Direct-Acting Antiviral Treatment of Patients with Hepatitis C Resolves Serologic and Histopathologic Features of Autoimmune Hepatitis

Camila C. Simoes,[1] Omar A. Saldarriaga,[1] Netanya S. Utay,[2] Ashley E. Stueck,[3] Sheharyar K. Merwat,[4] Shehzad N. Merwat,[4] Thomas D. Schiano,[5] Maria Isabel Fiel,[6] and Heather L. Stevenson [1]

Patients with hepatitis C virus (HCV) often have elevated serum markers and histologic features of autoimmune hepatitis (AIH). We evaluated an HCV-positive (HCV+) study group that had elevated serum markers of AIH before starting direct-acting antiviral (DAA) therapy (n = 21) and compared them to an HCV+ control group that did not have laboratory studies suggesting AIH (n = 21). Several patients in the study (17/21) and control (11/21) groups had liver biopsies before DAA treatment, and many were biopsied due to elevated serum markers of AIH. Evaluation of pre-DAA treatment liver biopsies showed histologic features suggestive of AIH in 64.7% (11/17) of the study group and 45.5% (5/11) of the control group. Patients who were HCV+ with elevated serum markers of AIH had significantly increased hepatitis activity ($P < 0.001$) and slightly increased fibrosis stages ($P = 0.039$) in their pretreatment liver biopsies compared to controls. We hypothesized that the elevated serum markers and histologic features of AIH would resolve following DAA treatment. Serum markers of AIH in the study group began decreasing by 6 months posttreatment, and 52.4% (11/21) had complete resolution. Alanine aminotransferase levels significantly decreased into the normal range for all patients (21/21). Even patients that had persistence of serum markers of AIH after DAA treatment had normal transaminases. Six patients from the study patient group and 4 patients from the control group had follow-up liver biopsies after DAA treatment, and all biopsies showed resolution of the histologic features of AIH. *Conclusion*: The majority of HCV+ patients that have serum markers and/or histopathologic features of AIH should initially be treated with DAA. (*Hepatology Communications* 2019;3:1113-1123).

Worldwide, hepatitis C virus (HCV) has a prevalence of 2%-3% and infects more than 170 million people. The majority of patients (75%-85%) develop chronic infections, and about 20% of these patients will progress to cirrhosis.[1-3] In addition to liver injury, up to 74% of patients develop extrahepatic manifestations.[4,5] Chronic HCV infection can induce autoimmunity[6-9]

Abbreviations: +, positive; AASLD, American Association for the Study of Liver Diseases; AIH, autoimmune hepatitis; ALT, alanine aminotransferase; ANA, antinuclear antibody; AST, aspartate aminotransferase; DAA, direct-acting antiviral; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IAIHG, International Autoimmune Hepatitis Group; IFN, interferon; IgG, immunoglobulin G; LKM-1, liver–kidney microsomal type 1; MHAI, Modified Hepatitis Activity Index; SMA, smooth muscle actin; SVR, sustained virologic response; UTMB, University of Texas Medical Branch.

Received March 13, 2019; accepted May 17, 2019.

Supported by a Moody Endowment Grant (award number 2014-07 to H.L.S.) and the National Center for Advancing Translational Sciences Clinical and Translational Science Award KL2 Scholars Program (award number KL2TR001441 to H.L.S.).

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep4.1388

Potential conflict of interest: Nothing to report.

that can lead to the development of antibodies, such as antinuclear antibody (ANA), smooth muscle actin (SMA)/F-actin, liver–kidney microsomal type 1 (LKM-1), soluble liver antigen (SLA), and immunoglobulin G (IgG), and some patients may develop frank autoimmune hepatitis (AIH).[10,11] These circulating nonorgan-specific autoantibodies (e.g., ANA, SMA/F-actin, and IgG) occur in about 50% of patients with chronic HCV infection.[12,13] The autoantibody profile observed in patients with chronic hepatitis C may arouse clinical suspicion for concurrent AIH and prompt unnecessary liver biopsy procedures and/or immune suppressive treatment.

HCV infection and AIH are two chronic inflammatory diseases that share several characteristics, including histopathologic features (e.g., active lymphocytic portal and lobular inflammation), elevated transaminases, and the typical sequelae of chronic liver injury, including cirrhosis. However, they have markedly different treatment approaches. Differentiating patients that have primary AIH from those that have secondary AIH due to infection with chronic hepatitis C may be clinically challenging and is of importance because immunosuppression may enhance viral replication. No standard therapeutic strategy for patients with chronic HCV and AIH overlap or elevated serum markers of AIH is currently available.

Interferon (IFN)-free or direct-acting antiviral (DAA) therapies have provided major advances in the treatment of HCV, with greater than 90% of patients achieving sustained viral response (SVR) with most regimens, and have been shown to be safe and effective in patients with associated autoimmune conditions.[14-17] The impact of DAA treatment on patients with HCV with elevated serum markers of AIH and transaminases, with or without the presence of histopathologic features of AIH, is not clear in the literature except for brief discussions in a few case reports.[16,18] The goal of this study was to determine if the observed elevation of serum markers and histologic features of AIH in patients with HCV would normalize following DAA treatment.

Patients and Methods

STUDY POPULATION

At the University of Texas Medical Branch (UTMB), we have an ongoing institutional review board-approved clinical study to collect blood and/or liver biopsy tissues from patients who were HCV positive (HCV+) before and after DAA treatment. At the time of this study, 103 patients consented and were enrolled and 91 had serologic testing to exclude possible AIH before treatment initiation. Of these 91 patients, 39.5% (36/91) had at least one abnormal test result. Nineteen of these patients were excluded due to lack of insurance approval for DAA treatment or absence of appropriate follow-up laboratory studies. Collaborators at Mount Sinai Hospital also observed similar laboratory abnormalities in 9 of their patients who were HCV+; 4 had pretreatment liver biopsies with appropriate follow-up laboratory testing and were included in the study group (total n = 21). All patients (from UTMB and Mount Sinai) were prospectively followed after DAA treatment to evaluate for SVR, persistence of serum markers of AIH, and normalization of transaminases. Demographic

ARTICLE INFORMATION:

From the [1]Department of Pathology, University of Texas Medical Branch, Galveston, TX; [2]Infectious Disease Division, Department of Internal Medicine, University of Texas Health Science Center at Houston, Houston, TX; [3]Department of Pathology, Dalhousie University, Halifax, Canada; [4]Department of Gastroenterology and Hepatology, University of Texas Medical Branch, Galveston, TX; [5]Department of Hepatology; [6]Department of Pathology, Icahn School of Medicine at Mount Sinai, New York, NY.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Heather L. Stevenson, M.D., Ph.D.
Assistant Professor, Department of Pathology
University of Texas Medical Branch
712 Texas Avenue
Clinical Services Wing, Room 5.506K
Galveston, TX 77555-0416
E-mail: hlsteven@utmb.edu
Tel.: +1-409-392-1568

data, pertinent clinical history, including presence of other autoimmune diseases, and DAA treatment type and duration were also recorded. Six study patients had follow-up liver biopsies after DAA treatment. All data collected from these patients have been de-identified.

