Original Article:

Immunoreactivity and intoxication syndrome in patients with chronic viral hepatitis c

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Abstract:

Method: Etiological, gender, age and laboratory characteristics of chronic viral hepatitis C (CVHC) were studied and analyzed. The distribution of patients depending on the virus genotype, the activity degree and the severity of liver fibrosis were found out. Indices of non-specific reactivity, endogenous intoxication and inflammation in the course of CVHC, their dependence on treatment were studied. Correlations between the values of non-specific reactivity, endogenous intoxication, inflammation and patients' affiliation to the group of injecting drug users, the APRI score, the stage of liver fibrosis and the virus genotype were estimated. Significant increase in endogenous intoxication (LII, HII, ISS), non-specific immunoreactivity (IR, LMR, I_{lymph}, IA) and inflammation (ILG) at decrease in some endogenous intoxication indices (ISL, NRR) and inflammation (KI, ILESR) in patients with CHCV compared with healthy persons were established. ***Result:*** The decrease in endogenous intoxication indices (LII; HII; ISL; ISS; NRR) were observed during antiviral treatment. Such unambiguous reaction of non-specific immunoreactivity indices and inflammation indices were not observed: some of them increased (I_{lymph}, IA), some of them decreased (IR, NLR; ELR). KI decreased during the treatment, but ILG increased. Direct correlation between patients' affiliation to a group of injecting drug patients and the values of HII, NRR ($p<0.05$), between the APRI score and I_{lymph} level ($p<0.05$) was identified. ***Conclusion***: No correlation between the indices values and fibrosis severity (according to METAVIR) and virus genotype was found.

Keywords: chronic viral hepatitis C; non-specific reactivity indices; endogenous intoxication indices; inflammation indices, fibrosis, genotype;

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Introduction:

Viral hepatitis C (VHC) is one of the urgent health issues in Ukraine and in the world. 180 million people suffer from chronic VHC infection and are prone to complications and die of liver cirrhosis and hepatocellular carcinoma [1]. The issue is not only medical, but also socio - economic due to the high rate of infection prevalence, the absence of specific prophylaxis, high frequency of infection chronicity (50.0 % - 85.0 %), frequent adverse disease sequelae, high-cost treatment of chronic viral hepatitis C (CVHC) and its complications [1-3].

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The possibility of hepatitis C virus contamination is very high, and due to the lack of a specific vaccine, it is even higher, than hepatitis B virus, where the level of HBV (Hepatitis B virus) seroprevalence in some regions was 4.97%[4]. Although the percentage of positive anti-HBsAg results remains low in some regions of the world[5]. Due to the progressive aging of the infected population, the issue of management of elderly patients with CVHC has emerged recently [6]. According to WHO, about 325 million people in the world live with chronic infection caused by hepatitis B or C. According to the Centre of Medical Statistics of Ministry of Health of Ukraine, the total number of 32,975 cases of chronic viral hepatitis C (CVHC) was revealed for the first time in Ukraine during 2013 – 2017. Acute viral hepatitis C is usually asymptomatic and it is only occasionally associated with a life-threatening disease. Approximately 15% -45% of infected persons were spontaneously free of the virus within 6 months after infecting without any treatment. The remaining 60% - 80% of people develop CVHC. The risk of liver cirrhosis development within 20 years is 15 % - 30 % in persons with this disease. In 2017, 46 284 cases of liver cirrhosis of various etiology were registered among adults (18 years and older) [7].

For a long time, recombinant alpha 2b-interferon combined with ribavirin has been the main and practically the only really effective drug for CVHC treatment. Treatment duration was 24-48 weeks [8-10]. When using these combinations, full recovery from hepatitis C could be achieved on average in 54-56% of patients with hepatitis, this value for HIV-positive patients was about 40 %. The success of treatment largely depended on VHC genotype, the 2nd and 3rd genotypes of the virus (up to 76-88% probability of cure) responded the treatment the best, and the 1st (up to 50%) responded the treatment significantly worse [11-14]. However, it should be emphasized that hepatitis C treatment is complicated and it is connected with a number of problems: complete elimination of the virus is achieved on average only in 30-50% of patients; the high cost of one treatment course, and often the necessity of a refresher course of treatment; significant percentage of disease recurrence after treatment cessation; the rapid development of drug resistance; pronounced side effects which in some cases cause the necessity to discontinue a drug.

