Biomarkers of Heavy Drinking

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In recent years significant advances have been made in biological assessment of heavy drinking. These advances include development of new laboratory tests, formulation of algorithms to combine results on multiple measures, and more extensive applications of biomarkers in alcoholism treatment and research.

Biomarkers differ from the psychometric measures discussed in other chapters of this *Guide* in at least four major ways. Most importantly, they do not rely on valid self-reporting, and, hence, are not vulnerable to problems of inaccurate recall or reluctance of individuals to give candid reports of their drinking behaviors or attitudes. They can thus add credibility to research dealing with alcohol treatment efficacy and can provide clinicians with an additional source of objective information on patients.

Second, although biomarkers are subject to many of the usual psychometric issues of validity and reliability, some, such as internal consistency and construct validity, are not relevant to their evaluation. Instead, major concerns in evaluating biomarkers deal with criterion validity, stability, test-retest consistency, and interrater reliability. These issues have a bearing particularly for new markers for which fully automated test procedures have yet to be developed.

Third, the expertise required to ensure valid results from biomarkers is somewhat different from that needed to obtain maximally valid self-report information, where rapport, assurance of confidentiality, motivation for honesty, current state of sobriety, and testing conditions are important considerations. The accuracy of biomarker information is rarely a function of sample collection, but rather is closely related to sample handling, storage, and transmittal; quality assurance of laboratory procedures for isolation of the biomarker; and methods for quantifying and interpreting results.

Finally, although often used as screens for diagnosis of alcohol abuse or dependence, strictly speaking, biomarkers are reflections of physiological reactions to heavy drinking. Self-report screening scales, on the other hand, generally use a diagnosis of alcohol dependence as the criterion against which they are evaluated. Assessment of drinking behavior per se and severity of alcohol dependence are both important, albeit somewhat non-overlapping phenomena.

This chapter addresses the following issues: criteria for selection of biomarkers, traditional

biomarkers, emerging biomarkers, use of biomarkers in combination, use of biomarkers in alcohol treatment research and clinical practice, and research needs. Although the chapter focuses only on biomarkers, it is, of course, important to recognize that their use is in no way in competition with informed use of other psychometric measures. Rather, clinicians and researchers need to know how to maximize the information value of each class of measures.

Selecting a Biomarker

Selecting the proper biomarker for a particular application involves several issues. Ideally, the biological test would yield values that would directly correspond to the amount of alcohol consumed over a defined period of time. The sample for the test would be easy to obtain, readily testable, and inexpensive to quantify. Results would be quickly available. Further, the procedure would be highly acceptable to patients and therapists. No currently available biomarker has all of these features. Tests that directly or indirectly measure alcohol blood levels approach these goals but are useful only in situations of acute alcohol ingestion. They do not provide information regarding drinking status prior to acute ingestion.

Several additional considerations should guide the choice for a biological test. First, the *window of assessment* (i.e., the amount of time that the marker remains positive following drinking) needs to be understood. In emergency room settings as well as in occupational contexts, to include transportation, public safety, or delivery of medical care, level of alcohol consumption in the immediate past is often the primary concern. On the other hand, in insurance and general health care treatment screening contexts as well as in alcoholism treatment efficacy trials, the emphasis is likely to be particularly on chronic heavy drinking.

An additional concern that should guide selection of the biomarker is the *nature of the population* being assessed. Biomarkers often perform differently as a function of age, gender, ethnicity, and health status of the respondent. So, too, biomarkers are likely to perform more accurately in distinguishing extreme groups than in determining at-risk or harmful use of alcohol in a population heterogeneous with respect to drinking behavior.

Psychometric characteristics should also be considered in choosing a biomarker. Most notable of these are sensitivity and specificity. *Sensitivity* refers to the ability of a test to accurately identify those with the trait of interest. *Specificity* reflects the ability of a test to accurately detect those individuals without the trait. A test with high specificity will produce a low percentage of false-positive results. In populations with low base rates of a particular trait, a test with high specificity is generally needed to minimize the number of people erroneously labeled as having the trait. When the prevalence of the trait is high, specificity is generally not as critical as sensitivity. Statistical properties of screening tests are addressed in more detail in the chapter by Connors and Volk in this *Guide*.

Traditional Biomarkers

Table 1 summarizes some characteristics of the traditional biomarkers discussed in this section.

