

Original paper

# The occurrence of autoantibodies in patients with chronic HCV infection, including patients dialyzed and after kidney transplantation

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## Abstract

**Introduction:** There are reports suggesting that hepatitis C virus (HCV) may stimulate the autoimmune process. Studies have been undertaken to evaluate the occurrence and type of autoantibodies in HCV-infected patients with and without immunosuppression. Results were analyzed according to HCV genotype, intensity of inflammation and liver fibrosis stage.

**Material and methods:** The study included 105 patients chronically infected with HCV, including 25 with immunological suppression administered for kidney disease or kidney transplantation. Blood samples were tested by immunoblotting for the presence of AMA-M2, SLA/LP, LKM-1, LC1, anti-F-actin, anti-desmin, anti-myosin, anti-gp210 and anti-sp100 autoantibodies, and ANA. All the patients were scored for autoimmune hepatitis.

**Results:** Autoantibodies were detected in 32.5% of patients without immunosuppression and in 16% with immunosuppression. Single types of autoantibodies were identified in 26% of patients. The most frequent ones were ANA (19%) and AMA-M2 (5.7%). The presence of antibodies in patients with genotype 1 was significantly higher in comparison to their occurrence in genotype 3. Autoimmune hepatitis was not diagnosed in any of the patients. Immunoglobulin G level was significantly higher in patients with detectable autoantibodies, compared to patients without antibodies (1.89 vs. 1.28 g/dl,  $p < 0.001$ ). No correlation between fibrosis stage or intensity of inflammatory state and the frequency of antibodies was found.

**Conclusions:** The antibodies are significantly more frequent in patients without immunosuppression and in patients infected with genotype 1 than genotype 3. The presence of these autoantibodies is not associated with the development of autoimmune hepatitis. Higher level of immunoglobulin G in the serum correlates with the presence of autoantibodies.

**Key words:** autoantibodies, HCV infection, immunosuppressive therapy.

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## Introduction

Hepatitis C virus (HCV) infection is a common cause of chronic inflammatory liver injury, but not only that. Hepatitis C virus infection may influence the composition of intracellular proteins. This fact is connected with the binding of viral proteins to host proteins, resulting in the change of their spatial and biochemical structure. Moreover, viral infections may influence production of proteins structurally similar

to those naturally occurring, but showing antigenic differences. This phenomenon, called molecular mimicry, constitutes one of the most important theories explaining the existence of autoimmune diseases. Presentation of proteins modified according to this mechanism may be responsible for the development of autoimmune processes leading to occurrence of different types of autoantibodies [1]. Autoantibodies are not the only active element in autoimmune processes. Actually, autoantibodies are readily available markers

**Table 1.** Control and study groups

Factor	HCV without immunosuppression	HCV with immunosuppression	Control group
Number of patients	80	25	27
Age (years)	44 (23-67)	46 (23-69)	37 (25-56)
Female/male (%)	30/50 (38/62)	11/14 (44/56)	13/14 (48/52)
Genotype 1/3 (%)	58/22 (73/27)	21/4 (84/16)	-

*There were no significant differences between the groups.*

of activation of such processes, but cannot be used as a definitive predictor of autoimmune disease, such as autoimmune hepatitis (AIH). Immunization by HCV may lead to autoimmune hepatitis, which overlaps with viral hepatitis [2]. This is extremely important from the therapeutic point of view, especially among patients with concurrent injuries to other organs, such as chronic kidney injury or kidney transplantation.

This study aimed to evaluate the frequency and type of autoantibodies among patients taking immunosuppressive therapy for chronic kidney injury or patients after kidney transplantation, compared to patients infected with HCV, in whom immunosuppression was not used. Occurrence of autoantibodies was correlated with HCV genotype, intensity of inflammatory state and the stage of fibrosis in the hepatic tissue. The relation between the presence of autoantibodies and the level of immunoglobulin G,  $\alpha$ -fetoprotein (AFP) and guanosine-5'-triphosphate (GTP) in the serum was tested. All the patients were scored for AIH.

