

Carbohydrate-deficient Transferrin as a Marker of Chronic Alcohol Abuse: A Critical Review of Preanalysis, Analysis, and Interpretation

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Background: Carbohydrate-deficient transferrin (CDT) is used for diagnosis of chronic alcohol abuse. Some 200–300 reports on CDT have been published in impact factor-listed journals. The aims of this review were to condense the current knowledge and to resolve remaining issues on CDT.

Approach: The literature (1976–2000) was searched using MEDLINE and Knowledge Server with “alcohol and CDT” as the search items. The data were reviewed systematically, checked for redundancy, and organized in sequence based on the steps involved in CDT analysis.

Content: The review is divided into sections based on microheterogeneity of human serum transferrin (Tf), definition of CDT, structure of human serum CDT, pathomechanisms of ethanol-induced CDT increase, preanalysis, analysis, and medical interpretation (post-analysis). Test-specific cutoff values for serum CDT and causes of false positives and negatives for chronic alcohol abuse are discussed and summarized.

Summary: Asialo- and disialo-Fe₂-Tf, which lack one or two complete N-glycans, and monosialo-Fe₂-Tf (structure remains unclear) are collectively referred to as CDT. Diminished mRNA concentration and glycoprotein glycosyltransferase activities involved in Tf N-glycan synthesis and increased sialidase activity most likely account for alcohol-induced increases in CDT. Knowledge about in vivo and in vitro effects on serum CDT is poor. Reliable CDT and non-CDT fractionation is needed for CDT measurement. Analysis methods with different analytical specificities and recoveries decreased the comparability of values and statistical parameters of the diagnostic efficiency of CDT. CDT is the most specific

marker of chronic alcohol abuse to date. Efforts should concentrate on the pathomechanisms (in vivo), preanalysis, and standardization of CDT analysis.

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Carbohydrate-deficient transferrin (CDT)¹ is widely used for laboratory diagnosis of chronic alcohol abuse. Since the first report by Stibler and Kjellin in 1976 (1), many studies on CDT have been published. The majority of the CDT literature concentrates on the diagnostic efficiency of CDT in different clinical settings and among various populations. At the same time, preanalytical and analytical issues, which are fundamental for reliable CDT analysis and thus for meaningful comparison of values, and the diagnostic specificities and sensitivities of CDT obtained in different studies by different analysis methods have not gained much attention. Appropriate data are rare, scattered through the reports, and mostly incomplete. However, routine laboratory diagnosis often involves preanalytical and analytical issues of CDT analysis (in addition to questions about the interpretation of CDT values). Therefore, the aims of this review are to summarize and condense current knowledge on the various aspects of CDT analysis, to resolve the remaining issues, and to encourage studies for assessing these issues. Finally, the review is aimed as a clear and easily accessible CDT reference for routine laboratory and clinical use. Therefore, the review follows the sequence of the analytical process: microheterogeneity of human serum transferrin (Tf), CDT isoform structure, pathobiochemistry, preanalysis, analysis, and interpretation (postanalysis) of CDT.

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¹ Nonstandard abbreviations: CDT, carbohydrate-deficient transferrin; CDG, carbohydrate-deficient glycoprotein; Tf, transferrin; pI, isoelectric point; IEF, isoelectric focusing; TIA, turbidimetric immunoassay; Allo A, lectin from *Allomyrina dichotoma*; TJA, lectin from *Trichosanthes japonica*; EIA, enzyme immunoassay; and γ -GT, γ -glutamyltransferase.

Microheterogeneity of Human Serum Tf

Tf, the most important iron-transport protein, is synthesized mainly in hepatocytes and consists of three substructural domains: a single polypeptide chain, two independent metal ion-binding sites (one within the N-terminal and the other within the C-terminal domain), and two N-linked complex glycan chains (Fig. 1). These Tf substructures show a distinct variability even under non-pathological conditions (2). Because of this, Tf is not a homogeneous molecule, but shows a distinct microheterogeneity and is, from the analytical point of view, a group of similar Tf isoforms collectively referred to as Tf. Human serum Tf appears in serum protein electrophoresis on cellulose acetate within the β -globulin fraction. With high-performance electrophoretic methods, e.g., isoelectric focusing (IEF) followed by immunoblot and staining, a multitude of Tf bands become visible (2, 3). Knowledge about Tf microheterogeneity is fundamental for correct analysis and interpretation of CDT.

VARYING IRON LOAD

Tf is known to be the most important Fe^{3+} -transport protein in humans. Each Tf molecule can bind a maximum of two metal irons, preferably Fe^{3+} . Depending on the Fe^{3+} supply of the organism, Tf molecules are iron free (Fe_0 -Tf or apo-Tf) or loaded with one ($\text{Fe}_{1\text{N}}$ - and $\text{Fe}_{1\text{C}}$ -Tf, where N and C indicate the N- and C-terminal regions, respectively) or two (Fe_2 -Tf) Fe^{3+} ions (2). In healthy controls, Tf iron saturation is $\sim 30\%$, and Fe_0 -, $\text{Fe}_{1\text{N}}$ -, and $\text{Fe}_{1\text{C}}$ -Tfs are detectable in serum (Fig. 1). In Fe^{3+} deficiency, Tf iron saturation decreases and higher amounts of Fe_0 - and $\text{Fe}_{1\text{N}}$ -Tfs occur in serum. In hemochromatosis (Fe^{3+} excess), Tf iron saturation increases and the isoforms found in serum are almost exclusively Fe_2 -Tfs. The isoelectric point (pI) of the Tf molecule decreases by ~ 0.2 pH units with each Fe^{3+} ion bound (2).

DIFFERING N-GLYCAN CHAINS

The two Tf N-glycan chains differ in their degree of branching, showing bi-, tri-, and tetraantennary structures (2, 4). Each antenna of the Tf N-glycan chains terminates with a (negatively charged) sialic acid molecule (Fig. 1). Because of this, asialo-Tf, and the sialylated forms monosialo- through octasialo-Tf can occur in serum (2, 4). The relative amounts of these Tf isoforms as a percentage of total serum Tf are $<1.5\%$ heptasialo-Tf, $1\text{--}3\%$ hexasialo-Tf, $12\text{--}18\%$ pentasialo-Tf, $64\text{--}80\%$ tetrasialo-Tf, $4.5\text{--}9\%$ trisialo-Tf, and $<2.5\%$ disialo-Tf in healthy persons (5–7). Asialo- and monosialo-Tf and octasialo-Tf are not detectable (5) or represent $<0.5\%$ (asialo-Tf) and $<0.9\%$ (monosialo-Tf), respectively, of total Tf under nonpathological conditions (7). The pI of the Tf molecule decreases by ~ 0.1 pH units with each sialic acid residue bound to the N-glycan chains (2, 4).

