World Journal of Gastroenterology

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DOI: 10.3748/wjg.v22.i14.3725

World J Gastroenterol 2016 April 14; 22(14): 3725-3734
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

REVIEW

Biomarkers for detection of alcohol consumption in liver transplantation

Katharina Staufer, Michel Yegles

Katharina Staufer, Division of Transplantation, Department of Surgery, Medical University of Vienna, 1090 Vienna, Austria

Michel Yegles, Laboratoire National de Santé, Service de Toxicologie médico-légale 1, rue Louis Rech, L-3555 Dudelange, Luxembourg

Author contributions: Staufer K contributed to conception and writing of article; Yegles M contributed to scientific advise.

Conflict-of-interest statement: No potential conflicts of interest.

Correspondence to: Katharina Staufer, MD, Assistant Professor, Division of Transplantation, Department of Surgery, Medical University of Vienna, A-1090, Waehringer Guertel 18-20, 1090 Vienna,
Austria. katharina.staufer@meduniwien.ac.at
Telephone: +43-1-4040068700
Fax: +43-1-4040068720

Received: December 21, 2015
Peer-review started: December 24, 2015
First decision: January 13, 2016
Revised: January 26, 2016
Accepted: February 20, 2016
Article in press: February 21, 2016
Published online: April 14, 2016

Abstract

Alcoholic liver disease is an established, yet controversial, indication for liver transplantation. Although an abstinence period of up to 6 mo prior to transplantation is mandatory, alcohol relapse after transplantation is a common event. In case of recurrence of heavy drinking, graft survival is significantly impaired. Guidelines on detection and surveillance of alcohol consumption in this patient cohort are lacking. This review summarizes the challenge of patient selection as well as the current knowledge on established and novel alcohol biomarkers with special focus on liver transplant candidates and recipients.

Key words: Ethyl glucuronide; Liver cirrhosis; Short-term alcohol markers; Long-term alcohol markers; Psychological support

Core tip: Currently, consensus statements on alcohol screening prior to and after liver transplantation are lacking. Routinely applied alcohol markers have certain limitations in the setting of liver disease and end-stage cirrhosis. Novel alcohol biomarkers, such as ethyl glucuronide in urine and hair as well as phosphatidylethanol, however, show promise to significantly improve the selection and surveillance of patients within the liver transplant setting.

Staufer K, Yegles M. Biomarkers for detection of alcohol consumption in liver transplantation. *World J Gastroenterol* 2016; 22(14): 3725-3734 Available from: URL: http://www.wjgnet.com/1007-9327/full/v22/i14/3725.htm DOI: http://dx.doi.org/10.3748/wjg.v22.i14.3725

INTRODUCTION

Detection of alcohol consumption and surveillance of alcohol abstention in patients with alcoholic liver

disease (ALD) are at the center of attention of transplant physicians. Liver transplantation (LT) is an established treatment of end-stage ALD, which presents the second most common indication for LT in Western countries[1,2]. Due to the prevalent perception within the population and among treating physicians that ALD is a self-induced disease, and possibly perpetuated by the evident lack of donor organs, this indication is controversial[3]. Therefore, ALD as an indication for LT all the more requires transparent, comprehensible selection criteria. Since alcoholism is a life-long disease and is not cured by LT, optimal selection of patients with a low risk of alcohol relapse as well as continuous monitoring and support after LT are essential.

In order to facilitate optimal patient selection for the life-saving opportunity of LT, patients currently have to undergo a thorough psychological evaluation by an experienced addiction specialist. In addition, an abstinence period of at least 3 up to 6 mo is required in most transplant centers. Nevertheless, alcohol relapse on the waiting list is detected in up to 25%[4-6] and occurs in up to 50% of liver transplant recipients[7,8]. Of these, up to 36% of patients resume heavy drinking[9-11]. Long-term outcome after LT is significantly reduced in the patient cohort with return to excessive alcohol consumption but is similar to other indications despite return to low to moderate alcohol consumption[12,13].

Therefore, our eligibility criteria to list patients need to be refined, and we need more reliable tools to predict the individual risk of relapse to heavy drinking. In addition, surveillance and concomitant psychological support after LT should take a central role. This requires continuous surveillance programs for LT recipients to avoid alcohol relapse as well as tools to maximize rates of early detection of alcohol relapse to prevent graft damage.

