Science of Drinking: Alcohol Use, Abuse and Alcohol Biomarkers
Session # 2000

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Disclosure

} Speaker Disclosure

*In the past 12 months, I have not had a significant financial interest or other relationship with the manufacturer(s) of the product(s) or provider(s) of the service(s) that will be discussed in my presentation.*

} *This presentation will not include discussion of pharmaceuticals or devices that have not been approved by the FDA or unapproved or “off-label” uses of pharmaceuticals or devices.*
Organization of the Talk

- Alcohol use and abuse
- Health benefits of moderate drinking
- Hazards of alcohol abuse
- Limitation of blood alcohol testing
- Alcohol biomarkers and their clinical application
History of Alcohol Use
History of Alcohol Use

- Professor Dudley's Drunken Monkey Hypothesis proposes that the human attraction to alcohol may have a genetic basis due to the high dependence of primate ancestors to fruit as the primary food about 40 million years.
- Yeasts on the fruit skin and within fruit convert sugars into ethanol during ripening and hungry monkeys capable of detecting alcohol smell survived better than others. Then “natural selection” favored monkeys with a keen appreciation for the smell and taste of alcohol.

Definition of Standard Drink

- Alcohol content of various beverages varies widely. The average alcohol content of beer is 5%, wine is 10% and whiskey is 40%.
- However, the serving size also vary according to the type of beverage. For example, beer usually comes in a 10 or 12 ounce bottle while a shot of tequila in a mixed drink is only 1.5 ounces.
- Therefore, regardless of the alcoholic beverage, a standard drink contains roughly the same amount of alcohol (0.6 ounce of alcohol or 14 gm of alcohol).
Guideline for Alcohol Consumption

- **Men:** No more than two standard alcoholic drinks per day (1 standard drink: 14 gm alcohol or 0.6 ounce of alcohol).
- **Women:** No more than one standard alcoholic drink per day.
- **Adults over 65 (both male and female):** No more than one drink per day
- Always consume alcohol with food.
- Women are more susceptible to the effect of alcohol than men and may even be more susceptible just before her period.

Zakhari S. Alcohol Res Health 2006; 29: 245-54.
Alcohol Consumption
Benefits for Moderate Alcohol Consumption

- Increased longevity and reduced risk of coronary heart diseases
- Better survival chance after a heart attack
- Reduced risk of stroke
- Reduced risk of developing diabetes
- Reduced risk of forming gallstone
- Reduced risk of developing arthritis
- Reduced risk of developing age related dementia and Alzheimer’s disease.
- Reduced risk of certain types of cancer
- Possible lowers chances of getting common cold
Definition of Heavy Drinking

- National Institute of Alcohol Abuse and Alcoholism sets threshold at more than 14 drinks per week for men (or more than 4 drinks per occasion) and more than 7 seven drinks per week for women (or more than 3 drinks per occasion) as risky practice.

- Hazardous drinking is defined as 21 or more drinks per week by men or more than 7 drinks per occasion at least three times a week. For women, more than 14 drinks per week or drinking more than 5 drinks in one occasion at least three times a week is considered as hazardous drinking.

- Binge drinking is drinking 4-5 drinks in one occasion.
Alcohol Abuse in U.S
Alcohol Metabolism

ADH

Ethanol $\rightarrow$ Acetaldehyde

NAD$^+$ $\rightarrow$ NADH + H$^+$

CYP2E1

Ethanol $\rightarrow$ Acetaldehyde

NADPH + H$^+$ + O$_2$ $\rightarrow$ NADP$^+$ + 2H$_2$O

ALDH2

Acetaldehyde $\rightarrow$ Acetate

NAD$^+$ $\rightarrow$ NADH
Number of hours needed for blood alcohol to become zero in men (women)

