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ORIGINAL ARTICLE

Variants in *LTA*, *TNF*, *IL1B* and *IL10* genes associated with the clinical course of sepsis

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Abstract The aim of this study was to explore the association between some SNPs of the *TNF*, *LTA*, *IL1B* and *IL10* genes with cytokine concentrations and clinical course in Colombian septic patients. We conducted a cross-sectional study to genotype 415 septic patients and 205 patients without sepsis for the SNPs −308(G/A) rs1800629 of *TNF*; +252 (G/A) rs909253 of *LTA*; −511(A/G) rs16944 and +3953(C/T) rs1143634 of *IL1B*; and −1082(A/G) rs1800896, −819(C/T) rs1800871 and −592(C/A) rs1800872 of *IL10*. The association of theses SNPs with the following parameters was evaluated: (1) the presence of sepsis; (2) severity and clinical outcomes; (3) APACHE II and SOFA scores; and (4) procalcitonin, C-reactive protein, tumor necrosis factor, lymphotoxin alpha, interleukin 1 beta and interleukin 10 plasma concentrations. We found an association between the SNP *LTA* +252 with the development of sepsis [OR 1.29 (1.00–1.68)]; the SNP *IL10* −1082 with sepsis severity [OR 0.53 (0.29–0.97)]; the *TNF* −308 with mortality [OR 0.33 (0.12–0.95)]; and the *IL10* −592 and *IL10* −1082 with admission to the intensive care unit (ICU) [OR 3.36 (1.57–7.18)] and [OR 0.18 (0.04–0.86)], respectively. None of the SNPs were associated with cytokine levels, procalcitonin and C-reactive protein serum concentrations, nor with APACHE II and SOFA scores. Our results suggest that these genetic variants play an important role in the development of sepsis and its clinical course.

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Introduction

Sepsis is an important public health problem in the whole world. It is considered the most common cause of death among critically ill patients in non-coronary intensive care units (ICU) [1]. In Colombia, it is also considered an important health problem. In a multicenter study carried out in 10 hospitals between 2007 and 2008, the researchers reported a monthly cumulative incidence rate of sepsis of 3.61 per 100 admissions and a monthly period prevalence of 18.6 % per hospital [2].

In the second and third international consensus definitions of sepsis, it is commonly recognized that sepsis is generated as a result of a host of systemic dysfunctional

responses to infection, and therefore, most of the clinical manifestations are not only a consequence of microbial pathogenicity, but are strongly influenced by the development of systemic inflammation [3, 4]. During a typical clinical course, most of the patients develop a pro-inflammatory stage meant to eliminate the pathogen, which may lead to significant tissue damage. The pro-inflammatory stage is accompanied by an anti-inflammatory response so as to restore immune homeostasis that may be associated with immunosuppression [5]. The balance between these stages determines the outcome of the patients; as it is an extremely flexible process, it depends on several variables including the genetic background of the host [6]. In fact, different studies have demonstrated the importance of genetic components, such as single-nucleotide polymorphisms (SNPs) in genes encoding inflammatory mediators with susceptibility to, and development and prognosis of sepsis [7–9].

Cytokines are mediators of inflammation associated with sepsis susceptibility and severity, since they stimulate most of the characteristic signs and symptoms of systemic inflammation in sepsis [10], and consequently, the differences in their expression as response to infection could be responsible for the diversity of clinical courses of the patients. Indeed, some studies have associated cytokine concentrations with clinical severity [11–14]. Based on previous evidences, the purpose of this study is to explore the association between certain genetic variants such as the tumor necrosis factor (*TNF*), lymphtoxin alpha (LTα), interleukin 1 beta (IL1β) and interleukin 10 (*IL10*) genes and cytokine plasma levels, with sepsis development, the severity and the clinical outcomes in Colombian patients at different clinical stages.

Materials and methods

Ethics

The study protocol and the informed consent were approved by the Ethical Committee at the Instituto de Investigaciones Médicas (Universidad de Antioquia) and at the Sede investigación Universitaria (CBEIH-SIU).

Study subjects

The study was conducted in the emergency department (ED) and in the ICU of the "Hospital San Vicente Fundación" (Medellín, Colombia), a fourth-level University Hospital with an admission rate of approximately 1800 patients per month through the ED. The study design was defined as a cross-sectional analysis nested in a cohort because we were considering here only time-fixed variables: some genetic variants of cytokines, measured once the patients were hospitalized, and simultaneously the clinical status at the time of admission by to the emergency room. Although we also explored the association between SNPs and mortality, the purpose of this was a secondary analysis that was only exploratory.

During our study, we genotyped 620 patients belonging to a larger cohort of patients examined in a previous study [15]. The cohort included patients that were > 18 years old with clinical suspicion of infection and were admitted in the ED or ICU within 24 h before recruitment for the study, between July 2007 and September 2008.

