

RESEARCH ARTICLE

Ethyl glucuronide in hair: A 5-year retrospective cohort study in subjects sanctioned for driving under the influence of alcohol and psychoactive substances

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Abstract

The evaluation of drinking behaviors can help in limiting high-risk situation, such as driving under the influence (DUI). We investigate ethyl glucuronide in hair (hEtG) levels to evaluate alcohol consumption behavior in subjects followed up after having been charged for DUI of psychoactive substances and/or alcohol. We performed a retrospective observational cohort study on 4328 subjects over 18 years old who underwent hEtG analysis in the period 2015–2019 in the Italian Province of Pavia. hEtG level was used as a proxy for the alcohol consumption behavior. Effects of age, sex, and district on alcohol drinking behavior were investigated with an ordinal logit model. A state sequence analysis was used to study people's alcohol consumption behavior over time. hEtG was found ≥ 7.0 pg/mg in 22.2% of the drivers (of which 7% has an hEtG ≥ 30.0 pg/mg). Among positive cases, a prevalence of males (96.3%) aged 35–44 (32.6%), coming from main city and hinterland (38.2%), was observed. The propensity to drink was higher for males (odds ratio [OR] ≈ 2.28 , $p < 0.001$) and for subject coming from the district devoted to the cultivation of vineyards. Young age classes have a reduced drinking risk if compared to the drivers over 55 years old ($p < 0.001$). A general decreasing trend over time in hEtG values was observed. Being male, age ≥ 55 years, and coming from rural areas are potential risk factors related to alcohol drinking habits among drivers. Ethyl glucuronide in hair test in the driving license reissuing protocol contributed to decrease alcohol misuse behaviors.

KEYWORDS

alcohol misuse, driving license renewal, driving under the influence, ethyl glucuronide in hair, propensity to drink

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1 | BACKGROUND AND AIMS

Alcohol abuse causes, nowadays, approximately 3 million deaths every year, in accordance to World Health Organization,¹ thus confirming ethanol as the most widespread psychoactive substance worldwide. Hence, during the last decades, different biomarkers have been studied to prevent and diagnose acute, chronic, and/or binge drinking habits. In particular, markers for monitoring a chronic excessive alcohol consumption are fundamental in many diagnostic fields related to either clinical and forensic toxicology, such as driving license renewal (DLR) issues.²

Alcohol is mostly (90–98%) metabolized in liver by alcohol dehydrogenase, whereas 2–8% is excreted unchanged, mainly through breath and urine, and about 1.5% goes through a non-oxidative metabolism to β -D-ethyl glucuronide (EtG), ethyl sulfate (EtS), ethyl phosphate (EtP), phosphatidyl ethanol (PEth), and fatty acid ethyl esters (FAEEs) (5). EtG, a small, non-volatile, water-soluble molecule, was found in hair in 1993 by Sachs³ and later by Skopp et al.⁴ EtG in hair (hEtG) has been studied as a potential biomarker for monitoring past and continuative alcohol ingestion.^{5–11}

In contrast with traditional biomarkers, age, gender, and body mass index (BMI) do not significantly affect hEtG diagnostic performance.¹²

Until 2015, the protocol used in the province of Pavia for DLR included only the evaluation of traditional markers and the analysis of carbohydrate deficient transferrin (CDT). Among indirect markers for chronic heavy drinking diagnosis, the most used ones are aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyl transferase (GGT). AST and ALT are not directly related to alcohol consumption but increase, generally, in liver diseases. GGT has a higher sensitivity for sensitive alcohol consumption, but a low specificity, because it could increase also in some conditions such as cholestasis. The main drawback is, thus, represented by the relatively low diagnostic sensitivity and specificity of these traditional markers, because they are related to a potential hepatic damage that could be due to other causes, rather than a chronic alcohol abuse. CDT isoforms increase after few weeks of alcohol chronic excessive consumption. Though diagnostic specificity of CDT is extremely high, sensitivity is limited, particularly because the least sialylated isoforms of transferrin tend to normalize after about 10- to 15-day abstinence from alcohol consumption.^{13–15}

Different studies, published in the period 2005–2015, demonstrated that hEtG is highly sensitive and specific in evaluating a chronic excessive alcohol consumption, that is, defined, according to the World Health Organization survey, as an average consumption of 60 g/day or more ethanol over a several months period.^{16–20} In particular, a concentration of hEtG higher than 30 pg/mg is related to a past and continuative excessive alcohol drinking habit.^{6,11,19,21,22} A consensus about the procedures and the cutoffs to be used for hEtG has been developed by a group of experts of the Society of Hair Testing (SoHT) in 2009 in Rome,²³ and it is currently revised about every 2 years. Before the 2019 revision, the consensus provided two different cutoffs. A cutoff set at 7.0 pg/mg was suggested to discriminate

an abstinence or an occasional use from a moderate continuative alcohol consumption, whereas the 30 pg/mg cutoff was chosen to identify those subjects at high risk of chronic excessive alcohol consumption. After the 2019 revision, the cutoff of 7.0 pg/mg was reduced to 5.0 pg/mg.²⁴

Thanks to the consensus, since 2015, hEtG has been included in the list of biomarkers to be checked and evaluated in the new medical committee DLR protocol of the Pavia districts, for drivers previously charged for driving under influence of psychoactive substances and/or alcohol (DUI).

