Inflammatory and immunogenetic markers in correlation with pulmonary tuberculosis*

Marcadores inflamatórios e imunogenéticos e sua relação com tuberculose pulmonar

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Abstract

Objective: To describe serum levels of the cytokines IL-10, TNF-α, and IFN-γ, as well as polymorphisms in the genes involved in their transcription, and their association with markers of the acute inflammatory response in patients with pulmonary tuberculosis. **Methods:** This was a descriptive, longitudinal study involving 81 patients with pulmonary tuberculosis treated at two referral hospitals. We collected data on sociodemographic variables and evaluated bacteriological conversion at the eighth week of antituberculosis treatment, gene polymorphisms related to the cytokines studied, and serum levels of those cytokines, as well as those of C-reactive protein (CRP). We also determined the ESR and CD4+ counts. **Results:** The median age of the patients was 43 years; 67 patients (82.7%) were male; and 8 patients (9.9%) were infected with HIV. The ESR was highest in the patients with high IFN-γ levels and low IL-10 levels. IFN-γ and TNF-α gene polymorphisms at positions +874 and −238, respectively, showed no correlations with the corresponding cytokine serum levels. Low IL-10 levels were associated with IL-10 gene polymorphisms at positions −592 and −819 (but not −1082). There was a negative association between bacteriological conversion at the eighth week of treatment and CRP levels. **Conclusions:** Our results suggest that genetic markers and markers of acute inflammatory response are useful in predicting the response to antituberculosis treatment.

Keywords: Tuberculosis; Cytokines; Immune system; Polymorphism, single nucleotide.

Resumo

Objetivo: Descrever os níveis séricos das citocinas IL-10, TNF-α e IFN-γ, assim como polimorfismos presentes em genes envolvidos na sua transcrição, e sua associação com marcadores de resposta inflamatória aguda em pacientes com tuberculose. **Métodos:** Estudo descritivo e longitudinal realizado em 81 pacientes com tuberculose pulmonar atendidos em dois hospitais de referência. Foram coletadas informações sociodemográficas, conversão bacteriológica na oitava semana de tratamento antituberculose, polimorfismos relacionados às citocinas estudadas, níveis séricos dessas citocinas, assim como de proteína C reativa (PCR). Também foram avaliados VHS e contagem de CD4+. **Resultados:** A mediana de idade dos pacientes era de 43 anos, sendo 67 (82,7%) do sexo masculino e 8 (9,9%) infectados por HIV. Os pacientes com níveis elevados de IFN-γ e baixos níveis de IL-10 apresentaram valores mais elevados de VHS. Não houve associação dos polimorfismos do gene IFN-γ na posição +874 e do gene TNF-α na posição −238 com os níveis das citocinas correspondentes. Houve uma associação entre polimorfismos do gene IL-10 nas posições −592 e −819 (mas não −1082) e baixos níveis de IL-10. Houve uma associação negativa entre a taxa de conversão bacteriológica na oitava semana de tratamento e níveis de PCR. **Conclusões:** Nossos resultados sugerem que marcadores genéticos e de resposta inflamatória aguda podem ser úteis na predição da resposta ao tratamento antituberculose.

Descritores: Tuberculose; Citocinas; Sistema imunológico; Polimorfismo de nucleotídeo único.

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Introduction

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis*, the most common clinical manifestation of which is pulmonary involvement; however, tuberculosis can affect other anatomical sites (extrapulmonary tuberculosis) or present as disseminated disease.[1]

Despite being a curable disease, tuberculosis remains a major public health problem worldwide. According to the World Health Organization, Brazil ranks 19th among the 22 countries that collectively account for 80% of all cases of tuberculosis worldwide and 108th among those in which the incidence of tuberculosis is highest. According to the Brazilian National Ministry of Health, 71,000 new cases of tuberculosis were added to the Brazilian Case Registry Database in 2010, corresponding to an incidence rate of 37.2/100,000 population.[2]

The systemic inflammation observed in patients with tuberculosis is mediated by the activation of the immune system, with excessive production of cytokines, such as IL-1, IL-2, IFN-γ, and TNF-α.[3] After the inflammatory process, there is an increase in the hepatic synthesis and serum levels of acute phase proteins, such as C-reactive protein (CRP), as well as in the ESR, which have been used in the diagnosis and follow-up of patients, given that their plasma levels directly reflect the intensity of the pathological process.[4]

