




ORIGINAL ARTICLE

B-cell activating factor (BAFF), BAFF promoter and BAFF receptor allelic variants in hepatitis C virus related Cryoglobulinemic Vasculitis and Non-Hodgkin's Lymphoma

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Abstract

Cryoglobulinemic Vasculitis (CV) is an autoimmune/lymphoproliferative disorder associated with HCV infection that in 5%–10% of cases evolves into a B cell Non-Hodgkin's Lymphoma (NHL). B-cell activating factor (BAFF) is a key regulator in B-cell development and survival. Particular genetic variants are responsible for BAFF signaling impairment in autoimmune and neoplastic diseases. We evaluated BAFF and BAFF-receptor (BAFF-R) polymorphisms in order to determine if they predispose to HCV-related CV and NHL. The analysis was performed on 416 HCV-chronically infected patients: 136 HCV without signs/symptoms of lymphoproliferations/autoimmunity (HCV), 166 HCV with CV (HCV-CV) and 114 HCV with NHL (HCV-NHL). Rs9514828 SNP on BAFF promoter, rs61756766 on BAFF-R and rs12428930 on the BAFF gene were evaluated by Real-Time PCR. Concerning rs9514828, the frequency of C/T genotype was significantly higher in HCV-CV than in HCV. The difference in the distribution of the T/T mutant genotype in HCV-CV compared to HCV was significant as well as the distribution of C/T and T/T genotype in HCV-NHL versus HCV. T minor allele was more frequent in HCV-NHL and HCV-CV than in HCV. The distribution of C/T + T/T (for the dominant model of penetrance C/T + T/T vs. C/C) was significantly higher in HCV-CV and HCV-NHL than in HCV. Genotyping of rs61756766 on BAFF-R coding gene, revealed C/T heterozygosity at a frequency of 11% in HCV-NHL versus 3% in HCV. The T minor allele frequency was higher in HCV-NHL than in HCV. No differences emerged by genotyping rs12428930 SNP on BAFF coding gene. Our results reinforce the hypothesis that BAFF/BAFF-R genetic pattern has a role in the pathogenesis of HCV-related lymphoproliferations. BAFF/BAFF-R variants could identify a risk haplotype for HCV related CV and NHL and a BAFF/BAFF-R genetic profile assessment could potentially contribute to tailoring anti-BAFF therapy by identifying patients with BAFF alterations in which the treatment could be more beneficial.

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KEYWORDS

B-cell activating factor, belimumab, cryoglobulinemia, hepatitis C virus, lymphoma, single nucleotide polymorphism

1 | INTRODUCTION

Hepatitis C virus (HCV) chronic infection results in liver damage and, in some cases, causes autoimmune/lymphoproliferative disorders (LPDs) ranging from clinically benign diseases such as Cryoglobulinemic Vasculitis (CV) to B cell Non-Hodgkin's Lymphoma (NHL).¹⁻⁵ HCV patients with CV suffer from mild to severe symptoms as consequence of a systemic vasculitis⁶ and have a 35 times higher risk to develop a NHL compared to the general population.⁷

Innate immunity factors may contribute to the pathogenesis of lymphoproliferative and autoimmune disorders, including those HCV-related.⁸

The B cell activating factor (BAFF), a cytokine member of TNF- α family produced by monocytes, is a key regulator in B-cell development and survival.⁹ BAFF binds to three different receptors on the B-cell surface: transmembrane activator and CAML-interactor (TACI), B-cell maturation antigen (BCMA) and BAFF receptor (BAFF-R).¹⁰ Among these three, BAFF-R is specific for BAFF while the others also bind to a proliferation-inducing ligand (APRIL).¹¹ BAFF-R is expressed on the surface of the B-cell,¹¹ the signal mediated by BAFF/BAFF-R binding is important for the survival and growth of normal B-cells¹² and it reduces B-cell apoptosis in B-cell NHL.¹³ Moreover, overexpression of BAFF contributes to the development of several autoimmune/LPDs such as Sjögren's Syndrome (SS),¹⁴ systemic lupus erythematosus (SLE)¹⁵ and HCV-related CV.^{16,17} Several studies report high levels of BAFF and/or its receptor in patients with autoimmune and neoplastic disease.^{18,19}

