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# Polyclonal and monoclonal B lymphocytes response in HCV-infected patients treated with direct-acting antiviral agents

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## Summary

Hepatitis C virus (HCV) chronic infection can be associated with extrahepatic manifestations such as mixed cryoglobulinaemia and lymphoproliferative disorders that are endowed with increased rates of morbidity and all-cause mortality. In this study, we used flow cytometry to evaluate the effect of interferon-free antiviral treatment on peripheral blood lymphocytes in HCV-infected patients with or without associated lymphoproliferative disorders. Flow cytometry analysis of peripheral blood lymphocytes was performed at baseline and at the end of treatment. In HCV-infected patients with lymphoproliferative disorders, we evaluated immunoglobulin (Ig) light chain κ/λ ratio variations as a measure of monoclonal B-cell response to antiviral therapy. Healthy volunteers were enrolled as controls. A total of 29 patients were included, nine with and 20 without lymphoproliferative disorders. Sustained virological response was achieved in 29 of 29 patients. We observed a significant reduction in the B-cell compartment (39% global reduction) in eight of nine HCV-infected patients with lymphoproliferative disorders after viral clearance. We recognized the same trend, even if less pronounced, in HCV-infected patients without lymphoproliferative disorders (9% global reduction). Among HCV-infected patients with lymphoproliferative disorders, three showed an improvement/normalization of the immunoglobulin light chain ratio, whereas in the remaining six patients monoclonal B cells persisted to be clonally restricted even 1 year after the end of treatment. Our data show that DAAs treatment can be effective in reducing the frequency of pathological B cells in the peripheral blood of HCV-infected patients affected by HCV-associated lymphoproliferative disorders; however, monoclonal populations can persist after viral eradication.

KEYWORDS
DAAs, HCV, Lymphoma

## 1 | INTRODUCTION

HCV infection is associated with both hepatic and extrahepatic manifestations. Among the latter, LPDs are correlated with HCV lymphotropism and oncogenic potential.[1]

**Abbreviations:** B-NHL, B-cell non-Hodgkin's lymphoma; DAAs, direct-acting antivirals; EASL, European Association for the Study of the Liver; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; Ig, immunoglobulin; LPD, lymphoproliferative disorder; MC, mixed cryoglobulinaemia; MELD, Model for end-stage liver disease; RBV, ribavirin; SVR, sustained viral response.

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Epidemiological studies have confirmed the association between HCV chronic infection and a broad spectrum of LPDs such as mixed cryoglobulinaemia (MC) and different B-cell non-Hodgkin's lymphoma (B-NHL) subtypes.[2-6]

However, the understanding of the molecular mechanisms linking HCV active infection to lymphoma development is still limited.

Several reports documented the ability of HCV to induce B lymphocytes proliferation by antigen stimulation and/or by interaction of the E2 viral envelope protein with the CD19/CD21/CD81 complex,[7,8] but a more recent study failed to demonstrate the presence of HCV-specific B-cell receptors on the surface of lymphoma cells derived from B-NHL associated with HCV infection. However, the restricted usage of immunoglobulin VH-169 in lymphoma cells supports antigen-driven proliferation and clonal selection as a causative mechanism for HCV-associated B-NHL development.[9]

Moreover, the high prevalence of monoclonal lymphocytosis in HCV-infected patients supports the notion that neoplastic transformation could be caused by chronic antigenic stimulation and clonal selection.[10,11]

Yet, the response of B-NHLs to antiviral treatment provides the strongest evidence for a potential causative role of HCV in lymphomagenesis. This was initially observed in nine patients with splenic lymphoma who underwent interferon (IFN)-α-based antiviral treatment. All patients who achieved sustained virological response (SVR) obtained lymphoma regression. Importantly, a correlation was observed between haematologic and virological response.[12] Later reports confirmed that IFN-α-based antiviral treatments can be efficacious for treating HCV-associated lymphomas.[13-19]

As IFN-free antiviral regimens became widely available, some case reports and data on a larger series of HCV patients affected by B-NHL treated with the new antivirals have been published, confirming that viral clearance can also be effective on the associated LPDs.[20-24]

However, all these interventional studies evaluated the haematologic response mainly by clinical criteria, whereas little is known at the cellular and molecular level.

In this study, we have investigated by flow cytometry the effect of HCV eradication following IFN-free antiviral therapy on peripheral lymphocytes of patients with and without HCV-associated LPDs.

