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Autoimmune liver serology before and after successful treatment of chronic hepatitis C by direct acting antiviral agents

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A B S T R A C T

Background and aims: Chronic hepatitis C virus (HCV) infection is associated with a wide range of immunopathological manifestations, which are significantly improved by successful interferon-based treatment. There is paucity of data on the impact of interferon-free HCV clearance on immunopathological manifestations, which might be expected to disappear more frequently as compared to what reported in interferon-induced HCV-clearance. We have investigated liver autoimmune serology before and after interferon-free clearance of HCV by treatment with direct acting antiviral agents (DAA).

Method: Patients within the Swiss Hepatitis C Cohort Study who underwent successful (SVR 12) HCV treatment with DAA were tested for autoimmune liver serology according to dedicated guidelines before and at least 6 months after end of treatment.

Results: A total of 235 patients were included; 62% males; median age 56 years; 27% with cirrhosis. Median time between end of DAA treatment and post-treatment serum sampling was 17 months. At least one autoantibody before treatment was found in 175 (74%) patients ; 32 (14%) were positive for 2 autoantibodies; no patient was positive for anti-SLA, anti-LC1 or typical AMA before or after DAA. ANA disappeared in 34%, SMA in 52% and anti-LKM1 in one of two patients after successful treatment, but, unexpectedly, one or more autoantibodies appeared in 27% of pre-treatment negative subjects.

Conclusion: HCV clearance by DAA is associated with autoantibody disappearance in more than one third of the patients who were positive before treatment. However, the majority of the patients remain autoantibody-positive and 27% of those who were negative before treatment developed autoantibodies after DAA-induced HCV clearance. These data confirm that HCV infection is associated with autoimmunity and show that the autoimmune imprint persists after viral clearance by DAA, suggesting that long-term follow-up may be warranted.

1. Introduction

Chronic hepatitis C virus (HCV) infection is associated with a wide range of immunopathological manifestations, ranging from autoantibody production to overt immune-mediated diseases, including mixed cryoglobulinemia, immune-complex vasculitis, B cell lymphoma, arthritis, Sjögren syndrome and autoimmune thyroid disorders [1–3]. Cryoglobulins are found in up to 60% of HCV-infected patients and disappear in 80–90% of them after HCV cure with pegylated interferon-alpha and ribavirin treatment [1]. Autoantibodies typical of autoimmune liver disease, such as anti-nuclear antibody (ANA), anti-smooth muscle antibody (SMA), anti-liver kidney microsomal type 1 (LKM1), anti-soluble liver antigen (SLA), anti-liver cytosol type 1 (LC1), and anti-mitochondrial antibody (AMA), have been reported in up to 70% of patients with chronic HCV infection [3–9]. Mechanisms suggested to be involved in autoantibody production include molecular mimicry between viral epitopes and autoantibody target antigens, decreased number of regulatory T cells, B cell monoclonal expansion and B-cell

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Table 1
Baseline demographic and clinical features of the study population (n = 235).

			Number of patients with missing data
Median age (range), y	56 (24–84)		0
Male (%)	146 (62)		0
Cirrhosis (%)	63 (27)		1
Diabetes (%)	14 (6)		3
History of alcohol consumption, any amount (%)	122 (52)		0
Median ALT level before DAA (IQR), IU/l	80 (50–139)		19
Median ALT level after DAA (IQR), IU/l	23 (17–31)		30
HCV genotype			31
	1 (%)	149 (63)	
	2 (%)	8 (3.4)	
	3 (%)	26 (11)	
	4 (%)	21 (9)	
DAA regimen			0
	SOF + LED ± RBV (%)	121 (51)	
	DAC + SOF ± RBV (%)	36 (15)	
	OMB + PAR ± DAS ± RBV (%)	33 (14)	
	SOF + RBV (%)	29 (12)	
	SOF + SIM ± RBV (%)	7 (3)	
	ELB + GRA (%)	5 (2)	
	Other (%)	4 (0.02)	

HCV, hepatitis C virus; ALT, alanine aminotransferase; DAA, direct-acting antiviral; SOF, sofosbuvir; LED, ledipasvir; RBV, ribavirin; DAC, daclatasvir; DAS, dasabuvir; OMB, ombitasvir; PAR, paritaprevir; SIM, simeprevir; ELB, elbasvir; GRA, grazoprevir.

activation [3,10]. It has been reported that the genetic background predisposing to autoimmune hepatitis (AIH) is associated with autoantibody production in chronically HCV infected patients [11,12]. Development of anti-LKM1, the serological hallmark of classical AIH type 2, during the course of chronic HCV infection has been described to herald the onset of overt AIH [13–15]. Collectively, these observations indicate that HCV can act as a trigger of autoimmunity.

Until recently, HCV treatment was based on interferon, whose side effects include exacerbating pre-existing autoimmune diseases or promoting a variety of immunopathological manifestations [16–20], ranging from autoantibody production to overt autoimmune disease. Anti-LKM1-positive HCV patients are at risk of developing acute hepatitis while on interferon, requiring treatment discontinuation and at times corticosteroid therapy [21,22]. The advent of direct acting antivirals (DAA) has dramatically changed HCV treatment, allowing to eliminate the infective agent safely in the vast majority of patients, including those with HCV-associated immunopathological manifestations or autoimmune diseases [23–26]. DAA treatment decreases the cryocrit and ameliorates mixed cryoglobulinemia-associated vasculitis [27–30].

The aim of the present study was to investigate the effect on serological autoimmune manifestations of successful DAA treatment of chronic HCV infection in a large cohort of HCV-infected adult patients.

2. Patients and methods

2.1. Patient population

Patients were recruited from the Swiss Hepatitis C Cohort Study (SCCS), initiated in 2000, which includes anti-HCV antibody positive subjects aged ≥18 years living in Switzerland [31]. Study sites include five University hospitals, three large non-university hospitals, and affiliated centres (http://www.swisshcv.org). To date, 5715 subjects have been enrolled. Data on demographics, HCV genotype, stage of liver disease, HCV therapy, HBV and HIV coinfection are collected by standardized questionnaires completed by physicians or trained study nurses during clinical visits at enrolment and at annual follow-ups. In addition, plasma/serum samples are collected and stored locally at −80 °C. The Ethics Committees of all participating centres granted approval for SCCS and all participants gave written informed consent.

