ASSESSMENT AND DETECTION

Use of Carbohydrate-Deficient Transferrin (CDT) and a Combination of GGT and CDT (GGT–CDT) to Assess Heavy Alcohol Consumption in Traffic Medicine

Vincenza Bianchi1, Alessandra Ivaldi1, Alessia Raspa1, Carlo Arfin1 and Matteo Vidali2,3,*

1Toxicology Laboratory, Department of Clinical Pathology, SS. Antonio e Biagio e C. Arrigo Hospital, Alessandria, Italy.
2Department of Medical Sciences, University “Amedeo Avogadro” of East Piedmont, Novara, Italy and 3Clinical Chemistry Unit, Maggiore della Carità Hospital, Novara, Italy.

*Corresponding author: Department of Medical Sciences, University “Amedeo Avogadro” of East Piedmont, Via Solaroli 17, 28100 Novara, Italy. Tel: +39-0321-660650; Fax: +39-0321-620421; E-mail: vidali@med.unipmn.it

(Received 26 June 2009; first review notified 21 August 2009; in revised form 11 September 2009; accepted 25 September 2009)

Abstract — Aims: Carbohydrate-deficient transferrin (CDT) has become widely used in traffic medicine to detect chronic alcohol abuse among subjects applying for driving-license renewal or regranting. By defining cut-off values in a large population of abstainers and moderate drinkers, we report on CDT, GGT–CDT (a combination of gamma-glutamylaminotransferase (GGT) and CDT) and the association between blood alcohol concentration (BAC) and CDT among Italian drivers. Methods: CDT was evaluated by a high performance liquid chromatography (HPLC)-based commercial kit in 652 abstainers or moderate drinkers, 603 drivers applying for driving-license regranting after a rehabilitation programme and 105 drivers involved in car accidents with blood alcohol concentration higher than the legal limit used in Italy (BAC >0.5g/l); GGT–CDT was calculated according to Sillanaukee and Olsson and Niemelä. Results: A common CDT cut-off (1.8%) and gender-specific GGT–CDT cut-off (4.15% for males, 3.56% for females) were calculated as 99.5th percentiles of the control population. Also, 3% and 27% of subjects were classified as CDT positive respectively among drivers applying for license regranting and drivers involved in car accidents. A significant association between BAC and both CDT values and CDT positivity was found, with a frequency up to 49% of CDT+ samples, suggesting chronic alcohol abuse, among drivers with BAC >2.5g/l. Concordance between CDT and GGT–CDT was only moderate (κappa = 0.44), with CDT performing better than GGT–CDT. Conclusions: A relevant proportion of drivers with high BAC are chronic abusers. GGT–CDT, previously validated with CDT immunoassays, should not be applied to traffic medicine in its current form and its performances re-evaluated with CDT measured by HPLC.

INTRODUCTION

Alcohol is widely recognized as one of the most important factors in road traffic accidents (del Rio and Alvarez, 1999; Smart and Osborne, 2000; Voas et al., 2000). Despite several public education campaigns and penalty worsening, alcohol-related disability and mortality are still increasing particularly among young adults. Nowadays, legislation of several European countries establishes that driving license cannot be issued or renewed to drivers who are chronic alcohol abusers. Moreover, a driver found to be driving under the influence of alcohol is heavily fined and subjected to license suspension. To have his license regranted, the offender must be evaluated with specific clinical and laboratory tests by a medical committee, and attend a rehabilitation programme if recidivist or high-risk driver, to prove a sober lifestyle.

Several studies have reported that a large proportion of apprehended drivers are repeat offenders and are more likely than others to be involved in fatal crashes (Brewer et al., 1994; Christophersen et al., 1996; Rio et al., 2001). The rate of recidivism was reported to be higher among heavy drinkers and correlated with blood alcohol concentration (BAC) at the time of the offence (Gjerde, 1987). Rehabilitation programmes may reduce both recidivism as well as alcohol-related crashes (DeYoung, 1997; Pratt et al., 2000). Therefore, the identification of high-risk offenders and the subsequent rehabilitation, by monitoring driver’s adherence to the abstinence programme, is a major concern for traffic safety with an important socioeconomic impact. In addition, it should be considered that drivers who are chronic alcohol abusers require both medical treatment and punishment (Dawson, 1999).

Among several laboratory tests routinely used in clinical and forensic medicine, carbohydrate-deficient transferrin (CDT) is considered the most specific biochemical marker for detecting chronic alcohol abuse and for monitoring abstinence during treatment (Salaspuro, 1999; Arndt, 2001; Golka and Wiese, 2004). CDT indicates collectively a group of minor glycoforms of human transferrin with a lower degree of glycosylation, namely asialo-, monosialo- and disialotransferrin.

