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ScienceDirect
Journal of Chromatography B, 841 (2006) 96–109
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Review

Carbohydrate-deficient transferrin (CDT) as a marker of alcohol abuse: A critical review of the literature 2001–2005☆

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Received 26 February 2006; accepted 4 May 2006
Available online 24 May 2006

Abstract

The diagnosis of alcohol abuse based on objective data is a necessary requirement in both clinical and forensic environments. Among the different biomarkers of chronic alcohol abuse, carbohydrate-deficient transferrin (CDT) is world wide recognized as the most reliable indicator. However, several problems about the real meaning of CDT and the reliability of its use for the diagnosis of alcohol abuses are still open, as reported by numerous research articles and reviews. The present article presents a critical review of the literature on CDT appeared in the period from 2001 to 2005 (included). The article is organized in the following sections: (1) introduction, (2) definition and structure of human serum CDT, (3) pathomechanisms of the ethanol-induced CDT increase, (4) preanalysis, (5) analysis, (6) data interpretation, (7) review papers, (8) conclusions. As many as 127 papers appeared in the international literature and retrieved by the search engines PubMed and Scopus are quoted.

Keywords: Carbohydrate deficient transferrin; Alcohol abuse; Biomarkers; Analysis

Contents

Abbreviations: CDT, carbohydrate-deficient transferrin; CSF, cerebrospinal fluid; CDGS, carbohydrate-deficient glycoprotein syndrome; CZE, capillary zone electrophoresis; DAB, diaminobutane; ESI, electrospray ionization; FDA, food and drug administration; EIA, elisa-immunoassay; GGT, gamma-glutamytransferase; HPLC, high performance liquid chromatography; ICP-MS, inductively coupled plasma mass spectrometry; MALDI, matrix assisted laser desorption ionization; MCV, mean corpuscolar volume; MS, mass spectrometry; RIA, radio-immunoassay; Tf, transferrin; TIA, turbidimetric-immunoassay; TOF, time of flight; VH-CDT, vitreous humour CDT

☆ This paper is part of a special volume entitled "Analysis of proteins, peptides and glycanes by capillary (electromigration) techniques", dedicated to Zdenek Deyl, guest edited by I. Miksik.

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doi:10.1016/j.jchromb.2006.05.005

1. Introduction

The objective diagnosis of alcohol abuse has a high relevance in different areas of clinical and forensic medicine, including gastroenterology, cardiology, neurology and psychiatry, occupational and traffic medicine, forensic pathology, etc.

In addition to the traditional diagnostic approach based on anamnestic information, clinical examination, questionnaires and a few clinical biochemistry indicators (liver enzymes, mean corpuscolar volume), in the recent years much attention has been paid to new markers of alcohol abuse, such as acetaldehyde protein adducts, fatty acid ethyl esters, 5-hydroxytryptophol, among which carbohydrate-deficient transferrin (CDT) has undoubtedly gained a neat prevalence in popularity. CDT indicates collectively a group of minor isoforms of human transferrin with a lower degree of glycosylation in comparison to the major isoform of this glycoprotein. On the basis of a sound body of literature, CDT serum concentration is closely correlated with the chronic intake of alcohol. Since its discovery in 1978 [1], CDT has soon become the objective of research in both pre-clinical and clinical areas and later has been widely used as the most reliable objective marker of chronic alcohol abuse. Although the prevalent literature supports the correlation between alcohol intake and CDT concentrations, several issues about the real meaning of CDT and the reliability of its use for the diagnosis of alcohol abuses are still open, as reported in several research articles and reviews. Because of the important personal and legal consequences of the diagnosis of alcohol abuse, it is important to follow periodically the scientific debate and the recent research achievements on these subject, in order to provide researchers and practitioners with updated information on this diagnostic tool.

A recent search on PubMed (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi) and Scopus (http://www.scopus.com/scopus/home.url) covering the period 1986–2005, using "carbohydrate deficient transferrin" as search item, found 535 and 671 articles, respectively. Among them, several reviews have been published concerning the different aspects of CDT analysis and interpretation. In particular, a review published by Arndt in 2001, covering the literature from 1976 to 2000, has become a fundamental reference paper in this time frame [2]. Consequently, the present work has been focused on the literature on CDT appeared in the period from 2001 to 2005 (included). Following the organization of Arndt's review, this article is divided in sections and sub-sections according to the different pre-analytical, analytical and interpretative aspects of CDT as a marker of alcohol abuse.

2. Definition and structure of CDT

Human transferrin (Tf) is a serum iron transporting protein composed of 679 amino acids with two potential glycosylation sites at Asn-413 and Asn-611, which usually bind two bi- and/or triantennary carbohydrate chains of variable composition, containing four different carbohydrates (*N*-acetylglucosamine, mannose, galactose and sialic acid terminals). Sialic acid is the only charged residue in these saccaride chains, and, when present, bears a negative charge. The major glycoform of transferrin contains two biantennary *N*-glycans with a total number of four sialic acid residues (tetrasialo-Tf, p*I* 5.4), but minor isoforms with two (disialo-Tf, p*I* 5.7), three (trisialo-Tf, p*I* 5.6), five (pentasialo-Tf, p*I* 5.2) and six (hexasialo-Tf, p*I* 5.0) sialic residues have been identified in normal human serum. Also, traces of asialo-Tf and monosialo-Tf can be determined in serum.

The transferrin glycoforms collectively referred to as CDT, on the basis of a large scientific consensus, include the isoforms with p*I*s $\geq$ 5.7, i.e. asialo-Tf, monosialo-Tf and disialo-Tf. However, since some immunometric methods for CDT analysis commercially available included 50% of trisialo-Tf, the inclusion of this glycoform in CDT definition was, in the past, proposed.

This point represented the subject of a fierce debate which took place in the years preceding the time covered by this review, which ended in the decision of excluding trisialo-Tf from CDT (see ref. [2]). In agreement with this conclusion, more recently, a comparison between two commercial methods, CDTri-TIA (Axis Shield, Dundee, UK), which includes trisialo-Tf in CDT results, and ChronAlcol.D (Sangui Biotech, Los Angeles, CA, USA), which reportedly does not include this isoform, showed a higher diagnostic sensitivity and accuracy of the latter method [3,4].

