Rīgas Stradiņa universitāte

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IMPACT OF POLYMORPHISMS IN THE ENDOBIOTIC AND XENOBIOTIC METABOLISM INVOLVED ENZYMES CODING GENES ON CHRONIC HEPATITIS C AND ACUTE TOXIC HEPATITIS

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CONTENTS

ABBREVIATIONS

ALT – alanine transaminase

AST – aspartate transaminase

BETA – regression coefficient

CI 95% – confidence interval 95%

CHC – chronic hepatitis C

DNA – deoxyribonucleic acid

GGT – gamma–glutamiltransferase

GST – glutathion S transferase

GSTA – glutathion S transferse alpha class

GSTM – glutathion S transferse mi class

GSTP – glutathion S transferse pi class

GSTT – glutathion S transferse teta class

HCV – hepatitis C viruss

MAF –minor allele frequency

MTHFR – methylene tetrahydropholate reductase

NAD – nicotinamide adenine dinucleotide

NADH – reduced form of nicotinamide adenine dinucleotide

OR – odds ratio

p – p value

PCR – polymerase chain reaction

RAKUS – Riga East University Hospital

RFLP – restriction fragment lenght analysis

RNS – ribonucleic acid

Rs# dbSNP – SNP number of SNP data base http://www.ncbi.nlm.nih.gov/snp/

SVR – sustained virological response

χ^2 – chi square

1. INTRODUCTION

1.1. Scientific novelty

The human body parallel is going metabolic pathways of endobiotics (body, normal metabolism in the case of a substance, such as bilirubin) and xenobiotics (taken from outdoor substances such as drugs, diet products used). There are many and varied proteins involved in these pathways, ranging from enzymes, which direct substrate is endobiotics or xenobiotics, followed by proteins that regulate genes encoding these enzymes activities, and proteins involved in the transport of substances in the cell and the body (transport protein receptor) [Omniecinski, 2011].

In the human body the main detoxification processes is going on liver, that protects other cells in the body and also the liver cells of direct or indirect harmful effects of endo– and xenobiotics, such as oxidative stress. If their metabolism is altered in the liver, it can affect hepatic injury and further development of the disease, including response to therapy.

One of the most common causes of liver injury in Latvia and worldwidely include alcohol and hepatitis C virus (Centre for Disease Prevention and Control and the World Health Organization data). Alcohol and hepatitis C virus causes both acute and chronic liver damage. The study included acute toxic alcohol hepatitis and chronic hepatitis C virus patients. The analysis of the functional polymorphisms in the genes encoding enzymes involved in alcohol acute toxic hepatitis and chronic hepatitis C virus could give more information about possible predictor factors for clinical gait. Latvian genetic studies have not been performed for acute toxic hepatitis patients, but in previous studies for chronic hepatitis C virus patients were including other genetic markers [Eglite, 2011; Jēruma, 2012; Tolmane, 2012].

1.1.1. Acute toxic alcohol induced hepatitis

Acute toxic alcohol induced hepatitis research is important because it is associated with high mortality in hospital ~ 65 % [Mathurin, 2003]. Currently, there are known several risk factors that affect the severity of liver damage in acute alcohol induced toxic hepatitis, such as the amount of alcohol used, supplements, diet, or lack thereof, as well as female gender is risk factor for more severe clinical symptoms [Stewart, 2001]. The acute toxic alcohol induced hepatitis is an important part of morbidity statistics also in Latvia. Analysing mortality rate (the number of deaths per 100 000 inhabitants) realated to alcohol usage, there was a peak in the 2006 (471 deaths 2006, 371 in 2005, 338 in 2004 (Central Statistical Bureau data)), that could be assotiated with extensive illegal alcohol poisoning in Eastern Europe, including Latvia, which may have been caused by poisoning with Polyhexamethyleneguanidine hydrochloride [Ostapenko, 2011], but this toxic admixture of illegal alcohol Latvian laboratory were not identified.

Acute alcohol induced toxic hepatitis develops of long–term or exess alcohol usage, or because of additives to legal or illegal alcohol. As well because the individual has reduced alcohol detoxification ability, determined both by genetic factors (enzymes involved in the alcohol metabolism directly and indirectly involved e.g. enzymes that reduce oxidative stress in the cell formed by alcohol) or non–genetic factors (amount of alcohol, food, etc.).

Alcohol metabolism in the body suggests that many biochemical reactions are leading to alcohol specific tissue damage and disease. This is due to hypoxia in liver tissues of various toxic compounds, formation of free oxygen radical that changes cell oxidation – reduction capacity, leading to oxidative stress in cells and subsequently causing the cell damage [Zakhari, 2006]. In order to prevent oxygen free radical created damage to cells there are mechanical and biochemical protection. Mechanical protection consists of

organelles and membranes. But the biochemical mechanisms of protection consists of reparation system, antioxidants, enzymes (superoxyddysmutase, catalase, peroxidase, etc.) and low molecular weight antioxidants (glutathione, uric acid, bilirubin, histidine dipeptide, lipoic acid, etc.) [Kohen, 2002].

For evaluation of acute toxic hepatitis severity are used multiple scales, including biochemical parameters such as bilirubin, prothrombin time/ INR ratio, creatinine/urea ratio, albumin, age [Mathurin, 2012].

Alcohol metabolising enzyme genetic studies in alcohol induced hepatic injury is controversial [Stickel, 2006], so the work was planned to study more indirectly involved enzyme coding genes: glutathione metabolising enzymes encoding genes (*GSTT1*, *GSTM1*, *GSTA1*, *GSTP1*), bilirubin metabolising enzyme encoding gene *UGT1A1*, homocysteine metabolism involved methylentetrahydroxypholate (MTHFR) encoding gene *MTHFR*, N–acetylation involved gene (NAT2), but also one directly involved enzyme – acetaldehyde dehydrogenase – encoding gene ALDH2 were included in the study.

1.1.2. Chronic hepatitis C

At present, the worldwidely could be ~ 3% (~ 130–210 million) of individuals having hepatitis C. Largest population study to determine the prevalence of hepatitis C in Latvia was done in 2008. It was found that the hepatitis C virus antibody (anti–HCV) is found in 2.4%, and 1.7% RNA Latvian population – which means that the approximate number of hepatitis C patients in Latvia are 39 000 [Tolmane, 2011, Tolmane, 2012].

Hepatitis C is caused by hepatitis C virus (HCV), which has six genotypes, and multiple subtypes [Simmonds, 2005]. The most common is the first genotype. Virus genotype plays a role in disease progression, as well as the

response to antiviral therapy, although its exact role is unknown [Poynard, 2003].

3/4 CHC patients during the acute infection period does not have any clinically significant symptoms of hepatitis. Approximately 80% of patients with acute hepatitis it is progressing to chronic hepatitis. 1/4 of chronic patients if left without treatment in 15–20 years will develop liver cirrhosis or cancer. CHC is characterized by varying degrees of liver inflammation and fibrosis progression, regardless of HCV genotype and viral load [EASL guidelines, 2011]. As risk factors are referred to the use of alcohol [Omniecinski, 2011], diabetes [White, 2008], advanced infectious age, co–infection with the human immunodeficiency virus (HIV) or other hepatotropic viruses. DepEnd on the different combinations of factors and in untreated case ~10–40% patients develop liver cirrhosis [Afdhal, 2004], death from cirrhosis complications is ~ 4% per year [Thompson Coon, 2007].

CHC antiviral therapy aim is viral eradication, that should prevent the further development of the disease and formation of complications. Treatment success is achieved sustained virological response (SVR) – 24 weeks after the end of treatment, there are no detectable HCV RNA in the blood [Ghany, 2011].

Standard treatment of all viral genotypes are still combined pegylated interferon and ribavirin [EASL guidelines, 2011] – sustained virological response is achieved in 40–50% of patients with viral genotype 1 and ~ 80% with the second and third viral genotype infected patients [Ghany, 2011].

In Latvia, as well as the world standard antiviral therapy consisting of pegylated interferon and ribavirin 2a or 2b in combination. Antiviral therapy influencing factors are both dependent on the genotype of the virus and the patient's genetic markers (such as IL28B [EASL guidelines, 2011; Ghany, 2011]), and other patient factors – weight [Bressler, 2003], alcohol

consumption [Anand, 2006], insulin resistance [Romeo–Gomez, 2005], liver damage and others [Manns, 2006].

CHC patients response to antiviral therapy also depends on the liver cell inflammation, that could be changed because of gene polymorphisms that are associated with a variety of metabolic changes in trace elements such as iron (*HFE* gene [Pietrangelo, 2004]), copper (gene *ATP7B* [Harris, 2000]), the various changes in synthesis of liver enzymes (e.g., alpha–1 antitrypsin, encoded by the gene *SERPINA1* [Kidd, 1983]), glutathione S transferase (e.g., genes *GSTM1* and *GSTT1*), low molecular weight antioxidants (e.g., bilirubin metabolism influencing the *UGT1A1* gene [Bosma, 1995; Datta, 2012]), and the leukocyte surface receptors, affecting imunity (for example, CC chemokine receptor is encoded by the gene *CCR5* [Katsounas, 2012]). The study identified the gene polymorphisms influence the clinical course of CHC.

1.2. Hypothesis

Endo–and xenobiotics metabolising enzyme activity changes caused by functional polymorphisms in the genes encoding, and their interaction affects the acute toxic hepatitis and chronic hepatitis C progress and clinical outcome, as well as the response to medicamentous (including antiviral) therapy.

1.3. Aim of the study

To clarify the genetic and non–genetic risk factors that affect the acute toxic alcohol hepatitis and chronic hepatitis C virus prognosis and treatment outcome.

