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Usefulness of hair analysis and psychological tests for identification of alcohol and drugs of abuse consumption in driving license regranting

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Highlights

- AUDIT score ≥ 8 , indicated hazardous and harmful alcohol use, in 52.4% of drivers.
- DAST-10 score ≥ 6 , showed drug abuse and dependence problem, in 21.3% of drivers.
- Hair EtG concentration proved chronic consumption of alcohol in 34.4% drivers.
- 58.8% hair samples were positive for drugs (n=17), being cocaine the most detected.
- Significant Spearman correlation between AUDIT score and hair EtG ($r=0.331$, $p<0.05$).

Abstract

The implementation of the points-based driving license helps to change the drivers' behavior and is related to a reduction of traffic accidents and fatalities. In Spain, when a driver loses all points, the driving license is revoked, so the driver must enroll on a Driver Awareness and Re-education (DARE) course. However, at the moment offenders are not submitted to any test to confirm absence of alcohol or drugs of abuse consumption, even when 9% of Spanish drivers lose their driving license for driving under the influence (DUI). The objective of this pilot study was the comparison of the usefulness of psychological tests and hair analysis to identify those individuals with a chronic consumption of alcohol and drugs of abuse among drivers performing DARE courses. Volunteers were submitted to the AUDIT and DAST-10 tests. Also a hair sample was collected and analyzed for ethylglucuronide (EtG) (LOQ 5pg/mg) and 35 licit and illicit drugs (LOQ 5-50pg/mg) by LC-MS/MS.

Sixty-one participants with a mean age of 37.2 ± 11.6 years, and mainly men (90.2%), were recruited and performed AUDIT and DAST-10 tests. All hair samples were analyzed for EtG and 17 samples for licit and illicit drugs. Mean AUDIT score was 9.6 (SD=7.5), showing a value ≥ 8 (indicator of hazardous and harmful alcohol use) in 52.4% of cases. Mean DAST-10 score was 2.9 (SD=3.3), but a score ≥ 6 was detected in 21.3% of cases (indicating drug abuse or dependence). Twenty-two samples were positive for EtG, 8 for drugs of abuse (8 cocaine, 2 opioids, 1 amphetamines, 1 cannabis), and 3 for medicines. EtG concentration (20.7-1254.1pg/mg) was higher than the Society of Hair Testing (SoHT) cut-off for chronic alcohol consumption (≥ 30 pg/mg) in 21 cases. All positive cases for methadone and cannabis, and half of positive cases for opioids and cocaine presented higher concentrations than SoHT cut-offs for chronic consumption. Higher AUDIT score and higher EtG concentration in hair were statistically associated with declaration of alcohol consumption ≥ 4 times/month and with previous fine for DUI of alcohol. In addition, AUDIT scores and EtG concentration in hair had a moderate but significant Spearman correlation ($r=0.331$, $p<0.05$). The combination of psychological tests and hair analysis seems to be a promising tool to identify individuals with chronic and problematic consumption of alcohol and drugs of abuse. Moreover, their application during driving license regranting procedures could increase the effectiveness of DARE courses, reduce recidivism and improve road safety.

Keywords: EtG; drugs of abuse; hair; AUDIT test; DAST-10 test; driving license regranting.

1. Introduction

The number of traffic accidents has decreased significantly over the past decades in Europe, with a reduction in the total number of injuries and fatalities for this cause [1]. Specifically, from 2005 to 2014 a reduction of 20% and 44% in injury accidents and fatalities, respectively, was observed [1]. However, traffic accidents are still a major public health problem and a leading cause of injury and death around the world.

The consumption of psychoactive substances (including alcohol) before driving is associated with a high risk of traffic accidents, due to the negative impact on the driving ability by disrupting normal cognitive behavior and psychomotor functioning [2]. According to the DRUID project in 2012, the EU mean prevalence of psychoactive substances in the general driving population has been estimated at 3.5% for alcohol >0.1 g/L and 1.5% for alcohol >0.5 g/L, 1.9% for illicit drugs and 1.36% for medicines [3]. However, in those drivers involved in a traffic accident, alcohol is detected in 24.4% of seriously injured drivers and 32.8% of killed drivers, and illicit and medicinal psychoactive drugs are found in 15.2% and 15.6% respectively [4].

