® Postepy Hig Med Dosw (online), 2017; 71: 1172-1176
e-ISSN 1732-2693

www.phmd.pl
Original Article

Received: 2016.09.21
Accepted: 2017.02.17
Published: 2017.12.29

Cryoglobulinemia and its correlation with clinical extrahepatic manifestations in chronic hepatitis C

Krioglobulinemia i jej związek z pozawątrobowymi objawami przewlekłego wirusowego zapalenia wątroby typu C

Joanna Jabłońska[1, A, B, D, E, F], Joanna Kozłowska[1, B, D, E], Jakub Ząbek[2, B], Bożena Walewska-Zielecka[3, B], Zbigniew Lewandowski[4, C], Alicja Wiercińska-Drapało[1, A, D]

[1]Department of Infectious and Tropical Diseases and Hepatology, Medical University of Warsaw/Hospital of Infectious Diseases in Warsaw
[2]Med Genetics Laboratory Warsaw
[3]Department of Public Health, Medical University of Warsaw, National Institute of Public Health/National Institute of Hygiene
[4]Department of Epidemiology, Medical University of Warsaw

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

Summary

Cryoglobulinemia is a condition with a confirmed relationship with HCV infection, but many other extrahepatic symptoms are also present in hepatitis C.

Aim: The aim of this study was to assess the incidence and type of extrahepatic manifestations and cryoglobulinemia as well as their correlation with clinical symptoms in Polish patients.

Material and methods: 246 consecutive patients with hepatitis C were studied. Clinical symptoms were assessed by anamnesis and a clinical investigation. Cryoglobulins were estimated by a method basing on their ability to reverse precipitation in temperature +4ºC. Monoclonality of cryoglobulins was estimated by agarophoresis immunotyping – capillary electrophoresis and immunofixation. The presence of classical rheumatoid factor (RF-IgM) was tested using ELISA. Anti-nuclear antibodies (ANA) were tested using the indirect immunofluorescence method. Anti-CCP, anti--SSA, anti-α-fodrin antibodies, RF IgG, IgM and IgA were assessed using commercial enzymatic tests. Statistical methods were: Fisher exact test, generalized additive models, odds ratios with 95% confidence intervals, aid of spline curve, SAS system version 9.4.

Results: Cryoglobulins were found in 93 persons (37.8%). Type II cryoglobulinemia was detected in 28 persons. Patients with cryoglobulinemia were elder ($p<0.0004$). 54 patients (21.95%) manifested clinical extrahepatic symptoms. Arthralgia was found in 27 cases (10.98%). Skin changes were found in 22 patients (8.94%). 12 persons had glomerulonephritis (4.88%). 11 patients (4.47%) had sicca syndrome. 6 patients developed peripheral polyneuropathy (2.4%). 4 persons developed B cell lymphoma (1.63%). There was no correlation between presence of symptoms and grading, staging, age, HCV genotype, and the presence of autoantibodies. Extrahepatic manifestations were present more frequently in women ($p<0.0008$).

Key words: cryoglobulinemia • chronic hepatitis C • extrahepatic manifestations

GICID: 01.3001.0010.7663
DOI: 10.5604/01.3001.0010.7663
Word count: 1719
Tables: 1
Figures: 1
References: 10

Author's address: dr n. med. Joanna Jabłońska Department of Infectious and Tropical Diseases and Hepatology, Medical University of Warsaw/Hospital of Infectious Diseases in Warsaw, 01-201 Warszawa, ul. Wolska 37; e-mail: joannaj108@hotmail.com

Background

HCV infection can cause numerous extrahepatic manifestations, mostly of immunologic origin, probably connected with longitudinal and excessive stimulation of the immune system [7]. Cryoglobulinemia is an extrahepatic manifestation with the most confirmed causal relationship with HCV infection. Knowledge about this disease has massively increased since its first description by Melzer and Franklin in 1966 [6], but still there are many questions. The question of why only some patients with hepatitis C develop this condition is still unresolved. The presence of negative stranded RNA in peripheral blood mononuclears of patients with symptomatic cryoglobulinemia was demonstrated, which confirms the direct role of HCV replication in developing cryoglobulinemia. There are also reports that show the impact of genetic host factors connected with the development of this syndrome [3,8].