The increased BAFF expression levels associated with NHL and autoimmune diseases seem to be attributable to the presence of different allelic variants in the BAFF coding sequence, BAFF promoter gene and BAFF-R gene.^{20,21} Previous data suggested that the single nucleotide polymorphism (SNP) rs9514828 (-871C/T) on the BAFF promoter was associated with an increased gene expression and a higher risk of lymphoproliferative disorders such as HCV-related CV.²²⁻²⁵

In addition, Nezos and collaborators reported a higher frequency of minor allele T of rs9514828 in patients with primary SS (pSS) and correlated it to a higher risk of the development of lymphomas compared to healthy controls.²⁶ In addition, two haplotypes containing rs9514828 SNPs were more frequent in pSS low risk patients' group.²⁶

An allelic variant on BAFF-R, the His159Tyr mutation (rs61756766), appears to confer an increased risk of the development of NHL through the NF- κ B pathway activation which induces apoptosis inhibition and magnifies all the described effects of BAFF.²⁷ An increased prevalence of rs61756766 has been reported in patients with SS and SS-related lymphomas.²⁸ This mutation has also been linked to a shorter interval prior to the development of lymphoma.²⁸

An increased risk of developing lymphoproliferative disorders has also been associated with other SNPs in an intronic region of BAFF gene: rs1224141, rs12583006 and rs12428930.²⁹ The presence of these risk alleles are strongly associated with a predisposition to follicular lymphoma and diffuse large B-cell lymphoma (DLBCL) and has been linked with high levels of BAFF in patient's serum.²⁹

The aim of this study was the analysis of previously described allelic variants of the BAFF/BAFF-R system in order to clarify whether they may lead to a predisposition of developing HCV-related LPDs and determine if their genetic pattern could be valuable in establishing a more rational, personalized therapy.

2 | PATIENTS & METHODS

2.1 | Patients

The study retrospectively analyzed 416 HCV-chronically infected patients: 136 HCV without signs/symptoms of LPD and/or autoimmune disease (HCV), 166 HCV with signs/symptoms of CV (HCV-CV) and 114 HCV with NHL diagnosis (HCV-NHL). Patients were recruited at the Center for Systemic Manifestations of Hepatitis Viruses (MaSVE) of the University of Florence, at the Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy and at the Department of Biomedical and Biotechnological Sciences, University of Catania, Italy. All patients were Caucasian and the main demographical, clinical and laboratory data are reported in Table 1.

As reported in Table 1, the NHL group included mainly DLBCL and marginal zone lymphomas and a smaller number of various subtypes (follicular lymphomas; mucosa-associated lymphoid tissue lymphomas; mantle cell lymphomas; small lymphocytic lymphomas; B cell chronic lymphoproliferative diseases).

HCV infection was confirmed by the detection of HCV RNA (AMPLICOR HCV Test, v2.0; Roche Diagnostics, Alameda, CA). The HCV genotype was determined by the VERSANT HCV Genotype 2.0 assay (Siemens Healthcare Diagnostics, Deerfield, IL). Cryoglobulinemia was diagnosed by the detection of circulating cryoglobulins in at least three metachronous samples. Vasculitis was diagnosed following previously published criteria.^{30,31} NHL type was defined according to the updated WHO classification of hematological malignancies.³²

2.2 | Ethical statements

This study was conducted in accordance with the ethical guidelines of the Helsinki Declaration. All subjects provided written informed consent and the protocol was approved by independent local ethics