According to a vast body of literature, after sustained alcohol intake, an increase of "total CDT" (i.e. asialo-Tf + monosialo-Tf + disialo-Tf) serum concentration occurs. However, it is well known that by using the current analytical techniques (IEF, capillary electrophoresis and HPLC), the fractions asialo- and monosialo-Tf are usually not identified in the serum of healthy subjects, whereas asialo-Tf is typically identified in the sera of alcohol abusers showing high increases of the disialo-Tf fractions. Despite its inclusion in the theoretical definition of CDT, monosialo-Tf, to the best of our knowledge, has never been clearly associated with alcohol abuse. On this basis, the use of asialo-Tf as a more specific alternative to "total CDT" has been proposed. Arndt [5] reported six reasons supporting the need for further investigating this hypothesis. The author pointed out that

the optimization of preanalytical and analytical conditions for asialo-Tf would be easier than for "total CDT", being the former a clearly defined analyte, and not a group of different molecules, as "total CDT". In particular, from an analytical point of view, asialo-Tf, reportedly shows two major advantages over disialo-Tf: the complete lack of carbohydrate chains and a higher p*I* difference (0.5 pH units) from tetrasialo-Tf (the major glycoform in serum). The former characteristic could ease the production of a specific antibody for a direct immonoassay, while the latter could lead to a neater separation between this isoform and the non-alcohol correlated isoforms (trisialo-Tf, tetrasialo-Tf, pentasialo-Tf, hexasialo-Tf).

Arndt's suggestions are supported by two papers by Legros et al. [6,7], in which, using capillary electrophoresis, asialo-Tf showed the best test diagnostic performance as compared with disialo-Tf or with the sum of asialo- and disialo-Tf for distinguishing not only between teetotalers and alcoholics, but also between moderate drinkers and alcohol abusers.

More recently, the same authors performed a 4-month trial on alcohol abusing and alcohol dependent patients by measuring the asialo-Tf by CZE. The authors reported a specificity of 100% for both groups and a sensitivity of 34 and 57% for alcohol abusers and alcohol dependent patients, respectively. Notwithstanding the great advantages in specificity offered by asialo-Tf, according to the authors, its low sensitivity limited the practical usefulness of this isoform as a marker of alcohol abuse [8].

Although thoroughly investigated in the past, the structure of human CDT is still known only partially. The main point to be clarified concerns the exact nature of the defective carbohydrate chains. Bergen et al., on the basis of analysis by ESI-MS of Tf isoforms previously analyzed by isoelectric focusing, concluded that the Tf glycoform pattern in the serum of chronic alcoholic patients reflected loss of entire glycan side chains and not of individual sialic acid moieties [9].

On the other hand, Flahaut et al., studying by MALDI-MS and fluorescent carbohydrate electrophoresis the oligosaccharides isolated from each transferrin isoforms, previously purified from patients with severe alcohol abuse, identified two different fractions of disialo-Tf, the major one resulting from the loss of entire N-linked oligosaccharides, the minor resulting from the loss of terminal sialic acids [10].

The role of glycosylation of Tf is still unclear, but a recent study by Valmu et al. has demonstrated a differential suceptibility to proteolysis by chymotrypsin for glycosylated and non-glycosylated recombinant human transferrin [11].

3. Pathomechanism of the ethanol induced CDT increase

Although an alcohol-induced increase of the less glycosilated isoforms of transferrin has been observed already in 1976, until now the exact mechanisms that underlie the production of CDT are not yet fully understood. Sillanaukee et al., on the basis of a review of the most relevant literature on CDT, concluded that the ethanol-induced effect on glycoprotein metabolism is a multistep process involving both protein transport and enzyme activity [12]. The effects of ethanol and its metabolites on growth, proliferation and synthesis of transferrin in hepatic cells have been investigated by culturing human hepatoblastoma cells (HepG2) in acetaldeyde containing media [13]. Acetaldehyde proved to facilitate growth retardation, inhibite phosphomannomutase activity and increase secretion of CDT.

Ramskogler et al. reported evidence of apoptosis as examined in 72 alcohol dependent patients using serum content of caspase-related M30 monoclonal antibody significantly correlated with GGT and liver enzymes, but not with CDT. These results suggested that CDT level was not affected by acute hepatocellular damage, but by derangement of hepatic metabolism [14].

On the basis of a large number of publications, there is a general agreement on a correlation between chronic alcohol intake above 60–80 g/day and increase of CDT above the "normal" values. Less agreement among authors exists on the correlation between CDT and alcohol intake when this is within the recommended limits (20–40 g/day). Sillanaukee et al. performed a 12-week, randomized, diet-controlled crossover trial according a 4×4 latin-square design on 11 apparently healthy, non-smoking middle aged men [15]. CDT, sialic acid, GGT and liver enzymes were analyzed after 3 weeks of daily intake of four glasses (40 g of alcohol) of red wine, beer, spirits (Dutch gin) or water (control). After 3 weeks' daily consumption of red wine, quite surprisingly, a significant decrease of serum CDT concentration was observed compared with water consumption. There was no effect of any alcoholic beverage on the other outcome measures. On the other hand, data from other authors seem to contradict these data by finding significant increases in serum CDT even after moderate wine use (375 ml/day for a month) [16] (our personal data from a recent comparison between alcohol abstainers and moderate drinkers is clearly in agreement with the hypothesis of CDT increases also after moderate alcohol intake (personal unpublished data)).

4. Pre-analysis

Among the different preanalytical conditions investigated as potentially affecting CDT results, including circadian fluctuations in serum, type of collection tubes, use and type of anticoagulant, duration and temperature of sample storage, the last one was reported to be the most relevant in laboratory routine practice [2]. In particular, the stability of CDT has been evaluated by Martensson et al. [17], Kohler et al. [18] and by Appenzeller and Wennig [19] at room temperature, +4 and −20°C. The common conclusions of these studies were: (i) the storage at −20 °C does not affect the CDT value even for long storage times (up to 8.4 years); (ii) freezing and thawing, even if repeated, does not affect significantly the results; (iii) room temperature (25 and 32 °C) is compatible with CDT stability only for storage times up to 3–5 days. On the other hand, there is not a general agreement about CDT stability at +4 °C. According to Appenzeller and Wennig the serum can be stored at this temperature for several weeks without CDT concentration changes [19], whereas Martensson et al. reported that CDT is substantially stable at +4 °C only up to 72 h [17]. A very particular study on CDT stability was performed by Kohler et al. [18] who carried out CDT analysis on

257 serum samples 1–2 days after collection and repeated the determination on the same samples after 7 months of storage at 4 °C. The authors found a great variation of CDT values between the two determination, but, in our opinion, the too drastic conditions of this study limit its interest and its applicability to the real laboratory conditions.

A further pre-analytical problem of some relevance is the possible influence of the time interval between sample collection and centrifugation. In a study performed on 152 blood samples drawn from 38 persons (four tubes for each person), divided into four groups on the basis of different time intervals between blood sample collection and centrifugation (1, 24, 48 and 144 h, respectively), the means and medians of CDT were reported to increase with the time of whole blood storage. ANOVA analysis of between-group differences was significant for mean CDT concentrations between 1 and 144 h of whole blood storage [20]. This poses an important warning to analysts who receive whole blood collected in other sites (e.g. hospital clinical chemistry laboratories) which is delivered to the laboratory for centrifugation and serum collection.

