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CHRONIC HEPATITIS C: BIOCHEMICAL AND IMMUNOGENETIC DIAGNOSTIC MARKERS FOR PREDICTING EFFICACY OF ETIOTROP THERAPY

Speciality - Infectious diseases

Summary
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CONTENT

1. ABBREVIATIONS

AFP	–	alpha fetoprotein
ALT	–	alaninaminotranspherasis
Anti-HCV	–	antibodies against hepatitis C virus
LPB	–	liver puncture biopsy
CK-18	–	cytokeratin18
Cyt.C	–	cytochrome C
DNA	–	deoxyribonucleic acid
ELISA	–	enzyme linked immunosorbent assay
EVR	–	early virological response
GGT	–	gamma-glutamyl transpeptidase
g/dL	–	grams per deciliter
g/L	–	gram per litre
Hb	–	hemoglobin
HCC	–	hepatocellular carcinoma
HCV	–	hepatitis C virus
HCV RNA	–	hepatitis C virus-ribonucleic acid
HLA	–	human leukocyte antigen
CHC	–	chronic hepatitis C
HA	–	hyaluronic acid
IFN	–	interferon
IL	–	interleukin
IR	–	insulin resistance
LIC	–	State Agency „Infectology Center of Latvia”
MHC	–	major histocompatibility complex
mil.	–	million
mmol/L	–	millimoles per litre
mkmol/L	–	micromoles per litre

N or n	–	total number of measurements or subjects
ng/L	–	nanograms per liter
ng/mL	–	nanograms per milliliter
OR	–	odds ratio
p	–	value
PEG IFN	–	pegylated interferon
PCR	–	polymerase chain reaction
WHO	–	World Health Organization
RBV	–	ribavirin
SD	–	standard deviation
SE	–	standard error of mean
IU	–	international units
SVR	–	sustained virological response
IU/L	–	international units per liter
U/L	–	units per liter
GSH	–	glutathione

2. IMPORTANCE OF THE PROBLEM

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.

Despite the fact that the ways of spreading and transmission of HCV are well known, according to WHO data, there are 3-4 million new HCV infection cases registered annually, approximately 80 % of those become chronic diseases.

It was detected in the epidemiological research done in Latvia in 2008, that 1,7% of the population of Latvia is HCV RNA positive (Tolmane I, et al.). 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 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 incidence among liver cirrhosis patients is 1,5 - 3,3%.

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

It is not insignificant that a medical treatment of CHC patients costs thousands of lats per person, without getting a desired result quite often.

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, morphological, immunological and immunogenetic parameters before the therapy. The determination of these characteristic parameters would allow to prescribe the CHC therapy individually.

By detecting negative prognostic factors, patients could be protected from uneffective, sometimes not advisable and even harmful courses of the treatment

3. OBJECTIVE OF THE STUDY

To find biochemical, morphological, immunogenetic and demographic parameters and markers allowing to predict the efficacy of the etiotrop therapy for CHC patients.

4. TASKS OF THE STUDY

1. To do biochemical and morphological examinations determining the condition of CHC patients' health.
2. To analyze the data of the biochemical and morphological examinations of CHC patients in the context of the efficacy of the received etiotrop therapy.
3. To study specific features of macroorganisms and the efficacy of the etiotrop therapy for CHC patients, by determining the HLA II class risk and protective alleles, the allocation of DR and DQ genotypes and haplotypes by study groups.
4. To set selection criterias for a possible positive result of the SVR etiotrop therapy by using discovered diferences.
5. Based on the achieved results, to create a pre-etiotrop therapy examination algorithm that would allow to predict the efficacy of the therapy and, having the risk of the negative prognosis, make possible corrections in the health condition of the patient during the pre-therapy stage.

5. SCIENTIFIC NOVELTY AND PRACTICAL VALUE OF THE STUDY

During the study the correlation between morphological changes in liver tissues, blood biochemical characteristic parameters and the efficacy of the CHC etiotrop therapy, as well as the relation between HLA II class DRB1, DQA1 and DQB1 gene alleles of CHC patients in Latvia and the efficacy of the CHC etiotrop therapy were found.

Based on the achieved results, recommendations were created for the improvement of the CHC patients' pre-therapy examination algorythm and the improvement of patients' health condition in case of potentially removable, laboratory detectable changes.

6. HYPOTHESES OF THE STUDY

1. It is possible that biochemical parameters of CHC patients – ALT, GGT, glucose, cholesterol, GSH, HA, CK-18, Cyt.C etc. allow to prognosticate the efficacy of the combined etiotrop therapy.
2. It is possible that there is a correlation between certain HLA DRB1, DQA1 and DQB1 gene alleles and the efficacy of the CHC etiotrop therapy.
3. It is possible that there is a correlation between the morphological changes of liver tissues and the efficacy of the etiotrop therapy for CHC patients.

7. STRUCTURE AND VOLUME OF THE STUDY

The Doctoral Thesis is written in Latvian language. It consists of 11 chapters: Introduction, Importance of the Problem, Objective of the Work and Terms of Reference, Hypotheses of the Work, Review of Publications, Materials and Methods, Results, Discussion, Conclussions, Practical References, List of Publications. It has 5 annexes.

Reading list comprises 188 references. The total volume of the Doctoral Thesis is 131 pages, including 49 pictures and 50 tables.

8. MATERIAL UN METHODS

8.1.Patients enrolled in the research study

During the process of the study the medical records of 450 CHC patients available in the Infectology Center of Latvia for the period from 1996 till 2009 were analyzed. The structure of the study is shown in Figure 8.1.

