

New evidence of high association between carbohydrate deficient transferrin (CDT) and alcohol-related road traffic accidents.

A retrospective study on 929 injured drivers

Nadia M. Porpiglia^{a,*}, Franco Tagliaro^{a,b}, Rocco Micciolo^c, Luisa Canal^d, Giacomo Musile^a, Federica Bortolotti^a

^a*Unit of Forensic Medicine, Department of Diagnostics and Public Health, University of Verona, Piazzale L. A. Scuro 10, 37134 Verona, VR, Italy*

^b*Institute Translational Medicine and Biotechnology, Sechenov First Moscow State Medical University, 2–4 Bolshaya Pirogovskaya Street, 119991 Moscow, Russia*

^c*Department of Psychology and Cognitive Sciences and Centre for Medical Sciences, University of Trento, Corso Bettini, 84, 38068 Rovereto, TN, Italy*

^d*Department of Psychology and Cognitive Sciences, University of Trento, Corso Bettini, 84, 38068 Rovereto, TN, Italy*

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*Correspondence to: Piazzale L. A. Scuro 10, 37134 Verona, VR, Italy.
E-mail address: nadia.porpiglia@univr.it (N.M. Porpiglia).

Abstract

Background: It is well known that traffic injuries still represent one of the main causes of death and that high blood alcohol concentrations while driving significantly increase the occurrence of accidents. However, only limited literature on the correlation between chronic alcohol abuse and accident risk is available. The aim of the present study was to investigate the hypothesis of an association between elevated concentrations of carbohydrate deficient transferrin (CDT) and the occurrence of alcohol-related traffic accidents.

Methods: The analytical determinations of BAC and CDT were performed following certified, routinely used methods in HS-GC-FID and HPLC, respectively. For BAC 0.5 g/L was used as cut-off, whereas 2.0% was used for CDT, according to the standardisation proposed by IFCC. A total of 929 drivers, tested for BAC at the time of hospital admission after a traffic accident, were classified in two groups: InjDr1 (BAC $\leq$ 0.50 g/L) and InjDr2 (BAC > 0.50 g/L); all drivers were also tested for CDT.

Results: InjDr1 included 674 individuals, only 2.5% showing a CDT above the cut-off, whereas InjDr2 group consisted of 255 subjects, 28.6% testing positive for CDT (Odds Ratio 15.5). When subdividing the InjDr group into increasing classes of CDT, a steady increase in the percentage of BAC-positive drivers was appreciated. Moreover, average BAC was found to parallel each class of CDT.

Conclusions: The reported data strongly support the use of CDT as a biomarker of increased risk of alcohol-related traffic accidents in the procedures of re-granting of the driving license upon confiscation for "drunk driving".

1. Introduction

It is well known that road traffic accidents still represent one of the main causes of death worldwide, also being the first cause of death in the population, aged 5–29 years [1]. The correlation between alcohol misuse and road traffic accidents has widely been investigated [2–5] and policies to contrast the phenomenon of “drunk driving” are adopted worldwide. A topic of general agreement among authors is that concentrations of alcohol in blood above 0.50 g/L strongly affect driving skills and compromise traffic safety.

In fact, since the Grand Rapids Study [6] until present, a strong body of literature has piled highlighting that high blood alcohol concentrations (BACs) drastically increase the likelihood of occurrence of road traffic accidents [2,4,7,8]. In particular, it has been estimated that for every 0.02 g/L of BAC increase, the risk of road traffic accidents doubles [5]. Such a vast and well-documented evidence-based consensus on the acute effect of alcohol on traffic safety is juxtaposed by a much weaker literature on the correlation between chronic alcohol abuse and the risk of traffic crashes. Nevertheless, the need of verifying the existence of such an association is of paramount importance in the process of testing the fitness of individuals to hold the driving license or to release other certifications attesting the suitability to safety-sensitive jobs, child custody and in other cases of civil litigation. Evidence that chronic alcohol abusers are “predisposed” to road traffic accidents already appeared in early studies carried out in the 1960s [9], but the lack of objective parameters of this condition hindered the practical significance of these studies. In fact, the diagnosis of chronic alcohol abuse relied only on clinical evidence, questionnaires, and non-specific biomarkers of alcohol-related organ damage (MCV, liver enzymes, etc.). Since the 1980s, however, the introduction of more specific biomarkers (CDT, FAEE, Peth, and EtG, just to mention a few) has offered better diagnostic tools and, consequently, the research in this field has steadily increased [10,11]. Among these biomarkers, by far the most studied is CDT, which has been widely applied to corroborate the diagnosis of chronic abuse of alcohol [12–15] and, in clinical contexts, this biomarker showed a diagnostic sensitivity and specificity of 70% and 98%, respectively [16].

