

Biochemical and Immunogenetic Diagnostic Markers Less Commonly Used for Predicting the Efficacy of Chronic Hepatitis C Etiotropic Therapy

Viksna L^{1,3*}, Jeruma A^{1,3}, Eglite J², Socnevs A² and Sondore V³

¹Department of Infectology and Dermatology, Riga Stradins University, 3 Linezera Street, LV-1006, Riga, Latvia

²Interdepartmental laboratory of Immunology and Immunogenetics, Riga Stradins University, 16 Dzirciema Street, LV-1007, Riga, Latvia

³Latvian Centre of Infectious Diseases, Riga East University Hospital, 3 Linezera Street, LV-1006, Riga, Latvia

*Corresponding author: Dr. Ludmila Viksna, Department of Infectology and Dermatology, Riga Stradins University, 3 Linezera Street, Riga, LV-1006, Latvia, Tel: 0037126604779; E-mail: Ludmila.Viksna@rsu.lv, ilze.zvera@rsu.lv

Rec date: Apr 15, 2014, Acc date: May 03, 2014, Pub date: May 08, 2014

Copyright: © 2014 Viksna L, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Introduction: 1,7% of the population of Latvia in 2008 was HCV RNA positive, thus approximately 38 thousands required the CHC antiviral therapy. The objective of the study was to evaluate some biochemical and immunogenetic parameters allowing predicting the efficacy of the etiotropic therapy for CHC patients.

Material and methods: Medical records of 213 CHC patients were analyzed. HCV RNA, anti-HCV, concentration of HA, GGT activity were detected and immunogenetic investigations were performed.

Results and discussion: Evaluation of GGT activity showed increase in 46,81% of non-responders and in only 21,57% responders. HA level in responders was significantly lower than in non-responders. Correlation between the incidence of HLA class II gene alleles in CHC patients and the type of CHC therapy was found. The results of the study confirmed the hypothesis that some biochemical and immunogenetic parameters characterizing the condition of the macro organism had an essential role in the efficacy of the CHC therapy.

Conclusions: The non-efficacy of the CHC etiotropic therapy was associated with increased GGT and HA levels upon the start of the therapy. The efficacy of PEG IFN+RBV combination therapy in comparison with the efficacy of Realdiron therapy was associated with different MHC HLA class II gene alleles.

Keywords: Chronic hepatitis C; Predicting efficacy of therapy; Biochemical and immunogenetic diagnostic markers

Introduction

Hepatitis C virus (HCV), although it was discovered in 1989, has become an essential public health problem nowadays because it is widely spread and affects the health and life quality of an individual. Today there are more than 170 million people infected with HCV and it is one of the most common initiators of blood born infectious diseases among humans.

It was detected in the epidemiological research done in Latvia in 2008, that 1,7% of the population of Latvia is HCV RNA (hepatitis C virus ribonucleic acid) positive [1]. That leads to conclusion that in total more than 38 thousand inhabitants of Latvia could be infected, for 50-80% of them or 19-30 thousands the CHC (chronic hepatitis C) antiviral therapy is required or needed.

Experts consider that one fifth of the patients with chronic HCV infection can develop liver cirrhosis within the period of 20-30 years. The prognosis of HCV-related cirrhosis is pessimistic, i.e., the 5-year survival rate is only 50%.

HCV-related liver cirrhosis has a risk of malignant transformation, the annual HCC (hepatocellular carcinoma) incidence among liver cirrhosis patients is 1,5 - 3,3%.

In spite of essential achievements in the field of the CHC etiotropic therapy after the implementation of PEG IFN (pegylated interferon) and RBV (ribavirin) in a clinical practice, only a little more than a half of the patients infected with HCV genotype 1, prevailing in Latvia due to the reason that it is identified in two thirds of all CHC patients, achieve SVR (sustained virological response) during the first course of the treatment.

Up to now a success in the treatment of CHC, doses of medication and its application time were mostly related to laboratory parameters characterizing the virus-HCV genotype and HCV RNA load before the therapy. However, more than 30% of CHC patients, undergoing the treatment with PEG IFN and RBV, are not able to get rid of HCV, and that means the development of a pathological process continues. So there are some more factors influencing the success of the HCV treatment.

For predicting the efficacy of the treatment in case of CHC, it is essential to specify the biochemical and immunogenetic parameters before the therapy. The determination of these characteristic parameters would allow prescribing the CHC therapy individually.

The objective of the study is to evaluate some biochemical and immunogenetic parameters and markers allowing predicting the efficacy of the current etiotropic therapy for CHC patients.

Material and Methods

Patients enrolled in the study

During the process of the study the medical records of 213 CHC patients treated in the Latvian Centre of Infectious Diseases were analyzed. The structure of the study is shown in Figure 1.

