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Further arguments against including trisialo-Fe₂-transferrin in carbohydrate-deficient transferrin (CDT): a study on male alcoholics and hazardous drinkers

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Background:	We attempted to determine whether including trisialo-Fe ₂ -transferrin in carbohydrate-deficient transferrin (CDT) affects the diagnostic accuracy of CDT as a marker of chronic excessive alcohol intake.
Material/Methods:	The criterion standard tests for the diagnosis of alcoholism and alcohol intake were the Composite International Diagnostic Interview (CIDI) and the Timeline-Followback (TLFB). The study groups (alcohol intake in each of the last 4 weeks before blood sampling) were comprised of 56 controls (≤280 g/week, no alcoholism), 54 hazardous drinkers (>280 g/week, no alcoholism), 63 alcoholics (>280 g/week, alcoholism diagnosis). CDT analysis was performed with %CDTri-TIA, which includes about 50% of trisialo-Fe ₂ -transferrin in CDT, and ChronAlcoI.D, which excludes this transferrin isoform from CDT.
Results:	Depending on the cut-offs for the CDT/transferrin ratio (upper or lower limit of the test-specific borderlines) and on the patient group, the diagnostic sensitivity was 28.1%–72.3% for %CDTri-TIA, as opposed to 50.0%–82.5% for ChronAlcoI.D. The diagnostic accuracy was 62.8%–78.5% for %CDTri-TIA and 71.8%–86.6% for ChronAlcoI.D. The latter test consistently showed higher diagnostic sensitivity and accuracy than %CDTri-TIA. The diagnostic specificity was 85.7%–98.2% for %CDTri-TIA and 91.1%–92.2% for ChronAlcoI.D. The areas under the ROC curve were 0.810–0.885 for %CDTri-TIA and 0.867%–0.896 for ChronAlcoI.D.
Conclusion:	The present study and data from the literature indicate that including parts of trisialo-Fe ₂ -transferrin by the %CDTri-TIA test significantly reduces the diagnostic sensitivity and thus accuracy of CDT as a marker of chronic excessive alcohol use.
key words:	alcohol • blood • carbohydrate-deficient transferrin • CDT • Receiver-Operator-Characteristics curve
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BACKGROUND

Carbohydrate-deficient transferrin (CDT) is widely used for laboratory diagnosis of chronic alcohol abuse [1]. A review of preanalysis, analysis and interpretation of CDT has been published recently [2]. According to the common and widely accepted definition of CDT, asialo-Fe₂-transferrin, monosialo-Fe₂-transferrin and disialo-Fe₂-transferrin are collectively referred to as CDT [1,2]. However, there has been controversy as to whether there is a diagnostic benefit from including (parts of) trisialo-Fe₂-transferrin in CDT [3-7]. This dispute was brought to a head recently in a published critique by Tagliaro et al. [8] of a paper by Wuyts et al. [9], which prompted a response by Delanghe et al. [10].

In assessing so-called 'trisialo-tests', CDT tests have most often been compared that not only exhibit different analytical specificity (including or excluding trisialo-Fe₂-transferrin), but also report different CDT units (absolute CDT concentrations vs. CDT/transferrin ratios). Thus the effects of using absolute or relative CDT concentrations may overlap with the effects of including or excluding trisialo-Fe₂-transferrin from CDT.

The aim of our study was to assess the effects of including trisialo-Fe₂-transferrin in CDT on the diagnostic accuracy of CDT as a marker of chronic alcohol abuse. For this purpose, we calculated the diagnostic sensitivity, specificity, and accuracy, as well as the area falling under the Receiver-Operator-Characteristics (ROC) curves, of 2 commercially available CDT tests: %CDTri-TIA (Axis, Norway) which includes about 50% of trisialo-Fe₂-transferrin in CDT, and ChronAlcoI.D. (Sangui BioTech Inc, USA), which excludes this isoform from CDT.

MATERIAL AND METHODS

The ethical committee of the St. Lucas Andreas Hospital approved the study protocol. All study participants gave informed consent, and the research was carried out in compliance with the provisions of the Helsinki Declaration of 1975, as revised in 1996.