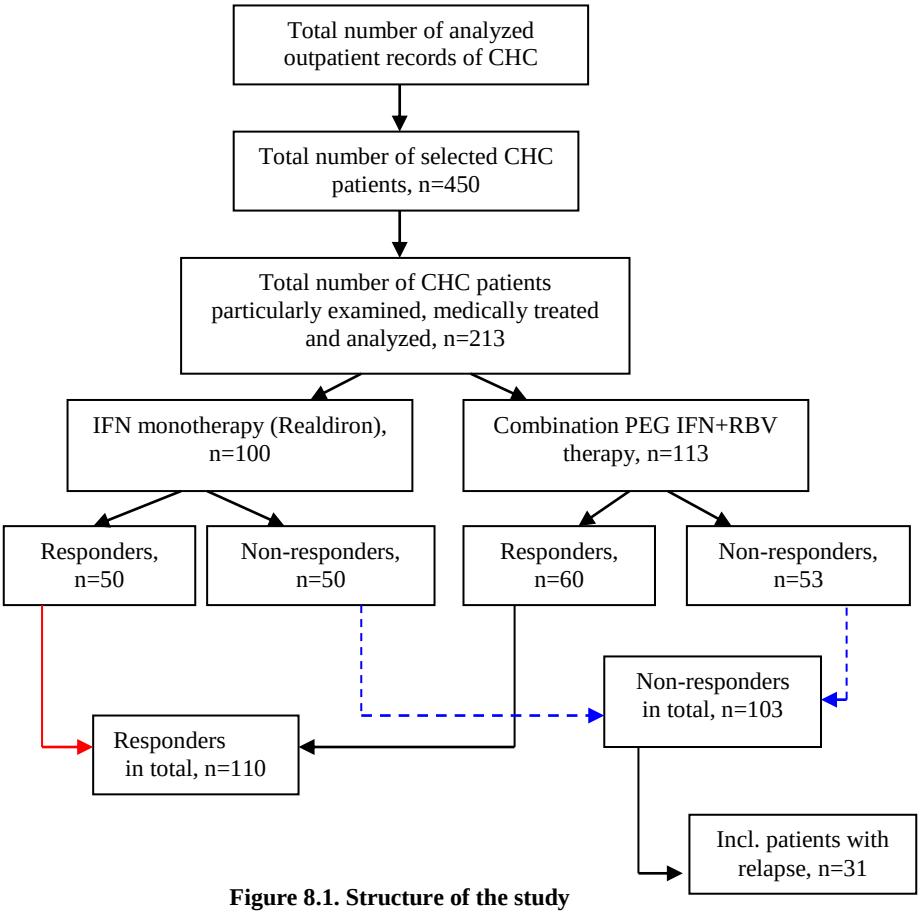

Figure 8.1. Structure of the study

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

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

Patients with the 1st and 3rd genotype of HCV, including 123 men and 90 women at the age 15-67, that had received CHC etiotrop therapy course during the period 1996 - 2007, were enrolled in the study after a careful selection.

According to the content of the applied CHC etiotrop therapy – monotherapy or combination therapy, patients were divided into two groups:

- the group of monotherapy includes patients who received IFN-alpha (realdiron) monotherapy. The standard dose of IFN-alpha medication was used – 3 million IU 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);
- 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 the 3rd genotype of HCV and 48 weeks in cases of the 1st genotype 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:

- 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**;
- 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 monotherapy and combination therapy, or in total, regardless of the content of the applied therapy.

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

- in the group of combination therapy - inability to achieve EVR. After 12 weeks of treatment, at least 2-log reduction in HCV RNA in a quantity test indicated EVR. That criteria was not used in the group of monotherapy because the determination of EVR has been included in clinical practice in Latvia only since approval of the guidelines of the etiotrop treatment of hepatitis C patients in 2005;
- in both groups, in the group of monotherapy and the group of combination therapy – HCV RNA detectable by the qualitative test after the treatment completion;

- 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 etiotrop 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 1st and 3rd genotype of HCV.

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.

8.2. Methods of the study

All specific and non-specific laboratory investigations and tests were carried out at the laboratory of State Agency „Infectology Center of Latvia" and the Immunology and Immunogenetics interdepartmental laboratory of RSU. The evaluation of obtained results was performed in accordance with the test systems manufacturers' instructions.

8.2.1. The proof of HCV infection

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

The determination of anti-HCV: anti-HCV in blood serum were determined by heterogeneous enzyme-linked immunosorbent assay (ELISA). Identical test systems comercially 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 comercially 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 quanitative 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.

8.2.2. Clinical blood tests

The full blood count was performed on an automated hematology analizer 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.

8.2.3. Biochemical blood tests

ALT activity was measured in U/L by a kinetic enzymatic reaction: GPT(ALAT) IFCC mod., Liqui UV Test, HUMAN, Germany; analyzer - Cobas Mira Plus.

GGT activity was measured in U/L by a kinetic reaction, using γ-GT liquicolor Colorimetric test, HUMAN, Germany; analyzer - Cobas Mira Plus.

Total protein concentration in blood was determined in g/L, using Total Protein Liquicolor, Photometric-Colorimetric Test for Total Protein, Biureth method, HUMAN, Germany; analyzer - Cobas Mira Plus.

Protein fractions were identified by electrophoresis method on cellulose acetate film, using electrophoresis fractionation analyzer ELPHOSCAN MiniPlus, SARSTEDT-Group, Germany.

