Prognostic Value of MMP-9 -1562 C/T Gene Polymorphism in Patients With Sepsis

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ABSTRACT

INTRODUCTION: Matrix metalloproteinase-9 (MMP-9) plays an important role in the pathophysiology of sepsis. A single-nucleotide polymorphism (SNP) at position -1562 (C/T) in the MMP-9 gene has been associated with differential MMP-9 expression, being higher when the -1562 T allele is present. We evaluated the association of the SNP MMP9 -1562 C/T with severity and mortality in patients with sepsis to establish whether the prognosis of the disease is affected.

MATERIALS AND METHODS: A case-control study exploratory was carried out in a cohort of infected patients. 540 individuals were selected in total, 270 patients with sepsis and 270 controls (infected but non-septic), classified according to the 2016 consensus (Sepsis-3). The presence of the single-nucleotide polymorphism (SNP; allele T and/or allele C) was determined through analyses of restriction fragment length polymorphism and plasma levels of MMP-9 were determined through enzyme-linked immunosorbent assay immunoassay.

RESULTS: SNP MMP-9 -1562 has two known alleles (T and C), with predominance of the C over the T allele; in the group of patients with sepsis, T allele was found in 7.2% of cases, while C allele in the rest (92.8%); in comparison, in the group of infected but non-septic patients, frequencies were 9.4% for T allele and 90.6% for the C allele ($P = .33$). Also, the presence of the polymorphic T allele was not related to the levels of MMP-9 in patients with sepsis in comparison with infected but non-septic patients 780 (397-1375) ng/mL vs 646 (172-1249) ng/mL ($P = .64$). There was also no association between the SNP and sepsis mortality ($P = .78$).

CONCLUSIONS: We concluded that there was no association between the SNP MMP9 -1562 C/T and sepsis or between the SNP MMP9 -1562 C/T and sepsis mortality in the Northeastern Colombian septic patient cohort. Further research is needed to clarify the correlation among sepsis, genetic factors with allele T and MMP-9 plasma concentration.

KEYWORDS: matrix metalloproteinase 9, prognosis, sepsis, single-nucleotide polymorphism

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Introduction

Sepsis is considered a public health problem and one of the leading causes of death worldwide.[1] The last consensus of sepsis-3 defined it as a "life-threatening organ dysfunction caused by a dysregulated host response to infection."[2] In Colombia, Ortíz et al[3] reported a monthly incidence rate of sepsis of 3.6 per 100 admissions per hospital and Leon et al reported a mortality of 18.5% and 45.6% in patients with severe sepsis and septic shock at 28 days, respectively.[4]

Several biomarkers have been described in the pathophysiology of sepsis, including matrix metalloproteinase-9 (MMP-9), which may represent a promising tool for the prognosis of patients with sepsis. MMP-9 is a proteinase that participates in the proteolytic regulation of intra- and extracellular matrix. MMP-9 is inhibited by TIMP1[5] and is involved in several processes such as the immune exacerbation,[6] chemotaxis,[7] and angiogenesis.[8] In

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sepsis, MMP-9 has been linked to an increase in vascular permeability due to the degradation of collagen present in the vascular basement membrane, facilitating the migration of inflammatory cells and modulating the inflammatory response.[9] Also, it is involved in vascular hyporeactivity, in animal models and cardiovascular disease,[10] lung injury,[11] in the coagulation/fibrinolysis system and in platelet function.[12]

Sepsis is a multifactorial and heterogeneous process that involves humoral and cellular immunological reactions. The stratification of the disease, the prognosis and the response to therapeutic management is difficult in these patients. For that reason, the International Sepsis Definitions Conference, in 2001, proposed a new scale of "predisposition, infection, response and organ dysfunction" (PIRO scale) which includes the study of genetic predisposition as a possible promising biomarker in the course of sepsis.[13] Some of these genetic variabilities have been reported by Villar et al.[14] This group and other authors reviewed single-nucleotide polymorphisms (SNPs) in CD14, TLR2, and TNF genes as factors that modulate clinical presentation, responses to medical treatment and susceptibility to sepsis or infection.[15,16] Similar studies have reported MMP-9 geners 3918242 playing an important role in the pathophysiology of sepsis. A single-nucleotide polymorphism at position -1562 in its gene has been associated with different MMP-9 levels of expression.

