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STUDY OF AXIS–SHIELD NEW %CDT IMMUNOASSAY FOR QUANTIFICATION OF CARBOHYDRATE-DEFICIENT TRANSFERRIN (CDT) IN SERUM

ANDERS HELANDER[1,2]*, MARIA FORS[2] and BIRGITTA ZAKRISSON[2]

[1]Department of Clinical Neuroscience, Karolinska Institutet and [2]Division of Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden

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Abstract — Carbohydrate-deficient transferrin (CDT) in serum has emerged as a useful biochemical marker for identifying current alcohol misuse and monitoring abstinence. This study evaluated the performance of Axis–Shield new %CDT turbidimetric immunoassay (TIA; microtitre and Cobas Mira applications). Comparison was made with the previous Axis %CDT-TIA immunoassay (reference value <5.5%) and %CDT with the high-performance liquid chromatography (HPLC) technique (reference value <1.2%). The new %CDT assay measures primarily the asialo, monosialo and disialo transferrin isoforms, and the result is expressed as the amount relative to total transferrin. The analytical precision (coefficient of variation: CV) of the %CDT assay ranged between 3.1 and 8.5% for kit controls and serum samples. The %CDT values in serum from healthy social drinkers [i.e. Alcohol Use Disorders Identification Test (AUDIT) score 1–7 for men, and 1–5 for women] were 2.07 ± 0.37% (mean ± SD, range 1.4–3.3%, n = 100) and this was not significantly different from healthy non-drinkers (1.88 ± 0.43%, 1.3–2.9%, n = 14), whereas abstinent alcohol patients showed slightly higher values (2.26 ± 0.41, 1.7–3.4, n = 25). In chronic heavy drinkers (mean daily intake 225 ± 137 g ethanol according to self-report), the %CDT values were markedly increased (6.33 ± 4.01%, 1.2–18.0%, n = 107). There was no significant difference in %CDT values between male and female social drinkers. The reference value of the new %CDT assay to be used in clinical practice was tentatively set at <3.0%, which is slightly higher than that obtained by receiver operating characteristics (ROC) curve analysis (<2.8%) and that proposed by the manufacturer in the Instruction Manual (<2.6%). The %CDT assay showed good overall correlation with %CDT-TIA (r = 0.986, P < 0.0001) and %CDT-HPLC (r = 0.978, P < 0.0001). The specificity of the %CDT assay in healthy social drinkers was 98% (%CDT-TIA 100%, %CDT-HPLC 99%) and the sensitivity for any drinking during last week in the alcohol patients was 75% (%CDT-TIA 71%, %CDT-HPLC 80%). The new Axis–Shield %CDT assay can be recommended for routine use. However, whenever a positive immunoassay test result could lead to serious consequences for the individual, it is recommended to confirm the CDT result by the HPLC technique.

INTRODUCTION

Carbohydrate-deficient transferrin (CDT) has emerged as a useful biochemical marker for identifying current alcohol misuse and monitoring abstinence (Stibler, 1991; Allen *et al*., 1994). CDT refers to an abnormal microheterogeneity of serum transferrin and is usually defined as the sum of the asialo, monosialo and disialo isoforms of transferrin. Individuals who have been drinking large amounts of alcohol (at least 50–80 g of ethanol per day) for a period of ~2 weeks or longer often show an increased level of transferrin molecules that lack one (disialo transferrin) or both (asialo transferrin) of the carbohydrate chains (Landberg *et al*., 1995; Peter *et al*., 1998; Henry *et al*., 1999). During abstention from alcohol, the serum CDT level declines with a half-life of 1.5–2 weeks (Stibler *et al*., 1991; Jeppsson *et al*., 1993) and normalization (i.e. return to within the reference interval) usually occurs within 3–4 weeks (Helander and Carlsson, 1996). The mechanism by which alcohol causes elevation of CDT has not yet been identified in detail, but apparently involves interference with the enzymes responsible for glycosyl transfer (Stibler and Borg, 1991; Lieber, 1999).

The major advantage of CDT compared with traditional laboratory tests used to indicate prolonged harmful alcohol consumption and associated liver damage, such as the mean corpuscular volume of erythrocytes (MCV), and γ-glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma or serum, is the higher specificity of CDT for alcohol exposure (Stibler, 1993; Meerkerk *et al*., 1998). However, a disadvantage is that, whereas measurement of the traditional alcohol markers has long been standardized, this is not yet the case for CDT. A multitude of analytical techniques, including various immunoassays, isoelectric focusing (IEF), high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE), and also different definitions of CDT (the transferrin isoforms covered vary between different methods, and the CDT value may be expressed as an absolute or relative amount) have been and are still in use. This is sometimes confusing and often hampers direct comparison of data between studies.

