ORIGINAL ARTICLE

Diagnostic performance of ethyl glucuronide in hair for the investigation of alcohol drinking behavior: a comparison with traditional biomarkers

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Abstract

Background Ethyl glucuronide (EtG) in hair has emerged as a useful biomarker for detecting alcohol abuse and monitoring abstinence. However, there is a need to establish a reliable cutoff value for the detection of chronic and excessive alcohol consumption.

Methods One hundred and twenty-five subjects were classified as teetotalers, low-risk drinkers, at-risk drinkers, or heavy drinkers. The gold standard for subjects' classifications was based on a prospective daily alcohol self-monitoring log. Subjects were followed for a 3-month period. The EtG diagnostic performance was evaluated and compared with carbohydrate-deficient transferring (CDT) and the activities of aspartate aminotransferase, alanine aminotransferase, and γ-glutamyl-transferase (γGT).

Results A cutoff of >9 pg/mg EtG in hair, suggesting an alcohol consumption of >20/30 g (at-risk drinkers), and a cutoff of >25 pg/mg, suggesting a consumption of >60 g (heavy drinkers), were determined by receiver operating characteristic analysis. The EtG diagnostic performance was significantly better ($P < 0.05$) than any of the traditional biomarkers alone. EtG, as a single biomarker, yielded a stronger or similar diagnostic performance in detecting at-risk or heavy drinkers, respectively, than the best combination of traditional biomarkers (CDT and γGT). The combination of EtG with traditional biomarkers did not improve the diagnostic performance of EtG alone. EtG demonstrated a strong potential to identify heavy alcohol consumption, whereas the traditional biomarkers failed to do so. EtG was not significantly influenced by gender, body mass index, or age.

Conclusion Hair EtG definitively provides an accurate and reliable diagnostic test for detecting chronic and excessive alcohol consumption. The proposed cutoff values can serve as reference for future cutoff recommendations for clinical and forensic use.

Keywords Alcohol consumption · Cutoff · Diagnostic performance · Ethyl glucuronide · Alcohol biomarkers

Abbreviations

ASAT	Aspartate aminotransferase
ALAT	Alanine aminotransferase
γGT	γ-Glutamyl-transferase
CDT	Carbohydrate-deficient transferring
EtG	Ethyl glucuronide
DASM log	Daily Alcohol Self-monitoring log
GC-MS/MS	Gas chromatography-tandem mass spectrometry
LOQ	Limit of quantification
ROC	Receiver operating characteristic
AUROC	Area under the ROC curve
BMI	Body mass index
OR	Odds ratio
CI	Confidence interval

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Introduction

Chronic and excessive alcohol consumption is recognized as a major public health concern. In Western Europe, it is estimated that 20% of the population have chronic and

excessive alcohol consumption, including drinkers categorized as at-risk and heavy drinkers. At-risk alcohol consumption (>20/30 g/day) is associated with a significant increased risk of disease and premature mortality, and it imposes a considerable social and economic burden [1, 2]. The early detection of at-risk alcohol consumption is of particular importance to help reduce alcohol-related injury.

The diagnostic value of the traditional and indirect biomarkers aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), γ-glutamyl-transferase (γGT), and carbohydrate-deficient transferring (CDT) is often limited by their lack of sensitivity and/or specificity, as increased values can be due to numerous other non-alcoholic disorders [3–5]. A combination of traditional biomarkers is thus recommended to improve the diagnosis [3, 6–10].

Ethyl glucuronide (EtG) is a promising biomarker of alcohol consumption. In contrast to traditional biomarkers, EtG is a direct metabolite of ethanol that is exclusively produced in vivo following alcohol consumption. EtG is incorporated into hair, which makes its analysis an effective and non-invasive method to monitor retrospective alcohol consumption over a long period of time (weeks/months). Several studies have investigated the potential of hair EtG as a marker of alcohol consumption [11–16]. Overall, EtG was not detected in the hair of teetotalers. Heavy drinkers (>60 g/day) displayed considerably higher hair EtG concentrations than the so-called social drinkers (≤60 g/day). In addition, the levels of hair EtG seemed to reflect the average daily amount of alcohol ingested [17, 18].