Gamma-Glutamyltransferase

Gamma-glutamyltransferase (GGT) is a glycoenzyme found in endothelial cell membranes of various organs. It appears to mediate peptide transport and glutathione metabolism. Elevated serum GGT level remains the most widely used marker of alcohol abuse. Levels typically rise after heavy alcohol intake that has continued for several weeks (Allen et al. 1994). With 2–6 weeks of abstinence, levels generally decrease to within

TABLE 1.—Characteristics of traditional markers

Marker	Time to return to normal limits	Type of drinking characterized	Comments
Gamma-glutamyl-transferase	2–6 weeks of abstinence	~ 70 drinks/wk for several weeks	Many sources of false positives
Aspartate aminotransferase	7 days, but considerable variability in declines with abstinence	Unknown, but heavy	Many sources of false positives
Alanine aminotransferase	Unknown	Unknown, but heavy	Many sources of false positives Less sensitive than aspartate aminotransferase
Macrocytic volume	Unknown but half-life ~ 40 days	Unknown, but heavy	Slow return to normal limits even with abstinence
Carbohydrate-deficient transferrin	2–4 weeks of abstinence	60+ g/d for at least 2 weeks	Rare false positives Good indicator of relapse

the normal reference range, with the half-life of GGT being 14–26 days. Laboratory tests for evaluating GGT are inexpensive and readily available.

GGT may elevate because of increased synthesis or accelerated release from damaged or dead liver cells. It seems to primarily indicate continuous, rather than episodic, heavy drinking, although a few moderate drinkers also produce elevated levels of GGT (Gjerde et al. 1988). Excessive drinking is not the only cause of elevated GGT levels; they may also rise as a result of most hepatobiliary disorders, obesity, diabetes, hypertension, and hypertriglyceridemia (Meregalli et al. 1995; Sillanaukee 1996). There are also large numbers of false negatives for GGT. For example, Brenner et al. (1997) observed that only 22.5 percent of construction workers drinking an average of 50–99 g/d had elevated GGT values, and even among those consuming >100 g/d, only 36.5 percent revealed high GGT levels.

Aminotransferases

The serum aminotransferases, aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT), are also often considered as screens for heavy drinking. ASAT catalyzes the reversible transfer of an amino group from aspartate to α-ketoglutarate to form glutamate and oxaloacetate. It is present in most eukaryotic cells, occurring in distinct isoenzymes in mitochondria (m-ASAT) and cytosol (c-ASAT). Both of these participate in the malate-aspartate shuttle, and in the liver the reaction transfers excess metabolic nitrogen into aspartate for disposal via the urea cycle (Nalpas et al. 1991).

Enhanced ASAT levels in alcoholics reflect liver damage, but alcohol consumption per se does not cause elevation (Salaspuro 1987). Serum ASAT does not correlate with the length of drinking (Skude and Wadstein 1977), but the highest ASAT values have been reported in alcoholics with a history of alcoholism exceeding 10 years. Other than with heavy drinking, serum ASAT also increases in a variety of liver diseases and may result from abnormal hepatocellular membrane permeability induced by ethanol (Zimmermann and West 1963).

The activity of mitochondrial ASAT can be analyzed by a rather simple immunochemical procedure (Rej 1980). The antibody against soluble ASAT is commercially available.

ALAT is found almost exclusively in the liver cytoplasm and is released to blood as a result of increased membrane permeability and breakage secondary to hepatocyte damage. ALAT appears to be the most sensitive and specific test for acute hepatocellular damage (Coodley 1971). Although in isolation ALAT is not particularly useful as a marker of chronic alcohol abuse or of chronic liver disease, the ratio ASAT/ALAT seems to provide meaningful information (Konttinen et al. 1970; Skude and Wadstein 1977; Reichling and Kaplan 1988). Usually a cutoff value of the ratio > 2 is assumed to reflect an alcoholic etiology of the liver disease (Matloff et al. 1980).

Macrocytic Volume

Elevated erythrocyte macrocytic volume (MCV) is common in alcoholic patients. This change results directly from the effect of alcohol on erythroblast development and persists as long as drinking continues (Buffet et al. 1975; Morgan et al. 1981; Whitehead et al. 1985).

