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Short Communication

CEACAM7 polymorphisms predict genetic predisposition to mortality in post-surgical septic shock patients

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Abstract We carried out a retrospective exploratory study on 173 patients who underwent major surgery and developed septic shock after surgery. Our findings suggest that *CEACAM7* rs1001578, rs10409040, and rs889365 polymorphisms could influence septic shock-related death in individuals who underwent major surgery.

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Introduction

The most severe stage of sepsis is septic shock, in which the underlying circulatory, cellular, and metabolic abnormalities are marked enough to increase mortality. Deaths related to sepsis and septic shock have decreased in the last decade, but they remain higher than for other pathologies. Moreover, the proper management of sepsis is based mainly on an early diagnosis and adequate treatment.[1]

Genetic polymorphisms influence the immune response that septic patients develop due to the infection, which is crucial to predict the outcome of these patients. In line with this, knowing the genetic background of these patients could help identify which of them present a high risk of dying, thus allowing the stratification and the early implementation of adequate treatments.

Carcinoembryonic antigen-related cell adhesion molecules (CEACAMs) are a group of immunoglobulin-related glycoproteins that play a role as modulators of diverse cellular processes such as differentiation, proliferation, cell adhesion, and survival.[2] *CEACAM* family genes are expressed across multiple cell types, including endothelial, epithelial, and immune cells such as macrophages and neutrophils.[3,4] The expression of CEACAMs on the host cell surface can provide a foothold for bacteria allowing pathogens to colonize and, in some cases, invade tissue and cause disease.[5] Moreover, several articles have proposed that some CEACAM family members play a role in innate immunity, particularly as pathogen receptors.[4,6,7] In a previous collaborative study carried out by our group,[8] we found *CEACAM7* gene expression in peripheral blood was directly linked to sepsis. Thus, we aimed to explore the association between *CEACAM7* SNPs and septic shock-related deaths in patients undergoing major surgery.

Methods

Study design

We performed a retrospective study between 2008 and 2012 on 173 patients recruited at the Hospital Clínico Universitario of Valladolid (Spain) who developed septic shock after undergoing major surgery. The study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee of Instituto de Salud Carlos III (PI05/09). Written informed consent was obtained from all patients before sample collection.

Demographic and clinical data were collected from medical records. Sequential Organ Failure Assessment (SOFA score) and Acute Physiology and Chronic Health Evaluation (APACHE II score) were calculated to assess the sepsis severity within the first 24 h following septic shock diagnosis.

Major surgery was defined as any surgical procedure (cardiac or abdominal) performed under general anesthesia, requiring respiratory assistance. Emergency surgery was indicated for life-threatening situations. The presence of infection was ruled out before surgery and microbiologically confirmed for all patients after surgery. The choice of empiric antibiotic treatment was made according to international guidelines and the distribution of the most common bacterial pathogens in our Intensive Care Unit. Septic shock was defined as sepsis with serum level of lactate $>$18 mg/dL ($>$2 mmol/L) and an acute circulatory failure with persistent arterial hypotension ($<$65 mmHg) despite adequate vasopressor therapy, according to the updated criteria of The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3).

CEACAM7 SNPs

CEACAM7 gene was significantly differentially expressed (fold change $>$3) in whole blood in septic patients compared to patients with no sepsis, according to a previous collaborative study carried out by our group.[8] For SNP selection, we used rSNPBase (http://rsnp.psych.ac.cn/) to filter for SNPs involved in proximal regulation regions in blood tissue (open chromatin regions, transcription factor binding sites, histone markers, or CpG regions), and selected those SNPs with a minor allelic frequency (MAF) over 15% in the European population. Before that, tagSNPs for those groups of SNPs with strong linkage disequilibrium (LD) were selected according to the pairwise r^2 LD criterion ($r^2 > 0.8$). Finally, we selected four SNPs in CEACAM7: rs1001578, rs10409040, rs59654817, and rs889365.

Genomic DNA was extracted using the High Pure PCR Template Preparation kit (Roche Diagnostics GmbH, Mannheim, Germany). Next, DNA genotyping was performed at the Spanish National Genotyping Center (CeGen; http://www.cegen.org/) using the Agena Bioscience's MassARRAY platform (San Diego, CA, USA) with the iPLEX® Gold assay design system.

