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Comparison of the Diagnostic Value of Phosphatidylethanol and Carbohydrate-Deficient Transferrin as Biomarkers of Alcohol Consumption

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Abstract

Background

The aim of this study was to compare the results of Phosphatidylethanol (PEth) and Carbohydrate-Deficient Transferrin (CDT) in blood as biomarkers of alcohol consumption in a large clinical cohort and to evaluate concentrations in relation to age and sex.

Methods

Results of PEth 16:0/18:1 in blood and CDT in serum were included, together with information of age and sex, which were extracted from a clinical chemistry database containing samples mostly from patients of primary care physicians and social care institutions. PEth concentrations were determined using Ultra Performance Convergence chromatography mass-spectrometer. CDT was quantified by electrophoretic Capillary System. CDT-values ≥ 1.7 %-units and PEth-values ≥ 0.31 µmol/L were considered to indicate heavy alcohol consumption.

Results

Samples from 6705 patients were included. The median age was 54.5 years, 34 % were females. Only 47 % of the patients with PEth ≥ 0.31 µmol/L had increased CDT ≥ 1.7 %-units examined in the same specimen (Cohen's kappa was 0.43, $p < 0.001$). Patients above 50 years had significantly higher concentrations for both CDT (1.0 %-units vs. 0.9 %-units, $p < 0.001$) and PEth (0.340 µmol/L vs. 0.200 µmol/L, $p < 0.001$) compared to younger patients. Concentrations of CDT were significantly higher in males compared to females ($p = 0.002$), while no significant sex differences were seen for PEth ($p = 0.465$).

Conclusions

A high fraction of the patients had PEth values above the suggested cut-off for heavy drinking and normal CDT values, verifying the superior sensitivity of PEth compared to CDT. The effect of age seems to be minor for both markers. Higher concentrations of CDT, but not

PEth, were seen in males, indicating that PEth, as opposed to CDT, might be formed equally in men and women. Therefore, the bias due to sex is possibly present only for CDT, not for PEth.

Key-words: Alcohol Biomarker, Phosphatidylethanol, Carbohydrate-Deficient Transferrin, Sensitivity, Detection Capability

Introduction

Consequences of alcohol consumption to public health are well documented. As much as 5.3 % of the total global deaths in one year (2016) may be attributed to alcohol consumption. This corresponds to approximately three million deaths every year caused by harmful alcohol use. Alcohol's impact on the global burden of disease and injuries, measured in disability-adjusted life years, is reported to constitute 5.1 % of lost healthy life years (World Health Organization, 2018).

An objective assessment of the patients' alcohol intake is therefore important. Traditionally, a wide range of both indirect and direct biomarkers have been used for the detection of high alcohol consumption. Indirect markers include mainly Carbohydrate deficient transferrin (CDT) as well as the enzymes aminotransferases (AST/SGOT and ALT/SGPT) and gamma-glutamyl transpeptidase (GGT) (Maenhout et al., 2013). Functionally, CDT is an iron transport glycoprotein and although it has traditionally been considered to be the most accurate biomarker for detecting heavy alcohol consumption, some studies have indicated that its sensitivity is low and varies greatly between patient groups, age and sex (Schröck et al., 2014, Wurst et al., 2010, Anton and Moak, 1994, Szabo et al., 2007). CDT levels above cut-off can be measured after approximately one week of heavy alcohol consumption, and the half-life is about 10–15 days (Stibler, 1991, Weykamp et al., 2014, Helander and Kenan Moden, 2013). One study concludes that the differences in sensitivity between these groups may not be statistically or clinically significant, and that it is unnecessary to adjust the reference intervals for CDT according to factors such as age, sex, ethnicity, BMI and smoking (Bergstrom and Helander, 2008). The specificity of CDT is on the other hand high compared to conventional liver enzymes such as ALT, AST and GGT which reflect liver damage in

general and not necessarily from alcohol consumption (Niemela, 2016). It should be taken into account that CDT levels usually increase during pregnancy (Bianchi et al., 2011).

In contrast to CDT, the direct biomarkers of alcohol abuse are formed only after the intake of ethanol and thus are more specific than all indirect biomarkers, which might be influenced by other factors and medical conditions (Schröck et al., 2014). Thus, phosphatidylethanol (PEth) seems to be one of a few promising direct biomarkers of alcohol abuse and has been widely used over the latest years due to its long detection window compared to other direct alcohol biomarkers (Gnann et al., 2014, Isaksson et al., 2011). Formation of PEth has been detected in blood within one hour after a single dose of 0.4 g/kg ethanol (Hill-Kapturczak et al., 2018), and a half-life of four days was observed (Varga et al., 2000). PEth has proved useful in a variety of settings including alcohol detoxification programs, occupational and pre-employment medical examination (Neumann et al., 2020), screening in emergency department (Kabashi et al., 2019), detecting heavy drinking among young adults, drug users, HIV positive patients (Bajunirwe et al., 2014), as well as for confirming abstinence from alcohol (Schröck et al., 2016).

