Research Article

EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

Image analysis of liver biopsy samples measures fibrosis and predicts clinical outcome

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Background & Aims: Histopathological scoring of liver fibrosis mainly measures architectural abnormalities and requires a minimum biopsy size (⩾10 mm). Liver collagen quantification may allow use of small size biopsies and improve the prediction of clinical outcomes. This study evaluated the ability of the collagen proportional area (CPA) measurement to predict clinical outcomes.

Methods: Clinical outcomes were determined using population based data-linkage for chronic hepatitis C (CHC) patients from 1992 to 2012. Quantitative digital image analysis of liver biopsies was used for CPA measurement.

Results: 533 patients with a biopsy size ⩾5 mm were included. Median follow up was 10.5 years. 26 developed hepatocellular carcinoma (HCC), 39 developed liver decompensation and 33 had liver related death. 453 had Metavir F0-F2 and 80 had F3-F4. CPA ranged from 1.3% to 44.6%. CPA and Metavir stage were independently associated with liver related death. Metavir stage, CPA stage and age were independently associated with HCC. CPA stage (C1: 0%–5%, C2: 5%–10%, C3: 10%–20%, C4: >20%) stratified risk and a significant difference in outcomes was present between all CPA stages for HCC and between C2-C3 and C3-C4 for decompensation and liver related death. The 15 year composite endpoint-free survival was 97% for C1, 89% for C2, 60% for C3, 7% for C4. C4 had significantly worse survival than ⩽C3 (p <0.001) in cirrhotic patients.

Conclusions: CPA stage gave additional information regarding risk stratification for adverse clinical outcomes independent of Metavir stage.

Keywords: Collagen proportional area; Chronic hepatitis C; Histological stage; Liver complication; Liver related death.

Received 19 September 2013; received in revised form 20 January 2014; accepted 22 February 2014; available online 6 March 2014

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Abbreviations: CPA, collagen proportional area; CHC, chronic hepatitis C; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; AUROC, area under receiver operating characteristic curves; HR, hazard ratio.

Introduction

Development of liver fibrosis is a critical feature of progressive chronic hepatitis C (CHC) and its severity is closely associated with adverse clinical outcomes [1]. The evaluation of liver fibrosis severity is essential to determine prognosis and guide management of CHC patients. Liver biopsy histological staging systems such as Metavir or Ishak have long been used to assess the severity of liver fibrosis [2] and the risk of liver related morbidity and mortality significantly accelerate after the development of cirrhosis (Metavir F4) [3]. However, the correlation between histological stage and clinical outcomes in patients with less severe fibrosis is not clearly determined. Additional histological features not incorporated into the classical fibrosis staging systems may also have important prognostic implications. A study showed that nodule size and fibrous septa thickness were independent predictors for the presence of clinically significant portal hypertension [4].

Quantitative digital image analysis is a newly developed method used to assess liver fibrosis. This technology segments digital images of liver biopsies and accurately measures the area of collagen and the area of remaining liver tissue and calculates the proportion of the biopsy occupied by collagen [collagen proportional area (CPA)] [5]. In contrast to histological staging systems CPA is a continuous measure of the amount of liver fibrosis and has minimal inter and intra-observer variability. Non standardized image capture techniques and image analysis methods have been used for CPA measurement and this has limited the use of CPA to a degree. Recently we evaluated and optimized the method for CPA measurement using small liver biopsy samples and achieved a high degree of accuracy and reproducibility [6].

Despite these limitations early studies have shown a significant correlation between CPA and histological stage as well as CPA and serum markers of liver fibrosis [7–10]. CPA also correlated with hepatic venous pressure gradient in CHC patients [10], with decompensation in cirrhotic patients [11], with transplantation free survival in children with biliary atresia [12], and post liver transplantation outcomes for recurrent hepatitis C virus (HCV) [13–15]. Therefore CPA has the potential to better predict clinical outcomes for CHC patients than established

histological scoring systems and allow improved management of these patients. The aim of this study was to compare the ability of the optimised method of CPA measurement with Metavir stage to predict long term liver related morbidity and mortality in a large well documented cohort of CHC patients with a range of fibrosis stages from none (F0) to cirrhosis (F4).

Patients and methods

All CHC patients who attended the Department of Gastroenterology/Hepatology, Sir Charles Gairdner Hospital and had a liver biopsy from 1992 to 2012 were included. An archived liver tissue block needed to be available for use. Exclusion criteria included co-infection with hepatitis B virus and human immunodeficiency virus; other liver diseases including hemochromatosis, α1-antitrypsin deficiency, Wilson disease and autoimmune liver diseases; and a history of liver transplantation, decompensation or diagnosis of HCC before liver biopsy. Patients who were successfully treated for HCV were also excluded. The study was approved by the Sir Charles Gairdner Hospital Human Research Ethics Committee and the Department of Health Human Research Ethics Committee.

Liver fibrosis evaluation

Liver biopsies were obtained percutaneously and routinely stained using Masson's trichrome. All biopsy slides were reviewed by an expert liver pathologist (BdB) who was blinded to clinical data. Liver fibrosis was staged using the Metavir staging system, namely: F0, no fibrosis; F1, portal fibrosis without septa; F2, few septa; F3, numerous septa without cirrhosis; F4, cirrhosis [16]. The size, fragmentation and number of portal tracts of each biopsy was recorded.

