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Phosphatidylethanol in patients with liver diseases of different etiologies: Analysis of six homologues and comparison with other alcohol markers

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ABSTRACT

Background and aims: Phosphatidylethanol (PEth) is a direct alcohol biomarker. Aim of the study was to evaluate the performance of six homologues of PEth in comparison to other alcohol markers in patients with liver diseases.
Methods: The study included 234 patients with liver disease, who gave statements about alcohol consumption during the three months prior to the doctor's appointment. Ethylglucuronide in urine (uEtG) and in hair (hEtG) and carbohydrate-deficient transferrin (CDT) were analyzed in addition to PEth.
Results: Of all patients 47% stated to have drunk alcohol during the past three months. UEtG, hEtG and CDT showed a sensitivity of 29% and a specificity of 92% together for ingestion of at least two standard drinks (24 g) per week. With PEth 16:0/18:1 in addition, sensitivity increased to 59%. For consumption in the last week uEtG's sensitivity and specificity was 28% and 100%, respectively. PEth's was 75% and 93%. When looking at patients who consumed at least two standard drinks per week during the past three months and of which a hair sample could be obtained, hEtG's sensitivity was 37% and specificity 90%. PEth had a sensitivity of 53% and specificity of 100%. Quotients of PEth 16:0/18:1 with 16:0/18:2, 16:0/20:4 and 18:0/18:2 were smaller when alcohol had been consumed more recently.
Conclusion: Despite the rather poor overall sensitivity of alcohol biomarkers in this study, PEth showed best sensitivity for all time periods of alcohol consumption.

1. Introduction

In multiple clinical and forensic settings an objective evaluation of the patients' alcohol consumption is important. In particular, in liver transplant candidates with alcoholic liver disease (ALD) abstinence checks are mandatory and required by law in Germany [1]. In addition, evaluating alcohol consumption behavior plays a role in treatment for patients with various liver pathologies [2].

To investigate the nature, extent and duration of alcohol exposure, alcohol biomarkers are measured from body fluids or keratinous tissue. Besides the traditional indirect biomarkers, which are rather insensitive (carbohydrate-deficient transferrin (CDT)) and non-specific (alanine transaminase (ALT), aspartate transaminase (AST), γ-glutamyl transpeptidase (GGT), mean corpuscular volume (MCV)), direct alcohol biomarkers can be measured [3]. These direct alcohol biomarkers are derivatives of ethanol, making them highly specific. For example, ethyl glucuronide (EtG) is synthesized when ethanol is glucuronidated by uridine 5′-diphosphoglucuronosyltransferase in the hepatocytes, the gastro-intestinal-tract and in the kidneys [4]. EtG is usually determined in urine (uEtG) and hair (hEtG). While the maximum detection window of EtG has been reported to be up to 5 days [5] in urine, it accumulates in hair and allows to detect alcohol consumption over the past months [3]. For patients with liver disease, sensitivity and specificity of uEtG have been reported to be 70–89% and 93–99% respectively for any alcohol consumption in the past 3–7 days [6]. Sensitivity and specificity of EtG in a 3 cm hair strand for detecting moderate and excessive alcohol consumption during the past three months were demonstrated to be as high as 85–100% and 97–100%, respectively [6].

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Phosphatidylethanol (PEth) is an abnormal phospholipid consisting of a phosphoethanol headgroup with a variety of fatty acid chains attached to a glycerol backbone. Because PEth production by the enzyme phospholipase D requires the presence of ethanol [7], it can be used as a direct alcohol marker. Previously, it was reported to have a sensitivity and specificity of 73–100% and 90–96%, respectively to determine any alcohol consumption in the previous one to four weeks [6]. The existence of at least 48 different homologues of PEth was described [8]. Simultaneous quantification of six of these homologues via LC/MS/MS has been established [9]. PEth was shown to have a half-life of 3–10 days [10,11]. Helander et al. [12] specified between homologues and reported half-lives of 3.7–10.4 days, 2.7–8.5 days and 2.3–8.4 days for PEth 16:0/18:1, PEth 16:0/18:2 and PEth 16:0/20:4, respectively. Therefore, PEth may have a detection window of several weeks [13]. A linear correlation between PEth concentrations in whole blood and ethanol intake was demonstrated [14,15]. It is generally accepted that it is impossible to reach complete specificity and selectivity for determination of alcohol consumption, but diagnostic certainty is increased by taking the results of different alcohol markers into consideration when evaluating an alcohol exposure [16].

Therefore, the aim of this study was to assess the diagnostic value of PEth homologues in comparison to other alcohol biomarkers regarding different consumption times and amounts.

2. Methods and materials

2.1. Analysis of alcohol biomarkers

For the analysis of uEtG the samples were measured via an enzymatic test (AU 480, Beckmann Coulter, Brea, California, USA). If the immune assay yielded a concentration above 300 ng/l, an aliquot from the same sample was quantitatively measured by LC/MS/MS as described previously [17]. Eventually a cut-off of 500 ng/ml was applied as it is set in the German legal transplant guidelines [1].