The implementation of the points-based driving license (PBDL) helps to change the drivers' behavior and is related to a reduction of traffic accidents and fatalities [5-8]. The PBDL has been adopted in different forms depending on the country, but in general terms, a certain number of points are assigned to every driver who commits a traffic violation and when the total number of points reaches the permitted limit, the driver's license is suspended or revoked.

Spain has a traditional high ratio of traffic accidents and road fatalities per population. But, while the number of traffic accidents has kept stable in the last years (91,187 injury accidents in 2005 and 91,570 in 2014), a significant decrease in the number of annual road fatalities has been observed (from 4,442 annual fatalities in 2005 to 1,688 in 2014) [1]. Spain adopted the PBDL in 2006 [9-10], showing a 14.3% reduction in road fatalities in the first year [11].

Driver Awareness and Re-education (DARE) courses are designed to raise awareness about the causes and the consequences of traffic accidents and to attempt to reduce the likelihood of reoffending [4]. The effectiveness of DARE courses showed an average recidivism reduction rate of 45.5%, but a large variation was observed (15%-71%) among the different studies [12]. In general, drivers with alcohol or drug dependence problem have a high ratio of recidivism [12], so some European countries perform toxicological analysis in various biological samples to probe abstinence before regranting driving license [13-15]. In Spain, tests to monitor alcohol or drug dependence problems are not mandatory at any point before granting or regranting driving license, even when 9% of the drivers performing DARE courses (and 17% of new drivers) have offenses for driving under the influence (DUI), and DUI offenses cause the loss of 27.4% of the total number of points [11,16].

In this pilot study, two different approaches have been used to detect alcohol and drug of abuse chronic consumption during driving license regranting, specifically psychological tests (easy to perform during DARE courses but some subjects may under-report to avoid legal consequences) and hair analysis (an objective measure of exposure but is time-consuming and requires complex analytical methodologies). The main objective of the pilot study was to compare the usefulness of both approaches (psychological tests and hair analysis) to identify those individuals with a chronic consumption of alcohol and drugs of abuse among drivers performing DARE courses. Also, sociodemographic parameters and drivers' behavior were studied. The identification of those individuals before driving license regranting could be useful to improve DARE courses and decrease recidivism.

2. Material and methods

2.1. Population

Subjects performing a DARE course for driving license regranting between April to July 2016 in three different Spanish cities (Ferrol, Ourense and Santiago de Compostela) were informed about the objectives of this pilot study and invited to participate. The pilot study was approved by the Ethics Committee of the University of Santiago de Compostela, Spain. All participants signed a written informed consent.

2.2. Data and sample collection

First of all, sociodemographic data (age, gender, educational level, professional status), frequency of alcohol and drugs of abuse consumption in the last 3 months, data related to driving license (type and date of issue) and other parameters related to driving behavior (number of previous fines, type of fines, number of previous car accidents, type of car accidents, mileage per month) were collected by a self-administered survey. Then participants answered two psychological tests. Finally, hair samples were collected from the *vertex posterior region* as close as possible from the scalp and tied up at the root. Hair samples were stored, protected from the light, at room temperature until analysis.

2.3. Psychological tests

The AUDIT (Alcohol Use Disorders Identification Test) is a 10-item psychological test developed by the World Health Organization (WHO) in 1989 as a simple method of screening to assess alcohol consumption, drinking behavior and alcohol-related problems [17]. AUDIT score ranges from 0 to 40 and a total score ≥ 8 is an indicator of hazardous and harmful alcohol use, and possible alcohol dependence [17]. On the other hand, DAST-10 (Drug Abuse Screening Test) is a brief version of the original test with 28-items, developed by Skinner in 1982. This test is used as a sensitive method for clinical screening and treatment evaluation research of the abuse of drugs other than alcohol in the

past 12 months [18]. A total score ≥ 6 is an indicator of drug abuse or dependence problem. Both psychological tests were administered by psychologists.

2.4. Hair analysis

Hair samples were cut as close as possible to the scalp from the posterior vertex region of the head. As recommended by the SoHT guidelines [19], the proximal segment (closest to the scalp), with a total length between 3 cm to 6 cm, was considered for analysis. The alcohol biomarker ethylglucuronide (EtG) was determined by ultra-performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS). Also, if the amount of specimen was enough, hair samples were analyzed by high-performance liquid chromatography tandem mass spectrometry (HPLC-MS/MS) for the determination of 35 licit and illicit drugs (opioids, amphetamines, cocaine, cannabis, ketamine, methadone, benzodiazepines, antidepressants and hypnotics).

