Received: 28 June 2018 | First decision: 26 July 2018 | Accepted: 5 September 2018

DOI: 10.1111/apt.14999

WILEY AP&T Alimentary Pharmacology & Therapeutics

Clinical relevance of serum non-organ-specific antibodies in patients with HCV infection receiving direct-acting antiviral therapy

Endrit Shahini[1] | Andrea Iannone[1] | Domenico Romagno[1] | Angelo Armandi[1] | Sonia Carparelli[1] | Mariabeatrice Principi[1] | Maria Teresa Viggiani[1] | Enzo Ierardi[1] | Alfredo Di Leo[1] | Michele Barone[1]

Gastroenterology Unit, Department of Emergency and Organ Transplantation (D.E.T.O.), University of Bari, Bari, Italy

Correspondence
Dr. Alfredo Di Leo, Gastroenterology Unit, Department of Emergency and Organ Transplantation (D.E.T.O.), University of Bari, Bari, Italy.
Email: alfredo.dileo@uniba.it

Summary

Background: Hepatitis C virus (HCV) infection is associated with production of different serum non-organ-specific antibodies (NOSA) and risk for developing autoimmune disorders. The clinical significance of these phenomena is not fully understood.

Aim: To assess non-organ-specific antibodies before and 24 weeks after the end of therapy with direct-acting antivirals in patients with HCV-related infection, to better clarify the clinical relevance of these antibodies in terms of treatment response and prognostic value.

Methods: Patients enrolled (191) were considered NOSA-positive for titres ≥1:40 on at least two determinations before treatment.

Results: At baseline, 46 patients were positive and 145 were negative for autoantibodies. The prevalence of autoimmune thyroiditis was significantly higher in the NOSA-positive than the NOSA-negative group (P = 0.02). HCV-RNA 24 weeks after the end of antiviral therapy was 100% negative in patients with antibody positivity and 98.6% in antibody-negative patients (P = 1.0). In the former group, autoantibodies disappeared in 30 of 46 patients (65.2%). On multivariate analysis, NOSA-negativity was significantly reduced in patients with hepatic hilar lymphadenopathy (OR = 0.17; 95% CI 0.02-0.94, P = 0.04). None of the adverse events occurring during antiviral therapy was related to autoimmune disorders.

Conclusions: HCV clearance frequently reduces NOSA positivity suggesting that they represent an epiphenomenon of the viral infection. However, in patients who did not become negative, long-term monitoring would establish whether they have an underlying process that may progress into a clear autoimmune or rheumatologic disease. (Trial registration number: NCT03566966).

The Handling Editor for this article was Professor Geoffrey Dusheiko, and it was accepted for publication after full peer-review.

1 | INTRODUCTION

Chronic hepatitis C virus (HCV) infection has been considered a major cause of mortality, morbidity, and resource utilisation in the world.[1] About 40%-70% of HCV patients develop at least an autoimmune extrahepatic disorder,[1–3] presumably due to the interaction between HCV E2 envelope protein and B lymphocyte CD-81 receptor.[2–5] In addition, the same interaction is responsible for the production of different serum non-organ-specific antibodies (NOSA), such as anti-nuclear (ANA), anti-smooth muscle (ASMA), and anti-liver-kidney microsome (LKM) antibodies.[4,5] Several autoimmune diseases (rheumatoid arthritis, mixed cryoglobulinemia, systemic lupus erythematosus, Sjögren syndrome, and autoimmune thyroiditis) may coexist with HCV infection.[6] Finally, the influence of HCV on immune system is further supported by its involvement in the development of B-cell non-Hodgkin lymphoma.[7]

The prevalence of circulating autoantibodies in HCV patients exhibits a large variation (ASMA 4%-78%, ANA 4%-54%, while LKM-1 ranged between 0% and 13%)[4] and is not associated to specific HCV genotypes.[8,9] However, the presence of circulating autoantibodies is also observed in 6%-10% of healthy subjects.[8,10]

