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The use of biological laboratory markers in the diagnosis of alcohol misuse: an evidence-based approach

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Abstract

Background: A large number of patients seen in clinical practice have an underlying alcohol problem. There is a pressing need for accurate methods to diagnose alcohol over-consumption objectively. Our aim was to determine how best to use biological markers to objectify alcohol problems in patients with clinical suspicion of alcohol misuse. *Methods:* A 6-month longitudinal multicenter trial was conducted, using four study groups (alcohol abusers, alcohol-dependents, healthy controls and consulting controls). CDT, GGT and MCV were measured. Statistical analyses used a computer learning system that created classification systems displayed in decision trees. *Results:* In 379 subjects the marker that best discriminated those with alcohol problems from controls was CDT. GGT then helped to differentiate between alcohol abuse and alcohol dependence in cases of high CDT. MCV, age and gender provided no extra information. *Discussion:* We recommend CDT as a first-line biological marker to confirm or disprove suspected alcohol misuse. High CDT plus GGT above normal points to alcohol dependence, while high CDT plus GGT below normal is evidence of alcohol abuse.

Keywords: Carbohydrate-deficient transferrin; Gamma-glutamyl transferase; Alcohol abuse; Alcohol dependence; Alcohol misuse; Decision tree; Decision making

1. Introduction

The powerful impact of alcohol-related illness on society is now widely acknowledged. Its total cost for France in 1996–1997 was estimated to be at least 12 thousand million euros, counting only declared illness (Reynaud and Parquet, 2001), and the total cost of alcohol-related deaths surpasses us$ 75 billion per year in the United States (Secretary of Health and Human Services, 1997; Substance Abuse and Mental Health Services Administration, 1993). Problem drinking is associated 'with increased morbidity and mortality (Lieber, 1995; Thun et al., 1997; Reynaud et al., 2001).

About 20% of patients seen in clinical practice present underlying alcohol misuse (Olfson et al., 2000; Manwell et al., 2002; Brienza and Stein, 2002). Primary care physicians can play a major role in identifying alcohol misuse and in initiating therapy. However, in the early phase of problem drinking the clinical signs are mild, and so subjects with alcohol misuse tend to be diagnosed at a later stage when medical complications have already appeared (Moore et al., 1989; Dawson et al., 1992; Hearne et al., 2002). There is, therefore, a compelling need for accurate methods for the objective diagnosis of alcohol over-consumption not only for doctors but also for patients, who very often deny alcohol misuse. Diagnosis first implies physicians' awareness of, and screening practice for, alcohol misuse among their patients, and then obviously relies on clinical examination based on knowledge of symptoms. Doctors can be helped by valuable tools such as standardized questionnaires, but no

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patient follow-up is possible with these. However, unlike personal interviews and standardized questionnaires, biological markers give unbiased information on alcohol consumption and drinking patterns. Consequently, biomarkers are helpful in screening alcohol misuse, motivating patients to change their drinking habits and monitoring changes in their alcohol consumption.

Biological indicators based on hematological characteristics, liver enzyme activities or lipids (triglycerides, transaminases, etc.) are more properly warning signs of alcohol misuse rather than true biological markers (Sharp, 2001). Mean corpuscular volume (MCV), gamma-glutamyl transferase (GGT) and carbohydrate-deficient transferrin (CDT) are the most common biomarkers currently used for this purpose.

Reported sensitivities of MCV in detecting alcohol dependence are between 34 and 85% (Unger and Johnson, 1974; Wu et al., 1974; Chick, 1981; Reynaud et al., 2000). Elevated GGT levels were found in 33–85% of alcohol-dependent patients (Fiellin et al., 2000; Reynaud et al., 2000). CDT seems to be the biomarker that performs best in detecting alcohol misuse. Sensitivities of up to 85% and specificities over 90% have been reported in alcohol-dependent patients (Anton and Moak, 1994; Sillanaukee et al., 1998; Yersin et al., 1995; Arndt, 2001; Reynaud et al., 2000; Schellenberg et al., 2001), and some studies indicate that combined measurement of CDT and GGT may enhance ability to detect alcohol abuse (Anton and Moak, 1994; Allen et al., 1994; Helander et al., 1996; Reynaud et al., 2000).

