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THE IMMUNOGENETIC ASPECTS OF HIV-ASSOCIATED TUBERCULOSIS

Summary of the Doctoral Thesis
for obtaining the degree of a Doctor of Medicine

Speciality – Infectology

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ABBREVIATIONS USED IN THE SUMMARY

AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
CD4 ⁺	T helper Cell
CD8 ⁺	T suppressor Cell
HIV	Human Immunodeficiency Virus
HIV-1/TB	HIV-1 Infection with Tuberculosis Co-infection
HIV RNS	Viral Load
HLA	Human Leukocyte Antigen
IFN- γ	Interferon- γ
IL-1 β	Interleukin-1- β
IL-10	Interleukin-10
IL-18	Interleukin-18
IRIS	Immune Reconstruction Inflammatory Syndrome
MHC	Major Histocompatibility Complex
NK cells	Natural Killer Cells
PCR	Polymerase Chain Reaction
Th ₀	T helper, which hasn't encountered an Antigen (naive T cell)
Th ₁	Type 1 T helper
Th ₂	Type 2 T helper

INTRODUCTION

HIV-associated tuberculosis is one of the most common opportunistic infections, which affects one third of HIV-1 patients (*WHO*, 2015). HIV-1 infection leads not only to the impairment of T helpers or CD4⁺ T cells, but to exhaustion of all the immune system, disruption of the cytokine profile and development of immune deficiency. It increases the risk of acute tuberculosis several times (*Kaplan et al.*, 2009), which enhances the replication of HIV-1 virus, reducing patient survival (*Goletti et al.*, 1996; *Orendi et al.*, 1998; *Lai et al.*, 2013). In Latvia, the number of HIV-associated tuberculosis cases rose along with the number of HIV-1 cases (*Database of Health and Healthcare Indicators in Latvia*, 2014). Late diagnostics of HIV-associated tuberculosis is acknowledged to be an essential problem, because typical clinical and radiological symptoms of a mycobacterial infection fade during immune deficiency (*Dierberg and Chaisson*, 2013).

The pathogenesis of HIV-associated tuberculosis is defined by various factors. On one hand, the risk of tuberculosis in patients with HIV-1 is associated with a compromised immune reaction, which manifests as decreased CD4⁺ T cell count (*Mendonca et al.*, 2007; *Arnold et al.*, 2015). Decreased CD4⁺ T cell count disorganizes the granuloma, which may be present in the lungs before the virus enters the organism (*Burman and Jones*, 2003; *Sharma et al.*, 2005). Moreover, a low CD4⁺ T cell count cannot ensure a sufficient production of Th₁ cytokines, which is required for the activation of macrophages and the elimination of mycobacteria (*Geldmacher et al.*, 2008; *Kalsdorf et al.*, 2009). However, contrary to other infections, which develop during HIV-1 infection, when CD4⁺ T cell count is under 200 cells/ μ l, tuberculosis may develop even when CD4⁺ T cell count is above 500 cells/ μ l, namely, before the transition to the AIDS stage (*Sonnenberg et al.*, 2005; *Lawn*

et al., 2009). It should be pointed that beneficial conditions for the development of mycobacteria occur also due to increased release of Th₂ cytokines (*Howard and Zwilling*, 1999; *Chinen and Shearer*, 2002; *Kalsdorf et al.*, 2009; *Kanwar et al.*, 2010).

On the other hand, development of HIV-associated tuberculosis is also determined by the patient's genetic factors, for example, Major Histocompatibility Complex (MHC) genes. The studies show that the risk of tuberculosis in patients with HIV-1 is associated with the structural variants of HLA class II genes (*Teran-Escandon et al.*, 1999; *Louie et al.*, 2004; *Selvaraj et al.*, 2006; *Figueiredo et al.*, 2008; *Saikia et al.*, 2015; *Volikova*, 2015). The main explanation for influence of genetic predisposition in the development of infectious diseases is associated with the HLA class II gene function, because the genes are responsible for the presentation of an antigen to the CD4⁺ T cells, thus affecting the efficiency of an immune reaction (*Male et al.*, 2006). However, the previous studies on the role of HLA class II genes in the development of HIV-associated tuberculosis present limited data on the association of HLA class II genes and the immune reaction indicators in humans, for example, CD4⁺ T cell count and a cytokine level.

