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Biochemical markers of alcohol abuse

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Summary

Excessive alcohol consumption is a major health problem in the UK leading to both serious morbidity and mortality. This study compared newer potential biochemical markers of excessive alcohol consumption [carbohydrate-deficient transferrin (CDT), mitochondrial AST (mAST) and alpha glutathione-s-transferase (α-GST)] with conventional markers (AST, ALT, GGT, MCV). Patients ($n=85$) were enrolled in the study and subdivided into several groups on the basis of alcohol consumption. Patients with non-alcoholic liver disease (NALD) ($n=40$) were also enrolled. All the markers, with the exception of the ratio mAST/total AST were significantly higher in heavy drinkers/alcoholics compared to teetotallers/social drinkers ($p<0.05$). mAST and AST/ALT ratio were significantly higher in alcoholics compared to NALD ($p<0.01$), whereas ALT was higher in the NALD group ($p<0.05$). Multivariate discriminant function analysis (Wilks method) demonstrated that the logarithmic functions of AST/ALT ratio and mAST could correctly classify 87.9% of cases into either the alcoholic or NALD groups. ROC plot analysis showed that AST, mAST and GGT were the best markers at distinguishing heavy consumption of alcohol from lesser levels and that AST/ALT ratio and mAST were the best in distinguishing alcoholics from NALD. In conclusion, none of the newer biochemical markers, with the exception of mAST, offers any major advantage over the conventional markers.

Introduction

Alcohol consumption has been steadily increasing in the UK, with a parallel increase in the prevalence of alcohol-related diseases. It is therefore important to have tests that facilitate the identification of alcohol abuse. Many patients do not give an accurate history of their alcohol consumption and are subsequently subjected to unnecessary and costly diagnostic procedures. In 1986, it was estimated that alcohol abuse was responsible for 25 000–40 000 excess deaths per year and the economic costs are enormous at £2–£4 billion per annum and 8 million lost working days.[1]

Biochemical markers for alcohol abuse are widely available, but none is 100% efficient. The ideal marker should have a high enough sensitivity and specificity to be useful as a screening test, should distinguish between social drinking and heavy alcohol consumption and should not be elevated by non-alcoholic liver disease (NALD). The liver enzymes, especially gamma glutamyl transferase (GGT) and mean corpuscular volume (MCV) have been widely used as potential markers. Studies using GGT have shown diagnostic sensitivities of 62% for hospitalized alcoholics and 43% for ambulatory alcoholics[2] with specificity around 80%[3]—thus GGT is not an ideal screening marker, but can be useful in confirming a clinical suspicion of alcoholism. Aspartate aminotransferase (AST) has a sensitivity of only 35%[2] with alanine aminotransferase (ALT) even lower. MCV has a sensitivity of approximately 50%[4,5] and a specificity of 90%.[5] No single marker has been shown to have sufficient diagnostic accuracy to be useful for alcoholism screening in ambulatory patients,[6] and because of this investigators have attempted to combine the markers to increase either sensitivity or specificity.

A number of new laboratory markers of alcoholism have been developed which are claimed to have

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increased diagnostic accuracy. Stibler and Borg[7] reported that chronic alcohol consumption will reduce the number of carbohydrate moieties attached to serum transferrin producing carbohydrate-deficient transferrin (CDT). Sensitivities approaching 80% have been reported in detecting recent heavy drinking.[7,8] It is not elevated by moderate alcohol consumption or by medications that can cause elevation of other markers of alcoholism.[7] Specificity of 98% has been reported[7] and, more importantly, studies have shown that CDT is not elevated in most types of NALD.[9,10] CDT therefore appears to be a very useful marker and may also find use in the monitoring of relapse.

