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Title: *IL-1B* rs16944 polymorphism is related to septic shock and death
Short title: *IL-1B* polymorphism and septic shock

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ABSTRACT

Background: IL-1β is a primary mediator of systemic inflammatory response syndrome (SIRS) and it may lead to shock septic. Our aim was to analyze whether *IL-1B* rs16944 polymorphism is associated with the onset of septic shock and death after major surgery.

Methods: We performed a case-control study on 467 patients who underwent major cardiac or abdominal surgery. Out of them, 205 patients developed septic shock (Cases, SS-group) and 262 patients developed SIRS (Controls, SIRS-group). The primary outcome variables were the development of septic shock and death within 90 days after diagnosis of septic shock. The *IL-1B* rs16944 polymorphism was genotyped by Sequenom's MassARRAY platform. The association analysis was performed under a recessive genetic model (AA vs. GG/GC).

Results: The frequency of septic shock was higher in patients with *IL-1B* rs16944 AA genotype than in patients with *IL-1B* rs16944 GG/AG genotype when all patients were taken into account (63.6% vs 41.8%; p=0.006), cardiac surgery (52.2% vs. 33.3%; p=0.072), and abdominal surgery (76.2% vs. 50.2%; p=0.023). However, the *IL-1B* rs16944 AA genotype was only associated with higher likelihood of septic shock in the analysis all population [adjusted odds ratio (aOR)=2.26 (95%CI=1.03; 4.97; p=0.042], but not when it was stratified by cardiac surgery (p=0.175) or abdominal surgery (p=0.467). Similarly, *IL-1B* rs16944 AA genotype was also associated with higher likelihood of septic shock-related death in all population [aOR=2.67 (95%CI=1.07; 4.97); p=0.035].

Conclusions: *IL-1B* rs16944 AA genotype seems to be related to the onset of septic shock and death in patients who underwent major surgery.

Key words: IL-1B; SNPs; septic shock; death; major surgery; systemic inflammatory response syndrome (SIRS)

INTRODUCTION

Sepsis is a deleterious host response occurring in patients following infection due to an uncontrolled immune response to microbial antigens [1, 2]. It is the primary cause of death from infection, especially if not recognized and treated promptly [3]. Septic shock (sepsis plus hypotension not reversed with fluid resuscitation) is the most advanced stage of disease severity [4], which partly determines the outcome and death [3, 5, 6]. In recent years, despite advances in treatment and supportive care, severe sepsis and septic shock are among the leading causes of death in patients admitted worldwide to an intensive care unit (ICU), with 28-day mortality above 25% [7, 8]. Within these patients, early and aggressive support treatment has not improved survival [4]. Therefore, it is essential, for early preventive care, to have predictive biomarkers to identify patients at risk of developing sepsis.

Sepsis is most commonly produced by bacteria; but fungi, viruses, or parasites may also trigger sepsis [9]. Therefore a high number of microbial factors may cause the typical septic inflammatory cascade with cytokine production such as interleukin 1(IL-1), interleukin 6 (IL-6), and tumor necrosis factor (TNF) [1]. These cytokines may lead to endothelial damage, the formation of blood clots in small blood vessels, multi-organ failure, development of shock and death [1].

The proinflammatory cytokines utilize the Janus kinase–signal transducer and activator of transcription (JAK–STAT) pathway to transduce their information into the cell nucleus for developing a specific response against microbial pathogens [10]. Moreover, these proinflammatory cytokines may activate the suppressor of cytokine signaling-3 (SOCS3) and may modulate the cytokine signaling, usually preventing, but in some cases aggravating the outcome of infections [10]. Thus, the immune dysfunction process in septic patients might be influenced by the cytokine profile and to promote an inefficient response at clearing invasive microbial pathogens [11], which may predict the survival of septic patients [12, 13].