Inclusion criteria for the present study were: positive plasma HCV RNA, successful HCV therapy with DAA (all interferon-free regimens), available serum or plasma samples collected before (up to five years) DAA treatment and at least six months after cessation of treatment. Successful treatment was defined as negative HCV RNA 12 weeks after the end of treatment [sustained virological response (SVR) 12] or absence of positive HCV RNA during the first year after end of treatment. Patients who were HBsAg and/or HIV positive were excluded. Diagnosis of cirrhosis was made on clinical, laboratory and imaging assessments.

2.2. Autoantibody testing

Autoantibodies were tested at Laboratorio Unico Metropolitano, Ospedale Maggiore, Bologna, Italy, according to the consensus statement issued by the International AIH Group (IAIHG) [32]. The following reactivities were investigated blindly: ANA, SMA, AMA, anti-LKM1, anti-LC1 and anti-gastric parietal cell (GPC) were tested by indirect immunofluorescence (IIF) on triple rodent tissue (Euroimmun Medizinische Labordiagnostika AG, Lübeck, Germany), titres ≥1:40 being considered positive; the ANA pattern was evaluated by IIF on HEp2 cells at a titre of 1:80 (Euroimmun). All sera were tested by ELISA (INOVA Diagnostics, Inc., San Diego, USA) for anti-SLA.

2.3. Statistical analysis

Statistical analysis was performed with PRISM 8 for Mac. Chi-square or Fisher exact tests were used for assessing the association between presence of autoantibodies pre-DAA, their appearance/disappearance after DAA and age, gender, transaminase levels, previous interferon/ribavirin treatment and presence of cirrhosis. The level of significance was set as $\alpha = 0.05$ two-tailed.

3. Results

Two-hundred-thirty-five patients fulfilling the inclusion criteria were identified (Table 1): 62% male, median age at DAA treatment start 56 years, one fourth with cirrhosis, three quarters infected with HCV genotype 1. The median time interval between the first and the second blood sample collection was 24 months (IQR 17.5–33.5, range 8–76); the median time interval between end of DAA treatment and collection of the second blood sample was 17 months (IQR 10.5–22, range 6–50). While still viraemic, 175 patients (74%) had at least one autoantibody

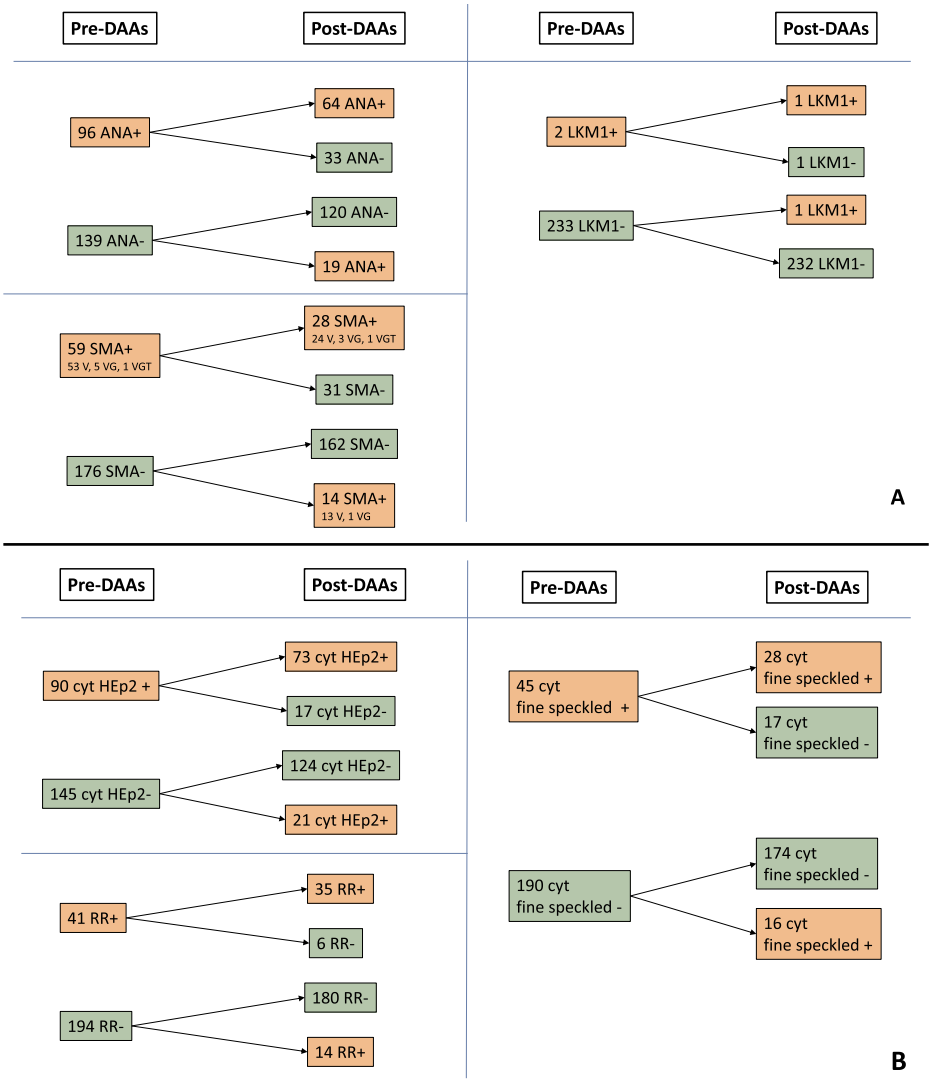

Fig. 1. Autoantibody behaviour pre and post direct acting anti-viral agents therapy in 235 patients with chronic hepatitis C virus infection. Panel A: Anti-nuclear antibody (ANA), smooth muscle antibody (SMA), anti-liver kidney microsomal (anti-LKM1) tested on triple rodent tissue; V, vessel; VG, vessel and glomerulus; VGT, vessel glomerulus and tubulus staining. Panel B: cytoplasmic antibodies tested on HEp2 cells; RR, rods and rings staining.

positivity, 42 (18%) being positive for two autoantibodies.