Glucose was measured in mmol/L by enzymatic colour reaction, using Glucosae liquicolor, GOD-PAP method, HUMAN, Germany; analyzer - Cobas Mira Plus.

Cholesterol was measured in mmol/L by enzymatic colour reaction, using Cholesterol liquicolor, CHOD-PAP method, HUMAN, Germany; analyzer - Cobas Mira Plus.

*C*oncentration of **iron** (Fe) in blood was determined in mmol/L by colorimetric reaction, using Iron Colorimetric Test with Ferrozine, Roche, Switzerland; analyzer - Cobas Mira Plus.

AFP was measured in ng/L by ELISA for quantitative determination of AFP (AxSYM system AFP, Abbott, USA).

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

CK-18 was measured in U/L by M30 test system - Apoptosense® ELISA(PEVIVA, Sweden).

Cyt. C was measured in ng/mL by human Cytochrome C ELISA kit (Bender MedSystems, Austria).

8.2.4. HLA genotyping

Immunogenetic investigations were carried out at the Immunology and Immunogenetics interdepartmental laboratory of RSU. Multiprimer polymerase chain reaction method was used for HLA genotyping. Molecular genotyping of HLA class II DRB1, DQA1 un 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).

8.2.5. Morphological examination of tissue specimens obtained by liver biopsy

Hematoxylin eosin standard staining method was used, 100-400- fold magnification, „CAPA" microscope, Germany.

8.2.6. Statistical processing of the results

The statistical analysis of the data was performed, using computerprograms 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 - Standard deviation (SD) un Standard error of mean (SE) were assessed.

Dispersion analysis - ANOVA was used to analyse 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 allelles 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+l)(2d+l)]/ [2b+l)(2c+l)]. The statistical significance was estimated according to Fisher's criterion. 95% confidence interval (95%CI) was calculated using the formula: 95% CI=InOR±l,96

The design of the study – retrospective and prospective.

9. RESULTS, ANALYSIS OF THE RESULTS

9.1. Demographic parameters of the patients

The age of the patients at the beginning of the therapy and the correlation between the gender of the patient and the efficacy of the therapy were analyzed during the study.

Non-responders in total figures were statistically credible older (p=0,025) than responders (Table 9.1.), which leads to the conclusion that it is preferable to start the CHC etiotrop therapy as early as possible.

Table 9.1.

Age of patients at the beginning of the therapy

	N	Age (in years) ± SE	p
Non-responders	103	36,5 ± 1,03	0,025
Responders	110	33,3 ± 0,98	

Among responders there was a higher frequency of women (p=0,008) than men, when analysing total data on the efficacy of the therapy (Table 9.2.).

Table 9.2.

Gender of patients in total

	N	The percentage of men	The percentage of women	p
Non-responders	103	67,0	33,0	0,009
Responders	110	49,1	50,9	
Total	213	57,7	42,3	

9.2. Morphological examination of liver tissues

Out of 213 patients enrolled in the study LPB was performed in 207 patients before the beginning of the therapy. It was not done on 3 patients with hemocoagulation disorders (1 patient had taken anticoagulants, 2 were hemophilia patients), 2 patients refused LPB, for one patient the manipulation was technically unsuccessful, because obtained liver tissue specimens were unsuitable for further examination.

Experts in morphology examined the morphological pictures of liver biopsy specimens and described the facts indicating inflammation, steatosis, apoptosis, fibrosis and cirrhotic changes.

Histological correlation with the efficacy of the therapy was observed in patients with liver fibrosis. Fibrotic changes were more observed in non-responders of the combination therapy group, where p=0.040 and the total data group, where p=0,018 (Table 9.3.).

Table 9.3.

Liver fibrosis in total

	N	Number of patients with liver fibrosis	Number of patients without liver fibrosis	p
Non-responders	102	89	13	0,022
Responders	105	78	27	
Total	207	167	40	

As regards fibrosis no statistical difference was observed between responders and non-responders in the Realdiron group, although the rest two groups had similar tendencies .

More frequent cases of fibrosis in patients that didn't achieve sustained virological response, i.e., in non-responders, probably point to the fact that the treatment of the patients must be started as early as possible, i.e., before fibrotic changes become irreversible.

9.3. Analysis of blood count parameters

Leukocyte count, platelet count and hemoglobin concentration were analyzed. There was no intergroup difference observed in leukocyte and platelet count between the group of responders and the group of non-responders.

It was observed (Table 9.4.) that in the group of patients undergoing combination therapy before the beginning of the therapy, as well as after the completion of it non-responders had comparatively higher level of Hb than responders.

Table 9.4.

Level of hemoglobin in the group of combination therapy

	N	Hemoglobin g/dL ± SE	p
Hb before the therapy			
Non-responders	53	15,20 ± 0,17	0,007
Responders	60	14,50 ± 0,18	
Total	113	14,83 ± 0,18	
Hb upon the completion of the therapy			
Non-responders	53	12,72 ± 0,21	0,012
Responders	60	11,95 ± 0,21	
Total	113	12,31 ± 0,15	
Hb 6 months after the completion of the therapy			
Non-responders	33	15,10 ± 0,28	0,043
Responders	55	14,40 ± 0,20	
Total	88	14,66 ± 0,17	

The concentration of hemoglobin in responders upon completion of the therapy was under the lower limit of reference range, which is a typical finding in the patients that have received RBV, because RBV causes red blood cell hemolysis. Unexpected finding is unchanged level of Hb in non-responders

upon the completion of the therapy. This leads to the possibility of a lack of compliance, i.e., a disuse of RBV or an insufficient intake of RBV as a reason for the inefficacy of the therapy. Similar changes in Hb level were observed in the total data group (p=0,012), though in that group the Hb level in responders did not exceed the limits of reference interval (12,66 g/dl), that was related to a summarization of patients' data from both groups, because the patients of the Realdiron group had not received RBV, therefore there was no reason for decrease in Hb level.