The genetic variation in single nucleotide polymorphisms (SNPs) at cytokine genes may influence the development of sepsis and their effect on clinical outcome. Specially, variations at the promoter region, where more of the transcription regulation takes place. [14]. IL-1β is a primary mediator of systemic inflammatory response syndrome (SIRS) and it may lead to septic shock and organ failure [15]. *IL-1B* SNPs have been associated with risk of inflammation, infection and sepsis. Concretely, the *IL-1B* rs16944 polymorphism, located on chromosome 2 at position 511 bp upstream of the transcriptional start site at *IL-1B* gene (*IL1B -511A>G*), seems to be associated with multiple inflammatory processes and autoimmune diseases such as periodontitis [16], systemic sclerosis [17] and pancreatitis [18]. Regarding infection, *IL-1B* rs16944 polymorphism has been associated with a higher risk of chronic HBV infection, severe influenza A virus infection, invasive mold infection and bone infection [19-22]. Whereas an absence of association has been found in other infections such as meningitis and skin infection [3, 23]. In regards to generalized infection and sepsis, *IL-1B* rs16944 polymorphism has been related to a higher risk of development of bacteremia and sepsis [24, 25]. However, Montoya-Ruiz et al. found that *IL-1B* rs16944 polymorphism is not associated with the clinical course of sepsis [26]. Besides, a recent meta-analysis did not find significant association between *IL-1B* rs16944 polymorphism and sepsis risk [27]. Thus, there are certain discrepancies about the relationship between *IL-1B* rs16944 and sepsis in the literature, and further studies would be needed.

OBJECTIVES

The aim of this study was to analyze whether *IL-1B* rs16944 polymorphism is associated with increased susceptibility for developing septic shock and septic shock-related death in patients who underwent major surgery.

STUDY DESIGN

Patients

We carried out a case-control study in 467 patients older than 18 who underwent major cardiac or abdominal surgery at the Hospital Clínico Universitario of Valladolid (Spain) between April 2008 and November 2012. The Case-group consisted of 205 patients that developed septic shock (SS-group) and the Control-group consisted of 262 patients with SIRS (SIRS-group). Furthermore, a substudy for analyzing the association with death within 90 days after diagnosis was performed in all septic shock patients. All patients were European white subjects.

The study was conducted in accordance with the Declaration of Helsinki. All patients gave their written consent for the study. The Ethics Committee of Hospital Clínico Universitario (Valladolid) and Instituto de Salud Carlos III (Majadahonda) approved this study.

Clinical data

Major surgery was considered as an operative procedure in which the patient was under general anaesthesia and respiratory assistance. Cardiac surgery included valve, coronary and mixed surgery. Abdominal surgery included wound dehiscence, perforation, biliary surgery, ischemia, pancreatic surgery, abdominal abscess and bladder perforation. Demographic and clinical data were obtained from medical records: age, gender, type of surgery, prior or pre-existing conditions such as diabetes, chronic obstructive pulmonary disease, hypertension, chronic kidney disease, cancer, liver disease and cardiomyopathy. Cardiopulmonary bypass was carried out in all cardiac surgeries. Severity of sepsis was evaluated by using two ICU scoring systems calculated within the first 24 hours after diagnosis: (i) Acute Physiology and Chronic health Evaluation (APACHE II score) [28], which consist of 12 physiological variables and 2 disease-related variables, and ranges from 0 to 71; (ii) Sequential Organ Failure Assessment (SOFA score) [29], which is made of 6 variables, each one representing an organ system failure, and ranging from 0 to 24.

Patients were evaluated for the presence of SIRS or septic shock after major surgery during hospitalization. The diagnosis of SIRS was made during the first 24 hours post-surgery and septic shock diagnosis during the whole follow-up time post-surgery, which were established according to the criteria laid down by the SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference [2]. The administration of catecholamines was performed according to the international guidelines for the management of severe sepsis and septic shock [4]. Baseline characteristics of patients were recorded within the first 24 hours after diagnosis. In regards to septic shock, the presence of infection was documented by positive microbiological findings and lactic acid was higher than 2 mmol/L. In those patients where infection was strongly suspected but not microbiologically confirmed, two experienced clinicians discussed and reached a consensus diagnosis according to physical and laboratory findings. Patients included in the SIRS-group were patients who had age and gender similar to the SS-group. Those patients who neither had SIRS nor septic shock were excluded.

DNA genotyping

Total DNA was extracted from peripheral blood with High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Mannheim, Germany). DNA samples were genotyped at the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) for *IL-1B* rs16944 polymorphism, by using Sequenom's MassARRAY platform (San Diego, CA, USA) and the iPLEX® Gold assay design system according to the published Illumina protocol [30]. The quality control was performed according to the CeGen criteria, which includes duplicated samples on each plate to check for technical replicates; negative and positive controls on each batch to exclude DNA contamination and ensure a technically correct laboratory process, respectively. Genotyping call-rate over 95% was used for all the SNPs.