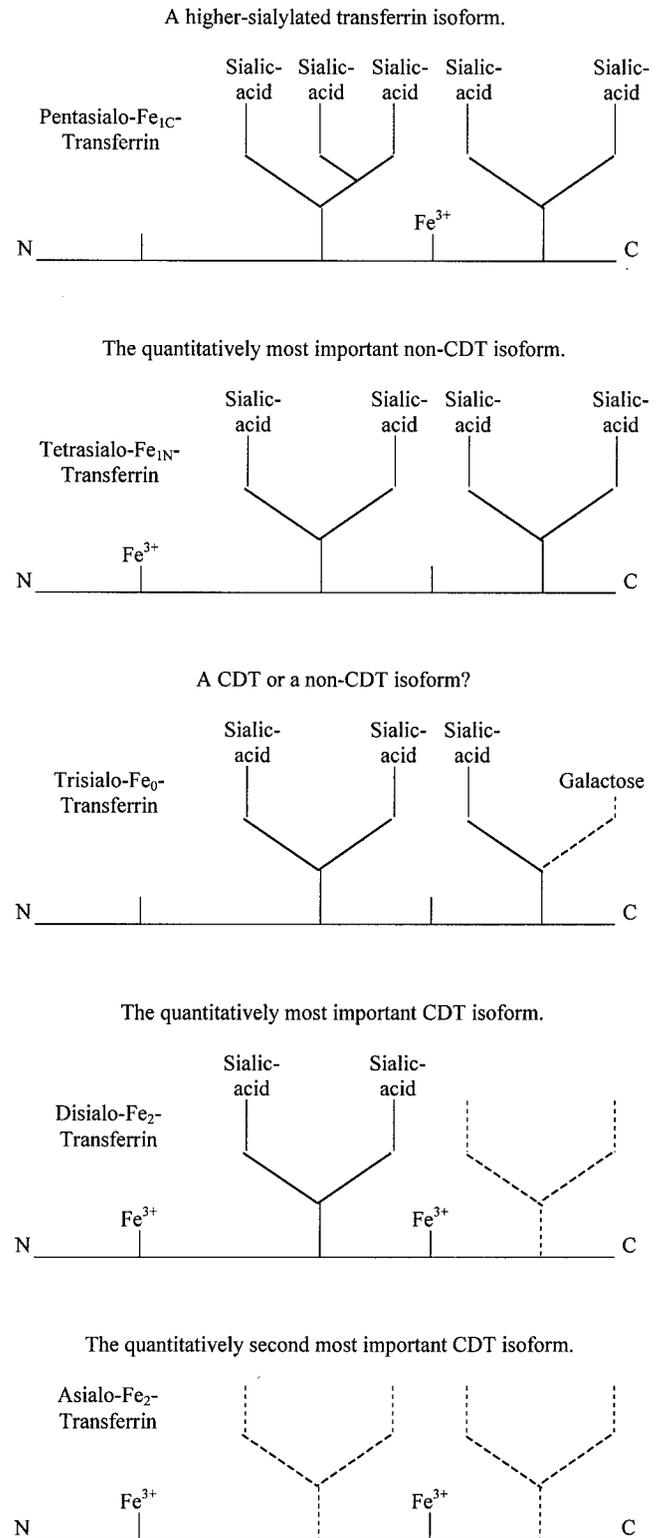


Fig. 1. Microheterogeneity of human serum Tf attributable to various Tf Fe^{3+} loads and different N-glycan chains.

The quantitatively most important Tf isoform is tetrasialo-Tf (second panel from the top). After chronic alcohol abuse, Tf isoforms lacking one or two complete N-glycan chains or showing incomplete N-glycan chains (asialo-, monosialo-, and disialo-Tf) appear with high prevalence in serum.

MODIFIED POLYPEPTIDE CHAIN (GENETIC Tf VARIANTS)
 Genetic Tf variants are attributable to substitutions of amino acid(s) in the polypeptide chain (2, 4). At least 38 Tf variants are known (8). However, only 4 of these show a prevalence of >1%. For Tf-C, the most important type in Caucasians, 16 subtypes have been reported. Of these, Tf-C1 shows the highest prevalence (>95%) in Caucasians (Fig. 2).

Tf-B ("busy") and Tf-D variants can interfere with CDT analysis. The pIs of non-CDT Tf-D isoforms are similar to the pIs of CDT Tf-C isoforms, which can lead to cofocusing (or coelution) and thus false-positive results for persons who are heterozygous Tf-CD and consume normal amounts of alcohol (9, 10). As we showed recently (11), Tf-D variants do not necessarily cause overdetermination of CDT; it depends on the pI of the Tf-D subtype and on the analytical specificity of the CDT analytical method. Tests incorporating trisialo-Fe₂-Tf into CDT are more strongly affected by Tf-D variants than tests using the classical CDT definition (11). Tf-B variants show diminished pIs and thus increased electrophoretic mobility compared with Tf-C. Cofocusing (or coelution) of the CDT isoforms of the Tf-B variant with non-CDT isoforms of the Tf-C variant can produce false-negative results for

persons who are heterozygous Tf-CB and chronically abuse alcohol.

PARALLEL CHANGES IN ALL THREE SUBSTRUCTURES OF Tf

Alterations in the three Tf substructures usually appear in parallel (2, 4). Thus, the distinct microheterogeneity of human serum Tf becomes even more pronounced. In addition, Tf molecules with various iron loads show differing sialic acid contents and/or a modified polypeptide chain (2). As pointed out above, iron load, sialic acid content, and modifications in the polypeptide chain affect the pI of the Tf molecule. Alterations in the pI, e.g., when one or two Fe³⁺ ions are bound or lost, can be compensated by the presence or absence of sialic acid residues or genetic Tf variants. Thus, Tf molecules with different amounts of iron and sialic acid but equal pIs, e.g., disialo-Fe₂-Tf (as the main CDT isoform) and tetrasialo-Fe₁-Tf (as the main non-CDT isoform), appear in serum (Fig. 2). A maximum of 36 (for the homozygous Tf type) or 72 (for the heterozygous Tf type) isoforms can be detected by IEF. Of these, only 3 (for the homozygous Tf type) or 6 (for the heterozygous Tf type) Tf isoforms are collectively referred to as CDT (see *Definition of CDT*

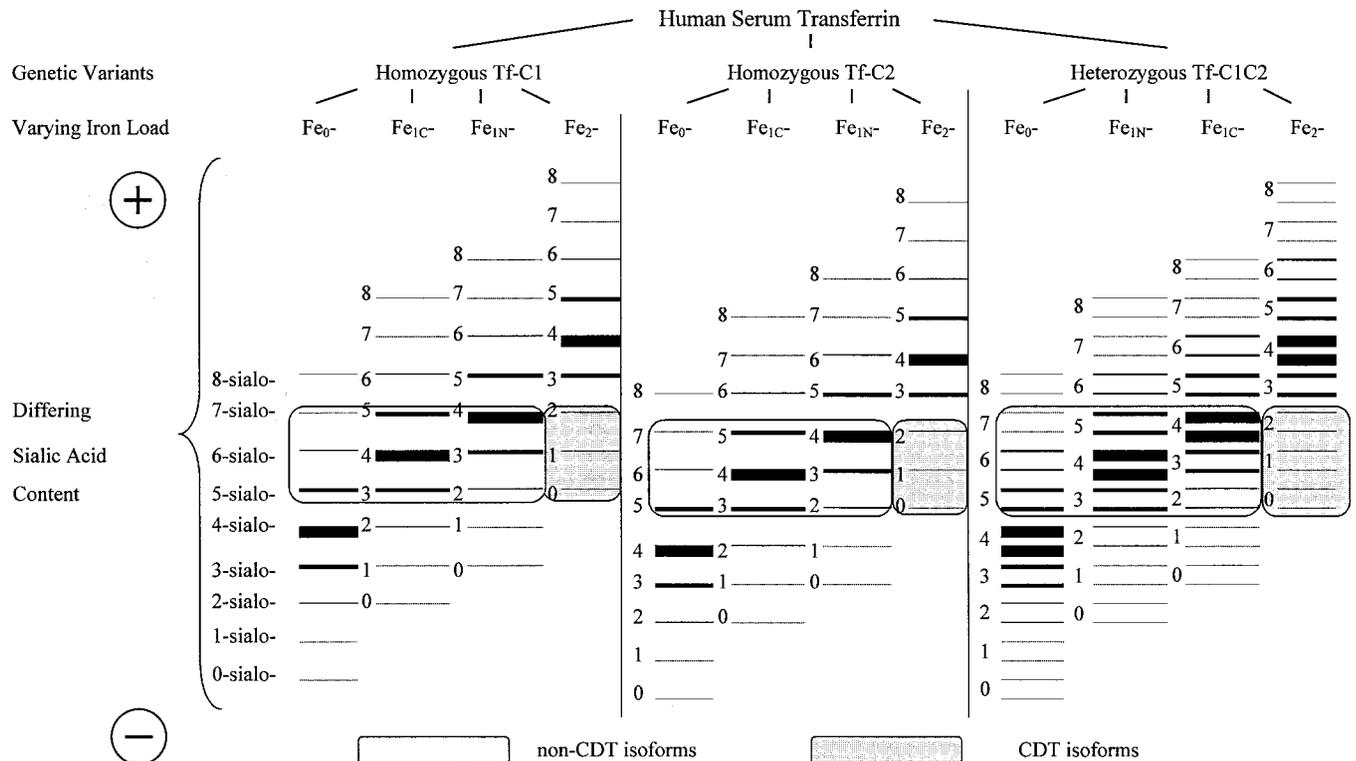


Fig. 2. Microheterogeneity of human serum Tf attributable to various Tf Fe³⁺ loads, different N-glycan chains, and genetic Tf variants (different polypeptide chains).