The goal of the study was to investigate hEtG levels and alcohol consumption behavior during the DRL monitoring period in a large cohort of DUI subjects from an administrative province in a northern part of Italy, between 2015 and 2019.

Specifically, we aimed to investigate the association between hEtG levels and sex, age, and concomitant drug abuse; to profile the alcohol drinking habits among people living in districts with different social and environmental characteristics; and to evaluate if the 2015 protocol regarding DRL and including recurrent hEtG monitoring could help in preventing chronic excessive alcohol consumption.

2 | DESIGN AND SETTINGS

2.1 | Study design, population, and inclusion criteria

We performed a retrospective observational cohort study on subjects who underwent DLR after been charged for DUI for alcohol or other substances of abuse in the Province of Pavia.

The study sample was constituted of drivers (aged over 18 years) who underwent at least an hEtG measurement, following the DLR protocol, by the Forensic Toxicology Laboratory of the University of Pavia, in the study period 2015–2019. Data were collected ensuring anonymity.

Exclusion criteria: Hair samples that were evaluated as dyed, bleached, or treated^{25,26} (assessment was carried out through self-declaration of the subjects together with an evaluation of the color of extraction solution), subjects with decreased kidney functions^{27,28} (during hair collection, we kindly ask some clinical information to each subject. The ones with a diagnosis of decreased kidney function were excluded from this study); all the subjects not resident in the Pavia districts.

At the time of signing, the consent for the collection and analysis of the hair, the subjects had also consented to the use of data and samples for statistical and research purposes.

2.2 | Study area

The province of Pavia is one of the 12 provinces in the north-western Italian region of Lombardy. The province is subdivided into three administrative districts and has 541,717 inhabitants (264,859 males and 276,858 females).²⁹

District I includes the main city (Pavia) with its hinterland, counting about 196,000 inhabitants; District II is an agricultural flat area of about 199,000 inhabitants; district III is a rural hilly area of about 147,000 inhabitants.

2.3 | Measurement

2.3.1 | Analytical method

The analytical procedure for hEtG test was previously published.⁵ Briefly, about 50- to 100-mg hair were incubated in bidistilled water, after addition of deuterated internal standard for 16 h and the sonicated for 2 h. The solution was directly injected in a liquid chromatographic tandem mass spectrometric system (LC-MS/MS). The chromatographic separation was achieved by means of a C18 column in reversed phase and isocratic mode, while mass spectrometric detection was performed with a triple quad operating in multiple reaction monitoring (MRM) mode and in negative polarization.

2.3.2 | Variables definition

hEtG level was categorized as a qualitative ordinal variable and was used as a proxy for the alcohol consumption behavior. Specifically, we defined:

1. abstinent or occasional drinker (AOC, hEtG < 7.0 pg/mg, if hEtG < 7.0, it was coded as 0);
2. moderate continuative alcohol drinker (MCD, 7.0 pg/mg ≤ hEtG < 30.0 pg/mg);
3. chronic excessive alcohol drinker (CED, hEtG ≥ 30.0 pg/mg).

CEDs are considered high risk alcohol consumers. Normally, DLR is granted to drivers who have at least three consecutive tests with an hEtG level less than 7.0 pg/mg. On the contrary, subjects providing positive results, even lower than 30.0 pg/mg, can be monitored for longer period and with higher frequencies, depending on the individual decision of the committee.

District of origin, sex, and age class were the independent qualitative categorical variables collected. For categorical variables with more than two categories, the one of major interest was chosen as reference category ("District III" for the district; "male" for the gender; "≥55" for the age class).

2.4 | Statistical analysis

Cohort characteristics at baseline are provided either overall or by drinker category. Categorical variables are reported as absolute/percentage frequencies and quantitative variables as measures of central tendency/location like mean (standard deviation) or median (first quartile; third quantile). The relationship between the dependent

variable and each independent variable is investigated with a chi-square test.

Effects of age, gender, and district on alcohol consumption behavior are investigated with an ordinal logit model. Parallel lines assumption is tested through the Brant's test (Appendix A), and a Partial Proportional Odds Model is chosen.³⁰⁻³² According to the drinker categories above, two model equations are studied:

- Equation 1 (AOC vs. MCD and CED): AOC are compared with habitual alcohol drinkers (MCD + CED).
- Equation 2 (AOC and MCD vs. CED): Low risk groups (AOC + MCD) are compared with high-risk alcohol consumers (CED).
- Results are presented in the form of odds ratio (OR), 95% confidence interval (CI), and *p* value (*p*) for each ordinal logit model equation. ORs and confidence intervals are displayed in forest plots as well. Significance is considered with a value of *p* < 0.05.

The analysis of variance (ANOVA) (Appendix B) is used to test the validity of this regression model. Significance is considered with a relaxed value of *p* < 0.10 or *p* < 0.15 when comparing multiple regression models. Akaike information criteria (AIC) is also assessed.

A state sequence analysis is performed^{33,34} (Appendix C) to study people's alcohol consumption behavior over time after following the first hEtG test (that could act as a test discouraging/deterrent event). The analysis is carried out on a subset of individuals having at least three consecutive hEtG measurements after the baseline hEtG measurement over a time span of 18 months.

All the analyses are performed using R 4.0.2. Graphs are produced with the ggplot2 package. Ordinal logistic analysis is performed using the VGAM package and state sequence analysis using the TraMineR package.

