Original Article

Biomarkers in sepsis at time zero: intensive care unit scores, plasma measurements and polymorphisms in Argentina

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Abstract

Introduction: A patient's response to sepsis is influenced by their genetic background. Our objective was to use plasma markers, such as protein C (PC), D-dimer, Plasminogen Activator Inhibitor-1 (PAI-1) levels, and the PAI-1 rs1799889 4G/5G and Tumor Necrosis Factor-α rs1800629 G/A polymorphisms to improve classical intensive care unit (ICU) scores.

Methodology: We studied 380 subjects, 166 with sepsis. We performed coagulation tests: plasma PAI-1 and PC levels were evaluated by chromogenic methods; and D-dimer was evaluated by immunoturbidimetric assay. Polymorphisms were performed using for polymerase chain reactions followed by digest with specific restriction enzyme. We acquired the APACHE and SOFA scores (time zero), sex, age, body mass index, associated co-morbidities, length of ICU stay (days), the severity of sepsis (sepsis, severe sepsis or septic shock), the HIV status and the ICU outcome (survival or death).

Results: We found significant differences between patients who died (n=80) and those who survived (n=86) in terms of the ICU length of stay (6 vs. 10 days), septic shock (64 versus 24%), age (51 versus 38 years old), HIV+ condition (34 versus 16%), SOFA (7 versus 4), APACHE (19 versus 13), D-dimer (4.32 versus 2.88 μg/ml), PC (46.0 versus 63.5 %) and PAI-1 (33.0 versus 16.5 UA/l). When we used a regression analysis with dichotomized variables, only the $SOFA^{4}$, $PAI\text{-}1^{16}$, HIV status and the PAI-1 4G allele proved to be predictors of death at time zero.

Conclusions: In the future, ICU scores may be further improved by adding certain genomic or plasma data.

Key words: PAI-1; TNF-α; SOFA; D-dimer; sepsis

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Introduction

The group of patients who are diagnosed with sepsis includes an extremely heterogeneous mix of cases with different underlying conditions, different anatomic sites of infection with a large number of different microorganisms of varying virulence and quantity, and a wide range of host inflammatory and immunologic capabilities [1-2]. Moreover, a causative organism is never found in a sizeable minority of patients with the clinical presentation of sepsis [3]. In spite of significant advances in both the supportive care and the understanding of the molecular basis of sepsis, it is still the most common cause of death among patients, both in the intensive care unit (ICU) [3-5] and elsewhere [6]. It is possible that the perpetuation of the inflammatory response results in severe sepsis or septic shock [7-8]. For instance, the excessive release of tumor necrosis factor-α (TNF-α) by macrophages into the bloodstream can produce a dangerous condition that leads to septic shock, organ failure and ultimately death [7,9,10]. Unfortunately, at present we cannot rapidly assess the patient's ability to produce an appropriate inflammatory response, nor do we have the tools to interpret and act on this information to benefit patients [1].

Multiple organ failure is a major cause of morbidity and mortality in critically ill patients. The Sequential Organ Failure Assessment (SOFA) score is now commonly incorporated into clinical practice and has been shown to have high accuracy in describing the course of organ dysfunction in patients with severe sepsis [11]. Whereas severity of illness scoring systems, such as the Acute Physiology and Chronic Health Evaluation II (APACHE), are based on the first 24 hours of ICU admission, the SOFA scoring system takes into account the time course of a patient´s condition during the entire ICU stay.

However, some publications have questioned the insufficient reliability of these scoring systems [12-13]. In fact, many publications focused their attention on using the plasma levels of certain factors, such as protein C (PC), procalcitonin, TNF-α, and plasminogen activator inhibitor-1 (PAI-1), as prognostic markers [14-16]. Other publications have investigated specific genetic polymorphisms in candidate genes [7,10,17-21].

During sepsis, fibrinolysis is inhibited while the activation of coagulation still proceeds, as shown by elevated D-dimer levels, which contributes to multiple organ dysfunction [8,22-24]. PAI-1 is considered the main cause for the downregulation of fibrinolysis during sepsis; high plasma levels of PAI-1 contribute to multiple organ dysfunction and thus to an increased risk of death [25]. In addition, PAI-1 has been implicated in other mechanisms, such as leukocyte migration [26]. Given its pivotal role in the coagulation and fibrinolytic systems, PC has been of particular interest as a cofactor in hemostatic abnormalities in patients with sepsis. Low levels of PC could be a predictor of mortality in septic adult patients [14,24,27]. Consequently, PC, PAI-1 and D-dimer levels could be good prognostic markers.

