INVITED REVIEW

DIAGNOSTIC TESTS FOR ALCOHOL CONSUMPTION

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Abstract — A variety of laboratory tests are available to assist in the diagnosis of hazardous alcohol consumption and related disorders. Standard tests, such as serum gamma glutamyltransferase activity and erythrocyte mean cell volume, have limited sensitivity, particularly in detecting non-dependent hazardous consumption. Most also have poor specificity in that results are affected by common diseases and medications. Over the past 10 years a number of new laboratory tests have emerged. One of these, carbohydrate deficient transferrin, has high sensitivity in detecting persons with alcohol dependence, and shows promise for identification of non-dependent hazardous drinking; it is also highly specific. Others such as measurement of bound acetaldehyde, serum β-hexosaminidase and the ratio of urinary serotonin metabolites offer promise in detecting recent heavy drinking. However, many issues remain unresolved. The newer markers have often been judged by contrasting their values in patients who are clearly alcohol dependent and abstainers or very light drinkers. It is now apparent that some are relatively insensitive markers of hazardous consumption. Future research needs to examine the performance of these markers among subjects with a range of alcohol intakes to fully determine their value in assessing drinking history. In addition, assays which are capable of some degree of automation need to be developed for analysing large numbers of samples.

'To find a man’s true alcohol intake, you double what he says and halve what his wife says'
Anonymous

THE SEARCH FOR AN ACCURATE MEASURE OF ALCOHOL INTAKE

There has long been doubt over the accuracy of drinking histories (Midanik, 1982). Heavy drinkers in particular tend to underestimate their consumption (Popham and Schmidt, 1981; Watson et al., 1984), so much so that after studying the validity of alcoholics' self-reports, Watson concluded ‘The results support a moratorium on the use of patients' self-reports in follow-up studies on alcohol consumption’ (Watson et al., 1984). Population surveys cast further doubt on the adequacy of self-report. Despite careful sampling and assessment of intake, surveys are usually able to account for only 40–60% of the known consumption of a country or region (Midanik, 1982; Nilssen et al., 1992).

There have been attempts made to explain and describe the under-reporting. Factors such as regularity of drinking occasions, interviewer characteristics, phrasing of questions and even the perceived status of the beverage consumed have been found to affect the accuracy of the intake assessment (Pernanen, 1974; Crawford, 1987). Memory problems are blamed for 10–20% of the underestimation (Pernanen, 1974; Wilson, 1981; Crawford, 1987). Conscious and unconscious denial is important (Cooke and Allan, 1983). Deliberate denial is likely to be a major problem if some disciplinary action may result from the assessment, as in the evaluation of drink drivers before licence restoration. Even patients in treatment, being reviewed by their treating clinician, may wish to please their therapist, and therefore understate their consumption. Orrego found that of persons with alcoholic liver disease who were reviewed weekly ‘patients with alcohol in their urine convincingly denied alcohol intake 52% of the times that they were questioned’ (Orrego et al., 1979).

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The perceived difficulty of obtaining an accurate drinking history may be one reason for the widespread under-diagnosis of hazardous alcohol use and related disorders. As few as 28% of problem drinkers are recognized by their doctor (Reid et al., 1986; Persson and Magnusson, 1988). The majority do not themselves seek help until there are established complications of their drinking (Bucholz et al., 1992). This late diagnosis is of particular concern because there are now available effective and low-cost methods of treating early stage problem drinking (Holder, 1991; Saunders and Foulds, 1992; Bien et al., 1993). Additionally, if a patient has presented with a medical complication of alcohol excess, failure to recognize the cause may result in incorrect treatment and unnecessary, perhaps invasive, investigations. For these reasons there has been much effort directed towards developing accurate laboratory markers of alcohol consumption.

While non-detection of problem drinking is all too often due to failure of the clinician to enquire into alcohol intake (Barrison et al., 1980), in other settings history taking is impossible. Examples of this are where the patient has marked memory loss or a severe withdrawal syndrome or is unconscious. In these situations, a laboratory test which pointed to hazardous alcohol consumption may provide a vital clue to diagnosis. Another potential role for such tests would be in community screening for hazardous alcohol consumption. This would enable early detection of hazardous alcohol consumption, and the opportunity for effective early treatment.

A variety of blood tests have been used to aid the assessment of drinking history. More recently, laboratory tests based on urine, breath and sweat analyses have been investigated. However, there has been a great deal of controversy over the usefulness of these markers. Many conventional tests have only limited sensitivity and specificity, and there have been doubts whether there is sufficient benefit to warrant their use.