Objective direct alcohol parameters could support evaluation by addiction specialists and improve patient selection prior to LT and optimize surveillance and early detection of alcohol consumption in liver transplant recipients.

This review summarizes the challenges of detection and correct assessment of alcohol consumption. It focuses on the current knowledge on alcohol biomarkers in liver disease and their particular value within the liver transplant setting and gives insight into possible future developments.

DIAGNOSIS OF ALD - A DILEMMA?

Correct diagnosis of ALD is critical for individual treatment approaches, but it is sometimes difficult, since specific features are lacking. After ruling out different liver diseases, diagnosis has to be based on patients' statement on alcohol consumption. Yet, reliable objective alcohol parameters for confirmation have not yet found their way into routine clinical use (see below).

According to the current guidelines of the European Association for the Study of the Liver (EASL) and the American Association for the Study of Liver Diseases (AASLD), ALD may diagnosed upon documentation of consumption of > 30 g/d of ethanol as well as the presence of clinical and/or biological signs of liver injury[14,15]. According to EASL guidelines, diagnosis should be based on "clinical" [such as gamma-glutamyl transferase (GGT), carbohydrate deficient transferrin (CDT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), mean corpuscular volume (MCV)], "biological" (serum fibrosis markers), and "ultrasound parameters" (detection of steatosis, grading of parenchymal alterations). Furthermore, liver biopsy might be required for confirmation of diagnosis.

However, all mentioned parameters may be non-diagnostic in the case of mild ALD or early cirrhosis[15,16], and patients are normally asymptomatic until an advanced state of liver disease has been reached. The documentation of regular alcohol intake, however, and especially the assessment of the alcohol amount is difficult. Commonly, patients do not admit alcohol consumption or underreport or do not indicate correct amounts of alcohol intake[17-19]. Furthermore, the amount of alcohol intake and development of liver alterations or severity of liver disease are not strictly linear[20]. Clear diagnosis of alcohol consumption and ALD is complicated by the lack of definite cut-off values of ethanol identified as harmful in certain populations.

In 60% of patients with regular alcohol intake > 60 g/d, hepatic steatosis was found[21,22]. In 29% of a large patient series, liver cirrhosis was detected by liver biopsy in 29% of a large series of patients with alcoholism[23]. Differential diagnosis to non-alcoholic fatty liver disease (NAFLD) (ethanol cut-off: 20 g/d for women, 30 g/d for men) and the assessment of alcohol as an additional hit to the liver are difficult. Even liver biopsy cannot safely discriminate ALD from NAFLD[24,25]. Moreover, in a meta-analysis, daily consumption of > 25 g of ethanol has been associated with an increased risk of liver cirrhosis and its complications[26]. Increased risk of mortality due to liver cirrhosis was recently found even below 25 g/d of ethanol (12-24 g/d)[27]. Thus, patients might be at risk at ethanol levels even below the current public recommendations for alcohol consumption.

REQUIREMENTS FOR SUITABLE ALCOHOL MARKERS IN LIVER DISEASE AND LIVER TRANSPLANTATION

Alcohol markers for detection of alcohol consumption and prediction of alcohol relapse need to fulfill certain features in the setting of ALD and LT. Only patients with a low risk of relapse are eligible for transplant. Patients in whom alcohol relapse is detected within the waiting period may be excluded from the life-

saving opportunity of LT. Since alcoholism is a life-long disease that is not cured by LT, LT recipients should receive close surveillance to provide the possibility of an early detection in the case of relapse. LT recipients with alcohol relapse are at risk for the development of alcoholic hepatitis and re-cirrhosis in the liver graft. Nevertheless, in case of alcohol relapse, patients may be denied liver re-transplantation.

Optimal alcohol biomarkers in the setting of LT should not be influenced by liver alterations, commonly present kidney dysfunction, changes in body composition (low fat and muscle mass in case of end-stage cirrhosis, increased body water in case of ascites), immunosuppressive medication, or multiple drug therapy and should be cheap and easily accessible. In LT candidates who get evaluated for LT or are on the waiting list, the markers need to be highly specific in order not to deny mistakenly a life-saving LT due to false positive alcohol tests. In LT recipients in whom early detection of relapse is the focus to preserve graft function, high sensitivity is of special interest.

