LIVER FUNCTION AND ASSOCIATED DISEASE STATES

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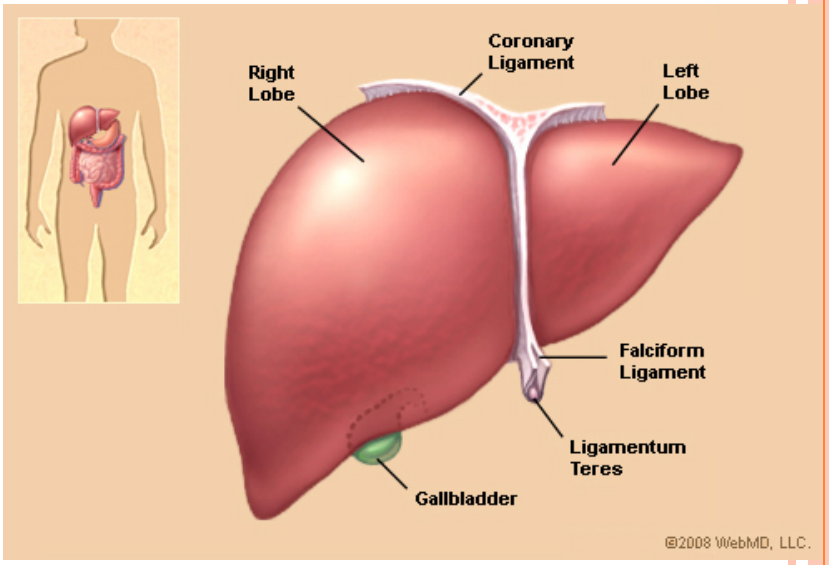
The Liver

- 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

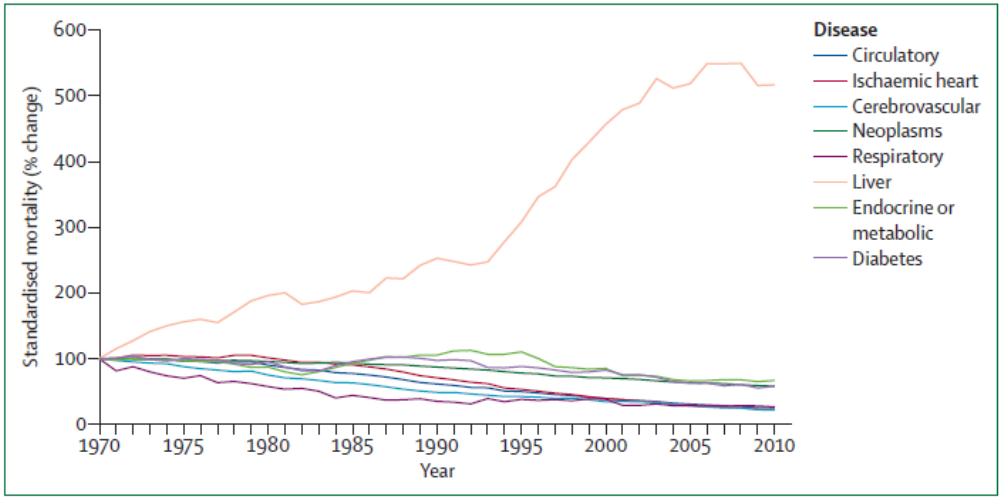
- **Protein Metabolism**:
  - Synthesis of most circulating proteins except δ-globulins (Igs)
  - Albumin, Transferrin, Caeruloplasmin, Acute phase proteins (e.g. CRP), α-1-anti-trypsin (α1AT), AFP, coagulation factors, components of complement.
- **Degradation**:
  - AAs degraded by transamination and 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 = H_2O, electrolytes, bile acids, cholesterol, phospholipids, conjugated bilirubin, small amounts protein).
- **Bile Acid Metabolism**:
  - Maintenance of cholesterol homeostasis (chol $\longrightarrow$ bile acids)
  - Bile acids = major organic anions excreted by liver
- **Bilurubin metabolism**:
  - UDPG Transferase conjugates bilirubin to bilirubin-glucuronide (H_2O-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/

