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Illicit drugs in Emergency Department patients injured in road traffic accidents

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Abstract
Urine and blood samples from 1730 drivers involved in road accidents (July 2012 – December 2015) were analyzed for the evaluation of driving under influence of drug of abuse according to the Lombardia Region guideline. The 22.5% (95% CI 20.5 to 24.5) of urine screenings tested positive for at least one class of drugs. 10.6% (95% CI 9.2 to 12.1) of the 1730 drivers were under the influence of drug, being blood concentration above the cut-off limit for at least one active substance; the proportion of illicit drugs in blood was cocaine 5.7 % (95% CI 4.7 to 6.9), cannabinoids 3.7 % (95% CI 2.9 to 4.7), opiates 1.4% (95% CI 0.9 to 2.1), methadone 1.4% (95% CI 0.9 to 2.1), amphetamines 0.2% (95% CI 0.04 to 0.5). Trend in proportion showed similar percentage (about 5%) of cocaine and cannabinoids consumption in the last two years. Poly-drug of abuse consumption emerged in the 10.4% (95% CI 6.4 to 15.7) of the positive blood and alcohol was above the legal limit in 47% (95% CI 39.6 to 54.5) of the subjects driving under the influence of drugs.

Key words
- drugs of abuse
- blood
- urine
- driving under the influence of drugs of abuse

INTRODUCTION

In Italy, driving under the influence of drugs of abuse (DUID) is illegal, but the law does not establish either the list of substances to be controlled or the punishable concentration limits for drugs of abuse. In order to regulate the analytical procedure in DUID cases, Lombardia Region issued, in 2012, a guideline to suggest a list of illicit drugs to be controlled and the blood concentration limits of the active substances (parent drug and/or metabolite) above which the driver can be prosecuted for DUID [1]. This procedure includes also the detection of inactive metabolites.

The aim of this study is to present the results of toxicological investigations carried out on blood and urine samples collected from drivers involved in road traffic accidents. The proportion of illicit drugs after the introduction of the Lombardia Region procedures is analyzed.

MATERIALS AND METHODS

Selection of cases

The population consisted of 1730 drivers involved in non-fatal traffic accidents and taken to seven Emergency Departments (ED) of Pavia and province and four hospitals of the Azienda Ospedaliera di Treviglio (BG), from July 2012 to December 2015. In case of road accidents, according to the Italian law, Police systematically requested the analysis for drugs of abuse, regardless of a clear suspect of DUID; a chain of custody for the collected samples was put into effect and the patients were asked for consent. Only in case of positive consent or when the patient was not able to provide a valid consent, biological samples were collected and sent to our lab. The laboratory proceeded to analyze biological matrices only in the presence of patient's consent or, if not available, of the magistrate's request. 17 patients were not included in this study, because they were not able to provide valid consent and the magistrate's request never arrived.

The mean age of population was 41.1 years + SD 15.7; males were heavily predominant, 80.5% *vs* female 19.5 %. The circumstances (i.e. type of the vehicle, suspected cause or victim of the accident etc.) of the road accidents were not available.

For this study, population data were anonymized and a unique, sequential number was assigned to each case.

Biological samples and storage

Blood was collected into glass tubes containing potassium fluoride as preservative and EDTA as anticoagulant. Urine was collected into polyethylene tubes. Samples were stored at 4 °C for periods not exceeding 72 h, otherwise were frozen at -20 °C. Analysis was routinely completed within 10 days, later only in case of absence of timely consensus.

Analytical procedures

The analytical strategy consisted of the following steps:

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Table 1
Cut-off of drugs of abuse/metabolites in urine and blood

Class	Analyte	Urine		Blood
		Screening cut-off	Confirmation cut-off	Cut-off
Opiates		150 ng/ml		
	Morphine		100 ng/ml	10 ng/ml
	6-acetylmorphine		10 ng/ml	10 ng/ml
	Codeine		100 ng/ml	10 ng/ml
Methadone		150 ng/ml		
	Methadone		100 ng/ml	10 ng/ml
	EDDP[1]		100 ng/ml	10 ng/ml
Buprenorphine		5 ng/ml		
	Buprenorphine		5 ng/ml	5 ng/ml
	Nor-Buprenorphine		5 ng/ml	5 ng/ml
Cocaine		150 ng/ml		
	Cocaine		50 ng/ml	10 ng/ml
	Cocaethylene		50 ng/ml	10 ng/ml
	Benzoylecgonine		50 ng/ml	50 ng/ml
Amphetamines		500 ng/ml		
	Amphetamine		200 ng/ml	50 ng/ml
	Methylamphetamine		200 ng/ml	50 ng/ml
	MDMA[2]		200 ng/ml	50 ng/ml
	MDA[3]		200 ng/ml	50 ng/ml
	MDE[4]		200 ng/ml	50 ng/ml
Cannabinoids		25 ng/ml		
	THC[5]			2 ng/ml
	THC-COOH[6]		15 ng/ml	5 ng/ml
Creatinine		> 20 mg/dl		