The use of PEth has increased considerably the latest years. In addition to having a high sensitivity and specificity among the direct biomarkers, one of its advantages is the ability to distinguish between moderate and heavy alcohol consumption (Walther et al., 2015, Helander et al., 2019a, Viel et al., 2012, Helander et al., 2019b). Suggested PEth concentrations of 20 ng/mL (~0.03 µmol/L) and 215 ng/mL (~0.3 µmol/L), respectively, have been used in clinical settings to distinguish moderate consumption from heavy drinking (Simon, 2018). Helander et al. stated that an average increase in PEth 16:0/18:1 of 0.10 µmol/L corresponds to an alcohol intake of 20 g a day (Helander et al., 2019b). A PEth concentration of 215 ng/mL (~0.3

106 µmol/L) would thus correspond to a daily intake of 60 g of alcohol, which is considered
107 harmful according to the World Health Organization “Guide for monitoring alcohol
108 consumption and related harm” (World Health Organization, 2000). Ulwelling and
109 colleagues, in a critical review, recommended a similar threshold of 200 ng/mL for
110 identifying heavy alcohol consumption. (Ulwelling and Smith, 2018). Whereas CDT has
111 shown possible variability between men and women, PEth seems to be more consistent
112 between the sexes (Wurst et al., 2010, Hill-Kapturczak et al., 2018). To our knowledge,
113 studies reporting sex- and age-specific sensitivity of PEth relative to CDT remain scarce, thus
114 warranting more research on this topic. Such studies can be performed based on large clinical
115 datasets. In the present study, we aimed to evaluate clinical utility of PEth and CDT in
116 relation to age and sex using a database of Fürst Medisinsk Laboratorium containing clinical
117 chemistry data from patients of south-eastern Norway treated at primary care centers or
118 alcohol and drug abuse institutions.

Material and Methods

Data collection

Results from PEth and CDT analyses performed over the period from September 2016 to April 2018 (Regional Ethics Committee, 2018/1041) at the Fürst Medisinsk Laboratorium were used for the present study. The study database contained anonymous and encrypted information on age and sex in addition to analytical results. Samples were mostly collected from patients of primary care physicians in addition to some from social care institutions. However, further information about the study population could not be obtained.

Sample preparation

Serum for CDT and ethanol analyses were collected in serum separating tubes (SST, Vacutainer, BD). Whole blood samples collected in Vacutainer (K2-EDTA, BD, NJ, US) were kept at room temperature close to 20 °C in sampling room and during transport. The samples were hemolyzed by freezing overnight at -20 °C after arrival in laboratory. After thawing and mixing, 100 µL sample and 900 µL 2-propanol solution (Rathburn, Walkerburn, UK) containing deuterated internal standard (D5-PEth 16:0/18:1, Chiron, Trondheim, Norway) were pipetted into deep well microtiter plates (DWP), (Porvair Sciences, Wrexham, UK) using a Hamilton MicroLab Star robot (Hamilton, Bonadoz, Switzerland). Subsequently the plates were sealed with Thermal sealing foil (Porvair Sciences) and centrifuged.

Analysis of PEth 16:0/18:1

PEth 16:0/18:1 analyses were performed on a Waters Acquity UPC2 (TM) Ultra Performance Convergence chromatography system connected to Waters TQ-S triple quadrupole mass-spectrometer (UPC2-MS/MS) (Waters, Milford, MA, USA) (van der Nagel et al., 2018).

The UPC2-MS/MS system was run in isocratic mode (70:30, A:B), A: CO2 5.0 ultra (Nippon Gases, Madrid, Spain), and B: Methanol (Fisher Scientific, Pittsburg, PA, USA) containing 5 mmol/L Ammonia (Sigma-Aldrich, St. Louis, MO, USA) with a flow of 1.0 mL/min. Chromatographic separation of PEth was achieved using a Waters Torus 2-PIC 1.7 µm – 2.1 x 50 mm column (Waters).

To enhance the signal, a make-up solution of Methanol (Fisher Scientific) containing 0.3 % formic acid (Rathburn) was continuously infused post-column into the mobile phase with a flow of 0.2 mL/min. The chromatographic cycle time was of about 2 minutes.

The mass spectrometer was operated in negative mode with ion-spray voltage of 2500 V, desolvation temperature 600 °C, source temperature 150 °C, cone voltage 30 V, collision energy 35 V, and gas flow 800 L/hour. The following transitions were used for PEth measurements: 16:0/18:01: m/z 701>281 (quantifier), m/z 701>255 (qualifier), and m/z 706>281 (internal standard D5-PEth 16:0/18:1).

Calibration curves of the 16:0/18:1 species were constructed based on PEth-calibrators at four levels ranging from 21.1 to 2106 ng/mL (0.03 to 3.0 µmol/L), prepared by spiking matrix with PEth 16:0/18:1 (Chiron).

Method validation was done according to guidelines (CLSI C62A). The lower limit of quantification (LoQ) was determined to 10.5 ng/mL (0.015 µmol/L), and the measuring interval from 7.0 to 14040 ng/mL (0.01–20 µmol/L). Concentrations below LoQ were set at zero. The reproducibility at 42.1 ng/mL (0.06 µmol/L) was CV 8.7 % (coefficient of variation) (N = 80) and reproducibility at 407.2 ng/mL (0.58 µmol/L) was CV 5.5 % (N = 80).

Low-level control and high-level control (Red Hot Diagnostics, Lund, Sweden) were run in front of and after the samples.

The ring test survey for the PEth blood analysis is run by Equalis (Uppsala, Sweden) and all samples have been within accept limits for the period of the study.

Analysis of CDT

Serum Carbohydrate Deficient Transferrin (CDT) was quantified by electrophoretic separation of the transferrin fractions using a "classic" Sebia Capillarys 2 (Lisses, France) without CDT-IFCC standardization (Schellenberg and Wielders, 2010). The LoQ for CDT (sum of disialo- and asialotransferrin) was 0.4 %-units. Concentrations below this limit were set to LoQ, due to CDT being an endogenous substance. The ring test survey for the CDT serum analysis is run by Referenzinstitut für Bioanalytik (Bonn, Germany) and all requirements have been met for the period of the study.