In this article we will provide an overview of recent advances in laboratory markers of alcohol consumption and illustrate some of the issues which challenge workers in the field. Areas for future research will be explored. The focus of our review will be markers of recent alcohol intake ('state markers'). We shall not consider markers of susceptibility to alcohol dependence ('trait markers'). Furthermore, we shall examine only those laboratory tests which can be performed on a single specimen and will therefore exclude metabolic studies which require a series of observations. It is also outside the scope of this paper to review electrophysiological or behavioural studies.

**CURRENTLY AVAILABLE MARKERS**

The most frequently used markers of alcohol intake are the serum enzymes gamma glutamyltransferase (GGT) and aspartate aminotransferase (AST) and the erythrocyte mean cell volume (MCV). Of these, serum GGT is the most sensitive indicator of hazardous alcohol consumption, and is usually the first test to become elevated (Rosalki, 1984). It is a biliary canaliculal enzyme which is induced by alcohol, and serum levels also rise in response to acute hepatocellular damage. Levels are especially high in patients with severe alcoholic liver disease (Wu et al., 1976), though they may fall in the later stages of cirrhosis.

Serum GGT has become the most widely employed marker of alcohol consumption. Serum levels are elevated in around 75% of persons with established alcohol dependence (Rosalki and Rau, 1972; Wu et al., 1976; Stetter et al., 1991). However, they are increased in as few as 30% of otherwise healthy, hazardous drinkers in a community setting (Tables 1 and 2) (Whitfield et al., 1978a; Poupon et al., 1989; Sillanaukee et al., 1992). GGT is rarely elevated in those aged less than 30 years (Whitfield et al., 1978b; Bliding et al., 1982) and is less sensitive in women than in men (Whitfield et al., 1978b). It is more likely to be elevated in regular rather than in episodic drinkers (Rosalki, 1984).

Another disadvantage of GGT is that it has only moderate specificity. There are many other causes of elevation, several of them, such as medications and hepatobiliary disease, relatively common (Salaspuro, 1986). The specificity of GGT is likely to be low in hospital practice (Stibler and Hultcrantz, 1987; Kapur et al., 1989; Schellenberg et al., 1989), though some community studies have demonstrated specificities of over 90% (Kärkkäinen et al., 1990; Sillanaukee et al., 1992). Because of this, even in a person who has previously been diagnosed as having a drinking problem, an elevated GGT does not necessarily signal
Table 1. Sensitivity and specificity of laboratory tests for detection of hazardous alcohol consumption and alcohol dependence/alcoholism

<table>
<thead>
<tr>
<th>Test</th>
<th>Hazardous consumption</th>
<th>Specificity (%)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>GGT</td>
<td>20–50</td>
<td>55–100</td>
<td>Baxter et al., 1980; Chick et al., 1981; Shaper et al., 1985; Kristenson, 1987; Poupon et al., 1989; Kärkkäinen et al., 1990; Sillanaukee et al., 1992</td>
</tr>
<tr>
<td>Dependence/alcoholism</td>
<td>60–90</td>
<td>55–100</td>
<td>Wu et al., 1976; Behrens et al., 1988; Schellenberg et al., 1989; Sillanaukee et al., 1992</td>
</tr>
<tr>
<td>MCV</td>
<td>20–30</td>
<td>64–100</td>
<td>Whitfield et al., 1978a; Baxter et al., 1980; Chick et al., 1981; Skinner et al., 1984; Bush et al., 1987; Sillanaukee et al., 1992</td>
</tr>
<tr>
<td>Hazardous consumption</td>
<td>40–50</td>
<td>64–100</td>
<td>Chick et al., 1981; Skinner et al., 1984; Poikolainen et al., 1985; Bush et al., 1987</td>
</tr>
<tr>
<td>Dependence/alcoholism</td>
<td>&gt;90</td>
<td>&gt;90</td>
<td>Bell and Steensland, 1987; Kapur et al., 1989; Sillanaukee et al., 1992</td>
</tr>
<tr>
<td>AST</td>
<td>10–30</td>
<td>&gt;90</td>
<td>Baxter et al., 1980; Sillanaukee et al., 1992</td>
</tr>
<tr>
<td>Dependence/alcoholism</td>
<td>35–50</td>
<td>&gt;90</td>
<td>Bell and Steensland, 1987; Sillanaukee et al., 1992</td>
</tr>
<tr>
<td>ALT</td>
<td>10–20</td>
<td>&gt;80</td>
<td>Baxter et al., 1980; Sillanaukee et al., 1992</td>
</tr>
<tr>
<td>Dependence/alcoholism</td>
<td>20–50</td>
<td>&gt;80</td>
<td>Bell and Steensland, 1987; Sillanaukee et al., 1992</td>
</tr>
<tr>
<td>CDT</td>
<td>26–62</td>
<td>&gt;90</td>
<td>Nilssen et al., 1992; Nyström et al., 1992; Sillanaukee et al., 1993; Godsell et al., 1995</td>
</tr>
<tr>
<td>Dependence/alcoholism</td>
<td>65–95</td>
<td>&gt;90</td>
<td>Stibler et al., 1986; Behrens et al., 1988; Gjerde et al., 1988; Kapur et al., 1989; Schellenberg et al., 1989; Kwoh-Gain et al., 1990; Stibler, 1991; Xin et al., 1992</td>
</tr>
<tr>
<td>β-HEX</td>
<td>86*</td>
<td>98*</td>
<td>Kärkkäinen et al., 1990</td>
</tr>
<tr>
<td>Dependence/alcoholism</td>
<td>66–95</td>
<td>&gt;95</td>
<td>Hultberg et al., 1980; Kärkkäinen et al., 1990; Wehr et al., 1991; Halvorson et al., 1993</td>
</tr>
<tr>
<td>Bound acetaldelyde</td>
<td>55</td>
<td>75–95</td>
<td>Niemelä and Israel, 1992; Sillanaukee et al., 1992</td>
</tr>
<tr>
<td>Dependence/alcoholism</td>
<td>40–97</td>
<td>75–99</td>
<td>Niemelä and Israel, 1992; Sillanaukee et al., 1992; Halvorson et al., 1993</td>
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*β-HEX may not distinguish between teetotallers and heavy drinkers in a young student population (Nyström et al., 1991).

