



# Addiction Medicine Unit

## **Knowledge Workbook IV**

### The Assessment of Alcohol Use Utilizing Biomarkers

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## The Assessment of Alcohol Use Utilizing Biomarkers Steven Kipnis MD FACP FASAM

Alcohol Dependence (alcoholism) is a chronic disease that affects 18 million adults in the United States and costs society more than \$185 billion annually. Approximately 100,000 Americans die annually as a result of alcohol-related events and between 20% and 40% of all hospital admissions are alcohol related (Wurst et al, 2005).

### WHAT ARE BIOMARKERS?

Biochemical substances in the body that can indicate the presence or progress of a condition, or any genetic predisposition toward it, are called biomarkers (Peterson, 2004). Biomarkers have been used as screening tools to help make a diagnosis (especially early in the disease process), as part of a work up of an abnormal liver profile, to monitor a patient's sobriety and in research to measure outcomes.

Depending on the type of biomarker, it can indicate the timeframe of alcohol use or as in trait markers, it can indicate if there is a genetic predilection to excessive alcohol use. An ideal biomarker needs to have features that make it a worthwhile test. These features include:

- Reliable, reproducible results
- Ability to distinguish small amounts of alcohol use versus heavy drinking
- Ability to accurately represent the amount of alcohol that was consumed over a period of time
- Must have a simple collection system
- Must be able to be collected in diverse settings
- Inexpensive
- Results should be available quickly

In addition to the features above, when choosing a biomarker, one needs to consider several factors:

- The amount of time that the marker remains positive for alcohol consumption
- The sensitivity of the biomarker (ability of a test to accurately identify those persons who have consumed alcohol)
- The specificity of the biomarker (ability of a test to accurately identify those persons who have not consumed alcohol). A high specificity is ideal as this leads to a low false positive rate.

No commercial biomarker at present has all of the above features and demonstrates 100% specificity and sensitivity. This shortcoming in biomarkers has led to the use of combination panels, where tests are combined to increase the likelihood of an accurate diagnosis. (Combination testing will be discussed later in this article.)

## TRADITIONAL BIOMARKERS

### **Blood Alcohol Concentration (BAC)**

The blood alcohol concentration or breath alcohol level is still the most accurate test for alcohol use if one is looking to determine very recent use. The level is easily determined, though does require simple equipment. The BAC can also be correlated with physical signs and symptoms:

- 20 - 99 mg%: loss of muscular coordination
- 100 - 199 mg%: neurological impairment, ataxia, prolonged reaction time, mental impairment, incoordination
- 200 - 299 mg%: nausea, vomiting, ataxia
- 300 - 399 mg%: hypothermia, dysarthria, amnesia, stupor
- 400 - > mg%: coma

The degree of impairment is affected by the level of tolerance, though a BAC greater than 150 if not showing signs of intoxication or any time the BAC is > 300 usually can indicate that alcohol dependence may be present.

The half-life of the BAC is 4 hours and a BAC level of 0.1% or 100 mg% (the legal limit in some states) returns to zero (alcohol completely metabolized and excreted) in most people in 8–10 hours, thus due to this rapid elimination, it is a poor test outside this timeframe.

### **Mean Corpuscular Volume (MCV) of Red Blood Cells**

The MCV is a measure of the volume of red blood cells. Alcohol appears to have a direct effect on erythroblast development, thus the MCV in heavy drinkers tends to exceed the average range and stays elevated for months after cessation of alcohol use. The MCV is an easy test to perform and is inexpensive. The drawbacks are that it has a low sensitivity, can give a false positive in cigarette smokers and returns to normal so slow that treatment programs cannot use it as a useful indicator of relapse. MCV is one of the tests that have been used in combination with other markers.

### **Serum Aminotransferases (AST, ALT, GGT)**

Commonly known as the liver enzymes, the serum aminotransferases include: aspartate aminotransferase (AST), alanine aminotransferase (ALT) and serum gamma-glutamyltransferase (GGT). These enzymes metabolize amino acids and due to liver cell turnover, are found in the blood normally. The ALT and AST are felt to be an indicator of liver disease in general and less specific to alcohol induced liver damage. ALT is more specific to alcohol induced liver cell injury than AST which is also found in heart, muscle, kidney and brain cells. Any injury or disease that can increase the level of cellular injury or death in these organs will cause an elevation of this marker. The ratio of AST to ALT seems to provide more meaningful information, especially if the ratio cut-off is greater than 2, where it is thought to reflect more specific liver disease as the etiology (Matloff, 1980).