A maximum of 36 (for the homozygous Tf types; left and middle) or 72 (for the heterozygous Tf type; right) isoforms can be detected by IEF. Of these, only 3 (for the homozygous Tf types) or 6 (for the heterozygous Tf type) isoforms are collectively referred to as CDT. At the same time, 9 (for the homozygous Tf types) or 18 (for the heterozygous Tf type) non-CDT isoforms (open boxes) with pIs almost identical to those of CDT isoforms (gray boxes) appear in parallel and can lead to overdetermination of CDT. To avoid this co-analysis and to reduce the number of Tf isoforms potentially occurring in human serum, a uniform Tf Fe³⁺ load is established by in vitro Tf Fe³⁺ saturation (elimination of Fe₀⁻ and Fe₁⁻Tfs by formation of Fe₂⁻Tfs).

below). At the same time, 9 (for the homozygous Tf type) or 18 (for the heterozygous Tf type) non-CDT isoforms with pIs similar to those of the CDT isoforms appear in parallel (Fig. 2). Because of this, the main problem in CDT analysis is the reliable separation of CDT isoforms from non-CDT isoforms (see *Analysis*).

Definition of CDT

Stibler and Kjellin (1) first reported the presence of Tf isoforms with pIs >5.7 in cerebrospinal fluid and serum from alcoholics. Increased amounts of these Tf isoforms appeared with high prevalence in serum from alcoholics and disappeared after abstinence, with a half-life of ~14 days (9, 12, 13). These Tf isoforms, corresponding to asialo-Fe₂-Tf, monosialo-Fe₂-Tf, and disialo-Fe₂-Tf, were later collectively referred to as CDT (9, 14).

TRISIALO-Fe₂-Tf AND CDT

There has been a debate as to whether there is an alcohol-induced increase of trisialo-Fe₂-Tf and/or a diagnostic benefit from including (parts of) of this isoform in CDT (7, 15–17). Dibbelt (18) recently demonstrated by HPLC that the trisialo-Fe₂-Tf concentration was statistically the same in serum samples with nonpathological and pathological CDT concentrations. Increased concentrations of the CDT isoforms were not associated in general with increased trisialo-Fe₂-Tf. Thus, Dibbelt stated that “trisialo-transferrin is obviously of no diagnostic value” and should not be included in CDT (18). This finding has been supported by two studies showing a diminished diagnostic accuracy (sensitivity) of so-called “trisialo-tests”, e.g., the %CDT-turbidimetric immunoassay (%CDT-TIA or %CDTri-TIA), which include ~50% of trisialo-Fe₂-Tf in CDT, compared with CDT tests that exclude this isoform from CDT, e.g., CDTECT (19) and ChronAlcoI.D. (this work group, submitted for publication).

Structure of Human Serum CDT

Serum samples from alcoholics showed decreased sialic acid content (20) and normal Tf isoform patterns after treatment with neuraminidase (complete removal of sialic acid residues from the Tf N-glycan chains) (13). Thus, a defective Tf carbohydrate structure, especially a sialic acid deficiency, in serum Tf from alcoholics was assumed. Subsequent investigations revealed not only missing sialic acid residues but also a lack of deeper links in the N-glycan chains (galactose, mannose, and N-acetylglucosamine) and an unaffected sequence of mannose, mannose, N-acetylglucosamine, N-acetylglucosamine (the latter bound directly to the polypeptide chain) (21). Recent studies showed that the main CDT isoforms, disialo-Fe₂-Tf and asialo-Fe₂-Tf, lack one or two complete glycan chains (22–24). According to Landberg et al. (22), disialo-Fe₂-Tf shows a single biantennary N-glycan chain with two sialic acid residues, whereas asialo-Fe₂-Tf has no carbohydrate structure (Fig. 1). Trisialo-Fe₂-Tf contains

two biantennary N-glycans, one with two terminal sialic acid molecules, the other with one terminal sialic acid and one terminal galactose (Fig. 2). Because of this, it is impossible for trisialo-Fe₂-Tf to form from pentasialo-Fe₂-Tf (containing one biantennary, disialylated N-glycan and one triantennary trisialylated N-glycan; Fig. 1) by loss of the biantennary N-glycan. Furthermore, the structure of monosialo-Fe₂-Tf, which is part of CDT, remains unclear. Landberg et al. (22) did not assess this isoform. Peter et al. (23) reported a fraction “Ib”, with molecular masses between fraction “Ia” (asialo-Fe₂-Tf) and “IV” (tetrasialo-Fe₂-Tf), low sialylation, and terminal galactose. The authors hypothesized that this fraction is a mixture “of transferrins with two N-glycan chains with shortened antennae” and that it “probably contains small amounts of transferrin with one N-glycan chain” (23). One might speculate that this fraction contains monosialo-Fe₂-Tf. It follows from these studies that the presence of biantennary, trisialo-Fe₂-Tf (22) and monosialo-Fe₂-Tf in serum after chronic alcohol abuse cannot be explained solely by the lack of one or two complete glycan chains. One might argue that monosialo-Fe₂-Tf is a quantitatively less important CDT isoform (7) and that trisialo-Fe₂-Tf is not a CDT isoform (18). Nevertheless, assessment of the structure of these Tf isoforms might be of value to further disclose the pathomechanisms of ethanol-induced increases in CDT. Summarizing the studies by Landberg et al. (22), Peter et al. (23), and recently by Henry et al. (25), it seems most likely that CDT isoforms can lack not only complete N-glycan chains (mainly asialo-Fe₂-Tf and disialo-Fe₂-Tf), but also parts of the N-glycan chains [as discussed previously by Stibler and Borg (21)]. If this is true, the production of specific CDT antibodies, which could be used in direct (homogeneous) CDT assays, becomes additionally complicated.