5. Analysis

Since the first identification of CDT isoforms by isoelectric focusing followed by immunofixation and staining of CDT-anti Tf complexes in cerebrospinal fluid of alcoholics in 1978, numerous analytical methods have been proposed for the determination of these glycoforms of transferrin in serum, including electrophoretic, chromatographic and immunometric methods [2]. More recently, sophisticated mass spectrometric methods have also been reported.

In this section, the most recent analytical approaches will be presented paying particular attention to the technical improvements and to the advantages and limits of each method in the real practice (see also Table 1 summarizing the methods and the corresponding references). The section is divided into four subsections: electrophoretic methods, chromatographic methods, immunometric methods and mass spectrometric methods.

5.1. Electrophoretic methods

Since CDT discovery, the fundamental analytical technique applied to its determination has been isoelectric focusing (IEF), often coupled to an immunometric recognition step (immunoextraction, immonofixation, immunoblotting, etc.) [2]. This analytical approach, although highly selective, shows an important drawback, represented by inaccuracy and imprecision of the quantitative evaluation, based onto off-line staining and densitometry. For this reason, it has been progressively substituted by more quantitatively accurate techniques, such as liquid chromatography and capillary zone electrophoresis. However, for its high selectivity, IEF is still a reference technique in CDT analysis, particularly for the resolution of closely related Tf isoforms. Welker et al. reported a case of a soccer player who caused a car accident, because of excessive alcohol consumption. The person's re-application for the driving licence was refused on the basis of several positive CDT test performed with immunometric methods. Since the subject denied any alcohol abuse, an IEF analysis was performed revealing the presence of a Tf-D-variant heterozygosity, which clearly interfered with the immunoassay causing a false positive CDT result [21].

Table 1
Analytical methods used for CDT analysis with respective references

Analytical techniques	References
Isoelectric focusing	[2,21]
Capillary electrophoresis	[2,6,22–31]
HPLC	[2,32,33]
Immunoassays	[2,34–46]
Mass spectrometry	[10,39,47–49]

Capillary electrophoresis was first proposed for the direct analysis of serum CDT isoforms by Tagliaro et al. [22]. The method was based on a capillary zone electrophoretic separation using bare fused-silica capillaries (20 μm i.d., 37 cm in length) and a buffer composed of 100 mM sodium tetraborate adjusted to pH 8.3. Direct detection of CDT zones was achieved by UV photometry at 200 nm. An improved version of this method was published in 2000 by Crivellente et al. [23]. The authors, by adding diaminobutane (DAB) to the separation buffer, managed to reduce the protein absorption to the capillary walls, achieving neat improvements in analytical sensitivity and selectivity.

More recently, Wuyts et al. reported a commercial CZE method using proprietary reagents, including a polycation and a polyanion (CEofix® CDT buffer system, Analis, Namur, Belgium), that provided a dynamic double coating of the capillary. The separation buffer contained also ferric iron, which reportedly avoided the need for an iron saturation pre-analytical step [24]. Although this method was simple and fast, the resolution between CDT and non-CDT isoforms was unsatisfactory and clearly insufficient for diagnostic use, as pointed out by Tagliaro et al. [25]. Shortly later, using an improved version of the same commercial reagent set and modified analytical conditions (including a sample pretreatment with ferric iron), Legros et al. [6] obtained the separation of all the major CDT glycoforms in human serum. Further improvements in resolution, using the same reagents, were obtained by Lanz et al. by fine tuning separation temperature and voltage [26,27]. The same authors reported a comparison study of different dynamic capillary coatings, including diaminobutane, spermine and the Ceofix® double coating. The last approach proved to be the best in terms of analytical sensitivity and reproducibility [28]. A new version of the commercial reagent kit was released by Analis in November 2003, which, according to our experience, provides a better resolution between disialo-Tf and trisialo-Tf peaks which was critical in the first release of the analytical method, as pointed out by Lanz et al. [26].

A further CZE method has been reported by Sanz-Nebot et al. [29]. The authors, after having tested different buffers (borate and monosodium glutamate) at pHs ranging from 7.0 to 9.5 and different dynamic coatings, including hydroxylamines, alkyldiamines, alfa-beta bis-quaternary ammonium salts, and organic solvents, concluded that the best resolution was obtained by using 100 mM borate buffer at pH 8.3 added with DAB 1 mM.

In our opinion, however, the quality of separations is clearly inferior to the results reported in previous papers by other authors (see refs. [26,27]).

A further dynamic coating was recently proposed by Fermo et al. [30]. The reported method was similar to the previous CZE methods (separation in 100 mM borate buffer pH 8.4), but for the use of 3 mM diethylenetriamine as buffer additive. Notwithstanding claimed advantages in terms of glycoforms resolution, the CDT measurements in normal subjects resulted much higher than those reported in the international literature. This suggested the existence of some interferences by other serum proteins, which were not excluded in the method validation [31]. No further validation/application of this method has been found in the recent literature.

In routine application, a relevant analytical improvement has been represented by the recent introduction of a multicapillary electropherograph (Capillarys, Sebia, Evry, France), which provides seven channel automated simultaneous separation and quantification of CDT isoforms, including sample pretreatment (iron saturation and dilution) and direct UV determination at 200 nm. Although proprietary reagents are used (CAPILLARYS^{TM} CDT) of unknown composition, the separation resembles those obtained with other CZE methods.

5.2. Chromatographic methods

The different transferrin glycoforms differ from each other in charge depending on the respective content of sialic acid, bearing a negative charge per residue. On this basis, Stibler et al. in 1986 proposed a chromatographic extraction of transferrin isoforms performed on anion exchange microcolumns followed by immunometric determination on the eluates [2]. Later, Jeppsson et al. reported a HPLC method also based on anion exchange separation of CDT isoforms on a Mono Q^{TM} HR 5/5 column with a salt gradient elution and direct detection at 460 nm wavelength (selective for the iron-transferrin complex) [32]. As already reported by Arndt [2], this method was adopted with minor changes by several authors with advantages over immunoassays in terms of analytical selectivity, accuracy and precision and a higher reliability of the identification of heavy drinkers. More recently, a further improvement of the same method was reported by Helander et al. by using a different anion exchange column (Source® 15Q PE 4.6/100, Amersham Biosciences, Piscataway, NJ, USA) and a modified salt gradient profile [33]. These changes produced a better resolution between disialo-Tf and trisialo-Tf, which is crucial for accurate CDT quantification, particularly in the presence of excess of trisialo-Tf. Recently, commercial reagents and HPLC columns for CDT analysis have been released by Recipe (Munich, Germany) and Bio-Rad (Hercules, CA, USA), with great potential advantages in inter-laboratory analytical standardization.

