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Increased mortality in septic shock with the *4G/4G* genotype of plasminogen activator inhibitor 1 in patients of white descent

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Abstract *Objective:* To evaluate the effect of the *4G/5G PAI-1* gene polymorphism on the development of organ failure and outcome in critically ill patients with septic syndromes. *Design and setting:* Prospective, observational study in a medical intensive care unit of a university hospital. *Patients:* 224 consecutively admitted patients. *Interventions:* Epidemiological data, severity scores, and the primary site of infection were recorded. DNA genotyping of the *PAI-1*, *TNF*-β, and *IL1-ra* genes, and measurement of plasma *PAI-1* antigen and D-dimer were carried out. *Measurements:* The primary outcome variables were organ dysfunction and mortality. *Results:* Eighty-eight subjects had septic shock at ICU entry or within 48 h from admission. Homozygotes for the *4G* allele exhibited higher plasma concentrations of *PAI-1* antigen and D-dimer than *4G/5G* and *5G/5G* subjects). ICU mortality was 44.0% in patients with *4G/4G*, 23.4% in *4G/5G* and 12.5% in *5G/5G*, mainly due to multiorgan failure. After adjusting for SAPS II at admission the genotypes independently associated with ICU mortality in septic shock were *TNF-B2/B2* (OR 2.83, 1.04–7.67) and *4G/4G* of *PAI-1* (OR 2.23, 1.02–4.85). The *PAI-1* genotype did not determine susceptibility to infection or the outcome in nonseptic systemic inflammatory response syndrome, sepsis, severe sepsis, and nosocomial septic shock. *Conclusions:* Homozygosity for *4G* of the *PAI-1* gene confers an increase in the risk of mortality in adult patients with septic shock due to a greater organ failure.

Keywords Plasminogen activator inhibitor 1 · Polymorphisms · Septic shock · Organ failure · Fibrinolysis

Introduction

Sepsis is the leading cause of death in critically ill patients, with a hospital mortality ranging from 30% to 50% in patients with septic shock [1, 2]. Epidemiological studies on inflammatory mediators have suggested a strong genetic relationship on the susceptibility and outcome of sepsis. *TNF*-α-*308A* and *TNF*-β-*252A* polymorphisms have been reported to be associated with susceptibility to meningococcal disease [3] and a high risk for septic shock in patients admitted with community-acquired pneumonia [4], respectively. Regarding the risk of mortality Mira et al. [5] found that the *TNF*-α-*308A* allele had a 3.7-fold increase in death among septic shock patients whereas homozygosity for *B2/B2* of *TNF*-β, which has been associated with a high tumor necrosis factor (TNF) response, increases the prob-

ability of severe posttraumatic sepsis in trauma by 3.4 [6]. With regard to the interleukin (IL) 1 pathway, the *IL-1raA2* allele seems to be more prevalent among patients with severe sepsis than among healthy individuals [7].

Inflammation and coagulation are closely linked and most patients with severe sepsis and septic shock have coagulation abnormalities [8]. In this setting nonsurvivors present a particular hemostatic profile characterized by a marked activation of coagulation and a more intense inhibition of fibrinolysis [9, 10]. *TNF*-α and *IL-1* increase the synthesis and release from endothelial cells of plasminogen activator inhibitor 1 (PAI-1), a key inhibitor of fibrinolysis and also decrease plasminogen activator synthesis [11, 12]. Thus, impaired fibrin degradation due to high circulating levels of *PAI-1* may contribute to enhanced microvascular fibrin deposition, which favors the development of organ dysfunction [13]. *PAI-1* may show a genomic variation in its promoter: a single base-pair deletion (*4G*) or insertion (*5G*). Homozygosity for the *4G* allele is reported to have higher basal and inducible concentrations of *PAI-1* and greater procoagulatory activity than one or two copies of the *5G* allele [14]. This has been associated with an increased risk of death from meningococcal sepsis in children [15–17] and a poor survival after severe trauma [18]. On the other hand, the *G4* variant of the *PAI-1* gene seems to be associated with a weak increase to the risk of coronary disease [19], and *G4/G4* genotype is associated with an increase in thromboembolic neurological complications after cardiac surgery [20]. Moreover, several reports claim for an involvement of the *G4* variant in a miscellany of other cardiovascular [21], and systemic diseases [22–24], although these studies need confirmatory evidence.