The literature reports show contradictory findings in behavior of polymorphism SNP MMP-9 -1562 C/T and its expression in sepsis. This study is the first to describe the possible genetic association of SNP MMP-9 -1562 C/T, in the largest cohort of a Colombian population, with sepsis. To assess the prognostic value of SNP MMP-9 -1562 C/T in sepsis we sought to (1) study the association of the SNP with presence and absence of sepsis; (2) stratify an analysis between the presence of the SNP with biomarkers for organic dysfunction, focusing on cardiovascular dysfunction, since the heart is the major affected organ to increase mortality; and (3) determine the relationship of the SNP with plasma levels of MMP-9 and (4) the association of the SNP with mortality in sepsis. This study was supported by the reporting of Genetic Association Studies (STREGA)—An Extension of the STROBE Statement.[17]

Materials and Methods

Type of study

Informed consent was obtained from all patients, or from their legal representative if the patient was unable to consent. The present study was approved by the Ethics Committee of the Autonomous University of Bucaramanga (0056/2009 and 0066/2015), Universidad Industrial de Santander UIS (0012/2014) (0029/2017), and the various hospitals that participated of the study. Design was defined as a cases and controls nested no match 1: 1 in a cohort, performed in the metropolitan area of Bucaramanga from 2009 (January) to 2013 (December).

Study design

The participants were followed up for 28 days to check your survival or severity, and the data were obtained by reviewing the clinical history and interviewing the patient or close family member.

Inclusion criteria ware as follows: (1) patients over 18 years old, admitted to the emergency department or intensive care unit; (2) patients were included if they fulfilled the sepsis criteria published in 2001 for sepsis, severe sepsis, or septic shock; then all the variables were subsequently reclassified using the 2016 sepsis. Exclusion criteria were as follows: (1) pregnancy; (2) patients with known chronic inflammatory diseases (cancer and autoimmune disease); (3) immunosuppression; (4) heart failure prior to the infectious process; and (5) diagnosis of sepsis or septic shock for longer than 72 h.

Clinical variables

We assessed severity and type of infection using severity scales (SOFA, APACHE II). We also used the CHARLSON co-morbidity index. Enrolled patients were followed up to 28 days after being enrolled in the study to evaluate mortality.

Clinical and biomarker data

We obtained clinical data, such as mean arterial pressure, platelet count, bilirubin, creatinine, blood urea nitrogen (to determine SOFA scale and organic dysfunction), troponin I, brain natriuretic peptide (NT-proBNP) (to identify cardiovascular dysfunction), plasma level MMP-9, metalloproteinase inhibitor TIMP-1, and determination SNP MMP-9 -1562 C/T (rs 3918242).

Peripheral blood collection was performed as soon as the patient was enrolled in the study, it means within the first 72 h of sepsis diagnosis. Tubes were centrifuged at 1000g for 15 min to get plasma or serum, respectively (with or without anticoagulant). Supernatants were aliquoted and stored at −80°C until analysis. We quantified the following biomarkers in serum: troponin I, NT-proBNP, BUN, creatinine, and bilirubin, by means of electrochemiluminescence using the COBAS 6000 analyzer (Roche, Bogota, Colombia). We measured MMP-9 and TIMP-1 levels in plasma by enzyme-linked immunosorbent assay (ELISA) using Quantikine kits (R&D Systems, Minneapolis, USA).

DNA extraction

We extracted DNA from 200 µL of peripheral blood from buffy coat using the QIAamp DNA blood kit, following the manufacturer's recommendations (QIAGEN, Germantown, USA). DNA quality was visually verified by electrophoresis using 1% agarose gels and stored at −80 in freezer.

