Chronic HCV infection and lymphoproliferative disorders: association or causal relationship?

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Chronic hepatitis C virus (HCV) infection has been associated with several extrahepatic manifestations. A causal relationship has been suggested for lymphoproliferative disorders (LPD) development in the setting of chronic HCV infection. The molecular mechanism of HCV-associated lymphomagenesis remains unclear, although several theories have been proposed. In a recent study, we evaluated mixed cryoglobulinemia syndrome, monoclonal gammopathy of uncertain significance (MGUS) and B-cell non-Hodgkin lymphoma (B-NHL) prevalence in a cohort of 1313 CHC infected patients and we evaluate the association of virological and clinical factors with the presence of LPD. A positive association was found between the presence of cirrhosis and MGUS (OR=2.8924, 95%CI 1.2693-6.5909; p=0.012) and between cirrhosis and B-NHL (OR=3.9407, 95%CI 1.7226-9.0153; p=0.001). Moreover, we reported that 66.7% of patients with indolent B-NHL responders to antiviral treatment obtained a complete onco-hematological remission, supporting both the use of a first-line antiviral treatment of HCV-related indolent lymphomas and the plausible causal relationship between HCV infection and lymphomagenesis.

Keywords: antiviral therapy; hepatitis C virus; mixed cryoglobulinemia; monoclonal gammopathy of undetermined significance; B-cell non-Hodgkin lymphoma


Introduction

Hepatitis C virus (HCV) is both a hepatotropic and lymphotropic virus and is estimated to affect over than 180 million individuals worldwide [1]. HCV infection becomes chronic in most of cases and, over time, people are at increased risk of developing cirrhosis and its complications, such as hepatocellular carcinoma [2]. However, several extrahepatic manifestations involving other organ systems have been reported in association with chronic HCV infection (CHC), including immune-mediated rheumatic disorders, endocrine disorders, renal complications and neoplastic disorders [3]. Hematological manifestations such as monoclonal gammopathy of uncertain significance (MGUS), mixed cryoglobulinemia (MC), and B-cell non-Hodgkin lymphoma (B-NHL) have been not only associated with HCV infection but a causal relationship has been suggested [4].

Chronic HCV infection and Monoclonal Gammopathy of Uncertain Significance

MGUS is a benign B-cell LPD characterized by a clonal proliferation of plasma cells secreting monoclonal immunoglobulin without evidence of malignancy at bone marrow level [5]. However, MGUS rarely evolve into multiple myeloma (MM). It has been reported an average rate of progression of 1% per year [6].
The prevalence of monoclonal gammopathies without cryoglobulinemia among CHC varies between 2% and 11% \cite{5, 7, 8} while in healthy population MGUS affects approximately 3% of individuals >70 years of age and about 1% of those >50 years old \cite{9}. In our study, 4.2% of CHC patients had a diagnosis of MGUS and the mean age was 61 years old \cite{10}. In fact, the frequency of MGUS in normal population appears to be lower and age-dependent, suggesting that HCV infection acts as significant risk factor for MGUS occurrence at an earlier age, and supporting the concept that a continuous antigenic stimulation may be the underlying cause of monoclonal gammopathy development \cite{5}.

**Chronic HCV infection and Mixed Cryoglobulinemia**

Cryoglobulins are cold-precipitable serum immune-complexes \cite{11}. According to immunoglobulins (Ig) configuration, MC can be classified in type I, II and III. MC type I consist of monoclonal IgG or IgM, but is not typically associated with HCV infection and is usually found in lymphoid tumors \cite{12}. In CHC patients, more than 50% of infected individuals have circulating mixed cryoglobulins formed by HCV, anti-HCV polyclonal IgG and monoclonal or polyclonal IgM in type II or type III MC, respectively, \cite{13}. In a study conducted by the MULTIVIRC group involving 1083 patients with CHC, it was reported a cryoglobulin prevalence of 40% and, interestingly, all cryoglobulins positive patients had either type II (65%) or type III (35%) MC \cite{14}. However, only a minority of infected patients (<5%) experiences a symptomatic MC syndrome (MCS), characterized by a combination of serological findings [MC especially type II with rheumatoid factor (RF) activity] and clinic-pathological features such as purpura, arthralgia, fatigue and diffuse vasculitis \cite{15}. In our study, 19 (76%) patients with MCS had type II cryoglobulinemia, while 6 (24%) showed type III. In agreement to previous published data, we found MCS prevalence=1.9% in our cohort of 1313 CHC patients \cite{10}.