Spectrum bias was avoided by assessing consecutive patients. To avoid reviewer bias, data collection on alcoholism diagnosis and alcohol intake was blinded to the results of the chemical analysis and vice versa. The ChronAlcoI.D. test [11] was performed without knowledge of the CDT results obtained by the %CDTri-TIA test and vice versa. Verification bias was avoided by applying the criterion standard tests to all subjects. The guidelines for studies of the diagnostic accuracy of diagnostic tests [12] were observed.

All subjects were male and able to communicate. Three study groups were formed: controls, hazardous drinkers, and alcoholics. The lower limit of hazardous alcohol use was used as the upper limit of harmless alcohol consumption. This limit, >280 g ethanol/week, is

defined as the level of persistent alcohol consumption being likely to result in adverse health effects [13,14].

56 controls were recruited from consecutive ambulatory psychiatric patients. Only patients with an alcohol consumption of ≤ 280 g ethanol/week in each of the last 4 weeks before blood sampling who had no prior diagnosis of Alcohol Use Disorder (AUD, 'alcoholism') were included in the control group. The mean alcohol consumption in the control group was 51 g ethanol/week (median 14 g/week). The mean age was 45 years (median 44 years).

63 alcoholics were recruited from treatment facilities (consecutively admitted to a detoxification ward or consecutively visiting an ambulatory alcoholism treatment center). Alcoholism in this study group was defined as AUD as defined in accordance with ICD-10 (International Classification of Mental or Behavioural Disorders) or DSM-IV (Statistical Manual of Mental Disorders, 4th edition) [15,16]. Only patients with a hazardous alcohol intake of >280 g ethanol/week in each of the last four weeks and a positive AUD diagnosis were included in the alcoholics group. The mean alcohol consumption in the alcoholics group was 1263 g ethanol/week (median 1060 g/week). The mean age was 44 years (median 44 years).

54 hazardous drinkers were recruited from a population of wine tasters. Only subjects with a hazardous alcohol use of >280 g alcohol/week in each of the last 4 weeks and a negative AUD diagnosis were included in the hazardous drinker group. The mean alcohol consumption in this group was 520 g ethanol/week (median 400 g/week). The mean age was 50 years (median 50 years).

Since criteria for the diagnostic accuracy of a laboratory parameter or an analytical procedure are almost always based on the correct identification of false positives and false negatives, we used widely accepted, reliable and validated diagnostic instruments as criterion standard tests for assessing alcoholism and chronic hazardous alcohol intake. Alcohol intake was assessed by Timeline-Followback (TLFB) [17]. The TLFB is a comprehensive retrospective self-report survey that allows the collection of reliable information up to 12 months before the interview date [18]. Alcohol use disorder (AUD) was assessed by means of the Composite International Diagnostic Interview (CIDI) 2.1: alcohol section. The CIDI is a valid and reliable, fully structured diagnostic interview, and enables diagnosis to be computer-generated according to ICD-10 and DSM-IV-criteria [15,16,19,20].

For serum samples, blood was collected into evacuated sterile gel-tubes (Becton-Dickinson, vacutainer). Serum was obtained by centrifugation at 2000g and 4°C for 10 min. Serum aliquots were stored at -20°C. Samples were thawed only once for assaying. To check the delay between CDT analysis by %CDTri-TIA and ChronAlcoI.D. (about 6 months for about half of the serum samples), %CDTri-TIA was repeated on a subset of 20 samples at the time of ChronAlcoI.D. analysis. Comparison

of the %CDTri-TIA CDT values from the first and the second measurement by t-test for paired samples ($p = 0.553$) and regression analysis by Passing and Bablok [21] yielded no significant differences. This confirms that CDT concentration in refrigerated serum samples remains stable for several months, as reported by other authors [22–24].

%CDTri-TIA was provided by AXIS Biochemicals ASA (Oslo, Norway), distributed by Orange Medical, The Netherlands. ChronAlcoI.D. was provided by Sangui BioTech, Inc. (Santa Ana, U.S.A.), distributed by Biodiagnosics (Kiel, Germany).

CDT/transferrin ratios were determined in Amsterdam by the %CDTri-TIA assay in accordance with the manufacturer's instructions. The test is based on anion-exchange chromatography for the fractionation of CDT isoforms (including 50% of trisialo-Fe₂-transferrin) and non-CDT isoforms, followed by a turbidimetric immunoassay (using transferrin antibodies) on microtiter plates (Array nephelometer, Beckman, and Array Flexisoft program). The results were reported as CDT/transferrin ratios in % of total transferrin. All measurements were done in duplicate, and the mean was calculated.

