LIVER FUNCTION AND ASSOCIATED DISEASE STATES

Jenna Waldron
5th January 2016
THE LIVER

- Largest solid organ in the body
- Major role in protein, carbohydrate & lipid metabolism
- ~80% hepatocytes (functional unit) with mitochondria – site of many metabolic pathways (e.g. glycolysis, Krebs cycle, AA synthesis/degradation, oxidative phosphorylation)
- Extensive reticuloendothelial system for synthesis and breakdown of blood cells.
- Detoxifies and excretes end products of metabolism, as well as exogenous compounds.
FUNCTIONS OF THE LIVER

- **Protein Metabolism:**
  - Synthesis of most circulating proteins except δ-globulins (Igs)
  - Albumin, Transferrin, Caeruloplasmin, Acute phase proteins (e.g. CRP), α-1-anti-trypsin (α1AT), AFP, coagulation factors, components of complement.

- **Degradation:**
  - AAs degraded by transamination and oxidative deamination
  - Ammonia production (excreted by kidneys in form of urea).

- **Carbohydrate metabolism:**
  - Gluconeogenesis
  - Glycogenolysis.

- **Lipid Metabolism:**
  - Clearance of TG-rich chylomicron remnants, synthesis of TG-rich VLDL, important source of HDL-C, receptor mediated elimination of LDL-C.
  - Bile salts excreted by liver into gut aid clearance of triglycerides (detergent action)
FUNCTIONS OF THE LIVER

- **Formation of Bile:**
  - (Components = H₂O, electrolytes, bile acids, cholesterol, phospholipids, conjugated bilirubin, small amounts protein).

- **Bile Acid Metabolism:**
  - Maintenance of cholesterol homeostasis (chol → bile acids)
  - Bile acids = major organic anions excreted by liver

- **Bilirubin metabolism:**
  - UDPG Transferase conjugates bilirubin to bilirubin-glucuronide (H₂O-soluble).

- **Metabolism of toxins/drugs:**
  - Drugs are metabolised and inactivated by enzymes of the endoplasmic reticulum system (some excreted in bile).
Liver Disease

- Only major cause of death still increasing year-on-year
- 5th ‘big killer’ in England & Wales, after heart, cancer, stroke and respiratory disease
- Since people can survive with 70% liver damage, there is a substantial burden of morbidity from liver disease, a high cost to the NHS and a huge economic and human cost from liver-related ill health.

The British Liver Trust: http://www.britishlivertrust.org.uk/
‘Liver Function Tests’

- Typical ‘LFT’ profile:
  - Bilirubin, Aminotransferases (ALT), ALP, Albumin
  - In some cases, total protein, δ-Glutamyl transferase (δ-GT), AFP, rarely AST.
  - Tend to be insensitive indicators of true hepatic function (considerable functional reserve).

- True tests of liver function:
  - Albumin - crude measure of liver’s synthetic capacity
  - Prothrombin Time (PT) – preferred test, marker of clotting efficacy
**Bilirubin**

- End product of haem metabolism
  - haem = derived from Hb and other haem containing pigments e.g. myoglobin.
**Bilirubin**

- Insoluble in H₂O, potentially toxic so in plasma bound to albumin (unconjugated bilirubin)
  - In health 95% unconjugated, not excreted in urine therefore bilirubininuria always considered pathological
- Transported to the liver and conjugated (via enzyme UDP-glucuronyl transferase) to form bilirubin-glucuronide (conjugated bilirubin)
**Bilirubin**

- Conjugated bilirubin excreted into bile.
- In terminal ileum and colon conjugated bilirubin form urobilinogens and stercobilinogen
  - mostly excreted in faeces, some absorbed and re-excreted in bile via the enterohepatic circulation.
Bilirubin and Jaundice

- Jaundice = Yellow discolouration of the skin or sclera (of the eye) due to the presence of plasma bilirubin > ~50 µmol/L (Normal bili < 22 µmol/L)
  - Visible in sclera > ~50 µmol/L
  - Yellow skin @ > ~100 µmol/L