## 2 | PATIENTS AND METHODS

### 2.1 | Patients

All consecutive HCV-infected patients with active HCV replication treated with any of the possible DAAs combinations who were referred to the Gastroenterology Unit of the University Hospital of Padua from July 2015 to October 2015 were considered eligible for the study and prospectively enrolled.

Exclusion criteria were as follows: baseline hepatocellular carcinoma (HCC), extrahepatic neoplastic disease, antineoplastic treatments, active infections and recent (3 months) or active variceal bleeding, HBV and/or HIV co-infection, patients who received any other antiviral treatment during the last 6 months before starting DAAs therapy.

Electronic medical records were reviewed for demographic information, clinical characteristics, laboratory and pathological findings.

Antiviral therapy was administered accordingly to the Italian Association for the Study of the Liver guidelines for HCV treatment.[25] SVR 12 or 24 weeks after the end of therapy (SVR12 or SVR24) was defined as serum HCV-RNA below the lower limit of quantification (LLOQ, 15 IU/mL). Patients were treated in accordance with the criteria of the Italian Medicines Agency.

The severity of liver disease was defined accordingly to clinical and biochemical parameters (Child-Pugh classification and Model for End-Stage Liver Disease [MELD] score). Furthermore, all patients underwent Fibroscan™ (before and 3 months after the end of DAAs therapy) as noninvasive method to evaluate liver fibrosis.

The study was conducted according to the Declaration of Helsinki. All patients gave written consent for data collection. The study was approved by the Padua University Hospital ethical committee (protocol number: 3103/A014).

### 2.2 | Immunophenotyping

All blood samples were EDTA-anticoagulated and analysed on the same day of the collection to avoid cellular death. Sample preparation and analysis were performed according to the CLSI guidelines.[26,27]

Total lymphocyte, neutrophil and platelet counts were measured with an XE-5000 haematology analyzer (Sysmex). The absolute frequencies of cell subsets were calculated on the basis of the relative frequencies on total lymphocytes.

Samples were probed before the beginning and at least 1 month after the end of treatment to avoid direct effects of the therapy.[28]

### 2.3 | Statistical analysis

Statistical analyses were performed using Prism 5 (GraphPad Software, San Diego, CA, USA). Nonparametric statistics were used because many of the data were not normally distributed. Tests used were the Mann-Whitney *U* test for unpaired comparisons and the Wilcoxon signed-rank test for paired comparisons. Two-tailed *P* values were calculated for all tests. An $\alpha$ of 0.05 was defined as the cut-off for significance.

## 3 | RESULTS

### 3.1 | Characteristics of the study population

Twenty-nine patients were enrolled (M/F 16/13, mean age 65 years old, range 43-78). Of these, nine presented with and 20 without evidence of monoclonal B lymphocytes in the peripheral blood, respectively.

Within the first group, five patients received a diagnosis of B-NHL, one of mixed cryoglobulinaemia syndrome (MCS) and three were classified as affected by HCV-associated monoclonal B-cell lymphocytosis (MBL) without further diagnostic evaluation (Tables 2 and 3).

**TABLE 1** Clinical characteristics of HCV/LPD− patients who underwent DAAs treatment

| Patient | Sex | Age, y | Liver stiffness (kPa) | HCV genotype | Anti-HCV regimen | Treatment history | SVR |
|---|---|---|---|---|---|---|---|
| 1 | M | 58 | 42.2 | 3a | Sofosbuvir/daclatasvir+rbv | 1 | SVR12 |
| 2 | F | 77 | 33.3 | 2a-2c | Sofosbuvir+rbv | Naive | SVR24 |
| 3 | M | 70 | 29.1 | 1b | Sofosbuvir/simeprevir+rbv | 2 | SVR24 |
| 4 | M | 75 | 28 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | 1 | SVR24 |
| 5 | F | 49 | 25.7 | 2 | Sofosbuvir+rbv | 2 | SVR24 |
| 6 | F | 68 | 20.1 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | Naive | SVR24 |
| 7 | M | 75 | 25.4 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | 3 | SVR24 |
| 8 | F | 52 | 33.8 | 4 | Sofosbuvir/simeprevir+rbv | Naive | SVR24 |
| 9 | F | 58 | 5.9 | 1a | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | 1 | SVR24 |
| 10 | F | 72 | 25.7 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | Naive | SVR24 |
| 11 | F | 56 | 13.8 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | 1 | SVR12 |
| 12 | F | 74 | 30.3 | 1B | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | Naive | SVR24 |
| 13 | M | 78 | 9.8 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir | Naive | SVR12 |
| 14 | F | 71 | 14 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | Naive | SVR24 |
| 15 | M | 57 | 27.7 | 4 | Simeprevir/daclatasvir+rbv | 2 | SVR24 |
| 16 | F | 72 | 26.3 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | 2 | SVR24 |
| 17 | M | 43 | 20.2 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | Naive | SVR24 |
| 18 | F | 56 | 21.3 | 1b | Sofosbuvir/ledipasvir+rbv | 2 | SVR12 |
| 19 | M | 69 | 43.8 | 2a-2c | Sofosbuvir/simeprevir+rbv | 2 | SVR24 |
| 20 | M | 66 | 26.3 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | Naive | SVR24 |