As a stand-alone alcohol abuse indicator MCV has somewhat low sensitivity, and its slow return to reference values diminishes its potential as a relapse marker. Nevertheless, several studies have recognized its screening value when it is considered with other markers of alcohol consumption (Mundle et al. 2000). Moreover, the testing methodology is easy and inexpensive.

Carbohydrate-Deficient Transferrin

Transferrin, a negatively charged glycoprotein, is metabolized in the liver, circulates in the bloodstream, and assists in iron transport in the body. It contains two carbohydrate residues and two *N*-linked glycans (MacGillivray et al. 1983). Six sialic acid moieties may be attached. With heavy alcohol intake, these moieties can lose carbohydrate content, hence the term "carbohydrate-deficient" transferrin (CDT) (Stibler and Borg 1988). The concentrations of asialo-, monosialo-, and disialo-transferrin are increased (Martensson et al. 1997).

CDT levels appear to elevate following alcohol consumption of 60–80 g/d for 2 or 3 weeks (Stibler 1991), and they normalize with a mean half-life of 2–4 weeks of abstinence (Lesch et al. 1996). Research on possible mechanisms underlying the effect of alcohol on reducing the carbohydrate content of transferrin has been reviewed by Sillanaukee et al. (2001). False-positive CDT results can be found in patients with an inborn error of glycoprotein metabolism or a genetic D-variant of transferrin. False positives can also occur in patients with severe non-alcoholic liver diseases (e.g., primary biliary cirrhosis), those with diseases characterized by high total transferrin, and individuals who have received combined kidney and pancreas transplants (Stibler and Borg 1988; Stibler 1991; Bean and Peter 1994; Niemelä et al. 1995; Arndt et al. 1997).

Two commercial kits to isolate and quantitate CDT in serum are available. CDTect and %CDT are both produced by Axis-Shield, ASA (Oslo, Norway). Although CDTect shows less sensitivity for females than for males (Allen et al. 2000), there does not appear to be a gender effect with

%CDT, a procedure that determines the *percent* of transferrin that is carbohydrate deficient, rather than the *absolute amount* of CDT as does CDTect. Despite the fact that the sensitivities of GGT and CDT appear approximately equal, CDT is far more specific than GGT and other liver function tests (Litten et al. 1995).

Emerging Biomarkers

Table 2 summarizes some characteristics of the emerging markers discussed in this section.

Hexosaminidase

Hexosaminidase (hex), also named *N*-acetyl-β-D-glucosaminidase, occurs in several major isoforms (commonly denoted as A, B, I, and P) (Price and Dance 1972). Although hex is found in most body tissues, its concentration is especially high in kidneys (Dance et al. 1969). Increased urine hex is also an indicator of diseases associated with renal malfunction, such as upper urinary tract infections (Vigano et al. 1983), hypertension (Mansell et al. 1978), diabetes (Cohen et al. 1981), and

Table 2.—Characteristics of emerging markers

Marker	Time to return to normal limits	Type of drinking characterized	Comments
Urine hexosaminidase	4 weeks of abstinence	At least 10 days of drinking > 60g/d	
Serum hexosaminidase	7–10 days of abstinence	At least 10 days of drinking > 60g/d	Many sources of false positives
Sialic acid	Unknown	Correlates with alcohol intake	Can be measured in serum or saliva
Acetaldehyde adducts	~ 9 days of abstinence	Hemoglobin-bound acetaldehyde adducts can distinguish heavy drinkers from abstainers	Can be quantitated in blood or urine but amount to be measured is quite small
5-HTOL/ 5-HIAA	6–15 hours postdrinking	Recent consumption of even fairly low levels of alcohol	Measured in urine
Ethyl glucuronide	3–4 days (half-life 2–3 h)	Identifies even low-level consumption	Can be measured in urine or hair
Transdermal devices	Not applicable	Records alcohol consumption continuously	Technical difficulties need to be overcome

Note: 5-HTOL/5-HIAAA = ratio of 5-hydroxytryptophol to 5-hydroxyindole-3-acetic acid.

preeclampsia (Goren et al. 1987*a*); it is also an indicator of rejection after kidney transplantation (Wellwood et al. 1973), and it is seen with the use of nephrotic drugs (Goren et al. 1987*b*). Moreover, children under 2 years of age and people over age 56 often have increased levels (Kunin et al. 1978).

