Carbohydrate-deficient transferrin (CDT) has been suggested as a specific marker of alcohol abuse. We designed this study to compare the conventional CDTect method (Pharmacia & Upjohn) and the new semiautomated Axis %CDT turbidimetric immunoassay (%CDT TIA) for their diagnostic performance to identify problem drinking. The sensitivities of the %CDT TIA and CDTect for correctly classifying heavy drinkers (n = 90) were 29% and 59% with the thresholds currently recommended by the manufacturers, respectively. In the control group (n = 114), which included hospitalized patients with abnormal serum transferrin concentrations, the CDTect assay gave 21 false-positive values (18%), whereas the %CDT TIA showed 100% specificity. With the cutoff limits based on the present healthy control group (mean ± 2 SD), the sensitivities of the %CDT TIA and CDTect were 61% and 86%, respectively. For men, the ROC plot area of the CDTect results in comparisons of alcohol abusers and healthy controls was significantly (P < 0.05) higher than that of the %CDT TIA results, whereas for women, there was no significant difference in this respect. The slope and intercept (with 95% confidence intervals) for linear regression between CDTect and %CDT TIA were 0.13 (0.12–0.15) and 0.16 (0.73–1.59), respectively (Sdly = 1.51, r = 0.744). CDTect results correlated positively with serum transferrin, whereas the %CDT TIA results showed a slight inverse correlation with serum transferrin (r = −0.132, P = 0.07). The data suggest that CDTect is more sensitive than %CDT TIA in detecting drinking problems. However, the %CDT TIA method yields more specificity when analyzing samples from patients with high serum transferrin concentrations.

Although several studies have shown that early medical intervention can be effective in promoting abstinence and reducing the harm associated with heavy alcohol intake, clinical identification of alcohol abuse continues to be problematic (1, 2). Because heavy alcohol consumption leads to the formation of a carbohydrate-deficient transferrin (CDT) fraction in biological fluids, serum CDT measurements have been suggested recently as a useful laboratory test for monitoring alcohol abuse (1, 2). There has also been growing interest in developing more convenient and cost-saving techniques for routine CDT determinations.

CDT consists of several subfractions with different amounts of sialylation (1, 3). To date, the most widely used method for measuring CDT in Europe has been based on ion-exchange microcolumn separation of the desialylated fraction from transferrin, followed by a transferrin radioimmunoassay for quantification (CDTect, Pharmacia & Upjohn). Transferrin isoforms have also been quantified by isoelectric focusing/immunoblotting and by HPLC (4–6), which was found recently to provide clinical sensitivity and specificity similar to those of the CDTect (7).

Recently, new approaches for CDT test kits have been introduced (8). In such modifications, the amount of CDT is reported as a relative amount to total transferrin (%CDT). Here, we report the first comparisons on the analytical characteristics and clinical value of the new semiautomated Axis %CDT TIA and the conventional CDTect method. Unlike in the CDTect method, the %CDT TIA determines both the desialylated fraction and total transferrin, using an automated turbidimetric procedure. These assays were shown to be markedly different with respect to the clinical value as alcohol markers.

**Materials and Methods**

**Patients and Controls**

Serum samples were obtained from 90 heavy drinkers with a well-documented history of continual ethanol consumption or binge-drinking, consuming 35–143 g/day.
during the 4 weeks prior to sampling. The duration of abstinence before sampling ranged from 0 to 7 days. There were 22 patients (9 women and 13 men) with biopsy-proven liver cirrhosis and 68 heavy drinkers (17 women and 51 men) with (n = 47) or without (n = 21) clinical and/or biochemical evidence of liver disease. Thus, the sample represented a wide spectrum of alcohol-induced health problems, including patients with early-phase drinking problems and patients with alcohol-induced liver cirrhosis and/or severe alcohol dependence. Among the heavy drinkers, there were 27 patients who were admitted because of ethanol intoxication (19 patients were intoxicated at the time of sampling) and 41 hospitalized patients, outpatients, or participants of voluntary health-screenings who were known to regularly consume excessive amounts of ethanol.