Hair samples were taken (if at least 3 cm long) by cutting it directly at the scalp and prepared as previously described and subsequently analyzed for EtG by a validated LC/MS/MS-method [18]. Hair could be sampled and analyzed from 91 patients. Reasons for not sampling or analyzing the hair samples were that the hair was too short/patients were bald (n = 51), the hair was chemically treated (n = 36) or that not enough material was available (strand too thin) (n = 7). Furthermore, patients refused hair sampling (n = 37) and in six cases the reason for missing hair sample analysis is unknown. According to international standards from the society of hair testing (SoHT) a cut-off of 5 pg/mg was used for abstinence [19]. Values >30 pg/mg suggest chronic, excessive alcohol intake. Analysis of 3 cm hair represents consumption of approximately the past three months.

CDT was analyzed by HPLC using a commercially available, fully validated, and IVD-CE-labeled kit (CDT in blood ClinRep© Komplettkit 'CDT im Serum– HPLC', Recipe, München, Germany). If the fraction of disialotransferrin exceeds 2.0% it indicates that alcohol was consumed excessively for two to six weeks [20]. MeOH and EtOH were measured via GC-FID as previously described [17]. EtOH was primarily analyzed to exclude the possibility of post-sampling formation of PEth [21].

PEth was analyzed from dried blood spots (DBS) that were volumetrically generated (20 μl) from EDTA-blood. For analysis one spot was processed as whole. Detailed information about sample preparation, instrument settings and validation results can be found in our previous work [9]. Additional validation for a calibration range up to 2000 ng/ml was performed and passed. PEth-homologues 16:0/18:1, 16:0/18:2, 16:0/20:4, 18:0/18:1, 18:0/18:2 and 18:1/18:1 were simultaneously quantified. Furthermore, the haematocrit (hct) of all blood samples was determined (haematology-analyzer ADVIA 2020i, Siemens, Munich, Germany). During validation of the applied method, matrix effect and recovery were inquired for hcts of 20%, 40% and 60% to exclude major analytical hct effects [9].

2.2. Patients

In the study 234 patients were included who presented to the outpatient liver and kidney clinic of the University Medical Center Hamburg- Eppendorf between October 2017-September 2018.

Of those, 87 had alcoholic liver disease (ALD), 124 had non-alcoholic fatty liver disease (NAFLD) or non-alcoholic steato-hepatitis (NASH) and 23 suffered from cryptogenic or other rare liver diseases (e.g., Wilson Disease).

To evaluate patients' alcohol consumption, a three-page questionnaire with adapted AUDIT elements was given out for self-assessment. Included in the form were questions about alcohol consumption over (I) the last three months, (II) the last four weeks and (III) the last week. All responses were kept anonymous. In parallel, alcohol markers were quantified in blood, urine and hair samples. Possible factors that might interfere with alcohol marker analysis were taken into consideration, such as consumption of alcohol-free beer or alcohol containing foods, the use of EtOH containing hygiene/cosmetic products and chemical treatment of hair. Informed written consent was given by all participating subjects and the study was approved by the local ethics committee (PV5068).

2.3. Clinical parameters

Creatinine, total bilirubin, liver enzyme activity and MCV were analyzed on the appointment day. Body mass index (BMI) was calculated using weight and height measured on the date of study entrance. Glomerular filtration rate (GFR) was calculated using the Modification of Diet in Renal Disease (MDRD) equation.

2.4. Statistical analysis

For statistical analysis, the program SPSS (IBM SPSS Statistics for Windows, Version 27.0) was used. Based on questionnaire responses an average estimated weekly alcohol intake was calculated. Table 2 presents the number of patients who made a statement about their consumption in the different time periods. Diagnostic accuracy was calculated based on questionnaire responses and as such, values were excluded if no response was given for alcohol consumption in the corresponding time-period (Table 2). Data was similarly excluded if at least two direct alcohol markers were positive while complete abstinence was claimed (n = 6). The PEth homologue 16:0/18:1 was used for comparison with other markers, as laboratories use this homologue primarily for analysis.

3. Results

3.1. Patient characteristics and alcohol consumption behavior

Characteristics of all 234 patients are summarized in Table 1. Of all included patients (n = 228), 50% (n = 114) stated that they had consumed alcohol at some point. As mentioned in 2.4 an overview of patient-declared alcohol consumption is given in Table 2. Significantly more patients with NAFLD/NASH admitted consumption of alcohol in the past three months and four weeks ($p < 0.001$) compared to patients with ALD. But the mean amounts of alcohol consumed during the four weeks and the three months prior to the appointment was significantly higher in patients with ALD compared to patients with NASH/NAFLD (factor 2.8, $p = 0.026$). Furthermore, the mean amount of alcohol consumed per week was significantly lower during the last week compared to the four weeks prior to the appointment ($p = 0.02$) (including all diagnosis).

Although there was no significant difference between males and females in terms of the percentage that admitted alcohol consumption during the past three months and four weeks, men stated a significantly higher consumption amount (factor of 2.1, $p = 0.006$). The consumed

Table 1
Patients' characteristics.