<table>
<thead>
<tr>
<th>Blood Alcohol Level</th>
<th>Time needed for zero level</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% (500 mg/dL)</td>
<td>27.7 (33.3) hours</td>
</tr>
<tr>
<td>0.2% (200 mg/dL)</td>
<td>11.1 (13.3) hours</td>
</tr>
<tr>
<td>0.15% (150 mg/dL)</td>
<td>8.3 (10) hours</td>
</tr>
<tr>
<td>0.12% (120 mg/dL)</td>
<td>6.6 (8) hours</td>
</tr>
<tr>
<td>0.1% (100 mg/dL)</td>
<td>5.5 (6.6) hours</td>
</tr>
<tr>
<td>0.08% (80 mg/dL)</td>
<td>4.4 (5.3) hours</td>
</tr>
<tr>
<td>0.05% (50 mg/dL)</td>
<td>2.7 (3.3) hours</td>
</tr>
<tr>
<td>0.03% (30 mg/dL)</td>
<td>1.6 (2) hours</td>
</tr>
</tbody>
</table>

Men metabolizes alcohol at 18 mg/dL/h, women: 15 mg/dL/h
Calculation of Blood Alcohol

- In 1932, the Swedish scientist Eric P. Widmark developed a formula for the calculation of the blood alcohol knowing the subject’s body weight and gender.

- $A = C \times W \times r$

- Where $A$ represents total amount of alcohol, $C$ the blood alcohol concentration in grams per liter, $W$ is the body weight of the person expressed in kilograms and $r$ is a constant which is assumed be roughly 0.7 for men and 0.6 for women. The modern version of this formula used in US is:

- $C = (\text{no of drinks} \times 3.1/\text{weight in pounds} \times r) - 0.015 \times t$
Calculation of Blood Alcohol

Because most standard drinks contain approximately the same amounts of alcohol, the type of drink does not matter. For example, if a 150 lb man drinks five beers in a 2 hour period, his blood alcohol should be:

\[ C = (5 \times \frac{3.1}{150} \times 0.7) - 0.015 \times 2 \]

\[ = 0.147 - 0.030 \]

\[ = 0.117\% (117 \text{ mg/dL}) \]

For a 150 lb woman blood alcohol should be:

\[ C = (5 \times \frac{3.1}{150} \times 0.6) - 0.015 \times 2 \]

\[ = 0.172 - 0.030 \]

\[ = 0.142\% \]
<table>
<thead>
<tr>
<th>Body Weight</th>
<th>Number of Drinks</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 lb</td>
<td>0.035 0.070</td>
</tr>
<tr>
<td>150 lb</td>
<td>0.030 0.060</td>
</tr>
<tr>
<td>175 lb</td>
<td>0.025 0.050</td>
</tr>
<tr>
<td>200 lb</td>
<td>0.022 0.044</td>
</tr>
<tr>
<td>225 lb</td>
<td>0.020 0.040</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Male Blood Alcohol Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>125 lb 0.105 0.140 0.175 0.210 0.245</td>
</tr>
<tr>
<td>150 lb 0.090 0.120 0.150 0.180 0.210</td>
</tr>
<tr>
<td>175 lb 0.075 0.100 0.125 0.150 0.175</td>
</tr>
<tr>
<td>200 lb 0.066 0.088 0.110 0.132 0.154</td>
</tr>
<tr>
<td>225 lb 0.060 0.080 0.100 0.120 0.140</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Blood Alcohol Concentration (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female 100 lb 0.051 0.102 0.153 0.202 0.255 0.306 0.357</td>
</tr>
<tr>
<td>125 lb 0.041 0.082 0.123 0.164 0.205 0.246 0.287</td>
</tr>
<tr>
<td>150 lb 0.034 0.068 0.102 0.136 0.170 0.204 0.238</td>
</tr>
<tr>
<td>175 lb 0.030 0.060 0.090 0.120 0.150 0.180 0.210</td>
</tr>
<tr>
<td>200 lb 0.026 0.052 0.078 0.104 0.130 0.156 0.182</td>
</tr>
<tr>
<td>225 lb 0.023 0.046 0.069 0.092 0.105 0.128 0.161</td>
</tr>
</tbody>
</table>

For a male drinking up to two drinks at dinner with food and spending about 1-2 h in dinner is safe because blood alcohol should be well below 0.08%. For women, it is only one to one and half drink.
Whole Blood Versus Serum Alcohol

- Legal limit of driving is 0.08% (80 mg/dL) whole blood alcohol. Sometimes medical alcohol is used in legal situation and converting serum alcohol to whole blood alcohol is important.

