Impact of haplotypes of *TNF* in the natural course of infective endocarditis

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Abstract

Based on previous findings for the role of single nucleotide polymorphisms (SNPs) of *TNF* for the predisposition for bloodstream infections, this study investigates the role of these SNPs at the promoter positions −376, −308, −238 in infective endocarditis (IE). In a case–control study, 83 patients with IE and 83 controls were enrolled. Blood genotyping for the presence of G or A alleles of the three SNPs was carried out using restriction fragment length polymorphisms. Haplotypes were calculated. Patients were mostly infected by *Staphylococcus aureus* (32.5%) and by species of enterococci (14.3%) and streptococci (14.3%). Carriage of the minor frequency A alleles at −238 of the promoter region of *TNF* was greater than in controls (8.4% versus 1.2%, p 0.003). The presence of any of the three GGA/GAA/AGA haplotypes was more frequent in patients with IE (OR 8.22, 95CI% 1.8–37.4, p 0.001). After multivariate logistic regression analysis, it was found that the only factor related to fatal outcome was carriage of the wild-type GGG haplotype (OR, 3.29, 95CI%, 1.05–10.29, p 0.04). GGA, AGA and GAA haplotypes were more frequent in patients with IE than in controls, suggesting a predisposition for IE and a potential protective role against fatal outcome, as the wild-type GGG haplotype was independently related with death.

Keywords: Genetic predisposition, infective endocarditis, single nucleotide polymorphisms, *TNF* gene
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Introduction

Infective endocarditis (IE) is a severe disease with significant mortality even in the era of cardiovascular surgery and antimicrobial treatment [1,2]. Microbiology, clinical course of IE and factors influencing outcome have been discussed in large epidemiological studies worldwide [2–10]. Staphylococci and streptococci have high affinity and adhesion to the cardiac structures, as already shown through *in vitro* studies and experimental animal models for IE [1,11]. Usually these bacteria interact with platelets, coagulation factors and matrix molecules on the already damaged tissue surfaces. Vegetations on valves or other cardiac structures are the characteristic formation of IE; they emerge as the end result of the dynamic interaction between host and microorganisms [12]. As a result of this interaction, a great bulk of pro-inflammatory cytokines is released from cells of the innate immune system, namely circulating monocytes and tissue macrophages [13]. These cytokines are encoded by genes that present with subtle differences from one host to the other, also known as single nucleotide polymorphisms (SNPs).

One of the most broadly studied cytokine genes is *TNF* encoding for tumour necrosis factor-α (TNF-α), which is one of the major inducers of pro-inflammatory phenomena [13]. Three SNPs are well recognized at the promoter region of *TNF*; a substitution of guanine by adenine at the −376 position (−376 *TNF* G/A, rs1800750); a substitution of guanine by adenine at the −308 position (−308 *TNF* G/A, rs1800629); and a substitution of guanine by adenine at the −238 position (−238 *TNF* G/A,

rs361525). The clinical impact of SNPs is not fully elucidated; many authors believe that carriage of minor frequency alleles in any of the above positions of the gene promoter is linked with predisposition to infections. To this end, many case–control studies have shown that carriage of these minor frequency alleles is linked with susceptibility for early development of ventilator-associated pneumonia after intubation [14], with HIV infection [15], and with H1N1-related pneumonia in patients with predisposing factors like obesity and chronic diseases of the airways [16]. No data on the role of *TNF* SNPs exist for the acquisition of IE. However, SNPs of other inflammatory mediators are reported to impact on the physical course of IE. SNPs of lipopolysaccharide-binding protein in patients with IE (c.291 C > T and c.613 A > G) were significantly more frequent compared with healthy controls [17]. Recently, SNPs of *TLR2* encoding for Toll-like receptor-2 (TLR2), which is the major pattern recognition receptor for structures of Gram-positive bacteria, was found to be significantly correlated with IE in 65 Spanish patients [18]. The *TLR2* R753Q co-dominant (OR = 13.33), recessive (OR = 9.12) and dominant (OR = 3.65) genotypes showed a positive association with IE [18]. The above conducted studies that demonstrate a link between SNPs of the gene encoding for molecules participating in the inflammatory response and IE generates the hypothesis that SNPs of *TNF* may also play some role.