Serum and urine activities of hex are increased in alcoholics and in healthy volunteers drinking > 60 g/d for at least 10 days (Hultberg et al. 1980; Kärkkäinen et al. 1990). Serum hex levels return to normal after 7–10 days of abstinence (Hultberg et al. 1980), whereas urine hex normalizes after 4 weeks of abstinence (Martines et al. 1989).

Other than as a result of heavy alcohol consumption, elevated levels of serum hex can occur with liver diseases (Hultberg et al. 1981; Hultberg and Isaksson 1983), hypertension (Simon and Altman 1984), diabetes mellitus (Poon et al. 1983), silicosis (Koskinen et al. 1983), myocardial infarction (Woollen and Turner 1965), thyrotoxicosis (Oberkotter et al. 1979), and pregnancy (Isaksson et al. 1984).

Kärkkäinen et al. (1990) reported sensitivities of 69 percent and 81 percent for serum and urine hex, respectively, in detecting heavy drinking among alcoholic subjects at admission to an inpatient detoxification program. Values for specificity were 96 percent for both markers. As an indicator of treatment progress, the urinary form demonstrated sensitivity of 72 percent in distinguishing heavy drinkers after 7 days of abstinence. This value exceeded the sensitivity of GGT, ALAT, or ASAT. Stowell et al. (1997*b*) also found that serum hex performed better than GGT, ASAT, ALAT, or MCV in identifying drinking in a group of alcoholics. The sensitivity of serum hex was 94 percent, and its specificity was 91 percent. In this study, serum hex also proved slightly more accurate than CDT.

Sialic Acid

Sialic acid (SA) refers to a group of *N*-acyl derivatives of neuraminic acid in biological fluids and in cell membranes as nonreducing terminal residues of glycoproteins and glycolipids. The range of normal serum values of SA is 1.58–2.22 mmol/L. In alcoholic subjects, however, higher SA values have been found both in serum and in saliva (Pönniö et al. 1999; Sillanaukee et al. 1999*b*).

Sillanaukee et al. (1999*a*) reported a positive relationship between alcohol intake and SA levels in serum. To date, neither the dose of alcohol needed to increase it nor the mechanism underlying its increase has been defined. Neither has the half-life time of SA been reported. However, it has been observed that concentrations in serum decrease after abstinence from alcohol (Pönniö et al. 1999). Clinical studies show that SA is elevated in alcoholic subjects as compared with social drinkers, demonstrating sensitivity and specificity values, respectively, of 58 percent and 96 percent for women and 48 percent and 81 percent for men (Sillanaukee et al. 1999*b*). In a similar study, SA produced an overall accuracy of 77 percent for females and 64 percent for males in distinguishing alcoholics from social drinkers. SA in saliva also performed quite well—72 percent and 53 percent for males and females, respectively (Pönniö et al. 1999).

SA levels also rise in conditions other than heavy drinking. Total SA and/or lipid-associated SA levels are elevated in patients suffering from tumors, inflammatory conditions, diabetes, and cardiovascular diseases (Sillanaukee et al. 1999*a*). Increase of SA also seems to correlate with level of tumor metastasis (Kokoglu et al. 1992; Reintgen et al. 1992; Vivas et al. 1992), and its levels appear to normalize after successful treatment of cancer (Polivkova et al. 1992; Patel et al. 1994).

Acetaldehyde Adducts

Acetaldehyde is the first degradation product of ethanol. This highly reactive metabolite is rapidly converted to acetate by aldehyde dehydrogenase. With chronic ethanol exposure, and in a non-enzymatic reaction, acetaldehyde can form stable adducts with a number of compounds, including proteins such as albumin and hemoglobin (Collins 1988; Goldberg and Kapur 1994; Niemelä 1999). Hemoglobin-acetaldehyde (HA) adducts have received more attention.

Adduct levels in blood or in urine indicate drinking behavior and have been proposed as potential markers of alcohol abuse (Tsukamoto et al. 1998). Early experiments in mice showed that both whole blood- and urinary-associated acetaldehyde levels were increased in ethanol-fed mice 24 hours after cessation of ethanol feeding (C.M. Peterson and Scott 1989; Pantoja et al. 1991). After 9 days of abstinence, levels of whole blood–associated acetaldehyde (WBAA) declined to control levels (C.M. Peterson and Scott 1989).

