Toxicology Case Studies
Acetaminophen and Liver Function

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Learning Objectives

• Describe Acetaminophen metabolism in normal and overdose settings
• Explain how Acetaminophen toxicity is treated
• Outline typical liver function test results seen in Acetaminophen toxicity
• Identify interferences with common acetaminophen assays
• Recognize the cause of hepatic toxicity given results to common laboratory tests
Case #1

• 39 year old Female presented to the ED awake and alert with mild Right Upper Quadrant pain.

• She reports that > 24 hours ago she ingested a large number of pills:
  – 20-30 Tylenol PM

• Past Medical History includes bipolar disorder and schizophrenia with previous suicide attempts, cocaine abuse
Case #1 Pertinent Laboratory Values

• Labs at ED Admission:
  - Na 134 (135-145 mEq/L)
  - K 3.3 (3.3 – 4.8 mEq/L)
  - Bicarb 14 (23 – 30 mmol/L)
  - Cl 103 (95 – 105 mEq/L)
  - BUN 9 (5-25 mg/dL)
  - Creatinine 1.17 (0.7 - 1.5 mg/dL)
  - Glucose 350 (70 – 110 mg/dL)
  - T. Bilirubin 2.1 (0.2 – 1.2 mg/dL)
  - AST 468 (4 – 40 U/L)
  - ALT 100 (4-40 U/L)
  - ALP 100 (4 – 110 U/L)
  - PT 21.7 (11.8-14.5 sec)
  - INR 2.0

• Urine Drug Screen: Acetaminophen positive (Cut-off 300 ng/mL) ; Tricyclic Antidepressants – positive (cut-off 300 ng/mL); Cocaine positive (cut-off 300 ng/mL)

• Confirmation – Acetaminophen, Diphenhydramine, and Cocaine

Ethanol, salicylates, opiates, Cannabinoids, and amphetamines negative
N-acetyl-p-aminophenol (paracetamol)
- Anti-inflammatory, analgesic, antipyretic
- Competitive inhibition of cyclooxygenase enzymes
- Liquids, tablets & capsules, pediatric formulations
- Combined with Diphenhydramine (Tylenol PM), codeine (Tylenol with Codeine), oxycodone (Percocet), and hydrocodone (Vicodin).
  - Jan 2011 FDA limits combination narcotics to 325 mg APAP
    • Constitutes >50% overdoses
  - May 2011 all OTC liquids standardized to 160 mg/5 mL
- Widely prescribed across all age groups
  - most common non-prescription medication
- Toxic dose about 150 mg/kg (7g in adult)
## Stages of Acute Acetaminophen Toxicity

<table>
<thead>
<tr>
<th>Stage</th>
<th>Time Post-ingestion</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0–24 h</td>
<td>Anorexia, nausea, vomiting</td>
</tr>
<tr>
<td>II</td>
<td>24–72 h</td>
<td>Right upper quadrant abdominal pain (common) AST, ALT, and, if poisoning is severe, bilirubin and PT (usually reported as the INR) sometimes elevated</td>
</tr>
<tr>
<td>III</td>
<td>72–96 h</td>
<td>Vomiting and symptoms of liver failure Peaking of AST, ALT, bilirubin, and INR Sometimes renal failure and pancreatitis</td>
</tr>
<tr>
<td>IV</td>
<td>&gt; 5 days</td>
<td>Resolution of hepatotoxicity or progression to multiple organ failure (sometimes fatal)</td>
</tr>
</tbody>
</table>

- Toxic dose about 150 mg/kg (7g in adult)
Acetaminophen Toxicity

Rumack-Matthew Nomogram

- Severe liver damage likely
- Probable risk: give antidote
- Increased risk in alcoholics, malnutrition or enzyme induced

APAP (μg/mL) vs. hours after ingestion

Courtesy of S. Dawling
Acetaminophen Metabolism

- **Acetaminophen**
  - Sulfotransferase: APAP-Sulfate (30% H$_2$O Soluble)
  - UDP-GT: APAP-Glucuronide (50 - 60% H$_2$O Soluble)
  - Hepatic oxidation (P450 (CYP2E1) 10%)

NAPQI

- Glutathione-S-transferase: APAP-Mercapturic Acid and APAP-Cysteine

- Hepatic and renal damage
Acetaminophen Toxicity - High-Risk Groups

- Glutathione depleted
  - malnutrition, anorexia, alcoholic, AIDS
- Cytochrome P450 (CYP2E1)-induced - increased production of NAPQI
  - ethanol
  - rifampicin (anti-TB)
  - anticonvulsants - phenytoin, phenobarbital, carbamazepine, oxcarbazepine

Inability to conjugate NAPQI with glutathione (depleted or too much NAPQI) results in toxicity.
Treatment of Acetaminophen Overdose

