

## Basic Study

**Autoantibody profiles in autoimmune hepatitis and chronic hepatitis C identifies similarities in patients with severe disease**

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**Abstract****AIM**

To determine how the auto-antibodies (Abs) profiles overlap in chronic hepatitis C infection (CHC) and autoimmune hepatitis (AIH) and correlate to liver disease.

**METHODS**

Levels of antinuclear Ab, smooth muscle antibody (SMA) and liver/kidney microsomal-1 (LKM-1) Ab and markers of liver damage were determined in the sera of 50

patients with CHC infection, 20 AIH patients and 20 healthy controls using enzyme linked immunosorbent assay and other immune assays.

### RESULTS

We found that AIH patients had more severe liver disease as determined by elevation of total IgG, alkaline phosphatase, total serum bilirubin and serum transaminases and significantly higher prevalence of the three non-organ-specific autoantibodies (auto-Abs) than CHC patients. Antinuclear Ab, SMA and LKM-1 Ab were also present in 36% of CHC patients and related to disease severity. CHC cases positive for auto-Abs were directly comparable to AIH in respect of most markers of liver damage and total IgG. These cases had longer disease duration compared with auto-Ab negative cases, but there was no difference in gender, age or viral load. KLM-1<sup>+</sup> Ab CHC cases showed best overlap with AIH.

### CONCLUSION

Auto-Ab levels in CHC may be important markers of disease severity and positive cases have a disease similar to AIH. Auto-Abs might have a pathogenic role as indicated by elevated markers of liver damage. Future studies will unravel any novel associations between these two diseases, whether genetic or other.

**Key words:** Autoantibody; Inflammatory diseases; Immune system; Hepatitis C virus; Smooth muscle antibody; Liver/kidney microsomal-1 autoantibody; Anti-nuclear antibody

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**Core tip:** This paper aims to determine what patients with chronic hepatitis C (CHC) and autoimmune hepatitis (AIH) produce autoantibodies. Disease duration in CHC is linked to disease severity and autoantibodies. Patients with severe CHC resemble AIH.

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## INTRODUCTION

Antibodies are a vital part of the immune response for recognition and elimination of invading organisms. However, when the immune system is dysfunctional, it can develop antibodies that react to self. The development of autoantibodies (auto-Abs) generally occurs during auto-immune disease, but their induction can also be a consequence of a chronic infection in

susceptible individuals. A number of auto-Abs with different specificities have been identified. As some auto-Abs occurrence in plasma is disease specific, they can be used in the diagnosis and classification of autoimmune diseases<sup>[1-3]</sup>. The hepatitis C virus (HCV) infection causes liver damage by inducing cirrhosis and can also lead to hepatocellular carcinoma. Recent studies have demonstrated that the virus may be involved in loss of tolerance to self-antigens and thereby promotion of auto-Ab production<sup>[4-6]</sup>. In particular, non-organ-specific auto-Abs (NOSAs) including smooth muscle ab (SMA), anti-nuclear ab (ANA) and liver/kidney microsomal-1 (LKM-1) ab are common and frequently found in sera of patients with chronic HCV (CHC)<sup>[7,8]</sup>. NOSA in HCV-infected patients correlate with the severity of necro-inflammation, fibrosis development, markers of liver damage: aspartate transaminase (AST) and alanine transaminase (ALT), alkaline phosphatase (AP) and levels of IgG<sup>[9]</sup>.

Clinical and laboratory features of CHC can sometimes lead to a mistaken diagnosis of autoimmune hepatitis (AIH). AIH is characterized by a liver-specific autoimmune response, infiltrating immune cells, auto-Abs in circulation, elevated immunoglobulin and serum transaminase level, and a favourable response to immunosuppression<sup>[10,11]</sup>. In AIH, ANA, SMA, and LKM-1 Abs can differentiate the severity of the disease. The existence of detectable hepatitis C viral load with or without circulating antibodies specific to HCV can often be used to differentiate CHC from AIH<sup>[12]</sup>. These two conditions, CHC and AIH involve different management strategies; chronic HCV infection has until recently often been treated with interferon- $\alpha$  (IFN- $\alpha$ ) which can provoke liver auto-immunity. The HCV infection can, in a few cases, develop into AIH, suggesting that the liver cells are damaged not only by the infection but also by an immune reaction to self<sup>[13,14]</sup>. AIH on the other hand requires immunosuppression, a treatment that could induce viral replication in cases of co-infection<sup>[11,14]</sup>.

