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

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).

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 autoimmunity6-9...
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
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, hepatic transaminase 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
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.
**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

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**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          | nd              |
| 2    | YES                 | 6                 | 0                             | 3                   | 0           | 2               | nd                  | nd                | nd                            | nd                  | nd          | nd              |
| 3    | nd                  | nd                | nd                            | nd                  | nd           | 3               | nd                  | nd                | nd                            | nd                  | nd          | nd              |
| 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          | nd              |
| 6    | YES                 | 5                 | 0                             | 3                   | 0           | 2               | nd                  | nd                | nd                            | nd                  | nd          | nd              |
| 7    | YES                 | 5                 | 10                            | 2                  | 3           | 0               | 2                   | YES               | 1                             | 10                  | 1           | 0               |
| 8    | YES                 | 8                 | 2                             | 4                   | 5           | 2               | YES                | 3                 | 0                            | 4                   | 0           | 0               |
| 9    | nd                  | nd                | nd                            | nd                  | nd           | 1               | nd                  | nd                | nd                            | nd                  | nd          | nd              |
| 10   | nd                  | nd                | nd                            | nd                  | nd           | 1               | nd                  | nd                | nd                            | nd                  | nd          | nd              |
| 11   | YES                 | 6                 | 0                             | 2                   | 0           | 2               | nd                  | nd                | nd                            | nd                  | nd          | nd              |
| 12   | YES                 | 5                 | 15                            | 2                   | 0           | 1               | nd                  | nd                | nd                            | nd                  | nd          | nd              |
| 13   | YES                 | 4                 | 20                            | 2                   | 0           | 3               | nd                  | nd                | nd                            | nd                  | nd          | nd              |
| 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          | nd              |
| 16   | YES                 | 8                 | 0                             | 2                   | 2           | 4               | nd                  | nd                | nd                            | nd                  | nd          | nd              |
| 17   | YES                 | 7                 | 10                            | 5                   | 2           | 5               | nd                  | nd                | nd                            | nd                  | nd          | nd              |
| 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          | nd              |
| 21   | YES                 | 9                 | 0                             | 2                   | 1           | 3               | nd                  | nd                | nd                            | nd                  | nd          | nd              |

*We used the MHAI to grade the amount of hepatitis activity, the Ishak criteria for fibrosis staging (scale of 0 to 6), and IAIHG simplified criteria to evaluate for histopathologic features of AIH. 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.

### Table 4. Control Cohort Pre-DAA and Post-DAA Treatment Biopsy Scores*

| Pt # | Biopsy Pretreatment? | MHAL Score (×/18) | Percent Macrovesicular Steatosis | Fibrosis Stage (×/6) | IAIHG Score | Total AIH Points | Biopsy Posttreatment? | MHAL 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                               | 1                   | 1           | nd             | 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 MHAL to grade the amount of hepatitis activity, the Ishak criteria for fibrosis staging (scale of 0 to 6), and IAIHG simplified criteria to evaluate for histopathologic features of AIH. 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.

°FUTMB patients; †Mount Sinai Hospital patients.

Abbreviations: nd, not done; Pt #, patient number.
(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, 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 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.

**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%) (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). 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 360 U/L). She had a typical history of AIH with features of lymphoplasmacytic portal inflammation, plasma cell-rich interface activity, and scattered lobular inflammation. A high-power magnification (×40) highlights the plasma cell-rich perivenulitis (B, arrow) and interface activity (C, asterisks). 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.

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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.

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