THE COMBINED USE OF THE EARLY DETECTION OF ALCOHOL CONSUMPTION (EDAC) TEST AND CARBOHYDRATE-DEFICIENT TRANSFERRIN TO IDENTIFY HEAVY DRINKING BEHAVIOUR IN MALES

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Abstract — The aim of this study was to determine the efficacy of the combined use of carbohydrate-deficient transferrin (CDT) and the Early Detection of Alcohol Consumption (EDAC) test to assess heavy drinking in a population of males (n = 187) drinking an average of 20 drinks per day. Heavy drinkers (n = 138) and light drinkers (n = 49) were analysed in three ways: using the EDAC test alone, using the CDT test alone and using the EDAC and CDT tests combined. The EDAC method uses linear discriminant function to analyse a battery of routine laboratory tests that generate a score for each subject and its associated probability value. This translates into the likelihood of each individual being a heavy or a light drinker. CDT uses ion-exchange chromatography to extract CDT in the serum and quantifies it by radioimmunoassay. The EDAC alone showed 88% (122/138) sensitivity rate when identifying heavy drinking males and 98% (48/49) specificity rate when assessing light drinkers. The CDT test alone showed a sensitivity rate of 58% (80/138) and a corresponding specificity rate of 96% (47/49). When analysed in parallel, 92% (127/138) of heavy drinkers showed abnormal EDAC and/or CDT tests and 94% (46/49) of light drinkers were negative for both tests. When analysed sequentially, the CDT test confirmed 61% (75/122) of the heavy drinkers identified by the EDAC test. Specificity rate for this testing strategy was 100%, because the only false positives for EDAC tested negative for CDT. This preliminary study shows that EDAC and CDT may react independently to alcohol intake and can be combined for maximum diagnostic accuracy.

INTRODUCTION

A number of blood tests have been developed to detect heavy alcohol drinking. These include traditional single laboratory tests such as liver enzymes (γ-glutamyltransferase, aspartate aminotransferase and alanine aminotransferase), as well as newer biochemical markers, such as carbohydrate-deficient transferrin (CDT) and the whole-blood-associated acetaldehyde (WBAA) assay (Conigrave et al., 1995; Bean, 1999, 2000). Also available are methods that use a multiple marker approach based on statistical analysis of common haematology and chemistry profiles (Hartz et al., 1997). The Early Detection of Alcohol Consumption (EDAC) test is an example of the latter (Bean, 2000; Harasymiw et al., 2000a). CDT, WBAA and the EDAC show slightly different sensitivity and specificity rates for identification of heavy drinking. The issue of which biomarker is best suited for screening, confirmation of a suspicion and/or monitoring abstinence and relapses is still a matter of debate. A biochemical marker suited for screening should have a high sensitivity rate to detect all heavy drinkers in the sample population but it should also show a high specificity rate to avoid false positives. A biochemical marker used for confirmation of a suspicion of heavy drinking should be very specific, in order to distinguish alcohol misuse from any other medical condition. For monitoring abstinence and relapses, biomarkers should show low intra-assay variability to maximize detection of meaningful changes from baseline values for each subject over time.

The ineffectiveness of traditional markers to screen for alcohol consumption in the general population has been recognized for many years. Sensitivity and/or specificity rates are far too low to propose their systematic use as screening tests in unselected medical populations. A biochemical marker with 60% sensitivity and 98% specificity rate for heavy drinking, when applied to a population with a 7% prevalence of alcohol misuse, has a positive predictive value of 0.66. This means that, if a patient has a positive test result, there is a 66% chance that this patient is a heavy drinker and a 34% chance that this patient is a false positive, rather than a true positive. Despite moderate sensitivity and specificity rates, this biochemical marker is not a good screening candidate. Tests with moderate diagnostic performance have serious drawbacks in employment, legal and insurance settings where false positive results can have serious consequences. However, if false positive results could be eliminated or greatly reduced, these tests might find greater acceptance and use in these settings.

