

Chapter 12

Diagnostic and Monitoring Investigations

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Synopsis

A number of biochemical or structural changes induced by chronic excessive alcohol intake have been used as biological markers of alcohol dependence or recent consumption. Such markers have been applied to diverse groups, including the general population or high-risk subgroups, patients presenting for treatment without known alcohol-related problems, and patients with known alcohol dependence who have a treatment goal of abstinence or controlled drinking.

The place of biological markers in relation to questionnaires and history-taking, and the points of difference between markers of alcohol use and other diagnostic tests, should be appreciated. Alcohol intake in the population is a continuum and, although risk of such adverse consequences as dependence or physical disease is increased by high levels of consumption, the population cannot be divided into "healthy" and "diseased" groups. Therefore, laboratory tests indicate the probability of current or future problems, rather than providing a diagnosis.

Of the currently available laboratory tests, carbohydrate-deficient transferrin (CDT) offers the best combination of specificity and sensitivity for clinical purposes. Measurement of 5-hydroxytryptophol also has high sensitivity and specificity, but values return to normal within a few hours of completion of ethanol metabolism and it is therefore mainly of research use. γ -Glutamyl transferase (GGT) lacks specificity, being abnormal in liver disease from any cause and also in patients taking enzyme-inducing drugs and in some people with obesity. However, GGT is the only marker of alcohol intake that has been shown in prospective studies to predict mortality and health-care or social security utilization. Erythrocyte mean cell volume (MCV) lacks both sensitivity and specificity, but may still be useful in alerting the doctor to alcohol abuse if a routine haematological profile returns an abnormal MCV with no obvious explanation.

Since the diagnosis will usually be evident in patients with severe alcohol dependence and massive current alcohol intake, the main uses of laboratory tests are to detect lesser degrees of hazardous or harmful use and to monitor abstinence in patients receiving treatment. Sen-

sitivity in detecting hazardous alcohol intake is low, but people with abnormal GGT values might benefit more from intervention. Monitoring abstinence with whichever test was abnormal at presentation or another time of known excessive drinking (usually CDT or GGT) is better than applying the same test to all patients. Detection of the medical complications of alcoholism relies on conventional liver (or other organ) function tests.

Progress is likely to come through further development of markers of risk, and their use in conjunction with questionnaires for the detection of hazardous or harmful alcohol use. Genetic markers of alcohol dependence risk may be discovered within the next 5 years, and their clinical use will raise important practical and ethical issues. Non-genetic markers, of the risk of death or disease among hazardous drinkers, are a more immediate prospect.

This chapter aims to provide a summary of the more useful biological markers of alcohol intake or alcohol-related disease, with information on the sensitivity and specificity of the tests in various situations related to alcohol problems. Where possible, information is given on the biochemical and pathological events leading to test abnormality, and on the prognostic significance of abnormalities. Briefer accounts are given of tests that are less effective or not yet widely available, and of the prospects for tests for genetic susceptibility to alcohol dependence or alcohol-related disease.

This chapter is aimed at clinicians, laboratory scientists and students, and is intended to provide:

1. An improved understanding of the role and limitations of laboratory tests in the assessment and management of people with alcohol-related conditions.
2. A resource for subsequent checking of key facts and for access to the original work on which these facts are based.

LABORATORY INVESTIGATIONS ON THE ASSESSMENT AND MANAGEMENT OF ALCOHOL-RELATED CONDITIONS

There is a substantial literature, dating back many years, on the pathological and biochemical changes that occur in people affected by alcohol dependence or alcohol-related disease. This knowledge can be applied to evaluation of individual patients, with a view to obtaining information which will help in their management. Nevertheless, there is little point in doing investigations for clinical purposes unless there is a clinical question to be answered and some action that may be influenced by the result.

The questions that may occur are, in principle, similar to those that occur in the management of any other physical or mental illness:

- Can a provisional diagnosis be confirmed or ruled out?
- Has the patient's condition improved or deteriorated with the passage of time or in response to treatment?
- Is the person at above-average risk for development of disease or for development of a complication of their existing disease or condition?

In addition, there is a question that needs to be answered in a research or clinical trial setting. This is basically the same as the second question above; have patients (on average) got better or worse after some intervention? Objective evidence of change or improvement is important in all trials, but it is particularly relevant when the main endpoint is behaviour change as reported by the patient or client.

The questions outlined above may occur at a number of points in the course of the disease or condition, for example:

- Before alcohol dependence or hazardous alcohol use commences.
- During periods of excessive alcohol consumption, with or without dependence.
- During treatment, whether the goal is abstinence or controlled drinking.
- After medical consequences of excessive drinking have developed.

DEFINITIONS AND CLASSIFICATIONS

A distinction should be made between a test that indicates current disease, and a test that evaluates the risk of a disease developing in the future. Most clinical laboratory tests are of the former type, in that they are normal before the disease develops and, generally, they revert to normal after the disease resolves. They reflect the current state of the patient and, in the context of markers of alcohol consumption, they have become known as *state markers*.

Other tests, of which cholesterol is probably the most widely known example, give a measure of the probability of some adverse event occurring in future. Variation in these *risk factors* may be due to multiple factors, such as genetic make-up or diet, but although the level of the risk factor and therefore of the risk may vary with time, they are not tied closely to the current state of the patient. In the extreme case of risk associated with a particular allele at a genetic locus, the genotype, and therefore the risk, are set at conception and remain constant (apart from age-related expression of the trait) throughout life. In the context of alcohol dependence, tests that measure risk have become known as *trait markers*; but at present there are few thoroughly validated trait markers for risk of alcohol dependence.

It is therefore possible to classify biological markers related to alcohol in two dimensions; the state/trait difference, and the major stages or events in the natural history of the condition. State and trait markers are potentially applicable to hazardous alcohol consumption, alcohol dependence or organ damage. Markers could exist in each of these six categories, but at present there are no examples of state markers for dependence. (Although dependence in a currently drinking person may be easily detectable, it might be useful to determine whether neurochemical changes associated with dependence are still present and the symptoms of dependence would reappear after any exposure to alcohol.)