Increasing evidence suggests that individual differences in infectious disease manifestation may result from the genetic predisposition of the patient. Specific genetic factors might predict the risk not only for developing severe infection, but also for developing organ dysfunction leading to death [18-20]. Among TNF-α variants, a polymorphism that affects TNF-α expression has been located at nucleotide position -308 (TNF-α rs1800629 G/A) and has been associated with morbidity and mortality in severe forms of fulminant purpura, mucocutaneous leishmaniasis and sepsis [28]. In addition, the PAI-1 gene contains a polymorphism (PAI-1 rs1799889 4G/5G, either 4 or 5 guanine bases) in which the PAI-1 4G allele produces more PAI-1 than the PAI-1 5G allele during an acute phase response, such as in sepsis. In fact, the PAI-1 rs1799889 4G/5G polymorphism has been found to correlate with a poor outcome in meningococcal sepsis [29-32]. Specific polymorphisms in the encoding of this gene have been suggested to correlate an increased mortality in septic shock, although contradictory results have been presented [33]. There are several factors that contribute to this discrepancy, and ethnicity is one of them.

Our objective was to use plasma markers, such as PC (%), D-dimer (μg/ml), PAI-1 (UA/l) levels, and the PAI-1 rs1799889 4G/5G and TNF-α rs1800629 G/A polymorphisms to improve classical ICU scores, such as the SOFA and APACHE, in predicting the outcome of septic patients at the time of admission to the ICU in Argentina.

Methodology

Patients

We studied 380 unrelated Caucasian adults (age greater than 18 years), 166 of whom had sepsis as defined by international guidelines (with or without identified pathogens), with no more than 48 hours of evolution, who were hospitalized in the ICU at an infectious disease hospital in Argentina. The criteria for ICU admission were based on the patient's clinical condition at the time of presentation (time zero). In addition, we analyzed 214 volunteer subjects without any known infection at the time of blood extraction to investigate the genotypic Hardy-Weinberg equilibrium (control group). Study subjects were all Argentineans who resided in the same geographic area. The study was approved by the appropriate ethics committees and subjects gave informed consent for inclusion. Subjects were excluded if they were pregnant or younger than 18 years of age.

Patients' data

We acquired the APACHE and SOFA scores at the time of diagnosis (time zero), in addition to information on sex, age, body mass index and associated co-morbidities (diabetes, hypertension, dyslipidemia, tobacco use, atherothrombotic diseases, coinfection, inactivity). We also recorded the length of ICU stay (days), the severity of sepsis (sepsis, severe sepsis or septic shock), the HIV status, and the ICU outcome (survival or death).

Laboratory tests

Blood was obtained by clean venipuncture without stasis and collected into plastic tubes containing sodium citrate (ratio 9:1). After double centrifugation at 2,500 g for 15 minutes, platelet-poor plasma was immediately stored at -40°C. The plasma was used to perform coagulation tests for PC (%), D-dimer (μg/ml) and PAI-1 (UA/l). The plasma PAI-1 and PC levels were evaluated by chromogenic methods (Diagnostica Stago, France). The D-dimer was evaluated by immunoturbidimetric assay (Liatest, Stago, France).

Blood collected into tubes and allowed to clot at 37°C, and then centrifuged at 1,500g, was used for

sera preparation. Sera were stored at -40°C until used to perform routine tests by standard techniques.

One portion of the blood collected into the plastic tubes containing EDTA was stored at -40°C for polymerase chain reactions (PCR). Genomic DNA was extracted from white blood cells using an automatic method (QIAmp Mini blood, Qiagen).