The aim of the study was to evaluate the effect of the *4G/5G PAI-1* gene polymorphism in the development of organ failure and outcome in adult patients with septic shock. In addition, we evaluated the association of genomic variations in *TNF*-β and *IL1-ra* on the influencing the *PAI-1 4G/4G* gene effect on the primary endpoints.

Patients and methods

Patients

During the 12-month period from January 2003 to January 2004, 301 patients were admitted to the medical ICU of the Hospital Clinic of Barcelona. The study population consisted of 224 consecutively patients (mean age 62.3 ± 15.9 years, range 18–85; 63.8% men) with a minimum ICU stay of 48 h and without short-term irreversible diseases, as reported in the Electronic Supplementary Material (ESM; Fig. 1). The patients were included and followed until hospital discharge. Furthermore, 80 healthy blood donors recruited consecutively were used as the control population. All the subjects were of white descent and living in or around Barcelona. Informed consent was obtained from the patients or their relatives within 24 h after admission, and none refused to participate. The protocol was approved by the institutional review board of the Hospital Clinic of Barcelona.

Epidemiological data, the primary site of infection, and infection-related organisms were recorded (Table 1 and ESM). Clinical infections were defined according to the Centers for Diseases Control and Prevention criteria [25]. Severe sepsis and septic shock were defined according to SCCM/ESICM/ACCP/ATS/SIS consensus conference [26], as described in the ESM. Septic shock was considered nosocomial (late) when observed after 48 h after ICU entry. Severity indexes including Acute Physiology and Chronic Health Evaluation (APACHE) II, Simplified Acute Physiology Score (SAPS) II, and Sequential Organ Failure Assessment (SOFA) were calculated at ICU admission and thereafter on a daily basis. Within 48 h of ICU entry 88 patients exhibited septic shock and 26 severe sepsis (Fig. 1); of the remaining patients 51 had sepsis and 59 had noninfectious systemic inflammatory response syndrome (SIRS). Mean APACHE II, SAPS II, and SOFA scores at entry were 16.8 ± 6.3, 37.4 ± 12.3, and 8.6 ± 3.1 at ICU entry, respectively. Multiorgan failure was considered in case of acute progressive dysfunction of two or more organs systems, with a minimum failure score of 3 points for each organ. Acute respiratory distress syndrome was considered according to the European Consensus score [27], with patients showing a Murray et al. [28] score of 2.5 or higher. Blood samples and the information used in the study were coded and patient confidentiality was preserved. Biochemical studies and genotyping were performed in the patients after completion of follow-up. Patients with septic shock required surgery more frequently than patients with sepsis. Coagulation parameters at ICU entry did not differ among the groups of patients, except for greater fibrinogen levels in septic subjects than in those with noninfectious SIRS (Table 1).

Biochemical and genotype studies

Collection of venous blood samples and processing are reported in ESM. Prothrombin and activated partial thromboplastin times, fibrinogen, and D-dimer levels were measured [29]. Plasma antigen related to *PAI-1* was measured by an enzyme-linked immunosorbent assay (Biopool, Umea, Sweden) based on a double-antibody principle [30]. Genomic DNA was extracted from 100 µl whole blood with a silica gel column method (QIAamp DNA blood mini kit, Qiagen, Hilden, Germany), and stored at –80 °C until further use. Genotyping was validated by direct sequencing of a group of random samples. We determined the *PAI-1 4G/5G*, *TNF*-β-*252G B1/B2*,

Table 1 Epidemiological and clinical data of the patients on ICU admission (*NA* not applicable)