For a state marker, it is important to understand the concepts of test *sensitivity* and *specificity* and the relevance of the prevalence of the condition in the population tested. Sensitivity is the proportion of people with the condition who will be detected by the test, while specificity is the proportion of people who do not have the disease who have normal or negative results. These two proportions are interdependent, because one can always improve the sensitivity at the cost of poorer specificity, or vice versa, by changing the cut-off point that defines a normal or abnormal result. For this reason, estimates of test performance should always quote both sensitivity and specificity, and comparisons are easiest if specificity is set at 95% for all the tests being compared or evaluated.

The prevalence of a condition among the population tested, together with the sensitivity and specificity, will determine the proportion of *false positives* and *false negatives* produced by testing.

The sensitivity and specificity should not vary with the prevalence of the condition sought, but they will be affected by the nature of the control and affected groups used in the test evaluations. A control group comprising hospital or laboratory staff will

often lead to higher specificity than can be expected during the application of the test to a mixed group of patients, while a severely affected patient group will produce a higher sensitivity estimate than the mixture of more and less severe cases usually seen in practice. Therefore evaluations of test performance should be assessed with the control and study groups' characteristics in mind.

For a qualitative risk factor marker, the concept of relative risk is more appropriate than sensitivity. One genotype (say the homozygote of the commonest allele) is chosen as the reference point and the increase or decrease in risk of having or developing the condition for each of the other possible genotypes is calculated. For quantitative risk factors, the difference between people in the highest and lowest quintiles, or the difference in risk between the 25th and 75th centiles, is sometimes quoted.

SPECIAL CONSIDERATIONS RELEVANT TO ALCOHOL

Several considerations apply to markers of alcohol consumption or dependence which are absent from the more common applications of laboratory tests:

- In contrast to diseases which are either present or absent, alcohol consumption is common in most countries, and the frequency distribution of alcohol intake is continuous. Many people drink at levels that are either harmless or beneficial; it is only the minority who drink at hazardous levels who may benefit from testing. Furthermore, although there are expert guidelines on what constitutes safe or hazardous drinking, it is very likely that the threshold for alcohol-related harm differs between individuals. What is safe for one person may be harmful for another. This adds to the difficulty of defining who is in the "abnormal" group and who is in the "normal" one.
- Even for dependence, which can be dichotomized, these are quantitative aspects. Some people will have multiple symptoms and frequent relapses, whereas for others alcohol dependence may only be present for a short time and cause comparatively minor problems. Studies based on subjects recruited from clinical sources are likely to show greater differences between the subjects and unaffected controls than studies based on community recruitment. As mentioned above, this will affect statistics such as test sensitivity.
- Because there is no entirely reliable method or "gold standard" for the measurement of alcohol consumption, it is hard to evaluate the absolute sensitivity or specificity of a test. Unreliable measures of the independent variable (alcohol intake) will decrease the correlation with marker results and diminish the separation between two contrasted groups. Only the relative performance of different tests can be compared.
- Ethanol metabolism is rapid compared to that of many other drugs, and essentially complete. The products of this metabolism are carbon dioxide and water, and are indistinguishable from the products of metabolism of foodstuffs or body energy stores. Therefore, it is necessary to use markers of the consequences of alcohol use, which are inherently less reliable than detection of drug metabolites—but which may, paradoxically, be more informative.

For all these reasons, the use of biochemical tests for estimation of alcohol intake has limitations, which should be understood and acknowledged. Besides, alcohol intake is irrelevant apart from its association with risk of problems and alcohol-induced disease; wherever possible, we should concentrate on detecting or predicting the harm that excessive alcohol use can cause. Because validation of markers of long-term harm requires long-term studies, such an approach has been rare.

Conversely, some people benefit from their alcohol intake. On a population basis, there

will be some level of intake at which the chance of an average person benefiting from alcohol is exceeded by the chance of sustaining damage. Some individuals will presumably differ quite widely from this average safe limit, and it might be possible to use biochemical tests to assess both benefit and harm and the balance between them. However, this does not appear to have been done.

It is reasonable to ask whether screening with laboratory tests is a useful activity. Perhaps interviewing techniques, or simple questionnaires, offer a cheaper substitute. Detection of dependence and problems arising from alcohol are more directly approached in this way. However, there are an increasing number of long-term prospective studies to show that testing can have predictive value, and that intervention based on results of laboratory tests can be beneficial. The optimum strategy may be to use a short verbal or written checklist first, followed by laboratory investigations in those found to be at high risk. Nevertheless, it must be appreciated that the group at risk from their drinking is wider than just the group meeting diagnostic criteria for dependence.

BIOCHEMICAL CONSEQUENCES OF ALCOHOL USE

In this section, we consider analytes whose mean values change in 'alcoholics' and which have been proposed as markers of alcohol intake. It is not necessary to consider the large number of tests where mean values can be shown to be affected by alcohol; for a diagnostic test it is necessary to have a minimum overlap between the frequency distributions for the affected and unaffected groups.

Potentially there are three groups of interest, which unfortunately have no absolute boundaries; control subjects or safe drinkers, hazardous drinkers, and dependent drinkers. It is necessary to distinguish between studies that have contrasted actively drinking alcoholics and control subjects, and those that have contrasted subjects believed to be taking safe quantities of alcohol and subjects taking hazardous amounts. For each test we will consider:

- Reference ranges and any factors affecting them.
- Test performance, including any sex and age effects on marker effectiveness.
- Information relevant to test interpretation.
- Prognostic value.

These topics have previously been reviewed by Goldberg & Kapur (1994), Conigrave et al. (1995), Sharpe et al. (1996) and, in part, by Stibler (1991).

γ -Glutamyl Transferase (GGT)

GGT is an enzyme with poorly understood physiological functions, but which seems to be associated with amino acid transport at cell membranes, and with glutathione uptake. It is easily measured in serum or plasma using chromogenic substrates, and inexpensive methods are available for automated photometric analysers and for some near-patient testing systems.

Reference Range

Most laboratories and textbooks quote a reference range up to 55 u/l at 37°C for men and 40 u/l for women. Our own unpublished data show that the 95th centiles of the GGT frequency distribution for people reporting consumption of up to 70 g alcohol in the past week

are 66 for men and 44 for women. Because of the effects of alcohol and of obesity on GGT, the reference range depends on the degree to which people with these characteristics are excluded.