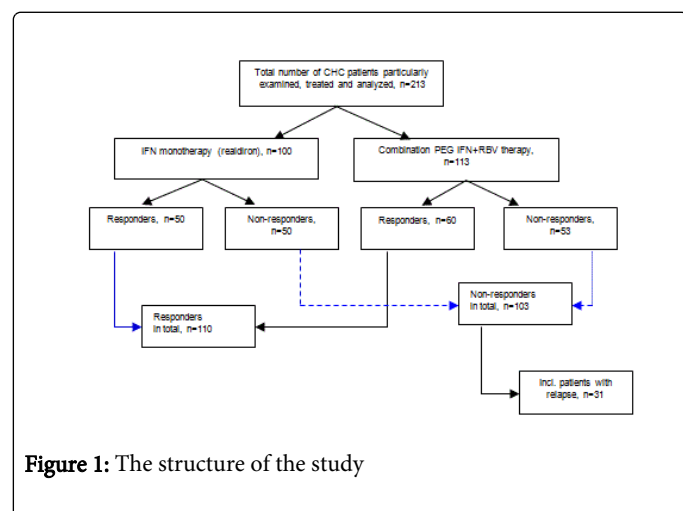


Figure 1: The structure of the study

The following facts and indicators were used as criteria for inclusion in the study:

1. HCV infection confirmed by molecular biological and serological tests;
2. increased ALT (alaninaminotransferase) activity before the treatment;
3. morphologically confirmed chronic hepatitis;
4. the complete, as regards medication doses and duration, CHC etiotropic therapy course.

Patients with HCV genotype 1 and 3, including 123 men and 90 women at the age 15-67, that had received CHC etiotropic therapy course, were enrolled in the study after a careful selection.

According to the applied CHC etiotropic therapy-monootherapy or combination therapy, patients were divided into two groups:

1. The group of monootherapy includes patients who received IFN-alpha (realdiron) monootherapy. The standard dose of IFN-alpha medication was used – 3 million IU (international units) 3 times a week subcutaneously, duration: 6-12 months. The total amount of patients in this group was 100, including 53 men and 47 women, aged 15-67 (mean age: 33,6 years);
2. The group of combination therapy includes patients who received PEG IFN alpha-2a 180 mkg a week subcutaneously or PEG IFN alpha-2b 1,5 mkg/kg a week subcutaneously and RBV 800-1200 mg/dm orally. RBV was dosed according to each patient's body weight. Both previously mentioned PEG IFN, according to the literature, are considered of equal value. The duration of the therapy – 24 weeks in cases of genotype 3 of HCV and 48 weeks in cases of genotype 1 of HCV. The total amount of patients in the group of combination therapy – 113, including 70 men and 43 women, aged 20-63 (mean age: 36,01 years).

According to the efficacy of the applied therapy, the patients of both groups were divided into two subgroups:

1. The patients, for whom the applied therapy was proven to be effective or patients that achieved SVR, as shown by undetectable HCV RNA, identified by qualitative HCV RNA test, the sensitiveness of which is 50 IU/ml, 6 months after completion of the full therapy course. This group of patients hereafter shall refer to as responders;
2. The patients, for whom the applied therapy was proven to be ineffective and SVR was not reached, hereafter shall refer to as non-responders.

Responders were compared to non-responders according to either the content of the applied therapy, separately in groups of monootherapy and combination therapy, or in total, regardless of the content of the applied therapy.

The following parameters were considered as criteria confirming the inefficacy of the treatment:

1. in the group of combination therapy - inability to achieve EVR (early virological response). After 12 weeks of treatment, at least 2-log reduction in HCV RNA in a quantity test indicated EVR. This criterion was not used in the group of monootherapy;
2. in both groups, in the group of monootherapy and the group of combination therapy – HCV RNA detectable by the qualitative test after the treatment completion;
3. in both groups – HCV RNA undetectable by the qualitative test upon the treatment completion, but detectable 24 weeks after the treatment completion, indicating the relapse of CHC.

The efficacy of the applied CHC etiotropic treatment in the group of combination therapy was also analyzed according to the HCV genotype of the patient, i.e., separately for the patients with the genotype 1 and 3 of HCV.

Methods of the study

All specific and non-specific laboratory investigations and tests were carried out at the laboratory of the Latvian Centre of Infectious Diseases and the Immunology and Immunogenetics interdepartmental laboratory of Riga Stradins University (RSU). The evaluation of obtained results was performed in accordance with the test systems manufacturers' instructions.

As the proof of HCV infection in all cases anti-HCV antibodies (antibodies against hepatitis C virus) and HCV RNA were identified. The HCV genotyping was performed for the patients with the applied CHC combination therapy.

The determination of anti-HCV

Anti-HCV in blood serum was determined by ELISA (heterogeneous enzyme-linked immunosorbent assay). Identical test systems commercially available from various manufacturers were used: ORTHO[®] HCV 3.0 Ortho-Clinical Diagnostics Inc, USA; AxSYM HCV version 3.0 Abbott, USA; INNOTEST HCV[®] Ab. IV Innogenetics, Belgium; MONOLISA anti-HCV PLUS version 2. BIO-RAD, France.