1.4. Tasks of the study

1. To recruite patients – with acute alcohol toxic hepatitis and chronic hepatitis C. From the patients' medical records and outpatient charts summarize disease severity and characteristics of biochemical and morphological parameters. Select the appropriate control group for each of the groups.
2. To determine the non–genetic risk factors that affect the acute toxic alcohol hepatitis clinical course by analysing biochemical and other nongenetic data that describes the course of the disease.
3. To identify functional polymorphisms in the genes *GSTT1, GSTM1, GSTA1, GSTP1, MTHFR, UGT1A1, NAT2* and *ALDH2* in acute toxic hepatitis patients and the corresponding control group, to analyse their association with biochemical characteristics and clinical course.
4. To identify the factors that influence the clinical course of CHC and efficacy of antiviral therapy by analyzing the biochemical, morphological and other indicators of out–patient cards.
5. To identify polymorphisms in the genes *GSTT1, GSTM1, UGT1A1, CCR5, HFE, ATP7B* and *SERPINA1* in CHC patients and appropriate control group; to analyze their association with biochemical parameters and other disease characteristics of the patients in the out–patient cards.

2. MATERIAL AND METHODS

2.1. Material

This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the Central Medical Ethics Committee of Latvia. All study participants signed an informed consent form that was issued according to the regulations of the Central Medical Ethics Committee of Latvia.

2.1.1. Patients with acute toxic alcohol induced hepatitis and control group

The study included 60 acute toxic hepatitis patients diagnosed during the period from 2006 to 2010 in Latvian Centre of Infectious Diseases, Riga East University Hospital.

Patient inclusion criteria:

– a history of alcohol abuse;

– liver damage confirmed by biochemical parameters (liver function tests AST, ALT, GGT, bilirubin, hyaluronic acid, alkaline phosphatase, cholesterol, prothrombin, red blood cells count and white blood cell count);

Patient exclusion criteria:

– other reason (not alcohol) induced hepatic toxicity;

– not given consent to participate in the study.

Acute toxicity of alcohol induced hepatitis diagnosis was confirmed based on anamnestic (alcohol consumption) and clinical examination and biochemical data. The patient population included 35 (58%) males and 25 (42%) females with a mean age 46.50 ± 10.03 years. 19 of the patients (9 males and 10 females) died from the toxic liver damage. For patients were

detected biochemical characteristics during hospitalisation (total and direct bilirubin, alkaline phosphatise, cholesterol, prothrombin, hyaluronic acid, count of white blood cells and red blood cells, lymphocytes and segmented neutrophils percentage, reticulocyte count, erythrocyte sedimentation rate, ALT, AST, GGT, cytochrome C and cytokeratin – 18 level), for analysis were chosen analysis that were taken in most severe disease period.

Of all 12 patients included in the study were from one massive poisoning event in 2006 which was identified a single origin of illegal alcohol by place (two of the patients died), and possibly toxic hepatitis caused by illegal alcohol with possible polyhexamethyleneguanidine hydrochloride admixture [Ostapenko, 2011], that were not investigated for Latvian patients.

The control group was formed according to the gender distribution of populations – 71 males (58%) and 51 females (42%) aged 21–25 years (mean age 22.3 ± 3.1 years). Control subjects were not available for biochemical analysis, because it was used to identify genetic markers population frequencies. The control group of individuals were themselves as noted as healthy subjects, there were no marked data on chronic diseases and alcohol abuse for those individuals.

2.1.2. Patients with chronic hepatitis C and control group

Patient inclusion criteria:

– at least six months had a positive HCV–RNA, detected in Latvian Centre of Infectious Diseases, Riga East University Hospital;
– are available biochemical and morphological data before therapy or at the time of diagnosis;
– if the patient has received antiviral therapy Latvian Centre of Infectious Diseases, Riga East University Hospital, data are available for treatment progress and outcome;

Patient exclusion criteria:

– not given consent to participate in the study.

Total study included 233 patients, who had positive HCV–RNA level: 125 (53.6%) males and 108 females (46.4%). Diagnosis of patients were confirmed in period from 1992 till 2009. Antiviral therapy was initiated in 160 patients from 2001 till 2013.

160 patients (70.1%) started a combined antiviral therapy – pegylated interferon and ribavirin. Patients characteristics are shown in Figure 2.1. Patients characterizing biochemical parameters were obtained from outpatient charts: before treatment was taken into account viral genotype, viral load – defined HCV–RNA (not been established in patients with viral genotype 3, because those patients has good response to antiviral treatment) ALT, AST, GGT, iron, ferritin, hyaluronic acid, cytokeratin, cytochrome C, white blood cell and red blood cell count, neutrophil percentage, hemoglobin, morphological examinations determining the Knodell Histologic Activity index (HAI) in assessing periportal, intralobular and portal infiltration and the severity of fibrosis.

Patients who started therapy, in the third month of treatment and six month after completion of therapy were determined for HCV–RNA qualitative or quantitative (viral load has not been established in patients with viral genotype 3), ALT levels, white blood cell count, neutrophil percentage, hemoglobin level and red blood cell count.

The control group comprised 307 unrelated individuals chosen to represent the general population of Latvia. The control group characteristics: subjects were between the ages of 21–25 years (mean age 23.3 ± 2.1 years); gender distribution – 74% females (n = 228) and 26% males (n = 79). Participants in the control group underwent polymorphism frequency determination only. Biochemical association analysis, clinical examination, and exclusion of HCV infection were not performed in this group.

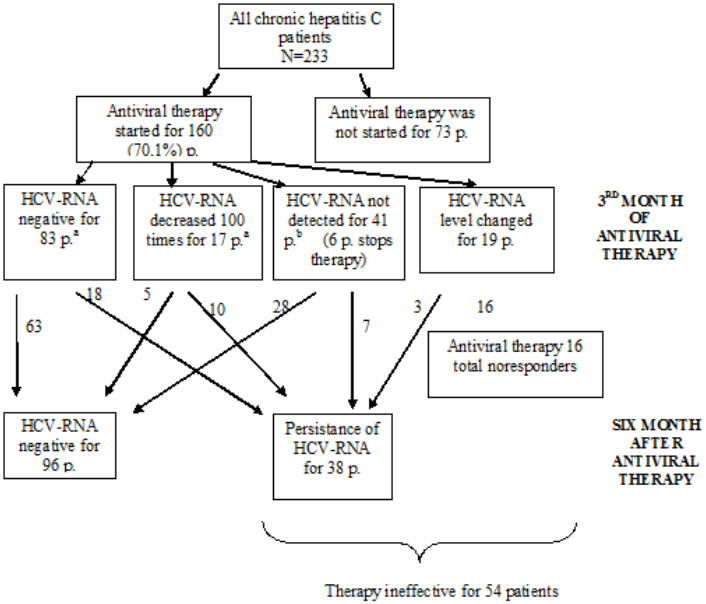

Figure 2.1. **Characteristion of chronic hepatitis C patients**
[a] two patients discontinued antiviral treatment;
[b] six patients discontinued antiviral treatment

2.2. Methods

DNA was isolated by adapted chloroform/ phenol extraction method [31].

For acute toxic hepatitis patients were selected functional polymorphisms analysis in the genes that encoded proteins are directly involved in the metabolism of ethanol (*ALDH2*) as well as those protein encoding genes that affect oxidative stress in the cell (*GST, NAT2, UGT1A1, MTHFR*). All polymorphisms are changing enzyme activity or involved in haplotype that determines altered enzyme activity. The selected markers functional activity and the methods used for analysis in 2.1. table.

Table 2.1.

Selected Genetic Markers and their Genotyping Methods for Acute Toxic Hepatitis Patients

Gene	Allele[1]	dbSNP	Enzyme activity compared to wild type allele	Method
ALDH2	ALDH2*2 (GLU504LYS)	rs671	No activity [Crabb, 1989]	PCR–RFLP (restrictase *AcuI*) [Wang, 2002]
UGT1A1	(TA)7	rs8175347	Lower activity [Bosma, 1995]	Fluorescent PCR, fragment analysis by ABI Prism 310 genetic analyser [Lin, 2006]
GSTA1	C69T	rs3957356	Lower activity [Coles, 2001]	PCR–RFLP (restrictase *EamI*) [Ping, 2006]
GSTP1	A333G	rs1695	Lower activity [Johansson, 1998]	PCR–RFLP (restrictase *Alw26I*) [Harries, 1997]
GSTT1	Null genotype	Gene deletion	No activity [Pemble, 1994]	Multiplex PCR [Kondo, 2009]
GSTM1	Null genotype	Gene deletion	No activity [Seidegard, 1988]	Multiplex PCR [Kondo, 2009]
MTHFR	C677T	rs1801133	Lower activity [Frosst, 1995]	PCR–RFLP (restrictase *HinfI*) [Safarinejad, 2012]
NAT2	C481T (NAT2*5[a])	rs1799929[b]	Normal activity [Leff, 1999; Agundez, 2008]	PCR–RFLP (restrictase *Taq1*) [Gelatti, 2005]
	A590G (NAT2*6[a])	rs1799930	Lower activity [Garcia–Closas, 2011]	PCR–RFLP (restrictase *KpnI*) [Gelatti, 2005]
	G857A (NAT2*7[a])	rs1799931	Lower activity [Garcia–Closas, 2011]	PCR–RFLP (restrictase *BamH1*) [Gelatti, 2005]

[a]nomenclature according to the *Human Genome Organisation* (www.gene.ucl.ac.uk/nomenclature/)
[b]SNP is in haplotype NAT2*5, but it is not a core SNP

In CHC patients were analyzed molecular markers that are assotiated with monogenic liver diseases (*SERPINA1* gene (alpha 1–antitrypsin deficiency), *UGT1A1* (Gilbert's syndrome), *HFE* (hereditary hemohromatose), *ATP7B* (Wilson's disease), as well as genes that might influence inflammatory processes in the liver cell, which has not previously been studied in Latvian population (*GSTM1* and *GSTT1* null genotypes markers in the *GSTA1, GSTP1* and *CCR5* genes). Selected markers, their functional activity and the methods used for analysis are summerised in the table 2.2.

Table 2.2.