Another factor, that needs to be considered in the assessment of risk of tuberculosis development, is a viral load (HIV RNA). It is known that HIV RNA differs in females and males at similar stages of HIV-1 (*Gandhi et al.*, 2002). The studies demonstrate that a viral load is the key factor, determining the CD4⁺ cell count and the progression of the HIV-1 infection (*Katzenshtein et al.*, 1996; *Mellors et al.*, 1997). Whereas *Benjamin et al.* (2013) conclude in their study, that, in patients with HIV-associated tuberculosis, a high viral load may be diagnosed irrespective of the CD4⁺ cell count. It means that, in the case of HIV-associated tuberculosis, the viral load can have relatively low predictive value (*Benjamin et al.*, 2013).

The description above demonstrates that the development of tuberculosis during HIV-1 infection is affected by several factors: CD4⁺ cell count, viral load, HLA class II genes, and the cytokine profile. However, it is not clear how exactly the mentioned factors contribute to the development of tuberculosis in the case of HIV-1 infection.

The Aim of this dissertation is to examine the association between the HLA class II gene alleles, the immune status indicators and the viral load in patients with HIV-associated tuberculosis in Latvia.

Research Objectives:

1. To detect the protective and risk alleles of the HLA class II genes DRB1*, DQA1*, and DQB1*, which affect the development of tuberculosis in the case of HIV-1 infection.
2. To assess the differences of the viral load and immunologic indicators among the HIV-1 patients with and without tuberculosis.
3. To assess the mechanisms of protective and risk alleles that affect the immune reaction in HIV-associated tuberculosis.
4. To assess the effect of the immunogenetic parameters on the indicators of the course of HIV-associated tuberculosis.

Novelty of the Dissertation

- Considering the specific nature of the HLA class II gene polymorphism and alleles associated with the predisposition towards infectious diseases in different populations and ethnic groups, those HLA class II gene alleles were identified in this dissertation that affect the risk of tuberculosis in HIV-1 patients in Latvia.

- Specific immune mechanisms that explain the effect of the HLA class II genes on the development of HIV-associated tuberculosis are demonstrated by revealing the association between the gene alleles and CD4⁺ T cell count.
- The associations of the HLA class II genes with the level of IL-10 and with the time period until the manifestation of tuberculosis are demonstrated, which confirms the importance of genetic factors in the development of tuberculosis in HIV-1 patients.

Practical Significance and Recommendations

Five protective and four risks alleles, affecting the development of tuberculosis in the case of HIV-1 infection, are identified in the dissertation. The identified alleles can be recommended for the assessment of risk of tuberculosis in patients with HIV-1 infection in any of its stages among the population of Latvia. Considering the latent course of the HIV-1 infection and its detection in the late stages, HLA class II gene typing and assessment can be recommended right after the confirmation of HIV-1 infection.

In order to minimize the development of tuberculosis, it is suggested to start prophylactic therapy with Isoniasidum regardless of immunological indicators after active tuberculosis is excluded.

Based on the cytokine levels in the sampled population with HIV-associated tuberculosis, assessment of the IL-18 level (in addition to the assessment of the CD4⁺ cell count) can be recommended for the assessment of patient's risk of death.

Structure of Dissertation

The dissertation is written in Latvian. There are eight sections in it: Introduction, Review of Literature, Materials and Methods, Results,

Discussion, Conclusions, References and Appendices. The review of literature describes the mechanisms leading to the impairment of the immune system during HIV-1 infection and HIV-associated tuberculosis, as well as the role of the genetic factors in the development of both infections, which supports the immunogenetic research of HIV-associated tuberculosis. 178 literature sources are used in this dissertation. This work contains 15 figures and 9 tables.

1. MATERIALS AND METHODS

1.1. Research Sample

The study was conducted in the Latvian Centre of Infectious Diseases of Riga Eastern Clinical University Hospital from 2012 to 2016. It was approved by the Ethics Committee of Rīga Stradiņš University (date of the decision: 27/09/2012).

The criteria for inclusion in the study were following:

- 18 years or older;
- confirmed HIV-1 infection and confirmed tuberculosis (*Mycobacterium tuberculosis*) co-infection (for HIV-1/TB group);
- confirmed HIV-1 infection without tuberculosis co-infection (for HIV-1 group).

Pregnant women were not included in the study.