Test Performance

The specificity of GGT as an alcohol intake marker is diminished by its abnormal values in many kinds of liver disease (including the increasingly common chronic hepatitis of viral origin), its induction by microsomal enzyme-inducing drugs (Rosalki et al., 1971), and increases in mean value associated with obesity (van Barneveld et al., 1989) and non-insulin-dependent diabetes (Barbieux et al., 1990). In the general population, lack of specificity is less of a problem than in a hospital environment.

Sensitivity, like specificity and the reference range, depends on the definition of the group studied. For dependent or alcoholic patients, sensitivity is in the range 60–90% (Conigrave et al., 1995). For people drinking hazardous amounts of alcohol but not known to be dependent, sensitivity values of 20–50% have been found. Interpretation of these values is made more difficult by the wide range of specificities reported (55–100%).

Test Interpretation

The limited sensitivity of GGT for detecting hazardous drinking has the corollary that some hazardous and heavy drinkers have an elevated GGT, while others do not, as can be seen in Figure 12.1 (adapted from Whitfield et al., 1978). The range of GGT results seen in the sampled population becomes greater as alcohol intake increases. Because the physiological function of GGT is uncertain, we cannot define the underlying difference between a heavy drinker with a normal GGT and one with an elevated GGT, but we can look for other differences between these groups and for differences in prognosis.

First, subjects with raised GGT are more likely to show abnormalities in the liver function tests, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Whitfield et al., 1981; Whitfield and Martin, 1985a). This is consistent with each test responding to alcohol-induced liver damage, and indeed, it has been shown that alcoholics with raised GGT are more likely to have evidence of liver steatosis, fibrosis or cirrhosis on liver biopsy (Wu, Slavin and Levy, 1976; Kryszewski et al., 1977; Frezza et al., 1989). For reasons that are not yet understood, subjects with excessive alcohol consumption and raised GGT are also likely to have increased serum triglycerides and urate (Whitfield et al., 1981), and increased blood pressure (Henningesen et al., 1980). Second, several groups have shown that subjects with raised GGT have an increased incidence of mortality or morbidity in prospective studies.

Prognostic Value

A number of independent studies have shown that an increased GGT is a predictor of increased mortality, morbidity or use of health and social security resources. Some studies have taken into account variations in alcohol use, obesity, or other risk factors and shown that the effect of GGT is independent of these. Some studies have classified causes of death or types of illness, e.g. into “alcohol-related deaths”, cardiovascular deaths and deaths from cancer. The overall picture is that GGT is a significant risk factor, but it is not so clear that it only predicts “alcohol-related” death.

The earliest studies were performed in Malmö, Sweden, from 1974 onwards (Kristenson et al., 1980; Peterson et al., 1980; Kristenson et al., 1983; Trelle, Kristenson & Petersson, 1985; Hood et al., 1990). Men aged in the 40s were screened for a number of health-related

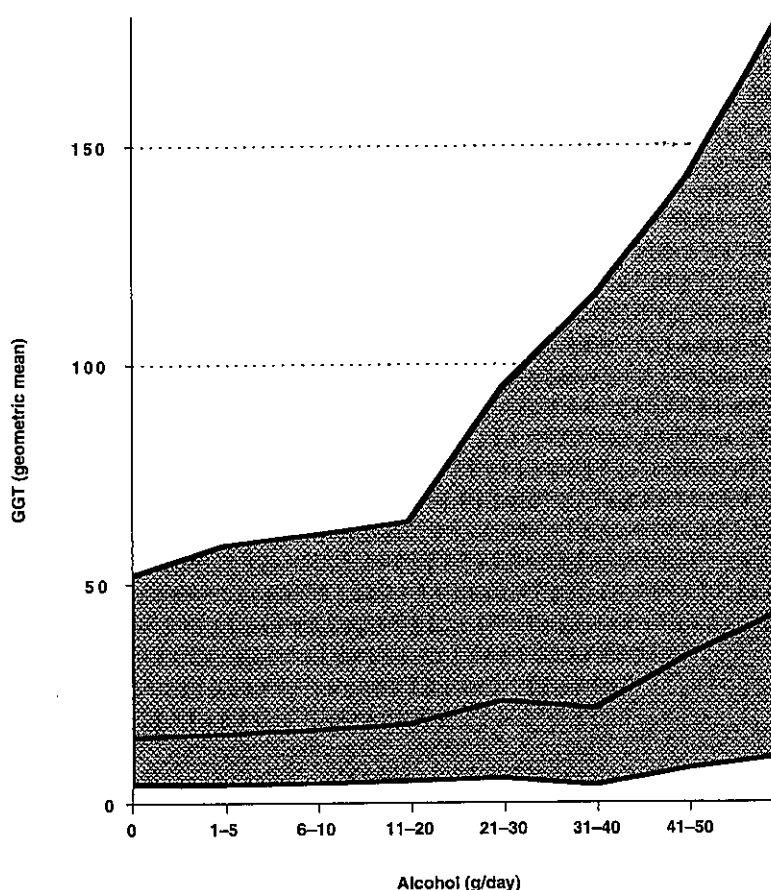


Figure 12.1 Geometric mean and 95% confidence intervals for GGT by self-reported average daily alcohol intake in men. Note that the variance of the GGT distribution increases progressively above 20g alcohol/day. Data from Whitfield et al. (1978)

variables, and those with a GGT result in the top decile were investigated further and, if appropriate, enrolled in an intervention trial that aimed to reduce drinking. Participants were followed-up after the screening process and a strong association between high GGT and mortality was established (see Figure 12.2). High GGT was also a good predictor of alcohol-related admissions to hospital, orthopaedic hospitalization, and other events that would be expected to occur more frequently in heavy drinkers. It was also, unexpectedly, a predictor of coronary heart disease.

Investigation of men admitted to hospital in Helsinki, Finland, following injury (Antti-Poikka & Karaharju, 1988) revealed that high GGT among heavy drinkers was a predictor of increased complications and longer hospital stay.

A study conducted in Sydney, Australia (Conigrave et al., 1993), also assessed GGT results as a predictor of mortality and healthcare utilization. Again, significant results were obtained and it could be shown that this was not solely due to GGT acting as a marker of alcohol intake; a high GGT adds information to that obtained from an estimate of alcohol consumption.

At least three other studies, in Germany, Japan and England, have investigated the relationship between cardiovascular risk and GGT. The Japanese study (Miura et al., 1994) showed a high GGT to be a predictor of the development of hypertension; the English study (Wannamethee et al., 1995) found an increased risk of ischaemic heart disease and deaths from all causes; and the German study (Brenner et al., 1997; Arndt et al., 1998) showed increased all-cause mortality and retirement from work due to disability in men with high GGT.