- Classification of Jaundice:
  - Pre-hepatic (predom. unconjugated) – Haemolysis (↑ bilirubin load), immature liver function, common in neonates
  - Hepatic (conj > unconj) - ↓ conjugation (e.g. Gilbert’s, Crigler-Najjar) or ↓ transport for excretion of bili into bile (e.g. hepatitis, cirrhosis, alcohol, drugs/paracetamol OD)
  - Post-hepatic (conjugated) – defective bile secretion/obstruction of bile ducts (e.g. ca head of pancreas, gallstones) or ductular disease (e.g. primary biliary cirrhosis).
# Jaundice – Differential Diagnosis

<table>
<thead>
<tr>
<th>Type of Hyperbilirubinaemia</th>
<th>Pre-Hepatic/ Haemolysis</th>
<th>Hepatic</th>
<th>Post-hepatic (Cholestatic)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lab Features</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• No bilirubinuria</td>
<td>• Bilirubinuria</td>
<td>• Bilirubinuria</td>
<td></td>
</tr>
<tr>
<td>• Normal ALT/AST and ALP</td>
<td>• Inc ALT/AST and ALP</td>
<td>• Pale stools (bilirubin does not reach gut)</td>
<td></td>
</tr>
<tr>
<td>• Increased AST and LDH</td>
<td>• Inc. PTT (reduced synthetic capacity)</td>
<td>• Modest inc. ALT/AST and LDH</td>
<td></td>
</tr>
<tr>
<td>• Reticulocytosis (inc. retics)</td>
<td>• Low albumin (in long-standing cases)</td>
<td>• Inc. ALP (usually &gt;3 x ULN)</td>
<td></td>
</tr>
<tr>
<td>• Low Hb (and abn RBC morphology)</td>
<td>• Urine may darken on standing (excess exc. urobilinogen)</td>
<td>• Inc. δGT, cholesterol and conjugated bile acids</td>
<td></td>
</tr>
<tr>
<td>• Low haptoglobin (binds free Hb)</td>
<td>• Pale stools (bilirubin does not reach gut)</td>
<td>• Pruritis (itching)</td>
<td></td>
</tr>
<tr>
<td>• Urine may darken on standing (excess exc. urobilinogen)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
INHERITED ABNORMALITIES OF BILIRUBIN METABOLISM

- **Unconjugated:**
  - *The Crigler-Najjar syndrome* – extremely rare, complete absence (type 1) or considerable reduction (type 2) of UDP-glucuronyl transferase. Deeply jaundiced within 1st few days of life. Unconjugated bili crosses B-B barrier, deposits in basal ganglia, leading to kernicterus. Phototherapy to reduce [Bili] and sometimes liver transplant.
  - *Gilbert’s Syndrome* – common (~7% of population), entirely benign, recurrent episodes of mild jaundice (plasma [Bili] <100 umol/L, all other LFTs normal. More pronounced jaundice when affected individual tired, intercurrent illness (e.g. flu) or fasting. Due to reduced conc of UGT1A isoform of UDP-glucuronosyltransferase responsible for bilirubin conjugation. Diagnosis of exclusion, genetic testing available.

- **Conjugated:**
AMINOTRANSFERASES (ALT/AST)

- (Transaminases) - a group of enzymes that catalyze the interconversion of amino acids and α-ketoacids/oxoacids by transfer of amino groups.

- **Alanine aminotransferase (ALT)**
  - Catalyses transfer of amino groups from alanine (α-amino acid) to 2-oxoglutarate (α-ketoacid):
    
    \[
    \text{Alanine} + \text{2-Oxoglutarate} \rightarrow \text{Pyruvate} + \text{Glutamate}
    \]

    - Key enzyme in gluconeogenesis

- **Aspartate aminotransferase (AST)**
  - Catalyses transfer of amino groups from aspartate to 2-oxoglutarate:
    
    \[
    \text{Aspartate} + \text{2-Oxoglutarate} \rightarrow \text{Oxaloacetate} + \text{Glutamate}
    \]
**Aminotransferases (ALT/AST)**