Among the whole cohort 26 of 29 (90%), patients had liver cirrhosis, being the vast majority of them classified as Child-Pugh A (25 patients, 86%). Of the entire study population, 19 (66%) patients had genotype 1 infection. Mean liver stiffness evaluated by Fibroscan™, Echosense, France was 18 kPa (range 4-43), and mean MELD score of cirrhotic patients before antiviral therapy score was 8 (range 6-15). Antiviral therapy was based on a "3D" combo regimen (Ombitasvir/Paritaprevir/Ritonavir/Dasabuvir plus Ribavirin) in half of the patients (15 patients, 51%). SVR12 was achieved in 29 of 29 patients. The patient affected by MCS also received Rituximab.

Mean liver stiffness after therapy was 16 kPa (range 4-38) with no statistically significant difference when compared to pre-antiviral therapy evaluation ($P$=.7).

Twenty healthy volunteers were included as controls. Cohort characteristics are summarized in Tables 1-3.

## 3.2 | Flow cytometry analysis

### 3.2.1 | HCV+/LPD− patients

All HCV+/LPD− patients presented with no evidence for the presence of monoclonal lymphocyte populations in the peripheral blood before and after antiviral treatment.

Patients total lymphocyte count did not significantly differ from that of a control group and was not affected by HCV eradication (Figure 1).

Regarding CD3+ T cells, no differences were found in terms of frequency and absolute count between patients and controls ($P$=.7 and $P$=.4, respectively).

CD3+ cell relative frequency increased significantly in HCV-infected patients after treatment. Distribution of helper and cytotoxic T cells is reported in Fig. S1.

**TABLE 2** Clinical characteristics of HCV/LPD+ patients who underwent DAAs treatment

| Patient | Sex | Age, y | Liver stiffness (kPa) | HCV genotype | Anti-HCV regimen | Treatment history | SVR |
|---|---|---|---|---|---|---|---|
| 1 | M | 73 | 21.8 | 2a-2c | Sofosbuvir+rbv | 2 | SVR24 |
| 2 | M | 66 | 14.9 | 3a | Sofosbuvir+rbv | - | SVR24 |
| 3 | M | 52 | 39.7 | 1b | Sofosbuvir+rbv | Naive | SVR24 |
| 4 | F | 67 | 27 | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | Naive | SVR12 |
| 5 | F | 74 | 12 | 1b | Sofosbuvir+ledipasvir | 2 | SVR12 |
| 6 | M | 51 | 27 | 1a | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | 2 | SVR12 |
| 7 | M | 47 | 11 | 1b | Sofosbuvir+simeprevir+rbv | 2 | SVR24 |
| 8 | M | 78 | 10 | 2 | Sofosbuvir+rbv | Naive | SVR12 |
| 9 | M | 75 | | 1b | Ombitasvir/paritaprevir/ritonavir/ dasabuvir+rbv | Naive | SVR24 |

**TABLE 3** Immunophenotypic characteristics of HCV/LPD+ patient B cells

| Patient | LPD | B-cell immunophenotype |
|---|---|---|
| 1 | MBL | CD20+CD5-CD23+/-CD10-CD11c+/-CD38+ |
| 2 | MBL | CD20+CD5-CD23-CD11c-CD38-CD10- |
| 3 | MCS | CD20+CD5-CD10-CD23-CD11c+CD103-CD25-CD38- |
| 4 | MBL | CD5-CD10-CD23-CD38+/-CD11c+/- |
| 5 | MZL | CD19+CD20+CD5-CD10-CD11c+CD38+CD103-CD25- |
| 6 | SMZL | CD20+CD5-CD10-CD23-CD11c+/-CD38-/+CD103-CD25- |
| 7 | MZL | CD20+CD5-CD23-/+CD10-CD11c-CD38+ |
| 8 | CD5+ B-NHL | CD20+CD5+CD23-CD10-FMC7CD11c-CD38+/-CD200- |
| 9 | CD5+ B-NHL | CD20+CD5+CD10-CD23-CD11c-CD38+ |

LPD, lymphoproliferative disorder; MZL, marginal zone lymphoma; MBL, monoclonal B-cell lymphocytosis; MCS, mixed cryoglobulinaemia syndrome; SMZL, splenic marginal zone lymphoma; B-NHL, B-cell non-Hodgkin lymphoma.