The detection of HCV RNA

For qualitative and quantitative assays of HCV RNA in blood serum commercially available reverse transcription polymerase chain reaction (PCR) method was used. The following tests were used for qualitative detection of HCV RNA: AMPLICOR® Hepatitis C virus (HCV) Test, version 2.0 Roche, USA; Cobas AMPLICOR Hepatitis C virus (HCV) Test, version 2.0 Roche, USA. The following tests were used for quantitative detection of HCV RNA: AMPLICOR® HCV Monitor™ Test, version 2.0 Roche, USA; Cobas AMPLICOR® HCV Monitor™ Test, version 2.0 Roche, USA. HCV genotypes were determined by reverse hybridization LiPa method: INNO-LiPa HCV II. Innogenetics, Belgium; The VERSANT HCV Genotype Amplification Kit (LiPa). Bayer Corporation, Germany.

The full blood count was performed on an automated hematology analyzer KX-21, Code No. 461-2261-1, SYSMEX Corporation, Kobe, Japan. During the study process the haematological parameters like hemoglobin, white blood cell count, absolute neutrophil count and platelet count were investigated more detailed.

GGT (Gamma-Glutamyl Transpeptidase) activity was measured in U/L (units per liter) by a kinetic reaction, using γ -GT liquicolor Colorimetric test, HUMAN, Germany; analyzer - Cobas Mira Plus.

Concentration of HA (Hyaluronic Acid) was determined in ng/ml (nanograms per milliliter), using Hyaluronic acid test kit (Corgenic Inc., USA).

Immunogenetic investigations

They were carried out at the Immunology and Immunogenetics interdepartmental laboratory of RSU. Multiprimer polymerase chain reaction method was used for HLA (Human Leukocyte Antigen) genotyping. Molecular genotyping of HLA class II DRB1, DQA1 and DQB1 locus gene alleles was performed by amplified two-stage DNA allele-specific amplification method. The genotyping of 10 DRB1 class alleles, 8 DQA1 class alleles and 10 DQB1 class alleles was performed during the study process. It was done using mixture of primers, manufactured by "ДНК -Технология" (Russia): with 10 versions of gene DRB1 alleles, 8 versions of gene DQA1 alleles and 10 versions of DQB1 alleles. The amplifying was performed by MC-2 multi-channel amplificator ("ДНК - Технология", Russia).

To compare the results of HLA test the material from RSU Immunology and Immunogenetics interdepartmental laboratory's database, respectively HLA defined in healthy blood donors, was used as a control group.

Statistical analysis

The statistical analysis of the data was performed, using computer programs SPSS and Microsoft Office Excel.

Standard descriptive statistical methods were used to describe the groups of the patients, the parameters of central tendency and indicators of dispersion - SD (Standard deviation) and SE (Standard error of mean) were assessed.

Dispersion analysis-ANOVA was used to analyze the quantitative parameters of the patients' groups. The qualitative variables were assessed by Pearson Chi-square and Fisher's exact test. The significance of difference between parameters was estimated with the 5% probability of statistic error.

The frequency of HLA alleles was calculated, using the formula: $f=n/2N$, where n - the frequency of alleles and N - number of patients enrolled in the study. OR or odds ratio was calculated according Woolf's method, using formula $(axd)/(bxc)$, where a - number of patients with the certain allele, genotype or haplotype; b - number of patients which do not have the certain allele, genotype or haplotype; c - number of healthy subjects with the certain allele, genotype or haplotype; d - number of healthy subjects without the certain allele, genotype or haplotype. In cases when one of the values - a , b , c , or d was zero, the odds ratio was calculated using Haldane modified formula for small groups of numbers, - $[(2a+1)(2d+1)]/[2b+1)(2c+1)]$. The statistical significance was estimated according to Fisher's criterion. 95% confidence interval (95%CI) was calculated using the formula: $95\% \text{ CI} = \ln OR \pm 1, 96$.

Results

As we analyzed the results of laboratory investigations of the patients' blood - the complete blood count, biochemical and immunological parameters, first of all we found out the proportion of the patients in general whose results exceeded the reference range. It was concluded that the results of laboratory investigations of the majority of the patients, irrespective of the effectiveness of the therapy, were within the reference range. The investigations' results of only 27.9% of the patients enrolled in the study deviated from the reference range.

GGT activity

Up to 80% of the patients infected with HCV develop chronic hepatitis in case of which an antiviral treatment is required in order to prevent from further progression of the pathologic process.

As the efficacy of the currently applicable CHC etiotropic therapy is not 100% and SVR is achieved by only 50-80% of patients, it is essential to detect which patients will have and which will not have positive outcome of the treatment. The objective of the present study was to find these differences and detect the characteristic facts by analyzing study outcomes of biochemical and immunogenetic diagnostic parameters less frequently used in a general practice.

The GGT activity before the start of the therapy was measured in 98 patients enrolled in the study. The mean GGT activity was 59, 3 U/L (SD=57,855).

