DIAGNOSTIC CHARACTERISTICS OF DIFFERENT CARBOHYDRATE-DEFICIENT TRANSFERRIN METHODS IN THE DETECTION OF PROBLEM DRINKING: EFFECTS OF LIVER DISEASE AND ALCOHOL CONSUMPTION

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Abstract — Aims: Due to methodological heterogeneity, conflicting views have been expressed on the validity of CDT measurements in the detection of alcohol misuse. Methods: We compared the characteristics of the conventional CDTect method and the Axis turbidimetric CDT assays in the assessment of 62 alcoholics, who were either with (n = 33) or without (n = 29) liver disease, as analysed by combined clinical, laboratory, and morphological indices. Controls were 45 healthy volunteers who were either social drinkers or abstainers. Results: In the total sample of alcoholics, the sensitivity of the %CDT method, which excludes the trisialotransferrin isoform from the measurement, was 63% for men and 46% for women, as compared to 65% and 36% of CDTect, respectively. Both of these methods showed higher sensitivities than the %CDT–TIA method, which reacts with trisialotransferrin (32% and 25%, respectively). The assay specificities were 100% for men and 91% for women with %CDT, and 96% and 87% with the CDTect, respectively. The correlation between the CDTect and %CDT method was higher in men (r = 0.86) than in women (r = 0.57). The presence of liver disease was found to influence the results of the CDTect method, such that the highest CDT concentrations were observed in patients with mild to moderate liver disease, especially among women, whereas the %CDT method was less sensitive to the effect of liver pathology. The self-reported alcohol consumption from the 4 weeks prior to sampling showed a higher correlation between the %CDT results (r = 0.64, P < 0.0001) than with the CDTect results (r = 0.40, P < 0.001). Conclusions: The data indicate that the new %CDT method offers advantages over the previous versions of the CDT methods. The improved characteristics may be most useful in assays for excessive alcohol consumption in female alcoholics, patients with liver disease, and in patients with abnormal serum transferrin concentrations.

INTRODUCTION

Although serum carbohydrate-deficient transferrin (CDT) has been widely used as a marker of alcohol misuse, the interpretations of the assay results have suffered from a lack of uniform international standardization (Arndt, 2001; Helander et al., 2001a; Conigrave et al., 2002; Helander, 2002; Tagliaro et al., 2002; Whitfield, 2002). In healthy people, the most common transferrin isoform is tetrasialotransferrin, whereas, as a result of ethanol misuse, various degrees of deficiencies in the sialic acid content of transferrin may be generated (Stibler, 1991; Allen et al., 1994; Helander et al., 2001a). CDT refers to those isoforms that have a reduced sialic acid content in oligosaccharidic chains bound to amino acids 413 and 611. Usually this definition means isoforms with 0–2 or 0–3 sialic acid side-chains (Stibler, 1991; Allen et al., 1994; Arndt, 2001; Helander et al., 2001a; Helander, 2002).

Recently, the relative importance of the various CDT isoforms in the clinical assays for detecting alcohol misuse has received increasing attention (Scouller et al., 2000; Whitfield, 2002). Although measurements with improved specificity towards such isoforms have been introduced, only a few studies are available on the comparisons between the different methods in clinical materials (Helander, 1999; Anton et al., 2001). The new assay approaches, which exclude the trisialo isoform (the isoform with three sialic acid side-chains) from the measurement, have recently shown high diagnostic accuracies in the detection of alcohol misuse (Legros et al., 2002). Previous studies have also indicated that the diagnostic characteristics of the CDT assays may be markedly different from each other. Studies with the conventional CDTect method have found high diagnostic sensitivities, although also a high rate of false-positive results among women and in patients with high serum transferrin levels (Vitlala et al., 1998). Concerns over the impact of liver status have also been raised (Tsutsumi et al., 1994; Niemelä et al., 1995; DiMartini et al., 2001).

We designed the present study to compare the conventional CDTect method and %CDT assays for their diagnostic value to identify problem drinking in alcoholic patients with or without liver disease. Separate versions, which have been made available for the %CDT assay, allow the comparisons between the assays with (%CDT–TIA) or without (%CDT) reactivity towards the trisialofraction. The data suggests that the %CDT assay, which is devoid of such reactivity, offers several advantages over the previous CDT methods.