For EtG analysis, 50 mg of hair were decontaminated with 2 mL of dichloromethane (3 times, 2 min each) and powdered using a ball mill (Precellys, Montigny le Bretonneux, France). Hair incubation was performed in 2 mL of water with sonication at room temperature for 2 hours. After centrifugation (4000 rpm, 10 min), hair samples were submitted to solid-phase extraction with Clean Screen EtG-UCT cartridges (United Chemical Technologies, Inc., Bristol, UK). Cartridges were conditioned with 2 mL of 1% formic acid in methanol and 2 mL of 1% formic acid in water. Samples were loaded and clean-up was performed with 2 mL of water. Cartridges were dried for 10 min under vacuum before elution with 2 mL of 1% formic acid in methanol. Eluates were evaporated to dryness with nitrogen at 35 °C, reconstituted in 50 μ L of 0.1% formic acid in water, and 10 μ L were injected into the UPLC-MS/MS. For chromatographic separation a Hypercarb column (2.1 x 100 mm, 5 μ m) (Thermo Scientific, San Jose, CA) at 40°C was employed, using 0.1% formic acid in water (A) and acetonitrile (B) as mobile phase at 0.3 mL/min. The gradient was programmed as follows: 0-6 min 20% B linearly increased to 95%, 6-6.2 min to return to initial conditions and 6.2-10 min column re-equilibration. EtG retention time was 2.6 min. A Xevo TQD (Waters Corp., Milford, MA, USA) mass spectrometer was employed, operating in electrospray in negative mode (ESI-), and using the following optimized settings: capillary voltage 2.5 kV; source block temperature 120°C; desolvation gas (nitrogen) temperature 350°C; desolvation gas flow rate 800 L/h; and cone gas (nitrogen) flow rate at 50 L/h. Data were acquired in MRM (multiple reaction monitoring) mode, using the following optimized precursor-product transitions: 220.9>85.0 (Cone: 35 V, Collision energy: 15 eV) and 220.9>75.0 (Cone: 35 V, Collision energy: 14 eV) for EtG; and 226.0>85.0 (Cone: 35, Collision energy: 16 eV) for the deuterated internal standard (EtG-d₅). The method was fully validated and applied routinely in our laboratory. The limit of quantification (LOQ) of EtG was 5 pg/mg.

A previously published method by Lendoiro et al. [20] was employed for determination of 35 licit and illicit drugs. Briefly, 50 mg of hair were incubated with 2 mL of acetonitrile at 50°C overnight and submitted to 2 consecutive extraction processes; first, to a liquid-liquid extraction (with a mixture of hexane and ethyl acetate), followed by a solid-phase extraction (Strata-X cartridges; Phenomenex, Torrance, CA, USA). Chromatographic separation was performed using an Atlantis T3 analytical column (2.1 x 100 mm, 3 μ m) (Waters Corp, Milford, MA) and acetonitrile and ammonium formate (pH 3) as mobile phase. The total run time was 32 minutes. One transition per analyte was monitored in MRM mode, and to confirm a positive result, a second injection monitoring 2 transitions was performed. The method was fully validated and applied to the analysis of hair specimens, including the proficiency test in hair organized by the Society of Hair Testing (SoHT). The LOQs were between 0.5 pg/mg for lysergic acid diethylamide and 50 pg/mg for Δ^9 -tetrahydrocannabinol (THC) and 5-20 pg/mg for the rest of the analytes.

2.5. Statistical analysis

For statistical analysis, participants were categorized depending on the score obtained in the psychological tests and the concentration found in hair. An AUDIT score ≥ 8 and a DAST-10 score ≥ 6 was considered as chronic exposure to alcohol and to drugs of abuse, respectively. For hair results, the cut-offs recommended by the SoHT in its guidelines were used. Hence, an EtG concentration ≥ 30 pg/mg was considered as chronic exposure to alcohol [19]. In addition, chronic exposure to drugs of abuse was established using the following values: ≥ 50 pg/mg for THC; ≥ 200 pg/mg for opiates and amphetamine derivatives; and ≥ 500 pg/mg and ≥ 50 pg/mg for cocaine and its main metabolite benzoylecgonine (BE), respectively [21].