The PAI-1 rs1799889 4G/5G polymorphism was amplified using the forward 5'-CACAGAGAGAGTCTGGCCACGT-3' and the reverse 5´-CCAACAGAGGACTCTTGGTCT-3' primers. A 50 µl reaction mix contained 100 ng de DNA, 0,5 µmol/l each primers, 0,1 mmol/l dNTP, 1x buffer, 1,5 mmol/l $MgCl_2$ and 1U Taq polymerase. The PCR conditions used were as follows: a denaturing step of 95°C for 5 minutes and then 37 cycles of 95°C for 1 minute, 62°C for 40 seconds and 72°C for 1 minute 45 seconds, with a final extension at 72°C for 10 minutes. The PCR product was analyzed by electrophoresis in 2% agarose gel and visualized by ethidium bromide staining. The amplification products were digested with the restriction enzyme Bsl-I (55°C for 2 hours). The digested fragments were separated by gel electrophoresis on a 4.5% agarose gel and visualized by ethidium bromide staining. The amplified product was expected to have a band of 99 bp for the PAI-1 5G allele and 98 bp for the PAI-1 4G allele, which were estimated from known molecular weight markers run in one of the streets. After the enzyme treatment, the PAI-1 5G allele presented two fragments of 77 and 22 bp, while the PAI-1 4G allele presented a single band of 98 bp.

The TNF-α rs1800629 G/A polymorphism was amplified using the forward 5'-AGGCAATAGGTTTTGAGGGCCAT-3' and the reverse 5´-ACACTCCCCATCCTCCCGGCT -3' primers. A 50 µl reaction mix contained 100 ng de DNA, 0,5 µmol/l each primers, 0,2 mmol/l dNTP, 1x buffer, 2,5 mmol/l $MgCl_2$ and 1U Taq polymerase. The PCR conditions used were as follows: a denaturing step of 95°C for 5 minutes and then 38 cycles of 95°C for 1 minute, 65°C for 45 seconds and 72°C for 1 minute 45 seconds, with a final extension at 72°C for 10 minutes. The PCR product was analyzed by electrophoresis in 2% agarose gel and visualized by ethidium bromide staining. The amplification products were digested with the restriction enzyme Nco-I (37°C for 4 hours). The digested fragments were separated by gel electrophoresis on a 4.5% agarose gel and visualized by ethidium bromide staining. The amplified product was expected to have a band of 100 bp for the TNF-α G allele and 107 bp for the TNF-α A allele. After the enzyme treatment, the TNF-α G allele presented two fragments of 80 bp and 20 bp, while the TNF-α A allele presented a single band of 107 bp.

Simultaneously three controls (homozygous normal, heterozygous, homozygous mutant) and white (lacking DNA) for each polymorphism were run.

Data analysis

Statistical analysis was performed by using SPSS for Windows (IBM, Chicago, IL, USA.). As the data were not normally distributed, they are reported as the median and percentiles (25 and 75) or percentage. Non-parametric tests, such as the U-Mann-Whitney test, were used to compare quantitative data, and the Chi-square test was used to compare proportions. A stepwise multiple regression analysis was performed to test the joint effect of different univariate predictors added to the model with $p < 0.01$ considered statistically significant**.**

Results

Among patients diagnosed with sepsis, the respiratory system was the most frequent site of infection (50.9%), followed by the central nervous system (20.5%), the skin and soft tissue (14.3%), and other sites (14.3%). The most frequent pathogens isolated were bacterial (59.0%: 27.3% Gram-positive, 13.7% Gram-negative and 18.0% *Mycobacterium tuberculosis*), followed by viral (6.2%), fungal (8.1%), and parasitic (0.6%). A microorganism could not be isolated in 26.1% of patients.

The distribution of both genotypes among the 214 control subjects was in Hardy-Weinberg equilibrium (PAI-1 rs1799889 4G/5G polymorphism: $\chi2 = 1.0156$, $p = 0.3136$; TNF-α rs1800629 G/A polymorphism: $\chi2 = 0.2232$, $p = 0.6366$).

Notably, the TNF-α A allele frequency (0.08 in sepsis versus 0.09 in controls) and the PAI-1 4G allele frequency (0.42 in sepsis versus 0.46 in controls) showed no statistical differences between septic patients and subjects in the control group (Table 1).

Table 2 describes the most relevant findings in our 166 septic patients, grouped according to their outcome (survival or death).

