

Epidemiological characteristics of HEPATITIS C and B viral infection in patients with lymphoproliferations sustain their role in lymphomagenesis

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ABSTRACT

Hepatitis B and C viruses (HBV, HCV), besides liver disease, are recognized causes of extrahepatic manifestations. There are sufficient, although controversial data to support HCV implication in lymphomagenesis. For HBV this association has never been proved.

Objectives: To investigate HCV and HBV association with lymphoproliferative disorders and to identify possible mechanisms of viral implication in the pathogenesis of these diseases.

Method: We designed a case control study comparing prevalence of HBV and HCV infection between patients with various types of lymphoproliferation and patients with other hematological diseases.

Results: HCV prevalence in patients with B cell lymphoproliferations was significantly higher than in controls. Non-Hodgkin lymphomas showed the strongest association with HCV, especially those with low grade histology. Multiple myeloma had a very high prevalence of HCV infection. Surprisingly HBV is also associated with lymphoproliferative diseases, particularly with B cell lymphoproliferations, indolent but also aggressive lymphomas.

Keywords: hepatitis C virus, hepatitis B virus, lymphoproliferation

BACKGROUND

Hepatitis B and C viruses are important pathogens not only because of their high prevalence, but also because of the severe complications of persistent infection, including cirrhosis, hepatocellular carcinoma and end-stage liver disease leading to hepatic transplantation. Worldwide

no less than 400 million people are chronic B virus (HBV) carriers and 170 million people are chronically infected with hepatitis C virus (HCV).

HCV infection is a recognized cause of extrahepatic disease, being associated with membranoproliferative glomerulonephritis, mixed cryoglobulinemia, porphyria cutanea tarda, leukocytoclastic vasculitis, focal lymphocytic

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sialadenitis, Mooren's corneal ulcers, lichen planus, rheumatoid arthritis, thyroid disease, non-Hodgkin's lymphoma and diabetes mellitus (1). This is not to surprise viewing HCV capacity to infect various cell types, besides the hepatocyte, such as peripheral blood mononuclear cells, dendritic cells or salivary glands epithelial cells. However, their occurrence is better explained by the chronic stimulation of the immune system induced by persistent viral infection, with initially polyclonal and then oligoclonal B lymphocyte expansion and with autoantibodies generation, including antinuclear antibodies, anti-smooth muscle antibodies, and anti-liver/kidney microsomal antibodies involved in the pathogenesis of many of these extrahepatic manifestations.

Acute or chronic B hepatitis have also been associated with extrahepatic manifestations, caused apparently by the aberrant immunologic response to extrahepatic viral proteins (2). Arthritis, dermatitis, glomerulonephritis, polyarteritis nodosa, mixed cryoglobulinemia, papular acrodermatitis and polymyalgia rheumatica are usually observed in association with circulating immune complexes that activate serum complement. An important consideration is that, although HBV replicates primarily in hepatocytes, replicative intermediates and virally encoded have been found in other structures, such as the adrenal gland, testis, colon, nerve ganglia and skin, suggesting the existence of a vast reservoir of extrahepatic replication (3). Moreover small amounts of HBV DNA have been demonstrated in peripheral mononuclear cells years after apparent resolution of chronic infection (4).

HCV implication as an etiological factor of type 2 mixed cryoglobulinemia (MC2) is supported by sufficient epidemiological data (5,6,7). MC2 is in fact a monoclonal gammopathy characterized by an indolent malign lymphoproliferation induced by chronic antigenic stimulation. In some patients continuing clonal expansion can lead to the development of a non-Hodgkin's lymphoma, even if this occurs rarely and after a long period of time.

The study of HCV implication in B cell lymphoma pathogenesis has generated controversial and apparently discordant results. The majority of studies showing a positive association come from Italy, which has a high prevalence of HCV infection. The strongest connection was established between HCV and

lymphoplasmacytoid lymphoma, usually complicating the course of a MC2 (8,9). Afterwards HCV has been associated with a much wider spectrum of B cell lymphoproliferations, including marginal zone splenic lymphoma, mucosa-associated lymphoid tissue lymphoma (MALT), follicular lymphoma, mantle lymphoma, and diffuse large cell lymphoma. Even multiple myeloma has demonstrated a higher prevalence of HCV infection (10).