Genetic factors have been associated with susceptibility to or protection against infection with *M. tuberculosis*.[5] In the immune response to *M. tuberculosis*, allele frequencies in cytokine gene polymorphisms vary considerably across populations, as reported in meta-analyses evaluating IFN-γ, IL-10, and TNF-α gene polymorphisms.[6,7] It has been proposed that serum cytokine levels and their role as markers of response to antituberculosis treatment be evaluated.[8] The maintenance of initially low serum levels of IFN-γ or high serum levels of TNF-α and of increased serum levels of IL-17 is associated with a worse prognosis, including a higher mortality rate and lower bacteriological conversion at the end of the 8th week of antituberculosis treatment. Recently, Lago et al.[9] described a possible association between recurrent tuberculosis and maintenance of high serum levels of IL-10 during antituberculosis treatment. The study of the genes involved in these processes and their interactions with the immune and inflammatory responses can aid in identifying better markers of protection against tuberculosis.

There have been few studies simultaneously evaluating the genotypic and phenotypic aspects of the human host immune response to infection with *M. tuberculosis*.[10,11] Given the paucity of data on the simultaneous evaluation of genetic, immunological, and inflammatory biomarkers in patients with pulmonary tuberculosis, we conducted the present study in order to determine the prevalence of IL-10 gene polymorphisms at positions −592, −819, and −1082; the prevalence of TNF-α gene polymorphisms at position −238; and the prevalence of IFN-γ gene polymorphisms at position +874. The study involved a sample of active pulmonary tuberculosis patients admitted to and treated at either of two referral hospitals in the city of Rio de Janeiro, Brazil. In addition, we measured the serum levels of the corresponding cytokines and analyzed the acute inflammatory response by determining CRP levels and CD4+ counts, as well as the ESR.

Methods

This was a longitudinal descriptive study involving 81 patients diagnosed with pulmonary tuberculosis and admitted to either of two referral hospitals for the treatment of tuberculosis in the state of Rio de Janeiro, Brazil (the *Hospital Estadual Santa Maria* and the *Instituto Estadual de Doenças do Tórax Ary Parreiras*), between March 23, 2007 and August 7, 2009. We included patients with positive smear microscopy and culture for mycobacteria, the presence of *M. tuberculosis* being subsequently confirmed by biochemical tests. We analyzed the following variables: CRP, ESR, CD4+, and bacteriological conversion at the 8th week of antituberculosis treatment.

For DNA extraction, a commercial kit (DNAzol; Gibco BRL/Life Technologies, Gaithersburg, MD, USA) was used in accordance with the manufacturer instructions. After DNA extraction, a DNA sample was analyzed by electrophoresis on 1% agarose gel in order to determine integrity and concentration, the sample being subsequently stored at −20°C.

For the analysis of TNF-α gene polymorphisms at position −238, 100 ng of DNA, 1× buffer, 1.5 mM $MgCl_2$, 200 µM dNTP, and 1 U Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA) were added to 15 pmol of each primer for polymerase chain reaction, which

