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STUDY OF FACTORS INFLUENCING CHRONIC VIRAL HEPATITIS C TREATMENT RESULTS

Summary of Doctoral Thesis to obtain PhD degree in medicine

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Promotion thesis has been developed at Riga Eastern University Hospital, hospital „Infectology Centre of Latvia".

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Great thanks!

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1. ABBREVIATIONS

a – regression constant
ALAT – alaninaminotransferase
ANA – antinuclear antibodies
ANOVA – ANalysis Of VAriance
Anti-HCV – antibodies against hepatitis C virus
b – regression coefficient
BMI – bone mass index
CC – interleukin 28B gene CC genotype
CI – confidence interval
CT – interleukin 28B gene CT genotype
e – Euler's number = 2.71828
ELISA – enzyme linked immunosorbent assay
Exp (B) – coefficient e^B or OR – odds ratio
F – Fisher's exact test
GGT – gamma glutamyltranspeptidase
gt – hepatitis C virus genotype
HAI – histological activity index
HBs antigen – superficial antigen of hepatitis B virus
χ^2 test – Chi-squared test
HCC – hepatocellular carcinoma
HCV – hepatitis C virus
HCV-RNA – hepatitis C virus ribonuclein acid
HIV – human immunodeficiency virus
HOMA-IR – homeostasis model assessment of insulin resistance
IFN – alpha interferon
LIC – Riga East University Hospital, hospital „ Infectology center of Latvia"
LR – Republic of Latvia
N or n – number
NABs – neutralizing antibodies to human interferon alpha

Non-CC – interleukin 28B gene CT, TC and TT genotypes
p – probability, p-value
PEG IFN – pegylated interferon alpha
PCR – polymerase chain reaction
RBV - ribavirin
r_s – Spearman's correlation coefficient
R^2 – determination coefficient
SD – standard deviation
SE, S.E – standard error of mean
Sig. – significance
SVR – sustained viral response
SPSS – Statistical Package of the Social Science
TC – interleukin 28B gene TC genotype
TSH – thyroid stimulating hormone
TT – interleukin 28B gene TT genotype
ULN – upper limit of normal
VHC – viral hepatitis C
VHB – viral hepatitis B
z – equation of regression
Wald – Wald's criterion
x – values of independent variables

2. TOPICALITY OF RESEARCH

Due to its distribution and clinical course, chronic viral hepatitis C has become one of the most common infectious diseases in the world. At present the number of the infected in the world is about 170 million, but in Europe it exceeds 9 million. The incidence of chronic viral hepatitis C in Latvia is relatively high. The antibody prevalence is 2.4%, HCV-RNA prevalence is 1.7%, it means that in Latvia there might be almost 40 000 chronic hepatitis C patients.

Hepatitis C viral infection in population has been found quite recently. The virus was discovered only in 1989 when its genome was identified. An opinion prevails that patients' getting infected started faster at the beginning of the 90ties of the past century (donors' blood was not tested) and is still going on due to the lack of vaccine. Chronic viral hepatitis C itself cannot essentially affect the patients' quality of life, however, about 20% of patients are known to develop liver cirrhosis within 10-20 years. Besides, it is impossible to say how long the patient has been infected, as well as whose disease is going to progress to develop cirrhosis and hepatocellular carcinoma (HCC). Since about 20 years have passed from the first diagnosed wave of infection, liver cirrhosis and hepatocellular carcinoma rate at present and in the nearest years is going to grow in the whole world.

When undergoing treatment, 54-63% patients can get rid of hepatitis C virus. Various factors are known to determine and affect the outcome of treatment. First of all, these are the patient's own factors and co-morbidities – age, sex, race, genetic factors, obesity, insulin resistance, diabetes mellitus, HIV infection, smoking, alcohol consumption, each individual's body reaction by developing neutralizing antibodies against alpha interferon, consequently, reducing its effectiveness, secondly, viral factors – genotype, viral load, thirdly, morphological changes before the therapy – fatty liver, degree of fibrosis, activity of inflammation, cirrhosis.

It is, therefore, important to find any factor influencing the treatment result and to correct it, as far as possible, prior to starting the therapy, in order to achieve maximum good therapeutic result.

3. NOVELTY OF RESEARCH

During the study there were found the factors influencing the result of chronic VHC treatment. On the basis of these factors, a treatment prognosis model for chronic viral hepatitis C was developed. The information obtained in the study can be used as the foundation for making important decisions when treating chronic VHC patients to improve the therapy result.

4. AIM OF RESEARCH

The aim of the study was to determine and analyze the factors influencing chronic viral hepatitis C treatment results in order to predict the possibility of SVR.

5. OBJECTIVES OF RESEARCH

1. To analyze the patient's factors (age, weight, BMI, smoking, alcohol consumption, co-morbidities, genetic factors) and the changes of analyses (insulin resistance, cholesterol level, neutralizing antibodies) in connection with the therapeutic effect.
2. To assess viral factors (genotype, viral load) as the indices affecting the treatment result.
3. To analyze morphological changes prior to undertaking the treatment.
4. To determine IL28B gene polymorphism in Latvia and its relation to the treatment result.
5. To develop the treatment prognosis model of chronic viral hepatitis C and to prepare recommendations for the practicing physicians in order to improve the treatment results of chronic VHC.

6. HYPOTHESES OF RESEARCH

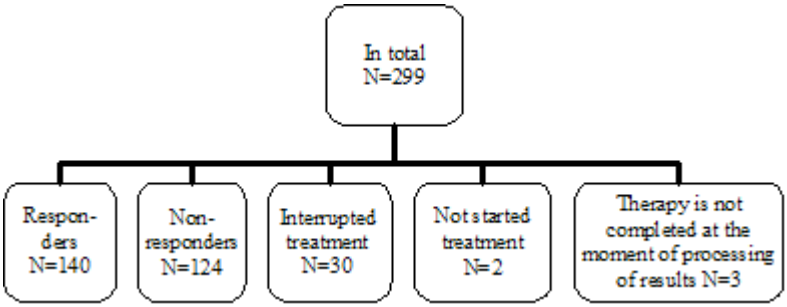
1. The patient's factors (age, BMI, IL28B genotype), liver functions, changes in metabolic and immunologic indices (GGT, insulin resistance, formation of neutralizing antibodies), morphological changes (degree of fibrosis, HAI), virus genotype and load affect chronic viral hepatitis C treatment result in Latvia in the same way as in other countries.
2. Finding out the factors influencing chronic VHC treatment result, one could predict the possibility of treatment, developing the treatment prognosis model.

7. MATERIAL AND METHODS

7.1. Study groups

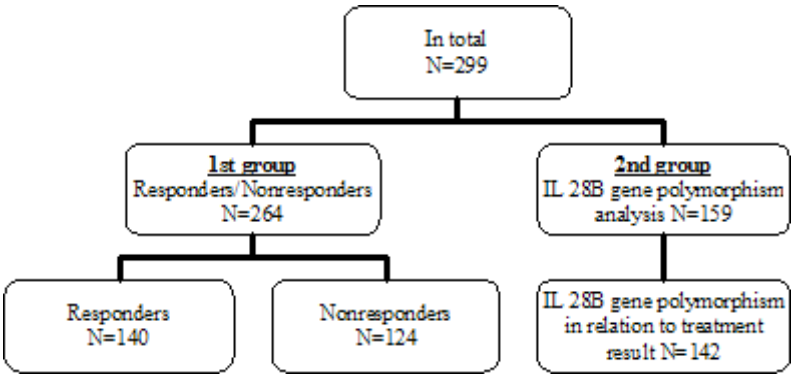
299 chronic viral hepatitis C patients were included in the study, who had attended LIC out-patient department in the period from 2009 till 2011. The diagnosis of chronic VHC was confirmed performing HCV-RNA test by PCR method. All patients were white race individuals, their average age was 38 years, males – 165 (55%), females – 134 (45%). Previously hepatitis C treatment was received by 34 patients (11.4%) being treated by various medicines – recombinant alpha IFN in monotherapy or in combination with RBV, or pegilated IFN in combination with RBV. Within this study 223 (74%) patients received pegilated interferon alpha 2a 180 μg/week, 71 (23.7%) patients received pegilated interferon alpha 2b 1.5 μg/kg/week in combination with ribavirin 800 – 1200 mg/day, 2 patients received interferon alpha 2a 180 μg/week in monotherapy, 1 patient received multiferon in combination with ribavirin, 2 patients did not undertake hepatitis C therapy.

From all 299 patients 140 (46.8%) responded to hepatitis C therapy, 124 (41.5%) – did not respond, 30 (10%) interrupted the therapy (24 – arbitrary, 3 – due to side effects, 3 – due to financial conditions), 2 – did not start therapy, 3 – have not yet accomplished therapy at the moment of processing of the results (Picture 7.1.).



Picture 7.1. Distribution of patients under study

In order to make the analysis of the factors influencing results of chronic VHC treatment, 2 patients' groups under study were organized (Picture 7.2.).



Picture 7.2. Patients' distribution into study groups

1st group – in order to analyze the factors influencing the treatment results, from all 299 patients under study there were selected 264 with chronic VHC who:

- ✓ Have received standard treatment with pegilated interferon and ribavirin,
- ✓ Have not interrupted the therapy arbitrarily,
- ✓ Have completed the treatment course and have been followed up untill 24 weeks after the end of treatment in order to assess the treatment result.