Selected Genetic Markers and their Genotyping Methods for Chronic Hepatitis C Patients

Gene	Allele[1]	dbSNP	Enzyme activity compared to wild type allele	Method
UGT1A1	(TA)7	rs8175347	Lower activity [Bosma, 1995]	Fluorescent PCR, fragment analysis by ABI Prism 310 genetic analyser [Lin, 2006]
GSTT1	Null genotype	Gene deletion	No activity [Pemble, 1994]	Multiplex PCR [Kondo, 2009]
GSTM1	Null genotype	Gene deletion	No activity [Seidegard, 1988]	Multiplex PCR [Kondo, 2009]
CCR5	32bp deletion	rs333	Lower activity [Samson, 1996]	PCR [Samson, 1996]
ATP7B	H1069Q	rs76151636	Lower activity [Thomas, 1995]	Bi Pasa PCR [Krumina, 2008]
SERPINA 1	PIZ	rs28929474	Lower activity [Sambrook, 2006]	Bi Pasa PCR [Rieger, 1999]
	PIS	rs17580	Lower activity [Curiel, 1989]	Bi Pasa PCR [Rieger, 1999]
HFE	C282Y	rs1800562	Lower activity [Feder, 1998]	PCR–RFLP (restrictase – *RsaI*) [Mura, 1997]
	H63D	rs1799945	Lower activity [Thomas, 1995]	PCR–RFLP (restrictase – *MboI*) [Wang, 2011]

[1]nomenclature according to the Human Genome Organisation (www.gene.ucl.ac.uk/nomenclature/)

2.3. Statistical analysis

Genotyping data quality control was performed using Plink software [Purcell, 2007]: the analysis were used with genetic markers that met the following criteria (exclusions are explained):

1) genotyping effieciency were at least 98% of individuals from the relevant patient and control groups;

2) minor allele frequency (MAF) > 5%, except for mutations that are causing monogenic disorders – for mutations PIZ, PIS, H169Q, C282Y were done statistical analysis because they are causing monogenic liver diseases and is essential for chronic disease, including CHC pathogenesis;

3) the distribution of genotypes compliance with Hardy Weinberg law ($p \leq 0.05$) – for GSTT1 and GSTM1 null genotypes it were not completed because chosen genotyping method allows to set only the homozygous genotypes.

For data processing was used PLINK software [Purcell, 2007]. The chi–square test was used to compare the patient and control groups with a significance threshold of $p < 0.05$ also analysing the odds ratio (OR) and 95% confidence intervals (CI 95%). As some of the genetic markers were within the same chromosome, it was also performed haplotype analysis, haplotype frequencies were compared using chi–square test, a reliable considering if $p < 0.05$.

SPSS software v.16.0 (SPSS Inc., Chicago, IL, United States) was used to compare mean biochemical marker values between the patient and control groups and between the two patient groups [with and without a sustained viral response (SVR), defined as the inability to detect viral RNA six months after therapy [EASL guidelines, 2011]. Parametric values were compared using ANOVA, and nonparametric data were evaluated with the Mann–Whitney test.

Genotype association analysis with biochemical markers was conducted using a full linear model comprising three genetic effects: additive effects of allele dosage, dominance deviation from additivity (a negative value indicates a recessive allele), and the 2–df joint test of both additive and dominance. Beta was evaluated as the regression coefficient. Data were accepted as statistically significant at a *P* value of < 0.05. To assess the impact of various factors on the resulting association data obtained was done (BETA coefficient and p–values) to correct the non–genetically different characteristics such as gender, age, acute toxic hepatitis after patient mortality (acute toxic alcoholic hepatitis patients), chronic hepatitis C virus patients after achieving SVR were used as covariates for p values adjustion [Purcell, 2007].

3. RESULTS

3.1. Characterisation of acute toxic hepatitis patients

The study included 61 patients (35 males (58%) and 25 females (42%)) with a diagnosis of acute toxic hepatitis who were hospitalized in Latvian Centre of Infectious Diseases, Riga East University Hospital. 19 patients (nine males and ten females) died in hospital from caused liver damage. Patient hospitalization ranged from 2 weeks to 3 months. The control group included 122 individuals (71 (58%) males and 51 (42%) females) with mean age 22.3 ± 3.1 years (21–25 years). Comparing the biochemical parameters which were set during the the disease culmination among females and males, statistically significant differences were found in cholesterol and prothrombin levels, and red blood cells count (statistically significant higher rates were for males, $p < 0.05$) (see results in table 3.1.).

Table 3.1.

Biochemical Indices Comparison between Genders in Acute Toxic Alcohol Induced Toxic Hepatitis

Biochemical parameter	Total in patients		Males		Females		P value
	Mean	SD	Mean	SD	Mean	SD	
Bilirubin (μmol/l)	432.12	199.51	446.41	211.81	407.44	179.4	0.515
Direct bilirubin (μmol/l)	315.35	183.89	336.68	201.99	278.61	145.6	0.291
Alkaline phosphatase (U/l)	673.84	776.59	761	827.32	521.31	676.6	0.331
Cholesterol (mmol/l)	6.29	5.86	7.84	6.34	3.75	3.9	0.021
Prothrombin %	61.82	34.78	73.07	35.47	43.28	24.8	0.004
Hialuronic acid (ng/ml)	566.28	459.63	581.5	424.31	554.1	508.5	0.904

End of Table 3.1.

Biochemical parameter	Total in patients		Males		Females		P value
	Mean	SD	Mean	SD	Mean	SD	
Leucocytes count (*10^9/l)	4.62	12.71	3.98	13.68	5.57	11.3	0.655
Lymphocyte (%)	18.67	11.28	19.44	12.32	17.37	9.5	0.641
Granulocyte (%)	63.53	13.04	62.44	15.22	65.38	8.3	0.483
Erythrocyte count (*10^{12}/l)	3.05	0.88	3.35	0.81	2.54	0.7	**0.001**
Hemoglobine (g/l)	120.09	110.18	125.12	127.30	112.43	79.6	0.686
Reticulocytes (‰)	24.77	13.56	21.85	13.09	28.78	13.5	0.090
Erythrocyte sedimentation rate (mm/h)	57.48	32.77	57.93	31.45	56.76	35.8	0.910
ALT (U/l)	81.58	142.99	75.58	53.34	91.37	225.3	0.709
AST (U/l)	138.08	121.67	121.58	71.26	165	174.9	0.224
GGT (U/l)	497.96	662.83	644.48	773.95	265.24	337.0	0.064
Cytohrome C (ng/l)	0.88	1.96	1.13	2.27	0.60	1.5	0.399
Cytoceratine–18 (U/l)	1125.9	499.79	1088.8	504.17	1172.2	503.3	0.584

The patients mean age was 46.5 ± 10.0 years. The comparison between the age of lethal and non–lethal cases were found statistically significant difference (respectively 43.9 ± 10.9 and 47.6 ± 9.5 years, p = 0.183, CI 95% 9.27–1.81) in patients (comparison of biochemical parameters between both groups are shown in table 3.2. – statistically differed only prothrombin, lymphocytes and cytochrome C level that were higher in surviving patients ($p \leq 0.05$)).

Table 3.2.

Acute Toxic Hepatitis Patients Characterisation by Outcome

Biochemical parameter	Non–lethal outcome		Lethal outcome		p value
	Mean	SD	Mean	SD	
Bilirubin (μmol/l)	418.89	201.51	468.69	184.54	0.446
Direct bilirubin (μmol/l)	295.25	192.92	371.00	148.83	0.206
Alkaline phosphatase (U/l)	700.16	800.24	611.08	744.33	0.733
Cholesterol (mmol/l)	7.11	6.23	4.06	4.16	0.124
Prothrombin %	68.27	34.29	45.95	31.83	0.050
Hialuronic acid (ng/ml)	435.08	388.34	828.67	512.41	0.086
Leucocytes count ($*10^9$/l)	3.39	8.673	7.86	19.96	0.250
Lymphocyte (%)	21.80	11.65	11.46	6.08	0.002
Granulocytes (%)	3.03	0.82	3.04	1.04	0.978
Erythrocyte count ($*10^{12}$/l)	103.74	69.25	161.53	172.77	0.085
Hemoglobine (g/l)	25.20	13.69	23.92	13.72	0.769
Reticulocytes (‰)	62.77	11.57	65.31	15.34	0.564
Erythrocyte sedimentation rate (mm/h)	60.16	32.82	51.08	33.06	0.408
ALT (U/l)	71.65	51.96	109.85	272.44	0.413
AST (U/l)	132.27	85.76	154.61	194.87	0.574
GGT (U/l)	570.32	749.72	325.38	351.15	0.268
Cytohrome C (ng/l)	21.80	11.65	11.46	6.08	0.04
Cytoceratine–18 (U/l)	1089.33	493.39	1192.19	520.62	0.515

3.2. Genetic marker association in acute toxic hepatitis patients

Analysis of genetic markers rs1799931 was excluded because MAF< 5% (MAF = 0.0092 for patients and the control group MAF = 0.0098). Hardy Weinberg law did not meet the only two markers – GSTM1 and GSTT1 null genotypes. Further analysis was not included also marker in the *ALDH2* gene because it was monomorph in all samples analyzed.

The analysis of genetic markers in association with acute toxic hepatitis, a statistically significant difference was found in the GSTT1 null genotypes (MAF = 0.2 for patients and the control group MAF = 0.1031, p = 0.0042, OR2.174, CI 95% 1.265–3.736) other results are not shown.

In case control analysis in acute toxic alcohol induced hepatitis patients *GSTA1* gene polymorphism rs3957356 showed statistically significant association with acute toxic hepatitis, that was most significant for recessive model (TT genotype frequency in patients 0.304, in control group 0.137, χ^2 = 4,329, p = 0.037), other results were not statistically significant (p> 0.05) and is not shown.

Separate analysis of GST encoding gene markers revieled no statistically significant differences between the combinations of GSTT1 and GSTM1 null/ non–null genotypes (statistically more likely to both non–null genotypes were found in the patient group (p = 0.004)). The analysis of GSTs four markers together, a statistically significantly higher were in the control group for combination of markers to determine the null GSTT1 activity, GSTM1, GSTP1 and GSTA1 normal activity. The results shown in Table 3.3.

Table 3.3.