## Material and methods

The study included one hundred and five patients infected with HCV, i.e. 79 infected with genotype 1b and 26 with genotype 3a. None of the patients was previously treated with antiviral therapy. Immunosuppressive therapy was used in 25 patients: 19 patients with end-stage renal disease (glomerulonephritis) and 6 after kidney transplantation, also due to being previously diagnosed with glomerulonephritis (Table 1). In 5 patients after kidney transplantation cyclosporine was administered, and in 1 tacrolimus. Fourteen patients with end-stage renal failure were hemodialyzed and periodically given glucocorticosteroids. Five patients with end-stage renal failure, who were not dialyzed, were constantly taking glucocorticosteroids.

Study inclusion criteria included diagnosis of chronic hepatitis C, exclusion of HBV and HIV, and no autoimmune hepatitis diagnosed in patient history.

The control group included 27 healthy volunteers.

Hepatitis C virus infection was diagnosed based on the presence of HCV RNA in the serum. The study was

performed using RT-PCR with starters specific for a non-coding viral 5'-terminal genomic fragment (5'-UTR). Viral genotype was determined with direct sequencing of the product obtained in the PCR reaction (Syngen Biotech, USA)<sup>1</sup>.

The presence of autoantibodies in the serum was detected with Liver-9-line test, by immunoblotting (membrane-fixed immunoblot test) purchased from Organtec, Germany. In the test, processed serum was transferred onto a nitrocellulose plate containing antigens binding antibodies against F-actin, desmin, myosin, liver cytosol antigen type 1 (LC1), liver-kidney microsome type 1 (LKM-1), soluble liver antigen/liver-pancreas (SLA/LP) antigens, Sp100, gp210 and antimitochondrial type M2 antibodies (AMA-M2). Moreover, antinuclear antibodies (ANA) were tested in the serum by ELISA, using a BEP 2000 A2/V instrument by Siemens<sup>2</sup>.

All patients were tested for HBV infection by evaluating the presence of HBs antigen in the serum, and for HIV by measuring anti-HIV antibodies (Abbott, USA).

All patients were evaluated for the level of gamma immunoglobulin, AFP and GTP activity in the serum<sup>2</sup>.

Fibrosis was measured in 16/25 (64%) immunosuppressed patients: in 6 liver biopsy was performed, and in 10 elastography (Aixplorer, Supersonic). From among 80 patients without immunosuppression, liver biopsy was performed in 58, and elastography in 22 patients. In patients subjected to liver biopsy, biopsates were stained with hematoxylin and eosin in order to determine the intensity of the inflammatory state, and with Syrian red to visualize connective tissue. Morphological state of the liver was described according to the Scheuer classification<sup>3</sup>. Results of liver fibrosis

<sup>1</sup>Analysis was performed at the Clinical Molecular Biology Department, Medical University of Białystok, Head: Prof. Nikliński.

<sup>2</sup>Tests were performed in the Laboratory of K. Dluski Memorial Regional Specialist Hospital in Białystok, Head: Jolanta Czajkowska, MSc.

<sup>3</sup>Morphological state of liver biopsates was evaluated by Prof. A. Panasiuk at the Clinics of Infectious Diseases and Hepatology, Medical University of Białystok.

determined by elastography were denoted in kPa and automatically converted into the Metavir scale.

After approval of the study by the Bioethical Committee at the Medical University of Bialystok, the patients expressed informed consent to participation in the research.

Statistical analysis was performed with  $\chi^2$ , Student's *t* and Mann-Whitney *U* tests. Significance was established at  $p < 0.05$ .