Although widely used, there is still no agreement on which CDT glycoform should be measured, how to express the results and which method should be preferred (Bianchi et al., 2008). Over the years, different analytical techniques have been applied for CDT quantification (Arndt, 2001; Bortolotti et al., 2006), including immununometric (Vitala et al., 1998; Helander et al., 2001; Delanghe et al., 2007), electrophoretic (Lanz et al., 2004; Helander et al., 2005; Tagliaro and Bortolotti, 2008), chromatographic (Helander et al., 2003) and mass-spectrometric methods (Flahaut et al., 2003; Kleinert et al., 2003; del Castillo Busto et al., 2005). Whereas some tests were lately abandoned because of poor accuracy and specificity, a number of commercial kits for CDT measurement have become readily available, together with different customized cut-offs. Moreover, CDT combined with classical chronic alcohol abuse markers was shown to have increased specificity and sensitivity in clinical settings than single markers alone (Sillanaukee et al., 2000; Sillanaukee and Olsson, 2001; Amtula et al., 2003).

In an effort to overcome the lack of standardization affecting CDT analysis (Bianchi et al., 2008), the Working Group on Standardization of CDT measurement (IFCC-WG-CDT) has recently suggested to use disialotransferrin (DST) as the primary target molecule for CDT measurement, expressed as relative amount (%CDT) to compensate for variable levels of total transferrin concentration. Moreover, high performance liquid chromatography (HPLC) was recommended as the best
interim reference method to be applied in CDT confirmatory analysis (Jeppsson et al., 2007).

Following IFCC-WG-CDT recent recommendations and defining a CDT cut-off in a large control population, here we report on the prevalence of chronic alcohol abusers, suspected on the basis of CDT test, among Italian drivers in relation to BAC at the time of the offence. The usefulness of GGT–CDT, a combination of gamma-glutamylaminotransferase (GGT) and CDT, has been also evaluated.

METHODS

Subjects and samples
The blood samples included in this study were collected at the Hospitals of Novara and Alessandria (Northern Italy). Analyses were performed at the Toxicology Laboratory of SS. Antonio e Biagio e C. Arrigo Hospital in Alessandria. Selected samples were confirmed, using the same Bio-Rad CDT kit, at the Clinical Chemistry Unit of the Maggiore della Carità Hospital in Novara, which together with Alessandria is part of the regional network for confirmatory-level drug and alcohol testing. All participants were interviewed about their drinking habits by trained medical personnel. Subjects were classified into: Group A including 652 (336 males, 316 females) abstainers or moderate drinkers (drinking <210 g/week for men, <140 g/week for women) as control population; Group B including 603 (552 males, 51 females) drivers applying for driving-license regranting after a rehabilitation programme; and Group C including 105 (78 males, 27 females) drivers involved in car accidents and found driving under the influence of alcohol (BAC higher than the Italian legal limit of 0.5 g/l). Blood samples were collected in Becton Dickinson Vacutainer Plastic Serum tubes for CDT and fluoride tubes (sodium fluoride and potassium oxalate) for ethanol analysis. After clotting, serum was separated by centrifugation at 3500 g for 5 min and stored at -20°C for CDT analysis. The study was planned according to the guidelines of the local ethical committee in conformity to the principles of the Declaration of Helsinki.

Procedure
CDT was measured by %CDT kit by HPLC produced by Bio-Rad, Munich. This HPLC-based commercial kit was recently validated by Helander in comparison with the reference candidate method and found to be appropriate both for routine and confirmatory CDT analysis (Helander and Bergstrom, 2006). Samples were prepared following manufacturer’s instructions and injected in an Agilent 1200 HPLC system with anion-exchange cartridge kept at 40°C, using a ternary buffer gradient with a flow rate of 1.4 ml/min and detection at 460 nm. After run, transferrin glycoform peaks were measured by baseline integration using the Chemstation software Rev. B. 03.01, and CDT was expressed as percentage of DST to total transferrin area. Two sets of controls (low control: 0.7–2.1%; high control: 2.6–4.4%) also supplied by Bio-Rad were run as triplicates and always found within the range declared by the manufacturer. Patients with CDT variants identified by HPLC were excluded by the analysis. With current settings (Agilent Workstation and
CDT and GGT–CDT Biomarkers in Traffic Medicine

RESULTS

CDT levels, measured in abstainers or moderate drinkers, showed a right-skewed distribution, with a median of 0.84% (95% CI 0.81–0.86%) and a Q1–Q3 interquartile range (IQR) of 0.68–0.99%. Skewness and kurtosis were respectively 0.87 (SE 0.10) and 1.04 (SE 0.19) (Figure 1). Males displayed significantly higher CDT values than females (0.87%, IQR 0.73–1.01% vs 0.80%, IQR 0.65–0.97%; P < 0.001). Conversely, age was not correlated with CDT and no significant difference in CDT values was observed when patients were divided in age classes neither in the whole population nor in sex subgroups.