Characteristic	Total (n = 234)	ALD (n = 87)	NASH/ NAFLD (n = 124)	Other/ unclear (n = 23)
Sex male (%)	130 (56)	55 (63)	63 (51)	12 (52)
Age (years), median (range)	58 (18–86)	62 (38–77)	56 (23–86)	53 (18–71)
Creatinine (mg/l), median (range)	9.8 (5–97)	12 (5–50)	9.4 (4.6–97)	8.7 (6.3–12)
Bilirubin (mg/l), median (range)	6 (2–77)	7 (2–77)	5 (2–29)	4 (3–39)
Albumin (g/l), median (range)	38 (19–47)	34 (19–45)	39 (24–47)	40 (24–47)
ASAT (U/l), median (range)	29 (4–266)	33 (9–266)	29 (4–230)	28 (18–130)
ALAT(U/l), median (range)	36 (6–259)	28 (9–256)	43 (6–259)	50 (30–239)
GGT (U/l), median (range)	83 (5–1772)	77 (5–1772)	80 (12–906)	126 (31–594)
BMI (kg/m^2), median (range)	27.9 (15.4–48.9)	27.5 (15.4–48.9)	28.4 (16.6–53.5)	28.1 (19.5–42.7)
GFR (ml/min), median (range)	78 (4.1–137)	53 (12–121)	85 (4.1–137)	91 (49–123)
MCV (fl) median (range)	89 (66.2–113)	92.7 (77.8–113)	87.9 (66.2–110)	89 (80–106)
Post-LTX	40	33	7	
Pre-LTX	156	53	103	
Pre-KTX	13	0	13	
Pre-KTX, Post LTX	2	1	1	
Liver cirrhosis	128	81	46	1

KTX = kidney transplantation.

amount did not differ between sexes during the week before the appointment.

3.2. Alcohol biomarkers

Of all 228 included patients, 33% (n = 76) had a positive alcohol biomarker in at least one of the three sample materials. Fig. 1 illustrates the number of cases with positive biomarkers, highlighting those with exclusively one positive marker. Interestingly, 46 (20%) patients admitted to alcohol consumption without having any positive alcohol biomarker. Positive alcohol consumption was defined as an ingestion of >24 g of alcohol per week, which is equivalent to two standard alcoholic drinks. The traditional markers uEtG, hEtG and CDT together showed a sensitivity of only 29% and a specificity of 92% for any alcohol consumption during the preceding three months. With PEth 16:0/18:1 in addition to those markers, sensitivity could be increased to 59%, and specificity remained similar with 93%.

3.2.1. PEth

PEth 16:0/18:1 was positive (≥10 ng/ml) in 63 cases (28%, total n = 228). In 32 cases it was the only positive alcohol marker (compared with hEtG, uEtG, CDT), with PEth concentrations from 12 to 772 ng/ml (mean: 66 ng/ml; median: 27 ng/ml). All 63 patients admitted to alcohol consumption within the last three months, so specificity is 100%. Sensitivity for alcohol consumption during that period was 53% for ≥24 g/week. Sensitivity and specificity of PEth 16:0/18:1 was 58% and 98% respectively when exclusively analyzing the four weeks before the appointment. The two patients who had a positive PEth but denied alcohol consumption in the four weeks prior to the visit, stated to have consumed alcohol in the preceding three months (60 g/week). When taking patients into consideration who drank at least 84 g of alcohol per week, which corresponds to seven standard drinks per week, sensitivity of PEth 16:0/18:1 was 92%, specificity 89%. Table 3 shows that this homologue has the highest sensitivity of the six homologues. Interestingly, PEth detected 50% of patients who claimed to have stopped alcohol consumption four weeks prior to the appointment (n = 8), but consumed alcohol in the months before, which demonstrates the potentially long detection window. In detail: Alcohol amounts the four patients with PEth <10 ng/ml stated to have drunk until four weeks prior to the appointment were 48 g/week, 60 g/week (twice) and 216 g/week. One patient who stated to have consumed 60 g/week had a PEth concentration of 14 ng/ml, another one 24 ng/ml. PEth 16:0/18:1

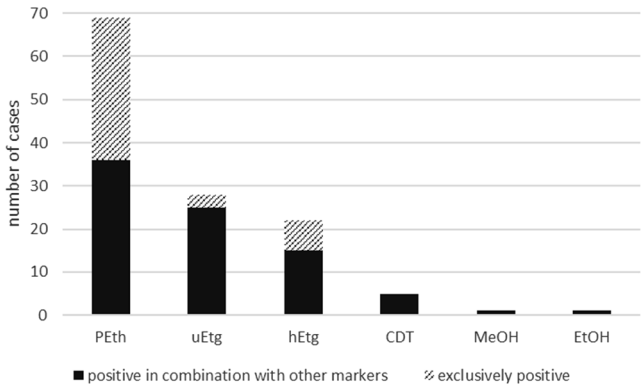

Fig. 1. Number of positive alcohol biomarkers using the applied cut-offs: 10 ng/ml PEth (n = 228), 0.5 mg/l uEtg (n = 228), 5 pg/mg hEtg (n = 91), 1.7% CDT (n = 224), 5 μg/ml MeOH (n = 228), 0.1‰ EtOH (n = 228).