Another 'new' marker is mitochondrial AST (mAST). Total AST consists of two isoenzymes: mitochondrial AST (mAST) and cytosolic AST.[11] As alcohol consumption results in selective mitochondrial injury, mAST may be preferentially released.[12] Nalpas *et al.*[13] reported that 84% of alcohol patients had elevated mAST levels, although 78.7% of patients with NALD had likewise. However, when the ratio mAST/total AST was used, only 11/61 NALD patients had an elevated ratio (>7%), conferring to this ratio a specificity of 81.9%. However, both mAST and mAST/tAST are less useful in non-selected populations.[14]

Alpha glutathione-s-transferase (α-GST) is distributed throughout the liver lobule, and is found in particularly high concentrations in centrilobular hepatocytes.[15] These are more susceptible to damage from hypoxia and toxins, so that GST is readily and rapidly released into the circulation following hepatic damage. Its half-life is much shorter (<90 min) than either AST (17 h) or ALT (47 h). GST measurement provides a better and more sensitive indication of hepatotoxicity in paracetamol-induced liver damage than does ALT.[16,17] It could therefore be a sensitive marker of alcohol-induced damage.

The aims of this study were to compare the more conventional markers of alcoholism (i.e. GGT, AST, MCV) with the newer markers and to calculate their sensitivities, specificities and efficiency at diagnosing excessive alcohol ingestion. We were particularly interested to assess which, if any, of the markers were able to distinguish between alcohol-induced hepatic toxicity and NALD.

Methods

Patients admitted to Shaftesbury Square Hospital (chronic alcohol and drugs of abuse centre) and by acute admission to the medical units of Belfast City Hospital (a major teaching hospital) were interviewed the following morning by the same registrar in clinical biochemistry (PCS) using a questionnaire with regard to alcohol intake and drinking habits. The questionnaire contained detailed questions with regard to alcohol consumption (exact name of drink and the quantity ingested) over the past 12 months prior to admission, but in particular concentrating on the previous 4 weeks. If possible, the information obtained from the patient was substantiated by interview with a relative. The following patients were excluded from the study: (i) those on enzyme-inducing medications such as anticonvulsants; (ii) patients >80 years or <18 years of age; (iii) those with acute or chronic liver disease from any other cause.

On the results of the questionnaire, the patients were assigned to the following groups. Group 1, teetotallers ($n=16$); group 2, social drinkers (<20 g alcohol/day) ($n=15$); group 3, moderate drinkers (20–39 g/day) ($n=16$); group 4; heavy drinkers (between 40–79 g/day) ($n=18$); group 5; alcoholics (≥80 g/day for at least 12 months) ($n=20$); group 6, binge drinkers (those consuming >160 g alcohol/day for greater than 7 days with a period of abstinence for at least one month prior to this) ($n=2$).

All the patients had venous blood collected on the day of admission for liver profile (ALP, AST, ALT, GGT), MCV, mAST, CDT and α-GST. Alcoholics had a repeat blood sample collected 7 days later, if still in hospital. Blood samples were also obtained from patients with NALD ($n=40$) attending the specialized liver clinic and from the medical wards (18 primary biliary cirrhosis, 14 chronic active hepatitis, 5 cryptogenic cirrhosis, 2 chronic persistent hepatitis, 1 haemochromatosis). None of the patients with NALD admitted to any consumption of alcohol in the past 12 months. All patients gave written consent to being investigated and the study was approved by the Regional Medical Ethics Committee.

Routine liver function tests (ALT, AST, GGT, ALP) were performed on a multichannel analyser (Prisma, Clinicon-AB) using standard commercial kits. MCV was calculated on a Coulter STKS cell counter. CDT was determined using a double antibody radioimmunoassay (CDTect, Kabi Pharmacia Diagnostics). mAST was determined using an immunochemical procedure described by Rej in 1980[18] (antibody directed against human cytosolic AST was kindly donated by Dr Rej). Serum α-GST levels were determined using an *in vitro* enzyme immunoassay (HEPKIT, Biotrin International).

Statistical analysis

Comparison between the different subgroups was performed using ANOVAR and unpaired t tests (following logarithmic transformation if necessary). Multivariate discriminant function analysis (Wilks method) was used to attempt to identify the most useful laboratory tests in distinguishing between

alcohol-induced hepatic disease and NALD. Receiver-operating characteristic (ROC) plot analysis was used to assess the best parameter in distinguishing those consuming $\geqslant 40$ g ethanol daily against those consuming <40g ethanol daily, those consuming $\geqslant 20$g against those <20g daily and alcoholics against those with NALD. Sensitivity, specificity and efficiency were also calculated.