This is the first study ever conducted trying to investigate the role of *TNF* SNPs in the natural course of patients with IE who were prospectively evaluated at a tertiary reference hospital.

Patients and Methods

Study design

This case–control study was conducted during the period September 2007 to June 2011. Inclusion criteria for cases were (1) Caucasian origin; and (2) diagnosis of endocarditis by the DUKE criteria [19]. Patients with neutropenia, HIV infection or chronic intake of corticosteroids were excluded. The study was approved by the Ethics Committee of the ATTIKON University General Hospital. Each participant completed a written informed consent, allowing the study's investigator to take and store blood sample for DNA extraction and analysis of *TNF* SNPs. A similar amount of blood sample was drawn from 83 healthy Greek blood donors of Caucasian origin after informed consent. They were well-matched for age, sex and cardiovascular risk factors and they had a negative history for IE. The SNPs of *TNF* of these individuals were used as a control group. A regression model of statistical analysis of predictors of outcome was applied via the SPSS.200 statistical software program (SPSS Inc., Chicago, IL, USA).

Patients' data collection

Following the participant's informed consent, data were recorded as follows: (1) demographics (age, gender, location), underlying diseases (diabetes mellitus, cardiovascular diseases including coronary heart disease, renal and liver disease, immunosuppression), previous history of IE or rheumatic fever, intravenous drug abusers, prosthetic valve or cardiac device (pacemaker/defibrillator), valve predisposition for IE (valve prolapsed, stenosis or bicuspid valve, congenital cardiopathy); (2) microbiology of IE (staphylococci, streptococci, enterococci, fungi and miscellaneous pathogens); and (3) clinical course of IE: persistent bacteraemia (more than 72 h despite adequate antimicrobial treatment), time from IE onset to complications of IE (i.e. valve abscess, peripheral emboli, stroke, sepsis [20], heart failure NYHA III–IV [21], renal failure, prosthetic valve dehiscence), cardiac surgery for IE and mortality. All patients were treated with antibiotics according to treatment guidelines for IE [22].

Blood collection and genotyping

Two millilitres of whole blood for genotyping analysis was collected in EDTA and stored at −70°C until processed. Genomic DNA was extracted by the Purigen Blood Core Kit C (Qiagen, Hilden, Germany) according to the instructions of the manufacturer. Typing for the three SNPs of *TNF* was performed by PCR on a Sensoquest thermal cycler LabCycler Gradient using 50 ng of genomic DNA at a final volume of 27 μL with 50 mM of $MgCl_2$ (New England Biolabs, Ipswich, MA, USA), 20 mM of dNTPs (New England Biolabs) and 1 mM of *Taq* polymerase (New England Biolabs). Selection of primers for SNP alleles of the *TNF*-gene and conditions of PCR were based on a previous publication [16].

For the –376 (G/A) SNP, a 148-base-pair (bp) fragment was amplified with forward primer (5-CCTCAGGACTCAAC ACAGC-3′) and reverse primer (5′-GGGGACCAGGTCT GTGGTCTGTTTCCTGTTAA-3′). For the −308 G/A SNP, a 147-bp fragment was amplified with forward primer 5′-GAG GCA ATA GGT TTT GAG GGC CAT-3′and reverse primer 5′-GGG ACA CAC AAG CAT CAA G-3′. For –238 G/A SNP a 165-bp fragment was amplified with forward primer (5′-CAG ACCACAGACCTGGTC-3′) and reverse primer (5′-AAG GATACCCCTCACACTCCCCATCCTCCCGGATC-3′).

All PCR consisted of one initial denaturation phase of 95°C for 10 min followed by 35 cycles; each cycle consisted of one annealing step of 94°C for 1 min, one polymerization step of 60°C for 1 min and one elongation step of 72°C for 1 min. Then another cycle of 72°C for 7 min was carried out before termination. Ten microlitres of the PCR product was digested after incubation for 4 h at 37°C with 0.5 U of the restriction enzyme *Hpa*I for the −376 G/A TNF SNP; *Nco*I for −308 G/A