Before DAA treatment, ANA was positive in 96 (41%) patients, with titres ranging from 1:80 to > 1:640, median 1:80 (Fig. 1A, Table 2); IIF patterns on HEp2 cells were: 54 speckled, 14 nucleolar, 16 homogeneous, 8 mixed, and one each anti-centriole, anti-centromere and anti-few nuclear dots. There was no association between pre-DAA ANA positivity or negativity with either gender, age, presence of cirrhosis, alanine-aminotransferase (ALT) levels or previous interferon/ribavirin treatment. No patient had the primary biliary cholangitis (PBC) associated rim-like/membranous or multiple nuclear dots patterns before or after HCV clearance. Of the 96 patients ANA positive before treatment, 33 (34%) became ANA negative after HCV clearance, and of 139 patients ANA negative before DAA, 22 (16%) became ANA positive (Table 2). ANA disappearance/appearance after DAA was not associated with gender, age or cirrhosis. The 33 patients with ANA titers > 1:80 pre-DAA were less likely to clear ANA post-DAA as compared to patients with a pre-DAA ANA titer of 1:80 ($p = 0.0004$); one third (11/33) of the patients who were still ANA-positive post-DAA had a decreased titer post-DAA.

SMA was positive before DAA in 59 (25%) patients, with a median titre of 1:80 and a range of 1:40–1:320 (Fig. 1A, Table 3). SMA positivity was not associated with gender, ALT levels or previous interferon/ribavirin exposure, while a significant association was observed with age younger than 50 years ($p = 0.021$) and with absence of cirrhosis ($p = 0.008$). Fifty-three patients had only the V (vessel) pattern on kidney tissue, while six had the AIH-characteristic VG (vessel and glomerulus) or VGT (vessel, glomerulus and tubulus) patterns (three male, none with cirrhosis, median age 56 years, range 43–71). Four of these six patients became SMA negative after HCV clearance, while two remained positive with only the V pattern at stable titres. Four patients (two male), who were SMA positive with the V pattern at a titre of 1:80 before DAA treatment, developed the VG/VGT pattern after DAA, with titres ranging from 1:80 to 1:160. Overall, of the 59 patients who were SMA positive before treatment, 31 (52%) became SMA negative after HCV clearance, and of the 176 patients who were SMA negative before DAA, 14 (8%) became SMA positive, 13 with the V pattern and one with the VG pattern. Post-DAA treatment SMA disappearance/appearance was not associated with gender, age or cirrhosis.

Anti-LKM1 was positive in two patients while viraemic: one 63-year old cirrhotic man and one 70-year old non-cirrhotic woman, with titres of > 1:640 and 1:320, respectively. The male patient was also ANA positive (1:80) with a speckled pattern, while the woman showed a cytoplasmic fine speckled staining on HEp2 cells (1:320). After HCV

Table 2
Anti-nuclear antibody: number of positive patients and titres.

Pre-treatment		Post-treatment	
Number of patients	Titre	Number of patients	Titre
139	< 1:80	117	< 1:80
		18 (6spe, 5nuc, 3 spi, 2hom, 2mix)	1:80
		2 (1spe, 1hom)	1:160
		1 (mix)	1:320
		1 (cen)	> 1:640
63 (45 spe, 7 nuc, 7 hom, 2 mix, 1crl)	1:80	29	< 1:80
		28 (16 spe, 6 hom, 3nuc, 2mix, 1crl)	1:80
		6 (2nuc, 2spe, 2mix)	1:160
		1 (nuc)	1:320
20 (5 spe, 5nuc, 5hom, 5 mix)	1:160	2	< 1:80
		8 (6spe, 2nuc)	1:80
		10 (5hom, 3nuc, 1spe, 1mix)	1:160
9 (3 hom, 2spe, 2nuc, 1mix, 1FND)	1:320	1	< 1:80
		3 (2hom,1nuc)	1:80
		3 (1spe, 1nuc, 1mix)	1:160
		2 (1spe, 1FND)	1:320
3 (2spe, 1hom)	1:640	1 (spe)	1:160
		2 (1spe, 1hom)	1:640
1 (cen)	> 640	1 (cen)	1:640

Spe, speckled; nuc, nucleolar; spi, mitotic spindle; hom, homogeneous; mix, mixted; cen, centromere, crl, centriole; FND, few nuclear dot.

Table 3
Anti-smooth muscle antibody: number of positive patients and titres.

Pre-treatment		Post-treatment	
Number of patients	Titre	Number of patients	Titre
176	< 1:40	162	< 1:40
		4 (V)	1:40
		7 (V)	1:80
		3 (1VG, 2 V)	1:160
5 (V)	1:40	3	< 1:40
		2 (V)	1:80
36 (33 V, 3VG)	1:80	19	< 1:40
		2 (V)	1:40
		9 (7 V, 1VG, 1VGT)	1:80
		6 (4 V, 2VG)	1:160
16 (13 V, 2VG, 1VGT)	1:160	9	< 1:40
		2 (V)	1:40
		3 (V)	1:80
		1 (V)	1:160
		1 (V)	1:320
2 (V)	1:320	1 (V)	1:80
		1 (V)	1:160

V, vessel, VG, vessel glomerulus; VGT, vessel glomerulus tubulus.

clearance, the male patient was still anti-LKM1 positive, but with a markedly lower titre of 1:40, whereas the female patient became anti-LKM1 negative (Fig. 1A); positivity for ANA and cytoplasmic staining on HEp2 cells persisted. One female patient aged 74 years without cirrhosis, developed low-titre (1:40) anti-LKM1 after HCV clearance; she was ANA (1:80, nucleolar pattern) and SMA (1:40, V pattern) positive pre-DAA, only the ANA positivity persisting after DAA, in association to the newly developed anti-LKM1.

Anti-GPC was detected in only one patient before HCV clearance, remaining positive after DAA but at lower titre. This reactivity appeared in three patients after successful DAA treatment (1:160).

Anti-SLA and anti-LC1 were absent in all patients before and after HCV clearance.

Before DAA, two patients, one female and one male, had the IIF pattern of AMA, though the antibody did not react with native pyruvate dehydrogenase complex and with the recombinant fusion protein BPO (encompassing the immunogenic domains of the E2 subunits of pyruvate, 2-oxoglutarate and branched-chain 2-oxo acid dehydrogenase complexes) and was therefore reported as atypical AMA. The antibody disappeared in both patients after HCV clearance.