3 | RESULTS

Characteristics of the sample are reported in Table 1.

A chart depicting the prevalence of total MCD and CED at baseline and by year is plotted in Figure 1 to assess the alcohol consumption general trend over the 5 years; an additional bar chart showing the percentages of both MCD and CED on the total numbers of habitual alcohol drinkers at baseline and by year is reported in Figure 2.

We present the results of propensity to drink in Table 2, adjusted for sex, age class, and district of origin, using the partial proportional odds (PPO) model. The results from the Brant test (see Appendix A) motivate the PPO model use, because the proportional odds assumption is reasonable only for the district and the sex.

Because the PPO assumption is met for district and sex, their coefficients do not vary across equations. In both equations, people living in District II are about 20% (OR ≈ 0.81, *p* < 0.05) less likely to drink or being heavy drinkers if compared with those living in District III, keeping fixed sex and age. The direction of the association is the same for the other district category: People living in District I are

TABLE 1 Baseline cohort characteristics by drinker category and overall

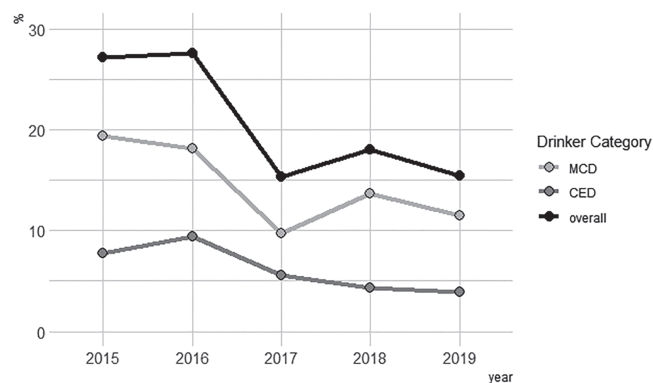
	AOC N = 3369 (77.80%)	MCD N = 658 (15.20%)	CED N = 301 301 (7.00%)	Overall N = 4328	p
EtG (pg/mg)					
(mean [ds])	-	20.0 (6.72)	93.4 (87.2)	9.54 (33.3)	
(median [min, max])	-	19.4 [8.20, 29.7]	67.8 [30.7, 914]	0 [0, 914]	
Repeated ^a EtG measurements (n [%])					
2	1744 (51.77%)	415 (63.07%)	216 (71.76%)	2,375 (54.88%)	
3	631 (18.73%)	200 (30.40%)	168 (55.81%)	999 (23.08%)	
4	243 (7.21%)	83 (12.61%)	134 (44.52%)	460 (10.63%)	
Tested for at least one type of drug ^b (n [%])					
and being positive to the test (n [%])	475 (14.10%)	72 (10.94%)	26 (8.64%)	573 (13.24%)	
	81 (17.05%)	19 (26.39%)	10 (38.46%)	110 (19.20%)	
Gender (n [%])					
M	2952 (87.60%)	617 (93.80%)	290 (96.30%)	3859 (89.20%)	<0.001
F	417 (12.40%)	41 (6.20%)	11 (3.70%)	469 (10.80%)	
Age class (n [%])					
18–24	342 (10.20%)	49 (7.40%)	5 (1.70%)	396 (9.10%)	<0.001
25–34	1,067 (31.70%)	168 (25.50%)	47 (15.60%)	1,282 (29.60%)	
35–44	978 (29.00%)	178 (27.10%)	98 (32.60%)	1,254 (29.00%)	
45–54	684 (20.30%)	163 (24.80%)	82 (27.20%)	929 (21.50%)	
≥55	298 (8.80%)	100 (15.20%)	69 (22.90%)	467 (10.80%)	
District (n [%])					
I	1,522 (45.20%)	275 (41.80%)	115 (38.20%)	1912 (44.20%)	0.011
II	826 (24.50%)	147 (22.30%)	77 (25.60%)	1,050 (24.30%)	
III	1,021 (30.30%)	236 (35.90%)	109 (36.20%)	1,366 (31.60%)	

Note: Categorical variables are presented as number of individuals and percentages N (%); numeric variables are presented as mean (sd) and median (min, max); p values refer to the chi-square test between the drinking category and each model predictor.

Abbreviations: AOC, abstinent or occasional drinker; CED, chronic excessive alcohol drinker; MCD, moderate continuative alcohol drinker.

^aRegular/non intermittent; for each EtG observed measurement at time t_k , there is a previous observed measurement at t_{k-1} .

^bDrugs tested in hair samples: morphine, 6-acetylmorphine, cocaine, benzoylecgonine, amphetamine, methamphetamine, MDMA, MDA, MDEA, cannabinoids, methadone, buprenorphine.

**FIGURE 1** Percentages of habitual drinkers and by category compared with the total subjects, analyzed each year at baseline

about 23% (OR \approx 0.77, $p < 0.01$) less likely to drink or being heavy drinkers if compared with those living in District III, keeping fixed sex and age.

Because age class does not fulfill the lines parallelism assumption, its coefficients are free to vary across equations.

Equation 2, focusing only on the risk of being a CED, shows smaller ORs if compared with Equation 1. The coefficient for age class 18–24 is very close to zero, indicating a 92% (OR \approx 0.08, $p < 0.001$) lower risk of being a CED for those in that class compared with those in the ≥ 55 years old class.

