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 accidences. 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.

*Cover Letter

Centro Regionale Antidoping
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Settori di attività
tossicologia *analitica – forense – industriale – veterinaria – dello sport*
sicurezza alimentare, prevenzione antidoping

Torino, October 21st, 2014

Dear Editor,

I'm pleased to submit the manuscript entitled "Driving under the influence of alcohol. A 5 year overview in Piedmont, Italy".

Aim of this study is to provide, for the first time in our Region, a comprehensive overview on the results of alcohol blood testing performed on over 2000 subjects, between 2008 and 2013, which were involved in vehicle accidents. Results can be compared with those of other geographical areas and of different time intervals. The present study highlights that alcohol abuse still represents a major problem that requires specific implementation of public policies for traffic crashes prevention. Once more, it is demonstrated that young drivers, both male and female, must be considered high-risk categories and should be addressed with targeted prevention campaigns. Therefore, we believe that these results can be of interest to the community of Forensic Toxicologists.

Best regards.

Yours faithfully,
Alberto Salomone

*Title Page (WITH Author Details)

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

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***Highlights (for review)**

- Driving under influence of alcohol is one of the main causes of traffic accidents
- Few prevalence studies connecting alcohol and accidents have been performed in Italy
- We describe blood samples analysis of injured drivers in Piedmont, Northern Italy
- Large majority of blood samples showed BAC concentrations above 1.5 g/L
- Young drivers, both male and female, must be considered high-risk categories

Dear **Journal of Forensic and Legal Medicine** editor,

I'm pleased to submit a **revised version** of the manuscript entitled "Driving under the influence of alcohol. A 5 year overview in Piedmont, Italy".

All the changes requested by reviewers were made to the manuscript and marked using a yellow highlighting. Furthermore, Table 4 and 5 were merged in Table 4a and 4b. An itemized list of these changes, in response to the referee's observations, is reported below.

Reviewer #1

- According to Italian Legislation, a zero-limit has been introduced for drivers <21 years. Introduction was modified in order to clarify this point
- Mean and standard deviation in Table 3 were replaced with media, quartiles and interquartile ranges. Text was also modified accordingly (lines 131-132)

Reviewer #2:

Introduction:

- L18: the sentence was modified
- L32: text was modified in order to clarify this point (lines 34-38 in the revised version). Reference #16 was added
- L37/38: text was modified as requested (lines 42-45 in the revised version)
- L46/47: this point was clarified (lines 53-54 in the revised version)

Material and Methods:

- L56: the preliminary blood screening was detailed (lines 65-66 in the revised version)
- More information was added (lines 58-62 in the revised version)
- L59: the point was clarified (line 72 in the revised version)
- The recalculation by using the P/B ratio was clarified and the reference #20 was added (lines 69-74 in the revised version)
- Validation data were added (lines 88-98 in the revised version)
- L80/81: The paragraph "Statistical analysis" was rewritten and the Results section was modified accordingly. Table 1 and 2 were harmonized as requested

Results:

- Normally, cars on the roads do not carry other passengers than the driver. Therefore, we assume that "Most subjects were likely to be car drivers". See also ref. #17
- L92: text was modified

- The minimum age is 18. Subjects <18 years are likely pedestrians or passengers
- Table 2 and table 3: the most relevant findings were highlighted (lines 137-145 in the revised version)

Discussion:

- L123: the point was clarified (lines 158-159 of the revised version)
- L128: Text and abstract were harmonized
- L144: text was modified and references #25-28 were added

ABSTRACT

Alcohol consumption represents a major health issue worldwide and a crucial factor in road accidences. 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.

Keywords

Alcohol; BAC; Traffic injures; Piedmont

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], 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.

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 autosampler 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 1.2:1[20]. A 100 µL aliquot is transferred into a 20 mL head-space vial. Afterwards, 10 µL 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 sec; injection time: 30 sec; 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 × 0.2 mm i.d. x 0.33 µm film thickness). The oven temperature was maintained isothermal at 70 °C for 8 minutes. 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 ions 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 ±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 ±10% at 0.5 g/L ethanol concentration, and within ±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).

Statistical analysis

Under the hypothesis of independent samples population, the Yates' chi-square test was selected for conformity assessment. The 2 x 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 H0 (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.

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 (Figure 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 Figure 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 Figure 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 even frequency for all groups of age, including the youngest drivers who are likely to show less tolerance to ethanol's effects.

Comprehensive data, including BAC median, 1st and 3rd quartiles and interquartile range (IQR), grouped according to sex and age, are reported in Table 3. It is evident that i) the majority of subjects involved in car accidents presented BAC above 1.5 g/L, and ii) when female subjects are involved, they show the same characteristics in terms of BAC.