- Oral activated charcoal if recent
- NAC (N-acetyl cysteine) or methionine
  - Glutathione substitute
  - Methionine can be given orally
  - NAC orally or intravenous
  - Most effective if given within 8 hours of ingestion
  - Efficacy declines 18 – 24 hours after ingestion
- NAC has other advantages
  - Combines directly with NAPQI
  - Enhances sulfate conjugation- provides sulfhydryl groups
  - Anti-inflammatory, anti-oxidant
  - Positive inotropic and vasodilating effects that improve microcirculation and oxygenation
Outcome/Prognostic Measures

**Poor Prognosis – 24 – 48 hours post ingestion**
- Encephalopathy-(Confusion, somnolence, or coma)
- Coagulopathy (INR >3)
- Elevated bilirubin
- Renal Failure (Creatinine >2.6)
- Metabolic Acidosis
- Hypoglycemia

**Good Prognosis**
Liver Function Tests Return to baseline
The Liver - Anatomy and Functions

**Synthetic**
- Carbohydrates
- Cholesterol
- Lipoproteins
- Hormones
- Serum Proteins
- Clotting Factors

**Metabolic**
- Ammonia
- Bilirubin
- Drugs
- Toxins
- Carbohydrates
- Fat
- Hormones
- Amino Acids
Liver Lobule Histology

Lobule

Portal Triad (Artery, Vein, Bile Duct)

Central vein

Connective Tissue

Courtesy of S. Dawling
APAP Toxicity Causes Centrilobular necrosis

• NAPQI binds hepatic cellular and membrane proteins inducing centrilobular damage

• NAPQI protein adduction results in oxidative and inflammatory damage leading to necrosis

• Staining of Rat liver post high dose APAP for NAPQI-protein adducts

Roberts, et al., AJP, 1991
Laboratory Analysis of Liver Function/Injury
Liver Function Tests (LFTs)

LFTs - misnomer – due to large functional reserve

- Conjugation or Excretion Impairment
  - Bilirubin
- Loss of Synthetic Capacity
  - Albumin
  - Prothrombin time (PT)
- Hepatocyte Damage
  - AST and ALT
- Canaliculular Membrane Damage
  - GGT and ALP

In Practice
1. Establish abnormal results
2. Elucidate cause by
   - Serology
   - Imaging
   - Liver biopsy
   - Tox Screen
# Enzymes and Liver Injury

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Tissue Expression</th>
<th>Sub-Cellular Localization</th>
<th>Half life (hours)</th>
<th>Clinical Utility</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>L, H, M, K</td>
<td>Cytoplasm, Mitochondria</td>
<td>16-18 (cyto), 87 (mito)</td>
<td>Cirrhosis, Early liver injury</td>
</tr>
<tr>
<td>ALT</td>
<td>L, K</td>
<td>Cytoplasm</td>
<td>40 - 48</td>
<td>Acute Liver Injury-(2 days+), Chronic liver disease</td>
</tr>
<tr>
<td>LDH</td>
<td>L, E, H, M, K, Others</td>
<td>Cytoplasm</td>
<td>4 - 6</td>
<td>Liver injury</td>
</tr>
<tr>
<td>ALP</td>
<td>L, B, I, PL</td>
<td>Canalicular Membrane</td>
<td>24</td>
<td>Bile duct obstruction, canalicular damage</td>
</tr>
<tr>
<td>GGT</td>
<td>L, P, K, PR</td>
<td>Canalicular Membrane, microsomes</td>
<td>336 (2 wks)</td>
<td>Canalicular damage, microsome inducing drugs</td>
</tr>
</tbody>
</table>

L, Liver; H, Heart; M, Muscle; K, Kidney; E, Erythrocytes; B, Bone; I, Intestine; Others (Leukocytes, tumors); PL, Placenta; P, Pancreas; PR, Prostate
Acute Toxic / Viral Hepatitis

Typically
- AST and ALT > 10x ULN
- AST > ALT (first 24 – 48 hours)
- ALT > AST (after 48 Hours)
- ALP < 3X ULN
- GGT > ULN with drug induced bile duct obstruction
- Liver damage is usually reversible
PT/INR and Acute Toxic Liver Injury

Sensitive measure of Impaired liver synthetic Function

Increase in PT/INR = Serious liver dysfunction

Prothrombin Time-
- Measures clotting time
- reflects the activity of factors I, II, V, VII, X

INR
- International Normalization Ratio
  \[ \text{INR} = \left( \frac{\text{Patient’s PT}}{\text{Normal PT}} \right)_{\text{ISI}} \]

AST and ALT During Therapy

NAC Therapy Begins

AST

ALT

Admission

Days
Synthetic Function With Therapy

N-Acetyl Cysteine Therapy Begins

PT (sec)
11.8 – 14.5

INR

Days After ED Presentation

Admission
APAP Concentration with Therapy

Days After ED Presentation

APAP μg/mL
(Normal: 5 – 30 μg/mL)
APAP Toxicity- Monitoring Therapy and Prognosis