B-cell frequency was increased in HCV-infected patients before DAAs treatment when compared to the control group (mean 17.55%, SD 8.2 vs mean 11.75%, SD 3.4, *P*=.01) and significantly decreased following HCV clearance (mean 15.7%, SD 7.6 vs mean 17.55%, SD 8.2, *P*=.01). However, B-cell absolute count did not differ between the three groups. Five HCV-infected patients in the pretreatment group and four at the end of treatment, but none in the healthy controls, had a B-cell frequency >21%, upper limit of the reference range for B lymphocytes (Figures 2 and 3)

The variance of CD19+ B cells was also higher in patients both before (*P*=.0003) and after (*P*=.0009) treatment when compared to controls.

In HCV-infected patients, the serum γ globulin concentration significantly decreased after the end of therapy (mean 14.69 g/L, SD 4.5 vs mean 17.21 g/L, SD 5, *P*=.001), whereas total proteins were unaffected.

CD16+ CD56+ double-positive NK cells were consistently reduced both before and after treatment in HCV-infected patients.

### 3.2.2 | HCV+/LPD+ patients

All HCV+/LPD+ patients showed the presence of monoclonal B-cell populations in the peripheral blood as revealed by surface immunoglobulin light chain expression unbalance (abnormal κ/λ ratio, normal reference range 1-3) with or without other immunophenotypic aberrancies (Table 3).

None of these patients presented with lymphocytosis, and the total lymphocyte count did not differ from that of the control group before treatment (*P*=.3). Nonetheless, total lymphocyte count was significantly reduced after DAAs treatment in HCV-infected patients ($1.36\times10^9$/L, SD 0.92 vs mean $1.83\times10^9$/L, SD 1, *P*=.01) (Figure 2).

Relative frequency and absolute count of CD3+ T cells were significantly lower in this group of patients than in controls and T-cell frequency, but not absolute count, recovered after treatment (mean 73.7%, SD 14.2 vs mean 64.4%, SD 11.4, *P*=.01). Helper and cytotoxic T-cell distribution is reported in Fig. S2.

In HCV+/LPD+ patients CD19+ B cells ranged from 10% to 44% and from 4% to 32% before and after treatment, respectively, with

