ORIGINAL ARTICLE

Improved detection of alcohol consumption using the novel marker phosphatidylethanol in the transplant setting: results of a prospective study

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SUMMARY

Phosphatidylethanol (PEth) is a new, highly specific alcohol marker. The aim of this study was to assess its diagnostic value in the liver transplant setting. In 51 pre- and 61 post-transplant patients with underlying alcoholic liver disease PEth, ethanol, methanol, carbohydrate-deficient transferrin (CDT), and ethyl glucuronide in urine (uEtG) and hair (hEtG) were tested and compared with patients' questionnaire reports. Twenty-eight (25%) patients tested positive for at least one alcohol marker. PEth alone revealed alcohol consumption in 18% of patients. With respect to detection of alcohol intake in the preceding week, PEth showed a 100% sensitivity. PEth testing was more sensitive than the determination of ethanol, methanol, CDT or uEtG alone [sensitivity 25% (confidence interval (CI) 95%, 7–52%), 25% (7–52%), 21% (6–45%) and 71% (41–91%), respectively], or ethanol, methanol and uEtG taken in combination with 73% (45–92%). Specificity of all markers was 92% or higher. Additional testing of hEtG revealed alcohol consumption in seven patients, not being positive for any other marker. Phosphatidylethanol was a highly specific and sensitive marker for detection of recent alcohol consumption in pre- and post-transplant patients. The additional determination of hEtG was useful in disclosing alcohol consumption 3–6 months retrospectively.

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alcoholic liver disease, carbohydrate-deficient transferrin, cirrhosis, ethyl glucuronide, methanol, phosphatidylethanol

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Introduction

Alcoholic liver disease (ALD) is one of the most common indications for orthotopic liver transplantation (OLT) in Western countries [1,2]. In order to assess the patient's willingness and ability to stay abstinent on a long-term basis, a psychological and/or psychiatric evaluation is performed, and if necessary, professional addiction treatment is offered. In addition, current German legal transplant guidelines require confirmation of

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alcohol abstinence by negative urine ethyl glucuronide (uEtG) tests prior to transplantation. Furthermore, determination of additional alcohol markers for assessment of sobriety, in particular EtG in hair and carbohydrate-deficient transferrin (CDT), is recommended [3].

Post-OLT monitoring of alcohol consumption by alcohol markers has also been found to be an important tool for detection of a relapse [4–7], which occurs in 5–21% of patients [8–11]. In case timely psychological support is not offered, severe graft injury occurs [12] resulting in poor patient survival prognosis [13].

Currently, a variety of direct and indirect alcohol markers is available. Both ethanol and methanol which can detect alcohol intake for up to 48 h [14] were found to have only a sensitivity of 22% in the transplant setting [4].

In comparison, uEtG is much more sensitive and reliable in pre- and post-Tx patients [4]. The ethanol conjugate ethyl glucuronide can be detected in the urine over a time period of up to 3–5 days [4,15–17] and allows also detection of small amounts of alcohol (<5 g ethanol) [18]. The sensitivity and specificity at the 0.5 mg/l cut-off range from 89% to 100% and 76% to 98%, respectively [4,17,19]. However, uEtG determination has several limitations including detection of extremely small, unintentionally ingested amounts of alcohol, alterations secondary to renal function, as well as false-negative or false-positive results related to urine sampling and urinary tract infections [19,20].

In contrast to the alcohol markers uEtG, ethanol and methanol, ethyl glucuronide detection in scalp hair (hEtG) allows retrospective assessment of chronic alcohol consumption over a time period of 3–6 months [21,22]. Hair EtG was found to be very sensitive (94–100%) and highly specific (99–100%) [22–26]. However, a hair sample of sufficient length (3 cm) may not be available. Furthermore, concentrations of EtG in hair may be reduced by hair treatment such as dyeing, perming [27], thermal straightening [28] and nonoxidative hair colouring [29] and may increase due to a reduced kidney function [30].

