Extrahepatic disorders of HCV infection: A distinct entity of B-cell neoplasia?

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Abstract. Some infectious agents have been associated with B-cell lymphoma development. In the last decades, it has been demonstrated that patients infected by hepatitis C virus (HCV) are more likely to develop B-cell non-Hodgkin's lymphoma (NHL) than those uninfected. The prevalence of HCV-infection among NHL patients is reported in this review of all Italian studies on NHL and HCV infection, both case-control and case series. From 18 studies, the prevalence of anti-HCV antibodies in 2736 NHL patients was 19.7% (range: 8.3-37.1%). The association of HCV-infection with each NHL histotype in case-control studies is discussed. Molecular mechanisms by which HCV infection promotes B-cell NHL development is also explored and indicate that HCV-associated lymphomas may be a distinct entity. Clarification of these mechanisms may improve diagnosis, classification and therapy of this subset of NHL. Finally, treatment of HCV-positive patients with lymphoproliferative disorders are herein summarized and further support the notion that HCV infection contributes to the development of these pathologic conditions.

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1. Introduction

Non-Hodgkin's lymphomas (NHL) are a heterogeneous group of lymphoproliferative malignancies with variable patterns of behavior and responses to therapy. Infectious agents have been associated with B-cell lymphoma development such as, human T-cell lymphotropic virus-1 (1), Epstein-Barr virus (EBV) (1,2), and, indirectly, human immunodeficiency virus (HIV) (3,4).

In the last two decades, several studies indicated that also hepatitis C virus (HCV) infection may contribute to the development of NHL (5-12). Interestingly, in a systematic review of 66 studies focused on HCV infection and NHL development, Negri *et al* (9), reported that the largest number of these studies were carried out in Italy, and they showed a high prevalence of HCV infection among NHL cases, ranging from 8.9 to 37.1%. However, most of these reports showed conflicting data on prevalence of HCV-infection among each NHL histotype. In this report, we discuss this feature through the review of all Italian case-control and case series investigation on NHL and HCV infection.

The mechanisms through which HCV infection promotes B-cell NHL development are still unclear. Accumulating evidence supports a model in which chronic stimulation of B-cells by antigens associated with HCV infection causes non-malignant B-cell expansion that may evolve into B-cell NHL. Elucidation of the mechanisms by which HCV promotes development of lymphoproliferative disorders is also discussed here, given their importance in improving diagnosis classification and treatment of HCV-associated lymphomas.

Table I. Prevalence of anti-HCV antibodies (Abs) and/or HCV-RNA among Italian case-control studies and case-series of non-Hodgkin's lymphoma patients.

Authors/refs.	Location	All NHL cases	NHL cases[a]	Anti-HCV Abs positivity		HCV RNA positivity	
				NHL	% (95% CI)	NHL	% (95% CI)[b]
All studies		2810	2736	539	19.7 (18.2-21.2)	206	14.7 (12.8-16.6)
Case-control studies		1195	1168	272	23.3 (20.9-25.7)	124	21.6 (18.2-24.9)
Talamini *et al* (10)	Pordenone/Naples	225	202	40	19.8 (14.3-25.3)		
Mele *et al* (6)	9 Italian towns	400	400	70	17.5 (13.8-21.2)	60	15.0 (11.5-18.5)
Guida *et al* (86)	Bari	60	56	12	21.4 (10.7-32.2)		
Montella *et al* (87)	Naples	101	101	25	24.8 (16.3-33.2)		
Vallisa *et al* (88)	Piacenza	175	175	65	37.1 (30.0-44.3)	64	36.6 (29.4-43.7)
De Vita *et al* (89)	Aviano/Pordenone	84	84	20	23.8 (14.7-32.9)		
Musto *et al* (90)	Foggia	150	150	40	26.7 (19.6-33.7)		
Case series		1615	1568	267	17.0 (15.2-18.9)	82	9.9 (7.9-12.0)
De Renzo *et al* (91)	Naples	61	61	12	19.7 (9.7-29.6)		
Pioltelli *et al* (92)	Northern Italy	300	300	48	16.0 (11.9-20.1)	41	13.7 (9.8-17.6)
Luppi *et al* (93)	Modena	157	157	35	22.3 (15.8-28.8)		
Catassi *et al* (94)	8 Italian towns	143	104	15	14.4 (7.7-21.2)		
Silvestri *et al* (17)	Udine	470	470	42	8.9 (6.4-11.5)	31	6.6 (4.4-8.8)
Pivetti *et al* (95)	Turin	47	47	7	14.9 (4.7-25.1)		
Pioltelli *et al* (96)	Milan	126	126	26	20.6 (13.6-27.7)		
Musolino *et al* (97)	Messina	24	24	2	8.3 (0.0-19.4)	5	20.8 (4.6-37.1)
Mazzaro *et al* (5)	NorthEast Italy	199	197	55	27.9 (21.7-34.2)		
Andriani *et al* (98)	Rome	38	32	8	25.0 (10.0-40.0)	5	15.6 (3.0-28.2)
Ferri *et al* (99)	Pisa	50	50	17	34.0 (20.9-47.1)		