Gamma glutamyltransferase (GGT), a glycoprotein found in the liver is the most widely used of all the liver enzymes to determine liver cell injury due to excessive alcohol consumption. When a person chronically consumes alcohol in excess of 4 drinks per day for 4 to 8 weeks, the GGT becomes elevated above the 54 U/l level in men and women. However, it is not a very sensitive test as it indicates continuous better than episodic drinking, and it is elevated in only 30–50% of the excessive drinkers when looking at the general population. In one study (Brenner et al 1997), only 22.5% of construction workers drinking 50–99 gms/day of alcohol had an elevated GGT. False positives can also be an issue as elevations of this enzyme are seen in nonalcoholic liver such as biliary cirrhosis, as well as in obesity, pancreatitis, prostate disease, diabetes, hypertension, hypertriglyceridemia and during the use of some medications (hormones and anticonvulsants).

## DIRECT METABOLITES OF ETHANOL

### **Acetaldehyde Adducts**

The first metabolic product in the breakdown of alcohol is acetaldehyde. Acetaldehyde binds to a number of proteins, such as albumin and hemoglobin producing compounds called adducts. Hemoglobin is a protein that is found in red blood cells, which have a lifespan of 120 days. If alcohol is consumed and acetaldehyde is produced, the number of acetaldehyde–hemoglobin products increase. This acetaldehyde adduct will remain high for at least one month after consumption stops (Halvorson et al, 1993).

Acetaldehyde adducts can be measured in the blood and the urine. The only common cause of a false positive is diabetes.

### **Ethyl glucuronide (EtG)**

EtG was introduced in the United States in 2003. It is a minor metabolite of alcohol and occurs when alcohol reacts with glucuronic acid, a substance that allows compounds to be water soluble. Made in the liver, less than .1% of alcohol is metabolized by this pathway. EtG can only be present if alcohol is consumed and it is measured using sophisticated laboratory equipment (Gas chromatograph/mass spectrometry).

EtG remains in the blood for up to 36 hours after alcohol is consumed; in the urine for up to 3–5 days after use; and in hair for months (Alt et al 2000). Drawbacks that have been noted when using this biomarker include:

- Hair levels are not well correlated with recent alcohol consumption
- One can get a positive test with incidental exposure as a result of food containing alcohol, the use of mouthwash or underarm deodorizing products and over the counter medications that contain alcohol, such as cough medications. These are not false positives, because alcohol has been absorbed into the body. However, the result can falsely accuse someone of relapsing or ingesting alcohol.

The cut-off for a reported positive has been set at 100–250 micrograms per liter to try to limit the incidental exposures results. Patients should be informed that these can occur

and that total abstinence should also include alcohol containing products. Clinical correlation is extremely important.

### **Ethyl Sulfate (EtS)**

Ethyl sulphate (EtS), a direct ethanol metabolite, appears to offer potential as a biomarker for recent alcohol consumption. Although its window of assessment is similar to that of ethyl glucuronide (EtG), there are differences between the two markers in their pathways for formation and degradation. The data from patients and volunteers in the work by Wurst et al (2006) suggest that the direct ethanol metabolite ethyl sulphate has the potential to serve as a biomarker of recent ethanol intake. Because EtG and EtS are formed via different pathways they might be used conjointly, thereby increasing sensitivity.

### **Fatty Acid Ethyl Esters (FAEE's)**

Fatty Acid Ethyl Esters are also breakdown products of alcohol and are formed by the esterification of fatty acids and ethanol. The clinical value of the serum to plasma ratio of FAEE's was described in 1996 (Doyle et al 1996) due to the finding that they appeared to mimic the appearance and decay of alcohol in the first few hours after consumption. It was also noted that they can be found in the serum up to 24 hours after cessation of ethanol intake and in hair for months (Wurst et al, 2004). Soderberg and colleagues found that men have two times the level of FAEE's than women when weight adjusted for ethanol content and that the FAEE level may be more useful if the result is divided by the triglyceride level. It has been found that there are subtypes of FAEE's, such as ethyl oleate, that could help to differentiate chronic alcoholism from episodic heavy drinking. Another subtype, ethyl arachidonate (Salem 2001), is only found in the liver and adipose tissue of post mortem individuals who drank pre-mortem and thus can be used to determine alcohol consumption in accident victims post mortem. Its greatest utility is in the first 12 hours after death (the alcohol levels tend to increase in the body after death). There are no false negatives or positives as yet though FAEE's are produced post mortem and also when specimens are stored at room temperature.

## **BIOMARKERS OF INTEREST**

### **Carbohydrate – deficient Transferrin (CDT)**

Transferrin is a glycoprotein that transports iron in the body. The normal person's Transferrin contains 4 to 6 Sialic acid (carbohydrate) molecules. Alcohol consumption interferes with Sialic acids ability to attach to Transferrin, thus the alcohol causes a deficiency in Sialic acid content, hence carbohydrate-deficient Transferrin. The usual way of stating the results is % of total transferrin and greater than 5% suggests heavy drinking. This test was approved by the FDA in 2001 and if a person consumes an average of 5 standard drinks (60 grams of ethanol) for 7–10 days and certainly for at least two weeks, there will develop a higher percentage of carbohydrate deficient Transferrin.

