Interleukin-10 rs2227307 and CXCR2 rs1126579 polymorphisms modulate the predisposition to septic shock

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Despite major improvements in its treatment and diagnosis, sepsis is still a leading cause of death and admittance to the intensive care unit (ICU). Failure to identify patients at high risk of developing septic shock contributes to an increase in the sepsis burden and rapid molecular tests are currently the most promising avenue to aid in patient risk determination and therapeutic anticipation. The primary goal of this study was to evaluate the genetic susceptibility that affects sepsis outcome in 72 sepsis patients admitted to the ICU. Seven polymorphisms were genotyped in key inflammatory response genes in sepsis, including tumour necrosis factor-α, interlelukin (IL)-1β, IL-10, IL-8, Toll-like receptor 4, CXCR1 *and* CXCR2. *The primary finding showed that patients who were homozygous for the major A allele in* IL-10 *rs1800896 had almost five times higher chance to develop septic shock compared to heterozygotes. Similarly, selected clinical features and* CXCR2 *rs1126579 single nucleotide polymorphisms modulated septic shock susceptibility without affecting survival. These data support the hypothesis that molecular testing has clinical usefulness to improve sepsis prognostic models. Therefore, enrichment of the ICU portfolio by including these biomarkers will aid in the early identification of sepsis patients who may develop septic shock.*

Key words: sepsis - septic shock - biomarkers - polymorphisms - inflammation - intensive care unit

Sepsis is a complex medical condition triggered by an infection that spreads through the bloodstream. This condition leads to a systemic and uncontrolled inflammatory response that causes multiple organ failure (Dellinger et al. 2013). Despite improvements in treatment and diagnosis, sepsis is still a leading cause of death. Approximately 750,000 individuals in the United States of America develop this condition annually and a third of the patients do not survive (Angus et al. 2001). Furthermore, sepsis is costly because patients who develop septic shock spend longer time in the intensive care unit (ICU) and have an increased risk of death (Schmid et al. 2002, Arnold et al. 2014).

Sepsis involves a complex interplay between pro and antiinflammatory mediators and an unpredictable host defence response. Despite recent efforts to elucidate biomarkers of the transition from severe sepsis to septic shock (Faix 2013, Alder et al. 2014), it is not yet clear why similar pathogen infections result in distinct outcomes (Maslove & Wong 2014). In this regard, variations in genes encoding proteins with significant immunological roles may be useful in determining the patient immunogenetic background, which could be responsible for interindividual susceptibility to sepsis complications. For example, an over-stimulated immune system in a sepsis patient could be related to genetic polymorphisms affecting protein function and gene expression, resulting in excessive proinflammatory responses (Arcaroli et al. 2005). Accordingly, single nucleotide polymorphisms (SNPs) within the tumour necrosis factor (TNF)-α gene promoter, such as -308 (A/G) (also known as allele TNF2), have been associated with increased promoter activity (Wilson et al. 1997) and increased TNF-α plasma levels in response to lipopolysaccharides (Louis et al. 1998). These biological changes increase the severity of sepsis, including mortality (Mira et al. 1999). Additionally, other SNPs that affect sepsis outcome have been identified in the Toll-like receptor (TLR) signalling pathway, heat shock proteins, coagulation and antiinflammatory cytokines [reviewed by Arcaroli et al. 2005)].

Current sepsis research has focused on quick molecular-based tests to identify subjects at a high risk of developing septic shock immediately after ICU admission. It is expected that this strategy will provide a rationale for early drug therapy and better sepsis prognosis (Reinhart et al. 2012). Although valuable insights have

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been gained from the study of genetic heterogeneity in the immune system on sepsis, only a few studies have addressed the clinical feasibility of molecular testing for risk stratification in an ICU. Here, we genotyped seven polymorphisms from pro and antiinflammatory genes in hospitalised sepsis patients to investigate their potential utility in future therapeutic trials.

SUBJECTS, MATERIALS AND METHODS

Patients and clinical data - From October 2012-October 2013, 72 randomly selected sepsis patients were followed. These patients were admitted to the Risoleta Tolentino Neves Hospital ICU in Belo Horizonte, Brazil. Sepsis diagnosis was based on the criteria established by the American College of Chest Physicians (Bone et al. 1992) and the 2001 International Sepsis Definitions Conference (Levy et al. 2003). Patients were further diagnosed with severe sepsis (acute organ dysfunction) or septic shock (refractory hypotension and hyperlactataemia) (Rivers et al. 2001). All subjects were monitored until hospital discharge or death.