## Results

The presence of autoantibodies was found in 4/25 (16%) immunosuppressed patients infected with HCV and in 32/80 (40%) patients without immunosuppression (the difference was statistically significant,  $p = 0.001$ ). In none of the patients with kidney injury or after kidney transplantation, and in all of those with diagnosed chronic glomerulonephritis, neither ANA, AMA-M2 nor LKM antibodies were found. Only single types of autoantibodies were detected in the group of patients receiving immunosuppression. Among the patients without immunosuppression, single types of autoantibodies were found in 21 patients, in 4 patients simultaneous presence of two autoantibodies was detected, and in one patient 3 autoantibodies. Among 27 healthy volunteers from the control group, autoantibodies were not detected (Fig. 1).

Antibodies against F-actin, LC-1 and sp-100 protein were not detected in any patient. The most frequently found antibodies were ANA (20/105; 19%) and AMA-M2 (6/105; 5.7%).

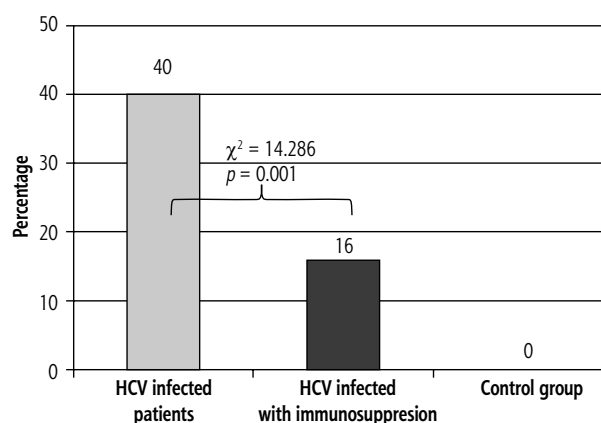


Fig. 1. Prevalence of autoantibodies in particular groups of patients

In the group of patients without immunosuppression there was a significant difference in the frequency of autoantibodies in patients infected by genotype 1 compared to those infected with genotype 3 (46.5% vs. 22.7%,  $p = 0.001$ ) (Table 2).

Scoring for autoimmune hepatitis was performed in the group of patients with detected autoantibodies. Evaluation was performed according to the guidelines of the International Autoimmune Hepatitis Group, where the minimal number of points indicating autoimmune disease is 15 [3]. The highest calculated number of points among the studied patients was 7 (HLA DR3 or DR4 was not taken into consideration when scoring, but omission of this factor may decrease the total result to 14 points).

Level of immunoglobulin G in the serum was significantly higher in patients with autoantibodies, com-

Table 2. Type of autoantibodies depending on the genotypes of the virus among HCV infected patients

Autoantibodies	Patients without immunosuppression			Patients with immunosuppression		
	Genotype 1 (n = 58)	Genotype 3 (n = 22)	Total (n = 80)	Genotype 1 (n = 21)	Genotype 3 (n = 4)	Total (n = 25)
	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
ANA	15 (25.9) <sup>x</sup>	2 (9.1) <sup>x</sup>	17 (21.3)	3 (14)	0	3 (12)
Anti-Myosin	2 (3.4)	1 (4.5)	3 (3.7)	0	0	0
Anti-Desmin	2 (3.4)	0	2 (2.5)	0	0	0
Anti-F-actin	0	0	0	0	1 (25)	1 (4)
LKM	1 (1.7)	0	1 (1.3)	0	0	0
SLA/LP	1 (1.7)	0	1 (1.3)	0	0	0
gp120	2 (3.4)	0	2 (2.5)	0	0	0
AMA-M2	4 (6.9)	2 (9.1)	6 (7.5)	0	0	0
Total	27 (46.5) <sup>*</sup>	5 (22.7) <sup>*</sup>	32 (40.0) <sup>#</sup>	3 (14)	1 (25)	4 (16) <sup>#</sup>

<sup>x</sup>Statistically significant difference ( $p < 0.05$ )

<sup>\*</sup>Statistically significant difference ( $p < 0.05$ )

<sup>#</sup>Statistically significant difference ( $p < 0.05$ )

