

Autoantibody positivity in chronic hepatitis C pre- and post-direct-acting antiviral therapy: a prospective multicenter south Korean study

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Background: Hepatitis C virus (HCV) infection causes extrahepatic manifestations involving B-cell dysregulation and autoantibody production. This study aimed to elucidate the positivity rates of four autoantibodies [anti-nuclear antibody (ANA), anti-smooth muscle antibody (ASMA), anti-liver kidney microsomal type 1 (anti-LKM1), and anti-mitochondrial antibody (AMA)] in patients with chronic hepatitis C (CHC) before and after direct-acting antiviral (DAA) therapy compared to those in healthy controls.

Methods: This study enrolled prospectively collected plasma samples from 201 CHC patients [median age, 62 years; 49.8% women; 100% sustained virological response (SVR)] from eight hospitals before and after DAA therapy and 127 healthy individuals. Autoantibodies were detected using indirect immunofluorescence.

Results: The positivity rate of ANA was higher in CHC patients than in healthy controls (32.3% *vs.* 21.3%, *P*=0.03) at pretreatment (PreTx) and decreased at SVR (32.3% *vs.* 23.9%, *P*=0.009). Female sex and higher globulin levels were related to ANA positivity in the control and CHC patient groups. Thirty-seven (57%) of 65 patients with ANA-positive HCV at PreTx maintained ANA-positivity at SVR. Among the 136 ANA-negative patients at PreTx, 11 (8%) showed newly positive ANA conversion at SVR. Patients with ANA positivity at SVR (*n*=48) were older and had a higher proportion of advanced liver disease than ANA-negative patients (*n*=153).

Conclusions: ANA positivity was observed in one-third of CHC patients at PreTx, which was significantly higher than that in healthy controls and decreased after SVR. CHC patients with ANA positivity after SVR were older and had more advanced liver disease than those with ANA negativity, suggesting persistent immune dysregulation after cure.

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Introduction

Hepatitis C virus (HCV) infection is a significant global health problem, with approximately 58 million individuals infected worldwide, and an estimated annual incidence of 1.5 million (1). In addition to liver disease, HCV infection causes extrahepatic manifestations, including mixed cryoglobulinemia, non-Hodgkin lymphoma, and autoantibody production (2-4). The frequency of circulating autoantibodies, especially rheumatoid factor or anti-nuclear antibody (ANA), is reportedly high among patients with chronic hepatitis C (CHC), suggesting a relationship between HCV and autoimmune processes.

However, the prevalence of circulating autoantibodies in patients with CHC is highly variable since ANA, anti-smooth muscle antibody (ASMA), and anti-liver kidney microsomal type 1 (anti-LKM1) positivity are observed in 4–54%, 4–78%, and 0–13% of cases, respectively; moreover, only a few studies have compared their prevalence in patients with CHC with that in healthy controls (6–10%)

(5-7). The mechanisms of autoantibody production and its clinical significance in CHC are unknown. Moreover, the alterations in the levels of these autoantibodies after direct-acting antiviral (DAA) therapy and viral eradication are not clear (8,9).

This study primarily aimed to compare the positivity rates of four conventional autoantibodies [ANA, ASMA, anti-LKM1, and anti-mitochondrial antibody (AMA)] in patients with CHC at pretreatment (PreTx) with those in healthy controls. The secondary aim was to investigate the change in their positivity rates before and after DAA therapy, and explore the clinical factors related to ANA positivity in patients with CHC and healthy controls. We present this article in accordance with the STROBE reporting checklist (available at <https://tgh.amegroups.com/article/view/10.21037/tgh-24-134/rc>).

Methods

Study population and method of plasma sampling

A total of 210 patients with chronic HCV (>18 years old) who underwent DAA therapy were prospectively enrolled from 8 university hospitals (Seoul National University Bundang Hospital, Inje University Busan Paik Hospital, Soonchunhyang University Bucheon Hospital, Jeonbuk National University Hospital, Chonnam National University Hwasun Hospital, Chungnam National University Hospital, Inje University Ilsan Paik Hospital, Keimyung University Daegu Dongsan Hospital) between 2020 and 2022, as part of the Korea HCV cohort study (10,11). Patients who met any of the following criteria were excluded: concurrent human immunodeficiency virus (HIV) or hepatitis B virus (HBV) coinfection (n=9), absence of blood samples before and after DAA therapy (n=0) and presence of active hepatocellular carcinoma (HCC) before DAA therapy (n=0) (Figure S1). A sustained virological response (SVR) was achieved after DAA therapy in all patients. Written informed consent was obtained from each patient before enrollment in the cohort study. This study was approved by the Institutional Review Board of

Highlight box

Key findings

- Anti-nuclear antibody (ANA) positivity was observed in one-third of patients with chronic hepatitis C virus (HCV) infection before treatment which is significantly higher than that in healthy controls.
- ANA positivity decreased after cure with antiviral treatment.

What is known and what is new?

- Western cohort studies reported that the ANA decreased after antiviral treatment.
- In this study, Asian HCV patients showed a similar phenomenon and patients with HCV who remained in the ANA-positive state after cure were older and had advanced liver disease compared to those who were ANA-negative.