There are 3 types of cryoglobulinemia by Bruet [2]: in type I, in most cases connected with malignant lymphoproliferative disorders, there is only monoclonal cryoprecipitating immunoglobuline. In type II cryoglobulinemia monoclonal IgM with rheumatoid factor activity and WA cross-reacting idiotype is present, exactly the same protein in all patients plus polyclonal IgG immunoglobulins. In type III cryoglobulinemia, there are polyclonal IgG immunoglobulins with the ability of cryoprecipitation.

The clinical expression of cryoglobulinemia varies because of the lack of symptoms, and can be observed as being anywhere from a chronic condition with Melzer triade (fatigue, palpable purpura and arthralgia) to fulminant life threatening complications [1,2,3,4,5,6,7,8,9,10].

Aim

Data regarding the extrahepatic manifestation of HCV infection are not homogenous and are dependent on the geographical area. We assessed the incidence and type of extrahepatic manifestations and cryoglobulinemia and their correlation with clinical symptoms in Polish patients.

Patients

246 consecutive patients with hepatitis C were studied. There were 147 women (60%) and 99 men (40%). The average age was 45 years (±12,6). HCV genotype was 1 in 88%, 3 in 11% and 4 in 1% of cases. 18 patients (7%) had liver cirrhosis. Liver biopsy was performed in 137 patients. Staging by Metavir Scale was S0 in 10%, S1 – in 36%, S2 – in 30%, S3 – in 11%, S4 – in 13%.

Methods

Clinical symptoms were assessed by anamnesis and clinical investigation.

Cryoglobulins were estimated by a method based on the ability of cryoglobulins to reverse precipitation in temperature of +4ºC. Serum after clotting and centrifugation was incubated 4-6 days in +4ºC. If turbid precipitate appeared, a sample was incubated for 1-2 h in temp. 37ºC and after this turbid sample became clear or precipitate dissolved, then the presence of cryoglobulins was confirmed. In order to validate the intensity of cryoprecipitation, the concentration of protein in cryoprecipitate, after washing cryoprecipitate 3 times with cold (+4ºC) PBS, was estimated using the Kalckar method.

Monoclonality of cryoglobulins was estimated by agarophoresis immunotyping – capillary electrophoresis and immunofixation with reagents and equipment by SEBIA/Horiba, System Capillares 2.

The presence of classical rheumatoid factor (RF-IgM) was tested by ELISA method (using Wampole Laboratories kits).

Anti-nuclear antibodies (ANA) were tested by indirect immunofluorescence method (IIF) based on slides covered by Hep-2 cells (produced and provided by Euroimmun (Germany).

RF IgG and IgA were assessed by commercial enzymatic tests by Cogent Diagnostics.

RF IgM was assessed with commercial enzymatic test by Wampole Laboratories.

Anti-CCP was assessed by ELISA Anti-CCP-ELISA (IgG) by Euroimmun.

Anti-SSA and anti-α-fodrin antibodies were assessed by ELISA by Pointe Scientific Aesculisa Anti-Fodrin-Check and Aesculisa SSA.

Statistical methods: Fisher exact test for comparing the frequencies of events were used. To estimate the risk of cryoglobulinemia depending on quantitative and qualitative factors, generalized additive models were used. The strength of these relations was expressed by odds ratios with 95% confidence intervals. The relation between age and risk of cryoglobulinemia was presented with the aid of a spline curve. SAS system version 9.4 was used for statistical calculation.

Results

Clinical symptoms

54 patients among the 246 investigated (21.95%) manifested clinical extrahepatic symptoms of HCV infection. In most cases, the patients had more than one extrahepatic manifestation.

