Immunonephelometric Carbohydrate-Deficient Transferrin Results and Transferrin Variants

To the Editor:

Carbohydrate-deficient transferrin (CDT)\(^1\) is a biomarker of growing importance in the assessment of alcohol abuse after conviction for drunk driving. CDT is a more specific indicator for alcohol than traditional liver function tests and is used for identification and follow-up of chronic high alcohol consumption (1). Various methods have been introduced for assaying CDT in serum, including isoelectric focusing, ion-exchange chromatography, HPLC, capillary zone electrophoresis (CZE), and latex enhanced immunonephelometry (1, 2).

Measuring the CDT percentage (%CDT) in a forensic context demands CZE or HPLC methodology, because it provides high-resolution separation of serum transferrin (Tf) isoforms and allows the detection of genetic variants and glycosylation disorders. In some cases, the interpretation of CDT results is hampered by the presence of mutant Tf (3). In addition to wild-type Tf (C), D variants (cathodal to C) and B variants (anodal to C) have been described (1). The allele frequencies of the Tf subtypes vary among populations of different ethnicities (4). Exact measurement of D variants of CDT is difficult, however, because disialo- and trisialylated D peak in HPLC or comigrate in CZE. Algorithms have been proposed to correct for the presence of mutant Tf in capillary electropherograms (4, 5).

Immunonephelometric tests in which highly specific monoclonal antibodies recognize the Tf glycosylation sites have been introduced. The N Latex CDT assay (Siemens) uses a monoclonal antibody that recognizes Tf glycoforms lacking 1 or 2 complete N-glycans (i.e., disialo-, monosialo-, and asialo-Tf) (2). In addition, total Tf is measured. There is limited evidence for the effect of mutant Tf on CDT test results, mainly because of the small number of participants who were enrolled in the evaluation study (2). Because Tf variants are common findings (particularly in non-Caucasian populations) (3, 4), knowledge of the performance of immunonephelometric tests in the presence of Tf mutants is of practical importance. In the present study, we compared the effect of Tf variants on CZE and nephelometric CDT testing. The study was approved by the Ethics Committee of Ghent University Hospital (EC/008-2012).

Within the framework of a driver’s license–regranting program, CDT was assayed on a Sebia CapilarySm 2 system (3). During a 1-year period, 4878 Caucasian drivers were enrolled, and 51 drivers (1.1%) were found to be heterozygous for Tf mutants. Samples carrying heterozygous Tf were stored at −20 °C until further analysis on a BN II nephelometer (Siemens) (2). All samples were measured with the same reagent batch to avoid lot-to-lot variation. In cases of Tf heterozygosity, CZE results were estimated by means of a correction factor (4, 5).

In 19 cases, a Tf B phenotype was present, and a D phenotype was found in 32 cases. D mutants could be divided into 3 subgroups according to their charge: the most common subtype (CZE retention time for mutant Tf, 130 s; n = 25), faster D variants (retention time, 120 s; n = 3), and slower D variants (retention time, 145 s; n = 4). The identity of the Tf mutants was confirmed by starch gel electrophoresis.

As a control group, 51 drivers with wild-type Tf were assayed in parallel. The Tf B and D subgroups had significantly lower (P < 0.0001, and P < 0.001, respectively) ratios of %CDT measured by the N Latex CDT method to %CDT measured by the CZE method, compared with the wild-type group (Fig. 1). The immunonephelometric assay produces higher %CDT values, approximately 2 times the CZE value for wild-type Tf. Tf B and Tf D heterozygotes, however, showed only about a 1:1 ratio of the %CDT measured by the N Latex CDT method to the %CDT estimated by CZE.

These data suggest that results obtained by various CDT techniques are not always interchangeable in the presence of mutant Tf. The immunonephelometric assay underestimates the %CDT in the presence of mutant Tf, compared with the %CDT estimated by CZE. This result could be due to less efficient binding of the mutant Tf by the monoclonal antibody used in the N Latex CDT assay. Because detection with the CZE test (based upon photometric absorption at 200 nm) is independent of protein sequence, this technique provides a more objective judgment than immunochromatographic methods. For forensic purposes, chromatographic/electrophoretic methods are mandatory for CDT analysis. This consideration could be of major importance for populations carrying a high frequency of the D allele, such as black Africans, African Americans, and Australian aboriginals (4). Correcting CZE results by a factor of 2 (4, 5) is an estimation, however, not an exact measure, and is possible only if the difference in the retention times of wild-type Tf and the Tf mutant permits sufficient separation of the Tf peaks. In cases of such interpretation difficulties, alternative markers (e.g., ethylglucuronide sulfate, phos-

\(^1\) Nonstandard abbreviations: CDT, carbohydrate-deficient transferrin; CZE, capillary zone electrophoresis; %CDT, CDT percentage; Tf, transferrin.
phoethanolamine) should be considered for the assessment of chronic alcoholism.

Author Contributions: All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors’ Disclosures or Potential Conflicts of Interest: No authors declared any potential conflicts of interest.

References


Fig. 1. Box-and-whisker plots for different subgroups of transferrin phenotypes for the ratio of the %CDT measured by the N Latex CDT assay to the %CDT measured by CZE.

*P < 0.001 and **P < 0.0001, Mann–Whitney U-test for independent samples compared with the wild-type subgroup. Data are presented as the median, interquartile range, and range.

Letters to the Editor

Thomas M. Maenhout2
Marc Uytterhoeven3
Elke Lecocq2
Marc L. De Buyzere2
Joris R. Delanghe2*

2 Department of Clinical Chemistry
Ghent University Hospital
Ghent, Belgium
3 Sonic Healthcare Benelux – Medical Laboratory
Antwerp, Belgium

*Address correspondence to this author at:
Department of Clinical Chemistry
Ghent University Hospital
De Pintelaan 185
B 9000 Gent, Belgium
Fax +32-9-3324985
E-mail joris.delanghe@ugent.be

Previously published online at DOI: 10.1373/clinchem.2012.195891