First Objective Association Between Elevated Carbohydrate-Deficient Transferrin Concentrations and Alcohol-Related Traffic Accidents

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Background: Carbohydrate-deficient transferrin (CDT) is a well-recognized highly specific marker of chronic alcohol abuse. The association of CDT with alcohol-related traffic accidents was evaluated to objectively validate the use of this marker for certifying the physical fitness for driving license regranting after its confiscation for drunk driving.

Methods: The study was carried out on 468 injured drivers (InjDr), who underwent mandatory blood alcohol concentration (BAC) and drug analysis in biological fluids. The InjDr group was divided into 2 subgroups on the basis of BAC legal limit adopted in Italy (BAC ≤ 0.5 g/l: InjDr1; BAC > 0.5 g/l: InjDr2). The control group (CntDr) included 236 subjects holding safety-sensitive job positions and undergoing mandatory toxicological analyses. The determination of BAC in blood and CDT in serum were performed using validated analytical methods based on head-space gas chromatography and high-performance liquid chromatography, respectively.

Results: The evaluation of CDT distribution in the 3 groups (CntDr, InjDr1, InjDr2) showed that CDT distribution in the InjDr1 group was similar to that observed in the CntDr group (p = 0.159) and different from that observed in the InjDr2 group (p < 0.001). Partitioning the CDT data of each group into “CDT positives” and “CDT negatives” on the basis of the cut off (1.90%), it was possible to calculate the odds of the 3 groups and then the odds ratios. The odds ratio of InjDr1 versus CntDr was 4.56 (p = 0.158), whereas the odds ratio of InjDr2 versus CntDr was 132 (p < 0.001). Furthermore, a dose-response effect was found only when comparing InjDr2 with CntDr.

Conclusions: The data of the present study strongly support the use of the CDT test to evaluate the risk of a subject to be involved in a road accident while driving under the influence of alcohol.

Key Words: Carbohydrate-Deficient Transferrin, Alcohol-Related Traffic Accident, Driving License, Drunk Driving.
most important actions adopted by the governments to reduce traffic accidents and the related injuries and deaths.

If there is a widespread consensus and a sound body of scientific evidence on the correlation of the impairment of the driving ability with the BAC, a much weaker objective evidence can be found in the current literature on the correlation between the chronic abuse of alcohol and an increased risk of causing traffic accidents. It should be stressed that this point is of the highest importance, particularly when persons, whose driving license has been withdrawn for “drunk driving” apply for reinstating of their license pretending to have quit abusing alcohol. In most countries, this delicate matter is under the responsibility of specialized medical committees, which have the task of verifying in every single case the reality of the alleged abstinence. In addition, the medical committee should exclude any reasonable risk of relapse to alcohol and consequently any risk of “driving while intoxicated.”

Traditionally, the diagnostic approach to this difficult problem was merely based on medical visit, patient interview, and few clinical laboratory data, including liver enzymes and mean corpuscular volume.

More recently, however, in most European countries, new biomarkers have been adopted to provide specific and objective evidence of the chronic alcohol intake of the subject (Bortolotti and Tagliaro, 2011; Illand, 1996; Mercier-Guyon, 1996; Musshoff and Daldrup, 1998).

Among these biomarkers, carbohydrate-deficient transferrin (CDT), the collective name of a group of minor glycoforms of serum transferrin, has found (alone or in combination with gamma-glutamyltransferase [GGT]) in driving license reinstating programs the most important application (Bean et al., 2009; Bortolotti et al., 2002; Maenhout et al., 2012; Morgan and Major, 1996). The widespread use of CDT in this context is explained by its well-recognized diagnostic specificity to identify sustained alcohol intake lasting for at least 10 to 15 days (Bortolotti et al., 2006; Delanghe and De Buyezere, 2009; Jones, 2008).

Although the usefulness of CDT in the clinical diagnosis of alcohol abuse has been reported in several independent studies, the trueness of the hypothesis that elevated CDT concentrations are also correlated to an increased risk of DUI, and more importantly, an increased risk of causing alcohol-correlated traffic accidents has so far found a weak support in the current literature.