Results

Table 1 shows the mean or median of the different laboratory measurements within the various subgroups. All of the data apart from MCV and mAST/tAST ratio required logarithmic transformation. In comparing the alcoholic and NALD groups, ALT was significantly higher in the NALD group (98.2 vs. 46.8 u/l (geometric means), $p<0.05$) whereas mAST and the AST/ALT ratio were significantly higher in the alcoholic group (12.8 vs. 5.5 u/l (geometric means) $p<0.001$, and 2.05 vs. 0.80 (geometric means) $p<0.01$, respectively). No other measurements demonstrated any other statistically significant differences. In comparing the combined heavy and alcoholic group ($n=38$) against teetotallers and social drinkers ($n=31$), all the laboratory markers, with the exception of the mAST/tAST ratio, were higher in the first group (all statistically significant, $p<0.05$).

To assess whether any of the tests could discriminate between 'normal' or 'safe' drinking and excessive alcohol consumption, subjects were divided into two groups; (i) teetotallers, social and moderate drinkers (<40 g/day alcohol); (ii) heavy drinkers and alcoholics ($\geqslant 40$ g/day alcohol). Results found to be above a particular decision level or 'cut-off' point were considered to be indicative of excessive alcohol intake. The cut-off points for ALT, AST, GGT and MCV were determined by using the laboratory's upper limit of the reference ranges. Cut-off points for CDT, mAST, AST/ALT ratio and GST were determined using the level at which maximum efficiency had been calculated, while the level for mAST/tAST was selected from the study by Nalpas *et al.*[13]

For CDT the maximum efficiency was found to be 78% at a cut-off of 12 u/l (sensitivity 68%, specificity 89%). For mAST the maximum efficiency was 86% at a cut-off of 5 u/l (sensitivity 79%, specificity 93%). For GST the maximum efficiency was 71% at a cut-off of 6 ng/ml (sensitivity 51%, specificity 92%), and for the AST/ALT ratio the maximum efficiency was 70% at a cut-off of 1.5 (sensitivity 59%, specificity 79%). The percentage of patients in each of the separate groups with values greater than the cut-off is shown in Table 2.

The sensitivity and specificity for each test was

Table 1 Distribution of results throughout the various subgroups

Marker	Teetotallers ($n=16$)	Social ($n=15$)	Moderate ($n=16$)	Heavy ($n=18$)	Alcoholic ($n=20$)	NALD ($n=40$)
GGT (u/l)	36 (12–58)	37 (20–46)	59 (16–87)	123 (26–749)	446 (33–1382)	353 (33–1176)
AST (u/l)	25 (14–34)	33 (15–47)	31 (15–42)	71 (23–174)	133 (16–368)	69 (28–382)
ALT (u/l)	21 (12–41)	25 (7–60)	32 (10–43)	49 (15–178)	60 (9–174)	162 (25–453)
AST/ALT	1.2 (0.66–2.11)	1.2 (0.72–4.5)	0.89 (0.51–2.2)	1.38 (0.58–3.7)	2.11 (1.06–5.13)	0.84 (0.49–2.71)
MCV (fl)	85.4 ± 9.1	85.5 ± 6.7	95.9 ± 6.9	96.3 ± 6.8	95.0 ± 4.9	97.1 ± 9.3
mAST (u/l)	3.4 (2.1–4.8)	4.0 (2.3–5.8)	4.1 (2.3–18.4)	7.8 (2.3–18.4)	13.8 (4.6–38.0)	6.1 (2.3–12.2)
mAST/tAST	0.16 ± 0.03	0.14 ± 0.07	0.16 ± 0.05	0.13 ± 0.05	0.13 ± 0.09	0.11 ± 0.07
CDT (u/l)	6.5 (4.4–10.1)	7.8 (3.3–18.5)	7.6 (4.6–14.6)	18.2 (4.4–55.8)	17.8 (4.8–55.3)	14.7 (7.9–26.4)
GST (ng/ml)	2.1 (0.9–10.7)	3.2 (0.12–5.1)	3.2 (0.4–8.6)	7.4 (0.5–23.4)	7.0 (0.4–32.5)	3.8 (0.7–14.3)

Results expressed at median (range) or mean ± SD.