current drinking. Alcohol dependent persons are more likely to be taking antiepileptic medication, to be diabetic or to have pancreatitis or heart failure. All of these relatively common conditions may elevate the GGT level.

As few as 50% of healthy people with an elevated GGT may actually be hazardous drinkers (i.e. positive predictive value = 50% (Behrens et al., 1988; Nilssen et al., 1992). An abnormal result, therefore, is difficult to interpret until other potential causes of elevation are excluded. Nonetheless, when other laboratory tests are normal, alcohol is the commonest cause of GGT elevation.

When a heavy drinker is denied access to alcohol, as when hospitalized or imprisoned, any elevation of GGT should gradually resolve. Values fall to approximately half within 2 weeks, and usually return to the reference range over 6–8 weeks. This provides useful confirmation that alcohol was the cause of the elevation. However, the fall may be delayed or incomplete if there is
underlying alcoholic hepatitis or cirrhosis or other medical disorders (Rosalki, 1984).

GGT levels have also been shown to have important prognostic value. In a study from Malmö, Sweden, middle-aged males with GGT values above 83 U/l experienced increased morbidity and mortality compared with those with low GGT results (Kristenson, 1987). These results have been confirmed and extended in an Australian study (Conigrave et al., 1993). Men attending a hospital emergency department who had a GGT result of >80 U/l were found to have a seven times increased risk of death over the next 3 years, a five times increased risk of liver disease and a 2-fold increased risk of trauma compared with men with a GGT <30 U/l. Women with higher GGT levels were also reported to have increased morbidity. In another study, elevation of GGT was found to be predictive of residual neurocognitive impairment in alcoholics commencing detoxification (Richardson et al., 1991). Trauma patients who have both elevation of GGT and questionnaire evidence of a drinking problem have been shown to have a 2-fold increased risk of experiencing a complication of their trauma (Jurkovich et al., 1993). These findings indicate that GGT values can be used to screen for those most likely to experience harm from their drinking.

GGT has also been used as a tool in intervention programmes (Kristenson et al., 1981; Persson, 1991). In the Malmö screening programme men with GGT values in the top decile were entered into a randomized trial of intervention, which included regular feedback of GGT results. The treatment group had significantly reduced morbidity and mortality compared with a control group over the subsequent 6 years (Kristenson et al., 1983).

Erythrocyte mean cell volume is slightly less sensitive than GGT, being elevated in ~50% of alcoholic inpatients (Skinner et al., 1984). As a screening test for non-dependent hazardous drinking it performs relatively poorly, in that elevated values are found in only 20–30% of hazardous drinkers in community settings (Chick et al., 1981; Skinner et al., 1984; Sillanaukee et al., 1992). Although a number of factors may increase MCV levels, for example folate deficiency, recent blood loss and a number of haematological conditions, its specificity is generally greater than that of GGT, being around 90% in general population samples (Chick et al., 1981; Behrens et al., 1988; Sillanaukee et al., 1992).