The clinical significance of this phenomenon has not been fully understood,[4] except for the presence of LKM-1 antibody, which is linked to a molecular mimicry between the cytochrome enzyme CYP2D6, primarily expressed in the liver, and HCV proteins in genetically predisposed subjects.[11] Despite the high prevalence of circulating autoantibodies in HCV patients, a liver-specific autoimmune disorder has been found in very few cases,[4,8] especially when a persistent elevation of liver cytolytic and/or cholestatic enzymes occurs even after an effective antiviral therapy.[5]

Guidelines published in 2011 recommended that serum NOSA profile had to be assessed before interferon treatment for HCV infection, since they could reflect the presence of active autoimmune diseases.[12] The scenario has now changed. In fact, direct-acting antivirals (DAAs) of second generation allow anti-HCV treatment also in patients with coexisting extrahepatic autoimmune disorders, being, rarely associated with adverse clinical events.[13,14] An interesting recent study has shown a significant reduction in pathologic B cells, in the peripheral blood of HCV patients with lymphoproliferative disorders after viral clearance.[15] This tendency was also demonstrated for HCV patients without lymphoproliferative disorders despite monoclonal B-cell populations can persist after viral disappearance.[15]

To the best of our knowledge, no data are available about the prevalence and clinical significance of NOSA in HCV patients treated with second-generation DAAs. Therefore, in the present study, we assessed NOSA before treatment and 24 weeks after the end of therapy, in order to better clarify their clinical relevance in terms of treatment response and prognostic value.

2 | MATERIALS AND METHODS

2.1 | Study design

This observational study was performed on Caucasian patients affected by chronic HCV infection who were consecutively admitted as outpatients to the Gastroenterology Unit, Policlinic Hospital, Bari, Italy, from July 2015 to August 2016 to receive a treatment with second-generation DAAs. The study was limited to patients with advanced fibrosis, cirrhosis, or extrahepatic HCV manifestations, since only these populations were suitable to receive the treatment by Italian Health System at that time period. Our research was carried out in compliance with the Declaration of Helsinki, and all procedures received local ethics committee approval (protocol# 61625/CE) (Trial registration number: NCT03566966). All patients gave informed consent to take part in the study.

Inclusion criteria were age >18 years, no history of autoimmune hepatitis and/or cholangitis, and no evidence on ultrasonography of active hepatocellular carcinoma, human immunodeficiency virus, or HBV coinfection. A previous treatment with interferon was not considered an exclusion criterion.

For patient enrolment, it was mandatory to insert a series of clinical and laboratory data on a web platform operated by the Italian Agency of the Drug in order to receive the approval for each treatment, as previously described.[16,17] At baseline, patient history, clinical examination, and routine pretreatment workup were collected. Coadministered drugs were recorded and possible interactions with all DAAs were carefully checked by consulting the Liverpool HEP interaction guidance.[18] All patients underwent the following laboratory investigations: haemogram, coagulogram, serum electrolytes, kidney, thyroid, and liver function tests, HBsAg, HBcAb, HCV-RNA with genotype, ANA, ASMA, LKM, and AMA, anti-mitochondrial antibody, and, when clinically indicated, other types of autoantibodies (anti-ENA, anti-extractable nuclear antigen; LA, lupus anticoagulant; ASCA, anti-saccharomyces cerevisiae antibodies; anti-dsDNA, anti-double-stranded DNA; APCA, anti-parietal cell antibodies). A titre ≥1:40 was considered the cut-off value for autoantibody positivity. In patients who undergone liver biopsy, "Simplified international scoring system" was used to exclude autoimmune hepatitis.[19] At the end of the treatment, patients were reassessed for the same laboratory parameters investigated in the pretreatment phase. In detail, NOSA pattern was evaluated after HCV clearance (HCV-RNA not detectable at 24 weeks after the end of therapy—SVR24, sustained virological response at 24 weeks) in baseline autoantibody-positive subjects.

