Insufficient Standardization of a Direct Carbohydrate-Deficient Transferrin Immunoassay

To the Editor:

Measurement of carbohydrate-deficient transferrin (CDT) can reveal alcohol-related changes in the serum transferrin glycosylation pattern. CDT is a more alcohol-specific indicator than liver-function tests and is used for identification and follow-up of chronic high alcohol consumption (1). Therefore stable calibration of the assays is very important. Comparison of CDT results between methods has often been hindered by method-dependent discrepancies in the definition of the measurand (the transferrin glycoforms covered) and the way results are expressed. With some methods there has been an increased risk for false-positive results (2). The lack of CDT standardization prompted initiation of a working group under the International Federation of Clinical Chemistry and Laboratory Medicine, whose aim was to define the measurand, select and validate a reference method, and work out procedures for the production of reference materials. The first recommendation was that the fraction of disialotransferrin to total transferrin (%disialotransferrin) should be the primary target for CDT testing, with HPLC as the candidate reference method (3).

The performance of individual laboratories and agreement of different methods can be determined through external quality assessment (EQA). A Swedish EQA scheme for CDT has been run by EQUALIS (External Quality Assurance in Laboratory Medicine in Sweden) since 1996. Each year, 10 samples (human serum pools without preservative) whose target %disialotransferrin values are set by selected expert laboratories that use HPLC (3) are distributed to the participants.

In-house or commercial HPLC assays are the most common CDT assays (n = 19) in the EQUALIS EQA scheme. HPLC methods involve separation of iron-saturated transferrin glycoforms by anion-exchange chromatography, and quantification by selective absorbance of the iron-transferrin complex at approximately 460 nm (4). The disialotransferrin fraction is calculated as relative peak area using baseline integration, in agreement with the recommendations of the CDT standardization work (3). The second most common method (n = 15) is the N Latex CDT direct immunonephelometric assay (Siemens, formerly Dade Behring). The N Latex CDT assay uses a monoclonal antibody that recognizes transferrin glycoforms lacking 1 or 2 complete N-glycans (i.e., disialo-, monosialo-, and asialotransferrin) and on a simultaneous transferrin immunoassay (5). The %CDT value (fraction of disialo-, monosialo-, and asialotransferrin to total transferrin) is calculated automatically.

In a multicenter evaluation of N Latex CDT (5), the %CDT results correlated well with the %disialotransferrin results by HPLC, but owing to the different measurements the numerical values were not interchangeable. For healthy controls, the upper 97.5th percentile for %disialotransferrin by HPLC was approximately 1.7% (4) compared with approximately 2.35% for %CDT by N Latex CDT (5). A discrimination limit of 2.5% (99th percentile) is proposed in the N Latex CDT package insert, and is commonly applied in clinical practice. However, starting in 2006, the relation between the HPLC and N Latex CDT values in the EQUALIS EQA surveys has gradually changed. At %disialotransferrin values around 2% by HPLC, the corresponding %CDT results by N Latex CDT were on average 0.4% higher in April 2006, but roughly identical in September 2007 (Fig. 1A). This change was observed over the entire measuring range (Fig. 1B).

Four serum pools with %disialotransferrin target values of 1.02%, 1.75%, 2.61%, and 3.52% that were stored frozen since their use in a Swedish CDT harmonization program in 2002–2003 were reanalyzed by HPLC in November 2007. The resulting values were almost identical to the original ones (1.00%, 1.83%, 2.58%, 3.53%; r² = 0.997), confirming that the...
HPLC target values were stable over the study period.

The change in the relation between HPLC and N Latex CDT values thus seems to be related to a change in the calibration of N Latex CDT when new reagent batches were released. During the study period, at least 6 lots have been in use. The results indicate that the manufacturer-recommended cutoff limit for N Latex CDT at 2.5% is currently too high and will produce false-negative clinical results (patients with harmful drinking habits remain undetected and untreated). Based on the comparison with the HPLC results in the EQUALIS EQA scheme, the current 99th percentile for %CDT by N Latex CDT should be approximately 1.9%–2.0% (Fig. 1B).

According to international directives, manufacturers of in vitro diagnostic products are required to provide reference intervals and control the conformity of each batch before placement on the market. However, the present observation highlights the need for laboratories to verify the cutoff limit for every new batch of N Latex CDT, and the value of EQA schemes. For an improved standardization of CDT, a reference method and reference materials are needed.