The diagnosis of HIV-1 infection was based on detection of specific antibodies and HIV-1 antigen (4th generation of *ELISA*), specific antibodies to HIV-1 and HIV-2 by using the *Immunoblot* and *Western blot* methods, and p24 antigen confirmed by neutralization assay. Real-time polymerase chain reaction was used for the confirmation of the diagnosis of HIV-1.

The diagnosis of tuberculosis was confirmed by the results of bacteriological examination of biological material, which included findings during microscopy of acid-fast bacteria and positive culture of the *M. tuberculosis* with identification of drug-resistance. Additionally, detection of the DNA of *M. tuberculosis* by real-time PCR method (*GeneXpert MTB/RIF*) was used. Chest x-ray radiography and computer tomography were performed for all patients. In the case of suspected extrapulmonary tuberculosis, other organs' radiography and computer tomography were performed.

The sample consisted of 258 patients with HIV-1 registered in the Outpatient Department of the Latvian Centre of Infectious Diseases of Riga Eastern Clinical University Hospital. Depending on the tuberculosis status, the sample was split into two groups.

The first group consisted of 158 patients aged 23 to 59 years (mean age = 36 ± 7 years) with HIV-1 infection and confirmed tuberculosis (HIV-1/TB group). It included 46 women aged 25 to 54 years (mean age = 35 ± 7 years) and 112 men aged 23 to 59 years (mean age = 36 ± 7 years). There was no statistically significant difference between the age of men and women ($t = 1.34$; $p = 0.18$). At the beginning of the study, stage C II – C III HIV-1 infections were detected in the HIV-1/TB group (*Revised Classification System for HIV Infection and Expanded Surveillance Case Definition for AIDS Among Adolescents and Adults*, 1993).

Tuberculosis in patients with HIV-1 infection (HIV-1/TB group) was diagnosed from 2012 to 2016 within the period of 0 to 16 years since the registration in the HIV-1 register. The average period since the patient was registered in the HIV-1 register until establishing the tuberculosis diagnosis was 6 ± 5 years. Unilateral or bilateral pulmonary tuberculosis without impairment of intrathoracic lymph nodes and pleuritis was detected in 48% of the patients. Pulmonary tuberculosis with pleuritis and impairment of intrathoracic lymph nodes, extrapulmonary and generalised tuberculosis constituted 52% of HIV-1/TB cases.

All patients in HIV-1/TB group received antiretroviral and antituberculous therapy according to the clinical guidelines for diagnostics, treatment and prevention of HIV-1 infection (*European Guidelines for treatment of HIV infected adults in Europe*, 2015). Considering the progressive course of HIV-1 infection, by 1 January, 2016, 33 patients in the HIV-1/TB group (21 %) had deceased due to tuberculosis or other complications caused by HIV-1.

The second group (HIV-1 group) consisted of 100 patients aged 18 to 48 years (mean age = 28 ± 7 years) with stages A I - A II of HIV-1 infection without tuberculosis. Among them, 30 were women and 70 – men (Figure 1.1).

Figure 1.1. **Number of patients in the HIV-1/TB and HIV-1 group**

Venous blood samples were taken from all the participants of the study to detect the $CD4^+$ cell count, viral load (HIV RNA) and to perform immunogenetic analysis, which included typing of HLA class II genes in three loci (DRB1, DQA1, and DQB1), and detection of four cytokines: interleukin-1- β (IL-1 β), interleukin-10 (IL-10), interleukin-18 (IL-18), and interferon- γ (IFN- γ).

Typing of HLA class II genes and detection of the cytokines was performed in Joint Laboratory of Clinical Immunology and Immunogenetics of Rīga Stradiņš University. The $CD4^+$ cell count and viral load were detected in the laboratory of the Latvian Centre of Infectious Diseases of Riga Eastern Clinical University Hospital.

1.2. Methods

1.2.1. Detection of the HLA Class II Alleles

To detect HLA class II alleles, 5 ml peripheral blood samples were taken in the tubes with the anticoagulant EDTA, then frozen in -20°C and stored in the laboratory until the testing.

The analysis includes extraction of human DNA from blood and HLA gene typing with low resolution polymerase chain reaction. Chromosomal DNA was extracted from leukocytes, using the commercial *QiaGen* kit for extraction of DNA from blood (*QIAamp® DNA Blood Kit*) according to the methodology approved by the manufacturer (*QIAamp DNA and Blood MINI Handbook*, 2016). The gene typing method includes detection of 13 HLA-DRB1* gene alleles, 8 HLA-DQA1* gene alleles, and 12 HLA-DQB1* gene alleles, using specific primers according to the methodology instructed by the manufacturer (*DNA-Technology*). The gene amplification was performed using a programmed thermocycler (*DTLite, DNA-Technology*).