Table 2 Percentage of patients in each subgroup with values greater than the cut-off

Assay	Cut-off	Teetotallers	Social	Moderate	Heavy	Alcoholic	NALD
ALT	42 (u/l)	0	13.2	0	22.4	40.0	65.0
AST	35 (u/l)	0	13.2	0	44.8	85.0	80.0
AST/ALT	1.5	25.0	33.4	12.5	38.9	70.0	22.5
CDT	12 (u/l)	0	13.2	25.0	55.0	85.0	65.0
GGT	60 (u/l)	0	0	25.0	44.8	85.0	90.0
GST	6 (ng/ml)	12.5	0	25.0	55.0	60.0	20.0
mAST	5 (u/l)	0	19.8	0	60.6	95.0	50.0
mAST/tAST	0.07	87.5	100	100	100	100	85.0

also calculated over the range of results observed and ROC curves plotted. These curves were plotted for those consuming $\geqslant 40$ g ethanol/day against those consuming <40 g ethanol/day (Figure 1), those consuming $\geqslant 20$ g/day against those consuming <20 g/day (Figure 2) and also between alcoholics and those with NALD (Figure 3). Plots for MCV, AST, ALT and mAST/tAST are not included in Figures 1–3 for clarity. The ROC curves would suggest that AST, mAST and GGT are the best markers for distinguishing between those who consume heavy amounts of alcohol ($\geqslant 40$ g/day) against those consuming lesser amounts (<40 g/day), with little evidence to suggest that mAST offers any further advantages over AST. The ROC plot to determine the best marker in distinguishing those consuming $\geqslant 20$ g ethanol daily against those consuming <20 g/day shows a similar picture. In the ROC plot to assess the best biochemical marker in distinguishing alcoholics from those with NALD, AST/ALT ratio and mAST were the most useful.

Multivariate discriminant functional analysis (Wilks method) demonstrated that using the logarithm of AST/ALT and mAST would correctly classify 87.9% of cases into either the alcoholic or NALD groups. Unfortunately, we were unable to obtain a sufficient number of day 7 samples from the alcoholic patients for statistical analysis ($n=3$) and only two binge drinkers were enrolled in the study.

Discussion

The aim of this study was to investigate the ability of both 'established' and newer biochemical markers to distinguish between those consuming alcohol at