	HCV (n = 136)	HCV-CV (n = 166)	HCV-NHL (n = 114)
Mean age (years)	55.3 ± 9.6	64.6 ± 10.9	64.5 ± 5.6
Sex (M/F)	77/59	61/105	42/72
Viral titer (IU/mL × 10 ⁶) ^a	2.9 ± 3.1	3.4 ± 6.6	3.5 ± 4.1
HCV genotype			
1	75 (55%)	91 (55%)	97 (85%)
2	38 (28%)	50 (30%)	13 (12%)
3–4	23 (17%)	25 (15%)	4 (3%)
Mean cryocrit ^b	/	12 ± 14.8	6.7 ± 4.2
Histology			
DLBCL			32 (28%)
MZL			56 (49%)
FL			6 (5%)
MALT lymphoma			3 (3%)
MCL			2 (2%)
Burkitt lymphoma			1 (1%)
SLL			5 (4%)
B-CLPD			9 (8%)

TABLE 1 Main demographical and clinical characteristics of the study sample

Abbreviations: B-CLPD, B cell chronic lymphoproliferative disease; CV, Cryoglobulinemic Vasculitis; DLBCL, Diffuse Large B-Cell Lymphoma; FL, Follicular Lymphoma; HCV, Hepatitis C Virus; IU, International Units; MALT, Mucose-Associated Lymphoid Tissue; MCL, Mantle Cell Lymphoma; MZL, Marginal Zone Lymphoma; NHL, Non-Hodgkin's Lymphoma; SLL, Small Lymphocytic Lymphoma.

^adata were available for 91/114 (80%) of lymphoma patients.

^bdata were available for 49/114 (43%) of lymphoma patients.

committees (approval code for the MaSVE center: BIO.16.014, approval code for Fondazione IRCCS Policlinico San Matteo 47493/2020).

Experimental procedures were conducted at the MaSVE laboratory (University of Florence, Florence, Italy) and at the Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy).

2.3 | Cell isolation and DNA extraction

Peripheral blood mononuclear cells (PBMCs) were isolated from fresh anticoagulated blood by gradient precipitation on Lymphoprep (Axis-Shield PoC AS, Oslo, Norway) according to the manufacturer's instructions. After the second wash, the cells were counted and stored at -80°C . Genomic DNA was extracted from PBMCs using the QIAamp DNA Mini Kit (QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions. QIAamp® DNA Blood Midi kit (QIAGEN GmbH, Hilden, Germany) for DNA extraction from whole blood was used when stored PBMC were not available and peripheral blood samples had been collected.

The rs9514828, rs12428930 and rs61756766 SNP genotyping was conducted using TaqMan SNP-Genotyping Assay (Life Technologies Corporation, Carlsbad CA-USA) based on allele-specific dual-

labeled probes on a Rotor Gene 6000 (Corbett Research, Sidney, Australia), as previously described.³³

2.4 | Measurement BAFF levels

Circulating plasma levels of BAFF were measured using a Human BAFF Quantikine ELISA Kit (R&D Systems, Inc) in 36/136 HCV, 36/166 HCV-CV and in 37/114 HCV-NHL retrospective plasma samples stored at -20°C .

2.5 | Statistical analysis

Statistical analysis was performed by GraphPad Prism statistical software. Continuous variables were expressed as mean \pm SD, categorical variables displayed as frequencies and Kruskal Wallis ANOVA (Dunn multiple comparison test) or X^2 /Fisher's exact test were applied to assess significance of the differences among subgroups, respectively. The Hardy-Weinberg Equilibrium (HWE) was analyzed by the X^2 -test. On the base of genotypes distribution in the compared groups (both HCV vs. CV + NHL and HCV vs. NHL) we also performed a dominant/additive model analysis by means logistic regression as previously

described.³⁴ All tests were 2-sided and, after adjustment for multiple measures, a p -value <0.01 was considered statistically significant. The correction was performed to make statistical tests more stringent, adjusting the statistical inference of multiple comparisons.

3 | RESULTS

We analyzed genotype distribution and allelic frequencies of rs9514828, rs12428930 and rs61756766 SNPs of BAFF/BAFF-R system in HCV positive patients with and without CV and NHL. Results are shown in Figure 1 Panel A-B-C. The genotype distribution fitted the Hardy Weinberg Equilibrium.