Table 3
Specificity, sensitivity and AUC-ROC of six PEth homologues for different minimum amounts of alcohol consumption during the past four weeks.

	specificity (%)		sensitivity (%)		AUC-ROC	
	≥24 g	≥84 g	≥24 g	≥84 g	≥24 g	≥84 g
PEth 16:0/18:1	98	89	58	92	0.78	0.93
PEth 16:0/18:2	98	88	53	84	0.76	0.89
PEth 16:0/20:4	98	90	44	71	0.71	0.82
PEth 18:0/18:1	99	92	40	68	0.70	0.82
PEth 18:0/18:2	98	93	40	68	0.70	0.82
PEth 18:1/18:1	99	95	33	60	0.66	0.78

Table 2
Alcohol consumption according to patients' statements in the questionnaire.

time period	alcohol consumption	Total	ALD	NAFLD/NASH	unclear/others
Last week	admitted in %	32 (n = 226)	15	41	52
	g/week EtOH mean (range)	65 (12–358)	108 (12–358)	53 (12–317)	66 (12–246)
Last four weeks	admitted in %	39 (n = 223)	22	48	57
	g/week EtOH mean (range)	199 (24–1792)	502 (24–1792)	116 (24–490)	166 (24–336)
Last three months	admitted in %	47 (n = 222)	29	55	70
	g/week EtOH mean (range)	195 (24–1792)	496 (24–1792)	108 (24–490)	159 (24–490)

concentration was 20 ng/ml with a reported consumption of 72 g/week and 39 ng/ml for 336 g/week until four weeks prior to the appointment.

Fig. 2 represents ranges and medians of PEth concentration in three different categories of alcohol amount consumed in the prior four weeks: 24–144 g/week (2–12 standard drinks), 156–336 g/week (13–28 standard drinks) and anything above 336 g/week, which equals the definition of excessive alcohol consumption (50 g/d). Although concentrations of all categories overlap, all PEth concentrations are significantly higher in the highest consumption category than in the others ($p = 0.038$, $U = 59$, $z = -2.1$).

Receiver-operating-characteristics (ROC) curves for all homologues are shown in Fig. 3 for different cut-off levels of alcohol consumption. AUC (area under the curve)-ROCs can be found in Table 3. For the consumption of ≥84 g/week in the previous four weeks, the AUC under the ROC curve for PEth 16:0/18:1 is 0.93. This result indicates PEth 16:0/18:1 is capable of differentiating between those who drink and those who abstain from alcohol or only drink occasionally.

All homologues showed correlation between their concentration and the claimed ethanol intake in the spearman ranks analysis ($p < 0.001$), with a correlation coefficient of 0.73 for PEth 16:0/18:1, 0.70 for 16:0/18:2, 0.61 for 16:0/20:4 and 18:0/18:2, 0.60 for 18:0/18:1, 0.56 for 18:1/18:1.

Despite women stating to have consumed significantly less alcohol (see 3.1), the concentrations of the PEth-homologues did not differ between the sexes ($p = 0.61$ for 16:0/18:1, $p = 0.41$ for 16:0/18:2, $p = 0.84$ for 16:0/20:4, $p = 0.76$ for 18:0/18:1, $p = 0.24$ for 18:0/18:2, $p = 0.34$ for 18:1/18:1). When comparing the ROC curves (alcohol ≥84 g/week), the AUCs of all homologues were closer in value to each other in females than in males (Fig. 4). Although the AUCs and sensitivities were higher in women compared to men, the differences in AUC-ROCs were not statistically significant ($p = 0.70$ for 16:0/18:1, $p = 0.35$ for 16:0/18:2, $p = 0.26$ for 16:0/20:4, $p = 0.07$ for 18:0/18:1, $p = 0.24$ for 18:0/18:2, $p = 0.05$ for 18:1/18:1) (Table 4).

For evaluating applicability of different cut-off values for PEth higher cut-offs were applied: specificity for consumption of ≥24 g/week and ≥84 g/week is 98% and 95% for 20 ng/ml respectively and 99% and 96% for 35 ng/ml. Sensitivity at a 20 ng/ml cut-off is 39% and 74% for ≥24 g/week and ≥84 g/week, respectively and 29% and 53% for 35 ng/ml.

It was evaluated if a combination of different PEth homologues can indicate how recently alcohol was consumed. In the group of patients who consumed ≥24 g of alcohol/week in the past four weeks, the ratios of PEth 16:0/18:1 to the other homologues were calculated. Subsequently, the quotients were compared with the Mann-Whitney-Test between (I) patients who stopped consumption one week before the appointment and (II) patients who drank consistently until the appointment. The median quotients were (II) 1.1, 2.0, 1.7 and (I) 1.6, 10.0, 3.3 for PEth 16:0/18:1 / PEth 16:0/18:2, PEth 16:0/18:1 / PEth 16:0/20:4 and 16:0/18:1 / PEth 18:0/18:2, respectively (Fig. 5). Thus, the quotients were significantly smaller if alcohol was consumed during the week before blood sampling ($p = 0.028$, $p = 0.002$, $p = 0.011$, respectively)

3.2.2. Urine-EtG vs PEth

The diagnostic accuracy of PEth was compared with uEtG regarding consumption during the week prior to the appointment. Altogether uEtG was positive (≥500 ng/ml) in 22 cases (10%, total n = 228). In two of those PEth was negative (<LOQ), although alcohol consumption was admitted by the patients (36 g/week and 24 g/week). Nonetheless, sensitivity of uEtG was very low (28%) for detecting alcohol consumption in the past week while specificity was very high (100%). Combination with PEth increases sensitivity strongly (77%) (Table 5).