5.3. Immunometric methods

The first immunometric method was reported by Stibler et al. [34]. Lacking specific antibodies for CDT, this method needed a preliminary extraction of CDT isoforms from whole serum by micro-column ion exchange chromatography followed by immunochemical quantitation of transferrin in the extracted material with anti human transferrin antisera. This method was further improved by the same authors and was later made commercially available in different diagnostic kits. These methods adopt a similar CDT extraction on anion exchange disposable cartridges, but differ in the immunochemical detection technique (RIA, EIA, TIA, etc.) [2]. In 2001, the FDA approved one of this immunoassays (%CDT-TIA, Axis Shield Plc, Dundee, UK) for the diagnosis of sustained and harmful alcohol use [35].

This analytical approach based on immunoassay has become extremely popular, especially in clinical laboratories, because of its simplicity and suitability for routine analysis, as proved by three multicentre validation studies [36–38]. However, from an analytical point of view, it should be stressed that, lacking specific antibodies for the CDT isoforms, the specificity of these immunoassays depends only on the selectivity of the disposable extraction cartridges, which is intrinsically low and heavily depending on environmental conditions and manual operation. The inaccuracy of immunometric methods has been recently demonstrated by Alden et al. [39]. The authors analyzed 430 serum samples with an immunoassay (%CDT, new version) from Axis-Shield. The eluates were pooled together and, after purification of transferrin by affinity chromatography, analyzed with HPLC to determine the ratios of the different isoforms with the following results: asialo-Tf 1.5%, disialo-Tf 70.7% and trisialo-Tf 27.8%. On the basis of these results, the authors concluded that the presence of trisialo-Tf in the eluates might generate falsely elevated CDT results. The possibility that elevated trisialo-Tf might cause false positive CDT data from immunoassay was also discussed by Helander et al. [40]. However, the authors, assuming that the "new" %CDT immunoassay from Axis Shield was not affected by trisialo-Tf, as claimed by the producer, attributed the falsely high results to an associated increase of monosialo-Tf. This hypothesis, in the opinion of the authors of the present review, looks extremely weak in view of the data reported by Alden et al. [39] and also in view of the minimal percentages of monosialo-Tf in sera which hardly could affect the computation of total CDT.

Another well known source of inaccuracy of immunoassays is represented by the genetic variants of transferrin, which are characterized by substitutions of aminoacids in the polypeptide chains, with corresponding potential changes in the overall charge of the molecule. To date, 38 Tf variants have been described, which are classified on the basis of their electrophoretic mobility as common (C) type, anodal (B) type and cathodal (D) type. In a study performed on 1614 individuals by using %CDT-TIA, Helander et al. [40] concluded that transferrin C subtypes (the most common in the population), because of their minimal difference in charge, did not interfere with the analysis, whereas transferrin BC and CD heterozigotes, regularly provided low or high results, respectively. Also, the authors pointed out that the transferrin B and D alleles occurred in <1% of the examined population.

The scarce accuracy of immunometric methods is also proved by several studies reporting on comparisons between immunoassays and different instrumental methods, such as CZE and

HPLC which, by providing a separation of all Tf glycoforms and direct determination of the separated peaks by UV radiation absorption, are intrinsically more specific. Bortolotti et al. [41] reported a study on 650 subjects applying to re-obtain their driving license, previously withdrawn for "drunk driving". The analysis performed with immunoassay and CZE, revelead a highly significant correlation ($P < 0.001$) between the results from two techniques. However, particularly in the samples with CDT values around the cut-off or moderately elevated, a wide dispersion of the correlation data was found. On this basis, the authors stressed the need to confirm by alternative techniques all the results from CDT immunoassays. Similar conclusions were drawn by Helander et al. [42] in a study conducted to validate the %CDT-TIA (Axis Shield, Dundee, UK).

More recently, two studies were published reporting on comparisons among immunoassays and separative techniques (i.e. CZE and HPLC). In both cases excellent correlation was found between CZE and HPLC, also in the presence of genetic variants, with a slight prevalence of HPLC in terms of sensitivity. When the immunoassay (%CDT-TIA) was compared to each one of the instrumental techniques, notwithstanding a good statistical correlation ($P < 0.001$), several "false positives" and a few "false negatives" were identified, most of which depending on the presence of D-variants or elevated trisialo-Tf values [43,44].

In conclusion, all authors recommend that, especially when CDT is used for forensic/administrative diagnostics, immunoassays are used only for a preliminary screening, whereas HPLC or CZE are applied for confirmation. This approach is in agreement with well established guidelines of forensic toxicology, which for drug analysis require that different methods are used for screening and confirmatory analyses, as explained by Arndt and Kropf [45]. Excluding any possibility that, after a screening immunoassay, confirmatory data are generated with a second immunoassay, the authors mention as realistic confirmatory techniques isoelectricfocusing, capillary electrophoresis and HPLC. However, since IEF is laborious and hard to use as a quantitative technique, CZE and HPLC look more suited for routine application, especially after commercial kits have been released.

In a more recent article, Arndt and Keller tested combination of commercially available CDT assays for their usefulness as screening and confirmatory techniques [46]. In particular, a set of 292 sera was analyzed by two immunoassays (both including a preliminary anion exchange extraction of CDT isoforms) and a HPLC method. Regardless the type of tests used approximately one-third of contradictory data (positive screening and negative confirmation or vice versa) was obtained. On this basis the authors quite surprisingly concluded that "we cannot recommend a combination of the three for screening and confirmatory analysis in forensic CDT testing", because of the lack of international CDT isoform standard material. The authors of the present paper disagree on this point for the following reasons: (i) Arndt and Keller in their considerations skip mentioning that HPLC is intrinsecally (does not need sample extraction, provides baseline separation of CDT isoforms, provides direct quantification at a highly selective wavelength) more reliable than any immunoassay and consequently a discordance between HPLC data and immunoassay data should be interpreted as a wrong result of the immunoassay and not merely a non-concordance between screening and confirmation; (ii) the measurement of CDT by HPLC is expressed by calculating the percentage area of CDT isoforms over total transferrin isoforms; this relative calculation does not require any external standard, but needs only that the peaks do not include interferent material (which can be easily checked by immunosubtraction) and that the molar absorbtivity of all transferrin isoforms is the same. In conclusion, the results of the paper by Arndt and Keller, in our opinion, should be interpreted as a further proof of the scarce reliability of immunoassays for CDT.