Over the past few years, the options and efficacy of treatment improved with the emergence of several classes of direct-acting antivirals. The targets of direct-acting antivirals are the components of virus assembly. For example, protease inhibitors bind to the non-structural (NS) site of NS3 to inhibit protease production; similarly, other various protein loci are exposed to nucleoside and non-nucleoside analogues, which are a barrier to replication by stopping transcription in NS5A and NS5B sites [15].

The main difficulties in predicting the treatment and endogenous intoxication are primarily due to the fact that it is not always possible to identify the degree of non-specific immunoreactivity, endogenous intoxication or inflammation based on the severity of clinical picture. As the presence of a toxic agent in a body is insufficient to explain the variety of clinical manifestations of endotoxicosis and determine the appropriate strategy for specific clinical setting [16,17].

Integrated laboratory parameters of endogenous intoxication (effective albumin concentration and reserve binding capacity of albumin) were studied in patients with CVHC in the context of data on disease signs and the nature of pathology and toxic effects. It was found that patients with CVHC developed significant changes in endogenous intoxication indices compared with the control group, where the indices were stable during traditional pathogenetic therapy. There was no correlation between clinical symptoms and indices of endogenous intoxication. More significant changes were found in CVHC patients with degenerative diseases, biliary system diseases and chronic constipation [18].

Hepatitis viruses develop endogenous intoxication syndrome due to the direct or immuno-mediated cytolysis of hepatocytes, which is the basic mechanism of pathogenesis. The enhancement of endogenous intoxication syndrome at viral hepatitis occurs due to the following pathological processes: various liver impairment, especially protein synthesis, activation of lipid peroxidation leading to the accumulation of free radicals, which increase in endotoxicosis; immune system imbalance. The endogenous toxic substances have additional damaging effect on cell membranes, organs and systems of an organism including liver. The laboratory manifestations of endogenous intoxication syndrome are registered on the background and after acute pathologic process, at chronic viral hepatitis without clinical signs and at liver cirrhosis development, which confirms the scale and depth of liver changes as the main organ and regulator of the detoxication system [19].

Objective: To determine the effect of interferon-

containing antiviral treatment at chronic viral hepatitis C on immunoreactivity and endogenous intoxication indices in patients and the correlation of these indices with the genotype and severity of liver fibrosis.

Patients and methods: 60 patients with CVHC, treated at Z. Y. Krasovytskyi Sumy Regional Clinical Hospital for Infectious Diseases from 2016 to 2018, were examined and the posthoc analysis of clinical and laboratory parameters were conducted. The average age of patients was (42.55±1.41). All patients were treated with interferon. The experimental group consisting of 44 apparently healthy individuals was taken for data comparison.

In order to determine the severity of the intoxication syndrome, complete blood count was studied; the absolute leukocyte count (10^9/l), ESR (mm/h) and leukocyte differential count were taken into account. The integrative indices of endogenous intoxication were calculated before and after 4 weeks of interferon-containing treatment with the help of Android application: "Blood Count. Indices of Endogenous Intoxication", which determined 15 special formulas that can be divided into 3 groups [13]:

Indices of non-specific reactivity were calculated according to the following indices: immunoreactivity index (IR), neutrophil-monocyte ratio (NMR), lymphocyte-monocyte ratio(LMR), the lymphocyte index was calculated from the lymphocyte-neutrophil ratio (I_{lymph}), eosinophils-lymphocytesratio(ELR); index of allergization (IA), nuclear index (NI) [16,17].

Intoxication indices were calculated using the following formules: leucocyte intoxication index (LII), hematological index of intoxication (HII), index of leukocytes shift (ISL), index of intoxication severity (IIS), neutrophil reactive response (NRR) [16,17].

Indices of inflammation activity were calculated according to the following parameters: Krebs index (KI), lymphocytic-granulocyticindex (ILG), index of leukocyte and ESR ratio (ILESR)[16,17].

The APRI score was calculated according to the formula: APRI=AST×100/ ((AST upper limit of normal)×platelet count (10^9/l)) [18].