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References


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Influence of L-Thyroxine Therapy on Parathyroid Hormone Concentrations

To the Editor:

In our RIA laboratory, we serendipitously observed that an unusual number of patients referred from the outpatient endocrinology clinic of the St. Savas Oncology Hospital had parathyroid hormone (PTH) concentrations exceeding the recommended upper reference limit of 6.90 pmol/L (65 ng/L) of our assay (ELSA-PTH, Cisbio International). Further work-up failed to show any evidence for hyperparathyroidism, but these patients were all receiving L-thyroxine for various thyroid problems, and their thyrotropin (TSH) concentrations were very suppressed (<0.4 mIU/L).

To investigate this phenomenon further, we obtained blood samples from all patients on thyroxine therapy (for goiter, hypothyroidism, thyroiditis, or thyroid cancer) referred to our laboratory. As a reference population, healthy blood donors, matched for age and sex and on no medication, were also included in our study. The study was conducted after institutional review board approval, and all participants gave their informed consent. The PTH was assayed in plasma samples obtained in EDTA-containing plastic tubes (Vacutainer®, Beckton Dickinson) and immediately centrifuged (1000g) for 10 min at 4 °C. Plasma was then separated and stored at −20 °C. The PTH assay (ELSA-PTH, Cisbio International) is a 2-step IRMA, with a monoclonal capture antibody specific for the mid region and the carboxyl-terminal part (39–84) of the PTH molecule and a radiolabeled polyclonal antibody that recognizes the N-terminal part (1–34) of the PTH molecule. Serum was also collected and sent to the high-volume hospital laboratory for calcium, phosphorus, albumin, creatinine, and hepatic enzyme determination, and another portion was assayed for TSH (IRMAZENco TSH-S, Zentech; detection limit: 0.025 mIU/L), thyroxine, and triiodothyronine (free and total). For PTH concentrations, 95% CIs were derived for each population using the MedCalc® software package. Owing to the known skewness in the distribution of the PTH values, logarithmic transformation of the data was used in calculating the 95% CIs. The patient population (n = 95, 9 males and 86 females, age range 42–75 years, mean age 58 years) on L-thyroxine therapy was subdivided (n = 64) into those with excessively suppressed TSH concentrations (<0.4 mIU/L, mean, 0.28 mIU/L) and those (n = 31) with TSH concentrations kept within normal limits (mean, 0.87 mIU/L). A 95% CI for PTH was also derived for healthy blood donors (n = 39). Patients on calcium-altering medications and/or with abnormal calcium/phosphorus concentrations, obese patients, and those with abnormal renal and/or hepatic function were excluded from this study (1).

Our results are shown in Fig. 1. The 95% CI for patients with excessively suppressed TSH concentrations was 2.12–12.27 pmol/L (20.0–115.7 ng/L), whereas for patients with normal TSH concentrations and healthy blood donors the 95% CIs were 1.37–7.54 pmol/L (12.9–71.4 ng/L) and 0.96–7.53 pmol/L (9.1–71.0 ng/L) respectively (mean PTH concentration ±2 SD, calculated from the log-transformed data). The means of the PTH values of the 2 L-thyroxine–treated groups of patients were compared using the unpaired t-test (with Welch correction), and the derived P value (P = 0.0197) indicated a statistically significant difference of the means of the 2 data sets. Comparison of the means of the PTH values of the healthy blood donors and the L-thyroxine–treated patients, with normal TSH values, showed no statistical significance (P = 0.4658).

Overtreatment of patients with L-thyroxine and hyperthyroidism is a well-known cause of accelerated bone loss with increased serum calcium, although the anticipated PTH suppression in these patients does not appear to be unequivocally supported in the literature (2, 3). The findings in this report could be attributable to a nongenomic action of the supraphysiological peak L-thyroxine concentrations on the parathyroid cells, possibly via mitogen-activated protein kinase, bypassing the calcium-sensing receptor regulation (4). Commercial kit–related parameters, such as the concurrent measurement of the intact PTH molecule along with its carboxyl-terminal degradation products in the plasma, may also play a role, although further studies are definitely required to clarify this issue, because PTH assays are known to vary in their specificity (5).

In conclusion, we have observed PTH concentrations to be higher in patients receiving L-thyroxine therapy who have highly suppressed TSH concentrations. Further studies of this phenomenon with other PTH assays are needed.