1.2.2. Detection of Cytokine Levels

To detect cytokine levels blood serum was used, then frozen at -20°C and stored in the laboratory until testing. The cytokine levels were detected, using a solid-phase enzyme-linked immunosorbent assay. To detect each cytokine individual *IFA-BEST* (*INF- γ* , *IL-1 β* , *IL-18*, and *IL-10*) reagent kits were used according to the methodology of the manufacturer.

1.2.3. Establishing the CD4⁺ Cell Count

The CD4⁺cell count was detected, using the reagent kit *CD4 % easy count kit* according to the methodology of the manufacturer. The prepared blood samples were analysed with *Partec CyFlow*.

1.2.4. Assessment of the Viral Load

To detect HIV-1 viral load, blood plasma was used and analysed with *COBAS® AmpliPrep/COBAS® TaqMHIV-1 Test, version 2.0*. HIV-1 RNA was extracted from the blood samples, using *AmpliPrep/COBAS® TaqMan® Specimen Pre-Extraction Reagent (SPEX) kits*. The detection of viral load was automated and performed, using *COBAS® TaqMan® Analyzer*. The detection range of virus RNA is 20–10,000,000 copies/ml.

1.2.5. Statistical Analysis of Data

The statistical analysis of data was performed in the programs *IBM SPSS* (version 22) and *Excel 2010 (Microsoft)*.

The comparison of the allele prevalence observed in the study groups was performed, using Pearson's chi-squared test. In the cases, when the expected incidence in one of the columns was less than five, Fisher's exact test was used to assess the statistical significance of the differences. Odds ratio (OR) was calculated, using the Cochran-Mantel-Haenszel (CMH) test. If the observed frequency equalled zero in at least one column, the Haldane's correction factor of 0.5 was used.

Conformity of the results to the normal distribution was tested with the Kolmogorov-Smirnov test and the Shapiro-Wilk test. Correlations were calculated, using the Spearman rank correlation coefficient. Logistic regression was used to differentiate the HIV-1 patients with and without tuberculosis.

2. RESULTS AND DISCUSSION

2.1. Analysis of Polymorphism of HLA Class II Genes

As a result of the HLA class II gene typing, 30 alleles were identified in the DRB1*, DQA1* and DQB1* loci. Then prevalence of the individual alleles was compared between the two study groups (the patients with HIV-1/TB vs. the patients with HIV-1). The results of the analysis show a statistically significant association between the individual alleles and development of tuberculosis in patients with HIV-1 infection (Table 2.1).

Table 2.1

Distribution of protective and risk alleles in HIV-1/TB (n = 158) and HIV-1 (n = 100) group

Alleles	HIV-1/TB Rel. freq.	HIV-1 Rel. freq.	χ^2	<i>p</i>	OR	OR 95% CI	<i>p</i>
HLA-DRB1*01	0.13	0.21	5.69	0.02	0.56	(0.35; 0.91)	0.02
HLA-DQA1*01:01	0.19	0.29	6.96	0.01	0.57	(0.38; 0.87)	0.01
HLA-DQA1*01:02	0.11	0.19	5.77	0.02	0.55	(0.33; 0.90)	0.02
HLA-DQA1*01:03	0.07	0.02	6.88	0.01	3.85	(1.31; 11.29)	0.01
HLA-DQA1*05:01	0.35	0.27	3.91	0.049	1.48	(1.01; 2.19)	0.048
HLA-DQB1*02:01	0.23	0.14	5.67	0.02	1.78	(1.10; 2.87)	0.02
HLA-DQB1*04:01	0.16	0.03	22.11	<0.001	7.16	(2.80; 18.30)	<0.001
HLA-DQB1*05:02	0.03	0.09	9.35	0.002	0.30	(0.13; 0.67)	0.004
HLA-DQB1*06:02	0.10	0.24	18.93	<0.001	0.34	(0.21; 0.56)	<0.001

Rel. freq. – relative frequency. χ^2 – chi-square. OR – odds ratio. CI – confidence interval

The comparison of the groups revealed that alleles HLA-DRB1*01, HLA-DQA1*01:01, HLA-DQA1*01:02, HLA-DQB1*05:02, and HLA-DQB1*06:02 are less common among patients with HIV-1 infection and

tuberculosis than in HIV-1 patients without tuberculosis. It indicates that the mentioned alleles have a protective effect against tuberculosis during HIV-1 infection. Whereas alleles HLA-DQA1*01:03, HLA-DQA1*05:01, HLA-DQB1*02:01, and HLA-DQB1*02:01 are identified as risk factors, because they are more common among patients with HIV-1 infection and tuberculosis than in those without tuberculosis. No association was detected between the other HLA class II alleles and predisposition or resistance to tuberculosis during HIV-1 infection.