9.4. Analysis of the results of biochemical blood tests

The following blood biochemical parameters were measured during the study: level of bilirubin, ALT activity, GGT, glucose level, total cholesterol level, total amount of protein, albumin, gamma globulins, level of iron, ferritin, GSH and AFP.

The total data obtained before the start of the therapy were used in the description of the results of biochemical assays.

9.4.1. GGT activity

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 9.5.) increase in 46,81% of non-responders and in only 21,57% responders.

Table 9.5.

Evaluation of GGT activity before the start of the therapy

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

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 9.1.) significantly higher mean GGT activity in the group of non-responders (75,681 U/L) than in the group of responders (44,353 U/L), exceeding the upper limit of reference range (10-66 U/L (men); 5-39 U/L (women)).

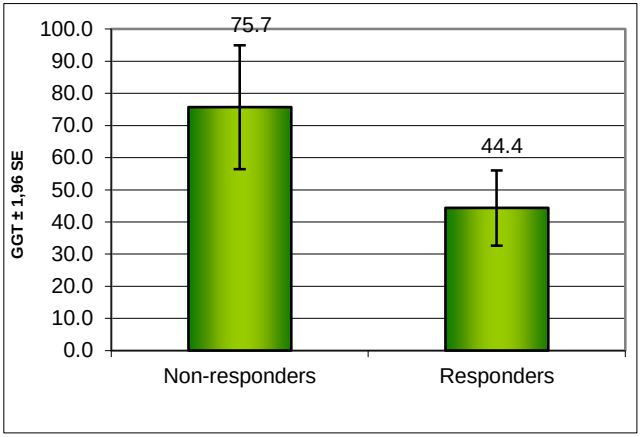

Figure 9.1. GGT level before the therapy

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 coexisting disease was observed and the present coexisting diseases

did not differ in the groups of responders and non-responders. Moreover, the CHC etiotrop therapy is usually not prescribed for patients with the history of active alcohol abuse.

9.4.2. Total cholesterol level

The total cholesterol level before the start of the therapy was measured in 29 patients enrolled in the study. The mean value of total cholesterol was 4,85 mmol/L (SD=1,148) and it did not exceed the limits of reference range.

The evaluation of the total cholesterol level in non-responders and responders showed (Table 9.6.) that the total cholesterol level remained unchanged in 81,25% of responders. In non-responders the total cholesterol level was within reference range in only 46,15%, but it was increased in 53,85% of cases.

Table 9.6.

Evaluation of cholesterol level before the start of the therapy

	N	Total number of patients with unchanged cholesterol level	Total number of patients with increased cholesterol level	p
Non-responders	13	6	7	
Responders	16	13	3	0,048
Total	29	19	10	0,064

The statistical significance was indicated by Pearson's index (p=0,048), but by Fisher's test the statistical significance was not reached (p=0,064).

9.4.3. Glucose level

The glucose level before the start of the therapy was measured in 57 patients. The mean value of glucose level was 5,18 mmol/L (SD=0,984), and it was within the limits of reference range.

In comparison with the glucose level of the patients in the non-responders group, the glucose level in responders was within the limits of reference range more often (80%), while in non-responders - in only 63,6%.

The evaluation of the mean values of the glucose level before the start of the treatment showed statistically significant (p=0,033) difference between the groups of responders and non-responders. The mean value of glucose in non-responders (5,53 mmol/L) was higher than in responders (4,96 mmol/L) as shown in Figure 9.2.

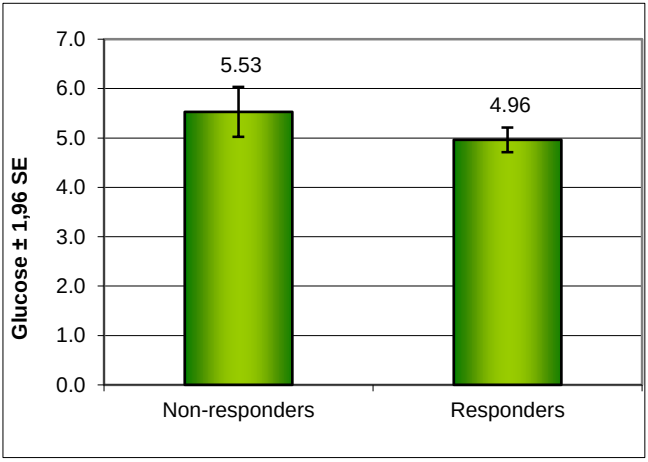

Figure 9.2. Glucose level before the therapy

The mean values of glucose level in both groups were within the limits of reference range. Nevertheless for clinical practice the fact is certainly useful

9.4.4. Level of iron

The mean iron concentration value determined in patients (n=35) before the start of the therapy was 25,64 mkmol/L (SD=9,555).

The difference in iron concentration was not observed betwen responders and non-responders. However there was a tendency among responders that most of the patients had unchanged level of iron, in 31,6% the concentration of Fe was increased, but in 5,3% - decreased. Among non-responders there were equal numbers of patients with elevated and with unchanged level of iron (Figure 9.3.).