What is the implication, and what should change now?

- The dysfunction of the immune system may persist in patients with HCV infection even after a cure is achieved with antiviral treatment.

Seoul National University Bundang Hospital [Institutional Review Board (IRB) No. B-0706-046-002] and conducted in accordance with the Declaration of Helsinki (as revised in 2013). All participating hospitals were informed and agreed with the study. The participants' plasma samples were prospectively collected and stored at -70°C till the analysis. The PreTx samples were collected between -4 and 0 weeks before starting DAA, and the SVR12 samples were collected between 12 and 16 weeks after completion of DAA medication. The control group comprised plasma samples from 127 healthy persons who were randomly selected from a biobank at Seoul National University Bundang Hospital under IRB approval (IRB No. X-2302-810-902) after age and sex adjustment to the general population of South Korea in 2021. The healthy controls also provided informed consent before their samples were collected in the biobank. Since the control samples from individuals aged >70 years were limited, we could not match their age to that of the patients with HCV. All healthy controls were tested negative for HCV, HBV, and HIV infection, without any active cancer, autoimmune, nor major organ diseases and had normal laboratory results on health examination.

Measurement of autoantibodies

ANA, ASMA, anti-LKM1, and AMA were detected using indirect immunofluorescence with cutoff levels of 1:80, 1:10, 1:10, and 1:10, respectively, according to the manufacturer's protocols, at the Seoul Central Laboratory in South Korea. ANA [detected with the Hep-20-10 EUROPattern (EUROIMMUN, Lübeck, Germany)], ASMA [detected with the Hep-2/Mouse Kidney/Stomach COMVI II (Immco Diagnostics, Inc., New York, NY, USA)], AMA [detected with the Hep-2/Mouse Kidney/COMVI I (Immco Diagnostics, Inc.)], and anti-LKM1 [detected using the Mouse Liver/Kidney/Stomach Kit (Immco Diagnostics, Inc.)] were assayed.

Collection of clinical data

Clinical data, including the patients' age, sex, body mass index, alcohol and smoking status, severity of liver disease categorized into chronic hepatitis, liver cirrhosis, and HCC; DAA regimens; comorbidities including autoimmune diseases (thyroid disease, diabetes, rheumatoid arthritis, systemic lupus erythematosus, mixed cryoglobulinemia, lymphoma, kidney disease, etc.); and laboratory results were collected from the medical records before and after DAA

treatment at the time of SVR evaluation. The laboratory data included the status of 4 autoantibodies (ANA, ASMA, anti-LKM1, and AMA), HCV RNA concentration, HCV genotype, white blood cell count, hemoglobin, platelet count, aspartate transaminase (AST), alanine transaminase (ALT), bilirubin, protein, albumin, calculated globulin derived from difference between the protein and albumin levels, alpha-fetoprotein, prothrombin time (international normalized ratio), creatinine, fasting blood glucose, cholesterol at PreTx and at SVR12 after DAA therapy. Using the collected data, indirect fibrosis markers, including the fibrosis-4 index, aminotransferase-to-platelet ratio index, Child-Pugh score, and Model for End-Stage Liver Disease score, were calculated at PreTx and at the time of SVR12. The results of all abdominal radiology and vibration-controlled transient elastography (Fibroscan[®]; Echosens, Paris, France), if available, were collected.

These data were entered into an established electronic case report form on the authorized website of the Korean Centers for Disease Control Korea HCV cohort study (<http://is.cdc.go.kr/>) by the research coordinators. All input data were quality-controlled by independent statistical researchers (D.B. and M.K.) at least four times per year.

Statistical analysis

Continuous variables were analyzed using Student's *t*-test for normally distributed data and the Mann-Whitney *U* test for non-normally distributed data. Categorical variables were analyzed using the Chi-squared test or Fisher's exact test. The McNemar test was conducted for paired data to compare the prevalence of autoantibody positivity before and after DAA treatment. To identify factors related to autoantibody positivity, multivariate logistic analysis was conducted, with adjustment for age, sex, and other clinically relevant and/or statistically significant factors identified by the univariate analysis. Statistical significance was established at $P < 0.05$. Data analyses were conducted using SPSS version 22 (IBM Corp., Armonk, NY, USA).

Results

Clinical characteristics of the study population

The clinical characteristics of the 201 patients with HCV at PreTx and SVR (median age, 62 years; women, 49.8%) and 127 healthy controls (median age, 55 years; women, 49.6%) are summarized in *Table 1*.

