Common variable immunodeficiency disorder (CVID)-related liver disease: assessment of the main histological aspects using novel semiquantitative scoring systems, image analysis and correlation with clinical parameters of liver stiffness and portal hypertension

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ABSTRACT

Aims – We aimed to investigate the relationship between T cell mediated sinusoidal injury, nodular regenerative hyperplasia like changes (NRH-LC) and fibrosis, clinical measures of fibrosis and portal hypertension, and progression rate in CVID-related liver disease.

Methods – This is a retrospective single centre study. Liver biopsies from CVID patients with liver disease were reviewed to assess for NRH-LC, fibrosis and elastosis, including collagen and elastin proportionate areas. CD3 positive T cells infiltration and sinusoidal endothelial changes by CD34 expression were quantified by image analysis and a semiquantitative method, respectively. These findings were correlated with liver stiffness measurements (LSM) and hepatic venous pressure gradient (HVPG).

Results – NRH-LC and pericellular elastosis were present in most biopsies (32/40 and 38/40, respectively). All biopsies showed fibrosis, which was limited to pericellular in 21/40 (52.5%) and included bridging fibrous septa in 19/40 (47.5%). 28/40 liver biopsies showed enhanced sinusoidal expression of CD34. There were more CD3 positive cells in biopsies with NRH-LC compared to those without. There was no significant correlation between LSM, HVPG and fibrosis/elastosis scores. Five of seven patients with at least two biopsies showed progression in fibrosis stage.

Conclusions – NRH-LC and fibrosis in CVID patients often co-exist along with the presence of sinusoidal endothelial changes and sinusoidal lymphocytic infiltration. Fibrosis progresses over time, and significant fibrosis can be observed in young patients (<30 years old), potentially reflecting a more aggressive form of CVID related liver disease. Further studies are necessary to investigate the relationship between histological findings, clinical measures of fibrosis and portal hypertension and outcome.

What is already known on this topic – Liver disease is a major complication of CVID, associated with NRH-LC as well as fibrosis.

What this study adds – Demonstration of the coexistence of fibrosis, NRH-LC and pericellular elastosis, quantification of liver fibrosis and elastosis by image analysis and illustration of sinusoidal endothelial changes by CD34 immunohistochemistry. Comparison of histology findings and clinical measures such as liver stiffness measured by fibroscan and HVPG.

How this study might affect research, practice or policy – Clinical parameters may not be sufficient to predict fibrosis in CVID patients, therefore histological assessment of the liver in CVID patients is informative and contributes to the investigation of the pathogenesis of CVID-related liver injury.

INTRODUCTION

Common variable immunodeficiency disorder (CVID) is the most common symptomatic adult primary immunodeficiency disorder, with a prevalence between 0.001-6.9 per 100,000 population, showing wide variations between geographical regions(1,2). Although the underlying pathogenesis remains largely unknown, current understanding suggests that primary B-cell dysfunction with defective T cell and antigen presenting cell functions are contributing factors (3,4). CVID is considered a heterogeneous group of diseases with variable phenotypic presentations hallmarked by antibody deficiency, recurrent infections, and failure to respond to vaccines. CVID may be accompanied by immune dysregulation resulting in autoimmunity and inflammatory complications (5).

The systemic manifestations of CVID may affect the liver, gastrointestinal tract, lungs, reticuloendothelial system and cause immune-mediated cytopaenias. Liver involvement is common with a reported prevalence ranging between 5-79% (6–12). Our recent study of 218 cases at the Royal Free London NHS Foundation Trust, a tertiary care centre in the UK, liver involvement was calculated at 42% (13). Nodular regenerative hyperplasia (NRH) is the most frequently described liver pathology among CVID patients, but granulomatous disease, infections, malignancies, cholangiopathy, autoimmune hepatitis and fibrosis are also associated with the disease (14–16).

There is a wide range of reported incidence of NRH in liver biopsies from CVID patients varying between 5% to 80% (9,17). However, there is only limited literature regarding the frequency and pattern of liver fibrosis. One biopsy series demonstrated co-existent cirrhosis in almost one third of CVID patients with NRH(6). Another study evaluating liver stiffness measurements (LSM) in CVID patients presenting with deranged liver function tests showed a LSM of ≥7.3 kPa in 33.8% of the study population, suggesting the presence of at least moderate fibrosis in a third of patients (10). A recent study from the United Kingdom that evaluated liver involvement in CVID patients and their survival rates showed that the outcomes of the patients with NRH is poor and further worsens when associated with cirrhosis and/or portal hypertension(18). We have shown recently (19) that fibrosis is invariably associated with the deposition of elastic fibres; and the deposition of elastic fibres along hepatocytes is often observed in the context of steatohepatitis and vascular disorders, suggesting that damage to the space of Disse stimulates pericellular/perisinusoidal fibrosis.

