UDC: 616.36-002.2-06-036:616.153.962.4:575.174.015.3]:57.088.7

Original research

Interaction of polymorphism of the interleukin-6 gene with immunological damages and their role in the development of mixed cryoglobulinemia in patients with chronic hepatitis C

K. V. Kalashnyk*[B,C,D], Yu. Yu. Riabokon[A,C,F], O. V. Riabokon[B,E]

Zaporizhzhia State Medical University, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

Aim. To determine the role of the relationship of immunological disorders with interleukin-6 gene polymorphism in the formation of HCV-associated mixed cryoglobulinemia.

Materials and methods. The study included 149 patients with chronic hepatitis C. The polymorphism of the IL-6 gene (rs1800795) was determined by the method of polymerase chain reaction, the quantitative content of IL-6, RF IgM and IgG by enzyme immunoassay, cryoglobulins by spectrophotometric method. The patients were divided into groups depending on the polymorphism of the IL-6 gene and the presence of mixed cryoglobulinemia.

Results. The frequency of formation of HCV-associated mixed cryoglobulinemia depended on the polymorphism of the IL-6 gene. In patients with chronic hepatitis C with mixed cryoglobulinemia, the frequency of registration of the CC genotype of the IL-6 gene was lower than in patients without mixed cryoglobulinemia, namely, in 9.7 % versus 28.6 % of patients. The presence of the G-allele, namely the CG/GG genotypes of the IL-6 gene polymorphism, was more often detected in patients with mixed cryoglobulinemia, namely, in 90.3 % of patients against 71.4 % of patients without signs of mixed cryoglobulinemia ($\chi^2 = 8.94$, $P = 0.003$).

In the presence of G-allele, the quantitative content of IL-6 in the serum of the general group of patients with CHC was higher than in healthy people ($P < 0.01$), and in the presence of the genotype, the CC did not differ from the control group ($P > 0.05$). The highest levels of IL-6 were recorded in patients with HCV-associated mixed cryoglobulinemia who had the G-allele. The content of IL-6 in the blood serum of these patients exceeded the indicators of both healthy people ($P < 0.001$) and the results of patients without mixed cryoglobulinemia ($P < 0.01$). In patients with chronic hepatitis C with mixed cryoglobulinemia, even in the presence of the CC genotype, the content of IL-6 in serum was higher both in comparison with healthy ($P < 0.01$) and in comparison with patients without signs of this extrahepatic manifestation ($P < 0.01$).

In patients with chronic hepatitis C with mixed cryoglobulinemia, the presence of CG/GG genotypes was associated not only with the highest serum IL-6 content, but also with the presence of more pronounced autoimmune disorders due to a higher content of RF IgM ($P = 0.04$) and mixed cryoglobulins ($P = 0.03$) in serum, in comparison with patients who had the CC genotype. Moreover, the presence of more pronounced immune disorders in patients with HCV-associated mixed cryoglobulinemia in the presence of CG/GG genotypes was accompanied by more frequent manifestation of severe general weakness ($P = 0.003$), arthralgia ($P = 0.02$) and the formation of Meltzer's triad.

Conclusion. The frequency of detection of the G-allele, namely the CG/GG genotypes of the IL-6 gene polymorphism, is the highest in patients with HCV-associated mixed cryoglobulinemia (90.3 %). The presence of CG/GG genotypes in patients with chronic hepatitis C with mixed cryoglobulinemia contributes to more pronounced immunological disorders due to the highest content of IL-6, mixed cryoglobulins, and RF IgM in serum, which causes the manifestation of the clinical symptoms of this hepatic manifestation.

Key words: chronic hepatitis C, mixed cryoglobulinemia, interleukin-6, genetic polymorphism.

Pathologia 2019; 16 (1), 27–32

DOI: 10.14739/2310-1237.2019.1.166189

***E-mail:** anopheles@ukr.net

Взаємозв'язки імунологічних порушень із поліморфізмом гена інтерлейкіну-6 та їхня роль у формуванні HCV-асоційованої змішаної кріоглобулінемії

К. В. Калашник, Ю. Ю. Рябоконь, О. В. Рябоконь

Мета роботи – визначити роль взаємозв'язків імунологічних порушень із поліморфізмом гена інтерлейкіну-6 у формуванні HCV-асоційованої змішаної кріоглобулінемії.

Матеріали та методи. У дослідження залучили 149 пацієнтів із хронічним гепатитом С (ХГС). Визначили поліморфізм гена ІЛ-6 (rs1800795) методом полімеразної ланцюгової реакції, кількісний вміст ІЛ-6, RFIgM та IgG методом імуноферментного аналізу та змішаних кріоглобулінів спектрофотометричним методом. Пацієнтів поділили на групи залежно від поліморфізму гена ІЛ-6 і наявності змішаної кріоглобулінемії.