was performed as follows: one cycle at 94°C for 1 min, followed by 5 cycles at 94°C, 67°C, and 72°C (60 s each), and 25 cycles at 94°C, 62°C, and 72°C (60 s each). For the genotyping of IFN-γ gene polymorphism at position +874, we used 200 mL of dNTP, 1.5 mM $MgCl_2$, 8.5% sucrose, 0.25 U of ThermoPrime Plus DNA polymerase (Thermo Fisher Scientific, Waltham, MA, USA), 5 mL of each specific primer, 0.5 mL of internal control primer, and 100 ng of DNA. The mixture was incubated at 95°C for 1 min; subsequently, 10 cycles were performed at 95°C for 15 s, followed by 10 cycles at 62°C for 50 s, 10 cycles at 72°C for 40 s, 20 cycles at 95°C for 20 s, 20 cycles at 56°C for 50 s, and 20 cycles at 72°C for 50 s. For the detection of IL-10 promoter gene polymorphisms at positions −819, −1082, and −592, the following steps were taken: for the −592 position, a 480-bp fragment was amplified and subsequently digested with the enzyme RsaI. For the −1082 and −819 positions, a 360-bp fragment was amplified and subsequently digested with the enzymes BseRI and MslI, respectively. In brief, 100 ng of DNA were added to each polymerase chain reaction, resulting in a final volume of 40 µL (−819 and −1082) or 30 µL (−592), consisting of 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 µM dNTP, 1.25 U AmpliTaq Gold DNA Polymerase (Perkin-Elmer Cetus, Norwalk, CT, USA) and specific primers for each mutation (10 pmol for the −592 position and 12.5 pmol for the −819/−1082 positions). All mixtures were incubated at 95°C for 10 min and submitted to amplification at 94°C for 30 s, at 60°C for 30 s, at 72°C for 40 s, and at 72°C for 7 min (IL-10 at position −592), followed by 35 cycles at 94°C for 30 s, at 58°C for 30 s, and at 72°C for 45 s, plus a final cycle at 72°C for 5 min (positions −819 and −1082). The amplified products were electrophoresed on 2% agarose gel containing ethidium bromide (0.5 mL/mL).

In the determination of cytokine levels, bead populations were visualized on the basis of their fluorescence intensities. In the cytometric bead array system, cytokine capture beads are mixed with detection antibody conjugated to the fluorochrome phycoerythrin and then incubated with the samples for the "conjugate" assay. The acquisition tubes were prepared with 50 µL of sample, 50 µL of the bead mixture, and 50 µL of detection reagent human Th1/Th2 phycoerythrin. The same procedure was performed in order to obtain the standard curve. The tubes were homogenized and incubated for three hours at room temperature in the dark. Subsequently, the reading was performed with a BD™ Cytometric Bead Array system (Thermo Fisher Scientific).[12]

Serum levels of CRP were used as a marker of the acute phase response (APR), i.e., as a marker of the systemic response to severe inflammation. A positive APR was defined as CRP levels > 0.3 mg/dL, whereas a negative APR was defined as CRP levels < 0.3 mg/dL. Serum CRP levels were measured by nephelometry.

The ESR was also used as a marker of the APR, a positive APR being defined as an ESR > 2 mm/h for females and as an ESR > 7 mm/h for males. The ESR was measured by the Westergren method.

We used descriptive statistics, including range (minimum and maximum values), mean, standard deviation, and 95% CI. We used the Kolmogorov-Smirnov test in order to test the normality of the variables and the Levene test in order to determine the equality of variances. Variables with non-normal distribution were log-transformed. For means with normal distribution, we used the Student's t-test. We used ANOVA in order to analyze the differences among quantitative variables. We used the chi-square test in order to identify associations among categorical variables. A value of $p < 0.05$ was considered statistically significant. We used the Statistical Package for the Social Sciences, version 16.0 for Windows (SPSS Inc., Chicago, IL, USA). In the analyses, we used the bacteriological conversion coefficient, which was calculated as the number of cases of patients who converted from a negative test result to a positive test result divided by the total number of patients at the beginning of treatment, and the mutation coefficient for polymorphisms, which was calculated as the number of cases of a given mutation divided by the total number of cases.

The present study was approved by the Research Ethics Committee of the Federal University of Rio de Janeiro School of Medicine Clementino Fraga Filho University Hospital on April 28, 2005 (Protocol no. 004/05).

Results

The median age of the patients was 43 years (range, 20-60 years). Of the 81 patients studied, 67 (82.7%) were male, 54 (66.7%) were non-White,

8 (9.9%) were co-infected with HIV, 52 (64.2%) reported regular alcohol use, 55 (67.9%) were smokers or former smokers, 20 (24.7%) reported illicit drug use, and 64 (79.0%) had normal CD4+ counts. All patients had smear-positive pulmonary tuberculosis.

In the analysis of the prevalence of IFN-γ gene polymorphisms at position +874, of TNF-α gene polymorphisms at position −238, and of IL-10 gene polymorphisms at positions −592, −819, and −1082, the mutant allele frequency was found to be 0.56, 0.56, 0.29, 0.43, and 0.68, respectively.