- Serum or plasma alcohol is higher than whole blood because serum/plasma has more water.

- The average serum to whole blood alcohol ratio is approximately 1.15. Therefore, many toxicologists divide serum alcohol with 1.15 to obtain whole blood alcohol level.
Blood Alcohol: Medical Vs Legal

- Medical alcohol is measured by using automated analyzer and enzymatic method where alcohol if present in serum is converted into acetaldehyde by alcohol dehydrogenase and in this process cofactor NAD (no absorption) is converted into NADH (absorbs at 340 nm).

- However, enzymatic method suffers from interference.

- Legal alcohol must be conducted by using GC and such methods are less susceptible to interference. Both whole blood and serum can be used.
Limitation of Enzymatic Method

- Although methanol and acetone do not interfere significantly with enzymatic alcohol determination, n-propyl alcohol may interfere significantly.
- High concentrations of lactate and LDH may produce false positive alcohol levels above legal limit.
- However, both lactate and LDH must be present in sufficient amount to cause this interference.
- Enzymatic methods are unsuitable for postmortem blood analysis.
Limitation of Enzymatic Method

Ethanol $\rightarrow$ Alcohol Dehydrogenase $\rightarrow$ Acetaldehyde $\rightarrow$ NADH + H$^+$ $\rightarrow$ Lactate $\rightarrow$ Lactate Dehydrogenase $\rightarrow$ Pyruvate

NAD$^+$ Witchcraft
# How Much LDH and Lactate?

<table>
<thead>
<tr>
<th>LDH</th>
<th>False Positive Ethanol (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>296 IU/L</td>
<td>0.6</td>
</tr>
<tr>
<td>2100 IU/L (postmortem)</td>
<td>14.9</td>
</tr>
<tr>
<td>6178 IU/L (postmortem)</td>
<td>221.1</td>
</tr>
<tr>
<td>9852 IU/L (postmortem)</td>
<td>30.1</td>
</tr>
<tr>
<td>16,677 IU/L (postmortem)</td>
<td>99.6</td>
</tr>
<tr>
<td>119,513 IU/L (postmortem)</td>
<td>&gt;600</td>
</tr>
</tbody>
</table>

Normal level <225 IU/L. Authors recommend that if LDH value is >2000 IU/L (U/L), enzymatic alcohol method may produce false positive result.

Elimination of this Interference

- Both lactate and LDH must be present in serum to cause this interference.
- LDH has a high molecular weight and is absent in the ultrafiltrate.
- Alcohol (protein binding 0%) is 100% present in the ultrafiltrate.
- Lactate, present in the ultrafiltrate alone, cannot interfere.
- EMIT alcohol measurement: unaffected by ultrafiltration.
Elimination of this Interference

- Patient 1: End stage renal disease, severe metabolic acidosis: LDH: 27,000 U/L, Lactate: 15 mmol/L.
- EMIT serum alcohol 690 mg/dL.
- Ultrafiltrate: 0 mg/dL.
- GC alcohol: 0 mg/dL.

- Patient 2: MI, acidosis was developed.
- LDH: 24,623 U/L
- Lactate: 5.6 mmol/L.
- EMIT serum alcohol: 440 mg/dL.
- Ultrafiltrate: 0 mg/dL.
- GC alcohol: 0 mg/dL.

Can Body Produce Alcohol?

- This is a very popular DWI defense: the defendant never drank alcohol but felt tipsy after eating a big meal and that caused the accident.