HBV association with lymphoproliferative diseases has not been investigated to date. □

MATERIAL AND METHOD

We conducted a case-control monocentric retrospective study which included all hematological patients of Coltea Clinical Hospital from Bucharest which have been tested for serological markers of HBV and HCV infection between January 2001 and December 2004. The study group was represented by patients with lymphoproliferative diseases and the control group was formed by the rest of tested patients.

For statistical analysis we used SPSS statistical package, applying χ^2 test with Yates correction, Fisher exact test and Mann-Whitney U test, whenever appropriate. □

RESULTS

The control group included 303 patients, with 169 women and 134 men. Median age was 53 ± 19 years. Age groups distribution showed the predominance of patients over 50 years (58%) (Figure 1). There were more women than men in all age groups, with the exception of extreme ages.

32 patients tested positive for anti-HCV antibodies, with a prevalence of 10.56%. More women than men are infected between 30 and 80 years of age. The prevalence of HCV infection increases with age, as theoretically predicted for this country.

Because of the heterogeneity of lymphoproliferations and of the reduced number of cases in each diagnostic category, we grouped non-Hodgkin's lymphomas by prognosis, according to the Working Formulation. Chronic lymphocytic leukemias, hairy cell leukemias, monoclonal gammopathies, mixed cryoglobulinemias and Waldenström diseases were assimilated as low grade lymphomas. Thus, from

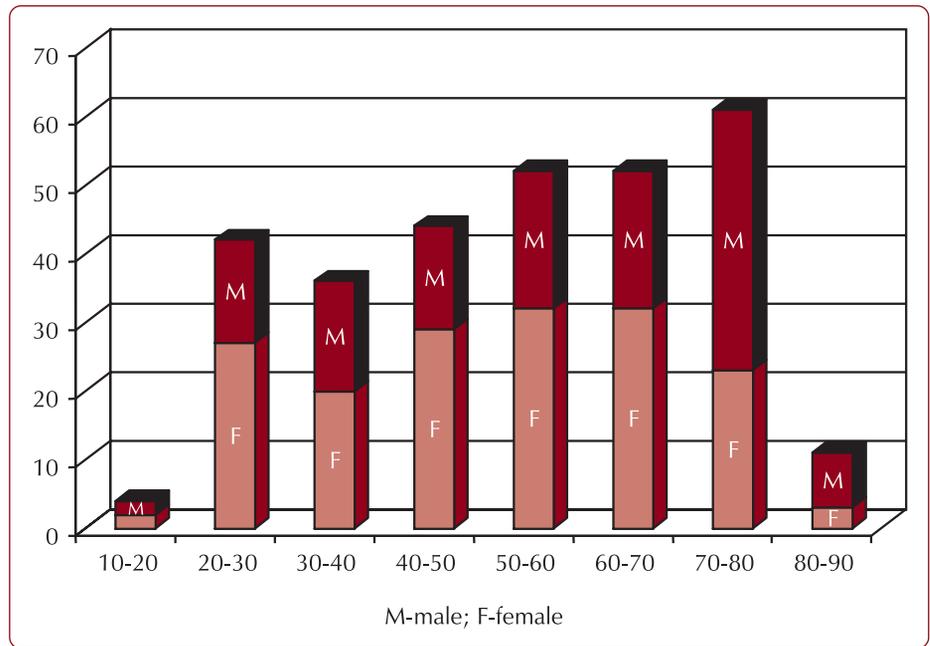


FIGURE 1. Sex and age distribution in control group

a total of 127 B cell lymphomas 76 were low grade (60%), 42 intermediate grade (33%) and 9 high grade lymphomas (7%). Other B cell proliferations considered for analysis were 19 Hodgkin’s diseases and 32 multiple myelomas, for a total of 178 patients representing the study group. 42 patients from this group tested positive for HCV, with a prevalence of 23.6%.

