



Short report

Driving under the influence of alcohol. A 5-year overview in Piedmont, Italy

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ABSTRACT

Alcohol consumption represents a major health issue worldwide and a crucial factor in road accidents. This study provides information on the prevalence of alcohol in blood testing performed on 2752 subjects involved in vehicle accidents, which occurred in Piedmont (northern Italy) between 2008 and 2013. Blood alcohol concentration (BAC) was determined by an ISO 17025 accredited GC/MS procedure. Fifty-one % of positive samples showed BAC concentrations above 1.5 g/L, with a legal cut-off fixed at 0.5 g/L (and 0 g/L for specified categories such as novice and professional drivers). BAC values proved statistically different regarding the day of sampling (week or weekend days), age and gender, with a prevalence of positive results that reflects different drinking habits of a multifaceted population of alcohol consumers.

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1. Introduction

Alcohol is a widely used legal drug in the western Countries, and driving under the influence of alcohol is considered as one of the main causes of traffic accidents. As a matter of fact, alcohol is responsible for a great number of traffic accidents due to its pharmacological action on the central nervous system, resulting in increased reaction time, decreased ability to estimate space and distances, increased feeling of self-confidence, and ultimately significant decrease in the ability to safely drive a motor vehicle.^{1–7} Several epidemiological and pharmacological studies show a significant positive correlation between the chances of a driver being involved in traffic accidents and his/her blood alcohol concentration (BAC),^{8–12} especially at very high BAC.¹³

Tolerance and political attitudes towards alcohol and drug use by vehicle drivers are different among Countries, and are reflected in assorted road-traffic legislation, law enforcement, and sanctions for offenders.^{14,15} In Italy, a driver is considered liable for driving under the influence of alcohol, if his/her BAC is higher than 0.5 g/L. In the interval 0.5–0.8 g/L, drivers are only liable for an administrative fine. Above 0.8 g/L, offenders are potentially convicted for criminal offence, with more severe sanction if the BAC is found

above 1.5 g/L. Furthermore, in 2010, the legal limit for BAC was fixed to 0 (zero) g/L for i) drivers <21 years, ii) drivers with less than 3 years' experience and iii) professional drivers of trucks, buses and taxis. In this case, it is reasonable to consider the limit of detection of the analytical method as the decision limit for assessing the zero-alcohol limit. However, considering the possible influence of endogenous production and other minor exogenous sources of ethanol which could provide a non-zero BAC level (and also to maintain a conservative approach), some authors have chosen 0.1 g/L as a cut-off to assess compliance with the zero-alcohol limit.¹⁶

In Piedmont, northern Italy, the Regional Government periodically issues the guidelines (last update: 28.07.2009) for monitoring drivers and pedestrians involved in traffic accidents. Currently, all drivers, passengers and pedestrians involved in road traffic accidents (fatal or not) are possibly tested for ethanol and illicit drugs. Therefore, whenever an injured person is admitted to a hospital for treatment, his/her blood is sampled after informed consent is given. If a driver refuses examination, the most aggravated sanctions, as in the case of BAC levels greater than 1.5 g/L, will be applied. Road accidents involving only cars are the most frequent and represent 75% of the total number of accidents with injured people, over the decade 2001–2010 in Piedmont (Italy). Among these, drivers represent 71% of the injured people, the remainders being front or rear passengers.¹⁷

In the last decade, few prevalence studies connecting alcohol consumption and road accidents have been performed in Italy,^{18,19}

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while more detailed reports were published in the context of international collaborative studies.¹² Aim of this study is to provide the first overview in Piedmont on the results of alcohol blood testing performed between 2008 and 2013, on over 2000 subjects involved in vehicle accidents. Although epidemiological studies are difficult to compare with each other because of the differences in study design,¹² the results described herein can be related with those collected from other geographical areas and in different time periods.

2. Materials and methods

All samples were taken from injured subjects involved in road traffic accidents and consequently admitted to local hospitals in the period 2008–2013. Generally, blood is sampled immediately after admittance in the hospital; however the time lapse between the accident and blood sampling is usually unknown, as well as the short-term storage conditions. Only samples taken from living subjects were considered. Post-mortem samples were excluded.

Our center is the reference laboratory in Piedmont for the execution of toxicological analyses, including those for the alcohol content in blood samples. The general procedure includes a preliminary blood screening by a colorimetric method using alcohol dehydrogenases and running on Abbott Architect c8000 analyzer. In case of BAC results above 0.5 g/L, further processing for confirmation, using a gas-chromatograph equipped with a headspace auto-sampler and interfaced with a mass-spectrometer (HS-GC-MS), is executed.