Two ways to improve assay performance are to increase the sensitivity and specificity rates (diagnostic accuracy) of the test, or to increase disease prevalence, i.e., to use the biochemical marker in a population with a higher proportion of heavy drinkers. To increase diagnostic accuracy, several recent studies have used parallel testing by combining two or more laboratory tests to identify alcohol misuse (Sillanaukee, 1992; Hartz et al., 1997; Harasymiw et al., 2000a). For instance, parallel testing using CDT and γ-glutamyltransferase (GGT) has been widely documented to improve the performance of either marker used alone (Anton and Moak, 1994; VanPelt et al., 2000). Similarly, a recent study showed the effectiveness of parallel testing using CDT and the Alcohol Use Disorders Test (AUDIT) to screen for alcohol use disorders in a routine workplace (Hermansson et al., 2000). CDT and mean corpuscular volume (MCV) have also been used jointly in parallel testing with optimal success in females.

Statistical methods that use combinations of 10 to 40 routine laboratory tests, such as the EDAC test, constitute the essence of a different combinatorial strategy (Hartz et al., 1997; Bean,
To assess alcohol consumption we used the Khavari Alcohol Test (Khavari and Farber, 1978). The heavy drinkers drank an average of 248 ± 153 g of alcohol per day (range 51–879 g) corresponding to a mean of 20.6 drinks per day. The 95% confidence interval (CI) for this group was 223–272 g daily. The light drinkers drank on average 1.8 ± 7.1 g of alcohol daily (range 0–45 g); the 95% confidence interval was 0–3.7 g daily.

### Laboratory analysis

Blood specimens (non-fasting) were obtained from each enrolled participant and sent to the laboratory for biochemical and haematological tests. Handling of specimens followed standard laboratory procedures, with daily collection by courier and overnight transportation to the testing site. Two tubes of blood were drawn for each patient, one for the blood counts and one for the chemistry panel. The tube for the chemistry panel was allowed to clot and serum separated after centrifugation at 2000 r.p.m. for 10 min. Serum was kept refrigerated until sent to the laboratory for analysis. Chemistry determinations were performed on standard equipment utilizing the Olympus AU5000 automated chemistry analyser, whereas haematology parameters were determined utilizing the Argos Cobas haematology instrument (LabCorp, Burlington, NC, USA).

### The CDT test

Frozen serum specimens were shipped to Dr Sillanaukee to perform the CDT analysis. One tube of serum from each subject was thawed and subjected to the CDTect procedure (Kabi-Pharmaecia, Uppsala, Sweden) as described previously (Stibler et al., 1991). Briefly, 50 μl of serum were pre-incubated with 200 μl of ferric citrate and 1 ml of elution buffer. The kit mini-columns were reconstituted and equilibrated with 2 ml of elution buffer. To each mini-column, 500 μl of iron-saturated serum sample was added and the eluate was collected and quantified by radioimmunoassay (RIA). The eluate contained CDT molecules with two, one or no sialic acid residues. The RIA was performed using rabbit anti-human transferrin antibody to bind CDT and sheep anti-rabbit antibody to precipitate the CDT–antibody complex. After centrifugation, the radioactivity of the precipitate was counted in a gamma counter. The amount of CDT in duplicate samples was calculated from a 5-point calibration curve derived from the displacement of radioactive CDT from the antibody by known amounts of human transferrin. The cut-off point used was 20 arbitrary units per litre (~20 mg/l).