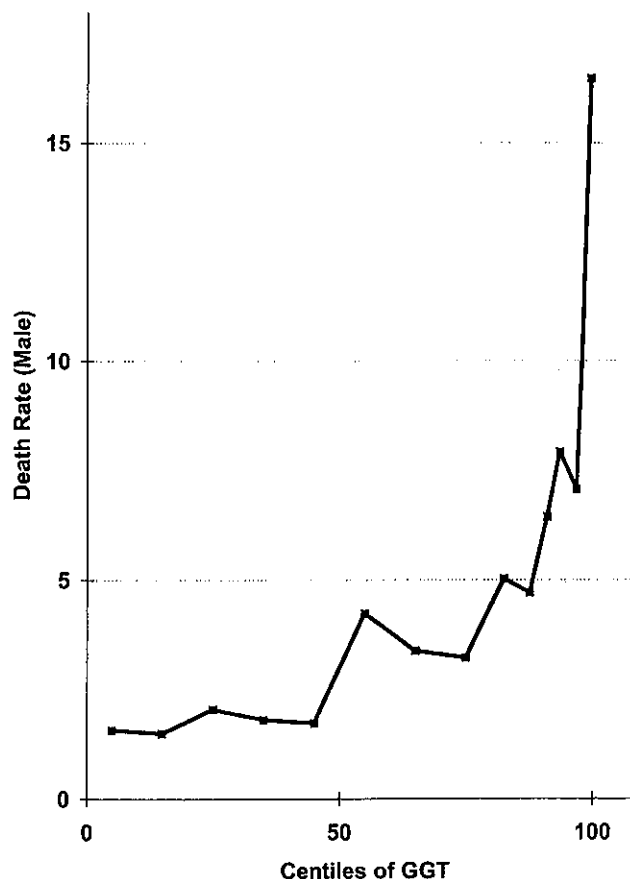


Figure 12.2 Deaths from all causes except cancer during 5–11 years follow-up of middle-aged men in Sweden, by centiles of GGT at screening. (Modified from Hood et al., 1990)

The exact relationships between elevated GGT, alcohol intake, coronary heart disease, alcoholic liver disease and all-cause mortality are uncertain. Nevertheless, it is clear that an alcohol-dependent person or a hazardous drinker with a high GGT has a worse prognosis than one with a normal GGT value. One must also conclude that drinkers with high GGT are not among the people who improve their cardiovascular risk through their alcohol use.

Erythrocyte Mean Cell Volume (MCV)

The size of erythrocytes is affected by genetic factors (Whitfield and Martin, 1985b) and also by a number of anaemias caused by deficiency of iron, folate and vitamin B₁₂. Alcoholism may cause both anaemia and macrocytosis, but the recognition that the mean cell volume was increased in a high proportion of people consuming large amounts of alcohol came after technical advances made it possible to measure MCV with much greater precision. The cause of this alcohol-related macrocytosis is unknown; folate deficiency or altered folate metabolism may play a role, but folate supplementation will not necessarily normalize MCV if drinking continues.

Reference Range

Ranges for MCV by automated haematology analysers vary, with values quoted as 83–98 fl (Rodger et al., 1987) or 82–95 fl (Davidson and Hamilton, 1978). Papers evaluating

MCV as a marker of excessive alcohol intake have generally set an upper limit of normal of 94 or 95 fl and have found that specificity at this cut-off point is around 90% (Bell et al., 1994; Wickramasinghe et al., 1994; Yersin et al., 1995).

Test Performance

The specificity of MCV for alcohol is affected by the population studied, and in particular by the presence of patients with megaloblastic anaemias. The review by Conigrave et al. (1995) found a range of sensitivities of 40–50% for “alcoholics” (alcohol-dependent subjects in treatment) and 20–30% for subjects with hazardous alcohol consumption. Results should be interpreted with knowledge of the subjects’ smoking status, which also increases MCV (Whitehead et al., 1995), but also with the knowledge that smoking and alcohol dependence are strongly associated.

Test Interpretation

The lack of knowledge about the cause of increased MCV in people with excessive alcohol consumption makes it difficult to assess its significance. Although there is experimental evidence that folate absorption and metabolism can be changed by alcohol (Blocker & Thenen, 1987), and some alcoholics will have low folate intake and definite folate deficiency, effects of alcohol on erythrocyte size through other mechanisms are thought to exist.

Prognostic Value

There is no information on relationships between MCV values and prognosis.

Glycoproteins

Chronic excessive alcohol consumption brings about a number of changes to plasma glycoproteins, particularly transferrin. For reasons which are not entirely clear, the terminal residues on the carbohydrate side-chains of transferrin are more often absent and, because the usual terminal carbohydrate residue is sialic acid, this leads to a change in charge and isoelectric point of the protein.

Animal experiments have provided evidence that both post-translational modification of transferrin is incomplete and normal hepatic uptake of asialoglycoproteins is impaired after chronic alcohol administration (Xin et al., 1995). Taken together, these hepatic changes increase the circulating concentration of carbohydrate-deficient transferrin (CDT).

Isotransferrins (varying in sialic acid content) can be separated by isoelectric focusing or by ion-exchange chromatography, and their concentration can be measured by immunoblotting and densitometry or by immunoassay. These are standard laboratory techniques but comparatively time-consuming and therefore expensive. Commercially available methods are also expensive.

Reference Range

The reference ranges depend on the method used and on the reference base for the result (by volume of serum, or as a proportion of total transferrin). Values are higher in women than men, and higher in younger (presumably premenopausal) women than in women aged over 50 (Whitfield et al., 1998). There have also been reports of higher values in pregnant

than in non-pregnant women (Godsell et al., 1995; Stauber et al., 1996). Some of this variation may be due to variation in serum total transferrin.

By the commonest method, based on anion exchange and transferrin immunoassay, the reference ranges are up to 20 u/l for men and 30 u/l for women. Older methods based on anion exchange gave reference ranges up to 80 or 100 mg/l because more of the trisialo-transferrin was included in the "carbohydrate-deficient" fraction. With methods that express results as a percentage of total transferrin, the reference ranges also vary according to the mixture of isotransferrins included; up to 2.5% for the %CDT RIA and up to 6% for the %CDT TIA, for both men and women.