Among inclusion criteria was the presence of at least one of the following characteristics: (A) suspected or confirmed infection according to the Center for Disease Control (CDC) definitions, (B) fever of unknown origin, (C) delirium or any type of encephalopathy of unknown origin, and (D) acute hypotension not explained by hemorrhage, myocardial infarction, stroke or heart failure. Exclusion criteria were: (A) refusal of the patients, their families or the attending physician to be part of the study, (B) antimicrobial treatment received in other medical institutions immediately before admission to the study, (C) medical decision to treat the patient ambulatory or in a different institution within 24 h after admission, (D) homeless or inability of the patient to follow-up and (E) previous participation in the same study. A clinical consensus of three experts with training and experience in internal medicine, infectious diseases and intensive care independently determined whether or not the patients presented an infection, and classified the study population into two groups, each one with two subgroups: (1) patients without sepsis: (A) patients with alternative diagnosis and (B) infected patients without sepsis, and (2) septic patients: (A) patients with sepsis but without organ dysfunction and (B) patients with severe sepsis. Then, in those cases that they did not reach an agreement, a formal meeting with discussion was required for a definitive consensus. The experts used the criteria of CDC for nosocomial infections and the International Sepsis Definitions Conference that took place in 2001 [3]. The criteria of each expert were defined during the first week, after knowing the results of microbiological tests (Fig. 1).

Clinical variables

Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA) score, procalcitonin (PCT) and C-reactive protein (CRP) serum levels were measured within 24 h after recruitment. PCT concentrations were measured by an immunoluminometric assay VIDAS B·R·A·H·M·S PCT (Biomérieux, Marcy-l'Étoile, France). CRP was measured

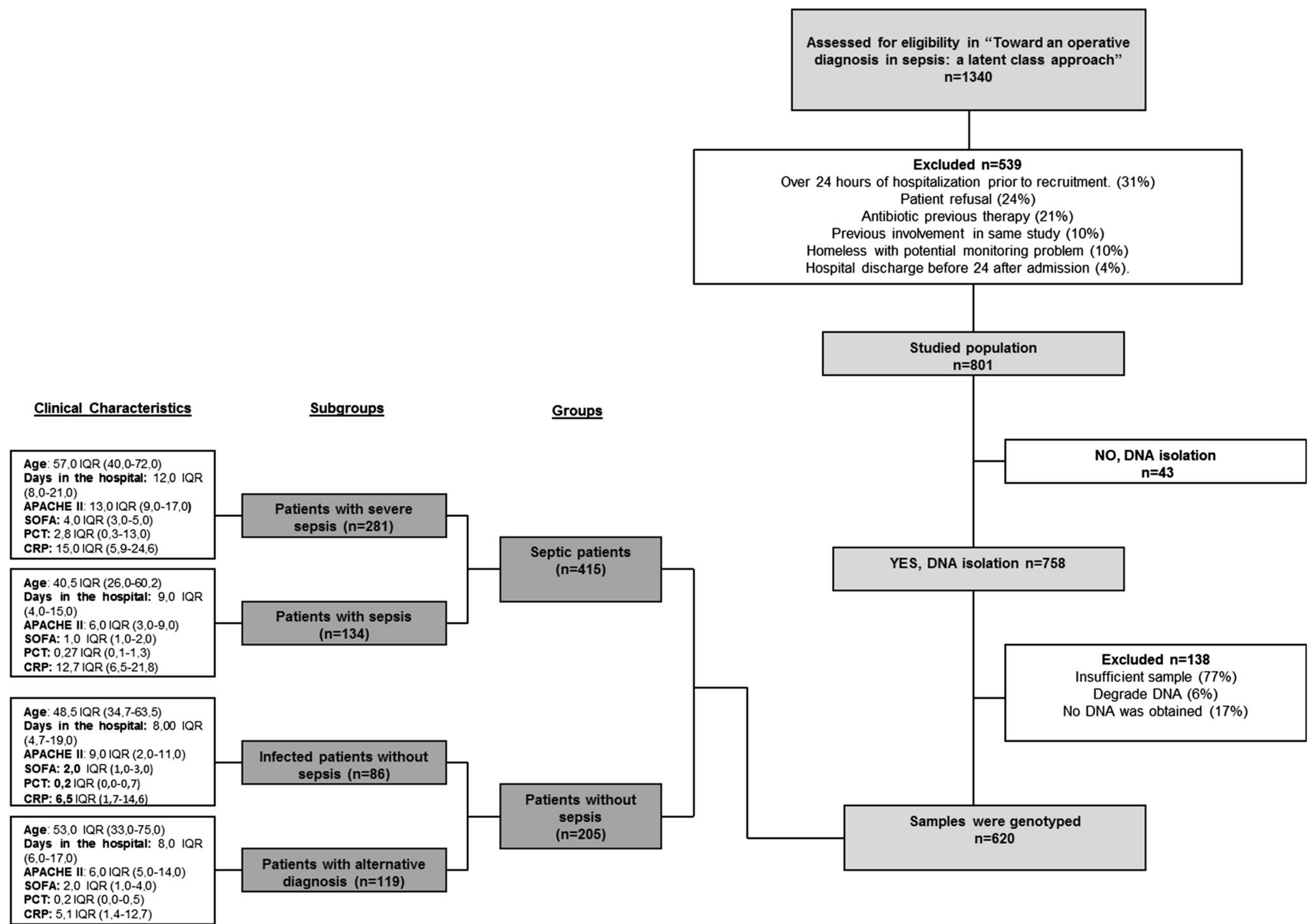

Fig. 1 Flowchart of recruitment, classification of the patients and clinical data

by an immunoturbidimetric assay using an ARCHITECT c-System (Abbott Diagnostics, Lake Forest, IL, USA).