Indeed, just a limited number of papers have investigated the scientific basis of the use of CDT in traffic medicine. These papers include both retrospective (Appenzeller et al., 2005a,b; Bianchi et al., 2010; Bortolotti et al., 2007; Brinkmann et al., 2002; Gjerde and Morland, 1987; Illand and Grassnack, 1995; Illand et al., 1994; Jaster and Wegener, 1993; Kristenson and Jeppsson, 1998; Marques et al., 2010) and prospective studies (Maenhout et al., 2014; Portman et al., 2010). The former group of works consistently report important percentages of “CDT-positive subjects” (i.e., subjects with CDT serum concentrations above the threshold limits of the “normal” population) among drivers apprehended for DUI, showing an association between CDT and drunk driving. Although these papers are highly suggestive, they are practically inconclusive as they lack a control group.

In the matter of the 2 prospective population studies, they evaluate the usefulness of the markers of chronic alcohol abuse, including CDT and γ-CDT (combination of CDT with GGT), as risk predictors for recidivism of DUI. Although in both papers the authors report a significant relationship of CDT and γ-CDT with DUI recidivism, they do not investigate the correlation of these markers with alcohol-related traffic accidents, which are the most important consequences of DUI.

On these grounds, the aim of this study was to evaluate the association of elevated CDT serum concentrations with alcohol-related traffic accidents with consequent hospitalization of the driver. The final aim of the study was to validate the use of CDT as a biomarker of chronic alcohol abuse for certifying the physical fitness for the driving license issuing and/or regranting after its confiscation for drunk driving.

MATERIALS AND METHODS

Subjects and Sample Collection

A total of 530 drivers admitted to the University Hospital of Verona (Verona, Italy) in the years 2010 to 2011 for injuries occurred in traffic accidents were preliminarily selected for inclusion in this study. According to the laws on traffic safety in force in Italy [art.5, law 214/2003], the injured drivers must undergo mandatory tests to assess the BAC and the presence of psychoactive drugs in the biological fluids. In this forensic/administrative frame, blood and urine samples of injured drivers included in this study were collected at the First Aid Department and rapidly forwarded to the Forensic Medicine Department for toxicological analyses, including blood alcohol and CDT determination and urine drug testing. To avoid interfering factors in data interpretation, 62 patients in which urine testing was positive for the presence of psychoactive drugs were excluded from the study.

The remaining 468 drivers (340 males, 128 females) were considered as the case group (InjDr). These subjects were divided into 2 subgroups according to BAC value. The subjects with a BAC value ≤0.5 g/l (the Italian legal limit to drive) were named InjDr1 subgroup involved in nonalcohol-impaired crashes. The subjects with a BAC value >0.5 g/l were named InjDr2 subgroup involved in alcohol-impaired crashes.

As control group (CntDr), 236 subjects (225 males, 11 females) holding safety-sensitive job positions (drivers and workers employed in the highway maintenance system) were included in the study. The law provision (GU # 266, 15.11.2007) requires that at least once per year, in a day randomly selected, the employees undergo clinical and toxicological analyses including CDT on blood and urine to exclude drug and/or alcohol abuse. All of subjects tested for the study held a driving license and did not have record of recent traffic accidents nor confiscations of the driving license. All of the CntDr showed negative results from drug screening in urine for the same panel of drugs and drug metabolites tested in the InjDr group.

After collection, blood samples as well as urine samples were stored at +4°C and processed within 1 to 2 days. In case of delayed analysis (in any case performed within 2 weeks), the specimens were stored at −20°C.
Whole blood was used for BAC determination, whereas CDT analyses were carried out on blood serum, which was obtained, after complete clotting of blood, by centrifugation at 1,730 × g at room temperature for 10 minutes.

Because of the compulsory environment in which sampling and analysis were performed, no consent from subjects was needed. The personal, clinical, and toxicological data of all the subjects included in the study were anonymized. Given the observational nature of the study without use of personal information, no authorization from the ethical committee was required in our jurisdiction.

**Analytical Instrumentation and Methods**

The determination of BAC was performed by a validated head-space gas chromatographic method using a 6000 Series 2 Gas Chromatograph equipped with a flame ionization detector (Young Lin, Anyang, The Republic of Korea) and with a Metablood wide bore column for volatile (30 m × 0.53 mm × 3 µm; Teknokroma, Barcelona, Spain). BAC value was expressed in g/L. The analytical sensitivity was 0.01 g/l.

