VERSITA
DOI: 10.2478/rrlm-2013-0007

Original article

Monoclonal B cell lymphocytosis in patients with hepatitis C virus infection: prevalence, demographic and laboratory correlations

Limfocitoză monoclonală B la pacienții cu virus hepatitic C: prevalență, corelații demografice şi de laborator

Tünde Tőrők-Vistai[1*], Manuela Sfichi[2], Anca Bojan[1], Cristina Pojoga[3]

1. The Oncology Institute Prof. Dr. Ion Chiricuță - Hematology Department
2. Emergency County Hospital Cluj-Napoca - Immunology Department
3. Regional Institute of Hepatology and Gastroenterology Prof. Dr. Octavian Fodor

Abstract

Hepatitis C virus is known to be a risk factor for the development of B-cell non-Hodgkin lymphoma. Studies investigating the prevalence of hepatitis C virus in lymphoma report controversial results, depending on the geographical area. Monoclonal B lymphocytosis is an asymptomatic condition which can evolve into malignant lymphoma, characterized by the presence of a circulating clonal B population. It can be detected by flow cytometry and it is found at higher prevalence in hepatitis C virus-infected patients than in the general population. In the literature, there are only a few studies investigating its prevalence in hepatitis C infected patients and in Romania, such a study hasn't been carried out before. We conducted a prospective study on 50 hepatitis C virus-infected patients from the Regional Institute of Gastroenterology and Hepatology Prof. Dr. Octavian Fodor. Clinical and laboratory data were collected from the medical files. Flow cytometric analysis was carried out at the Immunology Department of the Emergency County Hospital Cluj Napoca. We have found a prevalence of 22% of monoclonal B lymphocytosis. There were no statistical differences between patients with or without monoclonality, except for the lower leucocyte count (p=0.04) and the more increased liver echogenicity in patients with monoclonality (p=0.02). All of the 3 subtypes of monoclonal B lymphocytosis were found. Increased prevalence of monoclonal B lymphocytosis in patients with hepatitis C virus infection sustains the virus role in lymphomagenesis, but further studies are needed to analyze the rate of transformation into lymphoma in these patients.

Keywords: *monoclonal B lymphocytosis, hepatitis C virus, non-Hodgkin lymphoma, chronic lymphocytic leukemia*

Abstract

Este cunoscut faptul că infecția cu virus hepatitic C reprezintă un factor de risc pentru limfoamele non-Hodgkin cu celule B. Studiile referitoare la prevalența acesteia în limfoame raportează rezultate contradictorii, în funcție de zona geografică. Limfocitoza monoclonală B este o condiție asimptomatică care se poate transfor-

[*]**Corresponding author:** Tünde Tőrők-Vistai, The Oncology Institute Prof. Dr. Ion Chiricuță - Hematology Department, Bd. 21 Decembrie nr.73, Cluj-Napoca. E-mail: tunde.torok@yahoo.com, Tel.: 0729929275

ma în limfom malign, caracterizată prin prezența unei populații clonale B circulante. Poate fi detectată prin citometrie în flux şi s-a demonstrat că prevalența ei la pacienții cu virus hepatitic C este mai mare decât la populația generală. În literatură există doar câteva studii referitoare la prevalența limfocitozei monoclonale la pacienții cu virus C iar în România nu a fost condus un asemenea studiu. Am efectuat un studiu prospectiv pe 50 de pacienți cu virus hepatitic C internați la Institutul Regional de Gastroenterologie şi Hepatologie Prof.Dr.Octavian Fodor. Datele clinice şi de laborator au fost culese din foile de observație. Citometria în flux s-a efectuat în Laboratorul de Imunologie al Spitalului Județean de Urgență Cluj-Napoca. Am găsit o prevalență de 22% a limfocitozei monoclonale. Nu au existat diferențe statistic semnificative între pacienții cu şi fără limfocitoză monoclonală, cu excepția numărului mai mic de leucocite (p=0.04) şi a ecogenicității hepatice mai mari (p=0.02) la cei cu monoclonalitate. Am întâlnit toate cele 3 subtipuri de limfocitoză monoclonală B. Prevalența ridicată la pacienții cu virus hepatitic C susține implicarea virusului în limfomageneză, dar sunt necesare alte studii pe viitor, care să analizeze rata de transformare în limfom la aceşti pacienți.