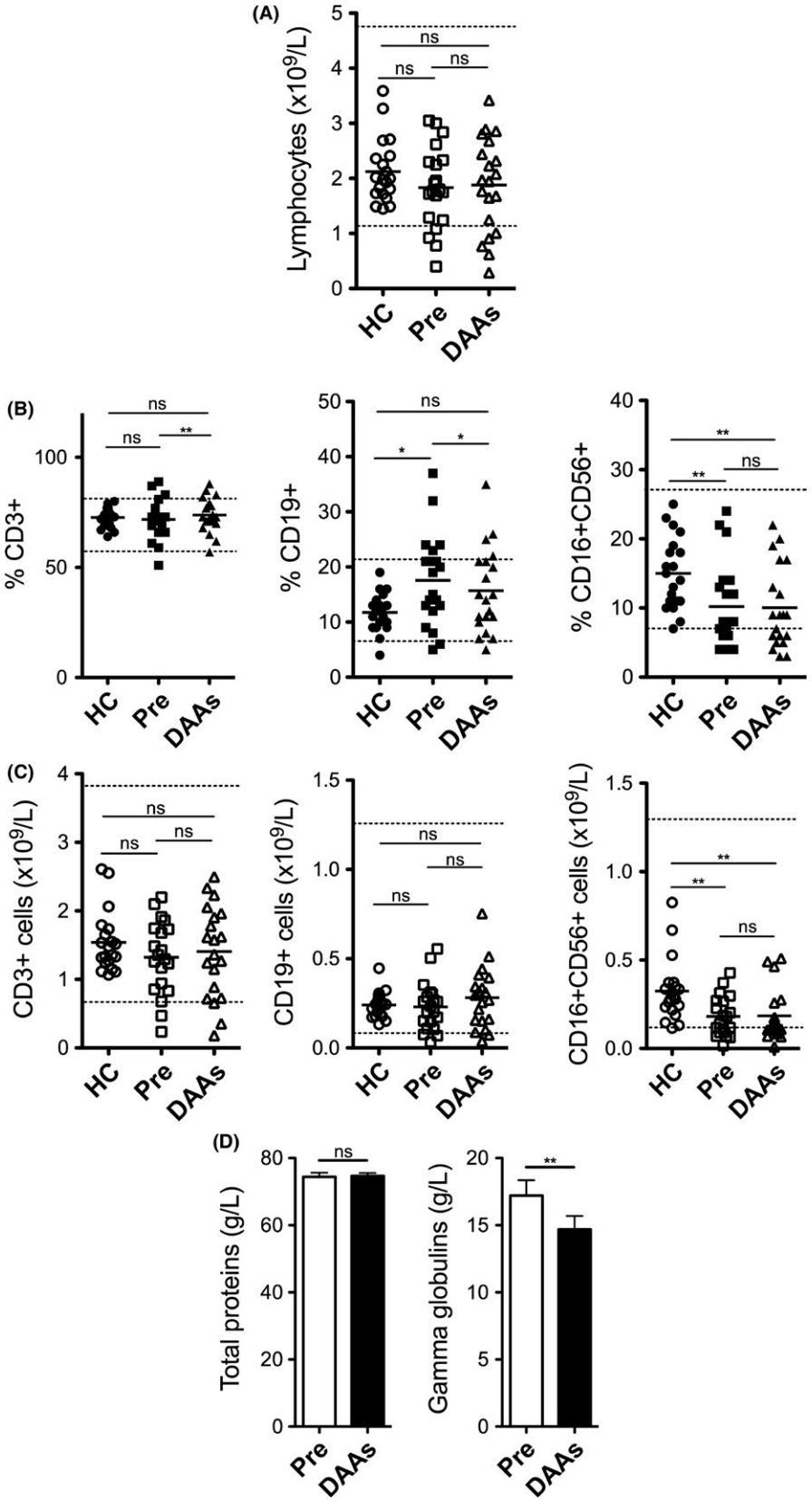


**FIGURE 1** Total lymphocyte count and subset distribution, serum protein and gamma globulin concentration in healthy controls and in HCV-infected patients before and after DAAs treatment. (A) Total lymphocyte count (reference range: 1.1-4.8x$10^9$/L) of HCV-infected patients did not differ from that of a healthy control group and was not affected by DAAs treatment. (B) Peripheral blood T-cell (CD3+, reference range 57%-81%), B-cell (CD19+, reference range 7%-21%) and NK-cell (CD16+CD56+, reference range 7%-27%) relative frequencies were determined by flow cytometry and expressed as percentages of the total gated lymphocytes. Note the broader range of CD19+ cells in HCV-infected patients and the increased frequency before treatment when compared to healthy controls. (C) Peripheral blood T cells (CD3+), B cells (CD19+) and NK cells (CD16+CD56+) expressed as absolute count. Note the persistent reduction in NK cells in HCV-infected patients both before and after antiviral therapy. (D) Serum protein electrophoresis showed that gamma globulin concentration was significantly reduced in HCV-infected patients after treatment, while total protein concentration was not affected. HC, healthy controls; Pre, pretreatment HCV-infected patients; DAAs, direct-acting antiviral agents; ns: *P*>.05; *: *P*<.05; **: *P*<.01. N=20 for each group. Black dotted lines show normal reference ranges

significantly increased variances compared to controls (*P*<.0001). Five HCV+/LPD+ patients in the pretreatment group and three at the end of treatment had a B-cell frequency >21%, upper limit of the reference range for B lymphocytes.

Before treatment frequency of CD19+ cells was higher in patients than in healthy controls (mean 23.5%, SD 9.5 vs mean 11.75%, SD 3.4, *P*=.0006), while after viral eradication, B-cell frequency was significantly decreased (mean 15.11%, SD 10.2, vs mean 23.5%, SD