DNA isolation and genotyping

Peripheral blood samples were collected during the admission; serum was separated to measure the cytokine concentration, and leukocytes were used for DNA isolation by the phenol–chloroform method. Genotyping was performed by PCR–RFLP (polymerase chain reaction restriction fragment length polymorphism). Specific primers were used for each SNP (Table S1), and PCR amplifications were carried out in Biometra T3000 Thermal Cycler (Biometra, Göttingen, Germany) using the BIOLASE^{TM} DNA polymerase (Bioline, London, UK), except for the SNP IL10 −1082, in which SAHARA^{TM} DNA polymerase (Bioline, London, UK) was used.

The PCR products were digested with the corresponding restriction enzyme: NcoI for *TNF* −308 (rs1800629) and *LTA* +252 (rs909253), Eco81I for *IL1B* −511 (rs16944), TaqI for *IL1B* +3953 (rs1143634), RsaI for *IL10* −592 (rs1800896), MsII for *IL10* −819 (rs1800871) and HpyAV for *IL10* −1082 (rs180072). The *IL10* −819 genotypes from some samples were verified by sequencing using a commercial service from Macrogen (Macrogen, Seoul, Korea).

Cytokine levels

Serum samples were stored at −80 °C until used. Tumor necrosis factor (TNF), lymphotoxin alpha (LTAlpha), interleukin 1 beta (IL1Beta) and interleukin 10 (IL10) concentrations were determined with the human ELISA kit (BD Biosciences, Franklin Lakes, NJ, USA), according to the manufacturer’s instructions. Levels below the detection limit of the assays were set as 0 pg/ml.

Data analysis

For all the continuous data, we tested the assumption of a normal distribution with the Shapiro–Wilk or the Shapiro-Francia tests. According to our findings, none of the variables fit a normal distribution, and therefore, we used nonparametric statistics for the comparisons. The data are

expressed as median and interquartile range (IQR) or proportions, depending on the type and distribution of the variable. Demographic and clinical variables were compared by the Mann–Whitney test and the Chi-square test using the SPSS software (version 18). Hardy–Weinberg equilibrium (HWE) was evaluated with the gPLINK software (version 2.050) using the methodology proposed by Wigginton et al. [16, 17]. Linkage disequilibrium was evaluated by the gPLINK and Haploview software (version 4.2), and r and D' values were calculated [18].

Genotypic and allelic frequencies were determined using the gPLINK software. Comparisons between groups were done by the Fisher exact test, and the odds ratios (ORs) and confidence interval (95 % CI) were calculated taking as control variant the genotype that had the highest allelic frequency or the one that was previously considered as a protective genotype [19–25]. In addition, the ORs for genotype frequencies were calculated according to different inheritance patterns.

Haplotype frequencies were obtained by gPLINK software using the Expectation Maximization (EM) algorithm. A p value <0.05 was considered statistically significant (uncorrected p value). SNPs association with (APACHE II) score, SOFA score, PCT, CRP and cytokine levels were explored by the Kruskal–Wallis test. Comparisons of cytokine concentration between patient subgroups were done by the Mann–Whitney test using STATA (version 12).

Results

Characteristics of the study population

During the study period, 1340 eligible patients were admitted to the emergency service and 539 were omitted based on the exclusion criteria described in Fig. 1. Eight hundred and one patients were followed and classified by the expert consensus in different subgroups according to their clinical status. The consensus among the experts had a kappa index of 0.69 between experts 1 and 2 and experts 1 and 3, and 0.64 between experts 2 and 3. For technical reasons, samples from 181 individuals were not available for genotyping (Fig. 1). Because of the large number of patients that were excluded of the genotyping process, the main demographic and clinical variables were compared between the original cohort and the genotyped patients, but no significant differences were observed, suggesting that our results could characterize the behavior of the original cohort (Table S2).

The final study population was composed of 620 patients, where 305 were females (49.2 %) with a median age of 52 years (IQR 34–70). The median value of SOFA was 2 (IQR 1–4), APACHE II was 10 (IQR 5–14), CRP levels were 10.9 mg/dl (IQR 4.2–21.6), and PCT levels were 0.50 mg/dl (IQR 0.11–5.08). The median hospital stay was 10 days (IQR 6–10); 13.5 % (84 patients) required treatment in ICU, and the 28-day mortality rate was 11.6 % (72 patients). The principal comorbidities were diabetes (n 118, 19.9 %), trauma (n 59, 9.5 %) and chronic obstructive pulmonary disease (CODP) (n 77, 12.4 %). Table S2 summarizes the classification of the patients and the main demographic and clinical characteristics of our study populations (Fig. 1).