Statistical analysis

Quantitative variables were expressed as the median and interquartile range (IQR), and categorical variables were shown as absolute count (percentage). Comparisons between groups were performed using the Mann—Whitney U test for continuous variables and the Chi-squared or two-tailed Fisher's exact test for categorical variables, including the Hardy—Weinberg equilibrium (HWE) for all *CEACAM7* SNPs that were analyzed.

The outcome variable was death within 28 days, which is the most appropriate for establishing sepsis-related death. Survival analyses were carried out for dominant, additive, and recessive models of inheritance. Survival probabilities were estimated by the Kaplan—Meier method, and the log-rank test assayed differences between groups. We used Cox proportional-hazards models to assess the risk of dying, adjusting by the most significant covariates with a stepwise forward selection method (pin $<$0.05 and pout $<$0.20). We included as covariates age, gender, comorbidities (hypertension, diabetes, obesity, heart disease, chronic kidney disease, chronic obstructive pulmonary disease, liver disease, and neoplasia), peritonitis, adequate antibiotic treatment, SOFA score, lactate, the urgency of surgery (scheduled or emergency) and type of surgery (cardiac or abdominal). We excluded spurious associations using multiple testing corrections with the false discovery rate (FDR)

using the Benjamini and Hochberg procedure (q-value). We considered a p-value or q-value less than 0.05 as significant.

LD was computed using Haploview 4.2 software, and statistical analysis was performed using Stata/IC 13.1 (StataCorp, Texas, USA).

Results

Patient characteristics

Baseline characteristics of septic shock patients, stratified by 28-days mortality, are shown in Supplementary Table 1. Briefly, patients who died were older, had higher percentages of chronic kidney disease, emergency surgery, peritonitis, and presented higher APACHE II scores (p-value <0.05). Additionally, patients who died developed earlier septic shock after surgery, had lower percentages of catheter-related bacteremia, cardiac surgery, and pneumonia (p-value <0.05).

Characteristics of *CEACAM7* SNPs

LD among most SNPs was high, but the maximum r^2 was 0.16 (Supplementary Figure 1). Rs59654817 and rs889365 were located in intronic regions of the *CEACAM7* gene, and the other two SNPs (rs1001578 and rs10409040) were located at the upstream of the *CEACAM7* gene. All SNPs had a MAF higher than 10% and fulfilled the HWE (p-value >0.05) (Supplementary Table 2).

Moreover, we performed a sub-analysis stratifying by the type of surgery and the presence of active neoplasm in our patients, but we did not find differences in genotype frequencies for *CEACAM7* SNPs (Supplementary Table 3).

Risk of septic shock-related death

Patients with *CEACAM7* rs10409040 CG/GG genotype (dominant model) and rs889365 AA genotype (recessive model) presented a higher mortality than patients with CC genotype (p-value = 0.019, q-value = 0.040) and GG/AG genotype (p-value = 0.020, q-value = 0.040), respectively. Furthermore, rs1001578 SNP was close to reaching statistical significance under the dominant inheritance model (p-value = 0.058, q-value = 0.077) (Table 1; Fig. 1).

Patients with rs10409040 CG/GG and rs889365 AA genotypes had a higher risk of death (adjusted hazard ratio [aHR] = 2.06 (q-value = 0.028) and aHR = 3.60 (q-value = 0.038), respectively), whereas rs1001578 CT/TT genotype was associated with a lower risk of death (aHR = 0.56 (q-value = 0.045)) (Table 1).

Discussion

Our study points out for the first time the relationship between *CEACAM7* SNPs and septic shock-related death. We found *CEACAM7* polymorphisms were linked to septic shock-related death. In this regard, rs10409040 CG/GG and rs889365 AA genotypes were associated with higher mortality, whereas rs1001578 CT/TT genotype was linked to increased survival.