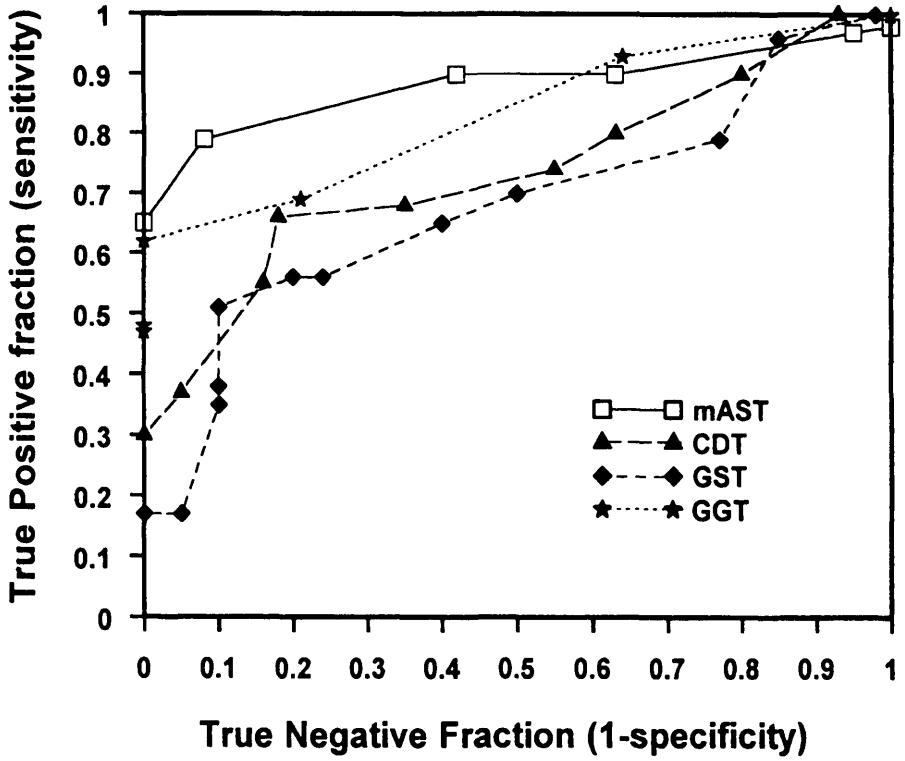

Figure 1. ROC plots for mAST, CDT, GST and GGT in distinguishing those consuming $\geqslant 40$ g ethanol/day ($n=47$) from those consuming <40 g ethanol/day ($n=38$).

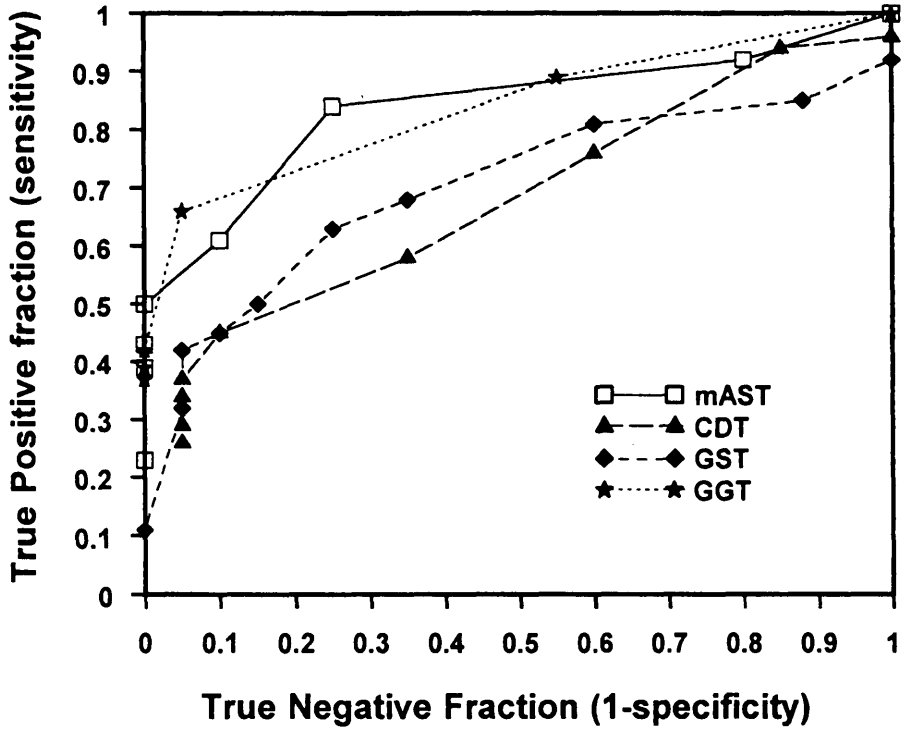

Figure 2. ROC plots for mAST, CDT, GST and GGT in distinguishing those consuming $\geqslant 20$ g ethanol/day ($n=54$) from those consuming <20 g/day ($n=31$).