CONTROL POPULATION

The study patient cohort (n = 21) was matched to a control group (n = 21) by age, sex, HCV genotype, and human immunodeficiency virus (HIV) status. These patients also consented to be participants in the clinical study in which blood and/or liver biopsy tissues were obtained from patients who were HCV+ before and after DAA treatment. Of the 91 patients who had serologic testing to exclude possible AIH before treatment initiation, 55 had normal results and 21 patients from this group were used to select the matched controls. Similar demographic and clinical data were recorded. Four control patients had follow-up liver biopsies after DAA treatment. All data collected from these patients have been de-identified.

LABORATORY DATA

For the purpose of this study, we investigated patients with chronic hepatitis C who had elevated levels of autoantibodies, including ANA, SMA, F-actin, and IgG. SLA and LKM-1 antibodies were not tested in these patients because all patients were adults. We used the following simplified diagnostic criteria of the International Autoimmune Hepatitis Group (IAIHG) published by Hennes et al.[19] to determine the level of antibodies that should be considered a positive result: for ANA and SMA, ≥1:40; F-actin, ≥20 mg/dL; and serum IgG, >1,600 mg/dL. Antibodies with levels below these established cutoffs were considered negative. At the UTMB, ANA and IgG levels were analyzed in-house in a College of American Pathologists (CAP)–Clinical Laboratory Improvement Amendments-accredited clinical chemistry laboratory. Serum ANA was assayed with an immunofluorescence-screening method and was reflexed to determine ANA titer and pattern if positive. IgG levels were determined by nephelometry. Levels of SMA/F-actin antibodies were determined by a reference laboratory (ARUP Laboratories, Salt Lake City, UT) in which IgG positivity was determined by enzyme-linked immunosorbent assay (ELISA) with reflex to smooth muscle antibody, IgG titer (catalogue [cat.] #0051174). At Mount Sinai Hospital, serum ANA analysis was performed by a reference laboratory (LabCorp Laboratories, Raritan, NJ). IgG was measured in-house on an Abbott Architect c16000 by turbidimetry in a CAP-accredited clinical chemistry laboratory. The SMA/F-actin antibodies were analyzed at LabCorp Laboratories; IgG positivity was determined by ELISA with reflex to SMA, IgG titer (cat. #006643). Laboratory data were collected for each patient at approximately 6 and 12 months posttreatment. Follow-up duration ranged from 6 to 33 months. We compared the serum markers of AIH and transaminase levels in the HCV+ study patient group (n = 21) (see Table 1) to a control group (n = 21). Other laboratory data, including HIV viral load, HCV viral load pre-DAA and post-DAA treatment, and HCV genotype, were also recorded (see Table 1).

LIVER BIOPSIES

To evaluate for the presence of features of AIH versus HCV-mediated hepatitis, two board-certified pathologists (Dr. Stevenson at UTMB and Dr. Fiel at Mount Sinai Hospital) were blinded to the patient's serology, clinical history, and prior pathology reports while reviewing the patient liver biopsies. Each patient's pre-DAA treatment liver biopsy (17 from the study cohort and 11 from the control cohort) was evaluated for histologic features of AIH, hepatitis inflammatory activity, and stage of fibrosis.[19-22] According to the simplified criteria established by the IAIHG, "typical" histopathologic features of AIH should include interface hepatitis with a lymphoplasmacytic infiltrate, rosettes, and emperipolesis. Liver biopsies should be assigned scores of 2 (typical), 1 (compatible), and 0 (atypical) (Tables 2-4)[19]; however, rosettes and emperipolesis have been shown to be nonspecific and difficult features to interpret.[23] Thus, we gave a score of "typical" or 2 points to liver biopsies that had plasma cell-rich interface/lobular activity and a score "compatible" or 1 point to cases that had mild plasma cell-rich interface/lobular activity. This approach is similar to the histopathologic features recommended in the American Association for the Study of Liver Diseases (AASLD) practice guidelines for diagnosis and management of AIH.[24] The original scoring

TABLE 1. LABORATORY RESULTS FOR STUDY PATIENTS

			Pretreatment						Posttreatment							
Pt #	Sex	Genotype	ANA	F-actin	SMA	IgG	ALT	AST	ANA	F-actin	SMA	IgG	ALT	AST	SM Normal* Post-DAA	ALT Normal Post-DAA
1†	F	1A	>1:1,280	Neg	nd	Neg	52	39	1:320	nd	nd	nd	30	18	NO	YES
2†	M	1A	1:160	Neg	nd	nd	54	61	1:160	nd	nd	nd	33	26	NO	YES
3†	F	1A	1:80	21	Neg	2,440	138	206	Neg	Neg	Neg	Neg	28	46	YES	YES
4†	F	1A	Neg	20	1:40	Neg	67	53	Neg	Neg	Neg	Neg	13	18	YES	YES
5†	M	1A	Neg	nd	nd	2,110	260	211	Neg	Neg	Neg	Neg	31	31	YES	YES
6†	M	1A	Neg	21	nd	1,680	63	56	Neg	Neg	Neg	Neg	36	41	YES	YES
7†	F	1B	1:80	Neg	nd	nd	48	53	Neg	nd	nd	nd	21	22	YES	YES
8†	F	1B	Neg	22	Neg	nd	122	133	Neg	23	Neg	nd	24	19	NO	YES
9†	F	1A/HIV+	1:80	Neg	nd	Neg	31	34	1:80	Neg	Neg	nd	31	28	NO	YES
10†	M	1A	Neg	27	Neg	Neg	26	26	Neg	30	Neg	nd	26	26	NO	YES
11†	M	1B/HIV+	Neg	20	Neg	1,600	138	210	Neg	Neg	Neg	Neg	24	35	YES	YES
12†	F	1A/HIV+	Neg	35	Neg	Neg	31	30	Neg	48	Neg	Neg	23	21	NO	YES
13†	F	1B/HIV+	Neg	39	Neg	2,230	58	70	Neg	Neg	nd	Neg	30	27	YES	YES
14†	F	1A	Neg	28	1:320	3,240	352	361	Neg	Neg	nd	Neg	22	16	YES	YES
15†	M	1A/HIV+	Neg	43	Neg	nd	66	50	Neg	Neg	Neg	Neg	43	36	YES	YES
16†	F	3B	Neg	50	Neg	1,710	240	126	nd	Neg	nd	Neg	18	23	YES	YES
17†	M	1A	Neg	29	Neg	1,950	91	93	Neg	Neg	Neg	Neg	17	28	YES	YES
18‡	M	1A	Neg	nd	nd	2,301	105	81	Neg	nd	nd	2286	23	27	NO	YES
19‡	F	1B	Neg	nd	1:1,280	1,711	39	46	nd	nd	1:40	Neg	31	24	NO	YES
20‡	F	4A	Neg	Neg	Neg	2,046	752	414	nd	nd	nd	1,660	20	15	NO	YES
21‡	M	2	1:2,560	nd	Neg	Neg	11	17	1:640	nd	Neg	Neg	11	18	NO	YES

