Carbohydrate-Deficient Transferrin Measured by Capillary Zone Electrophoresis and by Turbidimetric Immunoassay for Identification of Young Heavy Drinkers, Jean-Bernard Daeppen,1,2 Frederic Anex,3 Bernard Favrat,2 Alvine Bisseray,1 Joelle Leutwyler,1 Roland Gammeter,1 Patrice Mangin,2 and Marc Augsburger2 (1 Alcohol Treatment Center, CHUV, Lausanne, Switzerland; 2 Institute of Forensic Medicine, CHUV, Lausanne, Switzerland; 4 address correspondence to this author at: Alcohol Treatment Center, Mont-Paisible 16, CHUV, 1011 Lausanne, Switzerland; e-mail jean-bernard.daeppen@inst.hospvd.ch)

Carbohydrate-deficient transferrin (CDT) measured by capillary zone electrophoresis (CZE), particularly asialo-transferrin (Tf), is purported to better differentiate between excessive and moderate drinkers than does CDT measured by turbidimetric immunoassay (TIA) (1, 2). The use of biological markers such as CDT is of particular interest for identifying young heavy drinkers because other clinical signs of heavy drinking are generally absent and heavy drinking is a leading cause of morbidity and mortality in this age group (3, 4). Several authors have shown interest in the ability of CDT to identify nondependent heavy drinkers (5, 6); we therefore describe here the performance of CZE measurements of asialo- and disialo-Tf and TIA analysis of CDT in a large community sample of 19-year-old men, of whom 21% were heavy drinkers.

From a sample of 1018 men attending a mandatory 1-day army recruitment process for all Swiss males at age 19 years, 1004 (98.6%) agreed to complete a research questionnaire. Of these, 581 young men (57.9%) consented to give blood for the measurement of asialo-Tf (CZE), disialo-Tf (CZE), and CDT (TIA). The Ethics Committee of the Lausanne University Medical School approved the study protocol. Volunteers were compensated for participation in the study.

Volunteers gave written informed consent and then completed an instrument entitled “Health and Lifestyle Questionnaire”, which included questions assessing the typical quantity and frequency of alcohol consumption during the 12 months preceding the survey and the frequency of drunkenness over the last 30 days. One drink was defined as a 250-mL can or bottle of beer, a 120-mL glass of wine, or a 40-mL shot of liquor straight or in a mixed drink, and corresponded to ~12 g of pure ethanol.

A study investigator was present during administration of the questionnaire to verify that participants answered all items. Serum samples were obtained by centrifugation of peripheral blood collected in 10-mL tubes. Samples were stored at −20 °C before analysis.

Total CDT was measured by anion-exchange chromatography and TIA with the Axis-Shield CDT (TIA) reagent set (7). To separate and measure Tf isoforms, we used a previously described and validated CZE method (8, 9) with the Cefoxi CDT reagent set (Analis) on a Hewlett Packard (HP) 3D-CE instrument. The CZE conditions are described in Table 1 of the Data Supplement that accompanies the online version of this Technical Brief at http://www.clinchem.org/content/vol51/issue6/. CZE electropherograms showing the serum Tf profiles for a heavy drinker before and after addition of anti-Tf polyclonal antibody to the serum are shown in Fig. 1 of the online Data Supplement, and CZE electropherograms showing the Tf profiles of a teetotaler and of 2 heavy drinkers are shown in Fig. 2 of the online Data Supplement.

Peaks representing the different Tf isoforms were quantified as the amounts of the asialo-, disialo-, trisialo-, tetrasialo-, pentasialo-, and hexasialo-Tf (CZE) as a percentage of the total Tf content, in terms of valley-to-valley areas under the curve. The intraday CV values (n = 6) for “low” (0.6% by CZE) and “high” disialo-Tf (4.8% by CZE) were 9.8% and 1.2%, respectively, and the interday CVs (n = 5) for low (0.6% by CZE) and high disialo-Tf (4.8% by CZE) were 11% and 2.3%, respectively. The intra- and interday CVs for asialo-Tf (0.5% by CZE; n = 6) were 6.8% and 11%, respectively. The limit of quantification of each Tf (CZE) isoform was 0.1%, expressed a percentage of total Tf isoforms.

Continuous data are reported as the mean (SD) and the median (interquartile range). We used a χ2 test to compare categorical variables and Mann–Whitney U-tests to compare continuous variables because this nonparametric statistic makes no assumption about the distributional properties of variables. We also determined the areas under the ROC curves (AUROC), the sensitivity, and the specificity for disialo-Tf (measured by CZE) and CDT (measured by TIA) in identifying heavy drinkers.

There were 121 (20.8%) heavy drinkers in the sample: 31 (5.3%) who reported typical alcohol consumption of >21 drinks/week over the last 12 months, 52 (8.9%) who said they had been drunk at least 3 times over the last month; and 38 (6.5%) who reported both. Mean (SD) alcohol consumption in heavy drinkers was 26.4 (8.4) drinks (~300 g of ethanol) per week. Among the remaining participants, 435 (74.9%) were categorized as moderate drinkers, reporting, on average, 6.0 (4.7) drinks (~65 g of ethanol) per week, and 25 (4.3%) were considered abstinent (mean reported quantity and frequency = 0). The abstaining participants were retained as part of the moderate-drinker group.