<table>
<thead>
<tr>
<th>Patients admitted for treatment of 'alcoholism'</th>
<th>Family physician</th>
<th>Community screening</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>GGT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60–90 (Bell and Steensland, 1987; Behrens et al., 1988; Schellenberg et al., 1989)</td>
<td>40–50 (Baxter et al., 1980; Nalpas et al., 1989)</td>
<td>20–50 (Whitfield et al., 1978a; Shaper et al., 1985; Nilsson and Färde, 1991; Sillanaukee et al., 1993)</td>
</tr>
<tr>
<td><strong>MCV</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40–50 (Baglin et al., 1976; Chick et al., 1981; Rosalki, 1984)</td>
<td>20–30 (Baxter et al., 1980; Nalpas et al., 1989)</td>
<td>20–30 (Chick et al., 1981; Sillanaukee et al., 1993)</td>
</tr>
<tr>
<td><strong>AST</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 (Bell and Steensland, 1987)</td>
<td>10 (Baxter et al., 1980)</td>
<td>10–30 (Whitfield et al., 1978a; Sillanaukee et al., 1992)</td>
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<tr>
<td><strong>ALT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50 (Bell and Steensland, 1987)</td>
<td>10 (Baxter et al., 1980)</td>
<td>10–30 (Sillanaukee et al., 1993)</td>
</tr>
<tr>
<td><strong>CDT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>65–95 (Stüber, 1991)</td>
<td>45 (Poupon et al., 1989)</td>
<td>20–62 (Nilssen et al., 1992; Nyström et al., 1992; Sillanaukee et al., 1993; Godsell et al., 1995)</td>
</tr>
</tbody>
</table>
If both GGT and MCV levels are elevated, it becomes more likely that alcohol is the cause (Chick et al., 1981). However, antiepileptics and non-alcoholic liver disease may elevate both results. Because the red blood cell survives for 120 days after it has been released into the circulation, an MCV result may remain elevated for up to 3 months after a person has stopped drinking. For this reason it is less useful than GGT for monitoring alcohol intake in the weeks following treatment.

The serum transaminases, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are less often elevated than GGT, being raised in 50% of alcoholic inpatients (compared with 75% for GGT) (Bell and Steensland, 1987). The transaminases have not often been used in screening programmes because of their limited sensitivity. Indeed, as few as 10–30% of hazardous drinkers have increased values (Whitfield et al., 1978a; Sillanaukee et al., 1992). Like GGT, AST and ALT results have limited specificity and are elevated in any hepatic or biliary disease. AST can also arise from non-hepatic sites, particularly heart and muscle, and levels are increased in conditions such as myocardial infarction and skeletal muscle trauma.

AST and ALT are not generally used on their own for monitoring response to treatment; however, they can be useful in combination with GGT. When an alcoholic resumes drinking on discharge from hospital, the GGT and ALT levels are likely to rise by 20% or more and the AST by 40% or more from pre-discharge levels (Irwin et al., 1988). These authors report that monitoring GGT, AST and ALT in combination is a sensitive means of detecting resumption of drinking (95% sensitivity, 80% specificity) (Irwin et al., 1988).

Serum AST levels also have long-term prognostic value. Alcoholics with elevated levels are more likely to experience cirrhosis over the next 10 years (Sørenson et al., 1984). In men attending an emergency department, higher AST levels are associated with a greater risk of subsequent alcohol-related medical disorders (Conigrave et al., 1993).

A positive blood alcohol concentration (BAC) provides a highly specific indication of recent drinking. Furthermore, a high BAC in a person with little clinical evidence of impairment suggests tolerance induced by long-standing hazardous alcohol intake. Breathalysers provide an immediate result, and levels correlate well with blood alcohol. More recently passive alcohol sensors have become available, which can give a reading when held in front of a person who is speaking or breathing (Lestina and Lund, 1992). This allows measurement of BAC in the uncooperative or unconscious patient. Urine alcohol gives an accurate indication of the BAC at the time the urine was produced. It may therefore remain elevated for some hours after BAC has fallen to background levels. Transdermal alcohol sensors or sweat patches show promise as a means of prospectively measuring alcohol intake over several days (Phillips et al., 1984; Swift et al., 1992). However, there is an ongoing challenge in ensuring these are tamper proof.

Because the BAC detects alcohol intake in the past few hours, it is not necessarily a good indicator of chronic excessive drinking, particularly in medical settings where patients are likely to abstain from alcohol for some hours before the appointment. In general it shows a poor correlation with intake over recent weeks (Saunders and Ausland, 1987). In clinical settings it detects only 10% of hazardous drinkers (Persson and Magnusson, 1988) and is not a sensitive indicator of alcohol dependence (Wieczorek et al., 1992). When used in an emergency department where problem drinkers are more likely to present with consequences of intoxication, it performs better, detecting 54% of hazardous drinkers identified by a questionnaire (Robinson et al., 1992).