After sorting the *protective* factors according to their odds ratio effects, the list of alleles in descending order of their protective effect is following: HLA-DQB1*05:02 allele has the greatest protective effect (3.3 times lower risk), followed by HLA-DQB1*06:02 allele (2.94 times lower risk), then HLA-DQA1*01:02 (1.82 times lower risk), HLA-DRB1*01 (1.79 lower risk), and HLA-DQA1*01:01 (1.75 times lower risk) (Figure 2.1).

Figure 2.1. Assessment of risk and protective alleles of HLA class II genes

The protective allele effects are in blue and the risk allele effects are in red.

The negative numbers indicate risk reduction

Similarly, the *risk* factors are sorted according to the odds ratio effects. The greatest risk of development of tuberculosis for patients with HIV-1 infection is associated with HLA-DQB1*04:01 allele (7.16 times higher risk), followed by HLA-DQA1*01:03 allele (3.85 times higher risk), then HLA-DQB1*02:01 (1.78 times higher risk), and HLA-DQA1*05:01 (1.48 times higher risk).

The factors of genetic predisposition to tuberculosis was previously studied in HIV-1 patients among populations with high prevalence of HIV-1 and tuberculosis co-infection in Brazil, Mexico, Zimbabwe, Southern and Northern India (*Teran-Escandon et al.*, 1999; *Louie et al.*, 2004; *Figueiredo et al.*, 2008; *Selvaraj et al.*, 2008; *Saikia et al.*, 2015), as well as in a geographic region closer to Latvia – Ukraine (*Volikova*, 2015). The effects of the alleles identified in the dissertation in regards to tuberculosis are in accordance with the result acquired in the Ukrainian patient group (*Volikova*, 2015). Among Latvian and Ukrainian patients HLA-DRB1*01 allele has a protective effect. It is possible that this allele is important in reducing the risk of predisposition to tuberculosis among the HIV-1 patients in Eastern Europe.

The effects of alleles HLA-DQB1*05:02 and HLA-DQB1*06:02 did not conform to the previous studies. In the dissertation, both alleles are identified as protective, whereas they were risk factors among the Brazilian and Northern Indian patients (*Figueiredo et al.*, 2008; *Saikia et al.*, 2015). The discovered differences can be explained by the distinct genetic profiles of the populations.

2.2. Comparison of Viral Load and Immunologic Parameters in HIV-1/TB and HIV-1 Groups

Statistically significant differences were discovered between the HIV-1/TB and HIV-1 groups. The viral load (HIV RNA) is higher in the HIV-1/TB group than in the HIV-1 group (Table 2.2).

Table 2.2

Comparison of HIV RNA, CD4⁺, interleukins and IFN- γ in the HIV-1/TB and HIV-1 groups

Measurements	HIV-1/TB (n = 158)		HIV-1 (n = 100)		Mann-Whitney U value
	M	SD	M	SD	
HIV RNA, copies/ml	$7.44 \cdot 10^5$	$1.11 \cdot 10^6$	$8.11 \cdot 10^4$	$3.40 \cdot 10^5$	3313.00***
CD4 ⁺ , cells/ μ l	171.53	149.64	694.57	202.58	223.00***
IL-1 β , pg/ml	1.83	4.50	4.94	5.32	4234.00***
IL-10, pg/ml	12.51	9.57	11.00	8.95	6950.50
IL-18, pg/ml	572.16	276.88	606.46	274.07	7316.50
IFN- γ , pg/ml	10.21	29.03	4.22	10.28	7638.50

M – mean value. SD – standard deviation. *** p < 0.001

On the contrary, the CD4⁺ cell count is lower in the patients with HIV-1 and tuberculosis infections than in the patients with HIV-1 infection without tuberculosis. Whereas only a single biomarker out of four cytokines has shown a difference: the mean levels of IL-1 β are in average 2.5 times lower in the patients with HIV-1 infection and tuberculosis than in the patients with HIV-1 monoinfection.