Quite recently (January 2005), Dade Behring (Deerfield, IL, USA) has launched a new immunoassay, presented as the first Direct Immunoassay for CDT (N Latex CDT assay). Based on a specific antibody for immunological epitopes of the Tf molecule which are accessible only in the CDT isoforms, and, conceivably, protected by carbohydrate chains in the other more glycosylated Tf isoforms, this method may represent an important step forward in the immunometric determination of CDT. Being based on a highly specific antibody for CDT, the method does not require serum pre-treatment with anion exchange cartridges, as the previous immunoassays. Moreover, using latex agglutination for determination, it is compatible with most automated instrumentation for clinical chemistry. Because of its high specificity for epitopes directly correlated with the type of protein glycosylation, it intrinsically should not suffer from interferences from B and D Tf isoforms, which differ in the amino acid sequence. Unfortunately, so far little has been published on the characteristics and applications of this new and promising method.

5.4. Mass-spectrometric methods

The application of MALDI-TOF mass spectrometry to human transferrin analysis has been proposed for studying the molecular structure of transferrin glycoforms by characterizing the oligosaccharides released from the glycoprotein [10].

MALDI-TOF was also used to characterize the eluate from the extraction cartridges used in the Axis Shield immunoassay, showing the presence of two major peaks with molecular masses corresponding to the calculated molecular weight of disialo- and trisialo-Tf and a minor peak with a molecular weight corresponding to the asialo-Tf isoform [39].

Lacey et al. reported a determination of transferrin isoforms by liquid chromatography–ESI-mass spectrometry, after immunoaffinity purification of transferrin from serum and concentration on C4 columns [47]. The method was applied to study the abnormal transferrin isoforms present in the severe neurologic disorder known as carbohydrate deficient glycoprotein syndrome (CDGS). Later, in 2003, Kleinert et al. [48] presented a procedure, which differently from the previous method, did not require immunoextraction. The method included three steps: (i) isolation of transferrins from biological fluids (serum, plasma, CSF) using anion exchange HPLC with UV detection at 254 nm; (ii) concentration of the transferrin fraction; (iii) analysis of transferrin isoforms with liquid chromatography–electrospray

mass spectrometry. The method was successfully applied to samples from patients suffering from different forms of CDGS, but, unfortunately, the application to alcohol abusers (showing elevated levels of CDT) was too limited to draw conclusions on the usefulness of this technique for alcohol abuse diagnosis.

An alternative analytical approach to CDT separation has been reported by del Castillo Busto et al. [49]. The authors performed the separation of Tf isoforms on anion exchange columns by means of a linear gradient of ammonium acetate (0–250 mM in 45 min) buffered with 25 mM Tris-acetic acid (pH 6.0) solution. The detection was based on UV absorbance of proteins by using a UV–vis variable wavelength detector set at 280 nm and on specific atomic detection of Fe by online coupling the HPLC with an inductively coupled plasma mass spectrometer (ICPMS). The latter detection proved to be not only more selective than the former, being Tf the only iron transporting protein in serum, but also more sensitive with a detection limit ranging from 0.02 to 0.04 μM Tf. In a second part of the study, the authors presented a structural characterization of CDT by applying MALDI-TOF and ESI-Q-TOF to Tf fractions collected from HPLC. ESI-Q-TOF, although less sensitive than MALDI-TOF, reportedly provided better results in terms of mass accuracy.

6. Data interpretation

6.1. Diagnostic specificity and sensitivity of CDT

The great majority of the studies recently published on CDT concerns the evaluation of diagnostic sensitivity and specificity of this marker, often in comparison with other more traditional markers of chronic alcohol abuse, such as gamma-glutamyltransferase (GGT) and mean corpuscolar volume (MCV).

For the sake of conciseness and clarity, this vast literature has been summarized in tables. In particular, Table 2 collects the studies on diagnostic sensitivity and specificity of CDT for the identification of alcohol abuse. Table 3 summarizes the articles on pathological conditions different from alcohol abuse, which could affect CDT levels, thus representing potential sources of false positive results. Table 4 collects articles reporting conditions potentially leading to false negative results.

Although the consultation of the tables may looks self-explaining, some basic criticisms and warnings to the readers should be expressed in this text.

1. Often the individual alcohol consumption, a necessary prerequisite to calculate diagnostic sensitivity and specificity, is merely based on personal reports of the subjects or on different questionnaires of questionable reliability outside the clinical field. This approach may lead to a bias in the estimation of the alcohol intake.
2. The limit between “normal” and “abnormal” daily alcohol intake in different regions and cultures may vary dramatically.
3. In some studies the diagnostic performances of CDT are evaluated towards “heavy drinkers”, whereas in others on “light”, “moderate” or “hazardous drinkers”. These differences hamper the overall evaluation of the results from different studies.
4. In most studies, CDT is determined by using immunometric methods, whose complexity, scarce standardization, low analytical selectivity and poor precision, as above discussed, may explain discrepancies among different studies.

6.2. Gender dependence of serum CDT

In the past, several studies reported gender differences in CDT concentrations, without, however, providing satisfactory explanations of this phenomenon (see ref. [2]).

In the period covered by the present review, six papers on this topic have been found. Figlie et al. performed a study on 130 non-drinkers, 167 drinkers, and 183 alcohol dependent drinkers finding that alcohol dependent women presented a lower prevalence of abnormal values. On this basis, the authors concluded that the diagnostic sensitivity of CDT is lower in women than in men [50]. The same difference has also been reported in the studies by Gomez et al. [51], Anttila et al. [52], Mundt et al. [53] and Laatikainen et al. [54]. Also, in these more recent studies no scientific explanations of the differences were proposed. On the other hand, Rukstalis et al. in a comparison study on 96 women with a DSM-III-R diagnosis of alcohol dependence and 123 alcoholic males reported CDT levels higher in women than in men [55]. However, the authors pointed out that CDT levels in women reflected alcohol consumption as well as in men, without a demonstrable correlation with estradiol concentration. Once again, the reason of these discrepancies is unclear, but could be tentatively attributed to differences in the selectivity of the used analytical methods and on the weakness of the classification criteria.

