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Associations of genetic polymorphisms with clinical course and mortality in severe sepsis

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List of abbreviations

CI: confidence interval;

DNA: deoxyribonucleic acid;

ELISA: enzyme-linked immunosorbent assay;

H-W: Hardy-Weinberg equilibrium;

ICU: intensive care unit;

IL-10: interleukin 10;

IL-6: interleukin 6;

IQR: interquartile range;

OR: odds ratio;

$PaCO_2$: partial tension of carbon dioxide in arterial blood;

PCR: polymerase chain reaction;

SD: standard deviation;

SIRS: systemic inflammatory response syndrome;

SNP: single nucleotide polymorphisms;

SOFA: sequential related organ failure assessment;

TNF-α: Tumor necrosis factor α;

Introduction

Genetic variability plays an important role in the evolution of sepsis and its response to treatment. A detailed description of the human genome has opened the possibility to identify associations between single nucleotide polymorphisms (SNPs) and disease. Protein-coding genes involved in the innate immune system are potential candidates for influencing the differences in the expression of pro- and anti-inflammatory cytokines and the outcome of disease in individual patients, including the risks for complications [1]. Although several surveys have demonstrated that certain genetic factors might have unfavourable influence on outcome, a major part of the genetic variations have not yet been fully identified [2].

Bacterial products activate the transcription factor kappa beta in the cytosol of immune cells, which triggers the production of tumor necrosis factor-alpha (TNF-α), an initiating cytokine and key mediator of the inflammatory responses in sepsis. Taking into account its role in the pathogenesis [3, 4], genetic variations of TNF-A gene, coding for TNF-α, could potentially influence the clinical course and the outcome of disease. This assumption is supported by the demonstration of significant inter-individual differences in stimulated TNF-α production [5, 6].

The major histocompatibility complex class IV on chromosome 6 harbors 3 TNF genes, as well as several microsatellites. Variations in the TNF-A gene have been intensively studied revealing a relatively constant coding sequence for the gene, but several SNP have been described in the promoter region. Thus, a SNP has been identified in the -308 position of the promoter region (G to A, rs1800629). An association between the TNF -308 A allele and increased production of TNF-α has been demonstrated in lipopolysaccharide-stimulated human blood cells [7, 8], but searches for genetic associations between the TNF -308 A allele and human sepsis have given inconsistent results. Some studies have noticed an association between the TNF -308 A allele and a higher incidence and mortality of septic shock [9, 10]. However, no such association has been demonstrated in septic patients without shock [11, 12].

Interleukin-6 (IL-6) is instrumental in the regulation of innate and adaptive immune responses. The gene coding for IL-6 is localized in chromosome 7 (7p21p14). *In vitro* studies have revealed one single nucleotide polymorphism in the promoter region (-174 G/C, rs1800795) in connection with increased production of IL-6 [13, 14], but contradictory results were demonstrated after endotoxin infusion in healthy volunteers with GG and CC genotypes [15, 16].

The anti-inflammatory cytokine IL-10 is produced mainly in monocytes and macrophages and, to a lesser extent, in lymphocytes and epithelial cells. Its role in the pathogenesis of sepsis has been demonstrated in several clinical investigations [17, 18]. Variations in the production of IL-10 in LPS -stimulated blood cultures have been noticed and associations of IL-10 -1082 A/G (rs1800896) polymorphism with the transcription rate have been described [19].

Up to date genetic association studies in septic ICU patients are limited. No such investigations have been performed in Latvian ICU septic patients.

Study objective

In this study, **the aim** was to determine whether associations exist between promoter polymorphisms of cytokine genes and clinical course and outcome of severe sepsis.

The following **objectives** have been set for achieving this aim:

To assess association of the TNF -308 G/A (rs1800629), IL-6 -174 G/C (rs1800795) and IL-10 -1082 A/G (rs180089) polymorphisms with:

- Serum concentration of the corresponding cytokine
- Incidence of septic shock
- ICU mortality

The Structure and Extent of the study

The dissertation consists of 8 chapters: Introduction, Review of literature, Materials and methods, Results, Discussion, Conclusions, Clinical implications, References. The original version of this thesis is written in Latvian on 119 pages, including 71 tables and 34 figures. The list of references consists of 139 titles.

Approval of the work

Four articles have been published in Latvian peer-reviewed scientific journals. One paper was submitted to an international indexed peer-reviewed scientific journal. A patent on the use of "*Method of evaluating severity of patients' condition*" (G01N35/50 13496, 14.06.2006) was obtained.