In addition, determination of CDT can be used for detection of alcohol misuse with a daily intake of more than 60 g ethanol over a time period of 2–6 weeks. Previous studies evaluating CDT in the transplant setting revealed high specificity of 84–96%, respectively, but only limited sensitivity ranging between 41% and 66% [31–34].

Nowadays, also phosphatidylethanol (PEth), a homologue phospholipid group that is formed solely in the presence of ethanol, has been applied for detection of alcohol consumption [35,36]. PEth is measured in a whole blood sample and can detect alcohol consumption up to 3 weeks in alcohol misuse patients, and up to 3–12 days after a single drinking event yielding approximately 1.0 g/kg blood alcohol concentration [37]. The PEth level correlates well with the amount of alcohol consumed and was shown to have a high sensitivity of 94–100% [38–40]. Comasco *et al.* [41] described a specificity of 96% in an adolescent population of 200 healthy students. Also in a study of 222 patients with underlying liver disease and cirrhosis, specificity of PEth was extremely high with 96% [42]. To date, blood PEth level seems not to be influenced by age, gender, kidney diseases or drug intake [17,43,44].

In view of these advantages, the aim of this study was to evaluate the new alcohol marker PEth in a pre- and post-transplant setting and to compare its diagnostic value to the established alcohol markers.

Patients and methods

Patients

In this prospective study, patients were included who presented to the outpatient transplant clinic of the University Hospital Hamburg-Eppendorf between October 2015 and February 2016 either with liver cirrhosis due to ALD or for a yearly check-up visit after OLT for ALD. The study was approved by the local ethics committee (PV5068), and all patients gave written informed consent.

Questionnaire

In this study, a three-page questionnaire with adapted AUDIT elements was used. The questionnaire provided information on the patient's alcohol consumption (i) during the last 3 months, (ii) during the last 4 weeks or (iii) in the last week. In addition, factors possibly interfering with alcohol markers, for example use of alcohol-containing disinfectants, hair treatments or medication intake, were explored.

Determination of alcohol consumption markers

Phosphatidylethanol was determined from dried blood spots (DBS) prepared from lithium heparin blood samples. A previously described validated method by online solid-phase extraction followed by liquid chromatography–tandem mass spectrometry (online-SPE-LC-MS/MS)

in whole blood samples was modified for DBS analysis [37].

Briefly, blood samples were drawn and four spots of 20 μl of whole blood each were prepared on the day of sampling. After drying for at least 4 h, the DBS were stored at room temperature with desiccant for a maximum of 6 weeks until analysis. Two homologues of PEth (16:0/18:1 and 16:0/18:2) were analysed by online-SPE-LC-MS/MS with D_5-PEth 16:0/18:1 and D_5-PEth 16:0/18:2 as internal standards [45]. The calibration range was between 20 and 2000 ng/ml, and the limit of detection (LOD) and the limit of quantification (LOQ) were 10 and 20 ng/ml, respectively. Concentrations above 20 ng/ml were regarded as positive.

For hEtG determination, scalp hair samples were collected by cutting a 0.5-cm-thick hair strand close to the skin. The proximal 3-cm hair segment was analysed for EtG reflecting alcohol consumption 0–3 months prior to sampling (at maximum 6 months, considering hair in the resting state). In accordance with international standards [27], the following cut-off values for hEtG were applied: <7 pg/mg corresponds to a negative result indicating abstainers or rare drinking, 7–30 pg/mg corresponds to a positive result which strongly suggests repeated alcohol consumption, and >30 pg/mg corresponds to a highly positive result indicating excessive chronic consumption (consumption of an average of 60 g or more ethanol/day), respectively.

Ethyl glucuronide in hair was determined by a validated procedure [46]. In brief, hair samples were decontaminated and reduced to fine snippets. Aliquots of 50 mg were extracted with water at 60°C for 12 h and subsequent ultrasonication. After filtration and evaporation of extraction solvent, EtG was analysed by LC-MS/MS (Xevo TQ-S; Waters, Eschborn, Germany) using D_5-EtG as internal standard. Limits of detection and quantification were 1.7 and 4.7 pg/mg, respectively.