Cut-offs, evaluated in the whole population as 97.5th, 99th, 99.5th and 99.9th percentiles, were 1.4, 1.6, 1.7 and 1.8%, while in males and females were respectively 1.5, 1.6, 1.7 and 1.8% and 1.4, 1.5, 1.6 and 1.7%. Taking into account our results and following previous published studies, a common (not sex-related) cut-off of 1.8% (99.9th percentile) was selected for high specificity, as requested in a legal medicine context, where positivity could mean personal freedom restriction. The measured intermediate repeatability, estimated as coefficient of variation (CV)%, at this CDT level was 5.1% in both laboratories.

Chronic alcohol abuse detection has become increasingly relevant since it has been reported to be associated with hazardous driving and an increased risk of road traffic accident. In this study, both drivers applying for license regranting after a rehabilitation programme (median 0.90%, IQR 0.80–1.10), as well as drivers involved in car accidents with BAC >0.5g/l (median 1.20%, IQR 0.90–2.00), displayed CDT significantly (P < 0.001, corrected for multiple comparisons) higher than controls (Figure 1), with a prevalence of 3 and 27% of CDT-positive subjects among Groups B and C, respectively. Interestingly, BAC and CDT were found to be significantly associated (exact text P < 0.001), with a frequency of CDT+ samples, suggesting chronic alcohol abuse, ranging from 7 up to 49% of the drivers with BAC >2.5g/l (Table 1).

GGT was measured for all subjects investigated and then used for GGT–CDT calculation. In the control population, median GGT was 18 U/l (IQR 12–28). Males had higher GGT than females (24 U/l, IQR 17–35 vs 13 U/l, IQR 10–18; P < 0.001). Median GGT–CDT was 2.11% (IQR 1.64–2.53), with significantly higher levels in males than females (2.36%, IQR 1.98–2.82 vs 1.76%, IQR 1.37–2.21; P < 0.001). Due to the wide difference in GGT–CDT values (median difference 0.60%, 95% CI 0.49–0.68), different sex-specific positivity cut-offs (99.9th percentile) were selected for males (4.15%) and females (3.56%). Despite the highly significant association (Fisher’s exact test P < 0.0001), concordance was only moderate in both groups (Cohen’s k = 0.44). Among Group B, only nine out of 16 (56%) subjects with CDT above the cut-off were also positive for GGT–CDT, while 15 out of 24 (63%) subjects classified as positives for GGT–CDT had CDT below the cut-off, being nine patients with CDT even below the 90th percentile (1.20%) of control distribution. For subjects close to the cut-off (CDT ranging from 1.7 to 1.9%), GGT–CDT was pos-

### Table 1. Carbohydrate-deficient transferrin (%CDT) and blood alcohol concentration (BAC) cross-tabulation in 105 (78 males, 27 females) drivers involved in car accidents and found driving under the influence of alcohol (BAC higher than the Italian legal limit of 0.5 g/l)

<table>
<thead>
<tr>
<th>CDT pos</th>
<th>CDT neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5–1.5 g/l</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>1.5–2.5 g/l</td>
<td>6 (17%)</td>
</tr>
<tr>
<td>&gt;2.5 g/l</td>
<td>20 (49%)</td>
</tr>
</tbody>
</table>

%CDT pos/neg indicate a CDT value above or below the cut-off evaluated as 99.9th percentile of the abstainer and moderate drinker group (1.8%). Percentages are calculated according to row totals.

Bio-Rad CDT kit), six samples per hour were measured with possibility for programmable overnight batch analysis.

Serum GGT was measured on ADVIA2400 (Siemens Healthcare, Milan, Italy) by its own kit based on International Federation of Clinical Chemistry (IFCC) recommendations. GGT–CDT was calculated by combining %CDT and GGT according to the original published formula (Stillauanake and Olsson, 2001), lately modified with %CDT (relative amount as percentage) instead of CDT (absolute amount in units per litre) (Anttila et al., 2003): GGT–CDT = 0.8 × ln (GGT) + 1.3 × ln (%CDT).