To predict mortality at time zero, we used a multivariate model that included accessible variables at time zero, including the independent variables age

Table 1. Genotype (%) distribution and allele frequency among the control group and septic patients

	Control group n = 214	**Septic patients n =166**	*p*
PAI-1 Polymorphism (%)			
5G/5G	30.8	40.6	
4G/5G	46.3	35.2	0.068
4G/4G	22.9	24.2	
4G allele frequency	0.46	0.42	0.501
TNF-α polymorphism (%)			
TNF1/TNF1	83.4	85.9	
TNF1/TNF2	16.1	12.9	0.504
TNF2/TNF2	0.5	1.2	
TNF2 allele frequency	0.09	0.08	0.873

PAI-1: Plasminogen Activator Inhibitor-1; **TNF-α**: Tumor Necrosis Factor-α

Table 2. Characteristics of patients with sepsis, grouped according to their outcome

Median (quartiles) or %	**Survived** (n = 86)	**Died** (n = 80)	*p*
♦ Patients characteristics			
Gender female (%)	41.9	45.3	0.657
Age (years old)	38 (26-50)	51 (31-62)	0.001
HIV+ (%)	16.3	33.8	0.01
Associated co-morbidities (%)			0.517
Any	11.6	12.0	
1-2	55.8	45.3	
3-4	29.1	36.0	
5 or more	3.5	6.7	
♦ Sepsis characteristics			
ICU length of stay (days)	10 (7-14)	6 (3-12)	0.004
Septic shock (%)	24.4	64.0	<0.001
♦ Severity scores (at time zero)			
APACHE	13 (9-18)	19 (13-25)	<0.001
SOFA	4 (2-7)	7 (5-10)	<0.001
♦ Plasma tests (at time zero)			
D-dimer	2.88 (1.47-7.38)	4.32 (2.20-9.12)	0.017
Protein C (%)	63.5 (46.3-86.8)	46.0 (19.0-80.0)	0.004
PAI-1 (UA/l)	16.5 (9.6-30.2)	33.0 (18.8-77.5)	<0.001
♦ PAI-1 polymorphism			0.051
5G/5G (%)	48.8	31.1	
4G/5G (%)	32.6	37.8	
4G/4G (%)	18.6	31.1	
♦ Allele 4G frequency	0.35	0.50	0.078
♦ TNF-α polymorphism			0.400
TNF1/TNF1 (%)	84.3	86.7	
TNF1/TNF2 (%)	13.3	13.3	
TNF2/TNF2 (%)	2.4	0	
♦ Allele TNF2 frequency	0.09	0.07	0.861

HIV+: patients carrying human immunodeficiency virus; **ICU:** Intensive Care Unit**; APACHE:** Acute Physiology and Chronic Health Evaluation II score; **SOFA:** Sequential Organ Failure Assessment score**; PAI-1:** Plasminogen Activator Inhibitor-1; **TNF-α**: Tumor Necrosis Factor-α

(years old), HIV+, SOFA, APACHE, PC (%), PAI-1 (UA/l), D-dimer (μg/ml), and the PAI-1 4G allele (Table 2, $p < 0.1$). To obtain a useful model, we transformed some variables into categorical variables using cut-off values corresponding to the 50th percentile of the survived group: age[38], D-dimer[2.9], PC[63], PAI-1[16], SOFA[4] and APACHE[13]. To predict mortality at time zero, we used a conditional logistic regression analysis (forward conditional) with these dichotomized variables. The regression used four steps ($p < 0.01$). Step 1 included SOFA[4]; step 2 included SOFA[4] and PAI-1[16]; step 3 included SOFA[4], PAI-1[16] and HIV+; and, finally, step 4 included SOFA[4], PAI-1[16], HIV+ and the PAI-1 4G allele. Table 3 shows the odds ratios and the corresponding confidence intervals of the variables in the final model (step 4). In total, 76.4% of deaths but only 55.6% of surviving patients could be predicted with a SOFA score greater than 4 at time 0, with a global prediction of 65.4% (step 1). However, when we combined a SOFA of ≥ 4 and the presence of the PAI-1 4G allele, in combination with plasma levels of PAI-1 ≥ 16 (UA/l) and the HIV+ status, the global prediction rose to 71.9%, with a prediction of survival of 74.1% and a prediction of death of 69.4% (step 4, contingence table in regression analysis).