As can be seen intuitively from the forest plots (Figure 3), being male is a risk factor for the alcohol drinking behavior; conversely, living in District I or II and being younger represent protective factors.

The total number of people who underwent at least four repeated hEtG measurements is 460, in a time span of about 18 months (about 2 years from the moment of driving license withdraw). In Figure 4, we plotted the characteristics of these 460 subjects. From this full index plot, we notice a multiplicity of different trajectories, but the prevalent trajectories depict the AOC for the whole-time span

FIGURE 2 Percentages of moderate continuative alcohol drinker (MCD) and chronic excessive alcohol drinker (CED) on the total number of habitual drinkers at baseline, stratified by year [Colour figure can be viewed at wileyonlinelibrary.com]

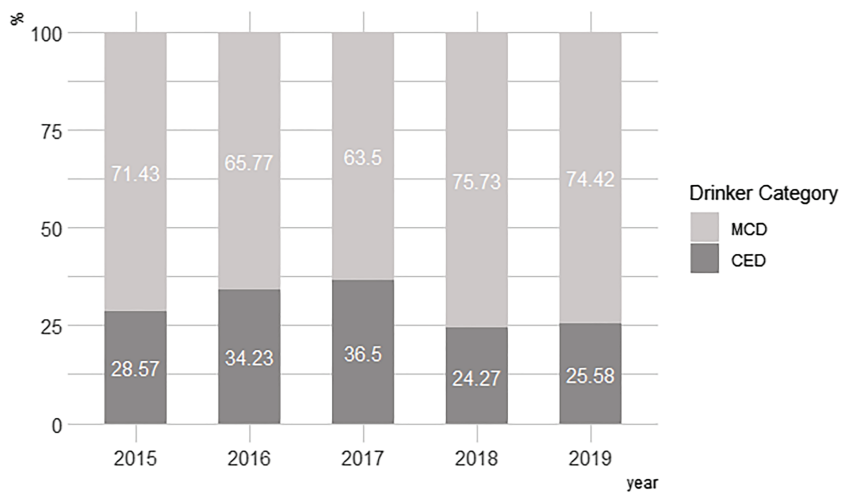


TABLE 2 Odds ratio estimates, 95% CI, and *p* values of the partial proportional odds (PPO) model of propensity to drink

	Propensity to drink					
	AOC vs. MCD and CED			AOC and MCD vs. CED		
	OR	95% CI	<i>p</i>	OR	95% CI	<i>p</i>
District						
I	0.7714	(0.6532; 0.9109)	0.0022	0.7714	(0.6532; 0.9109)	0.0022
II	0.8127	(0.6705; 0.9850)	0.0345	0.8127	(0.6705; 0.9850)	0.0345
III	1	-	-	1	-	-
Sex						
Male	2.2854	(1.6926; 3.0859)	<0.001	2.2854	(1.6926; 3.0859)	<0.001
Female	1	-	-	1	-	-
Age class						
18–24	0.2949	(0.2088; 0.4165)	<0.001	0.0783	(0.0312; 0.1962)	<0.001
25–34	0.3716	(0.2922; 0.4725)	<0.001	0.2307	(0.1565; 0.3402)	<0.001
35–44	0.5054	(0.4006; 0.6376)	<0.001	0.4990	(0.3590; 0.6935)	<0.001
45–54	0.6439	(0.5065; 0.8186)	<0.001	0.5721	(0.4062; 0.8057)	0.0014
≥55	1	-	-	1	-	-

Abbreviations: AOC, abstinent or occasional drinker; CED, chronic excessive alcohol drinker; MCD, moderate continuative alcohol drinker.

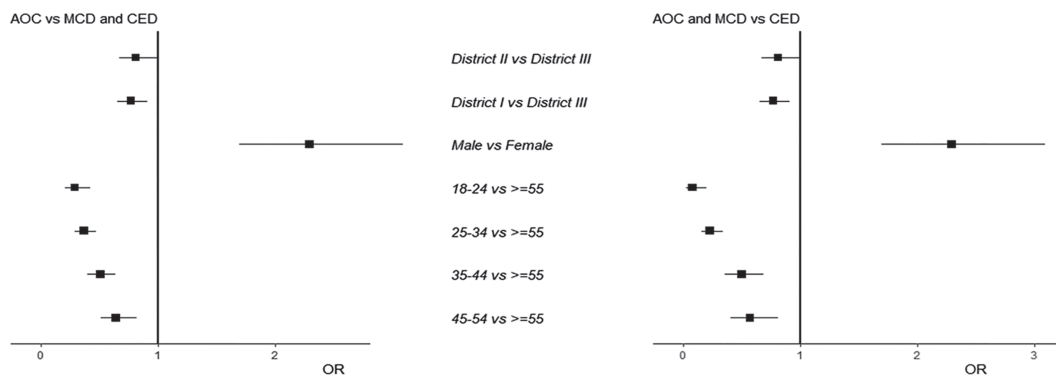


FIGURE 3 Forest plot for the predictors associated with the propensity to drink from the partial proportional odds (PPO) model. (a) Equation 1; (b) Equation 2

