Evaluation of the Cut-off for Serum Carbohydrate-deficient Transferrin as a Marker of Chronic Alcohol Abuse Determined by the ChronAlco I.D.TM-Assay

Ermittlung der Entscheidungsgrenzen für das Kohlenhydrat-defiziente Transferrin im Serum als Marker chronischen Alkoholmißbrauchs unter Verwendung des ChronAlco I.D.TM-Assays

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Summary: The aim of the study was to establish reliable cut-off values, indicating chronic alcohol abuse, of the relative (and absolute) serum carbohydrate-deficient transferrin (CDT) concentrations determined by the ChronAlco I.D.TM-assay. Serum samples from 88 women and 48 men, with the daily alcohol consumption, acute or chronic (liver) diseases and medication ascertained by means of a questionnaire, were analyzed. The cut-off values of the CDT/transferrin ratios and the CDT serum concentrations (95th percentiles) for women consuming < 20 g ethanol/day and men drinking < 50 g ethanol/day were 2.5% and 102 mg/l. Pathological CDT results were confirmed by isoelectric focusing. Taking into account the intra-individual variance of serum CDT and the analytical imprecision, the use of a borderline of 2.5-2.7% as decision criterion indicating chronic alcohol abuse is suggested.

Keywords: Alcoholism/serum; Transferrin/analogs and derivatives; Reference Values.

Zusammenfassung: Ziel der Studie war es, für die unter Verwendung des ChronAlco I.D.TM-Assays ermittelten und als Marker für chronischen Alkoholmißbrauch genutzten relativen (und absoluten) Serumkonzentrationen des Kohlenhydrat-defizienten Transferrins (CDT) zuverlässige Entscheidungsgrenzen zu etablieren. Wir untersuchten Serumproben von 88 Frauen und 48 Männern, von denen anhand eines Fragebogens Angaben zur täglichen Alkoholkonsumption, zu (Leber-)Erkrankungen sowie zur Medikation vorlagen. Die Entscheidungsgrenzen (95. Perzentilen) für die relativen (CDT/Transferrin) bzw. absoluten CDT-Konzentrationen von Frauen und Männern mit normaler Alkoholaufnahme (<20 g/Tag für Frauen und <50 g/Tag für Männer) waren 2.5% bzw. 102 mg/l. Pathologische CDT-Befunde wurden durch Isoelektrische

Fokussierung der Transferrin-Isoformen bestätigt. Unter Berücksichtigung der intraindividuellen Schwankungen der CDT-Konzentration und der analytischen Impräzision wird ein Grenzbereich von 2.5-2.7% als Entscheidungsgrenze für die Annahme eines chronischen Alkoholmißbrauchs vorgeschlagen.

Schlüsselwörter: Alkoholismus/Serum; Transferrin/Analogue und Derivate; Referenzwerte.

The measurement of carbohydrate-deficient transferrin (asialo-, monosialo- and disialotransferrin) in serum is used for the detection of chronic alcohol abuse (review in [1]). The first commercial CDT-test (CDTect-RIA; Pharmacia & Upjohn) was launched in 1993. Later, a non-radioactive variant of this test became available, CDTect-EIA. Although both tests have been used most frequently for determination of CDT, the production of the CDTect-EIA was terminated in January 1999. Thus, the laboratory has the choice of returning to the radioactive CDTect-RIA (which shows a good comparability with the CDTect-EIA [2]) or using a test incorporating (parts of) trisialotransferrin into the determination of CDT. However, whether trisialotransferrins occur with high prevalence in increased concentrations after chronic alcohol abuse and whether the incorporation of these transferrin isoforms improves the diagnostic performance of CDT is discussed controversially [3-5]. HPLC methods have been described [6, 7] and one method was recently launched commercially by Recipe Chemicals + Instruments GmbH (Munich, Germany). However, an analysis time of 20-30 min/chromatographic run restricts these methods to small series.

In 1998, a new test for determination of CDT became available, ChronAlco I.D.TM (Sangui BioTech, Inc., Santa Ana, U.S.A.) distributed by DPC/Biermann (Bad Nauheim, Germany). The test has 3 analysis steps: in vitro transferrin Fe³⁺-saturation, anion-exchange chromatography for separation of non-CDT- and CDT-transferrins and determination of CDT (and total serum transferrin) by a turbidimetric immunoassay. This test summarizes asialo-, monosialo- and

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disialo-Fe₂-transferrins as CDT (Huckler et al., 1999 in preparation), thus using the common CDT-definition given by Stibler [8]. The analysis results are expressed as a percentage of total transferrin (absolute CDT concentrations are optionally available).

As pointed out in [1], the different CDT analysis methods show varying specificities and recoveries of the CDT-transferrin isoforms. Thus, test-specific decision criteria have to be used when interpreting CDT values. Although the ChronAlco I.D.TM -test is increasingly used for routine laboratory diagnosis of chronic alcohol abuse, an independent study on the appropriate cut-off of the CDT concentration determined by this test is lacking so far.