[a]T-cell lymphoma, monoclonal gammapathies and unspecified were excluded. [b]Percentage accounted on 1401 NHL cases (575 from case-control studies and 826 from case series) tested for HVC RNA.

2. Methods

We identified reports on HCV and lymphomas published, in English, between 1996 and July 2009 through a MEDLINE search strategy based on the following words (all fields): 'hepatitis C virus' or HCV, and non-Hodgkin's lymphoma. For the analysis of NHL histotype by HCV positivity we used case-control or case series studies reporting information on: a) the prevalence of HCV infection in NHL patients and in control group; b) the availability of anti-HCV antibodies (Abs) and/or HCV-RNA test; c) Working Formulation (WF), or Revised European American Lymphoma (REAL), or World Health Organization (WHO) classification (13-15). In order to standardize these studies, the NHLs were recoded into the WHO and/or WF classification, whenever applicable. T-cell lymphomas, monoclonal gammopathies, unspecified and HIV-positive cases were excluded from the analysis.

3. Prevalence of anti-HCV Abs and/or HCV-RNA and HCV genotypes among Italian case-control studies and case series of non-Hodgkin's lymphoma patients

To analyze the prevalence of anti-HCV Abs among Italian case-control studies and case series of NHL patients, a total number of 2736 cases were studied. The number of anti-HCV Abs positive and/or HCV-RNA positive cases, reported in each study (case-control studies and case series) are shown in Table I. Overall, the prevalence of anti-HCV Abs in NHL patients was 19.7%, ranging from 8.3 to 37.1%. The prevalence was 23.3 and 17.0% for case-control and case series studies, respectively, but it was more homogeneous among case-control studies (range 17.5-37.1%) than among case series (range 8.3-34.0%). Selection bias may partly explain this difference. Indeed, NHL cases reported in case series may not represent the histological distribution seen in the general population, thus impacting on HCV prevalence. Conversely, multi-centric case-control studies usually pay great attention in reducing this kind of bias (16). The prevalence of HCV-RNA was 14.7% for all case (21.6 and 9.9% case-control studies and case series respectively), but consistent variability was observed for both case-control studies (range 15-36.6%) and case series (range 6.6-20.8%) (Table I).

To investigate the association of HCV-infection with each NHL histotype, we analyzed the prevalence of HCV antibodies according to both Working Formulation (Table II) and WHO classification (Table III). Although, the higher prevalence of HCV-positive cases was accounted among high-grade lymphomas (20.7%), no significant difference

Table II. Prevalence of hepatitis C virus (HCV) antibodies (aHCV) in NHL cases, according to Working Formulation classification.