SNP association with sepsis development

More than 84.1 % of the samples were genotyped for almost all SNPs, except for the *IL10* −1082A/G SNP, where only 49 % of the samples were genotyped. Most of the SNPs were in the HWE in the whole population and groups, suggesting no significant genotyping errors (Table S3). Nevertheless, the *IL10* −819(C/T) SNP was not in HWE in the entire population or study groups; the genotyping was confirmed by sequencing some samples (data not shown) and by repeating 20 % of the samples by PCR–RFLP, discarding genotype errors.

The linkage disequilibrium found between evaluated SNPs was: (1) SNP −308(G/A) and +252(G/A) in *TNF* and *LTA*, respectively, with a D′ 0.58 (0.35–0.75) and r^2 of 0.077; (2) SNP −511(A/G) and +3953(C/T) in *IL1B* with a D′ 0.41 (0.16–0.61) and r^2 of 0.037; and (3) SNP −819 (C/T) and −1082 (A/G) in *IL10* with a D′ 0.30 (0.09–0.49) and r^2 of 0.03.

We observed a significantly higher allelic frequency of SNP *LTA* +252G in septic patients compared to patients without sepsis (OR 1.29, IC 1.00–1.68) (Table 1). Also, a significant difference was observed for the haplotype *LTA* +252G—*TNF* −308G, with a higher frequency in septic patients, whereas the haplotype *LTA* +252A—*TNF* −308G was lower in septic patients (Table 1). These findings suggest that the G variant for SNP *LTA* +252 is most likely a risk factor associated with the development of sepsis.

Since our control group included patients with or without infection, we also performed a comparison taking only patients with infection. No allelic differences were detected between groups for SNP *LTA* +252, probably due to reduction in sample size. However, we observed a significant difference in genotypic frequency of SNP *IL1B* +3953(C/T), where septic patients exhibited a lower frequency of the TT genotype compared to infected patients without sepsis (OR 0.23, IC 0.08–0.67) (Table 1), suggesting that this genotype is associated as protective factor for sepsis development. Again, a higher frequency of haplotype *LTA* +252G-*TNF*-308G was found in septic patients (Table 1).

No significant differences were found in the allelic or genotype frequency of the others SNPs between septic patients and patients without sepsis (Table S4).

Table 1 Genotype and haplotype frequencies association with sepsis development

SNP	Variant	Frequency sepsis	Frequency without sepsis	p value	OR (CI 95 %)
Comparisons between septic patients ($n = 415$) *and without sepsis* ($n = 205$)					
Allelic frequencies					
LTA + 252(G/A)	G	0.3595	0.3020	0.0517*	1.29 (1.00–1.68)
	A	0.6405	0.6980		
Genotype frequencies					
LTA + 252(G/A)	GG	0.1368	0.0914	–	–
	GA	0.4453	0.4213	0.2704	1.23 (0.86–1.77)
	AA	0.4179	0.4873	0.0696	0.57 (0.32–1.03)
	GG+GA/AA	0.5821	0.7953	0.1153	1.32 (0.94–1.87)
	GG/GA+AA	0.1368	0.0914	0.1431	1.58 (0.22–17.6)
IL1B + 3953(C/T)	TT	0.0206	0.0513	0.0762	0.41 (0.16–1.06)
	TC	0.3033	0.2564	0.3776	1.21 (0.82–1.79)
	CC	0.6761	0.6923	–	–
	TT+TC/CC	0.3239	0.3077	0.7075	1.08 (0.74–1.56)
	TT/TC+CC	0.0206	0.0513	0.0717	0.39 (0.15–1.00)
Haplotype frequencies					
LTA + 252(G/A) *TNF*-308(G/A)	GA	0.0607	0.05814	0.8632	
	AA	0.0367	0.0333	0.7669	
	GG	0.3021	0.2349	0.0175*	
	AG	0.6005	0.6737	0.0164*	
SNP	**Variant**	**Frequency sepsis**	**Frequency infection**	**p value**	**OR (CI 95 %)**
Comparisons between septic patients ($n = 415$) *and infected patients without sepsis* ($n = 86$)					
Genotype frequencies					
LTA + 252(G/A)	GG	0.1368	0.1139	–	–
	GA	0.4453	0.3544	1.0000	1.05 (0.46–2.35)
	AA	0.4179	0.5316	0.3600	0.65 (0.30–1.43)
	GG+GA/AA	0.5821	0.4684	0.0642	1.58 (0.97–2.57)
	GG/GA+AA	0.1368	0.1139	0.7177	1.23 (0.58–2.61)
IL1B + 3953(C/T)	TT	0.0206	0.0875	0.0097*	0.23 (0.08–0.67)
	TC	0.3033	0.2375	0.4867	1.27 (0.72–2.25)
	CC	0.6761	0.6750	–	–
	TT+TC/CC	0.3239	0.3250	1.0000	0.99 (0.59–1.66)
	TT/TC+CC	0.0206	0.0875	0.0066	0.22 (0.08–0.62)
Haplotype frequencies					
LTA + 252(G/A) *TNF*-308(G/A)	GA	0.0611	0.0682	0.7389	
	AA	0.0364	0.0239	0.4398	
	GG	0.3017	0.2081	0.0195*	
	AG	0.6008	0.6998	0.0217*	