SUBJECTS AND METHODS

Study subjects

We studied 62 patients with alcoholism who had a well-documented history of continuous alcohol consumption or binge drinking. These included a sample of 33 with biopsy-proven liver disease (seven females; 26 males), who had a history of continuous alcohol consumption for at least 5 years in amounts exceeding 80 g/day. The severity of alcoholic liver disease was assessed according to previously established clinical and laboratory (CCL1 and morphological (CMI) criteria (Blake and Orrego, 1991). The CCLI index combines laboratory and clinical data, which have been shown to be significantly associated with the prognosis of alcoholic patients. When the
prognostic weights of each of such variables are added, the CCLI is obtained, which relates linearly to the mortality risk over its range of 0–25. The CMI index in turn summarizes the morphological findings of prognostic significance (necrosis, inflammation and Mallory bodies), which are graded on a 1+ to 3+ scale (Blake and Orrego, 1991). In addition, we studied 29 heavy drinkers (four females; 25 males), who were admitted for detoxification and had a well-documented history of heavy drinking consisting primarily of repeated inebriations, but were devoid of clinical and laboratory evidence of liver dysfunction. However, for ethical reasons these individuals were not biopsied at the time of blood sampling. Detailed interviews on the amount and pattern of alcohol consumption were carried out using a time-line follow-back method. The patients were asked how many drinks of alcohol (standard drink = 12 g of ethyl alcohol corresponding to one beer, one glass of table wine or three centilitres of 40% proof spirit) they had consumed during the (1) 24 h, (2) 1 week, and (3) 4 weeks preceding admission. These patients were found to have consumed a mean of 131 g alcohol per day (range 48–289 g/day) during the 4 week period prior to sampling. The mean duration of abstinence before sampling was 1.2 (range 0–4) days. The main clinical and laboratory characteristics of the study subjects are summarized in Table 1. The healthy controls were 45 volunteers (23 women; 22 men), who did not drink or who were social drinkers (<30 g ethanol per day on any occasion), as well as 23 women; 22 men), who did not drink or who were social drinkers (<30 g ethanol per day on any occasion), as well as examined by detailed personal interviews. These individuals were primarily hospital personnel, who volunteered for the study.

All serum samples were stored at −70°C until analysis. All participants gave their informed consent and the study was carried out according to the provisions of the Declaration of Helsinki.

### Table 1. Main clinical and laboratory characteristics of the study population

<table>
<thead>
<tr>
<th></th>
<th>Alcoholics with liver disease</th>
<th>Alcoholics without liver disease</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>33</td>
<td>29</td>
<td>45</td>
</tr>
<tr>
<td>Men/women</td>
<td>26/7</td>
<td>25/4</td>
<td>22/23</td>
</tr>
<tr>
<td>Age</td>
<td>48 ± 9</td>
<td>46 ± 12</td>
<td>51 ± 16</td>
</tr>
<tr>
<td>GT</td>
<td>309 ± 354***</td>
<td>123 ± 139</td>
<td>25 ± 10</td>
</tr>
<tr>
<td>Men</td>
<td>360 ± 371***</td>
<td>110 ± 114</td>
<td>29 ± 11</td>
</tr>
<tr>
<td>Women</td>
<td>84 ± 84</td>
<td>207 ± 267**</td>
<td>20 ± 8</td>
</tr>
<tr>
<td>MCV</td>
<td>99 ± 4.2***</td>
<td>96 ± 4.8***</td>
<td>92 ± 3</td>
</tr>
<tr>
<td>AST</td>
<td>82 ± 76***</td>
<td>41 ± 18</td>
<td>30 ± 24</td>
</tr>
<tr>
<td>Men</td>
<td>83 ± 81***</td>
<td>39 ± 16</td>
<td>36 ± 32</td>
</tr>
<tr>
<td>Women</td>
<td>73 ± 50***</td>
<td>53 ± 25</td>
<td>23 ± 5</td>
</tr>
<tr>
<td>ALT</td>
<td>99 ± 35***</td>
<td>46 ± 20**</td>
<td>28 ± 15</td>
</tr>
<tr>
<td>ALP</td>
<td>185 ± 59**</td>
<td>153 ± 48*</td>
<td>126 ± 30</td>
</tr>
<tr>
<td>BIL</td>
<td>19 ± 12**</td>
<td>12 ± 6</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>ALB</td>
<td>41 ± 5</td>
<td>43 ± 7</td>
<td>42 ± 3</td>
</tr>
</tbody>
</table>

The values are mean ± SD. For GT and AST with sex-specific reference intervals the data are shown separately for men and women.

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BIL, bilirubin; GT, gamma glutamyltransferase; MCV, mean corpuscular volume.

*P < 0.05, **P < 0.01, ***P < 0.001 (ANOVA, Bonferroni’s multiple comparison test).

### CDT analyses

The concentrations of CDT were first measured by a turbidimetric immunoassay (%CDT) after ion exchange chromatography (Axis-Shield, Oslo, Norway). This assay measures the transferrin variants with 0–2 sialic acid residues and excludes the trisialofraction containing 3 sialic acids. The concentration of CDT is expressed as a percentage of the total amount of serum transferrin. The measurements were carried out on a Behring Nephelometer II (Dade Behring, Behring Diagnostics, Marburg, Germany). Values exceeding 2.6% were considered to be high.