As in the control group females and males were equally represented, but median age was

58 ± 14 years, significantly higher than in the control group. There is an even more marked predominance of advanced ages than in the control group (Figure 2). HCV prevalence increases with age with the exception of the 30-50 years group, which includes a relatively high number of Hodgkin’s diseases, aggressive and intermediary lymphomas.

In the study group the median age is not varying with gender or viral infection status, but

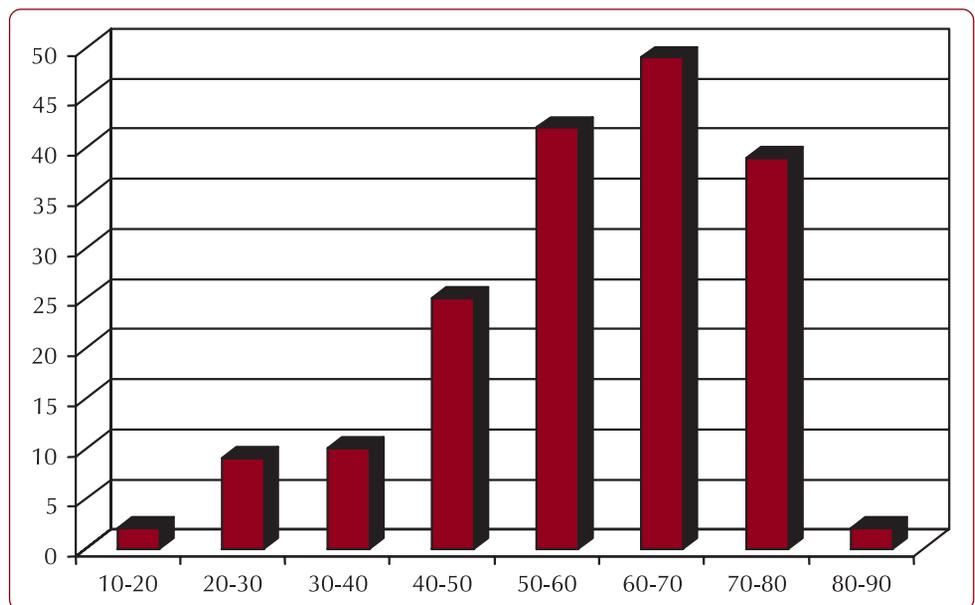


FIGURE 2. Age distribution in the study group

it varies with the type of lymphoproliferation. So Hodgkin's disease and aggressive lymphoma patients are significantly younger than the control group. Conversely indolent and intermediary lymphomas patients, along with multiple myeloma patients, are significantly older than control patients.

Table 1 shows the prevalence of HCV infection by sex and age for patients and controls.

Overall prevalence among patients with B cell lymphoproliferations was higher than in controls. In each sex and age group, the prevalence was higher in study patients than in controls, but statistical significance was reached only for women and age over 50.

Table 2 shows the prevalence of HCV infection among patients by type of B cell lymphoproliferation and by degree of histological differentiation of B-cell lymphoma.

We noted a high prevalence of HCV infection not only in the group of B cell lymphoproliferations as a whole, but also in all their subtypes with the notable exception of Hodgkin's disease. The relative risk of HCV infection in the study group was 1.83 (95%CI: 1.34-2.49).

In what concerns non-Hodgkin's lymphomas subtypes, HCV prevalence was significantly higher only in the indolent lymphomas group, with a relative risk of infection of 2.66 (95%CI: 1.79-3.93). In the case of intermediate grade lymphomas HCV prevalence was comparable with that of the control group. The high grade lymphoma group was too small for any meaningful considerations.

Very impressive is the prevalence of VHC infection in the multiple myeloma group.

HBV prevalence

From the 303 patients of the control group, 20 were HBsAg positives, the prevalence being 6.6%. It is somewhat smaller than HCV prevalence, probably because serologic diagnosis was available earlier than for HCV and because acute hepatitis B evolves more rarely into chronic infection.