	Low grade			Intermediate grade			High grade		
Autors/refs.	NHL	aHCV+	% (95% CI)	NHL	aHCV+	% (95% CI)	NHL	aHCV+	% (95% CI)
All studies	1132	214	18.9 (16.6-21.2)	276	48	17.4 (12.9-21.9)	801	166	20.7 (17.9-23.5)
Case-control studies	368	79	21.5 (17.3-25.7)	94	20	21.3 (13.0-29.6)	451	109	24.2 (20.2-28.1)
Talamini *et al* (10)	78	16	20.5 (11.6-29.5)	2	0	0.0 (-)	122	24	19.7 (12.6-26.7)
Mele *et al* (6)	150	24	16.0 (10.1-21.9)	15	2	13.3 (0.0-30.5)	215	41	19.1 (13.1-24.3)
Guida *et al* (86)	36	8	22.2 (8.6-35.8)	17	3	17.6 (0-35.8)	3	1	33.3 (0.0-86.7)
Montella *et al* (87)	31	3	9.7 (0.0-20.1)	48	11	22.9 (11.0-34.8)	21	10	47.6 (26.3-69.0)
Vallisa *et al* (88)	73	28	38.4 (27.2-49.5)	12	4	33.3 (6.7-60.0)	90	33	36.7 (26.7-46.6)
Case series	764	135	17.7 (15.0-20.4)	182	28	15.4 (10.1-20.6)	350	57	16.3 (12.4-20.2)
De Renzo *et al* (91)	13	3	23.1 (0.2-46.0)	10	2	20.0 (0.0-44.8)	38	7	18.4 (6.1-30.7)
Pioltelli *et al* (92)	100	17	17.0 (9.6-24.4)	73	10	13.7 (5.8-21.6)	136	22	16.2 (10.0-22.4)
Luppi *et al* (93)	102	27	26.5 (17.9-35.0)	20	0	0.0 (-)	35	8	22.9 (8.9-36.8)
Silvestri *et al* (17)	375	37	9.9 (6.8-12.9)	11	1	9.1 (0.0-26.1)	80	4	5.0 (0.2-9.8)
Musolino *et al* (97)	18	1	5.6 (0.0-16.1)	5	1	20.0 (0.0-55.1)	1	0	0.0 (-)
Mazzaro *et al* (5)	110	40	36.4 (27.4-45.4)	48	6	12.5 (3.1-21.9)	39	9	23.1 (9.9-36.3)
Andriani *et al* (98)	22	4	18.2 (2.1-34.3)				10	4	40.0 (9.6-70.4)
Ferri *et al* (99)	24	6	25.0 (7.7-42.3)	15	8	53.3 (28.1-78.6)	11	3	27.3 (1.0-53.6)

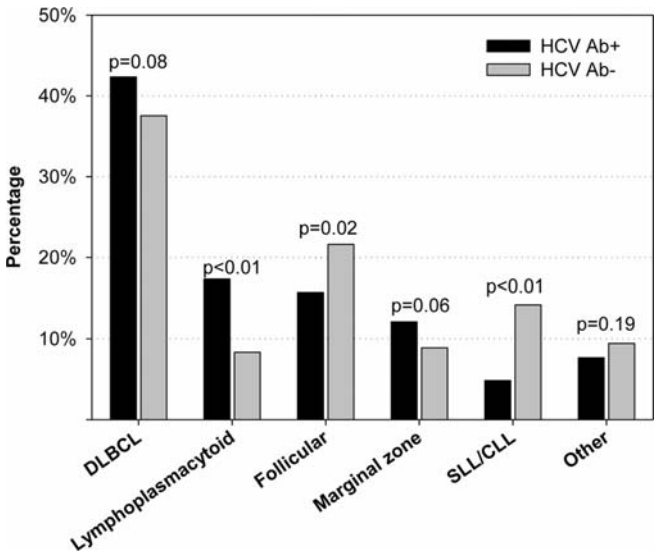

Figure 1. Distribution of NHL by histological subtypes according to positivity to hepatitis C virus (HCV) antibodies in case-control studies. Comparison between percentage was carried out through z-test. DLBCL, diffuse large B-cell lymphoma; SLL/CLL, small lymphocytic/lymphoma/chronic lymphocytic leukemia.

was observed with respect to low and intermediate grade (Table II). Intriguingly, among all case series, the prevalence of HCV-infected cases for each grade was lower then that observed among case-control studies (Table II). The prevalence of anti-HCV Abs among NHL histotypes, ranked by HCV prevalence was reported in Table III. The higher prevalence of anti-HCV Abs was observed among lymphoplasmacytoid/lymphoplasmacytic/immunocytoma histotype (32.1%) whereas the lowest was among small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) (7.5%) (Table III). Distribution of NHL by histological subtypes according to positivity to HCV antibodies in case-control studies is reported in Fig. 1. Lymphoplasmacytoid subtype was more frequently reported among anti-HCV Ab-positive cases than among anti-HCV Ab-negative counterparts. Conversely, follicular lymphoma and SLL/CLL were more frequent among anti-HCVAb-negative subjects.