The healthy controls were 42 volunteers (22 women and 20 men), who did not drink or who were social drinkers (<30 g of ethanol/day on any occasion). As additional controls, we analyzed 72 hospitalized non-drinking individuals (56 women and 16 men) who were patients with non-alcohol-related liver disease (n = 15), patients with abnormalities in their iron balance (n = 32), or pregnant women (n = 25). The nondrinking subjects with expected abnormalities in their serum transferrin concentrations were chosen for this study to obtain information on the effect of serum transferrin concentration on the specificity of the assays. One patient in the group with non-alcohol-related liver disease had increased serum transferrin. Among the patients with an abnormal iron balance, there were 20 patients with increased and 12 patients with decreased concentrations of serum transferrin. Among the pregnant women, there were 14 individuals with increased serum transferrin.

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

CDT analyses

CDT was analyzed by two different methods. In the first method, CDT was measured by anion-exchange chromatography followed by radioimmunoassay using a commercially available assay kit (CDTect, Pharmacia & Upjohn) according to the instructions of the manufacturer. In this procedure, serum transferrin isoforms are separated by a microcolumn, and the eluted transferrin fraction, which is deficient in its carbohydrate moieties, is subsequently quantified by a radioimmunoassay in which the CDT in the eluate competes with 125I-labeled transferrin for antibody binding sites. The reference range in this assay is 0–20 U/L for men and 0–26 U/L for women.

In the second method, CDT was analyzed by the Axis %CDT turbidimetric immunoassay (%CDT TIA, Axis Biochemicals AS), in which serum transferrin is first saturated with Fe³⁺ before the low sialic acid transferrin (the CDT) is separated by an ion-exchange chromatography minicolumn (8). In this procedure, the CDT content of the eluate and the total transferrin content of the Fe³⁺-saturated serum sample are measured separately by turbidimetric measurement, using the same anti-transferrin antibodies. The measurements are evaluated using a calibration curve, and the %CDT value is calculated. According to the manufacturer, amounts exceeding 6% are considered increased. In the present study, a Kone Optima Analyzer (Kone Instruments) was used for the measurements. To allow comparisons with previously published material (9), measurements were also performed by the %CDT radioimmunoassay (%CDT RIA, Axis Biochemicals AS). The upper reference range limit for the %CDT RIA is 2.5%.

Whereas the %CDT TIA method measures asialylated, monosialylated, disialylated, and 50% of the trisialylated serum transferrin isoforms (8), the %CDT RIA measures transferrin variants with 0–2 sialic acid residues. In the CDTect assay, serum transferrin isoforms with pl values higher than 5.7 (mono- and asialotransferrins) and minor amounts of isotransferrin with pl values of 5.7 (disialotransferrin) are detected (10).

TRANSFERRIN ANALYSES

Serum total transferrin concentrations were measured by the Array® Protein System (Beckman Instruments), which measures nephelometrically the rate of light-scatter formation produced by an immunoprecipitation reaction with the protein. The reference range for transferrin is 1.7–3.4 g/L. The method is not affected by the degree of transferrin desialylation.

STATISTICAL METHODS

Values were expressed as mean ± SD. The raw data of the CDT methods were subjected to a logarithmic transformation to yield gaussian nonskewed distributions, and ANOVAs were performed on the transformed values followed by the Bonferroni’s multiple comparisons procedure to test for statistical differences among pairs of groups. The differences were considered statistically significant at P < 0.05.

Linear regression analysis was used for the comparison studies. Additionally, the Bland-Altman plot (11) was used to monitor the agreement between the methods. To allow such comparisons between %CDT TIA and CDTect, the ratio CDTect/serum transferrin was first computed and transformed to the scale of %CDT TIA [referred to as (CDTect/Transferrin)’].

The 95% confidence intervals for observed indices were estimated (p ± 1.96 × (SE), where SE = √p × (1 – p)/n, where n >30, and p is the specificity or sensitivity) or the exact confidence ranges were calculated according to Armitage and Berry (12), when appropriate. The ROC plot areas ± SEs and the differences between the areas were calculated according to Hanley and McNeil (13, 14).
Results

Samples of patient pool sera with low and high CDT concentrations were first analyzed to determine the precision of the CDT methods. For the low concentration, the within-run precision was 7.2% for the %CDT TIA (mean, 3.5%, n = 13) and 6.2% for the CDTect (mean, 14.7 U/L, n = 10). For the high CDT content, the corresponding CVs were 4.8% for the %CDT TIA (mean, 4.6%) and 10% for the CDTect (mean, 33.6 U/L), n = 10. The day-to-day CVs for the low CDT concentration were 7.0% for the %CDT TIA (mean, 3.9%) and 22% for the CDTect (mean, 13.1 U/L), n = 9, whereas for the high CDT content, the corresponding CVs were 8.6% for the %CDT TIA (mean, 5.2%) and 12% for the CDTect (mean, 32.6 U/L), n = 9.