Immunological CDT assays are very convenient and time-saving when large numbers of samples are to be analysed on a routine basis. Most alcohol studies published to date have used the original CDTect radioimmunoassay which measures the sum of asialo, monosialo and part of disialo transferrin as an absolute amount (in units/l, with 1 unit of CDT equivalent to ~1 mg of transferrin). Having a very high or low total transferrin concentration might, however, lead to falsely high and falsely low CDT results when expressing the content as an absolute amount, but rarely when expressed as the ratio to total transferrin (Helander, 1999). Axis %CDT-TIA (variants of the %CDT-TIA and new %CDT immunoassays are distributed by Bio-Rad and Roche) turbidimetric immunoassay measures the sum of asialo, monosialo, disialo, and a portion (~50%) of trisialo transferrin as the relative amount to total transferrin. A specific drawback with this test is that having a very high or low relative amount of the trisialo transferrin isoform, which is found in a few per cent of the population (Helander *et al*., 2001), might yield falsely high and falsely low %CDT-TIA results, respectively.

*Author to whom correspondence should be addressed at: Alcohol Laboratory, L7:03, Karolinska Hospital, SE-171 76 Stockholm, Sweden.

The main objective in this study was to demonstrate the performance of Axis–Shield new %CDT turbidimetric immunoassay. The new %CDT assay measures primarily the asialo, monosialo and disialo transferrin isoforms, and the result is expressed as the relative amount to total transferrin. Comparison was made with the previous %CDT-TIA and %CDT by the HPLC technique (%CDT-HPLC) (Jeppsson *et al*., 1993).

MATERIALS AND METHODS

Subjects

The subject populations involved in this study are described in Table 1. Control subjects (healthy social drinkers) were recruited from among employees in a workplace in Stockholm, Sweden. During a company routine health examination, they were offered to check their alcohol habits with the Alcohol Use Disorders Identification Test (AUDIT) (Saunders *et al*., 1993) and by serum CDT measurement. Only those who screened negative on AUDIT (i.e. a score of 1–7 for men, and 1–5 for women) (Hermansson *et al*., 2000), and had no indication of excessive drinking in the alcohol consumption subscale (questions 1–3) (Seppä *et al*., 1995), were chosen for the study. Their alcohol consumption was not further recorded. Healthy non-drinkers were those employees who reported not drinking any alcohol and scored zero on AUDIT. Chronic heavy drinkers were recruited consecutively among the patients admitted for treatment of alcohol-related problems at the Centre for Dependency Disorders in Stockholm. According to a quantity–frequency questionnaire, which was filled out together with clinic staff members (Helander *et al*., 1999), most of them had been drinking regularly (mean 5.5 days/week, and 19.7 days/month) until 1–3 days prior to blood sampling (usually performed within 24 h of admission). Their mean ± SD alcohol intake was 225 ± 137 g daily (median 200 g, range 20–730 g) and there was no significant difference ($P = 0.569$) in average intake between men (mean ± SD: 227 ± 144 g, median 200 g, range 20–730 g) and women (mean ± SD: 204 ± 115 g, median 180 g, range 30–420 g). Abstinent alcohol patients were alcohol out-patients who reported zero alcohol intake in the last week prior to blood sampling. However, the vast majority of these patients had not been drinking any alcohol, or in a few cases small occasional amounts, in the last month according to self-report (mean 95 days since last intake) and negative random breath tests taken in connection with routine out-patient treatment visits at the clinic. The study was approved by the local ethics committee.

Serum samples

Blood samples were collected by venepuncture in vacutainer serum tubes. Serum was separated by centrifugation and stored at 4°C when analysed within 1 day, or at –20°C for longer periods. Samples were then thawed overnight at 4°C.

Methods

Measurements of serum %CDT with the new %CDT assay (all measurements were carried out with the microtitre application, but the analytical precision was also determined for the Cobas Mira application) and %CDT-TIA were carried out according to the manufacturer's instructions. Measurement of %CDT-HPLC was carried out according to Jeppsson *et al*. (1993), using valley-to-valley integration of the peaks representing asialo, monosialo, disialo and trisialo transferrins and baseline integration of isoforms with higher sialic acid content (mainly tetrasialo and pentasialo transferrin). Except for the performance study, single determinations were used with all methods. All CDT measurements were blinded in relation to the test results obtained with the other methods.

Statistics

The χ^2-test was used to test for normal distribution. Comparison between groups was made with *t*-test (parametric) or Wilcoxon test (non-parametric). Correlation tests were carried out with Pearson correlation coefficient (parametric) or Spearman rank correlation coefficient (non-parametric). Comparison of the overall sensitivity and specificity (threshold 60 g alcohol/day) of the CDT assays was made by receiver operating characteristics (ROC) curve analysis (Zweig and Campbell, 1993). The statistical calculations were carried out using MedCalc software.