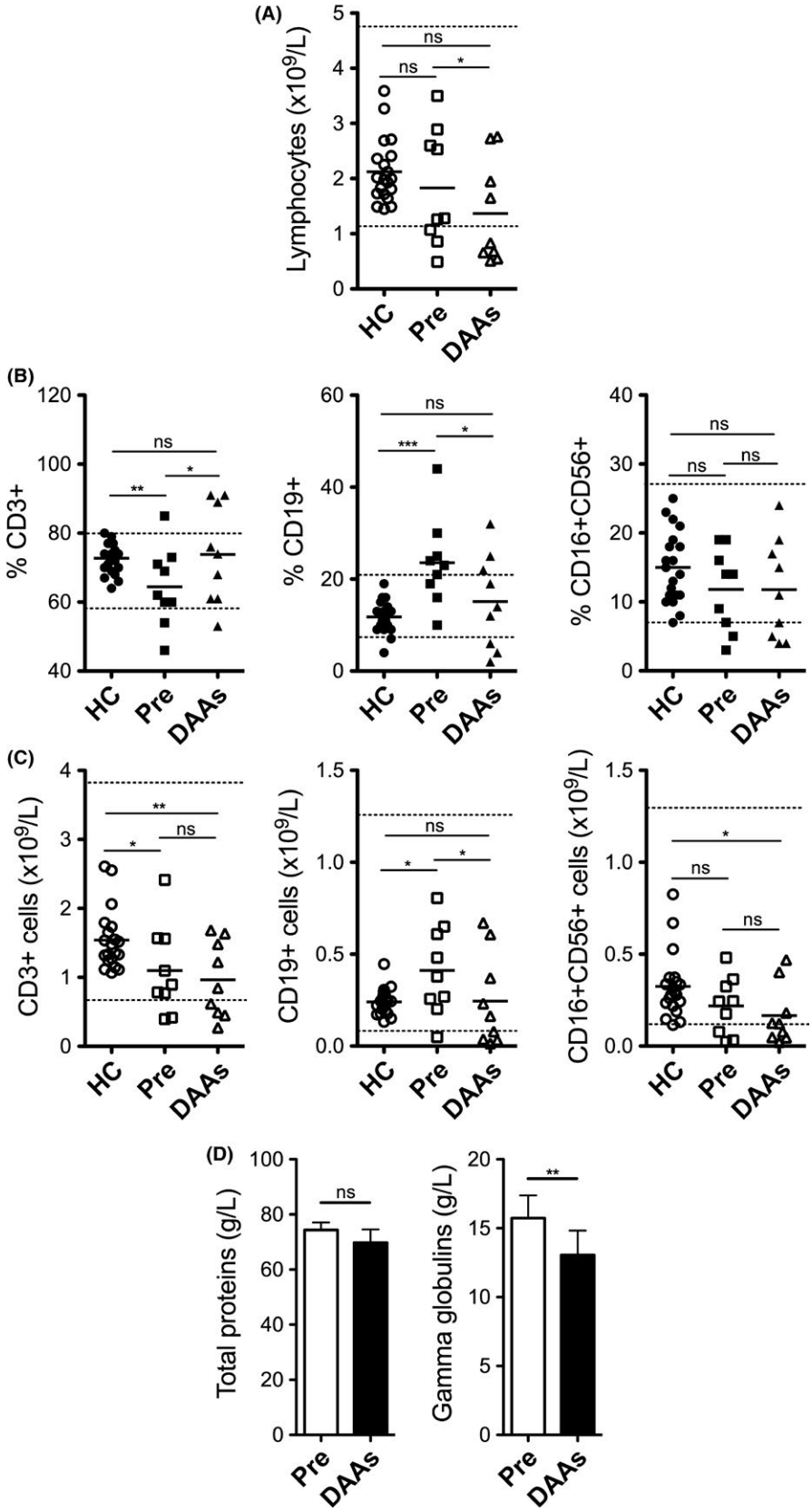


**FIGURE 2** Total lymphocyte count and subset distribution, serum protein and gamma globulin concentration before and after DAAs treatment in nine patients affected by HCV-associated LPDs. (A) Total lymphocyte count of HCV/LPD patients did not differ from that of healthy controls but was significantly lower after antiviral treatment than in the pretreatment group. (B) Peripheral blood T-cell (CD3+, reference range 57%-81%) B-cell (CD19+, reference range 7%-21%) and NK-cell (CD16+CD56+, reference range 7%-27%) relative frequencies were determined by flow cytometry and expressed as percentages of the total gated lymphocytes. Note the broader range of CD3+ and CD19+ cells in HCV/LPD patients compared to healthy controls. (C) CD3+ cells were lower in patients than in controls and increased as relative, but not absolute frequency after treatment. CD19+ cells were increased in patients before treatment and strongly reduced after the therapy. CD16+CD56+ NK cells were consistently reduced both before and after treatment in HCV-infected patients. (D) Serum protein electrophoresis showed that gamma globulin concentration was significantly reduced after treatment, while total protein concentration was not changed in HCV-infected patients. HC: healthy controls; Pre: pretreatment HCV-infected patients; DAAs: direct-acting antiviral agents; ns: $P>.05$; *: $P<.05$; **: $P<.01$, ***: $P<.001$. N=20 for healthy control and N=9 for HCV/LPD-infected patients. Black dotted lines indicate normal reference ranges