Pathomechanisms of Ethanol-induced CDT Increase

The pathomechanisms for the increase in CDT isoforms during chronic alcohol abuse are not completely understood at present. It is most likely that ethanol and/or its metabolite acetaldehyde affect N-glycan chain synthesis in the Golgi apparatus. Thus, Stibler and Borg (26) measured diminished activities of galactosyltransferase and N-acetylglucosaminyltransferase in serum from alcoholics. The addition of in vitro acetaldehyde to these samples further decreased the enzyme activities (26). Disulfiram (an antabuse for treatment of alcoholism), which inhibits aldehyde dehydrogenase, leading to an accumulation of serum acetaldehyde and subsequent nausea, did not affect serum CDT (27). Decreased incorporation of ³H-labeled leucine and N-acetyl-D-mannosamine into Tf, lower α2,6-sialyltransferase mRNA concentrations (because of destabilization by ethanol), decreased synthesis of α2,6-sialyltransferase, which is followed by lower sialyltransferase activity, and decreased sialylation of Tf in rats chronically fed alcohol were observed by Lakshman et al. (28). Xin et al. (29)

measured increased sialidase activity in liver plasma membranes and reduced sialyl, galactosyl, and *N*-acetylglucosaminyltransferases in Golgi homogenates from alcohol-treated rats. This loss in transferase activity was also observed when acetaldehyde was added to Golgi homogenates from normally fed control rats (29). In this connection, a finding by Fast et al. (30) is interesting. In assessing the role of the carbohydrate chains of sialyltransferase (EC 2.4.99.1) for enzyme activity, they observed diminished enzyme activity after partial removal of the *N*-glycans by *N*-glycanase. The presence of methanol or ethanol was necessary for complete deglycosylation. There was a correlation between the loss of catalytic activity of the enzyme and increased deglycosylation. One might speculate whether the diminished activities of sialyltransferase (galactosyltransferase and *N*-acetylglucosaminyltransferase) reported by Xin et al. (29) are attributable to incomplete carbohydrate structures of these enzymes and whether ethanol (or its metabolites) primarily affects the glycosylation of the carbohydrate transferases that are involved in Tf *N*-glycan synthesis. The results obtained by Lakshman et al. (28) point to primary effects of ethanol on sialyltransferase mRNA production.

In contrast to other glycoproteins, the lack of endstanding sialic acid residues in CDT isoforms does not cause accelerated hepatic clearance via the asialoglycoprotein receptor. Thus, the plasma half-life of CDT is ~14 days (9, 31–33), and that of Tf only ~7 days (34). Patients with liver cirrhoses, regardless of whether they were alcohol induced, showed normal arteriovenous CDT gradients (33). Because of this, ethanol-induced alterations of hepatic and/or renal CDT clearance seem to be unlikely as causes of increased CDT after chronic alcohol abuse. One reason for high CDT concentrations despite normal alcohol consumption is the carbohydrate-deficient glycoprotein (CDG) syndrome, a hereditary disorder of glycoprotein synthesis (35). Structural similarities between serum glycoproteins (Tf, α_1 -antitrypsin, and haptoglobin β chains) from patients with chronic alcohol abuse and patients suffering from the CDG syndrome were reported by Henry et al. (25). These findings suggest that alcohol-induced increases in CDT might also be attributable to an inhibition of the initial mannose-dependent steps of Tf *N*-glycan synthesis (25). Because some types of the CDG syndrome are attributable to deficiencies of phosphomannomutase (EC 5.4.2.8) or phosphomannose isomerase (EC 5.3.1.8), it might be interesting to measure the corresponding enzyme activities in patients with chronic alcohol abuse.

Our investigations revealed fluctuations between non-pathological and pathological CDT concentrations in serum from patients with combined kidney and pancreas transplantation and normal alcohol consumption. Patients with kidney transplantation alone always had CDT concentrations within reference values (36). It is known that pancreas transplantation causes hyperinsulinemia (by

connecting the endocrine pancreas to the blood system and thus avoiding the first-pass effect of the liver) and bicarbonate deficiency/metabolic acidemia (by draining the exocrine pancreas into the bladder). Because metabolic acidemia is common after alcohol abuse and persistent after chronic alcohol abuse, it might also be interesting to assess the acid-base balance and serum CDT from alcoholics in parallel.

CORRELATION BETWEEN SERUM CDT AND ALCOHOL INTAKE

Data concerning the correlation between the amount of ethanol consumed and serum CDT are inconsistent. This might be the result of ethical limitations on controlled drinking studies. According to Allen et al. (31), almost all studies on this subject are invalid except for those by Stibler and co-workers (13, 14) and Storey et al. (37). It is interesting that the critical alcohol intake [at least 50–80 g ethanol/day on 7 consecutive days (9)] for an increase in CDT is almost the same as for alcohol-induced liver cirrhosis (38).

Preanalysis

Several preanalytical conditions have been found to affect serum CDT concentrations. Stibler et al. (14) found that EDTA and heparin may disturb *in vitro* Fe³⁺-Tf saturation and/or anion-exchange microcolumn non-CDT and CDT isoform fractionation (substance-specific data not given). The authors also found that sample storage for 3 days at room temperature produced a 25% increase in CDT. In addition, lipemia can interfere with turbidimetric CDT assays. Delipidation reduces the serum CDT concentration by ~22% (14). Strong hemolysis can also lead to false-positive results (our unpublished data).

Preanalytical conditions that do not affect the serum CDT concentration include circadian serum CDT fluctuations [~8% (39); intraassay CV = 10% (11, 40, 41)]; collection of blood into containers with kaolin (coagulation accelerator) and/or polyacrylamide-gel separator (42); serum storage for ~30 h at room temperature (39), 7 days at 4 °C (39), and several months at –22 °C (14, 36, 39); repeated freezing and thawing (36, 39); diet (33); common drugs taken by patients of general practitioners (14, 43); and disulfiram (27).

Conditions that need to be studied include positioning of the patient during blood collection, duration of congestion, *in vivo* and *in vitro* drug effects, use of EDTA- or heparin-plasma, and stability of whole-blood samples.

Analysis

The analysis of CDT makes very high demands on selectivity, specificity, and sensitivity for three main reasons: (a) the distinct serum Tf microheterogeneity; (b) the distinct structural similarity of CDT and non-CDT isoforms; and (c) the low CDT isoform concentrations [<2.5 – 2.7% in healthy controls and $<20\%$ in alcoholics as measured by the ChronAlcoI.D. assay (44) in the presence of large

amounts of non-CDT isoforms with almost similar physicochemical properties].

CDT-specific reactions or CDT antibodies, and thus a homogeneous CDT assay, are not available at present. Thus, routine laboratory analysis of serum CDT requires separation of CDT from serum matrix constituents and from non-CDT isoforms. This can be achieved by chromatographic (e.g., anion-exchange) or electrophoretic (e.g., IEF) methods, using the different charges and pIs of CDT and non-CDT isoforms. As pointed out above, there are coexisting CDT and non-CDT isoforms with almost equal pIs, e.g., disialo-Fe₂-Tf (as the main CDT isoform), tetrasialo-Fe_{1N}-Tf, pentasialo-Fe_{1C}-Tf, and heptasialo-Fe₀-Tf (Fig. 2). To reduce the number of Tf isoforms occurring in the native serum sample and to preclude coexistence of CDT and non-CDT isoforms with equal pIs, CDT analysis usually starts with *in vitro* Tf Fe³⁺ saturation; this treatment establishes a uniform Tf iron load. Fe₁- and Fe₀-Tfs are transformed to Fe₂-Tfs, and only Fe₂-Tfs appear in the serum sample. The *in vitro* Tf iron saturation step is followed by fractionation of the CDT and non-CDT Tfs by electrophoresis or chromatography. Subsequently, the CDT isoforms are detected by immunological procedures. [CDT and non-CDT isoform separation can also be done after *in vitro* removal of Fe³⁺ from Tf by complexation with EDTA, which leaves Fe₀-Tfs as the only isoforms in serum. However, the selectivity of IEF is improved when focusing Fe₂-Tf isoforms (3)]. Regardless of which fractionation procedure and which immunoassay is used, a complete and stable Tf iron load is fundamental for reliable CDT analysis. Incomplete *in vitro* Fe³⁺ saturation or Tf iron loss during fractionation of the CDT and non-CDT isoforms inevitably leads to reformation of Fe₁- and/or Fe₀-Tfs, co-elution of CDT and non-CDT isoforms with equal pIs but different sialic acid and iron content, and overdetermination of CDT (3). This point must be assessed when developing (and launching) a new CDT analytical method (11, 40).