T-cell mediated sinusoidal endothelial injury is thought to be part of the pathogenesis of liver disease in CVID. The sinusoidal endothelial cells have a unique configuration of features that include the presence of fenestrae and the expression of a specific set of antigens(20). Changes to the configuration of sinusoidal endothelial cells to a more generic endothelial phenotype can be demonstrated by the presence of CD34 on the cell membrane.

As the pathology of liver disease in CVID has not been well characterised and the clinical outcomes of those with liver disease are impaired, including suboptimal outcomes from liver transplantation(21,22), early identification of liver fibrosis is important in understanding the natural history and provide optimal management for CVID patients.

We have therefore reviewed the histological patterns of liver injury in a large retrospective cohort of liver biopsies from CVID patients and correlated our findings with clinical data, as part of our multidisciplinary collaborative programme on CVID-related liver injury. The aim of our work was to 1. investigate the relationships between T-cell mediated sinusoidal injury and changes to the sinusoidal endothelium using IHC, 2. assess the presence of NRH and quantify fibrosis and elastosis 3. correlate these histological findings with clinical measures of fibrosis and portal hypertension and 4. assess the progression of liver fibrosis in CVID patients.

METHODS

Case selection and exclusion criteria

This is a retrospective single centre study. Data from 62 CVID patients who underwent at least one liver biopsy were collected from our institutional databases. CVID was defined following clinical diagnosis by a consultant immunologist, in line with current international guidelines, typically characterised by hypogammaglobulinaemia, recurrent infection and a failure to respond to pneumococcal or tetanus vaccination(5). Biopsy slides from all the cases were re-reviewed along with the clinical information. Twenty-two cases were excluded from the study (as they showed concomitant other pathologies or had insufficient slides and tissue. The remaining 40 cases were included in the final analysis (Figure 1).

Medical records from the selected patients were reviewed to collect demographic data and information about previous Fibroscan and hepatic venous pressure gradient (HVPG) results.

Histological assessment

All biopsies were evaluated with the Haematoxylin & Eosin stain (HE), picro-Sirius red (SR) for collagen, Victoria blue (VB) for elastic fibres and reticulin for NRH assessment. NRH was defined as parenchymal nodularity due to the presence in at least part of the biopsy sample of two populations of hepatocytes differing in size, because of nodules composed of thickened liver cell plates and with boundaries composed of compressed cell plates (23). Co-existent fibrosis was not considered an exclusion criterion for the diagnosis of NRH, as in our experience and the experience of others (6,24), features of NRH and fibrosis are often observed together in liver biopsies from CVID patients. In line with the proposal by Crotty et al (24) we applied the term NRH-like changes (NRH-LC) to this specific CVID setting and documented presence or absence of NRH-LC.

Fibrosis assessment

Fibrosis assessment was performed by a semi quantitative scoring system using the SR stain designed specifically for the purpose of this study and based on a preliminary assessment of the fibrosis pattern in this patient cohort.

1 : No fibrosis

2 : Patchy and focal pericellular fibrosis

3 : Diffuse pericellular fibrosis

3 : Bridging fibrosis

4 : Nodule formation/cirrhosis

Collagen proportionate area (CPA) was measured as described in previous studies (22,25) (see Supplementary).

Elastic fibres assessment

The presence of elastic fibres in a pericellular distribution and within fibrous septa was assessed as follows using the VB stain and adapted from our previous work (26). Pericellular elastic fibres were classified as stage 0 (absent), stage 1 (very occasional delicate

perihepatocyte strands visible only at high magnification 400x and after extensive search), or stage 2 (perihepatocytic elastic strands obvious at 100× or 200× magnification).
Parenchymal EPA was calculated for 40 biopsies with available VB stains using multispectral imaging (see Supplementary).

CD3 counts and CD34 expression

CD3 and CD34 immunostaining were performed on 28 cases out of the 40, these were the cases where the tissue was sufficient to assess the parenchymal T lymphocytosis and capillarization (see Supplementary).

CD34 immunostaining for the capillarization was graded as below (27).

0 - Not increased (i.e., staining only septal endothelium and endothelium of limiting plate of the nodule)

1- Marginal (non-confluent patches)

2- Focal (confluent patches showing diffuse CD34 immunostaining)

3- Diffuse incomplete (confluent areas showing diffuse CD34 immunostaining with very minimal non staining areas)

4- Diffuse complete (CD34 staining was seen in the whole biopsy)

Assessment of fibrosis progression in patients with multiple liver biopsies

Seven of the forty patients in our cohort had undergone a previous liver biopsy at least twelve months before the index biopsy. We assessed fibrosis/elastosis progression using the available connective tissue stains in this subgroup. Fibrosis in these biopsies was also classified using the same semi quantitative method as above.