Table 1 Clinical characteristics of HCV patients at pre- and post-DAA treatment and healthy controls

Variables	HCV at PreTx (n=201)	Healthy (n=127)	P value [†]	HCV at post-DAA	P value [†]
Age (years)	62 [52–70]	55 [42–64]	<0.001	NA	NA
Age group (years)			<0.001		NA
20–29	3 (1.5)	15 (11.8)		NA	
30–39	12 (6.0)	15 (11.8)		NA	
40–49	20 (10.0)	17 (13.4)		NA	
50–59	56 (27.9)	30 (23.6)		NA	
60–69	55 (27.4)	27 (21.3)		NA	
70+	55 (27.4)	23 (18.1)		NA	
Female	100 (49.8)	63 (49.6)	>0.99	NA	NA
BMI (kg/m ²)	23.8±4.62	NA	NA	NA	NA
History of alcohol use	47 (23.4)	NA	NA	NA	NA
History of tobacco use	105 (52.2)	NA	NA	NA	NA
Liver disease status			NA		NA
Hepatitis	170 (84.6)	NA		NA	
Cirrhosis	15 (7.5)	NA		NA	
Inactive HCC	16 (8.0)	NA		NA	
Child-Pugh class			NA		NA
A	192 (95.5)	NA		NA	
B	9 (4.5)	NA		NA	
C	0 (0.0)	NA		NA	
Log HCV RNA (IU/mL)	6.02 [5.29–6.54]	NA	NA	NA	NA
HCV genotype			NA		NA
1	93 (46.3)	NA		NA	
2	105 (52.2)	NA		NA	
Others	3 (1.5)	NA		NA	
DAA regimen			NA		NA
Glecaprevir/pibrentasvir	173 (86.1)	NA		NA	
Elbasvir/grazoprevir	11 (5.5)	NA		NA	
Ledipasvir/sofosbuvir	13 (6.5)	NA		NA	
Ledipasvir/sofosbuvir + ribavirin	3 (1.5)	NA		NA	
Sofosbuvir + ribavirin	1 (0.5)	NA		NA	
Type 2 diabetes	54 (26.9)	0 (0.0)	<0.001	NA	NA
Hypertension	98 (48.8)	28 (22.0)	<0.001	NA	NA
Thyroid disease	8 (4.0)	0 (0.0)	0.03	NA	NA

Table 1 (continued)

Table 1 (continued)

Variables	HCV at PreTx (n=201)	Healthy (n=127)	P value [†]	HCV at post-DAA	P value [‡]
Lab findings					
Hemoglobin (g/dL)	13.79±1.67	14.13±1.36	0.04	13.78±1.7	0.52
WBC (×10 ³ /μL)	5,847±1,798	5,442.13±1,492.34	0.04	6,338.51±2,119.59	<0.001
Platelet (×10 ³ /μL)	188.00±62.29	258.40±49.01	<0.001	198.02±63.39	<0.001
AST (IU/L)	61.36±45.07	30.78±15.70	<0.001	26.08±16.55	<0.001
ALT (IU/L)	62.35±62.13	30.04±22.56	<0.001	20.01±11.44	<0.001
Bilirubin (mg/dL)	0.80±0.48	0.86±0.32	0.20	0.80±0.60	0.70
Protein (g/dL)	7.42±0.60	7.23±0.34	<0.001	7.29±0.46	<0.001
Albumin (g/dL)	4.14±0.45	4.50±0.24	<0.001	4.33±0.46	<0.001
Globulin (g/dL)	3.28±0.50	2.74±0.32	<0.001	2.96±0.44	<0.001
Prothrombin time (INR)	1.03±0.94	NA	NA	1.04±0.10	0.51
Creatinine (mg/dL)	0.87±0.49	0.74±0.16	<0.001	0.96±0.92	<0.001
Cholesterol (mg/dL)	169.04±38.95	188.43±35.49	<0.001	175.72±41.63	<0.001
Glucose (mg/dL)	116.13±39.05	93.07±13.52	<0.001	111.37±32.63	0.02
AFP (ng/mL)	7.99±24.97	NA	NA	5.73±29.00	0.42
FIB-4	3.44±3.08	1.25±0.05	<0.001	2.19±2.18	<0.001
APRI	0.88±0.81	0.31±0.18	<0.001	0.42±0.72	<0.001
MELD score	3.28±4.22	0.24±0.29	<0.001	3.34±3.87	0.12
TE (kPa) (n=102)	7.50 [5.00–12.15]	NA	NA	NA	NA

Continuous variables are expressed as mean ± SD or median [IQR], and the categorical variable are indicated as n (%). [†], the P value between HCV patients at PreTx and healthy controls; [‡], the P value between HCV at PreTx and post-DAA. AFP, alpha fetoprotein; ALT, alanine aminotransferase; APRI, AST to platelet ratio index; AST, aspartate aminotransferase; BMI, body mass index; DAA, direct-acting antiviral; FIB-4, fibrosis-4 index; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalized ratio; IQR, interquartile range; MELD, Model for End-Stage Liver Disease; NA, non-applicable; PreTx, pretreatment; SD, standard deviation; TE, transient elastography; WBC, white blood cell count.

The proportions of liver cirrhosis and inactive HCC among the patients with HCV infection were 7.5% and 8.0%, respectively. HCV genotype 1 (46.3%) and genotype 2 (52.2%) were present in nearly all patients with CHC (98.5%). Common comorbidities, such as hypertension and diabetes, were present in 48.8% and 26.9% of patients, respectively. The following autoimmune comorbidities were found at PreTx: thyroid disease (4%), rheumatoid arthritis (1.5%), systemic lupus erythematosus (0%), mixed cryoglobulinemia (0%), lymphoma (0.5%), ankylosing spondylitis (0.5%), psoriasis (0.5%), and kidney diseases (6.5%). The most frequently prescribed DAA regimen was glecaprevir/pibrentasvir in 86.1% of cases, because sofosbuvir/velpatasvir and sofosbuvir/velpatasvir/

voxilaprevir were not approved in South Korea during the study period. Vibration-controlled transient elastography was performed in 102 patients (50.7%) at PreTx, which yielded a median liver stiffness value of 7.50 kPa. After SVR, most laboratory results related to liver function improved, including a significant reduction in plasma globulin levels and an increase in the total cholesterol levels (Table 1).