A study establishing cut-offs for serum CDT/transferrin ratios obtained by the %CDTri-TIA assay and indicating chronic hazardous alcohol use was not available from the literature. Therefore, the borderline of 5–6% CDT suggested by the manufacturer [25] was used.

The precision and accuracy of the assay were regularly checked by internal and external quality control material. In each run at least two controls were analyzed (normal and pathological CDT value, delivered with the %CDTri-TIA). The CVs for the low and high controls were <12.0% and <5.3% in the appropriate control period. The laboratory participated regularly in external quality control programs, with success.

Serum concentrations of CDT and CDT/transferrin ratios were determined in Ingelheim by the ChronAlco I. D. assay in accordance with the manufacturer's instructions. The test is based on anion-exchange chromatography for fractionation of CDT isoforms (excluding trisialo-Fe₂-transferrin) and non-CDT isoforms, followed by a turbidimetric immunoassay (using transferrin antibodies) on microtiter plates (Dynatec MR 5000 reader, Dynex Revelation 3.2 software; Dynex Technologies, Denkendorf, Germany). The results were reported as both CDT/transferrin ratios and CDT concentrations [11,26]. The cut-off used as decision criterion was 2.5–2.7% for the CDT/transferrin ratio [26]. Quality control was performed according to the recommendations found in the Guidelines of the German Federal Medical Association. Precision and accuracy of CDT measurement were checked by internal and external quality control material. In each analysis series, two control samples with normal and pathological CDT/transferrin ratio (CDT control set, Sangui BioTech Inc, USA) were placed at the beginning and at the end

of the run. The CVs for the low and high controls were <7.5% and <7.9% in the appropriate quality control period. A detailed study of the intra- and inter-assay variance of the ChronAlcoI.D. assay is given elsewhere in the literature [26]. The laboratory participated regularly in external quality control programs, with success.

Statistical analysis

Statistical analysis was done by use of the Analyse-it for Microsoft Excel program (Analyse-it Software, Ltd. Leeds, United Kingdom). Differences in the diagnosis of chronic alcohol abuse (Yes-diagnosis vs. No-diagnosis) of alcoholics and hazardous drinkers between the %CDTri-TIA and ChronAlcoI.D. were checked for significance by the McNemars test [27]. The two-tailed McNemars test was used to test the alternative hypothesis: changes of Yes-diagnosis by %CDTri-TIA to No-diagnosis by ChronAlcoI.D. \neq changes of No-diagnosis by %CDTri-TIA to Yes-diagnosis by ChronAlcoI.D. (or in other words: %CDTri-TIA and ChronAlcoI.D. have significantly different diagnostic accuracy). The one-tailed McNemars test was used to test the alternative hypothesis: changes of Yes-diagnosis by %CDTri-TIA to No-diagnosis by ChronAlcoI.D. changes of No-diagnosis by %CDTri-TIA to Yes-diagnosis by ChronAlcoI.D. (or in other words: %CDTri-TIA, including trisialo-Fe₂-transferrin, has a significantly reduced diagnostic accuracy).

RESULTS

Table 1 and 2 show the values for diagnostic sensitivity, specificity, and accuracy, as well as the areas under the ROC curves for %CDTri-TIA (which includes about 50% of trisialo-Fe₂-transferrin in CDT) and for ChronAlcoI.D. (which excludes this transferrin isoform from CDT) in both groups (alcoholics and hazardous drinkers). Since borderlines for CDT/transferrin ratios indicating chronic alcohol abuse are used by the two tests (5%–6% and 2.5%–2.7%) [25,26], the diagnostic criteria were investigated for each test and patient group at the test-specific lower (5% and 2.5%) and upper (6% and 2.7%) limits of the borderlines (Table 1 and 2).

The diagnostic specificity of the serum CDT/transferrin ratio was measured in a group of 56 psychiatric patients (controls) without alcoholism diagnosis and with an alcohol intake of ≤ 280 g/week during each of the last 4 weeks (see Material and methods). Using the lower limits of the test-specific borderlines (5.0% and 2.5% CDT), %CDTri-TIA showed 9 and ChronAlcoI.D. 6 false positives. Of these, 4 controls were tested false positive by both assays. Using the upper limits of the test-specific borderlines (6.0% and 2.7% CDT), 2 controls were tested false positive by %CDTri-TIA, and the same two subjects, plus 2 others from the control group (altogether 4, who were also false positive at the lower cut-off) were returned false positive by ChronAlcoI.D. As a result, the diagnostic specificity of the %CDTri-TIA varied between 85.7% (lower cut-off) and 98.2% (upper cut-off), and between 91.1% (lower cut-off) and 92.9% (upper cut-off) for the ChronAlcoI.D. assay (Tables 1 and 2).