Liver stiffness and hepatic venous pressure gradient measurements

Liver stiffness measured using Fibroscan and hepatic venous pressure gradient measurement (HVPG) were recorded as part of routine investigations. Values for each investigation were

obtained from clinical records. HVPG measures within a year of biopsy date (n=27) and Fibroscan measurements not more than four years from the biopsy date (n=17) were included in the analysis.

Statistical analysis

Ordinal and nominal categorical data are expressed as frequencies. Due to the limited size of the population the data was considered non-parametric for the purposes of reporting statistics, therefore continuous data are reported as medians and interquartile ranges. Categorical data was compared using Chi-square test or Fisher's exact test, when necessary. Mann-Whitney or Kruskal-Wallis tests were used to compare continuous data between groups. Spearman's rank order correlation was used to compare between continuous data. A critical alpha level was set as $p < 0.05$ (two-tailed). All statistical analyses were performed using IBM SPSS Statistics (version 29.0).

RESULTS

The median age of the patients in our cohort was 47.5 years (IQR 39 - 59.25) and 23/40 (57.5%) patients were female.

Overall histological findings

NRH-LC and fibrosis were commonly observed in patients with CVID: NRH-LC was present in most biopsy samples (32/40, 80%) and fibrosis was present in all 40 biopsy samples. Fibrosis was limited to pericellular in 21/40 (52.5%) samples, as either stage 1 (7/40, 17.5%) or stage 2 (14/40, 35%). Bridging fibrous septa were observed in 19/40 (47.5%) samples, either stage 3 (17/40, 42.5%) or stage 4 (2/40, 5%) (Figure 2). Of note, pericellular fibrosis co-existed in all of those with bridging fibrosis (stage 3 and 4).

Elastosis was present in nearly all cases of CVID that we assess. Pericellular elastic fibres were also present in most (38/40, 95%) samples, and graded as stage 1 in 29/40 (72.5%) and stage 2 in 9/40 (22.5%) (Figure 3).

Median parenchymal CPA, combined CPA and EPA were 4.26% (IQR 2.79%-6.73%), 9.02% (IQR 7.16%-12.82%) and 0.46% (IQR 0.17% - 1.97%), respectively.

CVID cases showed an increase in the number of CD3 positive cells in comparison to normal livers. Median parenchymal CD3 density in CVID liver biopsies was 206.52 cells/mm3, which was significantly higher than the median parenchymal CD3 density observed in the normal liver biopsies (61.12 cells/mm3, *p* 0.0017). Similarly, the median portal/stromal and combined CD3 density in CVID liver biopsies were 419.61 and 209.15 cells/mm3, respectively, which was significantly higher than the corresponding values in the normal liver biopsies (161.29 and 72.55, respectively, *p* 0.015 and 0.013) (Figure 4).

All twenty eight CVID liver biopsies assessed showed enhanced sinusoidal expression of CD34 at variable degrees. Sixteen out of twenty-eight cases (57.14%) showed a diffuse pattern of capillarization (grade 3 or 4) (Figure 5).

Comparison between cases with NRH-LC present (NRH-LC-P) and cases with NRH-LC absent (NRH-LC-A)

There were no significant differences observed between biopsies with NRH-LC present compared to those where NRH-LC were absent with regards to fibrosis and elastosis scores, CPA, EPA, CD34 sinusoidal stain, LSM or HVPG measurements (Table 1). Although all three CD3 density parameters were higher in the NRH-LC-P group compared to the NRH-LC-A group, there was not a significant difference between the two groups (Table 2).

Table 1 – NRH-LC comparison with histological/clinical parameters

Variable	N	NRH-LC absent (NRH-LC-A)	NRH-LC present (NRH-LC-P)	*P* value
Fibrosis stages	40			0.7916
Focal pericellular (Stage 1)		2	5	
Diffuse pericellular (Stage 2)		2	12	
Bridging fibrosis (Stage 3)		4	13	
Nodule formation (Stage 4)		0	2	
Pericellular elastic fibres	40			0.0557
Absent		2	0	
Stage 1		5	24	
Stage 2		1	8	
CPA*	40			
Parenchyma		3.87 (3.49)	4.26 (3.85)	0.4262
Combined		9.43 (4.03)	8.83 (5.98)	0.4529
CD34	28			0.4284
Not increased		0	0	
Marginal		1	1	
Focal		1	9	
Diffuse incomplete		3	12	
Diffuse complete		0	1	
Fibroscan measurement*	17	9.9	12.4 (11.7)	0.312
HVPG*	27	8.0 (6.0)	6.5 (4.0)	0.485

*Reported as median and IQR (in brackets), when possible.