The mechanism underlying MCS development is represented by a chronic lymphocytes stimulation that induces the B-cell clonal expansion and the subsequent production of antibodies, including RF, that are incorporated into cryoglobulins \cite{16}. Several studies reported MCS is usually associated with advantage, longer duration of HCV infections and type II MC. Sene D et al, retrospectively evaluated 125 CHC patients with MC and found that patients presenting MCS were older (p<0.01), had a longer history of HCV infection (p<0.05), higher prevalence of type II MC (OR=5, p<0.01), and a higher MC serum level (p<0.01) \cite{17}. Also Vallat L et al reported that HCV-infected patients with clonal B-cell proliferation were older (p=0.004) and more frequently symptomatic (p=0.03), and had longer history of CHC (p=0.009) with higher levels of cryoglobulinemia (p=0.001) and a more severe liver disease than patients without a clonal B-cell expansion (p=0.05) \cite{18}. Conversely, we did not find any association between virological and clinical factors with MCS diagnosis \cite{10}.

Evidence for a causal link between HCV and MCS comes from several clinical trials that reported a significant improvement in MCS in patients experiencing a sustained virologic response (SVR) to interferon (IFN)-α therapy \cite{19, 20}, and other study in which patients with CHC and MCS who relapsed after responding to antiviral therapy (AVT), also experienced a vasculitis relapse with the return of viremia \cite{21}.

**Chronic HCV infection and B-cell non-Hodgkin Lymphoma**

B-NHL can evolve from a pre-existing MC condition or can be an idiopathic form; it has been reported that 8-10% of MC type II evolve to lymphoma \cite{4, 22, 23}. Malignant evolution usually occur after a long-lasting CHC infection, as shown by the advanced age of patients who develop lymphoma \cite{4}. Usually, B-NHL arisen in cryoglobulinemic patients involves bone marrow and displays a low-malignancy phenotype, whereas B-NHL not associated to MC frequently expresses a high grade of malignancy from the beginning without involving bone marrow \cite{24}. Despite there is a large amount of data regarding the causal role of HCV in B-NHL development, the molecular mechanism of HCV-associated lymphomagenesis remains unclear.

**Pathogenesis of HCV-associated B-cell non-Hodgkin Lymphoma**

Currently most reliable theories include a continuous lymphocyte receptors external stimulation by HCV antigens leading to proliferation, a direct oncogenic effect mediated by HCV proteins following viral replication inside B-cell and a permanent genetic B-cell damage inducing a mutated phenotype by a transiently intracellular virus (“hit and run” theory) \cite{1}.

Several studies suggested an indirect role of HCV in LPDs pathogenesis highlighting the similar role of *Helicobacter pylori* inducing gastric mucosal-associated lymphoid tissue (MALT) lymphoma following a sustained antigenic stimulation \cite{25}. In fact, it has been reported that *Helicobacter pylori* eradication leads to complete remission in 81% and partial remission in 9% of low grade stage lymphomas \cite{26}. Similarly, the binding between HCV envelope protein E2 and CD81 receptor expressed on B-cells, that leads to the formation of a co-stimulatory complex with CD19 and CD21, induces a decrease in B-cell activation threshold and
subsequently triggers the JNK pathway leading to B-cells proliferation \[1, 27, 28\]. According to this mechanism, that polyclonal proliferation of B-lymphocytes in response to a sustained viral antigen stimulation could be the way that overtime leads to the development of the HCV-associated B-LPDs.

The hypothesis of a direct oncogenic role of HCV replication in B-cells has been investigated since it has been shown the presence of HCV RNA in peripheral blood mononuclear cells (PBMC) \[29\]. Moreover, Sung VM and coworkers established a B-cell line from an HCV-infected B-NHL and showed that cell line was able to produce HCV virions in culture that could infect primary human hepatocytes, PBMCs and other B-cell line \[30\]. However, data regarding evidence of active HCV replication in human lymphocytes \textit{in vivo} are still conflicting \[31-33\]. On the contrary, there are consistent data suggesting a potential lymphomagenic effect mediated by intracellular HCV proteins. In several animal models, the effect of HCV proteins (core, NS3 and NS5A) interaction with host cell signaling pathways on cell proliferation and viability has been investigated \[34\]. Ishikawa T and colleagues demonstrated that transgenic mouse model expressing HCV core protein develops malignant lymphoma with a higher frequency (4 out of 5 mice; 80%) compared to non-transgenic mouse model (1 out of 9 mice; 11%) \[35\]. Moreover, it has been demonstrated that HCV core protein inhibits B-cells apoptosis, down-regulates MHC class II molecules and up-regulates genes (MLLT3, BAL, and BMI1) associated with leukemia and B-NHL \[36\].