Discussion

Despite continuing improvements in the understanding of both the molecular basis for and treatment strategies in sepsis, mortality rates remain very high [4-6] in both adult and pediatric populations [34]. In this study, half of our septic subjects died. Most of these patients died shortly after admission, and mostly of septic shock. Differences in the rate and etiology of sepsis have been reported in different countries, mainly between developed and developing counties although differences can also be found among industrialized nations [31,34]. In a retrospective cohort study of adult patients with septic shock, the time of initiation of effective antimicrobial therapy was the single strongest predictor of in-hospital mortality [35-36]. In addition, the failure to initiate appropriate therapy within a given time period correlates with an increased morbidity and mortality; however, this critical point often cannot be met by ICU physicians as patients do not arrive at the hospital in a timely manner. In this situation, the clinical outcome depends to a great extent on the patients' genetics. Many studies have demonstrated the importance of underlying genetics in modulating the sepsis phenotype [7,17-21], and Sorensen *et al*. established the inheritance pattern of those predisposed to mortality due to infectious disease [37]. This is one of the reasons we decided to evaluate certain polymorphisms in this study.

The study of the association between the most common polymorphism, PAI-1 rs1799889 4G/5G, and the resolution of the septic process has been a predictable focus of interest [29-32]. In fact, our group has previously published an article reporting that the presence of the PAI-1 4G allele was a good predictor of a fatal outcome [38]. In concordance with other publications, we showed that the PAI-1 rs1799889 4G/5G polymorphism does not predispose to disease, but is associated with the severity and outcome of the disease. Although the association with diseases may always be due to linkage disequilibrium to other functional polymorphisms, this is unlikely in view of the extensive data confirming the functional effects of the PAI-1 rs1799889 4G/5G polymorphism.

Of all the molecules/factors involved in the septic process, TNF-α and IL-1 were considered critical mediators of septic shock. In fact, it was noted that the persistence of TNF-α and IL-6 in the serum, rather than peak levels of these cytokines, predicted a poor outcome in patients with septic shock. [7,9,39-40]. The TNF-α rs1800629 G/A polymorphism has been associated with variations in the TNF-α levels, and may, therefore, have an effect on the severity and outcome in the ICU. The TNF-α A allele is associated with an increased risk of death in children. Due to insufficient power, this finding could not be confirmed in a separate older patient group. The relative importance of this known polymorphism is still under discussion [35]. In fact, we found no association between TNF-α rs1800629 G/A polymorphism and either the disease outcome or the susceptibility to infection.

Any complex disease, such as sepsis, cannot be explained only by a variation in one or two genes alone, but by a variation in many genes, where the effect of any single variant is expected to be small (odds ratio < 2). Moreover, the sample size required to detect even moderate genetic effects is large [41-43]. However, in our study the PAI-1 4G allele conferred a greater than two-fold increase in risk of death. The PAI-1 rs1799889 4G/5G polymorphism (a common genetic variant) will emerge as a key component in a comprehensive understanding of many infectious diseases. In contrast, the contribution of the TNF-α rs1800629 G/A polymorphism to the

Table 3. Conditional Logistic Regression analysis (forward conditional) to predict death at time zero in 166 patients with sepsis

	Multivariate model	
	Odds ratio (95% CI)	**p values**
HIV+	3.41 (1.39-8.37)	0.008
Allele 4G	2.61 (1.22-5.58)	0.013
SOFA[4]	3.98 (1.84-8.59)	< 0.001
PAI-1[16]	3.88 (1.74-8.62)	0.001

HIV+: patients carrying human immunodeficiency virus; **SOFA:** Sequential Organ Failure Assessment score**; CI:** confidence interval; **PAI-1:** Plasminogen Activator Inhibitor-1

outcome, if any, may be too small and the sample size required to detect the same effect could be large.

As mentioned, in severe sepsis, and particularly in septic shock, fibrinolysis and coagulation inhibitors seem to be exhausted, as implied by a marked decrease in plasminogen and the main coagulation inhibitors, such as PC and antithrombin. In addition, PAI-1 levels were further increased [2,8,14,16]. The impairment in fibrinolysis and coagulation inhibitors appears to be independent of the causative infectious pathogen [22-24]. In fact, we showed that the D-dimer as a coagulation marker and the PAI-1 levels were markedly elevated in patients with poor outcome in comparison to patients who survived, and PC showed the lowest levels. However, only PAI-1 levels remain predictors of a fatal outcome at time zero, although the D-dimer or PC levels remain the known and favorite markers in many papers. PAI-1 is also a potent inhibitor of the PC pathway, and there are many reports connecting high levels of PAI-1 to the progression to septic shock and/or a poor outcome, mainly in meningococcal disease [10,16,22-23].

