Trisialo-Fe_2-Transferrin Does Not Improve the Diagnostic Accuracy of Carbohydrate-deficient Transferrin as a Marker of Chronic Excessive Alcohol Intake

Trisialo-Fe_2-Transferrin erhöht nicht die diagnostische Richtigkeit des Kohlenhydrat-defizienten Transferrins (CDT) als Marker chronisch exzessiver Alkoholaufnahme

A. Korzec[1], T. Arndt[2,4], M. Bär[1], M. W. J. Koeter[3]

Summary: We studied the diagnostic efficiency of two commercial tests for analysis of carbohydrate-deficient transferrin (CDT) as a marker of chronic alcohol abuse in alcoholics, %CDTri-TIA (which includes about 50 % trisialo-Fe_2-transferrin in CDT) and ChronAlcoI.D. (which excludes this transferrin isoform of CDT). TLFB (Timeline-Followback) and Composite International Diagnostic Interview (CIDI) 2.1-alcohol section, which are valid, reliable and fully structured diagnostic interviews, were used as gold standard for assessment of frequency and amount of alcohol intake. %CDTri-TIA showed a distinctly reduced diagnostic sensitivity (%CDTri-TIA, 52,8 %; ChronAlcoI.D., 71,7 %; p = 0,00) and accuracy (%CDTri-TIA, 66,2 %; ChronAlcoI.D., 77,9 %; p = 0,01). Diagnostic specificity was statistically not different between the tests (%CDTri-TIA, 95,8 %; ChronAlcoI.D., 91,7 %; p = 0,30). Inclusion of trisialo-Fe_2-transferrin in CDT does not improve its diagnostic efficiency.

Keywords: alcohol; carbohydrate-deficient transferrin; CDT; diagnostic accuracy.

Zusammenfassung: Es wurde die diagnostische Wertigkeit zweier kommerzieller Tests zur Bestimmung des Kohlenhydrat-defizienten Transferrins (CDT) als Marker chronischen Alkoholmißbrauchs in Alkoholabhängigen untersucht: %CDTri-TIA, der etwa 50 % des Trisialo-Fe_2-Transferrins in das CDT integriert und ChronAlcoI.D.™, der diese Isoform vom CDT ausschließt. Weit akzeptierte strukturierte Fragebögen (TLFB und CIDI-10) wurden als Goldstandard zur Ermittlung der Häufigkeit und der Menge der Alkoholaufnahme eingesetzt. Im Vergleich der beiden Tests zeigte der ChronAlcoI.D.™ eine signifikant höhere diagnostische Sensitivität (%CDTri-TIA, 52,8 %; ChronAlcoI.D., 71,7 %; p = 0,00) und Richtigkeit (%CDTri-TIA, 66,2 %; ChronAlcoI.D., 77,9 %; p = 0,01). Die diagnostischen Spezifitäten waren nicht signifikant voneinander verschieden (%CDTri-TIA, 95,8 %; ChronAlcoI.D., 91,7 %; p = 0,30). Die Einbeziehung von (Teilen) des Trisialo-Fe_2-Transferrins in das CDT verbessert nicht dessen diagnostische Aussagekraft als Marker chronischen Alkoholmißbrauchs.

Schlüsselwörter: Alkohol; CDT; Kohlenhydrat-defizientes Transferrin; Diagnostische Richtigkeit.

Carbohydrate-deficient transferrin (CDT) is widely used for laboratory diagnosis of chronic alcohol abuse. A review on CDT was published recently [1]. There is still controversy as to the diagnostic benefit from including trisialo-Fe_2-transferrin in CDT and/or using CDT concentrations or CDT/transferrin (CDT/Tf) ratios [1–5]. We investigated these issues by comparing diagnostic sensitivity, specificity and accuracy of two commercially available CDT tests: %CDTri-TIA (Axis, Norway) and ChronAlcoI.D. (Sangui Biotech Inc., USA).

Materials and Methods

The study was in accordance with the Declaration of Helsinki of 1975, as revised in 1996 and approved by the ethical committee of the St. Lucas Andreas Hospital. The guidelines for studies of the diagnostic accuracy of diagnostic tests [6] were observed: spectrum bias was avoided by assessing consecutive patients, reviewer bias by blinding case history on alcoholism and alcohol intake to laboratory results and vice versa, verification bias by applying the criterion standards to all subjects. Each test was performed without knowledge of the CDT results obtained by the other.