Detection of the SNP MMP-9 -1562 C/T

Amplification of the promoter region that contained the SNP was performed by PCR, using the forward primer 5′ GCC-TGG-TGG-CAC-ATA-GTA-GGC-CC-3′ and the reverse primer 5′ CTT-CCT-AGC-CAG-CCG-GCA-TC-3′ (Bionner, Alameda, USA) at a concentration of 0.2 µM in a final volume of 25 µL of master mix (Thermo Scientific, Waltham, USA). PCR reactions were run in a MJ Research PTC-100 thermocycler (Marshall Scientific, Hampton, USA), using the following cycles: 1 min of initial denaturation at 95°C, followed by 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s, with a final extension step of 10 min at 72°C. Amplification was verified by electrophoresis in 2% agarose gels by the presence of a band of 435 bp. For detecting the presence of the SNP, the amplicons were digested with 2 units of the restriction endonuclease SphI (Thermo Scientific, Waltham, USA) in 10 µL final volume for 17 h at 37°C. The resulting fragments were run by electrophoresis in 3% agarose gels and examined using the Gel Doc XR Gel Documentation System (Bio-Rad, Hercules, USA), and the bands were analyzed with ImagenLab software 15.2.1 (Bio-Rad, Hercules, USA) (Figure 1). The procedure was carried out in the facilities of the Laboratory of Genetic and Molecular Biology in Autonomous University of Bucaramanga, UNAB.

The 435-bp product obtained from PCR was digested by the restriction enzyme SphI, fragments generated by RFLP were separated by electrophoresis in 2% agarose gels. Lanes 1, 2, 3 and 6: homozygous CC; Lanes 4 and 7: heterozygous CT; Lane 5: homozygous TT; M: molecular weight marker (100 bp).

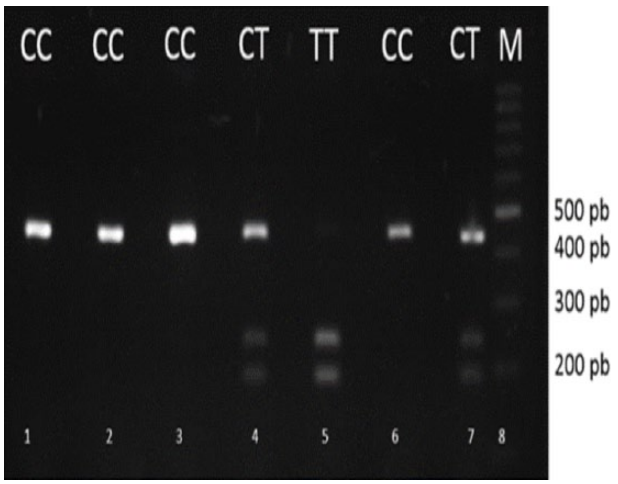

Figure 1. SNP MMP-9 -1562 C/T, is detected by RFLP. RFLP indicates restriction fragment length polymorphism.

Sample size

The original cohort was composed of 664 patients, classified according to the consensus of Sepsis 2001.[18] From this cohort, participants were subsequently reclassified according to the new Sepsis-3 criteria, yielding a total of 540 eligible participants, 270 of which had sepsis (cases) and 270 who, albeit infected, were determined as non-septic patients (controls).

Sample selection was non-probabilistic, using the largest available sample (Figure 2); however, sample size in the present project corresponds to a pilot study. Due to participant availability, sample size calculation was, therefore, done based on the largest available sample for matched populations. It is worth mentioning that the number of patients contemplated was based on the consultation estimates for this pathology in our institution and in published studies. This study will lay the necessary clinical and statistical bases for further studies on a larger scale, estimating the role of SNPs in a multifactorial disease such as sepsis.

Statistical analyses

We used STATA 14v software (StataCorp, Texas, USA) for the statistical analyses. Only bivariate analyzes were performed. First, we studied the distribution of quantitative variables by graphical representation analysis and found that these did not have a normal distribution. Hence, a descriptive univariate analysis was performed, and quantitative variables were reported as medians and interquartile ranges. Categorical variables were reported as frequencies and percentages. Comparisons of quantitative variables between groups were carried out using the Wilcoxon-Mann-Whitney test. Comparisons between groups on categorical variables were carried out with the chi-square test. SNP correlation with the different biomarkers was assessed using Kruskal-Wallis test. Also, the genotypic and allelic frequencies were calculated to determine the Hardy-Weinberg equilibrium using R Studio 3.3.3 software (RStudioInc, Boston, USA) We use in stratified logistic regression, between the presence of the polymorphic allele T (CT-TT vs CC), dominant inheritance model. To check whether a polymorphic allele copy is enough to generate the interest event, through the SNPstats platform (Institut Catalá dOncologia,Cataluña, España).