* Statistically significant differences

SNP association with disease progression

To associate SNP frequency with sepsis severity, comparisons were performed between patients with sepsis and severe sepsis (Table 2). Based on a dominant model for the variant G (GG+GA vs. AA), the frequency of the GG+GA genotype of the SNP *IL10* −1082 (A/G) was lower in patients with severe sepsis (OR 0.53, IC 0.29 −0.97) (Table 2). In addition, we evaluated the association of all SNPs with ICU admission during the sepsis; a significantly lower frequency of heterozygous genotypes for both *IL10* −592 (C/A) and *IL10* −1082 (A/G) SNPs was observed in patients admitted to ICU compared to non-admitted ICU patients, suggesting that these heterozygous genotypes

Table 2 SNPs association with sepsis severity

SNP	Variant	Frequency severe sepsis	Frequency sepsis	*p* value	OR (CI 95 %)
Comparison between patients with sepsis (*n* = 134) *and patients with severe sepsis* (*n* = 281)					
Genotype frequencies					
IL10 −1082 (A/G)	GG	0.0593	0.0476	–	–
	GA	0.3556	0.5238	0.5181	0.54 (0.13–2.2)
	AA	0.5852	0.4286	1.0000	1.09 (0.27–4.4)
	GG+GA/AA	0.4148	0.5714	0.0471*	0.53 (0.29–0.97)
	GG/GA+AA	0.0593	0.0476	1.0000	1.26 (0.32–4.92)

*Statistically significant differences

Table 3 SNPs association with the outcomes mortality and ICU admission of 28 day

SNP	Variant	Frequency affected	Frequency not affected	*p* value	OR (CI 95 %)
Comparison between patients admitted (*n* = 65) *and not admitted to ICU* (*n* = 350)					
IL10 −592 (C/A)	AA	0.2105	0.0736	–	–
	AC	0.3158	0.4571	0.0015*	0.24 (0.10–0.56)
	CC	0.4737	0.4693	0.0158*	0.35 (0.16–0.79)
	AA+AC/CC	0.5263	0.5307	10.000	0.98 (0.56–1.73)
	AA/AC+CC	0.2105	0.0736	0.0028*	3.36 (1.57–7.18)
IL10 −1082 (A/G)	GG	0.1154	0.0465	–	–
	GA	0.2308	0.4360	0.0500*	0.18 (0.04–0.86)
	AA	0.6538	0.5174	0.3971	0.51 (0.12–2.12)
	GG+GA/AA	0.3462	0.4826	0.2125	0.57 (0.24–1.34)
	GG/GA+AA	0.1154	0.0465	0.1618	2.67 (0.66–10.81)
Comparison between survivors (*n* = 441) *and non-survivors* (*n* = 47)					
Genotype frequencies					
TNF −308(G/A)	AA	0.0185	0.0071	0.4219	2.31 (0.23–22.62)
	AG	0.0741	0.1953	0.0366*	0.33 (0.12–0.95)
	GG	0.9074	0.7976	–	–
	AA+AG/GG	0.0926	0.2024	0.0642	0.4022 (0.1555–1.04)
	AA/AG+GG	0.0185	0.0071	0.3812	2.654 (0.2712–25.98)
Haplotype frequencies					
LTA + 252(G/A) *TNF*−308(G/A)	GA	0.0339	0.0661	0.1966	
	AA	0.0228	0.0361	0.4790	
	GG	0.3907	0.2745	0.01294*	
	AG	0.5527	0.6233	0.1600	

* Statistically significant differences

could be protector factors [(OR 0.24 (0.10–0.56) and 0.18 (0.04–0.86), respectively, Table 3]. Moreover, the CC and AA genotype frequency for *IL10* −592 was higher in patients admitted to ICU [OR 0.35 (0.16–0.79) and OR 3.36 (1.57–7.18) in a recessive pattern] (Table 3). No significant differences were observed in haplotype frequencies.

The association of all the SNPs with APACHE II and SOFA scores and also with PCT and CRP levels was evaluated; for this, the patients were classified into subgroups based on the genotype present. No significant associations were observed (Table 4).