Abbreviations: CPA, Collagen proportionate area; HVPG, Hepatic Venous Pressure Gradient; NRH-LC-A, Nodular regenerative hyperplasia-like changes absent; NRH-LC-P, Nodular regenerative hyperplasia-like changes present

Table 2 - Comparison of CD3 density* between NRH-LC-A and NRH-LC-P groups

CD3 density	NRH-LC absent (n = 5)	NRH-LC present (n = 23)	*P* value
Parenchymal	113.60 (186.41)	210.50 (208.0)	0.413
Portal/Stromal	295.90 (606.11)	445.31 (791.93)	0.479
Combined	166.97 (219.69)	220.60 (280.94)	0.493

*Reported as median and IQR (in brackets), when possible

Abbreviations: NRH-LC-A, Nodular regenerative hyperplasia-like changes absent; NRH-LC-P, Nodular regenerative hyperplasia-like changes present

Relationship between fibrosis score and EPA, CPA and CD3 density

Combined CPA and EPA values showed a significant gradual increase with increasing fibrosis stage. Median combined CPA values ranged between 8.22% to 29.34% for stages 1-4 and there was a significant difference in median CPA when comparing stages 1 and 4. EPA values were also significantly different between stages 1 and 3 (Table 3).

Similarly, median parenchymal CD3 density increased with fibrosis stages (stage 1: 145.81, stage 2: 206.03, stage 3: 276.65). The median parenchymal CD3 density in the normal livers was 61.12 (IQR 123.23 - 314.03). Tissue was not adequate for the additional stains in the two stage 4 cases (Table 3).

There was a significant association between fibrosis stage and CD34 expression (*p* 0.0377), with more diffuse changes observed with higher fibrosis stages (Table 3).

Table 3 - Fibrosis stage comparison with histological/radiological parameters

Variable		N	Stage 1	Stage 2	Stage 3	Stage 4	*P* value
Pericellular elastic fibres		40					0.423
	Absent		1	0	1	0	
	Stage 1		6	10	12	1	
	Stage 2		0	4	4	1	
CPA*		40					
	Parenchyma		4.18 (2.82)	4.71 (3.32)	3.28 (7.15)	9.32	0.321
	Combined		8.22 (4.25)	8.34 (4.72)	12.38 (8.49)	29.34	**0.0162**
CD3-positive cells density*		28					
	Parenchyma		145.81 (79.07)	206.03 (147.64)	276.65 (349.67)	-	0.197
	Portal/Stromal		295.90 (302.67)	393.90 (539.06)	743.00 (1198.02)	-	0.115
	Combined		144.99 (87.42)	208.40 (130.14)	304.50 (511.36)	-	0.125
EPA*		40	0.12 (0.23)	0.46 (1.35)	1.69 (3.72)	4.96	**0.0123**
CD34		28					**0.0377**
	Not increased		0	0	0	0	
	Marginal		1	0	1	0	
	Focal		4	3	3	0	
	Diffuse incomplete		0	6	9	0	
	Diffuse complete		0	1	0	0	

*Reported as median and IQR (in brackets), when possible

Abbreviations: CPA, Collagen proportionate area; NRH-LC-A, Nodular regenerative hyperplasia-like changes absent; NRH-LC-P, Nodular regenerative hyperplasia-like changes present; EPA, Elastin proportionate area

Correlation between histological features and LSM and HVPG measurements

LSM and HVPG values were available for 17 and 27 of the patients who underwent liver biopsy, respectively.

The median of available LSM was 10.9 kPa (IQR 8.1 kPa - 14.6 kPa) and the median HVPG measurement was 7 mmHg (IQR 5.5 mmHg - 9.5 mmHg), in the range of portal hypertension (≥6 mm Hg, ≤ 10 mmHg).

Median LSM was 9.9 kPa, 8.1 kPa, 11.75 kPa and 33.2 kPa in fibrosis stages 1-4, respectively, and median HVPG was 9.0 mmHg, 6.0 mmHg, 6.0 mmHg and 9.0 mmHg, in fibrosis stages 1-4, respectively (Table 4).

Table 4 – LSM* and HVPG* and fibrosis stages

	N	LSM (kPa)	N	HVPG (mmHg)
Fibrosis stage 1	1	9.9	5	9.0 (5.0)
Fibrosis stage 2	7	8.1 (5.2)	8	6.0 (5.0)
Fibrosis stage 3	8	11.75 (7.6)	13	6.0 (6.0)
Fibrosis stage 4	1	33.2	1	9

*Reported as median and IQR (in brackets), when possible. Abbreviations: LSM, Liver stiffness measurements; HVPG, Hepatic venous pressure gradient.

Interestingly, EPA and LSM showed moderate correlation (rs = 0.567) what was statistically significant (p = 0.018, Figure 6B). There was no correlation between combined CPA and LSM (p 0.171, Figure 6A) or HVPG (p 0.772) nor when comparing EPA and HVPG (p 0.240).