The median age (55 years) was lower in the healthy control group than that in the HCV group because of the non-availability of samples from normal older individuals, since the controls were age- and sex-matched to the general population of South Korea in 2021. The laboratory results of the control group were within the normal range, without a history of cancer, major cardiovascular or pulmonary,

Table 2 Positive rates of autoantibodies in HCV patients at pre-treatment and healthy controls

Autoantibody	HCV at PreTx (n=201)	Healthy (n=127)	P value
ANA	65 (32.3)	27 (21.3)	0.03
ASMA	4 (2.0)	3 (2.4)	>0.99
LKM1	0 (0.0)	0 (0.0)	–
AMA	0 (0.0)	1 (0.79)	0.39
Any autoantibody positive	66 (32.8)	29 (22.8)	0.052

Data are presented as n (%). AMA, anti-mitochondrial antibody; ANA, anti-nuclear antibody; ASMA, anti-smooth muscle antibody; HCV, hepatitis C virus; LKM1, liver kidney microsomal type 1; PreTx, pretreatment.

autoimmune, or liver disease (*Table 1*).

Positivity rates of four autoantibodies in the HCV and control groups

The comparison of the positivity rates of the four autoantibodies between the CHC and healthy control groups is summarized in *Table 2*. In patients with CHC, the ANA, ASMA, LKM1, and AMA positivity rates were 32.3%, 2.0%, 0%, and 0%, respectively; thus, the proportion of autoantibody-positive patients was 32.8%. In healthy controls, the ANA, ASMA, LKM1, and AMA-positivity rates were 21.3%, 2.4%, 0%, and 0.8%, respectively; thus, the proportion of autoantibody-positive patients was 22.8%. One healthy control with AMA-positivity showed alkaline phosphatase levels within the normal range. Therefore, the ANA-positivity rate in patients with CHC was significantly higher than that in the healthy controls ($P=0.03$).

Factors related to ANA-positivity in healthy controls and patients with HCV at PreTx

Among the healthy controls, the proportion of female patients was significantly higher in the ANA-positive subgroup than in the ANA-negative subgroup (66.7% *vs.* 45.0%, $P=0.046$). No other factors were significantly associated with ANA positivity (*Table S1*).

In patients with CHC, the ANA-positive subgroup showed a significantly higher serum globulin level (3.43 *vs.* 3.21 g/dL, $P=0.007$) than that in the ANA-negative subgroup, while the median age (65 *vs.* 60 years, $P=0.19$) and the proportion of women (44.6% *vs.* 52.2%, $P=0.31$) did not differ between these two subgroups. The plasma HCV RNA levels were lower in the ANA-positive subgroup than in the ANA-negative subgroup (log HCV RNA

titer 5.70 *vs.* 6.08 IU/mL, $P=0.06$; *Table S2*). Univariate and multivariate logistic analyses revealed that only high globulin level was an independent factor associated with ANA positivity among patients with HCV (odds ratio: 2.64; 95% confidence interval: 1.40–4.98; $P=0.003$; *Table 3*).

Change in autoantibody positivity after DAA therapy with SVR

The positivity rates for the four autoantibodies before and after DAA therapy (median sampling interval 5.1 months) are summarized in *Table 4*. The PreTx ANA positivity (32.3%) decreased significantly at SVR (23.9%, $P=0.009$), which was similar to that in healthy controls (21.3%). Among the four patients with ASMA positivity at PreTx, one patient achieved ASMA negativity at SVR. One anti-LKM1-negative patient converted to newly positive anti-LKM1 case at SVR. Any autoantibody prevalence at PreTx (32.8%) decreased significantly at SVR (25.4%) ($P=0.03$).

As shown in *Figure 1*, 57% (37/65) of patients with HCV who were ANA-positive at PreTx maintained ANA positivity at SVR12. Compared to the ANA-negative conversion group, the group that sustained ANA positivity was older (median 67 *vs.* 59 years, $P=0.01$) and had a higher proportion of liver cirrhosis or HCC (18.9% and 8.1% *vs.* 0% and 17.9%, $P=0.03$; *Table S3*). Meanwhile, 8% (11/136) of ANA-negative patients with HCV at PreTx converted newly positive ANA cases at SVR12, while 92% remained in the ANA-negative state (*Figure 1*). The newly positive ANA group was older (median age 70 *vs.* 58 years, $P=0.01$), with a higher proportion of patients with liver cirrhosis or HCC (27.3% and 18.2% *vs.* 16.8% and 4.8%, $P=0.08$) and higher globulin levels (3.45 *vs.* 3.19 g/dL, $P=0.08$) than the sustained-ANA-negativity group (*Table S4*).