Test Performance

Specificity for alcohol is better than older tests such as GGT or MCV, but CDT is abnormal in patients with some non-alcoholic forms of liver disease (Bell et al., 1993) and in a group of rare carbohydrate-deficient glycoprotein disorders (Stibler et al., 1998) and galactosaemia (Stibler et al., 1997). CDT, as measured by column anion-exchange methods, can be affected by uncommon variants of the transferrin protein, which vary in isoelectric point (Stibler et al., 1988).

The reported sensitivity of CDT in currently drinking alcohol-dependent subjects is 65–95%, and for hazardous alcohol intake 25–60% (Conigrave et al., 1995).

Test Interpretation

The reasons for variation in the sensitivity of CDT for alcohol abuse have recently become clearer. Initial reports were that subjects with insulin resistance or hypertension (Fagerberg et al., 1994a, b) were likely to show false negatives (poorer sensitivity). These results were confirmed in further studies (Whitfield et al., 1998) and effects of smoking, past alcohol dependence, obesity and dyslipidaemia and were also found, at least among subjects with hazardous alcohol use. Non-smokers showed substantially lower sensitivity of CDT for hazardous alcohol consumption, while obesity/high triglyceride/low HDL were also associated with lower sensitivity.

Prognostic Value

There is no information on the value of CDT in predicting morbidity or mortality in alcoholics or hazardous drinkers. In view of the reports of association between dyslipidaemia, hypertension and insulin resistance and a diminished CDT response to alcohol, it is likely that a raised CDT has no adverse implications, except as an indicator of excessive alcohol intake.

Acetaldehyde-Protein Adducts and Antibodies

Ethanol is metabolized to acetaldehyde by alcohol dehydrogenase, followed by conversion to acetate by aldehyde dehydrogenase. Acetaldehyde concentrations in the blood during ethanol metabolism are normally very low (in the micromolar range or below). Acetaldehyde can react with free amino groups in proteins to produce acetaldehyde-protein adducts, by a mechanism analogous to the formation of glycoproteins by glucose (Braun et al., 1997).

Measurement of such adducts as markers of alcohol intake is based on the premise that the more alcohol is ingested, the greater the time during which acetaldehyde is present

and the greater the degree to which reactions between acetaldehyde and proteins will occur. The reaction is believed to occur in two steps, the second of which is irreversible, so that the proteins retain their modified form for their lifetime in the circulation. In principle, any protein may become modified but most studies have focused on haemoglobin because of its high concentration in the blood.

These reactions were first demonstrated *in vitro* using unrealistically high concentrations of acetaldehyde, but there is now substantial evidence for the production of such adducts *in vivo* during alcohol metabolism, and for their persistence in the circulation after ethanol elimination is complete.

Because the chemical modification of the proteins changes their antigenic properties, antibodies to them are formed, and so the presence of either the modified protein or of the antibody may be used as a marker of high alcohol intake. Measurement of the modified protein has been by enzyme immunoassay, with antibodies directed against the acetaldehyde-modified epitopes on proteins (Worrall et al., 1998) or by ion exchange separation of modified haemoglobins (Sillanaukee et al., 1991; Chen et al., 1995). Measurements of the antibodies may distinguish between different classes of immunoglobulins (Viitala et al., 1997).

Reference Range

Ranges, and units of measurement, vary with the method used.

Test Performance

There appears to be no information on specificity, although industrial exposure or smoking might lead to formation of acetaldehyde-protein adducts. Sensitivity has been assessed in a small number of studies using differing analytical approaches (Sillanaukee et al., 1991, 1992; Lin et al., 1993; Worrall et al., 1996; Hazelett et al., 1998; Hurme et al., 1998), with results varying between 20% and 80%.

Test Interpretation

Since this test has not been widely used, there is little information on factors that might modify the results or their interpretation. The assumption is that this test would give an index of "ethanolic control" in the same way that glycosylated proteins give an index of glycaemic control in diabetics; a time-integrated measure of blood ethanol (in fact, blood acetaldehyde) concentrations. However, since acetaldehyde modification of proteins may have a role in the pathogenesis of alcohol-related organ damage, some interpretation as a risk factor may become possible.

Prognostic Value

There is no direct evidence that measurement of adducts or antibodies to them has value in the prediction of alcoholic liver disease or other consequences of alcohol use. However, they do appear to have significance in the pathogenesis of liver disease, because experimental animals treated with ethanol and immunized with acetaldehyde-modified proteins show more liver damage than animals treated with ethanol alone (Yokoyama et al., 1995). Moreover, alcoholic patients with ALDH deficiency, who will be more likely to form acetaldehyde-protein adducts, have a higher risk of alcoholic liver disease (Enomoto et al., 1991). There has been one report that pregnant women with increased acetaldehyde-haemoglobin adducts were more likely to give birth to babies affected by

fetal alcohol syndrome (Niemela, Halmesmaki & Ylikorkala, 1991), and another that antibodies to acetaldehyde-modified cardiac cytosolic proteins are detectable in alcoholic heart disease (Harcombe et al., 1995). Further prognostic studies of acetaldehyde adducts or antibodies to them are warranted.

Serotonin Metabolites

The actions of both alcohol dehydrogenase and aldehyde dehydrogenase lead to conversion of NAD to NADH in hepatocytes, and a consequent change in the equilibrium between any pair of compounds interconverted by an NAD-dependent dehydrogenase enzyme. Serotonin (5-hydroxytryptamine, 5-HT) is normally metabolized via an aldehyde intermediate to the carboxylic acid, 5-hydroxyindoleacetic acid (5-HIAA), with traces of 5-hydroxytryptophol (5-HTOL). The change in NADH:NAD ratio during ethanol metabolism leads to a relative increase in formation and excretion of 5-HTOL and an increase in the urine 5-HTOL:5-HIAA ratio (Voltaire et al., 1992). Being based on the effects of ethanol metabolism, this is a comparatively short-term marker, but abnormalities in the urine ratio persist for some hours after ethanol elimination is complete. The test has mainly been applied to intensive surveillance of patients in outpatient treatment programmes in Sweden, and to validation of other biological markers in patients under treatment.

Measurement of the 5-HTOL:5-HIAA ratio is performed using gas chromatography-mass spectrometry, which is now fairly widely available but still expensive for a routine test.