All statistical analyses were performed using SPSS software (Version 21, SPSS Inc., Chicago, IL, USA) employing descriptive statistics, Student t Test, Chi-Square Test and Spearman's correlation. The level of significance was set at $p \leq 0.05$.

3. Results and discussion

3.1. Population, sociodemographic data and driving parameters

One-hundred eight volunteers were recruited, all of them performing DARE courses for revocation of driving license after losing all points. All volunteers performed the 2 psychological tests (AUDIT test and DAST-10 test) but only 72 donated a hair sample. Of them, 11 cases were discarded because the amount of hair was scarce, and finally 61 samples were analyzed for determination of EtG. Moreover, in 17 cases the amount of hair was enough to be also analyzed for determination of drugs of abuse. Finally, the results of paired cases with completed AUDIT test and hair EtG analysis ($n=61$) were included in this pilot study for the determination of alcohol consumption. Also paired cases with DAST-10 and hair analysis for drugs of abuse ($n=17$) were included for determination of drugs of abuse consumption.

The mean age of the 61 participants was 37.2 ± 11.6 years (ranged 18-63 years). The majority were men (90.2%) and employed (83.6%). Women presented more unemployment than men (33% vs 14.5%). Their level of education was the following: 41.7% had primary education, 45% had secondary education (83.3% of women and 40.7% of men), and 13.3% (all men) had a university degree.

Declared frequencies of alcohol and drugs consumption are shown in Table 1. Alcohol and cannabis were the most consumed substances, as 25.4% and 17.5% of participants, respectively, declared their consumption more than 4 times per week.

In relation to traffic offenses, 91.2% of participants declared at least a previous fine, with an average number of 3.2 ± 2.4 (range 0-9), being DUI of alcohol (79.0%) and speeding (55.4%) the most common traffic offenses. Table 2 describes the type and number of previous fines declared by participants. Finally, 74.1% of participants declared a previous car accident, being in the 65.5% of cases a car accident with car damage and in a 20.0%, a car accident with injured and/or death people. Table 3 shows the type and number of previous car accidents declared by participants.

3.2. AUDIT test

The AUDIT test score ranged from 0 to 32 (mean \pm SD: 9.6 ± 7.5) in the 61 studied cases. A total score ≥ 8 was found in 52.4% cases ($n=32$), indicating hazardous and harmful alcohol use, and possible alcohol dependence [17]. No statistical significant differences in AUDIT scores were found between ages, genders, and professional status. However, drivers with primary education had a statistically significant lower AUDIT score compared with drivers with secondary education and university degree (7.0; 11.8; and 11.0, respectively, $p \leq 0.02$). Furthermore, AUDIT score was higher in those participants who declared consumption ≥ 4 times per month in the last 3 months than in those who admitted consuming < 3 times, for all the investigated substances: alcohol (14.5 vs 5.7, $p < 0.001$), cocaine (20.3 vs 8.9, $p = 0.009$) or benzodiazepines (21.7 vs 8.7, $p = 0.004$).

Finally, significantly higher AUDIT scores were also found in drivers who admitted a previous fine for DUI of alcohol (in comparison with drivers without fines) (11.5 vs 3.8, $p \leq 0.02$), declared at least a previous car accident with injured and/or death people (in comparison with drivers without this car accidents) (14.5 vs 8.3, $p \leq 0.01$), or admitted having previous problems for DUI (in comparison with those without problems) (10.7 vs 4.1, $p < 0.001$).

3.3. DAST-10 test

The DAST-10 score ranged from 0 to 10 (mean \pm SD: 2.9 ± 3.3). Thirteen out of 61 cases (21.3%) presented a total score ≥ 6 , indicating of drug abuse or dependence problem. No statistical significant differences in DAST-10 score were found between ages, genders, educational levels and professional status. However, DAST-10 score was higher in those participants with a declared consumption ≥ 4 times per month in the last 3 months of cannabis (compared with those who admitted consumption of < 3 times per month) (5.3 vs 2.0 , $p \leq 0.001$).

In addition, a higher DAST-10 score was observed in drivers who admitted a previous fine for not wearing a seatbelt (4.6 vs 2.5 , $p = 0.032$) and a previous car accident with car damage (3.6 vs 1.5 , $p = 0.023$).

