



Letter to the Editor

Valid carbohydrate-deficient transferrin testing

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Carbohydrate-deficient transferrin (CDT, asialo-+ mono-sialo-+ disialo-Fe₂-transferrin) is currently the most specific laboratory marker of chronic alcohol abuse [1,2]. In 1993, Pharmacia launched the first commercial CDT assay, based on CDT and non-CDT fractionation at anion-exchanger microcolumns. Since then, several modifications of this assay principle were or are still offered for CDT determination [2]. Evaluating the most recent external proficiency testing programs of the German United Society for Clinical Chemistry and Laboratory Medicine (DGKL) and the German Society of Toxicological and Forensic Chemistry (GTFCH) shows that the majority of participants use CDT tests based on CDT and non-CDT microcolumn fractionation followed by a turbidimetric immunoassay (DGKL March 2005 (No. AP2/05): 98 participants using immunoassay, 17 HPLC, 3 other unspecified method; GTFCH June 2005 (No. AMF 2/05): 23 immunoassay, 10 capillary electrophoresis (CE), 10 HPLC). It is known that tests based on CDT and non-CDT microcolumn fractionation followed by immunoassay can cause false-positive CDT results in the presence of genetic transferrin D variants (approx. 0.2% in [3]) and also in the presence of high trisialotransferrin concentrations (approx. total frequency 2–3% in [3]). False-negatives can be due to genetic transferrin B variants (approx. 0.7% in [3], approx. 2.5% (1–2 days) unpublished data from the author's laboratory) [1–3].

CDT measurements are widely used for identifying individuals with chronic alcohol abuse in employment, traffic and forensic medicine. The potentially strong social impact of an increased CDT value justifies the need for a maximum reliable analysis result. A system of screening and confirmatory analysis of CDT (in analogy to forensic drug analysis) has first been discussed in [4]. However, CDT analysis is still widely done by a single immunoassay without confirmatory analysis of borderline or pathological CDT results by a physico-chemical analysis method.

This is surprising, since HPLC [5] and CE [6,7] CDT testkits became commercially available during the last

years. These methods have the advantage of visualizing the transferrin isoform patterns. Nevertheless, both methods (HPLC and CE) are also affected by the presence of genetic transferrin variants, which can cause co-elution or co-migration of CDT and non-CDT isoforms (e. g. disialo-Fe₂-transferrin of the C phenotype and trisialo-Fe₂-transferrin of the D phenotype) [2,8]. As is true for immunoassays based on microcolumn CDT and non-CDT fractionation, this co-elution or co-migration can cause false-positive (D variants) or false-negative (B variants) CDT results. However, in contrast to the microcolumn tests, the presence of the genetic transferrin B or D variants will *always* be detected by HPLC or CE, whereas they will *never* be detected by the microcolumn assays. Using HPLC or CE for CDT analysis, a comment on the presence of genetic transferrin variants can be added to the report and the CDT analysis result can be interpreted with special care (or rejected for diagnosis of chronic alcohol abuse).

With HPLC and CE we have specific, sensitive, and from the microcolumn CDT assays independent analysis methods. HPLC and CE allow the detection of (a) abnormal transferrin isoform patterns most often due to chronic alcohol abuse, (b) the detection of genetic transferrin variants as causes for false-positive and false-negative CDT results, (c) the exclusion of false-positives by incomplete CDT and non-CDT fractionation at the anion-exchanger microcolumn, and (d) reliable exclusion of trisialo-Fe₂-transferrin from the CDT fraction. Due to the latter, there is no interference of CDT determination by high amounts of trisialotransferrin.

The requirements for screening and confirmatory CDT analytical methods can be derived from the general protocols for drug analysis (references in [4]). Almost all criteria for a confirmatory analysis method are fulfilled when using HPLC or CE for (confirmatory) CDT analysis.

Against this background (and with the comparable costs per analysis for HPLC, CE and microcolumn assays in mind), CDT analysis by an immunoassay without confirmatory analysis of borderline and pathological results is no longer justified. Arguments regarding the low prevalence of transferrin D variants in routine analysis (approx. 0.2% in [3]) are not valid as an excuse for not confirming borderline or pathological CDT results since: (a) statistics are valid only for groups or populations but not for the single

individual and (b) high trisialo-Fe₂-transferrin concentrations will more frequently cause false-positive CDT results.

Valid concepts for CDT analysis would be: Concept 1: Screening analysis by microcolumn CDT and non-CDT fractionation followed by immunoassay (using transferrin antibodies) or screening by the homogeneous Dade Behring CDT immunoassay (using CDT antibodies). In the case of borderline or pathological CDT screening results, confirmatory analysis by HPLC or CE. Laboratories which do not have HPLC or CE should send an aliquot of the serum to a reference laboratory for confirmatory CDT analysis. A normal CDT result by HPLC or CE has priority over the borderline or pathological screening result—CDT has to be reported as normal. In analogy to drug analysis, no confirmatory analysis of normal CDT screening results. Concept 2: CDT analysis by HPLC or CE (without the need of confirmatory analysis by a second independent method). For both concepts, the presence of genetic transferrin variants (detected by HPLC or CE) and their effects on CDT should be reported together with the CDT result. In such cases, the final CDT report should also draw the attention of the physician to an especially careful interpretation of the CDT result and to the utmost importance of the clinical background.

Most recently, Helander et al. [9] published a comparison of HPLC and CE for confirmatory analysis of CDT results obtained by microcolumn immunoassays. The results support the use of HPLC and CE as confirmatory CDT analysis methods [5]. This should encourage the implementation of concept 1 or 2 into routine CDT analysis—now (for improving the legal position of the patient and the laboratory). In any case, diagnosis of chronic alcohol abuse should never be made on a single CDT measurement but on the clinically background and longitudinal control of CDT and serum activity of γ -glutamyltransferase.

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