Prognostic role of liver biopsy in patients with severe indeterminate acute hepatitis

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All authors contributed to the manuscript for important intellectual content and approved the submission.

Competing interests: Rajiv Jalan has research collaborations with Takeda, and Yaqrit, and consults for Yaqrit. Rajiv Jalan is the founder of Yaqrit Limited, which is developing UCL inventions for treatment of patients with cirrhosis. Rajiv Jalan is an inventor of ornithine phenylacetate, which was licensed by UCL to Mallinckrodt. He is also the inventor of Yaq-001, DIALIVE and Yaq-005, the patents for which have been licensed by his University into a UCL spinout company, Yaqrit Ltd. Cornelius Engelmann has

on-going research collaboration with Merz Pharmaceutical and Novartis. He has received speaker fees from Novartis, Gilead and Merz Pharmaceuticals.

Abbreviations: IAH, indeterminate acute hepatitis; sIAH, severe indeterminate acute hepatitis; INR, international normalized ratio; MELD score, model for end-stage liver disease score; MELD-Na score, end-stage liver disease-sodium score; ALF-OFs, Acute Liver Failure-Organ Failure Score; BMI, body mass index; CT, computed tomography; LV, liver volume; LAAR, liver abdominal area ratio; LV/BMI, liver volume to BMI ratio; LV/BW, liver volume to body weight ratio; IQR, interquartile range; ALT, alanine aminotransferase; AST, aspartate aminotransferase; MRI, Magnetic resonance imaging; APTT, activated partial thromboplastin time; HR, Hazard ratio; CI, Confidence interval; PPV, positive predictive value

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Abstract

Background and aims: Severe indeterminate acute hepatitis (sIAH) is a poorly understood rare disease with no specific therapy. This study aims to define the clinicopathological characteristics of sIAH and the role of liver biopsy in determining prognosis.

Methods: Patients with sIAH admitted to a single centre between 2010 and 2019 were included. Histopathological patterns of liver biopsies were reviewed by two histopathologists and key findings further specified by multiplex immunofluorescence. Patients that died or underwent liver transplantation were analysed as 'non-survivors'.

Results: Of 294 patients with acute hepatitis, 43 with sIAH were included. Seventeen (39.5%) underwent liver transplantation and 7 (16.2%) died within 3-months. Multilobular necrosis was the predominant histopathological feature being significantly more frequent in non-survivors (62.5% vs.21.1%, p=0.016). Necrotic areas showed low HNF4α and Ki67 expression but high expression of CK19 and cell death markers identifying areas of severe tissue injury and inadequate regenerative response. Patients with multilobular necrosis had higher INR, MELD and MELD-Na scores compared to those without (p-values for all markers <0.05). Multivariate Cox analysis revealed that multilobular necrosis (HR=3.675, 95%CI: 1.322-10.211) and lower body mass index (HR=0.916, 95%CI: 0.848-0.991) independently predicted death/transplantation.

Conclusions: The results of this study provide novel insights into the important role of liver biopsy in sIAH patients suggesting that the presence of multilobular necrosis is an early indicator of poor prognosis.

Keywords: indeterminate hepatitis; histopathology; multiplex immunofluorescence

Introduction

In a significant proportion of patients with acute hepatitis the underlying aetiology cannot be determined. These patients are often labelled as having "indeterminate" acute hepatitis (IAH) [1]. IAH is a challenging rare disease due to lack of diagnostic criteria and specific medical treatment. About 10-15% of acute liver failure cases are thought to be due to severe IAH (sIAH) [2]. Spontaneous survival of sIAH is relatively lower compared with other acute hepatitis with known aetiologies [1]. In contrast to some of the other causes of acute liver failure, patients with sIAH often have a more protracted course allowing potential opportunities to prognosticate and intervene to prevent further progression. However, the poor understanding of pathogenesis of sIAH and the lack of systematic clinicopathological criteria that can predict future outcome limits our ability to treat these patients and prognosticate effectively.

