CTLA-4 Genetic Variants Predict Survival in Patients with Sepsis

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Abstract: Cytotoxic T lymphocyte-associated protein 4 (CTLA-4) is a coinhibitory checkpoint protein expressed on the surface of T cells. A recent study by our working group revealed that the rs231775 single nucleotide polymorphism (SNP) in the CTLA-4 gene was associated with the survival of patients with sepsis and served as an independent prognostic variable. To further investigate the impact of CTLA-4 genetic variants on sepsis survival, we examined the effect of two functional SNPs, CTLA-4 rs733618 and CTLA-4 rs3087243, and inferred haplotypes, on the survival of 644 prospectively enrolled septic patients. Kaplan–Meier survival analysis revealed significantly lower 90-day mortality for rs3087243 G allele carriers (n = 502) than for AA-homozygous (n = 142) patients (27.3% vs. 40.8%, p = 0.0024). Likewise, lower 90-day mortality was observed for TAA haplotype-negative patients (n = 197; compound rs733618 T/rs231775 A/rs3087243 A) than for patients carrying the TAA haplotype (n = 447; 24.4% vs. 32.9%, p = 0.0265). Carrying the rs3087243 G allele hazard ratio (HR): 0.667; 95% confidence interval (CI): 0.489–0.909; p = 0.0103) or not carrying the TAA haplotype (HR: 0.685; 95% CI: 0.491–0.956; p = 0.0262) remained significant covariates for 90-day survival in the multivariate Cox regression analysis and thus served as independent prognostic variables. In conclusion, our findings underscore the significance of CTLA-4 genetic variants as predictors of survival of patients with sepsis.

Keywords: sepsis; CTLA-4; single nucleotide polymorphisms; haplotypes; survival; predictors
1. Introduction

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [1] and remains a leading cause of death and critical illness worldwide [2]. The reported incidence of sepsis is increasing in aging populations with more comorbidities [1,3,4] and is accompanied by rising health care costs. The multifaceted pathophysiology of sepsis and sepsis-related organ dysfunction is highly diverse and therefore has been intensively investigated in the recent past [5]. Host genetic characteristics, such as gene variants encoding innate immune effectors or inflammatory mediators, seem to contribute considerably to the disease course and sepsis outcome [6–9]. The genetic component of host susceptibility to sepsis is clearly polygenetic [10], and identification of responsible variants will help elucidate the pathophysiology and biology of the disease. Identifying sepsis-associated genetic variants and evaluating their impacts on gene expression, protein function and host physiology will potentially reveal future targets for sepsis treatment [11].

CTLA-4 has gained recognition since James P. Allison and Tasuku Honjo were awarded the Nobel Prize for Physiology or Medicine in 2018 for their discovery of cancer therapy by inhibition of negative immune regulation [12]. CTLA-4 is a coinhibitory cell surface protein expressed on T cells that competes with CD28 for binding to CD80 and CD86 on antigen-presenting cells (APCs) [13,14] and thereby reduces T cell activation and proliferation [15]. Thus, an increased CTLA-4 expression has an inhibitory effect on the host immune reaction [16].

Coinhibitory immune checkpoint proteins, such as PD-1 and CTLA-4, have been reported to play key roles in the course of sepsis [13,17–19], and our working group has revealed genetic associations of their genes with the survival of septic patients [9,20]. Our recent study revealed that the CTLA-4 rs231775 GG genotype, which was reported to have an attenuated inhibitory effect on T cell reactions [21] and was associated with greater T cell activation and proliferation and autoimmune diseases [22], was also associated with favorable 90- and 28-day survival in septic patients compared to the survival rates of A allele carriers at this position [20].

The CTLA-4 gene is located on chromosome 2q33 and contains two further functional polymorphisms, the CTLA-4 rs733618 and CTLA-4 rs3087243, which are associated with enhanced T cell reactions and higher susceptibility to autoimmune diseases, such as myasthenia gravis [23], systemic lupus erythematosus [24,25], Hashimoto thyroiditis [26], latent autoimmune diabetes [27], multiple sclerosis [28] and acute liver transplant rejection [29]. This study aimed to investigate the impact of these two functional single nucleotide polymorphisms (SNPs) and inferred haplotypes compound of rs733618, rs231775, and rs3087243 on the 90-day mortality of septic patients in order to further reveal the role of CTLA-4 genetic variants in sepsis.

2. Methods

2.1. Patients

The present study included 644 prospectively enrolled adult Caucasian patients with sepsis. Patients were recruited from three surgical intensive care units (ICUs) through the GENOSEP database of the Department of Anesthesiology at the University Medical Center Goettingen [20]. Enrolled patients were identified by daily screening of the three ICUs according to the actual sepsis definitions and guidelines [1,30]. The exclusion criteria were defined in previous studies [20,31] and were an age below 18 years, pregnancy, therapy with immunosuppressive drugs or chemotherapy, myocardial infarction within six weeks before enrollment, human immunodeficiency virus (HIV) infection, chronic heart failure classified as New York Heart Association (NYHA) stage IV, end-stage incurable disease, a persistent vegetative state (apallic syndrome), and a “Do Not Treat” (DNT) or “Do Not Resuscitate” (DNR) order. Upon enrollment, blood was drawn within 72 h, and the patients were followed up for 90 days unless previously dismissed or deceased.