These observations have now been confirmed in humans. Moreover, the increase of WBAA following ethanol exposure suggests marked gender differences. Heavy-drinking male college students produced higher absolute values than their heavy-drinking female counterparts, although 74 percent of the women versus 44 percent of the men had levels above the 99th percentile for abstainers (K.P. Peterson et al. 1998).

Measurement of acetaldehyde adducts in blood is difficult. Initially, chromatography isoelectric focusing gel and affinity purifications were used. However, these methods failed to distinguish alcoholics from control subjects (Homaidan et al. 1984). The very low levels of adducts require more highly sensitive techniques such as ELISA, and studies using this technology have reported far better results. Unfortunately, no commercial ELISA kit is available yet.

Very little is known about sources of false-positive results for acetaldehyde adducts except that diabetics have levels of HA adducts and glycated hemoglobin twice as high as alcoholics (Sillanaukee et al. 1991).

Levels of HA adducts have also been noted to be higher in heavy drinkers than in abstainers (Gross et al. 1992). Sensitivity and specificity values of this potential marker among heavy-drinking males have been reported as 65 to 70 percent and 93 percent, respectively, with corresponding values for females of 53 percent and 87 percent (Worrall et al. 1991). On the other hand, Hazelett et al. (1998) did not find gender differences in the performance of HA adducts between genders and reported sensitivity and specificity values of 67 percent and 77 percent.

Immunoreactivity toward acetaldehyde-modified proteins was also found to be higher in plasma from alcoholics and patients with non-alcoholic liver disease. Nevertheless, the response in alcoholics was characterized by a higher IgA component than in patients with non-alcoholic liver disease or in control subjects (Worrall et al. 1991). Using mean values ± 2 standard deviations as a cutoff point, sensitivity and specificity in detecting alcoholic patients were 78 percent and 93 percent, respectively (Lin et al. 1993).

The possible utility of HA adducts as a marker of alcohol abuse during pregnancy has also been investigated. Sixty-three percent of mothers who delivered children with fetal alcohol effects were reported as having elevated levels (Niemelä et al. 1991).

Serotonin Metabolites

Serotonin (5-hydroxytryptamine [5-HT]) is a monoamine vasoconstrictor melatonin precursor. It is synthesized in the intestinal chromaffin cells or in the central or peripheral neurons and is found in high concentrations in many body tissues. Serotonin is produced enzymatically from

tryptophan by hydroxylation and decarboxylation. 5-Hydroxytryptophol (5-HTOL) and 5-hydroxyindole-3-acetic acid (5-HIAA) are end products in the metabolism of serotonin, with 5-HIAA being the major urinary metabolite. Alcohol consumption can alter the metabolism of serotonin by inducing a shift toward the formation of 5-HTOL. It is believed that the change induced by alcohol intake is due to a competitive inhibition of aldehyde dehydrogenase by acetaldehyde, which inhibits 5-HIAA formation, and through an increase of NADH levels, which favors the formation of 5-HTOL.

The response of 5-HTOL to alcohol is dose dependent, and the excretion of this metabolite does not normalize for several hours after blood and urinary ethanol levels have returned to baseline levels. Therefore, 5-HTOL has been regarded as a marker of recent alcohol consumption.

As 5-HTOL increases 5-HIAA decreases, so the ratio of 5-HTOL/5-HIAA has been proposed as an even more sensitive marker of rather recent alcoholic drinking than 5-HTOL in isolation (Voltaire et al. 1992). Use of this ratio would also correct for urine dilution as well as for fluctuations in serotonin metabolism due to dietary intake of serotonin (Feldman and Lee 1985).

In social drinkers, a fiftyfold increase in 5-HTOL/5-HIAA ratio was measured in the first morning void, when ethanol in breath was no longer measurable (Bendtsen et al. 1998; Jones and Helander 1998). Compared with other markers of recent alcohol intake, such as blood and urinary methanol, 5-HTOL/5-HIAA remains elevated for a longer time (6–15 hours vs. 2–6 hours for methanol) after blood alcohol levels have returned to normal levels. Increased levels of the 5-HTOL/5-HIAA ratio have been reported in association with disulfiram treatment, calcium cyanamide therapy, and glyburide treatment (Borg et al. 1992).