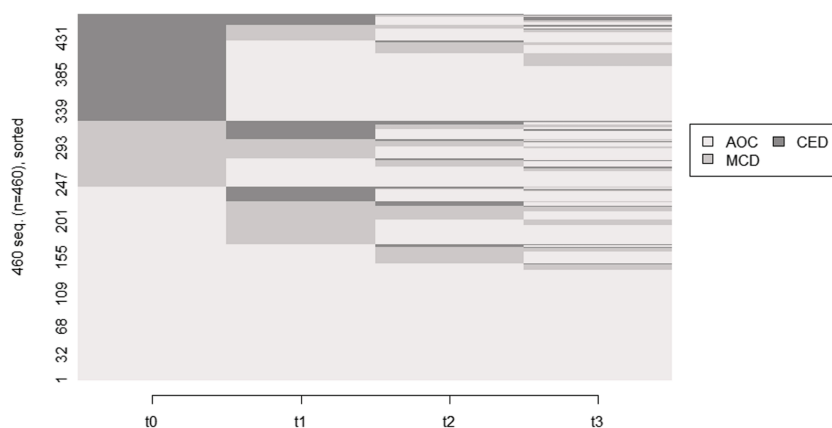


FIGURE 4 Full index plot of the drinking category over time of the 460 subjects who underwent at least four repeated ethyl glucuronide in hair (hEtG) measurements in a time span of about 18 months [Colour figure can be viewed at wileyonlinelibrary.com]

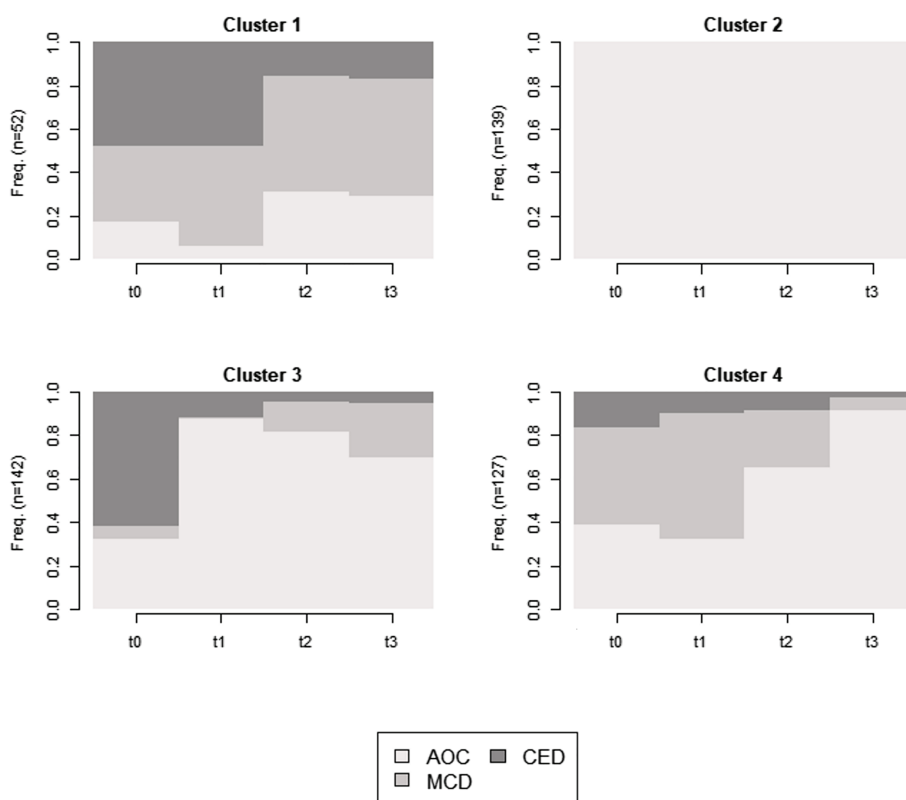


FIGURE 5 State distribution plots by cluster [Colour figure can be viewed at wileyonlinelibrary.com]

(e.g., continuous light gray line) or those with a state sequence “CED - AOC - AOC - AOC” like.

More data can be seen in Supporting Information.

With the analysis of the clusters, the hEtG trajectories are grouped according to their similarity in four clusters (Figure 5) (for more info, see Appendices C and D).

Clusters 2, 3, and 4 have a similar sample size (about 130/140 sequences), whereas Cluster 1 accounts for 52 similar sequences. Representative sequence plots may help in better interpreting and labeling the clusters (see Data S6).

Cluster 1 ($n = 52$) depicts habitual drinkers (MCD and CED), showing prolonged drinking habit. Two kinds of sequences with no change state are recognized: those who always remain CED and those who remain MCD.

There are also two kinds of sequences with one state change, such as: CEDs who lower their hEtG value after approximately 1 year; in the same way, the MCD resulted to have an hEtG value lower than 7.0 pg/mg after the first two measurements. People alternating periods of negativity with periods of moderate continuative drinking are also present.

Cluster 2 ($n = 139$) represents the AOC who always remain AOC.

Cluster 3 ($n = 142$) sees mainly CED at baseline, being negative to hEtG test from the second test onwards.

Cluster 4 ($n = 127$) accounts for people switching from active drinking behaviors to abstinence phases; all the MCDs have a state change in the lower category (AOC).