RESULTS

The analytical precision [total coefficient of variation (CV); four determinations per day on 5 separate days for three different kit lots] of Axis–Shield new %CDT assay ranged between 3.1 and 8.5% for kit controls and serum samples. The mean CV for the 'low' (%CDT value = 2.2%) and 'high' (%CDT = 3.3%) kit %CDT controls was 6.1 and 5.4%, respectively, with the microtitre application (Cobas Mira 5.7 and 5.3%), and 7.6, 7.1 and 5.7% (Cobas Mira 7.2, 5.0 and 5.1%) for the 'low' (%CDT value = 1.7%), 'medium' (%CDT = 2.9%) and 'high' (%CDT = 4.6%) CDT serum samples, respectively.

Table 1. Description of the populations involved in the study

Patient group	*n*	Females/males (%)	Age [mean ± SD, median (range)]	Statistics and probability
Healthy social drinkers	103	35/65	44.3 ± 9.8, 45 (27–63)	$\chi^2 = 20.393$ $P = 0.118$[a]
Healthy non-drinkers	14	7/93	43.5 ± 11.6, 43 (29–64)	$\chi^2 = 0.675$ $P = 0.413$[a]
Abstinent alcohol patients	25	24/76	48.4 ± 8.6, 47 (34–65)	$\chi^2 = 2.720$ $P = 0.437$[a]
Chronic heavy drinkers	110	23/77	49.9 ± 8.6, 50 (22–72)	$\chi^2 = 12.535$ $P = 0.325$[a]

[a]Accept normality.

Table 2. Distribution of serum %CDT values (Axis–Shield new %CDT immunoassay microtitre application) in the study populations

Patient group	*n*	%CDT (%) Mean ± SD	Median	Range	2.5th–97.5th percentile	Statistics and probability
Healthy social drinkers	100	2.07 ± 0.37	2.0	1.4–3.3	1.5–2.8	$\chi^2 = 10.074$ $P = 0.524$[a]
Healthy non-drinkers	14	1.88 ± 0.43	1.7	1.3–2.9	1.3–2.9	$\chi^2 = 1.509$ $P = 0.223$[a]
Abstinent alcohol patients	25	2.26 ± 0.41	2.2	1.7–3.4	1.7–3.3	$\chi^2 = 2.411$ $P = 0.300$[a]
Chronic heavy drinkers	107	6.33 ± 4.01	5.1	1.2–18.0	2.0–16.8	$\chi^2 = 63.909$ $P < 0.0001$[b]

[a]Accept normality.
[b]Reject normality.

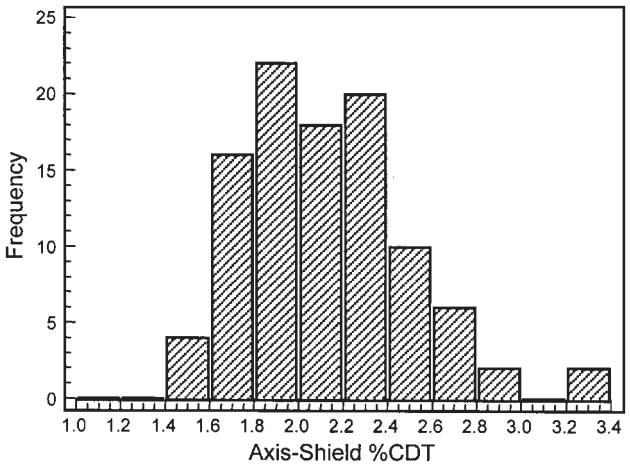

Fig. 1. Distribution of %CDT values obtained with Axis–Shield new %CDT turbidimetric immunoassay (microtitre application) in serum from healthy social drinkers.

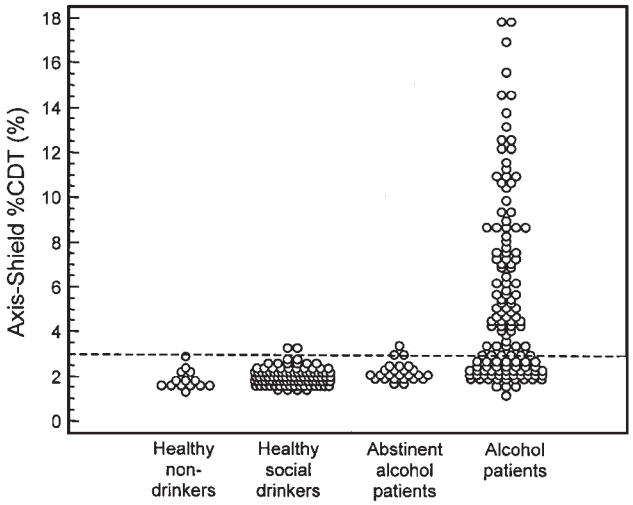

Fig. 2. Distribution of %CDT values obtained with Axis–Shield new %CDT assay (microtitre application) in healthy non-drinkers, healthy social drinkers, abstinent alcohol patients, and all actively drinking alcohol patients.
The tentative reference value (<3.0%) is indicated by a dashed line.