ELECTROPHORETIC METHODS

Because of its high selectivity, IEF is used as the reference method for serum Tf isoform analysis. The Tf isoforms are separated in a gel containing a pH gradient according to their characteristic pIs. Again, the first analytical step is *in vitro* saturation of Tf with Fe³⁺. After electrophoresis, the Tf bands are visualized by immunofixation and staining of the CDT-anti-Tf complexes (3). Finally, the Tf band patterns can be evaluated by densitometry. It should be taken into account, however, that application of a sufficient volume of serum for detection of CDT isoforms to the gel (1 μL diluted 400-fold) produces an overload of tetrasialo-Tf. It follows from this that the intensity of the main Tf fraction does not correlate with the amount of Tf focused in this band (3). This complicates quantitative evaluation of this and the other Tf fractions. From the analytical point of view, this drawback can be overcome by use of ratios of the different CDT isoforms, e.g.,

disialo-/asialo-Fe₂-Tf (11, 40, 45, 46). However, absolute CDT concentrations can also be obtained by IEF with a calibration curve generated by different amounts of asialo-Fe₂-Tf (47). Many IEF methods suggested for quantitative CDT analysis suffer from incomplete documentation of detection limits, recovery (e.g., by Western blotting), intra- and interassay CVs, and correlation between peak height (densitometry) and amount of the Tf isoform.

Because of its high selectivity, IEF can detect genetic Tf variants without *in vitro* neuraminidase treatment (10, 11). The latter is done for complete removal of Tf isoform sialic acid residues and thus formation of only asialo-Tfs (10, 13, 47). Assuming complete Tf iron load and Tf sialic acid loss, only one band of asialo-Fe₂-Tf would be detected in serum with a homozygous Tf type (e.g., Tf-C1), but two bands would be detected in serum with a heterozygous Tf type (e.g., Tf-CD or Tf-CB). IEF is suitable for visualization of Tf isoform patterns in the anion-exchange microcolumn eluates of (commercial) CDT tests. It is therefore an essential method for testing the reliability of the initial fractionation step used to separate CDT and non-CDT isoforms in current and upcoming CDT assays based on anion-exchange chromatography (11, 40). IEF is recommended for assessing unexpected CDT values (40, 48). IEF of serum Tf isoforms can also be used for diagnosis of the CDG syndrome [for a review, see Jaeken and Carchon (35)].

In addition to IEF in flat gels, capillary electrophoresis (49–51) and capillary zone electrophoresis (15, 52, 53) have been proposed for analysis of Tf isoforms. The main problem with these techniques is coating of the capillary surface to prevent protein adsorption and finding a coating-compatible, highly ultraviolet-transparent buffer (53). Only a tradeoff between these criteria is achievable at present. Thus, it is questionable that Tagliaro et al. (53) used uncoated (bare fused-silica) capillaries in their study without assessing the accuracy of the method, which was used for quantitative analysis of CDT.

Recently, Crivellente et al. (15) described improved analytical sensitivity and separation power of the capillary zone electrophoresis method described by Tagliaro et al. (53). The improved sensitivity and separation were obtained mainly by the addition of diaminobutane to the running buffer and an increase in column length (15). Nevertheless, the selectivity and sensitivity of capillary electrophoresis are still lower than IEF. When Tagliaro et al. (53) analyzed a serum sample with 61 units/L CDT (CDTect assay), indicating strong chronic alcohol abuse, asialo- (the second most common CDT isoform) and monosialo-Tf were not detected. Even with their improved method, Crivellente et al. (15) could not detect asialo-Fe₂-Tf. In contrast, IEF of serum samples with only 25 units/L (CDTect assay; cutoff, 18 units/L) clearly detected bands for mono- and asialo-Fe₂-Tf (36). Lectin affinity electrophoresis has been described for assessing Tf microheterogeneity in patients with alcoholic liver

disease (24). Whether these methods can gain wide acceptance for routine CDT analysis is still unclear.

CHROMATOGRAPHIC METHODS

Compared with IEF, chromatographic CDT methods are less sensitive (sample volumes of 100–500 μ L are needed) and less selective. Thus, genetic Tf variants cannot be detected by anion-exchange chromatography followed by immunoassay (a procedure usually used by commercial CDT tests). Using a HPLC method described by Jeppson et al. (54), Simonsson et al. (55) could reliably detect genetic Tf variants in serum from healthy blood donors. Thus, HPLC can also be used for assessing odd CDT values obtained by anion-exchange chromatography/immunoassay. However, IEF is still superior to HPLC in detecting and phenotyping genetic Tf variants with pI values close to that of tetrasialo-Fe₂-Tf, found in the most common Tf-C1 phenotype. Analysis times of ~20 min/sample (54–56) and a time-consuming HPLC column regeneration (54) further reduce the applicability of HPLC for large CDT analysis series.

Recently, Yoshikawa et al. (57) described a lectin affinity chromatography method for determination of CDT. Using Sepharose columns coated with *Allomyrina dichotoma* (Allo A) or *Trichosanthes japonica* (TJA) lectins, the authors were able to separate CDT-Allo A (corresponding to disialo-Tf) and CDT-TJA (corresponding to

asialo-Tf) fractions from serum. The diagnostic efficiency obtained with these fractions was higher than that of the %CDT-TIA (Axis) including trisialo-Fe₂-Tf.

COMMERCIAL CDT TESTS

In 1993, the first commercial CDT test (CDTect-RIA; Pharmacia & Upjohn) was introduced, followed by %CDT (Axis) and the CDTect enzyme immunoassay (CDT-EIA; Pharmacia & Upjohn). These methods use the common CDT definition when analyzing asialo-, monosialo-, and disialo-Fe₂-Tf. Later, a test incorporating part (50%) of the trisialo-Fe₂-Tf in CDT was developed by Axis and distributed by Axis and Bio-Rad (as the %CDT-TIA and %CDTri-TIA, respectively) and Roche (Tinaquant-%CDT/transferrin). Except for one HPLC method by Recipe, currently available commercial CDT tests, even the relatively new ChronAlcoI.D. assay, are based on fractionation of CDT and non-CDT isoforms on anion-exchange microcolumns. Table 1 lists the analytical specificities of commercial and noncommercial CDT tests and the corresponding test-specific cutoff values for CDT indicating chronic alcohol abuse.

NEED FOR STANDARDIZATION OF CDT ANALYSIS

The availability of CDT assays for routine laboratory diagnosis increased and accelerated the acceptance of CDT as one of the most specific markers of chronic alcohol

Table 1. Test-specific cutoffs or borderlines for serum CDT concentration indicating chronic alcohol abuse.

Method	Women	Men	Analytical specificity	Reference(s)
Anion-exchange chromatography and RIA (original Stibler method)	74 mg/L	74 mg/L	Asialo-, mono-, and disialo-Tf	(14)
Anion-exchange chromatography and RIA (CDTect-RIA; Pharmacia; modified Stibler method)	26–28 units/L	18–20 units/L	Asialo-, mono-, and disialo-Tf	(9, 41)
Anion-exchange chromatography and EIA (CDTect-EIA; Pharmacia)	26–28 units/L	18–20 units/L	Asialo-, mono-, and disialo-Tf	(9, 41)
CDTect:Tf ratio (calculated)	1.3%/1.0%	1.3%/0.6%	Asialo-, mono-, and disialo-Tf	(69)/(36)
Anion-exchange chromatography and RIA (%CDT; Axis)	2.5%	2.5%	Asialo-, mono-, and disialo-Tf	Manufacturer's test instructions
Anion-exchange chromatography and TIA (%CDT-TIA or %CDTri-TIA; Axis)	5–6%	5–6%	Asialo-, mono-, disialo-, and 50% trisialo-Tf	Manufacturer's test instructions
Anion-exchange chromatography and TIA (Tina-quant %CDT/transferrin; Roche)	6%	6%	Asialo-, mono-, disialo-, and 50% trisialo-Tf	Manufacturer's test instructions
Anion-exchange chromatography and TIA (ChronAlcoI.D.; Sangui)	2.5–27% 100–110 mg/L	2.5–2.7% 100–110 mg/L	Asialo-, mono-, and disialo-Tf	(44) (44)
Capillary zone electrophoresis	3%	3%	Asialo-, mono-, and disialo-Tf	(52)
HPLC	80 mg/L	80 mg/L	Asialo-, mono-, and disialo-Tf	(56)
HPLC	0.8%	0.8%	Asialo-, mono-, and disialo-Tf	(54)
HPLC (Clin-Rep-CDT im Serum; Recipe)	1.75–2.5%	1.75–2.5%	Asialo- and disialo-Tf	Manufacturer's test instructions
IEF-immunoblotting-laser densitometry	4 DU ^a	4 DU	Asialo-, mono-, and disialo-Tf	(10)
IEF-immunofixation	4.4%	4.4%	Asialo-, mono-, and disialo-Tf	(46)
IEF-Western blotting	100 mg/L	100 mg/L	Asialo-, mono-, and disialo-Tf	(47)
Lectin affinity chromatography	1.4%	1.4%	CDT-Allo A (separated on <i>A. dichotoma</i> agglutinin Sepharose columns)	(57)
Lectin affinity chromatography	1.3%	1.3%	CDT-TJA (separated on <i>T. japonica</i> agglutinin I Sepharose columns)	(57)

