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A BRIEF HISTORY OF THE ALCOHOL BIOMARKER CDT AND ITS APPLICATION IN FORENSIC MEDICAL EXPERTISE IN ROAD ACCIDENT INVESTIGATIONS

Porpiglia N., Wielders J., Tagliaro F.

Unit of Forensic Medicine, Department of Diagnostics and Public Health, University of Verona, Verona, Italy

It is well known that large-scale binge drinking is one of the leading risk factors for poor public health around the world. This is why an objective assessment of the current state of alcohol abuse is critical to protecting public health and safety, especially in clinical, professional context and in the context of road traffic. The article is devoted to the current problem of the history of the formation of the process of one of the most commonly used biostandards of consumed alcohol, namely carbohydrate-deficient transferrin. Taking into account the observed increase in road accidents while intoxicated, the article substantiates the importance of the CDT biomarker when conducting medical expertise.

Keywords: *CDT biomarker, road accident, forensic medical expertise, alcohol intoxication, public health protection.*

According to the most recent WHO global status report on alcohol and health, alcohol is currently consumed by 2.3 billion drinkers, which is more than half of the population in three WHO regions (the Americas, Europe and Western Pacific). In particular, current drinkers are largely represented by adolescents amounting to 155 million individuals aged 15-19 [1]. It is well known that the harmful use of alcohol still is one of the major causes of death and disability. Hence its early detection based on objective evidence appears ethically mandatory. The objective assessment of alcohol misuse can be performed either by directly determining the presence of ethanol in biological fluids, such as blood or breath, or by the determination of direct and indirect alcohol use biomarkers. The first class includes the metabolites of ethanol itself, whereas the indirect biomarkers are commonly related to the consequences of damage to organs and tissues due to excessive alcohol consumption. The group of these indirect biomarkers include liver enzymes (AST and ALT), GGT, MCV, and, quite improperly, CDT. In fact, the increase of CDT is not related to organ damage but simply to the interference of acetaldehyde with the glycosylation of transferrin [2] and this characterises CDT in between the direct and indirect biomarkers. It is worth noting that CDT proved to have the most specific and sensitive diagnostic power, when compared to the other most traditional markers resulting in a sensitivity of 67-84% and a specificity of 71-92% [3]. In particular, the diagnostic specificity power of CDT was highlighted by Bergström and Helander, who assessed that the

Correspondence address: Nadia Maria Porpiglia, BSc in Biological Sciences, MSc in Forensic Science, PhD in Forensic Science, Doctor, Postdoctoral researcher, Unit of Forensic Medicine, Department of Diagnostics and Public Health, University of Verona, Piazzale L.A. Scuro, 10 - 37134 Verona, Italy, email: nadia.porpiglia@univr.it

non-alcohol-related increase of CDT is rather unusual when using disialo-transferrin as the primary analyte to be determined as percentage of total transferrin using an HPLC reference method [4]. The same authors showed how there is no need to adjust the reference intervals for %CDT in relation to ethnicity, gender, age, BMI, and smoking habits and that the previous findings reported until then were only dependent on a weak methodology for the determination of CDT [5]. The new direct biomarkers that appeared in the most recent decades, such as hair EtG and PEth, are certainly noteworthy for being specific in determining alcohol abuse. However, it is not possible to make a direct comparison between these and CDT because they are detected in different biological matrices, hence covering different detection windows [6,7].

In the early stage of CDT analysis, Stibler et al. identified and measured the glycosylation pattern of transferrin (Tf) in serum by using the IEF technique by separation of the Tf glycoforms based on their different isoelectric points followed by their recognition with anti-transferrin antibodies [8]. This approach proved to be highly selective but also mostly relying on manual operation, very time-consuming and not suited for accurate quantitative determination of the different Tf glycoforms. A breakthrough toward a widespread use of CDT was represented, in the early 1990's by the introduction of a commercial kit, CDTect™ (Pharmacia Diagnostics), where small anion exchange columns allowed the preliminary separation of the less glycosylated glycoforms of Tf followed by their quantitative determination by a radioimmunoassay specific for human transferrin. This approach led to a quantification of CDT in terms of absolute amounts [9]. Later, in order to compensate for interindividual variability of serum Tf, directly impacting on CDT concentrations, a relative measurement of this parameter was preferred as a fraction of CDT concentration divided by concentration of total transferrin. Various non-radiometric technologies were, subsequently, developed to determine CDT, the most popular of which was the % CDT turbidimetric immunoassays (released by Axis-Shield and other producers), as well as direct CDT immunoassays [10,11]. The latest step forward among the immunometric methods was represented by the introduction of a specific antibody for asialo- and disialo-Tf, which allowed the production of an immunonephelometric assay, which did not need a preliminary separation of the CDT-related immunoreactive material (N Latex [11]). On the other hand, the need of alternative methods to immunoassay displaying higher analytical accuracy and a sharper visualisation of the entire Tf glycoform pattern emerged to meet the requirements of the forensic and administrative environments in which most of the results were used. For this purpose, two separative techniques were mostly applied such as HPLC and CE [12-15] which, at present, represent the majority of the used methodologies, the pros and cons of which will be briefly discussed later.