From all patients of this group 140 (53%) were males and 124 (47%) were females. There prevaled HCV 1st genotype – in 185 patients (70%), 2nd or 3rd genotype was seen in 79 (30%) patients. The mean age was 38 years. From the moment of making the diagnosis till starting the treatment there have passed on average 2.2 years (0.1 – 13.5). All these group patients were divided into subgroups depending on the treatment result:

1. Responders – patients, who have responded to therapy (N=140, 53%) – have reached SVR – HCV-RNA – negative at the end of therapy and 24 weeks after completing of therapy,
2. Nonresponders – (N=124, 47%) – have not responded to therapy, have not achieved SVR:

- ✓ Null response (N=51) – in 1st genotype patients there is not achieved a hunderedfold HCV-RNA viral load reduction within 12 treatment weeks, the treatment is interrupted, considering that it is going to be ineffective,
- ✓ Partial response (N=34) – is at least a hundredfold HCV-RNA viral load reduction within 12 treatment weeks, but at the end of treatment HCV-RNA – positive.
- ✓ Relapse (N=39) – after the treatment HCV-RNA is negative, but 24 weeks after completion of the treatment – positive.

Patient distribution is seen in Table 7.1.

Table 7.1.

Patient distribution in the 1st study group

Responders		**Nonresponders**					
140 (53%)		124 (47%)					
		Null response		Partial response		Relapse	
		51 (41,1%)		34 (27,4%)		39 (31,5%)	
1st gt	2nd, 3rd gt	1st gt	2nd, 3rd gt	1st gt	2nd, 3rd gt	1st gt	2nd, 3rd gt
75 (40.5%)	65 (82.3%)	51 (27.6%)	0	28 (15.1%)	6 (7.6%)	31 (16.8%)	8 (10.1%)

In the first group patients, in total 30 various factors, influencing the treatment, are identified and analyzed:

1. The patient's factors (age, sex, the time from diagnosing hepatitis C till starting the therapy, co-morbidities, abdominal circumference, weight, BMI, blood count, ALAT, GGT, cholesterol, triglycerides, TSH, glucose level, insulin, insulin resistance, ANA, formation of neutralizing antibodies against alpha interferon, interleukin 28B gene polymorphism, a patient's compliance to therapy, harmful habits – smoking, alcohol consumption).
2. Viral factors (genotype, viral load).
3. Morphological changes (fibrosis stage, activity of inflammation).

2nd group – in order to determine IL 28B gene polymorphism in Latvia, there were selected 159 chronic VHC patients from total 299 under study, who:

- ✓ Were the first from the queue included into the study. All second group patients were tested for interleukin 28B gene polymorphism in the 19th chromosome rs12979860 locus.
- ✓ 142 patients from this group, who had finished hepatitis C treatment, were determined and analyzed IL 28B polymorphism in relation to the therapy result.

Patient characteristic is seen in Table 7.2.

Table 7.2.

Second group patients' characteristics

Factors	**CC**	**Non-CC**	**All patients**
Mean age, years	35	37	37 (18 – 68)
Number of patients, >40 years	15 (33%)	40 (42%)	54 (39%)
Males	27 (59%)	57 (59%)	84 (59%)
BMI, kg/m^2	25.2	26.2	25.9
BMI, >30kg/m^2	5 (11%)	12 (13%)	17 (12%)
1st genotype	21 (46%)	66 (69%)	87 (61%)
2nd ., 3rd genotype	25 (54%)	30 (31%)	55 (39%)
HCV-RNA, x10^6 IU/ml (1st genotype)	2.78	2.19	2.33
HCV-RNA, >600,000 IU/ml (1st genotype)	18 (39%)	47 (49%)	65 (46%)
ALAT, U/l	112 (17 – 325)	104 (22 – 447)	106 (17 – 447)
ALAT, >ULN	43 (93%)	85 (88%)	128 (90%)
GGT, U/l	46.8 (9 – 228)	88 (6 – 526)	75 (6-526)
GGT, >ULN	11 (24%)	40 (42%)	51 (36%)
Cholesterol, mM/l	4.14 (2.09 – 7.35)	4.6 (2.46 – 8.17)	4.48 (2.09 – 8.17)
Triglycerides, mM/l	1.17 (0.28 – 4.06)	1.17 (0.3 – 5.44)	1.11 (0.28 – 5.44)
Liver fibrosis* (*Knodell*) F0 F1 F3 F4	0.975 (0 – 3) 9 (21.9%) 28 (68.3%) 4 (9.7%) 0	1.2 (0 – 4) 21 (24.7%) 47 (55.3%) 12 (14%) 5 (5.9%)	1.13 (0 – 4) 30 (23.8%) 75 (59.5%) 16 (12.7%) 5 (3.9%)
HAI index* (*Knodell*)	6.44 (1 – 12)	6.54 (2 – 13)	6.5 (1 – 13)
Steatosis* > 0 degree	34 (83%)	65 (76.5%)	99 (78.6%)
SVR	34 (74%)	50 (52%)	84 (59%)

* Missing data: histological examination n=11 (non-CC), n=5 (CC).

7.2. Material and methods

The research was confirmed by The Independent Ethics Committee for clinical investigation of drugs and pharmaceutical products performing its functions according to GCP and applicable regulatory requirements. The work was done in accordance with the international and LR laws and Helsinki declaration. Before undertaking the study, each patient got acquainted with the "Information to the patient" and gave a written consent for the participation in the study by signing "Statement of consent" and "Consent for data registration".

Most part of laboratory tests was done at LIC laboratory. Single tests were done at the laboratory of P.Stradins clinical university hospital (ANA), Genera genetics centre, Ltd. (IL 28B gene polymorphism) and P.Stradins clinical university hospital Department of Pathology (morphological examination of the liver tissue).

7.2.1. Chronic VHC diagnostics

In blood samples the following seromarkers were determined: anti-HCV and HCV-RNA, including HCV genotyping and identification of virus load (HCV in case of the 1st genotype).

For detection of anti-HCV in serum, ELISA tests by various companies were used (AxSYM system HCV version 3.0, Abbott, ASV; ORTHO HCV version 3.0, Ortho-Clinical Diagnostics Ltd., ASV; INNOTEST HCV Ab IV, Innogenetic, Belgium; MONOLISA Anti-HCV PLUS version 2, BIO-RAD, France).

For qualitative HCV-RNA detection in serum there were used commercially available reverse transcription polymerase chain reactions (PCR) method: Cobas AMPLICOR Hepatitis C Virus Test, v. 2.0, Roche Diagnostics, USA (sensitivity: >50 SU/ml, specificity: 100%).

HCV genotypes were determined using reverse hybridisation LiPA method (The VERSANT HCV Genotype Amplification Kit (LiPA), Bayer Corporation, Germany).

For quantitative HCV-RNA virus load detection there was used polymerase chain reaction: Cobas AmpliPrep/ Cobas TaqMan HCV test Roche, USA.

7.2.2. IL28B gene polymorphism detection

For testing IL28B gene rs12979860 polymorphism the standard molecular- biological methods were used in blood samples: classical DNA release from blood with phenolum, for amplification of polymerase chain reaction fragments, standard sequencing with Big Dye (Applied Biosystems). Genotypes were divided into CC, CT, TC, TT.

7.2.3. Detection of neutralizing antibodies against alpha interferon

For neutralizing antibody detection against alpha interferon there was used *iLite*^{TM} *antialpha assay* (BIOMONITOR, Ireland), in order to determine NABs in serum against human alpha interferon semiquantitatively, using lucipherase bioluminescence system.

7.2.4. Morphological examination

Morphological examination of the liver tissues was done at P.Stradins clinical university hospital Department of Pathology. For detection of inflammation activity and degree of fibrosis, Knodell's histological activity index was used.

7.2.5. Statistical analysis methods of results

The data statistical processing was done using the computer programmes SPSS v.15.0, MedCalc v12.0 and Microsoft Office Excel v.11. For the characteristics of patients' parameters the generally accepted descriptive statistical methods were used – summary tables with columns, bar graphs or histograms; indicators of central tendency and dispersion indicators – standard deviation (SD) and standard error (SE).

The meaning of parameter differences is estimated by 5% probability of statistical error, thus, if in the test results p-value was lesser or equal to 0.05, the differences between the study groups were recognized as statistically significant.

For the assessment of differences several statistical tests were used – if proportional data were conformed to the normal (Gaussian) distribution, for the quantitative difference analysis between several groups there was used the *analysis of variance (ANOVA)*, between two groups – *Student's t-test*. If the data were not conformed to the normal distribution, there was extra used a nonparametric *Mann-Whitney U test* for the comparison of two samples, or *Kruskal-Wallis H test* for the comparison or three and more samples. Conformity of proportional data to the normal distribution was determined by using *Kolmogorov-Smirnov test*.

Comparing the groups according to a certain qualitative parameter, Pearson's chi-squared (χ^2) or Fisher's exact criterion 2x2 tables were used. Considering χ^2 values and the number of freedom degrees (df), p value was stated.