The evaluation of the GGT activity showed (Table 1) increase in 46,81% of non-responders and in only 21,57% responders.

| | N | Total number of patients unchanged activity | Total number of patients increased activity | p |
|----------------|----|---|---|----------------|
| Non-responders | 47 | 25 | 22 | 0,008 0,010 |
| Responders | 51 | 40 | 11 | |
| Total | 98 | 65 | 33 | |

Table 1: Evaluation of GGT activity before the start of the therapy

The statistical significance of difference was high as assessed by either Pearson ($p=0,008$) or Fisher's test ($p=0,010$).

The evaluation of the mean GGT activity in non-responders and responders showed (Figure 2) significantly higher mean GGT activity in the group of non-responders (75,681 U/L; n=47) than in the group of responders (44,353 U/L; n=51), exceeding the upper limit of reference range (10-66 U/L (men); 5-39 U/L (women)).

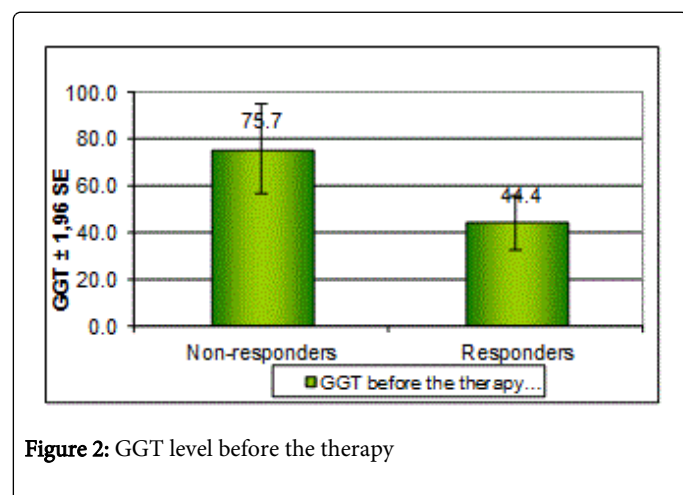


Figure 2: GGT level before the therapy

The increased GGT level in the group of non-responders, according to the available information, was not related to the use of alcohol or medications, because no co-existing disease was observed and the present coexisting diseases did not differ in the groups of responders and non-responders. Moreover, the CHC etiotropic therapy is usually not prescribed for patients with the history of active alcohol abuse.

The patients who had failed to get rid of HCV during the therapy course had higher frequency of the increased GGT activity and the mean GGT activity before the start of the therapy. This corresponds with literature data about the positive correlation between the low GGT level before the therapy and the patients' ability to achieve SVR [2-6].

The correlation of the GGT level with further advanced fibrosis and IR (insulin resistance) is described in the scientific literature [3,4,7,8]. In our study the presence of fibrosis was also more frequently observed in the patients that had failed to achieve sustained virological response, i.e., in non-responders. This could be related to the increased GGT level among non-responders.

The changes in the GGT level could not be related to the use of medication, because the majority of the patients enrolled in our study did not have severe coexisting disorders requiring the medication therapy affecting GGT. Just as there were no patients with the history of active alcohol abuse enrolled in our study.

Relationship/Association between markers of fibrosis and CHC and efficacy of the etiotropic therapy for CHC patients

The presence of fibrosis was observed in most cases of the patients enrolled in the study, but its incidence rate was higher in the patients that had not responded to the therapy. Advanced, for example, bridging fibrosis and cirrhosis, according to the literature data, are some of the most important independent prognostic factors for failure to achieve SVR [2]. The results of our study confirmed this statement.

During the study the marker of fibrosis – HA was measured in 22 CHC patients before the start of the etiotropic therapy and in 88 patients at least 6 months after completion of the CHC etiotropic

therapy. The evaluation of the relation between the marker of fibrosis and the efficacy of the applied therapy was done by dividing the patients into two groups: responders and non-responders.

Level of HA in relation to the efficacy of the CHC therapy

During our study the noninvasive marker of liver fibrosis - HA was measured and the evaluation of the relation between HA and the efficacy of the applied CHC therapy was done.

The comparison of the mean HA levels before the start of the therapy between both groups showed that the HA level in responders was significantly lower than in non-responders ($p=0,022$), furthermore the mean HA level in responders (28,72 ng/mL) did not exceed the limits of reference range (Table 2).

| | N | HA ng/mL ± SE | p |
|----------------|----|---------------|-------|
| Non-responders | 6 | 96,05 ± 43,95 | 0,022 |
| Responders | 16 | 28,72 ± 4,92 | |
| Total | 22 | 47,03 ± 13,44 | |

Table 2: Concentration of hyaluronic acid prior to the therapy

No significant difference was observed between both groups after the completion of the therapy.

The level of HA serves as an indicator or marker of fibrogenesis and therefore it increases with the development of severe liver fibrosis or cirrhosis [9-13]. We found that the level of HA in responders before the start of the therapy was within the limits of reference range and the mean HA level in responders was significantly lower than in non-responders, 28,72 ng/mL and 96,05 ng/mL, respectively. This finding could contribute to the statement that the absence of fibrosis is associated with higher probability or chance of achieving SVR.