[1]EDDP: 2-Ethylidene-1,5-DimethylDiphenyl-3,3-Pyrrolidine; [2]MDMA: 3,4-Methylendioxymethamphetamine;
[3]MDA: 3,4-Methylendioxyamphetamine; [4]MDE:3,4- methylendioxy-N-ethylamphetamine;
[5]THC: Delta9-Tetrahydrocannabinol; [6]THCCOOH = 11nor-9-carboxy -delta9- THC.

A) urine screening for six classes of drugs of abuse/metabolites (e.g. opiates, cocaine, cannabinoids, amphetamines, methadone and buprenorphine – *Table 1*) by immunological tests (Siemens, EMIT II Plus) and quantification of creatinine by enzymatic assay (Siemens) as index of urine suitability. Creatinine concentration < 20 mg/dl is consistent with over dilution of the sample and in this case, blood analyses is mandatory even if urine screening is negative;
B) blood identification and quantification of the parent drugs/metabolites tested positive in urine: drugs were isolated by solid phase extraction (SPE). Cocaine/benzoylecgonine blood analysis was performed by Gas Chromatography-Mass Spectrometry (GC-MS) in SIM mode after pentafluoropropionic anhydride derivatization, while the other drugs were analysed by Liquid Chromatography-Tandem-Mass Spectrometry (LC-MS/MS).
C) urine analysis in case of blood negativity for active and inactive compounds: an aliquot of urine, different from that used for the screening test, was processed to confirm the positive screening result. Urine confirmation test was performed by GC-MS analysis in SIM mode after SPE extraction for all drugs except for cannabinoids that were isolated by liquid/liquid extraction.

Cut-off values for the different classes and analytes are summarized in *Table 1*. According to the regional procedures, a sample is positive if the measured concentration for each analyte is above the cut-off level considering the confidence range of the methods (10% for urine screening and confirmation tests, 20% for blood quantification).

RESULTS AND DISCUSSION

The 22.5% (95% CI 20.5 to 24.5) of the screening tests in urine resulted positive for at least one class of drugs and in 10.6 % (95% CI 9.2 to 12.1) of the 1730 cases, subjects were driving under influence of drug, being blood concentration above the adopted cut-off limit for at least one active substance (parent drug and/or active metabolite).

Table 2 summarizes the positive results of the screening urine tests along with the positivities for active substances in blood and for inactive substances in blood and urine.

Cocaine and cannabinoids were the most frequently detected illegal drugs. In particular, cocaine/metabolites resulted positive in urine in the 11.4% (95% CI

Table 2
Percentage and 95% confidence interval (CI) of positive cases in urine and blood *vs* drug/class of drugs

Drug/class	Positive urine samples and percentage *vs* total cases (1730)	Positive blood samples for active substances *vs* positive urine samples and vs total cases	Positive blood samples for inactive metabolites only*
Cocaine	197 (11.4%; CI 9.9 to 13.0)	99/197 (50.2%; CI 43.1 to 57.4) 99/1730 (5.7%; CI 4.7 to 6.9)	27/197 (13.7%; CI 9.2 to 19.3) 27/1730 (1.6%; CI 1 to 2.3)
Cannabinoids	243 (14.0%; CI 12.4 to 15.8)	64/243 (26.3%; CI 20.9 to 32.3) 64/1730 (3.7%; CI 2.9 to 4.7)	67/243 (27.6%; CI 22 to 33.6) 67/1730 (3.9%; CI 3 to 4.9)
Opiates	56 (3.2%; CI 2.4 to 4.2)	24/56 (42.9%; CI 29.7 to 56.8) 24/1730 (1.4%; CI 0.9 to 2.1)	- -
Methadone	24 (1.4%; CI 0.9 to 0.21)	24/24 (100%; CI 85.7 to 100) 24/1730 (1.4%; CI 0.9 to 2.1)	- -
Amphetamines/ ecstasy	5 (0.3%; CI 0.09 to 0.7)	3/5 (60%; CI 14.7 to 94.7) 3/1730 (0.2%; CI 0.04 to 0.5)	- -
Buprenorphine	3 (0.2%; CI 0.04 to 0.5)	0/3 0/1730	- -

*Benzoylecgonine, THCCOOH, EDDP.