A total of 3 presentations have been prepared in relation to the subject in focus of the study. These were presented at different international scientific congresses and published in international indexed journals.

The list of publication is included at the end of summary.

Materials and methods

The study was performed in the Intensive Care Unit (ICU) of Pauls Stradiņš Clinical University hospital, Rīga after being approved by the Ethics committee of the Rīga Stradiņš University (Nr. 53-4/2, 14.02.2008.) and by the Central Medical Ethics Committee of Latvia (Nr. 13, 17.09.2008.).

Patients

103 consecutive patients were enrolled into a prospective observational study fulfilling the criteria of sepsis according to the International Sepsis Definitions [20]. All patients were of the Caucasian race. Patients less than 18 years of age were excluded from the study; as were patients with defined immunodeficiencies and those

who refused to participate. Informed consent was obtained from all the patients or from their next of kin in the case they could not respond on their own behalf.

In short, the sepsis diagnosis was based on the presence more than one of the criteria of systemic inflammatory response syndrome (SIRS) in combination with suspected or proven infection. SIRS criteria are the following:

1) a body temperature more than 38 ^{o}C or less than 36 ^{o}C;

2) a heart rate greater than 90 beats per minute;

3) tachypnea, manifested by a respiratory rate greater than 20 breaths per minute, or hyperventilation, as indicated by a $PaCO_2$ of less than 32 mm Hg; and

4) white blood cell count greater than 12,000/mm^3 or less than 4,000/mm^3 or the presence of more than 10 percent immature neutrophils ("bands").

To meet the criteria of septic shock, a documented systolic blood pressure of less than 90 mm Hg for at least 30 minutes in the absence of other causes of shock and at least 4 hours of inotropic support after adequate fluid replacement were required.

Basic demographic data (age, sex), primary site of infection and organ failure severity (based on the sequential organ failure assessment (SOFA) score) were noticed for all the patients on the day of inclusion into the study. The blood samples for the study of genetic polymorphisms of cytokines and for the investigation of the concentration of cytokines in serum, and for other laboratory tests were obtained during the first 24h after the diagnosis. All the patients were observed until discharge from ICU and the clinical outcome was noticed.

Cytokine serum concentrations

Cytokine serum concentrations (TNF-α, IL-6, IL-10) were determined. Blood was taken by venopuncture and sampled in vacutainers. The blood was then centrifuged and the serum was pipetted and frozen at -70°C until the analyses.

The serum concentrations of TNF-α, IL-6, IL-10 were determined by means of *enzyme-linked immunosorbent assay* ELISA (Biosource, Nivelles, Belgium), according to the manufacturers description.

Gene Polymorphism Analysis

Genomic DNA was extracted from ethylenediaminetetraacetic acid (EDTA) blood. Genomic DNA was extracted from blood sample using standard phenol-chloroform extraction method. Then region of interest was amplified using polymerase chain reaction (PCR). Oligonucleotide primers designed and synthesized for amplification and sequencing reaction are given in

Table 1. The reaction mix for polymerase chain reaction contained 28 ng DNA and 1 Mm oligonucleotides. Reactions were performed in 15 mkL of a 2× PCR solution of MasterMix (*Fermentas*, Lithuania).

Table 1. Sequences of Primers Used to Generate PCR Products

Primer name	Primer sequence	PCR product lenght	Amplified region
TNF-A-308F	5'-ACAGGCCTCAGGACTCAACA-3'		
TNF-A-308R	5'-GCACCTTCTGTCTCGGTTTC-3'	364bp	chr6:31650822+31651185
TNF-A-308seq	5'-AACACAGCTTTTCCCTCCAA-3'		
IL-6-174F	5'-TCGTGCATGACTTCAGCTTT-3'		
IL-6-174R	5'-GCCTCAGACATCTCCAGTCC-3'	328bp	chr7:22539729+22540056
IL-6-174seq	5'-TCATGGGAAAATCCCACATT-3'		
IL-10-1F	5'-TTCCCCAGGTAGAGCAACAC-3'		
IL-10-1R	5'-ATCCTCAAAGTTCCCAAGCA-3'	685bp	chr1:206946348-206947032
IL-10-1rsseq	5'-GATGGGGTGGAAGAAGTTGA-3'		

F = forward primer, R = reverse primer, bp = base pairs

Amplification was performed with an initial denaturation of 95°C for 5 mins, followed by 32 cycles of 95°C for 15 secs, 56°C for 30 secs, and 72°C for 30 secs; the reaction was completed with a final extension step of 72°C for 10 mins.

