HOW TO EVALUATE ELEVATED LIVER ENZYMES

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Presented at the Pre-workshop Educational Course, Milan, Italy, 1 June 2011
LABORATORY TESTS OF THE LIVER

PURPOSE

➢ To Assess if Liver Injury is Present

➢ To Evaluate the Functional Status of the Liver

➢ To Determine or Suggest the Etiology of Disease

➢ To Provide Prognostic Information About the Disease or the Treatment
LIVER CHEMISTRY PROFILES

- ALT (SGPT)
- AST (SGOT)
- Alkaline Phosphatase
- GGT
- Bilirubin
- Albumin
# CLASSIFICATION OF INJURY PATTERNS

<table>
<thead>
<tr>
<th>Hepatocellular</th>
<th>Cholestatic</th>
<th>Mixed</th>
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<tbody>
<tr>
<td>ALT</td>
<td>Bilirubin</td>
<td>All with Similar Abnormalities</td>
</tr>
<tr>
<td>AST</td>
<td>Alkaline Phosphatase</td>
<td></td>
</tr>
<tr>
<td></td>
<td>GGT</td>
<td></td>
</tr>
</tbody>
</table>

R score: ALT/Alk Phos expressed as multiple of upper limit of normal range
R>5= Hepatocellular; R= 2-4.99= Mixed; R<2= Cholestatic

Danan et. al. J CLIN EPI, 1993
ACUTE VS. CHRONIC

• Acute
  – Resolved within 6 months of onset or
  – Resolved following drug discontinuation

• Chronic
  – Persists beyond 6 months
<table>
<thead>
<tr>
<th>GRADE</th>
<th>DEFINITION</th>
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<tbody>
<tr>
<td>0</td>
<td>Normal-1.24 x ULN</td>
</tr>
<tr>
<td>1</td>
<td>1.25-2.5 x ULN</td>
</tr>
<tr>
<td>2</td>
<td>2.5-5 x ULN</td>
</tr>
<tr>
<td>3</td>
<td>5-9.99 x ULN</td>
</tr>
<tr>
<td>4</td>
<td>&gt;10 x ULN</td>
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</tbody>
</table>
HY’s RULE

• Bilirubin >3 mg/dl AND AST > 20 x ULN
• When present, chance of death from fulminant hepatic failure or need for liver transplantation =10%- 50%
• Validated by Bjornsson et. al (Hepatology August 2005;42(2);481-9)
THE HYPERSENSIVITY RULE

• ALT > 2 x ULN AND Hypereosinophilia
• Defines presence of hypersensitivity (immune mediated) liver injury
LIVER FUNCTION TESTS

- Prothrombin Time
- Bromosulphophthalein (BSP)
- Indocyanine Green
- MEG-X
- Aminopyrine
- Caffeine
- Midazolam
<table>
<thead>
<tr>
<th>ORDERING LOCATION: IM</th>
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<tr>
<td>ORDERING PHYSICIAN: SHERMAN, KENNETH E.</td>
<td>EPISODE ID:</td>
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<tr>
<td>PRINT DATE: 11/23/94</td>
<td>TIME: 2131</td>
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<table>
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<tr>
<th>PATIENT NAME</th>
<th>NUMBER</th>
<th>WARD</th>
<th>BED</th>
<th>AGE</th>
<th>SR</th>
<th>PHYSICIAN</th>
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<td></td>
<td>805059</td>
<td>IM</td>
<td>MAN</td>
<td>54</td>
<td>MC</td>
<td>SHERMAN, KENNETH</td>
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</tbody>
</table>

**CHEMISTRY**

<p>| | | | | |</p>
<table>
<thead>
<tr>
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</thead>
<tbody>
<tr>
<td>GAMMA</td>
<td>GT</td>
<td>8-40</td>
<td>HEPATIC</td>
<td>3.5-5.0</td>
</tr>
<tr>
<td>61</td>
<td>MIU/ML</td>
<td>HEPATIC</td>
<td>3.5-5.0</td>
<td>0.1-1.1</td>
</tr>
<tr>
<td>ALB</td>
<td>GM/DL</td>
<td>4.6</td>
<td>0.72</td>
<td>46.9</td>
</tr>
<tr>
<td>BILI</td>
<td>MG/DL</td>
<td>7-35</td>
<td>10-30</td>
<td>29.0</td>
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</table>

**DATE TIME**

| 11/23/910 | 166.9 |
| 11/23/910 | 89    |

(END OF REPORT)
SERUM TRANSAMINASES

Key Facts

- Injured Cells Leak Contents into Circulation
- ALT Found Primarily in Cytosol of Hepatocytes
- AST Found in Both Cytosol and Mitochondria
**TRANSAMINASE REACTION**

**ALT**

**Step 1**

\[
\text{Alanine} + \text{a – KETOGLUTERATE} \leftrightarrow \text{GLUTAMATE} + \text{PYRUVATE} \quad (\text{PLP})
\]

**Step 2**

\[
\text{NADH} \quad \text{NAD} \quad \text{PYRUVATE} \leftrightarrow \text{LACTIC ACID} \quad (\text{LDH})
\]
ENZYME ACTIVITY

Factors Affecting Analysis

- Concentration of Enzyme
- Concentration of Substrate
- Temperature
- Serum Solutes
- Interfering Substances
- “Control” Standards

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LABORATORY DETERMINATION OF NORMAL RANGE

Methods

➢ Samples are Taken from “Healthy” Population

➢ Arbitrary Cutoffs
  Standard Deviation Based
  Percentile Based
ALT: NORMAL DONOR POPULATION

Sherman et. al, JID 1982
EXTRINSIC FACTORS
Defining Normality

➢ Sex Distribution
➢ Age Distribution
➢ Season
➢ Race
➢ Weight
➢ Marital Status
ALT ABNORMALITIES
Geographic Distribution

Sherman et. al, JID 1982
DISTRIBUTION OF ABNORMAL ALT’S

AGE SPECIFIC PREVALENCE

Sherman et. al, JID 1982
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Updated ALT Ranges

Newly calculated healthy limits are indicated in each panel. A) Male participants. B) Female participants. To convert the alanine aminotransferase thresholds to nkat/L, multiply by 16,667.