Another mechanism proposed to explain lymphomagenesis is based proto-oncogenes and tumor suppressor genes mutations HCV induced, without evidence of intracellular viral replication \[37\]. It has been reported that (14;18) traslocation and bel-2 anti-apoptotic gene over-expression in lymphoid cells are frequent in chronic HCV infection \[38\]. In addition, the disappearance of Bel-2 rearrangements following AVT suggest that HCV plays a role in inhibition of B-cell apoptosis contributing to LPDs pathogenesis \[39\].

\textit{Subtypes of HCV associated B-cell non-Hodgkin lymphomas}

HCV has been associated with B-NHL, especially with indolent lymphomas such as marginal zone lymphoma (MZL), lymphoplasmaqcytic lymphoma (LPL), follicular lymphoma (FL), chronic lymphocytic leukemia (CLL) and small lymphocytic lymphoma \[40\]. However, several study reported an association of high-grade lymphomas such as diffuse large B-cell lymphoma (DLBCL) with CHC \[41-43\].

Our study included 41 patients with B-NHL (23M, 18F; mean age 62.5 ± 11.0) (10). The most prevalent form was MZL (36.6%) and DLBCL (24.4%) followed by FL (9.8%), LPL (2.4%), MM (2.4%), CLL (2.4%) and other B-NHL not otherwise specified (22%). Interestingly, type II cryoglobulins were associated with indolent B-NHL in 8 patients (17%) suggesting that these lymphoma cases might represent an MC HCV-related evolution. Virological and clinical features were analyzed in order to evaluate any possible association with the diagnosis of B-NHL: in our cohort, only cirrhosis showed a positive association with B-NHL (OR=3.9407, 95%CI 1.7226-9.0153; p=0.001) \[10\].

\textit{Therapy of HCV-associated B-cell non-Hodgkin lymphomas}

Despite epidemiological study reported a strong association between HCV infection and B-NHL development, the regression of indolent B-NHL following HCV eradication by AVT represents a clear demonstration of a causal relationship between HCV and lymphoma developing \[1, 40\].

Numerous studies evaluated AVT in patients with indolent B-NHL associated with HCV infection. Tursi A and colleagues treated with IFN and Ribavirin (RBV) 16 CHC patients with MALT obtaining SVR in 11 patients and in all of them a lymphoid tissue complete remission was achieved \[44\]. In another study, 13 CHC patients with histologically proven low-grade B-NHL (1 FL, 4 LPL and 8 MZL) underwent AVT with IFN and RBV. SVR was achieved in 6 patients and of these, 5 obtained a complete hematological response and 1 a partial remission. On the other hand, all non-responders had no virologic response \[46\]. Also a cohort of 18 CHC patients with splenic lymphoma were treated either with IFN alone or in association with RBV and, after HCV RNA clearance, 14 (78%) patients achieved a complete hematologic response \[47\]. Recently, Mazzaro C and colleagues treated with IFN and RBV 18 CHC patients with indolent B-NHL, mainly LPL, and reported that only patients that achieved SVR experienced a complete hematological response \[48\]. In our study, 17 out of 20 patients with indolent B-NHL were treated with IFN and RBV while 3 patients presenting clinical signs of advanced cirrhosis were not treated because their risk of liver failure as side effect of IFN-based therapy. In agreement to previous reports, 9 out of 17 patients experienced a SVR and 6 had a complete onco-hematological response (66.7%), 2 patients obtain a complete B-NHL remission after surgery and 1 patient obtain only a partial hematological response \[10\].
Conversely, in the management of HCV-associated high-grade B-NHL, immune-chemotherapy based on rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone (R-CHOP) on a 21-day schedule is the standard of care \[^{[49]}\]. Interestingly, it has been reported that AVT following immune-chemotherapy resulted in an improvement of disease-free survival indicating that eradication of HCV infection positively affects B-NHL outcome \[^{[50]}\]. Moreover, a recent retrospective study by Fondazione Italiana Linfomi showed that oncologic remission after AVT can be equally obtained both in indolent and aggressive lymphomas \[^{[51]}\]. Unfortunately, aggressive B-NHL requires therapy with prompt anti-tumoral effect and on the other hand simultaneous treatment with IFN-based AVT and R-CHOP is contraindicated due to hepatic and hematological toxicity \[^{[1]}\].

**Conclusion**

Currently, there is a large body of evidence that CHC infected patients are at increased risk of both benign LPDs, such as MGUS and MC, and malignant B-NHL. Despite the exact molecular mechanism of HCV-associated lymphomagenesis is still unclear, different theories have been proposed and argued without being mutually exclusive. Regarding therapy, AVT showed to play an important role in hematological remission; currently is considered the first line of treatment in indolent B-NHL whereas in high-grade B-NHL, R-CHOP is the standard of care. However, the recent availability of IFN-free direct acting antiviral drugs could considerably enhance SVR, safety and tolerability and consequently improve HCV-associated B-NHL therapy.

**References**