The PreTx ANA titers were significantly lower after

Table 3 Univariate and multivariate analysis for factors related to ANA positivity in HCV patients

Variables	Univariate analysis		Multivariate analysis [†]	
	OR (95% CI)	P value	OR (95% CI)	P value
Age <40 years (vs. ≥40 years)	0.74 (0.23–2.44)	0.63	0.94 (0.28–3.16)	0.93
Sex female (vs. male)	1.36 (0.75–2.46)	0.31	1.17 (0.63–2.17)	0.62
Liver disease HCC or cirrhosis (vs. hepatitis)	1.03 (0.51–2.07)	0.94	–	–
Child-Pugh class B (vs. A)	1.03 (0.51–2.07)	0.94	–	–
Log HCV RNA median ≥6 (vs. <6)	0.70 (0.38–1.26)	0.23	0.78 (0.42–1.44)	0.42
HCV genotype 2 (vs. genotype 1)	0.68 (0.37–1.25)	0.22	–	–
Globulin ≥3.5 g/dL (vs. <3.5 g/dL)	2.80 (1.50–5.20)	0.001	2.64 (1.40–4.98)	0.003
FIB-4 ≥3.25 (vs. <3.25)	1.02 (0.55–1.89)	0.96	–	–
TE ≥11.5 kPa (vs. <11.5 kPa)	1.75 (0.73–4.17)	0.21	–	–

[†], controlled for: age, sex, log HCV RNA, and globulin. ANA, antinuclear antibody; CI, confidence interval; FIB-4, fibrosis-4 index; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; OR, odds ratio; TE, transient elastography.

Table 4 Changes of autoantibody positivity in HCV patients receiving DAA therapy

Autoantibody	Pre-DAA (n=201)	Post-DAA (n=201)	P value
ANA	65 (32.3)	48 (23.9)	0.009
ASMA	4 (2.0)	3 (1.5)	>0.99
LKM1	0 (0.0)	1 (0.5)	>0.99
AMA	0 (0.0)	0 (0.0)	–
Any autoantibody positive	66 (32.8)	51 (25.4)	0.03

Data are presented as n (%). AMA, anti-mitochondrial antibody; ANA, anti-nuclear antibody; ASMA, anti-smooth muscle antibody; DAA, direct-acting antiviral; HCV, hepatitis C virus; LKM1, liver kidney microsomal type 1.

SVR, as shown in [Figure S2](#) ($P=0.006$). After excluding HCV patients with autoimmune disease ($n=14$), the PreTx ANA positivity (31.0%) decreased at SVR (24.1%, $P=0.12$; [Figure S3A](#)). In HCV patients with autoimmune disease, the PreTx ANA positivity (50.0%) decreased at SVR (17.6%, $P=0.008$; [Figure S3B](#)).

ANA pattern in patients with HCV at PreTx and SVR

The ANA patterns at PreTx and SVR in ANA-positive patients with HCV are indicated in [Figure 2](#). The ANA patterns tended to change before and after treatment ($P=0.09$). The proportions of the homogenous and speckled patterns were similar (12.9% *vs.* 12.4%) among the ANA-positive patients at PreTx. The patterns were followed in the order of nucleolar (2%), nuclear dot (1.5%), midbody (1.0%), and spindle fiber (0.5%). However, the proportion

of the homogenous pattern was stable (12.9%), while that of the speckled pattern decreased (8%) at SVR. In addition, the proportion of cytoplasmic antibody positivity decreased significantly from 18.4% at PreTx to 10.9% at SVR ($P=0.006$) ([Figure S4](#) and [Table S5](#)). In terms of cytoplasmic antibody patterns, 17.4% were speckled pattern and 1.0% were rod and ring pattern, and there was no fibrillar pattern at PreTx ([Figure S5](#)).

Among the patients with ANA-positive CHC at PreTx, those who maintained ANA positivity at SVR showed a higher frequency of homogenous and cytoplasmic patterns than those who lost ANA positivity at SVR12 ([Table S6](#)).

Discussion

In this study, 32% of patients with CHC showed ANA positivity at PreTx, which was significantly higher than