Confirmation alcohol analysis is performed in whole blood or plasma samples. Because the concentration of alcohol in plasma or serum is higher than in an equal volume of whole blood, in road-traffic cases an appropriate correction is necessary.²⁰ In the majority of our cases, sodium fluoride is used as blood anticoagulant, as regulated by regional guidelines, and therefore whole blood samples are analyzed. Nevertheless, in the rare cases when we receive plasma samples, ethanol concentrations are recalculated by using the plasma/blood ratio 1.2:1²⁰. A 100 μ L aliquot is transferred into a 20 mL head-space vial. Afterwards, 10 μ L of 2-isopropyl alcohol, used as internal standard at a final concentration of 1.0 g/L, is added and then the vial is crimped. The headspace equipment is a Dani 86.50 HS autosampler (DANI Instruments S.p.A., Cologno Monzese (MI), Italy), which was operating at the following conditions: vial equilibration time: 10 min; vial mixing: moderate; vial pressurize: 10 s; injection time: 30 s; oven temperature: 70 °C; sample loop temperature: 80 °C; transfer line temperature: 90 °C. The GC/MS analysis was carried out using an Agilent (Palo Alto, CA, USA) 5975 Mass Selective Detector interfaced to an Agilent 6890N gas chromatographer. Injections were made in the split mode into an Agilent HP-5 column (50 m \times 0.2 mm i.d. \times 0.33 μ m film thickness). The oven temperature was maintained isothermal at 70 °C for 8 min. Helium was used as the carrier gas. The injector and transfer line temperatures were set at 200 °C, and the split ratio was 50:1. Data were acquired in the selected ion monitoring (SIM) mode. The ions m/z 31 (quantification ion), m/z 45 and m/z 46 (qualifier ions) were selected for ethanol determination and the ions m/z 45 (quantitative) and m/z 59 (qualifier) for the internal standard. The method is internally validated and accredited in accordance with ISO/IEC 17025:2005 rules. Linear calibration was observed for ethanol in the range 0.1–3.0 g/L, with a squared correlation coefficient (R^2) of 0.9918. All back calculations of standards were found to lie within $\pm 5\%$ at each calibration level. Specificity tests proved successful. SIM chromatograms from negative whole blood samples showed no interfering signals at the ethanol retention time. Accuracy requirements were satisfied, as the percent bias was below 10% at all concentrations. Intra-assay precision was also

satisfying, as the CV% were within $\pm 10\%$ at 0.5 g/L ethanol concentration, and within $\pm 5\%$ at 0.8 g/L and 1.5 g/L. Experimentally verified LOD and LOQ values were 0.03 g/L and 0.1 g/L. The absence of carry-over effect was positively verified. Laboratory performances for ethanol analysis are constantly monitored through regular participation to inter-laboratory proficiency tests organized by LGC Standards Proficiency Testing (Lancashire, UK).

2.1. Statistical analysis

Under the hypothesis of independent samples population, the Yates' chi-square test was selected for conformity assessment. The 2×2 contingency tables were constructed by listing the number of male and female positive samples and male and female negative samples respectively. The chi-square test was performed, corrected by the Yates factor when a large discrepancy between the compared group populations was observed. Data are reported in Table 1. When the critical chi-square value at 95% confidential interval (CI) and 1 degree of freedom (df) proved larger than the calculated Yates' chi-square value, the null hypothesis H_0 (no significant differences between two groups) was retained. At a 95% CI and 1 df the critical chi-square value is 3.84.

The Kruskal–Wallis non-parametric hypothesis test was chosen to verify the occurrence of statistically significant differences between the independent populations divided by ranges of age and sex. The null hypothesis H_0 affirms that there are no significant differences between the independent populations under examination. A significant level (a two tailed P-value) of 0.05 (CI = 95%) was chosen for the statistical test. When the P-value proved smaller than the critical P-value, the hypothesis H_0 was rejected. All statistical analyses were conducted using the software KY PLOT v 2.0 beta 15.

3. Results

In the period 2008–2013, a totality of 2752 samples taken from injured subjects involved in car accidents was analyzed in our laboratory for confirmation analyses. Most subjects were likely to be car drivers, although this detail was not specified in the medical records accessible to laboratory personnel. Thus, the number of sample donors is expected to mainly comprise car drivers, and secondarily pedestrians, riders of bicycle or motorcycle, and car passengers. The majority of the samples (81.3%) was taken from male subjects, mainly of age 18–41 years. Across all ages, the number of blood samples collected from males exceeded those collected from females (Fig. 1). However, only for some groups of age (22–31, 32–41 and 42–51) the number of positive results (BAC > 0.5 g/L) was statistically different between males and females, as shown in Table 1. Nevertheless, gender of impaired drivers does not appear to represent a discriminating factor when the age is particularly low (≤ 21 yr) or high (> 51 yr).

During the 5-years period, no significant change was observed in the distribution of BAC violations. The situation is clearly represented in Fig. 2.

The majority (around 51%) of positive samples was found to have a BAC between 1.5 and 3.0 g/L. The extended interval BAC > 0.8 g/L summed up more than 85% of violations, all to be classified as criminal offence. More detailed description of the results, based on a subtler separation of age groups, is shown in Table 2. These independent subpopulations were compared by means of the Kruskal–Wallis test. All p-values are reported in Table S1. As also shown in Fig. 3, BAC levels between 0.5 and 1.5 g/L were more frequent for young drivers (aged less than 32 years). Most relevantly, the intervals 1.5–3.0 g/L and > 3.0 g/L both showed

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