Association of HLA class II alleles with the efficacy of the CHC etiotropic therapy

The immunogenetic investigations were carried out and the incidence or frequency of HLA class II DRB1, DQA1 and DQB1 alleles in CHC patients in general was analyzed. The correlation of these alleles with the type of CHC therapy, i.e., with the applied medication and the result or outcome of the treatment was found.

Within this part of the study 168 patients were examined and divided into the following groups according to the content and result of the applied therapy:

- the patients who received the combination PEG IFN + RBV therapy and for whom the applied therapy was proven to be effective (n=59),
- the patients who received the combination PEG IFN + RBV therapy and for whom the applied therapy was proven to be ineffective (n=45),
- the patients who received the IFN-alpha (realdiron) monotherapy and for whom the applied therapy was proven to be effective (n=30),
- the patients who received the IFN-alpha (realdiron) monotherapy and for whom the applied therapy was proven to be ineffective (n=34).

| HLA II class gene alleles | CHC (n=336) | Control group (n=200) | OR | p |
|---------------------------|-------------|-----------------------|------|--------|
| DRB1*03 | 43 | 14 | 1,95 | <0,035 |
| DRB1*05 | 83 | 33 | 1,66 | <0,026 |
| DRB1*07 | 42 | 4 | 7,0 | <0,000 |
| DQA1*0201 | 56 | 22 | 1,62 | <0,072 |
| DQB1*0201-2 | 74 | 28 | 1,74 | <0,022 |

Table 3a: Frequency of HLA II-DRB1 gene alleles

| HLA II-DRB1/DQB1/DQA1 haplotypes | OR | p |
|----------------------------------|-------|---------|
| 01/0201-2/0101 | 6,94 | <0,027 |
| 05/0301/0301 | 5,52 | <0,012 |
| 05/0502-4/0102 | 6,27 | <0,048 |
| 07/0201-2/0401 | 11,53 | <0,0001 |

Table 3b: Frequency of haplotypes among CHC patients versus control group

The frequency of HLA II-DRB1 gene alleles in CHC patients was statistically analyzed (Table 3), by combining data of all patients enrolled in the study, and it was found that DRB1*03 ($p<0,035$), DRB1*05 ($p<0,026$) and DRB1*07 ($p<0,000$) alleles had significantly higher frequency in the CHC patients. DRB1*06 ($p<0,0034$), DRB1*08 ($p<0,000$) and DRB1*15 ($p<0,020$) alleles occurred less frequently.

| HLA II class gene alleles | OR | p |
|----------------------------------|-------|---------|
| DRB1*01 | 2,58 | <0,071 |
| DQA1*0101 | 1,59 | <0,04 |
| DQB1*0502-4 | 2,82 | <0,089 |
| HLA II-DRB1/DQB1/DQA1 haplotypes | OR | p |
| 01/0201-2/0101 | 19,11 | <0,001 |
| 05/0301/0301 | 20,92 | <0,0001 |
| 05/0502-4/0102 | 16,50 | <0,001 |

Table 4: Frequency of HLA II class gene alleles and haplotypes among patients with effective IFN therapy

The frequency of HLA II-DRB1 gene alleles in the CHC patients in relation to the type of the treatment and its efficacy was analyzed (Table 4), and it was found that even the IFN-alpha (realdiron) monotherapy had been proven effective for the patients with DRB1*01 allele ($p<0,071$), but the combination PEG IFN + RBV therapy had been proven effective for the patients with DRB1*04 ($p<0,014$) and DRB1*06 ($p<0,003$) alleles (Table 5).

HLA II-DQA1*0201 gene allele was found more frequently ($p<0,072$), HLA II-DQA1*0102 allele – less frequently ($p<0,027$) among the CHC patients (Table 3).

The frequency of HLA II-DQA1 gene alleles in the CHC patients in relation to the applied therapy was also analyzed (Tables 4 and 5), and the following association was found: the IFN-alpha (realdiron) monotherapy had proven to be effective for the patients with DQA1*0101 allele ($p<0,04$), but the combination PEG IFN + RBV therapy had proven to be effective for the patients with DQA1*0301 allele ($p<0,05$).

| HLA II class gene alleles | OR | p |
|----------------------------------|-------|---------|
| DRB1*04 | 1,97 | <0,014 |
| DRB1*06 | 4,29 | <0,003 |
| DQA1*0301 | 2,42 | <0,05 |
| HLA II-DRB1/DQB1/DQA1 haplotypes | OR | p |
| 04/0301/0301 | 6,90 | <0,043 |
| 04/0302/0501 | 26,16 | <0,0001 |
| 05/0601/0103 | 28,11 | <0,000 |

Table 5: Frequency of HLA II class gene alleles and haplotypes among patients with effective combination therapy

Among the CHC patients (Table 3) HLA II-DQB1*0201-2 gene allele was found more frequently ($p<0,022$), but HLA II-DQB1*0303 ($p<0,029$) and HLA II-DQB1*0602-8 ($p<0,026$) alleles – less frequently.