Access to liver biopsy to determine the aetiology and to aid in clinical management is not widely available as it needs to be performed through the transjugular route to minimize the risk of bleeding. Many of these patients are coagulopathic or have end organ complications of liver failure. Studies of patients with drug-induced liver injury and acute on chronic liver failure showed that, in addition to the diagnostic utility, histological assessment can provide further prognostic information assisting clinical decision-making [3-5]. The literature on the role of liver biopsy in the management of patients with sIAH is scarce. In our unit, most patients with sIAH undergo liver biopsy through the transjugular route and this allows us to perform a systematic study to define the clinicopathological characteristics of sIAH and its role in predicting the outcome.

Methods

Patients

This report is part of the CARNATION study, which is a retrospective-prospective program based in the Liver Unit at the Royal Free Hospital, London that aims to evaluate clinicopathological characteristics of patients with acute hepatitis. The study was approved by the London - Hampstead Research Ethics Committee (07/Q0501/50) and was in compliance with the Declaration of Helsinki.

This study focuses on patients with sIAH and includes patients admitted between January 2010 and October 2019. sIAH was defined in a patient with less than 12-weeks history of jaundice, total serum bilirubin of more than 5 times the upper limit of normal and international normalized ratio (INR) of more than 1.2 at admission in the absence of any cause of liver injury diagnosed on extended liver screen, imaging and after exclusion of underlying chronic liver disease (Supplementary material 1). Patients were also excluded if they 1) fulfilled Kings College criteria for liver transplantation at admission; 2) had previous liver transplantation; 3) with chronic liver disease; 4) referral histology from another hospital; 4) no biopsy at baseline data in key parameters; 5) with missing original glass slides and insufficient remaining tissue for histology review.

Data collection, scores and management

The following data that were collected at the time of admission and on the day of histopathological examination: the model for end-stage liver disease (MELD) score [6],

MELD-sodium (MELD-Na) score [7] and Acute Liver Failure-Organ failure (ALF-OF) score[8].

All patients were managed according to a pre-defined protocol, which was modified to suit local practice but based upon the best available evidence and AASLD and EASL guidance on the management of acute liver failure [9-12]. The King's College criteria for poor prognosis were used to identify patients in need of urgent liver transplantation[13] and a multidisciplinary team made decisions on the eligibility of each patient for transplantation and the UK guidance on eligibility for super-urgent transplantation was strictly applied (http://www.odt.nhs.uk/transplantation/guidance-policies/).

Histopathological review and multiplex immunostaining

The histopathology review was carried out by two histopathologists with a special interest in liver disease (CA and AQ) who were blinded to the clinical data. Each sample was reviewed twice by the two histopathologists. All samples from each included patient were reviewed including any biopsy taken at presentation, during follow up and the available explanted liver tissue obtained at transplantation. Definition of histological terms is listed in Supplementary Material 2. Multiplex immunostaining on liver biopsy sections was performed as described elsewhere[14]. The following markers were used: HNF4α+ (hepatocyte nuclear factor 4 alpha) for hepatocytes, IBA1 (allograft inflammatory factor 1) for macrophages, CK19 (cytokeratin 19) for bile ductular cells, cleaved caspase 3 and RIPK3 (receptor-interacting serine/threonine-protein kinase 3) for cell death, αSMA (alpha-smooth muscle antigen) for hepatic stellate cell activation and Ki67 for cell

proliferation.

Statistical analysis

Student's t-test or Mann-Whitney U test was used for continuous variables. Pearson χ2 test or Fisher's exact test was used to compare categorical variables. The area under the receiver operator characteristic curve (AUROC) was used as a measure of the diagnostic accuracy. A Cox-proportional hazard regression analysis was performed to identify factors associated with outcome. Data analysis was performed using SPSS software version 24.0 (SPSS Inc., Chicago, USA) and R software (https://www.r-project.org/). A p-value <0.05 was considered statistically significant.

Results

Patient characteristics

From January 2010 to October 2019, 294 consecutive patients diagnosed with acute hepatitis or acute liver failure were identified and 230 patients were excluded for various reasons (Figure 1). Forty-three patients were included in the final analysis.