The prevalence of HCV genotypes in NHL cases is reported in Table IV. The most prevalent HCV genotype was 2a/2c followed by 1b. However, the difference between these two genotypes was not statistically significant. Analyses of HCV genotypes among infected B-cell NHL patients have yielded conflicting results. HCV genotypes 2a/2c were observed to be more frequent among B-cell NHL patients than either blood donors or individuals with chronic liver disease (17). Another study (10) reported a higher ratio of HCV genotypes 2a/2c to HCV genotype 1b among B-cell NHL patients than among control subjects, but this difference was not statistically significant. In contrast, Mele *et al* (6) did not observe any difference in the distribution of HCV genotypes between Italian B-cell NHL patients and control subjects. The HCV genotype distribution observed among both groups was similar to previous estimates of the HCV genotype distribution among the Italian population (18-20).

4. Genesis of B-cell HCV-associated lymphomas

Several Italian studies have suggested that type II mixed cryoglobulinemia syndrome (MCS), an autoimmune disease,

Table III. Prevalence of anti-HCV antibodies (Abs) in NHL cases, according to WHO classification.

NHL histotype/authors/refs.	Study type	NHL cases	Anti-HCV Ab positivity	
			NHL	% (95% CI)
Lymphoplasmacytoid/lymphoplasmacytic-immunocytoma		134	43	32.1 (24.2-40.0)
Talamini *et al* (10)	Case-control	10	3	30.0 (1.6-58.4)
Mele *et al* (6)	Case-control	13	4	30.8 (5.7-55.9)
Vallisa *et al* (88)	Case-control	25	16	64.0 (45.2-82.8)
Luppi *et al* (93)	Case series	16	2	12.5 (0.0-28.7)
Silvestri *et al* (17)	Case series	70	18	25.7 (15.5-36.0)
Marginal zone lympoma		127	30	23.6 (16.2-31.0)
Talamini *et al* (10)	Case-control	4	2	50.0 (1.0-99.0)
Mele *et al* (6)	Case-control	15	4	26.7 (4.3-49.0)
Vallisa *et al* (88)	Case-control	23	6	26.1 (8.1-44.0)
Luppi *et al* (93)	Case series	38	11	28.9 (14.5-43.4)
Silvestri *et al* (17)	Case series	47	7	14.9 (4.7-25.1)
DLBCL		515	105	20.4 (16.9-23.9)
Talamini *et al* (10)	Case-control	112	22	19.6 (12.3-27.0)
Mele *et al* (6)	Case-control	205	39	19.0 (13.7-24.4)
Vallisa *et al* (88)	Case-control	87	32	36.8 (26.6-46.9)
Luppi *et al* (93)	Case series	34	8	23.5 (9.3-37.8)
Silvestri *et al* (17)	Case series	77	4	5.2 (0.2-10.2)
MALT		84	17	20.2 (11.6-28.8)
Talamini *et al* (10)	Case-control	10	5	50.0 (19.0-81.0)
Mele *et al* (6)	Case-control	25	3	12.0 (0.0-24.7)
Guida *et al* (86)	Case-control	13	3	23.1 (0.2-46.0)
De Vita *et al* (89)	Case-control	15	3	20.0 (0.0-40.2)
Pioltelli *et al* (92)	Case series	16	3	18.8 (0.0-37.9)
Mazzaro *et al* (5)	Case series	5	0	0.0 (-)
Burkitt lymphoma		21	4	19.0 (2.3-35.8)
Talamini *et al* (10)	Case-control	10	2	20.0 (0.0-44.8)
Mele *et al* (6)	Case-control	10	2	20.0 (0.0-44.8)
Luppi *et al* (93)	Case series	1	0	0.0 (-)
Follicular lymphoma		275	39	14.2 (10.1-18.3)
Talamini *et al* (10)	Case-control	36	0	0.0 (-)
Mele *et al* (6)	Case-control	79	11	13.9 (6.3-21.6)
Vallisa *et al* (88)	Case-control	25	6	24.0 (7.3-40.7)
Luppi *et al* (93)	Case series	48	14	29.2 (16.3-42.0)
Silvestri *et al* (17)	Case series	87	8	9.2 (3.1-15.3)
Mantle cell lymphoma		60	7	11.7 (3.5-19.8)
Talamini *et al* (10)	Case-control	2	0	0.0 (-)
Mele *et al* (6)	Case-control	15	2	13.3 (0.0-30.5)
Vallisa et al. (88)	Case-control	12	4	33.3 (6.7-60.0)
Luppi *et al* (93)	Case series	20	0	0.0 (-)
Silvestri *et al* (17)	Case series	11	1	9.1 (0.0-26.1)
SLL/CLL		159	12	7.5 (3.4-11.7)
Talamini *et al* (10)	Case-control	18	6	33.3 (1.6-55.1)
Mele *et al* (6)	Case-control	10	2	20.0 (0.0-44.8)
Silvestri *et al* (17)	Case series	131	4	3.1 (0.1-6.0)