This investigation and the experimental protocols were approved under ethical project identification code 15/1/12 by the institutional ethics committee of the University of Goettingen in
Goettingen, Germany. The study was performed in accordance with the provisions of the Declaration of Helsinki and relevant guidelines and regulations. The methods were carried out in accordance with approved guidelines. Written informed consent was obtained from either the patient or their legal representative.

2.2. Data Collection

All patient data were recorded through the GENOSEP database. At enrollment, relevant baseline characteristics were obtained, such as comorbidities, preexisting medication, and the initial Sequential Organ Failure Assessment (SOFA) and Acute Physiology and Chronic Health Evaluation (APACHE II) scores. Patients were followed up for 90 days, and 90-day mortality was recorded as the primary outcome parameter. Within the first 28 days after sepsis onset, relevant clinical data were generated through a standardized clinical report form (CRF) on a daily basis. The data were collected from the electronic patient record system (Intellispace Critical Care and Anesthesia (ICCA), Phillips Healthcare, Andover, MA, USA). Parameters included SOFA score-relevant organ dysfunction variables; information about organ support requirements; and inflammatory, kidney and liver values.

2.3. CTLA-4 Genotyping and Haplotyping

DNA extraction, CTLA-4 rs733618 and rs3087243 genotyping, and haplotyping were performed entirely in the laboratories of the Department of Clinical Pharmacology of the University Medical Center Goettingen. The DNA extraction and genotyping methods were performed as previously described [20]. DNA was automatically extracted from either 200 µL of ethylene diamine tetraacetic acid (EDTA) blood using the QIAamp® DNA Blood Kit in the QIAcube®, 350 µL of EDTA blood using the EZ1® DNA Blood Kit in the BioRobot EZ1®, or peripheral blood mononuclear cells (PBMCs) using the AllPrep DNA Mini Kit according to the manufacturer’s instructions (all from Qiagen, Hilden, Germany). The quantity and quality of the DNA were determined by spectrophotometric measurement. Genotyping of the extracted DNA was performed via TaqMan polymerase chain reaction (PCR) using the predesigned TaqMan® SNP Genotyping Assays C_2415791_10 and C_3296043_10 and the 7900HT Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) according to the manufacturer’s instructions (Life Technologies, Darmstadt, Germany). The genotyping outcomes were generated using the 7900HT Fast Real-Time PCR System Software (SDS v2.4.1 for Windows 7, Applied Biosystems, Foster City, CA, USA). Twenty percent of the samples were genotyped in duplicate for reliability assessment and yielded concordant results.

The CTLA-4 haplotypes were inferred using the PHASE software (Matthew Stephens, Washington, DC, USA, version 2.1), which is a Bayesian statistical method that is used to reconstruct haplotypes from population genotype data [32,33]. CTLA-4 haplotype reconstruction was performed in 100 iterations to yield the most likely haplotype combination (best pair) for each individual, with a minimum probability for the most likely combination of 99.9%.

2.4. Statistical Analyses

The data analysis was performed using the STATISTICA 13 software (version 13.0, StatSoft, Tulsa, OK, USA). The patient baseline characteristics and disease severity data were evaluated in univariate analyses with Pearson’s Chi-square test, a two-sided Fisher’s exact test, the Mann–Whitney U test or the Kruskal-Wallis test, if applicable. The Kaplan–Meier time-to-event data were compared with the log-rank test. Multivariate Cox regression analyses were used to adjust for possible effect confounders. The appropriateness of the genotype distributions and allele frequencies of the study population was evaluated with a Chi-square test of the Hardy–Weinberg equilibrium. The CTLA-4 rs733618 CC and CT genotypes, as well as the rs3087243 GG and AG genotypes, were pooled to compare the clinical course of the functionally relevant rs733618 homozygous TT (n = 550) with C allele carriers (n = 94) and the rs3087243 G allele carriers (n = 502) with homozygous AA (n = 142) patients.
A *p*-value <0.05 was considered statistically significant. Data are presented as absolute numbers or percentages for categorical variables and the mean ± standard deviation for continuous variables.

2.5. Data Availability

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

3. Results

3.1. Baseline Characteristics

Our cohort of 644 prospectively enrolled adult Caucasian patients with sepsis, which was previously described [20], was examined in the present study. The average age was 63 ± 15 years; 66% were of male gender; and the average SOFA and APACHE II scores were 9.4 ± 3.9 and 22 ± 7, respectively, upon enrollment. A total of 93% of the patients were mechanically ventilated, 79% received vasopressor therapy, 21% received renal replacement therapy, and 51% were in septic shock during the observation period (Tables 1 and 2).

