

Genetic polymorphisms of the lymphotoxin alpha gene are associated with increased risk for lethal infections during induction therapy for childhood acute leukemia: a case-control study

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Abstract Specific mutations of the TNF- α (TNF- α) and Lymphotoxin- α (LT- α) genes are correlated to the outcome of patients during serious infections. This study aimed at correlating these polymorphisms to lethal infections during childhood acute lymphoblastic leukemia (ALL). A matched case-control study of 34 patients who died due to infections during ALL treatment and 68 ALL patients without lethal infections was performed. Genomic DNA was isolated from blood smears and specific fragments including the polymorphic site of each gene were amplified. In the total study population, 23/102 (22.5%) of the children carried at least two variant alleles (high-producer haplotype). The variant genotypes were equally distributed between cases and controls [relative risk (RR) 1.17 (CI 0.33–2.22, $P = 0.752$)]. With regard to infective organisms, no statistically significant differences could be detected between the groups for bacterial infections [RR 1.59 (CI 0.56–4.50), $P 0.379$]. Patients with a LT- α (10.5 kb/5.5 kb; 5.5 kb/5.5 kb) haplotype, however, seemed to have a significant higher risk of attracting a lethal infection during induction/consolidation chemotherapy (RR 2.98, CI 0.98–9.01, $P = 0.05$). These results support a role of specific genetic polymorphisms on lethal infections during induction chemotherapy of ALL treatment.

Keywords Infection · Polymorphism · Genetic · Leukemia · Childhood

1 Introduction

Infections in patients after chemotherapy remain a relevant risk, despite modern anti-infective treatment [1]. Childhood leukemia is regarded a principally “curable disease” these days, cure rates exceeding 80% in childhood acute lymphoblastic leukemia (ALL) underline the importance to avoid deaths due to side effects [27]. The incidence of infections in oncology patients is 14–60%, and up to 10% of the patients die in the context of the infections [2, 3]. Several risk factors for infections are well known, such as the duration of neutropenia, co-medication with steroids, parenteral nutrition and central venous lines [4–6]. Besides, genetic factors influence the susceptibility to infection: Several studies showed (conflicting) results with regard to the role of cytokine production and the outcome of patients during sepsis. Hence, genetic polymorphisms might influence the course of infections in critically ill patients [7–9]. Tumor necrosis factor (TNF) and lymphotoxin- α (LT- α) function as mediators of immune regulation and inflammation. Both lymphokines have similar biologic activities, and show 35% identity and 50% homology in amino acid sequence [10, 11]. The genes coding for TNF and LT- α are located tandemly on the chromosomal region 6p21.3–21.1 and are closely linked to the HLA-B locus within a highly polymorphic region of the major histocompatibility complex. Several single nucleotide polymorphisms of the TNF gene have been described. Exchange of guanine by adenine at position –308 of the TNF promoter region (TNF2 allele) is associated with higher serum levels of soluble TNF [12]. The TNF –308 (G \rightarrow A) polymorphism has been

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associated with susceptibility to cerebral malaria, lepromatous leprosy, mucocutaneous leishmaniasis, ulcerative colitis, Crohn's disease, fatal meningococcal disease, and septic shock [13–16]. A polymorphism in the coding region at position +252 of the LT- α gene (A \rightarrow G) leads to different alleles of LT- α , here referred to as LT- α (10.5 kb) for the wild-type allele, and LT- α (5.5 kb) for the variant allele. The LT- α (5.5 kb) allele was associated with higher levels of soluble LT- α in patients homozygous for LT- α (5.5 kb) [17]. A strong association between the LT- α (5.5 kb) allele and adverse outcome in autoimmune diseases (e.g. Crohn's disease) has been described. Biologic actions of both cytokines as well as their association with autoimmune and severe inflammatory disorders have drawn interest to their potential role in the prognosis of infections.