Our results indicate that asialo-Tf (CZE) could not differentiate between moderate and heavy drinkers because 574 (98.8%) of the participants had an asialo-Tf (CZE) value of 0% and only 3 moderate drinkers and 4 heavy drinkers had positive values. We did, however, find significant differences between heavy and moderate drinkers.
results may not hold true for samples of other individuals, such as women, older persons, or those recruited within medical settings. Our study sample consisted mostly of men, it is important to recognize several limitations when generalizing these findings to other populations. These results may broadly apply to young men, it is important to recognize several limitations when generalizing these findings to other populations. These results may not hold true for samples of other individuals, such as women, older persons, or those recruited within medical settings. Our study sample consisted mostly of Caucasians; thus, the findings may not apply to other ethnic groups. The differences we observed in drinking patterns between those who agreed to give blood and those who refused preclude generalizing the findings to the overall sample. Finally, although great effort was made to optimize the accuracy of these data, the information obtained regarding alcohol use and alcohol-related problems was based solely on the estimates and recollections of the participants.
We are grateful to Magali Dovat for skillful technical assistance with asialo-Tf (CZE) and disialo-Tf (CZE) measurements and to George Danko, PhD, for careful help in the editing of the manuscript.

References


DOI: 10.1373/clinchem.2004.044461

Comparison of the Unsaturated Iron-Binding Capacity with Transferrin Saturation as a Screening Test to Detect C282Y Homozygotes for Hemochromatosis in 101 168 Participants in the Hemochromatosis and Iron Overload Screening (HEIRS) Study, Paul C. Adams,1* David M. Reboussin,2 Cathie Leendecker-Foster,3 Godfrey C. Moses,4 Gordon D. McLaren,5 Christine E. McLaren,6 Fitzroy W. Dawkins,7 Ismhael Kasvoste,8 Ron T. Acton,9 James C. Barton,9 Dan Zaccaro,2 Emily L. Harris,10 Richard Press,11 Henry Chang,12 and John H. Eckfeldt3 (1 Department of Medicine, London Health Sciences Center, London, Ontario, Canada; 2 Department of Public Health Sciences, Wake Forest University School of Medicine, Winston-Salem, NC; 3 Department of Laboratory Medicine and Pathology, University of Minnesota, Minneapolis, MN; 4 MDS Laboratories, Toronto, Ontario, Canada; 5 Division of Hematology/Oncology, Department of Medicine, University of California, Irvine, CA, and Veterans Affairs Long Beach Healthcare System, Long Beach, CA; 6 Epidemiology Division, Department of Medicine, University of California, Irvine, CA, and Department of Medicine, Howard University, Washington, DC; 7 Department of Microbiology, Medicine, and Epidemiology and International Health, University of Alabama at Birmingham, Birmingham, AL; 8 Southern Iron Disorders Center, Birmingham, AL; 9 Kaiser Permanente Center for Health Research, Portland, OR; 10 Division of Blood Diseases and Resources, National Heart Lung and Blood Institute, NIH, US Department of Health and Human Services, Bethesda, MD; * address correspondence to this author at: Department of Medicine, London Health Sciences Centre, 339 Windermere Rd., London, ON N6A 5A5, Canada; fax 519-858-5114, e-mail padams@uwo.ca)

The diagnosis of hemochromatosis was previously based on a combination of clinical and laboratory assessments that included history and physical examination, increased transferrin saturation (TS) and serum ferritin, liver biopsy, the amount of iron removed by phlebotomy, and pedigree studies identifying other family members with iron overload (1). Since the discovery of the hemochromatosis gene (HFE) in 1996 (2), most studies from referral centers have shown that >90% of typical hemochromatosis patients are homozygous for the C282Y mutation of the HFE gene (3). Before the availability of DNA-based testing, it was assumed that most hemochromatosis patients have increased TS. However, recent population screening studies incorporating HFE genotyping have now shown that many C282Y homozygotes will have a normal TS and may never develop clinical signs and symptoms related to iron overload (4–8). TS has been recommended in many studies as the most clinically useful screening test for hemochromatosis because it is widely available and may be increased even in young adults with a genetic predisposition to hemochromatosis. Another potential advantage over DNA-based testing as an initial screening test is that TS may detect many types of iron overload other than those associated with HFE mutations. In addition, screening for iron overload instead of performing DNA-based testing may reduce the risks of potential genetic discrimination that some authors suggest is associated with identification of a C282Y homozygote with normal serum iron tests (9–11). The TS is a 2-step assay in which serum iron is the numerator and the denominator is either total iron-binding capacity (TIBC), [serum iron + unsaturated iron-bonding capacity (UIBC)] or an adjusted serum transferrin. The UIBC is a 1-step automated colorimetric assay that has been reported to have similar or better operating characteristics than TS for the detection of C282Y homozygotes (12–15). In this study, UIBC is compared directly with the TS (as measured by serum iron/serum iron + UIBC) for the detection of C282Y homozygotes in a large primary care population.

The study design and overall results of the Hemochro-