Case control study

Tumor necrosis factor and lymphotoxin-alpha genetic polymorphisms and risk of relapse in childhood B-cell precursor acute lymphoblastic leukemia: a case-control study of patients treated with BFM therapy

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Abstract

Background: Circulating levels of tumor necrosis factor (TNF) and lymphotoxin-α (LT-α) have been associated with outcome in solid and hematologic malignancies. Within the *TNF* gene and the *LT-α* gene, polymorphisms have been identified at nucleotide positions -308 and +252, respectively. The variant alleles for *TNF* are designated *TNF1* and *TNF2,* the ones for *LT-α LT-α (10.5 kb)* and *LT-α (5.5 kb)*. Of interest, *TNF2 and LT-α (5.5 kb)* were shown to be associated with higher TNF and LT-α plasma levels than their counterparts. In the present study, we investigated the associations of the above mentioned polymorphisms with risk of relapse in childhood acute lymphoblastic leukemia (ALL) treated according to Berlin-Frankfurt-Münster (BFM) protocols.

Methods: Matched case-control study of 64 relapsed and 64 successfully treated non-relapsed childhood B-cell precursor ALL patients of standard and intermediate risk for treatment failure.

Results: The odds ratio (OR) for the combined category of *TNF1/TNF2* and *TNF2/TNF2* genotypes in comparison to the *TNF1/TNF1* genotype was 1.17 (95 % confidence interval (CI) = 0.53 - 2.56, *P* = 0.697). The ORs for the *LT-α (10.5 kb/5.5 kb)* and the *LT-α (5.5 kb/5.5 kb)* genotypes with reference to the *LT-α (10.5 kb/10.5 kb)* genotype were 2.17 (95 % CI = 0.84 - 5.58, *P* = 0.107) and 0.5 (95 % CI = 0.09 - 2.66, *P* = 0.418), respectively.

Conclusions: Our results do not suggest a major role of the investigated genetic polymorphisms with regard to risk of relapse in standard- and intermediate-risk childhood B-cell precursor ALL treated according to BFM protocols.

Background

Tumor necrosis factor (TNF) and lymphotoxin-α (LT-α ; formerly TNF-β) are cytokines with pleiotropic biological activities including, for example, the induction of programmed cell death and the regulation of immune cell proliferation and differentiation [1,2]. In a variety of studies, plasma levels of TNF or LT-α have been associated with outcome of certain autoimmune and infectious diseases as well as solid and hematologic malignancies [[3–6]]. Of interest, the secretion of TNF and *LT-α* is believed to be influenced by genetic polymorphisms within their genes located tandemly on the long arm of chromo-

some 6 within the MHC class III region. One of the best described of these polymorphisms is located at nucleotide position-308 within the *TNF* promoter region and affects a consensus sequence for a binding site of the transcription factor AP-2 [7,8]. Guanine at position-308 defines the common *TNF1* allele and adenine the less common *TNF2* allele. 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. This single nucleotide polymorphism (A252G) affects a phorbol ester-responsive element and distinguishes two alleles that have been designated *LT-α (10.5 kb)* and *LT-α (5.5 kb)* [9]. Both the *TNF2* and the *LT-α (5.5 kb)* allele have been shown to correlate with elevated TNF or LT-α plasma levels. Besides a more severe outcome of autoimmune or infectious diseases and of particular interest to us, the *TNF2* and the *LT-α (5.5 kb)* alleles have been associated with an adverse outcome in lymphoid malignancies [[10–15]].

In the present study, we genotyped a matched case-control study group of 64 relapsed and 64 non-relapsed patients with childhood acute lymphoblastic leukemia (ALL) for the above described genetic polymorphisms within the *TNF* and *LT-α* genes in order to assess their predictive potential with regard to relapse in childhood ALL.