Table IV. Prevalence of anti-HCV antibodies (Abs) in NHL cases according to HCV genotype.

HCV genotype/authors/refs.	Study type	NHL cases	Anti-HCV Ab positivity	
			NHL	% (95% CI)
1a		127	3	2.4 (0.0-5.0)
Talamini *et al* (10)	Case-control	36	0	0.0 (-)
Mele *et al* (6)	Case-control	60	3	5.0 (0.0-10.5)
Silvestri *et al* (17)	Case series	31	0	0.0 (-)
1a/1b		168	1	0.6 (0.0-1.8)
Talamini *et al* (10)	Case-control	36	0	0.0 (-)
Mele *et al* (6)	Case-control	60	1	1.7 (0.0-4.9)
Pioltelli *et al* (92)	Case series	41	0	0.0 (-)
Silvestri *et al* (17)	Case series	31	0	0.0 (-)
1b		168	70	41.7 (34.2-49.1)
Talamini *et al* (10)	Case-control	36	18	50.0 (33.7-66.3)
Mele *et al* (6)	Case-control	60	25	41.7 (29.2-54.1)
Pioltelli *et al* (92)	Case series	41	13	31.7 (17.5-46.0)
Silvestri *et al* (17)	Case series	31	14	45.2 (27.6-66.3)
2a/2c		168	82	48.8 (41.3-56.4)
Talamini *et al* (10)	Case-control	36	16	44.4 (28.2-60.7)
Mele *et al* (6)	Case-control	60	28	46.7 (34.0-59.3)
Pioltelli *et al* (92)	Case series	41	23	56.1 (40.9-71.3)
Silvestri *et al* (17)	Case series	31	15	48.4 (30.8-66.0)
3a		96	1	1.0 (0.0-3.1)
Talamini *et al* (10)	Case-control	36	1	2.8 (0.0-8.1)
Mele *et al* (6)	Case-control	60	0	0.0 (-)
4c/4d		96	2	2.1 (0.0-4.9)
Talamini *et al* (10)	Case-control	36	0	0.0 (-)
Mele *et al* (6)	Case-control	60	2	3.3 (0.0-7.9)
Unknown		127	4	3.1 (0.1-6.2)
Talamini *et al* (10)	Case-control	36	1	2.8 (0.0-8.1)
Mele *et al* (6)	Case-control	60	1	1.7 (0.0-4.9)
Silvestri *et al* (17)	Case series	31	2	6.5 (0.0-15.1)

is a precursor of B-cell NHL in the context of HCV infection. Approximately, 90% of type II MCS patients are infected by HCV. This syndrome is also characterized by B-cell proliferation and the monoclonal IgM with rheumatoid factor activity is present in the serum of MCS patients.

It has been proposed that direct infection of B-cells by HCV is a step in some types of B-cell transformation. B-cells have been shown to be susceptible to HCV infection both *in vitro* and *in vivo* (21,22). RT-PCR, *in situ* hybridization, and immunochemistry studies have demonstrated that HCV colocalizes with low grade B-cell NHL tissue. In contrast, HCV-positive cells were primarily located along the edges of low grade B-cell NHL tissue and were only rarely observed to be interspersed within it (23-25). Additionally, neoplastic tissue biopsies from two HCV-positive patients with high grade B-cell NHL were found to be HCV-negative (26). Therefore, on the basis of theses findings, the direct infection of B-cells seems to be unrelated to progression of B-cell NHL among HCV-positive patients.