EtG in urine, and ethanol (EtOH), methanol (MeOH) and CDT in serum were determined as described previously [4], using cut-off values of ≥0.5 mg/l, ≥0.1 g/kg, ≥5 mg/l and >2.6%, respectively.

Determination of biochemical markers

Bilirubin, albumin, INR, gamma-glutamyltransferase (γ-GT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), mean corpuscular volume (MCV) and serum creatinine were analysed. The glomerular filtration rate (GFR) was calculated according to Cockcroft-Gault, the Model of End-Stage Liver Disease Score (MELD Score) was calculated using INR, serum bilirubin and serum creatinine.

Statistical analysis

Statistical calculations were conducted with Microsoft Excel (Microsoft Corporation, Redmond, WA, USA). *P*-values less than 0.05 were regarded as significant. McNemar test was conducted with SPSS (IBM Corp, Armonk, NY, USA). Adjustment for multiple comparisons was made using Holm's procedure.

For statistic calculation, missing samples were not included and were not defined as negative result.

Assessment of marker results as 'true positive'

As 'true alcohol consumption', we defined admitted alcohol intake within the respective defined time period (i.e. the week before testing for EtOH, MeOH, uEtG, PEth, last 4 weeks before testing for CDT and 3 months before testing for hEtG) or detection of more than one positive alcohol marker.

Results

Patient characteristics

A total of 112 patients were included in this study. Sixty-one patients had undergone liver transplantation (OLT) for ALD, 51 patients presented with ALD-related cirrhosis prior to a potential OLT, and seven of these patients were already on the OLT waiting list. Blood samples from these 112 patients were analysed for EtOH, MeOH, CDT and PEth (100%). Urine samples were available in only 107 cases (96%). A hair sample of a minimum length of 3 cm could be obtained from only 76 patients (68%) due to either objection of the patient ($n = 2$), lack of feasibility due to short hair ($n = 22$) or nonavailable samples ($n = 12$). The questionnaire was filled in by 98 (88%) patients. Detailed patient characteristics are given in Table 1.

Patient's statements

Forty-four of 51 pretransplant patients (86%) and 54 of 61 transplant recipients (89%) returned the questionnaire. Of the 98 patients who completed the questionnaire, 19 (19%) admitted alcohol consumption within the preceding 3 months. In addition, one of 14 patients, who did not fill in the questionnaire, admitted alcohol

Table 1. Patients' characteristics.

Characteristics	Total (n = 112)	Pre-transplant (n = 51)	Post-transplant (n = 61)
Sex, male no. (%)	69 (61.6)	31 (60.7)	38 (62.2)
Age (years), median (range)	59 (37–76)	58 (37–71)	63 (50–76)
Creatinine (mg/dl), median (range)	1.18 (0.56–6.22)	1.08 (0.56–6.22)	1.44 (0.7–5.99)
GFR stage 3b or worse, no. (%)	29 (25.9)	7 (13.7)	22 (36.1)
MCV (fl), median (range)	91 (74.4–118.3)	94.3 (80.2–118.3)	86.6 (74.4–108.8)
Bilirubin (mg/dl), median (range)	0.7 (0.2–22.7)	1.3 (0.3–22.7)	0.5 (0.2–6.6)
Albumin (mg/dl), median (range)	35 (14–42)	32 (14–41)	36 (24–42)
ASAT/GOT (U/l), median (range)	27 (5–182)	40 (7–182)	20 (5–74)
ALAT/GPT (U/l), median (range)	28 (9–209)	31 (11–117)	26 (9–209)
γ-GT (U/l), median (range)	66 (12–2519)	82 (30–2519)	46.5 (12–1184)
MELD Score, median (range)		12.1 (6.4–27.7)	
Time post-transplantation (years), median (range)			6 ± (1–20)

consumption when seen by his doctor. There was no difference between the declarations in the questionnaires of pretransplantation (confession of alcohol consumption n = 9) and post-transplantation patients (confession of alcohol consumption n = 10). Eleven patients admitted consumption of alcohol during the preceding week (six pretransplant and five post-transplant patients), while seven patients stated that they had consumed alcohol within the preceding 4 weeks, but not within the week prior to presentation to the outpatient clinic (three pretransplant and four post-transplant patients). Additionally, one pretransplantation patient claimed that he had not consumed alcohol within the preceding 4 weeks, but he admitted alcohol consumption within the three-months time period prior to presentation.