Analysis of ethanol

Serum ethanol was analyzed on Siemens Advia Chemistry XPT (ETOH_2), reported analytical range was 0.1–6.0 g/kg and CV was 5.6 %. We used values above 0.03 g/kg as detected ethanol in this study.

Statistics

SPSS IBM SPSS® Software version 25.0 was used for statistic evaluation of the data. Due to lack of normally distributed data, median and interquartile ranges were reported for continuous variables. Differences between groups of continuous variables were assessed by Mann-Whitney U test, while differences in proportions between dichotomized groups were assessed using Chi square test. Correlation between continuous variables was assessed using the Spearman´s correlation test. For assessment of the inter-rater reliability between CDT and PEth, the Cohen´s kappa correlation was used. For analyses of CDT and PEth according to age and sex and the interaction, two separate linear regression analyses were performed using CDT or PEth, respectively, as the dependent variable, and age and sex as independent variables. The concentrations of CDT and PEth, which were not normally distributed, were logarithmically transformed before insertion into the model. For visual assessment of the data, LOESS (locally estimated scatterplot smoothing) trend lines were used in the scatterplots. These lines represent a local non-parametric regression that fits the local median of the data. To assess the accuracy of CDT using PEth as reference, ROC-curve analysis was performed using Analyse-It ® version 5 for Microsoft ® Excel.

Ethics

Ethical approval was obtained from Regional Committee for Medical and Health Research Ethics, Region South-East, Norway (2018/1041).

Concentration Intervals for CDT and PEth

Interpretation of CDT and PEth concentrations varies between laboratories, but according to available documentation (Simon, 2018, Ulwelling and Smith, 2018); PEth values between 0.03 µmol/L (~20 ng/mL) and 0.30 µmol/L (~210 ng/mL) represent non-heavy alcohol consumption, while concentrations ≥ 0.31 µmol/L can be interpreted as heavy consumption. These are used as the main PEth categories in the present study. In addition, from a previous recent review (Ulwelling and Smith, 2018), it is also indicated that very heavy alcohol consumption is associated with PEth levels substantially higher than 0.31 µmol/L. Based on these data we also studied CDT levels in groups of patients showing PEth values in the intervals 0.31–1.00 µmol/L, 1.01–2.50 µmol/L and above 2.50 µmol/L (~1750 ng/mL), respectively (Figure 1).

Regarding CDT, we have used values ≥ 1.7 %-units defining heavy alcohol consumption, as stated in the kit description from the manufacturer (Sebia) for the non-standardized, "classic" Capillarys CDT (2) method used for all serum samples in the present study (Schellenberg et al 2010). This method measures the sum of disialo- and asialotransferrin, in contrast to the standardized Capillarys CDT-IFCC method which uses an IFCC approved HPLC method as reference measurement procedure (RMP) for calibration and the disialotransferrin fraction as the only measurand (Schellenberg et al., 2017, Helander et al., 2003). The IFCC-standardized and the "classic" Capillarys CDT (2) methods have different reference intervals and cut-off values, and their results are not directly comparable (Helander et al 2017).

Results

Six thousand seven hundred and five patients had PEth and CDT measured in the same blood sample. In patients with multiple measurements, the first sample was used. The median age was 54.5 years (54.5 for men and 54.4 for women, $p = 0.95$), 66 % were males and 34 % females. In the cohort, 1675 (25 %) had CDT values ≥ 1.7 %-units, while 3208 (48%) had PEth values ≥ 0.31 µmol/L.

The overall Spearman's rho correlation coefficient between CDT and PEth concentrations in all 6705 cases was 0.685 ($p < 0.001$). For men and women, the Spearman's rho correlation coefficients were 0.714 ($p < 0.001$) and 0.626 ($p < 0.001$), respectively. Among cases with PEth values < 0.31 µmol/L (n = 3497) the Spearman's rho correlation coefficient was lower (Spearman's rho = 0.427, $p < 0.001$).

Scatterplots of the individual values of CDT and PEth measured in the same sample are seen in Figure 2a and 2b for men and women, respectively.

Of the patients with PEth concentrations ≥ 0.31 µmol/L (n = 3208), 47 % (n = 1507) had a CDT value ≥ 1.7 %-units. Of patients with CDT concentrations ≥ 1.7 %-units (n = 1675), 90 % had a PEth value ≥ 0.31 µmol/L (Table 1). The three other groups were significantly different compared to the group with high CDT, but low PEth, which had a higher median age and comprised more males (Table 1). Only eight patients with a PEth concentration below LoQ had a CDT value ≥ 1.7 %-units.

Cohen's kappa between the two methods for determining heavy alcohol use (CDT ≥ 1.7 %-units or PEth ≥ 0.31 µmol/L) was 0.43 ($p < 0.001$) overall, 0.45 ($p < 0.001$) for men and 0.38 ($p < 0.001$) for women (Table 2). The kappa values were 0.43 ($p < 0.001$) for patients below 50 years and 0.42 ($p < 0.001$) for patients 50 years and older.

Figure 1 shows the number of cases with negative (CDT < 1.7 %-units) and positive (CDT ≥ 1.7 %-units) CDT results grouped by increasing PEth concentration intervals. The number of CDT positive cases increased from 0.6 % in the group with PEth values below 0.03 µmol/L to 78 % in the group with PEth values above 2.5 µmol/L.

To compare CDT at different cut-offs with PEth, ROC-curve analysis was performed to assess sensitivity and false positive proportion (Figure 3). Defining heavy drinking as PEth at 0.31 µmol/L or above, the sensitivity was only 31 % when the costs of false positive proportion was set as low as 2 %, which appeared at CDT 2.5 %-units (Table 3). However, since the positive likelihood ratio was high, CDT performed well in detecting heavy drinking, but the negative likelihood ratio demonstrated a poor capability to exclude heavy drinking.