*Normal ranges: ANA, <1:40; F-actin <20 units; SMA <1:40; IgG, 636-1,600 mg/dL; ALT, 9-51 U/L; AST,13-40 U/L.
†UTMB patients; ‡Mount Sinai Hospital patients.
Abbreviations: F, female; M, male; nd, not done; Neg, result within normal laboratory range; Pt #, patient number; SM, serum markers.

TABLE 2. SIMPLIFIED DIAGNOSTIC CRITERIA FOR AUTOIMMUNE HEPATITIS BY THE IAIHG[19,23]

Variable	Cutoff	Points*
ANA or SMA	Equal to or greater than 1:40	1
ANA or SMA	Equal to or greater than 1:80	2†
Or LKM-1	Equal to or greater than 1:40	
Or soluble liver antigen	Positive	
Serum IgG	More than upper limit of normal	1
	More than 1.10 times upper limit of normal	2
Liver histology (evidence of hepatitis is a necessary condition)	Compatible with autoimmune hepatitis	1
	Typical for autoimmune hepatitis	2
Absence of viral hepatitis	Yes	2

*For this study, we slightly modified the histologic criteria required for "typical" or "compatible" with autoimmune hepatitis. A score of "typical" or 2 points was given to liver biopsies that had prominent plasma cell-rich interface/lobular activity (see example in Fig. 2), and a score "compatible" or 1 point was given to cases that had mild plasma cell-rich interface/lobular activity. Total points: ≥6, probable autoimmune hepatitis; ≥7, definite autoimmune hepatitis.
†Total points for all autoantibodies cannot exceed 2 points.

system developed by the IAIHG in 1999 was not used for this study because it requires extensive clinical information that was not available for many of the patients.[25] Post-DAA treatment liver biopsies were reviewed in a similar manner when available; six were collected/evaluated from the study patient group, and four were collected/evaluated from the control group. Patients with evidence of active steatohepatitis, cholestasis, or chronic biliary obstruction in their liver biopsies were excluded from the study.

STATISTICAL ANALYSIS

Data were analyzed using SigmaPlot software (Systat Software Inc., San Jose, CA). Descriptive statistics (mean, median, and SD) were obtained, and *P* values were determined using the Student two-tailed *t* test (Shapiro-Wilk). When indicated, the Mann-Whitney rank sum test and Welch's *t* test were applied.

TABLE 3. STUDY COHORT PRE-DAA AND POST-DAA TREATMENT BIOPSY SCORES*

Pt #	Biopsy Pretreatment?	MHAI Score (×/18)	Percent Macrovesicular Steatosis	Fibrosis Stage (×/6)	IAIHG Score	Total AIH Points	Biopsy Posttreatment?	MHAI Score (×/18)	Percent Macrovesicular Steatosis	Fibrosis Stage (×/6)	IAIHG Score	Total AIH Points
1†	YES	9-10	10	4	2	4	nd	nd	nd	nd	nd	0
2†	YES	6	0	3	0	2	nd	nd	nd	nd	nd	0
3†	nd	nd	nd	nd	nd	3	nd	nd	nd	nd	nd	0
4†	YES	11	10	6	1	2	YES	3	5	6	0	0
5†	YES	8	10	6	2	4	nd	nd	nd	nd	nd	0
6†	YES	5	0	3	0	2	nd	nd	nd	nd	nd	0
7†	YES	5	10	2-3	0	2	YES	1	10	1	0	0
8†	YES	8	2	4-5	2	3	YES	3	0	4	0	0
9†	nd	nd	nd	nd	nd	1	nd	nd	nd	nd	nd	0
10†	nd	nd	nd	nd	nd	1	nd	nd	nd	nd	nd	0
11†	YES	6	0	2	0	2	nd	nd	nd	nd	nd	0
12†	YES	5	15	2	0	1	nd	nd	nd	nd	nd	0
13†	YES	4	20	2	0	3	nd	nd	nd	nd	nd	0
14†	YES	9	0	2	2	6	YES	2	0	2	0	0
15†	nd	nd	nd	nd	nd	1	nd	nd	nd	nd	nd	0
16†	YES	8	0	2	2	4	nd	nd	nd	nd	nd	0
17†	YES	7	10	5	2	5	nd	nd	nd	nd	nd	0
18‡	YES	9	0	6	2	4	YES	9	0	6	0	0
19‡	YES	8	0	6	1	4	YES	4	0	4	0	0
20‡	YES	8	5	4	1	3	nd	nd	nd	nd	nd	0
21‡	YES	9	0	2	1	3	nd	nd	nd	nd	nd	0

*We used the MHAI to grade the amount of hepatitis activity,[21,22] the Ishak criteria for fibrosis staging (scale of 0 to 6),[21] and IAIHG simplified criteria to evaluate for histopathologic features of AIH.[19] The IAIHG criteria were used to evaluate the liver biopsy for histopathologic features of AIH as follows: 0 points, no evidence; 1 point, compatible; 2 points, typical. Total AIH points were determined by adding points from the IAIHG scoring to points acquired from each patient's serologic results (refer to Table 1) as follows: ≤5, no AIH; ≥6, probable AIH; ≥7, definite AIH.
†UTMB patients; ‡Mount Sinai Hospital patients.
Abbreviations: nd, not done; Pt #, patient number.