Table 1 shows the distribution of polymorphisms in the patients under study, by serum cytokine levels. Serum IFN-γ levels were found to range from 0 (zero) pg/mL to 20.5 pg/ml, and there was no relationship between low serum levels of IFN-γ and the presence of mutations. Regarding TNF-α, although we found no homozygous mutations, we found a trend toward low serum levels of TNF-α among heterozygotes. We found a negative relationship between serum IL-10 levels and IL-10 gene polymorphisms at positions −592 and −819 ($p < 0.001$; Figure 1).

As can be seen in Table 2, there was a trend ($p = 0.08$) toward lower CRP production in the patients in whom serum IFN-γ levels were low (0.0-4.9 pg/mL) when compared with those in whom serum IFN-γ levels were higher (> 5.0 pg/mL). In the patients in whom serum TNF-α levels were low (0.0-4.9 pg/mL), there was a trend toward a higher ESR ($p = 0.04$). Low serum levels of IL-10 (i.e., serum IL-10 levels of 0.0-4.9 pg/

Table 1 - Distribution of the polymorphisms found in the patients under study, by serum cytokine levels.

Polymorphism	Patients, n (%)		
	Serum cytokine levels		
	0.0-4.9 pg/mL	5.0-9.9 pg/mL	10.0-39.9 pg/mL
IFN-γ			
TT	1 (1.2)	6 (7.4)	2 (2.4)
TA	14 (17.2)	29 (35.8)	10 (12.3)
AA	5 (6.1)	10 (12.3)	4 (4.9)
TA/AA	19 (23.3)	39 (48.1)	14(17.2)
TNF-α			
GG	10 (12.3)	1 (1.2)	0 (0.0)
GA	57 (70.3)	13 (16)	0 (0.0)
AA	0 (0.0)	0 (0.0)	0 (0.0)
GA/AA	57 (70.3)	13 (16)	0 (0.0)
IL-10 at position −592			
CC	22 (27.1)	12 (14.8)	0 (0.0)
CA	22 (27.1)	22 (27.1)	1 (1.2)
AA	1 (1.2)	1 (1.2)	0 (0.0)
CA/AA	23 (28.3)	23 (28.3)	1 (1.2)
IL-10 at position −819			
CC	7 (8.6)	4 (4.9)	0 (0.0)
CT	25 (30.8)	23 (28.3)	1 (1.2)
TT	1 (1.2)	8 (9.8)	0 (0.0)
CT/TT	26 (32)	31 (38.1)	1 (1.2)
IL-10 at position −1082			
GG	2 (2.4)	6 (7.4)	0 (0.0)
GA	22 (27.1)	13 (16)	0 (0.0)
AA	2 (2.4)	16 (19.7)	1(1.2)
GA/AA	24 (29.5)	29 (35.7)	1(1.2)

(IFN-γ): TT: wild-type homozygous genotype; TA: heterozygous genotype; and AA: mutant homozygous genotype. (TNF-α): GG: wild-type homozygous genotype; GA: heterozygous genotype; and AA: mutant homozygous genotype. (IL-10 at position −592): CC: wild-type homozygous genotype; CA: heterozygous genotype; and AA: mutant homozygous genotype. (IL-10 at position −819): CC: wild-type homozygous genotype; CT: heterozygous genotype; and TT: mutant homozygous genotype. (IL-10 at position −1082): GG: wild-type homozygous genotype; GA: heterozygous genotype; and AA: mutant homozygous genotype.

mL) were not associated with a higher ESR or with higher CRP levels. However, the ESR was negatively correlated with serum IL-10 levels ($p = 0.03$) and was positively correlated with serum IFN-γ levels ($p = 0.008$; Table 3).

Table 4 shows that lower bacteriological conversion was associated only with high serum levels of CRP. However, by applying the bacteriological conversion coefficient, we found a negative correlation between serum TNF-α levels and bacteriological conversion ($r = -0.43$; $p < 0.001$).