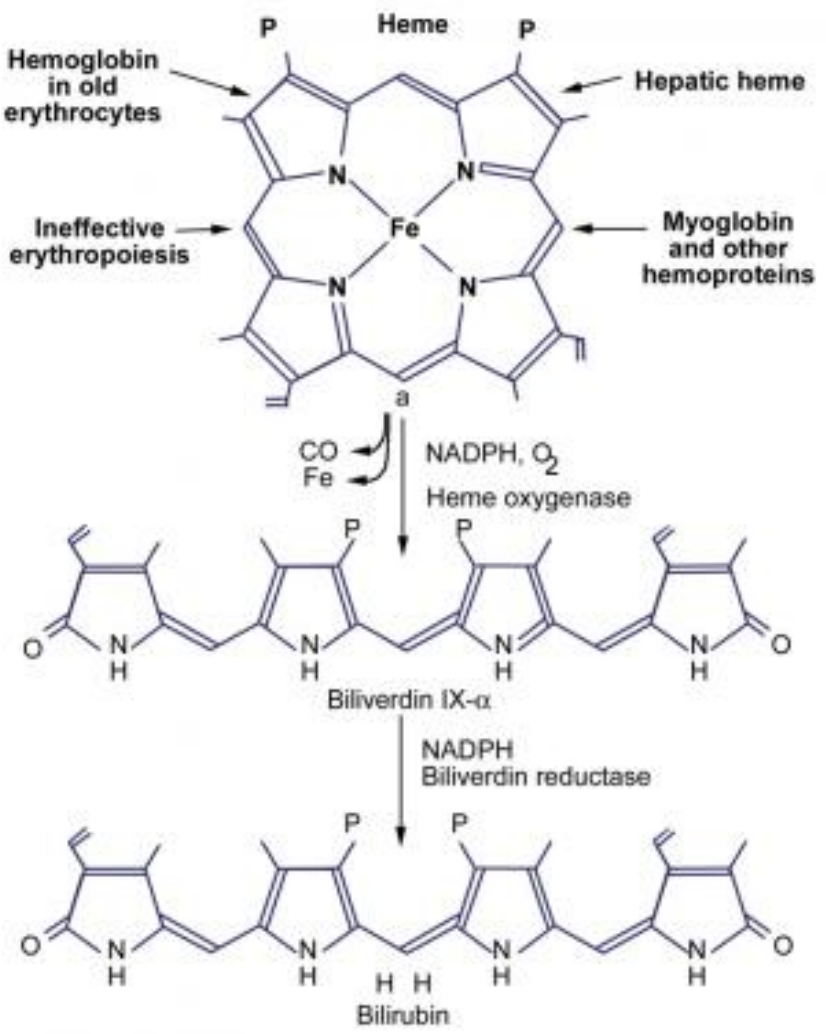
'Liver Function Tests'

- **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

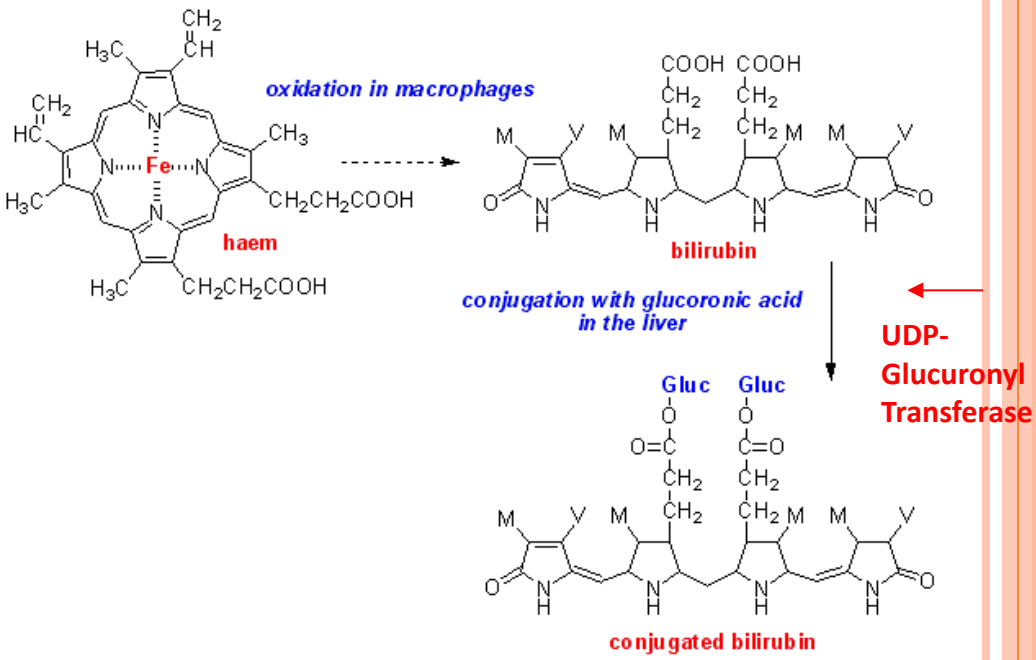
Bilirubin

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



BILIRUBIN

- Insoluble in H_2O , potentially toxic so in plasma bound to albumin (**unconjugated bilirubin**)
  - In health 95% unconjugated, not excreted in urine therefore bilirubinuria <u>always</u> 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 discoloration of the skin or sclera (of the eye) due to the presence of plasma bilirubin > ~50 µmol/L (Normal bili <22µmol/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

	Pre-Hepatic/ Haemolysis	Hepatic	Post-hepatic (Cholestatic)
Type of Hyper-bilirubinaemia	Unconjugated (usually)	Unconjugated and conjugated (conj > conj)	Conjugated
Lab Features	• No bilirubinuria • Normal ALT/AST and ALP • Increased AST and LDH • Reticulocytosis (inc. retics) • Low Hb (and abn RBC morphology) • Low haptoglobin (binds free Hb) • Urine may darken on standing (excess exc. urobilinogen)	• Bilirubinuria • Inc ALT/AST and ALP • Inc. PTT (reduced synthetic capacity) • Low albumin (in long-standing cases)	• Bilirubinuria • Pale stools (bilirubin does not reach gut) • Modest inc. ALT/AST and LDH • Inc. ALP (usually >3 x ULN) • Inc. δGT, cholesterol and conjugated bile acids • Pruritis (itching)

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 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:**
  - ***Dubin-Johnson syndrome*** – uncommon, benign condition. Decreased biliary transport of conjugated bili (mutation in multidrug resistance associated protein 2, MRP2).
  - ***Rotor's syndrome*** – uncommon, gene mutation not yet identified.