3.2.3. Hair-EtG vs PEth

Of the 91 hair samples that were obtained 22 (24%) tested positive for EtG (>5 pg/mg) (range: 9–292 pg/mg; mean: 70 pg/mg; median 54 pg/mg). In seven cases it was the only positive alcohol marker (range: 9–114 pg/mg; mean: 37 pg/mg; median 17 pg/mg). Five of the seven were classified as false-positive, because alcohol consumption was completely denied. Enhanced incorporation into the hair matrix and reduced rate of hair growth could have prolonged the detection window beyond three months. Sensitivity and specificity for detecting alcohol consumption in the three months before the appointment are shown in Table 5. PEth alone had a better sensitivity and specificity than hEtG. Combination of both markers improved sensitivity further. Of the 13 cases with positive PEth and hEtG both markers exceeded cut-off for excessive alcohol consumption (30 pg/mg for hEtG; 210 ng/ml for PEth) in four cases. In six cases hEtG concentrations indicated excessive alcohol consumption, while PEth concentrations did not; and in one case vice versa. Exact concentrations of both markers and the self-reported alcohol consumption can be found in the supplementary data (Table S1).

3.2.4. CDT vs PEth

CDT was positive (≥2.0%) in three (1%) cases (total n = 224). PEth was also positive in all three patients (183–473 ng/ml). Two more patients had CDT values between 1.7 and 2.0% which is suspicious for excessive alcohol consumption, PEth was positive in both (221 and 1141 ng/ml). Corresponding CDT and PEth concentrations are listed in the supplementary data, including the stated consumed alcohol amount (Table S2). Looking at patients with excessive alcohol consumption (at

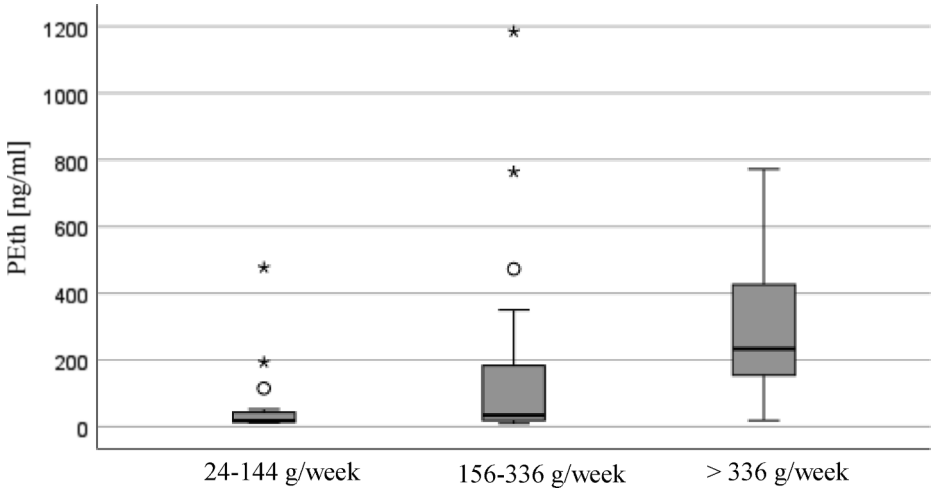

Fig. 2. Boxplots of PEth 16:0/18:1 concentrations corresponding to three different alcohol consumption amount groups.

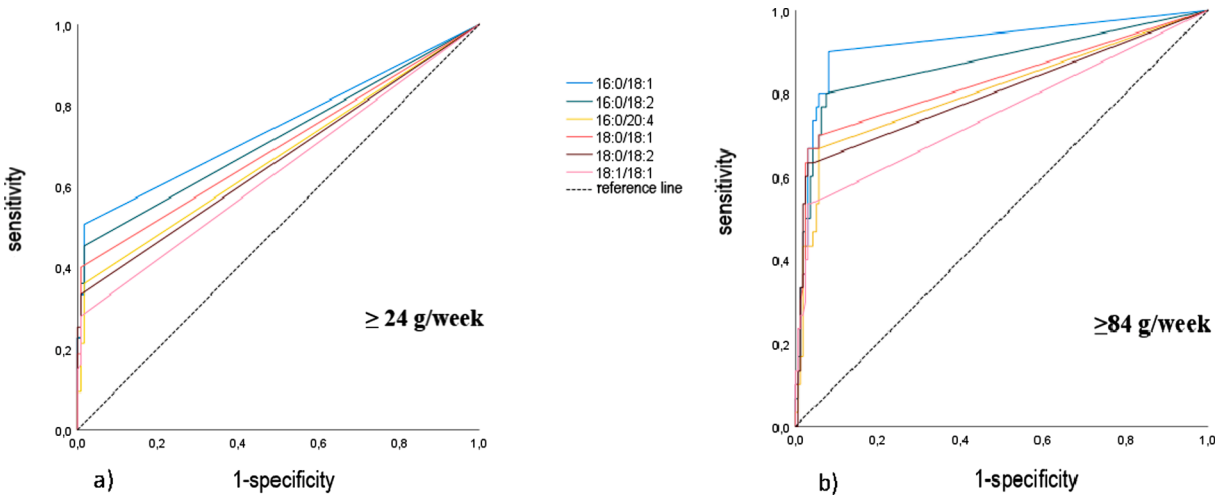

Fig. 3. ROC of PEth-homologues for a) ≥24 g alcohol/week, b) ≥84 g alcohol/week.