**Table 1.** Patient baseline characteristics with regard to the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) rs3087243 genotypes.

| Parameter                        | All (n = 644) | rs3087243          | p-Value |
|----------------------------------|--------------|--------------------|---------|
|                                  |              | AA (n = 142)       | GG/AG (n = 502) |
| Age (years)                      | 63 ± 15      | 67 ± 14            | 62 ± 15 | 0.0033 |
| Male (%)                         | 66           | 64                 | 67      | 0.5560 |
| Body mass index                  | 28 ± 6       | 27 ± 6             | 28 ± 6  | 0.2881 |
| Sepsis severity                  |              |                    |         |
| Septic shock (%)                 | 51           | 52                 | 51      | 0.8467 |
| SOFA score day 1                 | 9.4 ± 3.9    | 9.4 ± 3.9          | 9.4 ± 3.9 | 0.8047 |
| APACHE II score day 1            | 22 ± 7       | 22 ± 7             | 21 ± 7  | 0.2844 |
| Comorbidities, n (%)             |              |                    |         |
| Hypertension                     | 54           | 53                 | 55      | 0.7094 |
| History of myocardial infarction | 5            | 6                  | 5       | 0.9057 |
| Chronic obstructive pulmonary disease | 15       | 12                 | 16      | 0.2436 |
| Renal dysfunction                | 10           | 8                  | 11      | 0.3879 |
| Non-insulin-dependent diabetes mellitus | 9       | 8                  | 9       | 0.8492 |
| Insulin-dependent diabetes mellitus | 11       | 12                 | 10      | 0.5349 |
| Chronic liver disease            | 6            | 6                  | 6       | 0.9875 |
| History of cancer                | 16           | 15                 | 16      | 0.7830 |
| History of stroke                | 6            | 9                  | 5       | 0.0623 |
| Recent surgical history, n (%)   |              |                    |         |
| Elective surgery                 | 29           | 37                 | 27      |        |
| Emergency surgery                | 53           | 46                 | 55      |        |
| No history of surgery            | 18           | 17                 | 18      |        |
| Site of infection, n (%)         |              |                    |         |
| Lung                             | 62           | 64                 | 61      |        |
| Abdomen                          | 20           | 20                 | 20      |        |
| Bone or soft tissue              | 4            | 3                  | 4       |        |
| Surgical wound                   | 2            | 2                  | 1       |        |
| Urogenital                       | 2            | 1                  | 3       |        |
| Primary bacteremia               | 7            | 6                  | 7       |        |
| Other                            | 3            | 4                  | 4       |        |
Table 1. Cont.

| Parameter                     | All (n = 644) | rs3087243 | p-Value |
|-------------------------------|--------------|-----------|---------|
|                               | AA (n = 142) | GG/AG (n = 502) |         |
| Organ support (%)             |              |           |         |
| Used during observation period|              |           |         |
| Mechanical ventilation        | 93           | 96        | 93      | 0.1850  |
| Use of vasopressor            | 79           | 82        | 78      | 0.4063  |
| Renal replacement therapy     | 21           | 20        | 21      | 0.7173  |
| Used on sepsis onset          |              |           |         |
| Mechanical ventilation        | 86           | 86        | 86      | 0.9381  |
| Use of vasopressor            | 67           | 66        | 67      | 0.9048  |
| Renal replacement therapy     | 9            | 9         | 9       | 0.8851  |

SOFA, sequential organ failure assessment; APACHE, acute physiology and chronic health evaluation.

Table 2. Patient baseline characteristics with regard to the CTLA-4 haplotype H1: TAA.