Before treatment, 90 (38%) patients had a positive cytoplasmic staining at IIF on HEp2 cells (median titre 1:160, range 1:80–1:640). Patterns were: 41 rods & rings (RR; AC23 according to Chan et al. [33]), 44 fine speckled (AC20 or AC19 according to Chan et al. [33]), three filamentous, and two mitochondrial. Two patients had a low-titre mitotic staining pattern on HEp2 cells, i.e. anti-midbody antibody (AC27 according to Chan et al. [33]), disappearing after DAA. Seventy-three (81%) patients remained HEp2 cytoplasmic-positive after HCV clearance (Fig. 1B). Cytoplasmic staining at IIF on HEp2 cells was associated with presence of cirrhosis ($p = 0.028$) and previous interferon/ribavirin exposure (61 of 92 patients) ($p = 0.000$), while there was no association with gender, age or ALT levels. This strong association with previous interferon/ribavirin treatment was due to the fact that 38 of the 41 RR positive patients had been previously treated with interferon/ribavirin ($p = 0.000$), while the non-RR cytoplasmic patterns were equally present in patients with ($n = 22$) or without ($n = 27$) a history of previous interferon/ribavirin. Anti-RR antibody disappeared post-DAA in a significantly lower number of patients (15%) as compared to ANA (34%, $p = 0.026$) and SMA (52%, $p = 0.0001$). Anti-RR antibody appeared in 14 patients who were negative before DAA, nine of whom were treated with a ribavirin-containing DAA regimen; three of the five remaining patients had undergone interferon and ribavirin treatment 10, 11 and 13 years before DAA treatment (Fig. 1B). Fine speckled antibodies appeared in 16 patients negative before DAA. Appearance/disappearance of a cytoplasmic immunofluorescence pattern on HEp2 cells, including the RR pattern, was not associated with age, gender, or cirrhosis. In patients with a history of interferon and/or ribavirin treatment, the median time from end of the previous treatment and start of DAA treatment was 110 months (range: 6–243 months, IQR: 67–155), while the time from end of interferon/ribavirin treatment and testing of pre-DAA autoantibodies was 102.5 months (range: 0–241, IQR: 60–152).

Of the patients with a cytoplasmic staining pattern on HEp2 cells before DAA, one third had overlapping ANA positivity, and 22% had overlapping SMA positivity. Seven patients were positive for all three reactivities (Fig. 2).

Sixty (25%) patients were seronegative for all tested specificities before DAA, but 16 of them (27%) developed at least one autoantibody

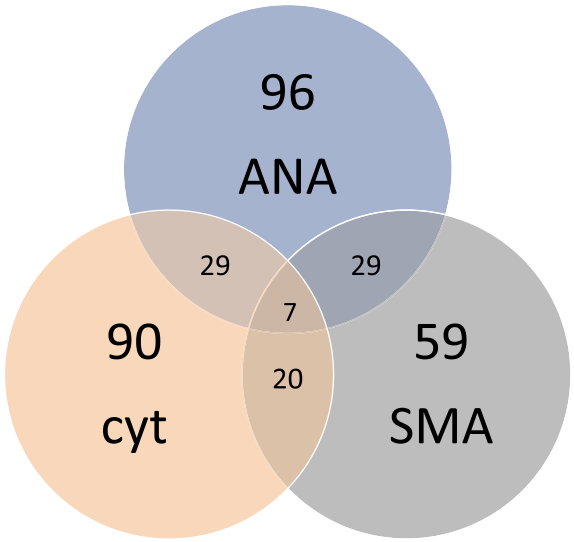

Fig. 2. Overlap of autoantibody positivity before direct acting anti-viral agents therapy in 235 patients with chronic hepatitis C virus infection. ANA, anti-nuclear antibody; SMA, anti-smooth muscle antibody; cyt, cytoplasmic antibodies tested on HEp2 cells.

Table 4
Autoantibodies giving a cytoplasmic staining on HEp2 cells: number of positive patients and titres.

Pre-treatment		Post-treatment	
Number of patients	Titre	Number of patients	Titre
145	< 1:80	124	< 1:80
		18 (12 fspe, 6RR)	1:80
		2 (1fspe, 1RR)	1:160
		1 (RR)	1:640
23 (16 fspe, 5RR, 2 fil)	1:80	10	< 1:80
		7 (5fspe, 1RR, 1fil)	1:80
		4 (2fspe, 2RR)	1:160
		2 (1RR, 1fspe)	1:320
28 (18fspe, 9RR, 1fil)	1:160	3	< 1:80
		3 (1fspe, 1RR, 1 cit)	1:80
		19 (12fspe, 7RR)	1:160
		3 (2RR, 1 fspe)	1:320
20 (10RR, 8fspe, 2 mit)	1:320	3	< 1:80
		3 (2fspe, 1RR)	1:80
		5 (4fspe, 1RR)	1:160
		6 (5RR, 1fspe)	1:320
		3 (RR)	1:640
19 (17RR, 2fspe)	1:640	1 (RR)	1:80
		1 (RR)	1:160
		6 (5RR, 1fspe)	1:320
		9 (8RR, 1fspe)	1:640
		2 (RR)	> 1:640

RR, rods and rings; fspe, fine speckled; fil, filamentous; mit, mitochondrial.

after HCV clearance: five ANA (titres 1:80–1:160, three speckled, two nucleolar), one anti-mitotic spindle (titre 1:80), two SMA (V pattern, titres 1:80 and 1:160), three cytoplasmic staining on HEp2 cells (two fine speckled, 1:80; one RR 1:640 having being treated with a ribavirin-containing DAA-regimen), one became positive for SMA and ANA (ANA 1:160 fine speckled, SMA 1:80 V pattern), one for SMA and cytoplasmic staining on HEp2 cells (SMA 1:80 V pattern, cytoplasmic 1:80 RR) and three for cytoplasmic/mitotic staining on HEp2 cells and ANA (cytoplasmic 1:80, two fine speckled and one RR; ANA 1:80, two speckled, one mitotic spindle) (Fig. 1A and B, Tables 1–4).