Outcome variables

The primary endpoint was to study the impact of the rs16944 polymorphism on the development of septic shock and septic shock-related death within 90 days after diagnosis of septic shock. The secondary endpoint was to study the impact of the rs16944 polymorphism taken into account the type of surgery.

Statistical analysis

For the description of the study population, p-values were estimated with nonparametric tests: Mann-Whitney U test was used for continuous variables and chi-squared/Fisher's exact test for categorical variables.

For the genetic analysis, logistic regressions were carried out to assess the relationship between *IL-1B* rs16944 polymorphism and outcome variables (SS-group vs. SIRS-group) under a recessive genetic model (AA vs. GG/AG), which was the model that best fit to our data. Each logistic regression test was adjusted by the most significant co-variables associated with the outcome variable, avoiding the over-fitting of the regression. We included the SNP (Enter algorithm (forced entry for the SNP)) and the most relevant characteristics by stepwise algorithm (at each step, factors are considered for removal or entry: a p-value for entry and exit of 0.15 and 0.20, respectively) according to the outcome of the variables analyzed. In the septic shock analysis, the following covariates were used: age, gender, smoker, and high alcohol intake, prior or pre-existing conditions (comorbidities), and type of surgery (cardiac or abdominal; emergency or scheduled). Regarding the death analysis, the following covariates were used: APACHE-II score, gender, antibiotic treatment, type of surgery (cardiac or abdominal), elective surgery (emergency or scheduled), activated partial thromboplastin time, procalcitonin, peritonitis, comorbidities (obesity, diabetes heart disease, chronic obstructive pulmonary disease (COPD), hypertension, neoplasia, and liver disease), smoker, and high alcohol intake.

All statistical analyses were performed by using the IBM SPSS Statistics for Windows, Version 21.0 (IBM Corp, Chicago, Armonk, NY, USA). All p-values were two-tailed. Statistical significance was defined as $p<0.05$. In addition, Hardy-Weinberg equilibrium (HWE) analyses were computed by Haploview 4.2 software, considering equilibrium when $p>0.05$.

RESULTS

Characteristics of the study population

The baseline characteristics of 205 cases (SS-group) and 262 controls (SIRS patients) are presented in **Table 1**. The SS-group had higher values of APACHE II and SOFA scores than the SIRS-group (p<0.001). Furthermore, the SS-group had a higher percentage of patients with abdominal surgery (p<0.001), emergency surgery (p<0.001), and chronic kidney disease (p<0.001), and lower percentage of cancer (p=0.005), and cardiomyopathy (p<0.001). Besides, the SS-group had the highest percentages of Gram-negative infections (52.7%), peritonitis (46.8%), and pneumonias (47.3%).

Table 1. Baseline characteristics of patients with systemic inflammatory response syndrome (SIRS-group) and septic shock (SS-group).

Characteristics	SIRS-group	SS-group	p-value
No. patients	262	205	
Gender (male)	166 (63.4%)	131 (63.9%)	0.904
Age (years)	72 (65-78)	73 (63-79)	0.127
Smoker	38 (14.5%)	36 (17.6%)	0.369
Alcohol intake	10 (3.8%)	15 (7.3%)	0.095
Time to diagnosis (days)	0 (0-0)	1 (0-4)	<0.001
Respiratory support (days)	0 (0-0)	4 (1-12)	<0.001
Surgery			
Cardiac	151 (57.6%)	82 (40.0%)	<0.001
Abdominal	111 (42.4%)	123 (60.0%)	-
Emergency	20 (7.2%)	130 (63.4%)	<0.001
Scheduled	247 (92.5%)	75 (36.6%)	-
Severity of disease score			
SOFA score	3 (3-4)	8 (7-10)	<0.001
APACHE II score	9 (8-9)	16 (13-19)	<0.001
Prior or pre-existing conditions			
Diabetes	50 (19.1%)	26 (12.7%)	0.063
Chronic obstructive pulmonary disease	35 (13.4%)	36 (17.6%)	0.209
Hypertension	161 (61.5%)	112 (54.6%)	0.138
Chronic kidney disease	15 (5.7%)	30 (14.6%)	<0.001
Cancer	93 (35.5%)	48 (23.4%)	0.005
Liver disease	5 (1.9%)	9 (4.4%)	0.119
Cardiomyopathy	160 (61.1%)	92 (44.6%)	<0.001
Obesity	34 (13.0%)	30 (14.6%)	0.605
Infection			
Gram-negative	-	108 (52.7%)	-
Gram-positive	-	102 (49.8%)	-
Fungus	-	41 (20.0%)	-
Catheter bacteraemia	-	70 (34.1%)	-
Surgical site infection	-	49 (23.9%)	-
Urinary tract infection	-	24 (11.7%)	-
Endocarditis	-	10 (4.9%)	-
Peritonitis	-	96 (46.8%)	-
Pneumonia	-	97 (47.3%)	-

