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Alcohol 46 (2012) 69–73

ALCOHOL

The diagnostic power of direct carbohydrate-deficient transferrin immunoassay in alcoholics. Absolute or relative values?

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Received 2 December 2010; received in revised form 22 July 2011; accepted 22 August 2011

Abstract

The objective of this study was to compare the diagnostic power of direct carbohydrate-deficient transferrin (CDT) immunoassay in alcohol abuse expressed in relative units with the diagnostic power of the results expressed in absolute units. Serum CDT was determined in 127 alcoholics using N Latex CDT direct immunonephelometric assay (Siemens Healthcare Diagnostics, Marburg, Germany). The diagnostic sensitivity, specificity, negative and positive predictive value, and also the positive and negative likelihood ratios do not differ between results expressed in relative or absolute units independently of cutoff chosen. Finally, the area under the receiver operating characteristic (ROC) curves for N Latex CDT test expressed in absolute units does not differ from the area for results expressed in relative units. We conclude that the diagnostic usefulness of N Latex immunonephelometric assay using the relative or absolute values is the same.

Keywords: Direct CDT immunoassay; N latex CDT; Units; Alcohol abuse; ROC curve analysis; Likelihood ratios

Introduction

The direct carbohydrate-deficient transferrin (CDT) immunoassay is the first routine method for CDT determination, which does not require preanalytical sample treatment. The biggest advantage is that the CDT antibodies used in the direct method are not influenced by transferrin genetic variants (Helander et al., 2004). Transferrin BC and CD heterozygotes, and C2C3 subtype, were found at frequencies about 0.7%, 0.2%, and 0.6%, respectively (Kamboh and Ferrell, 1987; Kasvosve et al., 2000). Total frequency of the rare transferrin variants in the white population was established on the level of about 2–3%. These variants may cause false (positive or negative) results of CDT determination with older indirect immunoassays (Helander et al., 2001). In the new direct method (N Latex CDT test), the transferrin genetic variants do not interfere with measurements (Delanghe et al., 2007). For example, it was shown that an abstinent student had a markedly increased %CDT value of 8.2% according to the photometric method, but measurement by the direct N latex CDT method gave the normal result of 2.1%. High-performance liquid chromatography (HPLC) analysis confirmed the presence of genetic variant in this subject. A disadvantage of the immunologic assays is that they do not distinguish single transferrin isoforms. The sensitivity and specificity of N Latex CDT assay using relative values calculated at threshold of 2.4% were 94% and 97%, respectively (Delanghe et al., 2007). To use for conforming a disease in the better way than sensitivity is the likelihood ratio of a positive result (LR+) and to rule out disease the better way is the likelihood ratio of a negative result (LR−) (Deeks and Altman, 2004; Okada and Rao, 2005). The LR summarizes information about the diagnostic power of the test combining the sensitivity and specificity of a test into a single information and is independent of the prevalence of the disease in the population (Akobeng, 2006). The better test is the one with the largest LR(+) and smaller LR(−). The extensive evaluation of CDT of older methods was done by Scouller et al. (2000), but the evaluation of new direct immunoassay method is not yet performed. The serum concentration of CDT can be expressed in absolute or relative units. Whether the relative values increase the diagnostic accuracy compared with absolute units is controversial (Helander, 1999; Keating et al., 1998; Sorvajarvi et al., 1996). The advantage of relative units is the improvement of the diagnostic specificity in patients with increased

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0741-8329/$ - see front matter
doi: 10.1016/j.alcohol.2011.08.010

transferrin concentration. However, it is necessary to remember that low transferrin levels can produce false-positive and false-negative results. Therefore, the problem of the CDT results expression is important. In this article, we compared the diagnostic power of direct CDT immunoassay results expressed in relative and absolute units. Additionally, with reference to possible acute-phase reaction (transferrin as a negative acute-phase protein) during alcohol abuse, we would like to evaluate the correlation between CDT and total transferrin concentration.