With the present work we wanted to establish reliable cut-off values of the relative (and absolute) CDT concentrations determined by the ChronAlco I.D.TM -assay that would indicate chronic alcohol abuse.

Materials and Methods

All procedures were in accordance with the Helsinki declaration of 1975, as revised in 1996.

Materials

All materials were delivered with the ChronAlco I.D.TM test kits (DPC/Biermann, Bad Nauheim, Germany).

Probands

Blood was drawn from healthy persons by venipuncture after an over-night fast, using Multifly Sets (Sarstedt, Nümbrecht, Germany). Serum was obtained after clotting for 30-45 min at room temperature followed by centrifugation at 2000 g for 10 min. Serum aliquots were stored at -22 °C until analysis. The daily alcohol consumption was determined by a structured questionnaire, stating the amount of beer, wine and spirits consumed during the previous 2 weeks. The persons were also asked for acute or chronic (liver) disorders and medication. Persons stating a potentially hazardous alcohol intake of more than 20 g ethanol/day for women and more than 50 g ethanol/day for men (suggestion by the WHO) and/or showing both elevated γ -GT and MCV (3 men and 6 women) were excluded from the control population.

ChronAlco I.D.TM-Assay

The CDT/transferrin ratio was determined in accordance with the instructions of the manufacturer. In short: For in vitro transferrin Fe³⁺-saturation, 100 μ l of serum were mixed with 500 μ l of ferric saturation reagent and incubated for 5-10 min at room temperature. CDT-transferrins and non-CDT-transferrins were

separated on anion exchange microcolumns by adding 500 μ l of the Fe³⁺-treated serum sample to the top of the column, rinsing the column with 1.5 ml of elution buffer, and eluting the CDT-transferrins with an additional 2.5 ml of elution buffer. The total serum transferrin concentration and the CDT-concentration were determined by a turbidimetric immunoassay using microtiter plates. The blanks and the final absorbance were measured at 405 nm (Dynatec MR 5000 reader and Dynex Revelation 3.2 software; Dynex Technologies, Denkendorf, Germany). The results were reported as CDT/transferrin ratio as well as CDT and total transferrin concentrations. Two internal quality control samples (CDT-controlset, DPC/Biermann, Bad Nauheim, Germany) were placed at the beginning and end of each analysis series.

Isoelectric focusing was used for verification of elevated CDT/transferrin ratios or CDT-concentrations. It was carried out as described previously [9] with minor modifications [10].

The intra- and inter-assay imprecision of the ChronAlco-assay was investigated by analyzing 5 serum samples with normal and elevated CDT fivefold in each series on 5 consecutive days.

Results and Discussion

ChronAlco I.D.TM is a new commercially available assay for the determination of CDT/transferrin ratios which are increasingly used for the detection of chronic alcohol abuse. In general, the diagnostic performance of a laboratory parameter depends on the test used and the (test-specific) cut-off values. Thus, the establishment of a reliable cut-off of the CDT/transferrin ratio determined by the ChronAlco I.D.TM-assay is a prerequisite for correct interpretation of the test results. We investigated 88 healthy women and 48 healthy men (students and employees of our institutes) regarding their daily ethanol consumption and their CDT-transferrin ratio in serum. Six women and 2 men reporting an ethanol consumption of more than 20 g/d (women) and more than 50 g/d (men) and 1 man showing an elevated γ -GT-activity and MCV were excluded from the control population. The main results of our study are given in table 1. As expected, the mean ethanol consumption of women was lower than that of men, regardless of whether it was calculated as g/d or g/d/kg. Calculating the 95th percentile of the CDT/transferrin ratios as determined by the ChronAlco I.D.TM-assay, we established a cut-off of 2.5% for women and for men. When 4 women with an alcohol consumption of >20 g/d and <50 g/d (upper amount for men) were included in the female control group, the cut-off of 2.5% for women remained unaffected. In combining women and men in 1 group, this cut-off was also 2.5%. Gender-independent cut-off values for CDT expressed as a percentage of total transferrin have been reported by others, using "%CDT-assays",

Non standardized abbreviations: CDT, carbohydrate-deficient transferrin; HPLC, high-performance liquid chromatography; IEF isoelectric focusing; RIA, radioimmunoassay.

Table 1 CDT/transferrin-ratio and CDT-concentrations in serum determined by the ChronAlco I.D.TM-assay from healthy persons with an ethanol consumption < 20 g/d (women) and < 50 g/d (men)

		Women	Men
Serum samples	n	82	45
Ethanol consumption (g/d)	range	0-18	0-49
	median	2.8	8.6
	mean	4.1	10.2
	standard deviation	5.5	10.6
Ethanol consumption (g/d/kg)	range	0-0.55	0-0.61
	median	0.04	0.09
	mean	0.07	0.13
	standard deviation	0.09	0.13
Serum CDT (% of total transferrin)	range	1.5-2.9	1.4-2.9
	median	1.9	1.9
	mean	1.9	1.9
	standard deviation	0.3	0.3
	95 th percentile	2.5	2.5
Serum CDT (mg/l)	range	54-126	48-123
	median	72	69
	mean	76	73
	standard deviation	14	15
	95 th percentile	102	102