- Although substantial alcohol may be produced endogenously in a decomposed body by the action of various microorganisms, our body does not produce enough endogenous alcohol.

- In one report, after a meal, negligible alcohol levels of 11.3 mg/dL (0.01%) and 8.2 mg/dL (0.008%) were detected in two out of eight patients with small intestinal bacterial overgrowth problem. Patients with liver cirrhosis often have small intestinal bacterial overgrowth.
Auto-Brewery Syndrome

- A 3-year old female patient with short bowel syndrome was operated and re-operated due to obstruction of small intestine. She also suffered from septemia due to bacterial overgrowth in her intestine.

- The patient was given Lactobacillus containing carbohydrate rich fruit drink when she was 3-year old and couple of weeks later her parents saw her walking erratically with a smell of alcohol.

- A breath analyzer showed an alcohol level of 22 mmol/L (101 mg/dL). When the carbohydrate rich fruit drink was discontinued her symptoms resolved but when the drink was reinstated, her symptoms returned with a blood alcohol level of 15 mmol/L (69 mg/dL).
Auto-Brewery Syndrome

- Liver enzymes and alcohol biomarkers were however normal. Culture of gastric fluid and feces showed the presence of Candida kefyr and she was treated with oral fluconazole for one week, and all her symptoms were resolved.

- A month later her symptoms reappeared and high alcohol level was again detected in her blood. New culture of gastric fluid showed the presence of Saccharomyces cerevisiae. Again the patient was treated with fluconazole and her symptoms were resolved.

- The cause of her alcohol was due to “Auto-brewery syndrome”. A diet less rich in carbohydrate was selected and she had no such symptoms for 2 years.

Alcohol Biomarkers
Clinical Application of Alcohol Biomarkers

- Identifying patients needing intervention for alcohol abuse.
- Monitoring patients undergoing alcohol rehabilitation and identifying any relapse of alcohol use.
- Outcome measures in studies evaluating new medication or behavior modification for intervening in alcohol abuse problems.
- Guiding patients in a positive manner to change their lifestyles and drinking habits.
- Documenting abstinence.
State Vs Trait Alcohol Biomarkers

- State markers are based on measurable biochemical changes following alcohol abuse. These markers can be either direct markers of alcohol abuse such as ethanol metabolites, or indirect biochemical markers that are elevated in chronic alcohol abusers.

- Trait markers are genetic markers indicating a degree of susceptibility of an individual for alcohol abuse.
## Commonly Used State Markers

<table>
<thead>
<tr>
<th>Slate Marker</th>
<th>Type of Marker</th>
<th>Cut-off Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gamma-glutamyltransferase (GGT)</td>
<td>Indirect</td>
<td>&gt;50 U/L</td>
</tr>
<tr>
<td>Mean Corpuscular Volume (MCV)</td>
<td>Indirect</td>
<td>&gt;96 fl</td>
</tr>
<tr>
<td>Carbohydrate-Deficient Transferrin</td>
<td>Indirect</td>
<td>&gt;1.6 -2.4%</td>
</tr>
<tr>
<td>β-Hexosaminidase</td>
<td>Indirect</td>
<td>Varies</td>
</tr>
<tr>
<td>Ethyl Glucuronide</td>
<td>Direct</td>
<td>&gt;500-1000 ng/mL in urine</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;7 pg/mg, hair for</td>
</tr>
<tr>
<td>Ethyl Sulfate</td>
<td>Direct</td>
<td>&gt;100 ng/mL in urine</td>
</tr>
<tr>
<td>Fatty Acid Ethylester</td>
<td>Direct</td>
<td>&gt;0.5 ng/mg of hair</td>
</tr>
</tbody>
</table>

Temporal window of biomarker detectability

EtS, ethyl sulfate; EtG, ethyl glucuronide; % CDT, carbohydrate-deficient transferrin; PEth, phosphatidylethanol; MCV, mean corpuscular volume; GGT, gamma glutamyltranspeptidase; FAEE, fatty acid ethyl esters