The determination of CDT was carried out using a method previously described and validated (Bortolotti et al., 2005). In short, the analysis was performed on a gradient high-performance liquid chromatography (Shimadzu, Tokyo, Japan) equipped with a UV-visible detector set at 460 nm. The separation of CDT glycoforms by chromatography (Shimadzu, Tokyo, Japan) equipped with a UV-visible detector set at 460 nm. The separation of CDT glycoforms was carried out using a 4.6 × 65 mm anion exchange column (Recipe, Munich, Germany) applying a salt gradient elution (buffer A: 10 mM BIS-TRIS pH 6.2; buffer B: 10 mM BIS-TRIS+500 mM NaCl; buffer C: 2 M NaCl). Sample preparation required ion saturation (with ferric chloride) and lipoprotein precipitation. CDT (including disialo-transferrin and, if present, asialo-transferrin) was expressed as percentage of the total transferrin content (including all the measurable transferrin glycoforms from asialo-transferrin to pentasialo-transferrin).

According to a consistent body of literature (Delanghe and De Buyzere, 2009; Kenan et al., 2010) and on the basis of our own experience, a cut off concentration of 1.90% was adopted for the classification of the CDT concentrations as “within the normal range” (including alcohol abstainers and “safe” drinkers) or “elevated,” indicating chronically sustained drinkers.

The toxicological screening of urine was performed with a commercial enzyme immunoassay (Cedia®, Beckman Coulter, Cassina De’ Pecchi, Milano, Italy) performed on an automated analyzer (Olympus AU400; Melville, NY), according to specifications provided by the manufacturer. The following cut off concentrations were used: opiates 300 ng/ml, cocaine metabolites 300 ng/ml, amphetamines 500 ng/ml, benzodiazepines 300 ng/ml, barbiturates 300 ng/ml, and cannabinoids 50 ng/ml.

**Statistical Methods**

The association between CDT and car accidents occurred under the influence of alcohol was quantified using the odds ratio. Exact 95% confidence intervals (CIs) were calculated employing the hypergeometric distribution. The Fisher’s exact test was used for hypothesis tests on the odds ratio. The distribution of CDT values in the InjDr1, InjDr2, and CntDr groups was compared via the nonparametric Kolmogorov–Smirnov test. To evaluate the dose–response effect of CDT on the risk of crashes, the chi-square for trend was calculated, after having categorized CDT into 5 ordinal groups (up to 1%, 1.01 to 1.20%, 1.21 to 1.40%, 1.41 to 1.90%, higher than 1.90%). The logistic regression model was employed to adjust for other covariates, and the area under the receiver operating characteristic (ROC) curve was calculated to evaluate the discriminative power of CDT. All the analyses were performed using R (R Core Team, 2014).

**RESULTS**

The determination of BACs in the InjDr group (n = 468) showed 341 subjects with BAC below 0.01 g/l (analytical sensitivity). Subjects with BAC values positive but below the Italian legal limit of 0.5 g/l were 27; the remaining 100 samples showed values >0.5 g/l. The BAC values’ range was from 0.01 to 3.92 g/l. All the subjects of CntDr group (n = 236) resulted negative at the BAC test.

The CDT values in the group of 468 drivers admitted for injuries occurred in traffic accidents (InjDr) that ranged from 0.1% to 14.57% (mean: 1.38%; SD: 1.49%; median: 1.07%). In the subjects with BAC ≤ 0.5 g/l (InjDr1 subgroup), the CDT ranged from 0.1% to 6.91% (mean: 1.06%; SD: 0.52%; median: 1.03%), while in the subjects with BAC > 0.5 g/l (InjDr2 subgroup), the CDT ranged from 0.58% to 14.57% (mean: 2.56%; SD: 2.76%; median: 1.46%). For the InjDr group, the nonparametric rho correlation coefficient between BAC and CDT was 0.440 (p < 0.001). The CDT values of the 236 subjects included in the CntDr ranged from 0.34% to 1.93% (average: 1.00%; SD: 0.27%; median: 0.99%).