3.4. Hair analysis

Hair analysis showed the presence of EtG in 22 out of 61 analyzed cases (36.1%), all of them men. EtG concentration ranged from 20.7 to 1254.1 pg/mg (mean \pm SD: 182.5 ± 264.2 pg/mg). Chronic alcohol consumption was detected in 21 out of 22 positive cases (34.4% of analyzed cases), as EtG concentrations were higher than the SoHT cut-off (≥ 30 pg/mg) [19]. Statistically significant differences in EtG concentrations were found between age groups (drivers older than 45 years old showed a higher mean EtG concentration than those under 45 years old: 150.5 pg/mg vs 24.5 pg/mg, $p = 0.001$) and educational levels (drivers with a university degree had higher mean EtG concentrations than those with primary education: 238.9 pg/mg vs 36 pg/mg, $p = 0.002$). Moreover, average EtG concentration was significantly higher in participants who declared consumption of alcohol ≥ 4 times per month in the last 3 months than in those admitting consumption of alcohol < 4 times (123.6 pg/mg vs 22.2 pg/mg, $p = 0.004$). No statistically significant differences in EtG concentrations were found for the consumption of other drugs. Drivers declaring a previous fine for DUI of alcohol showed a higher mean EtG concentration than individuals without this type of offense (88.5 pg/mg vs 2.7 pg/mg, $p = 0.04$). But surprisingly, declaration of a previous car accident was statistically significant associated with a lower EtG concentration in hair (42.5 pg/mg vs 136.6 pg/mg, $p = 0.001$).

For other licit and illicit drugs, hair analysis showed an overall 58.8% of positive results (10 out of 17 analyzed samples). Specifically, 8 cases were positive for drugs of abuse and 3 cases for medicines, with a prevalence of 47.1% for cocaine ($n=8$), 11.8% for opioids ($n=2$), 5.9% for amphetamine derivatives ($n=1$), 5.9% for cannabis ($n=1$), 11.8% for methadone ($n=2$), 5.9% for benzodiazepines ($n=1$) and 17.6% for antidepressants ($n=3$). So, proportionally, hair analysis showed to be more efficient to detect drug consumption than alcohol consumption. Regarding the detected drugs, it was surprising the high proportion of positive cases to cocaine and the unexpected low positive cases for cannabis, while in the general population the opposite has been observed. A possible explanation is cannabis consumption is predominant in young people, while 73.8% of the drivers in our sample were older than 30 years. In addition, all positive cases for methadone and cannabis, and half of positive cases for opioids and cocaine presented higher concentrations than SoHT cut-offs for chronic consumption [21] (Table 5). Moreover, polydrug consumption was common, and if alcohol consumption is included, 2 or more substances were detected in 7 out of 10 positive cases. As the number of hair samples

analyzed for the determination of drugs of abuse was low, statistical analysis was limited. However, all cases with a positive result in hair declared a previous car accident with injured and/or death people. Moreover, correlation between self-report of drug consumption in the last 3 months and hair analysis showed a 50% agreement.

3.5. Comparison between AUDIT score and hair analysis for alcohol consumption

Chronic consumption of alcohol was detected in 32 out of 61 cases using the AUDIT test and in 21 cases using the EtG concentration in hair. In 34 cases (55.7%) coincident results were found between AUDIT scores and EtG hair concentrations, while conflicting results were obtained in 27 cases (Table 4). Conflicting results were considered those cases with an AUDIT score of possible alcohol dependence (≥ 8) and a EtG concentration in hair < 30 pg/mg (19 cases, of which 18 cases had a EtG concentration $< \text{LOQ}$), or those cases with an AUDIT score < 8 and a EtG concentration in hair ≥ 30 pg/mg (8 cases). A possible explanation for those discrepancies could be the different window of detection measured by AUDIT (1 year) and by hair analysis (3-6 months). Having a university degree, declaring alcohol consumption ≥ 4 times per month and having a previous fine for DUI of alcohol were significantly associated with a higher probability of chronic consumption of alcohol using both approaches (AUDIT score and EtG concentration in hair). AUDIT scores and EtG concentrations in hair showed a moderate but significant Spearman correlation ($r = 0.331$, $p < 0.05$). Nevertheless, when considering only the group of drivers who admitted alcohol consumption ≥ 4 times per week, the correlation between AUDIT scores and EtG concentrations was much higher ($r = 0.51$, $p = 0.009$). Similar degrees of correlation were found between AUDIT scores and hair EtG concentrations by Dreher-Weber et al. [22] ($r = 0.361$, $p < 0.001$) in 344 nursing home residents, and by Marques et al. [23] ($r = 0.23$, $p < 0.01$) in 146 DUI drivers. Therefore, although in our pilot study AUDIT score was able to identify a higher number of cases with chronic consumption of alcohol, a combination of both tests would improve the detection of drivers with a chronic and probable problematic consumption of alcohol. Also Wurst et al. [24], after investigated whether biomarkers of alcohol consumption (EtG or FAEE) in hair would provide additional information to the use of the AUDIT test in pregnant women, concluded that the combined use of the AUDIT questionnaire and direct ethanol metabolites identify more potential alcohol consumers than does the sole use of the AUDIT questionnaire. The combined use of self-reports and direct ethanol metabolites seemed also promising to assess alcohol use among patients receiving maintenance treatment for opioid dependence [25,26]. Recently, Ferraguti et al. [27] agreed that the diagnosis of maternal alcohol consumption during pregnancy only based on indirect methods, such as questionnaires, might significantly underestimate alcohol use. In general, psychometric assessments require subject cooperation, yield potentially self-incriminating disclosures, and are expected to perform more poorly as risk indicators in real situations than under research conditions (when confidentiality is assured). So, the reasonably good capacity of the AUDIT test to detect chronic alcohol use in this pilot study might be different without those guaranties [23].