The values from the different subgroups of alcoholic and nonalcoholic patients are shown in Fig. 1. The mean %CDT TIA values in the alcohol abusers and in the healthy controls were 5.4 ± 2.5% and 2.6 ± 0.8% (mean ± SD), respectively. For the CDTect, the corresponding values were 27.5 ± 13.8 U/L and 11.5 ± 3.6 U/L, respectively. The differences were significant in both of the above comparisons. There were no gender differences in the %CDT TIA or in the CDTect values among alcohol abusers, whereas among the healthy controls, the CDTect values were significantly higher (P < 0.01) in women. In the group of hospitalized nondrinking patients, the mean values for the %CDT TIA and CDTect were 3.0 ± 0.9% and 19.9 ± 8.9 U/L, respectively, which were both significantly higher than those of the healthy controls.

The slope and the intercept for linear regression between CDTect and %CDT TIA results (with 95% confidence limits, n = 192) were 0.13 (0.12–0.15) and 1.16 (0.73–1.59), respectively. The S_yx was 1.51, and the correlation coefficient was 0.744 (Fig. 2). A considerable disagreement between the %CDT TIA and the CDTect results was also noted when they were examined by plotting the %CDT TIA data and the CDTect/Transferrin ratios according to the method of Bland and Altman (11) (Fig. 3).

The %CDT TIA method showed a significantly higher (P < 0.05) correlation with CDTect than the earlier %CDT RIA method (r = 0.629, n = 112). Serum transferrin was found to correlate with the %CDT TIA results in the subgroups of alcohol abusers (r = −0.248, n = 90, P < 0.05), hospitalized nondrinking controls (r = 0.274,
n = 60, P <0.05), and healthy controls (r = -0.297, n = 42, P <0.05), although not in the total study group (r = -0.132, P = 0.07; Table 1). The correlation of %CDT RIA with serum transferrin (r = -0.302, n = 112, P <0.01) was higher (P = 0.07) than that of %CDT TIA. CDTect results, in turn, were found to correlate significantly with serum transferrin in the total study group (r = 0.239, n = 192, P <0.001). The correlation was significantly higher (P <0.05) in women (r = 0.425, n = 104, P <0.001) than in men (r = 0.098, n = 100, not significant). The correlation between serum transferrin and CDTect results was particularly strong in the subgroups of hospitalized nondrinkers and healthy controls (r = 0.774, n = 72, P <0.001 and r = 0.546, n = 42, P <0.001, respectively).

The CDTect and the %CDT TIA results were also compared by ROC analysis. The results for both genders were analyzed separately. For men, the area under the curve (mean ± SE) was significantly (P <0.05) higher for CDTect (0.990 ± 0.009) than for %CDT TIA (0.941 ± 0.025), whereas for women, no significant differences (0.923 ± 0.040 and 0.901 ± 0.045, respectively) were found on the basis of the results obtained from the healthy controls and alcohol abusers (Fig. 4). The area under the ROC curve for CDTect results for men was significantly higher (P = 0.05) than that for women, whereas for %CDT TIA, no significant gender differences were found. For the total study population of women, including the hospitalized nondrinkers with high serum transferrin concentrations, the area under the curve (mean ± SE) was significantly (P <0.05) higher for %CDT TIA (0.861 ± 0.049) than that for CDTect (0.740 ± 0.061). For the total study population of men, including the hospitalized nondrinkers with high serum transferrin concentrations, the area under the curve (mean ± SE) was significantly (P <0.05) higher for %CDT TIA (0.916 ± 0.024) than for CDTect (0.887 ± 0.030).

Table 1. Correlation coefficients (r) of %CDT TIA and CDTect results with serum transferrin concentration in alcohol abusers and healthy controls.a

<table>
<thead>
<tr>
<th>Study group</th>
<th>%CDT TIA vs total transferrin</th>
<th>CDTect vs total transferrin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>-0.132b</td>
<td>0.224c</td>
</tr>
<tr>
<td>Women</td>
<td>-0.044d</td>
<td>0.425c</td>
</tr>
<tr>
<td>Men</td>
<td>-0.123d</td>
<td>0.098e</td>
</tr>
<tr>
<td>Alcohol abusers</td>
<td>-0.248a</td>
<td>-0.032d</td>
</tr>
<tr>
<td>Hospitalized nondrinking patients</td>
<td>0.274a</td>
<td>0.774c</td>
</tr>
<tr>
<td>Healthy controls</td>
<td>-0.297a</td>
<td>0.546c</td>
</tr>
</tbody>
</table>

a The results are given for the total study group, given separately for women and men, and given for the different study subgroups.
b P <0.1.
c P <0.01.
d Not significant.
e P <0.05.
population of men, including the hospitalized nondrinkers, no significant differences (0.921 ± 0.027 and 0.899 ± 0.031, respectively) were found.