Figure 7 illustrates the breakdown of biopsy findings according to the available Fibroscan and/or HVPG measurements. Of note, NRH-LC and diffuse pericellular fibrosis were observed

in one patient with normal Fibroscan reading, also, diffuse pericellular fibrosis and NRH-LC were observed in two patients who had liver stiffness measurements > 7kPa but normal HVPG measurements, and NRH-LC and bridging fibrosis in one patient with normal HVPG and no available liver stiffness measurements readings. Taken together, these cases suggest that the relationship between histological features including pericellular fibrosis, bridging fibrosis and NRH-LC and non-invasive liver stiffness measurements or HVPG is complex and not linear.

Relationship between age group and histology findings

The median age of patients without NRH-LC was 59.5 years (IQR 56.5 - 61.5) compared with 45.5 years (IQR 38.25 - 51.5) for the group with NRH-LC on biopsy (*p 0.033*).

Regarding age groups, NRH-LC was present in most patients (3/4, 75%) less than 30 years old, in all patients 31-49 years old and absent in most patients (7/8, 87.5%) above 50 years old. This correlation was significant (*p 0.016*).

In terms of fibrosis scores and age group, 75% (n=3) of the patients less than 30 years old had bridging fibrosis (stage 3). Whereas, 64.7% (n=17) of the patients over 50 years old had either focal or diffuse perisinusoidal fibrosis (stage 1 or 2) and 35.3% (n=17) had bridging fibrosis (Table 5), possibly suggesting a greater tendency for advanced fibrosis in younger patients. The distribution of CPA as well as fibrosis scores, EPA and CD3 density did not show a correlation with either age or age groups.

Table 5 - Fibrosis stage comparison with age group

	Fibrosis stages	

	N	Stage 1	Stage 2	Stage 3	Stage 4	*P* value
Age group	40					0.396
< 30 years old		0	1	3	0	
31-40 years old		1	5	3	0	
41-50 years old		1	2	5	2	
>50 years old		5	6	6	0	

Fibrosis progression over time

Seven patients had a previous liver biopsy specimen available. The median time between biopsies was 50 months (IQR 37.5-75.5 months).

The indications for the repeat biopsies fall into the two following groups: assessment of the cause of persistently or progressively abnormal liver function tests (LFTs) or progression of portal hypertension; with the exception of case 4, which was to investigate decompensation in the setting of disseminated *Cryptococcus* infection. A few patients had immunosuppressive treatment between biopsies, as noted in Table 6.

The biopsies showed at least mild progression in fibrosis stage in five (5/7), including 3/7 which progressed from mild pericellular fibrosis to bridging fibrosis. Two patients (ages 28 years old and 72 years old) did not show progression (Table 6).

Table 6 - Cases with more than one biopsy at least 1 year apart

Case	Age (years) at B1	Time gap months* B1-B2	Indication B1 and clinical information	Treatment received between biopsies	Comments on progression on histology
4	28	13	Worsening LFTs and cryptococcal infection	Budesonide, immunoglobulin replacement, antibiotics	No progression. Bridging fibrosis. Small gap between biopsies
14	40	86	Persistently abnormal LFTs, clinically portal hypertension. Biopsy to exclude other causes and ensure inflammation adequately suppressed	Immunoglobulin replacement, intermittent antibiotics for infection, prednisolone (oral) for choroidal granulomata / scleritis / central serous retinopathy initially and then maintained for liver as seemed to improve LFTs	Significant increase in fibrosis and elastosis
18	72	50	Cholestatic LFTs, and oesophageal varices. To assess fibrosis	Immunoglobulin replacement, antibiotics, PPI, ACE inhibitor	No progression. Patchy sinusoidal fibrosis in both biopsies
21	71	34	Abnormal LFTs, high fibroscan, to assess fibrosis and inflammation	Antibiotics, immunoglobulin replacement	Significant increase in fibrosis, small increase in elastosis
27	61	146	persistently elevated transaminases, splenomegaly	Immunoglobulin replacement, infliximab (Crohn's disease), antibiotics	Significant increase in fibrosis, small increase in elastosis
34	30	41	Persistent abnormal LFTs and increasing splenomegaly. Being assessed for lung transplant	Immunoglobulin replacement, antibiotics, inhaled bronchodilators	Small increase in fibrosis and elastosis
47	53	65	Progressive rise of LFTs, portal hypertension	Immunoglobulin replacement, antibiotics	Significant increase in elastosis, small increase in fibrosis

B1- index biopsy, B2 – previous biopsy, * time gap between biopsies in months, ACE - Angiotensin-converting enzyme, LFTs - liver function tests, NRH-LC - nodular regenerative hyperplasia-like changes, PPI - proton pump inhibitors.