The spreading of the diagnostic use of CDT in forensic and clinical contexts, as well as the availability of different analytical approaches for its determination, encouraged the International Federation of Clinical Chemistry (IFCC) to establish a Working Group on Standardisation of CDT (WG-CDT). The final aim of their work was to define and validate the CDT analyte, to select a reference method and reference materials, and to create a network of laboratories [17–20]. This action has established a sound ground for the adoption of this analyte as a biomarker of chronic alcohol abuse at international level, assuring an inter-laboratory comparability of the results.

On the other hand, the use of CDT (as well as any other alcohol abuse biomarker) to verify the fitness to drive (or to work), is only based on the generic assumption that a chronic drinker

consumes alcohol compulsively and consequently is likely to drive (or to work) under the effect of such substance, representing a risk to public safety. However, considering that the refusal of the certification of fitness to drive (or to work) can limit an individual's rights, this assumption needs to find specific validation and not merely lie in the realm of "plausibility".

Surprisingly enough, to date, the biomarkers of alcohol abuse have rarely found a diagnostic validation in this very context, being their application simply been drawn from the experience in the clinical diagnosis of alcohol misuse. However, quite recently CDT has been studied in terms of its specific association with the occurrence of alcohol-related road traffic accidents [21], proving more reliable than other traditional biomarkers such as MCV and GGT [22,23].

On these grounds, the present work was undertaken to investigate a possible association, more deeply, between the serum concentrations of CDT and the blood alcohol concentrations at the time of a car accident, with the intention of posing a ground of knowledge to support the diagnostic value of this chronic alcohol abuse biomarker in the delicate context of traffic medicine.

2. Methods

2.1 BAC analysis

BAC measurement was performed by head-space gas chromatography (HS-GC) equipped with a flame ionisation detector (on a model 6100 GC analyser purchased from Young Lin Instrument Co. Ltd., Anyang, Korea) with a method routinely used and compliant with the accreditation criteria of our laboratory (Health Authority of Regione Veneto, Italy). In brief, sample preparation was carried out by mixing whole blood (1:4 dilution) with an aqueous solution of tert-butyl alcohol (0.0975 g/L) as the internal standard (Carlo Erba Reagents, Cornaredo, Italy). After equilibration of the sample vials at 60 °C, a total volume of 1250 µL of the head space was automatically injected in the system at a temperature of 80 °C. Isotherm separation was carried out at 40 °C. An internal quality standard provided by ACQ Science GmbH (Rottenburg am Neckar, Germany) containing ethanol at a concentration of 0.50 g/L was measured at an interval of 5 test samples. The lower limit of quantitation (LOQ) of this technique is 0.01 g/L. The laboratory follows an external quality control programme for ethanol provided by LGC Proficiency Testing (Bury, United Kingdom).

2.2 CDT analysis

CDT determination was performed on a gradient HPLC system (LC-10 VP series, Shimadzu Europe, Duisburg, Germany) equipped with a UV-vis detector (model SPD-10AV VP, Shimadzu) set at a wavelength of 460 nm. The analytical procedure was performed following an edited version of the method reported by Jeppsson et al. [24]. In brief, starting buffer (phase A)