Demographic characteristics and clinical data, including Acute Physiology and Chronic Health Evaluation II (APACHE II), Sequential Organ Failure Assessment (SOFA), source of infection, laboratory results, microbiology and ICU mortality were obtained after sepsis diagnosis. Exclusion criteria were the following: under 18 years old, pregnancy, severe chronic respiratory disease, severe chronic liver disease (defined as a Child-Pugh score of 0.10), cancer, acquired immune deficiency syndrome and high dose immunosuppressive therapy. All enrolled subjects provided written informed consent approved by the Ethical Committee of Federal University of Minas Gerais (protocol 03182712.2.00005149/2011 - CAAE: 03182712.2.0000.5149/2012).

SNP selection, sample collection and genotyping - Seven SNPs were studied in genes encoding proteins implicated in systemic inflammatory responses to sepsis (Table I). Increased levels of TNF-α, interleukin (IL)-1β and IL-8 are thought to be key triggers of the initial hyperinflammation in sepsis, while IL-10 is a biomarker for sepsis-induced immunoparalysis, a clinical stage strongly associated with sepsis-related death (Boomer et al. 2011). SNPs in important cytokine (CXCR1 and CXCR2) and pathogen recognition receptors (TLR4) were also evaluated. The polymorphisms examined were rs1800629 (*TNF-α*), rs1143634 (*IL-1β*), rs1800896 (*IL-10*), rs2227307 (*IL-8*), rs1927911 (*TLR4*), rs16858811 (*CXCR1*) and rs1126579 (*CXCR2*).

Genomic DNA was extracted from peripheral blood using the Flexigene Kit (Qiagen, Germany) according to the manufacturer's instructions. DNA concentration and quality were evaluated using a NanoDrop 1000 (Thermo Scientific, USA). Genotyping was performed using Applied Biosystems TaqMan assays. Briefly, 50 ng DNA was used for the real-time polymerase chain reaction (PCR) genotyping. PCR was performed on the CFX96TM Real-Time PCR Detection System (Bio-Rad, USA) with the following parameters: 10 min at 95ºC and 50 cycles in two steps, 15 s at 95ºC and 1 min at 60ºC. Fluorescence was read after the completion of each PCR cycle and allele discrimination was performed by the CFX Manager software (Bio-Rad). Quality control was implemented to ensure data accuracy by retyping at least 10% of the d sample.

Statistical analysis - Because all data occurred in a Gaussian distribution, a student's *t* test and ANOVA were performed for the quantitative data and chi-squared tests were performed for categorical variables in SPSS v.20. Comparison of the observed genetic frequencies among groups was performed using UNPHASED (v.3.1.7) (Dud-

TABLE I

Characteristics of the genes and polymorphisms evaluated by this study

Gene	Function	SNP	Gene location	Major/minor allele	HWE p
CXCR1	Receptor α for the chemokine IL-8	rs16858811	Exon	C/T	**0.0045**
CXCR2	Receptor β for the chemokine IL-8	rs1126579	Utr 3'	G/T	0.1113
TLR4	TLR associated with pathogen recognition and activation of innate immunity	rs1927911	Intron	A/G	0.5242
IL-1β	Proinflammatory cytokine produced by activated macrophages	rs1143634	Intragenic	C/T	0.3461
IL-8	Chemotactic factor for neutrophils and other granulocytes	rs2227307	Intron	T/G	0.5242
IL-10	Antiinflammatory cytokine produced by monocytes and lymphocytes	rs1800896	Promoter	A/G	0.9025
TNF-α	Proinflammatory cytokine that attracts neutrophils	rs1800629	Intron	G/A	0.4092

Hardy-Weinberg equilibrium (HWE) p-value in bold was considered deviated from Hardy-Weinberg principle; IL: interleukin; SNP: single nucleotide polymorphisms; TLR: Toll-like receptor; TNF: tumour necrosis factor.

bridge 2008). Odds ratio (OR) values shown in the tables were obtained using the ancestral genotype or allele as a reference (displayed as OR = 1). HAPLOVIEW (v.4.1) was used to evaluate the deviation from the Hardy-Weinberg equilibrium (HWE) (Barrett et al. 2005). All tests were two-tailed and the p-level for significance was set at 0.05.