Patients with HBV infection are much more evenly distributed between age groups, suggesting an ongoing risk factor, other than transfusions, such as sexual transmission or other parenteral exposures (Figure 3).

	B cell lymphoproliferations			Controls			p
	No. patients	No. VHC+	%VHC+	No. patients	No. VHC+	%VHC+	
Sex							
Male	91	16	17,6	134	12	9	0,054
Female	87	26	29,9	169	20	11,8	0,0003
Age							
10-30	11	2	18,2	46	2	4,3	0,1
30-50	35	4	11,4	80	7	8,8	0,65
50-70	91	21	23,1	104	12	11,5	0,03
70-90	41	15	36,6	73	11	15,1	0,008
Total	178	42	23,6	303	32	10,6	0,0001

TABLE 1. HCV prevalence by sex and age among patients with B cell lymphoproliferations and controls

		No. patients	No. VHC+	%VHC+	p
Lymphoma	Low grade	76	24	31,6	<0,001
	Intermediate grade	42	4	9,5	NS
	High grade	9	2	22,2	NS
	Total	127	30	23,6	0,0004
Hodgkin disease		19	3	15,8	NS
Multiple myeloma		32	9	28,1	0,004
B cell lymphoproliferations		178	42	23,6	0,0001
Controls		303	32	10,56	1

TABLE 2. HCV prevalence by type of B cell lymphoproliferation

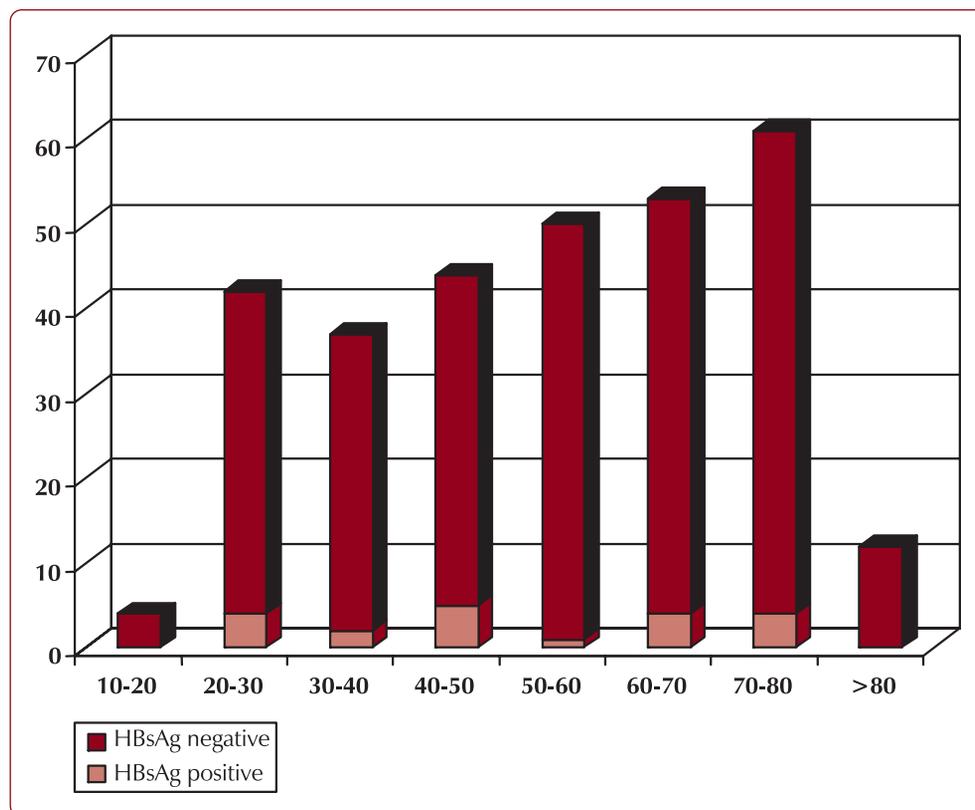


FIGURE 3. HBsAg positive patients by age group in controls

This time we included in the study group all the 206 patients with acute or chronic lymphoproliferation tested, irrespective of the type of cell involved. Median age and sex ratio remained unchanged. HBV infection prevalence was found to be 13.6% (28 patients infected), significantly higher than in the control group ($p=0.008$). Like in the control group distribution of infected cases between age groups is uniform.