Chronic co-morbid medical conditions are readily identifiable clinical variables associated with acute organ dysfunction and a worse prognosis. In fact, socioeconomic status is also an influential factor [3,31]. Nevertheless, our work showed that obesity and classical co-morbidities (diabetes, hypertension, dyslipidemia, tobacco use, atherothrombotic diseases, coinfection, inactivity) were similar in patients who survived in comparison to patients who died, though none of these variables were associated with death. On the contrary, an HIV+ status conferred a high risk of death. This is not surprising bearing in mind that our hospital is an HIV reference center and the HIV patients who died in our work never received highly active antiretroviral therapy, and consequently they had AIDS.

The APACHE and, mainly, the SOFA scores allow quantifying the severity of the patient's illness. The SOFA was developed based on the degree of organ dysfunction. However, some publications have questioned the reliability of these scoring systems [12-13]. As expected, both the APACHE and SOFA scores were higher in patients with a poor outcome than in patients who survived, but only the SOFA score proved to be a predictor of death at time zero. Moreover, the PAI-1 4G allele has proved to be a better predictor of death than the APACHE score at time zero. The SOFA score was determined to be a good predictor of survival or death, although the predictive value improved significantly when combined with the allele data. In addition, APACHE II is expected to be less useful than the SOFA score in predicting death at a time point in the first 24 hours.

One limitation of our prediction model is the fact that although the global prediction and prediction of survival increased, the prediction of death mildly decreased. These observations may therefore represent an important limitation of the described prediction mathematical model.

As a whole, at time zero, a SOFA score greater than 4, the presence of the PAI-1 4G allele, PAI-1 levels up to 16 (UA/l) and an HIV positive status could represent strong predictors of death. Neither the PC value, nor the D-dimer measurement, nor the APACHE score at time zero improved the prediction of death.

In the future, ICU scores may be further improved by adding data from certain genomic or plasma levels. The PAI-1 rs1799889 4G/5G polymorphism could represent a genetic marker for an increased mortality risk among septic patients; its predictive ability can be used to anticipate disease and recommend appropriate therapy, even outside critical care medicine. In the near future, knowledge

of genetic factors might help identify "patients at risk of death".