6.3. CDT and γ-glutamyltransferase

Gamma-glutamyltransferase (GGT) is a well known marker of chronic alcohol abuse, which, before the introduction of CDT, was considered one of the most reliable indicators. On this basis, in the last decade several studies have been published on the comparison of diagnostic efficiency of CDT and GGT. Also, some authors proposed the combined use of these markers in order to increase the overall diagnostic reliability. Sillanaukee and Olsson [56] reported a meta-analysis of six different clinical studies on alcohol abusers and social drinkers ($n = 1412$) in which the diagnostic value of CDT, GGT, AST, ALT and MCV was evaluated. The authors, on the basis of a predictive function [0.8 ln(GT) + 1.3 ln(CDT)] derived from the study, concluded that for discriminating between alcohol abusers and social drinkers the combined use of CDT and GGT was better than any of the single markers or than the combined use of CDT or GGT with other markers. This conclusion has been partially confirmed by a study from Chen et al. [57], who performed a study on 1684 subjects, recruited from general population, abstainer groups and alcohol treatment centers in order to draw

Table 2
Diagnostic accuracy of CDT for alcohol abuse (AUROC, area under ROC curve; PPV, positive predictive value; PPN, negative predictive value)

Reference	Subjects	Analytical method	AUROC	PPV	PPN	Specificity	Sensitivity
[98]	One hundred and eighty-three males drinking an average of 20 drinks/day	CDT-RIA				96%	58%
[99]	Forty-seven alcoholics drinking more than 100 g alcohol/day and 34 healthy teetotallers					91%	
[51]	Hospitalized patients with alcohol related problems		0.76			75%	71% (males) 65% (females)
[100]	Fifty-three control subjects (daily alcohol intake <20 g), 29 chronic alcoholic subjects (daily alcohol intake >40 g), 24 non-alcoholic patients with chronic hepatitis C, 22 patients with hepatitis C and alcohol abusers.	%CDT-TIA (Axis Shield)				87.8%	72%
[101]	One hundred and eighty-eight alcoholics and 132 control patients	%CDT-TIA (Axis Shield)				98%	48%
[102]	Hazardous Drinkers (25 males and 25 females) drinking 350 g (males) and 225 g (females) of alcohol per week or engaged in bouts of heavy drinking 1–2 days a month or more during the past 12 months	CDTect (Pharmacia)		14%		100% (males), 71% (females)	
[103]	Fifty-seven non-drinkers, 77 moderate drinkers (alcohol consumption <210 g/week), 139 heavy drinkers (alcohol consumption >210 g/week) classified on the basis of validated structured interview and daily anonymous diaries	HPLC	0.87			90%	63%
		CDTect (Biorad)	0.72			90%	33%
[104]	Twenty-nine medical male inpatients classified as alcohol abuser (19) and alcohol depen-dence (10) on the basis of DSM-III-R						10%
[105]	Males assessed for tumor surgery of the upper digestive tract. Diagnosis of alcohol misuse on the basis of DSM-III-R						CAGE question-naire + CDT 85%
[52]	Thirty-three alcoholics with liver disease, who had a history of continuous alcohol use >80 g/day and 29 alcoholics without liver disease consuming a mean of 131 g/day; 45 healthy social drinkers and abstainers	%CDT-TIA (Axis Shield)				100% for men; 91% for women	96% for men; 87% for women
		CDTect-RIA (Pharmacia)				63% for men; 46% for women	65% for men; 36% for women
[106]	Eighty control subjects and 33 alcoholics with the daily alcohol consumption >60 g alcohol in the course of last four weeks	ChronAlcol.D (Sangui Biotech Inc.)				Male: 96.7, female: 88	Male: 88.2, female: 60
[107]	Forty-seven patients with alcoholic liver disease (on the basis of biochemical and hystological analyses and on a history of drinking that exceeded 5 years with an average alcohol intake of more than 60 g/day); 26 patients with nonalcoholic steatohepatitis and 22 patients with other liver diseases and 21 healthy individuals	Affinity purification and ISF with densitometry of Coomassie stained transferrins	0.92				
[7]	Six hundred and forteen volunteers, classified after interviews, self-reported drinking habits and AUDIT scores as alcohol abusers (consuming >50 g/day ethanol for the previous 3 months or longer, $n = 413$) or moderate drinkers (<30 g/day ethanol; $n = 201$)	CZE (Ceofix Analis)	0.83				
		Cut-offs					
		1.2%				92%	73%
		2.6%				74%	77%
		2.8%				83%	67%
		3.0%				90%	63%
[108]	Thirty-six heavy drinkers admitted for detoxification and 30 healthy control subjects	%CDT-TIA (Axis Shield)					69%
		CDTect (Pharmacia)					61%
[109]	Multicenter study including patient groups “abusers”, “dependents” and “controls” on the basis of DSM IV criteria	%CDT-TIA (Biorad)				83%	Alcohol abuse: 80%, alcohol dependence: 91%

Table 2 (*Continued*))

Reference	Subjects	Analytical method	AUROC	PPV	PPN	Specificity	Sensitivity
[110]	Three hundred and ninty-six women and 403 men aged 30–60. Forty-five met the DSM-IV criteria for alcohol abuse (more than 14 drinks per weeks) and 17 met the DSM-IV criteria for alcohol dependence.	%CDT-TIA (Biorad), Cut-off: 2.6%	0.83			85%	61%
[111]	Cross-sectional health survey in northeast Germany with data collection from 1997 to 2001 of 4310 men and women asked for their recent alcohol consumption			For high risk drinking <50%			
[112]	Five hundred and eighty-one young men 19 years old attending a mandatory 1 day army recruitment process. On the basis of reported alcohol intake investigated by using "health and Lifestyle Questionnnaire", 20.8% were classified as heavy drinkers (>21 drinks/week over the last 12 months or drunk at least three times over the last month); 74.9% as moderate drinkers (6 drinks/week) and 4.3% as abstinent	%CDT-TIA (Biorad) Cut-off: 2.2%	0.66			63.2%	58.7%
		CZE (Ceofix, Analis) Cut-off: 0.62	0.58			60.3%	52.1%
[113]	Fifty alcohol dependent patients admitted to the Center of detoxification and 85 healthy teetotallers	%CDT-TIA Cut-off 2.2% for males and 2.5% for females	0.94				
[114]	Seventy-five patients treated for alcohol dependence	%CDT-TIA (Biorad)	0.98				
[115]	Random sample of 130 patients investigated by diagnostic interview			80%	85%	96%	47%
[116]	One hundred and one alcohol dependent patients, 115 social drinkers, 46 patients with unspecific increase of gamma-GT, 51 hepatitis patients and 20/31 patients with non-alcohol/alcohol dependent liver cirrhosis	Tina-quant %CDT-2nd generation (Roche) Cut-off: 3%				93.5% in patients with unspecific increase of gamma-GT, 88.2% in hepatitis patients and 70% in patients with non-alcohol dependent liver cirrhosis	73.3% for alcohol dependent patients
[117]	Forty-seven nonalcoholic patients (<280 g/ethanol/week and no alcohol use disorder); 67 alcoholic with harmful use (>560 g/ethanol/week and an alcohol use disorder)	ChronAlcol.D (Sangui Biotech) Cut-off: 3 U/L	0.91				63%
[118]	One hundred and two alcoholics, 34 healthy volunteers either social drinkers or abstainers	%CDT and CDTect	0.86				
[119]	Seventy-six patient with alcohol liver desease (criteria of the Chinese Medical Association), 55 patients with alcoholism, 32 patients with non-alcoholic liver disease	IEF with immuno-fixation and Comassie blue staining				71.9% in patients with alcoholic liver disease	93.4% in patients with alcoholic liver disease
[120]	Twenty-nine alcohol dependent patients, 28 alcohol abusers and 28 social drinkers identified on the basis of MAST, CAGE and AUDIT tests					93% for alcohol dependence and alcohol abuse	41% for alcohol dependence, 32% for alcohol abuse
[121]	Six hundred and thirty-three outpatients attending one outpatient care center, divided in five categories: 1, patients drinking more than 30 g/day for women and 50 g/day for men; 2, ralapse patients; 3, moderate drinkers; 4, patients weaned less than one month; 5, patients weaned more than one month					71–96%	32–92%