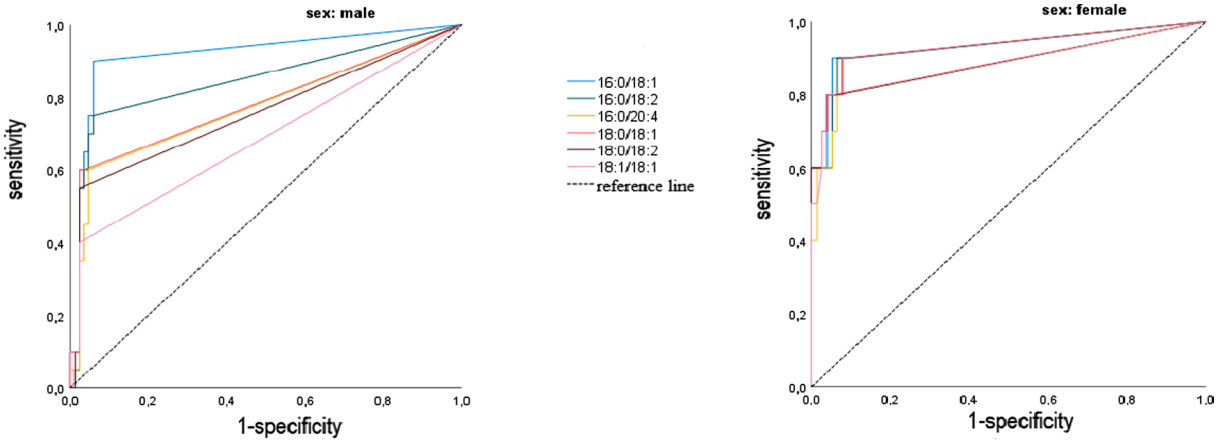

Fig. 4. AUC-ROC for ≥84 g alcohol/week for male and female patients.

Table 4
AUC-ROC and sensitivity of six PEth-homologues for ≥84 g alcohol/week in the past four weeks for male and female patients.

	AUC-ROC		sensitivity (%)	
	male (n = 114)	female (n = 91)	male (n = 38)	female (n = 23)
PEth 16:0/18:1	0.92	0.94	92	92
PEth 16:0/18:2	0.87	0.94	81	92
PEth 16:0/20:4	0.79	0.89	65	83
PEth 18:0/18:1	0.77	0.93	57	90
PEth 18:0/18:2	0.79	0.90	62	83
PEth 18:1/18:1	0.72	0.90	48	83

least 350 g/week, n = 10), PEth 16:0/18:1 and 16:0/18:2 were positive in all cases, whereas CDT was negative in all (<2.0%).

3.2.5. MeOH, EtOH

MeOH was found to be positive (≥5 μg/ml) in one sample with a value of 18 μg/ml. In this case PEth 16:0/18:1 and uEtG and were also positive with high concentrations. One patient was found to have a blood alcohol concentration of 0.2 ‰. Urine of the patient could not be sampled. Both PEth 16:0/18:1 (concentration 426 ng/ml) and hEtG (84 pg/mg) were positive.

4. Discussion

This study evaluated the diagnostic performance of PEth (six of its homologues) in comparison to other alcohol markers based on self-reported alcohol consumption in patients with liver diseases.

4.1. Specificity and sensitivity of PEth

The observed specificity of PEth was 98% for detecting alcohol consumption of >24 g/week in the past four weeks and even 100% when considering three months prior to blood sampling. This is especially important as false-positive results might wrongfully lead to denial of a liver transplant. On the other hand, in this study the observed sensitivity of PEth 16:0/18:1 for consumption of >24 g/week during the past week (75%) and past four weeks (58%) was rather low. This contrasts with a previous study of our group in pre- and post-transplant patients with alcoholic liver disease [22] which revealed a PEth 16:0/18:1 sensitivity of 100% despite of using a higher cut- off level of 20 ng/ml instead of 10 ng/ml. So, many more patients admitting alcohol consumption tested negative for PEth in this study. Due to poor chemical stability of PEth in whole blood, pre-analytical deterioration of the target analyte can reduce analytical outcome in PEth analysis [23] and impact sensitivity.