DISCUSSION

Our study is the result of a multidisciplinary collaboration caring for a large cohort of CVID patients in a tertiary care setting. We have demonstrated that liver disease is a common complication of CVID affecting 91/218 (42%) of our patients (13). In this study we have carried out a detailed histological assessment of liver biopsies from CVID patients. We have focused on features considered to be responsible for the development of liver complications in CVID patients, where an immune-mediated injury to the vascular endothelium is believed to trigger NRH-LC and fibrosis (28)(29). We also correlated our histological observations with clinical measures of fibrosis and portal hypertension and investigated fibrosis progression in patients who had multiple biopsies.

NRH is traditionally defined as 'presence of multiple 1 to 2 mm nodules separated by regions of hepatocyte atrophy with little or no fibrous septation' (17) and affects a variable proportion of CVID patients according to different studies (6–8). Changes consistent with NRH were present in the majority (80%) of our patients. In contrast to its traditional definition, and in line with other recent studies, NRH-LC and fibrosis in CVID patients are not mutually exclusive but tend to coexist. In a detailed recent histological study on liver biopsies from CVID patients, Crotty et al (24) described a distinctive pattern of delicate pericellular fibrosis in the majority (23/26) of their samples and proposed the term "NRH-like change" in the specific setting of CVID, to clarify the terminology. We believe that fibrosis starts at a pericellular level and progresses to the formation of septa later, based on the following observations: 1) the presence of focal or diffuse pericellular fibrosis in all biopsies; 2) the concomitant presence of bridging fibrous septa in some cases; 3) the interval change observed in those patients with multiple biopsies where pericellular fibrosis alone was observed in the first biopsy and bridging fibrosis in the second. The co-existence of NRH-LC, pericellular fibrosis, altered sinusoidal endothelium configuration and the prominent intrasinusoidal infiltrate of CD3 positive lymphocytes, all support the hypothesis of a T-cell mediated injury to sinusoidal

endothelial cells resulting in a vascular flow disturbance triggering NRH-LC and a concomitant fibrogenic response (12,28,29). It is also possible that CVID-related NRH-LC is different from NRH caused by other pathologies, such as obliterative vasculopathy.

Our digital CD3 counting method showed that CD3 positive lymphocytes are more numerous in CVID patients with NRH-LC than in those without. Further studies are required to demonstrate whether infiltrating CD3-positive lymphocytes predict fibrosis and clinical outcomes (29). This may be important, as it would instruct the use of immunosuppressive or immunomodulatory treatments in patients with high CD3 counts to prevent the development of worsening chronic liver disease.

Pericellular fibrosis in some of our cases was mild and focal. Conventionally, it has been challenging to differentiate the apparent enhancement of the ambient pericellular/perisinusoidal collagen strands due to hepatic plate atrophy between NRH-LC nodular areas from true fibrosis. In our approach, the presence of delicate pericellular elastic fibres, absent in normal conditions, allowed us to confirm the presence of pericellular damage, as has previously been shown in alcohol-related liver injury (26).

To our knowledge, this is the first study applying digital image analysis of histology specimens to quantify collagen and elastin fibres in liver biopsies from CVID patients. CPA has consistently been shown to correlate with semiquantitative fibrosis scores and predict clinical outcomes in various liver disorders (22,25,30). In our series there was a significant correlation between the increase in combined CPA values and fibrosis semiquantitative stage, and although there is a weak trend of LSM and CPA increasing together (Figure 6A), this was not statistically significant (r = 0.348, *p* 0.171*)*. We also could not find a correlation with CPA and HVPG measurements. The lack of association between HVPG and overall fibrosis stage may be due to the ubiquitous presence of pericellular fibrosis, which may not significantly contribute to increases in HVPG. The correlation between EPA and LSM is a novel finding and suggests the EPA could represent a more sensitive marker than CPA in the assessment of CVID liver injury. Further studies are necessary to confirm this observation. The partial correlation between histological and clinical measurements of fibrosis in our study could also be related to its retrospective nature, the relatively small number of patients with available HVPG and Fibroscan readings, the variable time interval between clinical readings and liver biopsy and sampling variation. Of interest, however, is the fact that of the three patients in our series

who had either a normal HVPG or Fibroscan, all showed pericellular fibrosis, two had NRH-LC, and one also bridging fibrosis. These observations support the role of liver biopsy in gauging liver injury in CVID patients, particularly in the assessment of patients with portal hypertension.

Finally, our study shows that NRH-LC tends to be more common in younger patients, as the majority of patients younger than 30 years had NRH-LC and bridging fibrosis. These findings suggest that the early manifestation of CVID related liver disease could reflect a more severe form of the disease.