- Timed APAP serum concentration to monitor for reduction
- ALT and AST begin to rise 24 hr., peak 48-72 hr.
  - severe toxicity >1000 U/L  ALT>AST
- PT/INR rise and Fall – poor prognosis if > 3 at 48 hours
- Bilirubin elevations – Our patient peaked at 2.3 (0.2 – 1.2 mg/dL)
- Check for signs of renal failure – elevated Creatinine, BUN, proteinuria
- **Metabolic acidosis** - develops once hepatic glycogen depleted and damaged hepatocytes cannot do oxidative phosphorylation, TCA cycle or make glucose
- Hypoglycemia
Acetaminophen Detection

Glynn & Kendall
• Measure formation of yellow colored product formed with nitrous acid
• Urine as is, plasma requires protein precipitation

Ortho-Cresol Condensation
• Many APAP metabolites are also hydrolyzed to p-aminophenol
  - increases sensitivity
  – Urine - use HCl
  – Plasma can be enzymatically converted
• Addition of copper sulfate improves color

Immunoassay
• APAP antibody - EMIT or FPIA for plasma
Acetaminophen Assay

Glynn & Kendall Color Test

Protein precipitation with 10% TCAA

\[
\begin{align*}
\text{Acetaminophen} & \\
\text{H-N-C-CH}_3 & \\
\text{OH} & \\
\text{O} & \\
\end{align*}
\]

\[
\begin{align*}
\text{H-N-C-CH}_3 & \\
\text{OH} & \xrightarrow{\text{HCl + NaNO}_2} \text{NH}_2 & \xrightarrow{\text{ammonium sulfamate}} \text{O}^- & \\
\text{Nitrous acid} & & \text{NaOH} & \\
\end{align*}
\]

Quantitate at 430 nm (yellow product)

Courtesy of S. Dawling
Acetaminophen Urine Assay

**O-cresol condensation-Color Test**

\[
\begin{align*}
\text{acetaminophen} & \xrightarrow{\text{HCl}} \text{p-aminophenol} \\
\text{o-cresol} & \xrightarrow{\text{Cu}^{2+}, \text{NH}_4^+} \text{Indophenol Dye (BLUE)}
\end{align*}
\]

**300 μg/mL Acetaminophen**

- **NAC**
- **+NAC**

**Also:**
- Ketones
- Ascorbic Acid

Quantitate at 600 nm

False Negative results with NAC

Courtesy of S. Dawling
NAC Interference with APAP Assays

- Enzymatic Assays Only
- Immunoassays are not affected
- Initial iv loading dose of NAC is 140 mg/kg
- Serum NAC can be >500 mg/L

Acetaminophen Serum Tests

**EMIT, FPIA or Enzymatic**

EMIT  | Antibodies  | APAP  
--- | --- | ---
FPIA | **Acetaminophen** | **Arylacylamidase**

\[
\text{H-N-C-CH}_3 \overset{\text{Arylacylamidase}}{\rightarrow} \text{H-N-C-CH}_3 + \text{CH}_3\text{COO}^{-}
\]

acetaminophen  \[\rightarrow\]  p-aminophenol

**Indophenol Dye (BLUE)**
quantitate at 600 nm

**Sodium Periodate**

\[
\text{O-H} \overset{\text{Sodium Periodate}}{\rightarrow} \text{O-H}
\]

\[
\text{CH}_3 \overset{\text{Sodium Periodate}}{\rightarrow} \text{CH}_3
\]

**Mn**

\[
\text{8-OHquinoline} \overset{\text{Mn}}{\rightarrow} \text{5-(4iminophenol)-8-OHquinoline}
\]

Courtesy of S. Dawling
Case #2

- 50 year old male presented to the ED with severe jaundice, and abdominal pain.
- Pertinent laboratory tests included:
  - AST 6370 (4 – 40 U/L)
  - ALT 6830 (4-40 U/L)
  - ALP 150 (4 – 110 U/L)
  - Tbili 198 (0.2 – 1.2 mg/dL)
  - PTT 39.7 (23 – 34 sec)
  - INR 3.3
- Acetaminophen 35 (5 – 30 μg/mL)
- NAC was administered. No improvement after 3 days
- Liver biopsy showed immune infiltrates indicative of autoimmune hepatitis
Bilirubin Interference- APAP Assays

- Interference is detected at Tbili > 10 mg/dL
- No significant affect on APAP concentration > 50 mg/L
- False positive APAP results may delay treatment for patients with liver failure

Case #3

• An 18yr old man presented to ED at 9am with complaint of nausea, vomiting, dizziness and abdominal cramps

• Claimed to have taken painkillers early yesterday afternoon and then proceeded to drink a bottle of wine