3.6. Comparison between DAST-10 score and hair analysis for drugs of abuse consumption

A limited number of hair samples ($n = 17$) were analyzed for the detection of 35 licit and illicit drugs due to the lack of hair specimen after EtG analysis. The average DAST-10 score in these participants was 3.7 (SD = 3.8), with a total score ≥ 6 (indicating of drug abuse or dependence problem) in 5 of them (29.4%) [18]. Regarding hair analysis, 10 samples showed a positive result to at least one substance included in the analytical method with a concentration $\geq \text{LOQ}$, but only 4 cases had concentrations higher than SoHT cut-offs for chronic consumption. The comparison between both tests (DAST-10 and hair analysis) showed consistent results in 13 cases (76.5%) and inconsistent results in 4 (Table 5), probing better agreement than AUDIT-EtG results. Two inconsistent results were especially surprising, Case 40 with the highest DAST-10 score (DAST-10 = 10) but testing negative for drugs, and Case 49 with the highest cocaine concentration in hair (Benzoylecgonine = 13,144.4 pg/mg, Cocaine = 12,069.5 pg/mg, and Cocaethylene = 1,049.9 pg/mg) and a DAST-10 = 0 (Table 5). Also Tassiopoulos et al. [28] found that 34.2% heroin users who tested positive for cocaine in hair did not report recent cocaine use, and concluded that confirmation of self-report with biochemical analysis may improve results.

Grekin et al. [29] found that the DAST-10 identified less than half of women using drugs during the last trimester of pregnancy, compared to biomarkers screening. In this context, under-reporting of illicit drug use remains a substantial concern despite the use of anonymity [29]. Self-report of drug use under driving licence regranting situations may also be expected to be under-reported.

3.7. Limitations

The main limitations of the present pilot study were the retrospective reporting of drug use (that may be subject to recall bias) and the differences in terms of windows of detection among psychological tests (past-year use) and hair analysis (three to six months past use). Moreover, the low number of hair samples analyzed for detection of drugs of abuse limited the statistical power for comparison between DAST-10 test and hair analysis. Finally, the participation in the pilot study was volunteer, anonymous and without consequences in driving license regranting procedure if any test showed a chronic or problematic consumption of alcohol and/or drugs of abuse. For that, participants may be expected to admit more drug use and abuse than in other more realistic circumstances.

4. Conclusions

The combination of psychological tests and hair analysis seems to be a promising tool to improve the identification of drivers with chronic and problematic consumption of alcohol and drugs of abuse. Nevertheless, similar studies in more realistic conditions should be conducted in order to obtain a better evaluation of these tools. If future investigations confirm our preliminary results, their application during driving license regranting procedures could increase the effectiveness of DARE courses, reduce recidivism and improve road safety.

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Table 1. Frequency of alcohol and drugs consumption declared by participants (n=61).