^a One DU is defined as 1% of the ratio of CDT:Tf exhibited by the strongly positive control run in each gel (10).

abuse to date. At present, ~200–300 reports on CDT have been published in impact factor-listed journals. However, the definition of CDT is increasingly vague (which Tf isoforms are CDT isoforms, i.e., asialo-, monosialo-, disialo- and/or trisialo-Fe₂-Tf; Table 1). Standardization of CDT analysis by an international CDT standard and quality-control material has not been attained [although it is possible to generate CDT enzymatically (55, 56, 58)]. Thus, different Tf isoforms are analyzed, with various recoveries, as CDT (Table 1), which exacerbates the already poor comparability of CDT values, diagnostic specificities and sensitivities, and predictive values obtained in different clinical settings and among different populations with different CDT analytical methods. To overcome this problem, redefinition of CDT (59) or consistent use of the common CDT definition introduced by Stibler (9) is needed. As a result of an international meeting on standardization of CDT analysis (in Berlin, May 2000), the aim is to develop a highly sensitive HPLC for analysis of CDT as a standard method (e.g., for calibration of CDT test-clinical analyzer applications) and to cease producing so-called “trisialo tests”.

The lack of standardization of CDT analyses should not discourage the use of CDT, or encourage the use of less specific markers, e.g., liver enzymes or uric acid. Hemoglobin A_{1C}, which is widely used as an integrative long-term marker of blood glucose concentration, is far from being well standardized. Nevertheless, measurement of an individual's hemoglobin A_{1C} concentration improves therapy for diabetes.

Medical Interpretation (Postanalysis)

NORMAL RANGES FOR SERUM CDT

Because of different analytical specificities and recoveries, normal ranges (reference values or cutoffs) for absolute and relative serum CDT are method-dependent. CDT values must always be interpreted with regard to the test-specific decision criteria (Table 1). Therefore, the laboratory should report the CDT value, the cutoff value, and the method of analysis. Such information is common in forensic drug analysis; it makes the comparison of reports from different laboratories much easier, is needed in follow-up studies, and should also be provided for CDT analyses. Changing the CDT test can cause a sharp increase or decrease of CDT values (Table 1), which can lead to a misinterpretation of the actual drinking status. Thus, it is valuable to announce an upcoming change in the CDT test and to give the CDT results by the “old” and the “new” CDT assay for a suitable time period in parallel. Taking into account the social consequences of high CDT values, it is advisable to use borderline values (95th percentile plus analytical imprecision) instead of cutoffs (44) (Table 1). Relatively constant serum CDT concentrations have been found in healthy persons, in patients with non-alcohol-related liver diseases, or during

abstinence (60, 61). Individual reference ranges were valuable for follow-up during alcohol withdrawal treatment (62).

GENDER DEPENDENCE OF SERUM CDT

Absolute serum CDT concentrations from healthy women typically are higher than those of healthy men (9, 31, 63–66). Recently, de Feo et al. (67) reported no gender dependence of serum CDT in nonabusers and alcohol abusers, but a positive correlation between CDT and Tf in nonabusers. The latter was also reported by van Pelt and Azimi (68). Because females frequently have subclinical iron deficiency and thus increased Tf concentrations, this might explain their higher CDT concentrations. However, these findings are surprising because it is commonly accepted that CDT and Tf do not correlate (see below). The reason for higher CDT concentrations in healthy women compared with healthy men remains unclear (63, 64), in spite of the findings of de Feo et al. (67) and van Pelt and Azimi (68). Serum CDT:Tf ratios are not gender-dependent [(44, 54, 69), and manufacturers' test instructions]. No correlation has been found between CDT and the menstrual cycle, serum estradiol, serum iron, or contraception (65, 66). Compared with premenopausal women, serum CDT was slightly increased (17.5 vs 19.3 units/L) in menopausal women receiving estrogen therapy (65, 66). Whether this slight difference is of practical importance [CVs of the CDT tests of ~10% must be considered (11, 40, 41)] and whether an association among menstrual function, CDT, and drinking behavior does really exist, as assumed by Oslin et al. (70), needs further investigation. Data pointing to a relationship between the amount and frequency of menstrual blood loss and CDT were recently presented by Leusink et al. (71).

Increased serum CDT concentrations during pregnancy have been reported. However, serum CDT did not exceed the gender- and CDTECT test-specific borderline of 26–28 units/L even in the third trimester (65, 66). Interestingly, de Jong and van Eijk (72) found a steady increase of tetrasialo-Tf and higher sialylated Tf isoforms and unaltered concentrations of sialic acid-deficient Tf isoforms in pregnant women during the second and third trimesters and also in women using contraceptives. In accordance with these observations, the CDT:Tf ratio should decrease (and not increase as usually discussed) in these women. This could be one reason for the diminished diagnostic sensitivity of serum CDT measurement in women compared with men (see below). Further efforts, in addition to those described by Mårtensson et al. (7), should be applied to identification of the Tf isoform patterns in healthy men and (pregnant) women. Another underlying cause can be gender-dependent differences in the drinking patterns and in the vulnerability of the liver to alcohol intoxication, as discussed recently (73).