Arthralgia and/or arthritis was found in 27 cases (10.98%). In 5 cases oedema of a few joints occurred periodically. In 2 patients, oedema and inflammation of knee joints developed. 8 persons had morning stiffness of joints. Radiological changes typical for rheumatoid arthritis were not found in any of the cases. In one case anti-CCP antibodies were found.

Skin changes, mostly palpable purpura on legs, were found in 22 patients (8.94%).

12 persons had glomerulonephritis (4.88%). In 6 cases a kidney biopsy was performed. Five patients had mebranoproliferative glomerolonephritis and one patient – mesangial proliferative glomerulonephritis. In 11 cases nephritic syndrome was observed.

11 patients (4.47%) had sicca syndrome and in one case xerophthalmia also occurred. Anti-SSB antibodies were not found in any of the cases, and in 2 cases anti-SSA antibodies and in one case anti-fodrine antibodies were found.

Six patients developed peripheral polyneuropathy (2.4%).

Four persons developed B cell lymphoma (1.63%).

There was no correlation between the presence of clinical extrahepatic manifestations and grading, staging, age, HCV genotype, presence of autoantibodies. Extrahepatic manifestations were more often found in women (29.3% vs 11.1%; p<0.0008). Sicca syndrome was found only in women (7.48%; p<0.004), so was lymphoma. Arthralgia was observed in 24 female patients and in 3 male patients (16.33% vs. 3.03%; p<0007). Skin changes (purpura on legs) were found in 19 women and in 3 men (12.93% vs. 3.03%; p<0,01). There was no correlation between gender and the presence of polyneuropathy (3.4% vs 1.01%) and nephropathy (3.4% vs 7.07%).

Immunological abnormalities

Cryoglobulins were found in 93 persons out of 246 (37.8%) – 57 female and 36 male (39 vs. 36%). Patients with cryoglobulinemia were older than patients without cryoglobulinemia (median 50.5 years vs. 43.1– p<0.0004). There was no connection between the presence of cryoglobulinemia and gender, HCV genotype, grading and staging. Type II cryoglobulinemia (with monoclonal IgM) was detected in 28 persons (30% of patients with cryoglobulinemia and 11% of all tested patients). It was more frequent in females than in males (47.46 vs. 21.43%; p<0.03).

There was no correlation between the presence of cryoglobulinemia and peripheral polyneuropathy and glomerulonephritis. There was a strong positive connection between cryoglobulinemia and sicca syndrome (found in 10.75% persons with cryoglobulinemia and 0.65% persons without cryoglobulinemia; p<0.0001), arthralgia (19.36% in the group with cryoglobulinemia vs 5.88% in the group without; p<0.0015) and purpura (22.58% in the group with cryoglobulinemia vs 0.65% in the group without; p<0.0001). In the group with cryoglobulinemia statistically more patients had any clinical extrahepatic manifestations of HCV infection (38.71% vs. 11.76%; p<0.0001).

There was a connection between the type of cryoglobulinemia and clinical symptoms, which were found in 20.34% of patients with cryoglobulinemia type III