Results of the alcohol markers

Twenty-five percentage (28/112) of patients tested positive for at least one alcohol marker (Fig. 1). There was no significant difference between pre- and post-OLT patients (25.4% vs. 24.5%; NS). None of the seven patients on the transplant waiting list tested positive for any of the alcohol marker.

The traditional markers EtOH, MeOH and CDT were positive in only seven of these 28 alcohol marker-positive patients (25%), and were never the only positive marker indicating alcohol consumption. In all these cases, alcohol consumption was also detected by PEth.

Urine EtG was detected in 11 of the 28 (40%) alcohol marker-positive cases and was in one case (a patient who denied alcohol consumption) the sole positive marker. All other 10 patients tested additionally positive for PEth.

A positive hEtG result indicating alcohol consumption of the preceding 3 months was present in 16 of the 28 cases (57%). It was the only positive marker in as many as seven (25%) patients.

Phosphatidylethanol was the marker which most often tested positive with 20 of 28 patients (71%) having a positive result. In all PEth-positive cases, PEth homologue 16:0/18:1 was the predominant homologue. Importantly, PEth was the only positive alcohol marker in six (21%) cases, but, in one of these patients, no urine sample was available for testing.

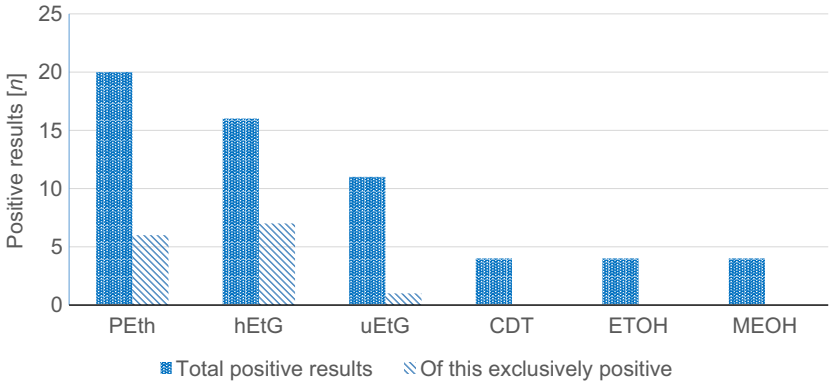

Figure 1 Distribution of positive results of alcohol marker.

In eight cases, alcohol consumption was not detected by PEth, but only by a positive uEtG ($n = 1$) or a positive hEtG ($n = 7$) result. However, hEtG and PEth reflect different time periods, so that these later seven patients probably had stopped alcohol consumption approximately 1–3 weeks prior to presentation.

Correlation between questionnaire and results of the alcohol markers

In 16 of the 19 patients (84%) admitting alcohol consumption, at least one marker was found to be positive (Fig. 2). All of the 11 patients who admitted alcohol consumption up to the week before presentation were found to be positive for PEth, while uEtG was positive in seven of these cases. Furthermore, hEtG tested positive in seven of seven available hair samples in this group. EtOH and MeOH were positive in only four patients.

Of the seven patients who admitted alcohol consumption within the preceding 4 weeks, but not within the preceding week, three patients tested positive for PEth. This marker is thought to stay positive for up to 3 weeks so that fewer patients tested positive than expected. On the other hand, only one of these seven patients had a positive CDT result, although the consumption had been within the window of detection of this marker. This may be due to consumption of smaller quantities of alcohol than 60 g/day. Furthermore, three of the seven patients were positive for hEtG indicating long-term alcohol consumption. As could be expected, none of the patients had a positive uEtG result.

The one pretransplantation patient who claimed alcohol consumption within 3 months prior to presentation showed, as should be expected, a positive hEtG result, while all other alcohol markers were negative (CDT sample was not available).