In a healthy group of volunteers who had ingested alcohol (3–98 g) the previous afternoon or evening, 87 percent of the men and 59 percent of the women evidenced increased 5-HTOL/5-HIAA in the first morning urine (Helander et al. 1996). Voltaire et al. (1992) proposed a 5-HTOL/5-HIAA ratio > 20 pmol/nmol as an indicator of recent alcohol consumption.

Ethanol

The physical presence of ethanol in urine, serum, or saliva can be easily determined (Tu et al. 1992) and was one of the first parameters considered as a marker for alcohol consumption. Additionally, by using ethanol as a marker to assess intake, false-positive results can be eliminated. Furthermore, a positive test result for blood ethanol per se as well as a demonstration of high alcohol tolerance has been considered as an index of heavy drinking (Hamlyn et al. 1975; Lewis and Parton 1981). Unfortunately, the rapid elimination of ethanol from the blood nearly always makes it impossible to assess alcohol ingestion beyond the most recent 6–8 hours and, hence, the test may be of limited value in assessment of chronic heavy drinking.

Accelerated alcohol metabolism has been observed in regular drinkers (Kater et al. 1969; Ugarte et al. 1977). Notably, ethanol elimination rate (EER) has been found to be 70 percent higher in alcoholics than in control subjects. Correlations between EER and self-reported alcohol consumption have been found, as have correlations between EER and several other markers of alcohol abuse. Sensitivity and specificity values for this potential marker in detecting alcohol consumption > 50 g/d have been reported as 88 percent and 92 percent, respectively (Olsen et al. 1989).

Transdermal Devices

Concentration of ethanol in transdermal fluid and mean concentration of ethanol in blood are related in a linear function. The “sweat patch” is a noninvasive method employing salt-impregnated absorbent pads protected by a plastic chamber with attached watertight adhesive that collects

transdermal fluid steadily for at least 10 days. This device has been designed to estimate the alcohol consumption of drinking subjects. Levels of ethanol in the sweat patch can identify individuals drinking > 0.5 g of ethanol/kg/d.

During an 8-day study in which healthy subjects consumed alcohol under controlled conditions, sweat patches were able to distinguish drinkers from nondrinkers with perfect sensitivity and specificity. It was also possible to distinguish different levels of alcohol consumption (M. Phillips and McAloon 1980). Unfortunately, field trials of the sweat patch have failed to replicate these results (E.L. Phillips et al. 1984). The primary difficulty has been with ethanol storage and losses due to evaporation, back-diffusion, and bacterial metabolism (E.L. Phillips et al. 1984; Parmentier et al. 1991).

The adaptation for transdermal detection of ethanol of the electrochemical technology used for many years in sensor cells such as the portable alcohol Breathalyzers has prompted development of an experimental transdermal alcohol sensor (TAS) by Giner, Inc. This device, which is currently being refined, detects ethanol vapor at the surface of the skin by using an electrochemical cell that produces a continuous current signal proportional to ethanol concentration. The device contains a system to monitor continuous contact with skin and records the data at 2- to 5-minute intervals, for a period of up to 8 days.

When tested among healthy subjects drinking under controlled conditions, it was determined that the sensor signal paralleled the blood alcohol concentration, although with some delay (Swift et al. 1992). The threshold sensitivity for the TAS was a blood alcohol concentration of approximately 20 mg/dL. No false-positive TAS signals were detected in sober subjects, including those with liver or renal disease.

Ethyl Glucuronide

Ethyl glucuronide (EtG) is a nonvolatile, water-soluble, direct metabolite of ethanol. It is present in various body fluids and hair. The detoxification pathway of alcohol elimination via conjugation with activated glucuronic acid represents about 0.5 percent of the total ethanol elimination. The glucuronidation of alcohol was first described in the beginning of the 20th century by Neubauer (1901); it was subsequently detected in human urine (Jaakonmaki et al. 1967; Kozu 1973).

EtG peaks 2–3.5 hours later than ethanol (Alt et al. 1997) and provides a timeframe of detection for up to 80 hours. The half-life of EtG is 2–3 hours (Schmitt et al. 1997). Results from a study on the kinetic profile of ethanol and EtG in healthy moderately drinkers who ingested a single dose of ethanol showed that a serum ethanol concentration less than 1 g/L and serum EtG higher than 5 mg/L was suggestive of alcohol misuse (Schmitt et al. 1997). Since investigations of EtG are preliminary in nature, no information is yet available about the minimal dose of alcohol needed to increase its levels, nor has a commercial kit yet been marketed.