ROC-curve analysis comparing sex, the male factor showed higher sensitivity at the expense of higher false positive proportion (AUC-curve difference 0.034 (confidence interval 0.013–0.054)).

When PEth was < 0.03 µmol/L, the distribution of CDT (%-units) was 0.6 (median), 1.1 (97.5 percentile) and 1.4 (99 percentile).

There was a weak association between CDT and age for men (Spearman's rho 0.186, $p < 0.001$), but not for women (Spearman's rho 0.028, $p = 0.183$). The correlation between PEth and age was similar for men and women (Spearman's rho 0.110 and 0.097, respectively, $p < 0.001$).

Patients above 50 years had overall significantly higher median concentrations for both CDT (1.0 %-units above 50 years vs. 0.9 %-units below 50 years, $p < 0.001$) and PEth (0.340 µmol/L above 50 years vs. 0.200 µmol/L below 50 years, $p < 0.001$). Males above 50 years had higher median concentration for both CDT (1.1 %-units vs. 0.9 %-units) and PEth (0.360 µmol/L vs. 0.200 µmol/L) than younger males ($p < 0.001$). Female patients had the same median CDT concentration of 0.8 %-units in both age groups, but women above 50 years had higher median PEth concentration than younger women (0.290 µmol/L vs. 0.170 µmol/L, $p < 0.001$).

Male subjects showed a higher number of both PEth values ($p < 0.001$) and CDT values ($p < 0.001$) above LoQ compared to females. Among subjects having values above LoQ, the overall median CDT concentration was significantly higher for males than for females. The median PEth concentrations, however, were not significantly different for men and women (Table 2). Similar results were obtained using a linear regression model on logarithmically transformed CDT or PEth values as dependent variable and age and sex as independent variable. Males had higher CDT values than females ($p = 0.002$), but not so for PEth ($p = 0.065$).

304 The same linear regression model, including both age and sex as independent variables, also
305 revealed significant interaction effect between age and sex for CDT ($p < 0.001$), but no such
306 interaction was seen for PEth ($p = 0.738$).
307
308 Ethanol in serum was analyzed in 990 (15 %) of the total 6705 samples, and 69 (7 %) of the
309 patients (23 women and 46 men) had ethanol detected in the sample. The median
310 concentrations of both PEth and CDT were higher in samples with detected ethanol than in
311 the samples where ethanol was not detected or not measured ($p < 0.001$). The median
312 concentration in samples with detected ethanol compared to samples without detected ethanol
313 was tenfold for PEth and twice as high for CDT (Fig. 4a and b).

Discussion

This study verifies the higher detection capability of PEth as a biomarker for alcohol consumption compared to CDT, as concluded in previous studies (Helander et al., 2019a, Helander et al., 2012, Andresen-Streichert et al., 2018, Winkler et al., 2013, Neumann et al., 2020). A high number of subjects showed elevated PEth but not CDT levels. Age seems to have a weak relation to CDT and PEth levels. Sex seems to have a weak, significant effect only on CDT levels, but no effect on PEth levels. Our findings also show higher levels of CDT and PEth in the samples where ethanol was detected compared to the other samples. As PEth levels increased tenfold compared to two-fold increase for CDT, however, in vitro formation of PEth could be suspected, which may be an important factor in the appraisal of an individual's alcohol use.

Previous studies have documented stronger correlations between PEth and CDT than between the biomarkers and self-reported alcohol consumption (Walther et al., 2015, Kechagias et al., 2015). Regarding the sensitivity of PEth compared to CDT, our results were in accordance with Kechagias and colleagues, who found that PEth correlated much better to alcohol consumption than CDT and other biomarkers did (Kechagias et al., 2015). It should be noted that although PEth showed increased detection capability compared to CDT in all PEth intervals, it was most pronounced in the moderate PEth levels, and thereby probably in drinkers with a more moderately increased consumption. The reason for the substantial number of patients showing high PEth values, but not high CDT values, could be the fact that PEth is formed after smaller intakes of ethanol compared to CDT and that formation occurs faster (Stibler, 1991, Hill-Kapturczak et al., 2018). The longer half-life of CDT (Brunton et al., 2011), however, could contribute to a higher number of positive CDT samples. On the

other hand, this could be one explanation for the eight subjects showing negative PEth, but high CDT levels. The possibility of ultra-rapid PEth metabolizers has been demonstrated in some individuals in previous research (Schröck et al., 2017a). Neumann and colleagues argue that 12 cases of low PEth, but positive CDT in their recent study possibly could be explained by relatively low PEth formation in some individuals (Neumann et al., 2020). In these cases, PEth must be considered a false negative result. They also discuss the possibility of slower elimination rate of CDT after ended alcohol intake. Genetically related increase in CDT without heavy alcohol consumption (Stibler, 1991, Zühlsdorf et al., 2016) could also be present in these subjects, which would imply that the PEth value is a true negative. De Wolf and colleagues convey an example of how a novel transferrin variant can interfere with CDT analyses using both HPLC and CZE methods (de Wolf et al., 2011).

In clinical practice CDT and PEth are used interchangeably which might depend on personal choice. Overall the ROC-AUC demonstrated that CDT performed fairly well using PEth as a reference standard, but at a CDT cut-off used (1.7 %-units), only 47 % of the subjects having a PEth of 0.31 µmol/L or above were detected. The selection of the reference standard, in this case heavy drinking at a PEth value of 0.31 µmol/L or above, defines the accuracy using blood tests to deem a person to one group or the other. Assessing the capability of PEth could also be done using CDT as the reference standard.