Frequency	Alcohol (%)	Cannabis (%)	Cocaine (%)	Amphetamine derivatives (%)	Other drugs of abuse (%)	Benzodiazepines (%)
Never	18.6%	57.9%	66.1%	92.8%	92.8%	83.9%
<1 time/month	18.6%	12.3%	16.9%	3.6%	3.6%	7.1%
2-4 times/month	20.4%	3.5%	11.9%	3.6%	0.0%	3.6%
2-3 times/week	17.0%	8.8%	1.7%	0.0%	0.0%	1.8%
≥4 times/week	25.4%	17.5%	3.4%	0.0%	3.6%	3.6%

Table 2. Type and number of previous fines declared by the participants (n=61).

Type of traffic offense	Number of previous fines	%
DUI of alcohol	0	21.0%
	1	43.9%
	2	17.5%
	3	8.8%
	>3	8.8%
DUI of drugs of abuse	0	90.7%
	1	3.7%
	2	5.6%
	3	0.0%
	>3	0.0%
Speeding	0	44.6%
	1	28.6%
	2	10.7%
	3	5.4%
	>3	10.7%
Not wearing a seatbelt	0	77.8%
	1	14.8%
	2	5.6%
	3	0.0%
	>3	1.8%
Failing a stop sign	0	87.0%
	1	9.3%
	2	0.0%
	3	3.7%
	>3	0.0%
Using a phone while driving	0	90.4%
	1	9.6%
	2	0.0%
	3	0.0%
	>3	0.0%

Table 3. Type and number of previous car accidents declared by the participants (n=61).

Type of car accident	Number of previous car accidents	%
Car damage	0	34.5%
	1	34.5%
	2	13.8%
	3	1.7%
	>3	15.5%
Injury and death people	0	80.0%

	1	16.4%
	2	3.6%
	3	0.0%
	>3	0.0%

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Table 4. AUDIT score and hair EtG concentrations of the studied cases with positive results (in bold are indicated the inconsistent results).

Case	AUDIT score	EtG (pg/mg)	Case	AUDIT score	EtG (pg/mg)
1	15	<LOQ	21	19	<LOQ
2	7	36.2	22	19	<LOQ
3	10	<LOQ	23	13	64.7
4	15	215.3	25	20	31.0
5	11	<LOQ	26	8	<LOQ
6	32	269.9	28	15	<LOQ
7	12	<LOQ	29	6	126.5
8	26	209.2	30	9	<LOQ
9	8	174.4	31	25	20.7
10	13	<LOQ	32	12	<LOQ
11	9	1254.1	33	22	<LOQ
12	11	43.8	36	4	32.4
13	27	464.3	39	8	123.1
14	6	80.4	41	5	325.0
15	12	<LOQ	43	8	<LOQ
16	12	53.5	44	8	<LOQ
17	9	<LOQ	47	2	73.4
18	12	<LOQ	49	4	54.7
19	22	43.2	60	0	166.5
20	15	150.7	61	10	<LOQ

Table 5. DAST-10 score and results of hair analysis in the 17 studied cases for drugs of abuse consumption.

Case	DAST-10 score	Drugs of abuse and medicines (pg/mg)	
1	9	Cocaine	BE: 776.7; COC: 761.6
2	5	Cocaine Opioids Methadone	BE: 1862.5; COC: 2499.1 6-AM: 151.8 MTD: 3195.6
3	9	Cocaine Opioids Methadone	BE: 1476.5; COC: 1536.2 MOR: 531.1; COD: 471.3 MTD: 3216.3
4	0	Antidepressants	CIT: 70.8
5	4	Negative	
16	6	Cocaine Amphetamine	BE: 67.6; COC: 105.2 AMPH: 50.8
17	0	Cocaine	BE: 49.6; COC: 68.0
26	7	Cocaine Cannabis	BE: 137.2; COC: 168.8 THC: 68.2
27	0	Negative	
28	0	Negative	
38	0	Cocaine	BE: 29.7
39	4	Benzodiazepines Antidepressants	DIA: 135.6; NORDIA: 130.4 CIT: 3059.1
40	10	Negative	
49	0	Cocaine Antidepressants	BE: 13144.4; COC: 12069.5; COCET: 1049.9 VEN: 93.0
50	0	Negative	
51	5	Negative	
60	0	Negative	

6-AM: 6-monoacetylmorphine; AMPH: amphetamine; BE: benzoylecgonine; COC: cocaine; COCET: cocaethylene; COD: codeine; CIT: citalopram; DIA: diazepam; MOR: morphine; MTD: methadone; NORDIA: nordiazepam; THC: Δ 9-tetrahydrocannabinol; VEN: venlafaxine