In calculations *odds ratio* (OR) was used. It is the ratio of probability of favorable outcome to probability of unfavorable outcome. If OR>1, then probability of favorable outcome is greater than probability of unfavorable outcome, if 0<OR<1, then probability of unfavorable outcome is greater than probability of favorable outcome. Odds ratio was calculated, using the computer program MedCalc ver. 12.0 by formula (A x D) / (B x C), where:

A – patient rate from the study group (without effect) with specific exposure;

B – patient rate from the control group (with effect) with specific exposure;

C – patient rate from the study group (without effect) without specific exposure;

D – patient rate from the control group (with effect) without specific exposure.

In case any of values A, B, C, or D were zero, odds ratio was estimated according to a modified formula which is meant for small groups of numbers – [(2A + l) x (2D + l)] / [(2B + l) x (2C + l)]. Statistical significance was determined by Fisher's criterion. 95% confidence interval (95% CI) was calculated by the formula: 95% CI = In OR ± l.96.

To determine the correlation between variables, the correlation analysis was used. The calculation method of the correlation depended on the variable scale. If variables were calculated by the linear scale, then *Pearson's correlation coefficient* was used. If one of the variables has the ordinals scale, then nonparametric *Spearman's range correlation coefficient* was used.

In the current study the following interpretation of the correlation coefficient was used:

0 = neither correlation exists;

0 – 0.2 = very low correlation;

0.2 – 0.5 = low correlation;

0.5 – 0.7 = moderate correlation;

0.7 – 0.9 = high correlation;

0.9 – 1.0 = very high correlation.

In order to find out the possible impact of independent factors on the effectiveness of therapy, *binary logistic regression* was used.

7.2.6. Binary logistic regression

Since the dependent variable of the study *Therapy result* is binary, the *binary logistic regression* was used. Contrary to the ordinary linear regression, where by means of equation one can predict the outcome of the dependent variable, the aim of binary logistic regressions is to state the probability of the event, in this case – whether a patient, due to the effect of some factors, will occur in one, or in another group, whether he/she will not respond to therapy (0), or respond (1). Probability is ranging from 0 till 1, where the border is 0.5, if probability is <0.5, then the prognosis is good for the 1st group, if ≥ 0.5, then it is good for the second group. Probability was calculated by the equation: $p=1/(1+e^{-z})$, where

$z = b_1x_1 + b_2x_2 + ... + b_nx_n + a$ (or the regression equation, x – values of independent variables, b – regression coefficients, a – regression constant).

e – mathematical constant (Euler's number) = 2.71828 (1828).

To acquire more precise results between the included independent variables there should not be any interrelations, therefore an extra correlation analysis for stating the correlation was performed.

Regression can have several methods of equation formations – *Enter*, *Forward* and *Backward*. All three were used. In the method *Enter* all independent variables are included in the equation, despite their impact strength on the dependent ones, thus the model is made by one step.

Methods:

✓ *Enter*

Regression model accepts only those patients where in the dependent variables there is no single missing variable. If there is the lack of at least one value, then the patient is excluded from the analysis.

R^2 value is calculated by two methods – Cox & Snell, which is used rarer, and Nagelkerke R^2, which is used more often. These indices show the influence of independent variables of the developed model on the dispersion of the dependent variable.

✓ *Backward Stepwise*

Using this method, by each step the one – the weakest independent variable is removed from the equation, while only the most significant independent variables remain in the equation.

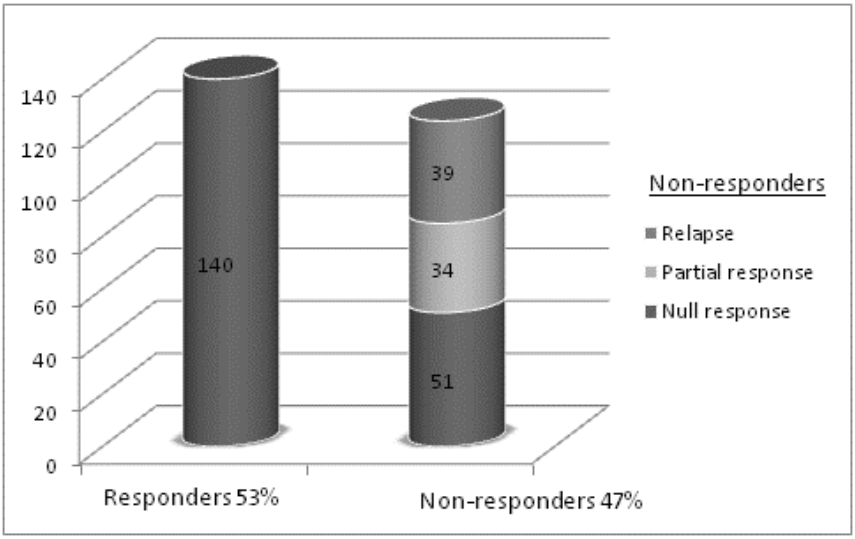
✓ *Forward*

According to this method the indices of precision of this model were lower, therefore it was not further analyzed.

8. RESULTS

Within the study 2 patient groups were analyzed.

1st group. To state the factors which affect the result of therapy, all treated patients were divided depending on the outcome of therapy – responders and nonresponders. The patient distribution is seen in Picture 8.1.



Picture 8.1. Patient distribution – responders/ nonresponders.

From all the included and treated chronic hepatitis C patients in the study –responders are 140 (53%), nonresponders – 124 (47%), who, as a result of therapy outcome are divided as follows: 51 patients were observed to have a null response – there is not achieved a hundredfold viral load reduction during 12 weeks, 34 patients were observed to have only a partial response – achieved at least a hundredfold HCV-RNA viral load reduction during 12 treatment weeks, but at the end of treatment HCV-RNA – positive, 39 patients – relapse – after treatment HCV-RNA negative, but 24 weeks after the end of treatment – positive.

In both patient groups 30 factors influencing treatment result were analyzed. Statistically significant differences between responders and nonresponders patient groups were seen in 11 parameters (factors), see Table 8.1.

Table 8.1.

Survey of results

Nr.	Parameters	Responders n=140, 53%	Nonresponders N=124, 47%	*p*
1.	Age, <45 years, %	58.6	41.4	0.005
	Age, ≥46 years	38.9	61.1	
2.	Body weight, kg	76.78	83.54	0.005
3.	BMI, kg/m^2	25.09	27.25	0.002
4.	GGT, U/l	36.0	63.5	0.000
5.	Insulin, μIU/ml	8.3	11.0	0.026
6.	IR, HOMA	1.78	2.51	0.031
7.	Fibrosis stage, *Knodell*	1.0	1.56	0.000
8.	HAI, *Knodell*	6.38	7.11	0.038
9.	Hemosiderosis, %	1.6	9.7	0.008
10.	HCV 1st genotype, %	40.5	59.5	0.000
	HCV 2nd, 3rd genotype, %	82.3	17.7	
11.	IL 28B CC genotype, %	89.7	10.3	0.001
	IL 28B non-CC genotype, %	59.8	40.2	

2nd group. Lately interleukin 28B coding gene polymorphism has been considered a significant influential factor on the treatment result, therefore patients of this group were determined IL 28B gene polymorphism and its effect on the therapeutic outcome.

Further on the most important factors in the results are analyzed separately.

8.1. Analysis of patient's factors

8.1.1. Age

All patients were divided into 2 groups according to the age – under 45 years and over 46 years. Further on there are summarized and analyzed the

differences in different age groups in relation to the therapy outcome. The data on 263 patients were analyzed whose age was mentioned, 1 patient's age was not known at the moment of the data processing.

In the group under 45 years the patients responded to therapy more often (n=112, 58.6%), but in the group over 46 years – rarer (n=28, 38.9%), *p=0.005, Fisher's exact test* (Table 8.2.).

Table 8.2.

Differences by age groups

Age on starting therapy (< 45 >)		**Therapy result**		**Total**
		Non-responders	**Responders**	
0-45	N	79	112	191
	% Age on starting therapy	**41.4%**	**58.6%**	100.0%
	% Therapy result	**64.2%**	**80.0%**	72.6%
> 46	N	44	28	72
	% Age on starting therapy	**61.1%**	**38.9%**	100.0%
	% Therapy result	**35.8%**	**20.0%**	27.4%
Total	N	123	140	263
	% Age on starting therapy	46.8%	53.2%	100.0%
	% Therapy result	100.0%	100.0%	100.0%

8.1.2. Body weight

From all 264 patients included in the study, 181 patients (68.5%) were measured the body weight (the weight for 83 patients was not measured). The mean weight for analyzed patients was 78.87 (±15.21) kg. The mean weight differences in responders and nonresponders groups were analyzed.

Table 8.3.

The mean weight in responders/ nonresponders groups

Therapy result	**Mean**	**Me-dian**	**Standard error**	**Standard deviation**	**Mini-mum**	**Maxi-mum**	**N**
Non-responders	**83.54**	84.50	2.221	16.624	52	140	56
Responders	**76.78**	76.00	1.261	14.103	49	112	125
Total	78.87	78.00	1.130	15.208	49	140	181

In the group of responders there was diagnosed a lower body weight, on average 76.8 (±14.1) kg, in comparison to a nonresponders group, where it was on average 83.5 (±16.6) kg, the difference is statistically significant, *p= 0.005, Student's t-test* (Table 8.3.)