The frequency of HLA II-DQB1 gene alleles in the CHC patients in relation to the applied etiologic therapy and its efficacy was analyzed, and it was found that the IFN-alpha (realdiron) monotherapy had been proven effective for the patients with DQB1*0502-4 allele ($p<0,089$) (Table 4).

When evaluating the incidence of HLA II-DRB1/DQB1/DQA1 haplotypes in the CHC patients and the association of these haplotypes with the type of the therapy, i.e., with the applied medication and the result of the treatment, it was concluded that:

- the most frequently found HLA II-DRB1/DQB1/DQA1 haplotypes in the CHC patients overall were 01/0201-2/0101 ($p<0,027$), 05/0301/0301 ($p<0,012$), 05/0502-4/0102 ($p<0,048$) and 07/0201-2/0401 ($p<0,001$);
- the IFN (realdiron) monotherapy had been effective for patients with HLA II-DRB1/DQB1/DQA1 haplotypes 01/0201-2/0101 ($p<0,001$), 05/0301/0301 ($p<0,0001$) and 05/0502-4/0102 ($p<0,001$);
- the combination PEG IFN + RBV therapy had proven to be effective for patients with HLA II-DRB1/DQB1/DQA1 haplotypes 04/0301/0301 ($p<0,043$), 04/0302/0501 ($p<0,0001$) and 05/0601/0103 ($p<0,000$).

When evaluating the incidence of HLA II-DRB1 gene alleles in CHC patients versus control group, i.e., healthy subjects, it was found that in CHC patients HLA-DRB1*07, HLA-DRB1*03 and HLA-DRB1*05 alleles had significantly higher frequency, but HLA-DRB1*06, HLA-DRB1*04 and HLA-DRB1*15 alleles were found less frequently. Therefore, a presumption could be made that among the population of Latvia DRB1*07, DRB1*03 and DRB1*05 alleles predispose to CHC infection, but DRB1*06, HLA-DRB1*04 and

DRB1*15 alleles protect or even exclude the possibility of being infected with HCV. Association of DRB1*0301, DRB1*07 and DRB1*0701 alleles with CHC infection has also been mentioned in other studies, but not regarding the population of Latvia [14-17]. The association of HLA-DRB1*15 and DRB1*1501 with the spontaneous release from HCV has been described [18,19].

More detailed analysis and evaluation of the association of HLA-DRB1 alleles with the outcome of the applied CHC etiotropic therapy confirmed that even the IFN-alpha (realdiron) monotherapy had been effective for the patients with DRB1*01 allele. According to the scientific literature, this allele is often observed in patients with the spontaneous recovery from HCV [14,15,18,20-22]. Perhaps due to this immunogenetic specificity the patients with HLA-DRB1*01 allele, if they for some reason, e.g., due to a specific HCV resistance, do not spontaneously recover, they still respond much better to a simple and for the patients with other alleles low-effective CHC etiotropic therapy. In the combination PEG IFN + RBV therapy group SVR was achieved by the patients with higher frequency of alleles HLA-DRB1*04 and HLA-DRB1*06. Chinese scientists also have described association between HLA-DRB1*04 allele and the inefficiency of the IFN + RBV therapy [23]. In the present study this allele was more frequently found among responders to the combination therapy. These differences could be explained by the incidence of HLA II class gene alleles among people of certain race, although there had been some differences in the therapy regimen as well: the IFN+RBV and the PEG INF-RBV.

The evaluation of the incidence of HLA II-DRB1 gene alleles in the CHC patients confirmed that HLA-DQA1*0201 allele was found more frequently, but HLA-DQA1*0102 allele – less frequently. A similar study conducted in Thailand describes totally opposite data: HLA-DQA1*0201 allele in the HCV patients versus the control group, i.e., the healthy subjects, is found infrequently, so the statement is made that this allele is protective against HCV infection [24]. Contradictions in the results, likewise as in case of HLA-DRB1, could be related to the ethnic differences of populations, e.g., in Poland haplotypes DRB1*0701/DQA1*0201/DQB1*02 are frequently found in young, as regards age, CHC patients who respond to the IFN therapy [14]. According to the data obtained by several scientists, allele HLA-DQA1*0103 is associated with the spontaneous recovery from HCV, but this association was not observed in our study.

When assessing the association of HLA-DQA1 gene alleles with the efficacy of the CHC etiotropic therapy, we found that the INF monotherapy had proven to be effective for the patients with HLA-DQA1*0101 allele, but for the patients with HLA-DQA1*0301 allele the combination PEG IFN + RBV therapy had proven to be effective. In the scientific literature regarding the association of HLA-DQA1* genes with the efficacy of the therapy, the most frequently mentioned is data obtained by polish scientists about high incidence of haplotypes DRB1*0701/DQA1*0201/DQB1*02 among responders to the IFN therapy [14,20].

Our evaluation of the incidence of HLA-DQB1 gene alleles in the CHC patients confirmed that HLA-DQB1*0201-2 allele was found more frequently, but HLA DQB1*0303 and HLA II-DQB1*0602-8 alleles – less frequently. According to the scientific literature data, HLA-DQB1*0201 allele is frequently found in the patients with chronic HCV infection, it is described that in France HLA-DQB1*0201 allele is more frequently found in the patients with liver cirrhosis [24]. While HLA DQB1*0303 allele is usually associated with milder form of HCV infection [25].