Reference Range

Values of 4–20 pmol 5-HTOL/nmol 5-HIAA were reported by Voltaire et al. (1992).

Test Performance

Because this test depends on a metabolic consequence of alcohol use, it would be expected that all occasions of alcohol consumption would increase the 5-HTOL:5-HIAA ratio within any individual subject. Sensitivity of 90% for men and 60% for women was reported by Helander et al. (1996a) for as little as 7g alcohol. When the ratio is used, foods containing 5-hydroxyindoles do not interfere and polymorphisms in alcohol and aldehyde dehydrogenases have no effect. However, ALDH inhibitors increase the ratio.

Test Interpretation

Results must be interpreted in the light of information on the time since drinking may have taken place; if testing is infrequent, then it will be difficult to assess the significance of normal results.

Prognostic Value

There is no information on relationships between 5-HTOL values and prognosis.

Alcohols and Alcohol Metabolites

Blood, plasma and urine ethanol concentrations rise after consumption of alcoholic beverages, and both the concentration and the time for which detectable amounts persist is

proportional to the ethanol intake. Ethanol is easily measured in blood or breath but, because most people consume alcohol from time to time, a positive result is of little value unless the subject denies taking alcohol. Presence of alcohol in the morning, or during a medical consultation, might be thought unusual and worthy of further investigation but there appears to be no data on this.

Tolerance to ethanol develops in people who habitually consume large amounts, and this takes two forms; metabolic tolerance (in which ethanol is metabolized more quickly than normal, possibly because of induction of hepatic microsomal systems) and neurological tolerance (in which symptoms or signs of intoxication are less than would normally occur at the blood ethanol concentration present). Neurological tolerance allows very high blood ethanol concentrations to occur while the patient remains conscious and this is a classical clinical indication of alcohol dependence.

Because of metabolic tolerance, ethanol metabolism is faster and concentrations of ethanol metabolites (particularly acetate) during ethanol metabolism are higher in alcoholics than in normal subjects (Olsen et al., 1989; Roine et al., 1988; Girela et al., 1994). Each of these has been proposed as a marker of excessive alcohol intake, but they suffer from the disadvantage of requiring alcohol to be administered or else performing the test while the subject is intoxicated. The main role for such tests would be after emergency admission to hospital. Although the principle has been validated, and Girela et al. (1994) reported sensitivity of acetate to be comparable to that of GGT or MCV, there are few studies on the practicality or effectiveness of such testing.

Methanol is present in small amounts in alcoholic beverages and may also be produced by gut flora. It is normally metabolized by alcohol dehydrogenase and, because ethanol is the preferred substrate for this enzyme, methanol metabolism is reduced after ethanol consumption. This has led to proposals for the measurement of methanol as a marker of alcohol intake but available data have not made the case for this (Roine et al., 1989; Buchholtz, 1993; Haffner et al., 1993; Helander et al., 1996a). In principle, methanol should only be detectable while ethanol is also present and for a short time afterwards.

Because ethanol distributes to all body fluids and tissues, measurement of ethanol in sweat has been exploited through the "patch test". An absorbent patch is attached to the skin of a subject and the ethanol content of the collected sweat is measured after 24 or 48 hours (Phillips and McAloon, 1980; Phillips, 1984; Phillips et al., 1995). Adhesive type, which reveals evidence of removal and re-application, helps to avoid the more obvious forms of deceit. A similar device based on a sensor which measures transdermal ethanol diffusion has also been developed (Swift et al., 1992). This approach may be the closest to a "gold standard" that is available. An accurate method of measuring alcohol intake in free-living subjects over a significant period of time would be valuable because it could be used to calibrate other methods of estimation, such as laboratory tests, but it would be difficult to recruit and retain sufficient heavy-drinking volunteers for such studies.

Fatty acid ethyl esters are formed enzymatically in the presence of ethanol. A small number of investigations have been conducted, mainly using tissue samples in experimental animals or post-mortem samples from humans. Such esters have been detected in human blood for up to 24 hours after consumption of ethanol (Doyle et al., 1994, 1996). Another minor metabolite of ethanol, ethyl glucuronide, has recently been found to persist for a few hours after ethanol elimination is complete (Schmitt et al., 1997; Wurst et al., 1999) and may be useful in a similar way to the measurement of 5-hydroxytryptophol.

Test Performance

There are few reports of evaluations of sensitivity and specificity of tests for ethanol or its metabolites, or other alcohols. Consequently, no summary of test performance can be given.

Test Interpretation

Results of test in this class will need to be interpreted in relation to the time of ethanol consumption, because false negatives will occur when, or soon after, ethanol elimination is complete.

Prognostic Value

There are no formal evaluations of prognostic value for any of this group of tests. Fatty acid ethyl esters are believed to be toxic and might play a role in organ damage (Laposata, 1998), but whether they have an important role in alcohol-related disease is still uncertain.

PRECURSORS OF HARMFUL ALCOHOL USE

There has long been a clinical impression that alcoholism runs in families. Adoption and twin studies have now shown that genetic factors account for most, if not all, of this family resemblance. The effect is reasonably strong; a large study of families in the USA (Dawson et al., 1992) estimated that life-time alcoholism risk was increased by 167% by having both first- and second-degree relatives with alcoholism, and in Australia, Heath et al. (1997) found that having an identical twin who was affected increased alcoholism risk around four-fold.

As a consequence of this evidence for genetic factors in alcoholism, many investigators have embarked upon the search for "genes for alcoholism" (more accurately, alleles for alcoholism). The main lesson learned so far is the need to reserve judgement until a number of independent reports have come to the same conclusions.

Genetic variation in the two major enzymes of ethanol metabolism, alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH), has been shown to affect alcohol dependence risk. There is also some evidence that *ADH* variation affects the risk of alcoholic liver disease among patients who are alcohol dependent. However, the polymorphism of *ALDH2* occurs only in Asians and the polymorphism in *ADH2* that affects alcohol use is present in only a small proportion (less than 10%) of Caucasian subjects. Although these enzyme variants are of considerable theoretical interest, showing some connection between alcohol metabolism and alcohol dependence, they do not have predictive or diagnostic value in the ethnic groups most prone to alcohol dependence.