The frequency of the C/T genotype in rs9514828 was significantly higher in HCV-CV than in HCV (50% vs. 42% $p = 0.01$). The difference in the distribution of the T/T mutant genotype in HCV-CV compared to HCV was 24% versus 16% respectively ($p = 0.02$ which is not significant after the adjustment for multiple testing). The distribution of C/T and T/T genotype in HCV-NHL versus HCV is respectively 52% HCV-NHL versus 42% HCV $p = 0.01$ and 24% HCV-NHL versus 16% HCV $p = 0.02$. The presence of the T minor allele was more frequent in HCV-NHL and HCV-CV than in HCV-controls (50% HCV-NHL vs. 37% HCV $p = 0.005$ OR = 1.71 95% CI = 1.18–2.48; and 49% HCV-CV vs. 37% HCV $p = 0.007$ OR = 1.62 95% CI = 1.15–2.28).

The distribution of C/T + T/T (for the dominant model of penetrance C/T + T/T vs. C/C) was significantly higher in HCV-CV and HCV-NHL than in HCV without lymphoproliferative disorders (74% HCV-CV vs. 58% HCV $p = 0.005$ OR = 2.06 95% CI = 1.27–3.35 and 75% HCV-NHL vs. 58% HCV $p = 0.005$ OR = 2.21 95% CI = 1.38–3.28).

On the base of genotype distribution in the compared groups (both HCV vs. HCV-CV + HCV-NHL and HCV vs. HCV-NHL), we also performed a dominant/additive model analysis by means logistic regression. When considering rs9514828 (BAFF-P) we obtained for HCV versus HCV-CV + HCV-NHL subjects a value of $\beta = 0.75 \pm 0.22$, $p = 0.001$, OR = 2.1 [1.4–3.3] and $\beta = 0.49 \pm 0.15$, $p = 0.001$, OR = 1.6 [1.2–2.2] - homozygote OR = 2.7, for dominant and additive model, respectively (similar results were obtained when comparing HCV vs. NHL patients - data not shown).

In addition, since age is considered a risk factor for lymphoproliferative disorders, we performed a correction for age, stratifying patients by median age (cut-off: 65 years). The statistical significance was maintained in the subgroup of younger patients ($p = 0.032$).

We subsequently analyzed the genotype distribution of rs61756766 SNP on BAFF-R coding gene. In HCV-NHL, the C/T heterozygote genotype had a frequency of 11% versus 3% in HCV ($p = 0.02$ which is not significant after the adjustment for multiple testing). As expected, we did not find patients with a T/T genotype. The T minor allele frequency was higher in HCV-NHL than in HCV-controls (6% HCV-NHL vs. 1% HCV $p = 0.004$ OR = 5.40 95% CI = 1.52–19.20).

Due to the low frequency of rs61756766 C/T genotype, it was not possible to perform the adjustment for patient age.

The genotype analysis of rs12428930 SNP on BAFF coding gene did not reveal any significant difference among the groups.

The genotype and allele frequency of all three SNPs were summarize in Table 2.

We assessed the BAFF protein levels in retrospective plasma samples, available for 36 HCV, 36 HCV-CV and 37 HCV-NHL patients. BAFF levels were significantly higher in HCV-NHL than in HCV (940.2 vs. 695.9 pg/ml $p = 0.0145$) and HCV-CV (940.2 vs. 686.4 pg/ml $p = 0.0087$). Results are reported in Figure 2.

The BAFF-P genotype distribution in the subsets of patients analyzed for BAFF serum levels did not allow a reliable statistical analysis due to low number of cases representing each genotype.

Interestingly when the samples analyzed for circulating BAFF levels are considered as a unique population of 109 patients, an increasing trend in the protein mean values going from wild-type homozygosis to mutated is observed (CC (19 patients) = 622 pg/ml; CT (53 patients) = 740 pg/ml; TT (37 patients) = 750 pg/ml).