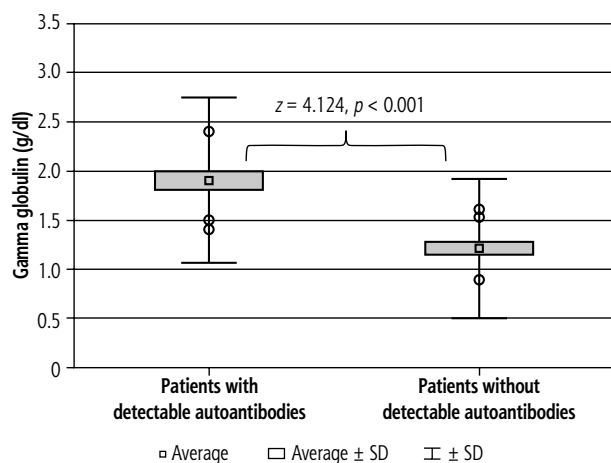


Fig. 2. Serum IgG concentration in the study groups

pared to patients without autoantibodies ( $1.89 \pm 1.82$  vs.  $1.28 \pm 1.2$  g/dl;  $Z = 4.124$ ;  $p < 0.001$ ) (Fig. 2). It has been demonstrated that IgG concentration above 1.6 g/dl is associated with the presence of autoantibodies, and is found in 87% of such patients.

No difference in AFP level or GPT activity was found among the patients with or without the presence of autoantibodies.

Average values of fibrosis in immunosuppressed patients were comparable to fibrosis patients without immunosuppression (average  $1.78 \pm 1.12$  vs.  $1.88 \pm 1.03$ ;  $p = 0.78$ ).

There was no difference in the progression of fibrosis in patients with the presence of autoantibodies compared to patients without autoantibodies ( $2.32 \pm 0.94$  vs.  $2.60 \pm 0.56$ ,  $p = 0.36$ ).

## Discussion

The influence of the liver on the mechanisms of tolerance and hyperreactivity is very important for the development of autoimmune diseases, autoimmune hepatitis in particular. Homology of HCV antigens' structure and the structure of hepatocyte proteins allows for consideration of the possibility of autoaggression as the effect of cross-antigenicity, leading to the development of autoimmune hepatitis.

Antinuclear antibodies (ANA) are directed against deoxyribonucleic acid and deoxyribonucleoproteins contained in the cell nucleus. Antinuclear antibodies are usually associated with autoimmune hepatitis type 1. In their study performed on 4754 adult Americans aged over 12, Satoh *et al.* detected ANA in 13% [4]. However, the occurrence of ANA in the healthy population is probably dependent on many factors, including geographical distribution. Retrospective analysis performed by Khairy *et al.* among 3673 Egyptians

infected with HCV and among healthy people demonstrated the presence of ANA in both of these groups in similar proportions, around 1.6% [5]. In a study by Molazadeh *et al.*, ANA were present in 0.9% (5/560) of healthy people in Iran [6]. In our research, in the control group of 27 persons we did not detect these autoantibodies, but among those infected with HCV they were detected in 19% of patients. Data presented by Basile *et al.* point to significantly more frequent occurrence of ANA in 65% of people infected with HCV, compared to healthy people [7]. It seems that HCV activates mainly the process of ANA synthesis.

According to the international diagnostic criteria of autoimmune hepatitis (International Autoimmune Hepatitis Group) [3, 8], AIH diagnosis could not be established in any of our patients. Among the patients with immunosuppression, autoantibodies were much less frequently found than in patients without immunosuppression. These observations point to activation of autoimmune mechanisms by HCV, but not to the influence on advanced immunological changes leading to the development of autoimmune diseases.