consisted of 10 mM Bis-Tris at pH 6.2 and phase B was made of phase A added with 500 mM NaCl at the same pH. The solutions were used to generate a linear gradient from 0 to 27% of phase B within 20 minutes. A solution of 2 M NaCl was used for regeneration. Upon preparation, all solutions were degassed and filtered through 0.45 µm membrane filters. Samples were injected at a volume of 100 µL and flow rate was maintained at 1 mL/min. The analytical anion-exchange column [65 × 4.6 mm (i.d.)] was kept at a temperature of 40°C during the separation and was part of a commercial kit named ClinRep® CDT in Serum provided by Recipe, Munich, Germany. Prior to HPLC analysis, sample preparation required ferric ion saturation and lipoprotein precipitation with CaCl₂, included in the same reagent kit. The results obtained in terms of %CDT were normalised according to the IFCC WG-CDT standardisation and the adopted cut-off limit between alcohol abusers and non-abusers was 2.00% [20]. The laboratory follows three external quality control programs for CDT provided by ARVECON GmbH (Walldorf, Germany), by IFCC Network on Standardisation of CDT (Winterswijk, The Netherlands), and by Biologie Prospective (Villers-Les-Nancy, France).

2.3 Subjects and specimen collection

The present study included a total of 929 subjects who were admitted to the University Hospital of Verona after being involved in road traffic accidents and suffering injuries of different severity during 2018–2019. The total of 929 individuals did not include those patients in which urine testing was positive for the presence of psychoactive drugs (i.e., cocaine, amphetamines and their metabolites, opioids like morphine, methadone and the major metabolites, benzodiazepines, and cannabinoids). As drivers implicated in a road traffic crash, the subjects were mandatorily tested for alcohol and psychoactive drugs as required by the Italian Road Traffic Code, art. 180 and 181. In this context, no ethical approval was needed by law. However, all the subjects, after being informed on the purpose of the sample collection, provided valid verbal consent.

After venipuncture, the whole blood samples for BAC analysis were collected and stored in oxalate fluoride tubes at 4 °C until analysed. Blood samples for CDT analysis were collected in test tubes without preservatives and anti-coagulants, let them clot and centrifuged at 3500 rpm for 10 min. The resulting supernatant was collected and stored frozen at -20°C until analysis.

3. Results and discussion

In the herein reported study, the 929 drivers were classified into two groups according to the measured BAC at the time of hospital admission:

- InjDr₁ group: BAC ≤ 0.50 g/L (i.e., within the Italian legal limit)
- InjDr₂ group: BAC > 0.50 g/L

InjDr₁ group included 674 individuals, whereas InjDr₂ group consisted of 255 subjects, who were considered by law “under the influence of alcohol” at the time of the crash. Overall, the BAC range was between 0.01 and 4.63 g/L (average 0.56 ± 0.98 SD). On the other hand, CDT values globally ranged from 0.13% to 14.47%. In the InjDr₁ group, CDT showed an average of 1.08% ($\pm 0.49\%$ SD), whereas in the InjDr₂ group the average value was 2.21% ($\pm 2.13\%$ SD).

When considering InjDr₁ group, only 2.5% of the subjects showed a CDT value exceeding the cut-off of 2%, whereas 28.6% of the subjects belonging to InjDr₂ tested positive for CDT (hence $> 2\%$). This high prevalence of CDT positives in the alcohol-positive injured drivers was already reported by our group [21], but in the present study, because of a larger group of drivers, we managed to better investigate quantitatively the association between BAC and CDT.

By taking into consideration the entire InjDr group ($n = 929$), the whole range of CDT values was divided into six classes and, within each class, subjects were classified as BAC positives or negatives, as shown in Table 1. The graphical representation of the table (Fig. 1) clearly shows a steady increase in the percentage of BAC positives in parallel with the increase of CDT in its different classes.

Table 1

Distribution of BAC negatives and positives according to selected classes of CDT.

%CDT range	Number of individuals	% individuals with BAC > 0.50 g/L	% individuals with BAC ≤ 0.50 g/L
0 – 0.5	20	0 ($n = 0$)	100 ($n = 20$)
0.5 – 1.0	319	12 ($n = 39$)	88 ($n = 280$)
1.0 – 1.5	431	23 ($n = 100$)	77 ($n = 331$)
1.5 – 2.0	69	62 ($n = 43$)	38 ($n = 26$)
2.0 – 4.0	56	75 ($n = 42$)	25 ($n = 14$)
> 4.0	34	91 ($n = 31$)	9 ($n = 3$)

It is remarkable to notice how the percentage of BAC-positive individuals increases along with the increase of CDT concentration. At CDT concentrations $\leq 0.5\%$, all the injured drivers showed BACs below the Italian legal limit of 0.50 g/L. In contrast, the CDT class ranging from 1.5 to 2% (i.e., above the average CDT concentrations in the population but still within the current CDT cut-off [25]) included 62% of subjects testing positive for BAC at the time of the car crash. This percentage rapidly increased to 75% when considering the CDT range between 2 and 4%. A non-parametric evaluation of the correlation between the two variables (CDT and BAC) in all the injured drivers, using the Spearman rho coefficient, provided a value of 0.455.