RESULTS

Out of the 72 sepsis patients included in this study (average age of 52.7 ± 19.0 years and 10.6 ± 8.4 days at ICU), 63.9% (46) were male (Table II). According to the clinical assessment, 20.8% (15) of the patients presented with severe sepsis and 79.2% (57) presented with septic shock. Clinical variables were not significantly different between these groups. Conversely, we identified robust influence of the clinical data on the death risk. Thirteen (18.1%) septic shock patients did not survive and they appeared to be predisposed due to increased age (66 ± 18.9 years; p = 0.005), female gender (30.8% vs. 10.9% of men; p = 0.035), fewer days in the ICU (4.31 ± 3.22; p = 0.002), higher APACHE II and SOFA scores [36.7 ± 5.7 (p = 0.0001) and 13.2 ± 3.1 (p = 0.0001), respectively] and laboratory results (Table II).

Depending on the SNP, we successfully genotyped approximately 95-99% of the samples. We found that only *CXCR1* rs16858811 deviated from the HWE (p < 0.01) (Table I). We first compared the genotype and allele frequencies between patients with severe sepsis and septic shock. According to our findings, the frequencies of the *IL-10* rs1800896 and *CXCR2* rs1126579 SNPs were heterogeneously distributed among the patient subgroups (Table III). For instance, patients carrying the AA genotype from *IL-10* rs1800896 had almost five times increased risk to develop septic shock compared to heterozygotes [p = 0.0083; OR = 4.85; 95% confidence interval (CI) = 1.67-14.03]. The risk was also greater for carriers of the A allele (p = 0.009; OR = 2.69; 95% CI = 1.26-5.72). For *CXCR2* rs1126579, we only found significant differences through allele comparisons, with carriers of the T allele at greater risk of septic shock (p = 0.0251; OR = 2.32; 95% CI = 1.10-4.91).

Contrary to clinical data, none of the genetic markers predisposed the patients to a higher risk of death, even though the ancestral genotype (p = 0.015) and allele (p = 0.0148) from *IL-1β* rs1143634 was frequently found in patients with an APACHE score greater than 25 (Table IV).

DISCUSSION

Understanding why certain patients are at greater risk for poorer sepsis outcomes goes far beyond the microorganism's infection and damage to the host tissues. Recent studies have suggested that inherited factors play a significant role in the predisposition to sepsis-related complications by affecting hyperinflammatory or hypoinflammatory responses to infection (Arcaroli et al. 2005, Baier et al. 2006, Abu-Maziad et al. 2010, Man et al. 2013). The primary goal of this study was to evaluate genetic predispositions that influence sepsis outcome. By genotyping polymorphisms in key inflammatory response genes in sepsis, we identified patients at a high risk to develop septic shock. Therefore, our findings support the hypothesis that molecular testing is clinically useful to improving sepsis prognostic models.

Sepsis remains a public health issue, perhaps due to the lack of valid biomarkers to assess an individual's risk for poor prognosis upon infection. Here, we found that homozygotes for the major A allele in *IL-10* rs1800896 have an almost five-times increased risk of developing septic shock compared to heterozygous patients. This finding is consistent with those of Baier et al. (2006) and Abu-Maziad et al. (2010), in which infants with the *IL-10* rs1800896 AA genotype had an increased incidence of late-onset sepsis, while Treszl et al. (2003) did not observe a similar association. IL-10 is a potent antiinflammatory cytokine that increases the severity of systemic infection in newborn infants at high levels (Romagnoli et al. 2001, Ng et al. 2003). Experimental evidence has shown that IL-10 is a preeminent therapeutic candidate for irreversible septic shock (Latifi et al. 2002). How the A allele of *IL-10* rs1800896 contributes to increased risk of septic shock is unclear. This SNP, also known as -1082 (A/G), is located within the *IL-10* promoter region and is therefore presumed to affect the gene's transcriptional activity. Because individuals carrying the A allele have decreased circulating levels of IL-10 (Schaaf et al. 2003), we hypothesise that AA homozygotes are prone to proinflammatory responses, which favour septic shock. However, further measurement of circulating IL-10 concentrations and genotyping of other IL-10 promoter polymorphisms [such as microsatellites, -819 (C/T) and -592 (C/A)] should be examined to refine this assumption.