Biomarkers in Combination

Since none of the biomarkers currently available offers perfect validity as a reflection of heavy drinking, considerable research has been undertaken to evaluate using them in combination. Originally, these investigations took the form of deriving multivariate combinations of a large number of markers to distinguish heavy drinkers from other groups or to identify whether or not an alcoholic patient in treatment had relapsed to drinking. One of the earliest and most successful attempts to use biomarkers in combination was by Irwin and colleagues (1988). They found that patients who had relapsed by 3 months after discharge from inpatient care generally had GGT

levels ≥ 20 percent, ASAT levels ≥ 40 percent, or ALAT levels ≥ 20 percent those measured at the time they left the facility.

More recently, researchers have attempted to develop screening or relapse-monitoring biochemical profiles by labeling as positive individuals who are above standard screening cutoff values on at least one of two or more biomarkers. The combination of CDT and GGT has most frequently been used for this purpose. In a review of these studies it was found that use of such a "binary inclusion rule" raised screening sensitivity by more than 20 percent above that achieved by either marker in isolation but resulted in minimal loss of specificity, suggesting that these two markers are validly identifying somewhat different groups of alcoholics (Litten et al. 1995). In general, although CDT has been shown to identify relapse far better than GGT, at least among males, the two markers in combination tend to yield even higher sensitivity (Allen and Litten 2001). CDT has also been combined effectively with ASAT (Gronbaek et al. 1995), B-hex (Stowell et al. 1997*a*), and SA (Pönniö et al. 1999).

With the exception of some early work using quadratic discriminant functions, all of these combinatorial strategies have involved a "multiple cutoff" approach (i.e., if any of the biomarkers is above its reference range, the case is termed positive). Recently, however, two "compensatory" models have been proposed (i.e., if the sum of the scores on the separate tests exceeds some pre-derived cutoff value, the test is regarded as positive).

Based on a community sample of more than 7,000 Finns, Sillanaukee and colleagues (2000) found that use of an additive combination of natural logs of GGT and CDT volumes

(8 x ln GGT + 1.3 x ln CDT)

distinguished heavy drinkers (> 280 g/wk) from individuals drinking at lower levels more effectively for males and as effectively for females as did either GGT or CDT alone.

Another compensatory model has been proposed by Harasymiw and Bean (2001), in which values on five biomarkers were combined to maximize separation between heavy drinkers recruited from substance abuse treatment centers and light drinkers or nondrinkers from religious groups (mainly Mormon) and 12-step programs.

Yet another approach to consideration of CDT and GGT was taken by Allen and colleagues (1999), who evaluated the likelihood of three types of relapse as a function of patients' quartile scores on CDT and GGT separately and in various combinations.

Although most combinatorial strategies involve evaluation of the biomarkers simultaneously, it is possible that use of them sequentially might prove more cost-effective. This is often termed *reflex testing*. Reynaud and colleagues (1998), for example, provided evidence supporting the use of CDT in individuals with GGT and MCV levels within normal limits. In distinguishing alcohol-dependent patients of this type from control subjects, the sensitivity and specificity of CDT were 84 percent and 92 percent, respectively.

Use of Biomarkers in Alcohol Treatment Research

Increasingly, laboratory tests are being used in studies to evaluate treatment efficacy. Despite the fact that they do not fully mirror the drinking behavior, they can enhance the credibility of the research because they are not vulnerable to dissimulation by the subject. (Mundle et al. [1999], for example, noted that 15 percent of the patients in an alcohol treatment study who denied drinking nevertheless had high levels of CDT, GGT, or both.) To the extent that biomarkers provide valid information about outcome beyond that yielded by self-report or other means, their use can also enhance statistical power in clinical trials. (Ironically, awareness by the subject that his or her laboratory test may corroborate drinking

status may itself also prompt more honest self-reporting, further enhancing statistical power). Some biomarkers, most particularly the liver function tests GGT, ASAT, and ALAT, provide important information on health status, a goal of alcohol treatment in its own right. Finally, biomarker changes may also inform data-monitoring boards on the safety of an intervention, especially a medication, under investigation.