There was no significant association between viral genotype and ANA, SMA and RR positivity before DAA, but three quarters of the study population were infected with genotype 1.

There was no association between seroconversion and time elapsed from end of DAA treatment and blood sample collection.

4. Discussion

By testing systematically autoantibodies relevant to autoimmune liver disease [32] in a large cohort of adult patients with chronic HCV infection before and after HCV cure with DAA-based interferon-free regimens, we confirm that a high proportion of HCV-infected patients are positive for autoantibodies pre-treatment [7–9,21,22,34]. Unexpectedly we found that the majority remain autoantibody positive after viral clearance and that, even more unpredictably, over a quarter of those who were negative pre-treatment develop de novo autoantibodies after DAA-induced elimination of the virus.

Before treatment we observed an ANA prevalence of 41% and of SMA of 25%, in line with previously reported prevalences [7,8,21,34]. Anti-LKM1 positivity, detectable in a low percentage of HCV infected individuals (up to 13%) [4,23], and associated with interferon-induced disease flares, was rare in our cohort (0.01%).

Mechanisms implicated in the autoimmune manifestations of HCV infection include molecular mimicry and direct HCV action over B lymphocytes [35]. Molecular mimicry, whereby immune responses to external pathogens become directed towards structurally similar self-components (33), has been documented for the production of ANA, SMA and anti-LKM1 in patients with HCV [10,36,37]. Clearance of HCV would be expected to be accompanied by disappearance of cross-reactive autoantibodies, as indeed was seen in a considerable proportion of patients in this study. However, two thirds of ANA and half of SMA positive patients remained positive after viral clearance and ANA and SMA appeared de novo in 16% and 8% of patients respectively after successful elimination of the virus. Moreover, some patients developed autoantibody patterns typical of autoimmune hepatitis. These observations suggest that the early encounter with the virus had triggered mechanisms linked to the late appearance of autoimmunity. Activation of B lymphocytes also plays a critical role in the development of autoimmune manifestations in HCV infection. Antigenic epitopes on the HCV particle are recognised by the B cell receptor, the viral particle interacting with the B cell in two additional ways: the HCV envelope glycoprotein E2 binds to the transmembrane tetraspanin CD81, and the C3d complement fragment, coating the HCV particle, binds to the C3d receptor, the CD21 molecule [3]. These multiple interactions lower the threshold of B cell activation by hundred times, favouring production and persistence of autoantibodies, as seen in our cohort, as well as cryoglobulins [3,38,39]. Thus, long-term persistence of cryoglobulins and B-cell clones after DAA-induced HCV clearance has been recently reported, at times associated with relapsing vasculitis [28,40–42].

Other mechanisms possibly involved in favouring autoimmune manifestations are exposure to intracellular antigens released following HCV-induced tissue injury, which may lead to loss of tolerance and production of autoantibodies, akin to the production of anti-myosin antibody after myocardial infarction [43], and loss of virus-induced anergy after viral clearance [51]. In the latter instance, in view of the similarities between viral and self amino acid sequences alluded to above, anergy of autoreactive lymphocytes is likely to be maintained by the presence of the virus, the sudden disappearance of HCV leading to anergy loss. In this context, it is of interest that three cases of lupus-like glomerulonephritis arising after DAA-induced HCV clearance have been reported [44]. However, it cannot be excluded that DAAs themselves induce autoimmune reactions, as two cases of AIH occurring during DAA treatment have been reported, though no information was provided on the autoimmune profile before starting treatment [45,46].

We observed positivity for HEp2 cytoplasmic antibodies in 39% of our patients, and in agreement with published reports we demonstrate a strong association between the RR pattern with previous interferon-alpha/ribavirin treatment [47,48]. In addition, we show that anti-RR positivity is present long-term after having been exposed to ribavirin and interferon-alpha, being detectable in our patients at a median of 8.5 years later. The main target of anti-RR is inosine-5′-monophosphate dehydrogenase 2 (IMPDH2), the rate-limiting enzyme involved in the guanosine triphosphate biosynthesis pathway. Inhibition of IMPDH2 by ribavirin induces the formation of RR structures, which become an autoantibody target, a process facilitated by the co-administration of the pro-inflammatory cytokine interferon-alpha. Indeed, anti-RR is considered to be drug-induced rather than virus-induced. In our series, anti-RR reactivity persisted after viral clearance in 85% of positive patients and appeared post treatment in 7% of those previously anti-RR negative, most of whom had been treated with ribavirin-containing DAA regimens, emphasising that exposure to ribavirin per se leads to anti-RR antibody production, even in absence of interferon-alpha. Of note, a fine speckled cytoplasmic pattern, hitherto described in connective tissue disorders [33], was present in 45 (19%) of our patients, remaining positive in two thirds. The clinical and prognostic significance of this antibody in the HCV setting remains to be determined.

Current guidelines do not advise autoantibody testing in the course of chronic HCV infection, either before or after DAA treatment [49,50], and patients without advanced fibrosis or without risk factors for re-infection, toxic or metabolic liver injury, are discharged from specialised centres after viral clearance, with no planned follow up [45,46]. Our findings indicate that autoimmune manifestations persist or arise de novo after HCV clearance. Though in the medium-term this was not associated with clinical manifestations, long-term it may lead to

autoimmune liver disease, as demonstrated by two cases where AIH developed 1.5 and 8 years after HCV encounter and autoantibody production, persisting after viral clearance [13–15]. This suggests that presence of autoantibodies may be considered a risk factor for liver injury and patients with protracted positivity or de novo appearance of autoantibodies after sustained viral clearance should be followed-up longer-term; correlation with clinical outcomes will be required.

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Conflicts of interest

None.

Authors' contributions

Study concept and design: BTBP, AC, DV, LM, GMV; acquisition of data: CDB, GD, AGG, TS, CT, LM, JM, NS, DS, DM; analysis and interpretation of data: BTBP, CDB, DV, GMV; drafting of the manuscript: BTBP; critical revision of the manuscript for important intellectual content: AC, LM, DV, GMV, DM, NS; obtained funding: AC, BTBP; study supervision: AC. All authors approved the final version of the manuscript.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jaut.2019.04.019.