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References

1. Carlet J, Cohen J, Calandra T, Opal SM, Masur H (2008) Sepsis: time to reconsider the concept. Crit Care Med 36: 964-966.
2. Riedemann NC, Guo RF, Ward PA (2003) The enigma of sepsis. J Clin Invest 112: 460-467.
3. O'Brien JM, Jr., Ali NA, Aberegg SK, Abraham E (2007) Sepsis. Am J Med 120: 1012-1022.
4. Dombrovskiy VY, Martin AA, Sunderram J, Paz HL (2007) Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003. Crit Care Med 35: 1244-1250.
5. Moreno RP, Metnitz B, Adler L, Hoechtl A, Bauer P, Metnitz PG (2008) Sepsis mortality prediction based on predisposition, infection and response. Intensive Care Med 34: 496-504.
6. Esteban A, Frutos-Vivar F, Ferguson ND, Peñuelas O, Lorente JA, Gordo F, Honrubia T, Algora A, Bustos A, García G, Diaz-Regañón IR, de Luna RR. (2007) Sepsis incidence and outcome: contrasting the intensive care unit with the hospital ward. Crit Care Med 35: 1284-1289.
7. Winning J, Claus RA, Huse K, Bauer M (2006) Molecular biology on the ICU. From understanding to treating sepsis. Minerva Anestesiol 72: 255-267.
8. Aird WC (2003) The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood 101: 3765-3777.
9. O'Dwyer MJ, Mankan AK, Stordeur P, O'Connell B, Duggan E, White M, Kelleher DP, McManus R, Ryan T (2006) The occurrence of severe sepsis and septic shock are related to distinct patterns of cytokine gene expression. Shock 26: 544-550.
10. Emonts M, Hazelzet JA, de Groot R, Hermans PW (2003) Host genetic determinants of Neisseria meningitidis infections. Lancet Infect Dis 3: 565-577.
11. Vosylius S, Sipylaite J, Ivaskevicius J (2004) Sequential organ failure assessment score as the determinant of outcome for patients with severe sepsis. Croat Med J 45 :715-720.
12. Arts DG, de Keizer NF, Vroom MB, de Jonge E (2005) Reliability and accuracy of Sequential Organ Failure Assessment (SOFA) scoring. Crit Care Med 33: 1988-1993.
13. Zygun DA, Laupland KB, Fick GH, Sandham JD, Doig CJ (2005) Limited ability of SOFA and MOD scores to discriminate outcome: a prospective evaluation in 1,436 patients. Can J Anaesth 52: 302-308.
14. Samransamruajkit R, Hiranrat T, Prapphal N, Sritippayawan S, Deerojanawong J, Poovorawan Y (2007) Levels of protein C activity and clinical factors in early phase of pediatric septic shock may be associated with the risk of death. Shock 28: 518-523.
15. H Heper Y, Akalin EH, Mistik R, Akgöz S, Töre O, Göral G, Oral B, Budak F, Helvaci S (2006) Evaluation of serum C-reactive protein, procalcitonin, tumor necrosis factor alpha, and interleukin-10 levels as diagnostic and prognostic parameters in patients with community-acquired sepsis, severe sepsis, and septic shock. Eur J Clin Microbiol Infect Dis 25: 481-491.
16. Mesters RM, Florke N, Ostermann H, Kienast J (1996) Increase of plasminogen activator inhibitor levels predicts outcome of leukocytopenic patients with sepsis. Thromb Haemost 75: 902-907.
17. Tang BM, McLean AS, Dawes IW, Huang SJ, Lin RC (2007) The use of gene-expression profiling to identify candidate genes in human sepsis. Am J Respir Crit Care Med 176: 676-684.
18. Texereau J, Pene F, Chiche JD, Rousseau C, Mira JP (2004) Importance of hemostatic gene polymorphisms for susceptibility to and outcome of severe sepsis. Crit Care Med 32 Suppl 5: 313-319.
19. Balding J, Healy CM, Livingstone WJ, White B, Mynett-Johnson L, Cafferkey M, Smith OP (2003) Genomic polymorphic profiles in an Irish population with meningococcaemia: is it possible to predict severity and outcome of disease? Genes Immun 24: 533-540.
20. Holmes CL, Russell JA, Walley KR (2003) Genetic polymorphisms in sepsis and septic shock: role in prognosis and potential for therapy. Chest 124: 1103-1115.
21. Tang BM, McLean AS, Dawes IW, Huang SJ, Cowley MJ, Lin RC (2008) Gene-expression profiling of gram-positive and gram-negative sepsis in critically ill patients. Crit Care Med 36: 1125-1128.
22. Raaphorst J, Johan Groeneveld AB, Bossink AW, Erik Hack C (2001) Early inhibition of activated fibrinolysis predicts microbial infection, shock and mortality in febrile medical patients. Thromb Haemost 86: 543-549.
23. Zeerleder S, Schroeder V, Hack CE, Kohler HP, Wuillemin WA (2006) TAFI and PAI-1 levels in human sepsis. Thromb Res 118: 205-212.
24. Mavrommatis AC, Theodoridis T, Economou M, Kotanidou A, El Ali M, Christopoulou-Kokkinou V, Zakynthinos SG (2001) Activation of the fibrinolytic system and utilization of the coagulation inhibitors in sepsis: comparison with severe sepsis and septic shock. Intensive Care Med 27: 1853-1859.
25. Kornelisse RF, Hazelzet JA, Savelkoul HF, Hop WC, Suur MH, Borsboom AN, Risseeuw-Appel IM, van der Voort E, de Groot R (1996) The relationship between plasminogen activator inhibitor-1 and proinflammatory and counterinflammatory mediators in children with meningococcal septic shock. J Infect Dis 173: 1148-1156.
26. Renckens R, Roelofs JJ, Bonta PI, Florquin S, de Vries CJ, Levi M, Carmeliet P,mvan't Veer C, van der Poll T (2007) Plasminogen activator inhibitor type 1 is protective during severe Gram-negative pneumonia. Blood 109: 1593-1601.
27. Yan SB, Helterbrand JD, Hartman DL, Wright TJ, Bernard GR (2001) Low levels of protein C are associated with poor outcome in severe sepsis. Chest 120: 915-922.
28. Mira JP, Cariou A, Grall F, Delclaux C, Losser MR, Heshmati F, Cheval C, Monchi M, Teboul JL, Riché F, Leleu G, Arbibe L, Mignon A, Delpech M, Dhainaut JF (1999) Association of TNF2, a TNF-alpha promoter polymorphism, with septic shock susceptibility and mortality: a multicenter study. JAMA 282: 561-568.
29. G Geishofer G, Binder A, Müller M, Zöhrer B, Resch B, Müller W, Faber J, Finn A, Endler G, Mannhalter C, Zenz W (2005) 4G/5G promoter polymorphism in the