Although a potential contribution of the TNF-308 (G \rightarrow A) and LT- α +252 (A \rightarrow G) polymorphisms to infectious processes remains unclear, it is of interest that so-called high-producer haplotypes [i.e. carriers of at least two variant alleles of TNF2 and/or LT- α (5.5 kb)] have been associated with higher risk for severe or even lethal infections [13, 14].

The role of TNF- α and LT- α in the context of infections has been subjected to investigation since long: TNF- α stimulates the production of a broad range of cytokines [10, 11]. As a result from this, a hostile environment as a line of defence against invading pathogens is created. Thus, TNF- α plays a fundamental role in the defence of any pathogenic invasion. However, during bacterial infections or even sepsis, an excessive or untimely TNF- α production proved to be extremely disadvantageous for patients and resulted in enhanced mortality and morbidity [18, 19].

Subsequently, genetic polymorphisms of the TNF- α and LT- α genes have been studied and variations of the cytokine production during infections and the role of specific genetic polymorphisms were elucidated. These studies indicated that certain genetic polymorphisms were related to a higher risk for severe or even lethal infections [12–14]. TNF- α polymorphisms at position –308 in the TNF gene (=guanine defines the TNF1 allele) are reported to enhance the TNF promoter activity and thus increase the TNF production whereas adenine defines the less common TNF2 allele [17].

With regard to the LT- α gene, a polymorphism at nucleotide position +252 within the first intron was reported to influence LT- α plasma levels. Here, a single nucleotide polymorphism (A252G) results in two different alleles (LT- α , 10.5 kb and LT- α , 5.5 kb) with the LT- α allele (5.5 kb) resulting in higher LT- α plasma levels [15]. In clinical studies, the TNF2 and the LT- α (5.5 kb) allele were associated with increased mortality risk in patients with established infections [15].

The current study was performed to challenge the hypothesis whether the above-mentioned genetic polymorphisms increased the risk to die from an infection during chemotherapy in children who were treated for ALL. Addressing this question, we genotyped a matched case-control study group of 34 children with lethal infections and 68 children without lethal infections and ALL for the above mentioned polymorphisms.

2 Patients and methods

From April 1, 1995 until June 30, 2000, 2169 children with ALL were enrolled into the ALL-BFM 95 multicenter trial. The details regarding study design and results have recently been published [20]. Briefly, treatment consisted of an intensive multi agent chemotherapy regimen resulting in profound and prolonged neutropenia. 6-year event-free survival (6y-pEFS) for all 2169 patients was 79.6% [20].

All patients with lethal infections during the ALL-BFM 95 trial were reported to the study centre located in Hannover (from 2004 onwards: Kiel). In total, 44 children died in the context of an infection (microbiologically proven or clinically suspected). In 34/44 patients, DNA was available and these children were included as cases into the study. These 34 cases were matched to 2 children each without lethal infections according to sex, age at diagnosis (\pm 6 months), white blood cell count (WBC) at diagnosis (\pm 10,000), immunophenotype, and risk group. The term risk group refers to the stratification system of this trial, which used age, white blood cell count (WBC) at diagnosis, and immunophenotype in addition to the prednisone response to induction treatment, and the unfavorable translocations $t(9;22)$ and $t(4;11)$.

Each case was matched with two controls for that the final study population consisted of 102 children (34 cases and 68 controls, Table 1).

3 Genotype analysis

Genomic DNA was isolated from remission bone marrow or peripheral blood smear as described before [21]. Genotypes for TNF- α and LT- α were determined by polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) analysis. The –308 TNF- α promoter polymorphism was analyzed by incorporating it into an NcoI restriction site that was created by introducing a single base exchange within the forward primer.¹⁴ Primer sequences were: forward 5'-AGGCA ATAGGTTTTGAGGGCCAT-3'; reverse 5'-CCTCCCTG CTCCGATTCCG-3'. The LT- α polymorphism at nucleotide position +252 was analyzed by PCR amplification of a