Positive alcohol markers in patients with negative alcohol statements

Of the 79 patients who claimed in the questionnaire that they had not consumed any alcohol in the preceding 3 months, 14% (11) had at least one positive alcohol marker. Furthermore, one of five patients not returning the questionnaire had a positive alcohol marker. Taken together, 12 of 83 (15%) of patients not admitting alcohol consumption tested positive for an alcohol marker (Fig. 3).

False-positive results

Analytical results of nine patients were declared as 'false positive' according to the above-mentioned definition, because the positive results (one patient only uEtG positive, five patients only hEtG positive, and three patients only PEth positive) were not supported by patients' statements for the relevant time period or by a positive result of a second marker.

Diagnostic value of the alcohol markers

As shown in Fig. 4, EtOH could be detected in 4% of the patients. No additional patient was detected applying the marker MeOH. When blood CDT values were also considered, an additional 2% of patients were found to have a positive result. The additional determination of EtG in urine revealed another 5% positive test

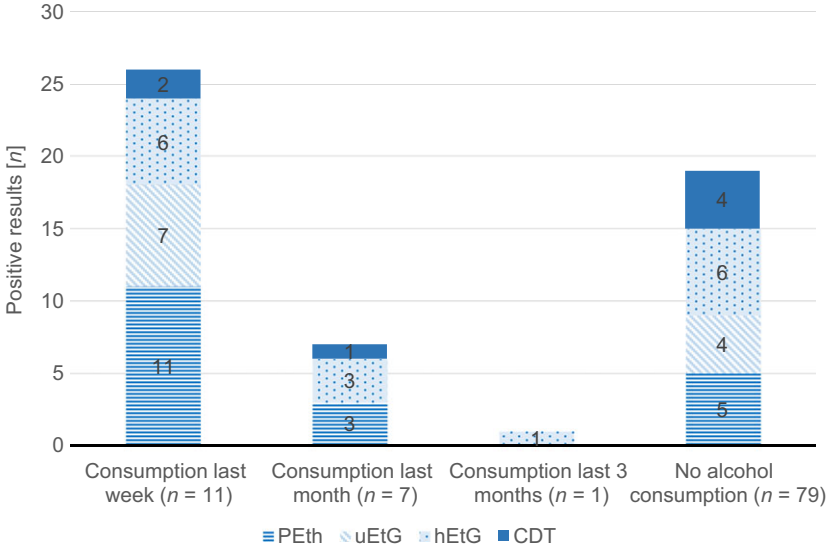

Figure 2 Interview statements compared with marker distribution (multiple mentions are possible).

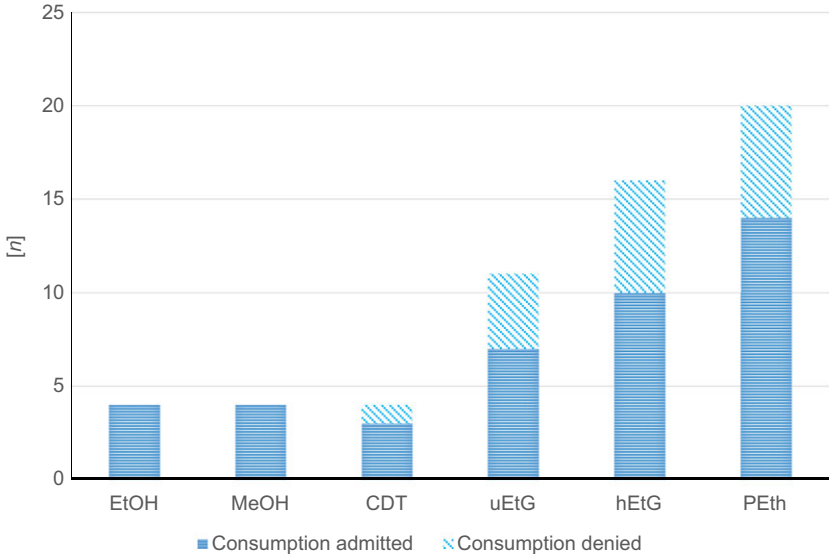

Figure 3 Alcohol detection through marker compared with interview statement (multiple mentions are possible).

