Supporting Information for the Liver Pathway

Box A: Metabolic Syndrome

The International Diabetes Federation (IDF) criteria to diagnose metabolic syndrome:

Metabolic syndrome may be diagnosed if the patient has a large waist circumference (≥94 cm in European men or ≥90 cm in South Asian men; ≥ 80 cm in European and South Asian women).
Plus any two of the following:

- HDL-cholesterol <1.0 mmol/L (men), <1.3 mmol/L (women)
- Triglycerides ≥1.7 mmol/L
- Blood pressure ≥130/85 mmHg
- Fasting plasma glucose ≥5.6 mmol/L

Box B: Quick Reference Guide to interpret specialist liver panel test results

Patients stay on the liver pathway if the specialist liver panel test results meet **ALL** of the following criteria:

- A1AT ≥1.10 g/L
- Caeruloplasmin ≥200 mg/L
- Negative Hepatitis B and C serology
- Transferrin saturation <50%
- Negative smooth muscle, mitochondrial (M2/non M2) and LKM antibodies

For any other results or for further information on each test, see boxes C-G.

Box C: Serum A1AT
Alpha-1-antitrypsin deficiency

Samples are referred for analysis at the Immunology Laboratory at the Royal Preston Hospital. Avoid sample collection if acute inter-current infection

Severe A1AT deficiency (0.6g/L) occurs with incidence 1:2000. Typical presentation includes COPD, emphysema and cirrhosis

Reference range (adults): 1.10 – 2.10g/L

- If the result is within the reference range or higher than the reference range, A1AT deficiency is excluded.
- If the A1AT is <1.00 g/L, the A1AT phenotyping will be performed.
  - The 'Z' allele is most frequently associated with liver disease.
  - PI*ZZ homozygotes occur in approximately 1 in 2,000-5,000 births in European populations.
  - Patients with PI*ZZ should be referred to Hepatology.

Box D: Serum Caeruloplasmin Wilson's disease

Samples are referred for analysis at the Biochemistry Laboratory at Manchester Royal Infirmary. Avoid sample collection if acute inter-current infection

Presentation of Wilson's Disease may be hepatic or neurological (clumsiness/ataxia).

Reference range (adults): 200-600 mg/L

- Further investigation is required if the serum caeruloplasmin <200 mg/L
  - Collect a 24 hour urine sample for copper analysis (please contact Clinical Biochemists if further information is required on 01254 734153/735927)
  - If 24 hr urine copper is increased, patients should be referred to Hepatology.

Box E: Hepatitis B/C Serology

Samples are analysed at the Royal Blackburn Hospital. Any positive results are referred for confirmation.

Patients with positive serology results should be referred to Hepatology.

Box F: Ferritin, Iron and Transferrin Saturation Hereditary Haemochromatosis

Samples are analysed at the Royal Blackburn Hospital.

Presentation of hereditary haemochromatosis includes abnormal LFT results (ALT), arthralgia/arthritis, late onset diabetes and bronze pigmentation.

Causes of increased ferritin include chronic infection/inflammation, malignancy and haematological conditions

- If the transferrin saturation >50% and ferritin >500ug/L in males/ >350ug/L in females suggest repeat fasting sample for iron and TIBC (exclude alcohol excess)
- If the above results are repeated on a fasting sample, send a sample for haemochromatosis (HFE) genotyping.
- Patients that are homozygous for the C282Y variant should be referred to Hepatology.

Box G: Liver Autoimmune Screen

Samples are referred to the Immunology Laboratory at the Royal Preston Hospital for analysis.

The liver autoimmune screen consists of 7 tissue autoantibody tests:

- Reticulin (R1) antibodies
- Gastric parietal cell antibody

- Smooth muscle antibodies
- Mitochondrial (M2) antibodies
- Mitochondrial (non M2 antibodies)
- LKM (Liver Kidney Microsome) Antibody
- Ribosomal antibody

All tests are reported as 'positive' or 'negative'- if the mitochondrial (M2) antibodies are positive, an antibody titre is also reported.

The interpretation of each test result, any cascade testing that is performed and the further action required is detailed in the rows below.

Test	Positive result
Reticulin (R1) antibodies	• A positive result is not associated with liver disease. • Reticulin antibodies can be associated with Coeliac Disease or Dermatitis Herpetiformis. *If clinically indicated, suggest send a sample for coeliac screen.*
Gastric parietal cell antibody	• A positive result is not associated with liver disease. *All positive results are automatically reflexed by the laboratory for intrinsic factor antibody.*
Smooth muscle antibodies	• If positive, the result is reported as either tubular (associated with type 1 autoimmune hepatitis) or vascular (commonly seen post viral infections). *All patients with positive **tubular** smooth muscle antibodies should be referred to Hepatology.*
Mitochondrial (M2) antibodies	• Positive results are associated with primary biliary cirrhosis (or less commonly autoimmune hepatitis) • If antibody titre is ≥640 units, further cascade tests are performed (liver blot including M2, LKM1, LC1, SLA and F-actin) and reported. *All patients with positive mitochondrial (M2) results should be referred to Hepatology.*
Mitochondrial (non M2 antibodies)	• May indicate liver disease. *All patients with positive mitochondrial (non M2) antibodies should be referred to Hepatology.*
LKM (Liver Kidney Microsome) Antibody	• Mainly present in type 2 autoimmune hepatitis (80% prevalence) • The presence of LKM can mask the presence of other antibodies so if LKM is positive, the liver blot is performed (same tests cascaded as for positive mitochondrial (M2) antibodies) *All patients with positive LKM antibody results should be referred to Hepatology.*
Ribosomal antibody	• A positive result is not associated with liver disease. • Ribosomal antibodies are associated with Lupus

	All positive results are automatically reflexed by the laboratory for ribosomal antibody (more specific test using pancreatic tissue).

Summary

Positive results for reticulin, gastric parietal or ribosomal antibody: patients stay on the NAFLD pathway.

Positive results for smooth muscle, mitochondrial (M2/non M2) or LKM: patients are referred to Hepatology for further investigation.

Box H: FIB-4

Samples are analysed at the Royal Blackburn Hospital.

The Fibrosis-4 score (FIB-4) is used to estimate the amount of fibrosis in the liver.

It is calculated using the following formula:

$$\text{FIB-4} = \frac{\text{age (years)} \times \text{AST (U/L)}}{\text{Platelet count } (10^9/\text{L}) \times \sqrt{\text{ALT (U/L)}}}$$

FIB-4 >3.25 has a 97% specificity and a positive predictive value of 65% for advance fibrosis.

This will be calculated and reported via ICE to the GP system.

FIB-4 auto-comments added by the laboratory:
<1.30: Review patient and repeat LFTs in 1 year
1.30-3.25: Please request the Enhanced Liver Fibrosis (ELF) test
>3.25: Refer to Hepatology for further assessment

Box I: Enhanced Liver Fibrosis (ELF) test

Samples are referred to the Blood Sciences Laboratory at Leeds General Infirmary for analysis.

The Enhanced Liver Fibrosis (ELF) test combines three serum biomarkers:

- Hyalouronic acid
- Procollagen III amino terminal peptide
- Tissue inhibitor of metalloproteinase.

The results from the three markers are used to calculate the 'ELF score'.
The ELF score correlates with the level of liver fibrosis assessed by liver biopsy so it is a non-invasive indicator of liver fibrosis.

ELF Score >9.5: High risk of advanced fibrosis. Refer patient to Hepatology

ELF Score ≤ 9.5: Low risk of advanced fibrosis. Review patient and repeat LFT in 1 year