CORRELATION WITH TOTAL Tf

Except for two recent studies (67, 68), no significant correlation between serum CDT and total Tf has been found (9, 14, 74, 75). A slightly improved diagnostic sensitivity was reported by Schellenberg and Weill (45, 76), who used a "Tf-index", referred to as the ratio of Tf isoform with a pI of 5.7 to the isoform with a pI of 5.4 (disialo-Fe₂-Tf/tetrasialo-Fe₂-Tf). Determining this ratio requires IEF, which is sometimes laborious and requires experienced personnel. This might be one reason why the Tf-index has not gained much acceptance. Automated IEF, such as that described by Hackler et al. (3) and Arndt and co-workers (36, 46), should be helpful in further evaluating the Tf-index. For routine purposes, the Tf-index has been replaced by the CDT:total Tf ratio. Whether this ratio increases the diagnostic accuracy compared with absolute CDT concentrations (and in each situation) is controversial (7, 19, 31, 44, 48, 77–81). Contradictory results were found in a single study using CDT:Tf ratios (67), which demonstrated improved diagnostic specificity in patients with iron-deficiency anemia but no decrease in false-negative results in patients with alcohol abuse. Similar diagnostic specificities for absolute CDT concentrations (units/L or mg/L) and CDT:Tf ratios were described by Lieber (48) and Helander (81). The main advantage of the CDT:Tf ratio is that it improves the diagnostic specificity of CDT in patients with increased Tf (although it is generally assumed that CDT and Tf do not correlate), e.g., for patients with iron deficiency (reduced iron stores) or iron-deficiency anemia but normal alcohol intake (67). However, low total serum Tf concentrations (e.g., from acute or chronic infection, hemochromatosis, nephrotic syndrome, or cancer) can produce both false-negative [e.g., in hemochromatosis (67)] and false-positive results. Thus, Lieber (48) found in some liver disease patients increased CDT:Tf ratios that were attributable to decreased serum Tf concentrations. Additionally, CDT:Tf ratios are less precise than absolute CDT concentrations (the CV of the CDT:Tf ratio is the sum of the CV of the CDT concentration plus the CV of the total Tf). Assuming a CDT concentration of 100 mg/L and a total Tf concentration of 4000 mg/L, the CDT:Tf ratio is 2.5%. Because of the analytical imprecision, which is ~10% for CDT and Tf, the CDT:Tf ratio shows an imprecision of at most 20% and varies between 2.2% (90 mg/L CDT, 4040 mg/L Tf) and 2.8% (110 mg/L CDT, 3960 mg/L Tf). When borderlines of 2.5–2.7% (CDT:Tf) and 100–110 mg/L (CDT) are used, the CDT:Tf ratio changes among normal (2.2%), borderline (2.5%), and increased (2.8%). At the same time, the absolute CDT concentration does not exceed the upper limit of the borderline. Knowing this is important for interpretation of CDT values in longitudinal studies or for control of relapse drinking. Our investigations revealed that measuring (and interpreting) absolute and relative CDT concentrations as well as the total serum Tf concentration improves the diagnostic efficiency of CDT as a marker of chronic alcohol abuse (unpublished data). It

might be interesting to reevaluate the data from the many clinical studies, taking into consideration absolute serum CDT concentrations, CDT:Tf ratios, and total serum Tf concentration.

CDT AND γ -GLUTAMYLTRANSFERASE

In contrast to CDT analysis, measurement of γ -glutamyltransferase (γ -GT) activity is highly standardized, automated, and inexpensive. Because of this, γ -GT is the most commonly used marker of alcohol abuse other than ethanol. It is obvious to ask whether the determination of γ -GT alone is sufficient and whether CDT values are really needed for laboratory diagnosis of alcohol abuse. Many studies on CDT investigated γ -GT activities as well. The correlation between γ -GT and CDT, the diagnostic sensitivities and specificities of CDT and γ -GT, and the diagnostic efficiencies of considering γ -GT and CDT were studied alone or conjointly. Reviews of the literature up to 1994 (82) and between 1966 and November 1998 (83) have been published. Since then, several studies on these subjects have appeared. Because of the different clinical settings with many different populations varying in sex, age, drinking amounts and patterns, and clinical backgrounds as well as the multiple analytical and statistical methods used, it is almost impossible to draw general conclusions from this vast amount of data: There was no distinct correlation between absolute or relative serum CDT concentrations and γ -GT (9, 75, 84–87). Therefore, parallel analysis of CDT and γ -GT makes sense; this analysis revealed additional diagnostic information for follow-up of alcoholics with normal γ -GT activities (84), for brief intervention among heavy drinkers in primary healthcare (88), for evaluating progress of patients in treatment for alcoholism (73), for assessing alcohol consumption in a general medical clinic (89), for monitoring alcohol consumption in men drinking 20–60 g of ethanol/day (90), and for detecting heavy alcohol consumption in female alcoholic outpatients and college students (91). As a general conclusion, CDT is currently the more specific and γ -GT the more sensitive marker of chronic alcohol abuse. The latter is true especially for women (70, 73, 91), but it is not clear whether it is related strictly to gender or to the higher vulnerability of a woman's liver to alcohol-induced damage (73).

Compared with CDT, γ -GT produces false-positive results regarding chronic alcohol abuse in many states of disease, e.g., obstructive liver disease, posthepatic obstruction, hepatitis, fatty liver, liver cirrhosis, liver cell carcinoma and liver metastases, cardiac insufficiency, mononucleosis, renal transplant, hyperthyroidism, myotonic dystrophy, diabetes mellitus, and pancreatitis (92, 93). In contrast to CDT (14, 43), γ -GT is highly affected by several medications and drugs of abuse, e.g., barbiturates, cephalosporins, estrogens, oral contraceptives, phenytoin, primidone, thyrostatics, anabolic steroids, phenothiazines, and antirheumatics (92, 93).

Table 2. Clinical conditions, regardless of the number of observations, that caused false positives when serum CDT was used as a marker of chronic alcohol abuse.

Diagnoses	Differential Diagnosis	Comments
CDG syndromes (9, 102) (new nomenclature: congenital disorder of glycosylation)	Distinct somatic (already post-natal) and psychological alterations (35); IEF of Tf isoforms (102)	Depending on the CDG syndrome type, the Tf isoform band patterns are similar to (25) or different from (103) those found in alcoholics; whether healthy parents can have (slightly) increased CDT [5 fathers and 1 mother in (102)] despite normal alcohol intake needs further investigation; it might have serious social consequences for such persons (who are obviously somatically and psychiatrically inconspicuous)
Genetic Tf-D variants (9, 10, 104)	IEF of Tf isoforms from in vitro Tf Fe ³⁺ - saturated, neuraminidase-treated (fully desialylated) serum samples	Because of analytical interferences, not by every Tf-D subtype and dependent on the CDT test (11)
Primary biliary cirrhosis (9, 105)	Mitochondrial autoantibodies (105); histology	
Chronic active hepatitis (9, 106)	Hepatitis antigens and antibodies by immunoassays; hepatitis viruses by PCR	CDT shows an overall reduced diagnostic accuracy in patients with advanced liver disease (48), decompensated liver cirrhosis, or end-stage liver disease (107)
Liver cell carcinoma (9, 79)	α -Fetoprotein; p53 tumor suppressor gene; histology	
Iron deficiency/and anemia (67)	Serum iron; Tf; ferritin, hemogram; erythrocyte indices	Not observed by Stibler et al. (14); CDT:Tf ratios of the same patients were normal (67)
Hemochromatosis, especially during intensive iron depletion (108)	Serum iron; Tf; ferritin	Temporary increase of CDT and CDT:Tf ratio, exceeding the gender-specific cutoffs for only 2 men and 1 woman; increase significantly correlated with intensity of iron mobilization from the liver, rather than with reduction of total body iron overload
Pancreas + kidney transplant (36)	Case history	Not in patients with kidney transplant alone (36)
Hypertension (109)	Blood pressure measurement; personal reviews (on medication)	Of 435 patients, 70 showed increased CDT, and of these 14 excessive alcohol use; generalization of the results not possible because mean age of 69.7 ± 4.7 years and definition of excessive alcohol as >24 g/day; discernible effects of hypertension on serum CDT concentrations were not confirmed by Whitfield et al. (85)
Cystic fibrosis (110)	Sweat test	No asialo-Fe ₂ -Tf in controls and patients detected, disialo- Fe ₂ -Tf increased: healthy individuals $0.735\% \pm 0.238\%$, patients $0.954\% \pm ?^a$ of total Tf (mean \pm SD), mean serum CDT concentration, 18.6 units/L (CDTect; cutoff <20 units/L) for male patients (n = 6) and 18.3 units/L (CDTect; cutoff <26 units/L) for female patients (n = 6); alcohol case history not assessed for controls; needs reevaluation
Patients with neuropsychiatric disorders (14); individual cases with lung, pancreas, and heart diseases without alcohol etiology and malignoma (111, 112)		Specific studies on cardiac congestion, acute cardiac infarction, coronary heart disease, stroke, chronic obstructive lung disease, asthma, pneumonia, nonalcoholic pancreatitis, and malignant disorders are not available at present
Individual case of a 41-year- old patient with achondroplasia (113)	Radiology	No causal link between achondroplasia and disturbed N- glycosylation of proteins detected