Convincing evidence suggests that chronic antigenic stimulation promotes B-cell NHL development among HCV-positive patients. Sequence analysis of rearranged Ig genes in malignant B-cells from HCV-positive patients reveals that certain combinations of heavy and light chain genes are frequently present. These common combinations include: IGHV3-23/IGHD3-22/IGHJ4, IGHV1-69/IGHD3-22/IGHJ4, or IGHV4-59/IGHD2-15/IGHJ2 with either IGKV3-20/IGKJ1 or IGKV3-20/IGKJ2 and IGHV3-7/IGHD3-16/IGHJ3 or IGHV3-7/IGHD3-22/IGHJ3 with IGKV3-15/IGKJ1 (25,27,28-31).

Many of the most common rearranged Ig genes present in malignant B-cells from HCV-positive patients with B-cell

NHL are identical to those that frequently encode monoclonal rheumatoid factors (mRFs) in individuals with type II MCS (28,29). It has also been observed that complementarity determining regions (CDRs) of rearranged Ig genes encoding RFs as well as those present in malignant B-cells from HCV-positive patients with B-cell NHL have a low ratio of replacement to silent mutations (26,28,32). This paucity of replacement mutations suggests that there is selective pressure against evolution of antibodies with higher antigen binding affinity in type II MCS as well as B-cell NHL among HCV-positive patients. The similarities shared by rearranged Ig genes present in B-cells from patients with type II MCS and malignant B-cells from HCV-positive patients with B-cell NHL support the possibility that the antigens involved in promoting type II MCS development are the same as those involved in promoting B-cell NHL development among HCV-positive patients. These similarities also support the possibility that type II MCS is a precursor of B-cell NHL. Antigens associated with HCV infection are suspected to contribute to development of type II MCS and B-cell NHL among HCV-positive individuals. This is supported by analysis of Ig genes used to express anti-HCV antibodies. IGHV1-69 was the VH gene most frequently used to encode anti-HCV antibodies that recognize E2 (33). Similarly, IGHV1-69 is commonly used to express mRFs with the WA CRI in individuals with type II MCS and is commonly present in malignant B-cells from HCV-positive B-cell NHL patients. Furthermore, an antibody encoded by mRNA isolated from an HCV-positive patient with DLBCL was observed to bind to E2 (11). These results provide evidence that E2 may be an important antigen involved in promoting development of type II MCS and B-cell NHL among HCV-positive individuals.

Cluster of differentiation-81 (CD81) has been proposed to be involved in chronic antigenic stimulation associated with HCV infection. CD81 is a cellular ligand for E2 that is expressed by most human cell types, but not red blood cells or platelets (34,35). CD81 is a member of the tetraspanin protein family and contains four transmembrane domains and two extracellular loops. Binding of E2 is mediated by the large extracellular loop (LEL) of CD81 (35-37). Two separate CD81 binding sites have been identified on E2 (38,39). Binding of HCV to CD81 alone is insufficient for viral infection. This is illustrated by the observation that tamarins from the Saguinus genus of New World monkeys are not susceptible to HCV infection even though tamarin CD81 binds E2 with higher affinity than human CD81 (40,41).

CD81 is a component of a multimeric protein complex on the cell surface of mature B-cells. Other proteins from this complex include CD19, CD21, and Leu-13 (42). Simultaneous binding of antigens to this complex and the B-cell receptor (BCR) has been suggested to facilitate B-cell proliferation (43). Binding of HCV to CD81 as well as the BCR may induce non-malignant polyclonal B-cell expansion. Genetic changes may later lead to malignant transformation of these B-cells and development of B-cell NHL.