We learn from the plots that one representative is necessary for each of the Cluster 2 and Cluster 3 to achieve the 30% coverage;

TABLE 3 Odds ratios for cluster memberships

		Cluster 1	<i>p</i>	Cluster 2	<i>p</i>	Cluster 3	<i>p</i>	Cluster 4	<i>p</i>
District	II	0.8531	0.6765	1.3435	0.2933	1.4462	0.1646	0.5324	0.0274
	I	0.7251	0.3645	1.4434	0.1409	1.0651	0.7966	0.7774	0.2947
Gender	Male	2.8505	0.3159	0.3951	0.0471	0.7346	0.5106	3.9631	0.0692
Age class	18–24	0.0912	0.0238	9.3183	<0.001	0.2173	0.0052	1.3714	0.5321
	25–34	0.3291	0.0131	3.7389	0.0015	0.4249	0.0104	1.7555	0.1482
	35–44	0.2597	0.0018	3.0623	0.0054	0.5388	0.0445	1.8842	0.0855
	45–54	0.5800	0.1870	1.6016	0.2853	0.5977	0.1202	2.1425	0.0518

2 and 4 representatives are, instead, needed for Cluster 4 and 1 for reaching the same coverage, and it has to be remembered that the actual coverage is, in order, in the % of $C_1(\%) = 34.6\%$, $C_2(\%) = 100\%$, $C_3(\%) = 67.9\%$, $C_4(\%) = 33.9\%$.

From logistic regression (Table 3), we confirm women are significantly more present in Cluster 1 ($OR = 0.3951$, $p = 0.0471$) and that people from District III are significantly more present than people from District II in Cluster 4 ($OR = 0.5324$, $p = 0.0274$).

Moreover, the chances to follow the trajectory pattern with 0 or very low hEtG level all over, depicted by Cluster 2, are multiplied by more than 9 for the youngsters. A similar explanation can be done for those aged 25–34 with chances multiplied by about 4 times.

The oldest cohort (≥ 55 years) is significantly more present in Cluster 1 than all the others ($ORs < 1$, all $p < 0.05$) except for the 45–54 years old class.

Among 4328 analysis, 188 tested positive also for at least one drug of abuse.

4 | DISCUSSION

In our study, we investigated the characteristics of the subjects who underwent toxicological analyses for driver's license regranting process after DUI. Our sample was mostly composed of men, aged between 25 and 34. These data are consistent with the national and international scenario,^{35,36} reporting that young men are the ones most frequently found driving under the influence of alcohol or substances of abuse.

The serial analyses of hEtG, thanks to its high diagnostic sensitivity and specificity in evaluating moderate and chronic excessive alcohol consumption, provided important information about the alcoholic habits of the sanctioned drivers over time and, because of its longer window of detection, also about the impact of the new protocol on the prevention from DUI of ethanol.

In our sample (4328 subjects), only 22.2% of the drivers provided positive hEtG values, suggesting, at least, habitual alcohol consumption (≥ 7.0 pg/mg) at T0 after the charge for DUI. This indicates that the subjects were occasional drinkers or that they decided to reduce/avoid alcohol use in view of the appointment.

Regarding those providing positive results for hEtG (MCD and CED) at T0, a prevalence of males (96.3%), aged 35–44 (32.6%), coming from District I (38.2%) was observed.

Alcohol chronic consumption and abuse is often found when facing a context of abuse by numerous and different drugs. The study confirmed that the highest percentage of subjects providing a positive result for drugs of abuse tested positive also for hEtG (see Table 2).

According to our results (see Table 2), males yielded to a risk of being positive for hEtG ($OR \approx 2.29$, $p < 0.001$) significantly higher than females, independently on other variables (district and age class). These data are supported by European and International statistics,^{37–39} reporting that men consistently exceeded women in typical drinking frequencies and quantities.

Though the number of females in our population is limited ($n = 10.8\%$), we observed that they were mostly aged 25–34 (36.5%), coming from District I (48.4%) and negative to hEtG (97.7%) at T0. Considering positive women (2.3%), an hEtG concentration above 30.0 pg/mg cutoff was measured for most of the tested subjects; yet, most of positive females were in the 45–54 years old range (54.5%), thus suggesting that drinking habits among young girls are more related to occasional and/or binge consumptions, rather than chronic or continuative exposure to ethyl alcohol.

When drinking habits were related to age, we observed that overall high risk grew with the increasing of age (see Table 2). This outcome confirms findings reported by previous research studies.³⁹

In our sample, all age classes have a reduced drinking risk if compared to the ≥ 55 -year old class: Subjects aged 18–24 have 70% ($OR \approx 0.29$, $p < 0.001$) lower risk, 63% ($OR \approx 0.37$, $p < 0.001$) lower risk for the 25–34, half ($OR \approx 0.51$, $p < 0.001$) for the 35–44, and 36% ($OR \approx 0.64$, $p < 0.001$) for the 45–54.

The characteristics of the territory are also associated with different drinking patterns: In the district mainly devoted to the cultivation of vineyards (District III), there is a higher propensity to drink compared with people living in Districts I and II. This result agrees with some studies⁴⁰ that show how alcohol consumption patterns are different between rural and urban areas. Indeed, the daily consumption of wine with meals is relatively common in District III, and it is associated with wine-making processes that involve an important percentage of the workers in this territory. Yet, it is important to highlight that EtG accumulation rate in hair, due to chemical characteristics of

this substances, could be more related to a continuative and daily alcohol consumption rather than binge drinking habits.