The prevalence of positive samples was evaluated further by considering the sampling day. As expected, car accidents related to the abuse of alcohol appears to be a major issue during weekends. Indeed, positive samples are equally divided between working days (sum of 5 days) and weekend days (two days only), as it shown in Table 4a. Interestingly, this imbalanced distribution polarizes further in the group of young drivers, who are more prone to incurring in car accidents under the influence of alcohol during the weekend, whereas for the group aged >41, positive samples are more often collected during working days. These data are shown in Table 4b.

Discussion

Over-consumption of alcohol and consequent impaired driving represent major public health issues worldwide, making alcohol the most dangerous drug in terms of harm to the individual and society[13,21,22]. The present study describes the prevalence of alcohol in blood testing performed on subjects involved in vehicle accidents and consequently admitted to local hospitals for medical examination. That alcohol consumption has a significant effect on the incidence of road accidents cannot be proved by the results of this study, but it can be inferred based on previous data and consensus-based assessments[8-12].

Despite the limitation that no information is available on the role played by blood donors in the accidents (driver, passenger, or pedestrian), and on the type of vehicles involved, it is likely that most samples were taken from car drivers[17]. While the legal cut-off concentration is 0.5 g/L (and 0 g/L for specified categories such as novice and professional drivers), the large majority of blood samples showed BAC concentrations above 1.5 g/L. As a matter of fact, such a high level of blood ethanol is likely to produce severe effects on driving impairment (and the younger the drivers, the more severe the effects observed), hence determining a crucial causal factor in many car accidents. Noteworthy, there are more people driving under the influence of alcohol during weekends rather than in working days, a known evidence that must guide the control policy. A notable finding of the present study is the high percentage of positive samples taken from female subjects, compared to the past. As shown also in studies conducted in different countries, drinking habits of women, especially young ones, are becoming increasingly similar to those of co-aged men, including the starting age of alcohol intake[13,22,23]. The differences between males and females apparently decrease in the group of elder subjects as well, but it is possible that, for aged people, the higher percentage of non-drivers among blood donors had leveled off the gender differences. Quite remarkably, a similar attitude between genders towards the abuse of alcohol

has been observed also in the post-accident monitoring phase, namely the driving re-licensing process[24].

Despite these data on alcohol abuse could not be fully combined with the incidence of associated abuse of psychotropic substances, scattered findings worldwide highlight that concurrent intake of alcohol and stimulating drugs is frequent, especially in car accidents caused by young subjects during weekend nights[25-28].

The present study highlights that alcohol abuse still represents a major problem that requires specific implementation of public policies for traffic crashes prevention. Once more, it is demonstrated that young drivers, both male and female, must be considered high-risk categories and should be addressed with targeted prevention campaigns.