Results

STUDY AND CONTROL COHORTS

The majority of the patients in the HCV+ study and control groups were identified due to their participation in a clinical study evaluating the effects of DAA treatment (38/42, 90.5%). Of the 91 patients who were HCV+ and enrolled in the clinical study, 39.5% (36/91) had at least one abnormal AIH-related laboratory test (e.g., IgG immunoglobulins and/or SMA/F-actin antibodies). Nineteen of these patients were excluded due to lack of insurance approval for DAA treatment or absence of appropriate follow-up laboratory studies after DAA treatment. Twenty-one patients who were HCV+ had adequate follow-up post-DAA treatment, and these were matched to a control group (n = 21) by age, sex, HCV genotype, and HIV status. The mean age was 56.2 ± 9.6 years in the study patient group and 55.4 ± 5.2 years in the control group. In both groups, the majority were female patients (57.0%) with HCV genotype 1A (13/21, 61.9%); 5 patients per group were co-infected with HIV, and all patients had HIV-1 RNA levels that were not detectable during the course of DAA treatment. None of the patients had known autoimmune diseases or a clinical history of AIH.

DAA TREATMENT REGIMENS

All study patients received DAA therapy with a duration ranging from 8 to 24 weeks (8 weeks, 1 patient; 12 weeks, 12 patients; 16 weeks, 2 patients; 24 weeks, 5 patients; and 1 patient, unknown). DAA type was Harvoni in 10 patients, Sovaldi plus Olysio

TABLE 4. CONTROL COHORT PRE-DAA AND POST-DAA TREATMENT BIOPSY SCORES*

Pt #	Biopsy Pretreatment?	MHAI Score (×/18)	Percent Macrovesicular Steatosis	Fibrosis Stage (×/6)	IAIHG Score	Total AIH Points	Biopsy Posttreatment?	MHAI Score (×/18)	Percent Macrovesicular Steatosis	Fibrosis Stage (×/6)	IAIHG Score	Total AIH Points
1†	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2†	nd	nd	nd	nd	nd	nd	YES	1	10	2	0	0
3†	YES	5	0	2	0	0	YES	5	0	3	0	0
4†	YES	3	15	2	0	0	YES	1	5	0	0	0
5†	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
6†	YES	6	5	6	2	2	nd	nd	nd	nd	nd	nd
7†	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
8†	YES	2	< 5	0	0	0	YES	2	0	1	0	0
9†	YES	1	5	1	0	0	nd	nd	nd	nd	nd	nd
10†	YES	5	0	0	1	1	nd	nd	nd	nd	nd	nd
11†	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
12†	YES	5	10	2	0	0	nd	nd	nd	nd	nd	nd
13†	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
14†	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15†	YES	7	0	3	2	2	nd	nd	nd	nd	nd	nd
16†	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
17†	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
18‡	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
19‡	YES	5	30	1	2	2	nd	nd	nd	nd	nd	nd
20‡	YES	7	0	4	1	1	nd	nd	nd	nd	nd	nd
21‡	YES	4	5	3	0	0	nd	nd	nd	nd	nd	nd

*We used the MHAI to grade the amount of hepatitis activity,[21,22] the Ishak criteria for fibrosis staging (scale of 0 to 6),[21] and IAIHG simplified criteria to evaluate for histopathologic features of AIH.[19] The IAIHG criteria were used to evaluate the liver biopsy for histopathologic features of AIH as follows: 0 points, no evidence; 1 point, compatible; 2 points, typical. Total AIH points were determined by adding points from the IAIHG scoring to points acquired from each patient's serologic results (refer to Table 1) as follows: ≤5, no AIH; ≥6, probable AIH; ≥7, definite AIH.
†UTMB patients; ‡Mount Sinai Hospital patients.
Abbreviations: nd, not done; Pt #, patient number.

in 5 patients, Viekira Pak in 3 patients, Epclusa in 1 patient, Zepatier plus ribavirin in 1 patient, and Sovaldi plus ribavirin in 1 patient. For the control patients, 15 received DAA therapy with a duration of 12 or 24 weeks (12 weeks, 13 patients; 24 weeks, 2 patients). DAA type was Harvoni in 5 patients, Sovaldi plus Olysio in 4 patients, Sovaldi plus Olysio plus ribavirin in 2 patients, Zepatier in 1 patient, and Sovaldi plus ribavirin in 3 patients. All achieved SVR with negative polymerase chain reaction results at 12 weeks posttreatment.

LABORATORY DATA

All study patients had one or more abnormal AIH-related laboratory test pre-DAA treatment according to the cutoff values in the simplified criteria for the diagnosis of AIH[19] (Table 1). Six of the patients (6/21, 28.6%) had elevated ANA, 12 (12/21, 57.1%) had elevated F-actin antibodies, 3 (3/21, 14.3%) had elevated SMA, and 11 (11/21, 52.4%) had elevated IgG immunoglobulins (Table 1). There was no significant difference in the pre-DAA treatment transaminase levels (alanine aminotransferase [ALT], $P = 0.191$; aspartate aminotransferase [AST], $P = 0.089$) when comparing the study and control patients (Fig. 1A). All patients were negative for the presence of serum cryoglobulins and had normal ceruloplasmin and alpha-1 antitrypsin levels.

Eleven of the 21 (11/21, 52.4%) patients with adequate follow-up showed complete normalization of serum markers of AIH (Table 1). Four of the patients (#1, 19, 20, and 21) with persistent elevation of serum markers of AIH showed decreases post-DAA treatment, and all of these patients had ALT levels in the normal range (Table 1). The 2 patients