Although about a hundred studies have evaluated the performance of these markers in patients with alcohol misuse, none has sought an evidence-based rationale (i) for the sequence in which these markers should be used in a clinical population suspected of alcohol misuse after clinical presentation, or (ii) to support diagnostic differentiation between alcohol abuse and alcohol dependence, despite the need for (i) a rational use of biomarkers (compounding of analytic errors and economic cost if all the markers are used every time), and (ii) accurate differential diagnosis to enable doctors to advise on subsequent treatment options.

In our study we set out to establish guidelines for the use of biological laboratory markers to objectify alcohol misuse and distinguish between alcohol abuse and alcohol dependence.

2. Materials and methods

We conducted a 6-month longitudinal multicenter study. We set up four study groups; alcohol abusers [a], alcohol-dependents [d], healthy controls [hc] and consulting controls [cc] to model various clinical situations. The study was approved by the ethics committee in 2000. The study was fully explained to the patients, and those who refused their informed consent to participate were excluded. All the patients subsequently received the results of their blood tests.

2.1. Subjects

The general exclusion criteria used were the following: declared pregnancy, patients for whom abuse or dependence was not the main diagnosis on axis I of DSM IV (mental retard, dementia, schizophrenia or other psychotic disorder, bipolar mood disorder, etc.), associated addiction(s), subjects with no health insurance cover, subjects benefiting from social protection measures (e.g., care orders), forcibly hospitalized subjects. Subjects were also excluded from the study for serious medical conditions, e.g., alcohol-induced coma, multiple trauma, delirium tremens, suicide attempts.

All the subjects were aged between 18 and 65 years. No patients in the abuser group or in the dependent group were taking any drugs liable to modify GGT, or had a health disorder liable to modify GGT. Everyone in these groups had consumed alcohol less than three days earlier.

The population of abusers studied was made up of patients who presented alcohol abuse as defined in DSM IV (F 305) and who were recruited in two outpatient treatment centers (CCAA Clermont-Ferrand and Tours, France). The dependent patients were recruited in specialized alcohol dependence units (CHU, Clermont-Ferrand and Nancy, France) and presented alcohol dependence according to the criteria defined in DSM IV (F 303.9). The diagnosis was made by clinicians specialized in alcohol-related pathology who were familiar with DSM IV. Diagnosis for alcohol abuse and alcohol dependence was checked by the corresponding MINI questions (Sheehan et al., 1998).

The first control population, which was age-matched and had a similar sex ratio, was recruited in a center for occupational preventive medicine. It comprised moderate drinkers whose daily alcohol consumption was less than 20 g per day, who were not pregnant and who had a negative CAGE score. This population was made up of subjects whose clinical and laboratory results were regularly monitored, who had no known illness and who could be trusted to answer questions truthfully.

A second control population (consulting controls) was made up of patients admitted to the emergency ward of Clermont–Ferrand University Hospital. The only selection criteria were: absence of abuse or dependence criteria as defined in DSM IV, declared alcohol consumption less than 20 g/day, and a negative CAGE result. This population was more representative of the general population of patients consulting in primary care. Some diseases or drugs can alter the values of GGT, MCV or CDT.

2.2. Blood sampling

CDT, GGT and MCV were measured at the first visit or on admission to hospital for withdrawal treatment or consultation for alcohol abuse (outpatient treatment center). Blood samples were shipped (frozen) as full blood (MCV) and centrifuged (CDT, GGT) from all the study sites to the laboratory in Clermont-Ferrand for analysis. Analysis of serum

samples was carried out blind: the analyst did not know whether a sample came from an abuser, a dependent or a control.

MCV was measured using a Technicon H2 system. Normal values ranged from 80 to 100 fl. Gamma GT was assayed by an enzyme method at 37 °C using a Bayer Diagnostic CHEM 1 automated analyzer. Normal values of GGT are less than 40 IU/l.