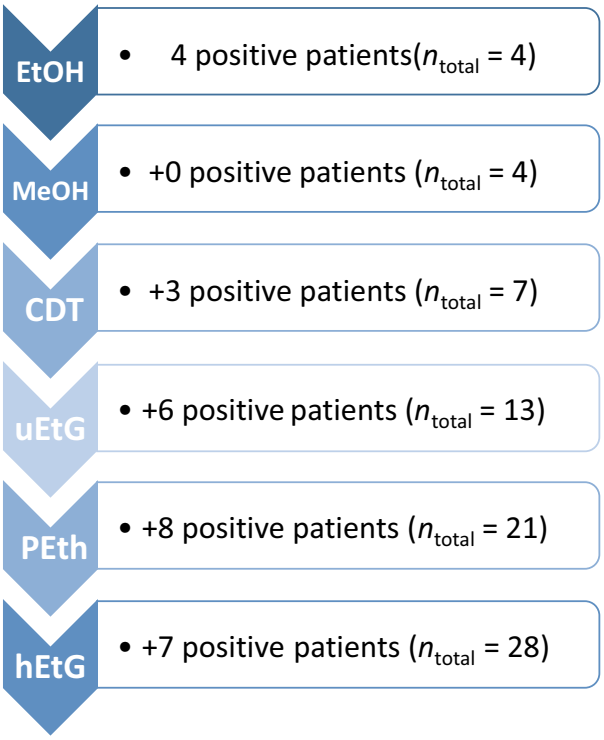

Figure 4 Additional positive alcohol consumption detected by an additional marker.

results. The analysis of PEth led to a further increase in the detection of alcohol intake by 7%. Because hEtG determination reflects a different detection period, the analysis of hEtG further increased the positive results by an additional 6%. Using only PEth and hEtG, alcohol consumption was detected in 27 of the 28 (96%) alcohol marker-positive cases.

Sensitivity and Specificity

To compare the different alcohol markers with regard to their diagnostic value, we calculated sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) (Table 2). Additionally, a comparison by McNemar test was conducted. As 'true alcohol consumption', we regarded either admitted alcohol intake within the respective defined time period or detection of more than one positive alcohol marker. Negatively stated, all cases with no admitted alcohol consumption in the respective detection period, and/or no second positive alcohol marker were regarded as 'false positive'. In fact, of course, this does not exclude alcohol consumption, it only indicates that alcohol consumption was not detected by the chosen testing procedures or the marker concentrations did not exceed the applied cut-offs.

Specificity of all markers was almost 100%, since often a second marker confirmed alcohol consumption (Table 2). For PEth, specificity was 96% and therefore slightly lower than that of the other traditional marker in blood and urine.

Phosphatidylethanol showed by far the highest sensitivity of all markers (100%). In comparison, the sensitivity of uEtG and hEtG was 71% and 84%, respectively. EtOH, MeOH and CDT showed by far the lowest sensitivity (25%, 25% and 21%, respectively). For this calculation, missing urine and hair samples were not included. If missing samples were to be treated as equivalent to a negative result, sensitivities for uEtG and hEtG would be clearly lower with 63% and 50%, respectively.

Table 2. Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) calculated with exact 95% confidence interval (95% CI) (Clopper–Pearson).

Marker	Sensitivity [%] (95% CI)	Specificity [%] (95% CI)	PPV [%] (95% CI)	NPV [%] (95% CI)
PEth	100 (79–100)	96 (91–99)	85 (62–96)	100 (96–100)
uEtG	71 (41–91)	98 (94–100)	90 (58–99)	95 (89–98)
hEtG	84 (54–98)	92 (82–97)	68 (41–89)	96 (88–99)
EtOH	25 (7–52)	100 (96–100)	100 (39–100)	89 (81–94)
MeOH	25 (7–52)	100 (96–100)	100 (39–100)	89 (81–94)
CDT	21 (6–45)	100 (96–100)	100 (39–100)	86 (78–92)
Combination of uEtG, EtOH, MeOH	73 (45–92)	98 (94–100)	91 (61–100)	95 (89–99)

Time periods of detection were defined as following: EtOH, MeOH, uEtG, PEth, alcohol consumption in the last week; CDT, alcohol consumption during the last 4 weeks; hEtG, alcohol consumption during the last 3 months.