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Figure 1

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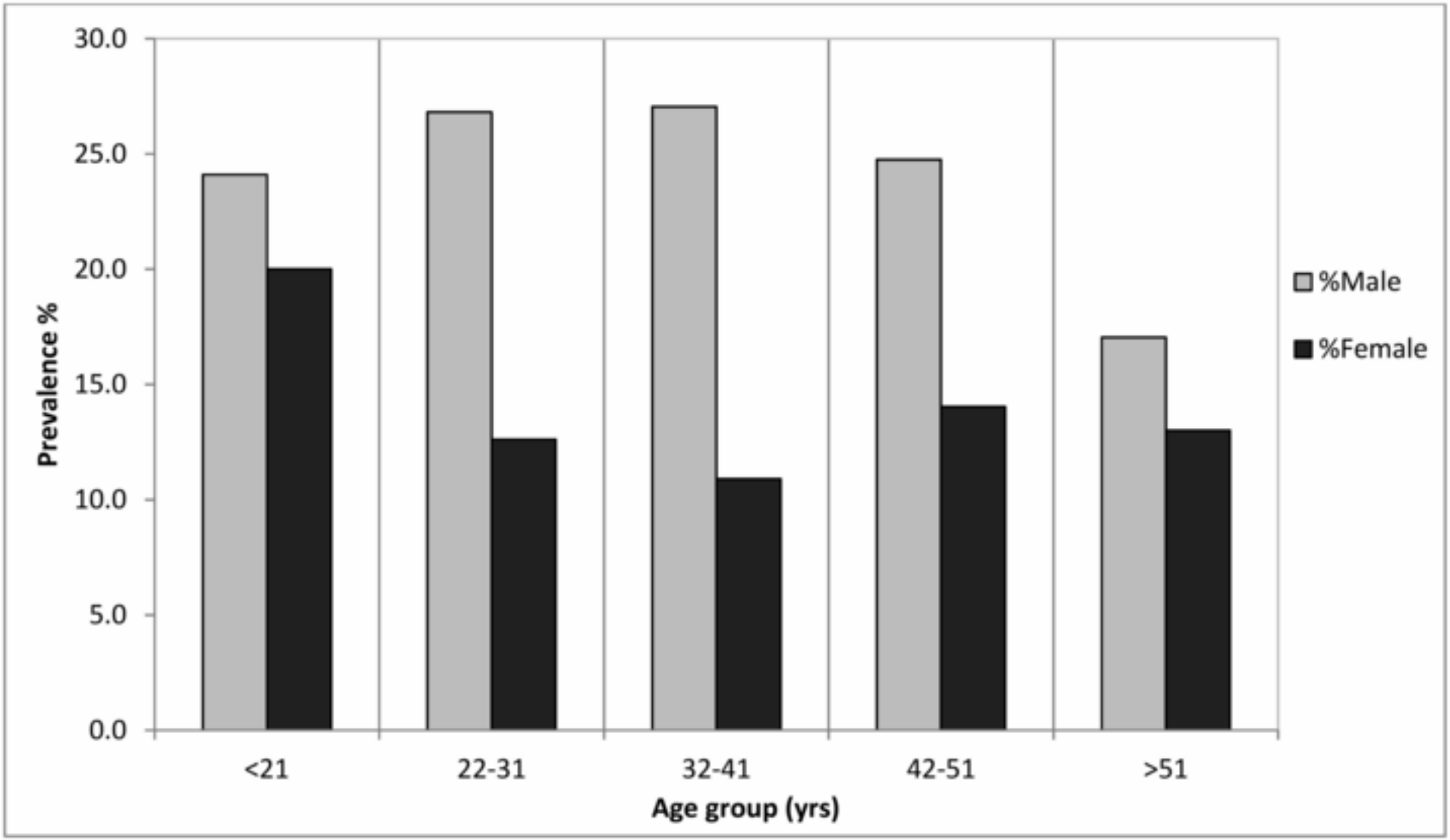

Figure 2

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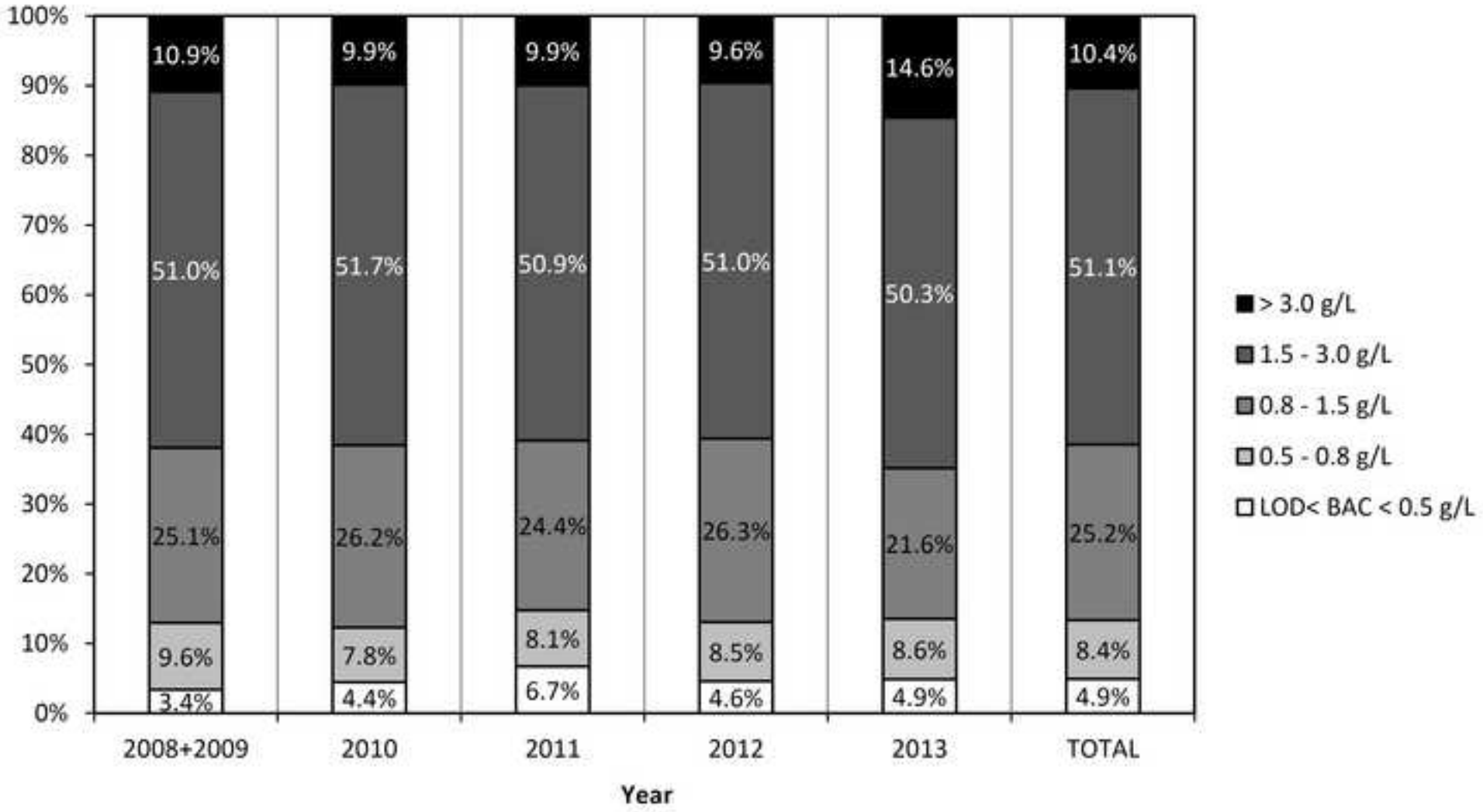