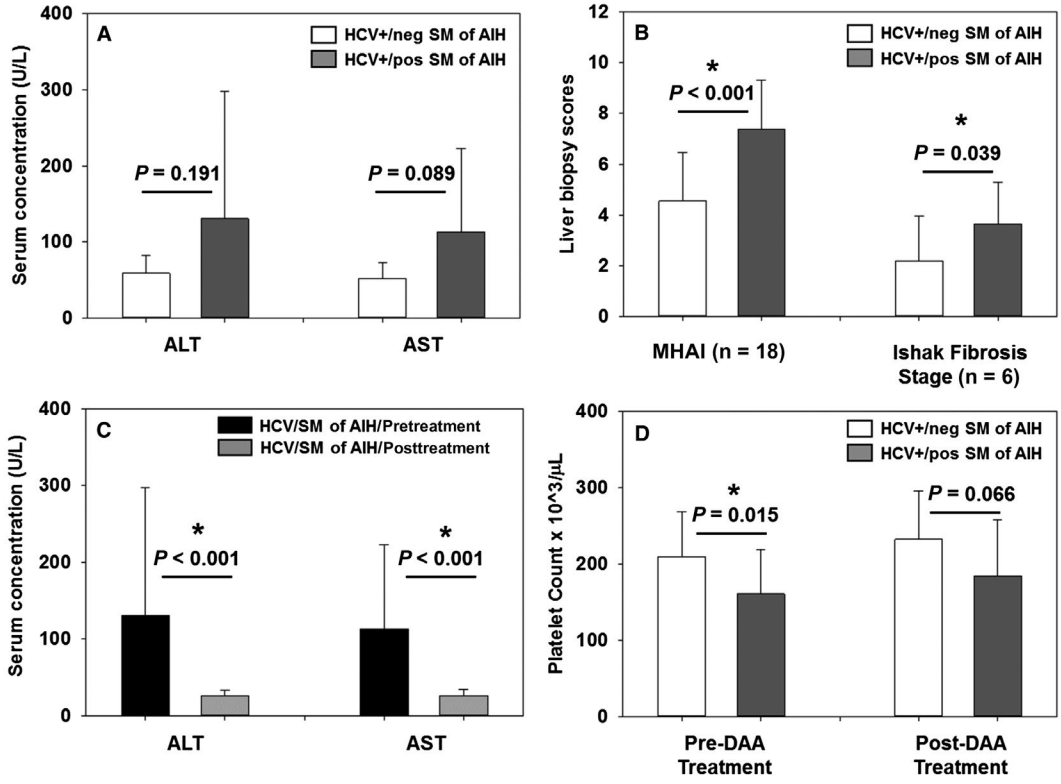

FIG. 1. Comparison of transaminases, liver biopsy scores, and platelet levels in study and control patients before and after DAA treatment. (A) Hepatic transaminase levels in the 21 study patients who were HCV+ with elevated serum markers of AIH were compared to an age-, sex-, HIV status, and genotype-matched HCV+ control group that did not have elevated serum markers of AIH. No significant difference was observed between these two groups (ALT, $P = 0.191$; AST, $P = 0.089$). (B) Liver biopsies were graded for inflammatory activity using the MHAI and staged for fibrosis using Ishak criteria.[21] The study patients who had elevated serum markers of AIH had significantly higher MHAI scores ($P < 0.001$) and slightly higher fibrosis stages ($P = 0.039$) compared to the control patients. (C) ALT and AST levels in the 21 study patients who were HCV+ after DAA treatment significantly decreased compared to before treatment ($P < 0.001$). (D) Platelet levels between the study and control patients were also compared before and after DAA treatment. Study patients with elevated serum markers of AIH had significantly lower platelet levels compared to control patients before treatment; however, this difference was no longer significant after DAA treatment (Table 4). Bar graphs and error bars represent mean ± SD. Abbreviations: neg, negative; pos, positive; SM, serum markers.

(2/21, 9.5%) with persistence of their elevated transaminase levels at 1-year posttreatment had only mild elevation (i.e., AST, 46 and 41 U/L for patients 3 and 6, respectively). Mean ALT and AST levels decreased significantly in the study patients before and after DAA treatment. Serum ALT levels decreased from a mean of 130.6 U/L (median, 66 U/L; SD, ±166.8) to a mean of 25.8 U/L (median, 24 U/L; SD, ±7.9; $P < 0.001$). AST decreased from a mean of 112.9 U/L (median, 61 U/L; SD, ±109.7) to a mean of 26.1 U/L (median, 26 U/L; SD, ±8.7; $P < 0.001$) (Fig. 1C). All control patients also had transaminase levels in the normal range following DAA treatment (data not shown). Four of the 10 patients that had persistently elevated serum markers of AIH were those with ANA positivity (Table 1). Because patients with autoimmunity may have immune-mediated thrombocytopenia,[26] we compared the platelet levels in the study and control patients before and after DAA treatment (Fig. 1D). Study patients with elevated serum markers of AIH had significantly lower platelet levels than the control patients; however, this difference was not statistically significant after DAA treatment (Fig. 1D).

LIVER BIOPSIES

Pretreatment liver biopsies were collected from 13 patients at the UTMB and from all 4 study patients from Mount Sinai Hospital (Table 3). These biopsies

were performed for fibrosis staging before DAA treatment approval or due to abnormal elevation of AIH-related serum markers. Seven pretreatment biopsies were performed for fibrosis staging (patient numbers 6, 7, 8, 11, 12, 13, and 17) and 10 were conducted due to elevated liver enzymes and/or abnormal AIH-related serologic tests (patient numbers 1, 2, 4, 5, 14, 16, 18, 19, 20, and 21) (Table 3). ALT levels between these two groups were similar ($P = 0.526$). Liver biopsies were evaluated blindly for features of AIH using a slight modification of the IAIHG-simplified criteria (Table 2) as described in Patients and Methods. Of these 17 patients, 11 had histologic features that were either "typical" or "compatible" with AIH (11/17, 64.7%)[19] (Table 3; Fig. 2). When the serologic data were combined with each patient's liver biopsy histology score, only 1 patient (#14; Tables 1 and 3) had an AIH score of "probable" and none had a score of "definite." If the patients had been able to receive two additional points for "absence of viral hepatitis" according to the IAIHG-simplified criteria, several more patients would have scored in the "probable" or "definite" for AIH range (Tables 1-3).

Pretreatment liver biopsies from the study patient group showed significantly increased hepatitis activity (i.e., Modified Hepatitis Activity Index [MHAI] scores) compared to the control patient group, with a mean of 7.4 and 4.5, respectively ($P < 0.001$) (Fig. 1B). However, fibrosis stages among the study and control patients were only slightly higher in the study patient cohort ($P = 0.039$) (Fig. 1B).[21] Patients with features of active steatohepatitis (e.g., hepatocyte ballooning degeneration) were excluded from the study; however, mild steatosis was present in several of the study and control patient liver biopsies and ranged from 5% to 30% and was not significantly different between the study and control patient groups ($P = 0.366$) (Tables 3 and 4).