Cuvinte cheie: *limfocitoză monoclonală B, virus hepatitic C, limfom non-Hodgkin, leucemie limfatică cronică*

Received: *19th January 2013;* ***Accepted:*** *10th May 2013;* ***Published:*** *15th June 2013.*

Introduction

Hepatitis C virus (HCV) is a predominantly hepatotropic virus, but is can also infect and replicate in the hematopoietic cells. It is an RNA virus, unable to integrate into the hosts' genome and does not encode any oncogenes. HCV can infect the B lymphocytes, leading to malignant lymphoma by a multistep pathonegetic pathway: first, HCV induces an oligoclonal proliferation of the infected B-cells, then the presence of HCV in lymphocytes could initiate growth dysregulation and predispose the lymphocyte to development of further molecular changes, leading eventually to malignant lymphoma. The HCV E2 envelope protein has been identified as a potential antigen that may drive the development of lymphoma (1-5). The prevalence of HCV infection is higher in patients with B-cell non-Hodgkin lymphoma than in the general population, studies reporting controversial results depending on the geographical area. The most frequent association is with lymphoplasmacytoid lymphoma, other histological types associated with HCV are: follicular, lymphocytic, marginal zone and diffuse large B-cell non-Hodgkin lymphoma (6-16).

Monoclonal B lymphocytosis (MBL) is an asymptomatic condition characterized by the presence in the peripheral blood of a clonal B-cell population which might evolve into malignant B-cell lymphoproliferative disease, like chronic lymphocytic leukemia (CLL) or indolent B-cell lymphoma. (17-19) Diagnostic criterias for MBL are:

1.Detection of a monoclonal B-cell population in the peripheral blood with kappa:lambda ratio >3:1 or <0.3:1, or greater than 25% of B cells lacking or expressing low level of surface immunoglobulin or a disease specific phenotype

2. B-cell count < $5x10^9$/l

3. Absence of features of a B-lymphoproliferative disorder.

There are 3 types of MBLv(20, 21):

- CLL-like MBL: CD5+, CD20dim
- Atypical CLL MBL: CD5+, CD20 bright
- Non-CLL: CD5-, CD20+.

Such a monoclonal B-cell population can be detected in approximately 3.5% of healthy individuals, with higher frequency in males and in elderly people. In HCV-infected patients MBL can be identified at a higher frequency than in the general population (22). There are only a few studies that investigated the prevalence of MBL in patients with HCV infection, with different results. The largest was conducted by Japanese authors, on 240 HCV-infected patients and 150 healthy controls, and they have found a prevalence of 2.9% of MBL in patients with HCV infection and none in controls. Another study was made in Italy, on 123 HCV-positive patients and it reported a prevalence of 28.5%. A study

made in Germany on 45 HCV-positive patients found a prevalence of 35.5% (23-25). In Romania, such a study hasn't been conducted before, therefore we aimed to quantify and immunophenotypically characterize monoclonal B-cell populations circulating in the peripheral blood of patients infected with HCV and to identify demographic and laboratory features of patients with HCV-associated MBL.

Methods

We conducted a prospective study on 50 consecutive patients with chronic hepatitis or cirrhosis, diagnosed and treated at the Regional Institute of Gastroenterology and Hepatology "Prof. Dr. Octavian Fodor" between October 2011 and April 2012. The study was carried out with the approval of the ethical committee of the hospital and the consent of the patients involved.

Clinical and laboratory data were obtained from the medical files of the patients. Hematological data (leukocyte count, white blood cell differential, thrombocyte count and hemoglobin level), erythrocyte sedimentation rate (ESR), liver function tests: liver enzymes: ALAT, ASAT, total bilirubin (TB), alkaline phophatase (ALP), gammaglutamyl-transferase (GGT), coagulation tests (prothrombin time-PT) were recorded. Ultrasonography parameters and fibroscan results were also recorded. The diagnosis of HCV infection was made by detection of anti-HCV antibodies by ELISA method. The diagnosis of hepatitis or cirrhosis was based on laboratory and imagistic findings (ultrasonography, fibroscan).