9.5, $P=.02$; 39% global reduction), becoming similar to what found in healthy controls ($P=.4$). B-cell absolute frequency did also significantly decrease after treatment in this group of patients (mean $0.24\times10^9$/L, SD 0.25 vs $0.41\times10^9$/L, SD 0.24, $P=.02$; 46% global reduction). B cell both relative and absolute count decreased in eight of nine patients after therapy, concomitantly with viral load reduction (Fig. S3).

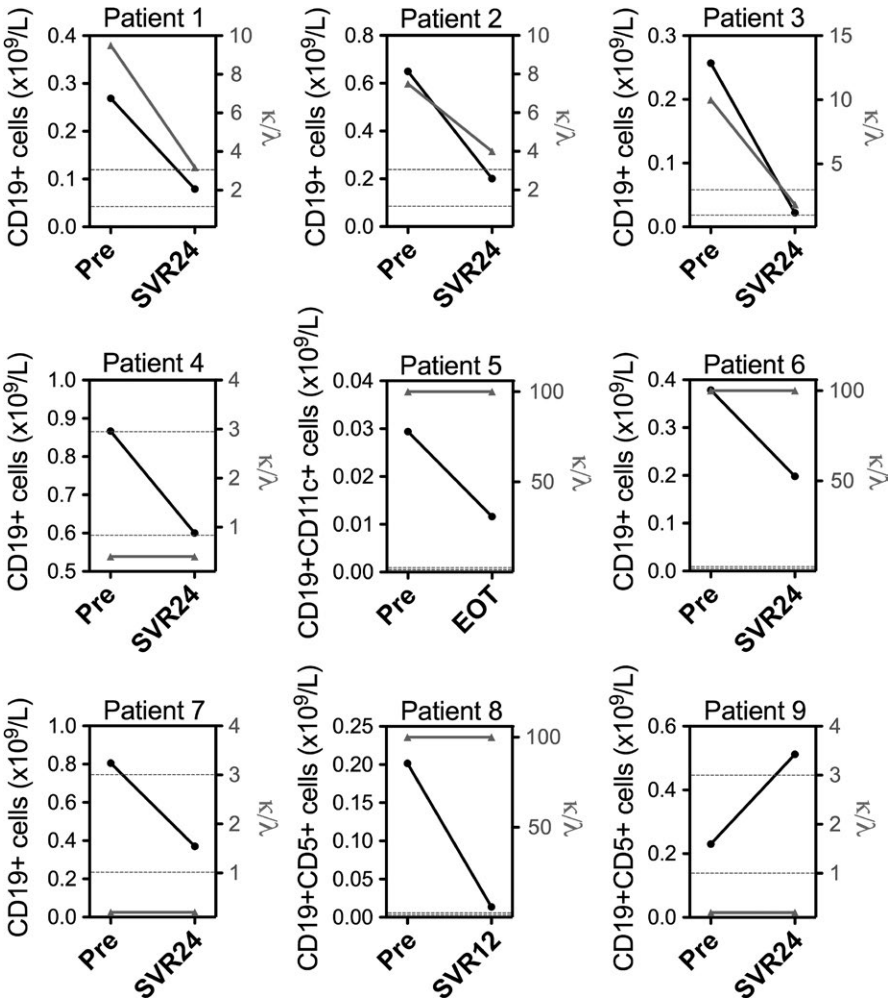


**FIGURE 3** Effect of direct-acting antiviral agents on peripheral blood monoclonal B cells in nine patients affected by HCV-associated LPDs. B-cell absolute count and κ/λ ratio before and after DAAs treatment are reported for each patient. On the left axis, molecular markers used to measure B-cell clonal populations (black lines) are shown. In Patient 5, CD19+CD11c+ cells were gated for the analysis. In patients 8 and 9, CD19+CD5+ cells were gated for κ/λ ratio measurements. Right axis shows κ/λ ratio values (grey lines). In patients 1-3, improved κ/λ ratio and reduced B-cell count are evident. Patient 3 (affected by MCS) received Rituximab after the end of the antiviral treatment. Patients 4-8 showed a reduction in B cells, but κ/λ ratios remained abnormal. In Patient 9, CD19+ CD5+ double-positive cells expanded and persisted to be clonally restricted. Grey dotted lines in each panel mark the normal reference range for κ/λ ratio (1-3). Pre, pretreatment; SVR, sustained viral response; EOT, end of treatment

Serum gamma globulins, but not total proteins, were significantly reduced after treatment (mean 13.05 g/L, SD 5.3 vs mean 15.7 g/L, SD 4.9, *P*=.007).