CDT was measured by immunonephelometric assay based on micro-isocratic anion-exchange chromatography, following a method described by Schellenberg et al. (1996). The method described and evaluated by Schellenberg et al. gives results in mg/l (as do some other commercial kits [CDTect™]). We chose Schellenberg's method because (i) in previous studies we encountered some analytical difficulties with the first %CDTTIA kit (Axis-Shield, Oslo, Norway) (Schellenberg et al., 2001), and (ii) the second modified %CDTTIA kit (with the same analytical characteristics as Schellenberg's method) had not yet been evaluated.

The serum was stored at −20 °C until assayed. Serum was saturated for 90 min by incubation with a solution of ammonium iron sulfate. A buffer (piperazine, 20 mmol/l, adjusted to pH 5.69 with formic acid) was used for sample dilution, column conditioning and elution. Serum transferrin fractions were separated according to their pH values by anion-exchange chromatography on microcolumns. Sialic acid-deficient transferrin fractions were collected in the eluate and then quantified by rate nephelometry on an Array 360 Protein System (Beckman Instruments). Human transferrin polyclonal Ig antiserum directed against human transferrin and control Vigil level 2 (Beckman) was used. The coefficient of variation was 4–5% within runs and 7–9% between runs. Normal values of serum CDT are less than 60 mg/l.

2.3. Statistical analysis

According to the distribution of the variables, data were expressed as mean ± SD or as mean with minimum and maximum, and comparisons used analysis of variance. We used a computer learning system that created classification systems displayed in decision trees (Answertree 2.0). The algorithm for performing classification and segmentation analysis was CHID (chi-squared automatic interaction detection) (Biggs et al., 1991; Kass, 1980). The goal of this method was to establish the relationship between a quantitative variable and a set of qualitative and quantitative variables. A decision tree, also called a segmentation, (AnswerTree 3.1) is a multivariate analysis that displays models visually with evaluation graphs used to discover significant groups, create profiles and help decision.

In our study, the variable to be explained was the group (categorical target: healthy controls, consulting controls, abusers and dependents) and we had five explanatory variables (gender, age, MCV, CDT and GGT). No cut-off level for biomarkers was entered into the statistical analysis.

At each step, the algorithm CHAID selects the predictor with the smallest measure of independence with the categorical target (highest F or chi-square). Split rules can give two or more segments (e.g., 8–42, 42–59, 59–76, 76–316). A subject can only appear once in the tree and split rules at different levels of the tree can give different segments for the same variable because the path from the top (initial segment) is different. The international convention for interpretation of borderline values is to include on the right and to exclude on the left. The significance threshold was set at $P < 0.05$.

3. Results

Eighty-four patients were included as abusers (77 (92%) men and 7 women), 82 patients as dependents (67 (82%) men and 15 women), 111 subjects as healthy controls (86 (77%) men and 25 women) and 102 subjects as consulting controls (76 (75%) men and 26 women). There were more men in the abusers and fewer men in the two controls ($\chi^2 = 10.577$ $P = 0.014$ ddl = 3). Even so, gender was taken into account in the segmentation (among the 5 explanatory data including CDT and GGT) and it did not appear in the two first levels (most discriminant) of the tree. This means that whether alone or associated with biological values, gender is not a major discriminant factor.

Among the four initial groups (healthy controls, consulting controls, abusers and dependents), the aim was to obtain 75 ± 10% of the subjects well-classified in these four groups by the segmentation or 90 ± 10% of the subjects well-classified by the segmentation in three groups (control groups, abusers, dependants). The calculations (level 5%) gave a number (upper limit) of 73 subjects for each group.

In the results, the numbers of subjects enabled us to reach these aims. We obtained 233 out of 372 (63%) well-classified subjects in the four groups (86% of the healthy controls, 81% of the dependants, 43% of the consulting controls and 42% of the abusers). We obtained 302 out of 372 (81%) well-classified subjects in three groups (97% for both control groups, 81% of the dependents and 42% of the abusers).