9.9 to 13.0) and 5.7% (95% CI 4.7 to 6.9) of cocaine blood concentrations exceeded the adopted cut-off; cocaethylene was positive in the 26.3% (95% CI 18-36) of cocaine blood positivities; one case tested positive for cocaethylene with cocaine concentration below the cut off. Blood cocaine positivities were associated with almost another different active drug in the 16.2% (95% CI 9.5 to 25).

The proportion of cocaine presented in this work differs from the data found in two previous Italian population studies [2, 3], where cocaine proportion in blood was, respectively, 2.7 and 7.1%, even if Legrand *et al*. [2] considered seriously injured drivers only, while our population is not selected on the basis of severity of injury**.**

Cannabinoids tested positive in 14% (95% CI 12.4 to 15.8) of urine samples and THC in blood was detected above the adopted limit in 3.7% (95% CI 2.9 to 4.7) of the total cases; six of the 64 positive cases for THC in blood (9.4%, 95% CI 3.5 to 19.3) were associated to cocaine positivity.

Low percentages of cocaine and THC blood positivity compared to proportion in urine can be due to the rapid decrease of these active compounds in blood and slow excretion of metabolites in urine [4-6]. In this study *(Table 3)*, the concentration ratios of THC/THCOOH and cocaine/benzoylecgonine measured in blood were low (0.21 + 0.11 and 0.16 + 0.13 respectively). The 25th percentile of THC blood concentration (3.1 ng/ml) resulted near to the cut off adopted in the guideline (2.0 ng/ml with 20% of analytical confidence). The same situation applied to cocaine, where the 10th percentile of blood concentrations was 16 ng/ml *versus* an adopted limit of 10 ng/ml with 20% of analytical confidence. It is possible that the time elapsed between the road accident and the specimen collection played an important role in the decrement of the active substances, but, unfortunately, this information was not available.

Table 3
Number of positive cases, mean concentrations, ranges and percentiles for the drugs, metabolites and their ratio detected in blood of drivers involved in nonfatal road accidents

Substance	LOQ[1] (ng/ml)	N (positive)	Mean + SD (ng/ml)	Range (ng/ml)	p10 (ng/ml)	p25 (ng/ml)	p50 (ng/ml)	p75 (ng/ml)	p90 (ng/ml)
Cocaine	10	99	70.9 + 71.6	12.6-378	16.0	26	45.5	85.3	159.8
Cocaethylene	10	27	33.6 + 16.5	12.9-90	14.2	20	31.6	44.5	53.1
Benzoylecgonine	50	126	561.7 + 687.4	62-3595	89.0	161	312	636	1209
Cocaine/benzoylecgonine		99	0.16 + 0.13	0.02-0.60	0.04	0.07	0.10	0.22	0.37
Cocaine/cocaethylene		43	2.14 + 1.39	0.52-5.65	0.88	1.15	1.71	3.02	4.58
THC	2	64	5.6 + 3.6	2.6-22	2.7	3.1	4.5	6.2	11.7
THCCOOH	5	131	24.3 + 22.4	6.2-120	7.8	10	15.8	30	54.4
THC/THCCOOH		64	0.21 + 0.11	0.03-0.57	0.1	0.12	0.20	0.27	0.36
Morphine	10	24	54.2 + 115.4	13.2-470	13.3	16.9	25	31.5	216
Methadone	10	24	187.8 + 152.4	16.9-582	32.3	93.2	122.5	279.5	448.7
MDMA	50	3	848 + 998	235-2000	235	235	309	2000	2000

