CDTect-RIA AND CDTect-EIA FOR DETERMINATION OF SERUM CARBOHYDRATE-DEFICIENT TRANSFERRIN COMPARED

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Abstract — CDTect-RIA and CDTect-EIA for determination of serum carbohydrate-deficient transferrin (CDT) by radioimmunoassay and enzyme immunoassay respectively were tested for equality and precision in four European laboratories. For correlational studies, serum samples with CDT concentrations up to 130 U/l were analysed in accordance with a uniform trial schedule. The regression of CDT values obtained by the two procedures was computed for each laboratory using the method of Passing and Bablok. Slopes and intercepts of the regression functions did not differ significantly from the values 1 or 0, as proved by the corresponding 95% confidence intervals. Precision studies were computed using analysis of variance. For CDT concentrations at the upper reference limit for men, the within-day coefficients of variation (CVs) ranged between 0.7 and 6.4% (median 5.2%) for CDTect-RIA and from 4.3 to 9.2% (median 6.2%) for CDTect-EIA. The corresponding pure between-day CVs were 5.0-18.5% (median 9.8%) and 3.5-14.5% (median 10.9%). The study demonstrates the equality of CDT values obtained by CDTect-RIA and CDTect-EIA. According to this study, the two methods can be used interchangeably without getting fluctuating CDT values, e.g. in longitudinal studies.

INTRODUCTION

Human serum transferrin shows a distinct microheterogeneity owing to: (a) altered protein moieties (genetic variants, e.g. transferrin-C, transferrin-D, and transferrin-B); (b) differing iron load (iron-free transferrin, Fe₀-transferrins; iron bound to the C-terminal or N-terminal binding site, Fe₁C or Fe₁N-transferrins; both binding sites loaded with iron, Fe₂-transferrins); and (c) different carbohydrate chains with 0 to 8 sialic acid residues (asialo-, monosialo-, ..., octasialo-transferrins) (de Jong and van Eijk, 1988; de Jong et al., 1990; van Noort et al., 1994) Using isoelectric focusing, Stibler et al. (reviewed in Stibler, 1991) found elevated concentrations of sialic acid-deficient transferrins (a-, mono-, and mainly disialo-transferrin) in the serum of alcoholics. The serum concentration of these isotransferrins, summarized as carbohydrate-deficient transferrin (CDT) (Stibler, 1991), is used for detection and follow-up of chronic alcohol abuse (e.g. Allen et al., 1994; Anton and Moak, 1994; Sillanaukee et al., 1994; Conigrave et al., 1995; Grønbæk et al., 1995) as well as for the detection of the carbohydrate-deficient glycoprotein syndrome, a hereditary disorder of serum glycoprotein metabolism (e.g. Jaeken and Carchon, 1993, Stibler and Cederberg, 1993; van Pelt et al., 1996).

Specific chemical reactions or antibodies for analysis of CDT are not yet available. Therefore, determination of CDT is usually done after elimination of Fe₀- and Fe₁-transferrins by iron saturation in vitro, followed by separation of CDT-isotransferrins from higher sialylated non-CDT-isotransferrins by chromatographic (Jeppesson et al., 1993; Simonsson et al., 1996;
Renner and Kanitz, 1997) or electrophoretic (e.g. Bean and Peter, 1994; Hackler et al., 1995; Arndt et al., 1997) methods. These procedures are usually sophisticated and not particularly suitable for large analysis series.

In 1992 the first set of reagents for determination of CDT, CDTect-RIA (Pharmacia & Upjohn, Sweden), became available commercially. Later, %CDT (AXIS, Norway), CDTect-RIA (Pharmacia & Upjohn, Sweden) and %CDT-TIA (also called CDTri-TIA) (AXIS, Norway) were launched commercially. These four sets of reagents use (after in vitro transferrin iron saturation for elimination of FeT- and FeO-transferrins) anion-exchange microcolumns for separation of CDT and the other isotransferrins. Subsequent quantification of CDT in the column effluxes or eluates is carried out by radioimmunoassays (CDTect-RIA, %CDT), an enzyme immunoassay (CDTect-EIA) or turbidimetrically (%CDT-TIA), using antitransferrin-antibodies.