Values are expressed as median (P25th – P75th) and absolute count (percentage).

P-values were calculated by Chi-square test or Fisher's exact test for categorical variables and Mann-Whitney test for continuous variables. Note that patients may have had more than one organism cultured.
Abbreviations: SIRS-group, patients with systemic inflammatory response syndrome; SS-group, patients with septic shock; SOFA, sequential organ failure assessment; APACHE, acute physiology and chronic health evaluation.

The demographic and clinical characteristics of septic shock patients according to the type of surgery are shown in **Table 2**. The septic shock in peritonitis was presented on the day of surgery; but in the other cases, it was presented a few days later of the surgery (p<0.001). The duration of respiratory support was higher in cardiac than abdominal surgery (p<0.010). Patients who underwent abdominal surgery showed higher frequencies of emergency surgery (p<0.001) and cancer (p=0.001) than patients who underwent cardiac surgery; whereas we found lower frequencies of hypertension (p=0.008), cardiomyopathy (p=0.001), and obesity (p=0.044). Regarding infection conditions, abdominal surgery group had a higher percentage of peritonitis (p<0.001) and lower percentage of catheter bacteraemia (p<0.001), endocarditis (p<0.001) and pneumonia (p<0.001) than cardiac surgery group.

Table 2. Baseline characteristics of patients with septic shock (SS-group) according to type of surgery.

Characteristics	**Cardiac**	**Abdominal**	**p-value**
No. patients	82	123	
Gender (male)	54 (65.9%)	77 (62.6%)	0.635
Age (years)	73 (65-79)	73 (63-80)	0.873
Smoker	14 (17.1%)	22 (17.9%)	0.881
Alcohol intake	5 (6.1%)	10 (8.1%)	0.584
Time to diagnosis (days)	2 (1-6)	0 (0-2)	<0.001
Respiratory support (days)	6 (3-14)	3 (1-8)	0.010
Surgery			
Emergency	29 (35.4%)	101 (82.1%)	<0.001
Scheduled	53 (64.6%)	22 (17.9%)	-
Severity of disease score			
SOFA score	9 (7-11)	8 (7-10)	0.339
APACHE II score	15 (13-18)	17 (14-21)	0.148
Prior or pre-existing conditions			
Diabetes	14 (17.1%)	12 (9.8%)	0.123
Chronic obstructive pulmonary disease	15 (18.1%)	21 (17.1%)	0.822
Hypertension	54 (65.9%)	58 (47.2%)	0.008
Chronic kidney disease	12 (14.6%)	18 (14.6%)	0.999
Cancer	9 (11.0%)	39 (31.7%)	0.001
Liver disease	2 (2.4%)	7 (5.7%)	0.266
Cardiomyopathy	56 (68.3%)	36 (29.3%)	0.001
Obesity	17 (20.7%)	13 (10.6%)	0.044
Infection			
Gram-negative	49 (59.8%)	59 (48.0%)	0.098
Gram-positive	47 (57.3%)	55 (44.7%)	0.077
Fungus	15 (18.3%)	26 (21.1%)	0.618
Catheter bacteraemia	44 (53.7%)	26 (21.1%)	<0.001
Surgical site infection	17 (20.7%)	32 (26.0%)	0.385
Urinary tract infection	11 (13.4%)	13 (10.6%)	0.535
Endocarditis	10 (12.2%)	0 (0%)	<0.001
Peritonitis	0 (0%)	96 (78.0%)	<0.001
Pneumonia	59 (72.0%)	38 (30.9%)	<0.001

Values are expressed as median (P25th – P75th) and absolute count (percentage).
P-values were calculated by Mann-Whitney test and Chi-square tests.
Abbreviations: SOFA, sequential organ failure assessment; APACHE, acute physiology and chronic health evaluation.