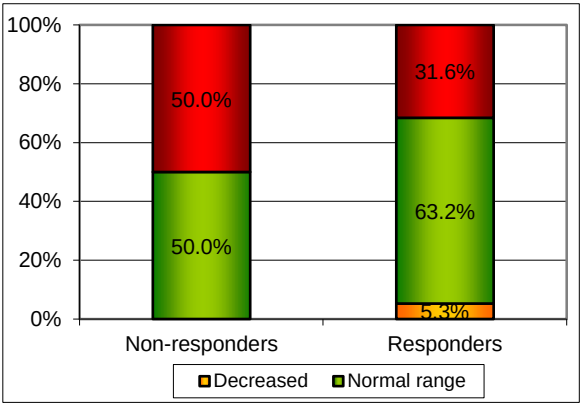

Figure 9.3. Iron assessment before the therapy

9.4.5. Gamma globulins

The gamma globulins were determined in 203 patients. The mean value of gamma globulins was 20,25% (SD=3,726), and it was within the limits of reference range.

Qualitative comparison of the gamma globulin levels was made and showed that there were more patients with unchanged γ globulin level (74,8%) in the group of responders in comparison with 62% of non-responders (p=0,036). Increased γ globulin level was more frequently observed in non-responders (34%) than in responders (25,2%) (Table 9.7.).

Table 7.9.

Evaluation of gamma globulin level before the start of the therapy

	N	Total number of patients with decreased γ globulin level	Total number of patients with unchanged γ globulin level	Total number of patients with increased γ globulin level	p
Non-responders	100	4	62	34	0,036
Responders	103	0	77	26	
Total	203	4	139	60	

9.4.6. Alpha fetoprotein

There was no difference in the AFP levels (n=39) between responders and non-responders before the start of the therapy. The mean values of both groups were within the reference range, i.e., 6,83 ng/mL in responders and 6,61 ng/mL in non-responders.

The re-evaluation of the AFP level (n=62) 6 months after the completion of the therapy showed the normal AFP level in responders, but elevated AFP level in 18,9% of non-responders (p=0,021). Statistically the mean APF value in non-responders (11,34 ng/mL) was significantly higher (p=0,035) than in responders (3,97 ng/mL) and exceeded the upper limit of reference range (Table 9.8.).

Table 9.8.

AFP level 6 months after the completion of the therapy

	N	AFP ng/mL ± SE	p
Non-responders	37	11,34 ± 2,78	0,035
Responders	25	3,97 ± 0,41	

This finding could confirm an ongoing progression of the fibrotic process in patients who failed to get rid of the virus.

9.5. Relationship between markers of apoptosis and fibrosis and CHC and efficacy of the etiotrop therapy for CHC patients

During the study the marker of fibrosis - HA, as well as the markers of apoptosis - CK-18 and Cyt.C were measured in 22 CHC patients before the start of the etiotrop therapy and in 88 patients at least 6 months after completion of the CHC etiotrop therapy. The evaluation of the relation between the markers of apoptosis and fibrosis and the efficacy of the applied therapy was done by dividing the patients into two groups: responders and non-responders.

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

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 9.9.).

Table 9.9.

Concentration of hyaluronic acid prior to the therapy

	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	

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

9.5.2. Correlation between the CK-18 neoepitop level and the efficacy of the CHC therapy

There was no difference in the CK-18 levels between both groups before the start of the therapy, in all patients the CK-18 was above the reference range.

After the completion of the therapy it was observed that in non-responders the CK-18 level in blood was almost two times higher than in responders (Table 9.10.). This difference was statistically significant (p=0,000).

Table 9.10.

CK-18 neoepitop level after the completion of the therapy

	N	CK-18 U/L ± SE	p
Non-responders	41	332,19 ± 33,56	0,000
Responders	47	184,36 ± 24,33	
Total	88	253,23 ± 21,70	

After the completion of the CHC etiotrop therapy the CK-18 level in all non-responders was above the upper limit of reference range. Among responders the proportion was considerably smaller: the CK-18 level was

increased in 78,7%, but in 21,3% of responders it was within the reference range (p=0,001).

As regards the other marker of apoptosis - Cyt.C, no significant difference was observed between the study groups neither prior to the therapy, nor after the completion of the therapy.

9.6. Patients from the group of non-responders with temporary undetectable HCV after the completion of the therapy

During the study we investigated the group of the patients, for whom the applied therapy had initially proven to be effective, because of undetectable HCV RNA after the completion of the therapy, but within 6 months after completion of the therapy, viremia returned, respectively, a relapse of the infection developed.

There were 31 patients who suffered the relapse. It was essential to evaluate that group of patients and search for differences, because early identification of relapse warning signs would make it possible to do corrections in the course of the treatment.

The patients of the HCV relapse group were compared with responders, as well as with the others, i.e., primary non-responders. The results of comparison of the patients from the group of relapse and responders did not significantly differ from the data of responders and the rest of non-responders.

Mutually comparing the patients with post-treatment HCV relapse and the others, i.e., patients - primary non-responders in whom the inefficacy of the therapy had already been detected on the 12th week of the treatment or upon the completion of the therapy, the only statistically significant difference before the start of the therapy was observed in the ALT activity (Table 9.11.).

Table 9.11.

ALT activity prior to the therapy

	N	ALT U/L ± SE	p
Non-responders	72	116,3 ± 7,75	0,012
Relapse	31	160,7 ± 19,50	

Before the start of the therapy the mean ALT activity of the patients with the post-treatment HCV relapse was 160,74 U/L (SD=108,58), that was significantly (p=0,012) higher than in the group of the rest of non-responders where the mean ALT activity value was 116,31 U/L (SD=65,77).