Quantitative image analysis

A new liver section was cut from the stored tissue block and stained using sirius red and this was used for CPA analysis. CPA was measured using the optimised method previously described [6]. Sections were scanned using the Aperio Scanscopie XT Digital Slide Scanner at 20× magnification (1.59×10^7 Pixels = 1 mm^2). The image was viewed using Aperio ImageScope software version 10.0. The liver capsule and large portal tracts were excluded as these did not represent disease related collagen [4]. The optimum threshold for positive pixels that corresponded to areas of sirius red staining was determined according to hue value and colour saturation using the original image for comparison. A binary image was produced by the software and CPA was expressed as a percentage of positive pixels to total pixels. The CPA measurement was calculated by the software. According to our previous study, a biopsy with a measurement area less than 5 mm^2 after exclusion of any large portal tracts or capsule was considered as insufficient and thus excluded from further analysis [6].

Data source

The long term follow up of patients was obtained from the Western Australia Data Linkage Unit. This is a validated population-based data linkage system that links multiple health related datasets including the state cancer register, the state hospital morbidity database and the state mortality records dating back to 1982, 1970, and 1969 respectively [17]. The Hospital Morbidity Data System has 100% coverage of data for hospital admissions throughout the state with a record linkage success rate of more than 99% [17]. The hospital admission diagnosis and the cause of death were recorded using ICD 9 (before 1997) and ICD 10 (after 1997) classification codes. Personal identifiers were encrypted and stored separately from the data used for analysis.

Endpoints and statistical analysis

The primary endpoint was liver related death or liver transplantation. Liver related death was defined as death from liver failure, variceal bleeding or hepatocellular carcinoma as well as death in which liver disease was the major contributing factor. The secondary endpoints were the first episode of liver decompensation (ascites, hepatic encephalopathy, variceal bleeding, hepatorenal syndrome, spontaneous bacterial peritonitis) or the development of HCC. Survival duration was determined from the time of liver biopsy to each endpoint. The correlation between CPA and Metavir stage was assessed by the Spearman correlation coefficient. Survival was assessed using Kaplan Meier curves and significance determined by the log rank test and cox regression analysis. Area under receiver operating characteristic curves (AUROC) was calculated for CPA and Metavir stage to predict each endpoint. Two sided p values of <0.05 were considered significant.

Results

844 CHC patients were initially included. The stored liver biopsy block used for sirius red staining and CPA measurement had been sectioned previously for routine histopathology assessment and the remaining stored tissue was therefore of smaller size and often <5 mm. Therefore, 208 patients were excluded due to insufficient liver biopsy size. 103 patients who had successful antiviral treatment were also excluded from the core analysis but were included in the sensitivity analysis. 533 patients were included in the final analysis with a median follow-up time of 10.5 years (range 0.1–20 years) (Table 1). The mean biopsy length used for Metavir staging was 15 mm (SD: 4.4 mm) and the mean portal tract number was 9 (SD: 4.0). Less than 1% of biopsies were fragmented. The mean biopsy length used for CPA measurement was 10 mm (SD: 3.7 mm). The range of CPA values varied from 1.3% to 44.6%. CPA value was well correlated with Metavir stage with a correlation coefficient of 0.7377, p <0.001. There was a wide range of CPA values within each Metavir stage with a median CPA of 3.7% for F0, 4.8% for F1, 7.2% for F2, 11.0% for F3, and 21.3% for F4. Considerable overlap of CPA values between Metavir stages was also observed (Fig. 1).

During follow up, 26 (4.9%) patients developed HCC, 39 (7.3%) developed liver decompensation, and 33 (6.2%) had a liver related death. The AUROC for Metavir stage was 0.88 (95% CI, 0.82–0.95) for predicting HCC, 0.80 (95% CI, 0.71–0.88) for liver decompensation, and 0.82 (95% CI, 0.74–0.90) for liver related death. CPA achieved a significantly higher AUROC of 0.92 (95% CI, 0.89–0.94) for predicting HCC (p = 0.0112) and a similar AUROC of 0.79 (95% CI, 0.71–0.88) and 0.84 (95% CI, 0.76–0.93) for decompensation and liver related death respectively. No significant improvement of AUROC was observed when CPA and Metavir were combined. 58 patients had the composite end-point of liver related death, HCC or liver decompensation during follow up. The AUROC for CPA to predict the composite end point was 0.83 (95% CI, 0.76–0.89). A cut point of 5% had a sensitivity of 87.9% and specificity of 51.4% in predicting the composite end point, whereas a cut point of 10% had a sensitivity of 62.1% and specificity of 90.3%, and a cut point of 20% had a sensitivity of 34.5% and specificity of 98.7% respectively. Using these cut points, CPA values were categorised into four stages: C1: 0%–5%, C2: 5%–10%, C3: 10%–20%, C4: >20%. 251 patients had C1, 201 had C2, 56 had C3 and 25 had C4.

Univariate analysis found that CPA stage, Metavir stage and age were significantly associated with liver decompensation, HCC development and liver related death (Table 2). Multivariate analysis found that CPA stage and Metavir stage were independently associated with liver related death; CPA stage, Metavir stage and age were significantly associated with HCC development and Metavir stage was significantly associated with liver decompensation (Table 2).

There was a significant difference in composite end point free survival between C1 and C2 (p = 0.01), C2 and C3 (p <0.001), C3 and C4 (p <0.001) (Fig. 2). The 15 year composite end point free