Characteristics of the *IL-1B* rs16944 polymorphism

The allelic and genotypic frequencies for *IL-1B* rs16944 polymorphism in the study population are shown in **Table 3**, which displayed missing values <5%. When we analyzed all types of surgeries together, the rs16944 polymorphism was not in HWE in the SS-group (p=0.037). Besides, the SS-group had higher frequency of rs16944 AA genotype than the SIRS-group (p=0.006). When patients were stratified by type of surgery, the rs16944 polymorphism was in HWE for cardiac surgery, except for abdominal surgery (p=0.007) (**Table 3**). Besides, the SS-group had higher frequency of rs16944 AA genotype than the SIRS-group for cardiac surgery and abdominal surgery, but the p-values did not reach the statistical significance (p=0.083 and p=0.058; respectively) (**Table 3**).

Table 3. Frequencies of alleles and genotypes for *IL1B* rs16944 polymorphism in the study population.

	G/A	All patients	SIRS-group	SS-group	p-value
All surgeries					
No.		467	262	205	
HWE (p-value)		0.500	0.280	0.037	
Alleles	G	70%	73%	68%	0.281
	A	30%	27%	32%	-
Genotypes	GG	50%	51%	49%	0.737
	AG	40%	43%	37%	0.223
	AA	10%	6%	14%	0.006
Cardiac surgery					
No.		233	151	82	
HWE (p-value)		0.550	0.340	0.999	
Alleles	G	67%	70%	62%	0.271
	A	33%	30%	38%	-
Genotypes	GG	44%	47%	38%	0.236
	AG	46%	46%	48%	0.877
	AA	10%	7%	15%	0.083
Abdominal surgery					
No.		234	111	123	
HWE (p-value)		0.090	0.610	0.007	
Alleles	G	74%	76%	72%	0.584
	A	26%	24%	28%	-
Genotypes	GG	57%	57%	57%	0.894
	AG	34%	39%	30%	0.190
	AA	9%	5%	13%	0.059

Values are expressed as absolute count and percentage. P-values were calculated by Chi-squared test.
Abbreviations: SIRS-group, patients with systemic inflammatory response syndrome; SS-group, patients with septic shock; HWE, Hardy-Weinberg equilibrium.

Association of *IL-1B* rs16944 polymorphism with septic shock and death

The relationship between *IL-1B* rs16944 polymorphism and development of septic shock under a recessive inheritance model (AA vs. GG/AG) is shown in **Table 4**. The frequency of septic shock was significantly

higher in patients with AA genotype than in patients with GG/AG when were taken into account all patients (63.6% vs 41.8%; p=0.006). However, when stratifying by type of surgery, only abdominal surgery showed significant differences (76.2% vs. 50.2%; p=0.023). On the other hand, the multivariate analysis showed that *IL-1B* rs16944 AA genotype was only significantly associated with higher likelihood of septic shock in all patients (adjusted odds ratio (aOR)=2.26; p=0.042). Other parameters, which were associated with the development of septic shock, were chronic kidney disease, cancer and emergency surgery (**Table 4**). When the population was stratified by type of surgery, the significance between rs16944 and septic shock disappeared.

Table 4. Summary of association analysis of *IL1B* rs16944 polymorphism (recessive inheritance) with development of septic shock in patients who underwent major cardiac or abdominal surgery.

	Univariate			**Multivariate**	
	GG/AG	**AA**	**p-value [(a)]**	**aOR (95%CI)**	**p-value [(b)]**
All patients (n=467)					
	41.8%	63.6%			
SS-group vs SIRS-group	(177/423)	(28/44)	0.006	2.26 (1.03; 4.97)	0.042
Cardiac surgery				0.60 (0.36; 1.01)	0.052
Chronic kidney disease				2.31 (1.04; 5.14)	0.040
Cancer				0.40 (0.23; 35.33)	0.002
Emergency surgery				20.14 (11.51; 35.33)	<0.001
Patients underwent cardiac surgery (n= 233)					
	33.3%	52.2%	0.072		
SS-group vs SIRS-group	(70/210)	(12/23)		1.96 (0.74; 5.18)	0.175
Cardiomyopathy				0.26 (0.12; 0.57)	0.001
Emergency surgery				14.09 (5.41; 36.70)	<0.001
Patients underwent abdominal surgery (n= 234)					
	50.2%	76.2%	0.023		
SS-group vs SIRS-group	(107/213)	(16/21)		1.65 (0.43; 6.41)	0.467
Gender (male)				1.79 (0.82; 3.91)	0.144
Cancer				0.26 (0.12; 0.55)	<0.001
Emergency surgery				27.23 (12.62; 58.74)	<0.001