The higher sensitivity of PEth in comparison with the other markers (EtOH, MeOH, uEtG) is statistically significant ($P < 0.001$; $P = 0.046$ and $P = 0.046$, respectively). Likewise, the observed higher sensitivity of uEtG in comparison with EtOH and MeOH can be considered to be significant ($P = 0.027$). Significances were analysed using a McNemar test with subsequent Holm's correction.

Discussion

In current practice, many transplant centres regularly test alcohol markers in ALD patients pre- and post-transplantation to monitor sobriety of their patients. In Germany, testing of uEtG in ALD patients on the transplant waiting list has even been made a legal requirement [3]. Despite its high sensitivity and specificity, uEtG has some disadvantages. False-negative uEtG results can occur due to bacterial degradation of EtG, in less than perfectly chilled storing conditions [21,22], because of high urine dilution caused intentionally or unintentionally by the patient, or by diuretic medication. Furthermore, false-positive uEtG tests may be a result of the high test sensitivity. Consuming ethanol-containing mouthwash solutions, sweets or excessive consumption of fruits/vegetables as well as baker′s yeast may produce a significant uEtG level [15].

Phosphatidylethanol, on the other hand, is a blood test with an extremely high specificity. The synthesis of PEth requires the presence of ethanol, and there are no known influencing factors [47]. Recent studies reported a high sensitivity between 70% and 99% for detection of alcohol consumption over a period of up to 3 weeks [48]. Therefore, the aim of this study was to test this promising direct alcohol marker PEth in comparison with the established alcohol markers uEtG, MeOH, EtOH, CDT and hEtG in pre- and post-transplantation patients.

In our cohort of 112 patients, 25% (13/112) of pre- and 26% (15/112) of post-OLT patients were found to test positive for at least one alcohol marker, while only 19 of 112 (17%) patients admitted consumption in the questionnaire.

Phosphatidylethanol tested positive in 20 (18%) patients and achieved a sensitivity of 100% for detecting alcohol consumption in the preceding week. Thereby, PEth outperformed EtOH, MeOH, uEtG and CDT which can also detect alcohol intake within this time period. Even when considering the markers EtOH, MeOH and uEtG together (sensitivity = 73%), the sensitivity of PEth alone (100%) was markedly higher. Applying a combination of EtOH, MeOH, CDT and uEtG, only 13 cases would have been detected. Analysing for PEth almost doubled the detection rate for alcohol consumption in the week prior to testing. Measurement of uEtG alone reached in this study a sensitivity of 71%. This is markedly worse than in our previous studies where we saw a sensitivity of 89% [4] and 86% [24]. A possible explanation could be the fact that patients in our outpatient transplant clinic are now being informed of the diagnostic window of uEtG, and therefore choose to remain abstinent more than 1 or 2 days prior to presentation. Additionally, in this study, urine sampling was carried out without visual control by the personnel, so sample manipulation or exchange cannot be excluded.

However, for testing transplant candidates, the specificity of a test is of upmost importance. It is crucial that a patient will not be wrongly accused of drinking alcohol and subsequently be taken off the waiting list

Table 3. Detailed information for three patients with only phosphatidylethanol (PEth) positive results.