4 | DISCUSSION

We evaluated the association of three allelic variants of BAFF/BAFF-R system in HCV patients with and without LPDs. We had previously shown that rs9514828 was more frequent in the HCV-CV group compared to HCV patients^{23,24} and our results were confirmed in other cohorts of HCV-CV patients.²⁵ Here we investigated the presence of this SNP in a larger population ($N = 416$ patients), confirming previous results^{23–25} and demonstrating, for the first time, a higher frequency of heterozygosis and T minor allele in HCV-NHL than in the HCV group. This association of rs9514828 T minor allele could potentially be a non-invasive biomarker of disease evolution in HCV patients with lymphoproliferative disorders, and the cause for the higher levels of BAFF cytokine that have been previously associated with these conditions.^{16–19}

Here we assessed circulating BAFF levels in a subset of serum samples for each patient subgroup, finding higher levels in the HCV-NHL compared to both HCV-CV and HCV subjects. Surprisingly, no difference was observed between HCV-CV and HCV, a conflicting result compared to previous data.^{16,23,24} This discrepancy could be mainly attributable to an unbalanced distribution of the BAFF-P genotypes among the three subsets as well as to the small numbers representing each genotype in the single subsets.

When the samples analyzed for circulating BAFF levels are considered as a unique population, an increasing trend in the protein mean values going from wild-type homozygosis to mutated is observed. This, despite the small sampling and the biased genotype distribution, seems to confirm the higher activity of the BAFF-P mutated allele as already reported by other authors.^{29,35} In fact, Novak and coworkers firstly demonstrated that mean serum BAFF level in those who carried the low-risk alleles was significantly lower compared to those with the high-risk alleles.^{29,35}

Our results concerning BAFF-R rs61756766 allele distribution are consistent with previous findings highlighting an association of