2.2 | Definition and follow-up of liver disease

Diagnosis of advanced liver fibrosis was based on liver transient elastography and histological evaluation, when available, as previously described.[16,17] Briefly, advanced fibrosis (F3) was indicated by liver stiffness measurement ranging from 10 to 14 kPa and platelets >100 000/μL, in the absence of hepatic decompensation and

ultrasound signs of portal hypertension while patients were considered to have cirrhosis when transient elastography values were >14.0 kPa and platelet counts ≤140 000/μL as well as when transient elastography values were 10-14 kPa and platelet counts ≤100 000/μL. Furthermore, patients were reviewed monthly during the antiviral treatment, and virological response was assessed by quantitative HCV-RNA at the 4th week, at the end of treatment, and 12 and 24 weeks after treatment.

2.3 | Statistical analysis

We assessed the normal distribution of continuous variables using the Shapiro-Wilk test and expressed them as mean and SD or median and interquartile range. We expressed categorical variables as percentages. We examined variables associated with negativity of NOSA following DAA treatment using logistic regression analysis. First, we examined the univariate association between each explanatory variable and the outcome (autoantibody negativity) using the likelihood ratio test. The continuous variable "HCV-RNA at baseline" had a highly skewed distribution and was transformed by taking logarithms. We included in the multivariate model all explanatory variables showing a $P < 0.25$ at univariate analysis. In the multivariate phase of analysis, we formally examined potential collinearity issues by fitting a linear regression model to the data and considering all type II tolerance values <0.1 as indicative of collinearity. We checked the linearity assumption for continuous variables in the multivariate model by plotting regression coefficients obtained from the inclusion of these variables as categorical in the multivariate model. We also assessed any biologically plausible interaction between explanatory variables using the forward method, considering a $P < 0.01$ as indicative of effect modification. For each variable included in the multivariate model, we estimated both unadjusted and adjusted odds ratios (OR), with their 95% confidence intervals (CI 95%) and the level of significance (using the likelihood ratio test).

We examined the fit of the final model, using the Hosmer-Lemeshow goodness-of-fit test for calibration and the *c*-statistic for discrimination. We also created regression diagnostic plots for the final model. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using the Statistical Analysis Software (SAS Institute Inc, Cary, NC).

3 | RESULTS

3.1 | Patients

A total of 197 patients affected by chronic HCV infection who were enrolled to receive a treatment with second-generation DAAs were screened for NOSA. Six patients were excluded from our analysis since two of them were affected by primary biliary cholangitis with AMA positivity, two dropped out treatment after 4 weeks for side effects occurrence, one assumed DAAs for only 4 weeks and then spontaneously stopped therapy, while the last one assumed DAAs for 16 weeks and then left our country. Finally, 191 patients were enrolled and were divided into two groups according to their NOSA status: 46 positive and 145 negative patients (Figure 1).

A comparison of baseline demographic and clinical characteristics of the two groups of patients is reported in Table 1. None of the parameters considered was statistically different in the NOSA-positive and negative patients with the exception of vitamin D serum levels, which were significantly lower in NOSA-positive group as compared to NOSA-negative patients.

3.2 | Virological response, autoantibody prevalence, and pattern before and after therapy

Sustained virological response at 24 weeks after the end of treatment (SVR24) was achieved in 143/145 (98.6%) patients without the presence of NOSA as compared to the 46/46 (100%) NOSA-positive patients ($P = 1.0$ by chi-squared).

In Table 2, the details of NOSA pattern before and after therapy are described. As reported, 39/46 patients (84.8%) were ANA positive, 6/46 patients (13.0%) ASMA positive, and 2/46 (4.3%) anti-LKM-1 positive. Furthermore, 6/46 patients showed additional autoantibody positivity: anti-ENA, LA, or ASCA positivity in 3/46 and anti-ENA, anti-dsDNA, or APCA in 3/46.