- Sensitive (but non-specific) marker of **acute** damage to cytoplasmic and/or mitochondrial membranes
  - **AST** – Liver/Heart/Skeletal/Brain. Liver AST = contained within cytoplasm and mitochondria
  - **ALT** – Predominantly liver. Liver ALT = cytoplasm only, more specific for liver damage

- Relative plasma activities of ALT and AST may help to indicate type of cell damage:
  - **Inflammatory/infective conditions** (e.g. viral hepatitis, drug OD) mainly damage to cyto membranes – **greater increase in plasma ALT**
  - **Infiltrative disorders** (e.g. malignancy) damage to mito and cyto membranes – **greater increase in plasma AST**
AMINOTRANSFERASES (ALT/AST)

- **AST/ALT >10 X ULN** - Acute hepatitis, crush injury, paracetamol OD

- **AST/ALT >5-10 X ULN** – MI, cholestasis, chronic/autoimmune hepatitis

- **AST/ALT <5 X ULN** – Other liver disease (e.g. NAFLD, Wilson’s disease, haemochromatosis, pancreatitis, haemolysis, alcohol abuse.)
ALKALINE PHOSPHATASE (ALP)

- A group of enzymes that hydrolyse phosphate esters at alkaline pH
- Main forms in serum from liver and bone (bone disease, growth, malignancy). Also from GI, placenta (pregnancy)
- Serum ALP rises in cholestatic liver disease due to increased ALP synthesis and the enzyme within the biliary tract is regurgitated into plasma
- If cause of raised ALP is not immediately apparent – ALP Isoenzymes determination (separation of bone and liver forms)
  - At City ALPI only processed if ALP >200 U/L
  - However, raised ALP together with raised ALT and/or δGT almost always indicates the ALP is of hepatic origin.
**Gamma-GT**

- A microsomal enzyme distributed in tissues of the liver and renal tubules (but serum levels mainly due to liver).
- Transfers glutamyl groups from gamma glutamyl peptides to other peptides or AAs.
- Increased $\delta$-GT activity in plasma whenever there is cholestasis.
- Also affected by ingestion of alcohol and drugs (e.g. phenytoin) – induce enzyme activity
- Therefore very sensitive index of liver pathology but not specific (raised levels may not necessarily indicate liver damage but simply reflect enzyme induction)
- In acute hepatic damage changes in $\delta$-GT parallel those of ALT/AST
PLASMA PROTEINS (ALBUMIN + TP)

- **Albumin:**
  - Major protein product of the liver
  - Long $T^{1/2} = \sim 20$ days – Sig. falls in albumin slow to occur if synthesis suddenly reduced
  - Hypoalbuminaemia = feature of advanced chronic liver disease (can occur in *severe* acute liver damage) but less specific than prolonged PT.
    - Note albumin = negative acute phase protein and can be low in non-hepatic diseases (e.g. nephrotic syndrome)

- **Total protein:**
  - Albumin + globulin (Igs)
  - Globulin (= TP minus Albumin) often reported with LFT results
  - Sometimes used as crude measure of severity of liver disease but generally identifies hypergammaglobulinaemia (e.g. myeloma) or sometimes immunodeficiency
PROTHROMBIN TIME (PT)

- Measure of activities of certain coagulation factors made by the liver.
- Used as a measure of true hepatic synthetic function.
- Prothrombin has a very short half-life – increased PT is an early indicator of reduced hepatic synthesis or if the liver cell mass is greatly reduced.
Types of Liver Damage

- **Hepatocellular:**
  - Typified by release of enzymes by damaged hepatocytes
  - \[^{\uparrow}\text{ALT/AST (and } \delta\text{-GT)}\]

- **Cholestatic:**
  - Cholestasis = Failure of adequate bile to reach the duodenum and therefore impaired biliary excretion of conjugated bilirubin
  - \[^{\uparrow}\text{Serum total bilirubin, ALP, } \delta\text{-GT, cholesterol and conjugated bile acids (more sensitive than total bili).}\]
  - Jaundice develops slowly, may be preceded by pruritis (itching)

- **Reduced mass of hepatocytes:**
  - If considerable, \[^{\downarrow}\text{albumin and prolonged PT(reduced prothrombin synthesis)}\]
**TYPES OF LIVER DISEASE**

- **ACUTE** (sudden onset):
  - Poisoning (e.g. paracetamol)
  - Infection (e.g. hepatitis)
  - Inadequate perfusion (reduced blood flow through liver)
  - Can progress to....