Conclusions

Our study shows that NRH-LC and fibrosis in CVID patients often co-exist along with the presence of sinusoidal endothelial changes and sinusoidal lymphocytic infiltration, which would fit with the hypothesis proposed by Malamut et al. (28) of a T-cell mediated injury to the sinusoidal endothelium resulting in vascular flow disturbance and NRH-LC as well as fibrogenesis. CPA correlates with our novel dedicated semiquantitative scoring system of CVID liver fibrosis and pericellular elastosis measurement is a valuable tool in the diagnosis of subtle pericellular fibrosis. The pathology observed in CVID is associated with progressive liver fibrosis, and significant fibrosis can be observed in young patients (<30 years old), potentially reflecting a more aggressive form of CVID related liver disease.

Funding

None.

Acknowledgements

None.

Competing interests

None.

Ethics approval

Research was carried out under ethical approval Ref: 07/Q0501/50 granted by the Hampstead NHS Research Ethics Committee.

Contributorship statement

HS (1st author) and AQ conceived the study design. HS (1st author), CGXB and AQ reviewed and scored all slides; HS (1st author) and CGXB acquired and analysed data and contributed equally as co-first authors to drafting the manuscript, AH performed image analysis, immunohistochemistry and contributed to the writing and revising of the manuscript. NE, HS (4th author), NB, SOB, DML, NH and DT collected clinical data. NH was involved in interpretation of results, editing the manuscript and contributing to data analysis. AQ, NH and DML critically reviewed the manuscript. All authors revised the content and have approved the final version for publication.

REFERENCES

1. Selenius JS, Martelius T, Pikkarainen S et al. Unexpectedly High Prevalence of Common Variable Immunodeficiency in Finland. Front Immunol. 2017 Sep 28;8:1190.
2. Weifenbach N, Schneckenburger AAC, Lötters S. Global distribution of common variable immunodeficiency (CVID) in the light of the UNDP Human Development Index (HDI): A preliminary perspective of a rare disease. J Immunol Res. 2020 Sep 1;2020:1–8.
3. Gereige JD, Maglione PJ. Current Understanding and Recent Developments in Common Variable Immunodeficiency Associated Autoimmunity. Front Immunol. 2019 Dec 10;10:2753.
4. Yazdani R, Habibi S, Sharifi L et al. Common Variable Immunodeficiency: Epidemiology, Pathogenesis, Clinical Manifestations, Diagnosis, Classification, and Management. J Investig Allergol Clin Immunol. 2020;30(1):14–34.
5. Bonilla FA, Barlan I, Chapel H. International Consensus Document (ICON): common variable immunodeficiency disorders. J Allergy Clin Immunol Pract. 2016 Jan-Feb; 4(1):38-59.
6. Azzu V, Fonseca M, Duckworth A et al. Liver disease is common in patients with

common variable immunodeficiency and predicts mortality in the presence of cirrhosis or portal hypertension. J Allergy Clin Immunol Pract. 2019 Apr 24;7(7):2484–6.e3.

7. Fuss IJ, Friend J, Yang Z et al. Nodular regenerative hyperplasia in common variable immunodeficiency. J Clin Immunol. 2013 May;33(4):748–58.

8. Ho HE, Cunningham-Rundles C. Non-infectious Complications of Common Variable Immunodeficiency: Updated Clinical Spectrum, Sequelae, and Insights to Pathogenesis. Front Immunol. 2020 Feb 7;11:149.

9. Ward C, Lucas M, Piris J et al. Abnormal liver function in common variable immunodeficiency disorders due to nodular regenerative hyperplasia. Clin Exp Immunol. 2008 Sep;153(3):331–7.

10. Crescenzi L, Pecoraro A, Fiorentino A et al. Liver stiffness assessment by transient elastography suggests high prevalence of liver involvement in common variable immunodeficiency. Dig Liver Dis. 2019 Nov;51(11):1599–603.

11. Globig AM, Strohmeier V, Surabattula R et al. Evaluation of Laboratory and Sonographic Parameters for Detection of Portal Hypertension in Patients with Common Variable Immunodeficiency. J Clin Immunol. 2022 Nov;42(8):1626–37.

12. Lima FMS, Toledo-Barros M, Alves VAF et al. Liver disease accompanied by enteropathy in common variable immunodeficiency: Common pathophysiological mechanisms. Front Immunol. 2022 Oct 20;13:933463.

13. Halliday N, Eden N; Somers H et al. Common variable immunodeficiency disorder (CVID)-related liver disease is a common complication frequently resulting in portal hypertension and is an independent predictor of mortality [Unpublished, under review].

14. Mehta N, Sadaps M, Lopez R et al. Spectrum of Liver Disease in Common Variable Immunodeficiency: 906. Official journal of the American College of Gastroenterology, ACG. 2017 Oct; 112:S507.