The mean age of the entire cohort was 43.6 ± 13.4 years and 20 (46.5%) were male. The median time from the onset of jaundice to liver biopsy was 21 (IQR 14-35) days. Liver function tests showed an average alanine aminotransferase (ALT) of 1393 ± 958 U/L, aspartate aminotransferase (AST) of 1307 ± 1232 U/L, total bilirubin of 362 ± 179 umol/L and INR of 2.2 ± 1.1.

Three patients were diagnosed with infection on admission, which were lower respiratory tract infection, cellulitis and bacteraemia, respectively. The one with bacteraemia had evidence of sepsis. Two more patients developed nosocomial infection, which were hospital acquired pneumonia and spontaneous bacterial peritonitis respectively. None of them developed evidence of sepsis.

Pathological features in sIAH

The pathological features finding in this cohort are presented in Supplementary Material 2. Nineteen patients had multilobular necrosis and 24 had milder necrosis (focal or confluent necrosis). Of the 19 biopsies with multilobular necrosis, 7 (36.8%) did not exhibit any preserved liver parenchyma. Examples of confluent and multilobular necrosis

are shown in Supplementary Figure 1.

According to the time from the onset of jaundice to biopsy, patients were divided into two groups: less than 2 weeks and more than 2 weeks. Ductular reaction (90.9% vs. 52.4%, p=0.013), ductular cholestasis (31.8% vs. 4.8%, p=0.046), and interface inflammation (100% vs. 76.5%, p=0.045) were more common in the patients with longer duration of jaundice (>2 weeks) compared to shorter period of jaundice (≤2 weeks). Mitotic activity was more frequently found in the liver of patients with shorter duration of jaundice (41.2% vs. 5.3%, p=0.016). There was no significant difference between the two groups in terms of the other clinical or histological features (Table 1).

The interval between the liver biopsy and liver transplantation ranged from 2 to 25-days (median 10-days). Most (14/17, 82.3%) explanted livers showed areas of multilobular necrosis. All the patients with multilobular necrosis on liver biopsy showed similar pathological features in the explanted liver. Five of the six patients without multilobular necrosis on biopsy were found to have multilobular necrosis in the explanted liver.

Relationship between biochemical parameters and pathological features

We explored the relationship between the pathological features and the results of biochemical examination at the time of liver biopsy (Table 2). Nineteen patients (44.2%) had multilobular necrosis and these patients had higher white blood cell counts, INR but lower ALT and albumin levels compared to those without (p-values for all markers <0.05). MELD and MELD-Na scores (both p<0.05) were higher in patients with multilobular

necrosis but the presence of hepatic encephalopathy and ascites were similar between the groups (p>0.5). Eight patients (18.6%) had ductular cholestasis on biopsy but only one of these patients had evidence of sepsis. The presence of ductular cholestasis was associated with lower ALT, AST and albumin levels (all p<0.05) but total bilirubin levels did not differ significantly from patients without ductular cholestasis (349.3 ± 186.7 vs. 390.6 ± 90.3 umol/L, p=0.336). Similar proportions of patients had hepatic encephalopathy and ascites in those with or without ductular cholestasis.

Clinical characteristics of survivors and non-survivors

Of the 43 patients, 17 (39.5%) underwent liver transplantation and 7 (16.2%) died within 3-months. They were defined as non-survivors. Nineteen patients (44.2%) who recovered spontaneously were defined as survivors (Table 3). The survivors had longer median duration of jaundice prior to biopsy than non-survivors (28 [IQR: 14-49] vs. 14 [IQR: 11-26] days, p=0.002). The median duration from the onset of jaundice to death or transplantation was 33.5 (IQR: 20.3-46.8) days. Gender, age, comorbidities, total bilirubin, ALT, AST and albumin levels were comparable between two groups (Table 3). Alpha-fetoprotein (AFP) data were available in only 26 patients in this cohort. The values in the survivors were 9.93±11.63 ng/ml and 204.28 ± 505.45 ng/ml in those that died or underwent liver transplantation (p=0.063). Non-survivors had significantly higher MELD, MELD-Na, ALF-OF scores and INR levels compared with the survivors (All p<0.05, detailed in Table 3). The BMI was significantly higher in survivors (31.3 ± 6.7 vs. 26.6 ± 5.5, p=0.018).