Ethanol (BAC) has been immediately evaluated after collection by Axsym (Abbott Laboratories, Abbott Park, IL, USA) on whole blood and, if >0.5 g/l (Italian legal limit for driving), confirmed by headspace gas chromatography–mass spectrometry (Agilent 6890 series) by home-made validated method.

Statistical analysis

Statistical analyses and graphs were performed by SPSS statistical software v. 15.0 (SPSS Inc., Chicago, IL, USA) and R software v. 2.9.0 (R Foundation for Statistical Computing). Differences between groups were estimated by non-parametric Kruskal–Wallis (with correction for multiple comparisons) or Mann–Whitney tests. Differences in proportions between %CDT positive and negative drinkers were estimated by chi-squared or Fisher’s exact test. Confidence intervals (CI) were calculated using CIA software v. 2.1.1 (by T Bryant, University of Southampton, UK). Normality distribution was assessed by the Kolmogorov–Smirnov and the Shapiro–Wilk tests and visually by histogram and kernel density plot. Concordance of %CDT and GGT–CDT was calculated by Cohen’s kappa index.

Table 2. Carbohydrate-deficient transferrin (%CDT) and GGT–CDT cross tabulation in 603 (552 males, 51 females) drivers applying for driving-license regranting after a rehabilitation programme (Group B) and in 105 (78 males, 27 females) drivers involved in car accidents and found driving under the influence of alcohol (BAC higher than the Italian legal limit of 0.5 g/l) (Group C)

<table>
<thead>
<tr>
<th>GGT–CDT pos</th>
<th>GGT–CDT neg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group B (drivers after rehab)</td>
<td></td>
</tr>
<tr>
<td>%CDT pos</td>
<td>9 (56%)</td>
</tr>
<tr>
<td>%CDT neg</td>
<td>15 (3%)</td>
</tr>
<tr>
<td>Group C (drivers with high BAC)</td>
<td></td>
</tr>
<tr>
<td>%CDT pos</td>
<td>19 (68%)</td>
</tr>
<tr>
<td>%CDT neg</td>
<td>15 (19%)</td>
</tr>
</tbody>
</table>

%CDT and GGT–CDT pos/neg indicate a CDT or a GGT–CDT value above or below the cut-off evaluated as 99.9th percentile of the abstainer and moderate drinker group, respectively 1.8% for CDT, 4.15% (males) and 3.56% (females) for GGT–CDT. Percentages are calculated according to row totals.
Because of legal implications related to CDT determination, both a validated and reliable method as well as reference values assessed in a suitable large population are mandatory. Previous studies have reported a sex difference only among moderate or high drinkers (Bergstrom and Helander, 2008b); although males and females in our sample were not significantly different in alcohol consumption, it is likely that a minor difference in moderate drinker prevalence among males and females, inflated by the size of the sample, may account for the statistically significant association observed between sex and CDT. Indeed, the observed difference in CDT values between males and females, although significant, was not clinically important (median difference 0.07%, 95% CI 0.03–0.10), particularly compared with the total precision of the test. Several cut-offs for CDT testing have been reported and are applied in many laboratories. Such a huge variability relies on the method used and on the glycoforms effectively measured. In this study, reference values suggested by the manufacturer were verified in a large sample of Italian abstainers or moderate drinkers, following IFCC-WG-CDT recommendations. Due to the wide departure of CDT values from Gaussian distribution, even after log transformation, cut-offs were calculated as percentiles.

Median CDT values in abstainers and moderate drinkers, here reported, were slightly lower than those described for different populations in previous papers using HPLC analysis (Bergstrom and Helander, 2008c). A reason could be found in the specific HPLC method used: in our daily experience, and according to a recently published study, HPLC Bio-Rad kit gives slightly lower values than the original method developed by Helander (Helander and Bergstrom, 2006). Moreover, a preliminary comparison between the Helander and Bio-Rad methods performed in our laboratory on 30 abstainers and moderate drinkers showed a mean bias of 0.20 (SD 0.17). This trend was confirmed when the laboratory participated with both methods to External Quality Assessment Schemes GTFC (Gesellschaft für Toxikologische und Forensische Chemie) and EQUALIS (External Quality Assurance in Laboratory Medicine in Sweden). Even that, the Bio-Rad method, validated against the interim candidate reference method, was preferred because of its rapidity (10 vs 40 min) without loss in specificity. This observation confirms that laboratory-specific cut-off definition is crucial, at least until a definitive CDT standardization.