- **CHRONIC** (slow process, persists over long time period, progressive destruction of liver):
  - Alcoholic liver disease/alcoholic fatty liver
  - Viral hepatitis
  - Primary Biliary Cirrhosis (autoimmune, progressive destruction of small bile ducts, relatively rare affecting up to 1 in 4000)
# Hepatitis

<table>
<thead>
<tr>
<th></th>
<th>Acute</th>
<th>Chronic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Definition</strong></td>
<td>Hepatitis of sudden onset</td>
<td>Hepatic inflammation for &gt;6 months</td>
</tr>
<tr>
<td><strong>Causes</strong></td>
<td>• Hepatitis A,B</td>
<td>• Hepatitis B,C</td>
</tr>
<tr>
<td></td>
<td>• Toxins (e.g. alcohol, paracetamol)</td>
<td>• Autoimmune Hepatitis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Alcohol</td>
</tr>
<tr>
<td>**Example(s)/</td>
<td><strong>Hepatitis A:</strong></td>
<td></td>
</tr>
<tr>
<td>Additional</td>
<td>• Transmitted by contaminated food/drink</td>
<td></td>
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<tr>
<td>information</td>
<td>• Never progresses to chronic</td>
<td></td>
</tr>
<tr>
<td></td>
<td>• Jaundice after few days, bilirubinuria, inc. ALT/AST, normal ALP</td>
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<tr>
<td></td>
<td>• Many cases resolve completely</td>
<td></td>
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<td></td>
<td>• Vaccine available but no treatment</td>
<td></td>
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<tr>
<td><strong>Hepatitis B:</strong></td>
<td>• Transmitted by maternal/sex, blood</td>
<td><strong>Hepatitis C:</strong></td>
</tr>
<tr>
<td></td>
<td>• Can be acute or progress to chronic</td>
<td>• Blood transmission (IV drug users)</td>
</tr>
<tr>
<td></td>
<td>• After incubation period of 1-6m, develop jaundice, slightly inc. ALT/AST, normal ALP</td>
<td>• No acute phase (no jaundice)</td>
</tr>
<tr>
<td></td>
<td>• Most make gradual recovery but can remain carrier &amp; progress to cirrhosis/liver cancer/liver failure</td>
<td>• Abnormal biochem as Hep B</td>
</tr>
<tr>
<td></td>
<td>• Vaccine and treatment available (interferon alpha)</td>
<td>• High risk of remaining carrier and developing cirrhosis/liver cancer/failure</td>
</tr>
<tr>
<td>**Autoimmune</td>
<td></td>
<td><strong>Autoimmune Hepatitis:</strong></td>
</tr>
<tr>
<td>Hepatitis:**</td>
<td></td>
<td>• Formerly ‘Chronic Active Hepatitis’</td>
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<tr>
<td></td>
<td></td>
<td>• Classic autoimmune disease in young women, strong association with IBD</td>
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<tr>
<td></td>
<td></td>
<td>• Present with jaundice, inc. AST/ALT, increased IgG/gamma globulins,</td>
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<td></td>
<td></td>
<td>autoantibodies (anti nuclear/smooth muscle)</td>
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<tr>
<td></td>
<td></td>
<td>• No vaccine but treatment available (interferon alpha/ribavirin)</td>
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<td></td>
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<td>• Requires education to prevent</td>
</tr>
</tbody>
</table>
CIRRHOSIS

- A irreversible consequence of all chronic liver diseases that are usually associated with recurrent episodes of necrosis, cell death and attempts by the liver to regenerate

- Characterised by:
  - Replacement of liver tissue by fibrous scar tissue
  - Liver shrinkage

- Complications include:
  - Hepatic encephalopathy
  - Ascites/bleeding/itching
  - Hepatorenal syndrome

- Diagnosis by liver biopsy (more helpful than Biochem)