In detail, among patients who were positive to ANA, 29/39 (74.3%) had speckled pattern, 7/39 (17.9%) homogeneous pattern, and 3/39 (7.7%) rods and rings pattern, and anti-actin specificity was not detected.

In 30/46 (65.2%) NOSA-positive patients, autoantibodies disappeared at 24 week after the end of antiviral therapy. In particular, a

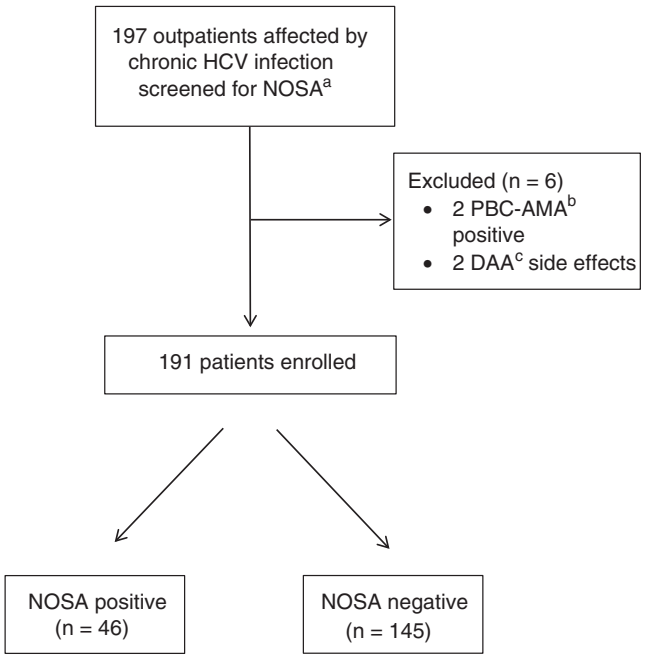

FIGURE 1 Flow diagram describing the process of patients selection. [a]NOSA, Non-organ-specific antibodies. [b]PBC-AMA, Primary biliary cholangitis-anti-mitochondrial antibody. [c]DAA, Direct-acting antiviral

TABLE 1 Baseline demographic and clinical characteristics of non-organ-specific antibodies (NOSA)-positive and -negative patients, before antiviral treatment

Characteristics	NOSA-positive (n = 46)	NOSA-negative (n = 145)	*P* value
Age, mean (SD) (y)	64.7 (10.8)	66.6 (11.7)	0.09
Sex, n (%)			
Male	28 (60.9)	86 (59.3)	0.72
Female	18 (39.1)	59 (40.7)	0.72
BMI, mean (SD), kg/m^2	27.9 (5.1)	28.4 (4.9)	0.47
Cirrhosis, n (%)	33 (71.7)	104 (71.7)	1.0
Fibrosis F3, n (%)	13 (28.3)	25 (17.2)	0.10
Extrahepatic manifestations, n (%)	0	7 (4.8)	0.20
HCV positive in OLT, n (%)	0	9 (6.2)	0.12
Genotype, n (%)			
1a	1 (2.2)	11 (7.6)	0.30
1b	30 (65.2)	93 (64.1)	0.89
2	11 (23.8)	29 (20)	0.52
3	2 (4.4)	5 (3.4)	0.67
4	2 (4.4)	7 (4.8)	1.0
Previous response to treatment, n (%)			
Naïve	25 (54.3)	69 (47.6)	0.42
Non-responder	13 (28.3)	50 (34.5)	0.43
Relapser	7 (15.2)	10 (6.9)	0.08
Stop for adverse events	1 (2.2)	0	0.24
Partial responder	0	5 (3.4)	0.34
Hepatic hilar lymphadenopathy, n (%)	11 (23.9)	21 (14.5)	0.13
Log HCV-RNA baseline, mean (SD), IU/mL	5.9 (1.0)	5.9 (0.9)	0.97
Previous use of ribavirin, n (%)	25 (54.4)	70 (48.3)	0.47
DAA regimen			
Sofosbuvir + Daclatasvir ± RBV	5 (10.9)	9 (6.2)	0.29
Sofosbuvir + Ledipasvir ± RBV	13 (28.2)	54 (37.2)	0.27
Sofosbuvir + Simeprevir ± RBV	12 (26.1)	26 (17.9)	0.23
Sofosbuvir ± RBV	9 (19.6)	26 (17.9)	0.80
Ombitasvir/Paritaprevir/Ritonavir + Dasabuvir ± RBV	7 (15.2)	30 (27.9)	0.41
Child-Pugh class A	33 (71.7)	99 (68.3)	0.52
Child-Pugh class B	0	5 (3.4)	0.34
MELD score, median (SD), points	7.6 (1.9)	7.3 (1.9)	0.12
Gamma globulin , mean (g/dL)	2.0 (2.8)	1.5 (0.5)	0.26
Vitamin D therapy, n (%)	10 (21.7)	23 (15.9)	0.36
Baseline vitamin D, mean (SD) (IU/mL)	19.6 (11.3)	29.5 (19.2)	0.02[a]
SVR24, n (%)	46 (100)	143 (98.6)	1.0