Because of the role of dopamine in neurotransmission and its postulated role in reward and dependence mechanisms, genetic variants of dopamine receptors, such as DRD2 and DRD4, have been investigated for associations with alcohol dependence. This has been a particularly controversial and contradictory area of research but it can reasonably be said that these genetic markers have no role in the diagnosis or management of alcohol problems.

Two enzymes have been claimed to be markers of genetic susceptibility to alcohol dependence. The most widely studied one, monoamine oxidase, now seems to be a state marker of smoking and its apparent role as a trait marker for alcohol dependence is due to the strong association between smoking and alcohol (Anthenelli et al., 1998). The other, adenylyl cyclase, has been less widely studied but appears to be a trait marker for alcohol dependence when measured after several days' abstinence, and may also have associations with depression (Menninger et al., 1998).

A comprehensive strategy for detecting genes affecting alcoholism risk requires a large number of subjects, related to each other in some way so that linkage analysis can be used, and results from around 300 genetic markers on each subject. So far, this approach has yielded suggestive, but not conclusive, results, which need replication and further refinement (Foroud et al., 1998; Long et al., 1998; Reich et al., 1998). Diagnostic applications

appear some years away and may require a profile of gene typings to build up an assessment of risk. This would be impractical at present but is likely to become technically feasible within such a time-frame. Genetic markers may be found that indicate subtypes of alcoholism, or likely co-morbidity from other psychiatric problems, or possibly susceptibility to specific types of alcohol-induced organ damage.

CHANGES EXPECTED DURING ABSTINENCE AND AFTER RELAPSE

By definition, state markers should return to normal when the abnormal condition has resolved. Some markers may remain elevated if liver damage is irreversible, and use of markers for characterization of patients as abstinent or relapsing is limited by the sensitivity of the tests since some patients will never show abnormal results.

The practicality of using tests as relapse markers will also depend on the half-life of their return to normal. As 5-hydroxytryptophol only remains abnormal for a few hours after drinking, most attention has been paid to GGT and CDT. MCV is very slow to return to normal, and there is no information on acetaldehyde-modified proteins in this role.

Over the past 20 years, at least 13 papers have explored the use of GGT and/or CDT as markers of abstinence or relapse. Earlier studies demonstrated a decline with abstinence, while more recent ones have concentrated more on identification of individual bouts of drinking. The topic has been reviewed by Borg (1996).

Initial papers reported that GGT decreased with abstinence in alcoholics, with a half-life of around 25 days. Some of these papers (Shaw et al., 1979; Montiero & Masur, 1986) found no decrease in MCV in the same patients. More recent papers have compared GGT and CDT directly; CDT decreases more quickly than GGT, with a half-life around 15 days. Nearly all these papers found CDT to be better (e.g. Helander et al., 1996b; Schmidt et al., 1997), but several recommended use of either as appropriate because not all patients had raised CDT. It became clear that even patients with normal CDT or GGT could show decreases with abstinence, and an individual reference range could be more useful than a universal one (Weill et al., 1988; Borg et al., 1995). The more recent papers have identified individual occasions of relapse by use of 5-HTOL and evaluated other markers by reference to this (Borg et al., 1995).

Ultimately, the use of test results in managing the treatment of alcohol-dependent patients will need to be justified by controlled trials measuring outcomes with and without test results.

EVALUATION OF ALCOHOL-RELATED DISEASE

The use of laboratory tests to detect and evaluate organ damage resulting from alcohol abuse is no different in principle from their use for other diseases. In many cases the tests are not specific for alcohol-related damage. The organs most often affected are the liver, heart, pancreas and brain and there are some important but rare vitamin deficiencies that can be investigated.

Liver Function Tests, and Markers of Fibrosis and Cirrhosis

Mild degrees of liver abnormality, such as fatty liver, produce no changes in serum markers beyond the increases in GGT and aminotransferases that may be present in any heavy

drinker. Alcoholic hepatitis will produce substantial increases in AST and ALT and, of course, bilirubin.

Cirrhosis or fibrosis are generally biochemically silent until very advanced stages are reached, but a number of markers of fibrosis have been advocated and evaluated. These are based on collagen metabolism and do show statistical associations with the presence or progress of fibrosis, but may not be reliable in the evaluation of individual patients. Fibrosis markers were reviewed by Plebani & Burlina (1991), and since that time more than a dozen reports have investigated single or multiple markers for fibrosis or cirrhosis in general, or alcoholic cirrhosis in particular. Tsutsumi et al. (1996) compared six assays in patients with alcoholic and non-alcoholic liver disease and found that five of them reflected the histologically assessed degree of fibrosis, with the triple-helix domain of Type IV collagen showing the most significant results in alcoholics. Values for this marker were higher in alcoholics, for any degree of fibrosis, than in non-alcoholic liver disease, and decreased to normal over eight weeks of abstinence from alcohol. This suggests that it is a marker of collagen turnover and active fibrosis rather than a marker of accumulated damage.

Hepatocellular carcinoma (HCC) is a serious complication of cirrhosis and might be detectable through measurement of α -fetoprotein (AFP) in serum. However, there are a number of obstacles to such screening programmes (see Khakoo et al., 1996). First, the increased risk is associated with cirrhosis and not alcohol *per se*, so defining the high-risk group encounters the difficulty of deciding which drinkers have cirrhosis. Second, treatable (small) tumours do not always produce elevated serum AFP values, particularly when the reference range is adjusted to allow for increased AFP in liver disease. Third, evidence so far suggests that ultrasound screening is more sensitive and specific than AFP screening, although combinations could be useful.

Other Complications of Alcohol Abuse

Thiamine deficiency may be detectable by measurement of erythrocyte transketolase activity (ETKA) in the absence and presence of added thiamine pyrophosphate. This requires a blood sample before any supplementation of the patient with thiamine is undertaken. The previous view, that alcoholics who become thiamine-deficient have a genetically abnormal form of transketolase, has not been confirmed.

Pancreatitis may arise from alcohol abuse. If the diagnostic question is whether pancreatitis is present, then amylase and lipase are used. If the question is whether the pancreatitis is alcoholic in origin, then CDT is reported to be the best test, with GGT abnormal regardless of aetiology. Some authors have suggested that amylase is less elevated in alcoholic than non-alcoholic pancreatitis and therefore that the lipase:amylase ratio might be relevant, but this is not generally felt to be useful.