Результати. Встановили, що частота формування HCV-асоційованої змішаної кріоглобулінемії залежала від поліморфізму гена ІЛ-6. У хворих на ХГС зі змішаною кріоглобулінемією частота реєстрації генотипу СС гена ІЛ-6 була меншою, ніж у пацієнтів без змішаної кріоглобулінемії – у 9,7 % проти 28,6 % хворих. Наявність G-алеля, а саме генотипів CG/GG поліморфізму гена ІЛ-6, частіше визначали у хворих зі змішаною кріоглобулінемією – у 90,3 % проти 71,4 % хворих без ознак змішаної кріоглобулінемії ($\chi^2 = 8,94$, $p = 0,003$).

За наявності G-алеля кількісний вміст ІЛ-6 у сироватці крові загальної групи пацієнтів із ХГС був вищим, ніж у здорових осіб ($p < 0,01$), а за наявності генотипу СС не відрізнявся від показника осіб контрольної групи ($p > 0,05$). Найвищий

Ключові слова: хронічний гепатит С, змішана кріоглобулінемія, інтерлейкін-6, поліморфізм генетичний.

Патологія. – 2019. – Т. 16, № 1(45). – С. 27–32

рівень вмісту ІЛ-6 зафіксували в пацієнтів із HCV-асоційованою змішаною кріоглобулінемією, які мали G-алель. Вміст ІЛ-6 у сироватці крові цих хворих перевищував показники як здорових осіб ($p < 0,001$), так і пацієнтів без змішаної кріоглобулінемії ($p < 0,01$). У хворих на ХГС зі змішаною кріоглобулінемією навіть за наявності СС-генотипу вміст ІЛ-6 у сироватці крові був вищим порівняно зі здоровими ($p < 0,01$) і хворими без ознак цього позапечінкового прояву ($p < 0,01$).

У хворих на ХГС зі змішаною кріоглобулінемією наявність генотипів CG/GG асоціювалася не тільки з найвищим вмістом ІЛ-6 у сироватці крові, але і з вираженішими автоімунними порушеннями внаслідок вищого вмісту RF IgM ($p = 0,04$) та змішаних кріоглобулінів ($p = 0,03$) у сироватці крові порівняно з пацієнтами, які мали генотип СС. Більш виражені імунні порушення у хворих із HCV-асоційованою змішаною кріоглобулінемією за наявності генотипів CG/GG супроводжувалися частішою маніфестацією вираженої загальної слабкості ($p = 0,003$), артралгій ($p = 0,02$) та формуванням тріади Мельтцера.

Висновки. Частота виявлення G-алеля – генотипів CG/GG поліморфізму гена ІЛ-6 – є найвищою у хворих із HCV-асоційованою змішаною кріоглобулінемією (90,3 %). Наявність у хворих на ХГС зі змішаною кріоглобулінемією генотипів CG/GG асоційована з вираженішими імунологічними порушеннями внаслідок найвищого вмісту ІЛ-6, змішаних кріоглобулінів, RF IgM у сироватці крові, що зумовлює маніфестацію клінічної симптоматики цього позапечінкового прояву.

Ключевые слова: хронический гепатит С, смешанная криоглобулинемия, интерлейкин-6, полиморфизм генетический.

Патология. – 2019. – Т. 16, № 1(45). – С. 27–32

Взаимосвязи полиморфизма гена интерлейкина-6 с иммунологическими нарушениями и их роль в развитии смешанной криоглобулинемии у больных хроническим гепатитом С

К. В. Калашник, Ю. Ю. Рябоконь, Е. В. Рябоконь

Цель работы – определить роль взаимосвязи иммунологических нарушений с полиморфизмом гена интерлейкина-6 в формировании HCV-ассоциированной смешанной криоглобулинемии.

Материалы и методы. В исследование включили 149 пациентов с хроническим гепатитом С (ХГС). Определен полиморфизм гена ИЛ-6 (rs1800795) методом полимеразной цепной реакции, количественное содержание ИЛ-6, RFIgM и IgG методом иммуноферментного анализа, а также криоглобулины спектрофотометрическим методом. Пациенты разделены на группы в зависимости от полиморфизма гена ИЛ-6 и наличия смешанной криоглобулинемии.

Результаты. Установлено, что частота формирования HCV-ассоциированной смешанной криоглобулинемии зависела от полиморфизма гена ИЛ-6. У больных ХГС со смешанной криоглобулинемией частота регистрации генотипа СС гена ИЛ-6 была ниже, чем у пациентов без смешанной криоглобулинемии – у 9,7 % против 28,6 % больных. Наличие G-аллели, а именно генотипов CG/GG полиморфизма гена ИЛ-6 чаще установлено у больных со смешанной криоглобулинемией – у 90,3 % против 71,4 % больных без признаков смешанной криоглобулинемии ($\chi^2 = 8,94$, $p = 0,003$).