The CD4⁺ cell count and viral load differences are associated with the different stages of HIV-1 progression between the groups. The higher level of viraemia among the patients in the HIV-1/TB group compared to the HIV-1 group is also associated with the presence of the tuberculosis infection

in the body, which enhances the replication of viruses (*Goletti et al.*, 1996). Similar connections have been discovered also in the study by *Benjamin et al.* (2013).

The analysis of the cytokine profile shows that, regardless of the presence of tuberculosis and the duration of the HIV-1 infection, the characteristics of the cytokine profile in the patient serum is similar. There were no differences in IL-10, IL-18 and IFN- γ parameters between the groups, considering that the cytokine profile in the HIV-1 group was detected at the initial phase of the disease, whereas, in the HIV-1/TB group – after the tuberculosis diagnosis was established and during the antiretroviral therapy. It may suggest the dysfunction of the immune system, maintained by impact of chronic HIV-1 infection and activation of *M. tuberculosis* (*Mihret et al.*, 2014; *Siddiqui et al.*, 2015; *Sullivan et al.*, 2015; *Kassa et al.*, 2016).

There was a difference in IL-1 β levels between the groups. It is one of the most important cytokines responsible for the activation of the antiviral immunity and the elimination of viruses and intracellular microorganisms (*Sharma et al.*, 2005). Therefore, a higher level of IL-1 β among patients in the HIV-1 group than in the HIV-1/TB group indicates activation of the immune reaction in the initial phase of the disease.

A comparison of the cytokine profiles among the HIV-1 patient groups with and without tuberculosis was also performed in the study by *Benjamin et al.* (2013). As in this dissertation, no difference was discovered between the patient groups in terms of IL-10; however, contrary to the results of the dissertation, a higher level of IFN- γ was discovered among the patients in the HIV-1/TB group than in the HIV-1 group (*Benjamin et al.*, 2013). Different results of the studies could be associated with the fact that the HIV-1/TB patients included in the dissertation study had received treatment for HIV-1 infection, whereas, *Benjamin et al.* (2013) tested participants' immune status parameters before the administration of the antiretroviral therapy.

It should be noted that the mean IL-10 parameters (Th₂ cytokine) were higher than the mean IFN- γ parameters (Th₁ cytokine) in both study groups. It might contribute to further development of tuberculosis in HIV-1 patients (Demissie *et al.*, 2004). However, the disease progression was not followed within the framework of this dissertation, considering the cross-sectional design of this study. A similar cytokine profile is characteristic to extrapulmonary or miliary tuberculosis (Sharma *et al.*, 2002; Hasan *et al.*, 2009), which comprised almost half of the cases in the HIV-1/TB group.

2.3. Contribution of Genetic Factors, Viral Load and Immunologic Parameters to the Development of HIV-Associated Tuberculosis

To assess the degree to which the genetic markers, viral load, cytokine levels and CD4⁺ cell count facilitates the development of tuberculosis in the case of HIV-1 infection, hierarchical regression analysis was used in the work. The role of the genetic markers in the differences between the HIV-1 and HIV-1/TB groups was tested at the first step of the hierarchical regression, assessing the protective and risk alleles, and the gender factor. Whereas the second step regression analysis included all the factors (genetic markers, viral load, CD4⁺ cell count, IL-1 β and gender). The results showed that the key variable determining the differences between the HIV-1/TB and HIV-1 groups is CD4⁺ cell count. Whereas the fact that the alleles lost their prognostic significance in the second step of the regression indicate that the effects of the alleles are directly or indirectly associated with the CD4⁺ cell count.

2.4. Association of Factors in the Combined HIV-1/TB and HIV-1 Group

To test the assumption about the direct or indirect association with the CD4⁺ parameters, a correlation analysis was performed. Considering that the conclusion about the allele effects was drawn as a result of the comparison of two patient groups, both group indicators were analysed together. The results confirmed a direct association between the CD4⁺ cell count and the HLA class II gene alleles that have been identified as protective or risk factors (Figure 2.3).

Figure 2.3. Association of the CD4⁺ cell count with other variables in the combined HIV-1 and HIV-1/TB group

The risk alleles are marked red. The protective alleles are marked blue.
The red arrows indicate a negative association between the parameters and the blue arrows – a positive one

A positive association was also found between the CD4⁺ cell count and IL-1 β . A weak negative correlation was discovered between the CD4⁺ and IL-10 parameters. There is a statistically significant negative correlation between the viral load (HIV RNA) and the CD4⁺ cell count.