In this study, we have assessed the prevalence of ANA, SMA and LKM-1 Abs in CHC and AIH patients and correlated this with markers of liver disease to determine any overlapping features. In the study, disease severity, immunoglobulin levels and disease duration were assessed. We found that the auto-Ab profile was directly associated with severity of disease in both groups of patients and that subgroups within the patients showed a significant overlap in respect to the laboratory markers assessed.

## MATERIALS AND METHODS

A total of 70 patients and 20 healthy controls were recruited during the duration of this study (Table 1). These included 50 patients diagnosed with CHC and 20 patients with AIH. Approval of the study was received from the Ethics board and the Office of the Vice President for Scientific Affairs and Postgraduate

**Table 1** Distribution of study group according to age and gender

Parameter	CHC ( <i>n</i> = 50)	AIH ( <i>n</i> = 20)	HC ( <i>n</i> = 20)
Age (yr), mean ± SD			
Range	10-65	16-69	16-69
mean ± SD	33 ± 2.38	37 ± 3.22	37 ± 3.22
Gender, <i>n</i> (%)			
Female	24 (48)	15 (75)	15 (75)
Male	26 (52)	5 (25)	5 (25)
Age (yr), mean ± SD			
Female	34 ± 2.28	35 ± 3.42	35 ± 3.42
Male	33 ± 2.38	47 ± 3.12	47 ± 3.12

CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; HC: Hepatitis virus.

Studies at the Sulimani University. CHC patients were diagnosed based on anti-HCV antibody positivity and by assessment of their HCV RNA viral load. Patients with liver damage due to excessive alcohol consumption, hepatotoxic drugs, or human immunodeficiency virus infections were excluded from the study. Patients diagnosed positive for hepatitis B surface antigen were also excluded. The diagnosis of AIH was based on the criteria established by the international AIH group. This includes predominant elevation of serum aminotransferase and IgG, exclusion of viral hepatitis, toxic or alcoholic liver injury and with a liver biopsy confirming lymphocyte infiltration indicative of autoimmune disease<sup>[15]</sup>. Some patients were diagnosed for the first time when enrolled in the study while others had been undergoing treatment for between two and 11 years. Information of age and gender was recorded for each patient. Twenty healthy gender- and age-matched blood donors served as controls.

#### Enzyme linked immunosorbent assay and other assays

SMA, ANA and LKM-1 Ab in serum were assayed by enzyme linked immunosorbent assay (ELISA) according to the manufacturer's instructions (CUSABIO, Wuhan, China). Total IgG and IgM was estimated quantitatively using a biochemical assay according to the manufacturer's instructions (VITAL Diagnostics, Puteaux, France). Serum AP, ALT, AST, serum albumin and total serum bilirubin (TSB) was quantitatively determined using a biochemical assay (BIOLABO, Maizy, France).

#### Statistical analysis

Analysis of data was performed by using software package SPSS (Statistical Package for Social Science) version 21. Normal distribution of the data was determined using D'Agostino & Pearson omnibus normality test. Results are expressed as mean ± SD. Statistical differences were determined by Duncan's test for multiple comparisons after analysis of variance. Significant differences between groups were determined using the  $\chi^2$  test. A *P* value less than 0.05, 0.01 or 0.001

**Table 2** Distribution and comparison of autoantibodies between the chronic hepatitis C and the autoimmune hepatitis patient group *n* (%)

Autoantibodies	CHC ( <i>n</i> = 50)	AIH ( <i>n</i> = 20)	HC ( <i>n</i> = 20)	HCV-AIH <i>P</i> value
SMA	4 (8)	0 (0)	0 (0)	
LKM-1	11 (22)	9 (45)	0 (0)	
ANA	16 (32)	13 (65)	4 (20)	
Total	31 (36)	23 (75)	4 (20)	0.0031 <sup>1</sup>

<sup>1</sup>Indicates significant difference on the 0.01 level. Statistically significant differences were determined using analysis of variance followed by Duncan's test. CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; HC: Hepatitis virus; HCV: Hepatitis C virus.

respectively, were considered statistically significant at the different levels.