Various cutoff values (4, 23, 25, 30, and 50 pg/mg) have been proposed to detect chronic and excessive alcohol drinkers [14, 17, 19–21]. Despite the >30-pg/mg cutoff consensus of the Society of Hair Testing (SoHT) from 2009 for heavy drinkers [22], there is no general agreement regarding the cutoff values that should be used.

A study conducted with 154 persons in a driving ability assessment used cutoff values of >7 pg/mg for social drinking between 20 and 40 g ethanol/day and >30 pg/mg for heavy drinking (>60 g/day) [23]. Hair EtG concentrations were compared with the plasma %CDT. In contrast to 53 persons tested with hair EtG >30 pg/mg, CDT was detected above the routinely used upper limit in 15 cases only.

Only one retrospective study based on self-reports integrated statistical components in order to validate the performance of EtG in hair [24]. Twenty-seven picograms per milligram has been proposed as cutoff value to identify heavy drinkers with a sensitivity of 0.92 and a specificity of 0.96.

A recently published Swedish study measured EtG in hair after the controlled daily ethanol consumption of 16 g (14 female volunteers) or 32 g (7 male volunteers) of over a 3-month period [25]. EtG could be detected in the hair of six from seven volunteers with a consumption of 32 g/day

and in lower concentrations in 6 of 14 cases with a consumption of 16 g/day. They concluded that EtG determination in hair can not totally exclude minor daily alcohol consumption and lacks sensitivity for the purpose of monitoring total abstinence.

An inaccurate estimation of alcohol consumption may lead to a misclassification of a subject and to unreliable cutoff values. The choice of a gold standard is thus a key element to ensure the validity of results. The assessment of alcohol consumption relies on either retrospective or prospective methods. Retrospective methods have been associated with a lack of compliance and missing data due to the need for subjects to recall alcohol consumption over extended periods [26]. Prospective alcohol self-monitoring logs represent a superior alternative to retrospective, self-reported methods. They allow the subject to collect more accurate alcohol consumption data on a daily or a drink-by-drink basis and do not require that subjects recall their alcohol consumption [27, 28]. To our knowledge, there have been no studies evaluating the diagnostic performance of hair EtG using a prospective, self-monitoring method as a gold standard.

The main objective of this prospective study was to assess the relevance of hair EtG as a marker of alcohol consumption in a well-characterized population. For this purpose, reliable cutoff values to identify chronic and excessive alcohol consumption were determined using statistical analyses. The diagnostic performance of hair EtG was evaluated and compared with traditional biomarkers alone, or in combination. Finally, the influence of intrinsic factors on the diagnostic performance of hair EtG was studied.

Materials and methods

Study population

This prospective study was conducted between March 2009 and June 2010 over a 3-month test period at the University Center of Legal Medicine, Lausanne. One hundred and forty subjects were recruited by advertisement in the local newspapers and by posters displayed in public places. Eligible subjects were above 18 years old, not pregnant, and with no cognitive impairment. Subjects with intravenous drug abuse were not authorized to participate in this study. Of the 140 subjects, ten did not meet the eligibility criteria, and five withdrew from the study. Full data were obtained from 125 subjects (69 women, 56 men) who completed the study. All participants provided written informed consent before enrollment. The study protocol was approved by the Clinical Research Ethics Committee of the University of Lausanne.

Daily alcohol self-monitoring log

The subjects' daily alcohol consumption was estimated using a Daily Alcohol Self-Monitoring log (DASM log) recorded over the 3-month study period. They were requested to report their alcohol consumption on a day-by-day basis using weekly calendars. The DASM log allowed to collect prospectively detailed quantitative and qualitative information about number, volume, frequency, and type of drinks (categorized as beer, wine, fortified wine, and spirit) consumed each day. The mean daily alcohol consumption (grams per day) was calculated based on alcohol content, reported volume, and frequency of drinking. Beer, wine, fortified wine, and spirit alcohol contents were defined as 5%, 12%, 20%, and 40% (v/v), respectively.