Table 1 Characteristics of 34 patients with acute lymphoblastic leukemia and lethal infections during chemotherapy and 68 controls out of the ALL-BFM 95 trial population

	Number of patients (%)	
	Cases: <i>n</i> = 34 (%)	Controls: <i>n</i> = 68 (%)
Sex		
Male	17 (50.0)	34 (50.0)
Female	17 (50.0)	34 (50.0)
Age (years)		
<1	1 (2.9)	2 (2.9)
1–6	15 (44.1)	30 (44.1)
>6 to <10	8 (23.5)	16 (23.5)
≥10	10 (29.4)	20 (29.4)
WBC at presentation (10 ³ μl)		
<10	13 (38.2)	26 (38.2)
≥10 to <50	11 (32.4)	22 (32.4)
≥50 to <100	3 (8.8)	6 (8.8)
≥100	7 (20.6)	14 (20.6)
Immunophenotype		
Pro-B-ALL	16 (47.1)	32 (47.1)
c-ALL	5 (14.7)	10 (14.7)
Pre-B-ALL	2 (5.9)	4 (5.9)
T-ALL	8 (23.5)	16 (23.5)
Not classified	3 (8.8)	6 (8.8)
Risk group		
Standard	9 (26.5)	18 (26.5)
Intermediate	22 (64.7)	44 (64.7)
High	3 (8.8)	6 (8.8)
DNA index ^a		
<1.16	24 (70.6)	38 (55.9)
≥1.16	1 (2.9)	8 (11.8)
Not examined	9 (26.5)	22 (32.4)
Infective organism		
Bacteria	18 (52.9)	–
Virus	3 (8.8)	–
Fungi	6 (17.6)	–
Unknown	7 (20.6)	–

Therapy stratification in risk groups was mainly based on initial leukemic cell mass estimate and initial treatment response

WBC White blood cell, c-ALL Common acute lymphoblastic leukemia, pre-B-ALL Precursor B-cell acute lymphoblastic leukemia

^a Ratio of DNA content of leukemic G0/G1 cells to normal lymphocytes

368 bp fragment using the following primer pair: forward 5'-CTCCTGCACCTGCTGCCTGGATC-3'; reverse 5'-GAAGAGACGTTTCAGGTGGTGCAT-3' [12]. The amplified PCR products were digested overnight with NcoI and analyzed on 3.0% Nusieve (TNF-α) or 3.0% conventional agarose gels (LT-α). In case of presence of the TNF1 allele, the amplified 107 bp fragment from the TNF-α promoter is

Table 2 Distribution of tumor necrosis factor alpha (TNF-α) and lymphotoxin-alpha (LT-α) genotypes and their association with the occurrence of lethal infections in 34 case subjects and 68 successfully treated matched control subjects with acute lymphoblastic leukemia from the trial ALL-BFM 95

	Cases (%)	Controls (%)	RR (95% CI)	<i>P</i>
TNF-α				
TNF1/TNF1	26 (76.5)	55 (80.9)		
TNF1/TNF2	6 (17.6)	11 (16.2)		
TNF2/TNF2	2 (5.9)	2 (2.9)	1.00 (0.39–2.60) ^a	1
LT-α				
10.5 kb/10.5 kb	14 (41.2)	41 (60.3)		
10.5 kb/5.5 kb	17 (50.0)	21 (30.9)		
5.5 kb/5.5 kb	3 (8.8)	6 (8.8)	1.83 (0.87–3.85) ^b	0.11
Haplotypes ^c				
Low-producer	26 (76.5)	53 (77.9)		
High-producer	8 (23.5)	15 (22.1)	1.17 (0.33–2.22)	0.752

RR Relative risk, CI confidence interval of TNF1/TNF2 and TNF2/TNF2 genotypes

^a Relative risk for the combined category of TNF1/TNF2 and TNF2/TNF2 genotypes with reference to TNF1/TNF1