Table 3 shows the prevalence of HBV infection by sex and age for patients and controls.

In contrast with the control group, where HBV infection is equally distributed between sexes, and HCV infection, which predominates in women, in the study group the majority of infected patients are males. Only patients 30 to 50 years of age have a significantly higher prevalence of HBV infection compared with the control group.

	Lymphoproliferations			Controls			P
	No. patients	No. VHB+	%VHB+	No. patients	No.VHB+	%VHB+	
Sex							
Male	105	22	21	134	9	6,7	0,001
Female	101	6	6	169	11	6,5	0,85
Age							
10-30	15	1	6,7	46	4	8,7	0,8
30-50	45	11	24,4	80	7	8,75	0,016
50-70	101	10	10	104	5	4,8	0,16
70-90	45	6	13,3	73	4	5,5	0,13
Total	206	28	13,6	303	20	6,6	0,008

TABLE 3. Prevalence of HBV infection by sex and age in patients and controls

		No. patients	No. VHB+	%VHB+	p
Lymphoma	Low grade	76	12	15,8	0,01
	Intermediate grade	42	6	14,3	0,07
	High grade	9	4	44,4	<0,0001
	Total	127	22	17,3	0,0006
Hodgkin's disease		19	1	5,3	0,8
Multiple myeloma		32	1	3,1	0,44
Acute lymphoblastic leukemia		16	3	18,8	0,067
B cell lymphoproliferations		194	42	23,6	<0,0001
T cell lymphoproliferations		12	1	8,3	0,8
Controls		303	20	6,6	1

TABLE 4. HBV prevalence by group of lymphoproliferations

Table 4 shows the prevalence of HBV infection among patients by type of lymphoproliferation and by degree of histological differentiation of B-cell lymphoma.

Surprisingly we found a very significant correlation of HBV infection not only with B cell lymphoproliferations (including acute disease) but also with non-Hodgkin's lymphomas, especially with low grade histology. However unlike HCV infection, we noted the unusually high frequency of positive patients in the case of aggressive lymphomas. If we consider this group of patients together with that of acute lymphoblastic leukemias, which is physiopathologically correct, HBV prevalence reaches an impressive 28% and becomes statistically significant.

Hodgkin's disease, multiple myeloma or T cell lymphoproliferations have apparently no connection with HBV infection.

DISCUSSION

As far as we know this is the only romanian case-control study of the prevalence of HCV and HBV infections in patients with lymphoproliferative diseases.

The fact that patients with chronic B cell lymphoproliferations have a relative risk of 1.69 to be anti-HCV antibody positive is an argument for a role of this virus in lymphomagenesis. The results are similar with those obtained in high prevalence areas.

Even if informations about the mechanism of HCV involvement in lymphomagenesis are lacking, the 2.66 relative risk of HCV positivity among indolent lymphoma favors the hypothesis of the chronic antigenic stimulation that the virus exerts on the immune system. The

first step in the development of the lymphoma would be mixed cryoglobulinemia and the next would be low grade lymphoma. Ongoing clonal selection will lead finally to aggressive form, which become independent form the antigenic stimulus. In our series aggressive lymphomas in infected patients are occurring at a younger age than low grade lymphomas, contradicting this hypothetically mechanism.

For multiple myeloma we found a very strong association with VHC infection.

Even if it has a high oncogenic potential, through DNA integration into host genome, there are no data regarding HBV association with the lymphoproliferations. Our study found a significantly higher prevalence of HBV infection, not only for whole group of B cell lymphoproliferations but also for the group of non-Hodgkin's B cell lymphomas.