^a ?, not given.**DIAGNOSTIC EFFICIENCY OF CDT**

Numerous clinical trials have been published regarding the diagnostic specificity and sensitivity of serum CDT as a marker of chronic alcohol abuse. Several reviews have been published (9, 31, 82, 94, 95). A systematic review of CDT comparing CDT and other markers of alcoholism based on a MEDLINE database from 1966 to November 1998 was provided by Salaspuro (83). Since then, many

new reports have appeared in journals listing impact factors. Most of these reports have already been discussed here. It is not the aim of this review to compare the diagnostic criteria (e.g., specificities, sensitivities, predictive values, ROC plots) obtained for the different analytes (e.g., γ -GT and CDT) in different populations (e.g., differences in sex, ethnicity, hospitalization, and no/mild/moderate/heavy/chronic alcohol abuse) by different

CDT methods (e.g., electrophoresis, chromatography, absolute/relative CDT concentrations, and different definition of the CDT group). For calculating parameters of diagnostic efficiency, reliable data regarding individual alcohol consumption are a prerequisite. Thus, for classifying false positives and true negatives, an objective "gold standard" is needed that determines the individual alcohol consumption without any error. Clinical background or personal reviews are customarily used for this purpose. However, if both were faultless, we would not need laboratory markers of chronic alcohol abuse. Because underestimating alcohol consumption is common, false true positives or false false negatives will occur and markedly affect the criteria of diagnostic efficiency of CDT obtained in a specific clinical study. Additionally, in one study, diagnostic sensitivities and specificities, and positive and negative predictive values varied by 12% when serum CDT concentrations for the same healthy controls and hospitalized patients were analyzed by different CDT tests (96). This should be considered when interpreting parameters of diagnostic efficiency.

DIAGNOSTIC SPECIFICITY OF CDT

The main causes for false-positive results in CDT analysis have been described by Stibler and co-workers (9, 14). Conditions currently known to potentially cause false positives and thus decrease the diagnostic specificity of CDT are summarized in Table 2. In interpreting these data, one should not generalize findings made on some patients of small (and in part highly selected) populations (see comments in Table 2). Altogether, there are fewer sources of false positives when CDT is used to monitor alcohol abuse than γ -GT. Some of these sources are relatively easy to differentiate from alcohol history (Table 2). Diagnostic specificities of CDT obtained in several different clinical studies have been published (9, 31, 82, 83, 94).

In summary, CDT is still the most specific laboratory marker of chronic alcohol abuse.

DIAGNOSTIC SENSITIVITY

There are conditions that can affect the diagnostic sensitivity of CDT as a marker of chronic alcohol abuse (Table 3). Differences in the drinking patterns during an appropriate period before blood sampling (long-term drinking pattern) and within the individual days of this period (short-term drinking patterns) must be assessed. Because the half-life of CDT is ~14 days, at least 14 days (4 weeks is better) before blood collection must be assessed. The results of a 3-week drinking experiment showed that chronic consumption of small amounts of alcohol affected serum CDT concentrations [confirmed by Whitfield et al. (85)], but short-time drinking of larger amounts do not (97). Because of ethical considerations, long-time drinking experiments are difficult to perform. However, it is known that ethanol blood concentrations are affected not only by the amount of alcohol consumed, but also by the

Table 3. Conditions reported to affect diagnostic sensitivity of serum CDT concentration as a marker of chronic alcohol abuse.

Variable	Findings/Comments
Age	<p>Reduced alcohol-dose CDT-response curve for women older than 50 years compared with younger women, no effect for men between 35 and 55 years (85)</p> <p>Significantly higher sensitivity in men older than 40 years (114)</p> <p>No correlation between serum CDT concentration and age (86, 98)</p>
Drinking patterns	<p>Amount of daily alcohol intake significantly affects serum CDT concentration, e.g. see Whitfield et al. (85)</p> <p>Long-term drinking of small alcohol amounts affected serum CDT concentration, short-term drinking did not (85, 97)</p> <p>Duration of abstinence before blood sampling affects serum CDT concentration, e.g. see Mundle et al. (86)</p> <p>Higher sensitivity in alcohol dependency compared with high alcohol consumption without alcoholism (117)</p>
Body mass	<p>Significant positive correlation between CDT and glucose disposal ($\mu\text{mol}/\text{min} \times \text{kg}$ of body mass) (98)</p> <p>Changes in body mass during the last 2 years did not correlate with serum CDT concentration (98)</p> <p>Reduced alcohol-dose CDT-response curve in patients with high body mass index (87); assessing the alcohol intake as $\text{g}/\text{day} \times \text{body mass}$ might rule out increased alcohol distribution volumes of these patients</p>
Hypertension	<p>Increased alcohol-dose CDT-response curve in patients with diastolic blood pressure >90 mmHg, no effect of diastolic blood pressure <90 mmHg (109)</p>
Smoking	<p>Higher diagnostic sensitivity of serum CDT concentration in smokers, most distinct in patients with alcohol dependence history (85)</p> <p>Significant positive association between smoking and mean CDT, mainly attributable to interaction between smoking and alcohol intake effects (85)</p> <p>Maternal serum CDT concentrations had a significant negative correlation with cigarette smoking (116)</p>
Sex	<p>Distinctly lower diagnostic sensitivity of serum CDT concentration from women compared with that of men (see "Gender Dependence of Serum CDT")</p>

amount per time period and by the body mass (distribution volume): the shorter the period in which a gram of alcohol is consumed and the lower the body mass, the higher the peak blood alcohol concentration. Thus, one might hypothesize that chronic short-term consumption of high amounts of alcohol by a lean person causes high peak blood alcohol concentrations and substantial liver injury, and thus affects serum CDT concentrations or the dose-response curve. A relationship between CDT insulin sensitivity and hypertension in men of a very specific patient sample were discussed by Fagerberg et al. (98).

The main concern about CDT as a marker of chronic alcohol abuse is its relatively low diagnostic sensitivity.

Sensitivities of >90% and up to 100%, as reviewed by Stibler (9), led to great hopes for CDT. Subsequent studies, reporting distinctly lower diagnostic sensitivities, could not meet these expectations [reviewed in Refs. (31, 83)]. Recently, a diagnostic sensitivity of 0% for women with or without liver diseases was reported (99). The authors' final statement that their data "underline the insufficient specificity of CDT" and that "it is not justified to base any medical decision on the measurement of CDT concentrations, . . . even more for forensic decisions" cannot be drawn from their own data. Thus, diagnostic specificities of 83.6% and 94.2% for men with or without liver disease and 96.9% and 91.9% for women are distinctly better than those found for γ -GT: 36.1% and 24% for men and 36.6% and 50% for women. The shortcomings of this study were discussed by Allen and Sillanaukee (100) and Arndt et al. (101). Except for this study, diagnostic sensitivities of 30–50% for women and 50–70% for men, depending on factors such as population, drinking patterns, and daily alcohol intake, seem to be a good average.

Conclusions

Our knowledge of in vivo and in vitro effects on serum CDT is poor but necessary for reliable CDT analysis. CDT analytical methods with different analytical specificities and recoveries decrease the comparability of CDT values and statistical parameters of the diagnostic efficiency of CDT obtained in different studies. A unique definition for and standardization of CDT are needed. CDT is not a screening tool for detection of increased alcohol consumption (and it was originally not aimed as such). CDT is the most specific marker of chronic alcohol abuse to date. Diagnosis of chronic alcohol abuse should always be made based on a clinical background, questionnaire, CDT, and γ -GT, and not on a single CDT value alone. Efforts should be directed toward the preanalysis of CDT, a uniform definition of CDT, and standardization of CDT analysis.

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