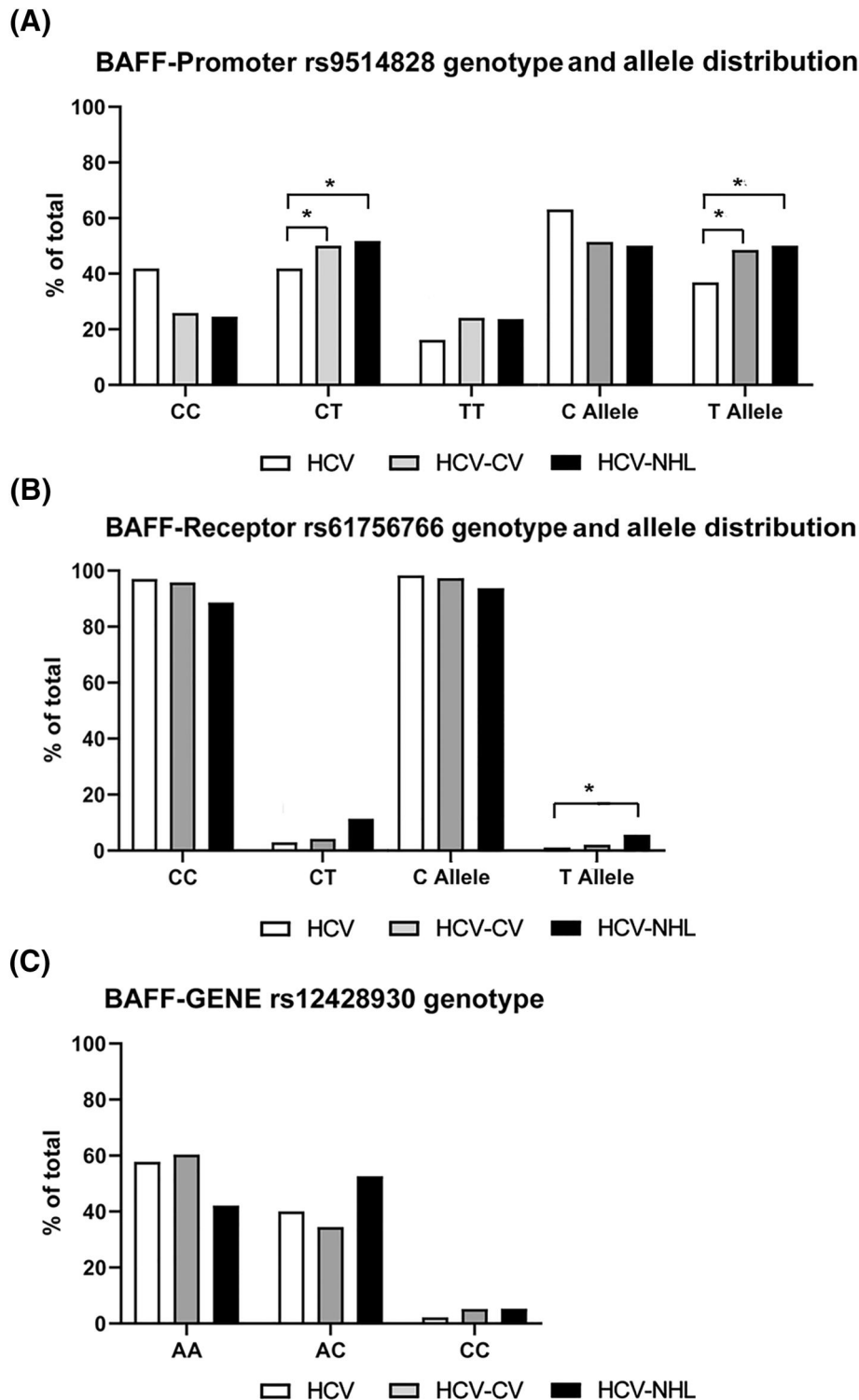


FIGURE 1 Genotype and allele distribution of rs9514828, rs61756766 and rs12428930 SNPs of BAFF/BAFF-R system in HCV, HCV-CV and HCV-NHL subgroups. Panel (A) Genotype and allele distribution of rs9514828 in HCV, HCV-CV and HCV-NHL sub-groups. Panel (B) Genotype and allele distribution of rs61756766 in HCV, HCV-CV and HCV-NHL subgroups. Panel (C) Genotype distribution of rs12428930 in HCV, HCV-CV and HCV-NHL subgroups. * $p \leq 0.01$. HCV, Hepatitis C Virus positive patients without signs/symptoms of lymphoproliferative disorders and/or autoimmune diseases; HCV-CV, Hepatitis C Virus positive patients with Cryoglobulinemic Vasculitis; HCV-NHL, Hepatitis C Virus positive patients with Non-Hodgkin's Lymphoma

the minor allele with autoimmune disorders such as SS.²⁸ As expected, we did not find any mutant homozygotes in BAFF-R rs61756766.²¹

Additionally, the higher lymphoma risk in HCV-CV patients attributable to BAFF-promoter germline variants resembles previous findings described in SS subjects with lymphoma.²⁶ In fact, SS

TABLE 2 Genotype and allele frequency of rs9514828, rs61756766 and rs12428930 SNPs of BAFF/BAFF-R system in HCV, HCV-CV and HCV-NHL subgroups

Polymorphism	HCV (n = 136)	HCV-CV (n = 166)	HCV-NHL (n = 114)	p	OR (95% CI)
BAFF-promoter rs9514828					
Genotypes					
CC	57 (42%)	43 (26%)	28 (24%)	n.s.	
CT	57 (42%)	83 (50%)	59 (52%)	HCV-CV versus HCV p = 0.01	1.93 (1.14–3.18)
				HCV-NHL versus HCV p = 0.01	2.10 (1.16–3.81)
TT	22 (16%)	40 (24%)	27 (24%)	HCV-CV versus HCV p = 0.02	2.41 (1.26–4.56)
				HCV-NHL versus HCV p = 0.02	2.49 (1.20–5.02)
CC:CT + TT	57 (42%):79 (58%)	43 (26%):123 (74%)	28 (25%):86 (75%)	HCV-CV versus HCV p = 0.005	2.06 (1.27–3.35)
				HCV-NHL versus HCV p = 0.005	2.21 (1.38–3.28)
CC + CT:TT	114 (84%):22 (16%)	126 (76%):40 (24%)	87 (76%):27 (24%)	n.s.	
Alleles					
C	171 (63%)	169 (51%)	115 (50%)	n.s.	
T	101 (37%)	163 (49%)	113 (50%)	HCV-CV versus HCV p = 0.007	1.42 (1.15–2.28)
				HCV-NHL versus HCV p = 0.005	1.71 (1.18–2.48)
BAFF receptor rs61756766					
Genotypes					
CC	132 (97%)	159 (96%)	101 (89%)	n.s.	
CT	4 (3%)	7 (4%)	13 (11%)	HCV-NHL versus HCV p = 0.01	4.24 (1.37–12.17)
Alleles					
C	268 (99%)	325 (98%)	215 (94%)	n.s.	
T	3 (1%)	7 (2%)	13 (6%)	HCV-NHL versus HCV p = 0.004	5.40 (1.52–19.20)
BAFF gene rs12428930					
Genotypes					
AA	80 (58%)	100 (60%)	48 (42%)	n.s.	
AC	54 (40%)	56 (34%)	59 (52%)	n.s.	
CC	2 (2%)	10 (6%)	7 (6%)	n.s.	
Alleles					
A	214 (79%)	256 (76%)	155 (68%)	n.s.	
C	58 (21%)	76 (24%)	73 (32%)	n.s.	

