no false negatives in active alcoholics (Wurst *et al.* 2004) and no false positives in sober subjects with a history of addiction (Wurst *et al.* 2003). Recently, *in vitro* formation of PEth at -20°C and at room temperature has been described in blood samples containing ethanol, in contrast to storage at -80°C or at $+4^{\circ}\text{C}$ (Aradottir *et al.* 2004a).

Our aim was to assess the usefulness of PEth [comparing sensitivity, specificity and the area under the curve (AUC)] as compared with carbohydrate-deficient transferrin (CDT), gamma-glutamyl transpeptidase (GGT) and mean corpuscular volume (MCV), calculating the results from sober patients against those from alcohol-dependent patients during withdrawal.

PATIENTS AND METHODS

Patients

Alcohol-dependent patients at day 7 of detoxification

Fifty-six alcohol-dependent patients (according to ICD-10 F 10.25) in detoxification, with a mean age of 43.1 years (median 43, SD 10.21, range 24–66), a mean blood alcohol concentration at hospitalization of 1.68 per mille (median 1.55, SD 0.97, range 0.01–3.39), a mean GGT of 161 U/l (median 81, SD 212, range 13–1240) and a mean MCV of 97.9 fl (median 96.4, SD 5.6, range 88.9–114) were included in the study. They had a self-reported mean consumption of 1570 g (median 1400, SD 911, range 280–5320) of ethanol in the last 7 days before hospitalization. They reported that this ethanol intake was representative for the last month. The exclusion criteria were severe diseases of the liver, kidney or brain; metabolic disorders; and intake of illicit drugs. To avoid potential *in vitro* formation of PEth in samples containing ethanol (like at the beginning of withdrawal), blood samples from the

alcoholics on day 7 of sobriety were used, which did not contain alcohol.

Sober patients with a substance use disorder

Over the time of 1 year, 52 samples of 35 sober forensic psychiatric in-patients in a closed ward who had committed a substance-related offense were used for comparison. All were sober for more than 4 weeks. Their self-reported ethanol consumption was 0 g. The complete time spectrum of possible alcohol detection was covered by the complementary use of breath and urinary ethanol (hours), urinary ethyl glucuronide (EtG) (days), %CDT and PEth (weeks) as well as GGT and mean MCV (weeks-months) (for details, see Wurst *et al.* 2003). The mean age of this group of patients was 34.3 years (median 34, SD 7.46, range 26–56), mean CDT was 1.02 (median 0.5, SD 1.37, range 0.2–4.7), mean GGT was 23.7 U/l (median 16, SD 22.6, range 6–94) and mean MCV was 89.9 fl (median 91, SD 5.3, range 67–98).

The study was approved by the ethics committees of the Universities of Vienna, Austria and Basel, Switzerland and the Bavarian Chamber of Physicians, Germany.

Methods

Phosphatidylethanol was measured in heparinized whole blood as described elsewhere (Varga *et al.* 1998) with high-performance liquid chromatography, combined with an evaporative light-scattering detector method. The limit of quantification was 0.3 $\mu\text{mol/l}$.

The determination of MCV and GGT were made using routine clinical laboratory methods.

The cut-offs for GGT and MCV were 28 U/l and 98 fl, respectively. The quantitation of CDT (cut-off: 2.6%) was performed using the CDT kit from Bio-Rad Laboratories (Philadelphia, PA, USA) according to the manufacturer's instruction.

Statistical analysis

For the statistical analysis, SPSS 13 (SPSS Inc., Chicago, IL, USA) was used.

Receiver operating characteristic (ROC) curve analysis (McFall & Treat 1999) was performed to analyze the AUC as well as the sensitivity and specificity for PEth, CDT, MCV and GGT. Data from the sober patients with a substance use disorder were calculated against the data from alcohol-dependent patients during withdrawal.

For that purpose, abstinence was used as state or criterion measure and PEth, CDT, MCV and GGT as test variables.

RESULTS

Phosphatidylethanol levels of between < limit of determination and 10.98 mmol/l (mean 2.47, median 1.83, SD