The evaluation of the CDT results in the 3 groups (CntDr, InjDr1, InjDr2) showed that the distribution of CDT values in the injured drivers with BAC values within the legal limit (InjDr1) resulted quite similar to that observed in the CntDr group. The similarity was demonstrated by both density estimate of 2 distributions (Fig. 1A) and empirical cumulative distributions (Fig. 1B). Furthermore, the nonparametric Kolmogorov–Smirnov test (a formal significance test for comparing 2 distributions) yielded a nonsignificant result (p = 0.159). All the quantiles of the 2 distributions were fairly similar. Indeed, the interquartile range was between 0.82% and 1.14% in the CntDr group, and between 0.83% and 1.17% in the InjDr1 group.

On the other hand, the distribution of CDT values of the subjects included in InjDr2 group (BAC > 0.5 g/l) was quite different from that observed in the CntDr group. The difference was quite evident observing both the density estimate of these 2 distributions (Fig. 1C) and the empirical cumulative distributions (Fig. 1D). The difference was confirmed by the Kolmogorov–Smirnov test, which yielded a highly significant result with a p-value well below 0.001. Furthermore, the quartiles of the 2 distributions were quite different; the interquartile range in the InjDr2 group was between 1.16% and 2.34%.

The partition of CDT data on the basis of the fixed cut-off of 1.90% showed that 43 of the 468 subjects (9.2%) included in the InjDr group had elevated CDT concentrations (i.e., above 1.90%), while only 1 of the 236 drivers (0.4%) of CntDr had CDT value >1.90%. Therefore, the odds of having elevated CDT concentrations in the InjDr and the CntDr groups were 0.1012 and 0.004255, respectively. The resulting odds ratio (InjDr vs. CntDr) was 23.8 (with a p-value at the Fisher’s exact test well below the 0.001 threshold).
Considering only the subjects included in InjDr₁ group (BAC ≤ 0.5 g/l), 7 of 368 (1.9%) showed elevated CDT concentrations on the basis of the cut off of 1.90%. Comparing the odds observed in this subgroup (0.01939) with those observed in the CntDr group, the odds ratio was 4.56, a value not significantly different from 1 at the Fisher’s exact test (p = 0.158).

On the other hand, 36 of 100 subjects included in InjDr₂ group (BAC > 0.5 g/l) showed elevated CDT concentrations on the basis of the same cut off. Comparing the odds observed in this subgroup (0.5625) with those observed in the CntDr group, the odds ratio was 4.56, a value not significantly different from 1 at the Fisher’s exact test (p = 0.158).

When subjects with BAC within the legal limit (InjDr₁) were considered, the “null” odds ratio value of 1 (i.e., 0 on a logarithmic scale) was included in the 95% CI for almost all the cut offs considered (Fig. 2A). On the other hand, when subjects with BAC above the legal limit were considered (InjDr₂), the “null” odds ratio value of 1 (i.e., 0 on a logarithmic scale) was not included in the 95% CI for almost all the cut offs considered (Fig. 2B). Examining the odds ratio of InjDr₂ versus CntDr, a fairly regular increase in the strength of the association for increasing values of CDT cut offs was observed. Indeed, using the first cut off of 0.58%, the odds ratio was 3.02, while using the cut off of 1.78%, the odds ratio was 49. However, at the next cut off of 1.80%, an evident jump emerged, as the sample odds ratio was as high as 142. A further increase in the cut off did not yield an increase in the odds ratios.

The dose–response effect of CDT on the risk of nonalcohol-impaired crashes was evaluated using InjDr₁ as the cases and CntDr as the controls. CDT was categorized into 5 ordinal groups (see Materials and Methods), and
the percentage of InjDr1 cases within each category was calculated. As there were 368 InjDr1 cases, the overall percentage of cases was 60.9%. As can be seen from Fig. 3, the percentage of cases was approximately constant in the first 4 categories (i.e., when CDT was up to 1.9); only the last category showed a numeric, although not significant, increase ($\chi^2 = 5.91; p = 0.21$). Therefore, no dose–response effect was found, and, consequently, CDT can be considered as not associated with the risk of nonalcohol-impaired crashes.