При наличии G-аллели количественное содержание ИЛ-6 в сыворотке крови общей группы больных ХГС было выше, чем у здоровых людей ($p < 0,01$), а при наличии генотипа СС не отличался от показателей лиц контрольной группы ($p > 0,05$). Самый высокий уровень содержания ИЛ-6 зафиксирован у пациентов с HCV-ассоциированной смешанной криоглобулинемией, у которых была G-аллель. Содержание ИЛ-6 в сыворотке крови этих больных превышал показатели как здоровых людей ($p < 0,001$), так и пациентов без смешанной криоглобулинемии ($p < 0,01$). У больных ХГС со смешанной криоглобулинемией даже при наличии СС-генотипа содержание ИЛ-6 в сыворотке крови было выше в сравнении и со здоровыми ($p < 0,01$), и больными без признаков этого внепеченочного проявления ($p < 0,01$).

У больных ХГС со смешанной криоглобулинемией наличие генотипов CG/GG ассоциировалось не только с самым высоким содержанием ИЛ-6 в сыворотке крови, но и с наличием более выраженных аутоиммунных нарушений за счет более высокого содержания RF IgM ($p = 0,04$) и смешанных криоглобулинов ($p = 0,03$) в сыворотке крови в сравнении с пациентами, которые имели генотип СС. Наличие более выраженных иммунных нарушений у больных с HCV-ассоциированной смешанной криоглобулинемией при наличии генотипов CG/GG сопровождалось более частой манифестацией выраженной общей слабости ($p = 0,003$), артралгий ($p = 0,02$) и формированием триады Мельцера.

Выводы. Частота установления G-аллели, а именно генотипов CG/GG полиморфизма гена ИЛ-6, самая высокая у больных с HCV-ассоциированной смешанной криоглобулинемией (90,3 %). Наличие у больных ХГС со смешанной криоглобулинемией генотипов CG/GG способствует более выраженным иммунологическим нарушениям за счет самого высокого содержания ИЛ-6, смешанных криоглобулинов, RF IgM в сыворотке крови, что обусловливает манифестацию клинической симптоматики этого непеченочного проявления.

A distinctive feature of chronic hepatitis C (CHC) is the high incidence of mixed cryoglobulinemia, which increases the risk of mortality [1,2]. The presence of mixed cryoglobulins in the blood is registered in almost 70 % of patients with CHC, but clinical manifestations develop only in every tenth patient [3]. It is known that the typical clinical triad of cryoglobulinemic syndrome is skin purpura, the general weakness of a significant degree of severity, joint pain (Meltzer`s triad). Also, in some patients, due to this syndrome, there is damage of the kidneys, sometimes the lungs, which causes difficulties not only in diagnosis but also in the treatment of these patients [4,5].

In the literature, in recent years, a significant number of studies have been conducted on the role of various cytokines in the course of CHC [6,7]. It is well known that the main importance in the development of HCV-associated mixed cryoglobulinemia is the tropism of the virus to B-lymphocytes, which leads to the formation of autoimmune disorders, namely, to the increase in production of autoantibodies and mixed cryoglobulins [8,9]. Taking into account the role of autoimmune disorders in the deve-

lopment of mixed cryoglobulinemia, attention is drawn to the role of interleukin-6 (IL-6) in the course of CHC. IL-6 is a multifunctional cytokine that contributes to the regulation of the immune system, hemopoesis and oncogenesis [10], and also affects the differentiation of B-lymphocytes into plasma cells [11,12]. Several studies have demonstrated the role of this cytokine in such pathological conditions as arterial hypertension [13], rheumatoid arthritis [14], hepatocellular carcinoma [15]. There are isolated studies to determine the role of IL-6 in the progression of liver fibrosis in patients with CHC [16].

When studying the immunopathogenesis of diseases, it is relevant to determine the quantitative content of some cytokines in conjunction with the polymorphism of the genes that they encode, because the polymorphism of the cytokines genes affects not only the natural course of the disease, but also the effectiveness of treatment [17]. Thus, the study [18] demonstrates the dependence of IL-6 products on the polymorphism of the encoding gene in patients with rheumatologic pathology. However, in literature available to us, no work was found on the role of the level of IL-6 in blood, depending on the polymorphism of the encoding gene in the development of HCV-associated mixed cryoglobulinemia.

Aim

To determine the role of the relationship of immunological disorders with interleukin-6 gene polymorphism in the formation of HCV-associated mixed cryoglobulinemia.

Materials and methods

The study was attended by 149 patients with CHC. The age of the patients was from 24 to 73 years. All the patients were examined in the communal institution "Zaporizhzhia Regional Clinical Infectious Disease Hospital" of the Zaporizhzhia Regional Council. Men were 98, women – 51. Duration of illness since the confirmation of etiology was 4.0 [1.0; 8.0] years. Most of the patients were infected with 1 genotype (101 – 67.8 %) or 3 genotype of HCV (45–30.2 %), 2 patients of them had infection with genotype 2, and one patient had simultaneous infection with 2 and 3 genotypes of the virus. Viral load was high (>400.000 IU/ml) in 95 patients and accordingly was low in 54 patients. The degree of liver fibrosis was determined by FibroTest (64 patients) and elastography (in 82 patients), liver biopsy was performed in 3 patients. In 87 (58.4 %) patients with CHC, the initial stages of liver fibrosis F 0-2 were diagnosed, and severe F 3-4 liver fibrosis was detected in 62 (41.6 %) patients. The normal level of ALT activity was observed in 24 (16.1 %), every third patient had minimal activity (55 – 36.9 %), one in five patients moderate (29 – 19.5 %), one in four patients high (41 – 27.5 %) activity of necroinflammatory process in the liver.