	Septic shock ($n=88$)	Severe sepsis ($n=26$)	Sepsis ($n=51$)	Noninfectious SIRS ($n=59$)
Age (years)	62.9 ± 15.0	64.3 ± 16.2	58.2 ± 17.1	63.9 ± 15.7
Sex: M/F	58/30	15/11	30/21	40/19
Prior hospital stay (days)	1.8 ± 3.4	1.8 ± 3.4	0.9 ± 2.3	1.3 ± 3.4
APACHE II score	18.5 ± 6.1	17.7 ± 6.5	13.3 ± 6.2^{*,5*}	17.0 ± 5.5^{7*}
SAPS II score	42.3 ± 12.0	40.2 ± 12.8	29.7 ± 11.7^{*,4*}	35.6 ± 9.1^{7*}
SOFA score	10.0 ± 2.9	9.1 ± 3.6	6.7 ± 2.7^{*,5*}	7.8 ± 2.4^{*,6*,8*}
Invasive mechanical ventilation	66 (75.0%)	15 (57.7%)	20 (39.2%)*	42 (71.2%)^{7*}
Prior antibiotics	15 (17.0%)	6 (23.1%)	10 (19.6%)	2 (3.4%)^{**,5*,8*}
Prior corticosteroids	11 (12.5%)	3 (11.5%)	6 (11.8%)	7 (11.9%)
Surgery [a]	26 (29.5%)	5 (19.2%)	7 (13.7%)***	22 (37.3%)^{7*}
Heart failure NYHA IV	2 (2.3%)	1 (3.8%)	0	10 (16.9%)^{**,7*}
Stroke	7 (7.9%)	2 (7.7%)	8 (15.7%)	11 (18.6%)
Chronic respiratory disease	13 (14.7%)	4 (15.4%)	7 (13.7%)	9 (15.3%)
Community-acquired pneumonia	43 (48.9%)	10 (38.5%)	21 (41.2%)	NA
Hospital-acquired pneumonia	3 (3.4%)	1 (3.8%)	3 (5.9%)	NA
Urinary tract infection	9 (10.2%)	4 (15.4%)	3 (5.9%)	NA
Bacterial Meningitis	3 (3.4%)	2 (7.7%)	3 (5.9%)	NA
Peritonitis	17 (19.3%)	3 (46.2%)	3 (5.9%)***	NA
Other infections	10 (11.4%)	4 (15.4%)	4 (7.8%)	NA
Platelet count ($\times 10^9$/l)	181 ± 124	216 ± 127	208 ± 119	172 ± 90
Prothrombin index	66 ± 16	70 ± 19	81 ± 17	73 ± 18
Activated partial thromboplastin time (s)	33.6 ± 5.2	32.4 ± 4.1	30.0 ± 4.2	33.4 ± 8.6
Fibrinogen (g/l)	5.75 ± 2.62	5.27 ± 1.63	5.29 ± 2.41	4.39 ± 1.72^{*,6*,8*}

$^{*}p < 0.001$, $^{**}p < 0.01$, $^{***}p < 0.05$ vs. septic shock, $^{4*}p < 0.001$, $^{5*}p < 0.01$, $^{6*}p < 0.05$ vs. severe sepsis, $^{7*}p < 0.01$, $^{8*}p < 0.05$ vs. sepsis,
[a] Surgery as cause of ICU entry or performed during stay