Case – Control Study for The Haplotypes in the GSTs Encoding Genes

Analysed genes	Allelic combination	Frequency in patients	Frequency in controls	χ^2	P value
GSTT1/ GSTM1	T0M1	0.1333	0.06278	6.565	0.0104
	T1M1	0.2333	0.3722	8.094	0.0044
GSTT1/ GSTM1/ GSTA1/ GSTP1	T0M1TA	0.05679	0.01605	5.23	0.0222
	T1M1CA	0.08914	0.1673	4.183	0.0408

In order to analysed whether genetic markers are assotiated with clinical severity there was done assotiation analysis in two patients groups – with lethal and non lethal outcome. No statistically significant association was found with markers except with – rs1799930 (MAF in lethal patients 0.395 and MAF in survived patients 0.218, p = 0.045, OR 2.340, CI 95% 1.006– 5.441). All genetic markers results shown in 3.4. table.

There was done also haplotype analysis for markers in the *NAT2* gene statistically significant assotiation was found with combination between TA (C481T/A590G) haplotype that is more frequent in patients with lethal outcome (frequency 0.3947) than in the control population (frequency 0.2179) (p = 0.045).

Table 3.4.

Genetic Marker Analysis with Outcome in Acute Toxic Hepatitis Patients

Rs# (dbSNP)	Gene	Minor allele	MAF lethal outcome (n = 19)	MAF non–lethal patients (n = 41)	P value	OR	CI 95%
rs1801133	*MTHFR*	T	0.472	0.312	0.098	1.968	0.878–4.413
–	*GSTM1*	Null genotype	0.421	0.341	0.400	1.403	0.637–3.088
–	*GSTT1*	Null genotype	0.263	0.171	0.239	1.735	0.689–4.367
rs4124874	*UGT1A1*	$(TA)_7$	0.368	0.402	0.723	0.866	0.392–1.915
rs3957356	*GSTA1*	T	0.394	0.451	0.561	0.793	0.363–1.735
rs1799929	*NAT2*	C	0.447	0.487	0.687	0.852	0.391–1.856
rs1799930	*NAT2*	G	0.395	0.218	0.045	2.340	1.006–5.441
rs1695	*GSTP1*	G	0.289	0.321	0.734	0.863	0.370–2.015

3.3. Genetic marker association with biochemical indices in acute toxic hepatitis patients

Using linear regression with the additive model was tested genetic markers association with biochemical parameters. No statistically significant association was found between GSTM1 and GSTT1 null genotypes and markers in the *NAT2* gene markers (statistically reliable results are shown in table 3.5.). GSTM1 null genotype was associated with elevated total bilirubin levels (BETA = 67.23, p = 0.014) – individuals with null genotypes had mean bilirubin level 362.62 ± 205.44 μmol/l, but with a non–null genotype –

497.09 ± 182.53 μmol/l (p = 0.014) as well as with direct bilirubin level (BETA = 53.89, p = 0.023) direct bilirubin for individuals with null genotype was 287.32 ± 173.28 μmol/l, but with a non–null genotype – 397.55 ± 169.13 (p = 0.20). Using covariates, the greatest impact was for the patient's age. GSTT1 null genotype were assotiated with the prothrombin level (BEAT = –11.05, p = 0.037) having prothrombin level of 40.87 ± 23.56 (comparing to individuals with a non–null genotype, who had mean prothrombin level 62.93 ± 33.67 (p = 0.037)) as well as with hyaluronic acid (BETA = 170.4, p = 0.014, individuals with null genotypes mean level of hyaluronic acid was 899.58 ± 443.15 ng/ml, but non–null genotype – 512.81 ± 444.34 ng/ml (p = 0.009)).

Table 3.5.

Genetic Marker Association with Biochemical Indices in Acute Toxic Hepatitis Patients

Bio–chemical parameter	Rs# (dbSNP)	Gene	BETA	P value (not adjusted)
Bilirubin	–	*GSTM1*	67.2	0.014
	rs1799930	*NAT2*	86.2	0.045
Direct bilirubin	–	*GSTM1*	53.9	0.023
GGT	rs1799929	*NAT2*	–261.3	0.018
	rs1799930	*NAT2*	325.8	0.011
Alkaline phosphatase	rs1799929	*NAT2*	–270.5	0.032
	rs1799930	*NAT2*	374.8	0.011
Holesterol	rs1799929	*NAT2*	–2.25	0.018
Prothrombin	–	*GSTT1*	–11.05	0.037
Hialuronic acid	–	*GSTT1*	170.4	0.014

NAT2 gene alleles rs1799929 and rs1799930 showed the opposite association – rs1799929 had a negative association with GGT (BETA = –261.3,

p = 0.018), alkaline phosphatase (BETA = –270.5, p = 0.032) and cholesterol level (BETA = –2.254, p = 0.018).

rs1799930 allele had a positive association with bilirubin (BETA = 86.17, p = 0.045), GGT (BETA = 325.8, p = 0.011) and alkaline phosphatase (BETA = 374.8, p = 0.011) levels. Biochemical parameters assotiation with the *NAT2* genotypes shown in Table 4.8. Association with GGT was influenced by clinical outcome of toxic hepatitis (p adjusted for treatment outcome = 0.004).

3.4. Chronic hepatitis C patients characterisation

Total study included 233 patients with hepatitis C – 125 (53.6%) males and 108 females (46.4%). Biochemical data were available for patients at the time whne diagnose where confirmed or at the beginning of treatment. The biochemical characteristics of the patients and the comparison between the genders are shown in table 3.6.

Table 3.6.

CHC Patient Characteristics at Time of Diagnosis

Parameter	Patients		Males		Females		p value
	Mean	SD	Mean	SD	Mean	SD	
CHC diagnosing age	38.73	12.88	37.62	13.52	40.03	38.73	0.156
HCV viral load	2.6 E+6	5.3 E+7	2.7 E+6	5.2 E+7	2.4 E+6	5.5 E+7	0.725
ALT (U/l)	102.8	86.13	116.88	92.83	86.72	74.95	0.008
AST (U/l)	65.49	61.86	71.68	73.01	58.56	45.67	0.123
GGT (U/l)	70.49	80.64	86.33	96.23	54.49	57.18	0.005
Fe (μmol/L)	24.26	9.82	26.43	9.21	19.92	9.75	0.006
Ferritin (ng/ml)	430.0	757.8	460.6	476.4	367.5	1150.3	0.640

End of table 3.6.

Parameter	Patients		Males		Females		p value
	Mean	SD	Mean	SD	Mean	SD	
Hyaluronic acid (ng/ml)	52.76	95.31	46.29	52.76	59.05	123.51	0.427
Cytokeratin e–18 U/l	340.9	342.2	352.2	399.2	330.0	278.74	0.685
Cytochrome (ng/l)	0.39	1.08	0.39	0.69	0.39	1.36	0.984
Leukocyte ($*10^9$/l)	5.89	1.63	6.11	1.66	5.65	1.56	0.036
Neutrophils (%)	54.47	9.56	55.13	9.27	53.71	9.88	0.263
Erythrocyte ($*10^{12}$/l)	4.72	0.49	4.91	0.48	4.5	0.40	0.000
Hb (g/l)	14.63	1.57	15.34	1.36	13.82	1.38	0.000
HAI	6.70	3.02	6.81	2.98	6.58	3.08	0.565

Males had statistically significantly higher ALT, GGT and iron levels and white blood cell count ($p < 0.05$) compared to females. Red cell counts and hemoglobin levels were higher in males, which can be explained by the different standards between genders.

For 182 patients were determined virus genotype – 71.4% had genotype 1, 5.5% had genotype 2 and 23.1% had genotype 3, for 51 patients (21.9%) genotype were not detected or was not marked on the in an hospital documentation. There were done comparison of the biochemistry parameters between patients with viral genotype 1 versus other genotypes – statistically differed ALT level – for patients with viral genotype 1 – 95.79 ± 68.48 U/l, and with other genotypes – 119 ± 159.42 U/l ($p = 0.018$).

As 3rd viral genotype is assotiated with prominent steatosis development in comparison with other genotypes than there were compared biochemical data at time of diagnosis between 3rd and 1st/2nd viral genotypes. Statistically

significant differences were found between the following values: ALT ($1^{st}/2^{nd}$ genotype 97.48 ± 77.8 U/l, 3^{rd} genotype 139.43 ± 11.84 U/l, p = 0.006), AST ($1^{st}/2^{nd}$ genotype AST 61.18 ± 53.08 U/l, 3^{rd} genotype 81.34 ± 75.62 U/l, p = 0.009).

12 patients had confirmed liver cirrhosis in liver biopsy. Comparing the biochemical parameters of patients in groups with and without cirrhosis, statistically differed levels of ALT, AST, GGT, cytokeratin –18 and hyaluronic acid levels prior to initiating therapy (statistically reliable results shown in Table 3.7.).

Table 3.7.

Comparison of Biochemical Parameters in Patients with/without Chirrosis

Biochemical parameters	Patients with chirrosis (n = 12)	Patients without chirrosis (n = 214[a])	p value [b]
ALT (U/l)	144.21 ± 66.10	101.82 ± 87.68	0.006
AST (U/l)	111.55 ± 39.09	63.91 ± 63.05	0.000
GGT (U/l)	128.08 ± 101.00	66.40 ± 78.86	0.003
Cytokeratyne–18 (U/l)	622.75 ± 362.58	333.49 ± 243.55	0.014
Hyaluronic acid (ng/ml)	210.55 ± 136.47	44.04 ± 87.74	0.000

[a] liver biopsy was not performed in 13 CHC patients [b]p value calculated using the Mann–Whitney test, which is suitable for non–parametric data

Comparing the incidence of cirrhosis among viral genotypes, no statistically significant differences ($p < 0.05$) were found neither comparing each genotype separately, nor compared the 3^{rd} viral genotype separately from the other two possible genotypes.

Antiviral treatment outcome was evaluated in 150 patients (excluded patients who discontinued therapy because of personal reason) – SVR was achieved in 46 (57.5%) males and 50 (71%) and females (OR = 1.488, p = 0.077, CI 95% 0.949 –2.332).