Muscle damage may occur in alcoholics, and measurement of creatine kinase may be useful. For other complications, such as cardiomyopathy or brain damage, laboratory tests have little to offer at present.

LABORATORY TESTS IN CLINICAL TRIALS

A number of treatments have been proposed or instituted for alcohol dependence. Whether these are based on psychological or pharmacological sciences, resource allocation considerations require that they should be evaluated and compared; and ethical considerations require that the most effective treatment should be offered. Such evaluations need to define desired outcomes, which may be abstinence, controlled drinking or reduction in harmful

effects of alcohol. If self-report of alcohol use is taken as a measure of treatment effectiveness, then optimism and bias may lead to incorrect conclusions but biological markers should be more objective.

As discussed earlier, application of biological markers to individual patients has limitations because of poor sensitivity or specificity. However, when the object is to compare two or more groups of people, then the individual differences in marker response to alcohol intake will become less important and differences between treatment groups will be readily detected. This approach has been applied to a number of trials of pharmacotherapy for alcohol dependence, of treatment based on counselling or combinations of each (Kristenson et al., 1983; Persson and Magnusson, 1989; Martinez Ruiz et al., 1995; Anton, Moak and Latham, 1996; O'Connor et al., 1997; Wilde and Wagstaffe, 1997).

CONCLUSIONS: WHAT INVESTIGATIONS, WHEN, AND IS THERE EVIDENCE OF EFFECTIVENESS?

A summary of the usefulness of biological markers in various clinical situations is attempted in Table 12.1. In each case, except for genetic markers of susceptibility, biological markers can presently provide useful information to be considered alongside clinical and interview data. The more expensive tests cannot be justified for universal application, but may play a role in particularly critical situations where alcohol use is denied and where public safety may be at risk if the patient successfully deceives his/her doctor. Continuing research using the more expensive tests is justified because a major clinical benefit from any of them would increase utilization and lead to development of cheaper methods.

Future research on biological markers may be most productive where it focuses on risk. The course of alcohol dependence and the development of complications is variable and some of the tests discussed (particularly GGT) have been shown to have predictive value. Other markers may also have this useful property, particularly acetaldehyde-modified proteins and fatty acid ethyl esters, and these, too, should be investigated in prospective studies.

Variation in risk of alcohol dependence may soon be assessable through use of genetic markers. This will undoubtedly raise ethical issues, but the same issues are already confronting clinical geneticists dealing with many diseases, and will become significant for many psychiatric conditions apart from alcohol dependence. A great deal of work will be required in order to understand the pathophysiology of the tests and the disease and to delineate the genotype-phenotype relationships with multiple genetic markers. As with state markers, the best test of whether genetic markers have clinical utility will be the effect on outcomes for the patients.

KEY WORKS AND SUGGESTIONS FOR FURTHER READING

Borg, S., Helander, A., Voltaire Carlsson, A. & Hogstrom Brandt, A.M. (1995). Detection of relapses in alcohol-dependent patients using carbohydrate-deficient transferrin: improvement with individualized reference levels during long-term monitoring. *Alcoholism: Clinical and Experimental Research*, **19**, 961-963.

This paper investigated one of the major uses for biological markers of alcohol intake, in the detection of continuing alcohol consumption in patients under treatment.

Table 12.1 Summary of the role of biological makers in various clinical situations

Phase	Question	Tests	Status
Prior to alcohol use or development of problems	Is this person at high risk for alcohol-related problems?	Genetic markers of susceptibility	None yet confirmed, except ALDH2 and ADH2 (only relevant in some populations). Others tentative and unlikely to become useful for several years
Drinking but no definite alcohol-related diagnosis	Is this person currently drinking excessive amounts of alcohol?	State markers: CDT and GGT	Well-documented test characteristics, less than 100% sensitivity. GGT may have predictive value. CDT has better specificity and possibly sensitivity, but is expensive
During treatment for alcohol dependence	Has the patient ceased drinking, or controlled his/her drinking to safe levels?	State markers: CDT and 5-HTOL	Both responsive to reduction in alcohol use and to its resumption; 5-HTOL short-term and CDT medium-term; both expensive tests
Alcohol dependence with continuing excessive drinking	Is there evidence of organ damage due to alcohol?	Organ damage markers	GGT and other LFTs; possibly amylase, CK, thiamine, depending on organ affected

Relapses were monitored by a combination of self-report (three times a week) and urine 5-HTOL (daily). The use of individual rather than collective reference ranges for CDT approximately doubled the number of relapses detected, and around 80% of these were verified by self-report or 5-HTOL.

Brenner, H., Rothebacher, D., Arndt, V., Schuberth, S., Fraisse, E. & Fliedner, T.M. (1997). Distribution, determinants and prognostic value of γ -glutamyl transferase for all-cause mortality in a cohort of construction workers from southern Germany. *Preventive Medicine*, **26**, 305–310.

This study covers sources of variation in, and predictive value of, GGT measurement in a large cohort of men from the working population. As expected, alcohol consumption, body mass index and hypertension had the greatest effect on serum GGT. However, GGT had predictive value which was independent of these variables, suggesting that it could be used to identify individuals or groups who are at highest risk.

Kristenson, H., Ohlin, H., Hulten-Nosslin, M.B., Trelle, E. & Hood, B. (1983). Identification and intervention of heavy drinking in middle-aged men: results and follow-up of 24–60

months of long-term study with randomized controls. *Alcoholism: Clinical and Experimental Research*, **7**, 203–209.

This is one of a series of valuable papers from the Malmö group, who investigated the importance of alcohol and the role of biological markers in an integrated public health screening project. Although it addresses the broad issues of early intervention, subjects were randomized to intervention or control groups after screening based on GGT results. Discussion of changes in GGT during treatment was used as a means of reinforcing reduced alcohol consumption in the intervention group.

Stibler, H. (1991). Carbohydrate-deficient transferrin in serum: a new marker of potentially harmful alcohol consumption reviewed. *Clinical Chemistry*, **37**, 2029–2037.

The author played a major role in the development of carbohydrate-deficient transferrin as a marker of alcohol intake. In this review she summarized the methods used and the characteristics and value of this test. Sensitivity and specificity data from 21 studies are tabulated and the causes of false positives are discussed. Although the paper was published some time ago, when the available data were mainly from severely affected currently drinking alcohol-dependent subjects, it still provides a good starting point.

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