References

[1] F. Negro, D. Forton, A. Craxì, M.S. Sulkowski, J.J. Feld, M.P. Manns, Extrahepatic morbidity and mortality of chronic hepatitis C, Gastroenterology 149 (2015) 1345–1360, https://doi.org/10.1053/j.gastro.2015.08.035.

[2] C. Palazzi, D. Buskila, S. D'Angelo, E. D'Amico, I. Olivieri, Autoantibodies in patients with chronic hepatitis C virus infection: pitfalls for the diagnosis of rheumatic diseases, Autoimmun. Rev. 11 (2012) 659–663, https://doi.org/10.1016/j.autrev.2011.11.011.

[3] D. Vergani, G. Mieli-Vergani, Autoimmune manifestations in viral hepatitis, Semin. Immunopathol. 35 (2013) 73–85, https://doi.org/10.1007/s00281-012-0328-6.

[4] N. Abuaf, F. Lunel, P. Giral, E. Borotto, S. Laperche, R. Poupon, P. Opolon, J.M. Huraux, J.C. Homberg, Non-organ specific autoantibodies associated with chronic C virus hepatitis, J. Hepatol. 18 (1993) 359–364.

[5] S. Vitozzi, P. Lapierre, I. Djilali-Saiah, G. Marceau, K. Beland, F. Alvarez, Anti-soluble liver antigen (SLA) antibodies in chronic HCV infection, Autoimmunity 37 (2004) 217–222.

[6] M. Lenzi, P. Manotti, L. Muratori, M. Cataleta, G. Ballardini, F. Cassani, F.B. Bianchi, Liver cytosolic 1 antigen-antibody system in type 2 autoimmune hepatitis and hepatitis C virus infection, Gut 36 (1995) 749–754.

[7] F. Cassani, M. Cataleta, P. Valentini, P. Muratori, F. Giostra, R. Francesconi, L. Muratori, M. Lenzi, G. Bianchi, D. Zauli, F.B. Bianchi, Serum autoantibodies in chronic hepatitis C: comparison with autoimmune hepatitis and impact on the disease profile, Hepatol. Baltim. Md 26 (1997) 561–566, https://doi.org/10.1002/hep.510260305.

[8] Y. Bayraktar, M. Bayraktar, A. Gurakar, T.I. Hassanein, D.H. Van Thiel, A comparison of the prevalence of autoantibodies in individuals with chronic hepatitis C and those with autoimmune hepatitis: the role of interferon in the development of autoimmune diseases, Hepato-Gastroenterology 44 (1997) 417–425.

[9] D. Buskila, A. Shnaider, L. Neumann, M. Lorber, D. Zilberman, N. Hilzenrat, O.J. Kuperman, E. Sikuler, Musculoskeletal manifestations and autoantibody profile in 90 hepatitis C virus infected Israeli patients, Semin. Arthritis Rheum. 28 (1998) 107–113.

[10] A.R. Kammer, S.H. van der Burg, B. Grabscheid, I.P. Hunziker, K.M. Kwappenberg, J. Reichen, C.J. Melief, A. Cerny, Molecular mimicry of human cytochrome P450 by hepatitis C virus at the level of cytotoxic T cell recognition, J. Exp. Med. 190 (1999) 169–176.

[11] A.J. Czaja, H.A. Carpenter, P.J. Santrach, S.B. Moore, Immunologic features and HLA associations in chronic viral hepatitis, Gastroenterology 108 (1995) 157–164.

[12] S.-J. Hwang, C.-W. Chu, D.-F. Huang, K.-H. Lan, F.-Y. Chang, S.-D. Lee, Genetic predispositions for the presence of cryoglobulinemia and serum autoantibodies in Chinese patients with chronic hepatitis C, Tissue Antigens 59 (2002) 31–37.

[13] S. Vento, F. Cainelli, C. Renzini, E. Concia, Autoimmune hepatitis type 2 induced by HCV and persisting after viral clearance, Lancet Lond. Engl. 350 (1997) 1298–1299, https://doi.org/10.1016/S0140-6736(05)62476-2.

[14] F.D. Mackie, M. Peakman, M. Yun, R. Sallie, H. Smith, E.T. Davies, G. Mieli-Vergani, D. Vergani, Primary and secondary liver/kidney microsomal autoantibody response following infection with hepatitis C virus, Gastroenterology 106 (1994) 1672–1675.

[15] D.P. Bogdanos, Y. Ma, N. Hadzic, B. Portmann, G. Mieli-Vergani, D. Vergani, P0295 virus-self crossreactivity inducing de novo autoimmune hepatitis eight-years after liver transplantation, J. Pediatr. Gastroenterol. Nutr. 39 (2004) S169.

[16] D. Serranti, G. Indolfi, G. Nebbia, M. Cananzi, L. D'Antiga, S. Ricci, S. Stagi, C. Azzari, M. Resti, Italian study Group for treatment of chronic hepatitis C in children, transient hypothyroidism and autoimmune thyroiditis in children with chronic hepatitis C treated with pegylated-interferon-α-2b and ribavirin, Pediatr. Infect. Dis. J. 37 (2018) 287–291, https://doi.org/10.1097/INF.0000000000001791.

[17] T. Zornitzki, S. Malnick, L. Lysyy, H. Knobler, Interferon therapy in hepatitis C leading to chronic type 1 diabetes, World J. Gastroenterol. 21 (2015) 233–239, https://doi.org/10.3748/wjg.v21.i1.233.

[18] S.L. DeMartelaere, M.K. Green, J.W. Shore, Exacerbation of Graves ophthalmopathy with interferon-alpha therapy, Ophthalmic Plast. Reconstr. Surg. 23 (2007) 319–321, https://doi.org/10.1097/IOP.0b013e3180a72711.

[19] A. Yurci, K. Guven, E. Torun, S. Gursoy, M. Baskol, H. Akgun, O. Ozbakir, M. Yucesoy, Pyoderma gangrenosum and exacerbation of psoriasis resulting from pegylated interferon alpha and ribavirin treatment of chronic hepatitis C, Eur. J. Gastroenterol. Hepatol. 19 (2007) 811–815, https://doi.org/10.1097/MEG.0b013e32816e070b.