15. Pecoraro A, Crescenzi L, Varricchi G et al. Heterogeneity of Liver Disease in Common Variable Immunodeficiency Disorders. Front Immunol. 2020 Feb 28;11:338.

16. Song J, Lleo A, Yang GX et al. Common Variable Immunodeficiency and Liver Involvement. Clin Rev Allergy Immunol. 2018 Dec;55(3):340–51.

17. Goldblum JR, Odze RD. Odze and Goldblum's Surgical Pathology of the GI Tract, Liver, Biliary Tract and Pancreas. Vol. 4th edition. Saunders; 2022.

18. Tranah TH, Cargill Z, Tavabie O et al. Challenges in liver transplantation for common variable immunodeficiency-related liver disease: a case series and systematic review. Journal of Liver Transplantation. 2021 Oct 1;4:100038.

19. Hall A, Cotoi C, Luong TV et al. Collagen and elastic fibres in acute and chronic liver

injury. Sci Rep. 2021 Jul 15;11(1):14569.

20. Dhillon AP, Colombari R, Savage K et al. An immunohistochemical study of the blood vessels within primary hepatocellular tumours. Liver. 1992 Oct;12(5):311–8.

21. Ramlaul N, Ooi J, Jeffrey GP et al. Liver Transplantation in Adults With Liver Disease Due to Common Variable Immunodeficiency Leads to Early Recurrent Disease and Poor Outcome. Liver Transpl. 2018 Nov;24(11):1622-1626.

22. Israelsen M, Guerrero Misas M, Koutsoumourakis A et al. Collagen proportionate area predicts clinical outcomes in patients with alcohol-related liver disease. Aliment Pharmacol Ther. 2020 Dec;52(11-12):1728–39.

23. Alastair D Burt, Linda D. Ferrell, Stefan G. Hubscher. MacSween's Pathology of the Liver. Vol. 8th. Elsevier; 2023.

24. Crotty R, Taylor MS, Farmer JR et al. Spectrum of Hepatic Manifestations of Common Variable Immunodeficiency. Am J Surg Pathol. 2020 May;44(5):617–25.

25. Buzzetti E, Hall A, Ekstedt M et al. Collagen proportionate area is an independent predictor of long-term outcome in patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther. 2019 May;49(9):1214–22.

26. Luong TV, Abou-Beih S, Watkins J et al. Elastic fibres in alcoholic liver disease. Sci Rep. 2020 Nov 18;10(1):20098.

27. Quaglia A, Jutand MA, Dhillon A et al. Classification tool for the systematic histological assessment of hepatocellular carcinoma, macroregenerative nodules, and dysplastic nodules in cirrhotic liver. World J Gastroenterol. 2005 Oct 28;11(40):6262–8.

28. Malamut G, Ziol M, Suarez F et al. Nodular regenerative hyperplasia: the main liver disease in patients with primary hypogammaglobulinemia and hepatic abnormalities. J Hepatol. 2008 Jan;48(1):74–82.

29. Ziol M, Poirel H, Kountchou GN et al. Intrasinusoidal cytotoxic CD8+ T cells in nodular regenerative hyperplasia of the liver. Hum Pathol. 2004 Oct;35(10):1241–51.

30. Calvaruso V, Dhillon AP, Tsochatzis E et al. Liver collagen proportionate area predicts decompensation in patients with recurrent hepatitis C virus cirrhosis after liver transplantation. J Gastroenterol Hepatol. 2012 Jul;27(7):1227–32.

Figure legends

Figure 1 - Case selection and exclusion criteria

Figure 2 - Fibrosis scoring in CVID liver biopsies using the Picrosirius red stain: (A) focal pericellular fibrosis (arrows), (B) diffuse pericellular fibrosis (arrows), (C) bridging fibrosis and (D) nodule formation (10x magnification).

Figure 3 - Liver biopsy showing diffuse pericellular fibrosis on the Picrosirius red stain (A) and pericellular elastosis on the Victoria blue stain (B) in the same area (20x magnification).

Figure 4 - CD3 positive cells in a normal liver (A) and a CVID liver biopsy (B) (20x magnification).

Figure 5 - CD34 expression in CVID liver biopsies: marginal (A), focal (B), diffuse incomplete (C) and diffuse complete (D) (10x magnification).

Figure 6 - Liver stiffness measurements and their relationship with (A) Combined (parenchymal and portal/stromal) Collagen Proportionate Area (CPA) and (B) Elastin proportionate area (EPA).

Figure 7 - Biopsy findings according to the available Fibroscan and/or HVPG measurements.

Abbreviations: NRH-LC-A, Nodular regenerative hyperplasia-like changes absent; NRH-LC-P, Nodular regenerative hyperplasia-like changes present; HVPG, Hepatic venous pressure gradient