CXCR2 is the IL-8β receptor that mediates neutrophil migration to inflammation sites. To our knowledge, this is the first report of the *CXCR2* rs1126579 SNP as a septic shock biomarker. Carriers of the minor T allele had a two-fold increased risk for developing septic shock compared to carriers of the C allele. The rs1126579 SNP is located in the 3′ untranslated region of the *CXCR2* gene and no data on its impact on CXCR2 activity have been described to date.

Recent advances in the understanding of sepsis suggested that after initial hyperinflammation, the immune system shifts to a profoundly suppressed state known as sepsis-induced immunoparalysis (Hotchkiss & Karl 2003, Boomer et al. 2011). Immunoparalysis accounts for the majority of sepsis-related mortality. None of the polymorphisms investigated here showed a significant effect on mortality rate of sepsis patients. These results suggest that these SNPs' scores have a weak, if any, influence on immunoparalysis mechanisms. Investigation into other genetic polymorphisms is important to identify biomarkers of sepsis-related death. Conversely, we found clinical, demographic and laboratory data associated with decreased survival. For example, most younger patients survived to hospital discharge. Importantly, age and genotype were independent risk factors, as multivariate analysis showed no significant influence of age on the genotype's effect (data not shown). Nevertheless, we expect that in a large-scale population, combining the individual SNP or gene-gene interaction risk score with clinical factors may increase the predictive ability of molecular testing (Man et al. 2013).

TABLE II
Demographic and clinical characteristics of the study

	Sepsis	Severe sepsis	Septic shock	p[a]	Survivor	Nonsurvivor	p[b]	APACHE > 25	APACHE < 25	p[c]
Subjects [n (%)]	72	15 (20.8)	57 (79.2)	NA	59 (81.9)	13 (18.1)	NA	36 (50)	36 (50)	NA
Age	52.74 ± 19.05	51.06 ± 20.10	54.15 ± 18.25	0.496	49.81 ± 17.95	66 ± 18.91	**0.005**	60.3 ± 17.05	45.1 ± 18.0	**< 0.0001**
Male [n (%)]	46 (63.9)	22 (66.7)	24 (61.5)	-0.652	41 (89.1)	5 (10.9)	**0.035**	20 (43.5)	26 (56.5)	0.141
Female [n (%)]	26 (36.1)	11 (33.3)	15 (38.5)		18 (69.2)	8 (30.8)		16 (61.5)	10 (38.5)	
Days at ICU	10.63 ± 8.44	11.88 ± 8.48	9.56 ± 8.35	0.249	12.02 ± 8.6	4.31 ± 3.22	**0.002**	11.69 ± 9.75	9.56 ± 6.85	0.285
APACHE II	26.65 ± 9.93	26.85 ± 9.81	26.49 ± 10.16	0.879	24.44 ± 9.3	36.69 ± 5.72	**0.0001**	34.56 ± 5.71	18.75 ± 6.26	**< 0.0001**
n	7.43 ± 4.13	7.58 ± 4.25	7.31 ± 4.08	0.786	6.15 ± 3.12	13.23 ± 3.08	**0.0001**	9.58 ± 4.23	5.28 ± 2.7	**< 0.0001**
Platelet (mm³)	214,027 ± 122,030	190,818 ± 10,497	233,666 ± 132,965	0.139	222,440 ± 119,113	175,846 ± 132,681	0.215	183,666 ± 130,551	244,388 ± 106,151	0.034
Neutrophils (mm³)	11,666 ± 6,683	11,906 ± 6,528	11,464 ± 6,889	0.782	12,407 ± 6,571	8,305 ± 6,373	**0.044**	10,926 ± 6,014	12,407 ± 7,300	0.351
Eosinophils (mm³)	94.96 ± 260.01	66.70 ± 145.5	118.87 ± 327.42	0.4	88.73 ± 235.2	123.23 ± 362.74	0.668	155.06 ± 350.84	34.86 ± 81.39	**0.049**
Basophils (mm³)	9.47 ± 51.01	16.82 ± 74.69	3.26 ± 8.83	0.264	3.02 ± 10.94	38.77 ± 116.98	**0.021**	15.33 ± 71.04	3.61 ± 12.72	0.333
Monocytes (mm³)	880.54 ± 875.84	954.70 ± 971.88	816.13 ± 790.69	0.510	958.73 ± 921.19	496.08 ± 461.64	0.096	931.71 ± 999.87	830.78 ± 746.90	0.631
Lymphocytes (mm³)	1,451 ± 1,097	1,525 ± 1,041	1,389 ± 1,153	0.604	1,536 ± 1,167	1,065 ± 590.44	0.163	1,548 ± 1,292	1,354 ± 869.27	0.455
CRP (mg/dL)	273.08 ± 131.79	259.3 ± 140.68	284.75 ± 124.43	0.418	274.30 ± 131.45	267.53 ± 138.61	0.868	249.26 ± 133.53	296.90 ± 127.43	0.126
Lactic acid (mg/dL)	3.23 ± 2.78	3.42 ± 3.07	3.06 ± 2.53	0.590	2.87 ± 2.58	4.84 ± 3.18	**0.02**	3.76 ± 3.16	2.69 ± 2.26	0.103