Flow-cytometric analysis

For detection of MBL we performed a four-color flow cytometric analysis of the peripheral blood. Flow cytometry was performed at the Immunology Laboratory of the Emergency County Hospital Cluj-Napoca. Diagnosis of MBL was made according to the IWCLL (International Workshop for Chronic Lymphocytic Leukemia) guidelines. Monoclonality was demonstrated by light chain restriction (kappa: lambda ratio >3:1 or < 0.3:1) and the monoclonal B-cell population was analyzed for expression of CD5 and CD20. The following combinations of monoclonal antibodies were used: anti CD20/CD5 (FITC/PE) and anti-kappa/lambda (FITC/PE). Peripheral blood was collected on EDTA. For light chain analysis blood was washed previously to prevent fixation of the antibodies on the plasmatic immunoglobulins. For this purpose, 100 µl of blood were mixed with 2 µl of CellWash liquid, centrifuged at 350RFC and the supernatant was disposed of. This procedure was repeated twice, then 50 µl of blood were incubated with 20 µl of monoclonal antibodies for 15 minutes at room temperature and in darkness. Erythrocytes were lysed, blood was centrifuged twice for five minutes at 300RFC, and then cells were resuspended in 500 µl Cell-Wash liquid. 10 000 cells were acquired and analyzed using CELLQuest software.

Statistical analysis

Was performed using the Statistical Package for Social Sciences (SPSS, ver. 20, Chicago, IL, USA). Data were classified as nominal and quantitative. For nominal variables we calculated frequencies and for quantitative variables we determined the central tendency (mean or median), as well as the minimum and maximum value. Quantitative variables were tested for normality of distribution using the Kolmogorov-Smirnov test. Differences of the mean between two groups were analyzed using the T test and the ANOVA test, for the analysis of three groups. A Chi-square test was used to compare categorical variables. The level of statistical significance was set at $p<0.05$.

Results

The study included 50 patients with HCV-infection, with age ranging between 21 and 76 years (average 56.72, median 57.5 years). There were 31 females (62%) and 19 males (38%). 34 patients had chronic hepatitis

Tabel 1. Immunophenotypic characteristics of patients with MBL

Patient no.	Kappa/lambda ratio	Immunophenotype
1	4	CLL
2	8	Atypical CLL
3	3.5	CLL
4	3.4	Atypical CLL
5	4	CLL
6	4	Atypical CLL
7	4	Atypical CLL
8	7	Non-CLL
9	3.6	Atypical CLL
10	3	Non-CLL
11	3.3	Atypical CLL

CLL: Chronic lymphocytic leukemia

and 16 cirrhosis. The time from the diagnosis of HCV infection ranged between 6 and 156 month (average 90.14, median 84).

Flow cytometric analysis revealed MBL in 11 patients (22%). All patients had the kappa:lambda ratio >3. All of the 3 MBL immunophenotypes were present: 55% atypical CLL phenotype, 27% CLL phenotype and 18% non-CLL phenotype. Immunophenotypical characteristics of the patients with MBL are presented in *Table 1*.

Prevalence of MBL depending of the liver disease is presented in *Figure 1*.

Demographic and laboratory data of the patients with or without MBL were compared and statistical significance of the differences were calculated (*Table 2*).

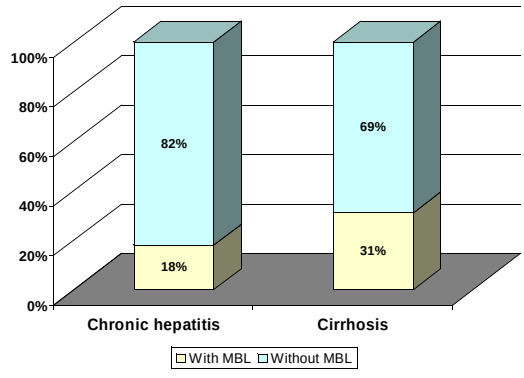

Figure 1. Prevalence of MBL depending on the liver disease

The white blood cell count was significantly lower in patients with MBL compared to those without MBL (p=0.04). There were no other statistically significant differences between the two groups.