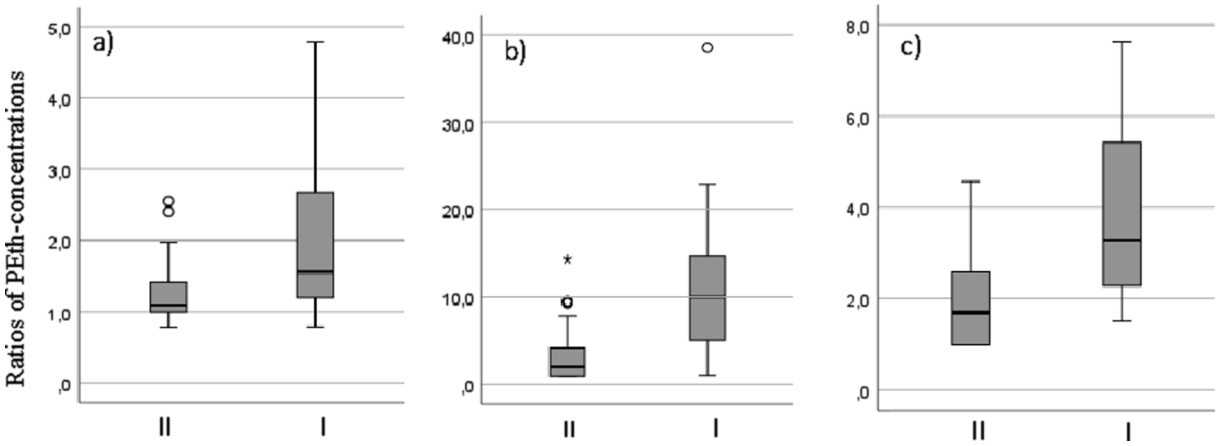

Fig. 5. Boxplots of the ratios of a) 16:0/18:2, b) 16:0/20:4 and c) 18:0/18:2 to 16:0/18:1 comparing I) patients who stopped consumption one week before the appointment and II) patients who drank consistently until the appointment.

Table 5
Specificity and sensitivity of uEtG (500 ng/ml), hEtG (5 pg/mg) and PEth (10 ng/ml).

		specificity (%)		sensitivity (%)	
		≥24 g	≥84 g	≥24 g	≥84 g
Last week	uEtG	100	96	28	41
	uEtG or PEth	93	79	77	88
	PEth	93	80	75	88
Last three months	hEtG	90	89	37	57
	hEtG or PEth	95	87	55	93
	PEth	100	90	53	90

In this study, DBS were generated at the site of sampling within four hours, so influence on sensitivity would be negligible. But there are other explanations for the lower sensitivity in this compared to our previous study. Firstly, it is possible that in the previous investigation the amount of alcohol intake of patients was higher and therefore more likely to be detected. The exact ethanol intake amount was not given in detail, so direct comparison is not possible. The previous study only included patients with ALD and according to the current study ALD patients were generally found to consume more alcohol than NAFLD patients. Secondly, it is possible that in the previous study, which included only patients in the transplant setting, patients were more likely to conceal their alcohol consumption out for fear of negative consequences. Indeed, overall, more patients (50%) admitted to alcohol consumption in this study compared to the previous one (19%). Other authors calculated a sensitivity of 79% for PEth for any drinking in the past four weeks (cut-off 8 ng/ml), with medians of alcohol amount being similar to the presented study (66 g/week and 70 g/week) [13].

Generally, referencing alcohol consumption to self-reports is one of the most critical issues in alcohol biomarker studies. Underreporting of alcohol consumption due to patients' fear of stigmatization is usually assumed. In addition, retrospective questionnaires on alcohol consumption might be difficult to fill out for some patients as estimating the amount of ingested alcohol after several weeks could be a challenge, especially for patients who drink moderately, and do not give special attention to their consumption behavior. This might especially apply to the NAFLD patients in this study. This is a general limitation to the study, which could be avoided by having participants fill out a drinking journal during the questioned time frame. In a study by Walther et al. [24] correlation of PEth was a lot better to alcohol consumption documented in a diary than to the retrospective consumption data, with correlations of 0.56 and 0.23 respectively.

A quantifiable PEth concentration excludes abstinence, but due to its relatively long half-life it might still be detectable after several weeks, depending on the concentration at the onset of abstinence. This was probably the case with patients in this study who claimed abstinence in the four weeks prior to blood sampling. Therefore, a patient's statement of abstinence for four weeks should not immediately be questioned because of detectable PEth.

4.2. PEth-homologues

There was a significant correlation of the amounts of ingested alcohol and all PEth homologue concentrations. This is in accordance with the results of other studies regarding PEth 16:0/18:1 [25,26] and supports its ability to estimate drinking patterns.

Concentration ratios of PEth 16:0/18:1 to the homologues 16:0/18:2, 16:0/20:4 and 18:0/18:2 could be promising in respect of estimating consumption time, since the concentration ratios were found to be markedly lower if alcohol was consumed during the week prior to blood sampling compared to abstinence during that week. This supports the use of PEth homologues in estimating timing of abstinence onset. Our data is in accordance with the observations of Javors et al. [27] and Hill-Kapturczak et al. [28] who studied synthesis and elimination of PEth 16:0/18:1 and 16:0/18:2. Since PEth 16:0/18:2 showed a faster initial synthesis rate and a shorter half-life than PEth 16:0/18:1, the authors concluded that this could be used to specify information about ingestion times.