Figure 3

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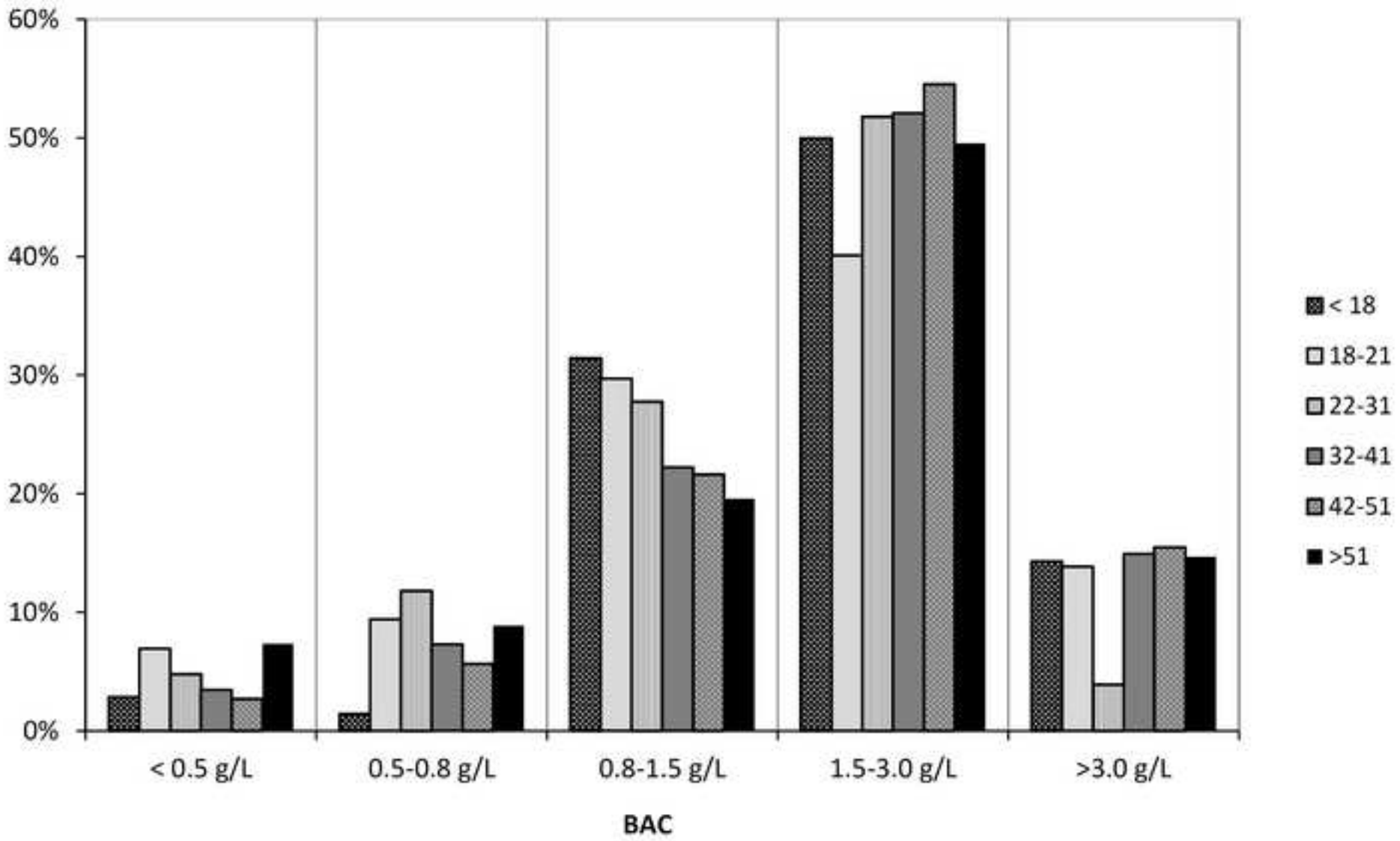