Our study indicates that, compared with 2015, in the last few years, the portion of heavy drinkers, among subjects tested for hEtG due to DUI issues, has decreased.

Focusing attention on the subjects who have undergone at least four repeated hEtG measurements ($n = 460$), a general decreasing trend in hEtG values over time was observed. A high percentage of subjects, in fact, seemed to have at least reduced alcohol consumption. The cause could be due to alcohol-focused traffic accident prevention programs, designed to prevent DUI and to inform the population about the dangers associated with this kind of risky behavior.⁴¹ Furthermore, the data obtained from this retrospective observational study suggested that the inclusion of hEtG testing in the protocol for DLR could have contributed to raise awareness among people about the new diagnostic tools available for monitoring alcohol chronic behaviors, thus partially limiting ethanol consumption habits associated with high-risk issues. In this sense, also the information exchanged between alcohol drinkers about the more sensitive and specific biomarker used, in particular through internet, could lead to a more moderate exposure to alcohol-based beverages.

A study of a research group from Zurich highlighted how hEtG measurement could be effective also on specific population. Indeed, the authors of the study observed that hair analysis could provide a benefit to some extent in monitoring not only chronic alcohol excessive consumption, but even moderate drinking habits, especially in subjects undergoing a zero tolerance when driving restrictions.⁴²

Only a small portion of subjects worsened their alcohol consumption over time, while some subjects maintained alcoholic habits despite the controls, especially those over 55 years of age and coming from District III. This can be explained with the fact that older people typically viewed alcohol risks and harm as belonging only to other heavier and more problematic drinkers. Modifying older people's drinking habit remains a challenge, as well-established patterns of behavior can be hard to change.⁴³

4.1 | Study drawbacks

The main limitation of the study is represented by the limited number of subjects that underwent a repeated hEtG evaluation. Indeed, only about 10% of the total have been tested at least four consecutive times. The reasons are mainly due to the individual decision of medical committees, the subject's decision of quitting drive license reissuing process, and the change of residence of the subjects.

Also, the lack of standardization of the elapsed time between two consecutive analyses could lead to a bias in the statistical evaluation.

Another important drawback is represented by the potential discrepancy between the evaluated subjects and the districts where they have been included. In fact, some individuals may be examined by a medical committee without being resident in the same district. However, this bias should impact on a relatively small percentage of subjects included in the study.

Finally, a potential correlation between hEtG and other biomarkers, such as CDT, could be of great interest for the study. Unfortunately, all the other biomarkers can be performed by clinical laboratories, and not only by forensic labs, and we could not obtain these data. Yet, the committee normally required a measurement of other biomarkers only once.

5 | CONCLUSIONS

Our study allowed to highlight some risk factors related to alcohol drinking habits among drivers such as being male sex, age ≥ 55 years, and coming from rural areas. A similar protocol could be applied to a larger territory, maybe at a national level, in order to evaluate other potentially at risk populations. Moreover, data obtained from this study suggested that the introduction of hEtG test in the DLR protocol led to benefits in terms of limiting the alcohol misuse habits among subjects being evaluated. Indeed, a significant decrease was observed among young people living in urban and suburban areas.

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CONFLICT OF INTEREST

No potential competing interest was reported by the authors.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

APPENDIX A: METHODOLOGICAL APPENDIX A

TABLE A1 Odds ratio estimates, 95% CI, and *p* values of the proportional odds model of propensity to drink

	Propensity to drink			Brant's test <i>p</i>
	OR	95% CI	<i>p</i>	
(Equation 1)/(Equation 2) AOC vs. MCD and CED/AOC and MCD vs. CED				
District				
II	0.8116	(0.6687; 0.9836)	0.0339	0.30
I	0.7711	(0.6531; 0.9107)	0.0022	0.90
Gender				
Male	2.2898	(1.7130; 3.1226)	<0.001	0.39
Age class				
18-24	0.2766	(0.1951; 0.3872)	<0.001	<0.001
25-34	0.3544	(0.2796; 0.4495)	<0.001	0.01
35-44	0.4966	(0.3951; 0.6249)	<0.001	0.92
45-54	0.6274	(0.4956; 0.7951)	<0.001	0.41
Overall	-	-	-	0.01

Abbreviations: AOC, abstinent or occasional drinker; CED, chronic excessive alcohol drinker; MCD, moderate continuative alcohol drinker.

APPENDIX B: METHODOLOGICAL APPENDIX B

TABLE B1 Results of ANOVA

	Model	Resid. df	Resid. dev	Test	df	Deviance	Pr (chi)	AIC
1	1	8654	5771					5775.5
2	District	8650	5759	1 vs. 2	4	12.9	0.012	5770.6
3	District + Gender	8648	5713	2 vs. 3	2	45.2	<0.001	5729.4
4	District + Gender + Age class	8643	5594	3 vs. 4	5	119.5	<0.001	5620

Abbreviations: AIC, Akaike information criteria; ANOVA, analysis of variance.

The goodness of fit of the model is assessed indirectly by comparing regression models with the regression models before adding variables. Significance is considered with a relaxed value of $p < 0.10$ or $p < 0.15$ when comparing multiple regression models. Akaike information criteria (AIC) is also assessed.