^b Relative risk for the combined category of LT-α (10.5 kb/5.5 kb) and LT-α (5.5 kb/5.5 kb) with reference to LT-α (10.5 kb/10.5 kb)

^c For definition of low-producer/high-producer see Sect. 3

cut into two fragments of 87 and 20 bp, a fragment amplified from TNF2 remains uncut [12]. The 368 bp fragment from LT-α is unaffected by NcoI digestion in case of presence of a LT-α (10.5 kb) allele while a PCR product amplified from a LT-α (5.5 kb) allele is cut into two fragments of 133 and 235 bp [22].

High-producer haplotypes are defined as patients with the following karyotypes: TNF1/TNF1-LT(5.5/5.5), TNF1/TNF2-LT (10.5/5.5), TNF1/TNF2-LT (5.5/5.5), TNF2/TNF2-LT (10.5/10.5) and TNF2/TNF2-LT (5.5/5.5), TNF1/TNF2-LT(10.5/5.5).

Patients with less than two variant alleles were referred as low-producer haplotype carriers: TNF1/TNF1-LT(10.5/10.5), TNF1/TNF2-LT (10.5/10.5), TNF1/TNF1-LT (10.5/5.5).

3.1 Statistical analysis

After frequencies were calculated for descriptive purposes, correlation analyses (contingency coefficients for nominal data, Spearman correlation coefficients for ordinal data, Pearson correlation coefficients for continuous data) were computed to investigate the interrelationships between TNF-α genotype, LT-α genotype and important clinical prognostic variables such as sex, age at diagnosis, WBC at diagnosis, immunophenotype and risk group. Differences in the distribution of categorical variables were analyzed by χ^2

or Fisher's exact test. The association between TNF- α and LT- α genotypes and lethal infection was examined by use of conditional logistic regression analysis to calculate relative risks and their 95% confidence intervals. Genotypes and genotype combinations were used as categorical variables in the analyses. The association of genotypes with lethal infections was analyzed by log rank tests. Computations were performed using SAS software (SAS-PC Version 6.04, SAS Institute Inc., Cary, NC).

3.2 Definitions of infections

A patient was regarded to have a fungal infection when fungi were isolated from blood or tissue biopsy. All bacterial infections were diagnosed using blood cultures taken from central-venous or peripheral punctures. Viral infections were diagnosed from blood (PCR-based diagnosis) or broncho-alveolar lavage. In the remaining children, an infection was regarded as *presumably causative* for the death in the clinical context (fever, infiltrates on chest X-ray, oxygen requirement, inotropic support).

3.3 Definition of sepsis

All children were febrile (body temperature above 38.5°C) and showed increasing C-reactive protein (CRP) levels with additional clinical signs of an uncontrolled infection, e.g. respiratory deterioration (accelerated respiratory rate or additional oxygen requirement) and/or hypotension (blood pressure < fifth percentile for age). Hence, all patients included in the analysis were suffering from sepsis or even severe sepsis or septic shock according to the definitions specified by Goldstein et al. and the Members of the International Consensus Conference on Pediatric Sepsis [23], as shown below:

1. Systemic inflammatory response syndrome (SIRS): presence of 2 of the following criteria
 - a. Core temperature >38.5°C or <36°C
 - b. Tachycardia (for definitions, see Goldstein et al.)
 - c. Mean respiratory rate >2 SD above normal or mechanical ventilation
 - d. Leucocyte count elevated or depressed (not secondary to chemotherapy)
2. Sepsis SIRS in the presence or as a result of a suspected or proven infection
3. Severe sepsis Sepsis plus one of the following:
 - a. cardiovascular dysfunction or
 - b. acute respiratory distress syndrome or
 - c. two or more other organ dysfunctions

3.4 Data collection

Clinical, microbiological, radiological and medical records were reviewed in detail in all cases to decide upon the cause of death. Autopsy data were also included when available.