a-c: *p*-values from comparison between severe sepsis vs. septic shock, survivors vs. nonsurvivors and Acute Physiology and Chronic Health Evaluation II score (APACHE) > 25 vs. APACHE < 25, respectively; CRP: C-reactive protein; ICU: intensive care unit; n: the absolute values; NA: not applicable, except where otherwise shown, values are displayed as average ± standard deviation; SOFA: Sequential Organ Failure Assessment score. Values in bold were considered statistically significant.

TABLE III

Association analysis of single nucleotide polymorphisms (SNPs) between sepsis patients

Gene (SNP)	Sepsis n (%)	Severe sepsis n (%)	Septic shock n (%)	OR (95% CI)	p
CXCR1					
rs16858811					
AA	49 (71.01)	22 (68.75)	27 (72.97)	1	0.700[a]
AC	20 (28.99)	10 (31.25)	10 (27.03)	0.81 (0.28-2.30)	
CC	0 (0)	0 (0)	0 (0)	NA	
A	118 (85.5)	54 (84.38)	64 (86.49)	1	0.725[b]
C	20 (14.5)	10 (15.62)	10 (13.51)	0.84 (0.32-2.17)	
CXCR2					
rs1126579					
CC	34 (49.28)	12 (37.5)	22 (59.46)	1	0.0617[a]
CT	29 (42.03)	15 (46.88)	14 (37.84)	0.51 (0.18-1.40)	
TT	6 (8.7)	5 (15.62)	1 (2.7)	0.11 (0.01-1.04)	
C	97 (70.29)	39 (60.94)	58 (78.38)	1	**0.0251**[b]
T	41 (29.71)	25 (39.06)	16 (21.62)	0.43 (0.20-0.90)	
TLR4					
rs1927911					
AA	16 (23.19)	8 (25)	8 (21.62)	1	0.1045[a]
AG	27 (39.13)	16 (50)	11 (29.73)	0.69 (0.19-2.38)	
GG	26 (37.68)	8 (25)	18 (48.65)	2.25 (0.62-8.14)	
A	59 (42.75)	32 (5)	27 (36.49)	1	0.109[b]
G	79 (57.25)	32 (5)	47 (63.51)	1.74 (0.88-3.44)	
IL-1β					
rs1143634					
GG	49 (71.01)	24 (75)	25 (67.57)	1	0.758[a]
GA	18 (26.09)	7 (21.9)	11 (29.73)	1.51 (0.50-4.53)	
AA	2 (2.9)	1 (3.1)	1 (2.7)	0.96 (0.05-16.23)	
G	116 (84)	55 (85.94)	61 (82.43)	1	0.573[b]
A	22 (15.94)	9 (14.06)	13 (17.57)	1.30 (0.51-3.28)	
IL-8					
rs2227307					
GG	20 (28.99)	7 (21.88)	13 (35.14)	1	0.459[a]
GT	38 (55.07)	19 (59.37)	19 (51.35)	0.54 (0.17-1.64)	
TT	11 (15.94)	6 (18.75)	5 (13.51)	0.45 (0.1-2.01)	
G	78 (56.52)	33 (51.56)	45 (60.81)	1	0.274[b]
T	60 (43.48)	31 (48.44)	29 (39.19)	0.69 (0.34-1.35)	
IL-10					
rs1800896					
AA	33 (47.83)	9 (28.1)	24 (64.86)	1	**0.0083**[a]
AG	51 (44.93)	20 (62.5)	11 (29.73)	0.21 (0.07-0.59)	
GG	5 (7.24)	3 (9.4)	2 (5.4)	0.25 (0.03-1.75)	
A	97 (70.29)	38 (59.38)	59 (79.73)	1	**0.009**[b]
G	41 (29.71)	26 (40.62)	15 (20.27)	0.37 (0.17-0.79)	
TNF-α					
rs1800629					
GG	54 (78.26)	26 (81.26)	28 (75.68)	1	0.323[a]
GA	14 (20.29)	5 (15.62)	9 (24.32)	1.67 (0.49-5.64)	
AA	1 (1.44)	1 (3.12)	0 (0)	NA	
G	122 (88.4)	57 (89.06)	65 (87.84)	1	0.822[b]
A	16 (11.6)	7 (10.94)	9 (12.16)	1.13 (0.39-3.22)	