Methods

Subjects

The tested group consisted of 127 alcohol-dependent patients (range: 23–66 years, mean: 44, men: 100, women: 27) from detoxification ward (Department of Detoxification, Psychiatric Hospital in Choroszcz). Patients were initially examined and interviewed regarding the history of disease and their use of alcohol, drugs, and smoking. The diagnosis of dependency was made on the basis of International Classification of Diseases-10th Edition criteria (World Health Organization, 1992). The self-reported mean alcohol consumption was 1,106 g of ethanol per week (range: 336–3,318) and mean time of dependency was 15 years (range: 2–37). The time after cessation of drinking was between 0 and 2 days.

The control group consisted of 52 healthy social drinkers (range: 26–61 years, mean: 45, men: 41, women: 11) with self-reported alcohol consumption of less than 20 g/daily on any occasion (<140 g/weekly). The research protocol was approved by the Bioethical Committee working at the Medical University in Bialystok (Approval No. R-I-002/94/2010).

Serum samples were collected from a peripheral vein once from each patient, immediately after admittance, before treatment. The sera were separated into two tubes and stored at −86°C until assayed.

Determination of CDT by direct immunoassay (N Latex)

CDT, as the percentage of total transferrin (%CDT), was assayed by particle-enhanced imunononephelometry using N Latex CDT test (Siemens Healthcare Diagnostics, Marburg, Germany) on BN ProSpec System (Siemens Healthcare Diagnostics, USA). According to information given by Siemens Healthcare Diagnostics, the reference interval for CDT was obtained from 561 healthy adults from Central Europe (1st to 99th percentile) in whom an elevated level of alcohol consumption has been excluded and ranged from 28.1 to 76 mg/L. The %CDT values in this population ranged from 1.19 to 2.47% (1st to 99th percentile). The concentration of transferrin (reference interval: 2.0–3.6 g/L) was determined by the method based on the measurement of the scattered lights by the complexes of transferrin with specific antibodies (N antisera to human transferrin and haptoglobin) (Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) on BN ProSpec System. The results were expressed in absolute units (milligram/liter) and in relative units (% of total transferrin concentration).

Statistical analysis

Results were expressed as medians and ranges. The GraphROC for Windows was used to evaluate the diagnostic accuracy and to compare the areas under two receiver operating characteristic (ROC) curves for different CDT units. LR(+) is a ratio between the probability of a positive test result given in the presence of the disease and the probability of a positive test result given in the absence of the disease. It was calculated as following: sensitivity divided by 1-specificity. LR(−) is the ratio between the probability of a negative test result given in the presence of the disease and the probability of a negative test result given in the absence of the disease and calculated as follows: 1-sensitivity divided by specificity. To calculate the correlation between CDT and transferrin, Spearman's rank correlation coefficient was used. P values less than .05 were considered significant.

Results

The mean value of CDT (range) for relative units from healthy control was 1.82% (range: 1.40–2.54%; 95th percentile: 2.29%) and for absolute 43.1 mg/L (range: 23.0–61.1; 95th percentile: 58.4 mg/L). The cutoff suggested by the ROC analysis (the highest accuracy) was 2.12% and 58.6 mg/L, respectively. There are no differences in CDT between male and female patients ($P = .513$ for the absolute units and $P = .525$ for the relative values).

Table 1 summarizes the results of diagnostic power of N Latex CDT test for alcohol abuse. Independent of the used cutoff, the sensitivity, specificity, and the positive predictive values (PPVs) and negative predictive values (NPVs) of N Latex CDT test were similar for results expressed in relative and absolute units. According to the analysis of positive likelihood ratios (LR+), the positive test result is from 9 to 15 times more likely in alcoholics than in healthy persons using relative units but from 15 to 22 times more likely in alcoholics than in healthy persons using absolute units. Using the absolute values, the individuals who do not alcohol abuse are about seven times more likely to have a negative results than individuals who abused alcohol, but using relative units above chance was about 10 (analysis of negative likelihood ratios).

As shown in Fig. 1, the area under the ROC curve (AUC) for relative and absolute CDT values was not statistically significant ($P = .769$). Generally, AUCs for N Latex CDT test expressed in different units were higher than 0.9. CDT results expressed in relative units correlated negatively with total transferrin concentration ($P < .05$) (Fig. 2).