PCR products were then purified (Sap-Exol) and further investigated by automated direct sequencing using ABI prism 3100 DNA (*Applied Biosystems)* sequencer according to manufacturer recommendations.

Statistical Analysis

Continuous variables were expressed as mean ± standard deviation (SD) if normally distributed or median (interquartile range) if asymmetric distribution was observed. Variables were tested for their association with mortality using Pearson χ^2 or Fisher's exact test (where appropriate) for categorical data (e.g. gender, polymorphisms) and Mann-Whitney U test for numerical data (e.g. age, SOFA, cytokine concentration). Conformity of genotype distribution to Hardy-Weinberg equilibrium was tested with Pearson χ^2 test between observed and expected ($p^2 + 2pq + q^2$, where p and q are frequencies of the common and the rare alleles, respectively). To examine potential associations between the alternative allele and the ratio of unfavorable outcome, allelic distribution was estimated in various genetic models (e.g. allelic, dominant and recessive models, respectively). The risk of lethal outcome resulting from the presence of individual alleles or genotypes was estimated with the odds ratio with 95% confidence intervals. We used SPSS software (version 16, SPSS, Chicago, IL) for statistical calculations. Two-tailed $P < 0.05$ was considered as statistically significant.

Results

Patient population

The initial cohort comprised 103 consecutive patients of Caucasian origin, diagnosed with severe sepsis, according to the American College of Chest Physicians/Society of Critical Care Medicine consensus criteria [21]. Complete clinical information was

available from 99 out of 103 patients. Four patients were not genotyped for TNF -308 G/A polymorphism.

Of the 103 patients recruited, 77 were men and 26 were women, with an age range of 21-83 yrs. A total of 44 patients died during their intensive care unit (ICU) stay (42.7%). Twenty-five patients (24.3%) developed septic shock.

Primary sites of infection were respiratory tract in 71% (n=73), abdominal organs in 26% (n=27) and other locations in 3% (n=3) of the patients. Microorganisms were identified in blood cultures, tracheal secrets or wound smears in 82 patients (80%). The most common Gram positive microorganism was *S. aureus* and correspondingly, the most common Gram negative bacterium was *E. coli* (Table 2).

Table 2. Microbiological characteristic of the study population

Gram-positive	**%**
S.aureus	43
MRSA	22
Enterococcus spp	31
Others	14
Gram-negative	**%**
E. coli	32
K. pneumoniae	24
P. aeruginosa	20
Others	14

As shown in

Table 3, no significant differences in demographical characteristics (age, gender), and length of ICU stay were observed between survivors and non-survivors. A more severe organ failure (SOFA) and higher incidence of septic shock was observed among the non-survivors. Cytokines TNF-α, IL-6 and IL-10 displayed significantly higher values in non-survivors compared with survivors.

Table 3. Demographical, clinical and laboratory characteristics of *survivors* and *non-survivors* of severe sepsis

Variable	Survivors n=59	Non-survivors n=44	P	Statistical test
Males, n (%)	45 (76,3%)	32 (72,7%)	0,7	Pearson χ^2 test
Age, years (± SD)	58 (±14)	59 (±12)	0,6	t-test
ICU LOS (IQR), years	10 (5-14)	15 (3-19)	0,2	Mann-Whitney U test
SOFA score (IQR)	6 (4-8)	9 (6-12)	0,002	Mann-Whitney U test
Septic shock, n (%)	10 (17%)	15 (34%)	0,045	Pearson χ^2 test
TNF-α, median (IQR), pg/ml	26 (18-40)	40 (28-63)	0,002	Mann-Whitney U test
IL-6, median (IQR), pg/ml	190 (95-430)	450 (235-790)	0,003	Mann-Whitney U test
IL-10, median (IQR), pg/ml	1 (1-7)	2 (1-39)	0,027	Mann-Whitney U test

SOFA = Sequential organ failure assessment score, ICU = Intensive care unit; TNF-α = tumor necrosis factor α; IL-6 = interleukin-6; IL-10 = interleukin 10; data given as mean ± standard deviation (SD) or as median and interquartile range (IQR), $P < 0.05$ is significant.

No significant differences in demographical characteristics (age, gender), and length of ICU stay were observed between septic patients with and without shock (

Table 4). A more severe organ failure (SOFA) and higher mortality was observed among the septic shock patients. Cytokines TNF-α, IL-6 and IL-10 displayed significantly higher values in septic shock patients compared with septic patients without shock.