LIVER TESTS

AST

Step 1

Aspartate + \( \text{AST} \) \( \rightleftharpoons \) Glutamate +
a – Ketoglutarate Oxaloacetate

Step 2

NADH NAD

Oxaloacetate \( \rightleftharpoons \) Malic Acid

MDH
RELATIVE TISSUE ACTIVITIES

Transaminases
LIVER TESTS
Alkaline Phosphatase

- A Group of Enzymes that Hydrolyzes Organic Phosphate Esters at an Alkaline pH
- Multiple Isoenzymes Exist
- Main Sources are Liver, Bone and Intestine
PATHOPHYSIOLOGY: LIVER TESTS

Alkaline Phosphatase

- Bile Acids (Increased in Cholestasis) Lead to de novo Synthesis of Alkaline Phosphatase

- Increased Alkaline Phosphatase Leaks Into the Circulation. The Mechanism for this is Poorly Understood
LIVER TESTS

Alkaline Phosphatase

\[ p - \text{Nitrophenol Phosphate} \iff p - \text{Nitrophenol} + \text{Phosphate} \]
ALKALINE PHOSPHATASE

Source of Elevation

- Liver, 80.8%
- Bone, 18.50%
- Intestine, 0.6%

Brensilver & Kaplan, GASTRO 1975
ALKALINE PHOSPHATASE
Liver Isoenzyme

Liver Disease, 65.6%
No Identifiable Liver, 34.4%

Brensilver & Kaplan, GASTRO 1975

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ALKALINE PHOSPHATASE
Relative Activity by Blood Group

Adapted from Bamford et. al., LANCET

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LIVER TESTS
Gamma Glutamyl Transpeptidase (GGT)

- Catalyzes the Transfer of Glutamyl Groups Between Peptides and Amino Acids
- Present in Many Tissues
  - Kidney
  - Pancreas
  - Liver
  - Spleen
  - Heart
  - Brain
  - Seminal Vesicles
LIVER TESTS

Pathophysiology: GGT

- Hepatic Microsomal Production Induced
  - Alcohol
  - Dilantin
  - Barbiturates

- Some drugs may cause leakiness of hepatocyte membranes

- Not a marker of liver damage. If elevated, helps confirm liver source for elevated alkaline phosphatase
LIVER TESTS

GGT

GGT

Gammaglutamyl – p – Nitroaniline $\iff$ p – Nitroaniline + Glycylglycine

p – Nitroaniline is a chromogen whose appearance is to be measured in a spectrophotometer.
BILIRUBIN

CONJUGATED
Biliary Obstruction
Hepatitis
Cirrhosis
Drug Reaction
Ductular Atrophy
BRIC

UNCONJUGATED
Hemolysis
Gilbert’s Syndrome
Crigler-Najjar Syndrome
PROFICIENCY TESTING
Unsatisfactory Rates

% Unsatisfactory

ALT | AST | Bilirubin | Alkaline Phosphatase

Hospital/Ind. Labs | Other Sites

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“I’ll want to run a few tests on you, just to cover my ass.”
Acute AST, ALT elevation

- Anti-HAV IgM
- HBsAg
  - HCV RNA
  - HBV DNA
  - Anti-HBc IgM
  - Anti-HDV if HBsAg+
- Consider
  - Nonhepatotrophic viruses
    - CMV, HSV, EBV, VZV
- Consider
  - drugs
- Examine for extrahepatic lesions
  - Blood culture for *Bartonella*, CMV, fungus, AFB
  - PCR blood for *Bartonella*, HSV, CMV, EBV, VZV
  - Consider Biopsy
- Amylase, lipase, lactate

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Chronic AST, ALT elevation
(>6 months)

- Anti-HCV
  - HCV RNA
    - Consider biopsy
  - Consider biopsy

- HBsAg or Anti-HBc alone
  - HBeAg
    - HBV DNA
      - Consider biopsy
      - Consider biopsy
  - Consider biopsy

- Review medications, alcohol use, temporal pattern
  - Consider discontinuing medication
  - No response at 3 months
    - Consider biopsy

- Fe studies
  - Alpha-1 antitrypsin
  - Ultrasound (Fatty liver)
  - Ceruloplasmin, ANA
  - Consider biopsy
Elevated bilirubin, and/or alkaline phosphatase, and GGT

- <50% direct
  - Gilbert Syndrome
  - Hemolysis
  - Protease inhibitors
  - Ducts dilated
    - ERCP with brushing, biopsy, dilation, stone removal, and/or stent placement
    - Discontinue medication
    - Observe for 3 months

- >50% direct
  - Ultrasound
  - Ducts not dilated
    - Duct associated with cholestasis
    - Diffuse change or increased echogenicity
    - ERCP

- Mass lesion
  - Biphasic CT or MRI
  - HCC, KS, lymphoma, metastatic CA, Cholangiocarcinoma
  - Hemangioma, adenoma, focal nodular hyperplasia, focal fat
  - Monitor q 4-6 months
  - Consider US; biopsy

Consider liver biopsy

Discontinue medication

Bile duct injury or bile stasis
TESTS WHEN HCV IS PRESENT

- Viral load
- HCV Genotype
- ?IL28b