Imagistic data were also compared and the only statistically significant difference was the increased liver echogenicity which was more frequent in patients with MBL (p=0.02) (*Table 3*).

Discussion

The prevalence of MBL in patients with HCV infection (22% in our study group) is significantly higher than that reported in the literature in the general population (3.5% in average) (22). Our finding is in accordance with other studies which have also found a high prevalence of MBL in HCV infected patients. These results sustain the pathogenetic role of HCV in B-cell lymphoproliferative disorders (23-25).

We have found a higher prevalence of MBL in patients with cirrhosis compared to those with chronic hepatitis (31% versus 18%). This finding is also in accordance with data from the

Table 2. Comparison of demographic and laboratory data of patients with or without MBL

Parameter	Patients without MBL	Patients with MBL	p
Gender, n (%)			
Female	26 (67)	5 (46)	p=0.35*
Male	13 (33)	6 (54)	
Age (years)#	56.17 (57, 20, 76)	58.63 (61, 33, 74)	p=0.56**
Age interval (years) No. (%)			
<50	7 (18)	3 (27)	p=0.21*
50-70	28 (72)	6 (55)	
>70	4 (10)	2 (18)	
Liver disease, n (%)			
Chronic hepatitis	28 (72)	6 (55)	p=0.47*
Cirrhosis	11 (28)	5 (45)	
Duration of HCV infection (month), n (%)			
<12	14 (36)	2 (18)	p=0.52*
12-60	13 (33)	6 (55)	
>60	12 (31)	3 (27)	
Leucocytes /mm3#	5818 (5520, 2570, 10290)	4602 (4500, 2150, 7650)	p=0.04**
Lymphocytes/mm#	1441 (1618, 642, 3171)	1395 (1379, 645, 2157)	p=0.18**
Thrombocytes /mm#	233820 (223000, 65000, 488000)	172272 (221000, 43000, 269000)	p=0.07**
Hemoglobin g/dl#	13.59 (13.9, 8.4, 16.7)	13.4 (13.9, 9.3, 16.7)	p=0.81**
ESR, n (%)			
Normal	18 (46)	2 (18)	p=0.18*
Elevated	21 (54)	9 (82)	
PT, n (%)			
Normal	18 (46)	6 (55)	p=0.88*
Increased	21 (54)	5 (45)	
ASAT (U/l)#	58.25 (41, 15, 184)	57.36 (42, 22, 151)	p=0.94**
ALAT (U/l)#	60.64 (51, 8, 199)	57.9 (42, 15, 167)	p=0.86**
Total bilirubin(mg/dl)#	1.03 (0.71, 0.33, 4.12)	1.03 (0.8, 0.48, 2.22)	p=0.98**
ALP (U/l)#	197.74 (177, 129, 410)	208.9 (182, 74, 522)	p=0.65**
GGT (U/l)#	52 (41, 21, 223)	65.63 (40, 24, 191)	p=0.42**

Average (median, minimum, maximum); *X^2 -test; ** T-test; MBL: monoclonal B lymphocytosis.

literature sustaining a higher prevalence of MBL in patients with advanced liver disease (19, 25).

Similar to results reported by other authors, the most frequent phenotype in our patients was the atypical CLL-type, confirming that, in contrast to the general population in which the CLL type is the most often seen, in patients with HCV infection, the most frequent immunophenotype is the atypical CLL (25).

Comparing patients with or without MBL, we didn't find a statistically significant difference concerning the gender, age and distribution of patients on age intervals. The duration of HCV infection didn't correlate with the prevalence of MBL. Laboratory data differed only in terms of the leucocyte count which was significantly lower in patients with MBL. Increased echogenicity of the liver was also found at a higher frequency in patients with MBL. The lower leucocyte count and the increased liver echogenicity in the MBL group could be explained by the more advanced liver disease, since there were more cases of cirrhosis in this group than in the group without MBL.