| Parameter                     | All (n = 644) | H1: TAA | p-Value |
|-------------------------------|--------------|---------|---------|
|                               | H1: TAA      | p-Value |
|                               | Positive (n = 447) | Negative (n = 197) |         |
| Age (years)                   | 63 ± 15      | 64 ± 15 | 62 ± 14 | 0.1014  |
| Male (%)                      | 66           | 67      | 64      | 0.4356  |
| Body mass index               | 28 ± 6       | 28 ± 6  | 28 ± 7  | 0.2388  |
| Septic shock (%)              | 51           | 51      | 53      | 0.6384  |
| SOFA score day 1              | 9.4 ± 3.9    | 9.3 ± 3.9 | 9.7 ± 3.8 | 0.1802  |
| APACHE II score day 1         | 22 ± 7       | 21 ± 7  | 22 ± 7  | 0.1474  |
| Comorbidities, n (%)          |              |         |         |
| Hypertension                  | 54           | 54      | 54      | 0.9671  |
| History of myocardial infarction | 5          | 6       | 3       | 0.0758  |
| Chronic obstructive pulmonary disease | 15         | 15      | 16      | 0.5779  |
| Renal dysfunction             | 10           | 10      | 11      | 0.6734  |
| Non-insulin-dependent diabetes mellitus | 9        | 9       | 8       | 0.6654  |
| Insulin-dependent diabetes mellitus | 11         | 9       | 13      | 0.1480  |
| Chronic liver disease         | 6            | 6       | 8       | 0.3893  |
| History of cancer             | 16           | 12      | 17      | 0.0731  |
| History of stroke             | 6            | 6       | 6       | 0.8209  |
| Recent surgical history, n (%)|              |         |         |
| Elective surgery              | 29           | 31      | 26      |         |
| Emergency surgery             | 53           | 52      | 54      |         |
| No history of surgery         | 18           | 17      | 20      |         |
| Site of infection, n (%)      |              |         |         |
| Lung                          | 62           | 64      | 58      |         |
| Abdomen                       | 20           | 19      | 21      |         |
| Bone or soft tissue           | 4            | 3       | 6       |         |
| Surgical wound                | 2            | 2       | 2       |         |
| Urogenital                    | 2            | 2       | 3       |         |
| Primary bacteremia            | 7            | 7       | 6       |         |
| Other                         | 3            | 3       | 4       |         |
| Organ support (%)             |              |         |         |
| Used during observation period|              |         |         |
| Mechanical ventilation        | 93           | 93      | 93      | 0.9580  |
| Use of vasopressor            | 79           | 79      | 80      | 0.6749  |
| Renal replacement therapy     | 21           | 18      | 26      | 0.0204  |
| Used on sepsis onset          |              |         |         |
| Mechanical ventilation        | 86           | 86      | 85      | 0.8341  |
| Use of vasopressor            | 67           | 66      | 69      | 0.4944  |
| Renal replacement therapy     | 9            | 6       | 15      | 0.0005  |
All patients were successfully genotyped, and the relevant haplotypes were inferred. The CTLA-4 rs733618 and rs3087243 genotype distributions were 490:550 (CC:CT:TT) and 142:305:197 (AA:AG:GG), with observed minor allele frequencies (MAFs) of 0.08 and 0.47, respectively. The observed frequencies were consistent with the Hardy–Weinberg equilibrium ($\chi^2$ test $p = 0.8789$ and $p = 0.2448$) and almost equaled the reported MAFs from reference populations, such as HapMap CEPH (CEU) with MAFs of 0.06 and 0.46 respectively (dbSNP database, accession numbers ss79540 [34] and ss44315036 [35]). The CTLA-4 haplotype analysis compound of the rs733618, rs231775, and rs3087243 SNPs revealed four common haplotypes (H1: TAA, H2: TGG, H3: TAG, and H4: CGG) (Figure 1).

**Figure 1.** Compound cytotoxic T lymphocyte-associated protein 4 (CTLA-4) haplotypes. SNP, single nucleotide polymorphism.

The corresponding haplotype distributions can be obtained from Table 3. Linkage disequilibrium (LD) analysis of the three SNPs revealed a squared correlation coefficient ($r^2$) of 0.39 for rs231775 and rs3087243, 0.23 for rs733618 and rs231775, and 0.09 for rs733618 and rs3087243.

**Table 3.** CTLA-4 haplotype distributions.

| Haplotype CTLA-4-1772 T>C/+49 A>G/+6230 G>A | Number of Haplotypes | $n$ (%) |
|--------------------------------------------|----------------------|--------|
| H1: TAA                                    | 0                    | 197 (30.59) |
|                                            | 1                    | 305 (47.36) |
|                                            | 2                    | 142 (22.05) |
| H2: TGG                                    | 0                    | 316 (49.07) |
|                                            | 1                    | 263 (40.84) |
|                                            | 2                    | 65 (10.09)  |
| H3: TAG                                    | 0                    | 451 (70.03) |
|                                            | 1                    | 178 (27.64) |
|                                            | 2                    | 15 (2.33)   |
| H4: CGG                                    | 0                    | 550 (85.40) |
|                                            | 1                    | 90 (13.98)  |
|                                            | 2                    | 4 (0.62)    |

Analysis of the baseline characteristics with regard to the genotype distributions revealed a significant difference in age between rs3087243 G allele carriers ($n = 502$) and AA homozygous patients ($n = 142$) at sepsis onset (62 ± 15 vs. 67 ± 14 years; $p = 0.0033$; Table 1). In addition, noncarriers of the inferred haplotype H1: TAA (TAA-negative, $n = 197$) received significantly more renal replacement therapy upon sepsis onset (15% vs. 6%, $p = 0.0005$; Table 2) as well as over the observation period (26% vs. 18%; $p = 0.0204$; Table 2) than carriers of the H1: TAA haplotype (TAA-positive, $n = 447$). These significant differences in patient baseline characteristics were adjusted by their inclusion as potential confounders in the Cox regression analysis.

### 3.2. Outcomes

Although Kaplan–Meier analysis of 90- and 28-day survival revealed no significant differences between the CTLA-4 rs733618 TT homozygous and C allele carriers ($p = 0.6864$ and $p = 0.4693$),
significant differences in survival were observed between the CTLA-4 rs3087243 G allele carriers and the AA-homozygous patients. CTLA-4 rs3087243 G allele carriers showed significantly lower 90-day mortality (27.3% vs. 40.8%, respectively; \( p = 0.0024 \); Figure 2) and 28-day mortality (17.9% vs. 27.5%; \( p = 0.0161 \); Figure 3) than AA homozygous patients.