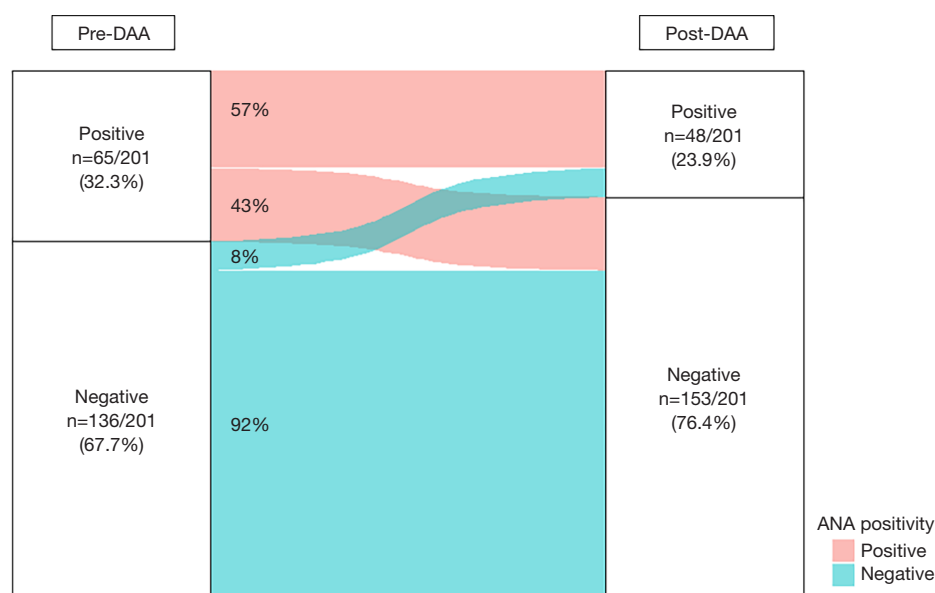


Figure 1 Changes in ANA positivity before and after DAA treatment. The alluvial plot shows changes in ANA positivity after DAA therapy with SVR. Although ANA positivity at PreTx (32.3%) decreased significantly at SVR (23.9%), 57% (37/65) of patients with ANA-positive HCV at PreTx maintained ANA positivity at SVR12, whereas 43% underwent conversion to ANA-negativity at SVR12. Meanwhile, 8% (11/136) of patients with ANA-negative HCV at PreTx converted to ANA-positivity at SVR12, whereas 92% remained in the ANA-negative state. ANA, anti-nuclear antibody; DAA, direct-acting antiviral; PreTx, pretreatment; SVR, sustained virological response.

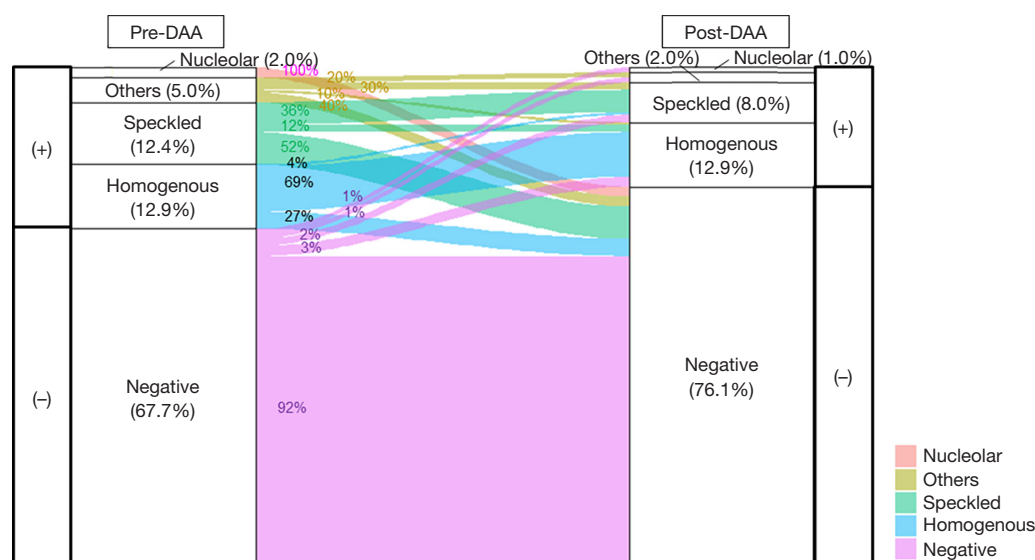


Figure 2 Changes in the ANA pattern in patients with HCV before and after DAA treatment. This alluvial plot shows the changes in the ANA pattern in patients with HCV before and after DAA therapy. The ANA patterns before treatment tended to change after treatment ($P=0.09$). Among patients with HCV at PreTx, the prevalence of homogenous and speckled patterns was comparable at 12.4% and 12.9%, respectively. After treatment, 69% retained the homogenous pattern, 4% transitioned to the speckled pattern, and 27% were converted to the ANA-negative state. After treatment, 36% maintained the speckled pattern, 52% shifted to the ANA-negative state, and 12% converted to a homogenous pattern. Notably, among patients initially negative for ANA at PreTx who transitioned to an ANA-positive status, the homogenous pattern was most frequently observed. +, positive; -, negative. ANA, anti-nuclear antibody; DAA, direct-acting antiviral; HCV, hepatitis C virus; PreTx, pretreatment.

that in the healthy control group (21.3%), and decreased to a rate similar to that in healthy controls at SVR. Female sex was related to ANA positivity in healthy controls, while elevated globulin levels were associated with ANA positivity in patients with CHC, irrespective of age or sex. Thirty-seven (57%) of 65 patients with HCV with ANA-positivity at PreTx maintained this status at SVR, whereas 11 (8%) of the 136 ANA-negative patients at PreTx exhibited newly positive ANA conversion at SVR. Patients with ANA positivity at SVR were characterized by older age and a higher prevalence of cirrhosis or inactive HCC compared to the ANA-negative patients; at PreTx, the homogenous and speckled ANA patterns showed a similar level of predominance, whereas the proportion of the homogenous pattern increased and that of the speckled pattern decreased at SVR. In addition, the proportion of cytoplasmic ANA positivity at PreTx significantly decreased at SVR.