Mean age was 41.1 years (±10.2) for the abusers, 40.7 years (±7.2) for the dependents, 39.3 years (±9.5) for the healthy controls and 38.7 years (±11.7) for the consulting controls. The ages of all the groups were comparable ($P = 0.28$). For the first step, the CHID used CDT (Fig. 1) as a discriminator to split the initial 379 subjects (level 1) into four separate groups (level 2). Subjects with CDT values between 8 and 42 mg/l were in the left node. Ninty two percent of them had no alcohol problems. There was no further segmentation for patients with CDT between 59 and 76 mg/l. Subjects with CDT values greater than 76 mg/l were in the right branch (level 2); 95% of these presented alcohol misuse (40% abusers and 55% dependents). This branch was split into three groups (level 3) using GGT as discriminator. Subjects with a GGT value below 41 IU/l (the normal value)

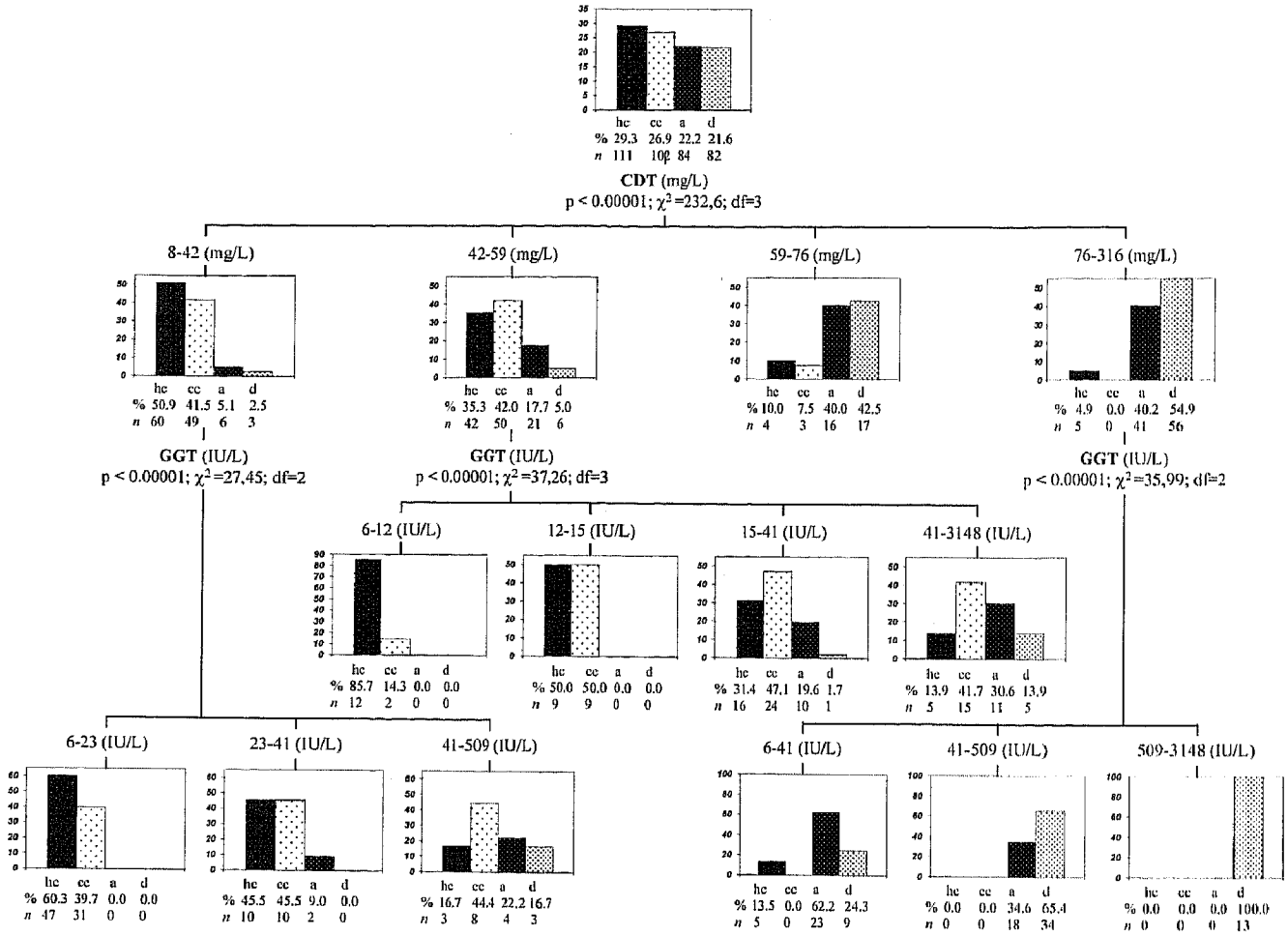

Fig. 1. Decision tree for the use of biological markers to confirm or disprove suspected alcohol misuse. The algorithm for performing classification and segmentation analysis was CHID (chi-squared automatic interaction detection); hc—healthy controls, cc—consulting controls, a—alcohol abusers, d—alcohol-dependent patients.

were essentially abusers. Subjects with a GGT value above 41 IU/l were mostly dependents.

Subjects with a CDT value between 42 and 59 mg/l (level 2) were split into four different groups also using GGT. Only the subjects with a GGT value below 41 IU/l could be considered as not displaying effects of alcohol misuse.

4. Discussion

To our knowledge, no studies have been carried out to form a scientific basis for diagnostic strategies using biomarkers in primary health care settings to deal with patients suspected of alcohol misuse. Our results show that the biomarkers CDT, GGT and MCV are not equally effective in distinguishing between subjects presenting alcohol misuse according to the DSM IV criteria and moderate alcohol consumers. CDT seems the most discriminative biomarker for this purpose.