DNA isolation and determination of polymorphism of the IL-6 gene (rs1800795) was performed using real-time polymerase chain reaction on the CFX-96 Touch product detection system (BIO-RAD, USA) using NP-512-100 kits (RU). The spectrophotometric method determines the concentration of mixed cryoglobulins in serum. The immune enzyme method in blood serum tested the concentration of IL-6 (Human IL-6 High Sensivity ELISA BMS213HS, Invitrogen, Austria), RF IgM and IgG (AESKULISA Rf-AGM No. 3116, AESKU, Germany) using the Sirio-S microplate reader (Seac, Italy). The control group consisted of 20 healthy individuals. When tested for viral hepatitis markers, they had negative results, the age of these individuals and the sex ratio was not statistically different from those included in the study patients. The research was conducted in the Training Medical Laboratory Center of the Zaporizhzhia State Medical University (headed by Professor A.V. Abramov).

For the analysis of data, 149 patients with CHC were divided into groups depending on the polymorphism of the IL-6 gene: the CC genotype (25 patients) and genotypes CG/GG (124 patients); depending on the presence of mixed cryoglobulinemia: patients with mixed cryoglobulinemia (93) and patients without this extrahepatic manifestation (56). In addition, 93 CHC patients with mixed cryoglobulinemia were divided into groups depending on the polymorphism of the IL-6 gene: the CC genotype (9 patients) and genotypes CG/GG (84 patients).

Statistical processing of the material was carried out using the software Excel (Microsoft, USA) and Statistica for Windows 13 (StatSoft Inc., JPZ804I382130ARCN10-J). In order to assess the validity of the differences between the quantitative features in the independent groups, the Mann–Whitney criterion was used, and the quality method χ^2 was used between qualitative features. Spearman correlation was used to detect relationships between quantitative features, while Kendall's correlation – between quantitative and ordinal values.

Results

As a result of the studies, it was found that the incidence of HCV-associated mixed cryoglobulinemia depended on the polymorphism of the IL-6 gene. In patients with CHC with mixed cryoglobulinemia, the incidence of genotype CC registration of the IL-6 gene was lower than in patients without mixed cryoglobulinemia, namely 9.7 % (9 out of 93) versus 28.6 % (16 out of 56) patients. The presence of the G allele, namely the genotypes CG/GG polymorphism of the IL-6 gene, was more often identified in patients with mixed cryoglobulinemia, namely in 90.3 % (84 out of 93) patients versus 71.4 % (40 out of 56) patients without signs of mixed cryoglobulinemia ($\chi^2 = 8.94$, $P = 0.003$).

An analysis of the relationships between the quantitative content of IL-6 in serum and the polymorphism of the gene encoding it has shown that, in the presence of G-allele, the content of IL-6 in the blood serum of the general group of patients with CHC exceeded the same rate in healthy subjects ($P < 0.01$). It should be noted that in patients with CHC with genotype CC, the quantitative content of IL-6 in serum did not have statistically significant differences from the index of healthy people ($P > 0.05$) (*Table 1*).

The highest level of quantitative content of IL-6 was observed in patients with HCV-associated mixed cryoglobulinemia who had G-alleles, in particular, patients with CG/GG genotypes. The level of IL-6 in the blood serum of these patients exceeded both

Table 1. The content of IL-6 in the blood of patients with CHC, depending on the polymorphism of the gene that it codes, and the presence of mixed cryoglobulinemia, Me (Q_{25}; Q_{75})

Indicator	Healthy people (n = 20)	Patients with CHC (n = 149)	Patients with CHC (n = 149)	
			with mixed cryoglobulinemia (n = 93)	without mixed cryoglobulinemia (n = 56)
Genotype CC	0.08 [0.08; 0.14]	0.22 [0.09; 0.28]	0.3 [0.26; 0.32]*,**	0.10 [0.08; 0.2]
Genotype CG		0.60 [0.22; 0.76]*	0.62 [0.20; 1.02]*	0.52 [0.24; 0.64]*
Genotype GG		0,31 [0.24; 0.67]*	0.64 [0.26; 0.94]*,**	0,24 [0.22; 0.30]*
Genotypes CG/GG		0.50 [0.24; 0.74]*	0.63 [0.23; 0.96]*,**	0.26 [0.24; 0.54]*

*: the difference is significant compared with healthy people (P < 0.001); **: the difference is significant compared to patients without mixed cryoglobulinemia (P < 0.01).