Discussion

To our knowledge, this is the first study to examine the relationships among biochemical markers, inflammatory markers, and immunogenetic markers in pulmonary tuberculosis patients in Brazil. The clinical features of the patients in our sample were similar to those of patients admitted to tuberculosis hospitals in developing countries.[13]

The genetic component of the host response to infection with *M. tuberculosis* in restricted ethnic groups is evident in the literature.[5] In the present study, the frequency of the mutant allele for IFN-γ gene polymorphisms at position +874 was 0.56, which is similar to that reported by other authors in various countries[14-16] but different from that reported by Fitness et al. in Africa.[17] In addition, the frequency of the mutant allele for TNF-α gene polymorphisms at position +238 was 0.56, which is similar

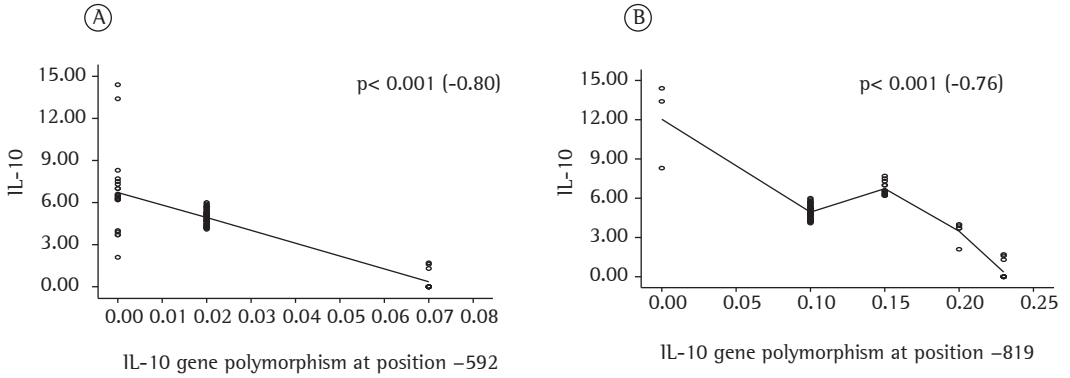

Figure 1 - Regression coefficient for IL-10 gene polymorphisms at positions –592 (in A) and –819 (in B).

Table 2 - Distribution of serum levels of IFN-γ, TNF-α, and IL-10 in the patients under study, by laboratory test results.

Results	Patients, n (%)								
	Serum cytokine levels								
	IFN-γ			TNF-α			IL-10		
	0.0-4.9 pg/mL	5.0-9.9 pg/mL	10.0-39.9 pg/mL	0.0-4.9 pg/mL	5.0-9.9 pg/mL	10.0-39.9 pg/mL	0.0-4.9 pg/mL	5.0-9.9 pg/mL	10.0-39.9 pg/mL
ESR									
Low	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Normal	1 (1.3)	6 (7.6)	1 (1.3)	4 (5.1)	4 (5.1)	0 (0.0)	4 (5.1)	3 (3.8)	0 (0.0)
High	19 (24.1)	37 (46.8)	15 (19)	61 (77.2)	10 (12.7)	0 (0.0)	41 (51.9)	30 (38)	1 (1.3)
CD4+									
Low	5 (6.6)	9 (11.8)	3 (4)	13 (17.1)	4 (5.3)	0 (0.0)	9 (11.8)	8 (10.5)	0 (0.0)
Normal	15 (19.7)	31 (40.8)	10 (13.2)	47 (61.8)	9 (11.8)	0 (0.0)	30 (39.57)	25 (32.9)	1 (1.3)
High	0 (0.0)	2 (2.6)	1 (1.3)	3 (3.9)	0 (0.0)	0 (0.0)	3 (3.9)	0 (0.0)	0 (0.0)
CRP									
Normal	1 (1.3)	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)	0 (0.0)	0 (0.0)	1 (1.3)	0 (0.0)
High	19 (23.8)	45 (56.3)	15 (18.8)	66 (82.5)	13 (16.9)	0 (0.0)	45 (56.3)	33 (41.3)	1 (1.3)

CRP: C-reactive protein.

to that reported in other studies.[18-20] In our sample, the allele frequencies for IL-10 gene polymorphisms at positions −592, −819, and −1082 were, respectively, 0.29, 0.43, and 0.68, being similar to those reported in most of the studies included in a meta-analysis.[6] Although our results are consistent with those of various studies, any differences regarding the frequency of these polymorphisms can be explained by ethnic differences among the study populations.

Table 3 - Correlation between serum cytokine levels and laboratory test results.