Identification of subjects' alcohol consumption profile

Subjects were classified into three categories (teetotaler, low-risk, and at-risk drinkers) based on the results from the DASM log. Teetotalers were defined as subjects who declared total abstinence from alcohol consumption (0 g/day) during the last 12 months. Low-risk drinkers were defined as subjects who consumed ≤20 g/day for women and ≤30 g/day for men. At-risk drinkers were defined as subjects who consumed >20 g/day for women and >30 g/day for men. These thresholds are based on WHO recommendations. To allow a direct comparison of our findings with results reported in the literature, subjects with an alcohol consumption of >60 g/day for both men and women were also classified and were referred to as heavy drinkers.

Study design

Subjects were required to attend three visits: at weeks 0, 6, and 12. The first interview consisted of introducing the purpose and design of the study. Information, including gender, age, body weight, body height, hair natural color, and hair cosmetic treatment, was collected. The DASM log was explained and handed out to each subject. Subjects were asked to complete and return the DASM log every week over the study period. Blood samples were taken at all visits, and hair at the first and the last one. The DASM logs were checked at week 6 and the end of the protocol.

Blood and hair collection

Blood samples were collected in Sarstedt S-Monovette® Serum Gel tubes, which were immediately centrifuged. Two milliliters of serum were separated and stored at −20°C until analysis. Hair samples were collected from the vertex posterior region, as close as possible to the skin. The 3-cm proximal hair segment, representing approximately 3 months of growth [29], was used for EtG analysis. Hair samples were stored dry and kept away from light at room temperature until analysis.

Serum and hair analysis

Traditional biomarkers (ASAT, ALAT, γGT, and CDT) were analyzed in all serum samples. ASAT, ALAT, and γGT were measured by immunoassay on a Dimension® Xpand® Plus clinical chemistry system (Siemens). CDT was analyzed by capillary zone electrophoresis, equipped with a UV detector set at 210 nm, using a commercial assay kit (CEofix™ CDT, Analis). CDT was expressed as the percent of asialo- plus disialo-transferrin isoforms in total transferrin.

Hair EtG was analyzed using a fully validated gas chromatography-tandem mass spectrometry (GC-MS/MS) method [30]. The limit of detection and the limit of quantification (LOQ) were 2 and 4 pg/mg hair, respectively.

Data analysis

Statistical analyses were performed using STATA® version 11 and R version 2.9.2 software. Mean, standard deviation, median, and interquartile range (25–75%) were used as descriptive statistics. Continuous variables were compared using the Kruskal–Wallis test and the Mann–Whitney U test (one-tailed); categorical variables were compared using the χ^2 test (two-tailed). The diagnostic performance of EtG, ASAT, ALAT, γGT, and CDT (sensitivity, specificity, positive predictive value, and negative predictive value) was calculated for the optimal cutoff values selected from the receiver operating characteristic (ROC) curves (i.e., the value on the ROC curve farthest from the diagonal line [chance] optimizing both sensitivity and specificity). The diagnostic performance of each test was compared using the area under the ROC curves (AUROC). An AUROC closer to 1 indicated a better diagnostic test, while an AUROC of 0.5 indicated no diagnostic ability. Univariate logistic regression analysis was performed to evaluate the independent effects of age, gender, and BMI on the EtG diagnostic performance, expressed as an odds ratio (OR). Multivariate logistic regression analysis was performed to determine the combination among the five biomarkers that could best predict excessive alcohol consumption. $P<0.05$ was considered statistically significant.

Results

Descriptive results

One hundred and twenty-five subjects were classified according to their self-reported alcohol consumption. The

demographic and reported alcohol intake characteristics and biomarker levels are presented in Table 1. Forty-three subjects did not report any alcohol consumption, 44 subjects reported an alcohol consumption ≤20/30 g/day (women/men), and 38 subjects reported an alcohol consumption >20/30 g/day (women/men). Twenty-one subjects had an alcohol consumption >60 g/day. There were no significant differences in age and BMI, whereas significant differences were noted in gender between the three groups. Women were less likely than men to report at-risk consumption.

The median EtG concentration varied among the groups and was significantly higher in at-risk drinkers than in low-risk drinkers (Mann–Whitney U test, $P<0.001$). The median values of γGT and CDT were also significantly higher in at-risk drinkers than in low-risk drinkers (Mann–Whitney U test, $P<0.001$). This difference, though significant, was less apparent with the median values of ASAT and ALAT (Mann–Whitney U test, $P<0.05$). Heavy drinkers showed significantly higher EtG, ASAT, ALAT, γGT, and CDT levels than the subjects who consumed ≤60 g/day (Mann–Whitney U test, $P<0.001$). Notably, significantly elevated biomarker values were observed in heavy drinkers, as compared with at-risk drinkers (Mann–Whitney U test, $P<0.01$).