Table 2. Comparison of indicators of autoimmune disorders and the frequency of HCV-associated mixed cryoglobulinemia clinical manifestations, depending on the IL-6 gene polymorphism

Indicator, units of measure	Healthy people (n = 20)	Patients with CHC with mixed cryoglobulinemia (n = 93)	
		Genotype CC (n = 9)	Genotypes CG/GG (n = 84)
Laboratory indices of autoimmune disorders			
Mixed cryoglobulins, optical units	less than 2.2	2.42 [2.36; 2.54]	2.66 [2.39; 3.13]**
RF IgM, IU/ml	1.43 [0.54; 2.13]	5.10 [1.75; 10,00]*	10.47 [5.10; 17.49]***
RF IgG, IU/ml	1.23 [0.10; 2.31]	5.74 [4.26; 10.20]*	7.27 [4.90; 8.84] *
Clinical manifestations of mixed cryoglobulinemia			
The general weakness, %		22.2 % (2 із 9)	71.4 % (60 із 84)**
Arthralgia, %		22.2 % (2 із 9)	63.1 % (53 із 84)**
Hemorrhagic vasculitis, %		–	15.5 % (13 із 84)
Meltzer's triad, %			15.5 % (13 із 84)

*: the difference is significant compared with healthy people (P < 0.01); **: the difference is significant compared with patients with CC genotype (P < 0.05).

indicators of healthy subjects (P < 0.001) and those of patients without mixed cryoglobulinemia (P < 0.01). In patients with CHC with mixed cryoglobulinemia, even in the presence of the CC genotype, there was an increase in the content of IL-6 in serum as compared to healthy people (P < 0.01) and in comparison with patients without signs of this extrahepatic manifestation (P < 0.01). In the absence of HCV-associated mixed cryoglobulinemia and the presence of the CC genotype, the content of IL-6 in serum is the same as in healthy people (P > 0,05) (*Table 1*).

To find out the role of IL-6 gene polymorphism in the progression of HCV-associated mixed cryoglobulinemia, we have analyzed laboratory parameters that characterize autoimmune disorders and the frequency of development of clinical manifestations of this extrahepatic symptom in 93 patients with HCV-associated mixed cryoglobulinemia, depending on the detected genotype IL-6 gene polymorphism. The analysis made it possible to note that in patients with mixed cryoglobulinemia, the presence of CG/GG genotypes was associated not only with the highest quantitative content of IL-6 in serum but also with more pronounced autoimmune disorders due to higher RF IgM quantitative content (P = 0.04) and a higher content of mixed cryoglobulins (P = 0.03) in serum, compared to patients who had the genotype CC. In addition, the presence of more pronounced immune disorders in patients with CHC with mixed cryoglobulinemia, in the presence of CG/GG genotypes, is associated with a more frequent manifestation of the clinical features of this extrahepatic signs than in patients with the genotype CC. The most commonly reported symptoms were weakness (χ^2 = 8.86, P = 0.003), arthralgia (χ^2 = 5.62, P = 0.02), and appearance of cryoglobulinemic vasculitis with the formation of Meltzer's triad was only observed in patients with genotypes CG/GG (*Table 2*).

The role of IL-6 gene polymorphism in the development of immune disorders and the formation of mixed cryoglobulinemia in patients with CHC also confirmed the association between the presence of certain IL-6 polymorphism with a quantitative content in the serum of RF IgM (τ = 0.27, P = 0.007). IL-6 gene polymorphism, namely the CG/GG genotypes, promotes higher levels of IL-6 in serum. According to the results of the Spearman correlation, a correlation was found between the content of IL-6 and RF IgM (r = 0.33, P = 0.03), between the content of mixed cryoglobulins and RF IgM (r = 0.37, P = 0.009).

Discussion

The data of modern literature on the cytokine genes polymorphism show a certain pathogenetic role of these parameters in the formation of CHC course variants. For example, the role of the T-like receptor 4 polymorphism in the chronicity of HCV infection and its subsequent progress is proved [19]. In our study, we found the relationship between the presence of the G-allele, namely the genotypes of the CG/GG IL-6 gene polymorphism. In this case, the genotype is observed by an increase in the quantitative content of mixed cryoglobulins in the serum of blood with the formation of HCV-associated mixed cryoglobulinemia and the appearance of relevant clinical signs. This allows us to consider the IL-6 gene polymorphism as a genetically determined risk factor for the development and progression of this extrahepatic manifestation in patients with CHC. In the available literature we did not find scientific research on the role of IL-6 gene polymorphism in the development of HCV-associated mixed cryoglobulinemia, but we found work that proves the role of IL-6 in the development and progression of rheumatologic diseases [20] and joint damage in CHC [21].