Likewise, Kaplan–Meier survival analysis revealed significantly lower 90-day mortality (24.4% vs. 32.9%; \( p = 0.0265 \); Figure 4) and 28-day mortality (14.7% vs. 22.4%; \( p = 0.0271 \); Figure 5) in TAA-negative patients compared to that of patients carrying the H1: TAA haplotype.
renal replacement therapy upon sepsis onset and during the observation period was included in accordance with findings from our previous study [36]. Application of baseline variables age, male gender, body mass index (BMI), and SOFA and APACHE II scores, statin characteristics and other potential confounders on 90- and 28-day mortality. In addition to the genotypes and the H1: TAA haplotype were performed to adjust for possible effects of baseline characteristics.

### 3.3. Cox Regression Analysis

Univariate and multivariate Cox regression analyses with regard to the CTLA-4 rs3087243 genotypes and the H1: TAA haplotype were performed to adjust for possible effects of baseline characteristics and other potential confounders on 90- and 28-day mortality. In addition to the baseline variables age, male gender, body mass index (BMI), and SOFA and APACHE II scores, statin therapy was included in accordance with findings from our previous study [36]. Application of renal replacement therapy upon sepsis onset and during the observation period was included in the Cox regression analysis for the H1: TAA haplotype based on our findings in the patient baseline

#### Figure 4. Kaplan–Meier survival analysis (90 days) with regard to the CTLA-4 haplotype H1: TAA.

#### Figure 5. Kaplan–Meier survival analysis (28 days) with regard to the CTLA-4 haplotype H1: TAA.

No other significant differences in mortality were observed for the remaining inferred haplotypes (H2: TGG, H3: TAG, and H4: CGG).

### 3.3. Cox Regression Analysis

Univariate and multivariate Cox regression analyses with regard to the CTLA-4 rs3087243 genotypes and the H1: TAA haplotype were performed to adjust for possible effects of baseline characteristics and other potential confounders on 90- and 28-day mortality. In addition to the baseline variables age, male gender, body mass index (BMI), and SOFA and APACHE II scores, statin therapy was included in accordance with findings from our previous study [36]. Application of renal replacement therapy upon sepsis onset and during the observation period was included in the Cox regression analysis for the H1: TAA haplotype based on our findings in the patient baseline
characteristics. Multivariate Cox regression analyses revealed that carrying the CTLA-4 rs3087243 G allele was a significant positive predictor for 90-day survival (hazard ratio: 0.667; 95% confidence interval (CI): 0.489–0.909; \( p = 0.0103 \); Table 4) and 28-day survival (hazard ratio: 0.676; 95% CI: 0.463–0.987; \( p = 0.0423 \); Table 4), as was not carrying the H1:TAA haplotype (for 90-day survival, hazard ratio: 0.685; 95% CI: 0.491–0.956; \( p = 0.0262 \); for 28-day survival, hazard ratio: 0.621; 95% CI: 0.407–0.947; \( p = 0.0270 \); Table 5).

### Table 4. Cox regression analysis with regard to the CTLA-4 rs3087243 genotypes.

|                      | Univariate Analysis | Multivariate Analysis |
|----------------------|---------------------|-----------------------|
|                      | 90 Days             |                       |
|                      | Hazard Ratio | 95% CI | p-Value | Hazard Ratio | 95% CI | p-Value |
| Age                  | 1.032       | 1.020–1.043 | 0.000000 | 1.025       | 1.013–1.038 | 0.000047 |
| Male gender          | 1.093       | 0.810–1.474 | 0.558922 | 1.076       | 0.796–1.454 | 0.633436 |
| Body mass index      | 0.985       | 0.961–1.009 | 0.218173 | 0.979       | 0.954–1.005 | 0.109574 |
| SOFA score day 1     | 1.104       | 1.065–1.143 | 0.000000 | 1.072       | 1.025–1.122 | 0.002432 |
| APACHE II score day 1| 1.069       | 1.046–1.092 | 0.000000 | 1.033       | 1.004–1.062 | 0.026207 |
| Statin therapy       | 1.114       | 0.807–1.536 | 0.511644 | 0.979       | 0.702–1.366 | 0.901706 |
| CTLA-4 rs3087243 G allele | 0.612 | 0.450–0.833 | 0.001760 | 0.667 | 0.489–0.909 | 0.010307 |

|                      | Univariate Analysis | Multivariate Analysis |
|----------------------|---------------------|-----------------------|
|                      | 28 Days             |                       |
|                      | Hazard Ratio | 95% CI | p-Value | Hazard Ratio | 95% CI | p-Value |
| Age                  | 1.029       | 1.015–1.043 | 0.000035 | 1.023       | 1.009–1.039 | 0.001824 |
| Male gender          | 1.278       | 0.875–1.866 | 0.204154 | 1.270       | 0.868–1.860 | 0.218759 |
| Body mass index      | 0.982       | 0.953–1.013 | 0.252686 | 0.976       | 0.945–1.008 | 0.135751 |
| SOFA score day 1     | 1.119       | 1.071–1.169 | 0.000000 | 1.085       | 1.026–1.146 | 0.003854 |
| APACHE II score day 1| 1.073       | 1.045–1.102 | 0.000000 | 1.033       | 0.998–1.070 | 0.063787 |
| Statin therapy       | 0.964       | 0.641–1.451 | 0.861906 | 0.841       | 0.552–1.281 | 0.420505 |
| CTLA-4 rs3087243 G allele | 0.624 | 0.428–0.908 | 0.013778 | 0.676 | 0.463–0.987 | 0.042323 |