Table 1: Characteristics of 64 relapsed case subjects and 64 successfully treated matched control subjects with acute lymphoblastic leukemia selected from trials ALL-BFM 86 and ALL-BFM 90

	Cases (%)	Controls (%)
Trial		
ALL-BFM 86	35 (54.7)	35 (54.7)
ALL-BFM 90	29 (45.3)	29 (45.3)
Sex		
Male	42 (65.6)	42 (65.6)
Female	22 (34.4)	22 (34.4)
Age (y)		
<1	1 (1.6)	1 (1.6)
1-9	56 (87.5)	56 (87.5)
10-14	6 (9.4)	7 (10.9)
15-18	1 (1.6)	-
WBC[a] ($10^3/\mu l$)		
<10	42 (65.6)	41 (64.1)
10-<50	21 (32.8)	22 (34.4)
≥ 50	1 (1.6)	1 (1.6)
Immunophenotype		
c-ALL[b]	54 (84.4)	54 (84.4)
pre-B-ALL[c]	10 (15.6)	10 (15.6)
Risk group[d]		
standard	23 (35.9)	23 (35.9)
intermediate	41 (64.1)	41 (64.1)
high	-	-
DNA index[e]		
<1.16	30 (46.9)	30 (46.9)
≥ 1.16	12 (18.8)	7 (10.9)
not examined	22 (34.4)	27 (42.2)
Genotype		
normal	4 (6.3)	10 (15.6)
11q23 abberations	-	1 (1.6)
t(1;19)	1 (1.6)	-
t(9;22)	-	-
other	15 (23.3)	13 (20.3)
not examined	44 (68.8)	40 (62.5)

[a] white blood cell count [b] common acute lymphoblastic leukemia [c] precursor B-cell acute lymphoblastic leukemia [d] therapy stratification in risk groups was mainly based on initial leukemic cell mass estimate and initial treatment response [16–18][e] ratio of DNA content of leukemic G_0/G_1 cells to normal diploid lymphocytes

Methods

Patients and study design

The present study utilizes patients and data from the ALL-BFM 86 and ALL-BFM 90 multicenter trials of childhood ALL, conducted by the BFM study group. Design, conduct, analysis, and results of the ALL-BFM 86 and ALL-BFM 90 trials are described in detail elsewhere [16,17]. In both trials treatment was stratified into three branches (standard, intermediate, and high risk), mainly according to the leukemic cell mass estimate and treatment response. Treatment (in most cases induction, consolidation, reinduction, maintenance) consisted of intensive multiagent chemotherapy regimens employing standard drugs (e.g. prednisone, vincristin, daunorubicin, L-asparaginase, cyclophosphamide, cytarabine, 6-mercaptopurine, 6-thioguanin, methotrexate). Parts of the study group received cranial radiotherapy.

The establishment of the present case-control study group has been described previously [18]. Briefly, relapsed patients from ALL-BFM 86 and ALL-BFM 90 with an available remission peripheral blood or bone marrow smear were included as cases into the study group if they could be matched to a successfully treated patient with an available remission peripheral blood or bone marrow smear (control individual) according to the following criteria: sex, age at diagnosis (± 6 months), white blood cell count (WBC) at diagnosis (± 10,000/μl), immunophenotype, trial, risk group, and treatment arm within the risk group of the respective trial. The latter criterion assured similarity of treatment between cases and controls. Controls had to have a minimum follow-up of 5 years. In case of relapses occurring later than 5 years of diagnosis, the follow-up for the control subject had to be at least as long as the time from date of initial diagnosis to date of relapse diagnosis in the case subject. If more than one control subject was available, the subject with the closest initial WBC at diagnosis with reference to the case subject was chosen. All spare remission peripheral blood or bone marrow smears were derived from official routine remission control examinations at time points during the first 6 month of treatment according to the study protocols of ALL-BFM 86 and 90.