Determination of optimal cutoff values and diagnostic performance of EtG

Table 2 lists the EtG diagnostic performance for each optimal cutoff value. ROC analyses were performed to determine the optimal EtG cutoff value, to allow distinguishing at-risk drinkers from the others. The optimal cutoff value was set at

Table 1 Demographic and alcohol intake characteristics and biomarker levels (EtG measured in hair; ASAT, ALAT, GGT, and CDT measured in plasma) for 125 subjects classified according to their self-reported alcohol consumption

|	Teetotalers (n=43)	Low-risk drinkers (n=44)	At-risk drinkers (n=38)	P -value*
Gender				<0.001
Women, n (%)	29 (67)	30 (68)	10 (26)	
Men, n (%)	14 (33)	14 (32)	28 (74)	
Age, years				0.051
Mean ± SD [(range)]	37.5±16.7 [(19–74])]	35.3±13.1 [(20–67])]	42.3±14.0 [(19–70])]	
Median (IQR)	32.0 (24.0–52.0)	30.0 (25.0–42.0)	41.5 (31.0–54.0)	
BMI (kg/m^2)				0.289
Mean ± SD [(range)]	22.8±3.4 [(15–32])]	23.7±3.0 [(18–33])]	23.9±4.2 [(19–40])]	
Median (IQR)	22.5 (20.0–24.6)	23.8 (21.6–24.8)	22.6 (21.2–25.6)	
Daily alcohol consumption (g/day)				
Mean ± SD [(range)]	0	8.7±7.2 [(0–30])]	64.4±34.5 [(21–150])]	
Median (IQR)	0	7.9 (3.1–11.3)	52.5 (36.1–88.0)	
Laboratory parameters				
EtG (pg/mg)				<0.001
Mean ± SD [(range)]	0.6±2.1 [(0–10])]	4.9±6.2 [(0–32])]	88.3±203.9 [(1–1,190])]	
Median (IQR)	0.0 (0.0–0.0)	3.5 (1.3–6.4)	31.9 (11.9–82.4)	
ASAT (U/l)				0.022
Mean ± SD [(range)]	19.7±6.3 [(9–38])]	18.2±6.2 [(5–37])]	27.1±16.3 [(12–82])]	
Median (IQR)	18.8 (15.7–25.0)	17.7 (13.6–20.8)	19.3 (15.9–34.9)	
ALAT (U/l)				0.027
Mean ± SD [(range)]	33.1±8.3 [(19–57])]	31.3±12.6 [(12–89])]	38.1±15.4 [(23–80])]	
Median (IQR)	31.0 (28.3–38.0)	27.5 (24.7–33.7)	32.2 (26.8–45.5)	
γGT (U/l)				<0.001
Mean ± SD [(range)]	26.7±10.0 [(9–50])]	33.1±14.1 [(10–81])]	67.6±55.1 [(14–215])]	
Median (IQR)	26.0 (19.0–33.7)	30.0 (25.2–35.9)	43.4 (30.2–96.7)	
CDT(%) (asialo- ± disialo-transferrin)				<0.001
Mean ± SD [(range)]	0.7±0.2 [(0.2–1.2])]	0.8±0.2 [(0.5–1.6])]	2.1±2.5 [(0.5–11.8])]	
Median (IQR)	0.6 (0.5–0.8)	0.8 (0.7–0.9)	1.0 (0.8–1.7)	

ASAT aspartate aminotransferase; *ALAT* alanine aminotransferase; *γGT* γ-glutamyl transferase; *CDT* carbohydrate-deficient transferring; *IQR* interquartile range

*Kruskal–Wallis test (used to compare the three groups together) and χ^2 test were statistically significant at a P- value <0.05.