In the pathogenesis of HCV-associated lymphoproliferations, involvement of the immune system also likely occurs. Cytokines might be of particular relevance in this context as they are involved in liver metabolism and in the immune response to viral agents (44). IL-1, with the two isoforms IL-1α and IL-1ß is a cytokine belonging to the innate immune system that plays an important role in initiating the cascade of events of immuno-inflammatory responses. The importance of IL-1 in the physiology of the immune system is highlighted by the presence of multiple endogenous regulators such as the IL-1 receptor antagonist, the interleukin receptor type I and type II and the IL-1 accessory protein that, when released into the bloodstream, may serve as naturally occurring inhibitors of IL-1 (45). Previous studies support the hypothesis that an imbalance between IL-1 and these inhibitors, either in blood or in tissue, may regulate the development and the natural course of chronic inflammatory diseases (45-50). In our previous report, we demonstrated a deregulated balance between IL-1 and its naturally occurring inhibitors in HCV-associated lymphoma with and/or without MCS reflecting an ongoing immuno-inflammatory response in which IL-1 is involved (51). A pathogenic role of IL-1 in lymphomagenesis in the context of HCV infection could open novel therapeutic approaches for prevention of NHL that are aimed at negating the action of endogenous IL-1 with specific inhibitors such as the IL-1ra. Of note, IL-1ra has already shown clinical efficacy in patients with rheumatoid arthritis (52). We have also demonstrated that osteopontin (OPN), another proinflammatory cytokine, may play a role in the development of HCV-associated lymphomas with and without concomitant type II MCS (53).

5. Genetic abnormalities associated with B-cell NHL among HCV-positive patients

Genetic mutation arising from aberrant somatic hypermutation (SHM) has been proposed to contribute to B-cell NHL. SHM is a process that enhances antibody affinity for a particular antigen by introducing nucleotide substitutions within the immunoglobulin variable (IgV) genes of germinal center (GC) B-cells (54). Specific features of SHM include the predominance of single base substitutions, preference for transitions over transversions, and specific targeting of AG/G/CT/AT (RGYW) motifs. Frequent aberrant SHM of PIM-1, PAX-5, RhoH/TTF, and c-myc have been demonstrated in lymphoid malignancies, in NHLs associated with acquired immunodeficiency syndrome (AIDS) as well as primary central nervous system lymphomas (PCNSLs) (55-57). In contrast, analysis of PIM-1, PAX-5, RhoH/TTF, and c-myc gene mutations within B-cell NHL tissue from HCV-positive patients did not reveal statistically significant clustering of mutations within RGYW motifs suggesting that HCV-associated malignancies have a distinct entity (58). However, clustering of bcl-6 mutations with RGYW motifs demonstrated that the SHM process remained active in the presence of HCV infection. The pattern of PIM-1, PAX-5, RhoH/TTF, and c-myc gene mutations was similar to the pattern of ß-catenin and p53 gene mutations in B-cell NHL tissue from HCV-positive patients analyzed by Machida *et al* (59). The authors proposed that genetic mutations in tumors from HCV-positive patients arise from induction of error prone DNA polymerase activity by HCV-infection instead of aberrant SHM process.

Among the molecular alterations, previous studies have shown that t(14;18)(q32;q21) translocation, that fuses the *bcl-2* proto-oncogene with *IGH* and consequently causes Bcl-2 overexpression, was common in peripheral blood

Table V. Published studies of rituximab treatment in patients with HCV-associated lymphoproliferative disorders.

Benign and malignant lymphoproliferative disorders	No. of patients	Authors/refs.
MALT lymphoma	1	Foxton *et al* (77)
Type II MCS and B-cell NHL	1	Lamprecht *et al* (78)
Type II MCS	20	Sansonno *et al* (79)
Type II MCS	15	Zaja *et al* (80)
Sjögren syndrome, and B-cell NHL	2	Ramos-Casals *et al* (81)
Type II MCS and glomerulonephritis	6	Roccatello *et al* (82)
Type II MCS and glomerulonephritis	5	Quartuccio *et al* (83)
Type II MCS	12	Roccatello *et al* (84)
B-cell BHL	10	Cervetti *et al* (85)

mononuclear cells (PBMCs) of HCV-positive patients with type II MC (60-62) suggesting that this genetic abnormality may contribute to B-cell lymphoma development of HCV-positive patients with concomitant type II MCS. This chromosomal aberration is associated with follicular lymphoma and only recently it has been also implicated in the pathogenesis of mucosa-associated lymphoid tissue (MALT) lymphoma of HCV-infected patients (63-65). Overall, these findings further support the notion that viral infection may influence the pathway of transformation and progression of lymphoma cells.