Even though the present study does not include information on alcohol consumption from self-reports or clinical assessments, it confirms a positive correlation between PEth and CDT values. However, the inter-rater reliability between CDT and PEth for determining heavy alcohol use might be considered surprisingly low in this study (Kappa 0.43, $p < 0.001$), considering that they are interpreted quite equally in clinical practice. In general, kappa values

below 0.21 indicate no agreement, 0.21–0.59 are suggested as minimal to weak, and values between 0.60 and 0.79 could be interpreted as a moderate inter-rater relationship (McHugh, 2012). To our knowledge, no comparable results regarding agreement between the two biomarkers as tests for heavy alcohol consumption are previously published.

Previous studies have found no significant differences in PEth values between male and female (Helander et al., 2019a, Hill-Kapturczak et al., 2018, Walther et al., 2015, Wurst et al., 2010). Higher CDT levels in male groups compared to female groups with the same alcohol consumption have been found, although several explanations exist (Walther et al., 2015). Effects of age and sex on alcohol biomarkers could be caused by higher alcohol concentrations in certain populations due to higher consumption. Changes in the total body water content and first pass metabolism could also differ according to age and/or sex (Norberg et al., 2003). An alternative explanation could be that there are differences in the formation of PEth and CDT levels from the same alcohol concentrations among male and female and age groups. This might be caused by e.g. reduced kidney function in the elderly (Denic et al., 2016) or unknown sex differences. In the present study we have no information about alcohol consumption; hence our design does not allow us to test the above mentioned hypothesis. Higher levels of CDT in males could be explained by increased consumption, but the lack of differences for PEth strengthens the notion that not only the amount of consumed alcohol is responsible for the observed differences. It should also be noted that although statistical significant sex effects could be found for CDT, it is possible that the clinical significance is small and that adjustment of the reference intervals is not required (Bergstrom and Helander, 2008).

388 Regarding the analytical method used for PEth in the present study, the physical and solvent
389 properties of the mobile phase CO_2 in supercritical state are very suitable for fat soluble
390 analytes like PEth. The procedure has proven to be reliable and robust. The UPC2-MS/MS
391 method has been reported as a reliable, flexible and suitable method for PEth measurements
392 (van der Nagel et al., 2018).
393
394 One challenge with PEth analyses is the possibility of in vitro formation of PEth in samples
395 containing ethanol (Aradottir et al., 2004a). A weakness of the present study is that ethanol
396 was measured in 15 % of the samples, which reflects the normal routine of the laboratory the
397 data were extracted from. It would be superior to analyze ethanol in all samples. However,
398 similar PEth and CDT values in samples where ethanol was not measured compared to the
399 samples where ethanol was measured but not detected, indicate that this does not represent a
400 major weakness. It is expected that higher PEth and CDT levels are seen in cases with
401 detected ethanol, but the bigger increase for PEth than for CDT concentrations between
402 samples with and without detected ethanol, could possibly be explained by in vitro formation
403 of PEth. This is in accordance with former experiments (Aradottir et al., 2004a). Different
404 storage conditions have previously been found to affect in vitro formation of PEth. In one
405 study (Aradottir et al., 2004b), blood samples with ethanol were stored at room temperature,
406 at 4°C, at -20°C and at -80°C, respectively. In these experiments PEth concentrations were
407 slightly elevated in samples stored at room temperature and at -20°C. Therefore, in vitro
408 formation of PEth in ethanol-containing samples may to some degree increase the PEth value
409 due to temperature conditions during transport and storage after sampling.
410
411 One strength of the present study is the inclusion of a large study sample size comprising
412 6705 cases. Also, the use of fully validated, robust analytical methods performed in the same

laboratory equal for all patients, represents a strength. The major limitation of our study is the lack of further clinical information about the patients, which could be utilized to adjust our results, and the lack of data on self-reported alcohol consumption through e.g. the Alcohol Use Disorders Identification Test (AUDIT). However, previous research has found significant correlation between PEth concentrations and AUDIT, (Helander et al., 2019b, Nguyen et al., 2018, Afshar et al., 2017, Schröck et al., 2017b, Piano et al., 2015, Kabashi et al., 2019). Even though we found a higher sensitivity for PEth compared to CDT, it is beyond the scope of this study to examine PEth in relation to CDT in detecting adverse alcohol consumption due to the lack of a predefined standard in our study, which might have been e.g. monitoring of alcohol consumption among the study participants. Nevertheless, another strength of this study is providing comparable data of two broadly utilized biomarkers on sex and age. Biomarkers seem to be a complementing objective measure to the self-reported data, on which to date most of alcohol research relies.

In conclusion, the present study showed that PEth in all concentration levels is more suitable compared to CDT when it comes to detection capability of heavy drinking. The inter-rater reliability between the two biomarkers is surprisingly low, considering that they are interpreted quite equally. Age does not seem to affect the concentrations of the two alcohol markers significantly. The fact that higher concentrations of CDT but not PEth are seen in males indicates that PEth, as opposed to CDT, might be formed equally in men and women. Therefore, the issue of sex bias that is possibly present for CDT, might be avoided for PEth. Consequently, this adds to the data on PEth serving as a reliable biomarker and a valuable tool in distinguishing between moderate and heavy drinking among male and female patients at various age.

Conflict of interest

None of the authors have any conflict of interests.

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609 Figure legends

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611 Fig. 1. Number of samples with CDT concentrations below 1.7 %-units and 1.7 %-units and
612 above in different PEth concentration intervals.