For comparisons, CDT was also measured with a competitive radioimmunoassay after microcolumn separation (CDTect). This assay excludes the trisialofraction and part of the disialofraction. Thus, the isoforms with 0–1 sialic acid residues (a- and monosialotransferrins) and minor amounts of isotransferrin with 2 sialic acid residues (disialotransferrin) are detected (Arndt et al., 1998; Helander et al., 2001a). The assay results are expressed as absolute CDT concentrations. The reference range in this assay is 0–20 U/l for men and 0–26 U/l for women. Measurements of the %CDT–TIA, which also measures the trisialylated fraction of serum transferrin were carried out on a Kone Optima Clinical Chemistry Analyzer (Kone Instruments, Espoo, Finland), as described previously (Viitala et al., 1998). The cut-off value for this assay is 6% of the total serum transferrin.

The cut-off values for all of the above assays were based on the manufacturers’ recommendations. For initial method evaluation, pooled sera from control and alcoholic patients with low and high CDT concentrations were first analysed to determine the analytical characteristics of the different methods. The within-run precisions (n = 10) for the %CDT were 5.2% for the low concentration and 4.4% for the high CDT content. The corresponding values for the CDTect method were 6.2 and 10%, respectively. The day-to-day CVs (n = 13) for the %CDT were 10% for the low and 5.6% for the high concentration, which were lower than those of the CDTect method.

### Other laboratory procedures

Serum gamma glutamyl transeptidase (GT), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), albumin, transferrin, bilirubin and mean corpuscular volume (MCV) of erythrocytes were measured by standard clinical chemical methods in an accredited (SFS-EN 45001, ISO/IEC Guide 25) laboratory of EP Central Hospital, Seinäjoki, Finland. For the parameters used as alcohol markers in this study, the following cut-off values were used: GT, 80 U/l (men), 50 U/l (women); ALT 50 U/l (men), 35 U/l (women); MCV 96 fl.

### Statistical methods

The data are expressed as mean ± SD. The statistical analyses were carried out using GraphPad Prism software (GraphPad Software, San Diego, CA, USA). The comparisons between the alcoholic and non-alcoholic groups were carried out using Student’s t-test or by analysis of variance (ANOVA), with the Bonferroni’s method for multiple comparisons. Correlations were calculated using Pearson’s product-moment correlation coefficients or Spearman’s rank correlations, as required. A P-value of less than 0.05 was considered statistically significant.
RESULTS

The mean CDT values in the total alcoholic population were significantly higher than those in the control group ($P < 0.0001$ for all methods). The (mean ± SD) %CDT values in the alcholics and in the healthy controls were 4.0 ± 2.3% and 2.0 ± 0.4%, respectively. For the CDTect, the corresponding values were 27.6 ± 12.2 U/l and 14.6 ± 5.6 U/l, and for the %CDT–TIA 5.4 ± 2.6% and 2.8 ± 1.2%, respectively. The data of the measurements from the alcoholic patients and the control population, as separated according to gender, are shown in Fig. 1. The sensitivity of the %CDT method was 63% for men and 46% for women, whereas for CDTect the corresponding values were 65 and 36%, respectively (Table 2). However, both of these methods showed approximately two-fold higher sensitivities than the %CDT–TIA method, which reacts with trisialotransferrin (32 and 25%, respectively). The corresponding specificities were 100% for men and 91% for women with %CDT and 95 and 87% with CDTect, respectively. The comparisons between the CDT methods and the conventional markers showed that CDT and GT gave the highest sensitivities in men, whereas in women the sensitivity of CDT, as assayed with either method, remained below that of GT (Table 2).

Separate analyses were subsequently made for the subgroups of alcholics with or without liver disease (Fig. 2). The presence of liver disease was found to influence the results of the CDTect assays leading to a high incidence of elevated values in the group of patients with liver pathology as compared to the corresponding group of healthy drinkers. Especially in women, the CDTect method did not differentiate between heavy drinkers and control patients, whereas the patients with liver disease had significantly higher values than those of controls ($P < 0.001$) and also higher than those of the heavy drinkers ($P < 0.05$) (Fig. 2). The %CDT method gave no such differences between these groups. In men, the alcohol misusing groups with or without liver disease were not significantly different from each other. Further analyses from the patients with CCLI and CMI data, including patients with a CCLI range of 0–14 (mean 5.1), revealed significant correlations between the CCLI index and %CDT ($r = 0.57; P < 0.01$), CDTect ($r = 0.55; P < 0.01$) and AST ($r = 0.61, P < 0.01$) results. For a CMI range of 0–7 (mean 2.4), the correlations between the CDT methods ($r = 0.27$ for %CDT; $r = 0.20$ for CDTect) or GT ($r = 0.23$) did not reach significance, whereas for AST the correlation was $r = 0.39; P < 0.05$.