The new %CDT values obtained with serum samples from healthy social drinkers are given in Table 2 and Figure 1. The corresponding mean and median %CDT values in serum from healthy non-drinkers (Table 2 and Fig. 2) were lower compared with healthy social drinkers, but the differences did not reach statistical significance ($t = 1.802$, $P = 0.074$). The %CDT values in abstinent alcohol patients were significantly higher, compared with the values in social drinkers ($t = –2.225$, $P = 0.028$). In the chronic heavy drinkers, the %CDT mean and median values were both markedly increased (Table 2 and Fig. 2).

No correlation between %CDT value and age was observed for the healthy social drinkers ($r = 0.061$, $P = 0.546$), nor with their AUDIT score ($r = 0.115$, $P = 0.252$). Moreover, there was no significant ($t = 1.244$, $P = 0.216$) difference in %CDT values between healthy female (mean ± SD: 2.13 ± 0.32%, median 2.2%, range 1.6–2.8%) and male (mean ± SD: 2.04 ± 0.39%, median 2.0%, range 1.4–3.3%) social drinkers (Figure 3). No correlation between %CDT values and age was observed for the chronic heavy drinkers ($r = –0.014$, $P = 0.884$). However, the male heavy drinkers had significantly ($t = –2.687$, $P = 0.008$) higher %CDT values (mean ± SD: 6.81 ± 4.12%, median 5.7%, range 1.7–18.0%), compared with the female heavy drinkers (mean ± SD: 4.40 ± 2.83%, median 3.1%, range 1.6–13.2%).

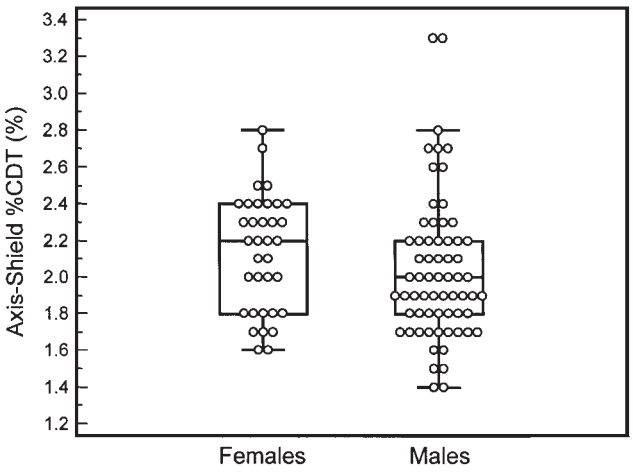

Fig. 3. Box-and-whisker plot [i.e. median, 25th and 75th percentiles, and range, with extreme values (outliers) indicated] for the distribution of %CDT values obtained with Axis–Shield new %CDT assay (microtitre application) in serum from healthy female ($n = 35$) and male ($n = 65$) social drinkers.

Table 3. %CDT values in serum from healthy social drinkers obtained with the %CDT-TIA immunological and %CDT-HPLC methods

CDT method	*n*	%CDT (%) Mean ± SD	Median	Range	2.5th–97.5th percentile	Statistics and probability
%CDT-TIA	100	3.91 ± 0.60	3.9	2.8–5.4	2.9–5.2	$\chi^2 = 7.534$ $P = 0.820$*
%CDT-HPLC	91	0.59 ± 0.24	0.60	0.14–1.27	0.17–1.11	$\chi^2 = 15.826$ $P = 0.148$[a]

[a]Accept normality.

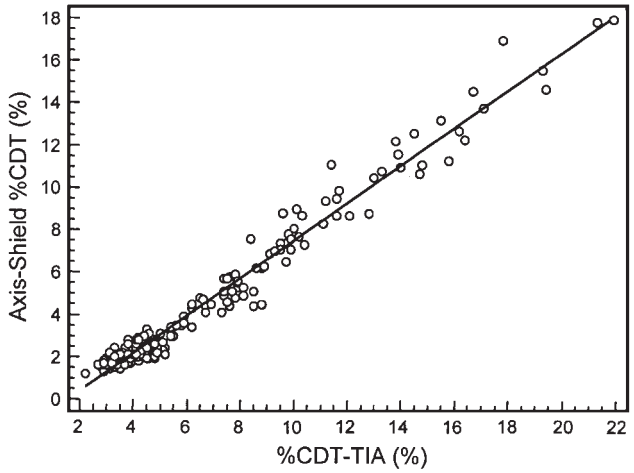

Fig. 4. Correlation between the serum %CDT values obtained with Axis–Shield new %CDT assay (microtitre application) and %CDT-TIA for all serum samples.
$r = 0.986$, $P < 0.0001$, $n = 248$. Regression equation: %CDT = –1.365 + 0.888 × %CDT-TIA.