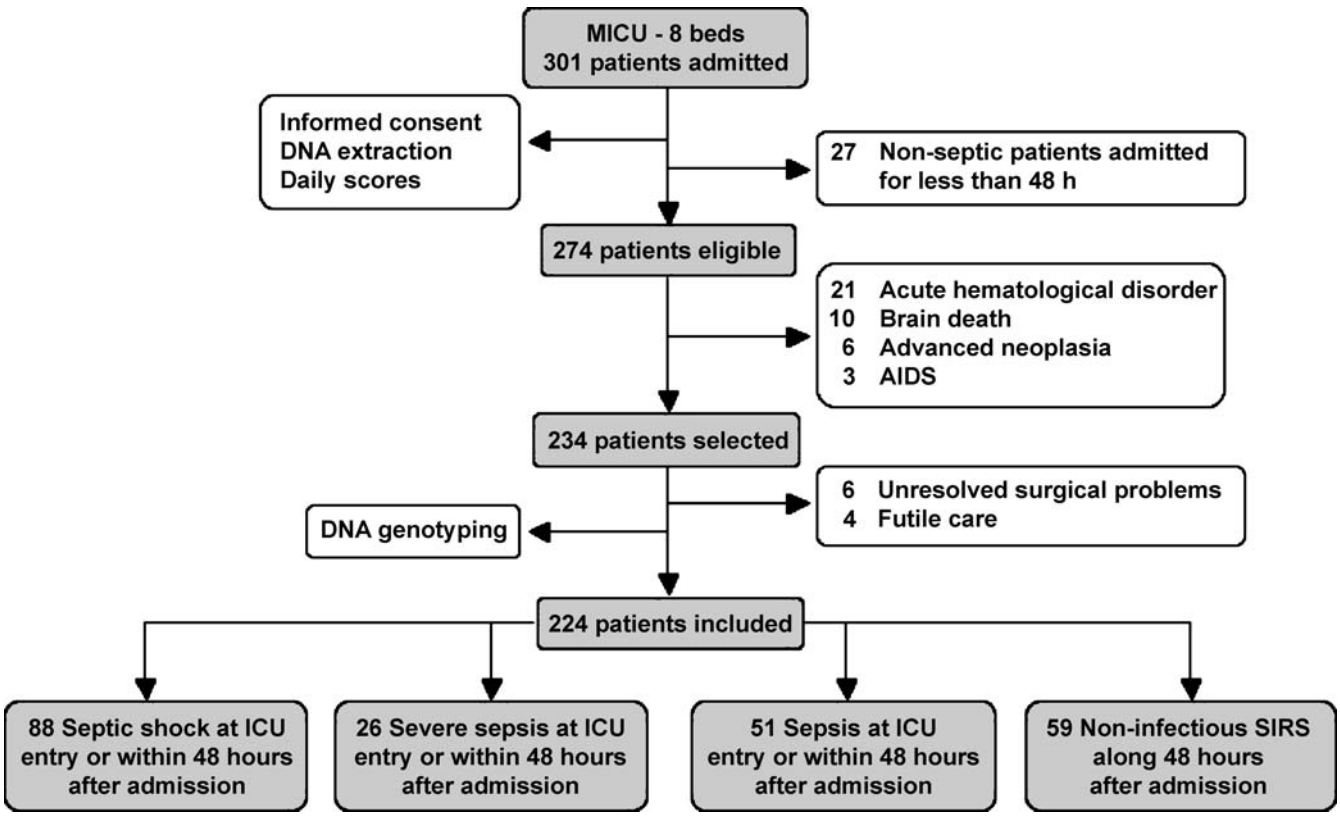

Fig. 1 Flowchart of the patient groups according to inclusion criteria

and *IL-1ra A2* polymorphisms [31–33], and methods for detection are shown in the ESM.

Statistical analysis

Results are given as means ± SD. Differences between groups were evaluated with the Fisher's exact and Student's *t* tests. Bonferroni's correction was applied to adjust the type I error in multiple comparisons. Pearson's correlation coefficients between continuous variables were calculated. Genotype distributions were tested for the Hardy-Weinberg equilibrium. To evaluate the variables independently associated with ICU and hospital mortality a logistic regression model (forward stepwise selection) was performed. In this model quantitative variables were introduced in its continuous form, and therefore the odds are referred to the natural unit of the predictor. All tests were performed with a bilateral significance level of $p < 0.05$. Statistical analysis was performed with SPSS statistical software (version 11.0.4; SPSS, Chicago, Ill., USA).

Results

Patient follow-up data

Data on the patients during ICU stay are summarized in Table 2. Patients with septic shock showed a significantly greater worsening in SAPS II score than those admitted because of sepsis or noninfectious SIRS. Thirty-two (14.3%) patients died in the ICU, mainly due to multiorgan failure. Mortality was higher in those with septic shock (27.3 vs. 5.8%, $p < 0.001$). Overall hospital mortality reached 20.1%, with higher post-ICU ward mortality in patients

Table 2 Patients characteristics during Intensive Care Unit stay

	Septic shock ($n = 88$)	Severe sepsis ($n = 26$)	Sepsis ($n = 51$)	Noninfectious SIRS ($n = 59$)
Length of ICU stay (days)	11.5 ± 10.4	12.3 ± 12.7	8.0 ± 5.8***	8.3 ± 8.0***
Highest SAPS II score	50.7 ± 16.2	45.7 ± 17.8	34.4 ± 14.9*,5*	39.4 ± 10.6*,5*
Δ SAPS II score	8.4 ± 10.8	5.4 ± 8.0	4.7 ± 6.2***	3.8 ± 6.1**
Highest SOFA score	11.6 ± 3.4	10.3 ± 3.1	8.4 ± 2.8*,4*	9.1 ± 2.5*
Δ SOFA score	1.6 ± 2.3	1.1 ± 1.9	1.4 ± 2.2	1.3 ± 1.7
Invasive mechanical ventilation	75 (85.2%)	20 (76.9%)	30 (58.8%)*	43 (72.9%)
Parenteral nutrition	24 (27.3%)	5 (19.2%)	9 (17.6%)	2 (3.4%)*,4*,5*
Renal replacement	17 (19.3%)	5 (19.2%)	2 (3.9%)	3 (5.1%)**
Late septic shock	6 (6.8%)	3 (11.5%)	3 (5.9%)	2 (3.4%)
ICU mortality	24 (27.3%)	3 (11.5%)	2 (3.9%)*	3 (5.1%)*
Multiorgan failure	22 (25.0%)	3 (11.5%)	2 (3.9%)*	1 (1.7%)*
Sudden cardiovascular collapse	2 (2.3%)	0	0	2 (3.4%)
Overall hospital mortality	29 (33.9%)	4 (15.4%)	2 (3.9%)*	10 (16.9%)***,5*