8.1.3. Body mass index (BMI)

From all 1st group patients, BMI was measured in 176 (67%) patients within the study. The mean BMI for the included patients was 25.75 (±4.36), which corresponds to the normal body weight. However, there are analyzed BMI differences between responders and nonresponders groups.

Table 8.4.

Differences of BMI in responders and nonresponders groups

Therapy result	Mean	Median	Standard error	Standard deviation	Mini-mum	Maxi-mum	N
Non-responders	**27.24654**	27.02641	.618274	4.543368	17.577	43.210	54
Responders	**25.09225**	24.18198	.374018	4.131162	17.998	38.104	122
Total	25.75322	25.07011	.328950	4.364011	17.577	43.210	176

In responders group there was observed a lower BMI, on average 25.09 (±4.13) kg/m^2 in comparison to nonresponders group where it is on average 27.25 (±4.54) kg/m^2, and the difference is statistically significant, *p=0.002, ANOVA* (Table 8.4.).

8.1.4. Gamma glutamiltranspeptidase

From all 264 patients included, 211 patients (80%) were determined GGT activity within the study. 110 patients (52.1%) within the study were found to have normal GGT activity, 101 patients (47.9%) – it was increased. Further on GGT activity data are analyzed in responders and nonresponders groups and the differences stated between groups.

During the study the differences were found in patients with normal or increased GGT activity. In the group with normal GGT activity 77 (70%)

patients responded to therapy, while in the group of increased GGT activity – 36 (35.6%) patients, *p=0.000, Fisher's exact test* (Table 8.5.).

Table 8.5.

GGT differences in responders and nonresponders groups

GGT		Therapy result		Total
		Nonresponders	Responders	
Within normal range	n	33	77	110
	% GGT	**30.0%**	**70.0%**	100.0%
	% Therapy result	**33.7%**	**68.1%**	52.1%
Above the norm	N	65	36	101
	% GGT	**64.4%**	**35.6%**	100.0%
	% Therapy result	**66.3%**	**31.9%**	47.9%
Total	n	98	113	211
	% GGT	46.4%	53.6%	100.0%
	% Therapy result	100.0%	100.0%	100.0%

✓ GGT linear data

Table 8.6.

Mean GGT differences in responders and nonresponders groups

Therapy result	Mean	Median	Standard error	Standard deviation	Mini-mum	Maxi-mum	N
Nonresponders	113.4184	**63.5000**	12.18232	120.59884	6.00	797.00	98
Responders	55.2389	**36.0000**	6.09517	64.79256	8.00	526.00	113
Total	82.2607	51.0000	6.81610	99.00959	6.00	797.00	211

Statistically significant difference is observed also when analyzing the linear data. In the nonresponders group the mean GGT activity was higher (Me = 63.5 U/l) in comparison to the responders group (Me = 36.0 U/l), *p=0.000, Mann-Whitney U test* (Table 8.6.).

In the nonresponders group there was observed statistically significant difference between patients with a null response, partial response and relapse. At GGT activities within the normal range less patients (n=9, 27.3%) had null response and more – (n=17, 51.5%) relapse, in comparison to the patients' group in which GGT activity was higher. In this group a null response was found in 31 patients (47.7%), relapse – 16 patients (24.6%), *p=0.026, Pearson's chi-squared test* (Table 8.7.).

Table 8.7.

Differences in GGT activities in nonresponders group

GGT		Therapy result			Total
		Null response	Partial response	Relapse	
Within the normal range	N	9	7	17	33
	% GGT	**27.3%**	**21.2%**	**51.5%**	100.0%
	% Therapy result	**22.5%**	**28.0%**	**51.5%**	33.7%
Above the norm	N	31	18	16	65
	% GGT	**47.7%**	**27.7%**	**24.6%**	100.0%
	% Therapy result	**77.5%**	**72.0%**	**48.5%**	66.3%
Total	N	40	25	33	98
	% GGT	40.8%	25.5%	33.7%	100.0%
	% Therapy result	100.0%	100.0%	100.0%	100.0%

A similar tendency was observed, inspecting the patients' groups with various therapy response: in a null response group the patients were found to have a higher GGT activity more often (n=31, 77.5%) and rarer – normal GGT activity (n=9, 22.5%), in comparison to the relapse group, where an opposite tendency was seen – more often a normal GGT activity (n=17, 51.5%) and rarer – increased (n=16, 48.5%), *p=0.026, Pearson's chi-squared test* (Table 8.7.).

8.1.5. Insulin

From all 264 patients included, 191 patients (72.3%) within the study prior to starting the therapy were tested for the insulin level in blood. The mean insulin level in the patients under study was 9.5μIU/ml (median), which is within the normal range. Further on the data are calculated separately for responders and nonresponders groups in order to find the differences.

Table 8.8.

Mean insulin level in responders and nonresponders groups

Therapy result	Mean	Median	Standard error	Standard deviation	Mini-mum	Maxi-mum	N
Nonresponders	17.9200	**11.0000**	2.90048	21.51050	3.20	130.10	55
Responders	13.0404	**8.3000**	1.35731	15.82881	2.40	132.70	136
Total	14.4455	9.5000	1.28307	17.73233	2.40	132.70	191

Analyzing the insulin level in both patients' groups, a statistically significant difference was found. The mean insulin level in nonresponders group was higher – Me=11.0 μIU/ml, in comparison to the mean insulin level in the responders group Me=8.3 μIU/ml, *p=0.026, Mann-Whitney U test* (Table 8.8.).

8.1.6. Insulin resistance (HOMA)

Median insulin resistance parameter in patients included in the study was 2.05, which is within the normal range. The data are separately analyzed in the responders and nonresponders groups.

Table 8.9.

Insulin resistance parameter in responders and nonresponders groups

Therapy result	Mean	Median	Standard error	Standard deviation	Mini-mum	Maxi-mum	N
Nonresponders	7.0915	**2.5100**	2.39138	17.73498	0.66	107.20	55
Responders	3.5691	**1.7800**	0.52753	6.15200	0.50	50.20	136
Total	4.5834	2.0500	0.78880	10.90144	0.50	107.20	191

The differences between the groups were observed comparing the mean insulin resistance parameters. In the nonresponders group IR HOMA was 2.51, but in the responders group – 1.78, *p=0.031, Mann-Whitney U test* (Table 8.9.).

Within the study 3 patients had diabetes mellitus as a co-morbidity, from them 1 – responded to therapy, 2 – did not respond.

8.1.7. Hemosiderin

From all 1st group patients 237 (89.7%) within the study were done morphological examination and hemosiderin's presence was tested in the liver tissues. All in all, 13 patients were found to have hemosiderin during morphological liver tissue examination. Hemosiderin's presence is analyzed separately in responders and nonresponders groups.

Table 8.10.

Differences of hemosiderin presence in responders/ nonresponders groups

Hemosiderin (qualitatively, is diagnosed/ not diagnosed)		Therapy result		In total
		Nonresponders	Responders	
Is diagnosed	N	11	2	13
	% HCV genotype	**84.6%**	**15.4%**	100.0%
	% Therapy result	**9.7%**	**1.6%**	5.5%
Not diagnosed	N	102	122	224
	% HCV genotype	**45.5%**	**54.5%**	100.0%
	% Therapy result	**90.3%**	**98.4%**	94.5%
In total	N	113	124	237
	% HCV genotype	47.7%	52.3%	100.0%
	% Therapy result	100.0%	100.0%	100.0%

Analyzing hemosiderin's presence in the liver tissues, the difference was observed in relation to the therapy effect. In the patients' group where hemosiderin was diagnosed in the liver tissues, proportionally a greater number of patients did not respond to therapy – 11 patients (84.6%), in comparison to the group where hemosiderin was not diagnosed – 102 patients (45,5%) did not respond to therapy. Besides, in the nonresponders group more often – 11 patients (9.7% cases) were diagnosed hemosiderin in the liver tissues in comparison to the responders group were it was observed only in 2 patients (1.6% cases), *p=0.008, Fisher's exact test* (Table 8.10.).

8.1.8. Formation of neutralizing antibodies against alpha interferon

From all 1st group patients included, 121 were determined neutralizing antibodies against alpha interferon. 21 patients were diagnosed NABs prior to starting the therapy (a control group), 20 responders and 80 nonresponders VHC patients were tested for NABs after the end of treatment. Positive NABs was found in 5 patients (5%) from 100 after the end of the therapy – one patient (5%) from 20 in responders group and 4 patients (5%) from 80, who yielded a negative treatment result. In the nonresponders group 3 patients with diagnosed NABs received a full treatment course – 48 weeks and they showed good effect

in the 12th therapy week. All 3 patients were seen to have a relapse after the end of treatment, 1 patient interrupted the treatment at the 12th week because no necessary viral load reduction was achieved – the therapy was considered to be ineffective – null response. Neither of the control group patients was seen to have positive NABs, Table 8.11.

Table 8.11.

Incidence of NABs in treated patients and control group

Determined NABs, n=121		
After therapy		Prior to therapy
Nonresponders, n=80	Responders, n=20	Control group, n=21
NABs, n=4 (5%)	NABs, n=1 (5%)	NABs, n=0

8.1.9. Other factors analyzed in the study

No statistically significant differences between responders and nonresponders groups were found analyzing the following patient's factors: patient's gender, smoking, alcohol consumption, skipped medicine doses, cholesterol and triglyceride level, ANA presence, US findings, presence of steatosis.