2.5. Association of Factors in HIV-1/TB Group

The correlation in the HIV-1/TB group is an extra confirmation of the association between the CD4⁺ cell count and the alleles that has been discovered in the combined group analysis (Figure 2.4).

Figure 2.4. Association of the CD4⁺ count with other variables in the HIV-1/TB group

The risk alleles are marked red. The protective alleles are marked blue. The red arrows indicate a negative association and the blue arrows – a positive one

Higher CD4⁺ cell count is positively associated with two protective alleles: HLA-DRB1*01 and HLA-DQA1*01:02. Additionally, a negative association between the CD4⁺ cell count and HLA-DQB1 04:01 allele has been discovered, which is a risk factor. In the previous studies about genetic predisposition to HIV-associated tuberculosis, the HLA class II gene alleles with a risk or protective effect were already discovered in various ethnic groups of patients (*Teran-Escandon et al.*, 1999; *Louie et al.*, 2004; *Figueiredo et al.*, 2008; *Selvaraj et al.*, 2008; *Saikia et al.*, 2015; *Volikova*, 2015). However, the specific immune mechanisms facilitating the resistance to tuberculosis in the case of HIV-1 infection were not described. The discovered correlations between the CD4⁺ cell count and the alleles, in addition to the confirmation of the key role of the CD4⁺ in the regression, confirm that the CD4⁺ cell count is not only a result of a direct and indirect influence of HIV-1 virus (*Ho et al.*, 1995; *Badley et al.*, 2000; *Herbeuval et al.*, 2005), but also a result of the immune reaction regulation, controlled by the HLA class II genes. Depending on the structural variation of the MHC II molecule, determined by the HLA class II allele, the efficiency of the immune response to the antigen is expressed by the CD4⁺ cell count, which, in turn, is the most important factor in the immune response to the tuberculosis.

The results of the study demonstrate that the patients with HIV-1 infection and tuberculosis have a statistically significant negative correlation between the CD4⁺ and viral load parameters. This association conforms to the results of the study by *Benjamin et al.* (2013) and reflects typical association of both parameters in the case of HIV-1 infection (*Mellors et al.*, 1997).

The results of the correlation analysis demonstrate several associations between the alleles and other variables. HIV-1 patients with tuberculosis have higher IL-10 levels, if they have risk allele HLA-DQB1*04:01 in their genotype, whereas lower IL-10 levels have been observed in patients with protective allele HLA-DQB1*06:02 in their genotype (Figure 2.5).

Figure 2.5. Association of the genetic markers with other variables in the HIV-1/TB group

The risk alleles are marked red. The protective alleles are marked blue. The red arrows indicate a negative association and the blue arrows – a positive one

Considering the previously discovered association between a higher level of IL-10 and progression of both infections (*Stylianou et al.*, 1996; *Dlugovitzky et al.*, 1997; *Mihret et al.*, 2014), it can be concluded that the association between IL-10 and the alleles confirms the effect of the genetic factors.

Other associations among the HIV-1 patients with tuberculosis were also discovered in the dissertation. The outcome of the disease, registered as “alive” or “deceased” in the documentation on 1 January, 2016, is associated with two variables. The patients, who were alive in this period, had higher CD4⁺ cell count and lower IL-18 levels.

It should be noted that the age and gender of the patients has practically

no association with the variables analysed in the dissertation. No association was detected between the viral load, CD4⁺, interleukin, and IFN- γ parameters, or the prevalence of the HLA class II alleles.

2.6. Viral Load and Immunologic Parameters in Patients with HIV-1 Infection and Tuberculosis Based on the Disease Outcome

Considering that, on 1 January, 2016, a part of the patients (33 people) from the sampled study population were deceased due to the HIV-associated diseases, including tuberculosis and other complications, the mean parameters were compared between the groups of living and deceased patients to understand the CD4⁺ cell count, viral load, interleukin and IFN- γ levels, which had not been assessed through correlation estimates.

The analysis revealed two statistically significant differences between the groups (Table 2.3).