Table 1. Alcoholics. Parameters of diagnostic performance of CDT (56 controls, 63 alcoholics) at the lower and upper limits of the test-specific borderlines indicating chronic alcohol abuse.

	ChronAlcol.D. (excluding trisialotransferrin)		%CDTri-TIA (including trisialotransferrin)	
	lower cut-off 2.5%	upper cut-off 2.7%	lower cut-off 5.0%	upper cut-off 6.0%
Sensitivity (95% CI)	82.5% (70.9–90.9)%	73.0% (60.3–83.4)%	72.3% (59.8–82.7)%	53.8% (41.0–66.3)%
Specificity (95% CI)	91.1% (80.4–97.0)%	92.9% (82.7–98.0)%	85.7% (73.8–93.6)%	98.2% (90.4–100)%
Accuracy	86.6%	82.4%	78.5%	74.4%
(p values are given in table 3)				
ROC area (95% CI)	0.896 (0.836–0.956)		0.885 (0.827–0.944)	
p=0.638				

Abbreviations: CI – confidence interval

Table 2. Hazardous drinkers. Parameters of diagnostic performance of CDT (56 controls, 54 hazardous drinkers) at the lower and upper limits of the test-specific borderlines indicating chronic alcohol abuse.

	ChronAlcol.D. (excluding trisialotransferrin)		%CDTri-TIA (including trisialotransferrin)	
	lower cut-off 2.5%	upper cut-off 2.7%	lower cut-off 5.0%	upper cut-off 6.0%
Sensitivity (95% CI)	55.6% (41.4–69.1)%	50.0% (36.1–63.9)%	50.9% (37.3–64.4)%	28.1% (17.0–41.5)%
Specificity (95% CI)	91.1% (80.4–97.0)%	92.9% (82.7–98.0)%	85.7% (73.8–93.6)%	98.2% (90.4–100)%
Accuracy	73.6%	71.8%	68.1%	62.8%
(p values are given in table 3)				
ROC area (95% CI)	0.867 (0.800–0.934)		0.810 (0.729–0.890)	
p=0.0497				

Abbreviations: CI – confidence interval

Table 3. McNemars test for checking the statistical significance of differences in diagnostic accuracy between ChronAlcol.D. and %CDTri-TIA (alcoholism and hazardous drinker diagnosis: established by questionnaires as criterion standard tests) at the lower (2.5% for ChronAlcol.D. and 5.0% for %CDTri-TIA) and upper (2.7% vs. 6.0%) limits of the CDT borderline.

a. Alcoholics, lower limit of test-specific CDT borderlines						
%CDTri-TIA	ChronAlcol.D.			delta	2-tailed	1-tailed
	Yes	No	Total			
Yes	44	3	47		Yes to No ≠ No to Yes	Yes to No ≤ No to Yes
No	9	7	16	95% CI	–0.170 to 0.027	–1.000 to 0.010
Total	53	10	63	p	0.1460	0.0730
b. Alcoholics, upper limit of test-specific CDT borderlines						
%CDTri-TIA	ChronAlcol.D.			delta	2-tailed	1-tailed
	Yes	No	Total			
Yes	34	1	35		Yes to No ≠ No to Yes	Yes to No ≤ No to Yes
No	16	12	28	95% CI	–0.269 to –0.115	–1.000 to –0.135
Total	50	13	63	p	0.0003	0.0001
c. Hazardous drinkers, lower limit of test-specific CDT borderlines						
%CDTri-TIA	ChronAlcol.D.			delta	2-tailed	1-tailed
	Yes	No	Total			
Yes	27	3	30		Yes to No ≠ No to Yes	Yes to No ≤ No to Yes
No	11	13	24	95% CI	–0.235 to 0.004	–1.000 to –0.018
Total	38	16	54	p	0.0574	0.0287
d. Hazardous drinkers, upper limit of test-specific CDT borderlines						
%CDTri-TIA	ChronAlcol.D.			delta	2-tailed	1-tailed
	Yes	No	Total			
Yes	15	3	18		Yes to No ≠ No to Yes	Yes to No ≤ No to Yes
No	12	24	36	95% CI	–0.254 to –0.011	–1.000 to –0.033
Total	27	27	54	p	0.0352	0.0176