a, *b*: genotype and allele comparisons, respectively; CI: confidence interval; IL: interleukin; n: absolute values; NA: not applicable; OR: odds ratio; TLR: Toll-like receptor; TNF: tumour necrosis factor. Values in bold were considered statistically significant.

TABLE IV

Association analysis of single nucleotide polymorphisms (SNPs) between survivors and nonsurvivors and APACHE > 25 of severe sepsis patients

Gene (SNP)	Nonsurvivor n (%)	Survivor n (%)	OR (95% CI)	p[a]	APACHE > 25 n (%)	APACHE < 25 n (%)	OR (95% CI)	p[b]
CXCR1								
rs16858811								
AA	7 (53.85)	42 (75)	1	0.142[c]	22 (62.86)	27 (79.41)	1	0.127[c]
AC	6 (46.15)	14 (25)	2.57 (0.73-8.94)		13 (37.14)	7 (20.59)	2.27 (0.77-6.69)	
CC	0 (0)	0 (0)	-		0 (0)	0 (0)	-	
A	20 (76.29)	98 (87.5)	1	0.189[d]	57 (81.43)	61 (89.71)	1	0.1643[d]
C	6 (23.08)	14 (12.5)	2.1 (0.72-6.12)		13 (18.57)	7 (10.29)	1.98 (0.74-5.33)	
CXCR2								
rs1126579								
CC	7 (53.85)	27 (48.21)	1	0.934[c]	18 (51.43)	16 (47.06)	1	0.933[c]
CT	5 (38.46)	24 (42.86)	0.80 (0.22-2.86)		14 (4)	15 (44.12)	0.82 (0.30-2.23)	
TT	1 (7.69)	5 (8.93)	0.77 (0.07-7.71)		3 (8.57)	3 (8.88)	0.88 (0.15-5.04)	
C	19 (73.08)	78 (69.64)	1	0.728[d]	50 (71.43)	47 (69.12)	1	0.7665[d]
T	7 (26.92)	34 (30.36)	0.84 (0.31-2.19)		20 (28.57)	21 (30.88)	0.89 (0.43-1.85)	
TLR4								
rs1927911								
AA	2 (15.38)	14 (25)	1	0.410[c]	8 (22.86)	8 (23.53)	1	0.314[c]
AG	4 (30.77)	23 (41.07)	1.21 (0.19-7.53)		11 (31.43)	16 (47.06)	0.68 (0.19-2.38)	
GG	7 (53.85)	19 (33.93)	2.57 (0.46-14.35)		16 (45.71)	10 (29.41)	1.6 (0.45-5.63)	
A	8 (30.77)	51 (45.54)	1	0.164[d]	27 (38.57)	32 (47.06)	1	0.3134[d]
G	18 (69.23)	61 (54.46)	1.88 (0.75-4.68)		43 (61.43)	36 (52.94)	1.42 (0.72-2.78)	
IL-1β								
rs1143634								
GG	11 (84.62)	38 (67.86)	1	0.129[c]	30 (85.71)	19 (55.88)	1	**0.015**[c]
GA	1 (7.69)	17 (30.36)	0.20 (0.02-1.70)		4 (11.43)	14 (41.18)	0.18 (0.05-0.63)	
AA	1 (7.69)	1 (1.78)	3.45 (0.19-59.84)		1 (2.85)	1 (2.94)	0.63 (0.03-10.74)	
G	23 (88.46)	93 (83.04)	1	0.481[d]	64 (91.43)	52 (76.47)	1	**0.0148**[d]
A	3 (11.54)	19 (16.96)	0.63 (0.17-2.34)		6 (8.57)	16 (23.53)	0.30 (0.11-0.83)	
IL-8								
rs2227307								
GG	2 (15.38)	18 (32.14)	1	0.412[c]	11 (31.43)	9 (26.47)	1	0.870[c]
GT	8 (61.54)	30 (53.57)	2.4 (0.45-12.57)		19 (54.29)	19 (55.88)	0.81 (0.27-2.42)	
TT	3 (23.08)	8 (14.29)	3.37 (0.46-24.29)		5 (14.29)	6 (17.65)	0.68 (0.15-2.98)	
G	12 (46.15)	66 (58.93)	1	0.238[d]	41 (58.57)	37 (54.41)	1	0.6221[d]
T	14 (53.85)	46 (41.07)	1.67 (0.70-3.94)		29 (41.43)	31 (45.59)	0.84 (0.43-1.65)	
IL-10								
rs1800896								
TT	27 (48.21)	6 (46.15)	1	0.306[c]	15 (42.86)	18 (52.94)	1	0.687[c]
TC	24 (42.86)	7 (53.85)	1.31 (0.38-4.45)		17 (48.57)	14 (41.18)	0.68 (0.19-2.38)	
CC	5 (8.93)	0 (0)	NA		3 (8.57)	2 (5.88)	1.6 (0.45-5.63)	
T	19 (73.08)	78 (69.64)	1	0.728[d]	47 (67.14)	50 (73.53)	1	0.4113[d]
C	7 (26.92)	34 (30.36)	0.84 (0.32-2.19)		23 (32.86)	18 (26.47)	1.35 (0.65-2.83)	
TNF-α								
rs1800629								
GG	10 (76.92)	44 (78.57)	1	0.1699[c]	27 (77.14)	27 (79.41)	1	0.5036[c]
GA	2 (15.38)	12 (21.43)	0.73 (0.14-3.80)		7 (0.2)	7 (0.2)	1 (3.0-3.24)	
AA	1 (7.7)	0 (0)	NA		1 (0.02)	0 (0)	NA	
G	22 (84.62)	100 (89.29)	1	0.5163[d]	61 (87.14)	61 (89.71)	1	0.6378[d]
A	4 (15.38)	12 (10.71)	1.28 (0.45-3.67)		9 (12.86)	7 (10.29)	1.28 (0.45-3.67)	