Table 4. Demographical, clinical and laboratory characteristics of septic patients with and without *septic shock*

	Shock n=25	Non-shock n=78	P value	Statistical test
Males, n (%)	20 (80%)	57 (73%)	0,49	Pearson χ^2 test
Age, mean (± SD), years	59,0 (15,7)	58,5 (12,7)	0,87	t-test
LOS, (IQR), day	12 (2-19)	11 (6-17)	0,76	Mann-Whitney U test
SOFA score (IQR)	12 (10-15)	6 (4-8)	<0,01	Mann-Whitney U test
Survivors, n (%)	15 (60%)	29 (37%)	0,045	Pearson χ^2 test
TNF-α, median (IQR), pg/ml	51 (35-130)	29 (18-40)	0,001	Mann-Whitney U test
IL-6, median (IQR), pg/ml	680 (400-2000)	210 (100-440)	0,001	Mann-Whitney U test
IL-10, median (IQR), pg/ml	12 (1-42)	1 (1-7)	0,014	Mann-Whitney U test

SOFA = Sequential organ failure assessment score, ICU = Intensive care unit; TNF-α = tumor necrosis factor α; IL-6 = interleukin-6; IL-10 = interleukin 10; data given as mean ± standard deviation (SD) or as median and interquartile range (IQR), $P < 0.05$ is significant.

Allele and genotype frequencies

Measured allele and genotype frequencies are shown in Table 5.

Table 5. Frequencies of single nucleotide polymorphisms in patients with severe sepsis

Polymorphism	Common alele	Rare alele	Measured frequence	SNP	Measured rare alele frequence	H-W test, p
TNF (-308)	G	A	0,36		0,19	0,51
IL-6 (-174)	G	C	0,71		0,45	0,84
IL-10 (-1082)	A	G	0,69		0,42	0,51

SNP = single nucleotide polymorphism; H-W = Hardy-Weinberg equilibrium; TNF-α = tumor necrosis factor α; IL-6 = interleukin-6; IL-10 = interleukin 10.

Table 6 summarizes the allele and the genotype distributions of SNPs in survivors and non-survivors. All alleles were in Hardy-Weinberg equilibrium.

Table 6. Frequencies of polymorphisms and alleles in subgroups of septic patients

	TNF -308 A/G	TNF -308 A	IL-6 -174 C/G	IL-6 -174 C	IL-10 -1082 G/A	IL-10 -1082 G
„Septic shock“	0,38	0,19	0,76	0,48	0,72	0,42
„Non-shock“	0,36	0,21	0,69	0,44	0,68	0,42
Survivors	0,38	0,20	0,66	0,39	0,61	0,40
Non-survivors	0,34	0,18	0,77	0,53	0,80	0,46

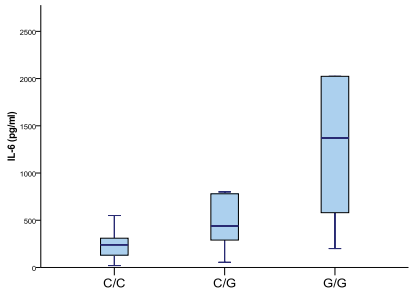
TNF = tumor necrosis factor; IL-6 = interleukin-6; IL-10 = interleukin 10.

Association „polymorphism – cytokine concentration”

We found no association between the SNPs under investigation and the corresponding cytokine serum level in septic ICU patients. Some associations between IL-6 -174 promoter polymorphism and the systemic IL-6 level were demonstrated in non-survivors and patients with septic shock.

Carriage of IL-6 -174 C/G polymorphism was associated with lower IL-6 serum concentration in **non-survivors** (Figure 1). Significant associations were observed in various genetic models.

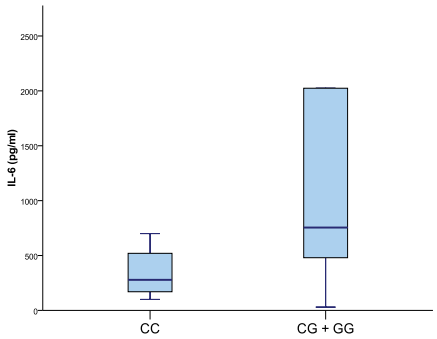
Figure 1. IL-6 levels in non-survivors according to IL-6 -174 C/G genotype



(P=0.01, Mann-Whitney U test, line=median, box=interquartile range).

In **septic shock patients** IL-6 -174 CC genotype carriage was associated with significantly lower IL-6 serum concentration as compared with IL-6 -174 G allele carriers (Figure 2).