In total, 21 (7.9%) patients were found to skip the medicine dose, but not more than 10 times during the therapy course. Neither patient had skipped the medicine more than 10 times. However, statistically significant differences were not seen between responders and nonresponders groups due to the number of skipped medicine doses.

Within the study 232 patients (88%) were determined HBs antigen in serum. One patient was found a positive HBs result. This patient, as a result of the therapy, responded to therapy from hepatitis C virus infection.

Within the study 191 (72%) patients were done the test for HIV infection, all of them had negative result.

8.2. Importance of viral factors

8.2.1. HCV genotype

From all patients included into the study HCV 1st genotype was found in 185 (70.1%) patients, 2nd or 3rd genotype – 79 patients (29.9%) – 2nd genotype in 5 patients, 3rd genotype – 72 patients. Since the therapy in the 2nd and 3rd genotype cases is similar, and the number of the 2nd genotype patients is small, the results of these genotype patients are analyzed together. Further on there are analyzed HCV genotype differences in different patient groups.

Table 8.12.

HCV genotype differences in responders and nonresponders groups

HCV genotype		**Therapy result**		**In total**
		Nonresponders	**Responders**	
1st group (1st genotype)	n	110	75	185
	% HCV genotype	**59.5%**	**40.5%**	100.0%
	% Therapy result	**88.7%**	**53.6%**	70.1%
2nd group (2nd, 3rd genotype)	n	14	65	79
	% HCV genotype	**17.7%**	**82.3%**	100.0%
	% Therapy result	**11.3%**	**46.4%**	29.9%
In total	n	124	140	264
	% HCV genotype	47.0%	53.0%	100.0%
	% Therapy result	100.0%	100.0%	100.0%

Patients with HCV 2nd or 3rd genotype responded more often to therapy – 82.3% (65 patients), comparing to the 1st genotype patients who responded rarer to therapy – 40.5% (75 patients), *p=0.000, Fisher's exact test* (Table 8.12.).

No statistically significant differences were found, analyzing viral load parameters in responders and nonresponders groups.

8.3. Assessment of morphological changes of the liver tissue

8.3.1. Fibrosis

179 (68%) patients included in the study were found to have fibrosis – 126 patients were found to have fibrous, expanded portal fields (HAI = 1 point), 38 patients – bridging fibrosis (HAI = 3), 15 patients– cirrhosis (HAI = 4). 58 patients were not diagnosed fibrosis (HAI = 0). 27 patients lack the data on fibrosis. The higher is HAI index, the more progressing is hepatitis C virus induced inflammatory process in the liver. In order to analyze all the data and to be objective, the method for the data processing is chosen for linear data with normal distribution – Student's t-test.

Analyzing stage of fibrosis, it was found that in the responders group there is more common 0 (no fibrosis) and the 1st (fibrous, expanded portal fields) fibrosis stage, while in the nonresponders group more commonly was seen the 3rd (bridging fibrosis) and the 4th (cirrhosis) stage of fibrosis. In the responders groups fibrosis is seen in 90 (64.3%) patients, in the nonresponders group – 89 (72%) patients, however, statistically significant difference between these groups was not found.

Table 8.13.

Differences as to fibrosis stage in responders and nonresponders groups

Therapy result	Mean	Median	Standard error	Standard deviation	Mini-mum	Maxi-mum	N
Nonresponders	**1.56**	1.00	0.125	1.327	0	4	112
Responders	**1.00**	1.00	0.086	0.959	0	4	125
Total	1.27	1.00	0.077	1.179	0	4	237

If these data are analyzed by the linear method, one can find statistically significant difference between both groups. The mean indicator in the responders group is 1.0, but in the nonresponders group – 1.56, *p=0.000, ANOVA* (Table 8.13.).

8.3.2. Cirrhosis

Further degree of fibrosis is cirrhosis. Analyzing the incidence of cirrhosis in responders and nonresponders groups, one can find a statistically significant difference. In the responders group cirrhosis is seen in 3 (2.4%) patients, but in the nonresponders group – 12 (10.7%) patients, *p=0.014, Fisher's exact test*, while patients without cirrhosis respond more often (n=122, 55%), in comparison to cirrhosis patients who respond rarer (n=3, 20%), *p=0.014, Fisher's exact test* (Table 8.14.).

Table 8.14.

Presence of cirrhosis in responders and nonresponders groups

Presence of cirrhosis qualitatively (is present/ is not present)		**Therapy result**		**Tota**
		Nonresponders	**Responders**	
No cirrhosis	N	100	122	222
	% Presence of cirrhosis	**45.0%**	**55.0%**	100.0%
	% Therapy result	**89.3%**	**97.6%**	93.7%
Cirrhosis is present	N	12	3	15
	% Presence of cirrhosis	**80.0%**	**20.0%**	100.0%
	% Therapy result	**10.7%**	**2.4%**	6.3%
Total	N	112	125	237
	% Presence of cirrhosis	47.3%	52.7%	100.0%
	% Therapy result	100.0%	100.0%	100.0%

8.3.3. HAI index

27 patients lack morphological data and HAI index is not determined. For the rest of 237 patients the linear method was used for HAI index analysis and the mean HAI index was determined in responders and nonresponders groups.

Table 8.15.

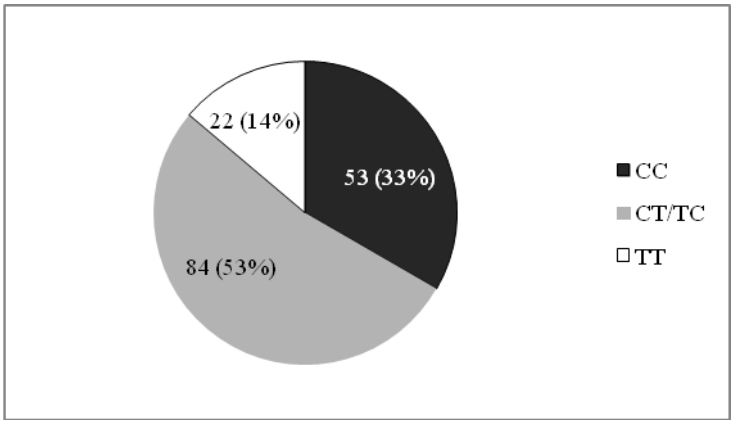
Differences of HAI index in responders and nonresponders groups

Therapy result	**Mean**	**Median**	**Standard error**	**Standard deviation**	**Mini-mum**	**Maxi-mum**	**N**
Nonresponders	**7.11**	6.00	0.282	2.985	1	14	112
Responders	**6.38**	6.00	0.208	2.327	1	12	125
Total	6.73	6.00	0.174	2.677	1	14	237

In the study there were found HAI index differences in relation to the therapy effect. In the responders group the mean HAI index is 6.38, in the nonresponders group – 7.11, *p=0.038, ANOVA* (Table 8.15.).

8.4. Interleukin 28B gene polymorphism

From all hepatitis C patients included into the 2nd group of the study, who were determined IL 28B gene polymorphism, 53 patients (33%, 95% CI 25.7-40%) were found CC genotype, 84 patients (53%, 95% CI 45-61%) – CT (83 patients) or TC (1 patient) genotype and 22 patients (14%, 95% CI 8.6-20%) – TT genotype (Picture 8.2.).



Picture 8.2. Interleukin 28B gene polymorphism

Further on there are analyzed the treatment results in 142 patients in relation to IL 28B gene polymorphism, to find out which IL 28B genotype is the most beneficial.

34 patients (73.9%) in CC genotype subgroup reached SVR (responders), in comparison to 50 patients (52.1%) in non-CC subgroup – 41 patients with CT/TC and 9 patients with TT, *p=0.002, Pearson's chi square test* (Table 8.16.).

Table 8.16.

Differences of therapy result in patients with various IL28B genotypes (CC, non-CC)

Therapy result		**IL28B genotype**		**Total**
		CC	**Non-CC**	
Nonresponders	N	4	35	39
	% IL28B	**8.7%**	**36.5%**	27.5%
Interrupted therapy /No result	N	8	11	19
	% IL28B	**17.4%**	**11.5%**	13.4%
Responded	N	34	50	84
	% IL28B	**73.9%**	**52.1%**	59.2%
Total	N	46	96	142
	% IL28B	100.0%	100.0%	100.0%

In CC subgroup 4 patients (8.7%) did not respond to therapy (all had relapse: 3 of them with HCV 1st genotype, 1 patient – with HCV 2nd genotype), comparing to 35 patients (36.5%), who did not respond to therapy in non-CC subgroup – 23 patients with CT/TC, and 12 patients with TT genotype (7 patients had a null response – all with HCV 1st genotype, 13 patients with partial response – 11 with HCV 1st genotype and 2 with HCV 3rd genotype, and 15 patients with relapse, all with HCV 1st genotype.

Table 8.17.