Table 2.3

Comparison of the Viral Load and Immunologic Parameters in HIV-1/TB Patients Based on the Disease Outcome on 1 January, 2016

Measurements	Alive (n = 125)		Deceased (n = 33)		Mann–Whitney U value
	M	SD	M	SD	
HIV RNA, copies/ml	$7.39 \cdot 10^5$	$1.14 \cdot 10^6$	$7.62 \cdot 10^5$	$1.03 \cdot 10^3$	1846.50
CD4 ⁺ , cells/ μ l	184.05	149.58	124.88	142.51	1454.00*
IL-1 β , pg/ml	2.02	4.88	1.14	2.51	2040.00
IL-10, pg/ml	11.82	8.90	15.14	11.54	1700.50
IL-18, pg/ml	546.86	266.58	667.99	279.94	1566.50*
IFN- γ , pg/ml	9.30	28.50	13.66	31.19	2002.50

M – mean value. SD – standard deviation. * $p < 0.05$

CD4⁺ cell count was lower in the HIV-1/TB patients who had *exitus letalis* by 1 January, 2016, than in those who were alive at that moment. Whereas, IL-18 levels were higher in the deceased patient group than in those alive. There was no statistically significant difference of the viral load or the levels of IL-1 β , IL-1 β and IFN- γ between the groups.

The association between the disease outcome (alive/deceased) and the level of IL-18 is demonstrated both by the difference between the groups and the correlation analysis. It allows considering this parameter as one of the negative prognostic indicators for HIV-1 infection in addition to those known previously (low CD4⁺ cell count and high viral load) (*Mellors et al.*, 1997). This association is in accordance with the results of the study demonstrated the association between the IL-18 level and development of immune reconstruction inflammatory syndrome (IRIS) in HIV-1 patients with tuberculosis (*Oliver et al.*, 2010). One cannot exclude that this indicator was a predictor of IRIS in the HIV-1/TB group, possibly leading to the patients' death. In order to verify the association of IL-18 with the lethal outcome in the case of HIV-associated tuberculosis, further studies are required.

3. CONCLUSIONS

1. The role of polymorphism of HLA class II genes in the development of tuberculosis in patients with HIV-1 infection was confirmed. In Latvia, HIV-associated tuberculosis most frequently develops when the genotype contains alleles HLA-DQA1*01:03 and 05:01, as well as HLA-DQB1* 02:01 and 04:01. The highest risk of HIV-associated tuberculosis is for people with allele HLA-DQB1*04:01. In the case of HIV-1 infection, resistance to tuberculosis is affected by the alleles HLA-DRB1*01, HLA-DQA1*01:01 and 01:02, HLA-DQB1*05:02 and 06:02. The most pronounced protective effect has been detected for allele HLA-DQB1* 05:02.
2. Patients with HIV-associated tuberculosis have higher viral load, lower CD4⁺ T cell count and lower IL-1 β levels than in HIV-1 patient without tuberculosis. Similar levels of IL-18, IL-10 and IFN- γ among patients with and without tuberculosis indicate the dysfunction of the immune system due to chronic HIV-1 infection and activation of tuberculosis.
3. CD4⁺ T cell count as the main factor affecting the co-occurrence of tuberculosis during HIV-1 infection is associated with the HLA class II genes polymorphism. The identified HLA class II risk and protective alleles affect CD4⁺ T cell count in two directions. The presence of the protective alleles in the genotype is associated with higher CD4⁺ T cell count, which enhances resistance to tuberculosis, whereas the risk alleles are associated with a lower CD4⁺ T cell count, which creates beneficial conditions for the development of tuberculosis co-infection. The identified association additionally confirms the potential of HLA class II genes to affect the efficiency of the immune reaction, which is reflected in the CD4⁺ T cell count changes in the case of HIV-1 infection.

4. The HLA class II genes are able to modify the production of IL-10, as well as to affect the viral load and the time until the manifestation of tuberculosis in the case of HIV-1 infection. A higher level of IL-10 was detected in the HIV-associated tuberculosis patients with the risk allele HLA-DQB1*04:01. Whereas lower IL-10 levels were detected when there was the protective allele HLA-DQB1*06:02 in the genotype. Furthermore, a lower viral load and a longer period until the manifestation of tuberculosis was observed in the patients with allele HLA-DQB1*06:02, which additionally confirms the protective effect of this allele.
5. The level of interleukin-18 (IL-18) can be a predictor of negative outcome for HIV-associated tuberculosis. A lethal outcome was observed among the HIV-associated tuberculosis patients with lower CD4⁺ T cell count and higher IL-18 levels.

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