CEACAM7 encodes a protein related to the immunoglobulin superfamily that regulates normal cellular differentiation and is mainly expressed in epithelial cells, especially pancreatic ducts and colorectal epithelial cells. The physiological function of CEACAM7 is not fully understood. However, as described in the Genotype-Tissue Expression Portal (GTEx Portal, https://gtexportal.org/), *CEACAM7* SNPs evaluated in this study seem to have an impact on the gene expression regulation of several *CEACAMs*, not only *CEACAM7*. In this setting, rs10409040 is located in an upstream region of several *CEACAMs* genes, such as *CEACAM4* and *CEACAM7*, being rs10409040 CG/GG genotypes linked to a lower *CEACAM4* expression in blood. CEACAM4 is a neutrophil receptor that triggers phagocytosis of bacteria,[4] and it has been reported that *CEACAM4* is under-expressed in patients with sepsis compared to non-septic patients. A decrease in *CEACAM4* expression levels in septic patients could lead to worse

Table 1 Survival probabilities (Kaplan–Meier product-limit method) and risk of death in septic shock patients who underwent major cardiac or abdominal surgery according to *CEACAM7* SNPs.

CEACAM7 SNPs				Kaplan–Meier estimation			Cox regression		
SNP	Model	Genotype	N	Deaths	*p*-value	*q*-value*	aHR (95%CI)	*p*-value	*q*-value*
rs1001578	Dominant	CC	90	37 (41.1%)	0.058	0.077	0.56	**0.034**	**0.045**
		CT/TT	83	23 (27.7%)			(0.33–0.96)		
rs10409040	Dominant	CC	91	24 (26.4%)	**0.019**	**0.040**	2.06	**0.007**	**0.028**
		CG/GG	82	36 (43.9%)			(1.21–3.49)		
rs59654817	Dominant	GG	81	29 (35.8%)	0.865	0.865	1.21	0.501	0.501
		GA/AA	92	31 (33.7%)			(0.70–2.08)		
rs889365	Recessive	GG/AG	168	56 (33.3%)	**0.020**	**0.040**	3.60	**0.019**	**0.038**
		AA	5	4 (80.0%)			(1.24–10.48)		

Statistics: Values are expressed as absolute count and percentage, and adjusted hazard ratio and 95% confidence interval. Cox regression analysis was adjusted by the most significant clinical, epidemiological and genetic characteristics (see statistical analysis section) (*), p-values were corrected for multiple testing using the false discovery rate (FDR) with Benjamini and Hochberg procedure (q-value). Significant differences are shown in bold.

Abbreviations: CEACAM7: Carcinoembryonic antigen-related cell adhesion molecule 7; aHR: adjusted hazard ratio; 95%CI: 95% confidence interval; p-value: level of significance; SNP: single nucleotide polymorphism.