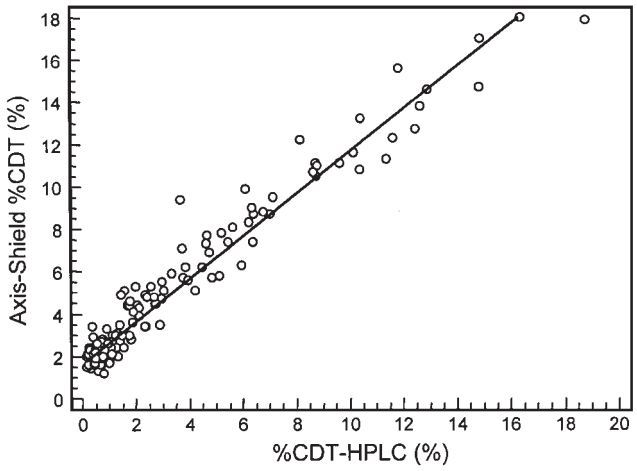

Fig. 5. Correlation between the serum %CDT values obtained with Axis–Shield new %CDT assay (microtitre application) and %CDT-HPLC methods for all serum samples.
$r = 0.978$, $P < 0.0001$, $n = 222$. Regression equation: %CDT = 1.632 + 1.002 × %CDT-HPLC.

The new %CDT values correlated significantly with average number of drinking days per week ($r = 0.492$, $P < 0.0001$) and month ($r = 0.471$, $P < 0.0001$), and with average daily alcohol intake ($r = 0.357$, $P < 0.0001$), according to self-report. A significant negative correlation was observed between %CDT value and time since last alcohol intake ($r = –0.510$, $P < 0.0001$).

Using ROC curve analysis, a cut-off of <2.8% was obtained for Axis–Shield new %CDT assay. However, based on the distribution of %CDT values in the healthy non-drinking (97.5th percentile = 2.9%) and socially drinking (97.5th percentile = 2.8%) populations (Fig. 2 and Table 2), the reference value to be used in clinical practice was tentatively set at <3.0%. This threshold secured a very high specificity of the test results in the present material.

The %CDT values in healthy social drinkers obtained with the %CDT-TIA and %CDT-HPLC methods are given in Table 3. It should be noted that the volume of some serum samples was not sufficient to be used with all three %CDT methods. The %CDT-TIA reference value originally proposed by the manufacturer was <6.0%, but the present results, like some previous publications (Bean *et al*., 1997; Viitala *et al*., 1998), indicate that this cut-off may be reduced to <5.5% or even lower (97.5th percentile in this study = 5.2%) with retained high specificity. The routine reference value for the %CDT-HPLC method is <1.2% which is in accordance with the present results (97.5th percentile = 1.11%). When all samples were included, the new %CDT assay showed good overall correlation with %CDT-TIA ($r = 0.986$, $P < 0.0001$, $n = 248$) (Fig. 4) and %CDT-HPLC values ($r = 0.978$, $P < 0.0001$, $n = 222$) (Fig. 5).

All three CDT methods showed very high specificity (98–100%) for the healthy social drinkers, as shown in Table 4. The sensitivity for ‘any drinking during last week’ in the alcohol patients (women and men combined) ranged from 65% and 71% for %CDT-TIA with <6.0% and <5.5% as cut-offs, respectively, up to 80% for %CDT-HPLC (Table 4). The sensitivity for the new %CDT assay was 75%, using the currently proposed reference value of <3.0%. With all three methods, and as demonstrated previously, the sensitivity values were markedly lower for female subjects. The sensitivity for ‘recent heavy alcohol consumption’ (defined as at least 4 drinking days during the last week) was 87% for the new %CDT assay, compared with 84% for %CDT-TIA (cut-off <5.5%). The area under the ROC curve (threshold 60 g alcohol/day) for the new %CDT assay was significantly higher compared with %CDT-TIA (Fig. 6).