>9 pg/mg. Based on this cutoff, the sensitivity of EtG was 0.82 (31 out of 38 at-risk drinkers were correctly classified), and the specificity was 0.93 (6 out of 87 subjects were misdiagnosed as at-risk drinkers). The positive and negative predictive values were 0.84 and 0.92, respectively, meaning that the probability for a subject with an EtG concentration of >9 pg/mg to be an at-risk drinker was 84%, and the probability for a subject with an EtG concentration of ≤9 pg/mg to be a low-risk drinker was 92%. This cutoff value is thus proposed as a good indicator of at-risk drinking. The optimal cutoff value for detecting heavy drinkers was set at >25 pg/mg (>60 g/day versus the others). EtG had a better diagnostic performance in detecting heavy drinkers than at-risk drinkers, with a false negative rate of 5% and a false positive rate of 3%. Low-risk drinkers showed in 58% of the cases EtG concentrations lower than the LOQ of the applied analytical method of 4 pg/mg. A reliable differentiation between teetotalers and low-risk drinkers was therefore not possible. A gain in the sensitivity of the analytical method may allow a distinction between these groups.

Comparison of diagnostic performances

The AUROC of each biomarker is represented in Fig. 1. EtG, ASAT, ALAT, γGT, and CDT had AUROC values greater than 0.5, where 0.5 indicates an ineffective test. The EtG diagnostic performance was significantly higher than the traditional biomarkers ($P<0.0001$). EtG showed the largest AUROC (0.95; 95% CI, 0.92–0.99) value, followed by CDT (0.80; 95% CI, 0.71–0.89), γGT (0.76; 95% CI, 0.66–0.86), ASAT (0.65; 95% CI, 0.54–0.76), and ALAT (0.60; 95% CI, 0.49–0.71). The diagnostic performance of each biomarker for the detection of heavy drinkers was also evaluated. All of the biomarkers were better predictors of heavy than of at-risk alcohol consumption (EtG, 0.99; 95% CI, 0.98–1.00; CDT, 0.84; 95% CI, 0.75–0.93; γGT, 0.79; 95% CI, 0.65–0.93; ASAT, 0.79; 95% CI, 0.68–0.90; ALAT, 0.73; 95% CI, 0.62–0.85). The AUROC value for EtG was significantly higher than that of the other traditional biomarkers ($P<0.001$).
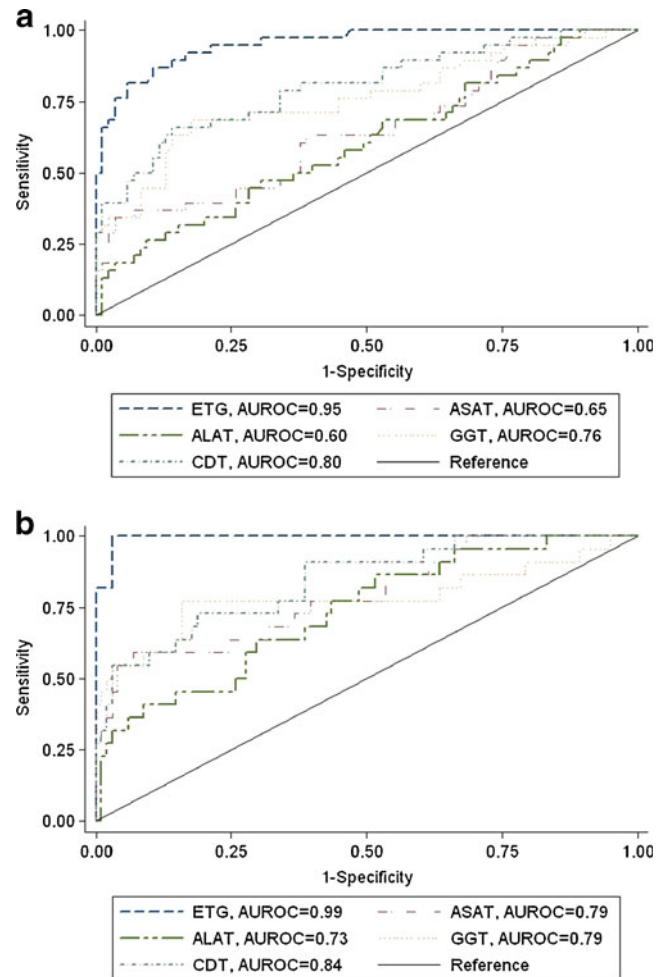
Fig. 1 A comparison of the diagnostic performance of ethyl glucuronide in detecting (**a**) at–risk alcohol consumption (>20/30 g/day; women/men) or (**b**) heavy alcohol consumption (>60 g/day; women and men) with traditional biomarkers (CDT, γGT, ASAT, and ALAT) using ROC curve analysis. *AUROC*, area under the receiver operating characteristics curve