Table 3. Comparison of imagistic data of patients with or without MBL

Parameter	Patients with MBL, n (%)	Patients without MBL, n (%)	p
1. Abdominal ultrasound			
Echostructure of the liver			
Normal	5 (45.5)	15 (38.5)	p=0.94*
Non-homogenous	6 (54.5)	24 (61.5)	
Echogenicity of the liver			
Normal	2 (18)	14 (36)	p=0.02*
Increased	9 (82)	25 (64)	
Liver dimensions			
Normal	9 (82)	18 (46)	p=0.1*
Increased	2 (18)	21 (54)	
Portal vein dimension			
Normal	7 (64)	28 (72)	p=0.88*
Increased	4 (36)	11 (28)	
Superior mesenteric vein dimension			
Normal	7 (64)	29 (74.4)	p=0.79*
Increased	4 (36)	10 (25.6)	
Splenic vein dimension			
Normal	7 (64)	29 (74.4)	p=0.79*
Increased	4 (36)	10 (25.6)	
Spleen dimensions			
Normal	7 (64)	29 (74.4)	p=0.79*
Increased	4 (36)	10 (25.6)	
2. Fibroscan			
Minimal/moderate fibrosis (F0-F2)	7 (64)	25 (74)	p=1*
Severe fibrosis (F3-F4)	4 (36)	14 (26)	

*χ^2 -test; MBL: monoclonal B lymphocytosis

Comparing the three subgroups of patients with MBL, there was a higher lymphocyte count in the CLL-type group. This is in accordance with the characteristics of CLL, which is defined by accumulation of lymphocytes in the bone marrow and the peripheral blood.

Conclusions

We have found an increased prevalence of MBL in patients with HCV infection, in accordance with other studies made on HCV- infected patients. Such a study hasn't been done before in Romania, and there are only a few studies on this topic published in the literature. While in the general population, it is known that the rate of progression of MBL to CLL is about 1.1% /year, there are no studies evaluating the rate of transformation of MBL in malignant B-cell lymphoproliferative disorders in patients with HCV infection. Another question which could be raised is if in all HCV-infected patients the appearance of lymphoma is preceded by MBL. If this would be the case, immunophenotyping of lymphocytes from the peripheral blood in patients with HCV infection, especially in patients with advanced liver disease, could be used as a screening method for early detection of lymphoma, raising the chances for cure of these patients. Longitudinal studies on HCV-infected patients are needed to analyze this theory.

Acknowledgements

There are no conflicts of interest.

Abbreviations

CLL - chronic lymphocytic leukemia
HCV - hepatitis C virus
MBL - monoclonal B lymphocytosis