Biochemical variable	p		
	IFN-γ	TNF-α	IL-10
CD4+	0.59	0.11	0.47
CRP	0.43	0.32	0.33
ESR	0.008 (+)	0.31	0.03 (−)

C-reactive protein; (+): positive correlation; and (−): negative correlation.

The functional role of allele −238A (TNF-α) in the regulation of TNF-α gene expression was described by Kaluza et al.,[21] whose in vitro studies showed an association between allele −238A and a downregulation of the TNF-α gene (and, consequently, a reduction in TNF-α protein production). Although we found no homozygous mutations, we observed a trend toward low serum levels of TNF-α in patients with a heterozygous genotype, as did Abhimanyu et al.[10] in a population of individuals from India whose ethnic characteristics were quite different from those of our study population. However, Haroon et al.[22] found no association between mutation and cytokine expression in a population of White individuals.

The presence of single-nucleotide polymorphisms in the first intron of the IFN-γ gene (at position +874) has been associated with tuberculosis[8,14] and severe tuberculosis.[23] The

Table 4 - Serum levels of C-reactive protein, ESR, CD4+, and cytokines, as well as frequency of genotypes, by bacteriological conversion.[a]

Variable	Bacteriological conversion		p
	Yes	No	
CRP, mg/dL	5.37 ± 3.96	8.6 ± 4.7	0.004
ESR, mm/h	60.3 ± 43.3	53.8 ± 37.6	0.57
CD4+, cells/mm^3	784.09 ± 699.44	657.5 ± 311.49	0.46
IL-10, pg/mL	4.6 ± 2.8	4.8 ± 1.21	0.73
TNF-α, pg/mL	3.6 ± 1.93	3.99 ± 1.36	0.42
IFN-γ, pg/mL	7.17 ± 4.84	7.69 ± 3.99	0.68
IL-10 at position −592[b]			
AA	2 (3.4)	0 (0.0)	0.42
CA/CC	57 (96.6)	18 (100.0)	
IL-10 at position −819[b]			
TT	8 (13.6)	2 (11.1)	0.78
CT/CC	51 (86.4)	16 (88.9)	
IL-10 at position −1082[b]			
AA	27 (45.8)	9 (50.0)	0.75
GA/GG	32 (54.2)	9 (50.0)	
TNF-α[b]			
AA	0 (0.0)	0 (0.0)	--
GA/GG	59 (100.0)	18 (100.0)	
IFN-γ[b]			
AA	11 (18.6)	6 (33.3)	0.18
TA/TT	48 (81.4)	12 (66.7)	

CRP: C-reactive protein; (IFN- γ): TT: wild-type homozygous genotype; TA: heterozygous genotype; and AA: mutant homozygous genotype. (TNF-α): GG: wild-type homozygous genotype; GA: heterozygous genotype; and AA: mutant homozygous genotype. (IL-10 at position −592): CC: wild-type homozygous genotype; CA: heterozygous genotype; and AA: mutant homozygous genotype. (IL-10 at position −819): CC: wild-type homozygous genotype; CT: heterozygous genotype; and TT: mutant homozygous genotype. (IL-10 at position −1082): GG: wild-type homozygous genotype; GA: heterozygous genotype; and AA: mutant homozygous genotype. [a]Values expressed as mean ± SD, except where otherwise indicated. [b]Values expressed as n (%).

gene encoding IFN-γ is highly conserved, and few polymorphisms are found in the intragenic region. In our sample, we found no association between IFN-γ gene polymorphisms at position +874 and serum IFN-γ levels, a finding that is similar to those of Abhimanyu et al.[10] and Vidyarani et al.[24] but different from those of Vallinoto et al.,[11] who found low serum levels of IFN-γ in patients with a homozygous mutant genotype at position +874A/A.

We found a significant relationship between high IFN-γ levels and a high ESR, a finding that is consistent with those of Peresi et al.[4] This is possibly due to the fact that the presence of this mutation has been associated with decreased production of IFN-γ (a cytokine that plays an important role in controlling the defense against the pathogen) and, therefore, a diminished acute inflammatory response.