The %CDT and CDTect results correlated significantly with each other ($r = 0.80; P < 0.0001; n = 107$) in the total study.
population. However, the correlation among women was weaker 
\( r = 0.57; P < 0.001 \) than that in men \( r = 0.86; P < 0.0001 \). Similarly, the correlation between \%CDT–TIA and the CDTect results in all alcoholics \( r = 0.74; P < 0.0001 \) and among women \( r = 0.54; P < 0.0001 \) was weaker than that in men \( r = 0.81; P < 0.0001 \). The self-reported alcohol consumption showed a stronger correlation with the \%CDT results \( r = 0.64; P < 0.0001 \) than with the CDTect results \( r = 0.40; P < 0.01 \).

**DISCUSSION**

Although CDT measurements have been widely used in alcohol screening programmes the data on their accuracy have remained conflicting, with sensitivities ranging from <20% to 100% (Stibler, 1991; Nyström et al., 1992; Allen et al., 1994; Bean et al., 1997; Salaspuro, 1999; Scouller et al., 2000; Arndt, 2001; Helander et al., 2001b; Sillanaukee et al., 2004).
Therefore, there is an urgent need for improved methodological standardization and comparative studies for the different methods in well-characterized clinical materials. Most of the current knowledge on CDT has been gathered based on the measurements with the CDTect method. Although the more recently introduced %CDT measurements have offered the benefit of expressing the CDT concentrations conveniently as percentages of total transferrin with similar cut-offs for both genders, the previous versions of the %CDT measurements (including %CDT–TIA) have shown poor sensitivities in clinical materials (Viitala et al., 1998).

The present data indicate that the new %CDT assay may offer several benefits for assessing excessive alcohol consumption. First, there is an improved sensitivity when compared to the previous %CDT assays. This finding appears to stem from the fact that the new assay is devoid of reactivity towards the trisialylated fraction of transferrin, which is consistent with the view that the generation of this isoform in vivo is not significantly influenced by ethanol consumption. This finding is also in line with other recent observations on transferrin desialylation as a result of alcohol misuse (Arndt et al., 2002; Legros et al., 2002, 2003). Second, the data show a higher accuracy of the new %CDT method when compared to the conventional CDTect method. This observation appears to be primarily due to the higher specificity among women and in patients with liver disease, which may be explained by previous findings indicating that the CDTect method may be sensitive to un-specific changes in serum transferrin metabolism (Sorvajärvi et al., 1996; Viitala et al., 1998; De Feo et al., 1999; DiMartini et al., 2001; Whitfield et al., 2001; Arndt and Kropf, 2002; Whitfield, 2002). Third, the analytical precision profiles of the %CDT method are also superior to those of CDTect, suggesting that this method should yield improved reproducibility and thus be more suitable for routine laboratory use.

The lack of sensitivity for CDT as a marker of alcohol misuse among women has been previously recognized in several reports (Anton and Moak, 1994; Löf et al., 1994; Reif et al., 2001). With the new %CDT method, despite this improvement, the diagnostic sensitivity in women continues to be lower than that in men. Our data further suggest that patients with mild to moderate alcoholic liver pathology, as judged by morphological indices, tend to show higher CDTect values than do alcoholics without liver pathology. These findings may be explained in the light of previous observations indicating that transferrin synthesis rate in (human) alcoholics may be accelerated in patients with early-stage liver disease but diminished in cirrhotic patients (Potter et al., 1985). In severe liver disease, CDT levels when measured with the CDTect method, are expected to decrease upon decreased transferrin protein synthesis capacity. The %CDT method in turn appears to be less sensitive to the effects of liver disease per se. The strong correlation between the %CDT results and the amount of ethanol consumed indicates that this method may reflect ethanol-induced changes in the process of desialylation more specifically than CDTect.

The majority of previous studies that have compared CDT and other markers of ethanol consumption (GT, MCV, AST) have concluded that the sensitivity of CDT exceeds that of the conventional markers (see Scouller et al., 2000). Although current data suggest high sensitivities for GT measurements, it should be noted that GT is known to lack specificity in assays of hospitalized materials where several conditions may lead to elevated values, including non-alcoholic liver diseases, diabetes, obesity or use of various drugs (Salaspuro, 1999). Despite the fact that CDT, as previously measured with the CDTect method, has also been reported to lack specificity, this may occur more rarely, including primarily conditions which affect body iron stores and thus alter serum transferrin levels (Viitala et al., 1998; De Feo et al., 1999; Arndt and Kropf, 2002; Whitfield, 2002). Because such effects are not expected to occur with the %CDT method, it should also further improve the clinical value of CDT measurements in alcohol screening programmes in hospitalized materials.

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REFERENCES


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