Differences of therapy result in HCV 1st genotype patients with various IL 28B gene polymorphism

			IL28B genotype		**Total**	**p value**
			CC	**non-CC**		
Therapy result	Nonresponders	n	3	33	36	
		% IL28B	15,79%	52,38%	43,90%	
HCV 1st genotype	Responders	n	16	30	46	**0.007367**
		% IL28B	**84,21%**	47,62%	56,10%	
Total		n	19	63	82	
		% IL28B	100%	100%	100%	

It has already been proved earlier that, in relation to the therapy result, the most unfavorable one is HCV 1st genotype. The therapy result has been

analyzed extra in relation to IL 28B gene polymorphism in HCV 1st genotype patients. Statistically significant difference was found between IL 28B genotype subgroups. In CC subgroup the patients with HCV 1st genotype responded to therapy more often – 16 patients (84.2%), in comparison to non-CC subgroup, where 30 patients (47.6%) underwent the treatment, *p=0.007, Fisher's exact test* (Table 8.17.).

8.5. Prognosis model of treatment result in chronic viral hepatitis C

While analyzing the differences, statistically significant differences were found ($p<0.05$) in 11 variables. These variables were selected for regression model as possible influential factors (independent variables), as well as HAI index, whose p-value is close to 5% border (Table 8.18.).

In the column „missing data" of Table 8.18. one can see what percentage of patients did not undergo this examination within the study. In this case from 264 patients 94 patients or 35.6% were chosen for the model from the total number of patients neither of whom had any missing variable.

Table 8.18.

Independent variables (factors possibly influencing therapy result)

Nr	Independent variable	P-value	Scale	Missing data (%)
1.	HCV genotype (1st – group 1; 2nd or 3rd – group 2)	0.000	Dichotomic variable	0.8%
2.	GGT	0.000	Lineary scale	20.1%
3.	IL28B genotype (1 – CC; 2 – non-CC)	0.001	Range scale	52.3%
4.	Body mass index	0.002	Lineary scale	33.3%
5.	Fibrosis	0.002	Range scale	10.2%
6.	Weight	0.005	Lineary scale	31.4%
7	Age group (1-<45 years; 2- >45 yrs.)	0.005	Dichotomic variable	0.4%
8.	Hemosiderin (1-is found; 2-not found)	0.008	Dichotomic variable	10.2%
9.	Presence of cirrhosis (1-no; 2-is)	0.014	Dichotomic variable	10.2%
10.	Insulin	0.026	Lineary scale	27.7%
11.	Insulin resistance HOMA	0.031	Lineary scale	27.7%
12.	HAI index	0.085	Range scale	10.2%

Since the calculation of the regression model and the structure differ from the classical difference analysis, including independent variables, then one can take also those parameters whose p value exceeds the classical 0.05, if it is close to it. Therefore also HAI index is included in the further calculations.

Odds ratio (OR) shows (Table 8.19.), how high is the prognosis not to respond to therapy at a certain independent variable.

Table 8.19.

Influence of independent variable to therapy result

Nr	Independent variable	Odds ratio (OR)	95% CI	P-value
1	HCV genotype 1st group	6.60	3.45 – 12.63	0.000
2	Hemosiderin is found	6.58	1.43 – 30.36	0.016
3	IL 28B genotype Non-CC	5.89	1.92 – 18.05	0.002
4	BMI extra weight and obesity	2.83	1.44 – 5.57	0.003
5	Cirrhosis is present	4.88	1.34 – 17.77	0.016
6	GGT is above the normal range	4.21	2.37 – 7.50	0.000
7	Age > 46 years	2.23	1.28 – 3.88	0.005
8	HAI index 8-12	1.60	0.95 – 2.69	0.078
9	Insulin is above the normal range	1.57	0.83 – 2.94	0.163
10	Insulin resistance HOMA above the normal range	168	0.89 – 3.15	0.109

Table 8.20.

Correlation analysis of independent variables

Spearman's correlation coefficient		Insulin (total)	Insulin resistance HOMA	Weight	BMI	HCV genotype 2	Hemosiderin	IL28B genotype	Age on starting therapy	GGT (total)	Fibrosis	Presence of cirrhosis
Insulin resistance HOMA	Correlation coefficient	**.987**										
	2-tailed significance (p-value)	**.000**										
	N	191										
Weight (dropped out)	Correlation coefficient	.238	.242									
	2-tailed significance (p-value)	.002	.001									
	N	174	174									
Body mass index	Correlation coefficient	.284	.296	**.859**								
	2-tailed significance (p-value)	000	.000	**.000**								
	N	169	169	176								
HCV genotype 2	Correlation coefficient	-.004	-.003	-.120	-.203							
	2-tailed significance (p-value)	.957	.972	.108	.007							
	N	189	189	179	174							
Hemosiderin	Correlation coefficient	-.009	-.003	.035	.007	.161						
	2-tailed significance (p-value)	.905	.964	.659	.934	.014						
	N	173	173	163	160	235						
IL28B genotype	Correlation coefficient	.090	.097	.005	.075	-.242	-.022					
	2-tailed significance (p-value)	.321	.282	.959	.419	.006	.810					
	N	124	124	120	117	126	118					

End of table

Spearman's correlation coefficient		Insulin (total)	Insulin resistance HOMA	Weight	BMI	HCV genotype 2	Hemosiderin	IL28B genotype	Age starting therapy	GGT (total)	Fibrosis	Presence of cirrhosis
Age on starting therapy (< 45 >)	Correlation coefficient	.172	.192	.023	.144	-.174	-.109	.024				
	2-tailed significance (p-value)	.017	.008	.758	.057	.005	.095	.792				
	N	190	190	180	175	261	236	126				
GGT (total)	Correlation coefficient	.152	.160	.368	.354	-.140	-.198	.181	.122			
	2-tailed significance (p-value)	.057	.045	.000	.000	.043	.006	.059	.076			
	N	158	158	154	151	209	193	110	211			
Fibrosis (dropped out)	Correlation coefficient	.194	.201	.175	.180	-.047	-.078	.014	.382	.279		
	2-tailed significance (p-value)	.011	.008	.026	.024	.473	.236	.885	.000	.000		
	N	172	172	161	158	235	233	116	236	191		
Presence of cirrhosis (dropped out)	Correlation coefficient	.168	.155	.168	.160	-.096	-.133	.140	.294	.252	**.463**	
	2-tailed significance (p-value)	.028	.042	.033	.044	.142	.042	.135	.000	.000	**.000**	
	N	172	172	161	158	235	233	116	236	191	237	
HAI index	Correlation coefficient	.173	.188	.179	.233	-.025	-.066	-.030	.269	.254	**.731**	.285
	2-tailed significance (p-value)	.023	.013	.023	.003	.706	.314	.747	.000	.000	**.000**	.000
	N	172	172	161	158	235	233	116	236	191	237	237

By applying Spearman's correlation test, it was found that there exists a close relationship between insulin resistance HOMA and insulin (r_s=0.987, p=0.000), BMI and weight (r_s=0.859, p=0.000), as well as between HAI index, presence of cirrhosis and fibrosis (r_s=0.731, r_s=0.463, p=0.000), thus for the regression analysis there was left one significant or more precise variable from two in each pair (Table 8.20.).

To calculate the regression there are used 3 equation formation methods – *Enter*, *Forward* and *Backward*.

✓ *Enter* results

In the equation of the method *Enter* model are included all independent variables, despite the impact strength on the dependent, thus the model was made by one step.

This model takes only those patients where in independent variables there is not a single missing variable, in Table 8.18. with all the independent variables there are calculated the percentages of missing data. In this case 94 patients are taken from 264 patients or 35.6% from the total number of patients who do not have any missing variable.

Table 8.21.

Calculation of model coefficient *Omnibus* test

Steps		**Chi-square**	**df**	**p value**
Step 1	Step	28.356	8	**0.000**
	Block	28.356	8	**0.000**
	Model	28.356	8	**0.000**

Chi square table (Table 8.21.) shows whether the influence of independent variables of the step, block and model on the dependent variable is statistically significant.

Table 8.22.

Summary of model

Step	**-2 Log possibility**	**Cox & Snell R^2 value**	**Nagelkerke R^2 value**
1	86.145	0.260	**0.370**

Summary of model contains the information about R^2 values by two methods – Cox & Snell, which is used rarer, as well as Nagelkerke R^2, which is used more often. These parameters show part of the influence of the independent variables of the developed model on dispersion of the dependent variable. In this case these are 37%; it is the mean result (Table 8.22.).

Table 8.23.

Classification table for *Enter* model

Observed			Predicted		
			Therapy result		Correct prognosis (%)
			Nonresponders	Responders	
Step 1	Therapy result	Nonresponders	**13**	**15**	46.4 %
		Responders	**8**	**58**	87.9 %
total (%)					**75.5 %**

In the classification table (Table 8.23.) the information is surveyed on precision of regression and the proportion of correct prognosis is indicated – 75.5%. This table shows correctly predicted (grey color) and incorrectly predicted number of patients.

In the Table 8.24. variables in equation summarizes the information on influential variables and their significance.

Table 8.24.