BMI, Body mass index; OLT, orthotopic liver transplantation; DAA, direct-acting antivirals; SVR24: sustained virological response at 24 weeks after HCV eradication; RBV, Ribavirin; MELD, Model for End-Stage Liver Disease.
[a]Significantly different by *t*-test.

complete disappearance of NOSA was observed in 25 over 39 (64.1%) patients that were initially ANA positive, in 4/6 (33.3%) initially ASMA positive, and 1/2 (50.0%) anti-LKM-1 positive ($P = 1.0$ by Fisher test). The negativity of NOSA was not influenced by the initial titre of autoantibodies.

3.3 | Comorbidities in patients with and without NOSA positivity

The comorbidities of NOSA positive were compared to those of NOSA-negative patients before DAAs treatment (Table 3). The

TABLE 2 Relationship between HCV infection clearance and non-organ-specific antibody pattern in autoantibody-positive patients at baseline and after direct-acting antivirals treatment

Variables	Before therapy (n = 46)	After therapy (n = 46)	*P* value
ANA titre, n (%)			
Negative	7 (15.2)	32 (69.6)	<0.0001[a]
1:80	19 (41.3)	4 (8.7)	<0.001[b]
>1:80	20 (43.5)	10 (21.7)	0.03[a]
ASMA titre, n (%)			
Negative	40 (87.0)	44 (95.6)	0.27
1:40	2 (4.3)	1 (2.2)	1.0
1:80	4 (8.7)	1 (2.2)	0.36
LKM titre, n (%)			
Negative	44 (95.6)	45 (97.8)	1.0
1:40	1 (2.2)	0	1.0
1:80	1 (2.2)	1 (2.2)	1.0
Anti-ENA or LA or ASCA positive[c]	3 (6.5)	0	0.24
Anti-ENA or Anti-dsDNA or APCA positive[c]	3 (6.5)	3 (6.5)	1.0

ANA, anti-nuclear antibody; ASMA, anti-smooth muscle antibody; LKM, anti-liver-kidney microsome antibody; ENA, extractable nuclear antigen; LA, lupus anticoagulant; ASCA, anti-Saccharomyces cerevisiae antibody; dsDNA, anti-double-stranded DNA; APCA, anti-parietal cell antibody.
[a]By chi-squared test
[b]By Fisher's exact test.
[c]Simultaneously positive to other non-organ-specific antibodies.

prevalence of patients with autoimmune thyroiditis was significantly higher in NOSA positive as compared to NOSA-negative patients ($P = 0.02$). The antiviral treatment did influence neither the clinical course nor the hormonal replacement therapy in both groups of patients for all the period of observation. As far as other autoimmune diseases, their distribution in the two groups of patient was not significantly different (Table 3).