9.7. Association of HLA class II alleles with the efficacy of the CHC etiotrop 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).

Table 9.12.

Incidence of HLA II-DRB1 gene alleles in CHC patients versus control group

DRB1* alleles	CHC (n=336)	Control group (n=200)	OR	p
*01	48	31	0.91	< 0,701
*15	49	45	**0,59**	**< 0,020**
*03	43	14	**1.95**	**< 0,035**
*04	39	23	1,01	< 0,970
*05	83	33	**1,66**	**< 0,026**
*06	29	28	**0,56**	**< 0,034**
*07	42	4	**7,0**	**< 0,000**
*08	3	14	**0,12**	**< 0,000**
*09	0	2	-	-
*10	0	5	-	-

The frequency of HLA II-DRB1 gene alleles in CHC patients was statistically analyzed (Table 9.12.), 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 occured less frequently.

Table 9.13.

HLA II-DRB1 gene alleles in CHC patients in relation to the type of treatment

DRB1* alleles	*01	*15	*03	*04	*05	*06	*07
Effective combination (PEG INF+RBV) therapy; n=59	0,14	0,14	0,13	**1,97/ p<0,014**	0,21	**4,29/ p<0,003**	0,03
Ineffective combination (PEG INF+RBV) therapy; n=45	0,16	0,16	0,12	0,08	0,62/ 0,01	0,06	0,18/ 0,005
Effective realdiron n=30	**2,58/ 0,071**	0,13	0,13	0,08	0,22	0,07	0,13
Ineffective realdiron n= 34	0,09	0,16	0,13	0,53/ 0,065	0,77	0,74	1,15
Control group n=100	0,16	0,23	0,07	0,12	0,17	0,15	0,02

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 9.13.), 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 9.14.

Frequency of HLA II-DQA1 gene alleles in CHC patients and control group

DQA1* alleles	CHC (n=336)	Control group (n=200)	OR	P
*0101	43	30	0,83	< 0,473
*0102	46	42	**0,60**	**< 0,027**
*0103	33	16	1,25	< 0,479
*0201	56	22	**1,62**	**< 0,072**
*0301	45	27	0.99	< 0,972
*0401	17	8	1,28	< 0,574
*0501	96	48	1,27	< 0,249**
*0601	-	7	-	-

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 9.14.).

Table 9.15.

HLA II-DQA1 gene alleles in CHC patients in relation to the etiotrop therapy

DQA1* alleles	*0101	*0102	*0103	*0201	*0301	*0401
Effective combination (PEG INF+RBV) therapy; n=59	0,14	0,13	0,11	0,14	**2,42/ p<0,05**	0,05
Ineffective combination (PEG INF+RBV) therapy; n=45	0,14	0,14	0,09	0,78/ 0,051	0,08	0,08
Effective realdiron n=30	**1,59/ p<0,04**	0,15	0,08	0,17	0,13	0,02
Ineffective realdiron n= 34	0,09	0,13	0,08	0,19	0,15	0,37/0,38
Control group n=100	0,15	0,21	0,08	0,11	0,13	0,04

The frequency of HLA II-DQA1 gene alleles in the CHC patients in relation to the applied therapy was analyzed (Table 9.15.), and the following correlation 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).

Table 9.16.

Frequency of HLA II-DQB1 gene alleles in CHC patients and control group

DQB1* alleles	CHC (n=336)	Control group (n=200)	OR	p
*0201-2	74	28	**1,74**	**< 0,022**
*0301	76	39	1,21	< 0,395
*0302	21	12	1,04	< 0,907
*0303	10	14	**0,41**	**< 0,029**
*0304	1	3	0.30	<0,292
*0401-2	14	9	0,92	< 0,854
*0501	41	25	0,97	< 0,919
*502-4	28	10	1,73	< 0.146
*0601	15	11	0,80	< 0,589
*0602-8	53	49	**0,61**	**< 0,026**

Among the CHC patients (Table 9.16.) 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.

Table 9.17.

HLA II-DQB1 gene alleles in CHC patients in relation to the etiotrop therapy

DQB1* alleles	*0201-2	*0302	*0303	*0502-4	*0602-8
Efective combination (PEG INF+RBV) therapy; n=59	0,17	0,06	0,029/0,05	0,05	0,19
Ineffective combination (PEG INF+RBV) therapy; n=45	0,23	0,06	-	0,35/ 0,036	0,15
Effective realdiron n=30	0,28	0,02	0,02	**2,82 /p<0,089**	0,06
Ineffective realdiron n= 34	0,23	0,15/0,045	0,06	0,04	0,33/ 0,062

The frequency of HLA II-DQB1 gene alleles in the CHC patients in relation to the applied etiotrop therapy and its efficacy was analyzed (Table 9.17.), 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).

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:

1. 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);
2. 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);
3. 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).

10. DISCUSSION

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 etiotrop 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 possitive outcome of the treatment. The aim of the present study was to find these differences and detect the characteristic facts.

When analyzing the demographic parameters it was already found that patients who had succeeded to clear the HCV during the therapy were younger than the patients which had not achieved SVR. This finding corresponds with the information described in the scientific literature about the correlation between the age of the patient and the efficacy of the therapy, i.e., the patients under the age of 40-45 is more likely to achieve SVR. Taking into account the correlation between the age of the patient and SVR, it is essential to start the antiviral therapy as soon as possible after timely clarification of CHC diagnosis.