Comparison of the patients for whom received antiviral therapy was successful (achieved SVR) with those to whom it was unsuccessful (non–

response and those with relapse) was statistically significantly lower levels of GGT and hyaluronic acid, fibrosis scores and total HAI index and higher white blood cell count ($p< 0.05$) (results shown in table 3.8.).

Table 3.8.

Patients with/ without Reached SVR

Parameter	Patients with SVR		Patients with persistant virus		p value
	Mean	SD	Mean	SD	
Age	37.75	11.94	38.98	11.94	0.545
HCV viral load	2.6E+6	5.5E+7	3.4E+6	6.2E+7	0.489
ALT (U/l)	112.71	97.09	110.47	81.98	0.888
AST (U/l)	63.23	60.69	74.28	59.22	0.304
GGT (U/l)	52.02	50.77	118.67	117.51	0.000
Fe(µmol/L)	22.35	8.93	25.59	10.58	0.238
Ferritine (ng/ml)	495.23	1099.06	518.15	552.03	0.938
Hyaluronic acid (ng/ml)	36.76	42.09	71.52	94.74	0.021
Cytokeratyn–18 (U/l)	387.23	424.39	355.85	356.60	0.714
Cytochrome (ng/l)	0.22	0.45	0.65	1.89	0.106
Leucocyte ($*10^9$/l)	5.98	1.66	5.32	1.19	0.012
Neutrofils (%)	53.68	10.27	53.46	9.29	0.989
Erythrocyte count ($*10^{12}$/l)	4.63	0.49	4.77	0.49	0.114
Hemoglobine (g/l)	14.41	1.50	14.76	1.49	0.184
Fibrose	1.2	0.97	1.78	1.34	0.003
HAI	6.63	2.7	7.73	2.99	0.027

Antiviral treatment received 87.5% from patients with 1st genotype, 90% with 2nd genotype to and 85.7% from patients with 3rd viral genotype. Comparing the effectiveness of treatment between viral genotypes were found

statistically significant difference (p = 0.001), and comparing also 1st genotype against 2nd and 3rd viral genotypes p = 0.003.

One of the efficacy of risk factors is liver fibrosis, that last stage liver cirrhosis. Among the patients who received treatment, 143 patients had underwent liver biopsy, and 9 patients had liver cirrhosis. SVR was achieved in 89 (66,42%) patients without cirrhosis, and in 3 (33.33%) patients without cirrhosis (p = 0.045).

3.5. Genetic marker analysis in CHC patients

Comparing the analyzed markers frequencies in CHC patients and control group statistically significantly differed frequency of GSTM1 non–null genotype (OR = 1.487, p = 0.0052), GSTT1 null genotype (OR 1.621, p = 0.0226) and CCR5 del32 allele (OR 1.675, p = 0.0026) (results shown in table 3.9.).

Table 3.9.

Genetic Marker Assotiation in CHC Case–Control Study

Rs# (dbSNP)	Gēns	Minor allele	MAF in patients	MAF in control	P value	OR	CI 95%
–	*GSTM1*	M1	0.536	0.438	0.005	1.487	1.12–1.97
–	*GSTT1*	T0	0.156	0.103	0.023	1.621	1.07–2.46
rs8175347	*UGT1A1*	$(TA)_7$	0.360	0.355	0.880	1.024	0.76–1.38
rs333	*CCR5*	D	0.245	0.162	0.003	1.675	1.19–2.35
rs1800562	*HFE*	Y	0.047	0.034	0.401	1.315	0.66–2.61
rs1799945	*HFE*	D	0.128	0.120	0.702	1.077	0.73–1.59
rs76151636	*ATP7B*	Q	0.016	0.007	0.192	2.228	0.65–7.66
rs28929474	*SERPINA1*	PIZ	0.016	0.024	0.489	0.666	0.21–2.12
rs17580	*SERPINA1*	PIS	0.016	0.000	0.066	NA	NA

Genetic marker analysis was conducted for 150 patients who started therapy and who did not interrupt non–medical indications. 96 patients had achieved SVR, while 56 patients had persistant virus (19 patients were totally non–response, and 16 of them discontinued treatment).

The comparison between the patients to achieve SVR and ineffective treatment (results shown in Table 3.10). Statistically significant only differed in the CCR5 gene deletion of 32bp, which was more frequent in patients with ineffective therapy (MAF with persistent virus = 0.3723, MAF for patients with SVR = 0.1625, p = 0.0002, OR = 3.057, CI 95% 1.69–5.13). If compared both genders for males – MAF ineffective therapy = 0.3167, MAF for patients with SVR = 0.1, p = 0.0013, OR = 4.171, CI 95% 1.678 –10.37 and females – MAF ineffective therapy 0.4706, MAF for patients with SVR = 0.225, p = 0.0087, OR = 3.062, CI 95% 1.304–7.19.

Table 3.10.

Genetic Marker Assotiation with SVR

Rs# (dbSNP)	Gene	MAF	MAF in non–SVR	MAF in SVR	p value	OR	CI 95%
–	*GSTM1*	M0	0.436	0.474	0.586	0.859	0.495–1.49
–	*GSTT1*	T0	0.128	0.132	0.943	0.971	0.430–2.189
rs8175347	*UGT1A1*	$(TA)_7$	0.396	0.355	0.503	1.192	0.713–1.995
rs333	*CCR5*	D	0.372	0.163	0.000	3.057	1.69–5.53
rs1800562	*HFE*	Y	0.041	0.073	0.287	0.540	0.171–1.704
rs1799945	*HFE*	D	0.100	0.115	0.693	0.852	0.384–1.888
rs76151636	*ATP7B*	Q	0.029	0.022	0.709	1.333	0.292–6.078
rs28929474	*SERPINA*	PIZ	0.010	0.028	0.309	0.343	0.039–2.974
rs17580	*SERPINA*	PIS	0.019	0.011	0.57	1.76	0.244–12.69

Compared totale non–responders – 19 patients (after three months of treatment, viral load has not decreased at least 100 fold) – statistically

significant had in in the CCR5 gene del32 allele (MAF nonresponders = 0.447, MAF responders = 0.204, OR = 3.165, p = 0.0012), other results showed in table 3.10.

Early response is defined as the disappearance of the virus in the third month of treatment. To find out if any of the analyzed genetic markers influence early response to treatment there were compared patients who achieved early response to treatment with patients who had a partial response and the null response to therapy (there were 35 patients was not determined by viral load in the third month of treatment). Statistically significantly difference were found with GSTM1 null genotype – MAF non responders = 0.3182, MAF patients early viral response = 0.4853, OR = 0.4949, p = 0.052, CI 95% 0.2414–1.015. Comparing the genotype frequency in males, the association was stronger with those who reached early viral response (OR = 0.4011, p = 0.042, CI 95% 0.164–0.981), for females tatistically significant association were not found (p > 0.05).

3.6. Genetic marker association with biochemical indices before antiviral treatment for chronic hepatitis C patients

By linear regression analysis were done the association analysis between genetic data and biochemical and morphological data at diagnosis, all data (BETA and p value) were adjusted for age, gender and viral genotype.

Statistically significant results were obtained in the association between PIZ allele with increased cytochrome and hyaluronic acid levels, PIS allele, which is associated with an increased hyaluronic acid and decreased hemoglobin level (statistically significant assotiation were lost after adjusted for sex, age and sex or gender, age and viral genotype), a GSTM1 non–null genotype were assotiated with elevated levels of cytokeratin–18, reduced HAI

index, and decreased hemoglobin level (statistically significant assotiation were lost after adjusted for sex, age and sex or gender, age and viral genotype) and the UGT1A1 gene (TA)7 allele, which was associated with increased levels of cytokeratin (statistically significant results shown in table 3.11).

Table 3.11.

Genetic Marker Assotiation with Biochemical Parameters

	SNP	Allele	BETA	p value
Cytohrome C	rs28929474	PIZ	1.657	3.969e–005
Cytokeratin–18	–	M1	86.41	0.0153
	rs8175347	(TA)7	99.92	0.0498
	rs17580	PIS	460.1	0.0005
Hyaluronic acid	rs28929474	PIZ	166.4	0.0001
	rs17580	PIS	103.8	0.0101
Hb before treatment	–	M1	–0.345	0.0266
	rs17580	PIS	–1.183	0.0521
Fibrose	rs28929474	PIZ	0.819	0.0753
HAI	–	M1	–0.7737	0.0083

4. DISCUSSION

Endo–and xenobiotics metabolising gene functional polymorphism analysis is carried out for many diseases, including liver damage, cardiovascular disease, diabetes, etc, because tissue damage is caused not only with direct chemical/ infectious lesion, but also by altered body's response to this damage. In this study were analysed the two most common causes of liver damage – alcohol and HCV [EASL guidelines, 2011; EASL guidelines, 2012, Mathurin, 2012], and the proposed disease – acute toxic alcohol damage and chronic hepatitis C. Both diseases have been widely studied, but are still looking for new biochemical and genetic markers that allow for better forecasting of the course of disease.

4.1. Clinical and biochemical indices characterising liver damage in patients with acute alcohol induced hepatitis

One of the biochemical indicators of liver damage that is included in many assessment scales is prothrombin is because it shows the synthesis of liver function [EASL guidelines, 2012]. When prothrombin is reduced, then it may be a sign of liver failure [Lisman, 2010]. In our study, the prothrombin level statistically differed between lethal and and nonlethal cases, confirming that it is an important indicator of liver damage. Since it also differed between the genders in our studied population for patients with alcohol induced acute hepatitis ($p = 0.004$), that could point to that women were probably developed more severe liver damage, similar to that previously described [Stewart, 2001], because there are not described differencies in the prothrombin level between geneders. More severe hepatic injury is attributed to the fact that the female body comparing to the males have more fat and less water and therefore with the same concentration of alcohol in the blood, is reached a higher alcohol

concentration as well as fact that excreted in the stomach has lower activity, and hence alcohol is less degraded compared to men [Lieber, 2000]. However, it should be noted that not all enrolled patients had known duration and amount of alcohol used, and those are also important non–genetically factors affecting alcohol metabolism.