6. Treatment of HCV-associated lymphoproliferative disorders

Treatment of HCV infection has been proposed as a complementary therapeutic approach to treat B-cell NHL among HCV-positive patients. A similar strategy has been used successfully to treat MALT lymphomas associated with *Helicobacter pylori* infection (66). The effectiveness of antiviral agents for treatment of type II MCS and B-cell NHL among HCV-positive patients has been documented by several studies. Interferon-α (IFN-α) 2b treatment reversed monoclonal B-cell expansion among HCV-positive patients with type II MCS (67). Loss of detectable t(14;18)(q32;q21) among PBMCs was frequently achieved in HCV-positive patients treated with either IFN-α alone or IFN-α plus ribavirin (68,69). These findings also provide evidences that t(14;18) translocation is strongly associated with B-cell transformation in the context of HCV infection and it is useful for monitoring progression of lymphoproliferative disorders and their response to therapy. IFN-α2b alone or IFN-α2b plus ribavirin also induced regression of splenic lymphoma with villous lymphocytes in all HCV-positive patients receiving treatment; in contrast, HCV-negative controls did not benefit from antiviral therapy (70). Treatment with pegylated IFN-α plus ribavirin was more recently shown to be useful for treatment of low grade B-cell NHL among HCV-positive patients (71). The effectiveness of antiviral treatment for type II MC syndrome or B-cell NHL among HCV-positive patients provides evidence that HCV infection contributes to the development of these conditions. Unfortunately, some HCV-positive patients with type II MCS or B-cell NHL do not respond to antiviral therapy alone. Cytotoxic agents may also be problematic for many patients. Treatment with cyclophosphamide may be ineffective, too toxic, or promote viral replication among HCV-positive patients (72,73).

Rituximab, a chimeric human/murine IgG1 monoclonal antibody that binds specifically to CD20, is a promising alternative to cytotoxic agents for the treatment of type II MCS or B-cell NHL among HCV-positive patients. In fact, the efficacy of rituximab for treatment of lymphoproliferative disorders among HCV-positive patients was documented (74-85). The most relevant studies on rituximab treatment of HCV-associated lymphoproliferative disorders are reported in Table V. Sansonno *et al* reported the largest study of rituximab for treatment of type II MCS. This study examined the efficacy of rituximab therapy among 20 HCV-positive patients with type II MC syndrome resistant to IFN-α. A complete response was achieved in 80% of patients and more than 60% of patients showed rapid improvement of disease symptoms (79). Zaja *et al* studied rituximab treatment of 15 patients with type II MCS that was previously unresponsive to other therapeutic regimens (80). Quartuccio *et al* investigated the efficacy and safety of rituximab in the treatment of 5 patients with HCV-related cryoglobulinaemic nephritis. A rapid and sustained renal response was observed in all patients; no major side-effects occurred and steroids were not required in the follow-up (83). Roccatello *et al* studied rituximab treatment of 12 symptomatic patients with HCV-associated MCS with signs of systemic vasculitis. Interestingly, the authors reported that HCV viral load remained stable during short- and medium-term observation and no acute or delayed side effects were observed after therapy (84). Similarly, Cervetti *et al* observed that 10 patients responded to anti-CD20 monoclonal antibody treatment and none exhibited significant toxic effects (85).

7. Conclusion

Epidemiological and experimental studies have demonstrated that HCV infection contributes to development of type II MCS, which may evolve into B-cell NHL. Many HCV-positive patients with type II MCS or B-cell NHL respond to antiviral

therapy. Numerous studies support the possibility that HCV infection promotes B-cell NHL development by an antigen driven mechanism. However, no specific antigen has yet been identified. Most of HCV positive patients may develop B-cell lymphoma. Overall, these studies strongly support the notion that HCV-associated lymphomas may be a distinct entity and further characterization of the mechanisms by which HCV infection contributes to B-cell NHL development may improve its diagnosis, classification, and treatment.

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