Abbreviations: HCV, Hepatitis C Virus positive patients without sign/symptoms of lymphoproliferative disorders and/or autoimmune diseases; HCV-CV, Hepatitis C Virus positive patients with Cryoglobulinemic Vasculitis; HCV-NHL, Hepatitis C Virus positive patients with Non-Hodgkin's Lymphoma; Odds Ratio; OR, n.s., not significant.

patients with a high risk of developing hematological malignancies were characterized by higher frequency of the minor T allele of the rs9514828 BAFF polymorphism compared to healthy controls.²⁶ Hence, a specific genetic pattern may be linked to the increased lymphoma risk in different autoimmune disorders and it is conceivable that the evolution toward malignancy shares common pathogenic pathways.

In a recent analysis, BAFF levels in the cerebrospinal fluid decreased after remission induced by therapy and increased after therapy failure in primary central nervous system B-cell

lymphomas.³⁶ The authors speculated that the level of surviving tumor cells may affect the cerebrospinal fluid levels of BAFF and that BAFF production may promote the lymphoma B-cells survival, unless remission is achieved. In fact, one patient presented clinical remission with decreased BAFF levels after treatment, while the patient with no therapeutic response had increased BAFF levels.

Following the evidence of its involvement in autoimmune/lymphoproliferative disorders, BAFF pathway is considered a potential therapeutic target.

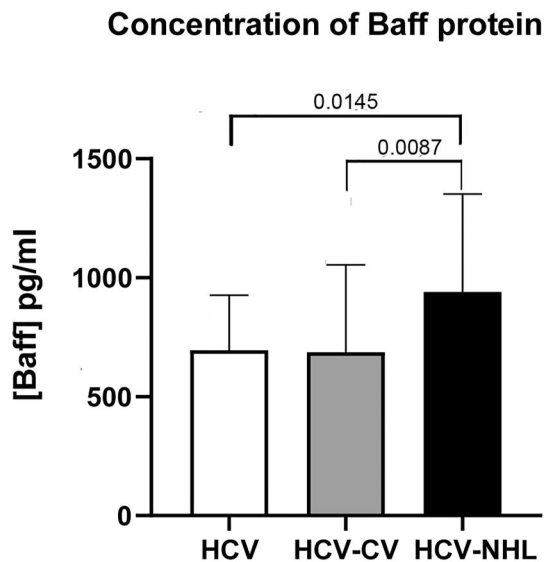


FIGURE 2 Concentration of plasma levels of BAFF protein in HCV, HCV-CV and HCV-NHL subgroups. BAFF protein concentration in plasma samples of HCV, HCV-CV and HCV-NHL subgroups. HCV, Hepatitis C Virus positive patients without sign/symptoms of lymphoproliferative disorders and/or autoimmune diseases; HCV-CV, Hepatitis C Virus positive patients with Cryoglobulinemic Vasculitis; HCV-NHL, Hepatitis C Virus positive patients with Non-Hodgkin's Lymphoma