Genotype analysis

Genomic DNA was isolated from remission bone marrow or peripheral blood smears as described before [18]. Genotypes for *TNF* and *LT-α* were determined by polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) analysis. The -308 *TNF* promoter polymorphism was analyzed by incorporating it into an NcoI restriction site that was created by introducing a single base change within the forward primer [7]. Primer sequences were: forward 5'-AGGCAATAGGTTTTGAGGGCCAT-3'; reverse 5'-TCCTCCCTGCTCCGATTCCG-3'. The *LT-α* polymorphism at nucleotide position +252 was analyzed by PCR amplification of a 368 bp fragment using the following primer pair: forward 5'-CTCCTGCACCTGCTGCCTGGATC-3'; reverse 5'-GAAGAGACGTTCAGGTGGTGTCAT-3' [10]. The amplified PCR products were digested overnight with NcoI and analyzed on 3.0 % Nusieve (TNF) or 3.0 % conventional agarose gels (LT-α). In case of presence of the *TNF1* allele, the amplified 107 bp fragment from the *TNF* promoter is cut into two fragments of 87 and 20 bp, a fragment amplified from *TNF2* remains uncut [7]. 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 [10].

Table 2: Distribution of tumor necrosis factor (TNF) and lymphotoxin-α (LT-α) genotypes and their association with the occurrence of relapse in 64 case subjects and 64 successfully treated matched control subjects with acute lymphoblastic leukemia from ALL-BFM trials 86 and 90

	Cases (%)	Controls (%)	OR[a] (95 % CI[b])	*P* value
TNF				
TNF1/TNF1	41 (64.1)	43 (67.2)	1.00	
TNF1/TNF2	21 (32.8)	20 (31.3)	1.17[c] (0.53-2.56)	0.697
TNF2/TNF2	2 (3.1)	1 (1.6)		
LT-α				
10.5 kb/10.5 kb	25 (39.1)	30 (46.9)	1.00	
10.5 kb/5.5 kb	32 (50.0)	23 (35.9)	2.17 [d] (0.84-5.58)	0.107
5.5 kb/5.5 kb	7 (10.9)	11 (17.2)	0.50 [d] (0.09-2.66)	0.418

[a] odds ratio [b] confidence interval [c] odds ratio for the combined category of *TNF1/TNF2* and *TNF2/TNF2* genotypes [d] odds ratio 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)* was 1.5 (95% CI = 0.68-3.32; *P* = 0.317)

Statistical analysis

After frequencies were calculated for descriptive purposes, correlation analyses (contigency coefficients for nominal data, Spearman correlation coefficients for ordinal data, Pearson correlation coefficients for continous data) were computed to investigate the interrelationships between TNF genotype, LT-α genotype and important clinical prognostic variables such as sex, age at diagnosis, WBC at diagnosis, and immunophenotype. Differences in the distribution of categorical variables were analyzed by X^2 or Fisher's exact test. The association between TNF and LT-α genotypes and relapse of leukemia was examined by use of conditional logistic regression analysis to calculate odds ratios and their 95 % confidence intervals. Genotypes and genotype combinations were used as categorical variables in the analyses. The association of genotypes with time to relapse was analyzed by log rank tests. Computations were peformed using SAS software (SAS-PC Version 6.04, SAS Institute Inc., Cary, NC).