### Table 5. Cox regression analysis with regard to the CTLA-4 haplotype H1: TAA.

|                      | Univariate Analysis | Multivariate Analysis |
|----------------------|---------------------|-----------------------|
|                      | 90 Days             |                       |
|                      | Hazard Ratio | 95% CI | p-Value | Hazard Ratio | 95% CI | p-Value |
| Age                  | 1.032       | 1.020–1.043 | 0.000000 | 1.025       | 1.013–1.038 | 0.000047 |
| Male gender          | 1.093       | 0.810–1.474 | 0.558922 | 1.076       | 0.796–1.454 | 0.633436 |
| BMI                  | 0.985       | 0.961–1.009 | 0.218173 | 0.979       | 0.954–1.005 | 0.109574 |
| SOFA score day 1     | 1.104       | 1.065–1.143 | 0.000000 | 1.072       | 1.025–1.122 | 0.002432 |
| APACHE II score day 1| 1.069       | 1.046–1.092 | 0.000000 | 1.033       | 1.004–1.062 | 0.026207 |
| Statin therapy       | 1.114       | 0.807–1.536 | 0.511644 | 0.979       | 0.702–1.366 | 0.901706 |
| Renal replacement therapy during observation period | 2.854 | 2.134–3.817 | 0.000000 | 2.724 | 1.867–3.974 | 0.000000 |
| Renal replacement therapy upon sepsis onset | 1.565 | 1.021–2.399 | 0.040064 | 1.973 | 1.192–3.268 | 0.008241 |
| H1: TAA negative     | 0.698       | 0.504–0.967 | 0.030812 | 0.685       | 0.491–0.956 | 0.026202 |

|                      | Univariate Analysis | Multivariate Analysis |
|----------------------|---------------------|-----------------------|
|                      | 28 Days             |                       |
|                      | Hazard Ratio | 95% CI | p-Value | Hazard Ratio | 95% CI | p-Value |
| Age                  | 1.029       | 1.015–1.043 | 0.000035 | 1.023       | 1.009–1.039 | 0.001824 |
| Male gender          | 1.278       | 0.875–1.866 | 0.204154 | 1.270       | 0.868–1.860 | 0.218759 |
| BMI                  | 0.982       | 0.961–1.009 | 0.218173 | 0.979       | 0.945–1.008 | 0.135751 |
| SOFA score day 1     | 1.104       | 1.065–1.143 | 0.000000 | 1.085       | 1.026–1.146 | 0.003854 |
| APACHE II score day 1| 1.073       | 1.045–1.102 | 0.000000 | 1.033       | 0.998–1.070 | 0.063787 |
| Statin therapy       | 0.964       | 0.641–1.451 | 0.861906 | 0.841       | 0.552–1.281 | 0.420505 |
| Renal replacement therapy during observation period | 3.028 | 2.131–4.301 | 0.000000 | 2.772 | 1.769–4.343 | 0.000009 |
| Renal replacement therapy upon sepsis onset | 1.616 | 0.970–2.691 | 0.065366 | 1.933 | 1.060–3.524 | 0.031464 |
| H1: TAA negative     | 0.632       | 0.418–0.955 | 0.029340 | 0.621       | 0.407–0.947 | 0.027008 |
3.4. Disease Severity

The average SOFA and organ-specific SOFA subscores, as well as inflammatory, kidney, and liver parameters were included in the disease severity analysis (Tables 6 and 7). Assessment of disease severity revealed that CTLA-4 rs3087243 G allele carriers had significantly lower average SOFA-Central Nervous System (SOFA-CNS) scores (1.9 ± 1.1 vs. 2.2 ± 1.0; p = 0.0094) over the observation period and a lower percentage of ventilated days as a fraction of observation days (65 ± 32% vs. 71 ± 32%; p = 0.0280) than AA homozygous patients.

The H1: TAA noncarriers showed highly significant higher average procalcitonin levels (5.1 ± 9.7 ng/dL vs. 3.5 ± 9.1 ng/dL; p = 0.0002) as well as significantly lower urine outputs (2792 ± 1242 mL/day vs. 3060 ± 1370 mL/day; p = 0.0229) over the observation period than the H1: TAA-positive patients.

Table 6. Disease severity with regard to the CTLA-4 rs3087243 genotypes.

| Variable                                      | All (n = 644) | rs3087243 p-Value |
|-----------------------------------------------|---------------|------------------|
|                                               | AA (n = 142)  | GG/AG (n = 502)  |     |
| SOFA                                          | 7.0 ± 3.5     | 7.4 ± 3.8        | 6.9 ± 3.5 | 0.3005 |
| SOFA-Respiratory score                        | 2.0 ± 0.8     | 2.0 ± 0.8        | 2.0 ± 0.8 | 0.6043 |
| SOFA-Cardiovascular score                     | 1.5 ± 1.0     | 1.7 ± 1.0        | 1.5 ± 1.0 | 0.1284 |
| SOFA-Central Nervous System score             | 2.0 ± 1.1     | 2.2 ± 1.0        | 1.9 ± 1.1 | 0.0094 |
| SOFA-Renal score                              | 0.8 ± 1.2     | 0.7 ± 1.1        | 0.8 ± 1.2 | 0.6669 |
| SOFA-Coagulation score                        | 0.4 ± 0.6     | 0.4 ± 0.7        | 0.3 ± 0.6 | 0.5521 |
| SOFA-Hepatic score                            | 0.4 ± 0.7     | 0.4 ± 0.7        | 0.4 ± 0.7 | 0.9780 |