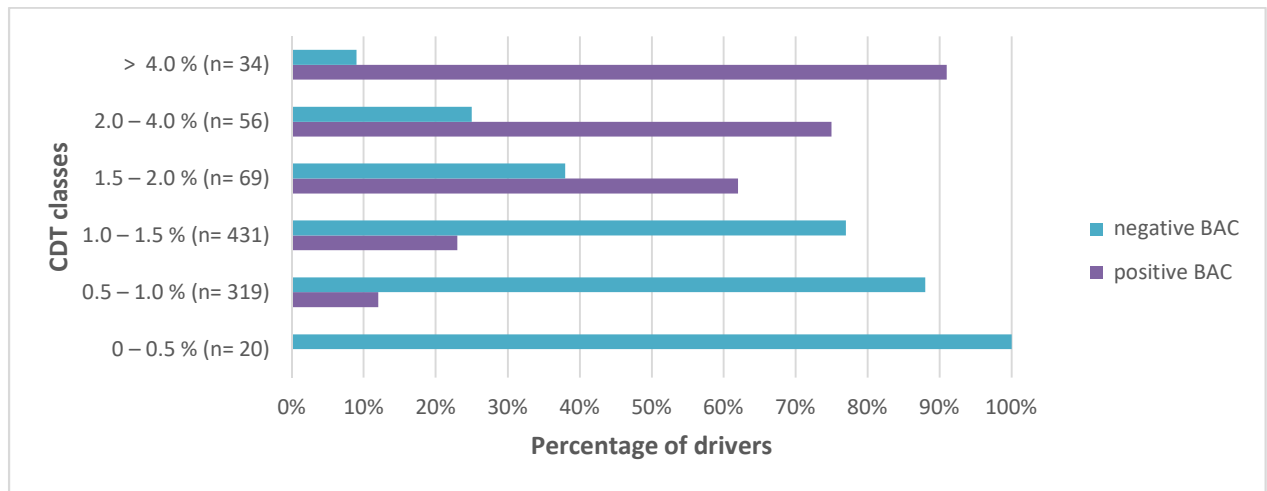


Fig. 1. Percentage of BAC negatives and positives according to selected classes of CDT.

It was also noteworthy to interpolate a logistic model employing CDT as the independent variable and the probability to test positive to BAC as the dependent one. The graphical representation of the estimated model is shown in Fig. 2, where the CDT value that corresponds to a 50% probability of being BAC positive is 1.96, i.e. substantially the CDT cut-off in use in many laboratories, including ours.

Another statistical tool to evaluate the strength of the association between CDT and BAC is to assess the odds ratios corresponding to different CDT values candidate to become suitable cut-offs in the context of traffic safety. The results are summarised as follows:

- CDT cut-off set at 1% -> odds ratio = 4.6
- CDT cut-off set at 1.5% -> odds ratio = 12.2
- CDT cut-off set at 2% -> odds ratio = 15.5
- CDT cut-off set at 4% -> odds ratio = 31.0

The odds ratio is the ratio between the odds of being positive at BAC analysis when CDT is “positive” (i.e. above the selected cut-off) and the odds of being positive at BAC analysis when CDT is “negative” (i.e. below or equal to the selected cut-off). For instance, considering the currently used cut-off of CDT (i.e., 2%), the corresponding odds ratio of 15.5 indicates that those individuals who, by law, cannot hold the driving license show a 15.5 times higher possibility to result positive to BAC determination with respect to subjects with $CDT \leq 2\%$.