The sensitivities of the methods for detecting alcohol abuse, based on the cutoff limits recommended by the manufacturers of the present tests, were 29 ± 9% for the %CDT TIA and 59 ± 10% for the CDTest, respectively, for 100% (92–100%) specificity in the healthy controls. The %CDT RIA method yielded sensitivities of 34 ± 10%, for 100% (88–100%) specificity. For the hospitalized non-drinking patients, the %CDT TIA gave a specificity of 100% (94–100%), whereas that for the CDTest was 71 ± 10%. In the 35 nondrinking patients with increased serum transferrin, there were 21 (60%) false-positive values for the CDTest and none for the %CDT TIA. In the patients with non-alcohol-related liver disease, there was one (7%) false positive for the CDTest and none for the %CDT TIA.

There were nine alcohol abusers with increased serum transferrin. In these subjects, %CDT TIA gave six (66%) and CDTest three (33%) false-negative results when the cutoff limits given by the manufacturers were used. When the cutoff limits based on the present healthy control group (mean ± 2 SD) were used, the sensitivities of the methods were 61 ± 10% for %CDT TIA and 86 ± 7% for the CDTest, respectively. The specificities were 98% (87–100%) and 95% (77–100%), respectively. However, with these cutoff limits, the specificities of the methods toward the hospitalized nondrinkers decreased to 88 ± 8% for the %CDT TIA and 53 ± 12% for CDTest.

Discussion

The present data indicate that the new semiautomated turbidimetric CDT assay (%CDT TIA) is markedly different from the CDTest method with respect to analytical characteristics and clinical value as a blood test for alcohol abuse. Although excessive ethanol consumption has long been known to reduce the sialic acid content of transferrin, the data on the chemical nature and the relative amounts of the various transferrin isoforms that exist in alcoholics has remained controversial. Nevertheless, assays measuring various carbohydrate-deficient isoforms of transferrin have been made available. The earlier %CDT method (%CDT RIA), detected disialo- and asialo-transferrins (transferrin variants with 0–2 terminal sialic acid residues), whereas the new %CDT TIA method measures transferrin isoforms with 0–3 terminal sialic acid residues. Apparently this may also explain the different cutoff limits between these %CDT assays (6% vs 2.5%, respectively). The CDTest method, in turn, measures transferrin fractions with pI values >5.7 and minor amounts of transferrin isoforms with pI values of 5.7. The pI value of 5.7 corresponds to disialotransferrin, and the higher pI values indicate mono- and asialotransferrin (10).

Unexpectedly, the present data shows that the correlation between CDTest and %CDT improves when the %CDT measurements are carried out with the %CDT TIA, the quantitation scheme of which includes 50% of the trisialotransferrins (8), which should not be measurable in the CDTest procedure (10). However, current data indicating a lack of correlation between the %CDT TIA and CDTest results supports the view that there are considerable differences in the transferrin isoforms detected by these two assays.

Many recent reports have indicated that 15–30% of all hospital admissions in general hospitals are related to alcohol abuse (3, 8, 15–17). Because of the high prevalence of alcohol-related problems and because these problems are associated with serious health and social consequences, screening for alcohol-related problems is most
important. However, the heterogeneity of alcohol disorders complicates the development of a “gold standard” that can be used to determine the predictive validity of screening tests. Studies have shown that alcohol-related health problems arise at a consumption rate of 60 grams (men) or 40 grams (women) of alcohol per day. Therefore, it is crucial to detect excessive drinking as the underlying cause of morbidity particularly in patients who are not obvious alcoholics. In the present study, we examined patients with a wide variety of alcohol-related problems to obtain a representative sample of consecutive admissions of patients with alcohol-related problems in general hospitals. This may also explain the present finding of lower sensitivities than those seen in many previous studies contrasting more extreme groups (3, 18–20).