Figure Captions

Figure 1 Distribution of positive samples in different intervals, according to sex and age.

Figure 2 Distribution of positive samples in different intervals of concentration, according to year of sampling.

Figure 3 Distribution of positive samples in different intervals of concentration, according to age (narrow separation).

Table 1

SEX	<18			18-21			22-31			32-41			42-51			>51		
	POS	TOT	%POS	POS	TOT	%POS	POS	TOT	%POS	POS	TOT	%POS	POS	TOT	%POS	POS	TOT	%POS
M	8	30	26.7	100	418	23.9	240	895	26.8	191	706	27.1	124	501	24.8	120	704	17.0
F	5	16	31.2	19	104	18.3	38	301	12.6	23	211	10.9	24	171	14.0	19	146	13.0
χ^2 difference	0.742			0.219			p<0.05			p<0.05			p<0.05			0.230		
Yates' χ^2 difference	0.988			0.272			p<0.05			p<0.05			p<0.05			0.282		

Table 2

Age	Blood alcohol concentration (BAC)					
	<0.5 g/L	**0.5-0.8 g/L**	**0.8-1.5 g/L**	**1.5-3.0 g/L**	**>3.0 g/L**	**N**
<18	3%	1%	31%	50%	14%	70
18-21	7%	9%	30%	40%	14%	202
22-31	5%	12%	28%	52%	4%	670
32-41	3%	7%	22%	52%	15%	549
42-51	3%	6%	22%	55%	15%	407
>51	7%	9%	20%	50%	15%	326
TOT	**5%**	**8%**	**25%**	**51%**	**11%**	2224

Table 3

Age (Male)	<18 yr	18-21 yr	22-31 yr	32-41 yr	42-51 yr	>51 yr
N	42	146	528	462	307	243
BAC (median)	1.65	1.61	1.66	1.96	2.01	1.89
1st quartile	1.36	1.13	1.19	1.33	1.43	1.20
3rd quartile	2.13	1.98	2.20	2.63	2.65	2.53
IQR	0.77	0.85	1.01	1.30	1.22	1.33
Age (Female)	**<18 yr**	**18-21 yr**	**22-31 yr**	**32-41 yr**	**42-51 yr**	**>51 yr**
N	26	39	110	65	89	60
BAC (median)	1.66	1.56	1.58	1.85	1.76	2.20
1st quartile	1.36	1.16	0.98	1.47	1.31	1.55
3rd quartile	1.94	2.26	2.18	2.48	2.63	2.67
IQR	0.58	1.10	1.20	1.01	1.32	1.12

Table 4

Table 4a

Year	%Working days	%Weekend days
2008+2009	49%	51%
2010	43%	57%
2011	51%	49%
2012	55%	45%
2013	53%	47%
TOTAL	**50%**	**50%**

Table 4b

Age	%Working days	%Week-end days
<18	39%	61%
18-21	47%	53%
22-31	38%	62%
32-41	53%	47%
42-51	61%	39%
>51	66%	34%
TOT	50%	50%

Tables captions

Table 1 Distribution of positive samples in different intervals, according to sex and age. Prevalence was statistically evaluated by means of test χ^2.

Table 2 Results of confirmation analysis, grouped according to age and BAC.

Table 3 Distribution of positive samples (BAC>0.5 g/L), according to sex and age.

Table 4a Trend over 5 years of percentage of positive samples, grouped according to week-day of sampling

Table 4b Trend of percentage of positive samples, grouped according to week-day of sampling and age.

Table S1

Click here to download Optional e-only supplementary files: Table S1.doc

Conflict of Interest Statement

We wish to confirm that there are no known conflicts of interest associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

We confirm that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. We further confirm that the order of authors listed in the manuscript has been approved by all of us.

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