* The definition of low and moderate drinking in pregnant women greatly varies among studies.
Direct Markers:
FAEE: Fatty acid ethyl ester
PEth: Phosphatidyl ethanol
EtG: Ethyl glucuronide
EtS: Ethyl sulfate
PLD: Phospholipase D

Maenhout et al.
Clin Chim Acta 2013; 415: 322-329
**Gamma-glutamyltransferase (GGT): Limitations**

- GGT may also be elevated in a person taking any barbiturate, in non alcoholic liver diseases, cardiovascular disease, obesity and individuals with high lipids.

- Therefore sensitivity of GGT is approximately 40% and specificity approximately 80%.

- However, GGT has more sensitivity and specificity than other liver enzymes.

Mean Corpuscular Volume (MCV)

- The normal range of MCV is 86-98 fl.
- Excessive alcohol consumption increases MCV by macrocytosis. If both GGT and MCV are elevated, it is likely that regular heavy alcohol consumption is the cause.
- Because, an erythrocyte remains in circulation on an average of 120 days it takes MCV about three months to return to normal level after abstinence.
- Upper limit of MCV in moderate drinkers was 98 fl (range 82-98 fl), abstainer was 96 fl (range 82-96) but as high as 104 fl in heavy drinkers according to one study.

Common Conditions that may Cause Macrocytosis

- Alcoholism
- Reticulocytosis
- Non alcoholic liver disease
- Hypothyroidism
- Vitamin B 12 deficiency
- Folate deficiency
- Multiple myeloma
- Myelodysplastic syndromes
- Drug therapy (anticancer, anticonvulsant, certain antibiotics)
Drugs Associated with Macrocytosis

- **Anticancer Agents**
  - Cyclophosphamide, hydroxyurea, Methotrexate,
  - Azathioprine, Mercaptopurine, Cladribine,
  - 5-Fluorouracil, Cytosine Arabinoside

- **Anticonvulsants**
  - Phenytoin, Primidone, Valproic Acid

- **Antimicrobial/Antiviral**
  - Pyrimethamine, Sulfamethoxazole, Trimethoprim,
  - Valacyclovir, Zidovudine,, Stavudine

- **Hypoglycemic**
  - Metformin

- **Other Drugs**
  - Triamterene, Sulfasalazine, Nitrous Oxide

Carbohydrate-Deficient Transferrin

- Transferrin consists of 679 amino acids and approximately 6% of the transferrin molecule is carbohydrate. Sialic acid, an integral component of transferrin, is a monosaccharide.

- Tetrasialotransferrin represents approximately 80% of all transferrin molecules in a healthy individual. Other transferrin molecules found in blood may have more (5-8 sialic acid moieties) or less (0-3 sialic acid moieties).

- Acetaldehyde interferes with the incorporation of sialic acid to transferrin, resulting in transferrin molecules containing zero, one, or two sialic acid moieties.

- Collectively disialotransferrin, monosialotransferrin and asialotransferrin are called carbohydrate deficient transferrin. In alcoholics carbohydrate deficient transferrin represents >1.6% of all transferrin.
Beta-Hexosaminidase

- N-Acetyl-β-hexosaminidase (β-Hex) is a lysosomal hydrolase which is present in most cells and involved in metabolism of carbohydrate and gangliosides.
- Heat stable β-Hex (isoform B, I and P containing two β chains; collectively called β-Hex B) in serum and total β-Hex (may also contain heat labile A and S isoforms) in urine are sensitive markers for alcohol abuse (sensitivity: 94%, specificity: 91%).
- Drinking 4-5 drinks per day (>60 gm of alcohol) for 10 days significantly increases β-Hex activities.
- Even after ingesting a large quantity of alcohol (120-160 gm), β-Hex activity increases significantly in serum urine and saliva.
- Levels return to normal in 7-10 days after abstinence.