Measurement of BAC has played a major role in laboratory and roadside studies on the effect of alcohol on driving performance. As a consequence of this type of research, random stopping of drivers by police for breath testing in some countries has been used in enforcing drink driving laws and as an effective deterrent (Loxley et al., 1992). Measurement of BAC in trauma and accident victims has helped quantify the huge impact that alcohol has on trauma and mortality, on the roads, in drownings, suicides and violent crime (Council on Scientific Affairs, 1986; Cherpetel, 1993a, b; Hingson and Howland, 1993).

High density lipoprotein cholesterol (HDL-C) levels correlate with recent intake; however, the sensitivity of an abnormal HDL-C in detecting hazardous drinkers being treated on an outpatient basis was found by Skinner et al. (1984) to
be only 26%. In the same study HDL-C levels were elevated in none of the general practice patients with hazardous consumption (≥60 g day); however, the sample size was relatively small.

Other standard biochemical tests have been shown to correlate with recent alcohol intake. These include alkaline phosphatase, bilirubin and plasma urate. However, none of these offers sufficiently sensitive or specific detection of hazardous alcohol intake to warrant use on its own. Several blood tests have been used as composite indices in the detection of hazardous alcohol consumption, sometimes aided by computer algorithms (Ryback et al., 1982; Lichtenstein et al., 1989; Beresford et al., 1990). While this provides higher sensitivity than any one test alone, it involves increased expense and complexity and has not become routine practice.

In summary, those markers of recent drinking which are currently widely available are limited by poor sensitivity and/or specificity when compared with expert clinical assessment. They are also less likely to be of value in patients with non-dependent hazardous drinking than in those with established alcohol dependence. This is a significant limitation because it is non-dependent hazardous drinking which is often difficult to detect and for which aids to diagnosis are more often needed. It is the non-dependent drinkers too, in whom intervention is more likely to be effective.

**NEWER MARKERS OF ALCOHOL USE**

The limitations of established markers of alcohol use have intensified the search for better tests. One of the most promising of these, carbohydrate deficient transferrin (CDT), has been reported to be a sensitive and highly specific marker of chronic excessive alcohol use. CDT is a collective term referring to isoforms of transferrin which are deficient in sialic acid residues. In persons with excessive alcohol consumption there is an increase, in particular, in the concentration of the transferrin isoform which has an isoelectric point at pH 5.7 (the principal isoform of transferrin normally focuses at pH 5.4). The mechanism by which excessive alcohol consumption causes elevated CDT levels is as yet undetermined. It seems that alcohol (or its principal metabolite, acetaldehyde) may interfere with several steps in the production, secretion and elimination of glycoproteins in the liver (Matsuda et al., 1983; Stibler, 1991; Ghosh et al., 1993).

The sensitivity of CDT in detecting alcohol dependence is 65–95%, and its specificity is ~97% (Stibler, 1991). The only recognized causes of false positive results are advanced chronic liver disease (primary biliary cirrhosis, chronic active hepatitis and drug induced hepatic insufficiency), a genetic variation in transferrin and a rare hereditary glycoprotein disorder (Stibler, 1991). Most patients with liver disease have a normal CDT result (Behrens et al., 1988; Stibler, 1991).

While CDT appears to be more sensitive than existing markers in the detection of alcohol dependence, there have been only a few studies of non-dependent hazardous drinkers. It has been reported that CDT, when measured using a microanion exchange method now commercially available, has very low sensitivity in detecting hazardous drinking (22–29%) (Nilsson et al., 1992; Nyström et al., 1992; Sillanaukee et al., 1993). However, in our laboratory, using isoelectric focusing combined with immunofixation and staining, we found over half of a sample of hazardous drinkers had an elevated CDT compared with 19% who had an increased GGT (Godsell et al., 1995). CDT levels are less likely to be elevated in young people and so the test may have limited value in this group (Chan et al., 1989). Females drinking < 15 g alcohol/day have slightly higher values than males, though still within the reference range (Stibler et al., 1988, 1991). Little is known about possible racial variation in CDT levels (Behrens et al., 1988).

Several issues concerning CDT need further attention. A crucial one is the method of separation of the isoforms. The isoelectric point of a transferrin isoform varies according to the number of sialic acid groups present. Several methods for separation of isoforms have been developed, based on the difference in charge. A commercial kit (CDTect, Pharmacia) is now available but one comparison of its results with other methods revealed substantial differences in reference ranges and, more worryingly, lower sensitivity in detecting excessive drinkers (Xin et al., 1992). In addition more false positives were recorded with the use of the kit than with the method of isoelectric focusing combined with Western blotting (Xin et al., 1992).
There is also inconsistency in the method of reporting results: some investigators presenting aggregate data for all isoforms with a pI of 5.7 or more, or in some cases > 5.7, and others reporting only the concentration of the major carbohydrate-deficient isoform. CDT has most often been reported as the quantity of the transferrin isoform at or above pI 5.7. Other authors have preferred to use the transferrin ratio (the ratio of the isoform at pI 5.7 to total transferrin) or the transferrin index (the ratio of the chief abnormal isoform at pI 5.7, to the major normal isoform at pI 5.4). It is not clear that the transferrin ratio or index offer any advantage over simple quantification of the carbohydrate-deficient isoforms except perhaps in situations where the total transferrin is likely to be abnormal.