The availability of sets of reagents for determination of CDT has accelerated the acceptance of CDT as the most specific marker of alcohol abuse available so far. However, these methods summarize different isotransferrins as CDT and report the results in different units, e.g. U/l for CDTect-RIA and CDTect-EIA vs CDT/transferrin ratios for %CDT and %CDT-TIA. This complicates comparison of CDT values and diagnostic specificities and sensitivities obtained in different studies. The majority of clinical studies published so far have used the CDTect-RIA for quantification of serum CDT. However, working with radioactive material requires special laboratory equipment and expensive disposal of the contaminated material. An alternative method to CDTect-RIA could be the CDTect-EIA, since these two assays differ only in the final CDT quantification step (radioimmunoassay vs enzyme immunoassay), but not in the isotransferrin fractionation procedure (the same isotransferrins are summarized as CDT) or the reported units (U/l).

The aim of our study was to investigate the equality and precision of CDT values obtained by CDTect-RIA and CDTect-EIA, since appropriate data on these aspects are not available. With the information presented here, we hope to contribute to a better comparability of CDT values obtained using both methods.

### MATERIALS AND METHODS

All procedures were in accordance with the Helsinki Declaration of 1975, as revised in 1983.

#### Materials

All materials were delivered with the CDTect-RIA and CDTect-EIA test kits (Pharmacia & Upjohn, Uppsala, Sweden). Blood was drawn after overnight fasting into tubes containing a gel separator (Gel-Monovette Sarstedt, Nümbrecht, Germany). After clotting at room temperature for 30 min, serum was obtained by centrifugation (2000 g for 10 min at 4°C). Serum aliquots were stored at −70°C and sent to the participating laboratories on dry ice.

#### Methods

**Assay of serum CDT concentration.** Serum concentration of CDT was determined by CDTect-RIA and CDTect-EIA in accordance with the instructions of the manufacturer. In short, 50 μl of serum sample were mixed with ferric citrate solution (200 μl) and elution buffer (1 ml) for in vitro transferrin iron saturation (elimination of FeT- and FeO-transferrins). An aliquot (500 μl) of this mixture was applied to the top of the anion-exchange microcolumns. In this step, the FeT-transferrins with isoelectric point values <5.7 (non-CDT-isotransferrins) are adsorbed at the anion-exchanger, whereas the Fe2-isotransferrins with isoelectric point values >5.7 (CDT-isotransferrins) occur within the column effluxes. They are finally quantified in the efflux by means of a competitive, double antibody immunoabsorbent assay (CDTect-RIA) or by a microplate enzyme immunoassay (CDTect-EIA). For the latter, only automated microplate washers were used.

**Trial protocol.** Four European laboratories took part in this multicentre study (laboratories I–IV). The time schedule for the study is given in Table 1. An internal CDT standard or quality control material were not available. The accuracy of the tests was studied therefore by analysis of the control sample which was delivered with each test kit in each test run.

On each day, CDTect-RIA and CDTect-EIA were performed (in parallel) as follows: (a) equilibration of the anion-exchange microcolumns in accordance with the directions for use; (b) in
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Table 1. Protocol for the multicentre trial (time schedule for each participating laboratory)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Day 1 and day 3</th>
<th>Day 2 and day 4</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CDTect-EIA (in duplicate)</td>
<td>CDTect-RIA (in duplicate)</td>
</tr>
<tr>
<td>Control</td>
<td>x x x x</td>
<td>x x x x</td>
</tr>
<tr>
<td>1-15</td>
<td>x x</td>
<td>x x</td>
</tr>
<tr>
<td>16-30</td>
<td>x x</td>
<td>x x</td>
</tr>
</tbody>
</table>

vitro Fe³⁺-saturation of the serum samples, one saturation for each sample used for correlation studies (nos 1-15 or 16-30) and one saturation for the control sample used for precision studies for CDTect-RIA and CDTect-EIA; (c) isotransferrin fractionation at the CDTect-RIA and CDTect-EIA anion-exchange microcolumns (in duplicate); (d) quantification by radioimmunoassay and enzyme immunoassay (in parallel) in accordance with the directions for use.

Statistics

The Astute statistical software package (DDU Software, University of Leeds, Leeds, UK) supplement for the Microsoft Excel spreadsheet programme and the Statgraphics statistical package (STSC Inc., Rockville, IL, USA) were used for statistical calculations. All calculations were made using the original analysis data, but not the means of the duplicate measurements.

Correlational studies. We used the method of Passing and Bablok (1983) for testing the correlation of CDT values for serum samples 1-30 obtained by CDTect-RIA and CDTect-EIA. After testing a linear relationship between X and Y, confidence limits were obtained for the slopes and the intercepts of the correlation functions for each laboratory.