Knowledge about Tf microheterogeneity is crucial for proper analysis and interpretation of CDT as a biomarker of alcohol abuse in clinical and forensic contexts [16]. This aspect, together with the very close similarity of disialo-Tf and non-CDT glycoforms as well as the low amount of disialo-Tf compared to the rest of the Tf glycoforms, explains why CDT analysis requires very high selectivity, specificity, and sensitivity [2]. In particular, the preanalytical conditions reported to affect serum concentrations of CDT are: i. the presence of EDTA, which may disturb in vitro iron saturation [17]; ii. delipidation and strong haemolysis (respectively, reduce and increase the % CDT) [2]. In contrast, preanalytical conditions that do not affect serum concentration of CDT are listed as follows: sample

collection into tubes containing polyacrylamide-gel separators and/or coagulation accelerators [18]; storage at i. room temperature for less than approximately 30 hours [19], ii. seven days at 4°C [19], and iii. several months at -22°C [19]; diet; and drug therapies [2].

A change regarding the interpretation of CDT in terms of alcohol abuse diagnosis is that, initially CDT was intended as a group of the following glycoforms: asialo-Tf, monosialo-Tf, and disialo-Tf. However, it was later demonstrated that monosialo-Tf, a very small portion of the total serum transferrin, was not related to alcohol use [5]. Moreover, the inclusion of the asialo- in addition to the disialo-Tf fraction in the measurement of CDT introduced method-dependent variables and, consequently, inter-laboratory interpretative issues (e.g., definition of cut-off values, difficulties to standardise a method on a mix of two measurands). Controversies on the practical use of CDT were based on a few analytical weak points including: covering different glycoforms of CDT, using different methodologies, applying different cut-offs, and expressing CDT as relative (percentage) or absolute amount [20].

CDT standardisation process

All the aforementioned inconsistencies were substantially related to the fact that the CDT measurement was not standardised. For this purpose, in 2005 the IFCC instituted the Working Group on Standardisation of CDT (WG-CDT) with the main goal of posing the bases on which different methods should produce identical results, permitting use of common cut-offs. Ultimately, this harmonisation would ease the interpretation of CDT results and boost its clinical and forensic use as an alcohol abuse biomarker.

The WG-CDT agreed to define a standard operation procedure, which was recognised in a specific qualitative and quantitative HPLC method for the determination of CDT that allowed to express the relative concentration of the less glycosylated forms of transferrin to total transferrin (% CDT) [12]. As previously mentioned, both CE and HPLC can be considered as efficient separative techniques with the ability of providing a full separation, quantification and spectrum of the Tf glycoforms profile, with the final achievement of allowing the discrimination of interferences imputable to genetic variants, CDG syndromes, and defects of glycosylation due to liver pathologies. However, HPLC was considered as the best candidate for a reference measurement procedure (RMP) because of its higher analytical specificity, being its detection based on the identification and measurement of iron-saturated transferrin at 470 nm. In contrast, CE, although displaying a higher separation efficiency, relies on the less specific UV detection at 200 nm representing the UV absorption of the peptide bond [21]. Agreement was also found on the use of the baseline peak integration approach instead of the valley-to-valley, proving to reduce the inter-laboratory variation coefficient [20].

On these grounds, the WG also decided to select one primary analyte for the determination of CDT, which meant making a choice between asialo- and disialo-Tf, which are both directly related to copious alcohol consumption through a dose-related mechanism [22]. However, disialo-Tf was preferred because of a higher diagnostic sensitivity, being asialo-Tf mainly detectable in the most severe abusers, while disialo-Tf is detectable over a much wider range of alcohol use [9].