Compared to other biochemical parameters between the genders a statistically significant different levels were found of cholesterol and red blood cell count. It is known that alcohol alters lipid metabolism in the body, more in females – reducing low density cholesterol level [Wakabayashi, 2009], our study statistically significant differences were found in total cholesterol level (women had statistically significantly lower level, $p = 0.021$), similar to already described gender difference in alcohol effects on lipid levels [61]. No statistically significant difference between genders were found in any biochemical parameters characterizing of liver injury as GGT, AST, ALT and alkaline phosphatase.

Patients who died from caused liver damage comparing with patients who survided were not found a statistically significant difference in liver injury/inflammatory markers, but were found in different parameters that characterize the hepatic synthesis function – prothrombin (statistically significantly higher in patients who survived $p = 0.05$) and lymphocyte count (which is higher in patients who survived, $p = 0.04$), which characterize both synthesis and inflammation process. *Marcos et al.* study has shown that a reduction in the number of lymphocytes is assotiated with decreased immunity/body's defences – there were found statistically significant differences when comparing lymophocyte count in patients with the alcoholic cirrhosis and alcoholics without liver disease (OR = 1.40 , CI 95% 1.11–1.77), but there were not found differencies when comparing patients with alcohol induced liver damage with the control group (OR = 1.09 , CI 95% 0.87–1.37) [Marcos, 2011]. Lymphopenia is also caused by liver failure in alcohol induced

liver cirrhosis, also it changes $CD4^+/CD8^+$ ratio. This ratio can be used as a non–invasive marker of fibrosis in alcochol induced liver damage [Marcos, 2011].

Alkaline phosphatise (the same as GGT) is described as a more specific indicators of alcohol induced hepatitis [Perrillo, 1978], but in our group of patients was not possible to assess its comparison with the control group. Level of alkaline phosphatase in patients were only compared with the reference range (0 – 117U/l), and the average values of the patient group were increased (673 U/l), but none of the currently widely used scales is not including alkaline phosphatase as an indicator to determine the severity of alcohol induced hepatitis [Mathurin, 2012]. Also in our study group showed no statistically significant differences in the levels comparing different patient subgroups – by gender or outcome (lethal/ non–nonlethal cases).

4.2. Genetic marker association analysis in acute toxic hepatitis patients

The study included 10 genetic markers that were studied in acute toxic hepatitis patients, two of them were excluded from further analysis G857A allele in the *NAT2* gene (rs1799931) because its minor allele frequency were < 0.05, and the *ALDH2* gene marker because it was monomorph, as previously described in Europeans descent populations [Wang, 2002].

The analysis of other genetic markers of acute toxic alcohol hepatitis patients, statistically significant results were found in the analysis of null genotypes in the genes *GSTM1* and *GSTT1*, and polymorphisms in the *NAT2* gene.

GSTs encoding genes are involved in oxidative stress reduction. In more details are studied null genotypes in the genes *GSTT1* and *GSTTM1*and their

role in alcohol liver disease pathogenesis. Our study revealed that in acute toxic hepatitis patients compared with the control group is more common *GSTT1* null genotype (OR = 2.174, p = 0.004), as well as combination of non–null genotypes of the genes *GSTT1* and *GSTM1* are more common in acute toxic hepatitis group compared with the control group (p = 0.004). *Marcos et al.* have performed a meta– analysis, gathering information from 15 different studies were was determined *GSTM1* and *GSTT1* null genotypes and Ile105Val allele (*GSTP1* gene) frequency in alcoholics with and without cirrhosis, as well as the control group. Statistically significant association was found only with *GSTM1* null genotype comparing alcoholics with and without alcoholic liver disease (OR = 1.43, CI95% 1.14–1.78) and in patients with alcoholic cirrhosis and alcoholics without liver disease (OR = 1.40, CI95% 1.11–1.77), but not comparing to the control group (OR = 1.09, CI 95% 0.87–1.37) [Marcos, 2011]. Our study did not confirmed such assotiation that could be related to the size of the selected group of patients, as well as the fact that our selected group of patients had not known history of alcohol usage, and they were analyzed as acute toxic hepatitis patients, rather than chronic alcohol users, as opposed to *Marcos et al.* reported studies, which included chronic alcohol users with/without alcoholic liver disease also cirrhosis [Marcos, 2011]. Analysing *GSTM1* genotype assotiation with biochemical parameters were found its association with elevated bilirubin level (total bilirubin and direct bilirubin). *GSTM1* null genotype is associated with neonatal hyperbilirubinemia that is explained by the similarity of GSTM1 and GSTA1 ensymes and their conjugation with bilirubin [Muslu, 2008]. In adulthood GSTM1 relationship with bilirubin are described in relation to the use of hepatotoxic drugs.

In *Marcos et al.*, conducted meta– analysis study was not found association between *GSTT1* null genotype and alcohol induced liver injury [Marcos, 2011]. Our study found *GSTT1* null genotype assotiation with acute toxic hepatitis comparing patients and control group, it might be related to toxic

additives in illegal alcohol, as one of the GSTT1 specific substrates is benzene [Qu, 2005], which can be formed in different drinks containing sodium benzoate and ascorbic acid, as well as the selected population size. *GSTT1* genotype in our study group was also associated with reduced levels of prothrombin, that could be possible explain that *GSTT1* null genotype promotes to more severe liver damage formation and prothrombin is used as a marker of liver damage in liver failure [Robert, 1996]. Relationship between *GSTT1* null genotype and liver damage are described in relation to medication induced idiosyncratic failure, but the relationship with the prothrombin levels were not examined in reported case [Lucena, 2008].

GSTT1 null genotype were also assotiated with the increased levels of hyaluronic acid that is marked as a good marker for the development of fibrosis in hepatitis C virus [Avola, 2010]. Our study group had no information on liver fibrosis because patients were hospitalized with acute toxic hepatitis, and liver biopsy was not available, because of patients status (acute liver failure) therefore it was not possible to assess the *GSTT1* null genotype assotiation or roll in liver fibrogenesis assotiated with alcohol use.

NAT2 gene polymorphisms are determing slow or rapid acetylation of xenobiotics [Sim, 2008]. In our study were analysed three polymorphisms, one of which was exluded from further studies because minor allele frequency in the population is < 0.05. Comparing the frequencies of alleles between patients and the control group were not found statistically significant differences, but when comparing patients with lethal and nonlethal outcome there were more frequent G allele of A590G polymorphism in lethal outcome group (OR = 2.34, p = 0.045), which provides a slow acetylation. Analysing haplotypes of the NAT2 gene there were found assotiation with haplotype that determines the normal acetylation (p = 0.045), although the C481T allele is involved in haplotype determining the slow acetylation. Described above, the slow acetylation is more common in alcohol users who have not developed severe

liver damage – cirrhosis [Rodrigo, 1999], which coincides with our research group carried out haplotype analysis, but contrary to the association of individual alleles obtained in our study. To determine the slow acetylation possible association with liver damage was performed by linear regression analysis patients with slow acetylation (associated with A590G polymorphism) in our study population is a statistically significant association with elevated liver injury indicators – GGT and alkaline phosphatase ($p < 0.05$) compared with normal acetylation, hence a more pronounced liver damage, which explains the slow acetylation alleles an accompanying increased presence of dead patients in our study.

Haplotype including C481T allele determines slow acetylation however its individual impact on acetylation is conflicting [Agundez, 2008]. Our study shows that the alleles of C481T and A590G has opposite activity, both by linear regression analysis, as well as comparing the mean biochemical parameters between different acetylators. Statistically significantly differed GGT and alkaline phosphatase levels between individuals with different genotypes. Both rates were higher in patients with slow acetylation, to support the development of more severe hepatic injury in patients with slow acetylation.

Our study did not found a link between the *UGT1A1* gene polymorphisms that after extensive genome association studies is a major genetic marker associated with bilirubin levels [Lieber, 2000] that could be explained with a small cohort of patients, as well as the fact that ethanol alters the gene *UGT1A1* transcriptional activity [Kardon, 2000], which could compensate for mutations (TA)7 caused reduced expression of the gene that causes benign hyperbilirubinemia [Muslu, 2008].

4.3. Clinical and biochemical indices characterising liver damage in patients with chronic hepatitis C

CHC diagnosis is corfimed if there are detected anti–HCV and HCV – RNA at least for 6 months, but in order to determine liver damage biochemical tests and a liver biopsy are carried out [EASL guidelines, 2011]. Although there are several non–invasive techniques investigated for liver injury determination (Fibrotest HCV Fibrosure, ActiTest [Poynard, 2004]), that is important to choose further tactic, but in Latvia "gold standard" for liver damage determination still remains liver biopsy [Santantonio, 2008; EASL guidelines 2011]. For the study population there were available such already known risk paramaters that is important in CHC as GGT, ALT and also patients' gender .

For HCV infection becomes chronic, one of the risk factors are male gender [Massard, 2006; EASL guidelines, 2011]. Worse prognosis is associated with the fact that women with estrogen inhibits the stellata cell division and fibrogenesis as well as it affects transforming growth factor and synthesis of inflammation mediators that influence the development of fibrosis in the liver cells. That leads to ten times faster development of cirrhosis in CHC males than females [Massard, 2006]. To determine whether males had worse biochemical parameters at diagnosis moment in our study, they were compared between both genders. Statistically significant differences were found in levels of ALT, GGT, iron, hemoglobin and red blood cell count. The fact that for males statistically significantly higher were ALT and GGT levels may indicate a more pronounced liver damage in males, which coincides with the previously described [EASL guidelines, 2011], but compared HAI index, which is morphologically characterising inflammation of the liver cells, statistically significant differences were not found ($p = 0.565$). Iron level difference is explained by the fact that women during menstruation have iron loss, so it is a

lower level [Zeuzem, 2000], although the average values of both sexes joined the iron levels in the normal range (6.6 to 28.3 μmol/l). For red blood cell count and hemoglobin levels are known gender differences, so also are used seperate reference intervals for both genders. There are described changes in the whole blood of CHC cases that notes reduced neutrophil and platelet counts [Streiff, 2002], the following changes were not assessed in this study group, as analyzed were only the absolute numbers of white blood cells and relative neutrophils proportion (both paramaters were in reference range) and were not available full blood count data in the control population.