Precision studies. Within-day and between-day coefficients of variation (CV) for CDT values near the upper reference limit for men (18–20 U/l) were computed for each participating laboratory from the control sample (target value 22 U/l) measurements. We used analysis of variance (ANOVA) as described by Bookbinder and Panosian (1986).

RESULTS AND DISCUSSION

Equality and precision of CDTect-RIA and CDTect-EIA for analysis of CDT in human serum were tested in four European laboratories. Table 1 shows the time schedule and organization of the study for each participating laboratory.

Comparability of CDTect-RIA and CDTect-EIA

For method comparison, 30 serum samples (nos 1–30; Table 1) with normal and above-normal (up to 130 U/l) CDT concentrations and one control sample, delivered with each test kit for quality control, were analysed. For each laboratory (I–IV), the analysis results and correlation functions were plotted in X–Y diagrams with the CDTect-EIA results on the X-axis and CDTect-RIA data on the Y-axis (Figs 1a–d). We used the statistical model of Passing and Bablok (1983) for assessment of the correlation functions. This procedure is not dependent on the assignment of CDTect-RIA and CDTect-EIA to X and Y and does not assume one of the two methods to be free of errors. It therefore meets the distributional requirements for method comparison better than for example linear regression based on least squares. Extreme CDT values (outliers) were not excluded from the calculations, because of the inherent robustness of the Passing and Bablok (1983) method. Moreover, outliers are not necessarily gross measurement errors, but may indeed be caused by different sensitivities of the two methods with respect to specificity or susceptibility to interferences (Passing and Bablok, 1983). In the case of statistically identical CDT results obtained by CDTect-RIA and CDTect-EIA, the values 1 for the slopes and 0 for the intercepts must be enclosed in the corresponding 95% confidence intervals (CI). Table 2 shows the slopes, intercepts, and the corresponding 95% CI for the regression functions computed for laboratories I–IV. Since all 95% CI included the values 1 (slope) and 0 (intercept), respectively, there was no significant difference in
Fig. 1. Correlation of carbohydrate-deficient transferrin (CDT) values obtained by CDTect-EIA and CDTect-RIA for 30 serum samples with normal and above-normal CDT concentrations. For each participating laboratory (I–IV), the regression functions (a–d) were obtained using the method of Passing and Bablok (1983).

serum CDT values obtained by CDTect-RIA and CDTect-EIA. Thus, alternation of the CDT analysis method, e.g. from the radioactive CDTect-RIA to the non-radioactive CDTect-EIA or vice versa, should not cause significant alterations of CDT values, in e.g. longitudinal studies.

**Precision study for CDTect-RIA and CDTect-EIA**

The within-day and pure between-day coeffi-
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Table 2. Comparison of carbohydrate-deficient transferrin (CDT) values (serum samples 1–30) obtained by CDTect-RIA and CDTect-EIA according to the method of Passing and Bablok (1983)

<table>
<thead>
<tr>
<th>Laboratory</th>
<th>Slope</th>
<th>95% CI</th>
<th>Intercept (U/l)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laboratory I</td>
<td>0.974</td>
<td>0.880–1.082</td>
<td>3.319</td>
<td>0.332–6.431</td>
</tr>
<tr>
<td>Laboratory II</td>
<td>1.023</td>
<td>0.964–1.091</td>
<td>1.441</td>
<td>0.570–3.420</td>
</tr>
<tr>
<td>Laboratory III</td>
<td>1.052</td>
<td>0.972–1.112</td>
<td>-0.662</td>
<td>-2.203–1.538</td>
</tr>
<tr>
<td>Laboratory IV</td>
<td>1.108</td>
<td>0.918–1.286</td>
<td>1.249</td>
<td>-2.936–4.676</td>
</tr>
</tbody>
</table>

Since all 95% confidence intervals (CI) included the values 1 (slope) and 0 (intercept), respectively, the equality of serum CDT values obtained by CDTect-RIA and CDTect-EIA is demonstrated.

coefficients of variation (CV) of CDTect-RIA and CDTect-EIA near the upper reference limit of serum CDT for men were computed by ANOVA (Table 3). Using this statistical method, the between-day imprecision does not include the within-day imprecision. In accordance with Bookbinder and Panosian (1986), we therefore used the term ‘pure between-day CV’. For CDTect-RIA, the within-day CVs for laboratories I–IV ranged between 0.7 and 6.4% (median 5.2%). The pure between-day CVs were between 5.0 and 18.5% (median 9.8%). For CDTect-EIA, the within-day CVs for laboratories I–IV ranged from 4.3 to 9.2% (median 6.2%) and the pure between-day CVs from 3.5 to 14.5% (median 10.9%). The assay imprecision was almost the same for both methods. Comparable CVs for the CDTect-RIA were reported by others (Bell et al., 1994). In contrast to laboratories I–III, laboratory IV had no preparative training for the use of the assays. It remains unclear, whether this is the (only) reason for the on average higher imprecision found in this fourth laboratory (Table 3). For CDT concentrations at the gender-specific upper reference limits (18–20 U/l for men, 26–28 U/l for women), high imprecisions may cause false positives or false negatives with respect to chronic alcohol abuse.