• Labs:
  – Na 149 mmol/L
  – K 3.6 mmol/L
  – Bicarb 30 mmol/L
  – BUN 36 mg/dL
  – Creatinine 1.4 mg/dL
  – pH 7.4
  – INR 1.6
  – AST 200 U/L
  – ALT 250 U/L

• Drug screen: Acetaminophen 85 mg/L; Ethanol 30 mg/dL

• salicylates, opiates, benzodiazepines, cocaine and amphetamines negative
APAP Interaction with Ethanol

- Increased NAPQI is produced in chronic alcoholics
- Acute ETOH ingesters less likely to suffer hepatotoxicity

Lee, WM. *NEJM*. 2003
Case #4

- 47 year old female brought by ambulance to the ED for lethargy.
- Family members reported a history of ingestion of 500 mg (18 tablets) APAP over the last 2 days.
- Pertinent Lab Results
  - BUN 9 (5 – 25 mg/dL)
  - Creatinine 0.9 (0.7 -1.5 mg/dL)
  - AST 5409 (4 – 40 U/L)
  - ALT 1085 (4 – 40 U/L)
  - Acetaminophen 12 (5 – 30 mcg/mL)
  - UDS: Opioids and Cannabinoids were positive

Case #4-Hospital Course

• Patient was promptly treated with NAC
• Developed fulminant hepatic failure
  – Elevated transaminases (AST peak = 11,840 U/L)
  – Hypoglycemia
  – Coagulopathy (INR peak 5.1)
• Sent for liver Transplant- LFTs better by day 12
• Acute Renal failure ensued
  – Elevated Creatinine  (8.1 mg/dL peak)
  – Elevated BUN    (106 mg/dL peak)

What is the cause of the renal Failure??
APAP induced Renal Toxicity

• Several Potential Mechanisms
  – Cytochrome P450 pathway (NAPQI)
  – Prostaglandin Synthetase (PGES)
  – N-Deacetylase enzymes

• Male predominance of nephrotoxicity – CYP2E1 is induced by testosterone

• NAC does not treat nephrotoxicity

• No relationship between APAP does or Hepatotoxicity and Nephrotoxicity

• Requires long term care – our patient was discharged at hospital day 25
# Hepatitis- Differential Diagnosis

<table>
<thead>
<tr>
<th>Feature</th>
<th>Toxic</th>
<th>Viral</th>
<th>Alcoholic</th>
</tr>
</thead>
<tbody>
<tr>
<td>% chronic</td>
<td>0%</td>
<td>variable</td>
<td>5 - 10</td>
</tr>
<tr>
<td>% Fatal</td>
<td>10%</td>
<td>&lt; 3</td>
<td>5 - 10</td>
</tr>
<tr>
<td>AST/ALT (diagnosis)</td>
<td>&gt; 1 *</td>
<td>&lt; 1</td>
<td>&gt; 2</td>
</tr>
<tr>
<td>Peak AST (X ULN)</td>
<td>&gt;100</td>
<td>10 – 100</td>
<td>1 – 10</td>
</tr>
<tr>
<td>LDH (x ULN)</td>
<td>10 – 40</td>
<td>1 – 2</td>
<td>1 - 2</td>
</tr>
<tr>
<td>Peak Bilirubin (mg/dL)</td>
<td>&lt; 5</td>
<td>5 – 20</td>
<td>3 - 20</td>
</tr>
<tr>
<td>Prothrombin time</td>
<td>&gt;15 S</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Adapted from Qazi and Dufour. *Contemporary Practice in Clinical Chemistry*. Ch 24. Clarke W Ed. 2011
Self Assessment Questions

1. Which Metabolite Causes Acetaminophen (APAP) induced hepatotoxicity?
   A. APAP-Glucuronide
   B. APAP-Sulfate
   C. N-Acetyl Cysteine
   D. N-Acetyl-p-benzoquinone imine

2. The Rumack-Matthew nomogram can be used to predict hepatic toxicity in which patients?
   A. Patients who overdosed on APAP 24 hours ago
   B. Patients with chronic APAP overdose
   C. Patients who overdosed on APAP 2 hours ago
   D. Patients who overdosed on APAP, Aspirin, and Opiates 24 hours ago

3. Some Acetaminophen assays are inaccurate in the presence of
   A. Bilirubin
   B. N-Acetyl Cysteine
   C. Ketones
   D. All of the Above

4. At 48 hours after APAP overdose, a patient is more likely to recover if:
   A. Total Bilirubin is >2.0 mg/dL
   B. AST > ALT
   C. The patients glucose is > 100 mg/dL
   D. The patient’s creatinine is > 2.5 mg/dL

5. Which of the following “liver function tests” is least likely to be elevated in drug induced toxicity?
   A. AST
   B. Bilirubin
   C. LDH
   D. PT