Figure 2. Serum concentration of IL-6 in septic shock patients carrying IL-6 -174 CC genotype and IL-6 -174 G allele carriers



(P=0.03, Mann-Whitney U test, line=median, box=interquartile range).

Association „polymorphism – incidence of septic shock"

We found no association between SNP and increased incidence of septic shock in septic ICU patients.

Association „polymorphism – clinical outcome"

Association of the IL-6 -174 G/C polymorphism carriage with clinical outcome was observed in the general population of patients with severe sepsis (Table 7), as well as in the subgroup of patients without septic shock (

Table 8).

Table 7. Association of IL-6 -174 G/C promoter polymorphisms with outcome in patients with severe sepsis

	Non-survivors	Survivors	P	OR (CI 95%)
C/G	47/41	46/72	0,04	1,79 (1,03-3,14)
CC+CG/GG	34/10	39/20	0,22	1,74 (0,72-4,24)
CC/CG+GG	13/31	7/52	0,03	3,12 (1,12-8,65)

Data are presented as allele (or genotype) frequencies, e.g. CC+CG/GG – 34/10 means that CC or CG genotypes were observed in 34 individuals, but GG in 10, and mortality risks are calculated as odds ratio (OR) with 95% confidence intervals; $P < 0,05$ is significant.

Table 8. Association of IL-6 -174 G/C promoter polymorphisms with outcome in septic patients without shock

	Non-survivors	Survivors	P	OR (CI 95%)
C/G	33/25	36/62	0,014	2,27 (1,17-4,41)
CC+CG/GG	23/2	31/18	0,008	6,68 (1,41-31,7)
CC/CG+GG	10/19	5/44	0,009	4,63 (1,39-15,4)

Data are presented as allele (or genotype) frequencies, e.g. CC+CG/GG – 23/2 means that CC or CG genotypes were observed in 23 individuals, but GG in 2, and mortality risks are calculated as odds ratio (OR) with 95% confidence intervals; P < 0,05 is significant.

Association of the IL-10 -1082 A/G polymorphism carriage with clinical outcome in ICU was observed in the general study population (Table 9), as well as in patients with septic shock (Table 10).

Table 9. Association of IL-10 -1082 A/G promoter polymorphisms with outcome in patients with severe sepsis

	Non-survivors	Survivors	P	OR (95% CI)
G/A	40/48	47/71	0,418	1,26 (0,72-2,20)
GG + GA/AA	35/9	36/23	0,044	2,49 (1,01-6,11)
GG/GA + AA	5/39	11/48	0,313	0,56 (0,18-1,75)

Data are presented as allele (or genotype) frequencies, e.g. GG+GA/AA – 35/9 means that GG or GA genotypes were observed in 35 individuals, but GG in 9, and mortality risks are calculated as odds ratio (OR) with 95% confidence intervals; P < 0,05 is significant.

Table 10. Association of IL-10 -1082 A/G promoter polymorphisms with outcome in septic shock patients

	Non-survivors	Survivors	P	OR (95% CI)
G/A	16/14	5/15	0,047	3,41 (0,992-11,884)
GG+GA/AA	14/1	4/6	0,007	21 (1,9-229)
GG/GA+AA	2/13	1/9	0,802	1,39 (0,108-17,7)

Data are presented as allele (or genotype) frequencies, e.g. GG+GA/AA – 14/1 means that GG or GA genotypes were observed in 14 individuals, but GG in 1, and mortality risks are calculated as odds ratio (OR) with 95% confidence intervals; P < 0,05 is significant.

Association of TNF -308 A/G polymorphism with clinical outcome in ICU was observed neither in the general population nor in the subgroups.

Discussion

In this study, attempts to clarify the role of genetic factors in the clinical course and survival of ICU patients suffering from sepsis were made. To do so, the author examined for possible associations between TNF -308 A/G, IL-6 -174 C/G, IL-10 -1082 G/A promoter polymorphisms and the corresponding cytokine serum concentrations (i.e. TNF-α, IL-6, IL-10), the incidence of septic shock and mortality in ICU patients with severe sepsis.

In the course of the study, the author focused on a population of adult septic ICU patients who were divided into subgroups based on clinical appearance and outcome (i.e. patients with and without septic shock, survivors and non-survivors). The main demographic characteristics of the study population were similar to those reported by previous investigators [22-24]. No significant differences in age or gender were found between the analyzed subgroups of patients. However, more patients with severe organ dysfunction were observed among the non-survivors. Significantly higher median serum concentrations of TNF-α, IL-6 and IL-10 were observed in septic shock patients and non-survivors.