At enrolment, one NOSA-positive patient had an IgM-type monoclonal peak of ɣ-globulins with lambda and kappa chains whereas two patients had an IgG-type monoclonal peak (one with lambda and one with kappa chains). The latter two patients became negative to autoantibodies after DAAs. Two NOSA-positive patients were affected by type-2 mixed cryoglobulinemia, and another one by type-1 mixed cryoglobulinemia and they all became negative to autoantibodies after therapy.

Moreover, one patient was previously affected by kidney cancer and another one by squamous cell cancer of the face.

3.4 | Adverse events during DAA therapy

Some adverse events occurred during DAA therapy. In detail, NOSA-positive patients experienced significantly higher prevalence of gastrointestinal symptoms ($P = 0.004$), while more episodes of infection were observed in NOSA-negative patients ($P = 0.01$). The other adverse events observed during DAA treatment occurred with similar frequency in NOSA-positive and negative patients (Table S1).

TABLE 3 Comorbidities in non-organ-specific antibodies (NOSA)-positive and negative patients

Comorbidity	NOSA positive (n = 46)	NOSA negative (n = 145)	*P* value
Autoimmune thyroiditis (hypothyroidism)	9 (19.6)	11 (7.6)	0.02[a]
Past *Helicobacter pylori*-related gastritis	4 (8.7)	5 (3.4)	0.22
Lichen ruber planus	0	3 (2.0)	1.0
Monoclonal peak of ɣ-globulins	3 (6.5)	10 (6.9)	1.0
Mixed cryoglobulinemia	3 (6.5)	6 (4.1)	0.45
Past neoplastic disease	2 (4.3)	17 (11.7)	0.25
Kaposi's sarcoma	1 (2.2)	0	0.24
Non-Hodgkin B-cell type lymphoma	0	2 (1.4)	1.0
Other autoimmune diseases[b]	3 (6.5)	7 (4.8)	0.7

Data reported are expressed as numbers and percentages (in parenthesis).
[a]By Chi-squared test.
[b]Ulcerative colitis, type 1 diabetes mellitus, rheumatoid arthritis and vitiligo, myasthenia gravis, autoimmune gastritis, psoriasis, discoid lupus erythematosus, Crohn's disease.

3.5 | Predictive factors for NOSA post-treatment negativity

As shown in Table 4, at univariate analysis, there was no evidence of association between each of the explanatory variables (age, sex, HCV genotype, previous response to antiviral treatments, DAA regimens, ASMA and LKM titres, body mass index [BMI], HVC-RNA at baseline, and MELD score) and NOSA negativity. Interestingly, at multivariate logistic regression analysis, after adjusting for all other explanatory variables in the model, there was a statistically significant association between hepatic hilar lymphadenopathy and NOSA negativity (Table 4). The probability of NOSA negativity after therapy in patients with hepatic hilar lymphadenopathy was significantly reduced as compared with patients without this condition (OR = 0.17; 95% CI 0.02-0.94, $P = 0.04$).

4 | DISCUSSION

At the best of our knowledge, this is the first study on the assessment of NOSA in HCV patients before and after DAA administration. We demonstrated that HCV patients with chronic liver disease and NOSA positivity clear their autoantibodies in a significant percentage of cases (65.2%) after DAAs treatment. The presence of NOSA did not influence the HCV clearance since 100% of the patients obtained SVR24. At the same time, NOSA-negative

TABLE 4 Probability of non-organ-specific antibody (NOSA) negativity at the univariate and multivariate analysis