a, *b*: p-values from comparison between survivors vs. nonsurvivors and Acute Physiology and Chronic Health Evaluation II score (APACHE) > 25 vs. APACHE < 25, respectively; *c*, *d*: genotype and allele comparisons, respectively; CI: confidence interval; IL: interleukin; n: the absolute values; NA: not applicable; OR: odds ratio; TLR: Toll-like receptor; TNF: tumour necrosis factor. Values in bold were considered statistically significant.

Overall, a limitation of this study was the lack of comprehensive clinical information, such as the causative pathogen, initial infection site and the gap before treatment initiation. However, this caveat reveals that the positive genetic markers identified may be useful in assessing septic shock risk even when these data are not yet established. Nevertheless, we encourage further study aimed at investigating the clinical usefulness of these SNPs in specific infections, which may reveal reliable predictive models. Moreover, considering that some of the patients' demographic and clinical factors influence sepsis predisposition, it would be interesting to stratify our data to enhance input score reliability. However, the sample pool we recruited was too small to enable such an approach.

In conclusion, our findings revealed that *IL-10* rs1800896, *CXCR2* rs1126579 and selected clinical features can be used as markers for septic shock development, but not for decreased survival. Therefore, sepsis prognostic models including these biomarkers appear can enable the early identification of sepsis patients who may become septic shock cases. Although other studies are required to evaluate the accuracy of these data in different populations, a recent meta-analysis found that IL-10 polymorphisms are associated with sepsis susceptibility in Caucasian and Asian populations (Pan et al. 2015). Furthermore, in addition to the risk of septic shock in this study possibly due to increased IL-10 plasma concentrations (Wang et al. 2011), it is now well-known that early sepsis diagnosis and correct clinical support compliance play a critical role in mortality (de Oliveira et al. 2013, van Zanten et al. 2014). In this context, we speculate that IL-10-induced immunosuppression may have a more important impact on the restriction of infection than on sepsis outcome. Finally, we believe that enrichment of the ICU portfolio by incorporating personalised strategies such as genetic testing for biomarkers of therapeutic response may improve the outcome of patients with sepsis and alleviate ICU demand in the future.

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