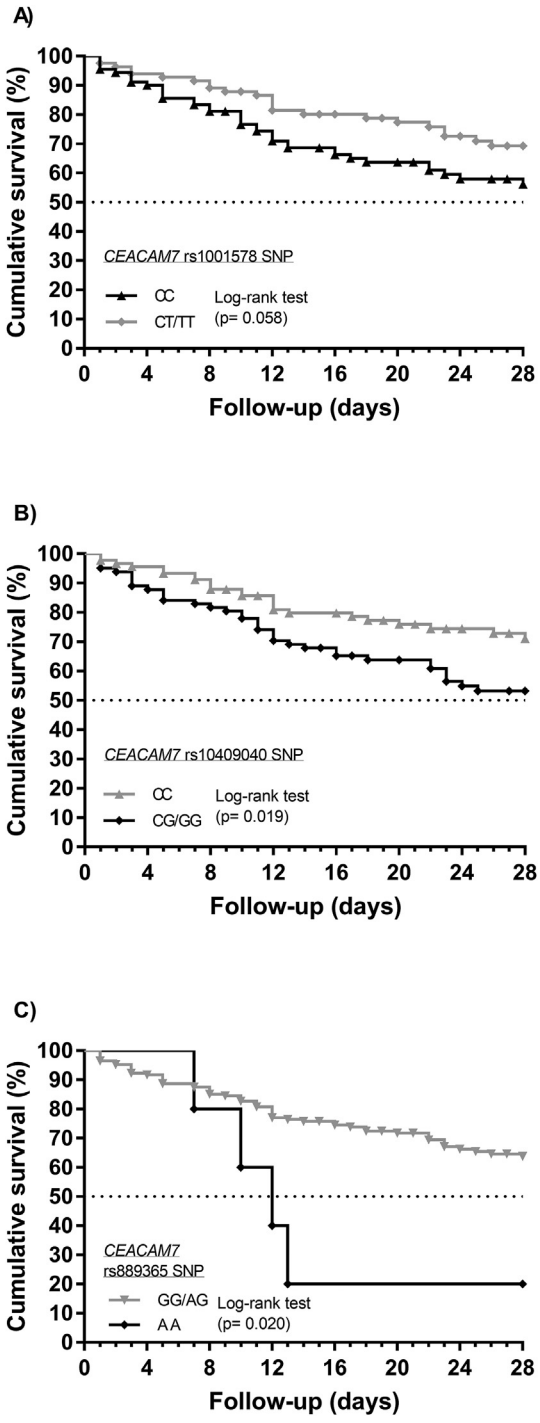

Figure 1. Survival analysis (Kaplan—Meier curve) regarding *CEACAM7* polymorphisms in septic shock patients who underwent major surgery. A) *CEACAM7* rs1001578 polymorphism; B) *CEACAM7* rs10409040 polymorphism; C) *CEACAM7* rs889365 polymorphism. **Statistics:** P-value was calculated by log-rank test. **Abbreviations:** CEACAM7, carcinoembryonic antigen-related cell adhesion molecule 7; SNP, single nucleotide polymorphism.

infection control, explaining higher mortality in patients with rs10409040 CG/GG genotypes.[9] On the other hand, rs1001578 CT/TT genotype is related to a lower *CEACAM7* expression in esophagus mucosa and lower *CEACAM6* expression in the blood (https://genenetwork.nl/bloodeqtlbrowser/),[10] which has been described as a pathogen receptor that mediates cellular adhesion and invasion.[7] In line with this, a decrease in *CEACAM6* expression in blood could decrease pathogenicity by reducing the docking sites for bacteria. Finally, the rs889365AA genotype is related to *CEACAM7* overexpression in esophagus mucosa. Although CEACAM7 had not yet been described as a receptor for pathogens, this role has been suggested for SARS-CoV-2.[6] Inversely to rs1001578, an increase in the mucosal docking sites for pathogens could increase the pathogenicity in septic patients.[2] However, although our hypotheses are supported by two large consensus databases that provide information about gene expression and regulation (https://gtexportal.org/ and https://genenetwork.nl/bloodeqtlbrowser/),[10] additional studies that analyze *CEACAM7* polymorphisms together with levels of expression of *CEACAM4*, *CEACAM6*, and *CEACAM7* in patients with septic shock would be needed to confirm these hypotheses.

Since some members of the CEACAM family play a key role in innate immunity,[4,6,7] it would be very interesting to extend the study of these polymorphisms to other pathologies. Both *CEACAM7* polymorphisms and gene expression levels of *CEACAM4*, *CEACAM6*, and *CEACAM7* could influence other inflammatory sterile pathologies such as autoimmune diseases. Another possibility would be to study its impact in the context of acute viral infections that present with clinical courses similar to sepsis, such as COVID-19 or influenza.

Our study presents several limitations. First, this is a retrospective study performed in one center, and we only included patients who underwent major surgery. Consequently, our conclusions could not be generalized to other kind of septic patients. Second, this study has an exploratory nature since the statistical power of our study could be limited due to the restricted sample size. Finally, the functional role of *CEACAM7* in immunity and the functional effects of selected SNPs are only partially understood; however, we did not have samples available for a RNA-seq study and to explore the potential role of the *CEACAM7* SNPs on gene expression.

Our preliminary study suggests that *CEACAM7* rs1001578, rs10409040, and rs889365 polymorphisms could influence septic shock-related death in individuals who underwent major surgery. However, more studies with larger sample sizes are needed to confirm our findings.

Ethics approval and consent to participate

This study was conducted following the Declaration of Helsinki. The Ethics Committee of Instituto de Salud Carlos III (PI05/09) approved the study. Written informed consent was provided by all participants before sample collection.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study may be available from the corresponding author upon reasonable request.

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Author contributions

Funding body, ET and SR. Study concept and design: MAJS, ET and SR. Patients' selection and clinical data acquisition: EGS, HGB, MLL, MHR, EGP, PMDP and ET. Sample preparation, DNA isolation and genotyping: MAJS. Statistical analysis and interpretation of data: FPG, MAJS and SR. Writing of the manuscript: FPG, MAJS and SR. Critical revision of the manuscript for relevant intellectual content: AFR and ET. Supervision and visualization: SR.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jmii.2021.09.006.