4 Results

4.1 Clinical characteristics of the patients

The clinical, microbiological and hematological data of the children are summarized in Table 1 and 5. The predominant causes of death were bacterial infection in 18/34 children, almost 20% of the documented infections were of fungal origin.

The details of the infections are outlined in Table 5. Here, the predominance of gram negative rods is obvious. Besides, gram positive infections and polymicrobial infections were seen. All patients had sign for sepsis or even severe sepsis before death.

Table 1 shows the distribution of matching variables and genetic analyses (DNA index) in the 34 cases and the 68 control subjects as previously described [21]. Within our entire study population of 102 patients, 81 (79.4%) patients were homozygous for the TNF1 allele, 17 (16.7%) patients were heterozygous (TNF1/TNF2) and 4 (3.9%) patients were homozygous for the TNF2 allele. With regard to LT- α , we observed 55 (53.9%) patients being homozygous for the LT- α (10.5 kb) allele and 38 (37.3%) patients being heterozygous [LT- α (10.5 kb)/LT- α (5.5 kb)]. Nine patients (8.8%) patients were homozygous for the LT- α (5.5 kb) allele. The prevalences observed in our study are similar to the ones reported by Demeter et al. in a healthy German control sample [24].

Table 2 shows the prevalences of the investigated TNF- α and LT- α genotypes by case-control status of our study population. In addition, Table 2 contains information on the association of the TNF- α polymorphism and the LT- α polymorphism with regard to the risk for lethal infections in our study subjects. For both genotypes, no particular associations with risk of a lethal infection were observed (Table 2). As reported in previous studies, the TNF1 allele was significantly linked to the LT- α (10.5 kb) allele as was the TNF2 allele to the LT- α (5.5 kb) allele (χ^2 test; $P < 0.01$) [19].

When we analyzed the risk of lethal infections conferred by so-called high-producer TNF- α /LT- α haplotypes (at least two high-producer alleles) in comparison to low-producer TNF- α /LT- α haplotypes (less than two high-producer alleles), we also failed to observe any meaningful

association in our study population [relative risk (RR) = 1.17; 95% confidence interval (CI) = CI 0.33–2.22; $P = 0.752$].

In Table 3, the polymorphisms under investigation and the infective organism were correlated. With regard to this subset analysis of our study, no association of bacterial, viral or fungal infection and high-producing phenotypes of TNF- α or LT- α could be detected (Table 3).

The final analysis of this study included the statistical correlation of the genetic polymorphisms under investigation and the point in time of the lethal infection (Table 4). Here, the study population was divided into those patients, where the infections occurred during the induction/consolidation chemotherapy (so called protocol I, days 1–64 of ALL-BFM therapy) and infections during all later periods of the antileukemic treatment. Here, a significant correlation between high-producing LT- α haplotypes ($P = 0.05$)

Table 3 Distribution of tumor necrosis factor (TNF- α) and lymphotoxin- α (LT- α) genotypes and their association with the infective organisms of lethal infections

	Cases (%)	Controls (%)	RR (95% CI)	P
Bacteria/TNF-α				
TNF1/TNF1	14 (41.2)	28 (41.2)		
TNF1/TNF2	3 (8.8)	7 (10.3)		
TNF2/TNF2	1 (2.9)	1 (1.5)	1.00 ^a (0.29–4.00)	n.c.
Bacteria/LT-α				
10.5 kb/10.5 kb	7 (20.6)	19 (27.9)		
10.5 kb/5.5 kb	10 (29.4)	13 (19.1)		
5.5 kb/5.5 kb	1 (2.9)	4 (5.9)	1.59 ^b (0.56–4.50)	0.379
Virus/TNF-α				
TNF1/TNF1	2 (5.9)	5 (7.4)		
TNF1/TNF2	1 (2.9)	1 (1.5)	–	–
TNF2/TNF2	–	–		
Virus/LT-α				
10.5 kb/10.5 kb	1 (2.9)	4 (5.9)		
10.5 kb/5.5 kb	2 (5.9)	2 (2.9)	2.73 (0.23–33.33) ^b	0.429
5.5 kb/5.5 kb	–	–		
Fungi/TNF-α				
TNF1/TNF1	4 (11.8)	11 (16.2)	–	–
TNF1/TNF2	1 (2.9)	1 (1.5)		
TNF2/TNF2	1 (2.9)	–		
Fungi/LT-α				
10.5 kb/10.5 kb	2 (5.9)	9 (13.2)		
10.5 kb/5.5 kb	2 (5.9)	2 (2.9)	2.73 (0.23–33.33) ^b	0.429
5.5 kb/5.5 kb	2 (5.9)	1 (1.5)		

