forms, especially non-CDT isoforms, is unlikely.

The sixth, and final, argument is that as an analyte, asialotransferrin can simplify the production of a specific antibody for a direct assay. With a complete lack of both transferrin N-glycans (2, 10, 11), the structure of asialotransferrin is different from the structure(s) of the non-CDT isoforms. Thus, there is the possibility of raising a specific antibody that binds strongly to the analyte (asialotransferrin), e.g., to an epitope near or at the original point of attachment of the carbohydrate chain(s). The availability of such an antibody against asialotransferrin would make a direct assay (without microcolumn fractionation of asialotransferrin from the other transferrin isoforms, as is currently needed for CDT and non-CDT fractionation by most of the commercial CDT assays) more probable. This would be an important step toward automation of CDT (or, more precisely, asialotransferrin) analysis.

Regardless of whether CDT or asialotransferrin is used for laboratory diagnosis of chronic alcohol abuse, the diagnosis should always be made based on inclusion of a clinical questionnaire and γ-glutamyltranspeptidase, and not on a single CDT value alone. Strategies for increasing the reliability of CDT results by use of a screening and confirmatory method have been discussed recently in this journal (14).

References

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Modification of the Colorimetric Assay for Serum Unsaturated Iron-Binding Capacity

To the Editor:

We reported recently that total iron-binding capacity (TIBC) values calculated from serum iron and unsaturated iron-binding capacity (UIBC) values were significantly lower than those obtained by a direct and fully automated TIBC assay (1, 2). We also reported that slopes of regression lines for calculated TIBC values plotted against serum transferrin (TRF) were ~7% lower than the theoretical ratio of TIBC to TRF (TIBC/TRF = 25.1 μmol/g). We found that this could be attributed to underestimation of UIBC values by colorimetric methods. One possible reason for underestimation of UIBC values was insufficient saturation of TRF. We modified the assay conditions of a colorimetric method for UIBC measurement to improve the correspondence between TIBC values converted from TRF and those calculated from serum iron and UIBC.

Both serum iron and UIBC were determined by colorimetric methods (Wako Pure Chemical Industries) with a Hitachi Model 7070 automated analyzer. Serum TRF concentrations were determined by a nephelometric assay on a Behring Nephelometer II analyzer (Dade Behring). UIBC values were determined by four modified methods (Table I). UIBC_A was measured by the original method, in which assay conditions were set by the manufacturer. Incubation time for saturation of TRF was extended from 5 min to 10 min for the measurement of UIBC_B. The ratio of iron provided to saturate TRF per serum sample (iron/serum) was increased from 0.195 μmol/L to 0.26 μmol/L for the measurement of UIBC_C by decreasing sample volume from 20 μL to 15 μL. Finally, both incubation and the iron/serum ratio were increased for the measurement of UIBC_D. TIBC values were calculated as the sum of serum iron and each UIBC value and designated Cal-TIBC_A, Cal-TIBC_B, Cal-TIBC_C, and Cal-TIBC_D, respectively. TIBC values converted from serum TRF concentrations with the theoretical TIBC/TRF ratio were designated Con-TIBC. We also calculated Cal-UIBC values as the difference between Con-TIBC and serum iron values. Correlations were assessed by principal component regression analysis. The 95% confidence interval (95% CI) for the slope of a regression line was estimated by the bootstrap method. A significance
level of 0.05 was used for all statistical tests, and a two-tailed paired t-test was applied.

Comparisons of the Cal-TIBC values and Con-TIBC and TRF concentrations are shown in Table 1. The within-run CVs for the modified UIBC assays were 2.6–3.9%. The values obtained by the UIBCA method and the UIBC method were significantly higher than those obtained by the UIBCA method \((P < 0.001)\), whereas there was no significant difference between the values obtained by the UIBCA and the UIBCC method \((P = 0.893)\). All slopes of the regression lines for correlations between the Cal-TIBC and Con-TIBC values were closest to 1.0, and the 95% CI for the slope included the theoretical TIBC/TRF ratio. The slope of the correlation between the Cal-TIBC and Con-TIBC values was lower than that between the Cal-TIBC and the Con-TIBC values, but the slope of the correlation between the Cal-TIBC and Con-TIBC values was lower than that between the Cal-TIBC and the Con-TIBC values.

![Table 1. Correlations between Cal-TIBC values and Con-TIBC and TRF concentrations.](image)

The rate of iron binding to TRF is affected by factors such as reaction temperature, pH, ionic strength, and anions. It has been reported that TRF binds iron with \(\text{HCO}_3^-\) as a coligand in the presence of \(\text{O}_2\) and that the concentrations of \(\text{HCO}_3^-\) and \(\text{O}_2\) affect the kinetics of the binding process \((3)\). Insufficient saturation of TRF by the effects of these factors leads to underestimation of the UIBC value.