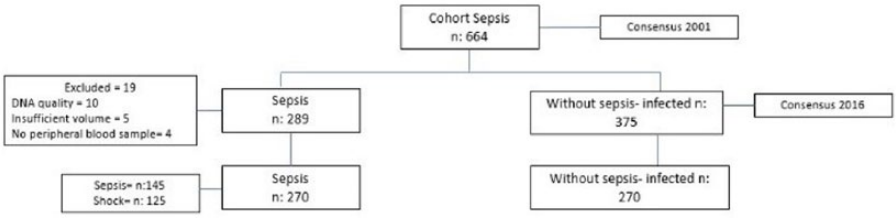

Figure 2. Population of study and classification according to consensus 2016.

Table 1. Sociodemographic characteristics of the study population.

VARIABLE	SEPSIS N = 270	INFECTED BUT NON-SEPTIC N = 270	*P* VALUE
Age (years)	61 (48-75)	57 (43-71)	.01
Male	135	141	.6
Female	135	129	.6
Creatinine (mg/dL)	1 (0.75-2.01)	0.78 (0.64-0.96)	<.001
BUN (mg/dL)	24.9 (16.3-44.5)	13.7 (10.42-19)	<.001
Bilirubin total (mg/dL)	0.4 (0.22-0.8)	0.2 (0.2-0.4)	<.001
NT-proBNP (pg/mL)	2099 (590-8937)	329 (153-867)	<.001
Troponin I (ng/mL)	0.1 (0.1-0.4)	0.1 (0.1-0.1)	.2
MAP (mmHg)	68 (62-79)	70 (68-75)	<.001
Platelets (mm^3)	232 (168-303)	248 (194-307)	.020
SOFA	2 (1-5)	0 (0-1)	<.001
APACHE	12.5 (8-18)	7 (5-10)	<.001
CHARLSON	1 (1-3)	1 (0-2)	.1
MMP-9 (ng/mL)	589 (229-1098)	196 (120-300)	<.001
TIMP-1 (ng/mL)	306 (170.5-579.2)	847 (615.8-1169)	<.001

Data presented as median (25th to 75th percentile).
Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; BUN, blood urea nitrogen; CHARLSON, Charlson Comorbidity Index; MAP, mean arterial pressure; MMP-9, matrix metalloproteinase 9; NT-proBNP, cerebral natriuretic peptide; SOFA, Sequential Organ Failure Assessment; TIMP-1, tissue inhibitor of metalloproteinase 1.

Results

540 adult patients were enrolled, 270 classified as patients with sepsis and 270 as infected but non-septic patients were used as controls. In septic group, 53% of the patients had sepsis and 47% had septic shock; 50% were women and 254 (94.1%) were mestizos; 76 (23%) of the septic group died within the 30 days of follow-up, and none of the control group died within this period. The most frequent diagnoses were urinary tract infection (34%), low respiratory tract infection (24%), soft tissue infection (26%), intraabdominal infection (8%), and others (7.5%). We found that the MMP-9 levels in the septic group were significantly higher than the controls (589 ng/mL vs 196 ng/mL, respectively, $P < .001$); on the contrary, TIMP-1 levels were lower in the patients with sepsis than in the controls (306 ng/mL vs 847 ng/mL, respectively, $P < .001$). Demographical and clinical data of the participants are detailed in Table 1.

No significant differences were observed between the proportions of the three possible genotypes (CC, CT, and TT) and the septic and control groups. Genotype distribution for the SNP MMP9-1562 C/T is summarized in Table 2.

We did not identify an allelic association with the plasma levels of MMP-9 and other biomarkers of organic dysfunction in patients with sepsis and none of these variables are confounding factors and infected individuals' group, significant differences were found between the presence of the T allele in TIMP-1, APACHE, and CHARLSON (Table 3). Similarly, no significant differences were observed between the presence of the polymorphic allele with survival at 30 days of follow-up and cardiovascular dysfunction (Tables 4 and 5).