The said RMP proved reproducible in the analysis of a candidate secondary reference material performed by an established network of reference laboratories using the HPLC RMP [23]. The

reference material, consisting of deep-frozen native human serum, proved stable throughout shipment and storage and allowed to achieve good and reproducible results within the laboratories of the network. The harmonisation procedure implicated that the refence material was tested with seven commercial methods (based on HPLC, CE, and immunonephelometric assay) through the use of multi-level secondary calibrators in order to reduce the method-dependent imprecision [24].

To the best of our knowledge, CDT is the only specific biomarker to have achieved international standardisation, including definition of cut-offs, availability of reference materials, etc.

CDT in traffic medicine

In the last decades, the use of CDT as a chronic alcohol abuse biomarker has found wide application in various forensic contexts, such as occupational accidents, safety-sensitive jobs, holding weapon licence, child custody, and other civil litigations. Nonetheless, probably the most widespread application of CDT in most European countries (e.g., Sweden, United Kingdom, Netherlands, Belgium, France, Italy) remains the field of certification of the fitness to hold the driving license. Notwithstanding such well-known applications of CDT, its interpretation and use in the prevention of alcohol-related road traffic accidents is still based on criteria developed in the clinical field, where the target is the prevention of alcohol-related pathologies and not the improvement of traffic safety.

In fact, although it is common belief that high blood alcohol concentration (BACs) severely increase the probability of occurrence of road traffic accidents [25,26], relatively scarce literature can be found on the correlation between chronic alcohol abuse and the risk of traffic crashes [27]. In contrast, this particular aspect of traffic medicine should be stressed since drivers whose driving license has been withdrawn for "drunk driving", in most cases, pretend to have ceased the abuse when applying for the regranting of their license. Although, in the European scenario, many groups have studied such correlation following both retrospective and prospective approaches, the methods to objectively verify the persistence or not of the alcohol abuse were, in most cases, unreliable for weaknesses in experimental design and/or in analytical methodology. It was not until 2015 that both acute intoxication and chronic abuse behaviour were investigated in the same subjects at the time of a road traffic accident [27,28]. In the first study, the authors measured BAC and CDT on 468 injured drivers by subdividing them according to BAC concentrations (below or above 0.50 g/L , i.e., the legal limit in most Western Countries) and comparing them to a control group (professional drivers mandatorily tested, without records of recent accidents) [28]. The results showed how the CDT distribution in the BAC-negative group of injured drivers was similar to that observed in the control group ($p = 0.159$) and different from that observed in the BAC-positive group ($p < 0.001$). In fact, the authors highlighted how their data strongly support the use of CDT to assess the risk of a subject to be involved in an alcohol-related road traffic accident [28]. The same group recently published a study where 929 drivers were tested for BAC and CDT at the time of hospital admission after a road traffic accident, with the aim of studying a potential correlation between CDT concentrations and the incidence of frequency and severity of alcohol intoxication of the driver. The results clearly showed that there is a high and almost linear correlation between % CDT and frequency of testing BAC-positive at the time of the accident. Moreover, strong correlation was also found between CDT and BAC of the injured driver. It is noteworthy to report that the range of CDT between 1.25 - 1.50 % (i.e., well below the currently adopted CDT cut-off) , is associated with BACs often above the Italian legal limit [27]. In both studies it was suggested by the authors that the cut-offs currently in use, should be

re-evaluated in terms of accident prevention.

In the same context, CDT proved more reliable than other traditional alcohol abuse indirect biomarkers such as MCV and GGT [29,30]. In particular, when MCV was studied in correlation with BAC in 6,244 drivers, the results showed that MCV increases were observed only in drivers involved in traffic accidents under severe alcohol intoxication (above 1.5 g/L). The authors concluded that MCV cannot be employed in the procedure of re-granting the driving license for a dramatic lack of sensitivity [29]. In the same framework, the degree of association of CDT, GGT and γ-CDT (i.e., the combination of GGT and CDT) with BAC was studied on 288 male drivers admitted to hospital after traffic accidents. The results, in terms of odds ratio, showed a much higher degree of association with BAC above 0.50 g/L for CDT (odds ratio 28) than for GGT (odds ratio 6). Eventually, the combination of CDT and GGT in the so-called γ-CDT parameter did not show any improvement in test performance compared to CDT alone [30].