4.3. Differences between sexes

In this study males consumed significantly more alcohol, but none of the PEth homologue concentrations differed significantly between the sexes. This may be because women's blood alcohol concentrations (bac) are averagely higher after consumption of equal amounts of alcohol, due to a lower distribution volume for ethanol. Higher bac leads to higher PEth concentrations. Sex was reported not to influence the diagnostic performance of PEth 16:0/18:1 in previous studies [6,29]. By comparing sensitivities and specificities between males and females, this was also observed in the current study. There was also no significant difference between the AUC-ROCs of the other homologues (p-value of 18:1/18:1 was 0.05 though). The sensitivities of all homologues, but 16:0/18:1, were higher in females, which means they detected more right positives in females than males. To our knowledge no other study has so far investigated these other homologues concerning sex.

4.4. Cut-off for PEth 16:0/18:1

In 3.2.1 it is shown that specificity was barely increased using 20 ng/ml or even 35 ng/ml as cut-off level when testing for abstinence. This

implies, that the currently recommended cut -off of 35 ng/ml could be lowered to improve sensitivity. Studies that investigate influence of ethanol uptake from alternative sources, like hygiene products or foods are still rare. Reisfield et al. [30] studied the influence of ethanol-containing mouthwash. In one of the 25 participants PEth 16:0/18:1 was 12 ng/ml after using the mouthwash four times per day for 12 days, which is above our suggested cut-off of 10 ng/ml. Several potential reasons for the increase in PEth are described by the authors, though. Nevertheless, as suggested in the study, potential heterogeneity in PEth response to small amounts of extraneous ethanol exposure should be further investigated.

4.5. UEtG vs PEth

Strikingly, sensitivity of uEtG for any consumption during the week prior to sampling was very low at 28%. In previous studies it was much higher at 71% and 86% [17,22]. Because EtG has a detection window in urine of approximately two to three days, the difference could arise from the day on which the alcohol was consumed during the week. Furthermore, the amount of ingested alcohol could have influenced the different outcomes. Bacterial infections of the urinary tract can cause degradation of EtG resulting in false-negative uEtG results [31,32]. Additionally, high urine dilution or medication with diuretics leads to reduced detection of EtG in urine [33]. Because there is no reason for a larger number of false negatives to exist in this study as compared to others, these are weak explanations for the low sensitivity. On the other hand, UEtG was the only positive marker in two patients. The amount of consumed alcohol (24 and 36 g/week) during the four weeks before blood sampling apparently was not enough in these patients for PEth to be quantified > LOQ. On the other hand, the consumption reported during the last week might have taken place in the days before the doctor's appointment, so urine was sampled within the detection window of uEtg. This demonstrates the benefit of uEtG analysis in addition to PEth's.

4.6. HEtG vs PEth

HEtG is a well-established alcohol-consumption marker. Its use however is limited because of sample availability, as a certain quantity and length of hair is required for analysis. Furthermore, hair that has been chemically treated is not suitable for EtG analysis [34,35]. In this study PEth 16:0/18:1 presented better sensitivity, specificity, and AUC-ROC than hEtG for detecting alcohol consumption in the three months prior to the appointment. As hEtG alone detected alcohol consumption in two cases there is value in testing hEtG in addition to PEth. HEtG has been shown to be influenced by kidney function [36]. In a study of Mosebach et al. [18] patients with suboptimal GFR had higher concentrations of hEtG. This is thought to be due to slow elimination secondary to inadequate kidney function, giving it more time to incorporate into hair matrix. This might have been the case for four patients in this study who had GFRs <50 ml/min and who tested positive for hEtG but claimed abstinence during the past three months. Other individual factors have been demonstrated to influence hEtG interpretation, such as obesity, which could have been the case for the other false-positive patient with a BMI of 31 kg/m^2, and a reduced rate of hair growth, which can be a symptom of kidney or liver disease [37]. As they primarily detect consumption in different time frames and are both known to be able to differentiate between excessive and light drinking, hEtG and PEth complement each other well and can be used together to potentially estimate drinking patterns.

4.7. CDT vs PEth

CDT did not have any additional use in detecting alcohol consumption in the context of abstinence testing, as it was never positive without PEth being positive as well. As such, these findings support the presumption of Arnts et al. [6] that PEth will soon gain importance over CDT.

5. Conclusion

All in all, sensitivity of the investigated alcohol consumption markers was lower than expected in this study. Nevertheless, PEth yielded the best sensitivity and specificity for consumption during all time periods prior to blood sampling. Especially the number of cases in which alcohol consumption was solely detected by PEth (n = 33), underlines the benefit of integrating PEth into standard alcohol marker measurement. This is supported by its easy sample handling and costs which align to other biomarker analysis.

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CRediT authorship contribution statement

Nadine Aboutara: Methodology, Formal analysis, Investigation, Writing – original draft, Visualization. **Anne Szewczyk:** Investigation. **Hilke Jungen:** Investigation. **Amadea Mosebach:** Methodology, Investigation. **Maria Rodriguez Lago:** Methodology, Investigation. **Eik Vettorazzi:** Formal analysis. **Stefanie Iwersen-Bergmann:** Conceptualization, Resources, Writing – review & editing. **Alexander Müller:** Conceptualization, Writing – review & editing, Supervision. **Martina Sterneck:** Conceptualization, Resources, Writing – review & editing, Supervision.

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.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cca.2021.11.013.

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