GOLD STANDARD FOR DETECTION OF ALCOHOL CONSUMPTION

To date, national and international guidelines for alcohol biomarker screening of patients within the transplant setting are still lacking. Many transplant centers in Europe and the United States routinely use ethanol (EtOH) and CDT as direct markers of alcohol consumption as well as GGT and MCV as indirect markers.

The recommendations of the National Institutes of Health/National Institute on Alcohol Abuse and Alcoholism[28] for ALD patients state: "For screening purposes in primary care settings, interviews and questionnaires have greater sensitivity and specificity than blood tests for biochemical markers, which identify only about 10% to 30% of heavy drinkers. Nevertheless, biochemical markers may be useful when heavy drinking is suspected but the patient denies it." GGT, MCV, and CDT are recommended, nevertheless, to show certain limitations within the transplant setting (see paragraphs on indirect and direct alcohol biomarkers).

ETHANOL METABOLISM

After ingestion, ethanol is absorbed *via* the oral, gastric, and small intestinal mucosa. About 2%-10% of ethanol are excreted *via* urine, sweat, and exhalation air without modification. EtOH detoxification starts within the stomach and is facilitated by alcohol dehydrogenase (ADH) (sigma-ADH, about 5% of ethanol metabolism). The major part of EtOH is metabolized to acetaldehyde within the liver by ADH, catalase, and the so-called microsomal ethanol oxidizing system (MEOS; *via* cytochrome P450 CYP2E1; especially in case of > 50 g/d alcohol intake or chronic alcohol intake). Acetaldehyde is further metabolized to acetic acid *via* acetaldehyde dehydrogenase. Acetic acid is metabolized *via* the citrate cycle and the mitochondrial respiratory chain and exhaled as CO_2. A very small amount (< 0.1%) of ethanol undergoes conjugation reactions with glucuronic acid in the presence of membrane-bound mitochondrial uridine diphosphate glucuronyl transferase to produce ethyl glucuronide (EtG) and with sulfate to produce ethyl sulfate (EtS), which are excreted in the urine[29-31].

INDIRECT BIOMARKERS OF ALCOHOL CONSUMPTION

State markers such as MCV[32] and the liver function tests GGT, ALT, and AST are widely used for routine screening of chronic alcohol consumption. These indirect markers of alcohol consumption may yet be elevated not only in case of ALD but all other forms of acute or chronic liver disease or vitamin B12 and/or folic acid deficiency. In a recent study on 210 non-excessive drinkers, 272 excessive drinkers, and 76 patients with alcoholic cirrhosis investigating the kinetics of alcohol markers during abstinence, the diagnostic accuracy of GGT to detect excessive drinkers was moderate [area under the curve (AUC) 0.68] and was associated with the amount of alcohol consumed within the past 30 d, which is in contrast to the AST/ALT ratio[33]. In this patient cohort consisting of ALD patients only, GGT reached a sensitivity and specificity of 49.6%, and 83.9%, respectively, for the detection of excessive alcohol consumption[33]. In contrast, in a setting of 141 liver transplant candidates and recipients, different markers were analyzed for their value in screening the consumption of any amount of alcohol, and the specificities of GGT, MCV, and AST were rather low[17]. Besides low specificity for chronic alcohol intake and detection of alcohol relapse[17], these markers have also low sensitivity for recent intake of excessive alcohol amounts[34]. When using these markers, we have to be aware of certain limitations, especially within a patient collective with already significant liver disease or on multiple drug therapy[35,36].

Carbohydrate deficient transferrin

Transferrin is a glycoprotein produced and secreted by the liver. The International Federation of Clinical Chemistry and Laboratory Medicine recommend that CDT should be expressed as CDT divided by the amount of total transferrin to account for individual variations in transferrin levels. In addition, the disialotransferrin glycoform of CDT (one of three isoforms) should be measured since it correlates best with the amount of ingested alcohol, as measured by high performance liquid chromatography (HPLC)[37].