In the healthy social drinkers group, %CDT-HPLC analysis identified one subject with a rare transferrin isoform type (tentatively identified as transferrin C2C3), which often causes high CDT results with the immunoassays, despite no current heavy drinking (Helander *et al*., 2001). Accordingly, the %CDT results obtained for this control patient with the new %CDT assay (3.3%) and %CDT-TIA (5.8%) were both

Table 4. Sensitivity for any drinking during last week in the alcohol patients, and specificity in healthy social drinkers, of Axis–Shield new %CDT assay in comparison with %CDT-TIA and %CDT-HPLC

CDT method	Sex	*n*	Cut-off limit	Sensitivity in drinking alcohol patients (%)	Specificity in healthy social drinkers (%)
New %CDT	F + M	107	<3.0%	75	98
	F	22		64	100
	M	85		78	98
%CDT-TIA	F + M	107	<6.0%	65	100
	F	22		45	100
	M	85		71	100
	F + M	107	<5.5%	71	100
	F	22		50	100
	M	85		76	100
%CDT-HPLC	F + M	93	<1.2%	80	99
	F	18		61	100
	M	75		84	99

elevated. Moreover, among the chronic heavy drinkers, %CDT-HPLC analysis identified two subjects with genetic transferrin BC variants, which often produce falsely low CDT results with the immunoassays (Helander *et al*., 2001). The corresponding %CDT results for these alcohol patients were 2.7% (normal) and 3.4% (elevated) with the new %CDT assay, and 5.0% (normal) and 5.5% (at cut-off) with %CDT-TIA, respectively.

DISCUSSION

The overall precision of Axis–Shield new %CDT assay was good and in the same range as reported for other CDT immunoassays, and both applications evaluated in this study (microtitre and Cobas Mira) therefore seem suitable for routine use. Based on the present results, the reference value of the %CDT assay to be used in clinical practice was tentatively set at <3.0%. This threshold gave a very high specificity (98%) in healthy social drinkers and yet a similar or improved sensitivity in the heavily drinking patients (self-reported intake ~200 g daily), compared with the %CDT-TIA assay. Especially for the female patients, the sensitivity tended to be higher with the new assay, but the difference in areas under the ROC curve was not statistically significant (new %CDT 0.929 ± 0.014, %CDT-TIA 0.870 ± 0.054: $P = 0.108$). A lower sensitivity in women is otherwise a general problem for the CDT methods, despite a similar level of alcohol intake as the men (Anton and Moak, 1994; Helander, 1999).

The proposed reference value for the new %CDT assay of <3.0% was slightly higher than that obtained by ROC curve analysis (<2.8%), and also higher than that proposed by the manufacturer in the Instruction Manual (<2.6%), which was based on a US population. By lowering the cut-off limit to <2.7% in our study, the sensitivity for recent heavy drinking among the chronic heavy drinkers was increased from 87 to 91%, but this gain in sensitivity was balanced by a reduction in specificity for the healthy social drinkers from 98 to 94%. It should be noted that the mean and median %CDT values obtained with the new %CDT assay (microtitre application) in our study were constantly 0.1–0.2% higher than those obtained in the US population (US data obtained from Axis–Shield ASA), both for healthy female and male social drinkers separately as for both groups combined. Whether this small difference resulted from methodological causes, or was due to differences between the Swedish and US control populations, remains to be elucidated.

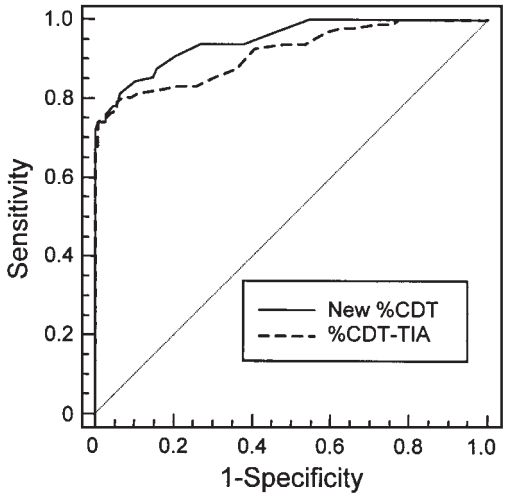

Fig. 6. Receiver operating characteristics (ROC) curve analysis for comparison of Axis–Shield new %CDT (microtitre application) and %CDT-TIA immunoassays (threshold 60 g alcohol/day, all samples included, $n = 206$).

The area under the ROC curve (± SEM) for the new %CDT assay (0.948 ± 0.017) was significantly ($P = 0.010$) higher than for %CDT-TIA (0.915 ± 0.021).