Patient 14, the only patient that met the criteria for a diagnosis of "probable" AIH, was a middle-aged woman who had some of the highest liver enzymes in the study patient group (ALT and AST, 352 and

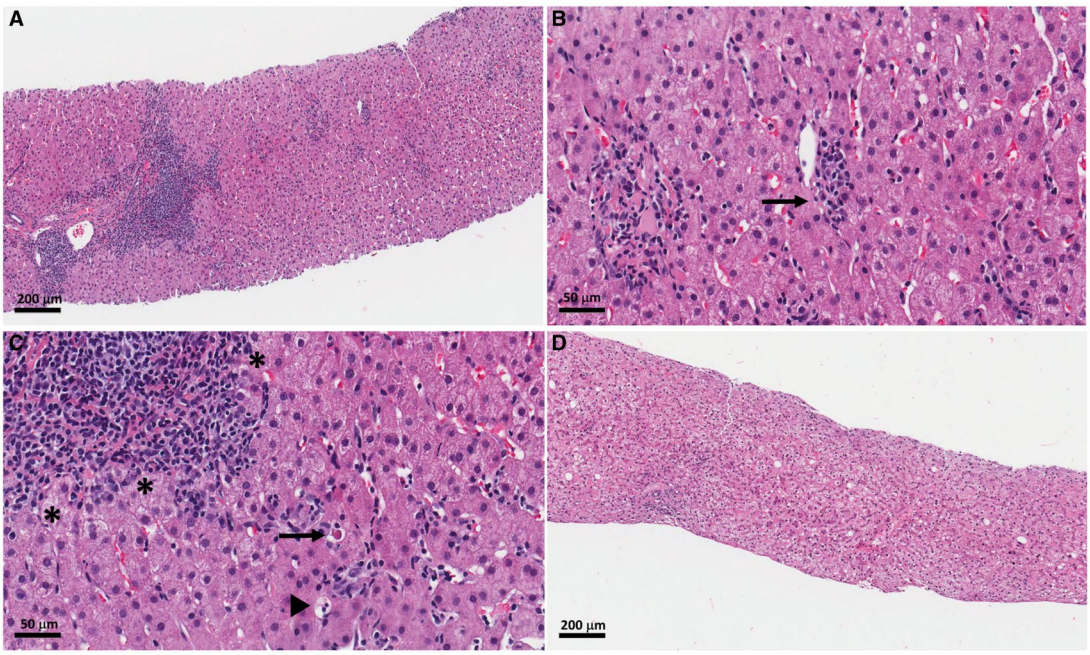

FIG. 2. Hematoxylin and eosin-stained pre-DAA and post-DAA treatment liver biopsies from a representative study patient (#14). (A) Percutaneous core needle biopsy of the liver shows histopathologic features consistent with AIH, including lymphoplasmacytic portal inflammation, plasma cell-rich interface activity, and scattered lobular inflammation (magnification ×10). (B,C) High-power magnification (×40) highlights the plasma cell-rich perivenulitis (B, arrow) and interface activity (C, asterisks). (C) Frequent lobular inflammation with apoptosis (arrow), hepatocyte rosetting (arrowhead), and focal emperipolesis-like regenerative changes (below arrowhead) was observed. (D) This patient returned for a post-DAA treatment liver biopsy that showed resolution of chronic hepatitis and histologic features of AIH.

361 U/L, respectively). Her elevated transaminases combined with elevated SMA/F-actin and IgG immunoglobulins (Table 1) prompted a liver biopsy (Fig. 2). The hepatic parenchyma showed all the characteristic histopathologic features of AIH, including plasma cell-rich interface activity, frequent lobular plasma cells, rosettes, and perivenulitis (Fig. 2A-C). Due to the patient's age, sex, and her classic presentation of AIH, immune suppression was considered in her management even though she was HCV+ with a viral load of over 5 million IU/mL. The case was discussed in detail at the UTMB's Liver Diseases Diagnostic Management Team conference, and the group decided to treat her active HCV infection with DAAs first. Her liver enzymes rapidly declined, and at 3 weeks post-DAA treatment, they were within the normal range for female individuals (ALT and AST, 20 and 16 U/L, respectively). She also had complete normalization of her serum markers of AIH, and a follow-up liver biopsy showed complete resolution of active hepatitis, including the histopathologic features of AIH (Fig. 2D). Because the majority of these patients were enrolled in a clinical study to collect blood and liver biopsy tissue pre-DAA and post-DAA treatment, 6 of the study patients and 4 of the control patients had posttreatment liver biopsies, including this patient. All the patients showed decreased MHAI scores and similar or slightly decreased fibrosis stages[21] post-DAA treatment, and histopathologic features of AIH were not seen[19] (Tables 3 and 4). Several of these patients had persistence of mild portal lymphocytic inflammation after DAA treatment, which has been reported.[27]

Discussion

The results of this study showed normalization of ALT and a decrease or normalization of serum markers of AIH in the majority of patients with chronic HCV infection following DAA therapy. The 6 study patients that had post-DAA treatment liver biopsies also had resolution of the histopathologic features of AIH (Table 3). These results are of clinical importance because no standard guidelines are available and serum markers of AIH are common in this population. Of the 91 patients who were HCV+ enrolled in this clinical study, 39.5% (36/91) had at least one abnormal AIH-related laboratory test (e.g., IgG immunoglobulins and/or SMA/F-actin antibodies). Even though many of these patients had only mild elevation, according to AASLD practice guidelines, low levels of autoantibodies or absence of plasma cells do not exclude a diagnosis of AIH.[24] These clinical features may prompt unnecessary liver biopsies and immune suppressive treatment in these patients.

ANA and SMA/F-actin are two of the main antibodies included in the simplified criteria score established by the IAIHG,[20] and elevations of these markers were commonly observed in the patients included in this study. In another study of 117 patients with chronic HCV infection, Clifford et al.[13] demonstrated that 14.0% and 66.0% of patients presented with positive ANA and SMA, respectively. Other studies observed a higher prevalence of SMA positivity (approximately 27%) compared to ANA (approximately 16%) in their cohorts of patients with HCV.[28-30] In this study, most patients who had persistently elevated serum markers of AIH after treatment were those with ANA positivity. At the time of this study, none of the patients had been diagnosed with other autoimmune diseases so the cause of the abnormally elevated ANA is not known.