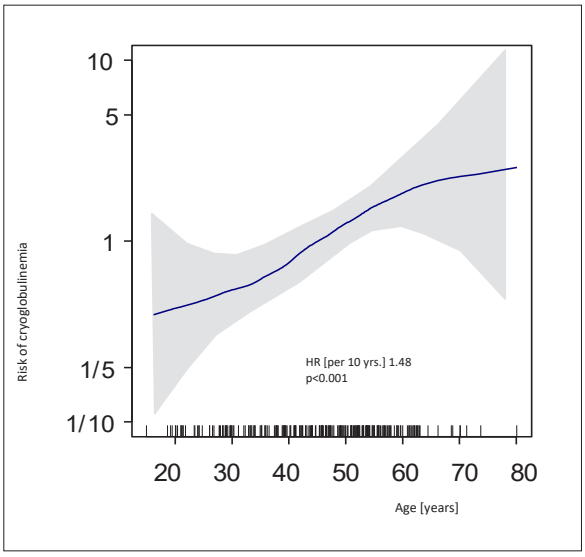

Fig. 1. Risk of cryoglobulinemia depending on age

Table 1. Risk of cryoglobulinemia depending on putative risk factors

Factors	Cryoglobulinemia& no (n=153) yes (n=93)	OR#	95% CI	p value
Age., median IQR [yrs.]	43.1 (33.1-53.1) 50.5 (42.9-57.1)	1.48§	1.18-1.85	< 0.001‡
Males	41.2 % 38.7 %	0.90	0.53-1.53	0.702
Stageing:				
0 - 2	71.4 % 67.2 %	1.00		
≥ 3	16.5 % 21.9 %	1.41	0.62-3.22	0.413
Cirrosis	12.1 % 10.9 %	0.96	0.35-0.68	0.941

& - distributions of factors or medians with IQR's (interquartile range) depending on presence of cryoglobilinemia, # - odds ratio and 95% confidence interval (CI), § - the risk was estimated for 10 yrs, ‡ - the relation was presented at figure 1.

and in 82.14% of patients with cryoglobulinemia type II (p<0.0001). The strongest connection concerned the presence of purpura on legs – it was observed in 53.57% of patients with cryoglobulinemia type II and in 8.47% of patients with cryoglobulinemia type III (p<0.0001). 25% of people with cryobulinemia type II and 3.39% with cryoglobylinemia type III had sicca syndrome (p<0.004). Nephropathy was observed in 21.43% of patients with cryoglobulinemia type II and 1.69% with cryoglobulinemia type III (p<0.004). Lymphoma was observed only in patients with cryoglobulinemia type II (10.71% vs 0%; p<0.03). Arthralgia developed more often in the group with cryoglobulinemia type II (35.71% vs 13.56%; p<0.02).

Out of four putative factors of cryoglobulinemia only age turned out to be a predictor of cryglobulinemia (Table 1). The risk of cryoglobulinemia was 1.48 fold increased per 10 years (p<0.001) (Fig. 1).

Disscusion

The connection of HCV infection with different immunological disorders is often suggested, but the question if these abnormalities result from HCV infection or accidentally coexist is frequently difficult to answer. Cryoglobulinemia is an extrahepatic condition with a confirmed relationship with HCV. HCV envelope protein E2 binds CD81, presented both on hepatocytes and Lymphocytes B and T, and stimulates chronically B cells [8,10].

We found the presence of cryoglobulins in sera in 38% of the patients, but only one third of them, mostly these with type II cryoglobulinemia, presented clinical symptoms. In other publications the range was similar. Lunel et al. found clinical symptoms in 25% of patients with detectable cryoglobulins [5].

The symptomatic cryoglobulinemia develops after years of HCV infection. The severity of symptoms also increases over time. Adinolfi assessed the annual incidence of cryoglobulinemia in hepatitis C patients as 3% [1]. In our group, the patients with cryoglobulinemia were older than the patients without this condition. The period of HCV infection was difficult to estimate because of the lack of evident acute hepatitis in most cases, but the data from anamnesis showed a probably longer history in patients with symptomatic cryoglobulinemia.

In the assessed group of patients, in most cases one person had more then one extrahepatic symptom. It was especially evident in patients with type II cryoglobulinemia, which demonstrated fatigue syndrome, arthralgia, skin lesions and sicca syndrome together. Genuine rheumatoid arthritis and Sjögren disease can be distinguished from HCV connected disorders by the use of newer markers: anti-CCP antibodies and anti-fodrin antibodies. Rheumatoid factor is not valuable in HCV infected patients, because it is frequently present in this population.