Organ support

|                                               | All (n = 644) | rs3087243 p-Value |
|-----------------------------------------------|---------------|------------------|
|                                               | AA (n = 142)  | GG/AG (n = 502)  |     |
| Ventilation days/observation days (%)         | 66 ± 32       | 71 ± 32          | 65 ± 32 | 0.0280 |
| Vasopressor days/observation days (%)        | 34 ± 30       | 37 ± 33          | 33 ± 29 | 0.2919 |
| Dialysis days/observation days (%)           | 9 ± 23        | 9 ± 23           | 10 ± 22 | 0.8287 |

Inflammatory values

|                                               | All (n = 644) | rs3087243 p-Value |
|-----------------------------------------------|---------------|------------------|
|                                               | AA (n = 142)  | GG/AG (n = 502)  |     |
| Leucocytes (1000/µL)                          | 13 ± 5        | 13 ± 5           | 13 ± 5 | 0.1026 |
| CRP (mg/L)                                   | 152 ± 87      | 137 ± 81         | 156 ± 88 | 0.1356 |
| Procalcitonin (ng/dL)                        | 4.0 ± 9.3     | 3.5 ± 10.1       | 4.1 ± 9.1 | 0.0655 |

Kidney values

|                                               | All (n = 644) | rs3087243 p-Value |
|-----------------------------------------------|---------------|------------------|
|                                               | AA (n = 142)  | GG/AG (n = 502)  |     |
| Urine output (mL/day)                         | 2978 ± 1337   | 2973 ± 1388      | 2979 ± 1324 | 0.9387 |
| Urine output (mL/kg/day)                      | 1.6 ± 0.8     | 1.6 ± 0.8        | 1.6 ± 0.78 | 0.9051 |
| Creatinine (mg/dL)                           | 1.2 ± 0.9     | 1.2 ± 0.9        | 1.3 ± 1.0 | 0.4218 |

Liver values

|                                               | All (n = 644) | rs3087243 p-Value |
|-----------------------------------------------|---------------|------------------|
|                                               | AA (n = 142)  | GG/AG (n = 502)  |     |
| AST (GOT) (IU/L)                              | 169 ± 598     | 127 ± 238        | 182 ± 672 | 0.7476 |
| ALT (GPT) (IU/L)                              | 94 ± 188      | 101 ± 193        | 92 ± 187 | 0.2135 |
| Bilirubin (mg/dL)                             | 1.2 ± 2.1     | 1.2 ± 1.8        | 1.2 ± 2.1 | 0.5972 |

CRP, C-reactive protein; AST, aspartate transaminase; GOT, glutamic oxaloacetic transaminase; ALT, alanine transaminase; GPT, glutamate-pyruvate transaminase.

Table 7. Disease severity with regard to the CTLA-4 haplotype H1: TAA.

| Variable                                      | All (n = 644) | H1: TAA p-Value |
|-----------------------------------------------|---------------|-----------------|
|                                               | Positive (n = 447) | Negative (n = 197) |     |
| SOFA                                          | 7.0 ± 3.5     | 7.0 ± 3.6       | 7.1 ± 3.5 | 0.5088 |
| SOFA-Respiratory score                        | 2.0 ± 0.8     | 2.0 ± 0.8       | 1.9 ± 0.8 | 0.9796 |
| SOFA-Cardiovascular score                     | 1.5 ± 1.0     | 1.5 ± 1.0       | 1.5 ± 1.0 | 0.7476 |
| SOFA-Central Nervous System score             | 2.0 ± 1.1     | 2.0 ± 1.1       | 1.9 ± 1.1 | 0.2731 |
| SOFA-Renal score                              | 0.8 ± 1.2     | 0.7 ± 1.1       | 0.9 ± 1.3 | 0.1230 |
| SOFA-Coagulation score                        | 0.4 ± 0.6     | 0.4 ± 0.6       | 0.3 ± 0.6 | 0.6741 |
| SOFA-Hepatic score                            | 0.4 ± 0.7     | 0.4 ± 0.7       | 0.4 ± 0.8 | 0.4264 |
Table 7. Cont.