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, is one of the most important independent prognostic factors for failure to achieve SVR. The results of our study confirm this statement.

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.

When analyzing the blood count parameters, we observed that non-responders in the group of the patients undergoing the PEG IFN+RBV combination therapy had comparatively higher level of Hb than responders before the start of the therapy, as well as after the completion of it. Those facts may suggest on the patients' adherence. Moreover, the data have been published in 2008 by japanese researchers that a high level of Hb stimulates the achieving of SVR.

According to the literature, there is a correlation between the reduction in Hb level and achieving of SVR in the CHC patients with genotype 1 treated with the PEG IFN+RBV, and these changes could be used as pharmacodynamic markers of the efficacy of the therapy.

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.

The correlation of the GGT level with futher advanced fibrosis and IR is described in the scientific literature. 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.

Among non-responders there were proportionally more patients with the increased cholesterol level before the start of the therapy, although the mean values of the total cholesterol between both groups did not differ.

Due to relatively small number of the investigated patients, the statistical evaluation using Pearson's index was performed and it indicated that the difference was statistically significant. This allows to make a presumption that having elevated total cholesterol level prior to the treatment is associated and predicts worse outcome of the therapy. More literature data is available referring to the association between SVR and the elevated total cholesterol level prior to the CHC therapy. In the patients who have low cholesterol level before the start of the therapy cholesterol increases if patients achieve SVR, while the increase of cholesterol level is not observed in patients who fail to get rid of HCV. The scientific literature provides data associating low cholesterol level with more advanced liver damage and therefore less effective CHC therapy.

The level of glucose in responders was more frequently within the limits of reference range, while 27,3 % of the patients among non-responders had increased level of glucose upon the start of the therapy. This finding only indicates a tendency in glucose level changes, because statistical significance was not detected. The evaluation of the mean values of glucose level showed that in non-responders the mean value of glucose was significantly higher than in responders, but it still was within the limits of reference range. The association between hyperglycaemia and worse probability of achieving SVR is described. This finding, among other things, is associated with IR, which is also considered as a marker predicting worse outcome of the therapy.

In the group of non-responders the γ globulin level was comparatively more frequently elevated, but the mean values of γ globulin were practically the same in both groups. When comparing the γ globulin levels of responders and

patients with post-treatment relapse, it was observed that the γ globulin level was increased in 40 % of the patients with the post-treatment relapse, and this frequency is higher than in responders and the rest of non-responders. This tendency could indicate a relatively more advanced fibrosis in the patients with the increased level of γ globulin that results in less efficacy of the therapy. The determination of γ globulins is included in the non-invasive fibrosis tests kit, e.g., FibroIndex, used for the non-invasive determination of expressed fibrosis in case of CHC.

When evaluating the AFP level before the start of the therapy we found no difference between responders and non-responders and no correlation between the AFP level and the outcome of the treatment. The re-evaluation of the AFP level 6 months after the completion of the therapy showed the unchanged AFP level in responders, but the elevated AFP level in 18,9% of non-responders. The difference was observed in the mean APF values as well: the mean AFP value in responders was within the reference range, but in non-responders it exceeded the upper limit of reference range. This finding could confirm the ongoing progression of the fibrotic process in patients for whom the CHC therapy was proven to be ineffective.

Several reports are available in the scientific literature stating that the elevated AFP level in the CHC patients without HCC indicates more advanced liver fibrosis and more severe progress of the disease. The APF level decreases during the course of the antiviral therapy. Taiwanese researchers have found that the APF value above 6 ng/mL indicates a higher stage of fibrosis. The results of our study confirm this statement.

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 level of HA serves as an indicator or marker of fibrogenesis and therefore it increases with the development of

severe liver fibrosis or cirrosis. 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.

The levels of the markers of apoptosis - CK-18 neoepitop and Cyt.C in blood were measured and the relation between these markers and the efficacy of the applied CHC therapy was evaluated. We found no difference in the levels of both markers between responders and non-responders before the start of the therapy. The evaluation 6 months after completion of the CHC etiotrop therapy showed the difference in the levels of CK-18. The CK-18 level in all non-responders was above the upper limit of reference range. Among responders the CK-18 level was increased in 78,7% of the patients. As regards the mean value of CK-18 the difference was statistically significant: it was observed that in non-responders the CK-18 level in blood was almost two times higher than in responders: 332,19 U/L and 184,36 U/L, respectively. This finding confirms literature data about significant reduction or decreasing of the CK-18 level after the successful CHC etiotrop therapy. This could be considered as an objective characteristic or hallmark of the reduction of apoptotic process. The evaluation of changes in the CK-18 level in dynamics would make it possible to evaluate during the treatment course the efficacy of the applied CHC etiotrop therapy by using the indirect marker, i.e., CK-18.

When evaluating the other marker of apoptosis - Cyt.C, we found no difference between both groups. This fact probably indicates that in the enrolled patients there is some other apoptotic mechanism, not the one induced directly by HCV, because normally Cyt.C is not present in blood and the level

of Cyt.C should normalize in the patients who have got rid of HCV if apoptosis is associated with CHC only.

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. The association of HLA-DRB1*15 and DRB1*1501 with the spontaneous release from HCV has been described.

More detailed analysis and evaluation of the association of HLA-DRB1 alleles with the outcome of the applied CHC etiotrop 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. Perhaps due to this immunogenetic specifity 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 etiotrop 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. 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. 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. 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 assesing the association of HLA-DQA1 gene alleles with the efficacy of the CHC etiotrop 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.