The UIBCB values were significantly higher than the UIBCA values, whereas there was no difference be-
between the UIBC_A values and the UIBC_C values. These results suggest that an extended incubation time for the saturation of TRF ensures more reliable UIBC values but the increase in iron/sérum ratio does not. The significant difference between the UIBC_O and UIBC_A values was not attributable to the increase in the iron/sérum ratio but to the extended incubation time. Larger UIBC values contributed to an improved correlation between the Cal-TIBC and Con-TIBC values; i.e., the upper limit of the 95% CI for the slope between the TIBC values; i.e., the upper limit of contributted to an improved correla- 

incubation time. Larger UIBC values

are positively correlated with the theoretical TIBC/TRF ratio, but to the extended incubation time for saturation of TRF should be increased to improve the agreement between the Cal-TIBC and Con-TIBC values. Although available ranges of incubation times for the TRF-saturation step differ among commercial analyzers, it is essential that the incubation time for saturation of TRF be set as long as is needed.

References


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Increased Carbohydrate-deficient Transferrin of Unknown Etiology in a 15-Year-Old Male Patient with Autoimmune Hepatitis Type 1

To the Editor:

Carbohydrate-deficient transferrin (CDT) is currently the most specific laboratory marker of chronic alcohol abuse (1). We report a 15-year-old boy with autoimmune hepatitis type 1, increased serum CDT, and no history of alcohol abuse. The case is particularly important because patients in the early phase of autoimmune hepatitis may be asymptomatic. The diagnostic criteria for autoimmune hepatitis (2), which were used for our patient, are summarized together with the patient's data in the Data Supplement that accompanies the online version of this letter at http://www.clinchem.org/content/vol49/issue6/.

The patient was admitted for evaluation of icterus ~1 week in duration, decreased performance, and fatigue for ~6 months. He had mild splenomegaly, and biopsy revealed developing micronodular cirrhosis. The patient's denial of alcohol intake was confirmed by his mother and by AUDIT (3) and MALT-F scores (4). One week before admission, the patient had herpes zoster treated successfully with Zovirax (orally) for 5 days. The patient had received no hepatotoxic medication and did not abuse drugs. Aspartate aminotransferase was increased ~30-fold, alanine aminotransferase 14-fold, bilirubin 8-fold, and direct bilirubin 27-fold. Serum concentrations of IgA and IgM were within the appropriate reference intervals. IgG was increased approximately twofold. For more details and other test results, see the Data Supplement.

The patient fulfilled the scoring criteria for definite autoimmune hepatitis type 1 at both pretreatment (minimum scoring points, 15; patient scoring points, 18) and posttreatment (minimum scoring points, 17; patient scoring points, 20; see the Data Supplement).

The CDT:transferrin ratio, measured by two turbidimetric immunoassays based on microcolumn CDT and non-CDT fractionation, was 3.2% [cutoff, 2.5%; borderline, 2.5–2.7% (1)] for the ChronAlcoL.D. assay (Sangui) and 3.2% (cutoff, 2.6%; borderline, 2.6–3.0%; manufacturer's test instructions) for the %CDT-TIA assay (Axis). Isoelectric focusing (IEF)-immunofixation-silver staining [slightly modified from the method of Hackler et al. (5)] showed an abnormal transferrin isofrom band pattern with increased amounts of disialo-Fe_2-transferrin, which led to a decreased triaio-/disialo-Fe_2-transferrin peak height (area) ratio of 1.20 (Fig. 1, lane 3) compared with 2.2 for the healthy control (Fig. 1, lane 2). Asialo transferrin, which is present with high prevalence in serum after chronic alcohol abuse (6) (Fig. 1, lanes 1 and 4) and genetic transferrin variants were not detected in our patient's serum (Fig. 1, lane 3). In summary, the IEF transferrin isofrom band pattern for our patient confirmed the immunologic CDT results qualitatively but was not typical for chronic alcohol abuse.

We excluded the currently known clinical conditions for false-positive CDT results (1) as etiologies of our findings (see the Data Supplement). We know of no evidence that Zovirax alters transferrin glycosylation. Liver cirrhosis has been reported to increase CDT (7). In this study (7), this was most probably attributable to active viral hepatitis, which is known to increase serum CDT concentrations (1, 8). Our patient had tested negative for acute and chronic viral hepatitis as well as asialoglycoprotein receptor antibodies. Thus, his increased CDT:transferrin ratio cannot be explained by viral hepatitis.

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