### The EDAC test

The EDAC test uses LDF to build a predictive model of group membership, based on observed characteristics of two different samples, in this case laboratory parameters of heavy drinkers and light drinkers. The LDF analysis generates a discriminant function based on linear combinations of the predictor variables that provide the best discrimination between the two groups (Johnson and Wichern, 1998). The functions are generated from the results of the laboratory variables and demographic parameters of subjects for which group membership (heavy drinkers versus light drinkers) is known. Discriminant analysis looks like the right-hand side of a multiple linear regression equation; using coefficients $a, b, c, d \ldots x$, for the
laboratory variables of the EDAC panel and demographic parameters such as gender and age, the function is:

\[ D = a \text{ (laboratory variable } X_i) + b \text{ (laboratory variable } X_j) + c \text{ (gender)} + d \text{ (age)}. \]

The \( D \) value obtained for each subject corresponds to a probability of the subject being a heavy or a light drinker, based on the subject’s alcohol consumption according to self-report. In this study the prior probabilities for a subject’s being a heavy drinker or a light drinker were set equal and the costs of misclassification for the two categories were also set equal. Therefore, we assumed equal costs associated with false positives and false negatives, because the prior probabilities for heavy and light drinking were set at 0.5 and 0.5, respectively. The cut-off point for the EDAC was set at a value of zero, with positive EDAC scores identifying heavy drinkers and negative EDAC scores identifying light drinkers. The derivation and validation of the discriminant equation used in this analysis has been described recently (Harasymiw and Bean, 2001).

The laboratory variables used to obtain the EDAC score were: albumin/globulin ratio, globulin, total protein, creatinine, blood urea nitrogen, blood urea nitrogen/creatinine ratio, alkaline phosphatase, triglycerides, total bilirubin, direct-reacting bilirubin, low density lipoprotein, uric acid, neutrophils, monocytes, lymphocytes and basophils.

## RESULTS

### Individuals identified as heavy or light drinkers by the EDAC test

First, we wanted to determine the diagnostic performance of the EDAC test used alone in this population. The EDAC showed a sensitivity rate of 88% (122/138) for identifying heavy drinking males and a specificity rate of 98% (48/49) for correctly assessing light drinkers. Accordingly, the false negative rate was 12%; 16 of the 138 self-reported heavy drinkers showed negative EDAC scores. The false positive rate was 2% with only one light drinker by self-report showing a positive EDAC score (Table 1).

We then used these performance parameters to calculate the positive predictive value of the EDAC test when applied to a population with 10% prevalence of alcohol misuse. If we use the EDAC test to screen 1000 individuals of whom 10% \( n = 100 \) are alcohol misusers, then 88% \( n = 88 \) of them will show a true positive result according to the EDAC’s sensitivity rate. Similarly, if we use the EDAC test to screen 1000 individuals of whom 90% \( n = 900 \) are light drinkers, then 96% \( n = 864 \) will show a true negative result and 4% \( n = 36 \) will be false positives. The PPV was 0.62 (58/94) which means that 62% of positive results will represent true heavy drinkers; the other 38% would represent false positives. The NPV was 0.95 (864/906) which means that most negative results (95%) represent non-heavy drinkers.

### The combined use of EDAC and CDT

The analysis of the combined use of EDAC and CDT was done in two ways. First, we assumed the clinician would have access to analyse the results from both tests simultaneously. The criteria for diagnosis were as follows. If both EDAC and CDT tests were positive or if only one of them was positive, then the clinician would diagnose heavy drinking. Only when both tests were negative, did the clinician assume lack of heavy drinking. When EDAC and CDT were analysed simultaneously the overall sensitivity was 92%, which means that 127 of the 138 heavy drinkers by self-report show at least one positive result for EDAC and/or CDT (Table 3). The corresponding specificity rate was 94% with 46 of the 49 light drinkers showing negative results for both tests (Table 3). The PPV for this combination of markers when used in a population with 10% prevalence of alcohol misuse was 0.63 (92/146), similar to the one obtained when using only CDT.