In patients with non-rheumatic diseases in the blood, RF can be detected, but CHC also is characterized by rather high percentage of patients with positive RF [22]. This is due to the fact that surface antigens of HCV interact with B-lymphocyte receptors and reduce the threshold for their activation, which leads to suppression of apoptosis. This causes the formation of somatic hypermutation of B-lymphocytes and increased production of both mixed cryoglobulins and RF [8,9,22]. The frequency of RF detection, according to various studies [23,24] in patients with CHC ranges from 40 % to 76%. According to the results of our study in patients with CHC with mixed cryoglobulinemia the presence of genotypes CG/GG leads to more severe immunological disorders due to the highest content of IL-6, mixed cryoglobulins, RF IgM in serum, which causes the manifestation of clinical signs of this extrahepatic manifestation. Literature data [25] suggest that the increased concentration of RF is more often found in patients with arthralgia and CHC than in patients only with the presence of hepatitis. At the same time, high blood levels of RF remain even after elimination of HCV [26].

In modern studies of immunopathogenesis of rheumatologic diseases considerable attention is paid to proinflammatory cytokines, including IL-6 [27]. IL-6 gene polymorphism in the position of 174 C/G (rs1800795) has been studied in patients with different rheumatologic pathologies. The study [28] has shown that patients with rheumatoid arthritis with the genotype CG have a higher degree of bone marrow deficiency, and the presence of the CC-genotype is associated with an early manifestation of the disease [29,30]. According to the results of our study in patients with HCV-associated mixed cryoglobulinemia and the presence of CG/GG genotypes, the highest levels of IL-6 in serum were observed and they most often have clinical signs of manifestation of autoimmune lesions. Also, the literature suggests that high levels of IL-6 in the blood are associated with greater activity of rheumatologic pathology [31].

Conclusion

1. IL-6 gene polymorphism influences the formation of mixed cryoglobulinemia in patients with CHC. The detection frequency of the G-allele, namely the CG/GG genotypes of IL-6 gene polymorphism, is the highest in patients with HCV-associated mixed cryoglobulinemia (90.3 %).

2. In patients with HCV-associated mixed cryoglobulinemia, who have CG/GG genotypes, the highest quantitative content of IL-6 in serum is determined, which is higher than healthy people ($P < 0.001$), and the rates of patients without mixed cryoglobulinemia ($P < 0.01$).

3. The presence of CG/GG in patients with CHC with mixed cryoglobulinemia is associated with more pronounced immunological disorders due to the high content of IL-6, mixed cryoglobulins, RF IgM in serum, which causes the manifestation of clinical signs of HCV-associated mixed cryoglobulinemia.

4. In patients with CHC it is expedient to determine the IL-6 gene polymorphism to solve the problem of the appointment of specific therapy in the first place.

Prospects for further research. Taking into account the data obtained, it is expedient to continue the research on the role of IL-6 gene polymorphism in relation to its quantitative content in predicting the effectiveness of various antiviral regimens in patients with HCV-associated mixed cryoglobulinemia.

Conflicts of interest: authors have no conflict of interest to declare.
Конфлікт інтересів: відсутній.

Надійшла до редакції / Received: 05.12.2018
Після доопрацювання / Revised: 01.02.2019
Прийнято до друку / Accepted: 07.02.2019

Information about authors:
Kalashnyk K. V., MD, Assistant Professor of the Department of Infectious Diseases, Zaporizhzhia State Medical University, Ukraine.
Riabokon Yu. Yu., MD, PhD, DSc, Associate Professor of the Department of Children Infectious Diseases, Zaporizhzhia State Medical University, Ukraine.
Riabokon O. V., MD, PhD, DSc, Professor, Head of the Department of Infectious Diseases, Zaporizhzhia State Medical University, Ukraine.

Відомості про авторів:
Калашник К. В., асистент каф. інфекційних хвороб, Запорізький державний медичний університет, Україна.
Рябоконь Ю. Ю., д-р мед. наук, доцент каф. дитячих інфекційних хвороб, Запорізький державний медичний університет, Україна.
Рябоконь О. В., д-р мед. наук, професор, зав. каф. інфекційних хвороб, Запорізький державний медичний університет, Україна.

Сведения об авторах:
Калашник К. В., ассистент каф. инфекционных болезней, Запорожский государственный медицинский университет, Украина.
Рябоконь Ю. Ю., д-р мед. наук, доцент каф. детских инфекционных болезней, Запорожский государственный медицинский университет, Украина.
Рябоконь Е. В., д-р мед. наук, профессор, зав. каф. инфекционных болезней, Запорожский государственный медицинский университет, Украина.