- Treatment of underlying cause, dialysis, liver transplant
**MALIGNANCY/LIVER INFILTRATION**

- The liver is the most common site for metastases from a primary tumour
  - Jaundice is the 1\(^{st}\) indication
- Hepatocellular carcinoma is associated with cirrhosis/hepatitis
  - Alpha-Fetoprotein (AFP) is a good marker of hepatocellular since it is raised in 80\% of cases
  - AFP essential for monitoring of response to treatment (conc falls and rises in relation to tumour mass)
**Other ‘Diagnostic’ Tests**

- **Hepatocellular carcinoma** – AFP (inc. in 80-90% of cases)
- **Hepatitis** – Viral serology
- **Primary biliary cirrhosis** – Tissue transglutaminase, endomesial/mitochondrial abs, liver biopsy
- **Alcohol abuse** - ↑δ-GT, AST:ALT (>2:1), ↑MCV (macrocytosis), ↑TGs, ↑Urate
- **Metabolic Liver Disease:**
  - Haemochromatosis – Ferritin, Transferrin saturation (Fe/UIBC), liver biopsy for Fe content (gold std)
  - Wilson’s Disease – Caeruloplasmin, 24hr urine Cu
  - α-1-Antitrypsin deficiency - Plasma α1AT/phenotyping
  - Porphyrias – Urine/plasma/faecal porphyrin measurement
OTHER ‘DIAGNOSTIC’ TESTS

- **Obstetric Cholestasis**
  - Total plasma bile acids (TBA)
  - Suggested when increased TBA in 3rd trimester of pregnancy associated with pruritis (can lead to fetal morbidity and mortality)
  - Elevation of ALT may follow increase in TBA.

- **HELLP Syndrome** – ‘Haemolysis, Elevated Liver enzymes and Low Platelets’
  - Associated with late pregnancy-induced hypertension (3rd trimester)
  - Measure LDH (↑), ALT (↑), FBC (↓ platelets, progressive anaemia) with BP monitoring.
Acute pancreatitis – due to necrosis of pancreatic cells
Release of enzymes into the retroperitoneal space & bloodstream
Presence of pancreatic juice in peritoneal cavity – severe abdo pain of sudden onset & shock

Causes:

- Gallstones, alcohol – account for >80% cases
- Pancreatic duct obstruction/regurgitation of bile
- HyperCa, hypertriglyceridaemia and drugs (e.g. opiates) may also evoke
**DIAGNOSIS AND MANAGEMENT OF PANCREATITIS**

- **Biochemical features:**
  - Plasma Amylase significantly increased (release from damaged cells) >5 x ULN within 2 - 12h of onset of symptoms
  - Uraemia/compromised renal function
  - Hypoalbuminaemia
  - Hypocalcaemia (formation of calcium salts with fatty acids released by pancreatic lipase in/around inflamed pancreas)
  - Metabolic acidosis
  - Abnormal LFTs

- **Severity can be assessed by the Ranson/Glasgow criteria:**

<table>
<thead>
<tr>
<th>At presentation</th>
<th>During first 48 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &gt;55 years</td>
<td>Plasma Urea rise &gt;10 mmol/L</td>
</tr>
<tr>
<td>WBC &gt;16 x 10⁹/L</td>
<td>Plasma Calcium &lt;2 mmol/L</td>
</tr>
<tr>
<td>Plasma glucose (non-diabetic) &gt;10 mmol/L</td>
<td>PaO2 &gt;8kPa</td>
</tr>
<tr>
<td>Plasma AST &gt;250 U/L</td>
<td>Plasma Albumin &lt;32 g/L</td>
</tr>
<tr>
<td>Plasma LDH &gt;350 U/L</td>
<td>HCT fall &gt;10%</td>
</tr>
<tr>
<td></td>
<td>Fluid sequestration &gt;6L</td>
</tr>
</tbody>
</table>
## Lab Measurement of Core ‘Liver Function’ Tests