The measurement of acetaldehyde has also been used as a marker of recent drinking. Since acetaldehyde is a reactive molecule, forming Schiff bases with amines, it binds readily to proteins. Chemical rearrangement can lead to an irreversible reaction, giving an acetaldehyde-protein adduct (Hoffmann et al., 1993). Two approaches have been taken to the detection of acetaldehyde as a marker of alcohol intake. The first approach is to detect acetaldehyde which is free or reversibly bound to plasma proteins or to blood cells (Eriksson and Fukunaga, 1993). The acetaldehyde is liberated from the blood and measured by gas or liquid chromatography (Halvorson et al., 1993). The second is to use an immunoassay to detect epitopes derived from acetaldehyde on proteins in the plasma (Niemelä et al., 1991; Niemelä and Israel, 1992). Neither approach has been examined sufficiently to fully determine its value in detecting and monitoring hazardous alcohol consumption but each shows promise.

Haemoglobin is another protein which forms adducts with acetaldehyde after ethanol ingestion (Niemelä et al., 1990; Sillanaukee et al., 1992). Haemoglobin-acetaldehyde adducts are detectable in 50% of hazardous drinkers (Niemiä et al., 1991; Sillanaukee et al., 1992) and the test is more sensitive than GGT (39%) and MCV (17%) in this setting (Sillanaukee et al., 1992).

Adducts between acetaldehyde and liver proteins are found in the serum of persons with alcoholic liver disease, particularly hepatitis (Israel et al., 1988). It is not yet clear whether the adducts between acetaldehyde and liver proteins are released as a result of liver damage or are themselves involved in inducing immunological damage (Niemelä et al., 1991).

Antibodies to acetaldehyde-modified epitopes have also been used as a marker of recent alcohol intake (Israel et al., 1988). In particular, the IgA response to acetaldehyde-modified epitopes has been reported to be a specific marker of alcoholic liver disease (Worrall et al., 1991).

The urinary ratio of the serotonin metabolites, 5-hydroxytryptophol (5HTOL) and 5-hydroxy-indoleacetic acid (5HIAA), has been found to reflect alcohol intake in the past 24 h (Voltaire et al., 1992; Voltaire Carlsson et al., 1993). As the test measures very recent alcohol intake, its clinical use in monitoring patients' intake requires frequent urine collections. It shows more promise as a research tool in settings where frequent follow-up of subjects is feasible.

Among alcohol dependent persons who are currently drinking, erythrocyte (cytosolic) aldehyde dehydrogenase (ALDH1) activity is significantly lower than in healthy controls (Agarwal et al., 1983; Lin et al., 1984; Johnson et al., 1992). Aldehyde dehydrogenase is the principal enzyme involved in oxidation of acetaldehyde. The isoenzyme found in the erythrocyte resembles the hepatic cytosolic isoenzyme, and has a low affinity for acetaldehyde (Lin et al., 1984). While activity levels are reduced in recently drinking alcoholics, there is a degree of overlap with control values (Johnson et al., 1992), which limits its clinical usefulness. Upon cessation of drinking, levels decline further, reaching their lowest following 2 weeks of abstinence, before returning to normal after 2–4 weeks' abstinence.

Beta hexosaminidase (β-HEX) is a lysosomal glycosidase. Levels increase significantly after drinking 60 g ethanol daily for 10 days (Hultberg et al., 1980; Isaksson et al., 1985). β-HEX has been found to have a higher sensitivity in the detection of hazardous alcohol intake than established markers of alcohol use (86% compared with 48% for GGT) (Hultberg et al., 1980; Kärkkäinen et al., 1990), and in one report good specificity (98% compared with 92% for GGT) (Kärkkäinen et al., 1990). However, levels of β-HEX also increase with liver disease of any cause, pregnancy, use of the oral contraceptive pill, and a number of other relatively common conditions.
(Kärkkäinen et al., 1990; Nyström et al., 1991). In addition β-HEX did not distinguish between teetotallers and the heaviest drinkers amongst
young university students (Nyström et al., 1991). To date, the number of studies on β-HEX remains small.