*$p < 0.001$, **$p < 0.01$, ***$p < 0.05$ vs. septic shock, 4*$p < 0.01$, 5*$p < 0.05$ vs. sepsis

Table 3 Distribution of genotype polymorphisms among the groups of patients; no statistical differences were observed between the admission groups

	Septic shock ($n = 88$)	Severe sepsis ($n = 26$)	Sepsis ($n = 51$)	Noninfectious SIRS ($n = 59$)	Healthy controls ($n = 80$)
PAI-1					
4G/4G	25 (28.4%)	5 (19.2%)	7 (13.7%)	16 (27.1%)	22 (27.5%)
4G/5G	47 (53.4%)	11 (42.3%)	27 (52.9%)	27 (45.7%)	42 (52.5%)
5G/5G	16 (18.2%)	10 (38.5%)	17 (33.3%)	16 (27.1%)	16 (20.0%)
TNF-β					
B1/B1	8 (9.1%)	3 (11.5%)	6 (11.7%)	7 (11.8%)	8 (10.0%)*
B1/B2	26 (29.5%)	9 (34.6%)	19 (37.2%)	20 (33.9%)	36 (45.0%)
B2/B2	54 (61.3%)	14 (53.8%)	26 (50.9%)	32 (54.2%)	36 (45.0%)
IL1-ra					
A1/A1	45 (51.1%)	13 (50.0%)	23 (46.0%)	22 (37.3%)	29 (36.2%)
A1/A2	37 (42.0%)	13 (50.0%)	23 (46.0%)	24 (40.6%)	41 (51.2%)
A1/A3	1 (1.1%)	0	2 (3.9%)	3 (5.1%)	1 (1.2%)
A2/A2	3 (3.4%)	0	2 (3.9%)	9 (15.2%)	9 (11.2%)
A2/A3	2 (2.2%)	0	1 (1.9%)	1 (1.7%)	0

*$p = 0.04$ vs. septic shock

admitted for nonseptic SIRS (12.5 vs. 3.6%, $p < 0.001$). Logistic multivariate analysis showed that the clinical variables independently associated with ICU mortality in patients admitted with septic shock were the SAPS II score at admission [odds ratio (OR) 1.08, 95% confidence interval (CI) 1.02–1.15) and the worsening of this score during ICU stay (1.16, 1.09–1.24). Moreover, 14 patients (range between the groups 3.4–11.5%) developed nosocomial septic shock while in the ICU.

Genetic analysis

The distribution of the *PAI-1* genotype was comparable in patients and controls and between the different subgroups of patients studied (Table 3). Likewise the overall allele frequency of *4G/5G* was 0.49/0.51 in all patients, which did not differ from that observed in controls (0.54/0.46). Regarding the *TNF*-β genotype, homozygosity for the *B2* allele was more prevalent in patients with septic shock than in controls (61.3 vs. 45.0%, $p = 0.04$). By contrast, the distribution of the *IL1-ra* genotype among the groups of patients did not differ from that of the healthy population. In patients with septic syndromes *PAI-1* alleles distributed evenly with *TNF*-β and *IL-1ra* alleles (Table 4). Except for the higher prevalence of homozygosity for the *B2* allele in patients admitted with septic shock, none of the genotypes analyzed was associated with diseases leading to ICU entry or the origin of infectious disease.

Coagulation abnormalities in organ dysfunction and septic shock

Plasma levels of the *PAI-1* antigen on admission were correlated with the Δ SOFA score ($r = 0.28$, $p = 0.02$) and were greater in patients who died than in survivors (45.3 ± 23.8 vs. 33.8 ± 16.7 ng/ml, respectively, $p = 0.02$). The *4G/4G* patients with septic shock had higher plasma concentrations of the *PAI-1* antigen than *4G/5G* and *5G/5G* subjects ($p < 0.001$, both; Table 5). Coagulation data at ICU entry were not related to the *PAI-1* genotype. However, *4G/4G* patients showed a twice the D-dimer concentration of those with other *PAI-1* genotypes ($p < 0.05$, both; Table 5). D-dimer levels at ICU entry were not correlated significantly with the SOFA score, but were also greater in patients with septic shock who died than in survivors (826 ± 919 vs. 513 ± 423 ng/ml, respectively, $p = 0.03$). A direct correlation between the *PAI-1* antigen and D-dimer upon ICU admission was observed ($r = 0.49$, $p < 0.001$).