Belimumab, a monoclonal antibody, binds to soluble form of BAFF preventing BAFF/BAFF-R binding and is both FDA and EMA approved for SLE treatment.³⁷ Pontarini and colleagues demonstrated a significant reduction of both BAFF and BAFF-R in SS patients treated with Belimumab, suggesting that it could regulate BAFF/BAFF-R interaction.³⁸ Belimumab has been successfully used in sequential therapy with Rituximab (RTX), an anti-CD20 monoclonal antibody, in patients with autoimmune disease who experienced previous therapy failure or relapse of symptoms.³⁹⁻⁴¹

Very recently, a French pilot study reported promising results in a case series study of 4 CV patients treated with Belimumab associated to RTX after having failed previous treatments.⁴² The authors described a good efficacy of the dual therapy paving the way to new therapy options for severe/refractory CV. Subjects who failed RTX usually experience higher BAFF levels after B-cell depletion and, in SLE patients, this caused a consequent increase of autoantibodies.⁴²

Similarly, a very recent paper described a case of refractory type II CV associated with Sjögren's syndrome, successfully controlled with Belimumab and hydroxychloroquine, even after discontinuation of RTX.⁴³

Therefore, a dual combination of RTX or other agents (i.e., hydroxychloroquine) plus Belimumab could assist in minimizing the risk of treatment failure and in preventing a BAFF rebound effect. In the light of this new CV treatment perspective suggested by previous reports,^{42,43} our study suggests that a genetic rationale may help a more targeted and successful approach. Further studies will clarify if genotyping of rs9514828 and rs61756766 SNPs could

identify CV patients with genetic linked impairment of the entire BAFF signaling pathway.

It is conceivable that a clear and complete picture of BAFF-P/BAFF-R genetic pattern could potentially contribute to tailoring the use of Belimumab, limiting its administration to patients with BAFF level alterations for which this therapy may be particularly beneficial, thus lowering treatment cost and minimizing side effects.

In particular, since the mutated BAFF receptor (rs61756766) is able to recruit TRAF2/3 and, in absence of BAFF binding, triggers a hyperactive and autonomous signaling,²⁷ Belimumab could be not effective in patients carrying this receptor variant. Diversely, the presence of BAFF promoter variant associated with increased protein expression, may be a good rationale for the use of the anti-BAFF treatment. Further dedicated studies will ascertain if subjects carrying both rs9514828 and rs61756766 mutated alleles could be the best candidates for the Belimumab therapy suggested by previous analyses.^{42,43}

5 | CONCLUSIONS

Our results clearly reinforce the hypothesis that BAFF/BAFF-R system is involved in the pathogenesis of HCV-related LPDs and suggest the need of further analyses to ascertain if a BAFF/BAFF-R genetic profile assessment could potentially contribute to tailoring the use of Belimumab by identifying patients with BAFF alterations in which this therapy could be more beneficial.

Further investigations aimed at screening all the known variants of the BAFF/BAFF-R system will be valuable in characterizing a risk haplotype for the development of HCV related NHL, consequently allowing the initiation of appropriate preventive measures.

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CONFLICT OF INTEREST

All authors declare that they have no conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization: Laura Gragnani; methodology: Laura Gragnani, Serena Lorini and Silvia Marri; validation, Serena Lorini, Enrico Di Stasio and Silvia Marri; formal analysis, Serena Lorini and Enrico Di Stasio; investigation Serena Lorini, Silvia Marri, Sara Rattotti, Silvia Zibellini, Umberto Basile and Francesco Madia; data curation, Monica Monti, Francesco Madia, Sara Rattotti and Silvia Zibellini; writing—original draft preparation Laura Gragnani and Serena Lorini; writing—review and editing, Laura Gragnani, Luca Arcaini, Massimo Libra and Anna Linda Zignego; supervision, Laura Gragnani, Luca Arcaini, Massimo Libra and Anna Linda Zignego.; project administration Laura Gragnani; funding acquisition, Laura Gragnani, Monica Monti, Luca Arcaini and Anna Linda Zignego. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable.

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PEER REVIEW

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