In the serum of healthy individuals mitochondrial AST (mAST) makes up < 10% of total
AST activity (Rej, 1978). However, following heavy alcohol consumption there is evidence of
mitochondrial damage, with an increase in the proportion of mAST to total AST (mAST:tAST)
in serum (Panteghini et al., 1983). In an early report, the mAST:tAST ratio was found to be
raised in 66–81% of patients with alcoholic liver
disease and to differentiate between alcoholic and
non-alcoholic liver disease (specificity 82%)
(Nalpas et al., 1986) However, its sensitivity for
detecting hazardous intake in the community is
lower (30–40%) (Nalpas et al., 1989; Schiele et
al., 1989; Nilssen et al., 1992) and its positive
predictive value as little as 13% (Nalpas et al.,
1989). Therefore as a screening test for the general
population its value seems to be limited.

METHODOLOGICAL CONSIDERATIONS

Several methodological issues are relevant
when evaluating any laboratory test assessing
alcohol use or abuse.

Selecting the index condition

There are several interconnected but con-
ceptually distinct entities related to alcohol con-
sumption. Alcohol dependence, a diagnostic term
which has now replaced the former one of
‘alcoholism’, is characterized by impaired control
over drinking, persistent drinking despite harmful
consequences, and frequently, withdrawal symp-
toms on cessation of drinking. It implies a drinking
problem of a severe nature. Most laboratory tests
have been evaluated for their ability to detect
alcohol dependent subjects. It is worth noting that
in the main they detect levels of alcohol intake
characteristic of alcohol dependent persons,
rather than the syndrome itself. Detection of haz-
ardous alcohol use is probably the most valuable
role for a laboratory test. Hazardous consumption
refers to alcohol use which confers a risk of
harmful consequences. A cut-off point (typically
40 g/day for men and 20 g/day for women is used
to define hazardous consumption). However, the
level of intake at which laboratory results become
abnormal will vary from person to person. Some
subjects with hazardous intake will have normal
test results, and some with ‘non-hazardous’ intake
will have elevated results. Several laboratory tests
can also be used to assess the presence and extent
of physical harm resulting from excessive con-
sumption. For example elevated values for GGT,
AST and ALT that do not fall as expected with
abstinence can reflect alcoholic hepatitis or cir-
rhosis.

Comparison of research results is rendered dif-
icult by the fact that many studies reported to
date have examined different target conditions.
Clearly, the sensitivity of a test varies according
to the condition it is being used to detect. A
test may have high sensitivity in detecting alcohol
dependence, but a low sensitivity in detecting non-
dependent hazardous alcohol consumption (Table
1). For most tests the highest sensitivities have
been obtained in settings where persons with more
advanced drinking problems and a higher fre-
quency of physical complications are likely to be
found (Table 2).

A further problem is that different criteria have
been used to define the index condition. Some-
times it has not been defined at all. For example,
the level of alcohol intake which is considered
hazardous has been steadily lowered over recent
years. A variety of criteria have been used to
diagnose alcohol dependence or ‘alcoholism’,
which are based either on clinical evaluation or
questionnaire-based assessment. The availability
of well-defined diagnostic criteria, such as those
set out by the American Psychiatric Association
or the World Health Organization, should allevi-
ate this problem (American Psychiatric Associ-

The subjects studied

The nature of the groups studied will have a
great influence on test performance. Currently
available laboratory tests are more likely to be
elevated in those with a long history of excessive
drinking. Accordingly, the best differentiation
(the greatest difference in mean values) and the
highest sensitivity can be expected when life-long
abstainers are compared with currently drinking
alcoholics. For similar reasons, the sensitivity of
these markers has been higher when an inpatient
treatment facility has been used for recruitment than when subjects are drawn from a community setting (Table 2). The age and sex distribution of the sample is also important. There are differences in the reference ranges and in response to alcohol between younger and older subjects, and between men and women (Whitfield et al., 1978b; Stibler, 1991).

The reference range

Both the level of alcohol intake and the results of laboratory tests in a population are continuous variables. The cut-off point which is selected to define 'normal' and 'abnormal' will obviously have a great effect on the sensitivity and specificity of a test. Studies on MCV illustrate the difficulties in comparing research results: increases in MCV have been reported in anywhere between 0% and 90% of alcoholics (Wu et al., 1974; Baglin et al., 1976; Chick et al., 1981; Bernadt et al., 1982; Rosalki, 1984; Skinner et al., 1984; Behrens et al., 1988) and in these studies the upper limit of normal for MCV ranged between 90 and 100 fl.