The observed distribution of polymorphisms in the study population was consistent with those reported in the literature (Table 11).

Table 11. Frequency of polymorphisms as compared with those reported in previous studies

Polymorphism	MPF	MPF in the previous studies (interval)	MAF	MAF in the previous studies (interval)
TNF (-308)	0,36	0,23-0,39[1]	0,19	0,12-0,21[1]
IL-6 (-174)	0,71	0,69-0,74[2]	0,45	0,41-0,49[2]
IL-10 (-1082)	0,69	0,46-0,93[3]	0,42	0,26-0,60[3]

MPF - measured polymorphism frequency, MAF – measured allele frequency

[1] Mira, Cariou et al. 1999; Tang, Huang et al. 2000; Gordon, Lagan et al. 2004
[2] Schluter, Raufhake et al. 2002; Michalek, Svetlikova et al. 2007
[3] Lowe, Galley et al. 2003; Shu, Fang et al. 2003; Schaaf, Boehmke et al. 2003; Stanilova, Miteva et al. 2006

In the present study, no association was found between **„polymorphism and cytokine concentration"** in the general population of septic ICU patients. At the same time, our results indicate a strong association between IL-6 -174 C/G polymorphism and IL-6 serum level in non-survivors and patients with septic shock. In non-survivors carriage of the IL-6 -174 C allele was associated with lower IL-6 serum concentration in all tested genetic models (i.e. genotype, dominant, recessive). In patients with septic shock IL-6 -174 CC genotype was associated with lower IL-6 serum level.

Our results suggest that the association of IL-6 -174 C/G polymorphism with IL-6 serum level is stronger in subgroups of patients with higher systemic levels of IL-6

(i.e. non-survivors and patients with septic shock). So we hypothesized that IL-6 -174 promoter polymorphism is more functionally significant in conditions of strong IL-6 gene expression.

Extrapolated to other SNPs, this hypothesis might partially explain the observed lack of association between TNF -308 and IL-10 -1082 promoter polymorphisms and the level of the corresponding cytokines. According to our results, the difference of TNF-α and IL-10 levels in the compared subgroups were much lower than with IL-6 levels. A possible explanation could be that in the present study the transcription rates of the TNF and IL-10 genes have not reached the respective "threshold levels" for detectable effects on cytokine concentration.

Alternative hypothesis to explain the lack of observed effects of TNF and IL-10 promoter polymorphism is the possible influence of other factors and mechanisms that regulate gene expression and final serum cytokine level. Effects of such molecular mechanisms are difficult to distinguish from SNP effect in genetic association studies.

A limitation of our study is that it does not allow any differentiation between the effect of the SNP under investigation and other polymorphisms in linkage disequilibrium. At the present, we could not distinguish whether the IL-6 -174 C allele exactly decreases the IL-6 transcription rate or whether it is a genetic marker in linkage disequilibrium with another functionally significant polymorphism.

Our results indicate association of IL-6 -174 and IL-10 -1082 polymorphisms with increased mortality. Carriage of IL-6 -174 C/G polymorphism strongly associated with increased mortality in septic patients without sign of shock in all analyzed genetic models. Carriage of IL-10 -1082 G/A polymorphisms associated with increased mortality in patients with septic shock in allelic and dominant genetic models. Our findings agree with data of the *Schluter et al* study of German ICU septic patients (Table 12).

Table 12. Association of IL-6 -174 C/G polymorphism with mortality in ICU septic patients in recent studies

	Studied population	Mortality	Frequency of IL-6 -174 in survivors and non-survivors (general group)	Frequency of IL-6 -174 in survivors and non-survivors (patient without septic shock)
Present study	Latvia, ICU septic patients, n=103	43% (n=44)	0,66 un 0,77 P=0,22 OR 1,74 (0,72-4,24)	0,63 un 0,92 P=0,008 OR 6,68 (1,41-31,7)
Schluter, Raufhake et al. 2002	German, ICU septic patients, n=50	50% (n=25)	0,56 un 0,92 P=0,004 OR 9,04 (1,7-46,9)	Not analyzed

In the general group of patients of the present study, we found no association of IL-6 -174 with mortality, but it was clearly demonstrated in the subgroup of patients without septic shock. The subgroup „patients without shock" of the present study is similar to the „ICU patient with severe sepsis" analyzed by *Schluter et al*. So, our data confirm the previously reported association of carriage IL-6 -174 C allele with increased mortality.