Our decision tree shows some borderline values. Borderline values are more indications than absolute values. The international convention for interpretation of borderline values is to include on the right and to exclude on the left. According to the variability of biological results, individual variability and precision of tests, if a subject presents a value close to a borderline, the two paths can be examined in the tree. The important results in this decision tree are:

1. Out of five explanatory factors (gender, age, MCV, CDT and GGT) to explain the initial groups, only two (CDT and GGT) are present in the first two levels (most discriminant) of the segmentation.
2. CDT appears first, and GGT adds to the information given by CDT.
3. 63% (four groups) or 81% (three groups) of the subjects are well-classified by this tree.
4. On the first level, over ≈59 mg/l of CDT, we go from a majority of controls to a majority of abusers-dependents.
5. On the second level and for subjects under ≈59 mg/l of CDT, abusers-dependents are present mostly over ≈41 U/l of GGT. For subjects over ≈76 mg/l of CDT, controls are present only under ≈41 U/l of GGT.

In current clinical practice, GGT and MCV measurement is used as a first-line test when alcohol misuse is suspected, and CDT is only used subsequently. Recently, Allen and Litten (2001) recommended that 'whenever possible CDT and GGT be used together and that the client be classified as positive if either test score is above the cut-off'. This recommendation is based on studies that have demonstrated the absence of any correlation between CDT and GGT (Conigrave et al., 2002; Sillanaukee et al., 1994; Reynaud et al., 1998) (the mechanisms responsible for their increase being different). The association of these two markers is considered useful because sensitivity will increase if both tests are done together (Litten et al., 1995). However, specificity decreases as a result, and it is difficult to know how much respective importance to give to high sensitivity and low specificity. Positive, negative or global predictive values always depend on the prevalence of alcohol misuse in the study population and are therefore also difficult to interpret (Weill and Schellenberg, 1993). In contrast, our analysis is independent of prevalence of alcohol misuse in our study population and so enables us to compare the performance of the biomarkers and their combinations.