In general the reference range for laboratory tests is chosen so that the specificity is 95% (i.e. 95% of the population fall within the reference range). Ideally studies examining the performance of markers should use this criterion for the reference range, and so enhance comparability. For screening purposes or in clinical practice it may be appropriate to select another cut-off point, depending on whether it is desirable to maximize sensitivity or to reduce the risk of false positives.

Lack of an objective 'gold standard'

At the present time, a clinical assessment or a diagnostic interview schedule provides the 'gold standard' against which the laboratory test performance can be compared. However, both methods are themselves subject to error or variation from many sources, and this will reduce the apparent strength of the association with laboratory test results. Outside of the laboratory setting, assessment of alcohol intake can only ever provide an estimate of true consumption. For this reason, no test which is examined in a 'real-life' setting is likely to have perfect sensitivity or specificity.

RESEARCH DIRECTIONS

The newer markers of alcohol intake show considerable promise as aids to detection of hazardous alcohol consumption. Several of these markers, and in particular CDT, appear to offer higher sensitivity and specificity than established markers. However, their advantages and deficiencies need to be better delineated. Many of the newer markers have been evaluated using small samples, often by comparison of heavy drinkers against abstainers or very light drinkers. This is a highly artificial situation. Different studies have produced conflicting results. In the case of CDT, studies using different assay techniques have resulted in widely differing estimates of sensitivity, and different cut-off points have been used to define hazardous alcohol intake. Studies using large cohorts and adequately considered clinical criteria and reference ranges for laboratory tests are required. In addition, studies are needed which measure alcohol intake and marker values repeatedly, without intervention between occasions, in order to estimate the importance of variation and measurement error. Methodological improvements, directed especially at reducing the complexity and costs of the newer assays, are also required. The development of successful commercial kits will be important in order to increase their availability.

For both currently available markers and the newer tests, females have been studied less often than males. While there is a lower prevalence of drinking problems in females, it remains important to understand the performance of the tests in this group. In pregnancy it is particularly important to detect hazardous alcohol consumption, and in this setting different reference ranges may be necessary, which take into account altered metabolism.

Several lines of investigation are suggested by recent findings on CDT. It is just one of a range of plasma proteins which are desialylated as a result of excessive alcohol consumption. Examination of the other desialylated proteins may provide a range of markers of alcohol intake, each potentially with a different half-life. Similarly, acetaldehyde may be associated with proteins and cells of differing lifespan, or may form adducts with plasma proteins with differing circulation half-lives. In principle, measurement of bound acetaldehyde could be used to give a profile reflecting drinking over different time periods. For example, it could be possible to retrospectively document a change from high to low level drinking. However, the practical application
of such a profile is some way off. Amongst other issues, it has yet to be determined whether acetaldehyde or other metabolites of alcohol change the half-life of the proteins or cells to be tested.

Not all persons with hazardous alcohol intake, dependence or related problems demonstrate abnormalities in laboratory tests. Research can help clarify which individuals are likely to show changes in these markers and whether those individuals with changes are more likely to experience morbidity related to their drinking (Conigrave et al., 1993).

These markers are not only useful as tools in clinical medicine and treatment evaluation, but also increase our understanding of the biochemical and pathological effects of alcohol. The formation of acetaldehyde adducts with liver proteins, for example, results in the formation of antibodies against these adducts. To what extent are these antibodies released as a result of liver damage, and to what extent are they the cause of immune-mediated damage?

CONCLUSIONS

Research over the past 10 years has revealed several markers with considerable potential for more accurate reflection of recent alcohol intake. These include CDT, β-hexosaminidase, acetaldehyde adducts and 5HTOL:5HIAA. These markers provide hope of more sensitive and specific aids to diagnosis and improved monitoring of intake. They should not only enhance early detection of hazardous alcohol consumption, but also facilitate monitoring of treatment outcomes. Laboratory tests are also particularly useful in settings where cooperativeness is suspect or when a history is not available. In the assessment of drink drivers, for example, a reliable laboratory marker of excessive intake could support or contradict a driver’s claim of light drinking. CDT has already been used in this setting (Gjerde and Mørland, 1987). However, before markers can be routinely used for a medico-legal purpose, they need to have a large body of research behind them, clearly establishing their reliability.

Both established markers and the newer ones have obvious limitations, which should serve as a caution to clinicians and researchers alike. Laboratory tests will never replace clinical judgement in the diagnosis of hazardous alcohol intake or dependence, and cannot provide a broad view of the effects of alcohol on a person’s life. Screening questionnaires in general offer higher sensitivity in detecting hazardous intake in the cooperative patient (Bernadt et al., 1982; Bush et al., 1987; Saunders et al., 1993). However, provided their limitations are clearly understood, laboratory tests provide useful aids in detection and objective monitoring of hazardous alcohol intake and many of its pathological sequelae.

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