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614 Fig. 2a. Scatterplot of individual values of PEth and CDT concentrations in 4448 male
615 patients with a LOESS trend line and reference lines for CDT and PEth values representing
616 heavy alcohol consumption. Note that the X-axis is base-10 log scale and the Y-axis is base-2
617 log scale.

618

619 Fig. 2b. Scatterplot of individual values of PEth and CDT concentrations in 2257 female
620 patients with a LOESS trend line and reference lines for CDT and PEth values representing
621 heavy alcohol consumption. Note that the X-axis is base-10 log scale and the Y-axis is base-2
622 log scale.

623

624 Fig. 3. ROC-curve analysis of CDT using PEth 0.31 μmol/L or above as the reference
625 standard for heavy drinking. The effect of using different cut-offs of CDT is shown in Table
626 3.
627 AUC: Area Under Curve. CI: Confidence Interval. TPF: True Positive Fraction. FPF: False
628 Positive Fraction. ROC: Receiving Operating Characteristics.

629

630 Fig. 4a. Concentrations of CDT (%-units) in cases where ethanol was not measured, not
631 detected and detected (> 0.03 g/kg). $P < 0.001$ comparing ethanol detected to not measured
632 and not detected. The box length is the interquartile range (25th to 75th percentile) of the
633 concentrations. The line across the inside of the box represents the median value. Whiskers

represent the largest or smallest value within 1.5 times the interquartile range. Circles and asterisks represent values exceeding 1.5 and 3 times the interquartile range, respectively. Y-axis: log-scale with reference line for the CDT value representing heavy alcohol consumption.

Fig. 4b. Concentrations of PEth in cases where ethanol was not measured, not detected and detected (> 0.03 g/kg). $P < 0.001$ comparing ethanol detected to not measured and not detected. The box length is the interquartile range (25th to 75th percentile) of the concentrations. The line across the inside of the box represents the median value. Whiskers represent the largest or smallest value within 1.5 times the interquartile range. Circles represent values exceeding 1.5 times the interquartile range. Y-axis: log-scale with reference line for the PEth value representing heavy alcohol consumption.

Table 1.

		PEth	
		Low (< 0.31 µmol/L)	**High (≥ 0.31 µmol/L)**
CDT	**Low (< 1.7 %-units)**	N = 3329 (49.6 %) Median age: 52.3 (p < 0.001)* Male 64 % (p < 0.001)*	N = 1701 (25.4 %) Median age: 55.2 (p < 0.001)* Male 63 % (p < 0.001)*
	High (≥ 1.7 %-units)	N = 168 (2.5 %) Median age: 61.8 Male 83 %	N = 1507 (22.5 %) Median age: 57.7 (p = 0.004)* Male 74 % (p = 0.011)*

* Compared to high CDT / low PEth group

Table 2.

	PEth (µmol/L) (median, IQR)	p	CDT (%-units) (median, IQR)	p	Kappa p < 0.001
Total (male and female)	**0.430 (0.850)**		**1.0 (1.1)**		**0.43**
Female	0.420 (0.880)	0.227	0.9 (0.7)	< 0.001	0.38
Male	0.430 (0.840)		1.0 (1.2)		0.45

Table 3.

CDT (%-units) cut-off values	True Positive Proportion (Sensitivity)	True Negative Proportion (Specificity)	False Positive Proportion	False Negative Proportion	Likelihood Ratio (Positive)	Likelihood Ratio (Negative)
1.3	0.62	0.90	0.10	0.38	6.24	0.42
1.7	0.47	0.95	0.05	0.53	9.78	0.56
2.5	0.31	0.98	0.02	0.69	16.63	0.70

Table legends

Table 1: Combinations of High and Low CDT Concentrations (≥ 1.7 %-units and < 1.7 %-units) and High and Low PEth Concentrations (≥ 0.31 µmol/L and < 0.31 µmol/L) With P-Values for Differences in Age and Sex Compared to the High CDT / Low PEth Group

Table 2: Male and Female Concentrations of PEth and CDT, and Kappa Values (Among Subjects with Values > LoQ)

Table 3: The Effect on Sensitivity and Specificity (and False Positive Proportion) Using CDT at Different Cut-offs to Indicate Heavy Alcohol Use. The Comparison Was Done Using PEth Concentration 0.31 µmol/L or Above as the Definition of Heavy Alcohol Use.

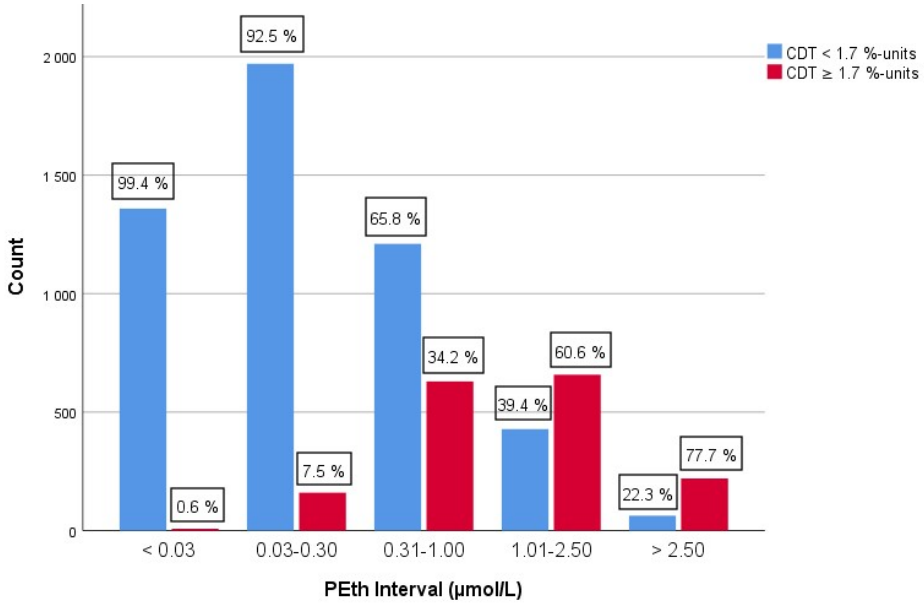


Fig. 1. Number of samples with CDT concentrations below and above 1.7 %-units in different PEth concentration intervals.