Acetaldehyde-Protein Adducts

- Acetaldehyde is highly reactive and forms stable adducts with albumin, and hemoglobin mostly in chronic alcoholics.
- Aldehyde-hemoglobin adducts are found in blood and urine.
- Aldehyde hemoglobin adducts formed within 30 minutes of drinking. This adduct is reversible adduct.
- The reversible adducts can be detected up to 48 hours after last drink. Then it is converted into irreversible adduct which does not break down into hemoglobin and acetaldehyde. The stable adducts accumulates in the blood of chronic drinkers and remains detectable up to 28 days.
Acetaldehyde-Protein Adducts

- Hemoglobin-acetaldehyde adduct concentration is higher in males than females because males usually have higher blood level of hemoglobin. In addition, hemoglobin-acetaldehyde concentrations are significantly higher in alcohol abusers than non-drinkers.

- Measurement of hemoglobin-acetaldehyde adduct in human blood is difficult. Initial techniques involved high performance liquid chromatography with isoelectric focusing gel and affinity chromatography, but very low level of such adduct pose a technical challenge. An ELISA assay is recently available.
Ethyl Glucuronide and Ethyl Sulfate
Ethyl Glucuronide and Ethylsulfate

- Ethyl glucuronide and ethyl sulfate are minor metabolites of alcohol which are found in various body fluids and in hair.
- After drinking ethyl glucuronide can be detected up to 36 h in serum and five days in urine.
- Regular drinkers may also have elevated levels of ethyl glucuronide in hair and may present for 4 weeks.
- Urinary ethyl glucuronide concentration of 500-1000 ng/mL could be from previous drinking and a value over 1000 ng/mL indicate recent drinking.

Ethyl Glucuronide

- In one study healthy people who ingested one standard drink showed a maximum ethyl glucuronide level of 3.7 mg/L in serum and ethyl glucuronide was detected eight hours after alcohol was completely eliminated from the body.

- In serum samples of non-drinkers, no ethyl glucuronide can be detected. In 37 out of 50 drivers suspected of driving under the influence of alcohol, serum ethyl glucuronide concentration ranged from 0.1 to 20 mg/Liter and if level exceeds 5 mg/L, it can be assumed that the person is abusing alcohol.

Ethyl Glucuronide and Ethylsulfate

- Because alcohol is produced by bacterial action after death, ethyl glucuronide and ethyl sulfate are postmortem markers of antemortem alcohol ingestion.
- If ethyl glucuronide especially ethyl sulfate (more stable) cannot be detected in postmortem body fluid but ethanol is positive, it indicates postmortem production of alcohol.

Ethyl Glucuronide : False Positive

- Use of ethanol based hand sanitizer can cause detectable level of ethyl glucuronide and sulfate in urine and excessive use of ethanol based hand sanitizer level may cause levels over cut off of 1000 ng/mL.

- Use of ethanol based mouthwash, non alcoholic drink (may contain small amount of alcohol) and even very ripe banana may cause false positive ethyl glucuronide level in urine.

- Ethyl sulfate are better indication of alcohol consumption.

Ethyl Glucuronide in Hair

- Ethyl glucuronide and sulfate in serum, urine and hair can be detected only by GC/MS or LC/MS/MS.

- In one recent study, using 12 males and 32 females, the authors concluded that persons who ingested 16 to 32 gm of alcohol (one to two glasses of wine each day), for 3 months had hair ethyl glucuronide level less than 30 pg/mg, the set limit of over-consumption of alcohol.

- Interestingly subjects who consumed only one drink a day (16 gm/day), had ethyl glucuronide concentration in hair < 7 pg/mg, the cut off abstinence.

Fatty Acid Ethylester

- Fatty acid ethyl esters are direct markers of alcohol abuse because they are formed due to chemical reaction between fatty acids and alcohol. The chemical reaction between alcohol and fatty acid is mediated by fatty acyl ethyl ester synthase (FAEE synthase).