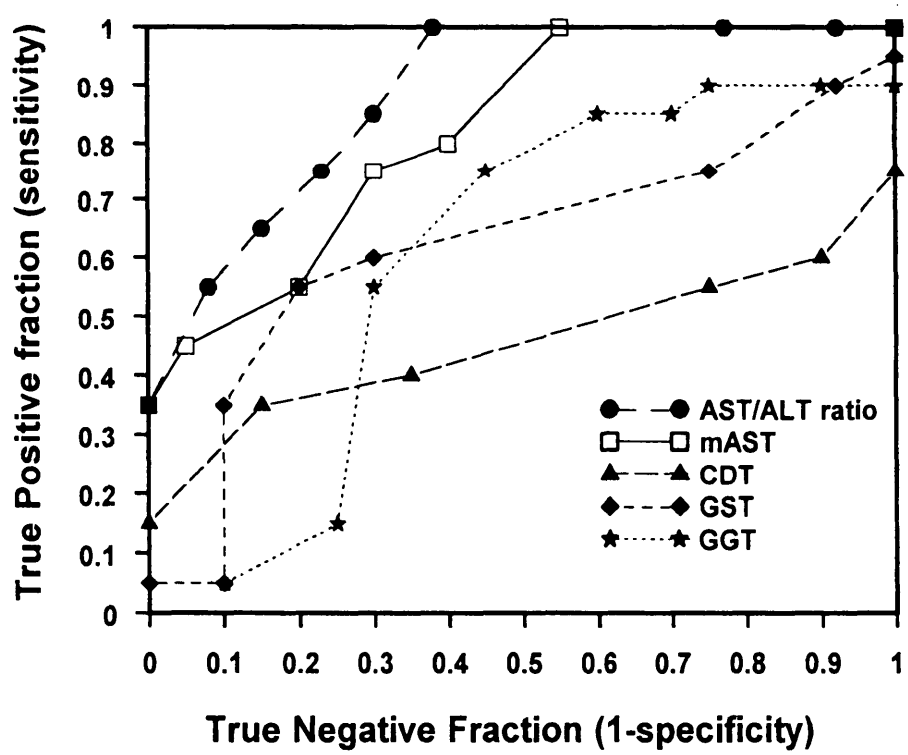

Figure 3. ROC plots for mAST, CDT, GST, GGT and AST/ALT ratio in distinguishing alcoholics ($n=20$) from NALD patients ($n=40$).

safe levels and those consuming excessive alcohol that is likely to lead to potential medical problems (both physical and mental). An ideal marker would be of considerable benefit, in that alcohol consumption is difficult to ascertain by interview with the patient alone and many patients fail to admit to their true consumption. As a result, many of these patients are subjected to an exhaustive diagnostic work-up to rule out possible aetiological factors in their illness. As an example, a study of patients admitted

to the medical and orthopaedic services of a community hospital showed that only 29% of patients with a history of alcohol abuse or alcohol dependence considered themselves to be alcoholics.[19] In another study, physicians were only able to identify 25% of alcoholics attending a general medical clinic.[20] Questionnaires such as CAGE[21] are widely used and easy to administer but have several limitations; they are dependent on patient co-operation and truthfulness, and their accuracy may be affected by socio-economic, gender and cultural factors.[22,23] In those patients who present with disturbances in liver function tests, it would be useful to distinguish between damage secondary to alcohol as opposed to other causes, and in this respect many of the biochemical markers are not so useful. The newer markers have been claimed to be better than the older methods in this respect, and we hoped to address this issue in our study.

Biochemical markers can be divided into two main groups—those useful for detecting chronic ingestion of alcohol and those useful for detecting acute consumption ('relapse' markers). The acute markers need to be sensitive to low levels of alcohol consumption to detect relapse, and our study shows that approximately 25% of subjects had elevations in CDT, GGT and GST at moderate levels of ethanol ingestion, whereas none had elevated transaminases or mAST. This may indicate that CDT, GGT and GST display better sensitivity at the lower levels of consumption.

Hepatic GGT is known to be induced by ethanol[24] and our results show a sensitivity of 66% and a specificity of 93% in distinguishing heavy drinkers and alcoholics from the others (teetotallers, social and moderate consumers). These results are similar to the results of others[2] but the major problem is that a raised serum GGT can be caused by other factors including microsomal inducing agents (e.g. anticonvulsants), NALD and biliary tract disease.[6] Nonetheless, GGT is a well-established laboratory assay, is cheap, and compares reasonably well with some of the newer more expensive assays.

Mean corpuscular volume (MCV) is known to be increased with excessive alcohol ingestion. This may be secondary to both a direct toxic effect of ethanol on the erythrocyte[25] as well as folic acid deficiency and liver disease. MCV suffers from low sensitivity, and the long half-life of the erythrocyte makes it useless for monitoring relapse.