CDT levels reflect continuous heavy drinking of

Table 1 Biomarkers for alcohol consumption used in the liver transplant setting

Biomarker	Biological compartment	Window of detection	Confounding factors	Cut off	Sensitivity	Specificity	Number of patients	Ref.
Indirect markers								
GGT	Serum	-	Liver disease	-	49.6	83.9	558	[33]
				< 55 U/l	85.7	39.7	141	[17]
MCV	Serum	-	Hematological diseases, vitamin deficiencies	< 94 fL	75.0	55.7	141	[17]
AST	Serum	-	Liver disease, muscle alterations	< 35 U/L	67.9	56.3	141	[17]
ALT	Serum	-	Liver disease	< 50 U/L	29.6	76.2	141	[17]
%CDT	Serum	1-2 wk	Hyperbilirubinemia, liver cirrhosis/ disease, smoking, sepsis, anorexia nervosa, airway diseases, rare genetic variants, transferrin levels	⩾ 2.5%	52.7	87.8	558	[33]
				> 2.6%	25.0	98.6	141	[17]
				> 2.6%	-	-	88	[18]
Direct markers								
Breath alcohol	Exhalation air	10-12 h	Alcohol containing mouth wash	⩾ 0.01 g/L	-	-	18	[6]
EtOH	Serum	10-12 h	Unknown	⩾ 0.1 g/kg	-[1]	-[1]	141	[17]
				-	-	-	41	[55]
MeOH	Serum	up to 48 h	Unknown	⩾ 5 mg/L	22.2	99.3	141	[17]
				⩾ 1.5 mg/L	-	-	41	[55]
EtG	Urine	up to 80 h	Heavily impaired kidney function, high amounts of baker's yeast/ sauerkraut, alcohol containing mouthwash, storage above 4 ℃ before measurement	⩾ 0.5 mg/L	-	-	18	[6]
					89.3	98.9	141	[17]
EtG	Hair	up to 6 mo	EtG containing hair treatment, heavily impaired kidney function	⩾ 7 pg/mg	76.0	91.0	88	[18]

[1]No sensitivity/specificity calculated, since only 1 of 141 patients had elevated EtOH. In the case of CDT, only studies using the HPLC method for determination of %CDT are reported. - not reported in the cited manuscript. GGT: Gamma-glutamyl transferase; MCV: Mean corpuscular volume; AST: Aspartate amino transferase; ALT: Alanine amino transferase; CDT: Carbohydrate deficient transferrin; EtOH: Ethanol; HPLC: High-performance liquid chromatography; MeOH: Methanol; EtG: Ethyl glucuronide.

50 to 80 g of EtOH per day over a period of 7 to 15 d[38,39]. After alcohol cessation, CDT normalizes within 2 to 3 wk ($t_{1/2}$: 10 d)[39]. Depending on the assay used, determination of CDT sensitivity and specificity varies. In patients with end-stage liver disease, specificity is as low as 70%, and sensitivity ranges between 46% and 73%[40-43]. A higher sensitivity and specificity of approximately 88% and 95%, respectively, has previously been achieved by combining CDT with MCV and GGT[36,40,43]. CDT is not yet suitable to detect short-term alcohol consumption, even at high doses of 80 g/d[44].

Confounding factors for the measurement of CDT besides liver cirrhosis, primary biliary cirrhosis, and hepatitis C infection are smoking, sepsis, anorexia nervosa, and airway diseases[45]. All of these conditions can lead to false positive test results. False negative results can be caused by obesity, female sex, and pregnancy[46,47]. Furthermore, genetic variants occurring in 1%-2% of patients can lead to impaired test results[48].

DIRECT BIOMARKERS OF ALCOHOL CONSUMPTION

Currently, a range of direct alcohol biomarkers are used that detect EtOH or EtOH metabolites in different body components. We can distinguish between short- and long-term alcohol markers (Table 1).

Short-term alcohol markers

Breath alcohol test: In general, median breath alcohol concentration (BAC) correlates well with blood alcohol concentration, bearing a sensitivity and specificity of 97% and 93%, respectively, in healthy volunteers, although the correlation is weak for individual cases[49]. The sensitivity and specificity of breath analyzers of different manufacturers often vary considerably. BAC may be influenced by consuming food within 4 h prior to testing, a longer time spent drinking, and the number of drinks consumed per hour[50]. Therefore, this test is mainly utilized in the context of drivers accused of driving under the influence and needs to be confirmed by a blood ethanol test if positive. Although breath alcohol testing has the advantage of low costs and ease of use, this method has certain limitations within the setting of LT.