Considering the social and personal consequences of a diagnosis of chronic alcohol abuse or alcohol dependence, in recent times several biomarkers of this condition have been proposed. Among these biological parameters, CDT shows important advantages over other existing diagnostic tools such as: high specificity, possibility of automation, international standardisation. Limitations include insufficient sensitivity to detect binge drinking, as well as interpretational difficulties in case of transferrin genetic variants and of advanced liver pathologies. However, in the context of traffic medicine, the most relevant feature of CDT is its proved association with the risk of occurrence of alcohol-related accidents, which has never been proved for other biomarkers.

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ԱԼԿՈԼԻ CDT ԲԻՈՄԱՐԿԵՐԻ ՀԱՄԱՌՈՏ ՊԱՏՄՈՒԹՅՈՒՆԸ ԵՎ ԴՐԱ ԿԻՐԱՌՈՒՄԸ ԴԱՏԱԲԺՇԿԱԿԱՆ ՓՈՐՁԱՔՆՆՈՒԹՅՈՒՆՆԵՐՈՒՄ ՃԱՆԱՊԱՐՀԱՏՐԱՆՍՊՈՐՏԱՅԻՆ ՊԱՏԱՀԱՐՆԵՐԻ ՀԵՏԱԶՈՏՈՒԹՅՈՒՆՆԵՐԻ ԴԵՊՔՈՒՄ

Պորպիլյա Ն.Մ., Ուիլդերս Ջ. Պ.Մ., Տալյարո Ֆ.

Հայտնի է, որ ալկոհոլի չարաշահումը ողջ աշխարհում համարվում է բնակչության առողջության վատթարացման առաջատար ռիսկային գործոններից մեկը: Սիա թե ինչու ալկոհոլի չարաշահման ներկա վիճակի օբյեկտիվ գնահատումը կարևոր է հանրային առողջության և անվտանգության պաշտպանության համար, հատկապես՝ կլինիկական, մասնագիտական համատեքստում, ինչպես նաև երթևեկության համատեքստում: Հոդվածը նվիրված է ներկայումս արդիական խնդիր համարվող սպառվող ալկոհոլում ամենահաճախ օգտագործվող բիոմարկերի՝ տրանսֆերինի կարբոհիդրատային դեֆիցիտի ստանդարտացման գործընթացի ձևավորման պատմությանը: Հաշվի առնելով ոչ սթափ վիճակում ճանապարհատրանսպորտային պատահարների թվի դիտարկվող աճը՝ հոդվածում հիմնավորվում է CDT բիոմարկերի կարևորության միտքը դատաբժշկական փորձաքննությունների անցկացման դեպքում:

Բանալի բառեր. *բիոմարկեր CDT, ճանապարհատրանսպորտային պատահար, դատաբժշկական փորձաքննություն, ալկոհոլային հարբածություն, բնակչության առողջության պաշտպանություն:*

КРАТКАЯ ИСТОРИЯ АЛКОГОЛЬНОГО БИОМАРКЕРА CDT И ЕГО ПРИМЕНЕНИЕ В СУДЕБНО-МЕДИЦИНСКОЙ ЭКСПЕРТИЗЕ ПРИ РАССЛЕДОВАНИИ ДОРОЖНО-ТРАНСПОРТНЫХ ПРОИСШЕСТВИЙ

Порпилья Н.М., Уайлдерс Дж.П.М., Тальяро Ф.

Общеизвестно, что неумеренное употребление алкоголя является одним из ведущих факторов риска пл охого состояния здоровья населения. Вот почему объективная оценка текущего состояния злоупотребления алкоголем имеет решающее значение для защиты здоровья общества и его безопасности, особенно в клиническом контексте и контексте дорожно-транспортного движения. Статья посвящена актуальной на сегодняшний день проблеме истории становления процесса стандартизации одного из наиболее часто используемых биомаркеров потребляемого алкоголя, а именно карбогидратного - дефицитного трансферрина. С учетом наблюдаемого роста дорожно-транспортных происшествий в состоянии алкогольного опьянения, в статье обосновывается мысль о важности бмар кера CDT при проведении судебно-медицинских экспертиз.

Ключевые слова: *биомаркер CDT, дорожно-транспортное происшествие, судебно-медицинская экспертиза, алкогольное опьянение, защита здоровья населения.*

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