Diagnostic performance of combined biomarkers

EtG, as a single biomarker, yielded a similar diagnostic discriminatory ability in detecting at-risk drinkers as the

Table 2 The diagnostic performance of ethyl glucuronide in detecting abstinence, at-risk alcohol consumption, and heavy alcohol consumption

	Daily alcohol consumption (g/day)		
	0 Teetotalers vs. others	>20/30 At-risk drinkers vs. others	>60 Heavy drinkers vs. others
AUC (95% CI)	0.95 (0.91–0.99)	0.95 (0.92–0.99)	0.99 (0.98–1.00)
Cutoff (EtG, pg/mg)	0	>9	>25
Sensitivity	0.93	0.82	0.95
Specificity	0.94	0.93	0.97
PPV	0.89	0.84	0.88
NPV	0.96	0.92	0.99

PPV positive predictive value; *NPV* negative predictive value; *CI* confidence interval

best EtG-included biomarker combination, which used EtG, ALAT, and γGT. The AUROC value of this combination was 0.96 (95% CI, 0.93–0.99), whereas the AUROC of EtG alone was 0.95 (95% CI, 0.91–0.99). As a single biomarker, EtG yielded a stronger diagnostic performance in detecting at-risk drinkers than any combination of the multiple traditional biomarkers. In particular, EtG was better in detecting at-risk drinkers than the best traditional biomarker combination, which included CDT and γGT ($P<0.05$). No significant differences in detecting heavy drinkers were observed when comparing EtG with the best traditional biomarker combination ($P=0.06$).

Effects of intrinsic factors on EtG

A univariate regression analysis of age, gender, and BMI revealed that none of these variables affected the diagnostic performance of EtG in identifying chronic and excessive alcohol consumption (age [OR, 0.95; 95% CI, 0.89–1.01; $P=$ 0.09], gender [OR, 0.86; 95% CI, 0.21–3.45; $P=0.83$], or BMI [OR, 1.04; 95% CI, 0.88–1.23; $P=0.62$]). Based on these results, no corrections were deemed necessary.

Discussion

Performance diagnostic and cutoff values

The main finding of this study was that hair EtG is a reliable indicator of chronic and excessive alcohol consumption, regardless of the subject's age, gender, or BMI.

Hair EtG demonstrates a high diagnostic performance in diagnosing chronic and excessive alcohol consumption. Even the best combination of EtG and traditional biomarkers does not significantly improve the diagnostic performance of EtG alone. Although EtG alone performed better in identifying at-risk drinkers than the best combination of traditional biomarkers, it showed a similar diagnostic performance in identifying heavy drinkers (as shown for CDT and γGT by Sillanaukee [7]). As expected, all of the biomarkers performed better in detecting heavy drinkers than at-risk drinkers. The highest diagnostic performance of the traditional biomarkers was obtained for the detection of heavy drinkers (0.73–0.84), whereas the lowest diagnostic performance was for the detection of at-risk drinkers (0.60–0.80), with minimum reported values that were not much higher than would be expected by chance. These findings are in agreement with previously reported results [3] and have been attributed to the known increase in the activity of traditional biomarkers following an alcohol consumption of over 60–80 g/day, sustained for a 2-week period [8, 31, 32]. Thus, the high amount of alcohol required to increase traditional biomarker levels makes the early detection of

alcohol misuse particularly difficult. In contrast, EtG is present even after low to moderate alcohol consumption, and it allows the identification of at-risk drinkers with a high diagnostic performance (0.95), as compared to traditional biomarkers.