| Variable                     | All (n = 644) | H1: TAA Positive (n = 447) | Negative (n = 197) | p-Value  |
|------------------------------|---------------|----------------------------|---------------------|----------|
| **Organ support**            |               |                            |                     |          |
| Ventilation days/observation days (%) | 66 ± 32      | 67 ± 32                    | 65 ± 32             | 0.3607   |
| Vasopressor days/observation days (%) | 34 ± 30      | 33 ± 30                    | 35 ± 30             | 0.6474   |
| Dialysis days/observation days (%) | 9 ± 23        | 9 ± 22                     | 11 ± 23             | 0.1246   |
| **Inflammatory values**      |               |                            |                     |          |
| Leucocytes (1000/µL)         | 13 ± 5        | 13 ± 5                     | 13 ± 4              | 0.4087   |
| CRP (mg/L)                   | 152 ± 87      | 147 ± 81                   | 162 ± 99            | 0.4562   |
| Procalcitonin (ng/dL)        | 4.0 ± 9.3     | 3.5 ± 9.1                  | 5.1 ± 9.7           | 0.0002   |
| **Kidney values**            |               |                            |                     |          |
| Urine output (mL/day)        | 2978 ± 1337   | 3060 ± 1370                | 2792 ± 1242         | 0.0229   |
| Urine output (mL/kg/day)     | 1.6 ± 0.8     | 1.6 ± 0.8                  | 1.5 ± 0.8           | 0.0529   |
| Creatinine (mg/dL)           | 1.2 ± 0.9     | 1.2 ± 0.9                  | 1.4 ± 1.1           | 0.0902   |
| **Liver values**             |               |                            |                     |          |
| AST (GOT) (IU/L)             | 169 ± 598     | 184 ± 687                  | 131 ± 272           | 0.9910   |
| ALT (GPT) (IU/L)             | 94 ± 188      | 99 ± 206                   | 81 ± 142            | 0.1307   |
| Bilirubin (mg/dL)            | 1.2 ± 2.1     | 1.2 ± 1.8                  | 1.3 ± 2.5           | 0.9584   |

4. Discussion

Identification of genetic variants associated with the outcome of sepsis is important for understanding the pathophysiology of the disease and identifying patients at a higher risk of death, and may even reveal future targets for personalized medicine. The present study aimed to identify associations between functional SNPs and haplotypes of the CTLA-4 gene with the outcomes of septic patients. The main finding was that in addition to our previous identification of the CTLA-4 rs231775 SNP as a prognostic variable for the outcome of septic patients, the functional CTLA-4 rs3087243 SNP and the inferred CTLA-4 haplotype H1: TAA were significantly associated with sepsis survival. Both CTLA-4 rs3087243 G allele carriers and CTLA-4 H1: TAA haplotype-negative patients had significantly better 90- and 28-day survival. To the best of our knowledge, this study is the first to report an association of these genetic variants with sepsis survival. The identified associations remained significant predictors for 90- and 28-day mortality in the multivariate Cox regression analysis, indicating that despite potential confounders, they were independent prognostic variables for the survival of septic patients.

The findings at the CTLA-4 rs3087243 position could be explained by observations that revealed lower CTLA-4 protein expression levels as well as higher susceptibility to autoimmune diseases in rs3087243 G allele carriers [24,26–28]. We can assume that lower expression levels of the coinhibitory regulatory CTLA-4 protein in rs3087243 G allele carriers result in an enhanced T lymphocyte host immune response [37], which may be beneficial in the immunosuppressive phase of sepsis. Our hypothesis that genetic variants associated with lower CTLA-4 expression levels and autoimmune disease susceptibility may be favorable during the course of sepsis is further supported by our findings for the H1: TAA haplotype. This most common CTLA-4 haplotype consists of the rs733618 T, rs231775 A, and rs3087243 A alleles, of which the second two are associated with unfavorable sepsis outcomes. Not carrying this haplotype was significantly associated with better survival during the course of sepsis.

Furthermore, our study revealed that CTLA-4 rs3087243 G allele carriers showed significantly better sepsis disease severity in terms of a lower average SOFA-CNS score and a reduced need for organ support, as represented by a reduced number of ventilation days as a fraction of observation days (Table 6). Significantly lower average urine outputs (mL/day) and higher procalcitonin levels were observed for the H1: TAA-negative patients (Table 7). Although the lower urine output levels
can be explained by the significantly higher administration of renal replacement therapy to the H1: TAA-negative patients in the patient baseline characteristics, we do not have a satisfactory explanation for the higher average procalcitonin levels.

The present study has some limitations. This study is a single-center study focusing on a relatively large and prospectively enrolled cohort of severely ill patients from surgical ICUs; no patients from medical ICUs were included in the study. Therefore, our observations may not be completely applicable to other ICU cohorts. In addition, our findings should be validated in additional patient cohorts, ideally including patients of other ethnicities and from other ICUs (i.e., neurological or medical ICUs).

5. Conclusions

Although a sepsis-specific biomarker is nonexistent at present, potential for the use of genetic biomarkers for prognostic purposes is high. In this context, our study provides further evidence implicating CTLA-4 polymorphisms and haplotypes in determining sepsis severity and mortality, and thus they may serve as prognostic variables. However, the molecular mechanisms by which CTLA-4 polymorphisms and haplotypes influence the sepsis disease severity and mortality risk remain to be identified. Our findings raise hope that identification of patients at risk and development of tailored therapeutic approaches may be enabled by the effective use of modern molecular diagnostics, including evaluation of genetic variants, such as the SNPs and haplotypes identified in our present study.

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