Importantly, when the simplified criteria score[19] was applied, only 1 of the 21 patients in this study had a diagnosis of "probable" AIH and none had a diagnosis of "definite." Several additional patients would have been in the "probable" or "definite" range if they had not been positive for HCV. Because rosettes and emperipolesis have been shown to be difficult features to interpret and therefore may lead to underdiagnosis of AIH,[23] we slightly modified the simplified criteria to be less stringent and used histopathologic features similar to those recommended in the AASLD practice guidelines for diagnosis and management of AIH[24] (Tables 3 and 4). It is also important to note that the three main hepatotropic viruses (i.e., hepatitis A virus, hepatitis B virus, and HCV) can all result in serologic and histopathologic features of AIH, which explains why their presence or absence is a critical component of most scoring systems.[24,25]

Stroffolini et al.[28] concluded that the presence of nonorgan-specific antibodies was likely a consequence of liver damage caused by the infection itself and suggested that they do not likely play an active role in the pathogenesis of liver disease. Results of this study support this hypothesis. SMA and ANA elevations may result from immune responses to damaged

hepatocyte contents, and these abnormal serum elevations have been reported to be an epiphenomenon in nonalcoholic steatohepatitis (NASH). Recent reports have shown that elevated markers for AIH are common in NASH (present in ~21% of patients) but are not associated with advanced histopathologic features or increased development of hepatic fibrosis.[31,32] Although several of the study and control patients had steatosis observed in their liver biopsies (Tables 3 and 4), we excluded patients with active steatohepatitis for this reason. Elevated serum IgG levels may occur due to hypergammaglobulinemia of cirrhosis, typically caused by diminished removal of immunoglobulins by the diseased liver. None of the study or control patients had cryoglobulinemia, and only 2 of the study patients had cirrhosis; 1 had elevated IgG, indicating that this is not the likely mechanism in this cohort of patients. This study did show that study patients with serum markers of AIH had significantly lower platelet levels than control patients before DAA treatment (Fig. 1D), which is evidence of an immune-mediated thrombocytopenia that improved after elimination of the virus.

Early studies showed that HCV could induce transient autoimmune responses leading to development of autoantibodies classically associated with AIH,[33,34] and it has been reported that chronic HCV infections that were autoantibody positive were more common in women, exhibited more severe transaminase elevation, and had histologic evidence of liver injury.[10,35] A study by Gilman et al.[36] also showed that autoantibody positivity in HCV infection was more common in women; their presence predicted a lower rate of achieving SVR when using IFN-based regimens, but their presence did not otherwise affect the natural history of chronic HCV or presence of extrahepatic manifestations. In the current study, the majority of patients who were HCV+ that had serum markers of AIH were female patients (12/21, 57.1%), and liver biopsies from these patients showed significantly more inflammatory activity and slightly increased fibrosis compared to controls. However, transaminase levels were not significantly different in the study patient and control groups. Importantly, regardless of the presence of serum markers of AIH in the study patients who were HCV+, they all achieved SVR when using these newer IFN-free regimens.

Recently, Sugiura et al.[16] reported a single patient diagnosed with chronic HCV–AIH overlap syndrome who was successfully treated with DAA therapy. Due to the lack of established criteria for this condition, they initially treated the patient with corticosteroids; when the patient did not respond, DAA therapy was initiated. The patient achieved SVR without any adverse effects, and serum markers of AIH normalized. Similar to the current study, their conclusion was that normalization of serum markers of AIH after HCV eradication indicates that HCV was entirely responsible for the autoimmune response. Another report by Sahebjam et al.[18] reported similar findings.

This study had a few limitations, including the small number of cases, and the inability to determine the mechanisms by which HCV results in elevated serum markers of AIH. The highest transaminase levels observed in this study cohort was 752 and 414 U/L for ALT and AST, respectively, in a single patient (#20). Even she had complete resolution of her elevated transaminases (20 and 15 U/L for ALT and AST, respectively) and serum markers of AIH with DAA treatment (patient 20; Table 1). However, if a patient with any viral hepatitis (i.e., hepatitis A-E) presents with features that suggest fulminant liver disease or a severe complication, such as a life-threatening HCV-induced vasculitis, immune suppressive therapy may be lifesaving. Despite these limitations and rare presentations, the results of the current study suggest that the majority of patients with HCV and elevated serum markers or histologic features of AIH should initially be treated with DAA. A prospective study in a larger number of patients would be useful to confirm these results.

Acknowledgment: We thank Dr. Jacob T. Wooldridge for his technical editorial assistance; Dr. Jeff East, Monica O'Donohoe, Dr. Muhammad Imran Ahmad, and the hepatology team for support with patient referral and follow-up; and Shana White for her skillful technical assistance. Most importantly, this work would have not been possible without the financial support of the Moody Endowment, Galveston, TX, and the National Center for Advancing Translational Sciences Clinical and Translational Science Award KL2 Scholars Program.

REFERENCES

1) Lanini S, Easterbrook PJ, Zumla A, Ippolito G. Hepatitis C: global epidemiology and strategies for control. Clin Microbiol Infect 2016;22:833-838.