Abbreviations: delta – difference between proportions; CI – confidence interval; p<0.05 indicates significant difference

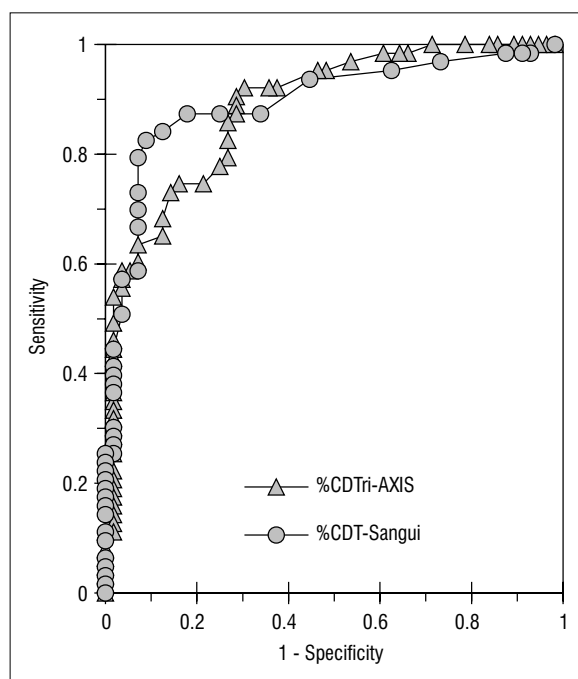


Figure 1. Receiver-Operating-Characteristic (ROC) curves for CDT as a marker of chronic alcohol abuse in 63 alcoholics and 56 healthy controls. %CDTri-TIA: including some trisialo-Fe₂-transferrin in CDT. ChronAlcoI.D.: excluding this isoform from CDT.

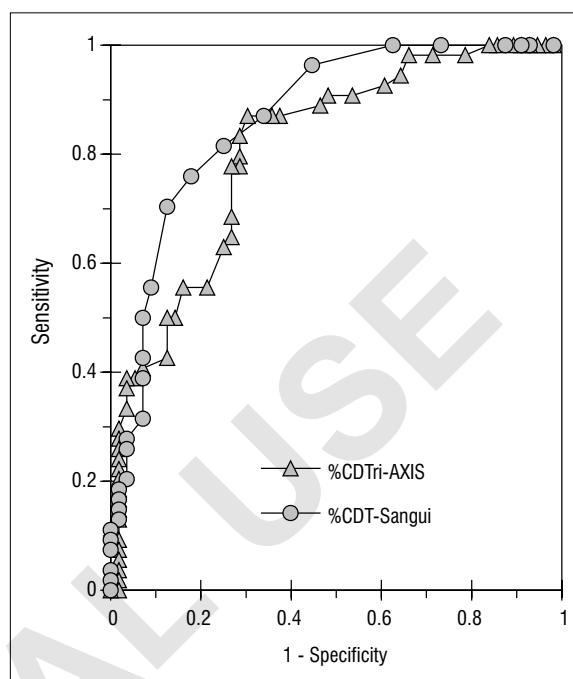


Figure 2. Receiver-Operating-Characteristic (ROC) curves for CDT as a marker of chronic alcohol abuse in 54 hazardous drinkers and 56 healthy controls. %CDTri-TIA: including some trisialo-Fe₂-transferrin in CDT. ChronAlcoI.D.: excluding this isoform from CDT.

Diagnostic sensitivity in alcoholics was examined in a group of 63 subjects with chronic alcohol intake of >280 g ethanol/week during each of the last 4 weeks before blood sample collection, previously diagnosed as alcoholic (see Material and methods). Using the lower limits of the test-specific borderlines, 16 patients were tested false negative by CDTri-TIA, and 10 by ChronAlcoI.D. (Table 3a). The number of false negatives increased for the two tests when using the upper limits of the test-specific borderlines (6.0% CDT for CDTri-TIA and 2.7% CDT for ChronAlcoI.D.). %CDTri-TIA failed to identify 28 of 63 alcoholics, while ChronAlcoI.D. failed for 13 alcoholics (Table 3b). Consequently, the diagnostic sensitivity was higher for both tests at the lower limit of the CDT borderlines, and in each case higher for the ChronAlcoI.D. (Table 1).