Among the different virus genotypes most significant differences are listed in the first/second and third viral genotype which typical marked fibrosis of the liver due to steatosis [Manns, 2006]. Comparing patients with third virus genotype with patients who had other genotypes statistically significant higher levels were for cell cytolysis characterising paramaters ALT ($p = 0.006$), AST ($p = 0.009$) and cell apoptosis characterising paramteres – cytokeratin–18 levels ($p = 0.005$). HCV has an important role in the pathogenesis of apoptosis for liver cells (encouraging the development of more severe hepatic injury) and leukocytes (more to the effectiveness of treatment – viral persistence) [Parfieniuk, 2013]. For that reason are analysed also apoptosis biochemical markers as cytochrome C and cytokeratin–18. Cytochrome C characterize non–specific tissue cell death, and cytokeratin – 18 is more specific on liver cell apoptosis and not mesenchymal –cell apoptosis. Liver cell apoptosis or necrosis increases ALT, AST (non–specific markers for cell death, not separating apoptosis or necrosis scores) and GGT (more specific enzyme in the liver cells, its level may increase either by the use of alcohol [Perrillo, 1978] or destruction of liver cells). Therefore, comparing the data may be determined that exposure to the third virus genotype is more marked hepatocellular apoptosis, accounting for more severe liver damage, corresponding to the above described [Poynard, 2003]. In our study group for patients with

morphologically confirmed liver damage – cirrhosis statistically significantly elevated were ALT, AST, GGT, and cytokeratin–18 levels, as well as hyaluronic acid ($p < 0.05$). Hyaluronic acid is synthesized in all tissues, the liver it is synthesized by Ito cells. Its levels are within the normal range in the absence of liver cell damage, but it's increase correlates with the severity of liver tissue fibrosis and this correlation is not pathogen–specific [McHutchinson, 2000].

Evaluating the obtained findings, it can be concluded that in addition to the traditionally used indicators of liver injury (ALT, AST, GGT) also in Latvia diagnostically important could be to determine hyaluronic acid and cytokeratin–18 level, which specifically characterize liver cell death and inflammatory processes.

The study group were compared with biochemical data before initiating therapy in patients with persistent virus and achieved SVR after antiviral treatment. Statistically significant differences were found for biochemical indicators of liver damage – GGT ($p < 0.001$), indicator for fibrosis – hyaluronic acid ($p = 0.021$), indicator for inflammation – white blood cell count ($p = 0.021$) and also when comparing morphological examinations – separate assessment of fibrosis ($p = 0.003$) and the total HAI ($p = 0.027$) showed statistically significant differencies. From tbiochemical parameters only white blood cell count was higher in patients achieving SVR, while other indicators were higher in patients with persistent virus after antiviral therapy. The higher white blood cells count provides better immunity which ensure achieving SVR and milder liver damage because the virus promotes apoptosis of liver cells and white blood cell [Parfieniuk, 2013]. Results in our study group is similar as it described previously – the antiviral therapy efficacy depends on liver fibrosis and inflammatory tissue development [EASL guidelines, 2011].

As a risk factor for treatment failure is referred male gender and older age [Manns, 2006; EASL guidelines, 2011], our study population was derived

almost statistically significant close relationship with the male gender (OR = 1.49, p = 0.077), but compared to the age of disease diagnosis, was found no statistically significant relationship – patients with SVR had mean age of 37.75 ± 11.94 years, but with persistent virus 38.98 ± 11.94 years (p = 0.545). A possible explanation could be that the patients were in the same age group, the population had a similar gender distribution, as well as the relatively small study group.

According to the literature, the SVR is achieved in 40–50% of patients with genotype 1 and ~80% of the genotypes 2 and 3 [Bosma, 1995]. In our study population effective therapy was for 57.3% of patients with genotype 1, but 15.5% were totally non–responders (that was reason for therapy discontinuation) and 27.2% of patients had relapse of the virus after 6 month of therapy were finished. In the study group of patients with other viral genotypes were not identify total non–responders and 16.7% had virus relapse after antiviral therapy were finished, the data coincides with the literature data [Bosma, 1995]. Higher SVR could be reached if in therapy is used protease inhibitors than there is possible to achieve up to 95% SVR in patients with viral genotype 1 [Chae, 2013].

4.4. Analysis of the genetic marker in chronic hepatitis C patients and its impact on disease

HCV infection are being sought better markers in order to predict more preciece antiviral treatment response and prognosis. As possible markers are analysed different biochemical and genetic markers. As the patient's immunity plays a role in persistence of infection, then studied are the *HLA* genes influence the development of CHC [EASL guidelines, 2011]. In Latvia were identifyed risk alleles of HLA class in haemophiliac patients infected with

CHC, it concluded that the *HLA* allele DRB1*07 is frequently found in patients were infection becomes chronic [Simanis, 2008] as well as CHC patients [Jeruma, 2012]. *HLA* markers were not included in the thesis.

In recent years widely are studies *IL28B* polymorphism, which allows accurately predict response to combination antiviral therapy (pegylated interferon and ribavirin) if the patient is infected with HCV genotype 1 [8Esteban, 2008; Wiegand, 2008; Tanaka, 2009]. There were done study in Latvian population which also confirmed that the *IL28B* polymorphism plays a role in the effectiveness of therapy in patients with viral genotype 1 [Tolmane, 2012], but these findings are not included in clinical guidelines because in Latvia there is no alternative therapy available yet, for that reason determination of *IL28B* allele could be used as possible predictive factor.

In the study were studied polymorphisms in genes, that products are involved in iron (*HFE* gene), copper (*ATP7B* gene) and bilirubin (*UGT1A1* gene) metabolism, as well as del32 polymorphism (*CCR5* gene), which alters leukocyte CCR5 receptor synthesis and also null genotypes of *GSTM1* and *GSTT1* genes that are associated with oxidative stress in the cell, which may affect the clinical course of CHC. The following are described separately for each of the analyzed polymorphisms.

In case – control analysis of the study group, a non–null *GSTM1* genotype was more frequent in CHC patients (OR = 1.487, p = 0.0052). The control group were not age and sex matched, but whereas these markers are located in autosomes, the result obtained from control population was used to determine the genetic markers of the incidence in the general population. About *GSTM1* null genotype assotiation with the development of CHC are published only a few studies, although it has been demonstrated the role of oxidative stress in HCV–induced liver damage development [Koike, 2006], as well as fact that HCV changes glutathione peroxidase activity in the body [Martinez, 2007] and the GSTM1 and GSTT1 ensymes have glutathione peroxidase

activity [Hurst, 1998]. Previously published study, which analyzed 135 CHC patients were found to be common GSTM1 null genotype in CHC patients, rather than as in our case a non–null genotype [Martinez, 2007]. The difference is explained by the null genotype frequency in different populations [Josephy, 2010].

The analysis of *GSTM1* null genotype impact on the effectiveness of antiviral therapy, showed no statistically significant differences between patients who achieved SVR and who not. The analysis of early viral response, *GSTM1* null genotype was less common in patients with persistent virus after antiviral therapy (OR = 0.4949, p = 0.052). The explanation could be that the *GSTM1* null genotype is associated with higher oxidative stress in hepatocytes affected by HCV, which causes more damage to the liver and, in turn, the liver damage is one of the risk factors for worse response to antiviral therapy [EASL guidelines, 2011].

Reasoning that in our patient group *GSTM1* null genotype is associated with liver cell damage, demonstrated by the fact that the *GSTM1* non–null genotype is related to the HAI (BETA = –0.7737, p = 0.0083), and cytokeratin–18, which is characterising by hepatocyte apoptosis (BETA = 86.41, p = 0.0153) – that would meant that the null genotype protect against apoptosis of hepatocytes, and promoting a different type of liver cell damage, such as inflammation and necrosis (as it positively correlated with the HAI). In order to explain and verify the results, it should be checked in larger population, as currently it is only possible to compare one study which was not found association with biochemical and morphological data CHC patients [Martinez, 2007]. On the other hand, conducted a meta– analysis study, which analyzed hepatocellular carcinoma relationship (one of the most common cause of it is CHC) with *GSTM1* null genotype, were included 3 studies of Europeans descent populations (443 patients, the control group – 408 individuals). It was obtained that *GSTM1* null genotype was less common in the patietnts with

hepatocellular carcinoma (OR = 0.79, p = 0.042, CI 95% 0.50–1.24). One study discovered that *GSTM1* null genotype has a direct assotiation with the CHC induced hepatocellular carcinoma pathogenesis, but the Indian population revealed that *GSTM1* null genotype has protective effect against the development of hepatocellular carcinoma [Kiran, 2008]. These results can be compared to those gained in our study, *GSTM1* genotype has been linked to liver damage in CHC, but further research is needed to better characterize their role.

Like GSTM1 also GSTT1 is involved in the regulation of cell oxidative stress and has glutathione peroxidase activity [Hurst, 1998]. In case – control analysis of *GSTT1* null genotype it was found to be more common in CHC patients (OR = 1.621, p = 0.0226), similar to that described in the Spanish population [Martinez, 2007], but analysis of the relationship with response to antiviral therapy a statistically reliable results were not obtained ($p > 0.05$). It was also found association with biochemical or morphological data in CHC patients. One of the explanations that the relationship to the biochemistry parameters in CHC patients are unable to find is offered by *Martinez S et al.* – that the GSTT1 and GSTM1 plays a role in the early development of the infection but not when infection becomes chronic [Martinez, 2007]. To confirm this, it is necessary to increase the investigational group, because there are only a few studies that examine this issue.

del32 allele in the gene *CCR5* was first described in connection with the human immunodeficiency virus infection, because the *CCR5* gene is encoding one of the lymphocyte receptors through which human immunodeficiency virus can infect lymphocytes and is therefore referred to as "human immunodeficiency virus resistance causing mutations" [Curiel, 1989]. Further studies revealed that this mutation although contribute to the development of HCV infection [Woitas, 2002]. There have also been studies in which this association was not found [Tommasi, 2006]. In our study population made case

– control study were found del32 allele higher rate in CHC patients (OR = 1.675, p = 0.0026), indicating that del32/del32 is the risk genotype of HCV infection.