Patients and Assessment of Alcohol Intake

All subjects were male. Elevated ("hazardous") drinking was defined as the level of persistent alcohol consumption being likely to results in adverse health effects: >280g ethanol/week [7, 8]. 57 *Controls* were recruited from consecutive ambulatory psychiatric pa-

[1]Sint Lucas Andreas Ziekenhuis, Amsterdam, The Netherlands
[2]Bioscientia GmbH, D-55218 Ingelheim, Germany
[3]Amsterdam Institute for Addiction Research, Amsterdam, The Netherlands
[4]Corresponding author: T. Arndt, Bioscientia GmbH, 55218 Ingelheim, Germany. Fax: +49-6132-781-428, E-mail: arndt@bioscientia.de
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tients. 24 patients with an alcohol consumption of ≤280 g ethanol/week in each of the last 4 weeks before blood sampling and who had no Alcohol Use Disorder (AUD, "alcoholism") diagnosis were included in the control group (mean and median of ethanol consumption and age were 47 and 21 g/week and 46.5 and 45.5 years). The remaining 33 patients had an AUD diagnosis in the last year or had been drinking >280 g ethanol/week in the last 4 weeks and were excluded from the study. 101 *Alcoholics* were recruited from treatment facilities: 72 patients consecutively admitted to a detoxification ward and 29 consecutive patients attending an ambulatory alcoholism treatment center. Alcoholism in this study group was defined as having an AUD diagnosis in accordance with ICD-10 (International Classification of Mental or Behavioural Disorders) or DSM-IV (Diagnostic and Statistical Manual of Mental Disorders, 4th edition) [9, 10]. 53 patients with an alcohol intake of >280 g ethanol/week in each of the last four weeks and with an AUD diagnosis were included in the alcoholics group (mean and median of ethanol consumption and age were 1326 and 1113 g/week and 42.3 and 43.0 years). The remaining 48 patients were excluded from the study due to cessation of drinking in the last 4 weeks.

Widely accepted, reliable and validated diagnostic instruments were used as *criterion standards* in assessing alcohol intake and alcoholism [11]. *Alcohol intake* was assessed from TLFB [12], a comprehensive retrospective self-report survey that allows the collection of reliable information up to 12 months before the interview date. *Alcohol use disorder (AUD)* was assessed by means of the Composite International Diagnostic Interview (CIDI) 2.1-alcohol section, which is a valid, reliable and fully structured diagnostic interview and enables diagnosis to be computer-generated according to ICD-10 and DSM-IV criteria [9, 10, 13, 14].

Blood samples

Blood was collected into evacuated sterile gel-tubes (Becton-Dickinson, vacutainer). Serum was obtained by centrifugation at 2600 x *g*, 5°C for 10 min. Serum aliquots were stored at –20°C. Samples were thawed only once for assay. To check if the delay between CDT analysis by %CDTri-TIA (summer 1999) and ChronAlcoI.D. (winter 1999) affected the CDT results, the %CDTri-TIA assay was repeated on a subset of 20 samples at the time of ChronAlcoI.D.: T-test for paired samples showed no significant differences between the "summer" and "winter" CDT values (mean CDT/Tf ratio 7.2 % ± 7.2 %.(summer) and 7.0 % ± 6.2 % (winter), mean summer-winter difference 0.19 % ± 1.18 % (p = 0.553 two tailed), correlation of test-retest 0.997 (p =0.000)). Passing and Bablok correlation [15] yielded no significant difference from zero for the intercept and from 1 for the slope, proving the CDT concentrations to be stable (statistically non-different) between summer and winter 1999. Unaltered CDT concentrations after freezing serum samples for several months were also reported in [16–18].

%CDTri-TIA- and ChronAlcoI.D.™-Assays

%CDTri-TIA Assay was provided by AXIS Biochemicals ASA (Oslo, Norway), distributed by Orange Medical, The Netherlands, and performed in Amsterdam. The test includes about 50 % of trisialo-Fe_2-Tf in CDT, and reports CDT/Tf ratios. *ChronAlcoI.D. Assay* was provided by Sangui BioTech, Inc. (Santa Ana, USA), distributed by Biodiagnostics (Kiel, Germany), and performed in Ingelheim. The test excludes trisialo-Fe_2-Tf from CDT and reports CDT concentrations and CDT/Tf ratios. Both tests are based on anion-exchange chromatography for fractionation of CDT isoforms and non-CDT isoforms, followed by nephelometric (Array nephelometer, Beckman/Array Flexisoft program by Beckman Coulter, Mijdrecht, The Netherlands, for the %CDTri-TIA) or turbidimetric (Dynatec MR 5000 reader/Dynex Revelation 3.2 software by Dynex Technologies, Denkendorf, Germany, for the ChronAlcoI.D.) quantification of CDT. Quality control was done by internal (delivered with the test kits and analysed in each series) and external quality control material (DGKC, Bonn; GTFCH, Heidelberg; Instand, Düsseldorf). The CV's for the low and high controls in the appropriate quality-control periods were <12.0 % and <5.3 % (%CDTri-TIA) and <7.5 % and <7.9 % (ChronAlcoI.D). Analytic specificity and precision of the ChronAlcoI.D. were assessed previously [19]. For both tests, borderlines indicating elevated alcohol consumption have been suggested: 5–6 % CDT for the %CDTri-TIA (test instructions) and 2.5–2.7 % CDT or 100–110 mg CDT/L for the ChronAlcoI.D. [20]. Taking into account the social consequences of false-positives regarding chronic alcohol abuse, we used the upper limits of these borderlines as decision criteria (cut-offs): 6 % CDT for the %CDTri-TIA and 2.7 % CDT or 110 mg CDT/L for the ChronAlcoI.D. (Table 1).