By applying the calculated cut-off of 1.8%, 3% of drivers in Group B continued to heavily drink (as shown by elevated CDT) after a rehabilitation programme and, together with drivers who quit programmes or those still driving after license confiscation, represent the ‘hard core’ offenders. Since follow-up data are not available, we cannot evaluate at this stage if the rehabilitation programme was helpful in decreasing recidivism. Moreover, 28 out of 105 (27%) drivers in Group C (with BAC >0.5 g/l) were heavy drinkers according to CDT positivity. Direct comparisons of studies on chronic alcohol use and driving is difficult, and minor differences with prevalence reported here may be due to different factors, among others the legal limit applied, the situation when the specimen was collected (driver stopped under alcohol influence, driver suspected for impaired driving, fatally or non-fatally injured drivers), demographic factors or concomitant use of illicit drugs (Brinkmann et al., 2002; Appenzeller et al., 2005). For the same reason, the prevalence of chronic abusers among drivers in Group C was a biased estimate to infer the effectiveness of the rehabilitation programme in reducing heavy drinking in subjects applying for license regranting.

Although high BAC should not be interpreted per se as evidence of chronic alcohol abuse or high risk of recidivism, the observed trend of increased CDT among Italian drivers with increasing BAC levels highlights the problem of alcohol abusers with respect to road safety and the need of effective strategies of prevention and rehabilitation. Moreover, as previously reported, it confirms that many apprehended drunk drivers are problem drinkers and need both medical treatment and punishment.

In recent years, a combination of CDT and GGT, namely GGT–CDT, was shown to have both higher specificity and sensitivity than single markers alone in detecting alcohol abusers. In this study, direct comparison between CDT and GGT–CDT based on alcohol consumption was not possible, as many drivers prefer not to talk about their alcohol habit, underreport it or often deny it. However, data displayed in Table 2 allowed for some speculative analysis. Whereas GGT was shown to be affected by gender, race, obesity, liver or non-liver-specific diseases and many medications (Niemela, 2007), specificity of CDT by HPLC in detecting chronic abusers was reported to be close to 100% (Bergstrom and Helander, 2008b). In fact, a recent study has clearly shown that, focusing on IFCC recommendations (DST as the primary analyte, measured in HPLC and expressed as relative amount), there is a low risk of CDT false positives (non-alcohol-related elevation of CDT), suggesting that previous reports on high frequency of CDT false positives were based on unspecified methods applied for CDT analysis (Bergstrom and Helander, 2008a). Since a specificity close to 100% implies a frequency of false positives close to 0%, virtually all drivers classified as positives by CDT were true positives, but GGT–CDT was able to identify only nine out of 16 (56%) and 19 out of 28 (68%) CDT-positive drivers, respectively, in Groups B and C. On the other hand, GGT–CDT was apparently able to detect more alcohol abusers than CDT (24 vs 16 in Group B and 34 vs 28 in Group C), with 15 CDT+/GGT– patients both in Group B and Group C (Table 2) that could be CDT false negatives. However, even if CDT sensitivity is not 100%, the observation that more than one-third of these CDT+/GGT–/CDT+ had CDT greatly lower than the 90th percentile (1.2% of CDT distribution indicates, at least in our experience, that many of these subjects (obviously not all) are instead GGT–CDT false positives, implying GGT–CDT to be less sensitive than CDT measured with this method and at the considered cut-off.

Our data, measured by a confirmatory HPLC method, suggest that GGT–CDT, originally evaluated and useful in a clinical setting, should not be applied to traffic medicine in its current form, at least without CDT, and its performances and calculation re-evaluated with CDT measured by a reference method and expressed as percentage of total transferrin. A current limitation of this study is the self-stated alcohol consumption, which, if underreported, might have slightly inflated the cut-off. However, it is worth noting that the cut-off here calculated is comparable with previous publications using
HPLC methods. Moreover, since few subjects in this study have CDT values close to the cut-off, minor changes of it (±0.1%) do not significantly modify the associations found (data not shown).

In conclusion, we reported here on chronic alcohol abuse in a wide sample of Italian drivers, showing that reliable results can be achieved by combining evidences from published studies, recent IFCC recommendations and a reference cut-off statistically verified in a large cohort of abstainers and moderate Italian drinkers. Moreover, our data confirm that a large proportion of drivers with high BAC are chronic abusers. To our knowledge, this is the first study, at least in Italy, where CDT was assessed in all drivers with a confirmatory HPLC method.

Note: This study has been presented, as oral presentation, at the 12th Congress of European Society for Biomedical Research on Alcoholism, held in Helsinki, 7–10 June 2009.

REFERENCES