	Univariate analysis	Multivariate analysis	
Variable	OR (95% CI)	OR[a] (95% CI)	*P* value
Hepatic hilar lymphadenopathy			
No	1.00[b]	1.00[b]	0.04
Yes	0.33 (0.08-1.35)	0.17 (0.02-0.94)	
ANA titre			
Negative	1.00[b]	1.00[b]	0.06
1:80	1.50 (0.21-10.82)	0.93 (0.06-12.90)	
>1:80	0.40 (0.06-2.57)	0.14 (0.01-1.40)	
Gamma globulin (g/dL)	0.62 (0.17-2.28)	2.51 (0.37-19.16)	0.64
Previous use of ribavirin			
No	1.00[b]	1.00[b]	0.26
Yes	0.40 (0.11-1.43)	0.37 (0.06-2.05)	
Vitamin D therapy			
No	1.00[b]	1.00[b]	0.39
Yes	0.44 (0.11-1.84)	0.25 (0.03-1.78)	
Baseline vitamin D (IU/mL)	0.95 (0.90-1.01)	0.96 (0.89-1.03)	0.51

ANA, Anti-nuclear antibody.
[a]Adjusted for all variables in the multivariate model.
[b]Reference group.

patients experienced a similar result reaching a SVR24 in 98.6% of the cases.

A meaningful impact on HCV disappearance has been previously attributed to the presence of autoantibodies. However, these results were obtained in course of interferon therapy, and this aspect probably led to different conclusions.[4,13,20–22]

Our study showed an overall prevalence of NOSA of 24.1%. In particular, ANA positivity was observed in 20.4% of HCV population. This value was comparable to that reported by previous Italian studies, showing an ANA prevalence at a titre >1:40 ranging from 6% to 25%.[8,23–26] Some studies from Egypt, China, and Taiwan reported that ANA prevalence (titre >1:40) varied between 1.6% and 22.9%,[23,27] while the prevalence of ANA (titre ≥1:40) in other western countries ranged between 3.6% and 54%.[4,8,23]

We found a prevalent ANA-speckled pattern, as commonly noted in HCV-positive patients. As far as the prevalence of ASMA and anti-LKM-1 positivity, our results were similar to those reported by others that used a titre ≥1:40 as autoantibody cut-off. In fact, 13.0% of our patients were ASMA positive and 4.3% were anti-LKM-1 positive, that is, within the range reported by previous studies in a similar population with HCV-related advanced liver disease (4.3%-74.5% in ASMA positive and 0.5%-20% in anti-LKM-1 positive).[4,8,23,24,27,28]

As concerning the other clinical significances of NOSA, before DAA therapy, we observed a significantly higher prevalence of patients with autoimmune thyroiditis in NOSA-positive compared to NOSA-negative group, as previously observed by others.[29] The clinical course and therapy as well as the level of anti-thyroid antibodies did not change significantly after DAA treatment. This was not surprising since, as it occurs for other autoimmune diseases, after the development of antigen-specific lymphocyte clones the level of autoantibodies is maintained by the presence of the antigens. Of interest, serum levels of vitamin D were significantly lower in NOSA-positive group compared to negative one at baseline. Vitamin D activates monocytes and macrophages, inhibits lymphocyte proliferation, and prevents the differentiation of dendritic cells into antigen-presenting cells reduces also immunoglobulin production and MHC class II expression.[30,31] It decreases cytokine production, increasing anti-inflammatory cytokines,[32] and prevents the development of autoimmune diseases in experimental models of autoimmune diseases.[33] However, the level of vitamin D did not influence both therapy outcome and clearance of NOSA.

Interestingly, in the NOSA-positive group, three patients reported a history of autoimmune disease, and therefore, they should be probably excluded from this group. However, the two subjects who suffered rheumatoid arthritis and ulcerative colitis did not obtain autoantibody negativity while the third patient, who was affected by type-1 diabetes, became NOSA negative after DAA treatment. This finding may be interesting on the light of the previous demonstration that there is a link between HCV infection and type-1 diabetes.[34] The onset of type-1 diabetes mellitus has been closely restricted to the genetic susceptibility demonstrated by the presence of the HLA DRB1-DQB1 haplotype in these patients.[35]

As compared to the data reported in patients treated with interferon-based regimens, and as previously observed by others, during and after the treatment with DAAs, we observed few mild adverse events.[36] In addition, no significant thyroid dysfunction or the development of severe systemic autoimmune disease was observed by us.