AMA-M2 autoantibodies, the second most frequently occurring type of autoantibodies among patients infected with HCV (7%), are characteristic in patients with primary biliary cirrhosis (PBC). Two subtypes of AMA antibodies, one against the proteins of cellular membrane pores gp210, and another against nuclear specific protein sp100, are currently thought to be more specific in the diagnostics of PBC than AMA-M2. Compared to AMA-M2, anti-gp210 and anti-SP100 are better markers of PBC exacerbation and risk of PBC development [9]. Thus, detection of these antibodies in only 2 patients seems justified. AMA-M2 are directed against mitochondrial internal membrane proteins. They are rarely detected in patients infected with HCV and usually occur in women during the treatment with interferon [10].

Antibodies directed against F-actin belong to the group of antibodies against smooth muscles. They may be produced in response to changes of filaments in the cells of the biliary tract and hepatic tissue matrix. Studies suggest an association of these proteins with HCV replication and formation of neoplastic lesions [11]. Hudacko *et al.* demonstrated F-actin antibodies in 16% of patients infected with HCV or HCV/HIV. Moreover, the authors documented the correlation between occurrence of these antibodies and severity of the inflammatory state in the liver [12]. In our research, antibodies directed against F-actin were not found in any of the patients.

Only in 3 patients were anti-myosin antibodies, usually occurring in patients with cardiac diseases, found. Antibodies against desmin were detected rarely, i.e. only

in 2 patients. Although desmin is a protein building Ito cells, such antibodies are found sporadically in autoimmune liver diseases [13].

Anti-SLA antibodies are thought to be a marker of AIH type III [14]. In our research these antibodies were found in one person, which agrees with reports suggesting that they are rarely found among people infected with HCV [15].

Antibodies against microsomal proteins of liver and kidneys, anti-LKM-1, are a marker of AIH type II [16]. It is suggested that these antibodies are formed mainly through molecular mimicry connected with the loss of immunological tolerance. The change of the structure of autoantigens, as the result of binding viral proteins with host proteins and forming a complex significantly differing from the primary antigen, usually underlies this phenomenon [17]. In our research the presence of these antibodies was demonstrated in only one person, which confirms the stimulating effect of the virus on autoimmune processes, but not the ability to initiate an autoimmune disease.

It is thought that the course of chronic HCV infection among dialyzed patients is more aggressive compared to the patients with healthy kidneys. However, clinical observations show a similar clinical picture and individual progress of the disease, regardless of dialysis therapy and other significant kidney injuries [18, 19]. Separate consideration of patients taking immunosuppressive agents because of kidney injury or after kidney transplantation in our studies aimed at confirming an inhibitory effect of immunosuppression on autoimmunization processes. We did manage to demonstrate such an effect in our research. However, such an effect is not important from the point of view of the patient, because the autoimmunization process, not leading to the development of autoimmune hepatitis, does not significantly influence the clinical course of HCV infection. Our results confirm the importance of immunological suppression in the absence of stimulation of the immune system. This is important because in any patients (even with positive autoantibodies) is not obtained AJH score and identifiable autoimmune disease.

The studies by Marconcini *et al.* suggest that the presence of autoantibodies (especially non-organ specific antibodies – NOSA) correlates with worse clinical course of chronic hepatitis type C and exacerbation of inflammatory changes in the liver [20]. Our research does not confirm such observations. Also the study by Mauss *et al.*, which analyzed the influence of the presence of autoantibodies on the efficacy of antiviral treatment among over 24 thousand patients infected with HCV, did not find any negative effect of ANA, LKM,

SMA or AMA on the clinical state and effect of antiviral treatment. In the opinion of the authors, routine testing for autoantibodies, before the treatment of patients with HCV, is redundant [21]. The results of our research seem to be in line with this statement, since they demonstrate that autoimmune diseases do not develop among patients infected with HCV.

## Conclusions

The antibodies are significantly more prevalent in patients without immunosuppression and in patients infected with genotype 1 than genotype 3. Use of immunosuppression significantly reduces the incidence of autoantibodies. Autoimmune stimulation by HCV appears to be insufficient for development of autoimmune hepatitis.

## Disclosure

Authors report no conflict of interest.

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