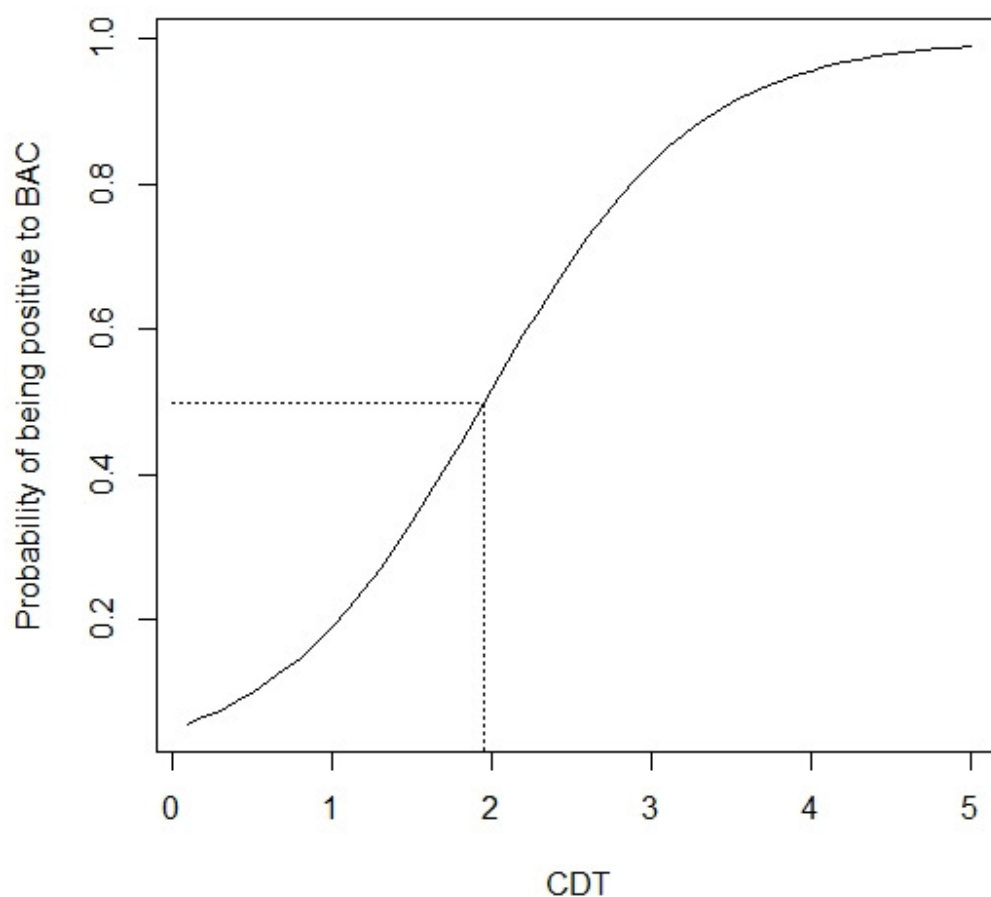


Fig. 2. Estimated probability of being BAC positive in relation to CDT values.

On the other hand, it is possible to consider the pattern of mean values of the BAC in the injured drivers in relation to different cut-offs of CDT. According to this approach, CDT values were subdivided into nine classes and the mean BAC was calculated within each class. The results are shown in Fig. 3. The strength of the association between the two variables was evaluated using the η^2 coefficient, which provided a value of 0.541. Looking at the fifth point in the figure, it is also clear that the class of CDT ranging from 1.25% to 1.50% already corresponds to an average BAC (0.58 g/L) which exceeds the legal limit adopted in Italy and in most Western countries. This could suggest a substantial reduction of the adopted CDT cut-off for certifying the fitness-to-drive. However, this crucial decision should be grounded also on specific studies focused on the calculation of the increased risk of alcohol-related road traffic accidents in the population with CDT values below the currently used cut-off.