References

[1] Lee, M., Yang, H., Lu, S., Jen, C., You, S., Wang, L. et al. (2012). Chronic Hepatitis C Virus Infection Increases Mortality From Hepatic and Extrahepatic Diseases: A Community-Based Long-Term Prospective Study. *Journal of Infectious Diseases*, 206(4), 469–477. doi: 10.1093/infdis/jis385

[2] Russi, S., Sansonno, D., Mariggiò, M., Vinella, A., Pavone, F., Laulletta, G., et al. (2014). Assessment of total hepatitis C virus (HCV) core protein in HCV-related mixed cryoglobulinemia. *Arthritis Research & Therapy*, 16(2), R73. doi: 10.1186/ar4513

[3] Roccatello, D., Sciascia, S., Rossi, D., Solfietti, L., Fenoglio, R., Menegatti, E., & Baldovino, S. (2017). The challenge of treating hepatitis C virus-associated cryoglobulinemic vasculitis in the era of anti-CD20 monoclonal antibodies and direct antiviral agents. *Oncotarget*, 8(25). doi: 10.18632/oncotarget.16986

[4] Mazzaro, C., Monti, G., Saccardo, F., Zignego, A. L., Ferri C., De Vita, S., et al. (2011) Efficacy and safety of peginterferon alfa-2b plus ribavirin for HCV-positive mixed cryoglobulinemia: a multicentre open-label study. *Clin Exp Rheumatol*., 29(6), 933–41.

[5] Terrier, B., Semoun, O., Saadoun, D., Sène, D., Resche-Rigon, M., & Cacoub, P. (2011). Prognostic factors in patients with hepatitis C virus infection and systemic vasculitis. *Arthritis & Rheumatism*, 63(6), 1748–1757. doi: 10.1002/art.30319

[6] Menegatti, E., Messina, M., Oddone, V., Rubini, E., Sciascia, S., Naretto, C., et al. (2016) Immunogenetics of complement in mixed cryoglobulinaemia. *Clin Exp Rheumatol*., 34(3 Suppl 97), 12–5.

[7] Yamagiwa, S., Ishikawa, T., Waguri, N., Sugitani, S., Kamimura, K., Tsuchiya, A., et al. (2017). Increase of Soluble Programmed Cell Death Ligand 1 in Patients with Chronic Hepatitis C. *International Journal of Medical Sciences*, 14(5), 403–411. doi: 10.7150/ijms.18784

[8] Holz, L., Yoon, J., Raghuraman, S., Moir, S., Sneller, M., & Rehermann, B. (2012). B cell homeostasis in chronic hepatitis C virus-related mixed cryoglobulinemia is maintained through naïve B cell apoptosis. *Hepatology*, 56(5), 1602–1610. doi: 10.1002/hep.25821

[9] Charles, E., Orloff, M., Nishiuchi, E., Marukian, S., Rice, C., & Dustin, L. (2013). Somatic Hypermutations Confer Rheumatoid Factor Activity in Hepatitis C Virus-Associated Mixed Cryoglobulinemia. *Arthritis & Rheumatism*, 65(9), 2430–2440. doi: 10.1002/art.38041

[10] Rose-John, S. (2015). The soluble interleukin-6 receptor and related proteins. *Best Practice & Research Clinical Endocrinology & Metabolism*, 29(5), 787–797. doi: 10.1016/j.beem.2015.07.001

[11] Naka, T., Nishimoto, N., & Kishimoto, T. (2002). The paradigm of IL-6: from basic science to medicine. *Arthritis research*, 4 Suppl 3(Suppl 3), S233–42. doi: 10.1186/ar565

[12] Kishimoto, T. (2005). INTERLEUKIN-6: From Basic Science to Medicine–40 Years in Immunology. *Annual Review Of Immunology*, 23(1), 1–21. doi: 10.1146/annurev.immunol.23.021704.115806

[13] O'Reilly, S., Ciechomska, M., Cant, R., & van Laar, J. (2014). Interleukin-6 (IL-6) Trans Signaling Drives a STAT3-dependent Pathway That Leads to Hyperactive Transforming Growth Factor-β (TGF-β) Signaling Promoting SMAD3 Activation and Fibrosis via Gremlin Protein. *Journal of Biological Chemistry*, 289(14), 9952–9960. doi: 10.1074/jbc.m113.545822

[14] Li, B., Xiao, Y., Xing, D., Ma, X., & Liu, J. (2016). Circulating interleukin-6 and rheumatoid arthritis. *Medicine*, 95(23), e3855. doi: 10.1097/md.0000000000003855

[15] Othman, M., Aref, A., Mohamed, A., & Ibrahim, W. (2013). Serum Levels of Interleukin-6 and Interleukin-10 as Biomarkers for Hepatocellular Carcinoma in Egyptian Patients. *ISRN Hepatology*, 2013, 412317. doi: 10.1155/2013/412317

[16] Salter, M., Lau, B., Mehta, S., Go, V., Leng, S., & Kirk, G. (2013). Correlates of Elevated Interleukin-6 and C-Reactive Protein in Persons With or at High Risk for HCV and HIV Infections. *JAIDS Journal of Acquired Immune Deficiency Syndromes*, 64(5), 488–495. doi: 10.1097/qai.0b013e3182a7ee2e

[17] Mechie, N. (2014). Predictability of IL-28B-polymorphism on protease-inhibitor-based triple-therapy in chronic HCV-genotype-1 patients: A meta-analysis. *World Journal of Hepatology*, 6(10), 759. doi: 10.4254/wjh.v6.i10.759