Variables in equation for *Enter* model

Step 1	B	S.E.	Wald	df	Sig.	Exp(B)
Insulin resistance HOMA	-.039	.050	.610	1	.435	.961
BMI	-.118	.071	2.775	1	.096	.889
HCV genotype	**2.128**	.858	**6.146**	1	**.013**	**8.394**
Hemosiderin	-.058	1.523	.001	1	.969	.943
IL28B genotype	-1.106	.774	2.042	1	.153	.331
Age group	.004	.643	.000	1	.995	1.004
GGT	-.001	.003	.079	1	.779	.999
HAI index	-.196	.127	2.363	1	.124	.822
Constant	4.919	4.269	1.327	1	.249	136.832

The column B includes regression equation coefficients which depict each independent variable's effect on the dependent one.

SE – Standard error – shows the variability of B coefficient.

Wald – Wald's significance criterion: the higher it is, the intense is the impact of the independent variable on dependent variable.

Sig. – significance by Wald's criterion. It shows whether the influence of independent variable is statistically significant.

Exp(B) – coefficient e^B or OR (odds ratio) parameter.

Thus, judging by Table 8.24., the greatest influence is for variable HCV genotype, it is confirmed both by B coefficient, Sig, and Exp(B). The impact of the rest of the independent variables is not statistically significant.

According to this model the regression equation would look like this: $p=1/(1+e^{-z})$,

where z = HOMA × (-0.039) + BMI × (-0.118) + HCV genotype × 2.128 + Hemosiderin × (-0.058) + IL28B genotype × (-1.106) + age group× 0.004 + GGT × (-0.001) + HAI index × (-0.196) + 4.919.

For example, a patient with the following independent variables:

HOMA – 1.36, BMI – 24.032, HCV genotype – 2 (grupa), Hemosiderin – 2 (not found), IL28B – 2 (non-CC), age group – 1 (< 45 years), GGT – 54, HAI index – 5

z = 1.36 × (-0.039) + 24.032 × (-0.118) + 2 × 2.128 + 2 × (-0.058) + 2 × (-1.106) + 1 × 0.004 + 54 × (-0.001) + 5 × (-0.196) + 4.919 = 2.92818.

Inserting in the formula, $p = 1/ (1 + e^{-2.92818}) = 1 / (1 + 0.0534) = 1 / 1.0534$ = **0.9493 or 94.93% possibility, that the patients will respond to therapy**.

✓ *Backward Stepwise* results

The model is formed by 6 steps – by each step the value of chi-square decreased, however, their changes are not statistically significant. Statistical significance of 6th model is very high, therefore this model can be practically useable.

Table 8.25.

6 steps of model

Step	-2 Log variability	Cox & Snell R^2	Nagelkerke R^2
1	86.145	.260	.370
2	86.145	.260	.370
3	86.147	.260	.370
4	86.226	.260	.369
5	86.696	.256	.364
6	89.125	.237	**.336**

R^2 parameter is slightly lower in comparison to the previous model – 0.336, however the difference is slight (Table 8.25.).

Table 8.26.

Classification table for *Backward* model

Observed			Predicted		
			Therapy result		Correct prognosis (%)
			Nonresponders	Responders	
Step 1	Therapy result	Nonresponders	13	15	46.4
		Responders	8	58	87.9
	Total (%)				75.5
Step 2	Therapy result	Nonresponders	13	15	46.4
		Responders	8	58	87.9
	Total (%)				75.5
Step 3	Therapy result	Nonresponders	13	15	46.4
		Responders	8	58	87.9
	Total (%)				75.5
Step 4	Therapy result	Nonresponders	13	15	46.4
		Responders	6	60	90.9
	Total (%)				77.7
Step 5	Therapy result	Nonresponders	13	15	46.4
		Responders	7	59	89.4
	Total (%)				76.6
Step 6	Therapy result	Nonresponders	**14**	**14**	50.0
		Responders	**6**	**60**	90.9
	Total (%)				**78.7**

Table 8.26. depicts each model's precision by steps. The highest precision – 79.7% is in the 6th step.

Table 8.27.

The variables in equation for *Backward* model

Steps		**B**	**S.E.**	**Wald**	**Df**	**Sig.**	**Exp(B)**
Step 1	Insulin resistance HOMA	-.039	.050	.610	1	.435	.961
	BMI	-.118	.071	2.775	1	.096	.889
	HCV genotype	2.128	.858	6.146	1	.013	8.394
	Hemosiderin	-.058	1.523	.001	1	.969	.943
	IL28B genotype	-1.106	.774	2.042	1	.153	.331
	Age group	.004	.643	.000	1	**.995**	1.004
	GGT	-.001	.003	.079	1	.779	.999
	HAI index	-.196	.127	2.363	1	.124	.822
	Constant	4.919	4.269	1.327	1	.249	136.832
Step 2	Insulin resistance HOMA	-.039	.050	.624	1	.429	.961
	BMI	-.118	.071	2.777	1	.096	889
	HCV genotype	2.127	.853	6.223	1	.013	8.389
	Hemosiderin	-.057	1.512	.001	1	**.970**	.944
	IL28B genotype	-1.106	.774	2.044	1	.153	.331
	GGT	-.001	.003	.079	1	.779	.999
	HAI index	-.195	.122	2.554	1	.110	.822
	Constant	4.920	4.264	1.332	1	.249	137.028
Step 3	Insulin resistance HOMA	-.039	.050	.622	1	.430	.962
	BMI	-.118	.071	2.785	1	.095	.889
	HCV genotype	2.125	.850	6.242	1	.012	8.370
	IL28B genotype	-1.104	.772	2.046	1	.153	.331
	GGT	-.001	.003	.079	1	**.779**	.999
	HAI index	-.196	.122	2.566	1	.109	.822
	Constant	4.802	2.909	2.725	1	.099	121.807
Step 4	Insulin resistance HOMA	-.039	.050	.596	1	**.440**	.962
	BMI	-.122	.069	3.067	1	.080	.885
	HCV genotype	2.154	.843	6.524	1	.011	8.620
	IL28B genotype	-1.142	.761	2.251	1	.133	.319
	HAI index	-.208	.115	3.298	1	.069	.812
	Constant	4.953	2.868	2.982	1	.084	141.576
Step 5	BMI	-.127	.068	3.492	1	.062	.880
	HCV genotype	2.056	.808	6.480	1	.011	7.817
	IL28B genotype	-1.113	.752	2.188	1	**.139**	.329
	HAI index	-.215	.114	3.557	1	.059	.807
	Constant	5.089	2.822	3.252	1	.071	162.259
Step 6	**BMI**	-.126	.067	3.569	1	**.059**	.882
	HCV genotype	2.377	.793	8.994	1	**.003**	10.774
	HAI index	-.191	.107	3.173	1	**.075**	.826
	Constant	2.502	2.094	1.428	1	.232	12.210

In the 6th step of the model only 3 variables left the regression – the most significant HCV genotype, BMI and HAI index. The last excluded variable is IL28B genotype, whose significance is 0.139. By increasing the selection border from 0.1 till 0.14, this variable remains in the equation, but then precision of the model decreases (Table 8.26., 8.27.).

Table 8.28.

Variables which are excluded from equation

			Significance	**df**	**Significance**
Step 2	Variables	**Age group**	.000	1	.995
	Total statistics			1	.995
Step 3	Variables	**Hemosiderin**	.001	1	.970
		Age group	.000	1	.998
	Total statistics			2	.999
Step 4	Variables	Hemosiderin	.001	1	.970
		Age group	.000	1	.989
		GGT	.079	1	.778
	Total statistics			3	.994
Step 5	Variables	**Insulin resistance HOMA**	.659	1	.417
		Hemosiderin	.001	1	.980
		Age group	.002	1	.963
		GGT	.060	1	.807
	Total statistics			4	.944
Step 6	Variables	Insulin resistance HOMA	.502	1	.479
		Hemosiderin	.017	1	.895
		IL28B genotype	2.316	1	.128
		Age group	.011	1	.915
		GGT	.281	1	.596
	Total statistics			5	.717

In Table 8.28. we can see the steps by which independent variables are removed from the model and equation. These are variables which in each model were of the least statistic significance – the lowest Wald's significance criterion in Table 8.27. (in column Wald).

According to this model the regression eaquation would look like this: $p=1/(1+e^{-z})$,

where z = BMI × (-0.126) + HCV genotype × 2.377 + HAI index × (-0.191) + 2.502.

If we take the same patient with the following independent variables: BMI – 24.032, HCV genotype – 2 (group 2), HAI index – 5

$z = 24.032 \times (-0.126) + 2 \times 2.377 + 5 \times (-0.191) + 2.502 = 3.272968.$

Inserting in the formula, $p = 1/ (1 + e^{-3.272968}) = 1 / (1 + 0.03789) = 1 / 1.03789 =$ **0.9635 or 96.35% probability that the patient will respond to therapy.**

✓ *Forward* results

The model by this method *Forward* R^2 (0.296) and precision parameters (74.5%) were lower, therefore it is not analyzed further on. According to this model only two independent variables – HCV genotype and BMI were included in the regression equation.

✓ The method chosen: *Backward*

Taking into account results of all three models, one can most precisely prognosis hepatitis C treatment result by using the method *Backward*, in which 3 clinical parameters (independent variables) are taking into account – BMI, HAI index, HCV genotype. Precision of this model prognoses reach 78.7%. The formula can be placed into Excel file, thus easing the calculation of prognosis.

Table 8.29.