The serum transaminases (ALT and AST) are a routine part of liver function tests, and excessive alcohol consumption can lead to raised levels due to increased cell membrane permeability and cell necrosis. The sensitivities of both transaminases are poor, with 35% reported for AST and lower still for ALT—our results display similar values (AST 65%, ALT 30%). Nonetheless, both ROC plot analysis and multivariate functional discriminant analysis demonstrated that the AST/ALT ratio was one of the two variables (along with mAST) that could be used to distinguish alcohol-induced liver disease NALD, in that the ratio tended to be lower in patients with NALD compared to alcoholics. The AST/ALT ratio therefore represents a simple, effective and inexpensive test in this context.

Many proponents of the newer biochemical markers would claim increased sensitivity and specificity as the main reason for their use both in screening and monitoring relapse. In this study we have used several different statistical processes to analyse the data, including ROC curve analysis. mAST appears to be the best new marker both in distinguishing heavy ethanol consumption from lesser amounts of consumption (sensitivity 79%, specificity 93%) and in distinguishing alcoholics from NALD (mAST elevated in 95% of alcoholics, and in 50% of NALD patients). Nalpas *et al.*[13] report that 84% of alcoholics have elevated mAST, and these results are similar to our own. Discriminant functional analysis shows that mAST is one of the two parameters that help to distinguish alcoholics from NALD, in that mAST tended to be higher in the alcoholic group. However, there is little evidence in this study to suggest that the measurement of mAST offers significant advantages over total AST in determining alcohol consumption. The mAST/total AST ratio was disappointing, in that there was little difference in the ratio between the various subgroups, contrary to the findings of other studies.[13]

CDT was generally not as good a marker as mAST, with lower sensitivity, and it failed to distinguish alcoholics from NALD (65% of NALD patients had values greater than the cut-off of 12 u/l). The manufacturers of the assay had recommended a cut-off level of 20 u/l, but only 11 patients had levels greater than this (i.e. 25% of alcoholics and 15% of NALD patients). Our results are disappointing, since elevations in CDT are reported to be specific to excessive alcohol ingestion and other studies have not demonstrated increased CDT concentrations in most types of NALD.[9,10] However, the exception is primary biliary cirrhosis[9] and this is of importance in this study as almost 50% of the NALD patients suffered from PBC. Despite this, CDT did not appear to be as good a marker as mAST, although it may be elevated at lower levels of alcohol consumption or over shorter time periods and could be a better marker for detecting relapse. A review of 20 studies using CDT as a potential marker for alcoholism found a sensitivity of 82% and a specificity of 97%.[26] However, more recently other studies have shown the diagnostic performance of CDT to be poorer than this.[27–31]

The measurement of GST did not offer any further advantages over the other markers, and lacked sensitivity. Although not statistically significant, there was an impression that the NALD patients may have lower levels of elevation of GST and this would require further, intensive investigation.

In conclusion, although the newer biochemical markers have good sensitivity in distinguishing excessive alcohol consumption from safer levels of consumption, none, with the exception of mAST, appeared to offer any benefit over established conventional tests (AST, GGT) in this study. None of the markers offered the possibility of clearly distinguishing between alcoholism and NALD. mAST again appeared to be the best new marker in this respect, although the AST/ALT ratio was equally useful. Discriminant analysis demonstrated that by using only two markers (i.e. the logarithm of mAST and AST/ALT ratio) almost 90% of patients could be correctly classified into one of the two groups (alcoholics or NALD). CDT displayed excellent sensitivity, but was also elevated in the NALD patients—this may have been due to the fact that almost 50% had primary biliary cirrhosis, which is known to be associated with increased CDT.

We are aware that our study is based on interviews with patients, and only in six cases was the information obtained confirmed by speaking to a relative. There is therefore a distinct possibility that a proportion of the patients may not have given a true reflection of their drinking habits and practices. The results and findings of this study need to be explored in a much larger trial to assess the true value of many of the newer potential markers of excessive alcohol consumption.

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