SNP association with mortality rate

The patients with infection (septic patients and infected patients without sepsis) were also divided into two subgroups, based on the 28-day mortality rate. Although there were no differences in the allelic frequencies, a statistically significant difference in the genotype frequency of *TNF*

Table 4 SNPs association with APACHE II, SOFA, CRP and PCT

SNP	Genotype	SOFA	IQR	APACHE II	IQR	CRP	IQR	PCT	IQR
TNF −308(G/A)	AA	2.00	(0.50–5.50)	13.00	(7.00–18.00)	19.90	(3.85–30.35)	0.18	(0.05–7.66)
	AG	2.00	(1.00–4.00)	10.00	(6.00–14.00)	9.75	(3.82–22.60)	0.50	(0.092–8.20)
	GG	3.00	(1.00–4.00)	10.00	(5.00–15.00)	11.40	(4.47–21.22)	0.50	(0.13–4.52)
LTA + 252(G/A)	GG	2.00	(1.00–4.00)	9.00	(5.00–14.00)	9.60	(3.80–19.80)	0.44	(0.10–5.13)
	GA	3.00	(1.00–4.00)	10.00	(5.75–14.00)	12.20	(4.80–23.20)	0.51	(0.12–5.74)
	AA	3.00	(1.00–4.00)	11.00	(5.50–16.00)	10.55	(3.55–20.88)	0.55	(0.10–4.40)
IL1B −511(A/G)	AA	3.00	(2.00–5.00)	9.00	(5.00–14.00)	9.95	(4.10–20.80)	0.43	(0.098–5.66)
	AG	2.50	(1.00–4.00)	9.00	(5.00–14.00)	10.80	(4.50–21.60)	0.53	(0.14–5.55)
	GG	2.00	(1.00–4.00)	10.00	(5.00–14.25)	10.85	(4.03–21.93)	0.32	(0.90–3.04)
IL1B + 3953(C/T)	TT	2.50	(1.00–4.50)	2.50	(1.00–4.50)	9.50	(3.50–12.25)	0.35	(0.50–3.89)
	TC	2.00	(2.00–4.00)	11.00	(6.00–14.00)	9.40	(4.13–22.70)	0.59	(0.10–7.10)
	CC	2.00	(1.00–4.00)	9.00	(5.00–15.00)	11.90	(4.28–21.48)	0.50	(0.12–4.23)
IL10 −592 (C/A)	AA	2.00	(1.00–5.00)	9.00	(5.00–14.00)	9.60	(4.40–21.50)	0.92	(0.17–8.20)
	AC	3.00	(1.00–4.00)	10.00	(5.00–15.00)	11.30	(4.10–22.40)	0.49	(0.10–4.49)
	CC	2.00	(1.00–4.00)	10.00	(5.50–14.00)	10.60	(4.22–20.70)	0.42	(0.12–4.61)
IL10 −819 (C/T)	CC	2.00	(1.00–4.00)	9.50	(5.00–16.00)	9.60	(3.95–22.75)	0.68	(0.19–5.70)
	CT	3.00	(1.00–4.00)	10.00	(6.00–15.00)	11.10	(4.40–22.30)	0.50	(0.12–5.12)
	TT	2.00	(1.00–4.00)	9.00	(5.00–14.75)	9.40	(3.65–17.30)	0.50	(.09–6.22)
IL10 −1082 (A/G)	GG	3.00	(2.00–4.00)	10.00	(5.00–12.00)	10.50	(2.70–18.30)	0.37	(0.15–6.45)
	GA	2.00	(1.00–4.00)	11.00	(6.00–16.00)	12.70	(5.03–22.20)	0.51	(0.16–4.40)
	AA	2.00	(1.25–4.00)	10.00	(5.00–14.00)	10.60	(4.33–22.33)	0.52	(0.11–5.10)

IQR interquartile range, *CRP* C-reactive protein, *PCT* procalcitonin, *SOFA* Sequential Organ Failure Assessment, *APACHE II* Acute Physiology and Chronic Health Evaluation II

−308(G/A) SNP was noted, where the proportion of the genotype AG was higher in the surviving patients than in the non-surviving patients (Table 3). Although the proportion of genotype GG was higher in non-surviving patients than in surviving patients, the *p* value and OR were not calculated since it previously served as a control genotype, based on a previous report [24]. Also, the frequency of the haplotypes *LTA* +252G—*TNF* −308G was lower in surviving patients, suggesting that the GG genotype is most likely a risk factor (Table 3).

SNP association with cytokine plasma levels

No significant differences were observed between the levels of cytokines and the different genotypes of SNPs evaluated. However, when comparing the median cytokine plasma concentrations between the different groups and subgroups according to the classification of the experts, we noted that plasma levels of IL1Beta and IL10 were significantly higher in septic patients than in patients without sepsis and infected patients without sepsis (Fig. 2a, b and data not shown). Furthermore, plasma levels of TNF, LTAlpha and IL1Beta were significantly higher in patients who were admitted to the ICU than in non-admitted patients (Fig. 2c–e).