The dose–response effect of CDT on the risk of alcohol-impaired crashes was evaluated using InjDr2 as the cases and CntDr as the controls. CDT was categorized into the same 5 ordinal groups previously considered. As there were 100 InjDr2 cases, the overall percentage of cases was 29.8%. However, as it can be seen from Fig. 3, this percentage was far from being constant and appeared regularly increasing from one category to the next. The chi-square yielded a highly significant result ($\chi^2 = 123; p < 0.001$); the chi-square for trend was also highly significant ($\chi^2 = 112; p < 0.001$). Therefore, when considering the risk of alcohol-impaired crashes, it should be concluded that a dose–response effect of CDT does exist.

These results did not change when a logistic regression model was fitted to the same data to adjust for other covariates (sex and age). When InjDr1 were considered as the cases, the discriminative power of CDT, measured by the area under the ROC curve, was negligible. Namely, the area was 0.548, a value quite near to 0.5, which indicates a total absence of discriminative power. On the other hand, when InjDr2 were considered as the cases, an important discriminative power of CDT was found, as the area under the ROC curve was 0.816.

**DISCUSSION AND CONCLUSIONS**

The need to use objective parameters supporting the medical decision in the process of regranting the driving license, after its suspension or confiscation for drunk driving, is well known at international level. For this reason in many Western countries, the medical visit is supported by the analysis...
of biomarkers of alcohol abuse, such as CDT, GGT (also in an integrated form, known as γ-CDT), liver enzymes, and mean corpuscular volume.

However, as already discussed in the introduction, even if a sound body of literature and a wide practical experience show the usefulness of CDT (as well as GGT and γ-CDT) for the identification of chronic/repeated alcohol abuse, the data supporting the correlation between these parameters and the real occurrence of traffic accidents are so far scarce and often scientifically weak.

In an attempt to unravel the significance of chronic alcohol abuse biomarkers in terms of increase in the risk of traffic accidents, the present study was focused on CDT, one of the most widely used indicators of chronic alcohol abuse in Europe. This biochemical parameter was determined in subjects injured in traffic accidents as well as in a control group of workers undergoing mandatory toxicological analyses. Being the aim of the study focused on the association of CDT with alcohol-related traffic accidents, all the subjects with evidence of recent psychoactive drug intake were excluded.

The results obtained from the evaluation of CDT, considering the whole distribution of CDT values, the odds ratios obtained using different cut offs, and the “dose–response” effect of CDT on the risk of crashes, suggest that the subjects injured in traffic accidents cannot be considered a homogeneous group. Indeed, when these subjects were divided into 2 groups on the basis of the BAC value (InjDr1 ≤ 0.5 g/l and InjDr2 > 0.5 g/l), only the latter group resulted significantly different from the CntDr.

As per the observed correlation between BAC and CDT concentrations, it should be pointed out that CDT kinetics is fairly slow (half-life of about 2 weeks) different from that of blood alcohol (half-life of about 2 hours). On this basis, the finding of a statistical correlation has to be very carefully considered in single cases.

Given the long half-life of CDT and its insensitivity to point abuses or short abstinence, the concentrations of this marker at the time of the medical visit for the driving license regranting reasonably reflects those at the time of the crash. On these grounds, the data of the present study strongly support the use of the CDT test to evaluate the risk of a subject to be involved in a road accident while DUI.

Moreover, on the basis of the pattern of increase in the odds ratios showed in Fig. 2, a decrease in the CDT cut off from 1.90% to 1.80 or 1.70% can be proposed, in order to avoid the exponential increase in the odds ratio.

The present study shows some limitations in the interpretation of the results. The CntDr, unlike the group of InjDr, included mainly skilled drivers. In addition, the cross-sectional nature of the study prevents us from drawing definitive conclusions on the causal association between increased CDT values and increased risk of having traffic accidents, even if the extremely high value of the odds ratio grants the trueness of an association between CDT and the risk of alcohol-related road crashes. In short, an elevated CDT does not predict future impaired driving crashes, but is associated with drunk drivers injured in a crash.

The present work shows also some undeniable strength points. To the best of our knowledge, it is the first study on the association between CDT value and alcohol-related traffic accidents. Indeed, the other cross-sectional studies are focused on the mere correlation between CDT and the risk of DUI of alcohol and not on the occurrence of a road accident, which is the real final event to be prevented by the adoption of restrictions in the granting of the driving license. An additional and final strength is that the “dose–response” effect of CDT was observed only in alcohol-impaired crashes.

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REFERENCES