Table 3
Clinical conditions leading to false positive diagnosis of alcohol abuse

References	Clinical conditions	Sample	Analytical method	Results
[122]	End-stage liver disease	Seventy-nine abstaining patients with end-stage liver severity (abstinence investigated by interview and by random blood alcohol levels)	%CDT-TIA	Fifty percent of patients showed positive results
[123]	Catabolic desease states due to psychiatric disorders	Eleven female patients reporting weight loss in recent weeks or showing clear clinical signs	%CDT-TIA Cut-off: 6%	6/11 patients showed elevated CDT levels
[124]	Antiepileptic drugs	Neurological patients undergoing a semistrctured clinical interview including ethanol consumption during the last 8 days	%CDT-TIA and CDTect	<false positives with %CDT-TIA than with CDTect
[125]	Autoimmune hepatitis Type 1	A 15-year old boy with autoimmune hepatitis type 1, whose denial of alcohol intake was confirmed by his mother and by AUDIT and MALT-F scores	ChronAlcol.D.	CDT 3.2%
			% CDT-TIA isoelectric focusing immunofixation—silver staining	CDT 3.2% Increase of disialo-TF, no asialo-Tf, no genetic transferrin variants
[126]	Advanced liver disease	Forty-four patients who underwent orthotopic liver transplantation for alcoholic cirrhosis. Pretransplant assessment by a specialist psycologist was negative for alcohol correlated problems. Pre and post-transplant CDT monitoring was performed	CDT-RIA	Eighty percent of patients showed significantly increased CDT values

clinical rules for the interpretation of GGT, AST and CDT data. The authors reported that, for men, the best accuracy for detecting daily consumption of 60 g ethanol or more in the past 30 days was provided by the use of the formula proposed by Sillanaukee and Olsson; for women, the best accuracy was provided by the use of GGT alone. The equation proposed by Sillanaukee and Olsson [56] has been also verified by Anttila et al. [58], who, in the assessment of 65 alcoholics, achieved a diagnostic sensitivity of 94% for men and 82% for women.

On the other hand, Schwan et al. [59], on the basis of a 6-month longitudinal multicenter trial on four different study groups (alcohol abusers, alcohol dependents, healthy controls and consulting controls), recommend the use of CDT as a "first line" biological marker and GGT as a support for differential diagnosis between alcohol abuse and alcohol dependence in patients with high CDT.

6.4. Clinical use of CDT

The usefulness of CDT in the detoxification treatments of alcohol abusers has been highlighted by Myrick et al. [60] and Walter et al. [61], who proved that this marker is more sensitive to relapses than the other traditional markers such as GGT and MCV.

On the other hand, an increasing number of researchers have proposed CDT as marker of other pathological conditions correlated or not to alcohol abuse. The most typical pathology strictly correlated with CDT is, as it is well known, the so called carbohydrate deficient glycoprotein syndrome (CDGS), a group of severe neurological disorders characterized by genetic defects in protein glycosylation [2].

As it is well known, one of the major causes of acute pancreatitis is alcohol abuse. Consequently, for formulation of prog-

Table 4
Conditions reported to lower serum CDT concentrations

Reference	Variable	Sample	Results
[53]	Therapy with angiotensin II receptor blockers	Seven hundred and ninty-nine care patients who were prescribed drug therapy for hypertension, diabetes and lipid disorders. For each patient were determined %CDT level, 30-day history of alcohol consumption, symptoms of alcohol abuse or dependence, health status and prescribed drugs	Lower %CDT levels
[127]	Total body water	Seven hundred and thirty men and 613 women participating in the WHO/ISBRA study on state and trait markers of alcoholism	Adjusting for differences in total body water significantly increase the diagnostic performance of CDT

nosis and for adequate treatment of this severe pathology, it is important to differentiate between alcoholic and non-alcoholic etiology. For this purpose, in the studies by Basterra et al. [62], Aparicio et al. [63] and Al Bahami and Ammori [64], CDT proved to be the most reliable marker in comparison with GGT, liver enzymes and MCV.

In the frame of the studies on the relationships between alcohol consumption and cardiovascular disease, a few papers on the correlation between CDT and cardiovascular risk have recently been published. In a study on a population of 3097 males and 2578 females divided in quartiles on the basis of CDT and GGT values, the highest CDT quartiles had higher HDL and lower triglycerides, whereas the highest GGT quartiles had higher total cholesterol and triglycerides and lower HDL (in men), suggesting that moderate consumption of alcohol may reduce mortality from vascular diseases [65]. On the other hand, epidemiological data reportedly show a typical "J curve" between alcohol intake and coronary artery disease mortality: non-drinkers have a higher risk of coronary artery disease versus moderate drinkers (daily consumption of 5–40 g in men and 5–20 g in women) with a risk reduction of about 30%. On this basis, Cambou suggested the use of CDT to differentiate between these two conditions [66]. Also Jousilahti et al. in a risk factor survey, including 3666 Finnish men aged 25–74 years, found an inverse association of CDT level with coronary heart disease [67]. Excessive alcohol consumption is also reported to be associated with hypertension. On this basis, the determination of CDT has been proposed as feedback of alcohol abuse in patients treated for Type 2 diabetes and hypertension [68,69].

Since alcohol abuse is recognized as the cause of one-third of seizure-related admissions, CDT has been proposed as biomarker of alcoholic etiology of epilepsy [70]. Also European Federation of Neurological Societies (EFNS) guidance regulations on diagnosis and treatment of alcohol related seizures suggest the use of CDT and GGT to support the clinician's suspicion [71].