Categorical variables are expressed in percentage (absolute count). (a) P-values were calculated by Chi-square tests. (b) P-values were calculated by logistic regression adjusting for the most important clinical and epidemiological characteristics (see **statistical analysis** section). **Abbreviations**: SIRS-group, patients with systemic inflammatory response syndrome; SS-group, patients with septic shock; aOR, adjusted odds ratio; 95%CI, 95% confidence interval; p-value, level of significance.

We also analyzed the association of *IL1B* rs16944 polymorphism with the type of infection occurred after surgery in patients with septic shock, but no significant association was found (**Supplemental Table 1**).
Table 5 shows the association between *IL-1B* rs16944 polymorphism and septic shock-related death under a recessive model of inheritance (AA vs. GG/AG). The mortality was slightly higher in patients with *IL-1B* rs16944 AA genotype than in patients with *IL-1B* rs16944 GG/AG genotype, but difference was not significant. However, the multivariate analysis showed that *IL-1B* rs16944 AA genotype was significantly associated with higher likelihood of septic shock-related death in patients (aOR=2.67; p=0.035), but significance was lost when data were stratified by type of surgery.

Table 5. Summary of association analysis of *IL1B* rs16944 polymorphism (recessive inheritance) with septic shock-related death in patients who underwent major cardiac or abdominal surgery.

	Univariate			Multivariate	
	GG/AG	**AA**	**p-value [(a)]**	**aOR (95%CI)**	**p-value [(b)]**
SS-group (n=205)					
death vs non death	53.1% (94/177)	67.9% (19/28)	0.209	2.67 (1.07; 6.65)	0.035
Emergency surgery				1.82 (0.91; 3.66)	0.092
APACHE II score				1.11 (1.04; 1.18)	0.002
Peritonitis				1.78 (0.88; 3.57)	0.105
Cardiomyopathy				1.95 (1.02; 3.77)	0.044
Cancer				1.96 (0.93; 4.13)	0.078
Patients underwent cardiac surgery (n= 82)					
death vs non-death	42.9% (30/70)	66.7% (8/12)	0.109	3.12 (0.83; 11.67)	0.091
APACHE II score				1.09 (0.98; 1.20)	0.088
Cancer				2.93 (0.65; 13.12)	0.159
Patients underwent abdominal surgery (n= 123)					
deathvs non-death	59.8% (64/107)	68.8% (11/16)	0.509	1.91 (0.54; 6.71)	0.313
Emergency surgery				3.80 (1.25; 11.52)	0.018
APACHE II score				1.14 (1.05; 1.25)	0.002
Diabetes				0.24 (0.56; 1.02)	0.053
Cardiomyopathy				3.09 (1.16; 8.25)	0.024
Cancer				2.09 (0.81; 5.42)	0.127

Categorical variables are expressed in percentage (absolute count). (a) P-values were calculated by Chi-square tests. (b) P-values were calculated by logistic regression adjusting for the most important clinical and epidemiological characteristics (see **statistical analysis** section).

Abbreviations: SS-group, patients with septic shock; APACHE, acute physiology and chronic health evaluation ; aOR, adjusted odds ratio; 95%CI, 95% confidence interval; p-value, level of significance.

DISCUSSION

In this study, we examined the influence of *IL-1B* rs16944 polymorphism on the development of septic shock and death in patients who underwent cardiac or abdominal major surgery. Our major finding was that the presence of *IL-1B* rs16944 AA genotype increased the odds of having septic shock and death in patients who underwent major surgery. However, no significant association was found when patients were stratified by the type of major surgery (cardiac or abdominal). To our knowledge, this study is the first description of the relationship of *IL-1B* rs16944 polymorphism with septic shock.