The positive relation or association between the CHC etiotropic therapy and DQB1*0502-4 allele was found. We observed that the frequency of DQB1*0502-4 allele was statistically higher in responders to the IFN (realdiron) monotherapy. In studies performed by other scientists the association between this allele and the outcome of treatment has not been found, although in Japan the researchers have found the association between haplotype DRB1*05*15/DQB1 and the effective IFN therapy [20,26-28].

The analysis of the haplotypes of the patients enrolled in our study identified the following association with the CHC etiotropic therapy: the Realdiron monotherapy was effective for patients with haplotypes HLA DRB1*01/DQB1*0201-2/ DQA1*0101, DRB1*05/DQB1*0301/DQA1*0301 and DRB1*05/DQB1*0502-4/DQA1*0102, but the combination therapy was effective for patients with haplotypes HLA DRB1*04/DQB1*0301/DQA1*0301, DRB1*04/DQB1*0302/DQA1*0501 and DRB1*05/DQB1*0601DQA1*/0103.

Thus, the results of the present study confirm the hypothesis that the condition of the macro organism, which is characterized by biochemical and immunogenetic parameters, plays the essential role in the efficacy of the CHC therapy.

Conclusions

The inefficacy of the CHC etiotropic therapy is associated with increased GGT level upon the start of the therapy and elevated level of HA upon the start of the therapy.

The most frequent MHC HLA II class gene alleles in the CHC patients in Latvia are DRB1*03, DRB1*05, DRB1*07, DQA1*0201, DQB1*0201-2 and haplotypes DRB1*01/DQB1*0201-2/DQA1*0101, DRB1*05/DQB1*0301/DQA1*0301, DRB1*05/DQB1*0502-4/DQA1*0102, DRB1*07/DQB1*0201-2/DQA1*0401.

MHC HLA II class gene alleles DRB1*01, DQA1*0101, DQB1*0502-4 and haplotypes, DRB1*01/DQB1*0201-2/DQA1*0101, DRB1*05/DQB1*0301/DQA1*0301, DRB1*05/DQB1*0502-4/DQA1*0102 are associated with the efficacy of the Realdiron therapy in Latvia.

MHC HLA II class gene alleles DRB1*04, DRB1*06, DQA1*0301 and haplotypes DRB1*04/DQB1*0301/DQA1*0301, DRB1*04/DQB1*0302/DQA1*0501 and DRB1*05/DQB1*0601DQA1*/0103 are associated with the efficacy of the PEG IFN+RBV combination therapy in Latvia.

The correlation of these parameters with SVR is statistically significant and considerable for predicting the result of the etiotropic therapy.

Acknowledgements

This study was financially supported by the Project No.9.1. of the National Program No.4.

Ethical Approval

All authors hereby declare that all human studies have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1946 Declaration of Helsinki.

Conflict of Interest

The authors state no conflict of interest.