## RESULTS

In the aim of discovering overlapping features comparing CHC and AIH cases such patients were recruited and sera gathered for analysis of auto-Ab levels and levels of markers of liver damage. Table 1 shows the demographics of the CHC-, the AIH- and the healthy control-group (HC). The CHC group included 50 patients (48% female) and the mean age were 33.4 ± 2.4 years. In agreement with other studies, AIH was more common among females (gender ratio 3:1) with males being older when diagnosed (47.0 ± 3.1 years). There were no significant differences in age or gender comparing patients and HC.

One or more of the auto-Abs was detected in 36% of CHC patients' serum (9 male, 9 female, Table 2). Reactivity for ANA was the most frequent (32%, 7 male, 9 female) while LKM-1 Ab was detectable in 22% (4 male, 7 female) and SMA in 8% (1 male, 3 female) of the cases. Also 20% of the healthy controls were positive for ANA (2 male, 2 female) but not for LKM-1 or SMA. A statistical analysis using the Chi-square test showed that there was no significant difference in the level of ANA comparing the control group and the CHC group. In AIH patients, 75% (3 male, 12 female) had at least one type of auto-Ab. ANA was also the most frequently detected, 65% (3 male, 10 female). LKM-1 Ab was identified in 9 cases (45%) all of which were female. SMA was not detected in any of the AIH patient's serum. A statistical analysis showed significant differences between the AIH and CHC groups of patients regarding auto-Ab prevalence (AIH patients were more often positive for the auto-Abs, *P* = 0.0031, Table 2). The level of ANA and LKM-1, however, tended to be highest in CHC patients plasma (for ANA level in CHC: 4.6 ± 2.7 pg/mL and in AIH, 3.3 ± 1.8 pg/mL, for LKM-1, level in CHC: 4.9 ± 2.8 and in AIH: 4.0 ± 1.4 pg/mL).

Next, we assessed the level of IgG in the patients' serum. As expected, the highest level was found in the serum from the AIH patients (Table 3). There was

**Table 3** Estimation of IgG and IgM and comparison between study groups (mean  $\pm$  SD)

Groups	IgG (mg/dL)	IgM (mg/dL)
CHC	1841 $\pm$ 66.44	176 $\pm$ 5.56 <sup>1</sup>
AIH	2054 $\pm$ 152.62	228 $\pm$ 5.56 <sup>1</sup>
HC	1098 $\pm$ 57.69 <sup>1</sup>	127 $\pm$ 4.80 <sup>1</sup>

<sup>1</sup>Denotes significant difference comparing with the other two groups,  $P < 0.05$ . Statistically significant differences were determined using analysis of variance followed by Duncan's test. CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; HC: Hepatitis virus.

no significant difference in IgG level comparing CHC and AIH, but the levels were significantly higher than the healthy controls (Table 3). In contrast, although significantly higher than the healthy controls, the mean concentration of IgM remained within the normal range in the CHC and AIH groups. The AIH patients IgM level was significantly higher than the CHCs' and the HCs'.

All of the markers of liver damage (AP, ALT, AST, and TSB) except serum albumin assessed in the serum were elevated above the level of the HCs' for both the AIH patients and the CHC patients (Table 4). However, in the AIH group, the levels of these markers were significantly higher than in the CHC group (Duncan's test,  $P < 0.05$ ). Only the level of TSB was at a similar level in both patient groups.

To determine whether the presence of auto-Abs in the CHC group was associated with worse disease progression we compared the level of liver damage markers from patients with and without auto-Abs. We found that the duration of infection was twice as long for patients with auto-Abs compared to without. Furthermore, patients with auto-Abs had significantly higher levels of IgG, AP, AST, ALT and TSB (Table 5). This finding is in agreement with that of a previous study<sup>[9]</sup>. The age and gender of the patients, viral load, IgM, and serum albumin did, however, not differ in the two CHC subgroups.

The CHC patients with auto-Abs, the longest duration of infection and the highest level of markers of liver damage were directly comparable with the AIH patients (Table 5). These two groups were remarkably similar in respect to IgG levels and most markers of liver injury (Table 6). Only the levels of IgM and TSB were higher in the AIH patients ( $P < 0.05$ ).