RR relative risk, CI confidence interval, n.c. not calculated

^a Relative risk for the combined category of TNF1/TNF2 and TNF2/TNF2 genotypes

^b Relative risk for the combined category of LT- α (10.5 kb/5.5 kb) and LT- α (5.5 kb/5.5 kb) with reference to LT- α (10.5 kb/10.5 kb)

and infections during protocol I was seen (Table 4.), whereas no correlation with regard to TNF- α or any other time was observed.

5 Discussion

In hematologic malignancies, in particular AML and ALL, infections occur more frequently than in patients with solid tumors [1, 2]. Generally, duration and degree of neutropenia and/or steroid medication are recognized risk factors [3, 4].

Current research supports the notion of the importance of TNF- α for the deleterious consequences of inflammatory reactions, e.g. hypotension, capillary leakage and acute respiratory distress syndrome or finally multi organ system failure [5–7].

With regard to the clinical impact of genetic variations and TNF- α , there is good evidence that genetic variation within regulatory regions of the genes coding only for TNF- α is strongly correlated to the amount of TNF- α produced [13, 14, 19].

Table 4 Distribution of tumor necrosis factor alpha (TNF- α) and lymphotoxin-alpha (LT- α) genotypes and their association to different points in time of lethal infections

	Cases (%)	Controls (%)	RR (95% CI)	P
Prot I/TNF-α				
TNF1/TNF1	14 (41.2)	30 (44.1)		
TNF1/TNF2	3 (8.8)	5 (7.4)		
TNF2/TNF2	1 (2.9)	1 (1.5)	1.50 (0.34–6.71) ^a	0.595
Prot I/LT-α				
10.5 kb/10.5 kb	6 (17.6)	23 (33.8)		
10.5 kb/5.5 kb	10 (29.4)	10 (14.7)		
5.5 kb/5.5 kb	2 (5.9)	3 (4.4)	2.98 (0.98–9.01) ^b	0.05
Other/TNF-α				
TNF1/TNF1	6 (17.6)	14 (20.6)		
TNF1/TNF2	2 (5.9)	3 (4.4)		
TNF2/TNF2	1 (2.9)	1 (1.5)	1.62 (CI 0.31–8.4) ^a	0.567
Other/LT-α				
10.5 kb/10.5 kb	4 (11.8)	9 (13.2)		
10.5 kb/5.5 kb	4 (11.8)	6 (8.8)		
5.5 kb/5.5 kb	1 (2.9)	3 (4.4)	1.18 (0.29–4.76) ^b	0.814

Prot I induction chemotherapy (day 1 to day 64 of ALL-BFM treatment schedule), other subsequent parts of the chemotherapy protocol (re-induction, maintenance), RR relative risk, CI confidence interval

^a Relative risk for the combined category of TNF1/TNF2 and TNF2/TNF2 genotypes

^b Relative risk for the combined category of LT- α (10.5 kb/5.5 kb) and LT- α (5.5 kb/5.5 kb)