Pathological features of survivors and non-survivors

Multilobular necrosis was more common in non-survivors than survivors (62.5% vs. 21.1%, p=0.016). Other histopathological features were not significantly different between the two groups. Multiplex immunofluorescence of liver tissue from both, a survivor and a non-survivor, confirmed necrotic areas with loss of hepatocytes, low HNF4α expression and high expression of cell death markers (cleaved caspase 3, RIPK3). High CK19 and low hepatocyte Ki67 expression resembled compensatory regenerative ductular reactions whilst hepatocyte proliferation was diminished. Necrotic areas from non-survivor were also characterized by reduced hepatic stellate cell activation (αSMA+ve) (Figure 2).

Multivariate analyses for outcome of sIAH

Multivariate Cox regression revealed the presence of multilobular necrosis (Hazard ratio (HR) =3.675, 95% Confidence interval (CI): 1.322-10.211, p=0.013), and lower BMI (HR=0.916, 95%CI: 0.848-0.991, p=0.029) independently predicted death/transplantation (Supplementary Table 1 and Figure 3). The positive predictive value (PPV) and the negative predictive value of multilobular necrosis for death/transplantation at 3 months were 62.5% and 78.75% respectively. A new model which comprised MELD score and multilobular necrosis was developed: MELD- multilobular necrosis = 1.16*MELD+ 3* multilobular necrosis (yes=1, no=0). The best cut-off value for predicting 3-month mortality was 31, with an AUROC of 0.833 and sensitivity of 70.83%, specificity of 88.89% and PPV of 88.9%.

Using competing risk analysis, we also evaluated the effect of multilobular necrosis on

death by defining liver transplantation as the competing event. The results showed that the impact of multilobular necrosis on death was marginally significant (HR=2.942, 95%CI: 1.000-8.657, P=0.050) (detail in Supplementary Table 2).

Discussion

The results of this study, which focuses on the role of liver biopsy in patients with sIAH, show for the first time that the presence of multilobular necrosis is an early, independent indicator of death or need for liver transplantation. The patient population in this study describes an incidence of sIAH amongst patients with severe acute hepatitis of about 21%, which is similar to what was observed in previous cohorts[1]. A particular problem with the umbrella term sIAH is that it is a collection of many poorly characterised aetiologies. This study did not attempt to define the underlying cause of sIAH but focused on characterising the syndrome from a clinicopathological stand point to determine whether there are any distinctive features on liver biopsy that may define the prognosis.

The results showed that, the presence of multilobular necrosis was associated with higher INR and MELD score and was an independent factor associated with death or transplantation. Though the predictive value of hepatic multilobular necrosis was shown for the first time shown in patients with sIAH, it was not an unexpected finding. This observation is similar to a previous study showing that multilobular necrosis could predict death of patients with severe autoimmune hepatitis [15]. Lefkowitch [16] also suggested that the presence of 50% or more of parenchymal necrosis in a liver biopsy should warrant discussions about transplantation. It is important to note that the liver biopsies were performed about a median of 10-days prior to transplantation, suggesting that multilobular necrosis on liver biopsy is an early predictor of poor outcome.

Sampling error is a potential concern in using liver biopsy for prognostication in patients

with acute liver failure [16, 17]. As shown in our series, there was often a discrepancy between the biopsy at presentation and the explanted livers. The most likely explanation is that the biopsy samples just represent a small area of liver tissue and thus might miss regenerative nodules in between areas of multilobular necrosis. The association between multilobular necrosis at biopsy and mortality or need for transplantation in our series could be due to the greater likelihood that multilobular necrosis is represented in a biopsy sample, if the necrosis is more extensive. To our knowledge, the reproducibility of the histological definition of multilobular necrosis in the prognostication of indeterminate hepatitis has not been formally tested. The use of reference images has been proposed to facilitate the reproducibility of histological interpretation[18] and could be applied to this setting.