Statistical processing was carried out using computer applications IBM SPSS Statistic 23 and Microsoft Office Excel 2010. Criteria were used to verify the validity of values (Wilcoxon-Mann-Whitney) and determine the correlation between two values (Spearman's correlation coefficient).

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Results and discussion:

60 CVHC patients were examined, their medical records were analysed. Among the patients, 70% were male (42 people) and 30% were female (18).

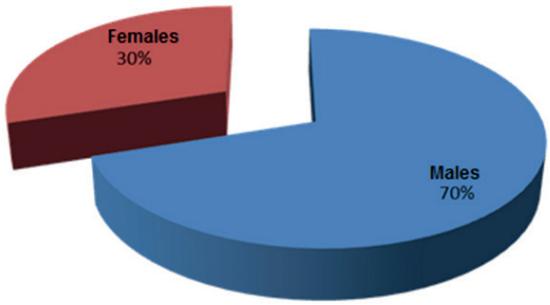

Figure 1 Sex distribution of investigated CVHC patients

The predominance of males among CVHC patients is typical for the epidemiological characteristics of this disease in Ukraine [7].

The number of young patients was 1,3 times bigger (33 people) than that of middle-aged people (25), and 16,5 times bigger than that of elderly patients (2).

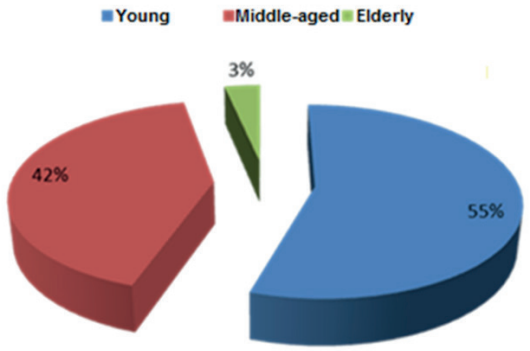

Figure2 Age distribution of investigated CVHC patients

The number of patients who have injected drugs (13,33%) was 6,5 times smaller than that of patients who have not (86,67%).

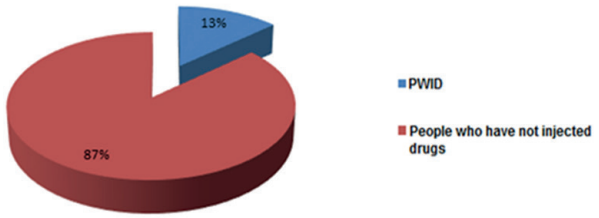

Figure 3 Distribution of patients in terms of affiliation to a group of injecting drugs people

The genotype distribution of CVHC patients was the following: the majority had genotype 1b (47 people), 4,2 times fewer patients had 3a (11 people), and the smallest number of patients had genotype 2 (2 people).

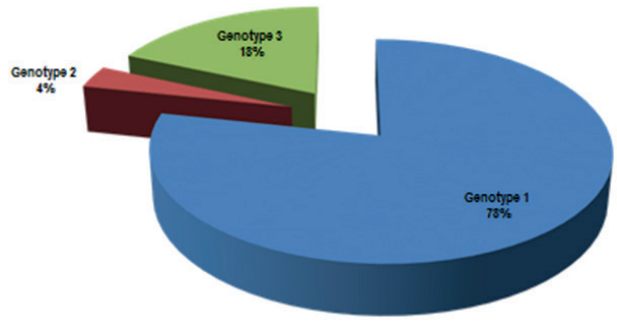

Figure 4 Distribution of patients by HCV genotypes

Sustained virologic response (SVR) was achieved in 61,11% of patients, while in 38,89% of patients the viral RNA was detected 6 months after the end of antiviral treatment.

Before the beginning of antiviral treatment, the values of the majority of endogenous intoxication indices (LII, HII, IIS), non-specific reactivity indices (IR, LMR, $I_{lymph.}$ IA) and inflammation activity indices (ILG) in CVHC patients were signicantly higher than those in the experimental group ($p<0,05$). The increasing tendency of endogenous intoxication indices among CVHC patients was also observed by other researchers using other methods to determine its value[19]. This fact is attributed to direct or immune-mediated hepatocyte cytolysis which is the root pathogenesis mechanism that forms the endogenous intoxication syndrome [20]. The values of some non-specific reactivity indices (ISL, NRR, KI, ILESR) and endogenous intoxication indices (ISL, NRR, KI, ILESR) were significantly higher than those in apparently healthy individuals ($p<0,05$). NLR, ELR, NI had the same values in two compared groups ($p<0,05$).