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, 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 frquently found in the patients with liver cirrhosis. While HLA DQB1*0303 allele is usually associated with milder form of HCV infection.

The positive relation or association between the CHC etiotrop 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 previously performed studies by foreign 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.

The analysis of the haplotypes of the patients enrolled in our study identified the following association with the CHC etiotrop 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/DQ A1*0301, DRB1*04/DQB1*0302/DQA1*0501 and DRB1*05/DQB1 *0601 DQA1*/0103.

The results of the present study confirm the hypothesis that the condition of the macroorganism, which is characterized by biochemical, immunogenetic and morphological parameters, plays an essential role in the efficacy of the CHC therapy.

The correlations of these parameters with SVR are statistically significant and considerable for predicting the result of the etiotrop therapy, as well as they are essential before the start of the treatment if a correction of these negatively affecting parameters is possible in order to improve the outcome of the etiotrop therapy.

11. CONCLUSIONS

1. The biochemical, morphological and immunogenetic status of the CHC patient plays an essential role in the efficacy of the etiotrop therapy.
2. The inefficacy of the CHC etiotrop therapy is associated with older age of the patient, affiliation to male gender, presence of fibrotic changes in liver tissues, increased GGT level upon the start of the therapy, increased glucose level upon the start of the therapy, increased γ globulin level upon the start of the therapy, increased cholesterol level upon the start of the therapy, elevated level of iron upon the start of the therapy, increased level of HA upon the start of the therapy, as well as increased level of Hb at the beginning and at the end of the therapy.
3. The elevated ALT acivity (4 times higher than the reference range) before the therapy might be associated with the risk of the relapse.
4. The clearance of HCV is associated with the decreasing of the CK-18 level.
5. In the patients who have failed to clear the HCV the elevation of AFP level is found during the further observation period and it confirms an ongoing progression of the pathologic process.
6. In Latvia the most frequently found in the CHC patients are MHC HLA II class gene alleles 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 *0 102, DRB1*07/DQB1*0201-2/DQA1*0401.
7. 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.

8. 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.

12. PRACTICAL RECOMMENDATIONS

1. When doing the examination of the patient before the start of the CHC etiotrop therapy, in addition to the currently evaluable parameters include in the examination algorithm the evaluation of the levels of GGT, glucose, cholesterol and HA.
2. To recommend before the start of the therapy to adjust or correct potentially amenable to change markers like glucose and lipid profile by means of diet and medication.
3. During the further observation period to evaluate AFP and CK-18 in the patients who have failed to clear the HCV in order to follow the further progression of the pathologic process.

13. APPROBATION OF THE PHD THESIS

The approbation of the Doctoral Thesis „CHRONIC HEPATITIS C: BIOCHEMICAL DIAGNOSTIC MARKERS FOR PREDICTING EFFICACY OF ETIOTROP THERAPY" took place on April 5, 2011 at the session of the Department of Infectology and Dermatology of RSU with the participation of the members of the Infectologists' Association of Latvia and representatives of the Department of Paediatrics of RSU.

14. SCIENTIFIC PUBLICATIONS

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10. L.Vīksna, V.Sondore, A.Jēruma, J.Keišs, A.Sočņevs, B.Rozentāle. Asins transmisīvās vīrusinfekcijas Latvijā: jauni priekšstati par šo slimību attīstību, jaunas diagnostikas un ārstēšanas stratēģijas. Valsts pētījuma programmas noslēguma izdevumam – monogrāfijai. Latvijas iedzīvotāju dzīvildzi un dzīves kvalitāti apdraudošas slimības zinātniskā analīze un galvenās rekomendācijas. Autoru kolektīvs Valda Pīrāga redakcijā, 2009:133.-142.
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15. PRESENTATIONS OF THE STUDY RESULTS

1. Oral presentation - Treatment of chronic hepatitis C: comparative study of the different interferons. 4th Nordic-Baltic Congress on Infectious Diseases, Tallin, Estonia, May 19, 2000.
2. Oral presentation - Hronisks C hepatīts narkotiku lietošanas gadījumos: klīniski morfoloģisks pētījums. RSU Scientific conference in Medicine, Riga, Latvia, March 5, 2004.
3. Oral presentation - Chronic hepatitis C in Latvia: facts and problems. The 1st International Digestive Diseases Workshop of Taiwan and the Baltis States, Riga, Latvia, May 31, 2004.
4. Oral presentation - Treatment of chronic viral hepatitis C in Latvia. The 1st International Digestive Diseases Workshop of Taiwan and the Baltis States, Riga, Latvia, May 31, 2004.
5. Oral presentation - Hepatīta C vīrusa (HCV) infekcija Latvijā: klīniskais, bioķīmiskais, morfoloģiskais, imunoloģiskais raksturojums, mūsdienu terapijas principi: preparātu pielietojums un efektivitāte. Meeting of the Infectologists' Association of Latvia, Riga, Latvia, June 27, 2002.
6. Oral presentation - Hronisks vīrushepatīts C: etiotropās terapijas pieredze v/a "Latvijas Infektoloģijas centrs". Meeting of the Infectologists' Association of Latvia, Riga, Latvia, December 18, 2008.
7. Oral presentation – Hronisks vīrushepatīts C: jauni priekšstati, jaunas diagnostikas un ārstēšanas stratēģijas. Meeting of the Infectologists' Association of Latvia, Riga, Latvia, November 26, 2009.