Diagnostic sensitivity in hazardous drinkers was examined in a group of 54 subjects with chronic alcohol intake of >280 g ethanol/week during each of the last 4 weeks before blood sample collection, but no diagnosis of alcoholism (see Material and methods). Using the lower limit of the test-specific borderlines (5.0% CDT for CDTri-TIA and 2.5% CDT for ChronAlcoI.D.), 24 patients were tested false negative by the %CDTri-TIA and 16 by the ChronAlcoI.D. (Table 3c). The number of false negatives increased for the two tests when using the upper limits of the test-specific borderlines (6.0% CDT for CDTri-TIA and 2.7% CDT for ChronAlcoI.D.). %CDTri-TIA failed to identify 36 of 54 hazardous drinkers, while ChronAlcoI.D. failed for 27 hazardous drinkers (Table 3d). Consequently, the diagnostic sensi-

tivity was higher for both tests at the lower limit of the CDT borderlines (most distinct for the %CDTri-TIA), and in each case higher for the ChronAlcoI.D. (Table 2).

In terms of diagnostic accuracy and area under the ROC curve in alcoholics and hazardous drinkers, both assays had higher diagnostic accuracy at the lower limit of the test-specific borderlines. ChronAlcoI.D. (which excludes trisialo-Fe₂-transferrin from CDT) consistently showed a higher diagnostic accuracy in comparison with %CDTri-TIA (including about 50% trisialo-Fe₂-transferrin in CDT) (Tables 1 and 2). Using the 2-tailed McNemars test, this difference was significant ($p < 0.05$) for alcoholics and hazardous drinkers only at the upper limit of the test-specific borderlines (Table 3a-d). Using the 1-tailed McNemars test, the diagnostic accuracy of the ChronAlcoI.D. was significantly higher for alcoholics at the upper test-specific borderline, and for hazardous drinkers at both the lower and upper test-specific borderlines (Table 3a-d). The differences in diagnostic sensitivity and accuracy were matched by analogous differences in the corresponding areas under the ROC curves. However, the difference in the area under the ROC curve was not statistically significant for the ChronAlcoI.D. and %CDTri-TIA when assessing alcoholics, though there was a significant difference in favor of the ChronAlcoI.D. when testing hazardous drinkers (Tables 1 and 2, Figs 1 and 2).

DISCUSSION

There is controversy as to whether or not trisialo-Fe₂-transferrin occurs in elevated concentrations after chronic alcohol abuse, and whether including (parts of) trisialo-Fe₂-transferrin improves the diagnostic accuracy of CDT [3–10]. On the one hand, this is scientifically interesting, e.g. for assessing the mechanisms that cause increased CDT concentrations after chronic hazardous alcohol intake. On the other hand, the availability of CDT tests with different analytical specificity further complicates the comparison of values and parameters of the diagnostic efficiency of CDT as a marker of chronic hazardous alcohol use [2]. The protocol used in the present study was designed to preclude overlapping of the effects of including or excluding trisialo-Fe₂-transferrin and the use of absolute serum CDT concentrations or CDT/transferrin ratios on the diagnostic performance of CDT. Thus, two commercial CDT tests that measure relative CDT concentrations were used.

Using the lower limits of the test-specific borderlines significantly improved the diagnostic sensitivity of both tests in both patient groups (Table 1 and 2). At the same time, the diagnostic specificity was reduced (although the change was not statistically significant) from 98.2% to 85.7% for the %CDTri-TIA, while it was only slightly affected in the ChronAlcoI.D. This may suggest that the cut-off values established and used for the ChronAlcoI.D. [26] were more accurate than those suggested for the %CDTri-TIA [25]. In establishing cut-off values for the ChronAlcoI.D. assay, the 95th percentile was 2.5% CDT. Given the analytical imprecision of ca. 10% and the social consequences of false positive diagnosis of alcohol abuse, it has been suggested that a borderline of 2.5%–2.7% should be used as a decision criterion [26]. In our study, the use of this borderline did not significantly increase the diagnostic specificity, as we expected [26], but it reduced the diagnostic sensitivity by 9.5% in the group of alcoholics (Table 1) and by 5.6% in the hazardous drinkers group (Table 2). Nevertheless, using a borderline for serum CDT may be useful when specificity is of primary importance, e.g. in traffic medicine cases or forensic medicine.