A recent review of the literature on the use of biochemical markers in alcohol medication development trials revealed that they have been used in the following ways (Allen et al. 2001):

- Description of the sample
- Determination of inclusion or exclusion of potential research participants
- Assessment of drug safety
- Specification of treatment outcome (usually as secondary outcome variables but occasionally as primary outcome variables)
- As a means of correcting for erroneous self-report of abstinence

To the extent that different individuals may vary on the biomarkers to which they respond, it is recommended that more than one measure be included in trials, particularly CDT and GGT. Although the ratio 5-HTOL/5-HIAA has rarely been used as an outcome measure, it too shows promise in this regard. As noted earlier, MCV, however, is generally not recommended for relapse monitoring since it returns to within normal limits rather slowly after onset of abstinence. Finally, if the technological difficulties can be resolved, the acetaldehyde adducts and transdermal devices might also be used in alcohol treatment efficacy trials.

Clinical Use of Biomarkers

Biomarkers in clinical practice have been generally used as a means of screening patients for a possible problem with alcohol. Although typically used in primary care settings, they have also been used in specialized medical settings such as emergency rooms, psychiatric clinics, gynecological clinics, and internal medicine practices. In most instances self-report procedures such as the Alcohol Use Disorders Identification Test will provide more accurate results, but in some situations, such as following trauma, it is possible that the patient may be unable to present an accurate drinking history. In still other instances, patients may be reluctant to acknowledge their level of consumption or its adverse consequences. Addition of biomarkers may thus identify some individuals in need of alcohol treatment who would not be discovered by a self-report. (As observed earlier, the patient's awareness that his or her self-report is subject to corroboration by laboratory tests may also prompt higher levels of candor on the self-report measures.) We would recommend that biochemical measures and self-report screening measures be used in combination. Further, we suggest that more than one biomarker be used for screening purposes. This combination might consist of, for example, GGT, CDT, and MCV.

A second potential clinical use of biomarkers is to assist in differential diagnosis to determine whether or not alcohol use may be prompting or exacerbating a presenting medical problem. This information can provide the clinician useful guidance on clinical management.

Third, giving patients feedback on biochemical measure levels in an empathic manner may help motivate positive drinking behavior change. For example, biomarkers were used in this way in the motivational enhancement strategy of Project MATCH (Miller et al. 1994).

Fourth, frequent monitoring of biomarker levels during the course of alcohol treatment may provide the clinician a means of early recognition of relapse which, in turn, may suggest the need to intensify or redirect efforts to prevent further drinking. In particular, several studies have considered the potential of CDT elevation as a means of recognition of relapse to drinking. All the projects produced positive results and, importantly, in two

of them CDT levels rose several weeks before patients admitted to their therapist that they had returned to drinking (Allen and Litten 2001). A combination of markers, such as CDT and GGT, is recommended for monitoring drinking status of patients in treatment. Testing should probably be quite frequent early in the course of followup, since risk of relapse appears highest then. Its frequency can then diminish as the patient's course of sobriety stabilizes.

More detailed recommendations for use of biomarkers in clinical contexts are offered by Allen and Litten (2001).

Research Needs

Despite the large number of studies (approximately 1,200) published on biomarkers, several fundamental questions remain and clearly warrant research.

Most importantly, dose-response relationships need to be specified. The markers should be better characterized by the drinking patterns required to elevate them. It is also important to determine underlying physiological differences and drinking pattern differences in patient responsiveness to alternative biomarkers.

Little research has been performed addressing the important issue of how to sequence a particular biological measure in a battery of other biomarkers and self-report measures. In screening for alcohol problems a particular "index of suspicion" might be appropriate before a specific biomarker is used. This index of suspicion might involve a questionable self-report or ambiguous findings on a clinical exam. Investigations of effective algorithms to quantify various indices of suspicion and the incremental informational value for clinical decisionmaking resulting from use of biomarkers are needed.

Since none of the existing biomarkers is optimal, research to identify an accurate, easy-to-measure, low-cost, nonreactive marker of drinking continues to be a priority. Research could also determine the best manner for combining and scoring relapse biomarkers.

Research is also needed to determine the impact of biomarker information as a source of feedback to patients and to devise treatment strategies that optimize this information as a means of enhancing motivation.

Finally, information on several applied usage parameters is needed to include the extent to which repeating laboratory tests is reactive (i.e., itself influences drinking or influences patient self-reports of drinking status).

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