Significant changes in all immunoreactivity and endogenous intoxication indices in comparison with the experimental group ($p<0,05$) were observed after the fourth week of antiviral treatment.

Table1 Changes in the indices of endogenous intoxication, non-specific immunoreactivity and inflammation activity in CVHC patients

Index	Group		
	Apparently healthy individuals	CVHC patients before starting AVT	CVHC patients 4 weeks into AVT
Endogenous intoxication indices			
LII	0,7±0,07	4,06±0,38 *	1,97±0,18 *, **
HII	0,64±0,06	3,26±0,34 *	1,16±0,12 *, **
ISL	1,68±0,10	1,24±0,09 *	0,83±0,04 *, **
IIS	0,16±0,02	1,27±0,15 *	0,81±0,11 *,**
NRR	12,75±1,82	7,16±0,97 *	3,35±0,62 *,**
Non-specific reactivity indices			
IR	4,65±0,36	6,33±0,45 *	4,54±0,07 *
NLR	8,88±0,91	7,64±0,57	5,60±0,70 *, **
LMR	4,77±0,45	5,91±0,42 *	5,86±0,44 *
Ilymph	0,59±0,04	0,89±0,06 *	1,15±0,05 *, **
ELR	0,08±0,01	0,08±0,01	0,03±0,004 *,**
IA	1,05±0,07	1,47±0,10 *	1,38±0,06 *
NI	0,06±0,01	0,06±0,003	0,11±0,02*, **
Inflammation activity indices			
KI	2,02±0,14	1,49±0,14*	0,99±0,05 *,**
ILG	4,85±0,45	8,26±0,50 *	11,16±0,54 *, **
ILESR	1,33±0,20	0,33±0,02 *	0,42±0,03 *

Note. Significant difference in comparison with: * - apparently healthy individuals; ** - parameter values before the beginning of treatment ($p<0,05$,calculated using Wilcoxon's criterion for paired samples– patients before and during AVT; calculated using Mann-Whitney criterion for independent samples)

The study of the influence of interferon-containing antiviral treatment on intoxication indices has shown that, in CVHC patients, LII and ELR decreased three times more frequently (75%) than it increased (25%) ($p<0,05$). IIS, NRR and NLR had an almost similar response to interferon-containing treatment - a decrease in the indices was more frequent (70%)

than an increase (30%) (p<0,05). After 4 weeks of treatment, HII also decreased more frequently (85%) than it increased (15%) (p<0,05). Similarly, ISL and KI decreased 4 times more frequently (80%) than they increased (20%) (p_{act}<0,05). The analysis of I_{lymph} values has demonstrated an opposite pattern, that is, the index increased more frequently (78,3%) than it decreased (20%), and remained constant only in 1,67% of examined individuals (p<0,05). A similar prevalence of index increases over their decreases was observed in NI (68,3% and 30%, respectively) and ILG (76,7% and 23,3%, respectively) (p<0,05). The influence of interferon-containing treatment on IR, LMR, IA and ILESR was not determined (p<0,05). No correlational relationship was observed between an increase or decrease of integrative indices in the dynamics and SVR of the patients. No relationship between the chosen indices and the presence of SVR was observed, however, it should be noted that the coefficient value for ILESR was practically certain (0,2; p=0,14).

The study of correlational relationships between the affiliation of patients to a group of people who have injected drugs (PWID) and the indices of non-specific immunoreactivity, endogenous intoxication and inflammation, has demonstrated a significant direct proportional relation between this risk group and HII (p<0,05) and NRR (p<0,05). Other indices did not demonstrate a significant correlation (p<0,05). The values of the Spearman's coefficient for some endogenous intoxication indices (LII, p=0,1; IIS, p=0,07) and non-specific immunoreactivity (ELR, p=0,09) were also observed to be practically certain.