HCV induces damage to the liver, and also causes a range of extrahepatic diseases, of which mixed cryoglobulinemia and non-Hodgkin's lymphoma are the most recognized diseases involving B-lymphocyte dysregulation. Cluster of differentiation 81 (CD81), also known as tetraspanin, was the first receptor identified for HCV entry. CD81 is expressed on both liver cells and B lymphocytes. HCV infection may stimulate B cells directly through the binding of HCV E2 envelope glycoprotein to cell surface CD81, leading to the polyclonal expansion of B cells, recruiting immune complexes, and culminating in a wide spectrum of autoimmune and lymphoproliferative disorders. In addition, the molecular mimicry of viral antigens may be a mechanism underlying the development of autoimmune phenomena (12).

ANA, a key biomarker of autoimmune liver disease and systemic rheumatic disease, is a diverse group of autoantibodies that recognize macromolecular components in the cell nucleus (DNA, RNA, proteins, and their complexes). Although ANA-targeting antigens are usually present in the nucleus, they can translocate to the cytoplasm or extracellular space, particularly during cell death (mostly apoptosis). In the extracellular space, nuclear antigens form immune complexes with ANAs that can stimulate an immune response. The immunofluorescence assay is regarded as the gold standard for ANA testing; however, the results vary widely according to the threshold for positivity (initial serum/plasma dilution of 1:40 or 1:80), cell types or reagents used for testing, observer's interpretation, and factors related to the study sample, including the genetic background of the participants. Therefore, the

interpretation of ANA test results requires appropriate controls (13).

However, 4–30% of healthy populations exhibit ANA positivity, depending on the study (3,14–17). ANA prevalence in the U.S. population aged above 12 years during 1999–2004 was 13.8% at a cutoff of 1:80 using Hep-2 ANA slides, and the prevalence was higher in women than in men. ANA positivity increased with age but not in a linear pattern, such that that peak age in women was 40–49 years, with a declining trend in older age groups (18). In our study, ANA positivity in healthy adult controls aged above 20 years was 21.3% and higher in women, in line with the results of the U.S. study. Although our sample size was small, to the best of our knowledge, this is the first study to report on ANA positivity in a healthy Korean population.

ANA-positivity rates in patients with CHC reportedly range from 20 to 50%, depending on the study. These differences may be related to age, prevalence of liver cirrhosis, and ethnicity. A Swiss HCV cohort study with 235 patients with CHC (median age, 56 years; men, 62%; cirrhosis, 27%) reported that ANA positivity at PreTx (cutoff, 1:80) was 41%, although it lacked data on healthy controls (19). An Australian study that included 127 diverse patients with CHC (mean age, 41 years; men, 61%; study period, 2005–2012) and 198 retrospectively recruited control samples (mean age, 50 years; men, 52%; study period, 1994) reported that the ANA/extractable nuclear antigen antibody positivity rates were higher in patients with CHC (50%) than in the controls (14%) using Hep2000 cells and a cutoff of >7 IU (15). A Taiwanese study that retrospectively enrolled 258 patients with CHC from 2020 to 2021 (mean age: 64 years, men: 44%, cirrhosis: 17%) reported that ANA positivity at PreTx was 28.7% (Hep-20-10 cells, cutoff 1:320) (20). This study showed that female sex ($P=0.023$), older age ($P=0.001$), lower HCV RNA titer ($P=0.06$), and more advanced fibrosis ($P=0.098$) were associated with ANA positivity. An American retrospective study that included 1,556 patients with CHC who underwent autoantibody testing during 1997–2016 reported that 388 patients (21.8%) showed ANA positivity, and that the proportion of women was higher and the rate of SVR was lower among the autoantibody-positive patients, although ANA positivity had no impact on the clinical outcomes (16). Studies that predominantly included Caucasian patients (15,16) tended to have a higher prevalence of ANA positivity than the current (32.3%) or previous Taiwan study (28.7%), which may be related to genetic differences. Moreover, higher serum globulin was

an independent factor related to ANA positivity in patients with CHC in this study, which has not been commonly reported in other studies. These findings suggest that chronic HCV infection may stimulate B cells, resulting in B-cell proliferation and increased globulin production. Interestingly, our results showed that patients with ANA-positive CHC had lower levels of HCV RNA in the blood, concurrent with the above-mentioned Taiwanese study, although the difference did not attain statistical significance. This may be related to the antiviral role of B lymphocytes, although a widespread and robust T-cell response is a well-known determinant of HCV clearance (21).

Historically, before HCV was identified in 1989, many HCV patients were misdiagnosed as autoimmune hepatitis (AIH) (22), because of clinical and pathological similarity (23). For example, interface hepatitis, one of the representative pathologic findings in AIH, is present in HCV as well (24). Recently, there have been interesting findings on the immune-modulatory effect of DAA treatment and the intricate interplay between HCV and AIH. Some case reports suggested that DAA treatment in HCV patients can bring improvement of concurrent AIH (25,26). Moreover, a well-designed prospective study with 96 weeks follow-up after DAA treatment showed that 79% (58/70) of cryoglobulinemia was regressed (27). Conversely, there is a hypothesis that the potential risks autoimmune disease following rapid viral clearance associated with the restoration of host immunity (22). Autoimmune markers usually disappeared after HCV clearance, but autoimmune conditions persisted in a subset of patients, and even new ones may appear after SVR. Therefore, attention may be needed in DAA-treated HCV patients even after achieving SVR.