Table 4 Distribution of *PAI-1* genotype polymorphisms according to *TNF*-β and *IL1-ra* genotype polymorphisms in patients with septic syndromes; no statistical differences were observed between the groups

	4G/4G ($n = 37$)	4G/5G ($n = 85$)	5G/5G ($n = 43$)
TNF-β			
B1/B1	6 (16.2%)	7 (8.2%)	4 (9.3%)
B1/B2	9 (24.3%)	31 (36.4%)	14 (32.5%)
B2/B2	22 (59.4%)	47 (55.3%)	25 (58.1%)
IL1-ra			
A1/A1	18 (48.6%)	41 (48.2%)	22 (51.1%)
A1/A2	17 (45.9%)	37 (43.5%)	19 (44.1%)
A1/A3	0	3 (3.5%)	0
A2/A2	2 (5.4%)	2 (2.3%)	1 (2.3%)
A2/A3	0	2 (2.3%)	1 (2.3%)

Effect of *TNF*-β and *IL-1ra* genotypes on organ dysfunction and mortality

We found no differences in severity scores with respect to baseline values and changes during ICU stay among the groups of patients homozygous to the *B2/B2* compared to other forms of the *TNF*-β gene (data not shown). However, the mortality of *B2/B2* patients with septic shock was higher than that in patients carriers of the *B1* allele (37.0 vs. 11.7%, respectively, $p = 0.01$). Mortality was not associated with the *TNF*-β polymorphism in the remaining groups of patients. On the other hand, neither was the *IL1-ra* genotype related to susceptibility to infection, clinical data or outcome in any group of the patients studied. Neither of these two genotypes was associated with the appearance of late (nosocomial) septic shock and survival.

Effect of the *PAI-1* genotype on organ dysfunction and mortality

The *PAI-1* genotype affected the clinical course of patients with septic shock, as *4G* homozygotes exhibited a greater worsening in clinical status (i.e., Δ SOFA) than other *PAI-1* genotypes ($p < 0.05$, all; Table 5). The presence of *4G/4G* in patients with septic shock was associated with an increase of 1 point in the SOFA score (OR 2.83, 95% CI 1.04–7.67). However, nonsignificantly greater supportive measures (i.e., mechanical ventilation, vasopressors administration, parenteral nutrition) were needed by the *4G/4G* patients. Clinical changes in the patients admitted for severe sepsis, sepsis, or noninfectious SIRS were independent of *PAI-1* genotype.

ICU mortality in septic shock reached 44.0% in patients with *4G/4G*, 23.4% with *4G/5G*, and 12.5% in *5G/5G* ($p = 0.03$ for *4G/4G* vs. other genotypes; Fig. 2), mostly due to multiorgan failure. The *4G* and *5G* allele distribution was 0.50/0.50 in alive and 0.69/0.31 in nonsurvivors ($p = 0.03$). After adjusting for SAPS II at admission the genotypes independently associated with ICU mortality in septic shock were of *TNF-B2/B2* (OR 2.83, 95% CI 1.04–7.67) and *4G/4G* of *PAI-1* (2.23, 1.02–4.85).

Table 5 Data of patients with septic shock according to the *PAI-1* genotype along ICU stay