[20] L. Muratori, D.P. Bogdanos, P. Muratori, M. Lenzi, A. Granito, Y. Ma, G. Mieli-Vergani, F.B. Bianchi, D. Vergani, Susceptibility to thyroid disorders in hepatitis C, Clin. Gastroenterol. Hepatol. Off. Clin. Pract. J. Am. Gastroenterol. Assoc. 3 (2005) 595–603.

[21] P. Muratori, L. Muratori, G. Verucchi, L. Attard, F.B. Bianchi, M. Lenzi, Non-organ-specific autoantibodies in children with chronic hepatitis C: clinical significance and impact on interferon treatment, Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am. 37 (2003) 1320–1326, https://doi.org/10.1086/379018.

[22] G.V. Gregorio, P. Pensati, R. Iorio, A. Vegnente, G. Mieli-Vergani, D. Vergani, Autoantibody prevalence in children with liver disease due to chronic hepatitis C virus (HCV) infection, Clin. Exp. Immunol. 112 (1998) 471–476.

[23] B. Terziroli Beretta-Piccoli, G. Mieli-Vergani, D. Vergani, The clinical usage and definition of autoantibodies in immune-mediated liver disease: a comprehensive overview, J. Autoimmun. (2018), https://doi.org/10.1016/j.jaut.2018.10.004.

[24] A. Sugiura, S. Wada, H. Mori, T. Kimura, Y. Matsuda, N. Tanaka, E. Tanaka, K. Kiyosawa, Successful treatment for chronic hepatitis C-autoimmune hepatitis Overlap syndrome due to daclatasvir and asunaprevir, Case Rep. Gastroenterol. 11 (2017) 305–311, https://doi.org/10.1159/000475752.

[25] K. Misaka, T. Kishimoto, Y. Kawahigashi, M. Sata, Y. Nagao, Use of direct-acting antivirals for the treatment of hepatitis C virus-associated oral lichen planus: a case report, Case Rep. Gastroenterol. 10 (2016) 617–622, https://doi.org/10.1159/000450679.

[26] M. Ramos-Casals, A.L. Zignego, C. Ferri, P. Brito-Zerón, S. Retamozo, M. Casato, P. Lamprecht, A. Mangia, D. Saadoun, A.G. Tzioufas, Z.M. Younossi, P. Cacoub, International Study Group of Extrahepatic Manifestations related to HCV (ISG-EHCV), Evidence-based recommendations on the management of extrahepatic manifestations of chronic hepatitis C virus infection, J. Hepatol. 66 (2017) 1282–1299, https://doi.org/10.1016/j.jhep.2017.02.010.

[27] C. Mazzaro, L. Dal Maso, L. Quartuccio, M. Ghersetti, M. Lenzi, E. Mauro, M. Bond, P. Casarin, V. Gattei, I.M. Crosato, S. De Vita, G. Pozzato, Long-term effects of the new direct antiviral agents (DAAs) therapy for HCV-related mixed cryoglobulinaemia without renal involvement: a multicentre open-label study, Clin. Exp. Rheumatol. 36 (Suppl 111) (2018) 107–114.

[28] M. Bonacci, S. Lens, Z. Mariño, M.-C. Londoño, S. Rodriguez-Tajes, J.M. Sánchez-Tapias, M. Ramos-Casals, J. Hernández-Rodríguez, X. Forns, Long-term outcomes of patients with HCV-associated cryoglobulinemic vasculitis after virologic cure, Gastroenterology 155 (2018) 311–315, https://doi.org/10.1053/j.gastro.2018.04.024 e6.

[29] L. Gragnani, A. Piluso, T. Urraro, A. Fabbrizzi, E. Fognani, L. Petraccia, A. Genovesi, L. Giubilei, J. Ranieri, C. Stasi, M. Monti, A.L. Zignego, Virological and clinical response to interferon-free regimens in patients with HCV-related mixed cryoglobulinemia: preliminary results of a prospective pilot study, Curr. Drug Targets 18 (2017) 772–785, https://doi.org/10.2174/1389450117666160208145432.

[30] D. Saadoun, S. Pol, Y. Ferfar, L. Alric, C. Hezode, S.N. Si Ahmed, L. de Saint Martin, C. Comarmond, A.S. Bouyer, L. Musset, T. Poynard, M. Resche Rigon, P. Cacoub, Efficacy and safety of sofosbuvir plus daclatasvir for treatment of HCV-associated cryoglobulinemia vasculitis, Gastroenterology 153 (2017) 49–52, https://doi.org/10.1053/j.gastro.2017.03.006 e5.

[31] L. Prasad, V.M. Spicher, M. Zwahlen, M. Rickenbach, B. Helbling, F. Negro, Swiss hepatitis C cohort study Group, cohort profile: the Swiss hepatitis C cohort study (SCCS), Int. J. Epidemiol. 36 (2007) 731–737, https://doi.org/10.1093/ije/dym096.

[32] D. Vergani, F. Alvarez, F.B. Bianchi, E.L.R. Cançado, I.R. Mackay, M.P. Manns,

M. Nishioka, E. Penner, International autoimmune hepatitis Group, liver autoimmune serology: a consensus statement from the committee for autoimmune serology of the international autoimmune hepatitis Group, J. Hepatol. 41 (2004) 677–683, https://doi.org/10.1016/j.jhep.2004.08.002.

[33] E.K.L. Chan, J. Damoiseaux, O.G. Carballo, K. Conrad, W. de Melo Cruvinel, P.L.C. Francescantonio, M.J. Fritzler, I. Garcia-De La Torre, M. Herold, T. Mimori, M. Satoh, C.A. von Mühlen, L.E.C. Andrade, Report of the first international consensus on standardized nomenclature of antinuclear antibody HEp-2 cell patterns 2014-2015, Front. Immunol. 6 (2015) 412, https://doi.org/10.3389/fimmu.2015.00412.

[34] M. Lenzi, S. Bellentani, G. Saccoccio, P. Muratori, F. Masutti, L. Muratori, F. Cassani, F.B. Bianchi, C. Tiribelli, Prevalence of non-organ-specific autoantibodies and chronic liver disease in the general population: a nested case-control study of the Dionysos cohort, Gut 45 (1999) 435–441.

[35] S. Ferri, L. Muratori, M. Lenzi, A. Granito, F.B. Bianchi, D. Vergani, HCV and autoimmunity, Curr. Pharmaceut. Des. 14 (2008) 1678–1685.