<table>
<thead>
<tr>
<th>Test (serum)</th>
<th>Units</th>
<th>Reference Range/ Interpretation</th>
<th>Method Principle</th>
<th>( \lambda ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bilirubin</td>
<td>( \mu \text{mol/L} )</td>
<td>&lt;21</td>
<td><strong>Diazonium salt/Diazo reaction</strong> (photometric) in presence of a surfactant, formation of azobilirubin</td>
<td>548</td>
</tr>
<tr>
<td>Conjugated (Direct) Bilirubin</td>
<td>( \mu \text{mol/L} )</td>
<td></td>
<td><strong>Diazo reaction</strong> (photometric) in presence of sulfamic acid, formation of azobilirubin</td>
<td>548</td>
</tr>
<tr>
<td>ALT</td>
<td>U/L</td>
<td>&lt;41</td>
<td><strong>Oxidation of NADH to NAD</strong> (photometric), measure rate of decrease in absorbance</td>
<td>340</td>
</tr>
<tr>
<td>AST</td>
<td>U/L</td>
<td>&lt;37</td>
<td><strong>Oxidation of NADH to NAD</strong> (photometric), measure rate of decrease in absorbance</td>
<td>340</td>
</tr>
<tr>
<td>ALP</td>
<td>U/L</td>
<td>20-130</td>
<td><strong>Para-nitrophophenyl phosphate</strong> (photometric), measure p-nitophenol (yellow)</td>
<td>404</td>
</tr>
<tr>
<td>( \delta \text{-GT} )</td>
<td>U/L</td>
<td>&lt;64</td>
<td><strong>L-gamma-glutamyl-3-carboxy-4-nitroanilide substrate</strong> (photometric)</td>
<td>416</td>
</tr>
<tr>
<td>Albumin</td>
<td>g/L</td>
<td>35-50</td>
<td><strong>Bromocresol Green</strong> (photometric) binds to albumin to produce coloured complex</td>
<td>628</td>
</tr>
<tr>
<td>Total Protein</td>
<td>g/L</td>
<td>60-80</td>
<td><strong>Biuret reagent</strong> (photometric) binds with protein nitrogen</td>
<td>572</td>
</tr>
</tbody>
</table>
## Lab Measurement of Other Tests to Investigate Liver Disease

<table>
<thead>
<tr>
<th>Test (serum)</th>
<th>Units</th>
<th>Reference Range/Interpretation</th>
<th>Method Principle</th>
<th>λ (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Bile Acids</td>
<td>µmol/L</td>
<td>&lt;14</td>
<td>Enzymatic colorimetric, formazan dye measurement</td>
<td>548</td>
</tr>
<tr>
<td>ALP Isoenzymes</td>
<td>N/A</td>
<td>‘predominantly bone’ or ‘predominantly liver’ etc</td>
<td>Sample treatment and separation by electrophoresis (utilises different degrees of sialation via wheat germ lectin)</td>
<td>N/A Qualitative</td>
</tr>
<tr>
<td>Amylase</td>
<td>U/L</td>
<td>&lt;110</td>
<td>CNPG3 Substrate, measure rate of formation of 2-chloro-4-nitrophenol (photometric)</td>
<td>404</td>
</tr>
<tr>
<td>AFP</td>
<td>kU/L</td>
<td>1-7</td>
<td>2-step immunoassay (chemiluminescent microparticle, CMIA)</td>
<td>N/A</td>
</tr>
<tr>
<td>α1-Antitrypsin</td>
<td>g/L</td>
<td>0.9-2.0</td>
<td>Aggregate/complex formation, measured by turbidimetry</td>
<td>604</td>
</tr>
<tr>
<td>Copper</td>
<td>µmol/L</td>
<td>11-25</td>
<td>ICP-MS (Sandwell), photometric</td>
<td>N/A</td>
</tr>
<tr>
<td>Caeruloplasmin</td>
<td>g/L</td>
<td>0.2-0.6</td>
<td>Immune complex formation, measured by turbidimetry</td>
<td>340</td>
</tr>
</tbody>
</table>
FURTHER READING

- Test kit inserts: Clin Chem Drive:\Automated Area\Kit Inserts\Abbott (Chemistry and Immunoassay Tests folders)
- SOPs!