In our study, low serum levels of IL-10 were found to be associated with IL-10 gene polymorphisms at positions −592 and −819 (but not −1082). This finding is similar to those of Abhimanyu et al.[10] and Edwards-Smith et al.,[25] who investigated IL-10 gene polymorphisms at position −1082 and showed that individuals carrying the AA genotype are low IL-10 producers, those carrying the GA genotype are intermediate IL-10 producers, and those carrying the GG genotype are high IL-10 producers; however, the ATA haplotype is associated with low IL-10 production. These discrepant results can be partly explained by the distinct and heterogeneous ethnic characteristics of the study populations.

We observed a trend toward a higher ESR among carriers of IL-10 gene polymorphisms at positions −592 (CA/AA) and −819 (CT/TT). The authors of a recent meta-analysis including 18 studies (none of which included patients from Latin America) were unable to confirm a higher risk of tuberculosis among patients with IL-10 gene polymorphisms at positions −592, −819, or −1082 but found a higher risk of tuberculosis among Europeans with IL-10 gene polymorphisms at position −1082.[6] In that meta- analysis, one of the studies assessing serum IL-10 levels also assessed serum levels of IFN-γ and IL-10. The authors demonstrated that a stronger relationship translated to less severe tuberculosis.

Jamil et al.[26] and Lago et al.[9] suggested that the maintenance of high serum levels of IL-10 during antituberculosis treatment is associated with an increased risk of recurrence, whereas low serum levels of IL-10 usually occur in mild forms of tuberculosis. The results obtained in the present study do not allow us make inferences regarding this issue, given that serum IL-10 levels were assessed only at time point zero and not during clinical follow-up (after completion of antituberculosis treatment).

In the present study, acute inflammatory response markers (CRP levels) were found to be higher in the patients in whom serum TNF-α levels were low (0.0-4.9 pg/mL) than in those in whom serum TNF-α levels were above 5.0 pg/mL. These data suggest that the presence of low concentrations of TNF-α at the time of the initial response against the disease is associated with a worse prognosis and clinical course; however, studies involving larger samples, as well as correlation studies, should be conducted in order to test these hypotheses in the Brazilian population, as mentioned in a review article by Wallis et al.[8] The role of TNF-α in the pathophysiology of tuberculosis has been associated with defense via macrophage activation and the subsequent inflammatory reaction.[3] Our findings reinforce the importance of this cytokine in the host response to *M. tuberculosis*.

In the present study, an association was found between elevated CRP levels and lower bacteriological conversion at the 8th week of antituberculosis treatment, showing the potential role of this mediator as a marker for monitoring the clinical course of the disease. Some authors have reported that ESR normalization is a marker of good response to treatment in subacute and chronic diseases, such as tuberculosis.[27,28] Various studies have shown increased levels of immune response markers, CRP, and ESR in the initial phase, all of which decrease during treatment.[29,30] Similar results were reported in a study by Peresi et al.,[4] in which CRP levels were significantly decreased only in the 3rd and 6th months of treatment. These findings suggest that CRP can be used in order to evaluate the APR in tuberculosis patients and as a marker of response to antituberculosis treatment, together with the clinical and epidemiological history of such patients.

In our study, there was no association between serum IFN-γ levels and bacteriological conversion, a finding that is similar to those of another study.[8] However, there was an association

between initially low serum levels of TNF-α and higher bacteriological conversion. These data are similar to those reported by Su et al.[30] Regarding bacteriological conversion (or lack thereof) and immunological and biochemical variables, we found a positive correlation between the inflammatory marker CRP and the absence of conversion. However, no such correlation was found for the remaining inflammatory markers (ESR and CD4+).

The limitations of the present study include the fact that we did not analyze IL-10 haplotypes, the fact that we did not include other cytokines that play a relevant role in the immune response to active tuberculosis, and the fact that we did not monitor the clinical and bacteriological response of the patients throughout the antituberculosis treatment period.

It is of note that, to our knowledge, this is the first study in Brazil to investigate the presence of proinflammatory and anti-inflammatory cytokines, acute inflammatory response mediators (by measuring serum CRP levels and the ESR), and the genetic background of patients in an attempt to elucidate certain mechanisms of the immunopathogenesis of tuberculosis. Given that this was a descriptive study, there was no control group, which is why we were careful to present the statistical associations without referring to the variables as "risk factors" for any given event.

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