Discussion

MMP-9 is secreted by cells of innate immunity such as neutrophils; therefore, this molecule is involved in the immune exacerbation in sepsis. However, literature presents conflicting reports regarding the possible role of plasma MMP-9 in sepsis. A study conducted by Lorente et al[19] found that the levels of MMP-9 were elevated in patients who had severe sepsis that survived and recently a study developed by Niño et al[20] found no association between MMP-9 levels and mortality. On the other hand, Hoffmann et al[21] demonstrated that plasma levels MMP-9 in patients with severe sepsis were significantly higher compared with healthy individuals. Also, interestingly, the inhibition of MMP-9 significantly reduces the bacterial load and the resulting immunopathology in a co-infection model. This highlights the inhibition of MMP-9 as a possible therapeutic agent.[22]

The SNP MMP-9 -1562 C/T polymorphism has been associated with the development of diabetic microvascular complication and coronary artery disease.[23–25] However, the study of polymorphism and its relationship with sepsis is not clear. On one hand, Martin et al[26] found that there was no association between the

Table 2. Genotype and allele frequency distribution.

VARIABLE	SEPSIS	INFECTED BUT NON-SEPTIC	*P* VALUE	OR	CI 95%
SNP MMP-9 -1562					
CC, n (%)	231 (85.87)	223 (82.66)	.30	1	
CT, n (%)	38 (13.75)	43 (15.87)		1.21	0.75-1.95
TT, n (%)	1 (0.37)	4 (1.48)		0.50	0.46-3.3
SNP MMP-9 -1562 allele					
C, n (%)	501 (92.8)	489 (90.6)	.33	0.32	0.23-1.32
T, n (%)	39 (7.2)	51 (9.4)	0.33	0.45	0.53-1.34

CT and TT, polymorphic genotype; T, polymorphic allele; MMP-9, matrix metalloproteinase 9.

Table 3. Correlation of SNP MMP-9 -1562 genotype with plasma levels of MMP-9 and biomarkers of organ dysfunction in sepsis and controls.

VARIABLES	SEPSIS N=270			INFECTED BUT NOT SEPTIC N=270		
	CC (N=231)	CT+TT (N=39)	*P* VALUE	CC	CT+TT	*P* VALUE
MMP-9 (ng/mL)	577 (218.1-1083)	780 (397-1375)	.14	884 (535-1474)	646 (172-1249)	.64
TIMP-1 (ng/mL)	305 (169-555)	304 (186-640)	.63	222 (139-355)	66 (34-162)	.05
MAP (mmHg)	68 (61-80)	69 (67-72)	.39	70 (68-75)	70 (68-73)	.72
Platelets (mm^3)	230 (170-304)	237 (168-300)	.93	245 (193-309)	253 (204-299)	.59
Creatinine (mg/dL)	1.09 (0.74-1.95)	0.98 (0.78-2.74)	.84	0.8 (0.6-0.9)	0.6 (0.6-0.7)	.50
BUN (mg/dL)	25 (16.33-45.27)	24 (16.3-36.9)	.40	14.5 (11.8-21.2)	10.1 (9.4-11.7)	.1
Bilirubin total (mg/dL)	0.15 (0.07-0.23)	0.12 (0.07-0.19)	.59	0.3 (0.2-0.5)	0.2 (0.2-0.2)	.25
NT-proBNP (pg/mL)	2102 (613-8776)	1656 (413-9554)	.79	323.6 (161.2-866.5)	373 (138.2-876.2)	.83
Troponin I (ng/mL)	0.1 (0.1-0.19)	0.1 (0.1-0.18)	.90	0.1 (0.1-0.1)	0.1 (0.1-0.1)	.70
SOFA	2 (1-5)	2 (1-4)	.36	1 (1-1)	0 (0-0)	.1
APACHE	13.8 (8-19)	12.65 (9-15)	.46	8 (6-10.5)	4 (3.7-4)	.02
CHARLSON	2 (1-3)	1 (1-4)	.78	1 (1-2)	0 (0-0)	.03

Data presented as median (25th to 75th percentile).
Abbreviations: APACHE, Acute Physiology and Chronic Health Evaluation; BUN, blood urea nitrogen; CHARLSON, Charlson Comorbidity Index; MAP, mean arterial pressure; MMP-9, matrix metalloproteinase 9; proBNP, cerebral natriuretic peptide; SOFA, Sequential Organ Failure Assessment; TIMP-1, tissue inhibitor of metalloproteinase 1.