The test is optimal within 3 days of the last drink. There are several problems with this biomarker however:

- It is valid only when the daily alcohol consumption is greater than 60 gms
- The methodology is time consuming
- Expensive equipment is needed to run the test
- Women have higher levels than men irregardless of the drinking history (Allen 2000)
- False positives are seen due to primary biliary cirrhosis, combined kidney and pancreatic transplants, inborn errors of glycoprotein metabolism and genetic variants of transferring.
- There is also a high rate of false negatives especially due to the insensitivity of the test in women.

The CDT if elevated due to heavy drinking will normalize after abstinence in 2–4 weeks. CDT has also been combined with other biomarkers to increase its usefulness.

### **Total Serum Sialic Acid (TSA) and Plasma Sialic Acid Index of Apolipoprotein J (SIJ)**

Sialic acid is a derivative of neuraminic acid which is a component of glycoproteins and glycolipids. Sialic acid can be measured as a direct measurement amount (TSA) or as a useful indicator of alcohol use when it is decreased in other molecules (CDT, SIJ). While it is unknown how much alcohol is needed to be consumed to raise the TSA level and how this occurs, it has been found that alcoholics have elevated levels of serum sialic acid. It takes longer to decrease the total amount during periods of abstinence as compared to CDT or GGT, so that it may not be a good marker at the beginning of abstinence. Once levels are normalized, it then could be utilized to evaluate for relapse. False positives do occur, especially in patients with tumors, metastatic disease, inflammatory conditions, diabetes and cardiovascular disease (Sillanaukee et al 1999).

Apolipoprotein J is another glycoprotein involved in the transportation of lipids in the blood and contains four times the amount of sialic acid than transferrin. Alcohol consumption reduces sialic acid levels in apolipoprotein j and due to the greater amount found in this molecule, it is easier to measure changes as compared to CDT. SIJ returns to normal levels after 8 weeks of abstinence.

### **N - Acetyl B- Hexosaminidase (Beta – Hex)**

Beta – Hex is a compound that is found in many cells and may be indicative of liver cell damage. It can be found in the serum and urine and indicates heavy alcohol consumption when elevated. It normalizes in 7 to 10 days on serum testing and at approximately 4 weeks on urine determinations (Martines et al, 1989). It remains a difficult test to obtain in the United States and has significant false positives showing elevated levels. Some causes of these false elevations include: diabetes, hypertension, silicosis, myocardial

infarction, thyrotoxicosis, pregnancy and pre-eclampsia, kidney infections and kidney disease, and in patients over the age of 56.

## BIOMARKERS RELATED TO NEUROTRANSMITTERS

### **Urinary Derivative of Serotonin (5 – HTOL/5 – HIAA)**

5 – Hydroxytryptophol (5 – HTOL) and 5 – hydroxyindole – 3 – acetic acid (5- HIAA) are metabolites of serotonin (5 – hydroxytryptamine). Both metabolic products are excreted in the urine, but measurements of the ratio of 5 – HTOL to 5 – HIAA may be better to show previous alcohol consumption than if used alone. Initially, alcohol and its metabolite acetaldehyde affect serotonin metabolism whereby more 5 – HTOL is produced when drinking than 5 – HIAA. This shift with an increase in 5 – HTOL is seen for at least 6 – 15 hours and may be indicative of alcohol consumption over the last 24 hours (Beck et al 2003). This test can only be done at certain laboratories and false positives have occurred with the use of antabuse and glyburide.

### **Salsolinol**

Salsolinol is a compound that is formed when the neurotransmitter dopamine, reacts with either acetaldehyde or with pyruvate, a metabolite of glucose. Early work shows that if one measures the salsolinol levels in the brain, there is no discernable difference in alcohol and non-alcoholic patients. However, if the levels are measured in the urine, lower levels are found following acute alcohol consumption (Haber et al 2002). This test appears to be research based at present.

## TRAIT MARKERS

Trait markers are an entirely different type of marker whereby the marker is genetic in origin. The characteristics of a trait marker are that it can be passed from parent to child genetically, that it is associated with the specific disease or disorder and it is not dependent on the activity of the disease or disorder. Several markers have been looked at and these include: adenylyl cyclase (AC) activity, gamma – aminobutyric acid (GABA), dopamine (DA), beta – endorphin and serotonin. The neurotransmitters in this group, GABA, DA, Serotonin and beta – endorphins, have some common features. GABA, Beta-endorphins and Serotonin levels all seem to be decreased in alcohol dependent patients. Dopamine has been researched and found to have contradictory levels in alcohol dependent patients (Peterson 2004).