Statistics

Diagnostic sensitivity, specificity, accuracy, ROC curves, confidence intervals (CI) and inter-assay variation coefficients were computed with the *statistics* software SPSS base 10.0 for Windows NT (SPSS Inc., Chicago, U.S.A.). Differences in the criteria of diagnostic efficiency between %CDTri-TIA and ChronAlcoI.D. were checked for significance by the McNemar test for paired samples. Confidence intervals were calculated with a formula given in [21]. P-values <0.05 indicate significant differences.

Results and Discussion

The parameters of diagnostic efficiency obtained at cut-offs of 6 % CDT for the %CDTri-TIA and 2.7 % for the ChronAlcoI.D. assay are summarized in Table 1. Compared with %CDTri-TIA, ChronAlcoI.D. showed significantly higher diagnostic sensitivities and accuracies. There were no significant differences in the diagnostic specificities between %CDTri-TIA and ChronAlcoI.D. (Table 1). Using cut-offs of 5 % CDT (instead of 6 % CDT, Table 1) for the %CDTri-TIA

Table 1 Parameters of diagnostic efficiency of %CDTri-TIA and ChronAlcoI.D. for 53 patients (alcohol intake of >280g ethanol/day and alcoholism diagnosis) and 24 controls (alcohol intake ≤280 g ethanol/day).

	ChronAlcoI.D. cut-off 2.7 %	%CDTri-TIA cut-off 6.0 %	Difference	95 % CI[a]	p[b]
Diagnostic sensitivity	71.7 %	52.8 %	18.9 %	7.2 % – 30.6 %	0.00
Diagnostic specificity	91.7 %	95.8 %	–4.1 %	–12.0 % – 3.8 %	0.30
Diagnostic accuracy	77.9 %	66.2 %	11.7 %	2.9 % – 20.5 %	0.01

[a] Confidence interval for difference between the two tests
[b] p values based on McNemar test without continuity correction [21]

and 2.5 % (instead of 2.7 %, Table 1) for the ChronAlcoI.D. improved the diagnostic sensitivities (from 52.8 % to 69.8 % for %CDTri-TIA, from 71.7 % to 81.1 % for ChronAlcoI.D.) and accuracies (from 66.2 % to 75.3 % for %CDTri-TIA, from 77.9 % to 84.4 % for ChronAlcoI.D.) for both tests, but diminished the diagnostic specificity of the %CDTri-TIA assay (from 95.8 % to 87.5 %). The diagnostic specificity of the ChronAlcoI.D. was unaffected (91.7 % at the low and the high cut-off).

Compared with CDT/Tf ratios (ChronAlcoI.D.), absolute CDT concentrations obtained by the same assay (ChronAlcoI.D.) showed a significantly reduced sensitivity (45.3 % for absolute vs 71.7 % for relative CDT concentrations; 95 % CI –40.0 % – –13.4 %, p=0.00) and accuracy (61.0 % for absolute vs 77.9 % for relative CDT concentrations; 95 % CI -26.6 % – –7.1 %, p=0.00).