Table 4. SNP MMP-9 -1562 C/T and survival in sepsis.

ALLELE	SURVIVOR, N (%)	NON-SURVIVOR, N (%)	OR (CI 95%)	*P* VALUE
CC (n=232)	168 (86.60)	64 (85.33)	1	.78
CT+TT (n=38)	26 (13.40)	12 (14.67)	1.47 (0.68-3.17	

Abbreviations: CI, confidence interval; OR, odds ratio.
The population is grouped with the presence or absence of the polymorphic allele T and its association with survival in sepsis.

presence of MMP-9 -1562 C/T polymorphism and the development of sepsis. In contrast, the study done by Collazos et al[27] reported that the presence of the polymorphic allele T is related to a higher concentration of MMP-9 in plasma in severe sepsis.

Given the discrepancies between the studies of plasma levels of MMP-9 in sepsis, this study aims to clarify the impact of the polymorphism 1562 C/T in the promoter region of the MMP-9 gene with sepsis. The epigenetic studies carried out found that

Table 5. SNP MMP-9 -1562 C/T with echocardiographic variables (diastolic dysfunction).

ALLELE	DDVI ALTERED, N (%)	DDVI NOT ALTERED, N (%)	*P* VALUE
CC (n = 226)	65 (80.2)	161 (85.2)	.47
CT + TT (n = 44)	16 (19.8)	28 (14.8)	

Abbreviation: DDVI, Left Ventricular Diastolic Dysfuntion.
CT and TT, polymorphic genotype; T, polymorphic allele.

region 1562 of the MMP-9 gene is a binding site for transcription factors that increase gene expression. Furthermore, in this position, the presence of the SNP rs3918242, which is generated by replacing a cytosine nucleotide (C) with one of thymine (T),[28] has been indicated. When the C allele is present, the rate of transcription decreases because the binding of the transcription factors is inhibited; therefore, the presence of the polymorphic T allele generates a higher level of MMP-9 in blood.[28]

In the present study, we evaluated the association of MMP-9 -1562 C/T gene polymorphism with development of sepsis and for the first time, the polymorphism was evaluated as a potential tool for prognostic and clinical outcomes in patients with sepsis according to the new classification of sepsis-3. We found that the MMP-9 -1562 C/T gene polymorphism was not associated with development of sepsis and there was no significant association between the polymorphism of MMP-9 with sepsis mortality, or with cardiovascular dysfunction or changes in plasma levels of MMP-9 in patients with sepsis.

On the other hand, from the allele frequencies, we found that the studied population shows Hardy-Weinberg equilibrium conditions. Likewise, our results suggest that the allele frequencies behave according to chance pairing rather than genetic drift, panmixia, or any other selective force that would affect random distribution of the alleles. Our results are also in line with those of León et al,[29] showing that the population of Bucaramanga does not present a homogeneic structure (Fst = 0.0038), as there are no subpopulations in the six polymorphisms studied and the association results are not seen with the studied outcomes.[29]

In our results, the presence of the -1562 C/T polymorphism of MMP-9 was not associated with mortality in patients with sepsis. Like our study, a multicenter study developed by Martin et al[26] in 90 ICU patients with severe sepsis and 91 uninfected controls patients with trauma and brain stroke found that there was no association between the presence of the polymorphism in non-survivors in comparison with the survivors in patients with sepsis ($P = .1$). Undoubtedly, we have a larger sample and a higher mortality rate in patients with sepsis, which increases the power of our results. Consequently, it is very likely that there is no real association between this polymorphism and the outcomes studied in our population. On the other hand, the control group of our study uses, infected non septic patients unlike Martin et al who examined patients without infection, which highlights the diagnostic value of sepsis and not merely infection.

The association of the MMP-9 -1562 C/T gene polymorphism and development of sepsis is poorly understood due to the low number of studies in patients with sepsis. We found that the polymorphism of MMP-9 did not have a significant association with the development of sepsis compared with non-septic infected patients. As we did, Martin et al[26] found that there was no association between the MMP-9 -1562 C/T gene polymorphism and the presence of sepsis ($P = .3$).