The most common extrahepatic symptom was arthralgia. It was more frequently observed in women and was correlated with the presence of cryoglobulinemia. In 7 cases, oligoarthritis was diagnosed and in 3 – polyarthritis. Even patients with the most severe joint problems and with clinical symptoms similar to rheumatoid arthritis did not fulfill the diagnostic criteria for this disease.

Ferri demonstrated worse prognosis for patients with cryoglobulinemia [4]. The quality of life was poorer in patients with symptomatic cryoglobulinemia and the outcome of the disease was worse. We also observed the same findings in our group.

In the majority of our patients with cryoglobulinemia treated with interferon and ribavirin, the treatment was ineffective. In many cases adverse events occurred and therapy had to be terminated. In the present era the treatment of both hepatic and extrahepatic manifestations of HCV infection with DAA will hopefully be more potent. Extrehepatic manifestations should be taken into consideration as a factor qualifying for immediate treatment.

Conclusions

1. Presence of cryoglobulins and symptomatic cryoglobulinemia are different clinical phenomena.

2. Liver disease and extrahepatic manifestations develop independently during the history of HCV infection.

3. Patients with extrahepatic manifestations of HCV infection and particularly type II cryoglobulinemia should be treated immediately with directly acting antiviral drugs.

4. Patients with cryoglobulinemia should be monitored for lymphoma development.

References

[1] Adinolfi L.E., Utili R., Attanasio V., Zampino R.,Ragone E.,Tripodi M.F., Ruggiero G.: Epidemiology, clinical spectrum and prognostic value of mixed cryoglobulinaemia in hepatitis C virus patients: a prospective study. Ital. J. Gastroenterol., 1996; 28: 1-9

[2] Brouet J.C., Clauvel J.P., Danon F., Klein M., Seligmann M.: Biologic and clinical significance of cryoglobulins. A report of 86 cases. Am. J. Med., 1974; 57: 775-788

[3] Cacoub P., Gragnani L., Comarmond C., Zignego A.L.: Extrahepatic manifestations of chronic hepatitis C virus infection. Dig. Liver Dis., 2014; 46: S165-S173

[4] Ferri C., Sebastiani M., Giuggioli D., Cazzato M., Longombardo G., Antonelli A., Puccini R., Michelassi C., Zignego A.L.: Mixed cryoglobulinemia: demographic, clinical, and serologic features and survival in 231 patients. Semin. Arthritis Rheum., 2004; 33: 355-374

[5] Lunel F., Musset L., Cacoub P., Frangeul L., Cresta P., Perrin M., Grippon P., Hoang C., Valla D., Piette J.C., Huraux J.M., Opolon P.: Cryoglobulinemia in chronic liver diseases: role of hepatitis C virus and liver damage. Gastroenterology, 1994; 106: 1291-1300

[6] Meltzer M., Franklin E.C., Elias K., McCluskey R.T., Cooper N.: Cryoglobulinemia – a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. Am. J. Med., 1966; 40: 837-856

[7] Pawełczyk A.: Cosequences of extrahepatic manifestations of hepatitis C viral infection (HCV). Postępy Hig. Med. Dośw., 2016; 70: 349-359

[8] Ramos-Casals M., Stone J.H., Cid M.C., Bosch X.: The cryoglobulinaemias. Lancet, 2012; 379: 348-360

[9] Sene D., Ghillani-Dalbin P., Thibault V., Guis L., Musset L., Duhaut P., Poynard T., Piette J.C., Cacoub P.: Longterm course of mixed cryoglobulinaemia in patients infected with hepatitis C virus. J. Rheumatol., 2004; 31: 2199-2206

[10] Trejo O, Ramos-Casals M., Garcia-Carrasco M., Yagüe J., Jiménez S., de la Red G., Cervera R., Font J., Ingelmo M.: Cryoglobulinemia: study of etiologic factors and clinical and immunologic features in 443 patients from a single center. Medicine, 2001; 80: 252-262

The authors have no potential conflicts of interest to declare.