References

1. Tolmane I, Rozentale B, Keišs J, Arša F (2009) C virushepatita izplatība Latvija: epidemiologiska pētījuma rezultāti. *Latvijas Arsts* 5: 36-38.
2. Kau A, Vermehren J, Sarrazin C (2008) Treatment predictors of a sustained virologic response in hepatitis B and C. *J Hepatol* 49: 634-651.
3. Villela-Nogueira CA, Perez RM, de Segadas Soares JA, Coelho HS (2005) Gamma-glutamyl transferase (GGT) as an independent predictive factor of sustained virologic response in patients with hepatitis C treated with interferon-alpha and ribavirin. *J Clinical Gastroenterol* 39: 728-730.
4. Parise ER, Oliveira AC, Figueiredo-Mendes C, Lanzoni V, Martins J, et al. (2006) Noninvasive serum markers in the diagnosis of structural liver damage in chronic hepatitis C virus infection. *Liver Int* 26: 1095-1099.
5. Taliani G, Gemignani G, Ferrari C, Aceti A, Bartolozzi D, et al. (2006) Pegylated interferon alfa-2b plus ribavirin in the retreatment of interferon-ribavirin non-responder patients. *Gastroenterology* 130: 1098-1106.
6. Tan J, Lok AS (2007) Update on viral hepatitis: 2006. *Curr Opin Gastroenterol* 23: 263-267.
7. Silva IS, Ferraz ML, Perez RM, Lanzoni VP, Figueiredo VM, et al. (2004) Role of gamma-glutamyl transferase activity in patients with chronic hepatitis C virus infection. *J Gastroenterol Hepatol* 19: 314-318.
8. Benini F, Pigozzi MG, Baisini O, Romanini L, Ahmed H, et al. (2007) Increased serum gamma-glutamyl-transpeptidase concentration is associated with non-alcoholic steatosis and not with cholestasis in patients with chronic hepatitis C. *J Gastroenterol Hepatol* 22: 1621-1626.
9. Mehta P, Ploutz-Snyder R, Nandi J, Rawlins SR, Sanderson SO, et al. (2008) Diagnostic accuracy of serum hyaluronic acid, FIBROSpect II, and YKL-40 for discriminating fibrosis stages in chronic hepatitis C. *Am J Gastroenterol* 103: 928-936.
10. Lackner C, Struber G, Liegl B, Leibl S, Ofner P, et al. (2005) Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. *Hepatology* 41: 1376-1382.
11. Halfon P, Bourlière M, Pénaranda G, Deydier R, Renou C, et al. (2005) Accuracy of hyaluronic acid level for predicting liver fibrosis stages in patients with hepatitis C virus. *Comp Hepatol* 4: 6.
12. McHutchison JG, Blatt LM, de Medina M, Craig JR, Conrad A, et al. (2000) Measurement of serum hyaluronic acid in patients with chronic hepatitis C and its relationship to liver histology. Consensus Interferon Study Group. *J Gastroenterol Hepatol* 15: 819-821.
13. Wong VS, Hughes V, Trull A, Wight DG, Petrik J, et al. (1998) Serum hyaluronic acid is a useful marker of liver fibrosis in chronic hepatitis C virus infection. *J Viral Hepat* 5: 187-192.
14. Łoś-Rycharska E, Szaflarska-Popławska A (2009) Influence of selected HLA tissue compatibility antigens on the course and efficacy of viral hepatitis C treatment--actual knowledge position. *Adv Med Sci* 54: 14-19.
15. Yee LJ (2004) Host genetic determinants in hepatitis C virus infection. *Genes Immun* 5: 237-245.
16. Alric L, Fort M, Izopet J, Vinel JP, Charlet JP, et al. (1997) Genes of the major histocompatibility complex class II influence the outcome of hepatitis C virus infection. *Gastroenterology* 113: 1675-1681.
17. Thursz M, Yallop R, Goldin R, Trepo C, Thomas HC (1999) Influence of MHC class II genotype on outcome of infection with hepatitis C virus. The HENCORE group. Hepatitis C European Network for Cooperative Research. *Lancet* 354: 2119-2124.
18. McKiernan SM, Hagan R, Curry M, McDonald GS, Kelly A, et al. (2004) Distinct MHC class I and II alleles are associated with hepatitis C viral clearance, originating from a single source. *Hepatology* 40: 108-114.
19. Lechmann M, Schneider EM, Giers G, Kaiser R, Dumoulin FL, et al. (1999) Increased frequency of the HLA-DR15 (B1*15011) allele in German patients with self-limited hepatitis C virus infection. *Eur J Clin Invest* 29: 337-343.
20. Wawrzynowicz-Syczewska M, Underhill JA, Clare MA, Boron-Kaczmarek A, McFarlane IG, et al. (2000) HLA class II genotypes associated with chronic hepatitis C virus infection and response to alpha-interferon treatment in Poland. *Liver* 20: 234-239.
21. Barrett S, Ryan E, Crowe J (1999) Association of the HLA-DRB1*01 allele with spontaneous viral clearance in an Irish cohort infected with hepatitis C virus via contaminated anti-D immunoglobulin. *J Hepatol* 30: 979-983.
22. Cangussu LO, Gerbase-DeLima M, Mingoti SA, Campos EF (2008) The influence of HLA in the clinical outcome of patients with chronic hepatitis C. *Hepatology* 48: 528.
23. Jiao J, Wang JB (2005) Hepatitis C virus genotypes, HLA-DRB alleles and their response to interferon-alpha and ribavirin in patients with chronic hepatitis C. *Hepatobiliary Pancreat Dis Int* 4: 80-83.
24. Vejbaesya S, Songsivilai S, Tanwandee T, Rachaiyubun S, Chantangpol R, et al. (2000) HLA association with hepatitis C virus infection. *Hum Immunol* 61: 348-353.
25. Hüe S, Cacoub P, Renou C, Halfon P, Thibault V, et al. (2002) Human leukocyte antigen class II alleles may contribute to the severity of hepatitis C virus-related liver disease. *J Infect Dis* 186: 106-109.
26. Airoidi A, Zavaglia C, Silini E, Tinelli C, Martinetti M, et al. (2004) Lack of a strong association between HLA class II, tumour necrosis factor and transporter associated with antigen processing gene polymorphisms and virological response to alpha-interferon treatment in patients with chronic hepatitis C. *Eur J Immunogenet* 31: 259-265.
27. Yu ML, Dai CY, Chen SC, Chiu CC, Lee LP, et al. (2003) Human leukocyte antigen class I and II alleles and response to interferon-alpha treatment, in Taiwanese patients with chronic hepatitis C virus infection. *J Infect Dis* 188: 62-65.
28. Dai CY, Chuang WL, Hsieh MY, Huang JF, Lin YY, et al. (2010) Human leukocyte antigen alleles and the response to pegylated interferon/ribavirin therapy in chronic hepatitis C patients. *Antiviral Res* 85: 396-402.