As chronic alcohol consumption is a known risk factor for spontaneous intracranial haemorrhage (SICH), a reliable diagnosis of alcohol abuse at admission of SICH patients is required. In a study performed on 105 SICH patients and 105 control patients suffering for dorsalgia, Herzig et al. found CDT positive in 25.7% of SICH patients versus 7.6% of control group subjects [72]. On the other hand, only 13% of SICH patients with positive CDT test stated regular and high consumption of all types of alcoholic beverages. On this basis, the authors concluded that CDT can be a marker of chronic alcohol consumption more sensitive than GGT and clearly better than the evaluation of patients' anamnestic data.

In a study including 24 patients, reportedly non-alcoholics, suffering from anorexia nervosa, Reif et al. [73] observed in 57% of cases a pathological increase of CDT value. Since during therapy CDT value normalized in association with the increase of body mass index, the authors concluded that CDT might serve as a parameter indicating prognosis and severity of anorexia.

CDT has been also proposed to identify protein glycosylation defects underlying some pathologies. Nihlen et al. in a study on 15 patients affected by chronic obstructive pulmonary disease (COPD) using multiple logistic regression analyses found a significant relationship between the diagnosis of COPD and CDT [74]. These findings, according to the authors, suggested that glycosylation defects of proteins may occur in COPD and could be involved in the pathogenetic mechanism of the disease.

On the basis of the premise that inflammatory process is associated with alterations in iron metabolism and that transferrin is an acute-phase glycoprotein, Piagnerelli et al. [75] hypothesized that CDT may increase rapidly in septic patients. To investigate this hypothesis blood samples were obtained from critically ill patients with ($n = 15$) and without ($n = 14$) documented sepsis and compared with healthy volunteers. CDT was significantly increased in septic patients compared with non-septic patients and volunteers. The authors referred this increase to degradation of transferrin by bacterial neuroaminidase.

On the basis of a reported generic association of CDT with rheumatoid arthritis and of the association of GGT/CDT with hypertension in males and with asthma in females, Sillanaukee et al. [76], suggest that CDT might also serve as health risk indicator.

6.5. Forensic use of CDT

A particular application field of CDT analysis is represented by the determination of this marker in the biological fluids collected during autopsy for diagnosis of pre-mortal alcohol misuse.

Simonnet et al. performed a study on the different factors potentially affecting the post-mortem CDT concentrations, including haemolysis, site of collection and storage conditions [77]. The authors reported that site of collection and storage temperature do not affect the CDT values, whereas haemolysis and repeated freezing and thawing may decrease level of CDT.

Because of post-mortal changes occurring in blood and other tissues, vitreous humour has been proposed as an alternative matrix. Berkowitz et al. determined CDT in the vitreous humour (VH-CDT) collected during autopsies on 21 alcoholic subjects and on seven controls. The authors reported a diagnostic sensitivity of 95% and a diagnostic specificity of 71% [78]. More recently, the same authors performed a study on the stability of VH-CDT investigating also the possible influence of time-dependent changes of total transferrin on VH-CDT. The authors concluded that VH-CDT is stable and that the time-dependent changes of VH-transferrin do not affect significantly VH-CDT, at least in the early post-mortem time interval [79].

In the forensic environment CDT, as reported by Seidl [80], has also been used to evaluate alcohol misuse in subjects applying for the re-granting of their driving license, after its confiscation for drunk driving. However, the author points out that CDT, possessing high specificity, but low negative predictive value, can be used as marker of alcohol misuse, but not to confirm an alleged teetotalism.

In traffic safety context, CDT has also been used to investigate the prevalence of chronic alcohol abusers among drunk drivers apprehended and submitted to blood alcohol analysis [81,82]. The authors observed that few alcohol abusers were present in the category below 0.5 g of alcohol per liter, whereas in the

category ranging from 3.0 to 3.5 g/l the prevalence of alcohol abusers was 47% and in the category above 3.5 g/l the prevalence was 67%. On the basis of these data the authors concluded that increased levels of CDT revealed not only a condition of chronic alcohol abuse, but also an increased risk to drive under alcohol influence.

The correlation between CDT and blood alcohol concentration has also been investigated by Reynaud et al. in a study performed on 166 patients admitted to hospital emergency services for acute alcohol intoxication as principal or additional diagnosis [83]. The authors found that 65.7% of patients presented pathological levels of CDT, drawing the conclusion that the patients admitted for drunkenness at emergency services should not be considered occasional abusers, but chronic alcohol abusers or alcohol dependents.

7. Review papers

The great interest of the scientific world devoted to CDT is witnessed by the numerous review papers published on this topic in the recent years. From 2000 until December 2005 at least 13 review articles on CDT have appeared in the international literature, although with different focuses. Some of these papers included CDT in general overviews of methods for diagnosing chronic alcohol abuse reviewing also the other traditional biomarkers of alcohol abuse and the screening tests (CAGE, AUDIT, etc.) [84–89]. The other reviews are specifically focused on CDT and include general reviews [90,91] and reviews on specific aspects of CDT as a marker of alcohol abuse, such as its diagnostic accuracy [92], its diagnostic specificity [93,94] and its suitability for monitoring alcoholics receiving treatment [95]. Last but not least, two interesting reviews have been dedicated specifically to the analytical aspects of the biomarkers of alcoholism including CDT [96,97].

8. Conclusions

An overall evaluation of the most recent achievements in CDT analysis permits to enucleate two main areas in which research has been particularly successful.

By far the most important advancement is in the analytical area in which sound and reliable commercially available instrumental methods based on capillary electrophoresis and HPLC show practicability, costs and productivity suitable for application in the real practice (although with some caution, we should also mention the introduction of the first direct immunoassay for CDT, using a specific antibody). These methods have proved to be by far more accurate, specific and precise than the currently used immunoassay kits, on which, however, the majority of clinical and forensic studies on CDT have been based. The diffusion of this more sophisticated research tools will prompt a re-evaluation of the diagnostic use of CDT, which undoubtedly will lead to a better understanding of pros and cons of this biomarker.

The refinement of the analytical techniques with a consequent increase of accuracy and the increase of methodological standardization will also help to overcome the problems arising from differences between the cut-off values adopted in laboratories which today hinder the interlaboratory interpretation of results.

Another very interesting and promising field of investigation can be identified in those attempts to study CDT in pathological conditions outside the traditional application field of this biomarker (i.e. diagnosis of alcoholism and monitoring of detoxification treatments and diagnosis of CDGS). In this group of potential new applications we may mention certification of physical fitness to drive vehicles, monitoring anorexia, and studying a possible alcoholic origin of severe illnesses detailed in Section 6.4.

In conclusion, CDT, particularly after the introduction of new accurate analytical methods, is and will be in the near future a field of intense research and important application in many field of clinical and forensic medicine.

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