	4G/4G ($n=25$)	4G/5G ($n=47$)	5G/5G ($n=16$)
Age (years)	66.3 ± 13.9	61.2 ± 13.9	62.9 ± 16.3
Length of ICU stay (days)	12.6 ± 10.6	8.9 ± 6.9	10.1 ± 4.6
APACHE II score on ICU admission	19.1 ± 6.7	17.4 ± 4.8	20.0 ± 7.1
SAPS II score on ICU admission	42.0 ± 11.1	40.7 ± 10.9	47.5 ± 12.7
SOFA score on ICU admission	9.3 ± 2.1	10.0 ± 3.3	10.3 ± 2.9
Δ SOFA score	2.34 ± 2.70	1.24 ± 1.76**	0.87 ± 1.28**
Acute respiratory distress syndrome	15 (60.0%)	19 (40.4%)	5 (31.2%)
Late septic shock [a]	2 (8.0%)	3 (6.4%)	1 (6.3%)
Invasive mechanical ventilation (days)	21 (84.0%)	39 (82.9%)	15 (93.8%)
Invasive mechanical ventilation, mean (days)	14.3 ± 12.7	8.9 ± 8.4**	10.8 ± 10.1
Vasopressors at alpha dose (days)	6.3 ± 7.0	4.0 ± 5.2	3.5 ± 2.0
Corticosteroids use (mg/day) [b]	19 (76.0%)	27 (57.4%)	11 (68.8%)
Corticosteroids use, mean (mg/day)	43.1 ± 36.9	50.6 ± 22.0	47.2 ± 40.3
Renal replacement (days)	5 (20.0%)	10 (21.3%)	3 (18.8%)
Renal replacement, mean (days)	4.4 ± 2.0	5.0 ± 4.5	4.6 ± 2.0
ICU mortality	11 (44.0%)***	11 (23.4%)	2 (12.5%)
Multiorgan failure	11 (44.0%)***	9 (19.1%)	2 (12.5%)
Sudden cardiovascular collapse	0	2 (4.3%)	0
Overall hospital mortality	13 (52.0%)***	13 (27.7%)	3 (18.8%)
Plasma PAI-1 on admission (ng/ml)	57.4 ± 23.3	31.6 ± 12.9*	24.5 ± 5.9*
D-dimer on admission (ng/ml)	829 ± 895	505 ± 368**	417 ± 410**

*$p < 0.001$, **$p < 0.05$ vs. *G4/G4* genotype; ***$p = 0.03$ vs. *5G* carriers, [a] Shock criteria reappeared 48 h after resolution of shock, [b] corticosteroid dose equivalent to methylprednisolone

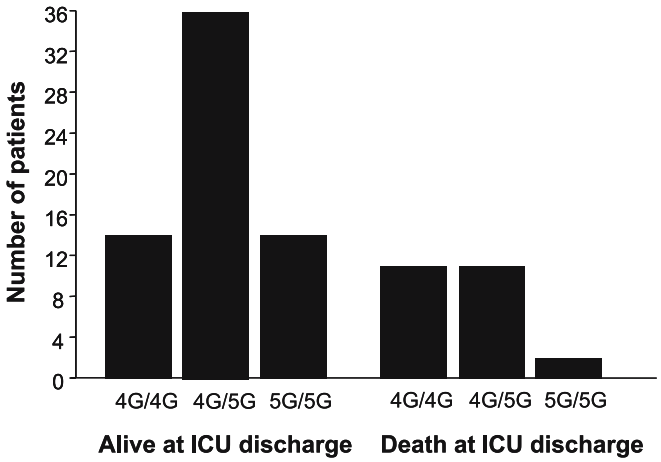

Fig. 2 *PAI-1* genotype distribution among patients with septic shock according to ICU survival. Patients homozygous for the *4G/4G* polymorphism had higher mortality than those with other *PAI-1* genotypes ($p = 0.03$)

Taking all the genotypes into consideration, we observed that 37.7% of the subjects with septic shock carrying *4G/4G* or *B2/B2* died, whereas the mortality decreased to 3.7% when both these polymorphisms were absent ($p < 0.001$). No additive effect on mortality was observed between the genotypes. The *4G* allele of the *PAI-1* gene was not related to the appearance of nosocomial septic shock and its mortality, although the number of patients evaluated was too small.

Discussion

In the present study we observed greater organ dysfunction and a twofold increase in the risk of mortality in patients with septic shock who were homozygous for the *4G*-deletion polymorphism of the *PAI-1* gene. In addition, similarly to previously published studies [6, 34], we observed that *B2/B2* of the *TNF*-β gene confers a higher susceptibility to septic shock and these subjects have a worse outcome. Moreover, we confirmed the lack of association between the genomic variations in *PAI-1*, *TNF*-β, and *IL-1ra* with each other. The study was underpowered to identify a clinical significant difference in the supportive case among the groups of patients.