IL-1β is a pro-inflammatory cytokine, which is involved in the response to infections, inflammation and coagulation [13]. IL-1β is able to activate the vascular endothelium and the coagulation system, and to down-regulate important physiologic anticoagulant pathways [13]. This process is critical in the pathogenesis of sepsis and septic shock. Moreover, sepsis susceptibility or resistance has been related to genetic variations within this gene [27]. In our study, the presence of the minor rs16944 A allele in homozygosis was related to the onset of septic shock and death (septic shock-related death). Such relationship has been previously studied by Gupta *et al* who described a significant association of rs16944 with the development of multiple organ failure and mortality (AG genotype was predominant in non-survivors) [25]. Moreover, Gu *et al* found that rs16944 G allele is implicated in the development of sepsis and organ dysfunction in major trauma patients [31] and Wan *et al* provided evidence about the association between rs16944 GG and the susceptibility to bacteremia after kidney transplantation [24]. On the other hand, a recent meta-analysis did not detect significant association between *IL-1B* rs16944 polymorphism and sepsis risk; but heterogeneity between studies was found and authors could not explore potential sources of between-study heterogeneity by meta-regression due to the small number of included studies. In this setting, several hypothesis could explain the discrepancies found between our study and other studies previously published. On the one hand, we may have found significant associations because our target population consisted of the more critically ill patients (septic shock). On the other hand, the significance level reached in our analysis was not very high, possibly because the size of our study population was limited. In addition, it may be because the *IL-1B* rs16944 polymorphism is not directly responsible of the variations in IL1β production and the increased susceptibility to infection and sepsis. The rs16944 is located upstream of the transcription start site, and several studies have suggested that its functional role may be due to the linkage with the causal mutation on the IL1B promoter region [32-34]. Another factor to be considered is the different distribution of the A and G alleles in different populations worldwide; where, the *IL-1B* rs16944 A risk allele is less frequent in Africans and more frequent in Europeans and Asians (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=16944). This could explain the discrepancies of *IL-1B* rs16944 association between different populations.

Regarding the association with septic shock susceptibility, no previous articles have been published to our knowledge. In this regards, our results suggested that genotyping of rs16944 may help to the prediction of septic shock and death after major surgery and to orientate clinicians about who are the most vulnerable patients. However, it needs to be studied more deeply, especially its role on the development of septic shock after different types of surgery, where we failed to find any association, probably because of the limited sample size after stratifying patients by the type of surgery.

Excessive IL-1 production is directly linked to the development of shock, multi-organ system failure, and death in patients with sepsis, which may be therapeutically targeted to improve the clinical outcome of sepsis [35, 36]. Regarding to the influence of rs16944 polymorphism on IL-1β levels, several studies have showed that *IL-1B* rs16944 AA genotype was associated with: higher gastric mucosal IL-1β levels, severity of *Helicobacter pylori* infection [10], and higher susceptibility to invasive mold infection after solid-organ transplantation. Conversely, AA genotype has showed a reduced Aspergillus-induced interleukin 1β production and tumor necrosis factor α secretion by PBMCs [21]. However, there are some discrepancies as some studies did not find any genetic association of rs16944 with IL-1β levels [20], infection or sepsis [3, 23, 27, 37]. In non-European population, the rs16944 AA genotype has been related to lower risk of infection [19], and the GG genotype was associated with production of IL-1β [38] and higher risk of infection [24, 38]. Additionally, we performed an *in silico* analysis for evaluating the possible functional implication of rs16944

polymorphism by using rSNABase (http://rsnp.psych.ac.cn/) [33]. This type of analysis allows us to analyze whether the polymorphism is located within a regulatory region and therefore could have a putative transcriptional regulatory effect. The *IL-1B* rs16944 polymorphism shows an eQTL (Expression quantitative trait loci) effect, meaning that there is a functional relationship between the rs16944 polymorphism and the measured expression levels of different genes. Thus, rs16944 polymorphism could influence the expression of IL1 gene at the level of translation.