The study of the correlation between the APRI score and the indices under investigation has demonstrated a significant relation between the chosen fibrosis marker and I_{lymph}(p<0,05). This, the higher the observed APRI score was, the higher the lymphocyte index value.

The calculation of the correlational relationship between the degree of liver fibrosis and the chosen indices has demonstrated that the value of the mutual relevance of the latter and the former is above the permissible level, that is, no significant relationship between the degree of liver fibrosis and the indices of non-specific immunoreactivity, endogenous intoxication and inflammation has been determined (p>0,05). Therefore, the indices of immunoreactivity and endogenous intoxication in patients under antiviral treatment do not correlate to (do not significantly depend on) the degree of fibrosis.

Table2 Correlational relationships between the indices of endogenous intoxication, non-specific immunoreactivity, inflammation activity, affiliation to PWID, and the APRI score

Index	PWID	APRI-test	SVR
Endogenous intoxication indices			
LII	0,21 (p=0,1)	-0,09 (p=0,51)	-0,10 (p=0,47)
HII	0,26 (p=0,04*)	-0,09 (p=0,47)	-0,09 (p=0,51)
ISL	0,05 (p=0,72)	-0,14 (p=0,29)	0,05 (p=0,70)
IIS	0,24 (p=0,07)	0,05 (p=0,73)	0,07 (p=0,63)
NRR	0,26 (p=0,04*)	-0,10 (p=0,47)	-0,13 (p=0,35)
Non-specific reactivity indices			
IR	-0,16 (p=0,21)	-0,04 (p=0,79)	0,14 (p=0,33)
NLR	-0,04 (p=0,75)	0,09 (p=0,51)	0,06 (p=0,67)
LMR	-0,18 (p=0,18)	-0,30 (p=0,84)	0,14 (p=0,32)
Ilymph	-0,06 (p=0,66)	0,27 (p=0,04*)	-0,004 (p=0,98)
ELR	0,22 (p=0,09)	0,01 (p=0,94)	-0,10 (p=0,46)
IA	-0,02 (p=0,86)	0,16 (p=0,22)	0,04 (p=0,76)
NI	0,16 (p=0,21)	-0,14 (p=0,30)	-0,14 (p=0,31)
Inflammation activity indices			
KI	0,06 (p=0,66)	-0,18 (p=0,18)	0,004 (p=0,98)
ILG	-0,08 (p=0,56)	0,17 (p=0,21)	0,02 (p=0,90)
ILESR	0,12 (p=0,36)	0,16 (p=0,23)	0,20 (p=0,15)

Note. Significant correlation between two parameters* (p<0,05, the correlation value was calculated using Spearman's correlation criterion)

The evaluation of the correlational relationship between the genotype and the indices of non-specific immunoreactivity and endogenous intoxication has determined that no sifnificant relationship exists between these parameters (p>0,05).

Conclusions:

1 .The conducted research has generalized and given further insight into the notion of non-specific immunoreactivity and endogenous intoxication in CVHCpatients, namely, a significant increase (LII, HII, IIS, IR, LMR, I_{lymph}, IA, ILG) or decrease (ISL, NRR, KI, ILESR) in the value of the majority of indices compared to those in apparently healthy individuals was determined.

2 . Interferon-containing antiviral treatment causes significant changes in the majority of endogenous intoxication indices, leading to their decrease (LII – by 2 times; HII – by 2,8; NRR – by 2,1; ISL, IIS – by 1,5). A portion of non-specific reactivity indices increases (I_{lymph} – by 1,3times; NI – by 1,8), while another portion decreases (IR, NLR – by 1,4; ELR – by 1,3 times). During treatment, KI decreases 1,5 times more frequently, while ILG increases (1,4 times). This data indicates a decrease of endogenous intoxication under the influence of the treatment.

3 . A direct correlational relationship between the affiliation of patients to the PWID group and the values of HII and NRR ($p<0,05$) has been determined.

4 . A direct correlation between the APRI score and the I_{lymph} level ($p<0,05$) was determined.

5 . A lack of correlation was determined between the degree of liver fibrosis (according to METAVIR), virus genotype, achievement of SVR and computable parameters of immunoreactivity, endogenous intoxication and inflammation indices in the given sample of patients ($p>0,05$).

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