On the bases of these results, autoantibodies could be considered HCV-related epiphenomena other than part of immune-based disease.[5] This hypothesis may be supported by our multivariate analysis, which showed a significant correlation between autoantibody negativity following antiviral therapy and the absence of hepatic hilar lymphadenopathy.

Before drawing definitive conclusions, it is important to point out that in nearly 34.8% of NOSA-positive patients, some degree of immunologic activation still remained present at the end of follow-up and after SVR24. After the exclusion of a clinical B-cell lymphoma development, the possible explanation of this finding could be the persistence a virus-independent B-cell polyclonal expansion or residual B-cell clones, maybe in a specific (unexplored) genetic background of the host. In this case, it would be necessary a longer follow-up to observe a complete B-lymphocyte clonal disappearance.

In conclusion, our study demonstrates that HCV clearance frequently reduces NOSA positivity in patients with advanced chronic liver diseases. At the same time, our results suggest a long-term monitoring in patients who did not became NOSA negative to establish whether they could hide an underlying process which may progress into a clear autoimmune or rheumatologic disease.

ACKNOWLEDGEMENTS

Declaration of personal interests: All authors declare no financial, professional, or personal conflict.

Declaration of funding interests: This research did not receive any financial support from funding agencies in the public, commercial, or not-for-profit sectors. Endrit Shahini, Andrea Iannone, Domenico Romagno, Angelo Armandi, Sonia Carparelli, Mariabeatrice Principi, Maria Teresa Viggiani, Enzo Ierardi, Alfredo Di Leo, and Michele Barone declare that they do not have any existing financial arrangements with a company whose products are reported in the submitted manuscript or any organisation sponsoring the research reported in the submitted manuscript.

AUTHORSHIP

Guarantor of the article: None.

Author contributions: Endrit Shahini, Domenico Romagno, and Michele Barone contributed to the conception and the protocol. Alfredo Di Leo provided supervision, coordination, and guidance of the analysis plan. Endrit Shahini, Michele Barone, Enzo Ierardi, and Maria Teresa Viggiani drafted the initial and final manuscript. Domenico Romagno and Michele Barone assigned patients to the appropriate therapeutic regimen, managed them in the study, and provided content expertise. Endrit Shahini, Angelo Armandi, and Sonia Carparelli enrolled patients and collected in a database baseline and on-treatment information. Endrit Shahini and Andrea Iannone systematically reviewed the data entries for completeness and consistency, checked for completeness of information, and performed the statistical analysis; Domenico Romagno, Mariabeatrice Principi, Maria Teresa Viggiani, Enzo Ierardi, and Alfredo Di Leo reviewed and approved the final draft. As submision's guarantor, Michele Barone declares that all autors approved the final draft and none of them had any personal or funding interest during the last 2 years to declare.

ORCID

Andrea Iannone http://orcid.org/0000-0002-5468-9515
Mariabeatrice Principi http://orcid.org/0000-0003-0545-5656
Maria Teresa Viggiani https://orcid.org/0000-0002-8689-9103
Alfredo Di Leo http://orcid.org/0000-0003-2026-1200
Michele Barone http://orcid.org/0000-0001-8284-5127

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section at the end of the article.

How to cite this article: Shahini E, Iannone A, Romagno D, et al. Clinical relevance of serum non-organ-specific antibodies in patients with HCV infection receiving direct-acting antiviral therapy. *Aliment Pharmacol Ther*. 2018;48:1138–1145. https://doi.org/10.1111/apt.14999