Another fact to take into consideration in our study is that *IL-1B* rs16944 polymorphism was not in HWE in patients with septic shock (including cardiac and abdominal surgery patients) and in abdominal surgery subgroup. Deviations from HWE may be due to inbreeding, population stratification or selection; but it also suggests that this disequilibrium can result from a true disease association [39]. In fact, there are some authors that use deviations from HWE as a tool for identifying susceptibility loci [39]. Thus, the deviations from HWE found in our study could be due to disease association. Moreover, other issues should be taken into account for the correct interpretation of our results. Firstly, the study design and the limited sample size might be responsible for the lack of statistical significance in some comparisons, as our results showed after stratifying patients by the type of surgery. Secondly, the rs16944 polymorphism may act synergistically with other polymorphisms, which may be all epistatic factors contributing to septic shock. Thirdly, SIRS group was selected to be similar to SS-group in age and gender; however, other clinical and epidemiological variables were not taken into account. Differences between groups could introduce some bias in the analysis and in order to correct it, the most significant clinical and epidemiological covariates were used to adjust regression models. Fourthly, the impact of some polymorphisms within genes encoding cytokines on the risk of septic shock and mortality has been previously described in the literature [40-42]. Further studies combining these polymorphisms together with rs16944 could be useful to design strategies to predict the more vulnerable patients and to prevent the occurrence of such serious event.

In conclusion, the *IL-1B* rs16944 AA genotype seems to be related to the onset of septic shock and death in patients who underwent major surgery. Our data suggest that *IL-1B* polymorphism might play a major role in pathogenesis of septic shock. Further researches are necessary to corroborate these findings.

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The authors do not have a commercial or other association that might pose a conflict of interest.

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The study was approved by the Research Ethic Committee of the Instituto de Salud Carlos III (ISCIII). This study was conducted in accordance with the Declaration of Helsinki and patients gave their written consent for the study.

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Study concept and design: MAJS, ET, and SR.
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Patients' selection and clinical data acquisition: ET, PL, MHR, and EGS.
Sample preparation, DNA isolation and genotyping: MAJS, LMM, and AFR.
Statistical analysis and interpretation of data: MAJS, LMM, and SR.
Writing of the manuscript: MAJS, LMM, and SR.
Critical revision of the manuscript for important intellectual content: LMM, AFR, and ET.
Study supervision: SR.
All authors read and approved the final manuscript.

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SUPPLEMENTAL MATERIAL

Supplemental Table 1. Association of *IL1B* rs16944 polymorphism (recessive inheritance) with the type of post-operative infection in patients with septic shock.

	Multivariate	
	aOR (95%CI)	**p-value [(b)]**
All patients (n=205)		
Gram-negative	0.87 (0.38; 2.00)	0.745
Gram-positive	0.60 (0.36; 1.01)	0.369
Fungus	1.92 (0.76; 4.89)	0.170
Catheter bacteraemia	0.91 (0.36; 2.29)	0.842
Surgical site infection	2.07 (0.86; 4.99)	0.106
Urinary tract infection	0.86 (0.23; 3.21)	0.827
Endocarditis	NA	NA
Peritonitis	0.82 (0.22; 3.04)	0.773
Pneumonia	0.97 (0.39; 2.38)	0.941
Cardiac surgery (n= 82)		
Gram-negative	1.89 (0.48; 7.43)	0.363
Gram-positive	0.51 (0.14-1.88)	0.315
Fungus	3.67 (0.70; 19.15)	0.123
Catheter bacteraemia	0.74 (0.19; 2.98)	0.676
Surgical site infection	2.40 (0.53; 10.87)	0.256
Urinary tract infection	2.12 (0.36; 12.42)	0.403
Endocarditis	NA	NA
Peritonitis	NA	NA
Pneumonia	2.45 (0.26; 22.94)	0.432
Abdominal surgery (n= 123)		
Gram-negative	0.59 (0.20; 1.76)	0.344
Gram-positive	4.35 (1.2-15.79)	0.025
Fungus	1.18 (0.32; 4.39)	0.804
Catheter bacteraemia	0.99 (0.24; 4.17)	0.994
Surgical site infection	2.22 (0.71; 7.01)	0.172
Urinary tract infection	NA	NA
Endocarditis	NA	NA
Peritonitis	0.82 (0.22; 3.04)	0.773
Pneumonia	0.65 (0.19; 2.24)	0.494

P-values were calculated by logistic regression adjusting for the most important clinical and epidemiological characteristics (see **statistical analysis** section).
Abbreviations: aOR, adjusted odds ratio; 95%CI, 95% confidence interval; p-value, level of significance; NA, not applicable (small sample size).