Further, we found that the LKM-1 Abs present only in sera of female patients in the AIH group. LKM-1 Ab positive AIH patients are often young females with worse prognosis<sup>[16,17]</sup>. In our study, 22% of the CHC patients were positive for LKM-1 Ab. However, such positivity was found among both male and female subjects (5 male, 6 female). Importantly, our immunological and biochemical analysis revealed that there were no significant differences between these two subgroups of LKM-1<sup>+</sup> CHC and AIH in respect of IgG, AP, AST, ALT, albumin or TSB. The only difference

was the concentration of IgM (Table 7).

## DISCUSSION

In this study, we have shown that certain patients with chronic hepatitis C (CHC) infection develop an auto-Ab profile similar to that of AIH. This group of patients had longer disease duration and more extensive liver damage, thereby sharing many features of AIH. The comparison of LKM-1 Ab positive patient emphasised the similarity between severe cases of CHC and the AIH group. Only IgM levels discriminated these two groups of patients. Such knowledge is important when interpreting biomarker results from patients with liver disease. Furthermore, the findings suggest an underlying similarity in disease aetiology in these cases of auto-Ab positive CHC and AIH patients.

It is important to differentiate between CHC and AIH as their treatment is completely different<sup>[18]</sup>. Thus although auto-Abs and elevated liver enzymes might suggest a diagnosis of AIH, patients require further investigation to exclude CHC. When comparing NOSA production from the CHC and AIH patients in our study there was a significant difference, underscoring the overall more severe progression of the disease in the patients with autoimmune disease. In other studies of CHC 6%-41% was positive for ANA, 5%-66% for SMA, and up to 86% for LKM-1 Abs<sup>[12,19-22]</sup>. There are several reasons to explain why such a variation in levels of auto-Abs has been observed. ANA measured by indirect immune-fluorescence of Hep-2 cells or by different ELISAs produce variable results dependent on the method used<sup>[23]</sup>. Regional differences in prevalence of autoimmune manifestations in HCV might influence the results<sup>[5,24]</sup>. Furthermore, differences in auto-Ab levels might be due to the local ethnic background. Hence, in HCV infected patients from Crete or Sweden, very few showed positivity for LKM-1<sup>[25,26]</sup>.

It is not known why 36% of the CHC infected patients in our study develop auto-Abs. Liver damage in CHC cases results in release of auto-antigens to which the immune system can react<sup>[27]</sup>. As stated above, infection duration might be one factor in the development of auto-Abs. Other studies have, however, failed to identify an association between infection duration and NOSA levels<sup>[9,28]</sup>. Other factors such as presence of the HLR-DR3 genotype might be more important for auto-Ab development<sup>[29]</sup>.

AIH is known to induce liver damage<sup>[11]</sup>. CHC patients with auto-Abs and long-standing disease had liver damage at the same level as the AIH cases. The appearance of auto-Ab positive CHC patients can show such high similarity to AIH that they can be misdiagnosed. This is especially the case for patients with extrahepatic symptoms<sup>[10,11]</sup>. The presence of circulating antibodies specific to HCV is chief for correct diagnosis<sup>[12]</sup>. The liver damage experienced during AIH is induced by infiltrated inflammatory cells which can

**Table 4 Comparison of the markers of liver injury among study groups (mean ± SD)**

Groups	AP (UI/L)	ALT (UI/L)	AST (UI/L)	ALB (g/dL)	TSB (mg/dL)
CHC	296 ± 19.90 <sup>1</sup>	30 ± 1.32 <sup>1</sup>	45 ± 1.71 <sup>1</sup>	3.8 ± 0.11 <sup>2</sup>	1.0 ± 0.27 <sup>2</sup>
AIH	373 ± 32.10 <sup>1</sup>	37 ± 3.20 <sup>1</sup>	56 ± 4.10 <sup>1</sup>	3.3 ± 0.15 <sup>1</sup>	4.0 ± 1.57 <sup>1</sup>
HC	171 ± 8.30 <sup>1</sup>	19 ± 1.00 <sup>1</sup>	28 ± 1.28 <sup>1</sup>	4.0 ± 0.06 <sup>2</sup>	0.6 ± 0.05 <sup>2</sup>

<sup>1</sup>Denotes significant difference comparing with the other two groups,  $P < 0.05$ ; <sup>2</sup>Indicate significant difference comparing with the group indicated with an <sup>1</sup>only. Statistically significant differences were determined using analysis of variance followed by Duncan's test. CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; HC: Hepatitis virus; ALB: Albumin; AP: Alkaline phosphatase; AST: Aspartate transaminase; TSB: Total serum bilirubin; ALT: Alanine transaminase.