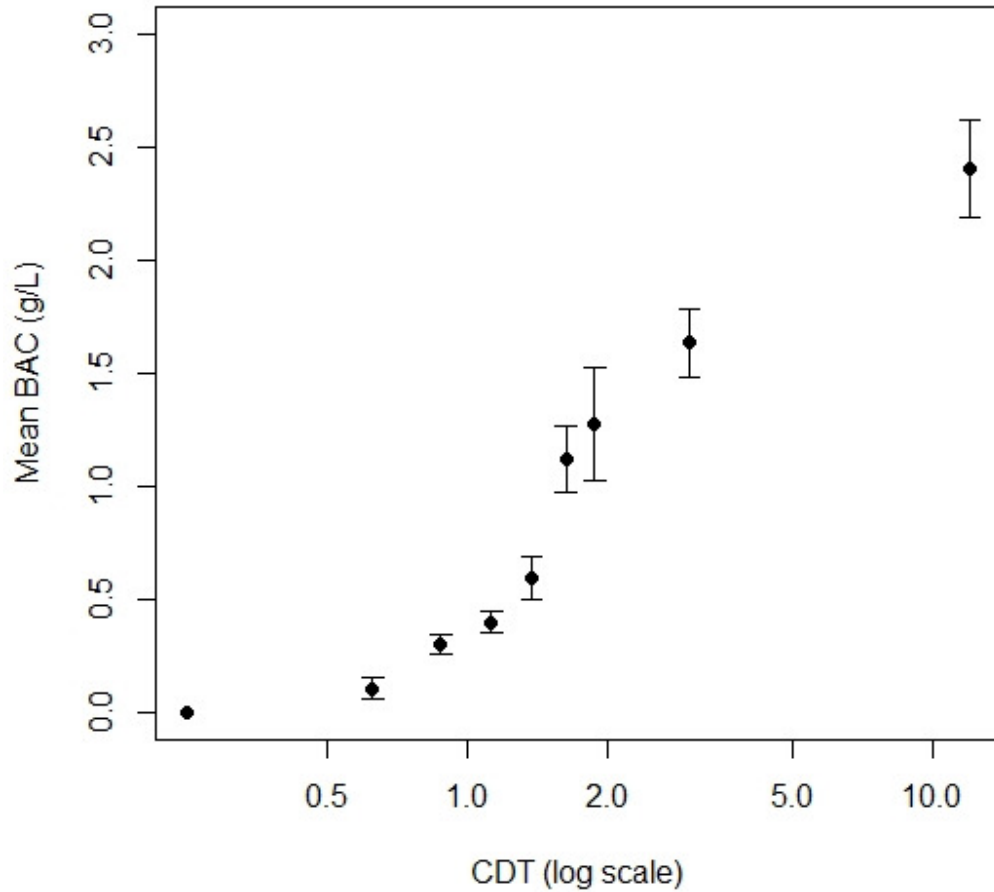


Fig. 3. Mean BAC values within nine selected classes of CDT. The classes were: ≤ 0.5 , 0.5-0.75, 0.75-1, 1-1.25, 1.25-1.5, 1.5-1.75, 1.75-2, 2-4, >4. CDT is plotted on a logarithmic scale. Bars represent standard errors.

4. Conclusions

Only a few authors have investigated, retrospectively, the usefulness of CDT in the context of traffic safety, generally reporting a correlation between CDT and BAC but mostly based on data not collected on the occasion of an occurred accident [26–34]. Also, the research groups of Maenhout and Portman reported on CDT as a potential predictor of recidivism in drunk driving [35,36]. In contrast, the present work, stemmed from previous articles from our group on the association between CDT and elevated BAC in road traffic crashes [21,23], and was aimed at studying this relationship on a quantitative basis, as a better confirmation of the reliability of CDT as a biomarker indicating the risk of being involved in a road accident while drink driving.

The presented data clearly confirms “quantitatively” the association of CDT with the occurrence of alcohol-related road traffic accidents. Notwithstanding the inherent limitations of a cross-sectional study, such as the present one, and considering the huge logistic and ethical problems of a longitudinal study, we believe that the overall information at present available on CDT and traffic crashes supports once again with sound evidence the usefulness of this biomarker for the certification of the fitness-to-drive, in case of a pre-existing alcohol related driving license confiscation. These considerations could reasonably be extended also to accidents occurring in other contexts, and primarily to the fields of occupational accidents and safety-sensitive jobs. Last-but-not-least, the present data show also that an average BAC above the common legal limit of 0.50 g/L can be found also in subjects showing CDT percentages below the currently adopted cut-off of 2.0%. This might suggest the opportunity of a re-evaluation of the present cut-off established on the basis of clinical studies, far from the field of traffic safety. Of course, this action would require adequate field studies in a multicentre context.

Declaration of interests

We declare no competing interests.

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