[1]Analytical confidence: 20%

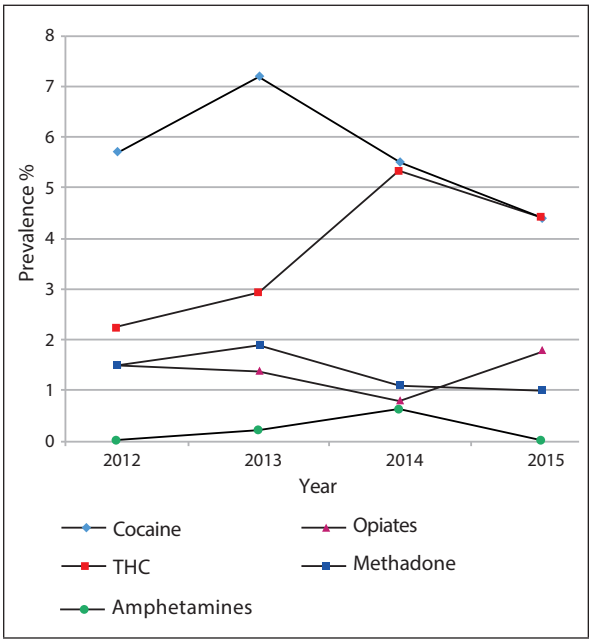

Figure 1
Prevalence in percentage of active drugs/metabolites positive in blood: distribution by year in the sampling period, July 2012- December 2015.

As regard the positive cases for opiates in blood (n = 24), the analyses revealed morphine in 23 subjects (presence of 6-monoacetylmorphine in 1 case) and only codeine in 1 case.

24 subjects resulted positive for methadone in blood and no information about patient's methadone therapy was available.

Amphetamines/ecstasy were positive in blood in 3 cases: two cases were positive for MDMA in association with cocaine, 1 case was positive for amphetamine in association with THC.

Figure 1 shows the distribution of the proportion of drugs in blood over the considered period: one notable finding is the higher proportion of cocaine compared to cannabinoids in the period 2012-2013, while in the last two years the data is similar.

Poly-drug consumption emerged in the 17.5% (95% CI 13.8 to 21.6) of positive urine screenings and in the 10.4% (95% CI 6.4 to 15.7) of the cases positive in blood for active substances. Moreover, alcohol was above the legal limit (0.5 g/L) in 47 % (95% CI 39.6 to 54.5) of the subjects driving under the influence of drugs, and the proportion of the population positive for ethanol only was 28.2% (95% CI 26 to 30.5).

Table 4 and *Figure 2* show the distribution of the drugs tested positive in blood samples versus different age groups: THC was mostly encountered in the age group 18-30, while cocaine was frequently detected in a wider age range (18-50 years). The 86% (95% CI 65 to 97) of poly-drug abuse cases was found in the population 18-40 year old. As regard to gender, the 91.9% (95% CI 84.7 to 96.4) and 84.4% (95% CI 73.1 to 92.2) of the population positive for cocaine and THC respectively, are males.

CONCLUSIONS

We analyzed urine and blood samples of 1730 drivers (July 2012-December 2015) involved in nonfatal road accidents in order to verify the driving under influence of drugs of abuse according to Lombardia Region procedure: urine immunoassay screening for illicit drugs and quantitative analysis in blood by chromatography coupled to MS detector methods. In the considered period, the most common drug of abuse quantified in blood above legal limits was cocaine (5.7%), followed by THC (3.7%); however, in the last two years the proportion of cocaine and cannabinoids resulted similar (about 5%). These data mostly differ from those collected in other countries were the most common drug found in blood was THC [2, 6-13]. However, in all these reports, the cut-off for THC in blood is lower (from 0.4 to 2.2 ng/ml) then ours (2 ng/ml with 20% of analytical confidence). Methadone, opiates and amphetamines resulted less frequently involved (1.4%, 1.4% and 0.2% respectively).

The percentage of urine samples positive for cocaine, cannabinoids and opiates was significantly higher than the blood one (see *Table 2*). This difference may arise, as

Table 4
Age and sex distribution of the patients and the results of detected proportions of drug use by substance in the specific age-sex groups