**Table 5 Comparison of different parameters in chronic hepatitis C with and without autoantibodies (mean ± SD)**

Parameters	CHC with autoantibodies M/F (9/9)	CHC without autoantibodies M/F (17/15)	P value
Mean age (yr)	33 ± 16	34 ± 14.3	0.36 NS
Viral load (copy/mL)	$8.3 \times 10^5 \pm 10.4 \times 10^6$	$3 \times 10^5 \pm 19 \times 10^5$	0.52 NS
Duration of infection (mo)	21.3 ± 12.3	11 ± 7.3	0.002 <sup>2</sup>
IgG (mg/dL)	2109 ± 462.4	1630 ± 323	0.001 <sup>1</sup>
IgM (mg/dL)	190 ± 25	172 ± 45.3	0.3 NS
AP (U/L)	428 ± 91.5	221 ± 104	0.001 <sup>1</sup>
AST (U/L)	52 ± 12.7	41 ± 9.81	0.007 <sup>1</sup>
ALT (U/L)	36 ± 9.7	27 ± 7.2	0.007 <sup>1</sup>
S. Albumin (g/dL)	3.8 ± 0.87	3.9 ± 0.72	0.47 NS
TSB (mg/dL)	1.5 ± 3.1	0.8 ± 0.58	0.001 <sup>1</sup>

<sup>1,2</sup>Indicate significant difference on the 0.01 and the 0.001 level respectively, NS indicates non-significance. Statistically significant differences were determined using analysis of variance followed by Duncan's test. CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; AP: Alkaline phosphatase; AST: Aspartate transaminase; TSB: Total serum bilirubin; ALT: Alanine transaminase.

**Table 6 Autoantibody positive chronic hepatitis C and autoimmune hepatitis, comparison of different parameters (mean ± SD)**

Parameters	Autoantibody positive CHC (n = 18)	Autoantibody positive AIH (n = 20)	P value
IgG (mg/dL)	2109 ± 249	2053 ± 710.5	0.42 NS
IgM (mg/dL)	190 ± 26.8	227 ± 63.1	0.027 <sup>1</sup>
AP (U/L)	428 ± 37.9	373 ± 166.1	0.33 NS
AST (U/L)	52 ± 8.42	57 ± 18.64	0.42 NS
ALT (U/L)	36 ± 5.5	37 ± 15.6	0.81 NS
TSB (mg/dL)	1.5 ± 4.1	4.0 ± 7.0	0.0033 <sup>2</sup>
Albumin (g/L)	3.8 ± 0.78	3.3 ± 0.69	0.12 NS

<sup>1,2</sup>Indicate significant difference on the 0.05 and the 0.01 level respectively, NS indicates non-significance. Statistically significant differences were determined using analysis of variance followed by Duncan's test. CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; AP: Alkaline phosphatase; AST: Aspartate transaminase; TSB: Total serum bilirubin; ALT: Alanine transaminase.

be visualized by a liver biopsy<sup>[11]</sup>. Indeed a liver biopsy is necessary for confirmation of AIH. Immune cells are, however, present in the liver albeit to a lesser extent also in CHC patients with serum auto-antibodies<sup>[30]</sup>.

**Table 7 Comparison between liver/kidney microsomal-1 positive patients in chronic hepatitis C and autoimmune hepatitis groups (mean ± SD)**

Parameters	Groups		P value
	LKM-1 <sup>+</sup> in AIH M/F (0/9)	LKM-1 <sup>+</sup> in CHC M/F (5/6)	
Mean age (yr)	31 ± 10	35 ± 19	0.51 NS
IgG (mg/dL)	2205 ± 566.1	2522 ± 295.6	0.34 NS
IgM (mg/dL)	262 ± 41.21	185 ± 26.26	0.001 <sup>1</sup>
AP (U/mL)	404 ± 146.7	472 ± 3	0.57 NS
AST (U/mL)	64 ± 12.71	57 ± 37.92	0.22 NS
ALT (U/mL)	41 ± 9.64	40 ± 5.41	0.45 NS
S. Albumin (g/dL)	3.4 ± 0.8	3.9 ± 0.9	0.5 NS
TSB (mg/dL)	3.4 ± 4.97	1.9 ± 3.90	0.06 NS

