Biological markers for abuse and addiction

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Denial is the feature of the alcoholism

- Patient’s history.
- Family
- Psychologic examination
- Laboratory markers of alcohol abuse.
Conventional laboratory markers of alcohol abuse

- GGT.
- AST/ALT ratio.
- mean erythrocytes corpuscular volume (MCV).

sensitivity 27-52%
specificity 85-90%
Innovative markers of alcohol abuse

- **Sialic acid deficient protein:**
  - transferin, \( \alpha \)-acidglycoprotein

- **Enzymatic systems:**
  - phosphatidylcholine hydroperoxide (PCOOH)

- **Direct ethanol metabolites**
  - fatty acid ethyl ester
  - ethyl glucuronide
  - phosphatidyl ethanol
  - ethyl sulfate
Carbohydrate-deficient transferrin - CDT

- CDT – one of the most sensitive and specific laboratory markers of alcohol abuse.
- Alcohol causes deficiency of sialic acid - measurement of this defect is marker of alcohol abuse.
- Half-time: 12 days cut-off value: 2.5% – 3% of CDT
CDT – characteristics

- Specificity 70-80%
- False positivity 20-30%
- Stability in serum 4C – week -20C 6 months
  - Serum separated - preferably during 4 hr
- RIA, HPLC, turbidimetry
- CDT – more specific then GGT
- CAVE - genetic variation, congenital disorders of glycosilation
- Disorders with transferin increase – pregnancy, oestrogen use, contraceptive use, iron deficiency anemia, anti-epileptic drug therapy – hepatic drug affects
%CDT in cirrhotic patients active alcohol abuse, control patients with cirrhosis

Box Plot
Split By: etyl

P=0.003
%CDT in patients without cirrhosis - active alcohol abuse, control patients

Box Plot
Split By: etyl

%CDT

P = 0.0009

controls  active abuse
MCV in patients without cirrhosis -active alcohol abuse, control patients

Box Plot
Split By: etyl

controls  active abuse

MCV

Units
70 75 80 85 90 95 100 105 110 115

P = 0.0348
GMT in patients without cirrhosis - active alcohol abuse, control patients

Box Plot
Split By: etyl

GMT

P = n.s. (0.5802)
Alcoholic hepatitis

- Alcoholic hepatitis diagnosis
  - Physical examination
  - ALT, AST, bilirubin elevated
  - Na, albumin low
  - INR and leukocytes elevated
New markers I

- **Fatty acid ethylester**
  - Non-oxidative metabolites of esterification of ethanol with free and bound fatty acids GC-MS
  - FAEES separate social drinkers from heavy
  - Detection serum up to 24 hrs (hairs – months)

- **Ethylglucuronide**
  - Direct metabolite of ethanol
  - Detection urine up to 8 hrs (hairs – months)

- **Phosphatidylethanol**
  - Ethanol-phosphatidyl adduct via action with phospholipase D
New markers II

- **5-OH-tryptophol**
  - Minor metabolite of serotonin
- **Sialic acid**
  - Total and Free sialic acid in serum increase
- **Beta-hexosaminidase**
- **Blood acetate**
- **Acetaldehyde adducts**
  - MS/MS methods for acetaldehyde modified Hb
- **Dolichol**
CDT and EtG

- CDT and EtG – established indicators of chronic alcohol abuse
  - Positivity 40-60 g EtOH per day
  - 1-2 weeks increase CDT
  - In 50-60% heavy drinkers
  - Increasing to 70-80% in alcoholics
Other biochemical parameters

GGT – WHO project increases 58% alc dependent,
  • Not sesnsitive to screen

  • IgA increase 69% alcohol liver diseases

  • Lipids – HDL, TG 80% increase heavy drinkers
  • Uric acid
Usefull parameters

- Combination GGT and CDT – most useful
- CDT – more specificity
- GGT – low sensitivity
- Specificity – negative test – negative diagnosis
  \[ \frac{TP}{TP + FP} \]
  0.95 very good, higher 0.7 acceptable
- Sensitivity – positive test – positive diagnosis
  \[ \frac{TP}{TP + FN} \]
Drug abuse analysis I

• **Basic laboratory parameters**
  • Sodium, potassium, creatinine, glucose, creatine kinase etc.

• **Toxicological analysis**
  – Only in some drugs
  – Metabolites
Drug abuse analysis II

- Saliva, Urine, Blood, Hair

- Point-of-care testing
  - Oriented, not analytically precise
Hair analysis I

- Diagnosis of drug abuse
- Control of treatment
- Doping control
Hair analysis II

- Hair analysis for drug and alcohol abuse
  - Canabis – THC
  - Morphine – heroine
  - Codeine – heroine
  - Methadon
  - Cocaine – benzoylecgonine
  - Crack – AEME
  - Amphetamine
  - LSD
  - GHB
  - Ethanol – FAEE, Etg
Candidate genes and alcoholic diseases

Mechanisms
- Alcohol toxicity
- ROS
- Immune response/inflammation
- Activation of stelate cells
- Collagen – synthesis/degradation

Candidate genes
- ADH, AIIDH, CYP2E1
- CYP2E1, GST, MPO, MnSOD
- TNFα, INFγ, IL-10, IL-1β, CD14
- TGFβ, leptin, CTGF, angiotensinogen
- TIMP-1, MMP-3,9

Results conflicting - Not reproduceable data

Initial euphoria has faded

Stickel 2006
Signal transduction and ethanol toxicity

Signal transduction mechanisms of ethanol toxicity are not well understood

Central role of MAPK and NF-κB

- Differentiation
- Development
- Apoptosis
- Inflammation

• Proapoptotic – JNK, c-jun, p38 via AP-1
• Antiapoptotic – ERK, NF-κB (IAP-inhibitory apoptosis protein)
Proteomics

- Biomarkers – 24 studies MALDI or SELDI-TOF MS
- Alcohol abuse
  - Increase fibrinogen α chain
  - Decrease apolipoprotein A-I and AII
    - Nomura et al 2004
- Hepatocellular carcinoma
  - Decrease des-Ala-fibrinopeptid A
    - Orvisky et al 2006
Conclusions

- %CDT is the most suitable biochemical marker of alcohol abuse in routine practice and combination with basic biochemistry and hematological examination can increase its credibility.
Abstinence from alcohol

• The knowledge of an abstinence is a basic information for decision in the treatment strategy.

• The prognosis in long term sobriety is excellent.
Thank you for your attention