- Fatty acid ethyl esters are formed primarily in the liver and pancreas and then are released into circulation. These compounds are also incorporated into hair follicle through sebum and can be used as a biomarker of alcohol abuse.

- There are four major fatty acid ethyl esters; ethyl myristate, ethyl palmitate, ethyl stearate, and ethyl oleate.
Meconium fatty acid ethyl esters in ship fetus

Concentration of different FAEE species in meconium from ethanol-exposed and control fetuses. Data are presented as mean +/- 2 SEM, n = 15 per treatment group (**p<0.01, ***p<0.001), ethanol administered daily in third trimester in ship at a dose of 0.75 g/kg.

Zelner et al. PloS one 2013; 8: e59168
Oxidative and non-oxidative pathways of ethanol metabolism

Oxidative

Ethanol

- Alcohol Dehydrogenase
- Microsomal Ethanol Oxidizing System
- Catalase

Acetaldehyde

- Aldehyde Dehydrogenase

Acetate

Non-oxidative

Fatty Acid

- FAEE Synthase
- Fatty Ethyl Ester
- Phosphatidylcholine

Phosphatidylethanol

Phospholipase D

Average FAEE in chronic alcohol abuser was 15,086 ng/mL compared to Chronic abuser, 4,250 ng/mL. Controls had no detectable FAEE. Ethyl palmitate and ethyl oleate were major components. Ethyl oleate is found in higher amounts in chronic alcohol abuser than binge drinkers.

Kaphalis et al. Alcohol 2004; 34: 151-158.
In one study, the authors concluded that strict abstinence is excluded at FAEE >0.2 ng/mg of hair, or ethyl glucuronide at >7 pg/mg.

FAEE consists of ethyl myristate, palmitate, oleate and stearate as determined by GC/MS. Ethyl glucuronide was determined by liquid chromatography/mass spectrometry.

Moderate social drinkers should have FAEE <0.5 ng/mg of hair and ethyl glucuronide <25 pg/mg.

Above these cut-offs alcohol abuse is probable.

Pragst et al. Ther Drug Monit 2008; 30: 255-263.
Emerging Biomarkers
Emerging Biomarkers of Alcohol

- Phosphatidyl ethanol
- 5-Hydroxytryptophol
- Plasma Sialic acid index of Apolipoprotein J
- Total Sialic acid in plasma
Phosphatidyl ethanol

- Phosphatidyl ethanol is a unique direct alcohol biomarker formed in cell membranes only in the presence of ethanol.
- The reaction is catalyzed by phospholipase D.
- In blood phosphatidyl ethanol is associated with erythrocytes and measured in whole blood.
- This marker is not detectable after a single drink, the threshold of detectable level in blood is 1000 gm of alcohol with a mean daily intake of 50 gm.
- Once positive, it remains positive for 2-3 weeks.
- This test is considered positive at a value over 20 ng/mL.

Skipper et al Alcohol Clin Exp Res 2013 [e-pub ahead of print].
5-Hydroxytryptophol

ó 5-Hydroxytryptophol (5-HTOL) is a minor metabolite of serotonin (5-hydroxytryptamine) but the major metabolite is 5-hydroxyindoleacetic acid (5-HIAA). 5-HTOL is excreted in urine mostly as glucuronide conjugate but 5-HIAA is secreted unchanged.

ó Alcohol and its metabolite acetaldehyde affect the metabolism of serotonin causing increased level of 5-HTOL.

ó Increased level of conjugated 5-HTOL in urine indicates recent drinking and this marker is elevated up to 24 h after recent drinking.

ó Sometimes ratio of 5-HTOL conjugate and 5-HIAA is measured in urine to compensate for urine dilution and this marker is a measure of sobriety.
5-Hydroxytryptophol/5-hydroxyindoleacetic acid Ratio

- These markers are usually measured by liquid chromatography combined with tandem mass spectrometry.