To identify heavy drinkers, a robust cutoff value has been determined: a cutoff value of 25 pg/mg offered the best combination of sensitivity (0.95) and specificity (0.97). These results are in agreement with a previous study, which suggested a cutoff of 27 pg/mg to identify heavy drinkers; the sensitivity and specificity were 0.92 and 0.96, respectively [24]. Using the 30-pg/mg cutoff recommended by the SoHT [22], we would have obtained a similar specificity (0.97); however, sensitivity would have decreased (0.81). In clinical and forensic practice, ensuring high specificity is important to reduce false positive results. In this study, the best specificity was obtained with a cutoff of 39 pg/mg: no subjects would have been misclassified as heavy drinkers, though only 81% would have been correctly identified as heavy drinkers. Similarly, a robust cutoff value was determined to detect at-risk drinkers. The optimal cutoff value was 9 pg/mg and yielded a sensitivity of 0.82 and a specificity of 0.93. To our knowledge, this is the first study investigating the usefulness of EtG in detecting at-risk drinking, which is often an early sign of chronic and excessive alcohol consumption.

A distinction between low-risk drinking and abstinence under the given analytical limits is not possible because of the high percentage of negative results in the group of low-risk drinkers. This limitation may be partially solved in the near future by upcoming analytical equipments offering a better sensitivity. However, when EtG is detected in hair, abstinence can generally be excluded.

Several studies have reported an effect of gender, age, or BMI on traditional biomarker levels. Interestingly, in this study, EtG was not affected by gender, age, or BMI. In addition, earlier studies demonstrated that EtG was likely not influenced even by hair color [18, 33]. These data support the relevance of EtG as a reliable biomarker because it does not require any gender, age, or BMI adjustments. These findings confirm the trends previously reported [24].

However, worries about artificial positive EtG results due to cosmetics or food cannot be neglected. A recent study carried out by Martins et al. [34] did not show false positive results after hair treatment with ethanol-containing hair care products. Nevertheless, a contamination by EtG-containing products cannot be excluded, even if that is exceptionally as reported in a case presented by Sporkert et al. [35]. Therefore, the hair treatment should always be recorded during the sampling. Positive EtG cases in hair due to food or none-hair cosmetics like mouthwash or hand sanitizers have not yet been reported in contrast to positive EtG urine results [36–41]. That can be explained by the small amount of ethanol ingested or absorbed leading to

EtG concentrations in urine insufficiently high to produce a positive EtG findings in hair.

Interpretation and practical considerations

In clinical and forensic practice, EtG has shown a high potential in monitoring abstinence, identifying a relapse to drinking, or in diagnosing chronic and excessive alcohol consumption. The analysis of hair EtG (0–3 cm, a proximal segment) should be interpreted according to the following criteria: (1) a hair EtG concentration of 0 to ≤LOQ suggests an absence of alcohol consumption (teetotalers), although a low-risk alcohol consumption cannot be ruled out; (2) a EtG concentration of >LOQ to ≤9 pg/mg suggests low-risk consumption and can exclude abstinence; (3) a EtG concentration of >9 to ≤25 pg/mg indicates at-risk consumption; and (4) a EtG concentration >25 pg/mg indicates heavy consumption.

In addition, care should be taken in the interpretation of hair EtG results to demonstrate a recent abstinence from alcohol. Because hair growth is irregular, and approximately 15–20% of the hair follicles remain in an inactive cycle phase, residual EtG can still be detected (rate, approximately 15–20%) even after the complete cessation of alcohol consumption [42]. Depending on the previous alcohol consumption behavior, a waiting period, corresponding to the inactive phase of hair growth, might be recommended when monitoring abstinence in order to avoid misinterpretations. For the abstinence control days or a few weeks after the cessation of alcohol consumption, the detection of EtG and ethyl sulfate in urine [43, 44] or phosphatidylethanol in blood [45–47] are therefore a better choice.

In conclusion, hair EtG demonstrates a strong diagnostic performance in detecting both at-risk and heavy alcohol consumption, and it clearly outperforms the diagnostic performance of the standard traditional biomarkers. In contrast with traditional biomarkers, age, gender, and BMI did not affect the EtG diagnostic performance. The analysis of hair EtG definitively provides an efficient clinical diagnostic tool to detect chronic and excessive alcohol consumption. Reliable cutoff values have been determined which can serve as reference for future cutoff recommendations in practice to identify alcohol-drinking behaviors.

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