The second analysis strategy was to assume the clinician had access to the EDAC test only and EDAC positive results were then confirmed by the CDT test. If an EDAC positive result were followed by a CDT positive test, then the clinician would

## Table 1. Individuals identified as heavy or light drinkers by the Early Detection of Alcohol Consumption (EDAC)

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>EDAC+</th>
<th>EDAC–</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy drinkers</td>
<td>138</td>
<td>122</td>
<td>16</td>
</tr>
<tr>
<td>self-report (%)</td>
<td></td>
<td>(88)</td>
<td>(12)</td>
</tr>
<tr>
<td>Light drinkers</td>
<td>49</td>
<td>1</td>
<td>48</td>
</tr>
<tr>
<td>self-report (%)</td>
<td></td>
<td>(2)</td>
<td>(98)</td>
</tr>
</tbody>
</table>

## Table 2. Individuals identified as heavy or light drinkers by carbohydrate-deficient transferrin (CDT)

<table>
<thead>
<tr>
<th>Group</th>
<th>( n )</th>
<th>CDT ≥20 U/l</th>
<th>CDT ≤20 U/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy drinkers</td>
<td>138</td>
<td>80</td>
<td>58</td>
</tr>
<tr>
<td>self-report (%)</td>
<td></td>
<td>(58)</td>
<td>(42)</td>
</tr>
<tr>
<td>Light drinkers</td>
<td>49</td>
<td>2</td>
<td>47</td>
</tr>
<tr>
<td>self-report (%)</td>
<td></td>
<td>(4)</td>
<td>(96)</td>
</tr>
</tbody>
</table>
assume heavy drinking. However, if an EDAC positive test were followed by a CDT negative test, the clinician would assume lack of heavy drinking. As stated in Table 1, there were 122 heavy drinkers and one light drinker with a positive EDAC test. The CDT test confirmed 61% (75/122) of the heavy drinkers identified by the EDAC test. Thirty nine per cent (47/122) of the self-reported heavy drinkers went undetected by the CDT test (Table 4). The only false positive for the EDAC test was negative by CDT, thus confirming the high specificity of the CDT test. Using both markers combined eliminated all false positives, because the only false positive result for the EDAC test was negative by the CDT test. This testing strategy yielded a PPV of 1.

DISCUSSION

It is quite clear that an effective method to detect heavy drinking in a variety of settings could provide large benefits for the patient, family members and society. Biochemical markers have been used in a number of studies to discriminate between light and heavy drinkers (Anton and Moak, 1994; Hartz et al., 1997; Hermansson et al., 2000; vanPelt et al., 2000). In this study, the EDAC test provided optimal predictive values to potentially make it a good candidate as a screening tool for males. Some of the factors that may have contributed to the optimal performance of the EDAC test relate to the extreme high and low rates of alcohol consumption in the groups represented in this population. The performance of the EDAC test in less extreme groups was recently described in at-risk college students with encouraging results (Harasymiw et al., 2000a).

Similar to most biochemical markers, additional sources of variation for the EDAC test include differences in the amount, duration and type of alcohol consumed, subjects’ drinking patterns and nutritional or health characteristics that affect individual responses to alcohol. The analysis of a larger population of subjects representing less extreme groups showed that the EDAC worked best when classifying populations according to gender and age (Harasymiw et al., 2000b).

The CDT test showed a low PPV when used hypothetically as a screening tool even though this biochemical marker has been tested successfully for its ability to detect heavy drinking in males. A well-known drawback of the CDT kit used in this study relates to the fact that it measures absolute CDT concentrations without taking into consideration the contribution of total serum transferrin. In this scenario, low serum transferrin could lead to false negative results for a poor overall performance of the test. Newer CDT tests with better performance, such as the %CDT Turbidimetric immunoassay (Axis Shield ASA, Norway) recently approved by the FDA may be better-suited for generalized use.