Several studies have found that high levels of blood MMP-9 are associated with sepsis. We found that patients with sepsis with CT + TT showed a greater median of MMP-9 plasma concentration; however, this difference was not significant ($P = .14$) (Table 3). In contrast, a study carried out by Collazos et al[27] in 90 patients with severe sepsis, 102 with HIV monoinfection, 111 with HIV-hepatitis C infection, and 86 non-infected controls (45 stroke and 41 trauma patients) demonstrated that the presence of the T allele is associated with a higher plasma concentration of MMP-9 in patients with sepsis ($P = .042$), due to the size of the sample and the dispersion of the data.

Although the association of elevated levels of MMP-9 with cardiac dysfunction and loss of vascular tone has been demonstrated in animal models of sepsis,[18] the role of MMP-9 in sepsis with cardiovascular disease in humans remains understudied. We could not show an association between the SNP of MMP-9 and markers of cardiac dysfunction (serum troponin I, NT-proBNP, mean arterial pressure and DDVI) of individual alleles of polymorphism in patients with sepsis.

In addition, the allelic frequencies of the SNP MMP-9 -1562 C/T of the present investigation were compared with the 1000 Genomes database, finding the same percentage in the present study of the polymorphic T allele (15.52% vs 7.3%), for which it is presented that the wild allele C in the studied population is fixed. Due to the contradictions in the levels of MMP-9 in sepsis between this and other studies, we believe that it is likely there is a polymorphism or mutation in the MMP-9 gene that may play a synergistic role with other causing elements of mortality and severity in sepsis, leaving it as a poor predictor on its own.

However, the literature on this topic is quite limited, preventing us to firmly conclude the above. Still, there are other plausible explanations for the observed absence of association: (1) the interaction of the MMP-9 gene with other genes involved in the mechanisms of the sepsis, (2) the presence of other relevant influencing haplotypes of SNPs in the MMP-9 gene or, (3) epigenetic and other genetic mechanisms of control mediated by the existing microenvironment, including inflammatory mediators and their byproducts. All these hypotheses fall beyond the scope of this study, which emphasizes the need to design future

studies to address and identify them, and thus clarify the role of SNP MMP-9 -1562 C/T in sepsis.

Limitations of study: (1) classification under the new Sepsis-3 criteria affected the number of participants in each group; (2) given the stratification by survival, and the subgroups in genotypes, statistical power is limited; (3) using a single SNP as a biomarker can constitute a disadvantage; however, the role of such a polymorphism with a larger sample than presented in previous studies was a stronghold; (4) the study clarifies the non-association of SNP and encourages investigators to study other polymorphisms to generate predictive models accompanied by clinical variables; (5) it is important to mention that the number of enrolled patients was based on the consultation estimates for this pathology, both within our institution and in previous published studies; and (6) exploratory analyzes were made for the formulation of hypotheses, but not to discard them or confirm the association of the SNP with the event studied.

This is the first study of which we are aware on the genotypic frequency distribution of the SNP MMP-9 -1562 C/T assessing the relationship between it and sepsis and its prognostic role in patients with sepsis in Latin America and, until now, the one with the largest sample size. Also, statistical, and computational studies are required to better comprehend the prognostic value of MMP-9 SNPs in the outcome of sepsis, both on its own and in combination with other markers.

Conclusions

The present study is the first one to genotype the MMP-9 polymorphism -1562 C/T in a Latin American population, finding the possible no association between the SNP and sepsis in infected patients and between the SNP and mortality of patients with sepsis. Also, this variant did not correlate with MMP-9 plasma levels, or with of various biomarkers of cardiovascular disease.

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Conceptualization: CB-M, SB-B, CMP, CIV, MEC, IB, DT-D
Data curation: CB-M, SB-B, CMP, SES
Formal analysis: CB-M, SES, CIV
Methodology: CB-M, SB-B, CMP, MEC, IB
Writing ± original draft: CB-M, SB-B, MCP
Writing ± review & editing: MFT-C, MEC, RM-V, RS, RI, APF.

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