It is already known that virtually all patients with sepsis have coagulation abnormalities, with widespread microvascular fibrin deposition, which contributes to organ dysfunction and profuse bleeding from various sites [35]. Inhibition of the fibrinolytic system is a key element of the pathogenesis of fibrin deposition during severe inflammation. In sepsis-induced generalized activation of coagulation the generation of thrombin also initiates fibrinolysis through the release of tissue plasminogen activator. However, the activation of the fibrinolytic system is transient due to a strongly proinflammatory cytokine-induced *PAI-1* expression and secretion by endothelial cells [36, 37]. Thus the net result is a proinflammatory and prothrombotic disorder with exhaustion of fibrinolysis and coagulation inhibitors [38]. In agreement with previous findings [39] we found that both plasma *PAI-1* and D-dimer were elevated

in patients with septic shock, mainly in those who died, and was correlated with the first to organ failure.

In addition to proinflammatory cytokines, the circulating plasma *PAI-1* level is affected by a deletion/insertion (*4G/5G*) polymorphism in the promoter of the *PAI-1* gene. Although both alleles bind a transcriptional activator, only the *5G* allele binds a repressor protein at an overlapping site. Therefore homozygosity for the *4G* allele renders this negative regulator unable to bind, resulting in greater transcription of the *PAI-1* gene [9, 11, 14]. In this regard we documented that plasma *PAI-1* concentrations in *4G* homozygotes doubled than that observed in *5G* carriers. As this increase in *PAI-1* may show circadian changes [40], we determined the *PAI-1* antigen from morning samples. Previous studies have shown that homozygosity for the *4G* allele of the *PAI-1* gene exerts a harmful effect on the prognosis of some subsets of critical patients, such as children with meningococcal sepsis, in whom the relative risk of dying from meningococcal sepsis increases from 2.0 to 2.4 compared with *5G* carriers [15, 17]. In these patients logistical regression indicated a 40% reduction in the probability of death for *4G/5G* patients and a 91% reduction for *5G/5G* patients compared to *4G* homozygotes [15]. Moreover, in patients with severe trauma, Menges et al. [18] found that the *4G* allele not only has increased levels of *PAI-1* during 14 days of observation after multiple traumas but also entailed a higher prevalence of sepsis and multiple organ failure. As a result 11 of 19 patients with the *4G/4G* genotype did not survive, whereas only 8 of the 29 *4G/5G* patients, and 2 of 13 patients with *5G/5G* of the *PAI-1* gene died. No studies have yet determined the effect of the *PAI-1* genotype in adult patients with septic shock of different origin. In the present study we observed that patients with septic shock without unsalvageable disease resulted in a hospital mortality of 52.0% for *4G/4G*, 27.7% for *4G/5G* and 18.8% for *5G/5G* patients, mainly due to multiorgan failure. This association with *PAI-1* gene polymorphism occurred independently of the causative infectious pathogen and was not observed in patients with sepsis and severe sepsis or in those with late (nosocomial) septic shock. However, this study has some limitations due to number of subjects and power of calculation, as well as derived from the complexity of the disease [41], and these initial positive findings needs to be confirmed in a large independent cohort in order to verify that the genetic background or environmental exposures did not affect the results. The *G4* variant of the *PAI-1* gene has also been implicated in many other diseases [42–49], as is reported in ESM.

On the other hand, in the present study we evaluated other genotypes that coded for the production of *TNF-α* and *IL-1ra* and found septic shock to be more prevalent in patients homozygous for the *TNF-B2/B2* polymorphism. However, in contrast to the report by Fang et al. [7], the prevalence of *IL-1raA2* among patients with severe sepsis and septic shock did not differ from that observed in healthy individuals. More importantly, *TNF-B2/B2* was associated with a 2.8-fold increase in the risk of mortality in patients with septic shock. Other candidate genes which regulate the inflammatory response and which may be implicated in the appearance and end result of septic shock are those that code for the heat shock protein, *IL-6*, *IL-10*, *CD-14*, and Toll-like receptors [50], were not evaluated in the present study.

In summary, we observed that patients admitted to the ICU with septic shock had greater organ dysfunction and exhibited a higher mortality when homozygous to the *4G* form of the *PAI-1* gene. This observation may be related to a procoagulant state in sepsis, as a consequence of fibrinolysis inhibition by *PAI-1*. However, the evidence from this single association study needs to be replicated to provide insurance against errors and biases. In addition, further studies of combined gene effects and gene-gene interactions as well as therapeutic approaches based on genetic risk in patients with both sepsis and a derangement of coagulation are needed.

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