In view of the present ROC analysis based on comparisons of alcohol abusers and healthy controls, the overall diagnostic performance of %CDT TIA is weaker than that of CDTect in detecting alcohol abuse in men, whereas for women, there is no significant difference. It should also be noted that, for CDTect, the ROC plot area is significantly higher for men than that for women, which is in line with previous observations on CDTect assays, which indicated higher sensitivities for men (7, 21, 22). However, the ability of CDTect to make diagnostic distinctions decreases markedly when comparisons are made between alcohol abusers and controls with increased serum transferrin concentrations. In nondrinking patients who have high serum transferrin concentration, CDTect results are frequently increased. This should be noted particularly in studies concerning women with a high prevalence of iron deficiency. This phenomenon may also account for the higher mean CDTect values in women, for the need of higher cutoff values, and for the apparent lack of diagnostic accuracy for alcohol-consuming women (21, 23–26). In contrast to the CDTect method, high serum transferrin may sometimes be associated with false-negative results in the %CDT TIA, such as in patients with early phases of alcohol-induced liver disease, when transferrin synthesis is active (23). Interestingly, the group of nondrinking patients with high serum transferrin concentrations showed higher %CDT TIA values than those of healthy controls, suggesting differences in the sialylation process or glycoprotein uptake in these populations.

In contrast to the present data, a recent study by Stowell et al. (20), who used %CDT RIA for %CDT measurements, achieved (at the cutoff values given by the manufacturers) sensitivities of 78–94% for the %CDT RIA and 83–88% for CDTect, which were not different from each other. This may be because Stowell et al. (20) reported findings on alcoholics who had been actively drinking for 2 weeks before sampling in amounts ranging from 120 to 342 g of ethanol per day, whereas our patients represent problem drinkers with a mean continual alcohol consumption of 35–143 g per day and 0–7 day abstinence before sampling. However, when the patients with the most severe alcohol dependence were analyzed separately, the sensitivity of both assays in the present study material were also found to be equal and markedly higher (64–73%) than in the total population, indicating that the different CDT assays may be equally effective in detecting an advanced stage of alcoholism (data not shown). Apparently, the number of carbohydrate moieties attached to serum transferrin may also change as a function of the amount of alcohol consumed and/or as a function of severity of liver disease (1, 23). Thus, the assays may be more different from each other in the detection of early-phase drinking problems or binge-drinking than in detecting more severe alcoholism.

A summary of the practical characteristics of the %CDT TIA and CDTect assays is given in Table 2. The precision of both assays appears to suffer from the multistep procedure. In fact, the performances of %CDT TIA and the CDTect method were comparable despite the fact that the former procedure is semiautomated. The instability of the eluted samples in the %CDT TIA procedure may complicate routine work, whereas corresponding samples in the CDTect procedure are stable for >1 week after ion-exchange elution, when stored refrigerated. The %CDT and the CDTect methods appear, however, to differ with respect to several analytical characteristics and, therefore, can not readily replace each other in routine laboratory work.

This study was supported by a grant from the Finnish Foundation for Alcohol Studies. We thank Erling Sundre-Hagen and Christina Westby, Axis Biochemicals AS, Oslo, Norway, for providing the %CDT TIA kits for this study.

<table>
<thead>
<tr>
<th>%CDT TIA</th>
<th>CDTect</th>
</tr>
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<tbody>
<tr>
<td>Economical, partly automated sample processing</td>
<td>Expensive, manual sample processing</td>
</tr>
<tr>
<td>Minor interference by serum transferrin</td>
<td>Significant interference by serum transferrin</td>
</tr>
<tr>
<td>Results of patients with early-phase drinking problems are usually similar to those of nondrinkers</td>
<td>Diagnostic performance is higher than that of %CDT TIA in men (higher ROC area in comparisons of alcohol abusers and healthy controls)</td>
</tr>
<tr>
<td>Eluted samples are not stable and must be analyzed immediately</td>
<td>Eluted samples are stable and may be analyzed after more than a week (if stored refrigerated)</td>
</tr>
<tr>
<td>Immunoturbidimetric method is convenient for personnel and environment</td>
<td>Because CDT fraction and total transferrin are determined separately, risk for errors in sample handling increases</td>
</tr>
</tbody>
</table>

Table 2. Summary of the practical characteristics of the %CDT TIA and the CDTect methods as markers of alcohol abuse.
References