Annotation. The ranges of measurements (raw data of laboratories I–IV) for two randomly selected serum samples at the upper reference limits for men and women were 15–24 U/l and 18–30 U/l for CDTect-EIA and 19–25 U/l and 20–38 U/l for CDTect-RIA respectively. The corresponding ranges of the ‘reported’ means (calculated from the duplicate measurements) were 16–23 U/l (men) and 20–28 U/l (women) for CDTect-EIA and 20–25 U/l (men) and 23–35 U/l (women) for CDTect-RIA.

As one group of us showed earlier (Arndt et al., 1998), false positives can also arise in rare cases from incomplete separation of CDT- and non-CDT-isotransferrins at the anion-exchange microcolumns, due to reduced elution velocity (CDT

Table 3. Precision study for CDTect-RIA and CDTect-EIA

<table>
<thead>
<tr>
<th></th>
<th>CDTect-RIA</th>
<th></th>
<th>CDTect-EIA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within-day CV (%)</td>
<td>Pure between-day CV (%)</td>
<td>Within-day CV (%)</td>
<td>Pure between-day CV (%)</td>
</tr>
<tr>
<td>Laboratory I</td>
<td>0.7</td>
<td>5.0</td>
<td>9.2</td>
</tr>
<tr>
<td>Laboratory II</td>
<td>5.8</td>
<td>6.1</td>
<td>7.3</td>
</tr>
<tr>
<td>Laboratory III</td>
<td>4.6</td>
<td>13.6</td>
<td>4.3</td>
</tr>
<tr>
<td>Laboratory IV</td>
<td>6.4</td>
<td>18.5</td>
<td>5.1</td>
</tr>
</tbody>
</table>

For each laboratory, the within-day and pure between-day coefficients of variation (CV) were calculated from repeated measurements (see Table 1) of a control sample (target value 22 U/l), using analysis of variance as described by Bookbinder and Panosian (1986).
values obtained from such columns must be rejected). For these reasons and because of the possible social consequences of false positives for the person tested, analysis should always be done in duplicate (as suggested by the manufacturer). Imprecisions >10% for the duplicates should be checked by repeated analysis.

An international CDT standard or quality control material is required for testing the accuracy of CDT analysis. A first step towards improvement of quality control of CDT analysis is the external quality control programme organized by Pharmacia & Upjohn. The need for standardization and a unified definition of CDT is discussed in detail by Arndt and Hackler (1998). The question of which isotransferrins are summarized as CDT (and to which extent) is not just a theoretical one, but also has practical importance, e.g. a patient may change doctors or hospitals (and thus CDT analysis method). CDT is increasingly used, e.g. in forensic and employment medicine (e.g. Gilg et al., 1994; Iffland, 1996; Morgan and Major, 1996; Sadler et al., 1996). Thus, evidence and counterevidence of chronic alcohol abuse supported by CDT values may depend on the analysis method. The lack of a unified definition of CDT undoubtedly complicates the interpretation of CDT values and the evaluation of the clinical performance of CDT in different clinical settings. This was an important issue at the Sixth Congress of the European Society for Biomedical Research on Alcoholism (ESBRA, 1997). With the present study, we hope to contribute to a better comparability and interpretation of CDT values obtained by CDTect-RIA and CDTect-EIA.

GENERAL CONCLUSIONS

This multicentre study has demonstrated the equality and good precision of CDT values obtained by CDTect-RIA and CDTect-EIA. The methods can be used interchangeably for reliable analysis of serum CDT. In our experience, CDTect-EIA is a useful non-radioactive alternative to the widely used CDTect-RIA. Regardless of which method is used for analysis of CDT, the diagnosis ‘alcoholic’ or ‘chronic alcohol abuse’ should not be made from a single CDT measurement, but also from anamnestic data (e.g. a structured questionnaire), repetition of CDT measurement, e.g. after 2-3 weeks of alcohol abstinence, and additional laboratory markers such as y-glutamyl transferase and mean corpuscular volume of erythrocytes.

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REFERENCES