[36] M.P. Manns, K.J. Griffin, K.F. Sullivan, E.F. Johnson, LKM-1 autoantibodies recognize a short linear sequence in P450IID6, a cytochrome P-450 monooxygenase, J. Clin. Investig. 88 (1991) 1370–1378, https://doi.org/10.1172/JCI115443.

[37] G.V. Gregorio, K. Choudhuri, Y. Ma, P. Pensati, R. Iorio, P. Grant, J. Garson, D.P. Bogdanos, A. Vegnente, G. Mieli-Vergani, D. Vergani, Mimicry between the hepatitis C virus polyprotein and antigenic targets of nuclear and smooth muscle antibodies in chronic hepatitis C virus infection, Clin. Exp. Immunol. 133 (2003) 404–413.

[38] E. Toubi, S. Gordon, A. Kessel, I. Rosner, M. Rozenbaum, Y. Shoenfeld, E. Zuckerman, Elevated serum B-Lymphocyte activating factor (BAFF) in chronic hepatitis C virus infection: association with autoimmunity, J. Autoimmun. 27 (2006) 134–139, https://doi.org/10.1016/j.jaut.2006.07.005.

[39] D.-A. Landau, M. Rosenzwajg, D. Saadoun, D. Klatzmann, P. Cacoub, The B lymphocyte stimulator receptor-ligand system in hepatitis C virus-induced B cell clonal disorders, Ann. Rheum. Dis. 68 (2009) 337–344, https://doi.org/10.1136/ard.2007.085910.

[40] M. Visentini, M. Del Padre, S. Colantuono, B. Yang, Y.A. Minafò, S. Antonini, M. Carnovale, A. De Santis, A. Pulsoni, G.M. De Sanctis, L. Gragnani, A.L. Zignego, M. Fiorilli, M. Casato, Long-lasting persistence of large B-cell clones in hepatitis C virus-cured patients with complete response of mixed cryoglobulinaemia vasculitis, Liver Int. Off. J. Int. Assoc. Study Liver. (2019), https://doi.org/10.1111/liv.14053.

[41] M. Visentini, M. Del Padre, S. Colantuono, B. Yang, Y.A. Minafò, S. Antonini, M. Carnovale, A. De Santis, A. Pulsoni, G.M. De Sanctis, L. Gragnani, A.L. Zignego, M. Fiorilli, M. Casato, Long-lasting persistence of large B-cell clones in hepatitis C virus-cured patients with complete response of mixed cryoglobulinaemia vasculitis, Liver Int. Off. J. Int. Assoc. Study Liver. (2019), https://doi.org/10.1111/liv.14053.

[42] M. Visentini, L. Quartuccio, M. Del Padre, S. Colantuono, Y.A. Minafò, M. Fiorilli, S. De Vita, M. Casato, Late relapses of hepatitis C virus-cured mixed cryoglobulinaemia associated with infection or cancer, Rheumatol. Oxf. Engl. 57 (2018) 1870–1871, https://doi.org/10.1093/rheumatology/key157.

[43] T.J. O'Donohoe, R.G. Schrale, S. Sikder, N. Surve, D. Rudd, N. Ketheesan, Significance of Anti-myosin Antibody Formation in Patients with Myocardial Infarction: A Prospective Observational Study, Heart Lung Circ, 2018, https://doi.org/10.1016/j.hlc.2018.03.008.

[44] M.E. Sise, J. Wisocky, I.A. Rosales, D. Chute, J.A. Holmes, K.M. Corapi, J.L. Babitt, J.S. Tangren, N. Hashemi, A.L. Lundquist, W.W. Williams, D.B. Mount, K.L. Andersson, H.G. Rennke, R.N. Smith, R. Colvin, R.I. Thadhani, R.T. Chung, Lupus-like immune complex-mediated glomerulonephritis in patients with hepatitis C virus infection treated with oral, interferon-free, direct-acting antiviral therapy, Kidney Int. Rep. 1 (2016) 135–143, https://doi.org/10.1016/j.ekir.2016.06.006.

[45] G. Covini, E. Bredi, S. Badalamenti, M. Roncalli, A. Aghemo, M. Colombo, Autoimmune hepatitis during ledipasvir/sofosbuvir treatment of hepatitis C: a case report, Hepatol. Commun. 2 (2018) 1179–1183, https://doi.org/10.1002/hep4.1248.

[46] K. Matsumoto, K. Kikuchi, Y. Kajiyama, Y. Takano, M. Mabuchi, S. Doi, K. Sato, H. Miyakawa, I. Yasuda, Development of autoimmune hepatitis during direct-acting antiviral therapy for chronic hepatitis C virus infection, Intern. Med. Tokyo Jpn. 57 (2018) 2669–2673, https://doi.org/10.2169/internalmedicine.0613-17.

[47] G.D. Keppeke, S.J. Calise, E.K.L. Chan, L.E.C. Andrade, Anti-rods/rings autoantibody generation in hepatitis C patients during interferon-α/ribavirin therapy, World J. Gastroenterol. 22 (2016) 1966–1974, https://doi.org/10.3748/wjg.v22.i6.1966.

[48] M. Alsius, M.J. Ferri, M. Buxó, C. López, I. Serra, X. Queralt, D. Acero, Autoantibodies to cytoplasmic rods and rings in patients with hepatitis C virus infection treated with direct-acting antivirals: the role of prior treatment with interferon plus ribavirin, Gastroenterol. Hepatol. (2018), https://doi.org/10.1016/j.gastrohep.2018.08.005.

[49] EASL - Clinical Practice Guidelines, (n.d.).http://www.easl.eu/research/our-contributions/clinical-practice-guidelines/detail/easl-recommendations-on-treatment-of-hepatitis-c-2018 (accessed December 2, 2018).

[50] D. Moradpour, J. Fehr, D. Semela, A. Rauch, B. Müllhaupt, Treatment of Chronic Hepatitis C - August 2018 Update SASL-SSI Expert Opinion Statement, (n.d.) 8.

[51] B. Rocha, C. Tanchot, H. Von Boehmer, Clonal energy blocks in vivo growth of mature T cells and can be reversed in the absence of antigen, J. Exp. Med 177 (1993) 1517–1521.