More recent studies have proposed improving diagnostic classification of alcohol abuse by combining CDT and GGT using the formula $\gamma\text{-CDT} = 0.8 \times \ln(\text{GGT}) + 1.3 \times \ln(\text{CDT})$ (Sillanaukee and Olsson, 2001; Anttila et al., 2003; Chen et al., 2003). In their retrospective study Sillanaukee and Olsson processed heterogeneous data sets from six clinical studies (CDT was analyzed using CDTect™). They found that γ-CDT had a higher sensitivity in discriminating alcohol misusers from social drinkers. Despite the methodological problems encountered in their study, the authors showed that the simple combination of CDT and GGT did not perform well.

For treatment decisions in alcohol problems, Sobell and Sobell (2000) proposed a stepped care approach. They proposed that 'the treatment used should be (a) individualized, (b) consistent with the research literature and supported by clinical judgment, and (c) least restrictive but still likely to be successful'. In analogy, our findings argue for a stepped screening approach to protect health care resources without sacrificing quality of screening. Our present work is perhaps a first step towards a practical decision tree for a stepped screening approach. For this purpose, we suggest that in the screening process for alcohol misuse (using biomarkers) CDT should be used (i) in the first line and (ii) probably alone.

As a second-line test GGT should be used. GGT can provide further information if CDT levels are high (>76 mg/l) or if CDT is borderline (42–59 mg/l). In cases of high CDT levels positive GGT points to alcohol dependence (65.4%) and negative GGT levels increase the probability of diagnosing alcohol abuse (62.2%). Some false-negatives can be detected by positive GGT (>41 IU/l) in borderline CDT.

These results are unsurprising, GGT and MCV showing a high sensitivity only in alcohol-dependent patients, and a very low sensitivity in alcohol abusers. However, an unexpected finding was that MCV, age and gender failed to provide any extra information for the detection of alcohol misuse or to support differential diagnosis between alcohol abusers and alcohol-dependent patients. Consequently our results do not justify MCV assay to objectify alcohol misuse or to support differential diagnosis.

One limitation of our study is that we did not include hazardous alcohol drinkers. The spectrum of alcohol misuse commonly comprises hazardous use (or, better, misuse), alcohol abuse and alcohol dependence. Alcohol abuse and alcohol dependence are clinical diseases also described by international classifications (DSM IV, ICD-10). In contrast, hazardous alcohol misuse seems to be a sub-threshold disease (Saunders and Lee, 2000) and its 'diagnosis' is not grounded on clinical signs but on consumption levels. However, despite the necessity of earliest intervention (in terms of secondary prevention), the absence of hazardous alcohol misusers in our study is probably of no consequence for our decision tree (GGT performs very badly in hazardous alcohol misuse), although further research is needed.

Our study also encountered the classic difficulty of identifying women with alcohol problems (Amodec et al., 1996), with only seven women (10%) in this group of consecutively recruited abusers, while there were 15 women to 67 men in the dependent group. Although classically CDT seems to be a less effective marker of alcohol problems in women (Löf et al., 1994; La Grange et al., 1994), the poor sensitivity reported by Löf was probably linked to the consumption standards adopted in that study (<210 g per week), which are insufficient to produce a positive effect on CDT.

A strong point of our study is that we examined a healthy control group and a consulting control group. We found high GGT levels in the consulting controls, confirming the low specificity of GGT described in several studies.

In conclusion CDT performs well as an initial screening test, but does not help differential diagnosis. In contrast, GGT performs well as a support for differential diagnosis between alcohol abuse and alcohol dependence in patients with high CDT. MCV provides no additional information and should consequently be considered as a biological indicator rather than a biomarker for alcohol misuse.

Future research (i) performed on a larger number of patients (ii) that includes hazardous alcohol misuse and (iii) that uses the logarithmic combination of GGT and CDT is needed.. One important point for future research is that the clinical objective that an alcohol misuse marker is expected to achieve has to be exactly defined: is it evidence of alcohol dependence, early detection of alcohol abuse and hazardous alcohol misuse, or early detection of relapse after alcohol weaning? Perhaps in future we will have (i) different biomarkers for each of these objectives, and (ii) different evidence-based screening and diagnostic steps to accomplish these aims.

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