The new %CDT immunoassay showed good overall correlation with the %CDT-TIA immunoassay and the %CDT-HPLC reference method. A major advantage of the new %CDT assay, compared with %CDT-TIA, is that it focuses principally on the asialo, monosialo and disialo transferrin isoforms, whereas %CDT-TIA also measures part (~50%) of trisialo transferrin. Hence, because trisialo transferrin normally makes up ~4–6% of total transferrin (Mårtensson *et al*., 1997; Dibbelt, 2000), the %CDT values obtained with the new method were typically 2–3% lower than the corresponding %CDT-TIA values. Although including part of trisialo transferrin in the CDT measurement was originally suggested to improve diagnosis of elevated alcohol intake (Heggli *et al*.,

1996), other studies have found trisialo transferrin not to be correlated to chronic alcohol consumption (Mårtensson *et al*., 1997), nor to the amount of disialo transferrin (Dibbelt, 2000) which is considered the main CDT isoform. On the contrary, because the amount of trisialo transferrin may vary considerably from <2% to >10%, having a very high or low relative amount of trisialo transferrin might lead to falsely high and falsely low %CDT results, respectively, with the %CDT-TIA method in identification of alcohol abuse (Helander *et al*., 2001). However, this risk will be markedly reduced with the new %CDT assay.

A common weakness with the immunoassays for CDT is that, unlike most methods based on HPLC, IEF and CE, they do not distinguish single transferrin isoforms. It is therefore difficult to establish whether the test result really indicates the true amount of CDT isoforms, or may be influenced by genetic B and D variants of transferrin, even though these are rare in most populations (Kamboh and Ferrell, 1987), or other chromatographic interference such as a very high or low amount of trisialo transferrin. As also demonstrated in this study, genetic transferrin variants may be an underlying cause of incorrect determination of CDT in detection of alcohol abuse with the immunoassays (Helander *et al*., 2001). For this reason, although the immunoassays mostly yield accurate determination of CDT in clinical practice (Helander, 1999), it is recommended to verify a positive analytical result to exclude the risk for inaccurate determination due to genetic variants. This should be done at least when a positive test result might lead to serious consequences for the individual, such as in regranting of driving licenses or workplace testing (Helander and Jones, 2000; Bjerre *et al*., 2001). In accordance with the decision of a recent meeting on CDT standardization (held in Berlin, Germany, in May 2000, chaired by Dr J.-O. Jeppsson and including clinical chemists and alcohol researchers from seven European countries), an ion-exchange HPLC method may be recommended for verification because, unlike IEF, the separation method is more similar to that used in the microcolumns in the CDT immunoassay.

In summary, Axis–Shield new %CDT turbidimetric immunoassay (the microtitre and Cobas Mira applications evaluated in this study) showed good overall analytical precision. The new assay also showed good correlation with the previous %CDT-TIA immunoassay and the %CDT-HPLC reference method, with the added advantage over %CDT-TIA that it measures primarily the asialo, monosialo and disialo transferrin isoforms. The reference value to be used in clinical practice was tentatively set at <3.0%, which gave a very high specificity (98%) in healthy social drinkers. The new %CDT assay can be recommended for routine use.

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REFERENCES

Allen, J. P., Litten, R. Z., Anton, R. F. and Cross, G. M. (1994) Carbohydrate-deficient transferrin as a measure of immoderate drinking: remaining issues. *Alcoholism: Clinical and Experimental Research* **18**, 799–812.

Anton, R. F. and Moak, D. H. (1994) Carbohydrate-deficient transferrin and gamma-glutamyltransferase as markers of heavy alcohol consumption: gender differences. *Alcoholism: Clinical and Experimental Research* **18**, 747–754.

Bean, P., Liegmann, K., Løvli, T., Westby, C. and Sundrehagen, E. (1997) Semiautomated procedures for evaluation of carbohydrate-deficient transferrin in the diagnosis of alcohol abuse. *Clinical Chemistry* **43**, 983–989.

Bjerre, B., Borg, S., Helander, A., Jeppsson, J.-O., Johnsson, G. and Karlsson, G. (2001) CDT a valuable marker for over-consumption of alcohol. Principles for use in testing prior to obtaining a drivers license. *Läkartidningen* **98**, 677–683.

Dibbelt, L. (2000) Does trisialo-transferrin provide valuable information for the laboratory diagnosis of chronically increased alcohol consumption by determination of carbohydrate-deficient transferrin? *Clinical Chemistry* **46**, 1203–1205.

Heggli, D.-E., Aurebekk, A., Granum, B., Westby, C., Løvli, T. and Sundrehagen, E. (1996) Should tri-sialo-transferrin be included when calculating carbohydrate-deficient transferrin for diagnosing elevated alcohol intake? *Alcohol and Alcoholism* **31**, 381–384.

Helander, A. (1999) Absolute or relative measurement of carbohydrate-deficient transferrin in serum? Experiences with three immunological assays. *Clinical Chemistry* **45**, 131–135.

Helander, A. and Carlsson, S. (1996) Carbohydrate-deficient transferrin and gamma-glutamyl transferase levels during disulfiram therapy. *Alcoholism: Clinical and Experimental Research* **20**, 1202–1205.