Discussion

The pathogenesis of sepsis is highly influenced by host immune responses. Although several studies have evaluated the association of genetic variants with the development of sepsis, the interpretation of the observations are complicated if one takes into account the differences in the genetic background among populations such as allelic frequencies of SNPs that vary between different ancestry populations as shown in the 1000 genomes project (http://www.1000genomes.org/home). In addition, factors such as sample size and the criteria used for the diagnosis of sepsis may be inadequate to provide statistical power or homogeneity between the groups evaluated, affecting the identification of such associations and the extrapolation of the results to different populations.

Since few reports exploring the association of SNPs with the development of sepsis have been published in Latin American populations, in this study, 620 Colombian patients were genotyped for 7 SNPs located in genes that code for TNF, LTAlpha, IL1Beta and IL10, increasing the number of patients previously evaluated in different Latin America cohorts [21, 26–30]. We also classified the patients using a consensus of three experts, based on the criteria of the CDC for nosocomial infections and the

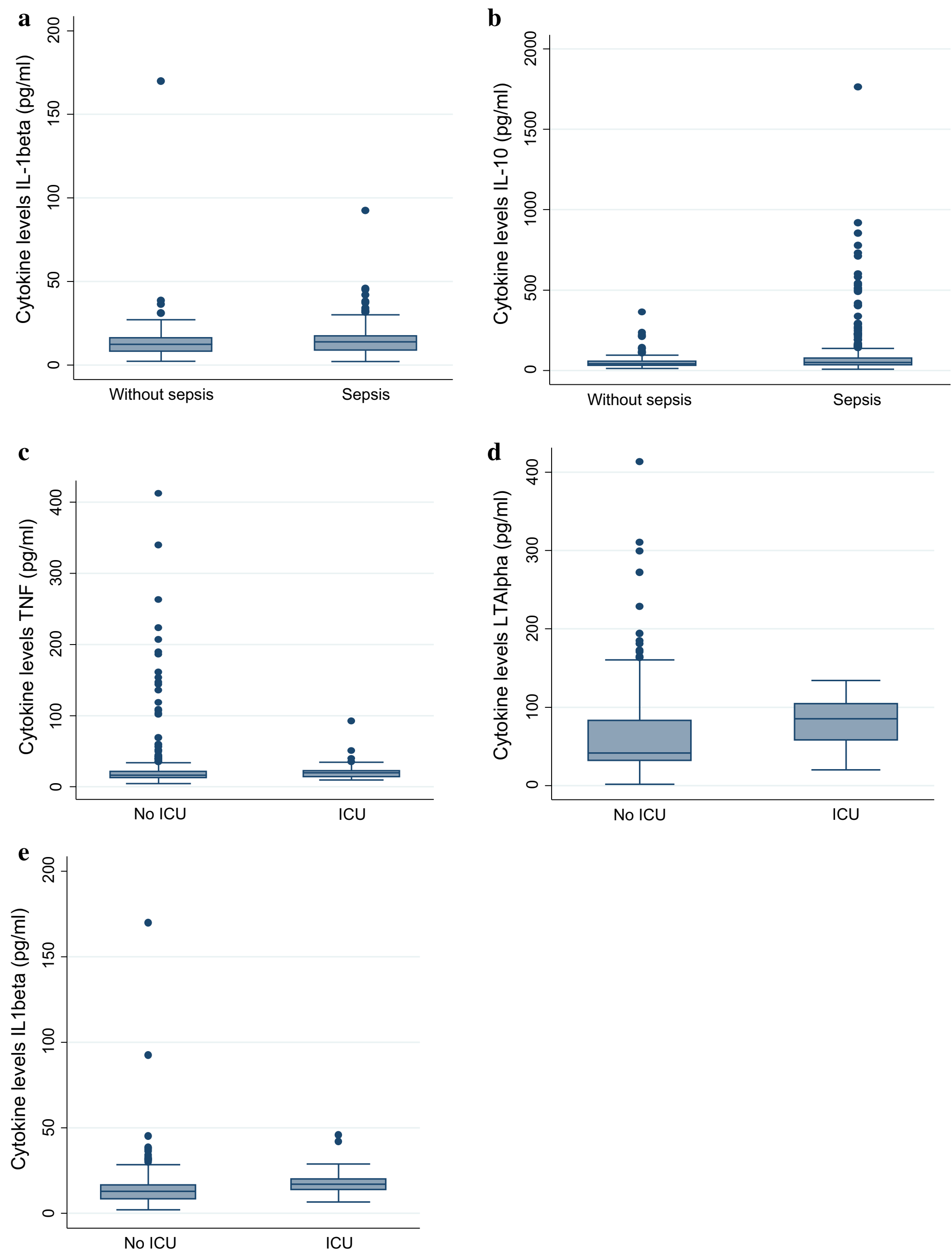

Fig. 2 Box plots showing the comparison of cytokine levels between patients group. **a**, **b** Median of IL1β and IL10 between patients with sepsis and without sepsis, respectively. **c**–**e** Median of TNF, LTα and IL1β between patients admitted to ICU and not admitted, respectively. *Dots* represent the outliers above the 90 % percentile. All comparisons were statistical significant at a *p* value <0.05

International Sepsis Conference [3]. In our study, the control population included patients with alternative diagnosis and infected patients without sepsis instead of healthy persons, since this population is more suitable to evaluate associations with potential risk factors. Our genotyping was validated because the SNP frequencies in the control group were similar to those of previous reports in the general Colombian population and with populations with similar ancestral components [31–34].

