Introduction

As a trainee clinical biochemist, the common core list (CCL) is a list of attributes to be developed in the course of our training. It is important to consider this at the outset of new projects in order to maximise the benefit obtained from the project, with respect to career development. Thus, I present the data from my MSc project with a summary of how this relates to my training and the CCL.

Non-Alcoholic Fatty Liver Disease (NAFLD)

Non-alcoholic fatty liver disease (NAFLD), the liver manifestation of the metabolic syndrome includes a spectrum of liver disease featuring hepatic fat deposition from benign steatosis to non-alcoholic steatohepatitis (NASH), where the liver exhibits hepatocellular damage, inflammation and fibrosis. Liver fibrosis is an important feature to define in NASH as it is associated with (silent) progress to cirrhosis and hepatocellular carcinoma. Sub-classification of the severity of NAFLD and making prognoses are dependent upon histological analysis of liver biopsy samples using complex biopsy scoring systems, e.g. METAVIR and the NAFLD Activity Score. Liver biopsy is an invasive procedure that is very unpleasant for patients. There is a 1% risk of complications, including pneumothorax, peritonitis and haemoperitoneum. It is also expensive and has recognised technical difficulties (1). Consequently, a biomarker that can assess the severity of disease in NAFLD patients would benefit in terms of patient safety and convenience. Patients with non-significant fibrosis and also those with cirrhosis (in whom a biopsy may worsen the condition of the liver) could avoid liver biopsy. In the longer term, a serum biomarker may also permit monitoring from primary care and so contribute to improved patient care. The efficacy of a promising serum biomarker, hyaluronic acid, was investigated.

Hyaluronic Acid in Liver Fibrosis

Hyaluronic acid (HyA) is a high molecular weight glycosaminoglycan made by hyaluronan synthase proteins in the plasma membrane of mesenchymal and inflammatory cells. The production and roles of HyA in the body are summarized in Figure 1. The elevation of HyA in the liver occurs via a mechanism of dual regulation of increased synthesis and clearance production.

Aims

1. Verification of an automated serum HA assay.
2. Measurement of serum HA in a cohort of NAFLD patients and comparison with gold standard liver biopsy analysis to ascertain if serum HA can predict liver fibrosis level.

Method

Assay Verification

Clinical laboratories have responsibility for ensuring that manufacturer’s claims are verified and any tests implemented are fit for purpose. This is regulated by International Standards (e.g. ISO 15189) and by CPA (UK) Ltd, who are responsible for accreditation of NHS laboratories. Verifying the analytical performance of new assays prior to introduction is vital to ensure accurate results are obtained and patients are subsequently treated in the most appropriate and safe manner.

Pooled patient serum samples with low, medium and high levels of HA were used to assess intra-assay and inter-assay imprecision, linearity, limit of detection, recovery and the influence of common interferences (haemolysis, lipaemia and icterus). The HA assay was implemented according to manufacturer recommendations (Siemens Diagnostics Ltd), specific to the Siemens Centaur XP analyzer. The protocol used is shown in Figure 2. The assay was calibrated every 14 days and three levels of quality control material were run on each day the assay was used.

Results

Assay Verification

The results of the assay verification studies are shown. Imprecision, recovery and interferences were assessed in the low, medium and high patient pools. Linearity was checked via linear dilution and measurement of HA in 4 patient samples. Limit of detection taken as 3 standard deviations above the mean blank (0.9% saline) measurement (n=10).

NAFLD Cohort

The samples that had HA concentrations below the lower cut-off of 50 ng/mL demonstrated strong consistency to METAVIR staging of the liver biopsies with 91.3% (42/46 samples) of cases agreeing. The 4 individuals with discordant results from HA analysis and the liver biopsy were classified as ‘significant fibrosis’/F2 by the hepatologist.

The HA concentrations of the samples above the upper threshold showed good correlation with the outcome of liver biopsy with 8/10 patients identified as cirrhotic via both means. The two patients who were ‘incorrectly’ classified as cirrhotic via serum HA concentrations were found to have severe fibrosis (F3) by liver biopsy. Only one patient with a liver graded as F4 was not encapsulated by the >200 mg/mL cut-off.

Conclusions

• The automated HA assay was suitable for clinical use.
• The 50 ng/mL cut-off was able to confirm the absence of liver fibrosis in 91% of individuals below the cut-off.
• The 200 ng/mL confirmed cirrhosis in all cases as found by biopsy, but identified two patients with severe fibrosis as cirrhotic.
• Serum HA measurement reduced the need for liver biopsy in 79% of the cohort.
• Larger studies are needed to fully assess the use of serum HA as a means to diagnose and monitor liver disease in NAFLD.

CCL Outcomes

I worked with clinicians from the hepatology and biochemistry departments in order to design & perform the study. Practical laboratory skills were utilised in preparing and economical factors.

References

(3) Burrett D (2010). Measurement verification in the clinical laboratory: A guide to assessing performance and acceptance testing of methods (quantitative examination procedures) and/or analyses 1-2-4.

Laura Russell
Dept. of Blood Sciences, Ninewells Hospital, Dundee

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