Results and Discussion

Table 1 shows the distribution of matching variables and genetic analyses (DNA index and karyotype) in the 64 relapsed case subjects and the 64 successfully treated control subjects as previously described [18]. Within our entire study population of 128 patients, 84 (65.6%) patients were homozygous for the *TNF1* allele, 41 (32.0%) patients were heterozygous *(TNF1/TNF2)* and 3 (2.3%) patients were homozygous for the *TNF2* allele. With regard to LT-α, we observed 55 (43.0%) patients being homozygous for the *LT-α (lo.5 kb)* allele and 55 (43.0%) patients being heterozygous *(LT-α (10.5 kb)/ LT-α (5.5 kb))*. Eighteen (14.1%) 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 [15]. Thus, it seems unlikely that *TNF* or *LT-α* genotypes are associated with the pathogenesis of childhood B-cell precursor ALL in the German population. With regard to clinically important variables, no particular associations between the *TNF* and *LT-α* genotypes and sex, age at diagnosis, WBC at diagnosis, immunophenotype, and risk group were observed (data not shown). Table 2 shows the prevalences of the investigated *TNF* and *LT-α* genotypes by case-control status of our study subjects. In addition, Table 2 contains information on the association of the -308 *TNF* promoter polymorphism and the intronic *LT-α* polymorphism at nucleotide position 252 with risk of ALL relapse in our study subjects. For both genotypes, no particular associations with risk of ALL relapse were observed (Table 2). As reported in previous studies, the *TNF1* allele was significantly linked to the *LT-α (lo.5 kb)* allele as was the *TNF2* allele to the *LT-α (5.5 kb)* allele (X^2 test; $P < 0.01$) [10,13]. The strong association between polymorphic *TNF* and *LT-α* alleles made Warzocha and colleagues to use haplotypes for their outcome analysis in a study on lymphoma outcome related to *TNF* and *LT-α* alleles [10]. In their study, the authors found that the presence of at least two *TNF* or *LT-α* high-producer alleles *(TNF2 or LT-α (5.5 kb))* was an independent risk factor for progression-free survival in adult diffuse large-cell lymphoma patients [10]. When we analyzed the risk of relapse conferred by so-called high-producer *TNF/LT-α* haplotypes (at least two high-producer alleles) in comparison to low-producer *TNF/LT-α* haplotypes (less than two high-producer alleles) we also failed to observe any meaningful association in our study population (odds ratio (OR) = 1.08; 95% confidence interval (CI) = 0.51 - 2.30; $P = 0.841$). However, based on the discordant pairs observed in our study, we only had a power of 80% to detect risks greater than 3.0 conferred by the *TNF1/ TNF2* and the *LT-α (10.5 kb)/LT-α (5.5 kb)* genotypes or by the high-producer *TNF/LT-α* haplotypes. Therefore, we neither can exclude smaller effects on treatment outcome mediated by the above mentioned exposures nor reasonably exclude clinically important effects of the rare *TNF2/TNF2* or the *LT-α (5.5 kb)/ LT-α (5.5 kb)* genotype. Furthermore, we investigated possible influences of the analyzed *TNF* and *LT-α* genotypes on time to relapse or on site of ALL relapse (isolated bone marrow relapse; combined and isolated CNS relapse; combined and isolated testis relapse) and, as above, did not detect any associations.

From the data presented in this study, we are not able to generalize our findings to childhood ALL patients of all immunophenotypic subgroups since we only investigated common and pre-B-cell ALLs. Similarly, we were not able to assess an association of the investigated *TNF* and *LT-α* genotypes on risk of relapse in high-risk childhood ALL patients since also these patients were not part of our study group. The latter point may be interesting to pursue as Demeter and colleagues, in a study on *TNF* and *LT-α* polymorphisms in chronic lymphocytic leukemia (CLL), detected an increase of the *LT-α (10.5 kb)* allele at more advanced disease stages [15]. Thus, additional investigations including childhood ALL patients of all clinically relevant subgroups are needed to lead to more conclusive results. However, for the subgroup of childhood B-cell precursor ALL of standard and intermediate risk treated according to BFM regimens that was analyzed in the present study, the investigated genetic *TNF* and *LT-α* polymorphisms do not seem to play a major role with regard to risk of relapse.

Conclusions

In a matched case-control group of 64 relapsed and 64 successfully treated childhood B-cell precursor ALL patients (all at standard or intermediate risk), the *TNF* gene polymorphism at nucleotide position -308 and the

LT-α gene polymorphism at nucleotide position +252 were not significantly related with risk of ALL relapse. Our results do not suggest a major role of the investigated genetic polymorphisms with regard to risk of relapse in childhood B-cell precursor ALL of standard and intermediate risk treated according to BFM protocols.

Competing interests

None declared.

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