CD16+ CD56+ double-positive NK-cell frequency and count were not affected by DAAs treatment and were always lower than in healthy controls.

### 3.2.3 | Monoclonal B-cell populations

To study the effect of DAAs on monoclonal B-cell populations, immunoglobulin light chain κ and λ surface expression were evaluated before and after the treatment as a marker for clonality (Figure 3). By combining B-cell count with κ/λ expression data, three different response groups were identified:

1. in three patients (two MBL and one MCS patients), both pathological B cells reduction and κ/λ ratio improvement/normalization were observed.
2. in five patients (one MBL and four B-NHL patients), despite a reduction in the B-cell compartment, no improvement in κ/λ ratio was found even after 1 year from the end of treatment.
3. in one patient (CD5+ B-NHL), an increased pathological CD5+ B-cell count with no improvement in the κ/λ ratio at 1 year after the end of treatment was observed.

## 4 | DISCUSSION

HCV-related B-NHL response to antiviral therapy has been extensively documented.[12-19]

Haematologic response to antiviral treatment was usually defined mainly by clinical parameters.[29,30] In our series, we observed that in eight of nine HCV/LPD+ patients, B cells decreased concomitantly with HCV eradication and independently from other virological features such as HCV genotype and type of antiviral regimens.

We also observed a similar trend in twenty HCV-infected patients without LPDs. These results suggest that HCV infection can induce, in a fraction of patients, an expansion of the B-cell compartment and that this effect can be at least partially rescued by viral clearance.

Polyclonal, antigen-driven expansion of the B-cell compartment can be the substrate on which the emergence of monoclonal populations occurs, similarly to the model proposed for linking gastric mucosa-associated lymphoid tissue (MALT) lymphoma development and *Helicobacter pylori* infection.[31] Our analysis suggests that a subset of chronically infected HCV-infected patients develop a polyclonal expansion of the B-cell compartment; B cells can then occasionally shift, probably under antigenic pressure, towards monoclonal proliferation or overt B-NHL.

Recently, Gragnani et al.[32] showed that in two HCV-infected patients affected with indolent lymphoma monoclonal B cells persisted after viral eradication. We found that in eight of nine HCV+/LPD+ patients, viral eradication with DAAs resulted in a consistent reduction in pathological B cells in the peripheral blood, but had variable effects on B-cell clonality. For example, in patients 1 and 2 (monoclonal lymphocytosis), B-cell absolute number decreased and the light chain expression progressively improved towards normal values after viral eradication. On the other hand in Patient 6 (splenic marginal zone lymphoma), monoclonal B cells were drastically reduced after viral clearance, but 6 months after the end of treatment, B lymphocytes were still all clonally restricted. Therefore, HCV-driven polyclonal B-cell expansion, monoclonal lymphocyte populations and early-phase LPDs, but not more advance B-NHL, responded well to HCV eradication with DAAs.

Persistence of laboratory and/or clinical stigmata of LPDs after viral eradication were not uncommon in IFN-based antiviral era.[33] Indeed, as proposed by Zignego et al.,[34] the persistence of the above-mentioned stigmata can be, in some cases, related to short follow-ups as well as to the overcoming of pathogenetic no-return points.

Overt diseases, probably because of further unfavourable oncogenic events, acquire autonomous growth ability and consequently partial or complete resistance to antiviral treatment, as suggested also by our experience.

Our study has some limitations. First, the follow-up period is relatively short. Certainly, a longer follow-up is needed to verify the long-term effects of viral eradication on monoclonal B-cell populations. Second, the sample size, a wider sample and other centre experiences are indispensable to confirm these preliminary findings. Finally, no molecular evaluation underpinning the association between HCV status and lymphocyte populations was carried out.

In conclusion, HCV eradication following DAAs treatments is associated with a rapid and consistent reduction in circulating pathological B cells in the majority of patients. However, monoclonal B-cell populations can persist after viral eradication. Further studies are needed to better understand the molecular relationship between HCV and B-cell transformation.

## CONFLICT OF INTEREST

None.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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