<sup>1</sup>Indicates significant difference on the 0.01 level, NS indicates non-significance. Statistically significant differences were determined using analysis of variance followed by Duncan's test. CHC: Chronic hepatitis C; AIH: Autoimmune hepatitis; AP: Alkaline phosphatase; AST: Aspartate transaminase; TSB: Total serum bilirubin; ALT: Alanine transaminase; LKM-1: Liver/kidney microsomal-1.

We speculate that the severe disease seen in AIH and CHC with auto-Abs is mediated by similar mechanisms and that the auto-Abs can contribute to liver damage in these cases.

Sixty-five percent of the AIH patients had any of the three auto-Abs investigated in this study. As previously reported, ANA was the most prevalent auto-Ab<sup>[9]</sup>. LKM-1 Ab is a serological marker for one subtype of the autoimmune disease, AIH-2 which is more prevalent among adolescent women and young girls<sup>[16,17]</sup>. In this study LKM-1 Ab was detected in 45% of samples which is an unusually high proportion. This might be explained by that the study contained many young women. It is also possible that the genotype associated with the development of LKM-1 antibodies is more prevalent in the area from where patients were recruited<sup>[31]</sup>. LKM-1 has been shown to recognize CYP2D7 which is expressed on the surface of hepatocytes. Such self-recognition, unique for LKM-1 among NOSA, could explain the worse disease in these patients<sup>[32]</sup>. Possibly of importance, epitopes within CYP2D7 share homology with HCV proteins<sup>[33]</sup>. CYP2D6 epitopes can induce both poly-reactive B cells and T cells<sup>[34]</sup>. It has been proposed that polymorphisms in the CYP2D6 gene lead to altered amino acids sequences and more immunogenic epitopes<sup>[35]</sup>. It is not known whether CYP2D6 polymorphisms could differentiate

AIH-1 and AIH-2. There are, however, other differences between the AIH subtypes; AIH-2 share an antibody profile with autoimmune polyendocrine syndrome type 1 which is caused by a mutation in the *AIRE* gene, leading to a break in tolerance<sup>[31]</sup>. In this study the levels of both ANA and LKM-1 tended to be higher in CHC than in AIH. Other studies have detected higher level of LKM-1 in AIH-2, than in CHC<sup>[36]</sup>. Further studies are required to explain why LKM-1 and ANA is higher in sera from the CHC patients than from AIH patients in this study. High level of LKM-1 Ab in CHC has previously been reported in paediatric cohorts<sup>[37]</sup>. Similar to our findings, LKM-1 Ab have previously been reported in HCV where the titre is associated with disease severity<sup>[38]</sup>. It is not known whether there is any relationship between patients with CHC, AIH and LKM-1 Ab or whether these CHC cases are more prone to develop AIH. Although no longer commonly used, it has been proposed that IFN $\alpha$  therapy for CHC, can induce autoimmune symptoms in LKM-1<sup>+</sup> individuals<sup>[39]</sup>.

SMA was detected in none of the patients in the AIH group, which is in disagreement with previous studies. As many as 70% to 80% of AIH cases have been described as SMA positive<sup>[40]</sup>. The successful treatment of our patients might be one explanation for the lack of SMA<sup>+</sup> patients as this is associated with the disappearance of serum ANA and SMA<sup>[41]</sup>. It should be pointed out that seronegativity in AIH have been described in 1%-34% of cases (in our study 25%) underscoring the heterogeneity of this autoimmune disease<sup>[42]</sup>.

High IgG levels are a distinctive feature of AIH<sup>[43-45]</sup>. In our study, however, there was no significant difference in levels comparing AIH and CHC. Other studies have reported elevated levels of IgG in both AIH and CHC patients<sup>[38]</sup>. The level of IgG has previously been associated with severity of disease among chronic HCV infected patients which is in line with our findings. Both diseases are characterized by activation of B cells and a large number of plasma cells<sup>[6]</sup>. We speculate that many of these released auto-Abs are of the IgG subclass which can explain the elevated level of these Abs in serum from both patient groups. This polyclonal activation is likely taking place either as a consequence of chronic antigen stimulation or due to loss of immune regulation<sup>[46,47]</sup>. Our interest is in Th17 cells that have an established pathogenic function in autoimmune diseases<sup>[48]</sup>. Th17 cells are present in the liver of both AIH and CHC patients<sup>[30]</sup>. Further studies will determine whether these cells could contribute to B cell activation or generation of an inflammatory environment that promotes auto-Ab production<sup>[49,50]</sup>.