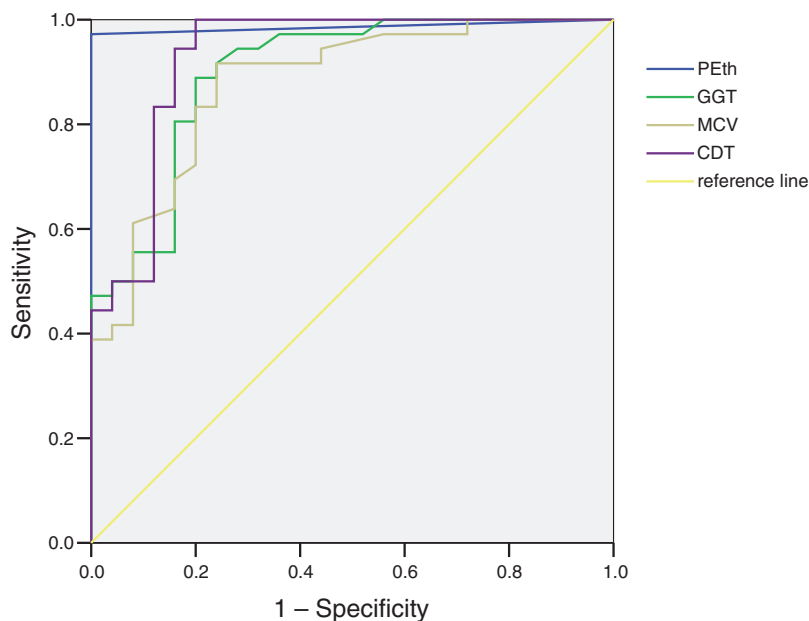


Figure 1 Receiver operating characteristics curve analysis for sober patients against alcohol-dependent patients during withdrawal, with phosphatidylethanol (PEth), mean corpuscular volume (MCV), gamma-glutamyl transpeptidase (GGT) and carbohydrate-deficient transferrin (CDT) as test variables

2.2) were found for the alcohol-dependent patients during withdrawal and levels below the limit of determination for the sober patients. An ROC curve analysis for the drinkers against the sober patients, with PEth, CDT, MCV and GGT as test variables, was calculated (Fig. 1). The resulting AUC was 0.974 [$P < 0.0001$, confidence interval (CI) 0.932–1.016] for PEth. At a cut-off of 0.36 $\mu\text{mol/l}$, the sensitivity was 94.5% and specificity 100%.

For CDT, the AUC was 0.931 ($P < 0.0001$, CI 0.866–0.955), for GGT 0.894 ($P < 0.0001$, CI 0.815–0.972) and for MCV 0.883 ($P < 0.0001$, CI 0.801–0.965). For CDT, the sensitivity was 77.1% and the specificity 88%. For GGT, the sensitivity and specificity were 94% and 72%, respectively. MCV reached a sensitivity of 40% and a specificity of 96%.

A significant Spearman's rank correlation was found between PEth and GGT ($r = 0.739$), CDT ($r = 0.643$), MVC ($r = 0.639$) and grams of ethanol consumed in the last 7 days ($r = 0.802$).

DISCUSSION

Our main finding is that a sensitivity and specificity of 94.5% and 100%, respectively, for PEth are very high and comparable to the results found for fatty acid ethyl esters (FAEEs) in hair with a sensitivity and specificity of 94.4% and 90%, respectively (Wurst *et al.* 2004).

Whereas in hair ethanol intake can be monitored over months and in urine for days, PEth in blood can give information on the very recent weeks. Like in an earlier study (Wurst *et al.* 2004), PEth was found to perform better than MCV and GGT. These findings are consistent with data from 12 volunteers consuming high amounts of ethanol over 3 weeks: PEth was increased in eight of them, but

GGT and CDT only in two of them (Varga *et al.* 1998). As *in vitro* formation of PEth in blood containing alcohol, at -20°C and at room temperature, has been described (Aradottir *et al.* 2004b), samples should be drawn when blood alcohol concentration (BAC) has reached zero.

During the last decade, attention has focused on *direct* ethanol metabolites because traditional *indirect* biological state markers are limited in two basic respects: (1) confounds due to age, gender, other ingested substances and non-alcohol-associated diseases; and (2) time spectra for previous drinking that they reflect (e.g. serum ethanol detects only recent use within hours) (Laposata 1999; Conigrave *et al.* 2002; Helander 2003). Promising direct ethanol metabolites include EtG, ethyl sulphate, fatty acid ethyl esters and PEth (Wurst *et al.* 2005a,b). Each of these drinking indicators remains positive in serum and urine for a characteristic time spectrum after the cessation of ethanol intake—EtG and EtS in serum for up to 12 h, EtG and EtS in urine for up to 7 days, PEth in whole blood more than 2 weeks after ethanol has left the body. As more and more the direct ethanol metabolites are used not only in studies but also in routine tests, we found it timely to compare some of the medium-term markers of the traditional kind (GGT, MCV, CDT) with this emerging biomarker, PEth.

Our preliminary data suggest that PEth has potential to be a sensitive and specific marker, reflecting longer lasting intake of higher amounts of alcohol. To fully understand the potential of PEth, further studies should also include PEth levels in social drinkers. If our promising findings are confirmed in future studies, applications for the use of PEth could include, for example, screening, motivational feedback, improving knowledge on drinking patterns, differentiating moderate/social drinking

from drinking above recommended levels, evaluating treatment programs and drug trials, monitoring before and after liver transplantation and opioid maintenance patients with hepatitis C and co-morbid risky alcohol use.

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