Our data show that the inclusion of (parts of) trisialo-Fe₂-transferrin in CDT by the %CDTri-TIA assay reduces the diagnostic accuracy of CDT in alcoholics and hazardous drinkers. In fact, ChronAlcoI.D. (which excludes this isoform from CDT) showed significantly higher diagnostic accuracy in alcoholics and hazardous drinkers as compared to the results obtained from the %CDTri-TIA. This was mainly due to higher diagnostic sensitivity (Table 1–3).

These findings are in accordance with an earlier study by Viitala et al. [28], comparing two commercial CDT assays: %CDT-TIA (identical with %CDTri-TIA, including about 50% of trisialo-Fe₂-transferrin, measuring CDT/transferrin ratios) and CDTECT (excluding trisialo-Fe₂-transferrin from CDT and measuring absolute CDT concentrations). In this study [28], %CDT-TIA showed a lower overall diagnostic accuracy for detecting alcohol

abuse in men, which was mainly due to diminished diagnostic sensitivity. In our study, absolute CDT concentrations (data not shown) had an overall weaker diagnostic accuracy in comparison with CDT/transferrin ratios. It follows from this that the findings by Viitala et al. [28] cannot be explained solely in terms of the different units used by the two tests (CDT/transferrin ratio by the %CDT-TIA and absolute CDT concentrations by the CDTECT). As we have previously shown, the analytical specificity is identical for the CDTECT [29] and the ChronAlcoI.D. [11] assay. Thus, CDTECT and ChronAlcoI.D. are very similar, since both, in contrast to %CDTri-TIA (also marketed under the trade name %CDT-TIA), exclude trisialo-Fe₂-transferrin from CDT. It is most likely (although not completely proven by our data, see below), that the diminished diagnostic accuracy of so-called 'trisialo-tests' is indeed due to the inclusion of trisialo-Fe₂-transferrin in CDT. This hypothesis is supported by the findings of Mårtensson et al. [5] and Dibbelt [30]. Mårtensson et al. [5] found no increase of trisialo-Fe₂-transferrin concentration after chronic alcohol consumption, but significant increases for asialo-Fe₂-transferrin (219% of normal serum concentration), monisialo-Fe₂-transferrin (28% increase) and disialo-Fe₂-transferrin (148% increase). Using HPLC, Dibbelt [30] was able to detect unambiguously increased concentrations of asialo- and disialo-Fe₂-transferrin in serum samples with pathological CDT/transferrin ratio, which were in general not accompanied by high trisialo-Fe₂-transferrin concentrations. Indeed, the trisialo-Fe₂-transferrin concentrations were almost identical in serum samples with normal and pathological CDT/transferrin ratio [30]. When the relative CDT concentrations obtained from ChronAlcoI.D. (excluding trisialo-Fe₂-transferrin from CDT), from %CDT TIA (including some parts of trisialo-Fe₂-transferrin in CDT; this is not the AXIS %CDT-TIA or %CDTri-TIA test, but rather a previous Sangui BioTech Inc. product) and from HPLC (excluding trisialo-Fe₂-transferrin from CDT; in-house method) were classified as either normal or pathological, 22% discrepancies were found between %CDT TIA and HPLC, but only 9% between ChronAlcoI.D. and HPLC [30].

There are strong arguments that the increased diagnostic sensitivity of the ChronAlcoI.D. obtained by excluding trisialo-Fe₂-transferrin from CDT is not achieved at the expense of diagnostic specificity: of 72 serum samples with CDT values of 2.7%–4.0% obtained by ChronAlcoI.D., 69 showed isoelectric focusing transferrin isoform patterns, which are typical for alcohol abuse [31]. (Isoelectric focusing is usually used as the reference method of transferrin isoform, i.e. CDT, analysis [32].)