In this study, we observed an association of the allele G of the SNP *LTA* +252 with risk of sepsis development; this finding is supported by the high frequency of the *LTA* +252G—*TNF* −308G haplotype in patients with sepsis in comparison with patients without sepsis. In contrast, previous studies in non-Latin American populations have found an association of the allele A with risk [35, 36]; this discrepancy could be explained by the differences in the ancestral component of our population and the previously evaluated population.

We did not identify an association of SNP *TNF* −308 (G/A) with risk of sepsis development that was commonly associated in previous studies [23, 24]. The absence in the identification of such an association could be explained by the low frequency of this SNP in our population (Table S4).

However, we found linkage disequilibrium between the evaluated SNPs in *TNF* and *LTA* and haplotype association with sepsis development, highlighting the importance of haplotype analysis. Furthermore, the SNP *TNF* −308(G/A) was associated with protector factor for mortality, with a dominant inheritance model for the A allele. Likewise, we also identified that the haplotype *LTA* +252G—*TNF* −308G was associated with a higher risk of fatal outcome, supporting the importance of these SNPs in the clinical course of sepsis.

We found that the genotype TT for SNP *IL1B* +3953 behaves as a protective factor for sepsis development. In a previous study in Colombia, the T allele was associated with lower cytokine production by monocytes in response to LPS stimulation, and with protection in the development of inflammatory diseases such as systemic lupus erythematosus and rheumatoid arthritis [34], suggesting a link between this genotype and a lower ability to establish a proinflammatory response.

With respect to disease severity, we noted that genotype AA of SNP *IL10* −1082 is a risk factor for severe sepsis development, with a dominant inheritance model for allele G. In contrast, previous studies have found that variant G is a risk factor for severe sepsis development [37], with increased mortality in patients with severe sepsis associated with higher IL10 production [38]; this discrepancy could be explained by the different ancestries between our population and the previously evaluated populations, or by differences in the diagnostic criteria. We did not find differences in the cytokine serum levels between sepsis and severe sepsis patients; however, the dynamic levels of the cytokines during the time course of the infection could explain this phenomenon [39].

In addition, the homozygous genotype of SNPs *IL10* −592 (A/C) and *IL10* −1082 (G/A) is associated with risk to be admitted to ICU. Because it is likely that patients heterozygous for both SNPs exhibited intermediate levels of IL10 in comparison with homozygous patients, the intermediate levels may improve the balance between the inflammatory and anti-inflammatory responses.

To clarify the association with the clinical course of sepsis, we also evaluated the SNPs association with the APACHE II and SOFA scores and with CRP and PCT concentrations, but we did not find any association in spite of previous observations. However, our results are in accordance with those of Paskulin et al. [21] who found no association of SNP *TNF* −308 with SOFA and APACHE II scores in critically ill patients using a similar sample size. It is noteworthy that our study population has lower SOFA and APACHE II scores in comparison with populations of similar studies [29, 40], being more difficult to find associations.

Finally, we measured the cytokine serum levels and found differences in patients with and without sepsis, and patients admitted and not admitted to ICU, underlying the importance of the cytokine levels in the clinical prognosis of septic patients. In contrast, we observed no correlation between having a particular genotype and the cytokine concentration, questioning the previous association between SNPs and cytokine production. However, it is important to consider that the design of our study has many disadvantages in attempting to explore such an association, since many factors affect cytokine levels in patients, such as the time course of infection, different treatments, the infectious agent and age.

In spite of the results obtained, we acknowledge several limitations in our study. First, we cannot discard a potential selection bias, as the population was enrolled with wide inclusion criteria and the hospital is a reference center in the region. Second, some kind of measurement bias and misclassification in the study groups is unavoidable, because clinical criteria for sepsis may be subjective and it is remarkable that a gold standard for this diagnosis does not exist. Finally, as commonly mentioned in genetic analysis, the underpowered sample size may preclude the ability to detect small but relevant associations especially in the association of mortality rate because few patients had a fatal outcome, and the analysis for the SNP *IL10* −1082 that had a low genotyping rate due low performance in the amplification.

In conclusion, our findings indicate that variants of the TNF locus and the genes that encode *IL1B* and *IL10* influence the inflammatory response during sepsis, based on their association with sepsis development and different clinical outcomes, which could to some extent explain the heterogeneity of the clinical course among patients. Further studies are required to understand the use of these variants in the clinical follow-up of septic patients and to determine the risk of a fatal outcome.

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Compliance with ethical standards

Conflict of interest All authors declare that they do not have a financial relationship with the organization that sponsored the research and have no conflict of interest.

Ethical approval "All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or of the National Research Committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

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