In this study, PreTx ANA positivity decreased after SVR to a level similar to that in the healthy controls. Our paper is in line with several other studies. A Swiss cohort study reported that ANA positivity at PreTx (41%) decreased to 35.3% at SVR (19). Two retrospective Italian studies reported that PreTx ANA positivity (85%) decreased to 29% at SVR (17) and PreTx ANA positivity (40%) disappeared in 50% at SVR24 (28). Recent Italian prospective cohort study with 96 weeks of follow-up, 50% of HCV CHC showed autoimmune manifestation including cryoglobulinemia and autoantibody positivity (27). Although only a few studies have reported on the change in ANA positivity after DAA therapy, their findings support our result of decreased ANA positivity after SVR.

Meanwhile, the current and previous studies consistently

reported that more than half of patients with ANA-positive CHC at PreTx remained in an ANA-positive state after SVR and about one in 10 ANA-negative patients at PreTx showed conversion to newly positive ANA state after SVR (19,27). Interestingly, our results showed that patients with ANA-positive CHC at SVR were older and had more advanced liver disease than the ANA-negative patients. Recent experimental studies have reported that despite virus eradication with DAA therapy, monoclonal B-cell expansion and intraclonal diversification persist in patients with HCV with lymphoproliferative disorders (29), and HCV-specific memory B cells live for long after HCV clearance (30). Moreover, B cells in patients who are cured of HCV exhibit persistent perturbations, showing that highly self-reactive immune signatures may contribute to a suboptimal vaccine response with the hepatitis B vaccine (31). Therefore, our results suggest that possibility of incomplete recovery of the B-cell response with possible self-reactive features even after viral eradication, especially in older patients with advanced liver disease.

Although the cytoplasmic antibody patterns changed after SVR, as shown in this study, their significance remains unclear. Among 37 cytoplasmic antibody-positive patients, a rod and ring pattern was found in two patients, in them one had interferon and ribavirin treatment experience. Though anti-rods and rings antibody induction was observed and suspected to viral relapse (32). Brazilian prospective cohort study evaluated the occurrence of anti-rod and ring antibody in DAA-treated HCV patients (33). The anti-rod and ring antibody was observed in 21% (11/52) and only 3.4% (1/29) of the interferon-naïve patients developed anti-rod and ring after DAA treatment. In our study, 1.6% of DAA-treated HCV patients developed anti-rod and ring antibody.

The current study has some limitations. First, the sample size of our study was not large, although this is the largest prospective study of Asian CHC. Although a healthy control group was appropriately included, age could not be matched because of the unavailability of plasma samples. Second, our study was conducted at a tertiary-care center, which could have potentially resulted in referral bias. However, to date, DAA therapy has mostly been performed at tertiary centers because of the high cost and strict reimbursement policy for DAA therapy in Korea. Third, number of healthy controls is relatively small in this study and ANA positivity in general Korean population need to validate. Fourth, the long-term outcomes after SVR according to ANA positivity were not observed. Additional

studies with a longer follow-up period of 48 to 96 weeks or longer after SVR are needed. Fifth, preplanned screening of asymptomatic, uncommon autoimmune condition may be helpful using extractable nuclear antigen antibodies test, it may further elucidate the role of ANA in patients with HCV (34). In addition, cryoglobulin was not detected in this study. Previous studies showed that cryoglobulinemia was the most common autoimmune disease associated with HCV (35,36). Therefore, further study including cryoglobulin measurement is warranted.

Conclusions

ANA positivity was observed in one-third of patients with CHC at PreTx, which was significantly higher than that in healthy controls and decreased after SVR. CHC patients with ANA positivity after SVR were older and had more advanced liver disease compared to those with ANA negativity. This suggests that B-cell dysfunction in patients with HCV may persist after SVR with DAA. Thus, investigation of the long-term outcomes, including the occurrence of HCC and the development of other autoimmune diseases according to ANA positivity, is warranted.

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Footnote

Reporting Checklist: The authors have completed the STROBE reporting checklist. Available at <https://tgh.amegroups.com/article/view/10.21037/tgh-24-134/rc>

Data Sharing Statement: Available at <https://tgh.amegroups.com/article/view/10.21037/tgh-24-134/dss>

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. This study was approved by the Institutional Review Board of Seoul National University Bundang Hospital [Institutional Review Board (IRB) No. B-0706-046-002] and conducted in accordance with the Declaration of Helsinki (as revised in 2013). All participating hospitals were informed and agreed with the study. Written informed consent was obtained from each patient before enrollment in the cohort study. The control group comprised plasma samples from 127 healthy persons who were randomly selected from a biobank at Seoul National University Bundang Hospital under IRB approval (IRB No. X-2302-810-902). The healthy controls also provided informed consent before their samples were collected in the biobank.

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