Patient no.	Questionnaire statement	PEth in blood (ng/ml) (16:0/18:1| 16:0/18:2)	hEtG (pg/mg)	uEtG (mg/l)	EtOH (g/kg)	MeOH (mg/l)	CDT (%)	Remarks	Our clinical assessment verdict
61	Last week: denied Last month: denied Last 3 months: denied	140|n.n	Hair length too short	0.23	0	<1	1	uEtG detected, but level <0.5 mg/l	True positive Reason: Low positive uEtG
105	Last week: denied Last month: denied Last 3 months: denied	316|82.5	Hair length too short	<0.1	0	<1	1.5	Recent history of consumption and alcohol relapse	True positive Reason: Very high PEth Other positive marker in recent visits
71	Last week: denied Last month: admitted Last 3 months: admitted	122/77.5	Refused	0.1	0	1.1	0.9	False positive only regarding test period of 1 week	True positive Reason: Consumption admitted last month

[49,50]. To date, no proven false-positive PEth values have been recorded either as a consequence of alcohol-containing medications, or as a consequence of comorbidities [17,41,43,44]. Also, in patients with liver diseases and cirrhosis, the validity of PEth remains high [42].

In this study, PEth showed a specificity of 96%. There were three patients who denied alcohol consumption in the preceding week and who only tested positive for PEth, but not for any other alcohol marker. Therefore, by definition, the results were regarded as false positive. However, in all of these cases, we seriously doubt alcohol abstinence (Table 3):

Patient no. 61 had an uEtG level of 0.23 mg/l [determined by immunoassay screening, result was confirmed by LC/MS-MS (0.16 mg/l)], which in most institutions regarded as positive (standard cut-off 0.1 mg/l). Only in the transplant setting, the cut-off level has been increased to 0.5 mg/l to exclude positive uEtG results from accidental alcohol intake. So, the positive PEth is confirmed by an elevated uEtG.

Patient no. 105 was tested highly positive for both homologues of PEth (16:0/18:1 = 316 ng/ml; 16:0/18:2 = 86.2 ng/ml) and in addition tested positive for other alcohol marker at several routine clinical visits to the outpatient clinic before the study visit.

Patient no. 71 admitted alcohol consumption within the last 4 weeks, but not within the last 1 week. As PEth is known to have a detection window of up to 3 weeks, the test result is confirmed by the patient's statement and not false positive. However, by definition we tested the performance within a time period of only 1 week.

If the above-mentioned three PEth results were regarded as truly positive, a specificity of 100% for PEth would be calculated.

The established marker hEtG also performed well in our study. This marker is the only tool to reliably detect alcohol consumption retrograde up to 3 months. In a previous study, we found high specificity (91%) and sensitivity (86%) in liver transplant candidates [24]. For this investigation, hair samples were only available in 68% of patients. On the other hand, EtG in hair detected alcohol consumption in seven additional patients who were not positive for any other marker. Only two of these patients admitted any alcohol consumption, which led to an overall specificity of 92%. Nonetheless, influencing factors such as the impact of thermal hair straightening and other external influences [28] and practical issues should be kept in mind when using this marker.

Regarding PEth analysis, the pre-analytic processing procedure is one important factor which has to be strictly controlled, because dried blood spots should be produced on the day of sampling. Alternatively, the specimen should be stored at −80°C within a very few hours after drawing the blood samples; otherwise, a loss of PEth concentrations can occur. Furthermore, the PEth analysis is not, at this time, a common procedure, and is only performed by a few specialized laboratories in Germany, although the costs are in fact the same as analysing uEtG via LC-MS/MS.

Conclusions

In conclusion, the determination of PEth is a valuable tool which could further improve the assessment of alcohol abstinence and detection of alcohol consumption. PEth outperformed all other markers in terms of sensitivity and number of detected patients and is a reliable alcohol marker. The determination made from a medically drawn blood sample as opposed to a collected urine sample, and the lack of influencing factors are clear advantages in comparison with uEtG testing. Determination of EtG in hair is a valuable additional test to detect alcohol consumption dating back more than several weeks.

Authorship

HA-S: designed study, toxicological analysis of specimen, analysed data and wrote the manuscript. YB: performed study, collected data, analysed data and wrote the manuscript. WW, AS, AM and GS: performed toxicological analysis of specimen and improved the manuscript. SP: performed investigation and interview of patients. EV: provided statistical advice and statistical calculation. AL: added ideas for improving the manuscript. BN: helped in writing the manuscript. MS: designed study, performed study, analysed data, wrote the manuscript.

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Conflict of interest

The authors have declared no conflicts of interest.

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