References

1. Turner NC, Dusheiko G and Jones A. Hepatitis C and B-cell lymphoma. Ann Oncol 2003;14:1341-1345
2. Zignego AL, Gianinni C, Ferri C. Hepatitis C virus-related lymphoproliferative disorders: an overview. World J Gastroenterol 2007;13(17):2467-2475.
3. Vlădăreanu A-M. Limfoamele în corelație cu virusurile limfotrope. Editura Amaltea 2007.Cap. Limfoamele maligne și virusurile hepatitice. Pag. 63-93
4. Ferri C, Caracciolo F, Zignego AL, La Civita L, Monti M, Longombardo G et al. Hepatitis C virus infection in patients with non-Hodgkin's lymphoma. Br J Haematol. 1994;88(2):392-394.
5. Pozzato G, Mazzaro C, Crovatto M, Modolo ML, Ceselli S, Mazzi G, et al. Low-grade malignant lymphoma, hepatitis C virus infection, and mixed cryoglobulinemia. Blood. 1994 Nov 1;84(9):3047–53.
6. Mele A, Pulsoni A, Bianco E, Musto P, Szklo A, Sanpaolo MG et al. Hepatitis C virus and B-cell non-Hodgkin lymphomas: an italian multicenter case- control study. Blood. 2003;102(3):996-999.
7. Duberg A-S, Nordstrom M, Torner A, Reichard O, Strauss R, Janson R et al. Non-Hodgkin's lymphoma and other neoplastic malignancies in Swedish patients with hepatitis C virus infection. Hepatology. 2005;41(3):652-659.
8. Anderson LA, Pfeiffer R, Warren JL, Landgren O, Gadalla S, Berndt SI et al. Hematopoietic malignancies associated with viral and alcoholic hepatitis. Cancer Epidemiol Biomarkers Prev.2008;17(11):3069-75.
9. Del Masso L, Franceschi S. Hepatitis C virus and risk of lymphoma and other lymphoid neoplasms: a meta-analysis of epidemiologic studies. Cancer Epidemiol Biomarkers Prev.2006;15(11):2078-2085.
10. Imai Y, Oshawa M, Tanaka H. High prevalence of HCV infection in patients with B-cell non-Hodgkin's lymphoma: comparison with birth cohort-and sex-matched blood donors in a Japanese population. Hepatology. 2002;35:974-6.
11. Al-Ghiti AAO, Arbanas T, Diculescu M. Epidemiological characteristics of hepatitis C and B viral infection in patients with lymphoproliferations sustain their role in lymphomagenesis. Medica. 2006;4(1):5-11.
12. Yenice N, Gulluk F, Arican N, Turkmen S. HCV prevalence in Hodgkin and non-Hodgkin lymphoma cases. Turk J Gastroenterol 2003;14(3):173-176.
13. Kuniyoshi M, Nakamuta M, Sakai H, Enjoji M, Kinukawa N, Kotoh K et al. Prevalence of hepatitis B or C virus infection in patients with non-Hodgkin's lymphoma. J Gastroenterol Hepatol 2001;16(2):215-9.
14. Kang J, Cho JH, Suh SW, Lee DH, Oh HB, Sohn YH et al. High prevalence of hepatitis B and hepatitis C virus infections in Korean patients with hematopoietic malignancies. Ann Hematol 2011;90(2):159-64.
15. De Rosa G, Gabbo ML, De Renzo A. High prevalence of hepatitis C virus infections in patients with B-cell lymphoproliferative disorders in Italy. Am J Hematol 1997;55:77-82.
16. Zucca E, Roggero E, Maggi-Solca N, Conconi A, Bertoni F, Reilly I et al. Prevalence of Helicobacter Pylori and hepatitis C virus infections among non-Hodgkin's lymphoma patients in Southern Switzerland. Haematologica 2000;85:147-153.
17. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Döhner H, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008 Jun 15;111(12):5446–56.
18. Mowery YM, Lanasa MC. Clinical aspects of monoclonal B-cell lymphocytosis. Cancer Control. 2012 Jan;19(1):8–17.
19. Ghia P. Another piece in the puzzle: is there a "nodal" monoclonal B-cell lymphocytosis? Haematologica 2011;96(8):1089-1091.
20. Matos DM, Falcão RP. Monoclonal B-cell lymphocytosis: a brief review for general clinicians. Sao Paulo Med J. 2011 May;129(3):171–5.
21. Marti G E, Rawstron A C, Ghia P, Hillmen P, Houlston RS, Kay N et al. Diagnostic criteria for monoclonal B-cell lymphocytosis. Br J Haematol 2005;130:325-332.
22. Rawstron A C, Green M J, Kuzmicki A, Kennedy B, Fenton JA, Evans PA et al. Monoclonal B lymphocytes with the characteristics of "indolent" chronic lymphocytic leukemia are present in 3.5% of adults with normal blood counts. Blood 2002;100:635-639.
23. Ohtsubo K, Sata M, Kawaguchi T, Morishige S, Takata Y, Oku E, Imamura R et al. Characterisation of the light chain-restricted clonal B cells in peripheral blood of HCV- positive patients. Int J Hematol 2009;89(4):452-459.
24. Johansson P, De Dechene E M, Eisele L, Kieruzel S, Horn P, Schmucker U et al. Increased prevalence of monoclonal B lymphocytosis in patients with chronic hepatitis C. Journal of Hepatology 2010;52(1):274.
25. Fazi C, Dagklis A, Cottini F, Scarfo L, Bertilaccio MT, Finazzi R et al. Monoclonal B cell lymphocytosis in hepatitis C virus infected individuals. Cytometry B Clin Cytom 2010;78(1):61-8.