Analytes	Age groups (years) and sex vs number of positivities in blood									
	16-18		19-30		31-40		41-50		50	
	M	F	M	F	M	F	M	F	M	F
Cocaine	1 /99 (0.1%)	0/99	33/99 (33.3%)	3/99 (3%)	34/99 (34.3%)	3/99 (3%)	23/99 (23.2%)	2/99 (2%)	0/99	0/99
THC	3/64 (4.7%)	1/64 (1.6%)	32/64 (50%)	5/64 (7.8%)	7/64 (11%)	2/64 (3.1%)	8/64 (12.5%)	2/64 (3.1%)	4/64 (6.2%)	0/64
Opiates	0/24	0/24	10/24 (41.7%)	0/24	6/24 (25%)	0/24	6/24 (25%)	0/24	2/24 (8.3%)	0/24
Methadone	0/24	0/24	8/24 (33.3%)	0/24	8/24 (33.3%)	1/24 (4.2%)	5/24 (20.8%)	1/24 (4.2%)	1/24 (4.2%)	0/24
Amphetamines	0/3	0/3	2/3 (66.6%)	0/3	1/3 (33.3%)	0/3	0/3	0/3	0/3	0/3
Poly-drugs	0/22	0/22	11/22 (50%)	1/22 (4.5%)	6/22 (27.3 %)	1/22 (4.5%)	3/22 (13.6%)	0/22	0/22	0/22

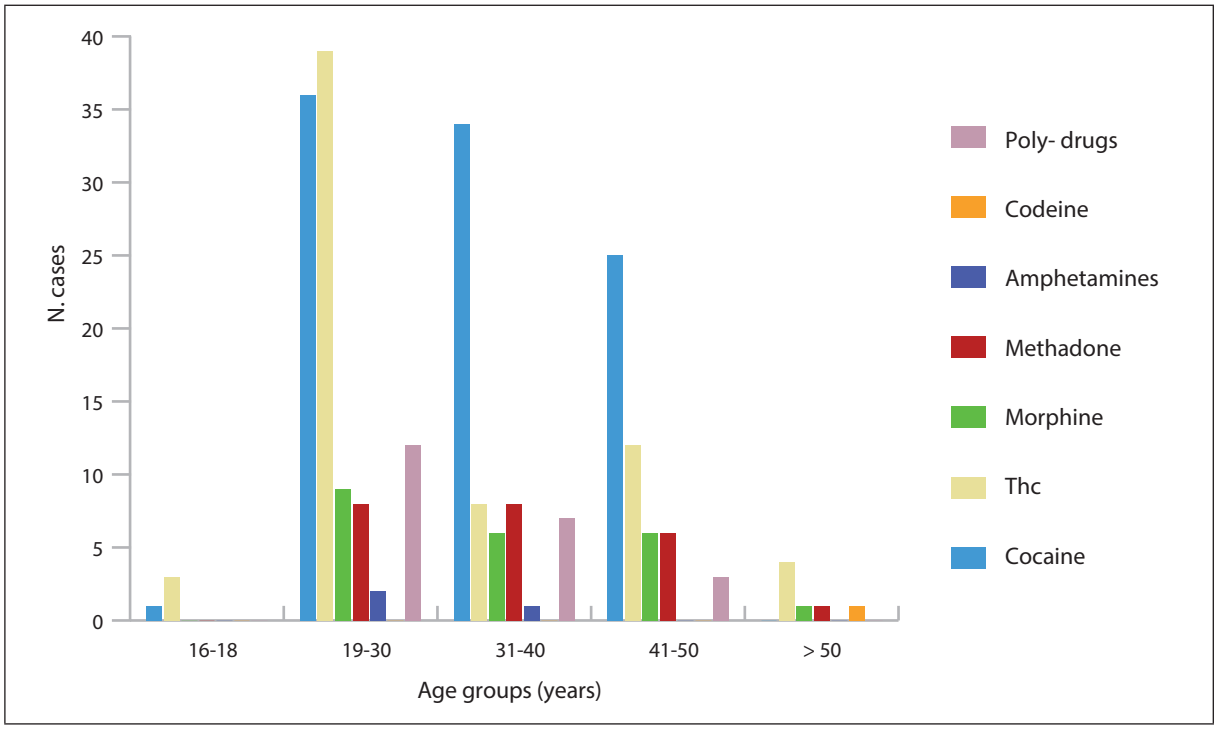

Figure 2
Positive blood samples for drugs of abuse *vs* age groups.

well as from a previous drug consumption, also due to the time elapsed between the road accident and sample collection that plays a decisive role in the outcome of the analysis.

The presented data confirm the low value of urine results for DUID aim, even if immunoassay urinary screening remains a simple and reliable tool to select the cases for the more complex quantitative blood analysis.

Conflict of interest statement

There are no potential conflicts of interest or any financial or personal relationships with other people or organizations that could inappropriately bias conduct and findings of this study.

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