Explanation of found assotiation could be that CCR5 is involved in the immune response, which is of importance for the infection persitantance as well as antiviral treatment efficacy. *CCR5* gene encoding a chemokine receptor and chemokines are hemostatistic cytokines determining cells migration to the site of inflammation. CCR5 chemokine is one that is important for HCV progression and pathogenesis [Zeremski, 2011]. CCR5 chemokine synthesis reinforce HCV infected cells, and there is demonstrated that they contribute to the development of fibrosis in the liver cells affected by HCV [Zeremski, 2007]. In our study group linear regression analysis in order to determine the association with biochemical and morphological characteristics of our patient population however statistically significant assotiation were not found (p> 0.05).

Compared with patients to achieve SVR, del32 was more frequent in patients with ineffective therapy (OR = 3.057, p = 0.0002) and more frequent in patients with no response to antiviral therapy (OR = 3.165, p = 0.0012), which was more pronounced in males (OR = 4.492, p = 0.00098). The results are contrary to those described previously [Glas, 2003], but the above negative associations with treatment outcome were found in smaller groups of patients (n = 78 [Ahlenstiel, 2003], n = 59 [Glas, 2003]), one of the studies were described in the del32 mutation has an effect in monotherapy with ribavirin [Ahlenstiel, 2003].

Although the role of chemokines is demonstrated in HCV infection, however, there are relatively few published studies on the most common mutation in the *CCR5* gene that affects the protein synthesis and CVH clinical course and the response to antiviral therapy, so it would need to continue to study most patients.

ATP7B gene coding product is involved in copper metabolism and mutations in this gene is causing autosomal recessive metabolic disease – Wilson's disease [Mura, 1997]. The study included H1069Q mutation analysis. Although its incidence is less than 0.05, it was included in the analysis because it leads to metabolic disease and its carrier frequency Latvian is 1:80, which is slightly higher than the average in Europe is marked – 1:90 [Krumina, 2008].

In the association study, a statistically significant association was detected only by virus persistence (not reached SVR) in males (OR = 6.2, p = 0.045). Wilson's disease is described in copper accumulation differences between the sexes [Mura, 1997], but women have a better response to antiviral therapy in conjunction with estrogen synthesis [Bosma, 1995; EASL guidelines 2011]. The resultant of our result can be explained by the fact that the mutation leads to increased accumulation of copper in liver tissue and also more pronounced inflammation of the liver [Marikovsky, 2002], and it is one of the risk factors of antiviral treatment efficacy [EASL guidelines, 2011], but in our surveyed population H1069Q mutation was not associated with any of the inflammation or fibrosis indicators.

In connection with the clinical course of the CHC publications suggests that the Wilson disease causing mutations and causes some protection against HCV infection were included in the study, 60 molecular approved Wilson's disease patients and 94 individuals who are mutation carriers in the *ATP7B* gene [Liggi, 2012]. The role of chronic copper CHC course being studied [Ko, 2005], because the copper in the body involved in the regulation of immunity, although the exact mechanism is still unknown [Lucena, 2008]. And it is known that CHC patients are found elevated copper levels, but more is not related to the accumulation of copper, but with the release of hepatocyte apoptosis during [Guo, 2013], but the increase was more pronounced exactly in the acute infectious process [Rashed, 2011].

The literature refers to the conflicting data on copper and Wilson's disease association with CHC, which would be necessary to study the largest population , including the power level of CHC patients. In the power level to reduce the availability of effective drugs such as D– penicillamine.

SERPINA1 gene alleles PIS and PIZ is causing inherited metabolic diseases – alpha 1– antitrypsin deficiency which is traditionally characterized by a childhood hepatitis and the development of emphysema in adulthood [Chappell, 2008], but subsequent studies have shown that it can cause liver damage in adulthood , but mutation carriers develop liver damage ~66 years of age [Topic, 2012]. Both the mutation frequency of CHC patients and the effect on clinical symptoms was analyzed, despite the fact that the mutation frequency of < 0.05. PIZ mutation were included in the study because of pre–existing mutant frequency studies have shown that it is very common in Latvian population [Lace, 2008].

In association analysis, a statistically significant correlation was found between the PIS mutation and null response to the antiviral response (OR = 7.118, p = 0.0254, CI 95% 0.9705–52.2), which was more reliable in women (OR = 10.82, p = 0.042, CI 95% 0.632–185.1). Analysis of the relationship with biochemical parameters were found statistically significant association with the characteristics of a cell apoptosis (cytochrome C) and fibrosis (with hyaluronic acid and morphological examinations). The variability should be interpreted with caution because of both mutation frequency is < 0.05 and the association could be found to occur only due to the small number of patients (indicated by the wide 95% confidence interval), but they explain each other, the more inflammation and scarring of the liver is associated with a poorer response to antiviral therapy [Bosma, 1995; EASL guidelines, 2011]. The association with alpha 1–antitrypsin deficiency and the clinical course of CHC has been little studied and are obtaining conflicting results [Settin, 2006; Gharib, 2011].*SERPINA1* gene mutations may play a role in the pathogenesis

of CHC, as has been demonstrated altered alpha 1–antitrypsin synthesis of HCV infection – it is reduced, and it is not associated with the most common mutation – PIZ and PIS [Elzouki, 1997], so it would be necessary to study the largest population and the including antitrypsin level.

CONCLUSIONS

1. More severe alcohol induced liver damage is characterised by elevated prothrombin and cytochrome C level in serum.
2. More sever clinical outcome in acute toxic alcohol induced hepatitis is determined by polymorphisms in the genes *NAT2* and null genotypes in the genes *GSTT1* and *GSTM1*, while for functional polymorphisms in the genes *ALDH2*, *UGT1A1*, *GSTA1*, *GSTP1* and *MTHFR* has no effect.
3. Antiviral therapy efficiency in case of chronic hepatitis C is influenced by liver damage stage when therapy is started – if it is milder (characterised by hyaluronic acid, cytokeratin–18, leukocyte count and liver biopsy data) there is higher possibility to reach SVR.
4. Genetic markers is influencing chronic hepatitis C pathogenesis and antiviral therapy efficiency:
    a. chronic hepatitis C genetic risk factors are null genotypes in the genes *GSTM1* and *GSTT1* and del32 allele in the gene *CCR5;*
    b. antiviral treatment efficiency risk factor is del32 allele in the gene *CCR5;*
    c. liver damage in chronic hepatitis C patients is influenced by null genotype in the gene *GSTM1* and functional alleles in the genes *UGT1A1* and *SERPINA1;*
    d. liver damage in chronic hepatitis C patients is not influenced by functional polymorphisms in the genes *HFE, ATP7B.*

APPROBATION OF THE PhD THESIS

Scientific publications:

1. **Piekuse L**., Lace B., Kreile M., Sadovska L., Kempa I., Daneberga Z., Mičule I., Sondore V., Keiss J., Krumina A. Impact of the genes UGT1A1, GSTT1, GSTM1, GSTA1, GSTP1 and NAT2 on acute alcohol–toxic hepatitis. // Central European Journal of Biology, 2014, 9(2): 125–130. doi: 10.2478/s11535–013–0249–y .
2. **Piekuse L**., Kreile M., Zarina A., Shteinberga Z., Sondore V., Keiss J., Lace B., Krumina A. Association of inherited liver disorders with chronic hepatitis C. // World Journal of Hepatology, 2014, 6(2): 92–97. doi: 10.4254/wjh.v6.i2.92.
3. Krumina A., Keiss J., Sondore V., Chernushenko A., Cernevska G., Zarina A., Micule I., **Piekuse L.**, Kreile M., Lace B., Krumina Z., Rozentale B. From clinical and biochemical to molecular genetic diagnosis of Wilson disease in Latvia. // Genetika, 2008, 44(10): 1379–1384.

Abstracts in the material of scientific conferences:

1. **Piekuse L.**, Kreile M., Lace B., Keiss J., Sondore V., Krumina A. *NAT2* gene polymorphism influence on clinical outcome in acute alcohol induced toxic hepatitis. Proceedings of the Golden Helix Symposium "Genomic Medicine: Translating genes into health", 2012, p23.
2. **Piekuse L**., Keiss J., Sondore V., Micule I., Kreile M., Lace B., Krumina A. Gilbert syndrome molecular diagnostics and clinical characterization in Latvia. European Journal of Human Genetics, 2011, 19 (2) Suppl. 2: p334.
3. Kreile M., **Piekuse L**., Krumina A., Keišs J., Sondore V. Hereditārās hemohromatozes mutāciju biežums C hepatīta slimniekiem Latvijā. Riga Stradins University Scientific Conference, Abstracts, 2011, p. 197.

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6. **Piekuse L**., Keišs J., Sondore V., Kreile M., Lāce B., Krūmiņa A. Ksenobiotiķu atindēšanas enzīmu kodējošo gēnu polimorfismu raksturojums pacientiem ar akūtu toksisku hepatītu. Riga Stradins University Scientific Conference, Abstracts, 2010, p. 89.
7. **Piekuse L**., Kreile M., Zariņa A., Lāce B., Sondore V., Krumina A., Keiss J. Inherited liver disorder impact on CVH infection in Latvia. European Journal of Human Genetics, vol. 18., Suppl.1., 2010, p.265.
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9. Zariņa A., Kreile M., **Piekuse L**., Čerņevska G., Keišs J., Mičule I., Krūmiņa A. Vilsona slimības izraisošās mutācijas H1069Q biežums bērniem vecumā līdz 18 gadiem Latvijas populācijā. Riga Stradins University Scientific Conference, Abstracts, 2010, p. 240.
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