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):

Alanine + 2-Oxoglutarate ⟶ Pyruvate + Glutamate

  - Key enzyme in gluconeogenesis

- **Aspartate aminotransferase (AST)**
  - Catalyses transfer of amino groups from aspartate to 2-oxoglutarate:

Aspartate + 2-Oxoglutarate ⟶ Oxaloacetate + 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 δ-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 δ-GT parallel those of ALT/AST

Plasma Proteins (Albumin + TP)

- **Albumin:**
  - Major protein product of the liver
  - Long $T^{1/2}$ = ~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
  - ↑ ALT/AST (and δ-GT)
- **Cholestatic:**
  - Cholestasis = Failure of adequate bile to reach the duodenum and therefore impaired biliary excretion of conjugated bilirubin
  - ↑Serum total bilirubin, ALP, δ-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, ↓ 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

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

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 1st 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 biposy
- **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

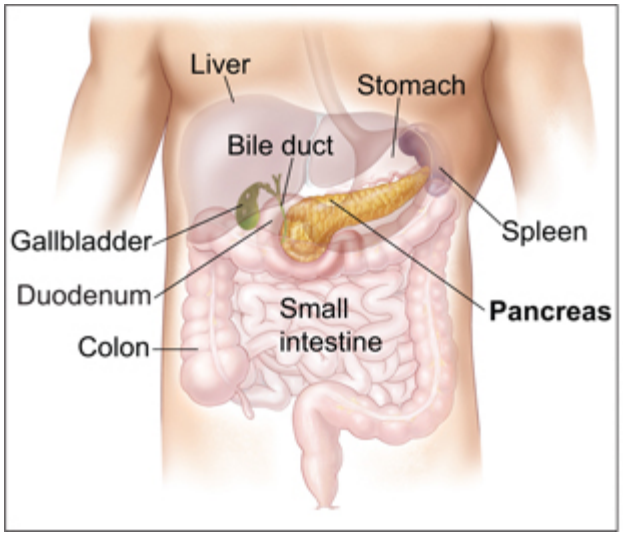
Other 'Diagnostic' Tests

- **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** – '**H**aemolysis, **E**levated **L**iver enzymes and **L**ow **P**latelets'
  - Associated with late pregnancy-induced hypertension (3rd trimester)
  - Measure LDH (↑), ALT (↑), FBC (↓ platelets, progressive anaemia) with BP monitoring.

Pancreatitis

- **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 realeased by pancreatic lipase in/around inflamed pancreas)
  - Metabolic acidosis
  - Abnormal LFTs

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

At presentation	During first 48 hours
Age >55 years	Plasma Urea rise >10 mmol/L
WBC >16 x 10^9/L	Plasma Calcium <2 mmol/L
Plasma glucose (non-diabetic) >10 mmol/L	PaO2 >8kPa
Plasma AST >250 U/L	Plasma Albumin <32 g/L
Plasma LDH >350 U/L	HCT fall >10%
	Fluid sequestration >6L

Lab Measurement of Core 'Liver Function' Tests

Test (serum)	Units	Reference Range/ Interpretation	Method Principle	λ (nm)
Total Bilirubin	µmol/L	<21	**Diazonium salt/Diazo reaction** (photometric) in presence of a surfactant, formation of azobilirubin	548
Conjugated (Direct) Bilirubin	µmol/L		**Diazo reaction** (photometric) in presence of sulfamic acid, formation of azobilirubin	548
ALT	U/L	<41	**Oxidation of NADH to NAD** (photometric), measure rate of decrease in absorbance	340
AST	U/L	<37	**Oxidation of NADH to NAD** (photometric), measure rate of decrease in absorbance	340
ALP	U/L	20-130	**Para-nitrophenyl phosphate** (photometric), measure p-nitophenol (yellow)	404
δ-GT	U/L	<64	**L-gamma-glutamyl-3-carboxy-4-nitroanilide substrate** (photometric)	416
Albumin	g/L	35-50	**Bromocresol Green** (photometric) binds to albumin to produce coloured complex	628
Total Protein	g/L	60-80	**Biuret reagent** (photometric) binds with protein nitrogen	572

Lab Measurement of Other Tests to Investigate Liver Disease

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

Further Reading

- Clinical Biochemistry: Metabolic and clinical aspects, William J Marshall, Stephen K. Bangert (Chpts 13 & 14)
- Tietz Textbook of Clinical Chemistry and Molecular Diagnostics 4th Edition, Cal A Burtis *et al.*
- Test kit inserts: Clin Chem Drive:\Automated Area\Kit Inserts\Abbott (Chemistry and Immunoassay Tests folders)
- SOPs!