The Determination of Carbohydrate Deficient Transferrin (CDT) with N Latex CDT: Comparison with a Validated HPLC Method

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AIMS: Carbohydrate Deficient Transferrin (CDT) is the collective name of a group of a minor glycoforms of transferrin (Tf) (namely, asialo-, monosialo-, disialo-Tf), whose serum concentration increases after repeated excessive alcohol intake (i.e. 60 - 80 g/day for at least 7 - 10 days). At present, CDT is considered the most reliable indicator of chronic alcohol abuse, showing a diagnostic specificity > 90% and a diagnostic sensitivity >70%. For several years CDT analysis has routinely been performed by immunometric methods based on a preliminary extraction of the CDT isoforms with disposable anion exchange cartridges followed by immunometric detection using antibodies anti-total Tf. This method, however, suffers from poor analytical specificity and scarce precision, as demonstrated by different studies [1,2]. Quite recently, a new immunometric method (N Latex CDT, Dade Behring, Deerfield, IL, USA) has become available, which, differently from the previous immunoassay, is based on antibodies specific for the CDT related isoforms of Tf. The aim of the present study was to compare the analytical performances of this new analytical approach with a validated HPLC method, at present the candidate reference technique for CDT analysis.

METHODS: Sera from 150 subjects, applying for the driving license after its confiscation for drunk driving, have been analyzed with both techniques. The immunoassay analysis was performed on BN ProSpec(R) system (Dade Behring) using proprietary reagents (N Latex CDT, Dade Behring)). N Latex CDT is a ready to use latex-particle enhanced assay that contains polystyrene particles coated with a monoclonal antibody against CDT that are agglutinated by CDT-coated polystyrene particles. CDT in the sample inhibits the reaction between antibody-coated and CDT-coated particles in a dose dependent manner. The agglutination reaction is monitored by measuring light scattering. HPLC analysis was performed on a gradient HPLC (Shimadzu Europe, Germany), using an anion exchange column (Recipe, Munich, Germany) eluted with a gradient of NaCl (from 0 to 135 mM in 20 minutes) concentration in 10 mM BIS-TRIS buffer, pH 6.2. Detection was by radiation absorbance at 460 nm. Prior to injection, serum samples were saturated with a ferric solution. The results from both methods were expressed as percentage ratio of CDT isoforms on total transferrin (% CDT).

RESULTS AND CONCLUSIONS: With the immunoassay %CDT ranged from 1.03 to 4.5% with mean value of 1.96% (SD 0.63). With HPLC %CDT ranged from 0.8% to 5.6% with mean value of 1.62% (SD 0.92). The difference of the mean values (0.34%) was found statistically significant (p < 0.001) using the Student t-test. The quantitative comparison of the results of the two methods showed a significant correlation described by the following equation: \( y = 0.5925x + 0.997 \) (r = 0.86) where y = data from the immunoassay and x = data from the HPLC. The analysis of about 100 subjects with normal CDT concentrations, based on the HPLC analysis [2] (cut-off = 1.9%), were used to evaluate a cut-off level of the N Latex CDT assay, which at the 97.5% percentile resulted to be 2.58%.

Keywords: CDT analysis, Immunometric methods, HPLC