[18] Shah, S., Ma, Y., Scherzer, R., Huhn, G., French, A., Plankey, M., et al. (2015). Association of HIV, hepatitis C virus and liver fibrosis severity with interleukin-6 and C-reactive protein levels. *AIDS*, 29(11), 1325–1333. doi: 10.1097/qad.0000000000000654

[19] Sizova, L., Koval, T., Kaidashev, I., Ilchenko, V., & Dubinskaya, G. (2016). Rol' geneticheskogo polimorfizma Toll-like receptorov 4 i 7 v razvitii khronicheskogo gepatita C i gendernye osobennosti ikh raspredeleniya [The role of genetic polymorphisms toll-like receptor 4 and 7 in the chronic hepatitis C and gender features of their distribution]. *Georgian medical news*, 1(250), 51–55. [in Russian].

[20] Karimifar, M., Pourajam, S., Tahmasebi, A., & Mottaghi, P. (2013). Serum cryoglobulins and disease activity in systematic ц‌lupus erythematosus. *Journal of research in medical sciences : the official journal of Isfahan University of Medical Sciences*, 18(3), 234–238.

[21] Riccio, A., Postiglione, L., Sabatini, P., Linvelli, M., Soriente, I., Sangiolo, M., et al. (2012). Similar Serum Levels of IL-6 and its Soluble Receptors in Patients with HCV-Related Arthritis and Rheumatoid Arthritis: A Pilot Study. *International Journal of Immunopathology And Pharmacology*, 25(1), 281–285. doi: 10.1177/039463201202500132

[22] Palazzi, C., Buskila, D., D'Angelo, S., D'Amico, E., & Olivieri, I. (2012). Autoantibodies in patients with chronic hepatitis C virus infection: pitfalls for the diagnosis of rheumatic diseases. *Autoimmunity Reviews*, 11(9), 659–663. doi: 10.1016/j.autrev.2011.11.011

[23] Örge, E., Çefle, A., Yazıcı, A., Gürel-Polat, N., & Hulagu, S. (2010). The positivity of rheumatoid factor and anti-cyclic citrullinated peptide antibody in nonarthritic patients with chronic hepatitis c infection. *Rheumatology International*, 30(4), 485–488. doi: 10.1007/s00296-009-0997-1

[24] Wu, Y., Hsu, T., Chen, T., Liu, T., Liu, G., Lee, Y., & TSAY, G. (2002). Proteinase 3 and dihydrolipoamide dehydrogenase (E3) are major autoantigens in hepatitis C virus (HCV) infection. *Clinical & Experimental Immunology*, 128(2), 347–352. doi: 10.1046/j.1365-2249.2002.01827.x

[25] Lormeau, C., Falgarone, G., Roulot, D., & Boissier, M. (2006). Rheumatologic manifestations of chronic hepatitis C infection. *Joint Bone Spine*, 73(6), 633–638. doi: 10.1016/j.jbspin.2006.05.005

[26] Reyes-Avilés, E., Kostadinova, L., Rusterholtz, A., Cruz-Lebrón, A., Falck-Ytter, Y., & Anthony, D. (2015). Presence of Rheumatoid Factor during Chronic HCV Infection Is Associated with Expansion of Mature Activated Memory B-Cells that Are Hypo-Responsive to B-Cell Receptor Stimulation and Persist during the Early Stage of IFN Free Therapy. *PLOS ONE*, 10(12), e0144629. doi: 10.1371/journal.pone.0144629

[27] Scott, D., Wolfe, F., & Huizinga, T. (2010). Rheumatoid arthritis. *The Lancet*, 376(9746), 1094–1108. doi: 10.1016/s0140-6736(10)60826-4

[28] Marinou, I., Healy, J., Mewar, D., Moore, D., Dickson, M., & Binks, M., et al. (2007). Association of interleukin-6 and interleukin-10 genotypes with radiographic damage in rheumatoid arthritis is dependent on autoantibody status. *Arthritis & Rheumatism*, 56(8), 2549–2556. doi: 10.1002/art.22814

[29] Pascual, M., Nieto, A., Matarán, L., Balsa, A., Pascual-Salcedo, D., & Martín, J. (2000). IL-6 promoter polymorphisms in rheumatoid arthritis. *Genes & Immunity*, 1(5), 338–340. doi: 10.1038/sj.gene.6363677

[30] Lee, Y., Bae, S., Choi, S., Ji, J., & Song, G. (2012). The association between interleukin-6 polymorphisms and rheumatoid arthritis: a meta-analysis. *Inflammation Research*, 61(7), 665–671. doi: 10.1007/s00011-012-0459-1

[31] Ripley, B., Goncalves, B., Isenberg, D. A., Latchman, D. S., & Rahman, A. (2005). Raised levels of interleukin 6 in systemic lupus erythematosus correlate with anaemia. *Annals of The Rheumatic Diseases*, 64(6), 849–853. doi: 10.1136/ard.2004.022681