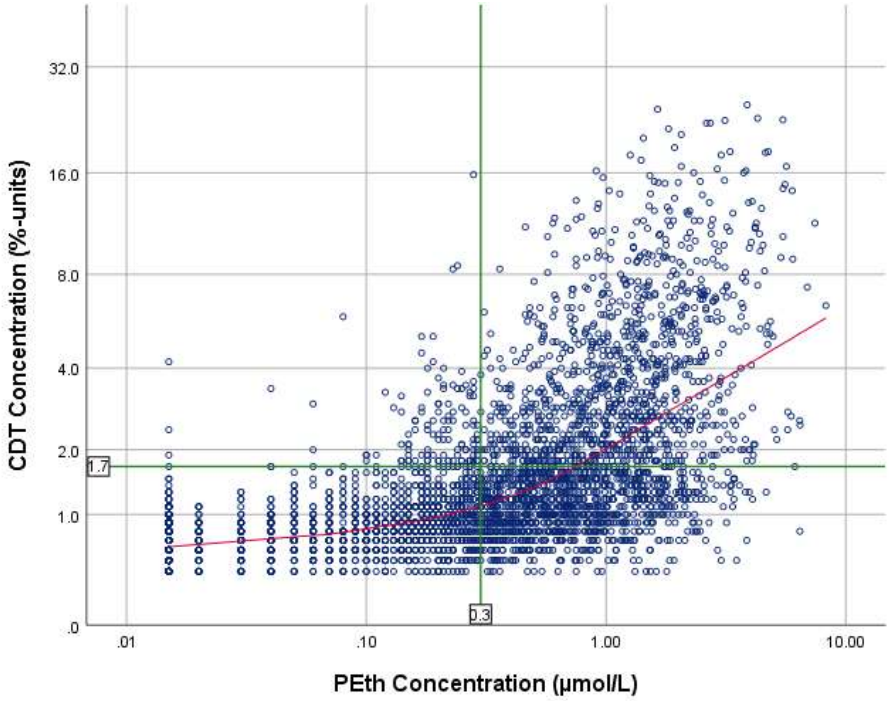
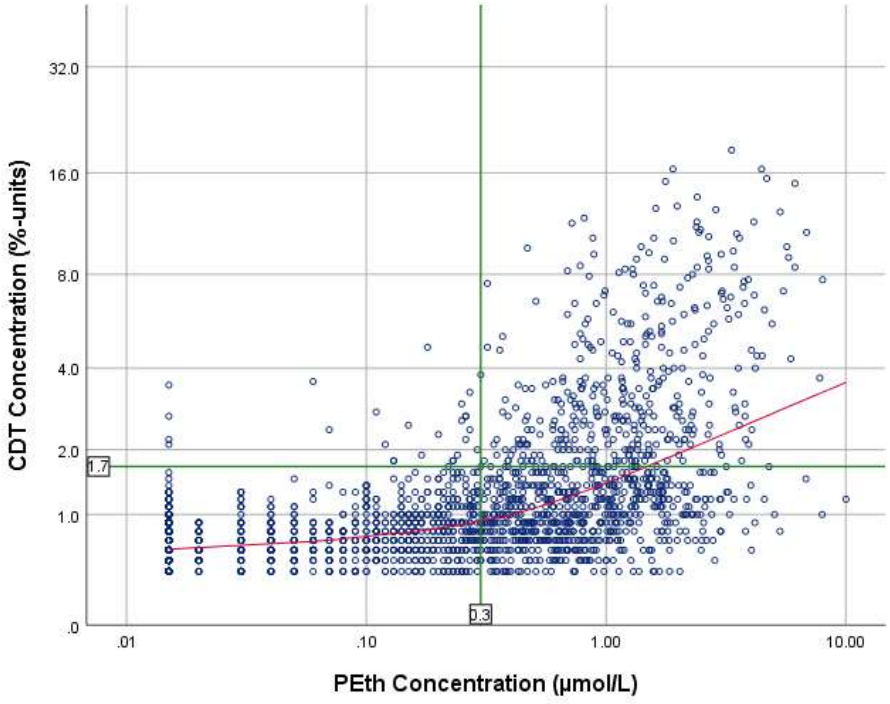


Fig. 2a. Scatterplot of individual values of PEth and CDT concentrations in 4448 male patients with a LOESS trend line and reference lines for CDT and PEth values representing heavy alcohol consumption. Note that the X-axis is base-10 log scale and the Y-axis is base-2 log scale.