Table 5 Details of infections in 32 patients with lethal infection and ALL

Patient	Genotype (high risk/low risk) ^a	Age (years)	Type of infection	Sepsis (according to sepsis criteria [23])	Interval (days) diagnosis–death
1	High	17	<i>Staph epidermidis</i>	Severe sepsis	n.a.
2	Low	13	<i>Streptococci</i> in blood culture, suspected PCP	Severe sepsis	185
3	Low	19	<i>Enterococci, Candida krusei, Clostridien, koag.-neg. Stapholococci</i>	Severe sepsis	94
4	High	9	<i>Aspergillus</i>	Severe sepsis	92
5	Low	9	Clinically infection (fever, elevated CRP)	Severe sepsis	6
6	Low	3	<i>E.coli</i>	Severe sepsis	39
7	Low	0.5	<i>Pseudomonas ssp.</i>	Severe sepsis	25
8	Low	3	<i>E.coli</i>	Severe sepsis	22
9	Low	4	Clinically infection suspected (fever, elevated CRP)	Severe sepsis	9
10	High	11	<i>Pseudomonas</i>	Severe sepsis	30
11	High	0.5	PCP, <i>E.coli</i>	Severe sepsis	267
12	Low	2	CMV	Severe sepsis	216
13	Low	3	<i>E.coli</i>	Severe sepsis	26
14	High	3	CMV, Adenovirus, <i>Aspergillus</i>	Severe sepsis	327
15	Low	13	<i>Aspergillus</i>	Severe sepsis	182
16	Low	6	<i>E.coli</i>	Severe sepsis	50
17	Low	9	Clinically infection suspected (fever, hypotension, elevated CRP)	Severe sepsis	14
18	Low	10	<i>E.coli</i>	Severe sepsis	220
19	Low	13	<i>Aspergillus</i>	Severe sepsis	208
20	High	9	<i>Klebsiella</i>	Severe sepsis	57
21	Low	13	<i>E. coli, Strept. salivarius, Bacteroides distasonis and vulgatus, Bifidobacterium spp</i>	Severe sepsis	434
22	Low	2	<i>Pseudomonas ssp.</i>	Severe sepsis	213
23	Low	16	<i>Morganella morganii</i>	Severe sepsis	154
24	Low	1	<i>Enterococci, Staphylococci, Aspergillus</i>	Severe sepsis	207
25	Low	13	<i>E. coli</i>	Severe sepsis	219
26	Low	2	<i>Pseudomonas ssp.</i>	Severe sepsis	21
27	Low	6	n.d. (clinically sepsis, pneumonia)	Sepsis	69
28	High	4	n.d. (clinically fever, renal failure, ARDS)	Severe sepsis	54
29	Low	15	n.d. (clinically fever, respiratory failure, ARDS)	Severe sepsis	397
30	High	3	<i>Aspergillus</i>	Severe sepsis	79
31	Low	8	“Fungi”	Severe sepsis	571

PCP pneumocystis jiroveci pneumonia, n.d. not determined, CMV cytomegalo virus, ARDS acute respiratory distress syndrome, CRP c reactive protein

N = 31, in 3 patients detailed information on the infection was lacking

^a For definitions of high risk/low risk genotype, see Sect. 3

This study is the first to explore the role of TNF- α and LT- α genotypes for risk of lethal infection in a cohort of patients with ALL during therapy, where all patients were treated similarly. These homogeneous conditions in a matched pair setting made it possible to explore the role of TNF- α and LT- α as genetic susceptibility factors for infections in the context of chemotherapy induced immunosuppression.

According to our results, a statistically significant genetic influence of the polymorphisms under investigation was found only for LT- α during the induction/consolidation chemotherapy (so-called ‘protocol I’ of the ALL-BFM trial). All other analyses (addressing different infective organism or genetic polymorphism of TNF- α) yielded no statistically significant results. We speculate that this genetic influence was only detectable in the early

phase of chemotherapy because during the subsequent courses of the treatment, other factors might obliterate individual risk factors for infections (e.g. days of neutropenia, insertion of a central venous access device, parenteral nutrition) and these risk factors were not included in the analysis. However, the lack of correlation between TNF- α polymorphism and lethal infection cannot be concluded from this hypothesis but might be due to the relatively low number of infections included in this study.