Aside from optimizing the sensitivity and/or specificity rates of any single test, another way to increase the positive predictive value is by combining markers in either parallel testing or reflex testing. Biomarkers that are poorly correlated should be selected for parallel testing and biomarkers that are highly correlated should be selected for reflex testing. For instance, AUDIT and CDT both have value for identifying a different segment of the high risk drinking population and, therefore, have been considered as complementary instruments for parallel testing during alcohol screening (Hermansson et al., 2000). In contrast, the insurance industry in the USA uses reflex testing when it screens its applicants using high density lipoprotein (HDL)-cholesterol and liver enzyme tests. Samples from subjects with abnormal HDL undergo further testing by CDT and subjects with abnormal liver enzymes undergo further testing by haemoglobin-associated aldehyde (HAA). Thus, CDT and HAA are used as reflex tests (Daniel, 1997). When using reflex testing, the medical professional needs to combine tests that are highly correlated with each other so that a subject who tests positive in the screen would also test positive in the reflex test.

In our study, we combined EDAC and CDT in two ways. When both tests were used in parallel, sensitivity increased, but specificity decreased, compared to the use of each marker alone. This loss of specificity resulted in a concomitant decrease in PPV to resemble the one obtained when using only CDT. In contrast, when the EDAC-positive samples were submitted to reflex testing by the CDT test, we maximized specificity as evident by the elimination of all false positives. This preliminary study shows that EDAC and CDT may react independently to alcohol intake and that they can be combined for maximum diagnostic accuracy.

Alcohol misuse is a major factor in morbidity and mortality worldwide. It was estimated in the early 1990s that the USA alone the costs associated with the consequences of alcohol misuse surpassed $148 billion per year (Lewin Group, 1992). Rates of frequent heavy drinking and alcohol-related problems between 1984 and 1995 remained especially high among African American and Hispanic men, suggesting that men of these two ethnic groups should be specifically targeted for renewed prevention efforts (Caetano, 2000). However, diagnosis and treatment usually occur only after the onset of either physical morbidity or a personal crisis resulting from mounting psychosocial problems (Bucholz et al., 1992). Most hazardous and harmful drinkers can be identified with simple

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**Table 3. Sensitivity and specificity for the Early Detection of Alcohol Consumption (EDAC) and carbohydrate-deficient transferrin (CDT) tests combined**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>EDAC+ CDT+</th>
<th>EDAC+ CDT-</th>
<th>EDAC- CDT+</th>
<th>EDAC- CDT-</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heavy drinkers, self-report (%)</td>
<td>138</td>
<td>127</td>
<td>11</td>
<td>127</td>
<td>11</td>
</tr>
<tr>
<td>Light drinkers, self-report (%)</td>
<td>49</td>
<td>3</td>
<td>46</td>
<td>3</td>
<td>46</td>
</tr>
</tbody>
</table>

**Table 4. Early Detection of Alcohol Consumption (EDAC) screen confirmed by the carbohydrate-deficient transferrin (CDT) test**

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>CDT ≥20 U/l</th>
<th>CDT &lt;20 U/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDAC+, heavy drinkers</td>
<td>122</td>
<td>75</td>
<td>47</td>
</tr>
<tr>
<td>(%)</td>
<td>(61)</td>
<td>(39)</td>
<td></td>
</tr>
<tr>
<td>EDAC+, light drinkers</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>(%)</td>
<td>(0)</td>
<td>(100)</td>
<td></td>
</tr>
</tbody>
</table>
verbal screening, but some will not acknowledge heavy drinking. Denials occur more frequently in settings where there may be negative consequences due to diagnosis. Indeed, misuse of alcohol creates many situations in which individuals may find themselves in conflict with their employers, spouses or the courts. The costs associated with lost productivity, family distress and legal problems are substantial (Lewin Group, 1992). A cost-effective biological marker that is also accurate in identifying heavy alcohol consumption would facilitate timely assessment of the patients and promote earlier interventions for optimal clinical benefits and treatment results.

Based on this study, the EDAC test may represent a practical candidate as a screening tool, because of the broad availability of the routine laboratory panel, optimal performance and reduced costs. Ongoing studies are currently evaluating CDT as an additional independent predictor variable in the discriminant analysis. The addition of CDT to the EDAC panel may further improve the performance of the EDAC and it may allow the use of a reduced number of routine laboratory tests.

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REFERENCES