Our findings are in accordance with an earlier study [2], comparing the %CDT-TIA (identical with %CDTri-TIA, including about 50 % of trisialo-Fe_2-Tf, measuring CDT/Tf ratios) and the CDTect (excluding trisialo-Fe_2-Tf, measuring absolute CDT concentrations). Compared with CDTect, %CDT-TIA showed an overall reduced diagnostic accuracy for detecting alcohol abuse in men, this being mainly due to a diminished diagnostic sensitivity [2]. For ChronAlcoI.D., absolute CDT concentrations (as used by the CDTect) showed an overall weaker diagnostic accuracy when compared with the corresponding CDT/Tf ratios (see above). Thus, the findings in [2] cannot solely be due to the different units used by the two tests (% of total Tf by the %CDT-TIA and U/L by the CDTect). CDTect [22] and the ChronAlcoI.D. [19] show a similar analytic specificity. The fact that both tests exclude trisialo-Fe_2-Tf from CDT makes the greatest difference in comparison with the %CDTri-TIA. Thus, it is more likely that the diminished diagnostic accuracy of so-called "trisialo-tests" (%CDTri-TIA or %CDT-TIA[1]) is due to the inclusion of trisialo-Fe_2-Tf in CDT. This conclusion is supported by findings by others [3, 5, 23]: No increase of trisialo-Fe_2-Tf concentration after chronic alcohol consumption, but significant increases for asialo-Fe_2-Tf (by 219 % of its normal serum concentration), monosialo-Fe_2-Tf (28 % increase) and disialo-Fe_2-Tf (148 % increase) were described in [23]. Increased concentrations of asialo- and disialo-Fe_2-Tf in serum samples with pathological CDT/Tf ratio and almost identical trisialo-Fe_2-Tf concentrations in serum samples with normal and pathological CDT/Tf ratio were reported in [3]. Classifying relative CDT concentrations obtained by ChronAlcoI.D., %CDT TIA (including 50 % of trisialo-Fe_2-Tf into CDT; Sangui BioTech Inc., Santa Ana, USA) and HPLC as either normal or elevated, Lipkowski et al. found 22 % discrepancies between %CDT TIA and HPLC, but only 9 % between ChronAlcoI.D. and HPLC [5]. The authors strongly recommend not to include trisialo-Fe_2-Tf into CDT [5].

The significant differences in diagnostic sensitivity and diagnostic accuracy between %CDTri-TIA and ChronAlcoI.D. were not matched by analogous differences in the corresponding areas under the ROC curve (AUC, see Fig. 1). This discrepancy is most probably

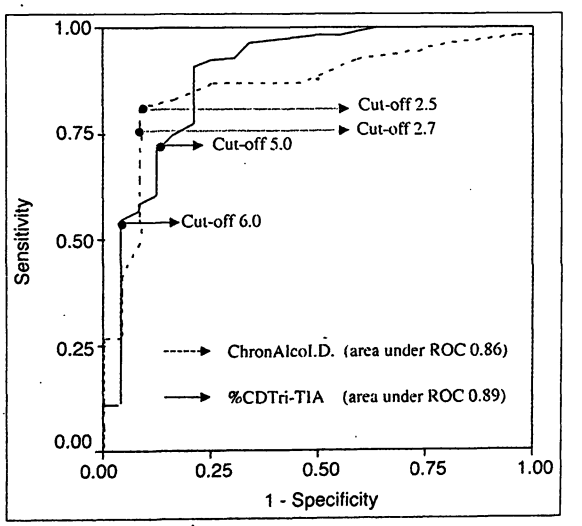

Figure 1 ROC plot for two commercial CDT tests for laboratory diagnosis of chronic excessive alcohol intake for 53 alcoholics and 24 healthy controls: %CDTri-TIA (including about 50 % of trisialo-Fe_2-transferrin in CDT), ChronAlcoI.D. (excluding this transferrin isoform).

[1] Unfortunately, the new product by Axis, excluding trisialo-Fe_2-Tf from CDT and using the common CDT definition, has the same name, %CDT TIA.

caused by an intersection of both curves outside the clinically important part: In the cut-off area where in normal clinical practice the diagnostic measurements or decisions are made (at the recommended cut-offs), %CDTri-TIA performs worse than ChronAlcoI.D. In the cut-off area where diagnostic decisions will never be made (because of the corresponding unacceptable low diagnostic specificities), %CDTri-TIA performs better than ChronAlcoI.D. and thus gains AUC. However, ROC analysis seems less suitable for comparing the tests under study because this method assumes that the choice of cut-off is made only from data plotted, without information from previous published work suggesting what is the best cut-off value [24].

This is in contrast to the concept of our study (use of non-arbitrary, recommended and widely accepted cut-offs). Comparisons of sensitivities of diagnostic tests are usually made on the same level of specificity or vice versa. In our study, this approach would mean comparison of the test performance for one test at the recommended (optimal and widely used) and for the other at a non-recommended (non-optimal and never used) cut-off. Therefore, we have assessed the diagnostic efficiency of both tests at their recommended cut-offs. The relatively small number of controls may limit the significance of our study. However, it does not explain the significant differences in diagnostic accuracy between %CDTri-TIA and ChronAlcoI.D.

Conclusion

If trisialo-Fe_2-Tf increases at all after chronic alcohol abuse, its proportional change might be less than that for the common CDT isoforms. If this is true, the comparably less affected but large amounts of trisialo-Fe_2-Tf might mask the alcohol-induced increases in the CDT isoforms and thus lower the diagnostic sensitivity of CDT. As a consequence, the production of so-called "trisialo-tests" by Axis has been terminated recently.

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