APPENDIX C: METHODOLOGICAL APPENDIX C

Index plots are initially generated to render individual sequences of hEtG results coded as AOC, MCD, CED, and their diversity. Sequences are plotted considering the overall sample and stratified by district, gender, and age class. The transversal state distribution is computed to evaluate the percentage of drinkers at each time spot.

Agglomerative hierarchical clustering is the exploratory method used to identify distinct groups (clusters) of sequences with similar pattern. This strategy aims to build a typology of the observed sequences with each cluster grouping people with similar alcohol drinking behaviors over time. The approach for categorizing the patterns consists of computing pairwise distances between them by sequence alignment algorithms and other suitable metrics, using this information for clustering the sequences. The algorithm chosen to measure the dissimilarity between two state sequences is the optimal matching (OM) algorithm with an indel cost set at 0.5 and a

substitution cost matrix based on the observed transition rates. A low indel cost relatively to substitution costs is dictated by the interest in taking into account for similar shifted patterns: In this way, indel operation is favored over substitution.

The optimal number of clusters is chosen to be 4 among the potential solutions with 4, 5, 6, and 7 clusters. The number represents a good trade-off between the sample size and the multiple indices performance, as shown below in Appendices D and E.

The way used to summarize the clusters is extracting a set of representative sequences from the clusters themselves according to a search algorithm: Original sequences are sorted according to a density criterion. The representative set includes the sequences with the densest neighborhood. The *neighborhood density* (coverage) is defined as the number (density) of sequences in the neighborhood of the sequence elected to be a representative. The neighborhood radius is set as 10% of the maximum theoretical dissimilarity ($D_{max} = 4$), and the size of the representative set is controlled by fixing the minimal expected coverage of the representative set at 30%.

Explorative descriptive statistics (absolute frequencies and percentages) are computed for each cluster to investigate the individual profile.

After having labeled the clusters, a common further step is to examine how the cluster membership depends on covariates by means of logistic regressions.

APPENDIX D: METHODOLOGICAL APPENDIX D

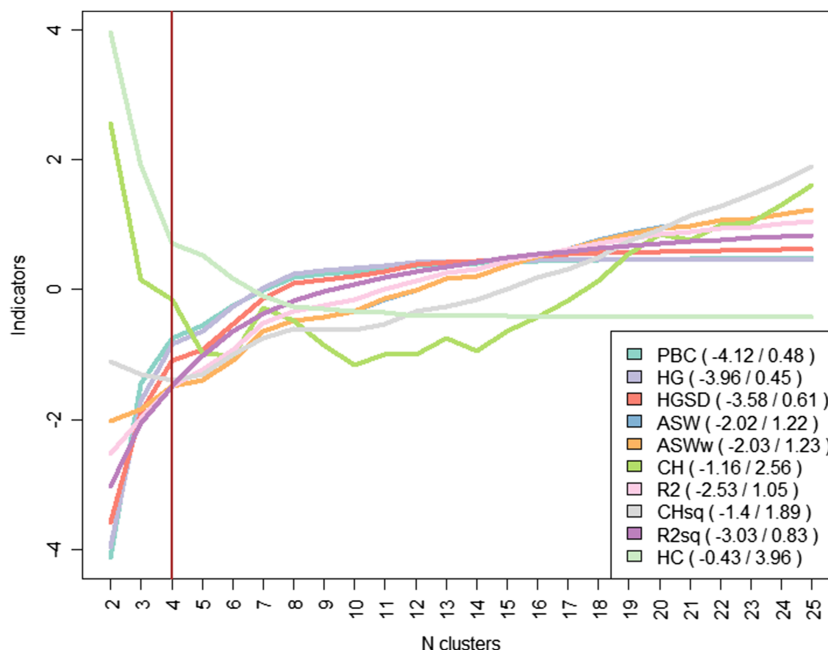


FIGURE D1 Indices for clusters comparison [Colour figure can be viewed at wileyonlinelibrary.com]

Legend:

- PBC. Point biserial correlation. Correlation between the given distance matrix and a distance, which is equal to zero for individuals in the same cluster and one otherwise.
- HG. Hubert's gamma. Same as previous but using Kendall's gamma coefficient.
- HGSD. Hubert's gamma (Somers' D). Same as previous but using Somers' D coefficient.
- ASW. Average Silhouette width (observation).
- ASWw. Average Silhouette width (weighted).
- CH. Calinski-Harabasz index (pseudo F statistics computed from distances).
- R2. Share of the discrepancy explained by the clustering solution.
- CHsq. Calinski-Harabasz index (pseudo F statistics computed from squared distances).
- R2sq. Share of the discrepancy explained by the clustering solution (computed using squared distances).
- HC. Hubert's C coefficient.

APPENDIX E: METHODOLOGICAL APPENDIX E

TABLE E1 Sample size by cluster solution

Cluster solution	Sample size
4	$C_1 = 52; C_2 = 139; C_3 = 142; C_4 = 127;$
5	$C_1 = 32; C_2 = 139; C_3 = 142; C_4 = 127;$ $C_5 = 20;$
6	$C_1 = 32; C_2 = 139; C_3 = 142; C_4 = 60;$ $C_5 = 20; C_6 = 67;$
7	$C_1 = 32; C_2 = 139; C_3 = 74; C_4 = 60;$ $C_5 = 20; C_6 = 67; C_7 = 68;$