Using multiplex immunofluorescence, we histologically determined the molecular expression of several biomarkers targeting molecular markers of cell death, stellate cell activation and regeneration in sIAH patients with different prognosis. This is an attractive method to stain tissues that are scarce such as in this case. Also, staining multiple biomarkers in the same liver section helps to identify biological processes that may be relevant such as those related to cell death and regeneration. Multiplex immunofluorescence staining of liver tissue confirmed a loss of hepatocytes and poor regenerative capacity in a patient that died. This finding was consistent with previously established idea that the prognosis of severe liver injury is associated with the failure of hepatic regeneration [19].

Ductular cholestasis is traditionally considered by histopathologists to be due to superimposed infection [20]. In this study, only one out of 8 patients with ductular cholestasis was diagnosed with infection not supporting this view. Also, ductular cholestasis is thought to reflect the severity of clinical cholestasis [20]; yet the presence of ductular cholestasis in this cohort did not correlate with serum bilirubin level. Ductular cholestasis could therefore be related to other factors such as altered bile secretion due to the parenchymal damage and may be a hallmark of the degree of surviving liver parenchyma rather than necrosis [20]. The correlation between ductular cholestasis and lower albumin indicates that the ductular cholestasis could also reflect the loss of functional liver parenchyma. Possible explanations for this association include overload of bilirubin on residual functioning parenchyma leading to clogging of the ductules or alteration of bile composition due to reduced availability of bile salts resulting in a form of "inspissated bile"[21]. These, in turn could worsen hepatocyte function.

Another intriguing finding of this study was that higher BMI was independently associated with better outcome of patients with sIAH. Increasing evidence has shown obesity reduces the mortality of severe and end-stage liver disease [22, 23]. BMI>25 kg/m^2 had been shown to be predictive in acute on chronic liver failure [23]. This kind of 'obesity paradox' has also been found in critically-ill patients and those with other chronic illnesses [24]. Evidence from the sepsis literature showed altered pro-inflammatory (interleukin -8) to anti-inflammatory (interleukin -10) cytokine ratio in sepsis, which correlated with higher visceral adipose tissue [25]. The adipocytes can release adipokines and anti-inflammatory factors, such as leptin and interleukin -10, which may

improve survival during acute critical illness by mitigating the deleterious inflammatory response [26, 27]. This function of adipocytes could be one of the plausible explanations for the 'obesity paradox'. Furthermore, better nutritional status in patients with higher BMI could be another possible reason [28, 29].

There are some limitations of this study. First, although the retrospective nature of the study brings with it the elements of bias and incomplete data, our study was based on liver biopsies, which were carefully archived and the individual patient data were collected from prospective databases and electronic records minimising this limitation. The retrospective nature also provide long term follow-up, which, in this study has provided important insight. Second, the overall sample size was relatively small but this is not surprising as the study addresses a rare disease. Finally, this study does not allow us to provide insights into the underlying cause of sIAH nor does it defines the mechanisms why some patients recover and others do not.

In conclusion, the results of this study provide novel insights into the important role of liver biopsy in sIAH patients suggesting that the presence of multilobular necrosis is an early indicator of poor prognosis.

Figure legends

Figure 1. CONSORT diagram showing the flow-chart of cases selection.

Figure 2. Multiplex immunofluorescence of liver tissue from a survivor (A) and non-survivor (B). Sample was assessed for markers of proliferation (Ki67), stellate cell activation (αSMA), cell death (cleaved caspase 3, RIPK3) and cell type (HNF4α, CK19, IBA-1). Marker expression is shown as relative positive area (%).

Figure 2. Kaplan Meier curve for the outcome of severe acute indeterminate hepatitis. A: According to presence or absence of multilobular necrosis. B: According to body mass index (BMI).

Table legends

Table 1 Chronology of clinicopathological features in severe acute indeterminate hepatitis stratified by the interval between onset of jaundice and the time of liver biopsy.

Table 2 Comparison of biochemical indicators at the time of biopsy in patients with different pathological features

Table 3 Baseline characteristics of patients with different outcomes

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