### **Adenylyl Cyclase (AC) Activity**

AC activity may be the most promising of the trait markers at present. Adenylyl cyclase is a protein that is found in cell membranes and is involved in the generation of energy of cells. This enzyme does not fulfill all of the criteria of a perfect trait marker. It is less active in platelets of abstinent alcoholics than non-alcoholics, increased when alcoholics drink and other drugs, like cannabis, may increase its levels as well.

## BIOMARKERS USED IN COMBINATION

Due to the lack of the perfect biomarker or trait marker, researchers have looked at combinations of tests that would generate a result with increased specificity and sensitivity. Early work by Irwin et al in 1988, looked at a combination of tests in alcohol dependent men after their discharge from a treatment program. The findings were that the predictive value of using three tests (GGT, ALT and AST) was better than single tests in determining relapse.

The CDT has been combined with several different tests, though the most useful may be with the GGT (Chen et al, 2003) if one is looking to combine only blood results. Martin et al in 2002 looked at combining lab markers (CDT and GGT) along with the CAGE questionnaire. They were able to improve detection rates in surgical patients.

### **EDAC**

A panel of routine laboratory tests (from 12 to 36) could detect early alcohol consumption as shown by the EDAC score. The baseline tests include: monocytes, high density lipoprotein, GGT, AST, ALT, Direct Bilirubin, MCV and others. This scoring system constructs a “mathematical fingerprint” that is calculated by combining the results of routine laboratory tests using linear discriminant analysis. The identified patient’s “fingerprint” pattern is compared to previously developed “stereotype” fingerprints (Harasymiw et al 2005). The EDAC score shows good performance when looking at men who drink more than 48 grams (4 beers) per day for a month or women whose drinking history is one of 36 grams (3 beers) per day for a month. This test is available through Alcohol Detection Services. The identified patients’ test results are submitted and an analysis is returned. The analysis looks at patterns of scores over time and determines a current P – positive value. If the current P – positive score exceeds the lowest previous score by 25% or more, then the result indicates an apparent relapse. P-positive scores that decrease by 25% or greater on two consecutive periods indicate abstinence during that time period. The EDAC can identify heavy drinkers more than one month after cessation of alcohol (Hartz et al 1997).

## CONCLUSION

To date, there are over a thousand studies of biomarkers. Biomarkers are only one part of a clinician’s complete evaluation of a patient, which should include an extensive biopsychosocial evaluation. Objective measurements certainly have a place not only in the initial evaluation, but also they can be used in a significant way as part of the treatment process. A treatment provider should never lose site of the fact that testing can be of therapeutic value in the recovery process as a validation of the patient’s sobriety and recovery. However, one also needs to never loose site of the fact, that there is no perfect marker at present and a positive test could be detrimental to one’s recovery if it indeed is a false result.

COMMON MARKERS AND FALSE POSITIVES THAT CAN OCCUR

MARKER	TIME TO RETURN TO NORMAL LEVELS FROM START OF ABSTINENCE	FALSE POSITIVES
GGT	2 – 6 WEEKS	BILIARY CIRRHOSIS OBESITY DIABETES HYPERTENSION PANCREATITIS PROSTATE DISEASE HYPERTRIGLYCERIDEMIA SOME HORMONES SOME ANTICONVULSANTS
ALT, AST	7 DAYS (VARIABLE)	ANY LIVER DISEASE CAN OCCUR IN HEART, MUSCLE, BRAIN AND KIDNEY DISORDERS
MCV	MONTHS	SMOKING
CDT	2 – 4 WEEKS	END STAGE LIVER DISEASE BILIARY CIRRHOSIS GENETIC VARIABLES KIDNEY/PANCREAS TRANSPLANT
BETA - HEX	URINE – 4 WEEKS SERUM 7 – 10 DAYS	DIABETES HYPERTENSION SILICOSIS MYOCARDIAL INFARCT THYROTOXICOSIS PREGNANCY
SIALIC ACID	UNKNOWN	TUMORS TUMOR METASTASIS INFLAMMATORY DISORDERS DIABETES CARDIOVASCULAR DISEASE
ACETALDEHYDE ADDUCTS	~ 9 DAYS	DIABETES
5-HTOL/5-HIAA RATIO	6 – 15 HOURS	ANTABUSE TREATMENT GLYBURIDE TREATMENT
ETHYL GLUCURONIDE	3 – 4 DAYS	INCIDENTAL EXPOSURES
FAEE	12 HOURS AFTER DEATH	ALCOHOL LEVELS INCREASE NORMALLY IN THE POST - MORTEM PERIOD

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