Recently, Lausen and colleagues analyzed the influence of a polymorphism of the mannose-binding lectin (MBL) with regard to infections during induction therapy. Their study showed no significant correlation between infections and MBL. The difference to our study might be explained by the fact that in our study a different pathway of the human response to infections was investigated illustrating the plethora of lines of defence against infections. [25].

In a study, McArthur et al. found a significant association between a “high producing” TNF genotype, higher serum TNF- α levels and mortality in children admitted to the intensive care unit with sepsis and positive blood cultures [26]. The authors hypothesized that the outcome of a life-threatening event in response to bacterial infections (i.e. systemic inflammatory response syndrome) might be related to the amount of TNF- α secreted, which in turn may be influenced by the individual’s genetic makeup.

These data find good support by data from Stueber et al. [19]. According to their data, there was a strong correlation between the outcome of adult postoperative patients, a “high producing” TNF- α genotype and mortality during sepsis. Not all studies have demonstrated an association between TNF- α polymorphisms and complications: No increased risk of septic shock or mortality was observed in a cohort of postoperative surgical adults as reported by Tang et al. [8]. Another study examining the polymorphisms of the LT- α gene demonstrated no association between the genotypes at this site and survival in women with severe sepsis [16].

Finally, a pilot study of high-risk term and premature infants with sepsis and culture-proven bacteremia did not demonstrate a risk of worse outcome [9]. Hence, although most evidence appears to support an association between certain genotypes in the regulatory region of the gene coding for TNF- α , levels of TNF- α production, and mortality in patients with sepsis, there are in part conflicting results that might be explained by different study designs or heterogeneous study end points.

Nevertheless, our study has some limitations: first, not all the patients included in the study had a microbiologically

proven infection, e.g. a positive blood culture or were only “autopsy-proven” cases included. Here, the judgement of the clinician in charge and the opinion of the study centre together with the clinical, radiological and microbiological data resulted in the stratification of the patient. With regard to fungal infections, where diagnostics are still somewhat limited, the stratification might have been therefore subject to inaccuracies.

Second, the study was heterogeneous with regard to the pathogens: children with different infections were studied and especially with regard to viral and fungal infection only very small patient numbers were included in the study. Whether or not TNF-polymorphisms might play a role in the course of these infections in ALL patients cannot be answered with this study, as no significant correlation was observed. Third, no TNF- α levels were measured in the study population, hence the conclusion whether or not the genetic profile resulted in significantly different TNF serum levels cannot be answered from our data. With regard to this aspect, however, several studies have unveiled that “high-producer” polymorphisms such as the LT- α allele (5.5 kb) and the TNF2 allele are strongly related to high elevated TNF levels in vivo [17, 19].

On the other hand, these data support in a matched-pair fashion the role of genetic factors possibly influencing the course of an infection in patients with ALL during therapy. Thus, bearing in mind that infections besides relapse remain the major threat for children with malignancies, our study adds information on the “profiling” of high risk patients as one. However, these results are only preliminary data of a small cohort and need confirmation on a larger scale. In patients with a so-called high risk genotype, one might speculate as to which prophylactic measures could be beneficial. Here, any interventions to reduce the number of (serious) infections should be explored in a randomized fashion to ensure its benefit for the children.

To conclude, the results of this matched-pair analysis show a significant correlation between lethal infections and a genetic polymorphism of the LT- α gene presumably related to higher production of TNF- α . The influence did reach statistical significance only during protocol I—induction/consolidation chemotherapy of the ALL-BFM trial—but not with regard to different infective organisms which might be explained by the low numbers of lethal infection in the population under investigation.

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