Mainly due to low sensitivity resulting from rapid elimination of EtOH from the body (about 0.1 g/kg per hour), patients might not be detected[51]. In a study by Erim *et al*[6] alcohol breath tests were used as a screening method for alcohol consumption along with self-reports in LT candidates participating in an alcohol dependence group therapy. Nobody admitted alcohol consumption, and breath testing was positive in only one out of 18 patients. Comparing these results to urinary EtG (uEtG) revealed that 50% of patients had positive results. Wetterling *et al*[52] found similar results

by comparing the detection rate of alcohol relapse in patients during a long-term alcohol dependence program: by breath alcohol tests, personal interviews, and uEtG 4.4%, 5.7%, and 37.7% of alcohol relapses were discovered. In 15.6% (265 cases) of patients, alcohol consumption was detected only by uEtG. In three cases, on the other hand, alcohol consumption was only detected by breath alcohol test[52]. These results illustrate that breath alcohol testing in the setting of LT might not be an ideal tool for monitoring abstinence or early detection of relapse.

Blood ethanol levels: EtOH blood concentration is a measure for recent alcohol consumption and remains positive for only a few hours after alcohol intake[51]. This elimination kinetic has to be considered when performing abstinence monitoring. Carbonneau *et al*[4] described the utility of random measurement of blood alcohol levels in 134 liver transplant candidates on the waiting list. Of these, eight patients had detectable serum alcohol levels, three patients admitted alcohol consumption in the presence of negative EtOH levels, and 12 patients refused blood withdrawal. A higher number of random blood alcohol tests was independently associated with a lower risk of alcohol use on the waiting list[4]. Nevertheless, within an LT outpatient setting with scheduled visits, detection of alcohol relapse by elevated EtOH levels may have been limited due to an adaptation of drinking habits to scheduled visits[17,18]. Overall, blood alcohol level is a useful marker in cases of suspected alcoholization.

Blood methanol levels: Methanol (MeOH), a direct metabolite of EtOH, is detectable for up to 2 d but may accumulate in body fluids in the case of continuous heavy drinking[51,53,54]. Very little data are available on patients with liver dysfunction. The sensitivity and specificity of MeOH for detection of alcohol relapse using a cut off of 5 mg/L (HS-GC/FID, Perkin-Elmer) in 141 liver transplant candidates and recipients were reported to be 22% and 99%, respectively[17]. In another study on 41 liver transplant candidates, a cut off of 1.5 mg/L was indicative of probable recent alcohol intake (ruling out other confounding factors) or 3 mg/L (regarded "highly positive") was used[55]. By measuring MeOH, more relapsers were identified than with blood EtOH levels and self-reports by Alcohol Use Disorders Identification Test (AUDIT; 32 *vs* 3 patients; sensitivity and specificity not given by the authors)[55]. MeOH, however, can also be endogenously produced and, therefore, might show false positive results that mistakenly deny transplantation due to suspected alcohol relapse on the waiting list[56]. More data are necessary to reliably rate the value of MeOH within the LT setting.

EtG and ethyl sulfate in urine: EtG is an ethanol conjugate that can be detected up to 36 h in the blood and up to 80 h in the urine, even after complete elimination of EtOH from the body[57,58]. uEtG is detectable after consuming very small amounts of alcohol ($\leqslant$ 5 g)[58,59]. The higher the amount of alcohol intake, the longer the detection window[30,60,61]. It can easily be measured by an immunoassay (lower detection limit 0.1 mg/L) or liquid chromatography-tandem mass spectrometry (LC-MS/MS). uEtG and uEtS show good correlation[62,63].

Since uEtG levels are, to a great extent, independent of liver and kidney function, uEtG is a useful marker within the setting of liver disease and LT. A sensitivity and specificity of 84% and 68%, respectively, were reported with a cut off of 0.145 mg/L in a large cohort of drinkers and non-drinkers with and without liver cirrhosis[59]. Sensitivity and specificity increased to 91% and 77%, respectively, when a cut off of 0.435 mg/L was used in low to moderate drinkers compared to heavy drinkers[59]. In a cohort of LT candidates and recipients using a cut off of $\geqslant$ 5 mg/L of the DRI-EtG-enzyme immunoassay [(EIA; Thermo Fisher Scientific Inc.; Passau, Germany] a sensitivity and specificity of 89% and 99%, respectively, could be reached[17]. In this study, uEtG significantly outperformed all other tested markers, such as GGT, AST, ALT, MCV, CDT, and MeOH in predicting alcohol consumption[17]. The vast majority of patients admitted alcohol consumption only after confrontation with positive test results. A study in 109 LT candidates with ALD showed positive uEtG or uEtS in 20% (cut offs 0.2 mg/L, and 0.1 mg/L), although only 3% of patients indicated alcohol consumption[64]. Another study comparing uEtG, AUDIT-C, EtOH in blood and urine, CDT, and AST, ALT, GGT, and MCV in 121 LT candidates and recipients confirmed the high sensitivity and specificity of uEtG of 89.2% and 98.8%, respectively. Combining uEtG with AUDIT-C further improved detection of alcohol consumption (AUC = 0.94 *vs* 0.98), and additional assessment of CDT had no further benefit (AUC = 0.98)[65].