- In one study, the mean ratio of glucuronide conjugate of 5-HTOL to 5-HIAA was 5.1 nmol/μmol (range: 2/5-10.5) in non-drinkers vs 88.5 nmol/μmol (range: 15.3-382) in drinkers.

- The authors speculated that a ratio over 15 nmol/μmol indicates recent drinking.

Plasma Sialic acid index of Apolipoprotein J

- Apolipoprotein J which can transfer cholesterol from one lipoprotein to another.
- Apolipoprotein J contains high amount of sialic acid and alcohol consumption interferes with incorporation of sialic acid in apolipoprotein J molecule.
- Sialic acid index of apolipoprotein J is a sensitive and specific marker for heavy alcohol consumption but additional research is needed to fully understand its utility as an alcohol biomarker.
Total Sialic acid in plasma

- Sialic acids are family of 36 acetylated derivatives of neuraminic acid which are found in carbohydrate chains of glycoprotein in biological fluids such as blood and cell membranes. The most abundant sialic acid is N-acetylneuraminic acid.

- Because alcohol consumption interferes with incorporation of sialic acid in various glycoproteins, measuring total sialic acid in plasma may be useful as an alcohol biomarker, but more research is needed.
# Application of Various State Markers

<table>
<thead>
<tr>
<th>State Marker</th>
<th>Application</th>
</tr>
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<tbody>
<tr>
<td>Gamma-glutamyl transferase</td>
<td>Heavy Consumption</td>
</tr>
<tr>
<td>Mean corpuscular volume</td>
<td>Heavy Consumption</td>
</tr>
<tr>
<td>Carbohydrate deficient transferrin</td>
<td>Heavy Consumption</td>
</tr>
<tr>
<td>Beta-Hexosaminidase</td>
<td>Heavy Consumption</td>
</tr>
<tr>
<td>Apolipoprotein J</td>
<td>Heavy Consumption</td>
</tr>
<tr>
<td>Fatty acid ethyl ester</td>
<td>Recent Heavy Use</td>
</tr>
<tr>
<td>Ethyl Glucuronide</td>
<td>Monitoring Sobriety</td>
</tr>
<tr>
<td>Ethyl Sulfate</td>
<td>Monitoring Sobriety</td>
</tr>
<tr>
<td>5-Hydroxytryptophol</td>
<td>Monitoring Sobriety</td>
</tr>
</tbody>
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Tait Markers of Alcohol

- Adenyl Cyclase Activity: The activity of this protein found in cell membrane and platelets is lower in non-drinkers than drinker. However, marijuana can decrease the activity.

- Gamma-Aminobutyric Acid: Alcoholics have lower level of this neurotransmitter than non-drinkers.

- Beta-Endorphin: Alcoholics have lower level of this neurotransmitter than non-drinkers. In addition, children of alcoholics have fewer opiate receptors.

- In addition polymorphism in alcohol dehydrogenase. Acetaldehyde dehydrogenase, dopamine receptors, glutamate, serotonin, neuropeptide Y and its receptor genes and ACN9 gene all may play important role in genetics of alcohol dependance.
Conclusions

- One standard drink contains approximately 14 gm alcohol. Moderate drinking (up to 14 drinks per week for males and 7 for females, but up to 7 drinks for both sex over 65 years of age) has many health benefits but exceeding these levels may cause alcohol related organ damage most commonly liver damage.

- Consuming two standard drinks by a male or one by a female with food in one evening should produce blood alcohol well below 0.08%.

- Among all biomarkers of alcohol abuse, GGT and MCV are routinely determined in routine blood analysis.

- %CDT is a good marker for screening alcoholics as well as monitoring abstinence and there are several easy to use FDA approved assays for application in clinical laboratories.

- Assays are also available for determining beta-hexoaminidase B activity using spectroscopy but determination of other markers such as FAEE, ethyl glucuronide and ethyl sulfate require chromatographic methods.

- There are several emerging slate markers and genetic markers for alcohol abuse which are under development.
THANK YOU