Figure 1 Isoelectric focusing was used for confirmation of the %CDT results obtained by the ChronAlco I.D.TM-assay. Lane CSF: transferrin isoform band pattern of a cerebrospinal fluid which was used as an internal standard for the identification of the different transferrin bands. Lane 1: serum transferrin isoform band pattern of a healthy man stating an elevated alcohol consumption. This person was excluded from our male control group. Lane 2: serum transferrin isoform band pattern of a healthy control with normal alcohol intake. The corresponding %CDT values are given below lanes 1 and 2. In comparison with lane 2, lane 1 shows a more intense band of disialo- and additional bands of mono- and asialo-Fe₂-transferrin. The transferrin band pattern in lane 1 is typical of persons with chronically elevated alcohol consumption. Similar band patterns were also obtained for randomly selected serum samples from women and men with %CDT-values (>2.5-2.7%), confirming the borderline for the CDT/transferrin ratio determined in our study.

too [11]. We suppose that slight gender-dependent differences in the total serum transferrin concentration level cut the differences between absolute serum CDT in women and men as known from the literature. It remains unclear why a cut-off of >2.8% is suggested by the manufacturer. In their own study on 60 serum samples from healthy controls, the mean +2SD was 2.3% (test instructions).

Surprisingly, the cut-off values for the absolute CDT concentration, which is optionally available by the ChronAlco I.D.TM-assay, was similar between women and men in our study. This is in contrast to the literature on CDTest assays, which reports higher CDT concentrations for women [2, 8, 12, 13]. One reason for this observation could be the different recovery of the CDT-transferrins by the CDTest- [14] and the ChronAlco I.D.TM-assay (Hackler et al., in preparation).

The intra- and inter-assay coefficients of variation for CDT/transferrin ratios determined by the ChronAlco I.D.TM-assay were <9% and <11%. Taking into account this analytical imprecision and the intra-individual variance of serum CDT (Helander et al., 1998), we suggest using a borderline of 2.5-2.7% as a decision criterion for laboratory diagnosis of chronic alcohol abuse. Using this borderline and analyzing the serum samples with increased CDT/transferrin ratios by isoelectric focusing (IEF), we obtained transferrin isoform band patterns which are typical of patients with chronically elevated alcohol consumption: increased amounts of disialo-Fe₂-transferrin and additional bands of asialo- and monosialo-Fe₂-transferrin

(Fig. 1, lane 1). Thus, the positive ChronAlco I.D.TM-results and the cut-off established in this study were confirmed by the reference method of transferrin isoform analysis, IEF.

An elevated diagnostic specificity of the CDT/transferrin ratio in comparison with absolute CDT concentrations has been reported for patients with increased total transferrin concentrations, e. g. during pregnancy or iron deficiency anemia [16-18]. However, in using the CDT/transferrin ratio for detection of chronic alcohol abuse we have to take into account serum samples with low transferrin concentrations (e. g. due to acute or chronic infections, hemochromatosis, distinct proteinuria, cancer) which might cause false-positives. ChronAlco I.D.TM optionally offers the possibility of parallel determination of absolute CDT (and transferrin) concentrations. Out of 128 controls with normal alcohol intake 4 showed normal absolute CDT concentrations (78-84 mg/l) but elevated CDT/transferrin ratios (2.9-3.1%). The total serum transferrin concentrations from these 4 controls were all at the test- and gender-specific lower reference range limit. Of these 4 serum samples only 2 showed bands of carbohydrate-deficient transferrins in IEF. Increased absolute CDT concentrations (111 and 126 mg/l) but normal CDT/transferrin ratios (1.9 and 2.2%) were observed in 2 controls, showing total serum transferrin concentrations at the test-specific upper reference range.

Thus, in our experience the interpretation of both the CDT/transferrin ratio and the CDT concentration can improve the diagnostic performance of CDT for patients with pathological (elevated and diminished) total transferrin concentrations. A very similar diagnostic performance when using the CDT/transferrin ratio or absolute CDT concentrations has been reported [19]. This might be a statistical effect which, under distinct circumstances, might be not attributable to the single individual. Further studies on this subject are needed.

N.B. Due to varying analytical specificities and recoveries of the different CDT test sets, a conversion of relative to absolute CDT (and transferrin) concentrations, and vice versa is not possible.

Conclusions

The cut-off of the serum CDT/transferrin ratio determined by the ChronAlco I.D.TM-assay is 2.5% both for women and men. The use of a borderline of 2.5-2.7% is indicated. Other commercial tests for determination of CDT, including parts of trisialotransferrin into CDT, report cut-off values of 6%. Thus, the test-specific cut-off values must always be observed when interpreting CDT values. A standardization of CDT analysis, regarding which transferrin isoforms should be collectively referred to as CDT and is urgently needed regarding the units (e.g. % for CDT/transferrin ratios and mg/l for absolute CDT concentrations).

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