Although highly sensitive and specific, influencing factors that might lead to false positive or negative uEtG results have to be taken into account. False negative results might occur in the presence of bacterial degradation in case of urinary tract infections (uEtS not altered)[66] but also might occur because of post collection synthesis of bacteria in the urine[67]. However, this *in vitro* reaction can be prevented by refrigerating, freezing, or collecting urine samples in NaF containers[59] or using dried urine on filter paper[68]. Cannabinol, ingestion of high amounts of baker's yeast, sauerkraut, non-alcoholic beer, or alcohol containing mouth washes as well as severe kidney disease can cause false positive results[59,69-71]. Recently, Høiseth *et al*[72] showed that chronic kidney disease increased the detection window of uEtG and uEtS. In 14 patients with an estimated glomerular filtration rate < 60 mL/min per 1.73 m^2 and moderate use of alcohol (up to seven standard drinks per week), uEtG could be detected up to as twice as long as in

healthy volunteers ingesting comparable doses of alcohol.

Long-term alcohol markers

EtG and fatty acid ethyl ester in hair: EtG and fatty acid ethyl ester (FAEE) detection in hair allows for assessment of alcohol consumption for up to 6 mo (about 1 cm length of scalp hair strand per month). According to the recommendations of the Society of Hair Testing, patients with EtG concentrations of < 7 pg/mg are regarded as teetotallers or very rare drinkers, ⩾ 7 pg/mg to 29 pg/mg strongly suggest repeated alcohol consumption, and concentrations of ⩾ 30 pg/mg strongly suggest chronic excessive alcohol intake[73]. These cut-offs are valid for 0-3 cm up to 0-6 cm of proximal scalp hair segments. The analysis of FAEEs alone is not recommended to prove alcohol abstinence but may be used in cases of suspected false negative EtG results (FAEEs cut offs: 0.2 ng/mg for a 0-3 cm, 0.4 ng/mg for a 0-6 cm proximal scalp hair segment). The correlation between hEtG and the amount of alcohol consumed are linear[74]. In addition to a time period of up to 6 mo, which is covered by hEtG, it has the advantage of doubled sensitivity over CDT despite equal specificity[75].

Sterneck *et al*[18] presented promising results for the application of hEtG within the transplant setting. Investigating 63 LT candidates with alcoholic liver cirrhosis and 25 patients with cirrhosis due to other reasons, hEtG was compared to uEtG, blood EtOH, MeOH, CDT, and psychological interviews. Although only 30% of patients admitted alcohol consumption, 62% of patients tested positive for any alcohol marker. Alcohol abstention was disproved in 83% of cases by hEtG only. Sensitivity and specificity were 85% and 97%, respectively, using a cut off of 30 pg/mg in 6 cm hair strands. hEtG results were independent of liver and kidney function. Recent work by the same study group analyzed the value of hEtG in 104 LT recipients (31 ALD, 73 non-ALD patients) in comparison to uEtG, EtOH, MeOH, CDT, patients' self-reports, and physicians' assessments[76]. By applying hEtG, the detection rate of 7% by any alcohol markers could be increased to 17%.

hEtG test results can be confounded by heavily impaired kidney function leading to higher hEtG results[72]. Further, hair tonics, which might include EtG, can result in false positive results[77,78]. False positive FAAE results were found after use of EtOH containing lotions[79], whereas these lotions had no impact on hEtG[80]. False negative or decreased hEtG results can be found after bleaching, dying, and thermal hair straightening (only tested *in vitro*)[81-83].