In conclusion, we found AIH-related auto-Abs associated with HCV infection. The emergence of auto-Abs in CHC might be infection duration dependent, but it is not related to gender, the age of patients and serum viral load. Auto-Abs, especially LKM-1, in CHC cases might have pathogenic role leading to more

severe disease which is indicated by an alteration of liver function tests and elevation of total IgG. It is not known whether auto-Ab prevalence is due to prolonged disease or whether certain patients are more susceptible for their development. We conclude that auto-Ab levels in CHC may be important markers of disease severity and that these patients have a disease similar to AIH. Future studies will unravel any further associations between these two diseases, whether genetic or other.

## COMMENTS

### Background

Worldwide, 130-200 million individuals are infected with hepatitis C. Although current therapies control the disease rather well, 80% of the infected patients develop chronic hepatitis C (CHC). It is not known why some of the patients develop more severe disease. Autoimmune hepatitis (AIH) is a disease of the liver that has a prevalence of 10-20/100000 individuals. This disease can be controlled by using immunosuppressive therapies. In this paper the authors show that the presence of non-organ specific autoantibodies (NOSA) in both CHC and AIH is associated with severe disease. Although overall CHC is less damaging to the liver than AIH cases, a subgroup can be defined with more severe pathology. This CHC subgroup is defined by liver/kidney microsomal-1 (LKM-1) positivity.

### Research frontiers

In the field of autoimmunity, novel therapies have recently been developed as well as better understanding of the aetiology of disease. Recent discoveries of novel immune cells and their dysregulation in the autoimmunity have increased the knowledge of disease aetiology and provided prospect for development of novel therapies. Novel genome-, transcriptome- and epigenome-sequencing techniques have given important insight of autoimmune associations to certain genomic regions and the immunological heterogeneity underlying disease. As some subpopulations of CHC patients have antibodies that react to self, we speculate that the analysis of novel immune cells and comparison of immune cells from CHC patients with AIH using novel genomic and epigenomic tools can provide disease related knowledge useful for novel future treatment-strategies of these patients.

### Innovations and breakthroughs

Th17 cells have recently been implicated in the development of AIH and response to hepatitis C virus infection in the liver. Although it is not known how these cells confer pathology, studies from other autoimmune diseases has suggested that the cells support B cell germinal centre formation and production of auto-antibodies. This can be both through IL-21 production by Th17 cells and through transdifferentiation of Th17 cells to follicular T cells. Studies have shown that polymorphisms in the TNF $\alpha$  gene are associated with development of AIH. We are currently analysing how TNF $\alpha$  is suppressing Th17 cell expansion in rheumatoid arthritis patients. Future studies by authors would aim to determine the role of Th17 cells and possibly TNF $\alpha$  in the development of autoantibodies (auto-Abs) in AIH and LKM-1<sup>+</sup> CHC.

### Applications

The current study has characterized the immune response in CHC and AIH in homogenous patient cohorts. The findings differentiated CHC and AIH patients into different subgroups. These findings open the way for future studies of immune cell mediated induction of autoimmunity in CHC and AIH. We aim to characterize immune cells present in the liver and the peripheral blood of the patient cohorts. Further, genome wide association studies, analysis for expression quantitative trait loci and whole genome epigenome will be undertaken to gain better understanding of the autoimmune disease process which is regulating the immune cells. Findings from these studies will be correlated to disease severity, auto-Ab levels and other markers of liver disease. Importantly the knowledge gained will provide the possibility to discover overlapping genomic/epigenomic features between AIH and the

subgroup of LKM-1 positive CHC patients with severe disease.

### Peer-review

The manuscript by Amin *et al* compares the incidence of auto-Abs associated with AIH, immunoglobulin levels and markers of liver disease in groups of age-matched subjects with autoimmune hepatitis, CHC infection and healthy controls.

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