It is important to emphasize, that association of IL-6 -174 polymorphism with increased mortality was observed in the subgroup of patients which did not show association between „SNP and cytokine". Our observations suggest that the effect of IL-6 promoter polymorphism on mortality is not reflected by IL-6 level. Possibly IL-6 -174 polymorphism is in linkage disequilibrium with other functionally significant polymorphism. This hypothesis is supported by previous publications that which found no effect of IL-6 on mortality.

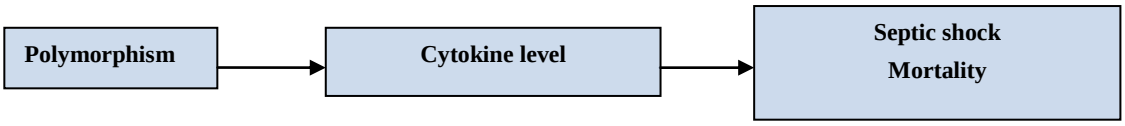
The present study demonstrates an association between IL-10 -1082 polymorphism and mortality in ICU patients. The association for the greater part was observed in patients with septic shock. In fact, the author of the present thesis was unable to find any previous publication describing such an association.

Table 13. Association of IL-10 -1082 G/A polymorphism with mortality in the present and previous studies

Author	Population	Mortality	Frequencies of IL-10 -1082 in survivors and non-survivors	Frequencies of IL-10 -1082 in survivors and non-survivors (in patients with septic shock)
Present study	Latvia, ICU septic patients, n=103	overall 43% (in septic shock patients 60%)	0,61 un 0,79 P=0,04 OR 2,49 CI 95% (1,01-6,11)	0,40 un 0,93 P=0,007 OR 21,0 CI 95% (1,9-229)
Schaaf, Boehmke et al. 2003	German, patients with pneumococcal disease, n=69	7%	0,20 un 0,60 P=0,65 OR 2,40 CI 95% (0,25-22,7)	Not reported
Stanilova, Miteva et al. 2006	Bulgaria, ICU septic patients, n=33	64%	0,33 un 0,57 P=0,18 OR 2,67 TI 95% (0,61-11,7)	Not analyzed
Lowe, Galley et al. 2003	UK, ICU patients, n=67	33%	Association not observed (distribution of genotypes not reported)	Not analyzed
Shu, Fang et al. 2003	China, septic patients, n=116	Not reported	~0,5 un ~0,5	Not analyzed

Taken together, our analysis shows an association between IL-6 -174 and IL-10 -1082 promoter polymorphisms with mortality in certain subpopulations of septic ICU patients (i.e. with/without septic shock). However, we did not observe a higher serum concentration of the corresponding cytokine in the same subpopulations, as we would expect according to our initial hypothesis (Figure 3).

Figure 3. Initial hypothesis of the study



It should be emphasized, that the proposed initial model "SNP-cytokine level - septic shock/clinical outcome" was not confirmed for the tested polymorphisms, neither in the general study population nor in the subgroups.

Table 14. Summary of the observed associations

	Study population	"Shock"	"Non-shock"	Non-survivors	Survivors
SNP – cytokine	Not observed	IL-6	Not observed	L-6	Not observed
SNP - mortality	IL-6, IL-10	IL-10	IL-6	xxxxx	xxxxx
SNP – septic shock	Not observed	xxxxx	xxxxx	Not observed	Not observed

Our findings indicate some functional effect of the tested SNPs in septic ICU patients. The study confirms some previously reported associations and shows some new associations requiring confirmation in future investigations. Although our study gives no clear answers concerning the functional significance of the examined polymorphisms, we have demonstrated an association of TNF-α, IL-6 and IL10 polymorphisms with cytokine level, incidence of septic shock and mortality in certain subpopulations of ICU septic patients.

Conclusion

- IL-6 -174 C/G polymorphism is associated with lower IL-6 serum level and higher mortality, but not associated with increased incidence of septic shock,
- IL-10 -1082 G/A polymorphism is associated with increased mortality, but is not associated with IL-10 serum level and incidence of septic shock,
- TNF -308 A/G polymorphism is not associated with increased TNF-α serum level, and not associated with incidence of septic shock and mortality,
- Evidence of functional effects of IL-6 and IL-10 promoter polymorphism on clinical course and outcome of sepsis in ICU patients was demonstrated in the present study.