Helander, A. and Jones, A. W. (2000) Application of alcohol markers in traffic medicine. *Proceedings of The 15th International Conference on Alcohol, Drugs and Traffic Safety*, pp. 1501–1506. Stockholm.

Helander, A., von Wachenfeldt, J., Hiltunen, A., Beck, O., Liljeberg, P. and Borg, S. (1999) Comparison of urinary 5-hydroxytryptophol, breath ethanol, and self-report for detection of recent alcohol use during outpatient treatment: a study on methadone patients. *Drug and Alcohol Dependence* **56**, 33–38.

Helander, A., Eriksson, G., Stibler, H. and Jeppsson, J.-O. (2001) Interference of transferrin isoform types with carbohydrate-deficient transferrin quantification in the identification of alcohol abuse. *Clinical Chemistry* **47**, 1225–1233.

Henry, H., Froehlich, F., Perret, R., Tissot, J. D., Eilers-Messerli, B., Lavanchy, D., Dionisi-Vici, C., Gonvers, J. J. and Bachmann, C. (1999) Microheterogeneity of serum glycoproteins in patients with chronic alcohol abuse compared with carbohydrate-deficient glycoprotein syndrome type I. *Clinical Chemistry* **45**, 1408–1413.

Hermansson, U., Helander, A., Huss, A., Brandt, L. and Rönnberg, S. (2000) The Alcohol Use Disorders Identification Test (AUDIT) and carbohydrate-deficient transferrin (CDT) in a routine workplace health examination. *Alcoholism: Clinical and Experimental Research* **24**, 180–187.

Jeppsson, J.-O., Kristensson, H. and Fimiani, C. (1993) Carbohydrate deficient transferrin quantitated by HPLC to determine heavy consumption of alcohol. *Clinical Chemistry* **39**, 2115–2120.

Kamboh, M. I. and Ferrell, R. E. (1987) Human transferrin polymorphism. *Human Heredity* **37**, 65–81.

Landberg, E., Påhlsson, P., Lundblad, A., Arnetorp, A. and Jeppsson, J.-O. (1995) Carbohydrate composition of serum transferrin isoforms from patients with high alcohol consumption. *Biochemical and Biophysical Research Communications* **210**, 267–274.

Lieber, C. S. (1999) Carbohydrate deficient transferrin in alcoholic liver disease: mechanisms and clinical implications. *Alcohol* **19**, 249–254.

Mårtensson, O., Härlin, A., Brandt, R., Seppä, K. and Sillanaukee, P. (1997) Transferrin isoform distribution: gender and alcohol consumption. *Alcoholism: Clinical and Experimental Research* **21**, 1710–1715.

Meerkerk, G. J., Njoo, K. H., Bongers, I. M., Trienekens, P. and van Oers, J. A. (1998) The specificity of the CDT assay in general practice: the influence of common chronic diseases and medication on the serum CDT concentration. *Alcoholism: Clinical and Experimental Research* **22**, 908–913.

Peter, J., Unverzagt, C., Engel, W.-D., Renauer, D., Seidel, C. and Hösel, W. (1998) Identification of carbohydrate deficient transferrin forms by MALDI-TOF mass spectrometry and lectin ELISA. *Biochimica et Biophysica Acta* **1380**, 93–101.

Saunders, J. B., Aasland, O. G., Babor, T. F., de la Fuente, J. R. and Grant, M. (1993) Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO Collaborative Project on Early Detection of Persons with Harmful Alcohol Consumption—II. *Addiction* **88**, 791–804.

Seppä, K., Mäkelä, R. and Sillanaukee, P. (1995) Effectiveness of the Alcohol Use Disorders Identification Test in occupational health screenings. *Alcoholism: Clinical and Experimental Research* **19**, 999–1003.

Stibler, H. (1991) Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed. *Clinical Chemistry* **37**, 2029–2037.

Stibler, H. (1993) Diagnosis of alcohol-related neurological diseases by analysis of carbohydrate-deficient transferrin in serum. *Acta Neurologica Scandinavica* **88**, 279–283.

Stibler, H. and Borg, S. (1991) Glycoprotein glycosyltransferase activities in serum in alcohol-abusing patients and healthy controls. *Scandinavian Journal of Clinical and Laboratory Investigation* **51**, 43–51.

Stibler, H., Borg, S. and Joustra, M. (1991) A modified method for the assay of carbohydrate-deficient transferrin (CDT) in serum. *Alcohol and Alcoholism* **26** (Suppl. 1), 451–454.

Viitala, K., Lahdesmäki, K. and Niemelä, O. (1998) Comparison of the Axis %CDT TIA and the CDTect method as laboratory tests of alcohol abuse. *Clinical Chemistry* **44**, 1209–1215.

Zweig, M. H. and Campbell, G. (1993) Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. *Clinical Chemistry* **39**, 561–577.