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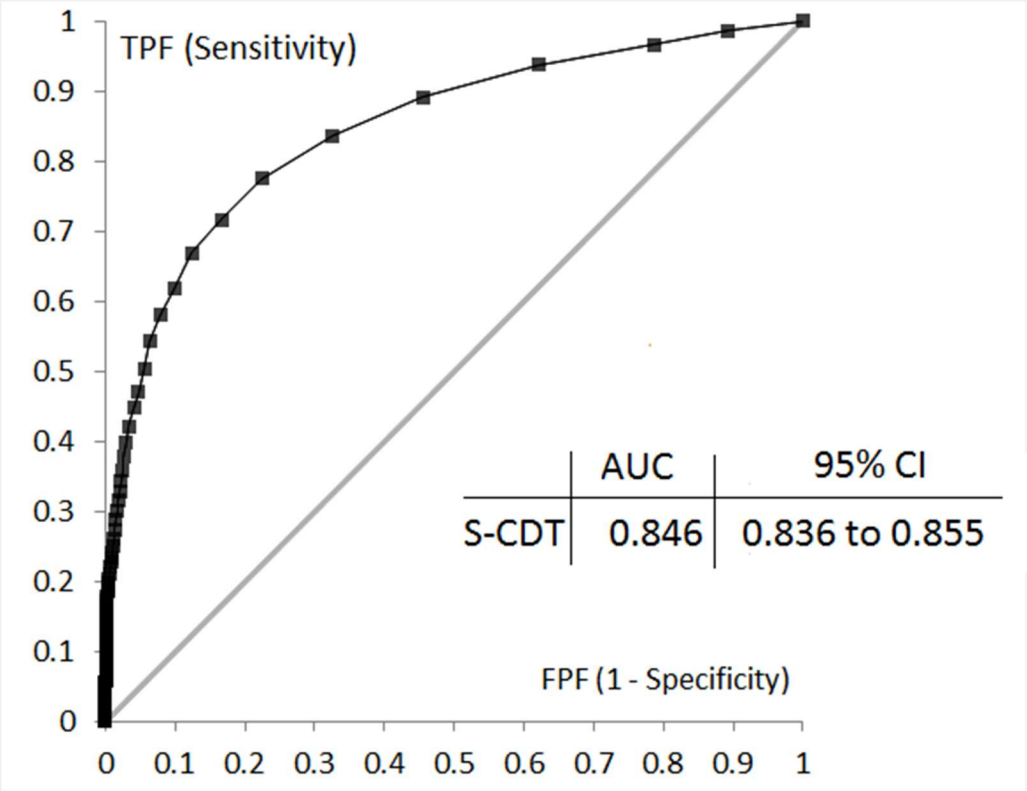
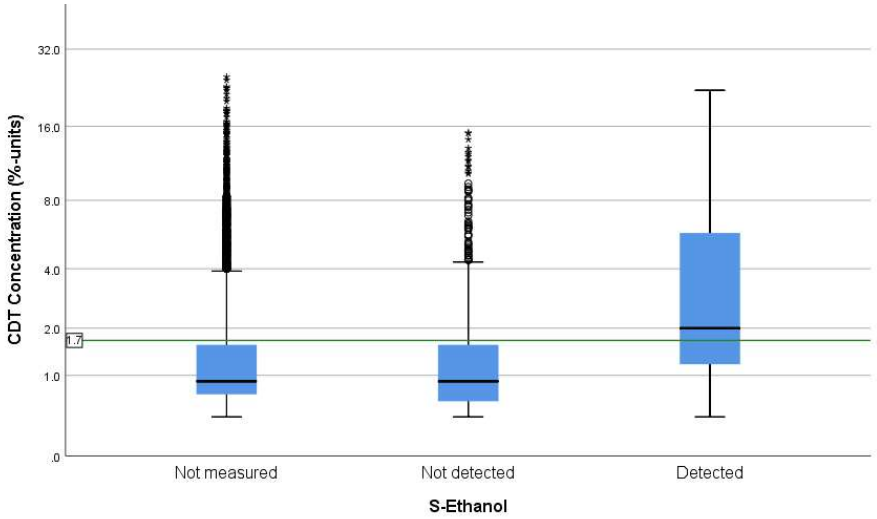


Fig. 3. ROC-curve analysis of CDT using PEth 0.31 µmol/L or above as the reference standard for heavy drinking. The effect of using different cut-offs of CDT is shown in Table 3.

AUC: Area Under Curve. CI: Confidence Interval. TPF: True Positive Fraction. FPF: False Positive Fraction. ROC: Receiving Operating Characteristics.



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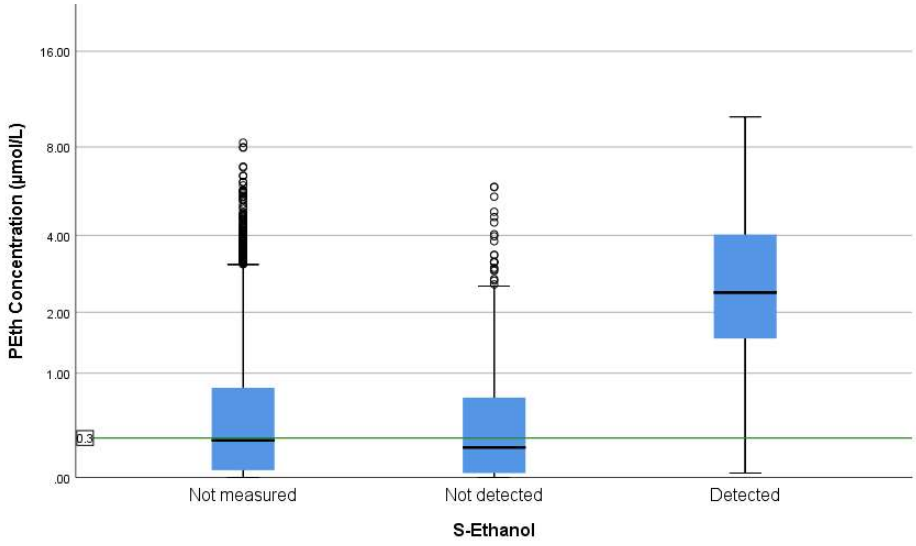

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