Publication on the subject of the thesis

Publications in peer-reviewed journals

1. **O. Sabelnikovs**, L. Nikitina-Zake, A. Krumina, Z. Jaunberga, J. Klovins, L. Viksna, L. J. Bjertnaes, I. Vanags "Associations between TNF-α, IL-6 and IL-10 promoter polymorphisms and mortality in severe sepsis" [*paper submitted to Crit Care*]
2. **O. Sabelnikovs**, L. Nikitina-Zake, I.Vanags „Association of interleukin 6 promoter polymorphism (-174G/C) with IL-6 level and outcome in severe sepsis", Proc Latvian Acad. Sci. Section B, Vol. 62 (2008), No. 4/5, p.162-164
3. **O. Sabeļņikovs**, I. Jaunalksne, I. Vanags, P. Oš̄s "sTNF-R2 un TNF-α attiecības saistība ar slimības iznākumu pacientiem ar smago sepsi" RSU Zinātnisko rakstu krājums, 2006, p.160.-162.
4. **O. Sabelnikovs**, I. Vanags, L. Nikitina-Zake, I. Jaunalksne, P. Oss, A. Krauze "Association of TNF -308 A/G polymorphism with TNF-α level, illness severity and outcome in septic patient", Acta Chirurgica Latviensis 2006(6) p.86.-88.
5. **O. Sabeļņikovs**, I. Vanags „Method of evaluating severity of patients condition"; J. Patenti un preču zīmes" 2006; N10; p.1386
6. **O. Sabelnikovs** „Role of TNF and TNF receptors interactions in regulation of systemic inflammation" , Acta Chirurgica Latviensis; 2005(5); p.59.-61.

Abstracts published in internationally indexed journals

1. **O. Sabelnikovs**, L. Nikitina-Zake, J.Zhuravleva, E.Sama, I.Vanags „Association of IL-10 promoter polymorphism -1082 G/A with adverse outcome in severe sepsis and septic shock", Critical Care, 2009, Vol 13, Suppl. 1, p S144-145.

2. **O. Sabelnikovs**, L. Nikitina-Zake, I. Vanags "Association of interleukin 6 promoter polymorphism -174C/G with outcome in severe sepsis", Critical Care, 2008, Vol 12, Suppl. 2, p S181.

3. **O. Sabelnikovs**, I. Vanags, L. Nikitina-Zake, I. Jaunalksne, P. Oss "Association of TNF -308 A/G polymorphism with TNF-α level, illness

severity and outcome in septic patient" EJA, Volume 24, Suppl. 39, p. 149, 2007

Abstracts published in other publications or proceedings of conferences

1. **O. Sabelnikovs**, I. Jaunalksne, I. Vanags, P. Oss. „Association of sTNF-R2/TNF-α ratio with clinical outcome in severe sepsis". Abstracts of the 2nd International Baltic Congress of Anaesthesiology and Intensive Care (Tallinn, Estonia, 2006).

2. **O. Sabeļņikovs**, P. Oš̌s „Septisko stāvokļu marķēšana" 1.Starptautiskā Baltijas anesteziologijas, intensīvas terapijas kongresa (2005.g.) tēzes.

Presentation at conferences and meetings

1. „**Association of IL-10 promoter polymorphism -1082 G/A with adverse outcome in severe sepsis and septic shock**" poster presentation on the „29th International Symposium on Intensive Care and Emergency Medicine" (Brussels, Belgium, 2009).

2. "**Association of interleukin 6 promoter polymorphism -174C/G with outcome in severe sepsis**" poster presentation on the „28th International Symposium on Intensive Care and Emergency Medicine" (Brussels, Belgium, 2008).

3. "**The Role of Gene in Sepsis**", 4th International Baltic Congress of Anaesthesiology and Intensive Care (Rīga, Latvia, 2008)

4. "**Association of TNF -308 A/G polymorphism with TNF-α level, illness severity and outcome in septic patient**" poster presentation on the "Euroanaesthesia 2007" international congress (Munich, Germany, 2007).

5. „**What's new in the diagnosis of sepsis?**", oral presentation on the 3rd International Baltic Congress of Anaesthesiology and Intensive Care (Vilnius, Lithuania, 2007).

6. „**Association of sTNF-R2/TNF-α ratio with clinical outcome in severe sepsis**" oral presentation on the 2nd International Baltic Congress of Anaesthesiology and Intensive Care (Tallinn, Estonia, 2006).

7. „Septisko stāvokļu marķēšana" („**Markers of sepsis**") poster presentation on the 1st International Baltic Congress of Anaesthesiology and Intensive Care (Rīga, Latvia, 2005)

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