Prognosis model of hepatitis C response to therapy in Excel file

	BMI	**1 – 1st gt. 2 – 2nd, 3rd gt. HCV genotype**	**HAI index**
Multiply by coefficient B			
z-value			
z-value * -1			
e	2.718281828		
e^-z			
e^-z + 1			
Probability			

In Table 8.29. we can see the calculation formula in Excel file. In tinted table Windows are placed the patient's clinical parameters (independent variables) – BMI, HCV genotype and HAI index and acquire probability of response to therapy in percentage.

Table 8.30.

Probability of response to therapy for specific patient (1)

	BMI	**1 – 1st gt. 2 – 2nd, 3rd gt. HCV genotype**	**HAI index**
	24	2	7.11
Multiply by coefficient B	-3.024	4.754	-1.35801
z-value	2.87399		
z-value * -1	-2.87399		
e	2.718281828		
e^-z	0.056473149		
e^-z + 1	1.056473149		
Probability	**94.65%**		

Table 8.31.

Probability of response to therapy for specific patient (2)

	BMI	**1 – 1st gt. 2 – 2nd, 3rd gt. HCV genotype**	**HAI index**
	32	2	7.11
Multiply by coefficient B	-4.032	4.754	-1.35801
z-value	1.86599		
z-value * -1	-1.86599		
e	2.718281828		
e^-z	0.15474294		
e^-z + 1	1.15474294		
Probability	**86.60%**		

In Tables 8.30. and 8.31. we can see how probability of response to therapy is changing in HCV 3rd genotype patients with HAI 7.11 and BMI in the normal range, comparing it to obesity. Obese patient's probability of response to therapy decreases from 94.65% to 86.6%.

Table 8.32.

Probability of response to therapy for specific patient (3)

	BMI	**1 – 1st gt. 2 – 2nd, 3rd gt. HCV genotype**	**HAI index**
	24	1	7.11
Multiply by coefficient B	-3.024	2.377	-1.35801
z-value	0.49699		
z-value * -1	-0.49699		
e	2.718281828		
e^-z	0.60835907		
e^-z + 1	1.60835907		
Probability	**62.18%**		

Table 8.33.

Probability of response to therapy for specific patient (4)

	BMI	**1 – 1st gt. 2 – 2nd, 3rd gt. HCV genotype**	**HAI index**
	32	1	7.11
Multiply by coefficient B	-4.032	2.377	-1.35801
z-value	-0.51101		
z-value * -1	0.51101		
e	2.718281828		
e^-z	1.66697399		
e^-z + 1	2.66697399		
Probability	**37.50%**		

In Tables 8.32. and 8.33. we can see how probability of response to treatment changes in the patient with HCV 1st genotype and HAI index 7.11. If the patient is with normal BMI, the probability of his/her response to therapy is 62%, but in the obese patient the probability of response to therapy considerably decreases – up to 37.5%.

In Table 8.34. we can see that in an obese patient with HCV 1st genotype and considerable inflammation activity/fibrosis, the probability of response to therapy is slight – only 13.86%.

Table 8.34.

Probability of response to therapy for specific patient (5)

	BMI	1 – 1st gt. 2 – 2nd, 3rd gt. HCV genotype	HAI index
	32	1	14
Multiply by coefficient B	-4.032	2.377	-2.674
z-value	-1.827		
z-value * -1	1.827		
e	2.718281828		
e^-z	6.21521302		
e^-z + 1	7.21521302		
Probability	**13.86%**		

Table 8.35.

Probability of response to therapy for specific patient (6)

	BMI	1 – 1st gt. 2 – 2nd, 3rd gt. HCV genotype	HAI index
	24	1	2
Multiply by coefficient B	-3.024	2.377	-0.382
z-value	1.473		
z-value * -1	-1.473		
e	2.718281828		
e^-z	0.22923674		
e^-z + 1	1.22923674		
Probability	**81.35%**		

In Table 8.35. we can see that the patient with HCV 1st genotype, but normal BMI minimal inflammation activity (HAI = 2), probability for response to therapy is high – 81.35%. This result justifies to start the therapy even at slight changes in the liver not expecting when the disease progresses and the probability for treatment reduces.

Using this model, each specific patient is able to expect the possibility of response to therapy.

9. CONCLUSIONS

1. The patient's factors were found which affect the possibility of chronic C hepatitis response to therapy.
   1.1. Age – patients of younger age (under 45 years) responded to therapy more often (58.6%), comparing to much older patients (over 46 years) (38.9%).
   1.2. BMI – patients with normal BMI responded to therapy more often – 80.5%, comparing to patients with increased BMI (extra weight) – responded to therapy 61.5% or with a marked BMI – obesity – responded to therapy in only 52% of cases.
   1.3. Patients with a normal GGT activity responded to therapy more often – 70% cases, comparing to patients with increased GGT activity – in this patient group almost two times less patients 35.6% responded to therapy. In the patient group with increased GGT activity a zero response was observed more often, rarer – relapse, comparing to patients with normal GGT, where an opposite tendency was observed.
   1.4. In the responders group there was a lower mean insulin level (8.3) and the insulin resistance parameter (1.78), comparing to nonresponders group, 11.0 and 2.51 respectively.
   1.5. Presence of hemosiderin in the liver tissues was found comparatively rarely – 5.5% patients, while in nonresponders group it was seen more often (9.7%), comparing to responders (1.6%).
   1.6. Neutralizing antibodies against alpha interferon were found in 5% responders – similar in both groups (nonresponders and responders).

2. Viral factor, which affects the possibility of therapy, is the viral genotype – patients with HCV 2nd or 3rd genotype responded to therapy more often – up to 82%, comparing to patients with HCV 1st genotype, who responded to therapy on average in 40.5% cases.

3. Possibility of response to therapy also determines morphological changes in the liver tissues and the presence of cirrhosis:
   3.1. In the responders group there were lower mean parameters of fibrosis (1.0) and HAI index (6.38), comparing to nonresponders, 1.56 and 7.11 respectively.
   3.2. Cirrhosis patients responded to therapy rarer (20%), comparing to those chronic VHC patients, who did not have cirrhosis (55% responded to therapy).
4. In the current study in Latvia more commonly patients were found to have IL 28B gene CT genotype – 53% cases (n=84), CC genotype – 33% cases (n=53), most rarely – TT genotype of 14% patients (n=22). Patients with CC genotype responded to therapy more often – 74%, comparing to those with non-CC genotype subgroup, where 52.1% patients responded to therapy. HCV 1st genotype patients with IL 28B gene CC genotype responded to therapy up to 84%.
5. On the basis of 3 significant factors influencing the treatment (HCV genotype, BMI and HAI), it is possible to predict the possibility to respond to therapy for each patient by formula:

$$p = \frac{1}{1+e^{-z}}$$

z = BMI × (-0.126) + HCV genotype × 2.377 + HAI index × (-0.191) + 2.502.

HCV genotype:

1st genotype -1,

2nd, 3rd genotype -2.

Using this formula, prognosis for response to therapy can be calculated by 78.7% precision.

10. PRACTICAL RECOMMENDATIONS

1. Prior to starting chronic hepatitis C treatment, one has to state the factors influencing the therapy result – BMI, virus genotype and HAI.

2. Considering the factors influencing the therapy result, one has to predict the possibility of SVR, using the developed model for chronic hepatitis C treatment prognosis.

3. In connection with the prognosis result, it is important to choose the individualized tactics:
   3.1. Prior to starting chronic hepatitis C therapy, one has to correct the factors which can be influenced (weight, BMI).
   3.2. To treat patients with slight HAI changes and normal BMI precociously.
   3.3. To consider the usefulness of the therapy for the 1st genotype patients with fibrosis/cirrhosis and obesity. To consider adding protease inhibitor to standard treatment.

4. All patients, attending the family doctor, should be examined for the possible viral hepatitis C (to determine anti-HCV), in order to diagnose the disease as early as possible. In a case of negative result, to repeat the examination every 5 years within one's lifetime.

11. PUBLICATIONS ON RESEARCH THEME

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4. I.Tolmane, B.Rozentāle, J.Keišs, L.Ivančenko, Z.Reinholde, N.Šubņikova, Ņ.Sumļaņinova, I.Reinholde , R.Sīmanis „Patient's Perception: Hepatitis C Virus Can Be Transmitted During Medical Manipulations", RSU Zinātnisko rakstu krājums 2011

12. ABSTRACTS AND PRESENTATIONS ON RESEARCH THEME

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2. I.Tolmane, B.Rozentale, J.Keiss, A.Jeruma, L.Ivancenko, Z.Reinholde, N.Shubnikova, N.Sumlaninova, R.Simanis „Chronic Hepatitis C Patient's Perception on Routes of Transmission of Hepatitis C Virus" *Hepatol Int* 2010;4(1):170
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4. I.Tolmane, B.Rozentāle, J.Keišs, L.Guseva, J.Storoženko, A.Jēruma, L.Ivančenko, N.Šubņikova, Z.Reinholde, Ņ.Sumļaņinova, I.Reinholde, G.Sīpola, R.Sīmanis „Neitralizējošo antivielu nozīme C hepatīta neizārstēšanās gadījumā" – stenda referāts, tēzes publicētas PLZK tēžu grāmatā, Rīgā, 24.27.10.2011.
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