FUTURE DIRECTIONS

Phosphatidylethanol (PEth) is a phospholipid formed only in the presence of EtOH *via* a transphosphatidylation catalyzed by phospholipase D in the cell membrane of peripheral blood cells[84-86]. It is a long-term alcohol parameter that becomes positive after repeated consumption of ⩾ 50 g/d EtOH over a period of 2 to 3 wk measured by HPLC in whole blood and can be detected up to > 2 wk (probably up to 6 wk in some cases[87]) after cessation of alcohol consumption[88,89]. Using LC-MS/MS, elevated PEth levels can be found after a single day of excessive alcohol intake[90]. A recent study also reported PEth to be a valuable tool for the detection of moderate alcohol consumption[91]. Novel assays even allow qualitative and quantitative measurement of PEth in dried blood spots[92,93]. PEth was described to bear higher sensitivity than CDT, GGT, or MCV (sensitivity of 99% *vs* 40% to 77%)[94]. Another study found an excellent diagnostic accuracy (AUC = 0.974, sensitivity 94.5%, specificity 100%) superior to that of CDT, GGT, or MCV in patients with excessive alcohol consumption taking part in a detoxification program[95]. Helander *et al*[96] showed the superiority of PEth over CDT for monitoring alcohol consumption in an outpatient alcohol detoxification program. In 43% of these patients alcohol consumption was detected only by PEth, in 38% by PEth plus CDT, and in 21% by EtG/EtS only. False positive or high test results can occur if samples are stored at room temperature[92]. PEtH levels are not affected by sex or age[97], and in contrast to CDT, PEth is not influenced by the presence or severity of liver disease[87,98]. Although already first described in the 1980s to be synthesized in peripheral blood[84,85], PEth still has not found its way into routine diagnostics. Data on LT candidates and recipients are currently lacking.

Platelet monoaminoxidase-B (MAO-B) protein levels showed promising accuracy in detection of heavy drinking measured by immunoblotting or enzyme-linked immunosorbent assay (AUC = 0.72)[99]. If combined with CDT, its performance can be improved (AUC = 0.84). Formerly investigated as a genetic trait marker of alcohol dependence, MAO-B presents an interesting target. Protein levels, in contrast to MAO-B enzymatic activity, are not impaired by smoking[100]. This marker, although promising, needs to be further validated.

A recent study investigating a study cohort of 53 excessive drinkers, a control group of 49 individuals, and a validation cohort of 40 excessive drinkers and 40 controls discovered four novel proteins for the detection of excessive alcohol intake by serum proteomic analysis[101]. These proteins were AT-rich interactive domain-containing protein 4B, phosphatidylcholine-sterol acyltransferase, hepatocyte growth-factor like protein, and ADP-ribosylation factor 6. They are indirect markers of alcohol consumption and are superior to routinely used markers such as AST, ALT, GGT, MCV, and CDT (AUC = 0.70 to 0.86 *vs* 0.21 to 0.67). They might be involved in inflammation processes, cellular organization, protein

transportation, and cell proliferation mechanistically linked to alcohol consumption and metabolism. However, to date it remains unclear whether the secretion and excretion of these proteins are influenced by liver function.

CONCLUSION

Alcohol biomarkers significantly improve detection of alcohol consumption. To account for various drinking patterns, we always examine several markers in combination to cover different time periods. In the setting of LT, sensitivity and specificity are of special concern, since a false positive alcohol test may deny a patient the opportunity of a life-saving LT. During the evaluation process for LT, hEtG seems to be an attractive marker, since a time frame of up to 6 mo is covered and false positive results are unlikely. However, data demonstrating improved relapse rates after LT are missing, and optimal cut offs in this setting are unclear. For surveillance of patients on the waitlist for LT, a combination of uEtG, PEtH, and GGT seem to be the most appropriate tools. In LT recipients, a combination of uEtG, GGT, and CDT or PEtH might be valuable. Long-term follow-up after LT seems to be well covered by additional hEtG concentrations. Currently, MeOH does not seem to have advantages over the mentioned parameters. Importantly, routinely assessed alcohol biomarkers prior to and after LT may help guide treatment decisions for LT and allow early referral for psychological support and alcohol detoxification programs.

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P- Reviewer: Ruiz-Margain A **S- Editor**: Ma YJ
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ISSN 1007-9327