2) Lavanchy D. Evolving epidemiology of hepatitis C virus. Clin Microbiol Infect 2011;17:107-115.
3) Sarna A, Panda S. HCV in people who inject drugs: a neglected epidemic. Lancet Infect Dis 2015;15:4-5.
4) Rosenthal E, Cacoub P. Extrahepatic manifestations in chronic hepatitis C virus carriers. Lupus 2015;24:469-482.
5) Ferrari SM, Fallahi P, Mancusi C, Colaci M, Manfredi A, Ferri C, et al. HCV-related autoimmune disorders in HCV chronic infection. Clin Ter 2013;164:e305-e312.
6) Ramos-Casals M, Munoz S, Medina F, Jara LJ, Rosas J, Calvo-Alen J, et al.; HISPAMEC Study Group. Systemic autoimmune diseases in patients with hepatitis C virus infection: characterization of 1020 cases (The HISPAMEC Registry). J Rheumatol 2009;36:1442-1448.
7) Paroli M, Iannucci G, Accapezzato D. Hepatitis C virus infection and autoimmune diseases. Int J Gen Med 2012;5:903-907.
8) Ferri C, Sebastiani M, Giuggioli D, Colaci M, Fallahi P, Piluso A, et al. Hepatitis C virus syndrome: a constellation of organ- and non-organ specific autoimmune disorders, B-cell non-Hodgkin's lymphoma, and cancer. World J Hepatol 2015;7:327-343.
9) Himoto T, Masaki T. Extrahepatic manifestations and autoantibodies in patients with hepatitis C virus infection. Clin Dev Immunol 2012;2012:871401.
10) Cassani F, Cataleta M, Valentini P, Muratori P, Giostra F, Francesconi R, et al. Serum autoantibodies in chronic hepatitis C: comparison with autoimmune hepatitis and impact on the disease profile. Hepatology 1997;26:561-566.
11) Pavic S, Simonovic J, Boricic I, Svirtlih N. Autoantibodies characteristic for autoimmune hepatitis found in chronic hepatitis C. [In Serbian] Srp Arh Celok Lek 2003;131:437-442.
12) Luo JC, Hwang SJ, Li CP, Lu RH, Chan CY, Wu JC, et al. Clinical significance of serum auto-antibodies in Chinese patients with chronic hepatitis C: negative role of serum viral titre and genotype. J Gastroenterol Hepatol 1998;13:475-479.
13) Clifford BD, Donahue D, Smith L, Cable E, Luttig B, Manns M, et al. High prevalence of serological markers of autoimmunity in patients with chronic hepatitis C. Hepatology 1995;21:613-619.
14) Gragnani L, Fabbrizzi A, Triboli E, Urraro T, Boldrini B, Fognani E, et al. Triple antiviral therapy in hepatitis C virus infection with or without mixed cryoglobulinaemia: a prospective, controlled pilot study. Dig Liver Dis 2014;46:833-837.
15) Urraro T, Gragnani L, Piluso A, Fabbrizzi A, Monti M, Fognani E, et al. Combined treatment with antiviral therapy and rituximab in patients with mixed cryoglobulinemia: review of the literature and report of a case using direct antiviral agents-based antihepatitis C virus therapy. Case Reports Immunol 2015;2015:816424.
16) Sugiura A, Wada S, Mori H, Kimura T, Matsuda Y, Tanaka N, et al. Successful treatment for chronic hepatitis C-autoimmune hepatitis overlap syndrome due to daclatasvir and asunaprevir. Case Rep Gastroenterol 2017;11:305-311.
17) Nguyen HH, Khathlan A, Fritzler MJ, Swain MG. A case series evaluating the impact of Hepatitis C eradication using direct acting antivirals on primary biliary cholangitis-associated autoimmunity. BMC Gastroenterol 2018;18:97.
18) Sahebjam F, Hajdu CH, Nortey E, Sigal SH. Direct acting antiviral therapy is curative for chronic hepatitis C/autoimmune hepatitis overlap syndrome. World J Hepatol 2016;8:632-636.
19) Hennes EM, Zeniya M, Czaja AJ, Parés A, Dalekos GN, Krawitt EL, et al. International Autoimmune Hepatitis Group. Simplified criteria for the diagnosis of autoimmune hepatitis. Hepatology 2008;48:169-176.
20) Batts KP, Ludwig J. Chronic hepatitis. An update on terminology and reporting. Am J Surg Pathol 1995;19:1409-1417.
21) Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696-699.
22) Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431-435.
23) Balitzer D, Shafizadeh N, Peters MG, Ferrell LD, Alshak N, Kakar S. Autoimmune hepatitis: review of histologic features included in the simplified criteria proposed by the international autoimmune hepatitis group and proposal for new histologic criteria. Mod Pathol 2017;30:773-783.
24) Manns MP, Czaja AJ, Gorham JD, Krawitt EL, Mieli-Vergani G, Vergani D, et al.; American Association for the Study of Liver Diseases. Diagnosis and management of autoimmune hepatitis. Hepatology 2010;51:2193-2213.
25) Alvarez F, Berg PA, Bianchi FB, Bianchi L, Burroughs AK, Cancado EL, et al. International Autoimmune Hepatitis Group Report: review of criteria for diagnosis of autoimmune hepatitis. J Hepatol 1999;31:929-938.
26) Calvaruso V, Craxi A. Immunological alterations in hepatitis C virus infection. World J Gastroenterol 2013;19:8916-8923.
27) Putra J, Schiano TD, Fiel MI. Histological assessment of the liver explant in transplanted hepatitis C virus patients achieving sustained virological response with direct-acting antiviral agents. Histopathology 2018;72:990-996.
28) Stroffolini T, Colloredo G, Gaeta GB, Sonzogni A, Angeletti S, Marignani M, et al. Does an 'autoimmune' profile affect the clinical profile of chronic hepatitis C? An Italian multicentre survey. J Viral Hepat 2004;11:257-262.
29) Fried MW, Draguesku JO, Shindo M, Simpson LH, Banks SM, Hoofnagle JH, et al. Clinical and serological differentiation of autoimmune and hepatitis C virus-related chronic hepatitis. Dig Dis Sci 1993;38:631-636.
30) Narciso-Schiavon JL, Schiavon Lde L. Autoantibodies in chronic hepatitis C: a clinical perspective. World J Hepatol 2015;7:1074-1085.
31) Ravi S, Shoreibah M, Raff E, Bloomer J, Kakati D, Rasheed K, et al. Autoimmune markers do not impact clinical presentation or natural history of steatohepatitis-related liver disease. Dig Dis Sci 2015;60:3788-3793.
32) Vuppalanchi R, Gould RJ, Wilson LA, Unalp-Arida A, Cummings OW, Chalasani N, et al.; Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN). Clinical significance of serum autoantibodies in patients with NAFLD: results from the nonalcoholic steatohepatitis clinical research network. Hepatol Int 2012;6:379-385.
33) Kawamoto H, Sakaguchi K, Takaki A, Ogawa S, Tsuji T. Autoimmune responses as assessed by hypergammaglobulinemia and the presence of autoantibodies in patients with chronic hepatitis C. Acta Med Okayama 1993;47:305-310.
34) McFarlane BM, Bridger C, Tibbs CJ, Saleh MG, Fuzio A, Verucchi G, et al. Virus-induced autoimmunity in hepatitis C virus infections: a rare event. J Med Virol 1994;42:66-72.
35) Hsieh MY, Dai CY, Lee LP, Huang JF, Tsai WC, Hou NJ, et al. Antinuclear antibody is associated with a more advanced fibrosis and lower RNA levels of hepatitis C virus in patients with chronic hepatitis C. J Clin Pathol 2008;61:333-337.
36) Gilman AJ, Le AK, Zhao C, Hoang J, Yasukawa LA, Weber SC, et al. Autoantibodies in chronic hepatitis C virus infection: impact on clinical outcomes and extrahepatic manifestations. BMJ Open Gastroenterol 2018;5:e000203.