If trisialo-Fe₂-transferrin increases at all after chronic alcohol abuse, its proportional change may be less than that for the other CDT isoforms. If this is true, the comparably less affected but large amounts of trisialo-Fe₂-transferrin (although only about 50% is included in CDT by the %CDTri-TIA) may mask the alcohol-induced increases in the CDT isoforms, and thus lower diagnostic sensitivity. Other variables, such as different

antibodies, dilution factors etc. may account (at least in part) for the lower diagnostic accuracy of the %CDTri-TIA in our study. It is unlikely, however, that the inclusion of trisialo-Fe₂-transferrin in CDT had no effect at all on the diagnostic accuracy of CDT as a marker of chronic alcohol abuse. Indeed, the introduction of so-called 'trisialo-tests' was aimed to improve the diagnostic accuracy of CDT. Heggli et al. reported a benefit from including this isoform [7], which is in contrast to our data and those reported by Dibbelt [30] and Lipkowski et al. [3]. Dibbelt concludes: 'Because trisialo-transferrin is obviously of no diagnostic value, I strongly recommend not including this isotransferrin in the CDT fraction measured for laboratory diagnosis of alcoholism.'

The area under the ROC curve has been suggested to be an effective means for studying the diagnostic utility of different tests for a particular disease [33]. Accordingly, we calculated the ROC curves for our study groups (Tables 1 and 2; Figs. 1 and 2). CDT obtained from the ChronAlcoI.D. had greater areas under the ROC curve (alcoholics and hazardous drinkers). Using the 2-tailed McNemars test (Tables 1 and 2), a significant difference was obtained only for hazardous drinkers. It might be argued that %CDTri-TIA (and thus the inclusion of parts of trisialo-Fe₂-transferrin in CDT) is thus equivalent to ChronAlcoI.D. in diagnosing chronic alcohol abuse, at least in alcoholics. Our argument against this conclusion runs as follows:

ROC curves were originally established to characterize the performance of electronic devices, e.g. for characterizing the ability of a receiver ('diagnostic test') to detect a signal ('disease state of a patient'). ROC curves consider extreme experimental as well as routine situations and cover the whole range of statistical events. Thus, ROC curves in clinical chemistry are especially useful for assessing the optimum trade-off between false positives and false negatives [33]. However, two ROC curves can have identical areas and may even intersect at several points, but their shape, and thus their ability to discriminate between healthy and sick subjects at the test-specific cut-off, can be quite different [34]. According to Kazmierczak [34], a test with a smaller ROC curve area can be more powerful. This is confirmed by our data, at least for alcoholics. The %CDTri-TIA test yields some ROC curve area at very low levels of diagnostic specificity, where clinical decisions will not be made (Fig. 1). This is not relevant for routine use of CDT. We conclude that the diagnostic accuracy of the test that excludes trisialo-Fe₂-transferrin from CDT (ChronAlcoI.D.) is superior to that of the test which includes parts of trisialo-Fe₂-transferrin in CDT (%CDTri-TIA) (Tables 1–3, Figs. 1 and 2), despite statistically equivalent ROC curve areas for alcoholics (Table 1, Fig. 1).

Our study was designed to evaluate whether the diagnostic accuracy of CDT is improved by including (parts of) trisialo-Fe₂-transferrin in CDT (as claimed by the manufacturer of the %CDTri-TIA assay). Therefore, statistical analysis can also be done by the 1-tailed McNemars test. This clearly shows that there was no

diagnostic benefit from using the 'trisialo-test' %CDTri-TIA in our study. The p-values were, as expected, lower (closer to the significance level of 0.05), and one additional significant result was obtained (lower limit of the borderline, hazardous drinkers, Table 3c) in comparison with the data from the 2-tailed McNemar test.

Our data clearly support the critique by Tagliaro et al. [8] of capillary electrophoresis for CDT determination as described by Wuyts et al. [9] and defended by Delanghe et al. [10]. Tagliaro et al. [8] conclude: 'Analytical selectivity and diagnostic effectiveness should not be sacrificed in favour of operative simplicity, throughput, and automation.' We would like to add, in reply to Delanghe et al. [10], this is true regardless of whether or not an unspecific test has been approved by the FDA or not.

CONCLUSION

Including trisialo-Fe₂-transferrin in CDT reduces the diagnostic sensitivity of CDT and does not improve the diagnostic accuracy of CDT as a marker of chronic alcohol abuse in men. CDT has been a valuable marker of chronic alcohol abuse in alcoholics. Its diagnostic sensitivity has been limited in hazardous drinkers.

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