There are however some important differences. For example HCV prevalence gets higher with age both in the study and in the control group, reflecting probably the higher risk of transmission in the distant past trough transfused blood before serologic screening. In contrast HBV prevalence is relatively constant with age reflecting an ongoing risk of infection, probably by another mechanism, such as sexual transmission. Female predominance for HCV and male predominance for HBV could have the same explanation.

The patients' age and type of lymphoproliferation are also different between the two viruses. HCV prevalence is significantly higher for patients over 50 years of age, while HBV is more frequent in patients below that age. Moreover HCV is strongly associated with indolent lymphomas and multiple myelomas,

which tend to occur in older patients, whereas HBV infection is more frequent in aggressive lymphomas and acute leukemias, which affect generally younger patients. All these differences are better explained if we consider a different model of oncogenic transformation for each virus. In the case of HCV chronic antigenic stimulation is the most plausible one, while for HBV it could be direct oncogenesis through insertions of viral genetic material in strategic areas of the genome. This could explain also the rapidity with which the lymphocytic clone acquires the malign phenotype. Intuitively the proposed models are analog to the development of hepatocellular carcinoma, which in the case of HBV can appear in young patients with mild hepatic disease, while in the case HCV it involves usually the cirrhotic liver of an older patient, after a long evolution of the infection.

Conclusion

1. In our study HCV infection prevalence in patients with B cell lymphoproliferations is significantly higher than in controls.
2. Among B cell lymphoproliferations, the non-Hodgkin's lymphomas showed the strongest association, especially the indolent ones.
3. Chronic HCV infection is not associated neither with T cell lymphoproliferations, nor with Hodgkin's disease, like in other studies.
4. HCV prevalence was unexpectedly high in the case of multiple myeloma patients.
5. The most surprising finding of this study was the strong association between HBV infection and lymphoproliferations, particularly with B cell lymphoproliferation and, as a subtype, with non-Hodgkin's lymphomas. Unlike HCV, B viral infection is common not only in low grade lymphoma, but in high grade lymphoma as well.

REFERENCES

1. **Medina J, Garcia-Buey L, Moreno-Otero R** – Hepatitis C virus-related extra-hepatic disease: Aetiopathogenesis and management. *Aliment Pharmacol Ther* 2004; 20:129-141
2. **Prysopoulos NT, Reddy KR** – Extrahepatic manifestations of chronic viral hepatitis. *Curr Gastroenterol Rep* 2001; 3:71-78
3. **Mason A, Wick M, White H et al** – Hepatitis B virus replication in diverse cell types during chronic hepatitis B virus infection. *Hepatology* 1993; 18:781-789
4. **Mason A, Yoffe B, Noonan C et al** – Hepatitis B virus DNA in peripheral-blood mononuclear cells in chronic hepatitis B after HBsAg clearance. *Hepatology* 1992; 16:36-41
5. **Ferri C, Greco F, Longombardo G et al** – Antibodies to hepatitis C virus in patients with mixed cryoglobulinemia. *Arthritis Rheum* 1991; 34:1606-1610
6. **Agnello V** – The aetiology of mixed cryoglobulinaemia associated with hepatitis C virus infection. *Scand J Immunol* 1995; 42:179-184
7. **Dammacco F, Sansonno D, Cornacchiulo et al** – Hepatitis C virus infection and mixed cryoglobulinemia: A striking association. *Int J Clin Lab Res* 1993; 23:45-49
8. **Ferri C, Caracciolo F, Zignego AL** – Hepatitis C virus infection in patients with non-Hodgkin's lymphoma. *Br J Haematol* 1994; 88: 392-394
9. **Silvestri F, Barillari G, Fanin R et al** – Impact of hepatitis C virus infection on clinical features, quality of life and survival of patients with lymphoplasmacytoid lymphoma/immunocytoma. *Ann Oncol* 1998; 9: 499-504
10. **Maurizio Montella, Anna Crispo, Filippo et al** – Hepatitis C virus infection and a new association with extrahepatic disease: multiple myeloma. *Haematologica* 2000; 85(8):883-884