plasminogen-activator-inhibitor-1 gene in children with systemic meningococcaemia. Eur J Pediatr 164: 486-490.
30. Haralambous E, Hibberd ML, Hermans PW, Ninis N, Nadel S, Levin M (2003) Role of functional plasminogen-activator-inhibitor-1 4G/5G promoter polymorphism in susceptibility, severity, and outcome of meningococcal disease in Caucasian children. Crit Care Med 31: 2788-2793.
31. García-Segarra G, Espinosa G, Tassies D, Oriola J, Aibar J, Bové A, Castro P, Reverter JC, Nicolás JM (2007) Increased mortality in septic shock with the 4G/4G genotype of plasminogen activator inhibitor 1 in patients of white descent. Intensive Care Med 33: 1354-1362.
32. H Hermans PW, Hibberd ML, Booy R, Daramola O, Hazelzet JA, de Groot R, Levin M (1999) 4G/5G promoter polymorphism in the plasminogen-activator-inhibitor-1 gene and outcome of meningococcal disease. Meningococcal Research Group. Lancet 354: 556-560.
33. Jessen KM, Lindboe SB, Petersen AL, Eugen-Olsen J, Benfield T (2007) Common TNF-alpha, IL-1 beta, PAI-1, uPA, CD14 and TLR4 polymorphisms are not associated with disease severity or outcome from Gram negative sepsis. BMC Infect Dis 7: 108.
34. Wolfler A, Silvani P, Musicco M, Antonelli M, Salvo I (2008) Incidence of and mortality due to sepsis, severe sepsis and septic shock in Italian Pediatric Intensive Care Units: a prospective national survey. Intensive Care Med 34: 1690-1697.
35. G Garnacho-Montero J, Aldabo-Pallas T, Garnacho-Montero C, Cayuela A, Jiménez R, Barroso S, Ortiz-Leyba C (2006) Timing of adequate antibiotic therapy is a greater determinant of outcome than are TNF and IL-10 polymorphisms in patients with sepsis. Crit Care 10(4): R111.
36. Kumar A, Roberts D, Wood KE, Light B, Parrillo JE, Sharma S, Suppes R, Feinstein D, Zanotti S, Taiberg L, Gurka D, Kumar A, Cheang M (2006) Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 34:1589-1596.
37. Sorensen TI, Nielsen GG, Andersen PK, Teasdale TW (1988) Genetic and environmental influences on premature death in adult adoptees. N Engl J Med 318: 727-732.
38. Wingeyer SP, de Larranaga G, Cunto E, Fontana L, Nogueras C, San Juan J (2010) Role of 4G/5G promoter polymorphism of Plasminogen Activator Inhibitor-1 (PAI-1) gene in outcome of sepsis. Thromb Res 125: 367-369.
39. Imahara SD, O'Keefe GE (2004) Genetic determinants of the inflammatory response. Curr Opin Crit Care 10: 318-24.
40. Menges T, König IR, Hossain H, Little S, Tchatalbachev S, Thierer F, Hackstein H, Franjkovic I, Colaris T, Martens F, Weismüller K, Langefeld T, Stricker J, Hempelmann G, Vos PE, Ziegler A, Jacobs B, Chakraborty T, Bein G (2008) Sepsis syndrome and death in trauma patients are associated with variation in the gene encoding tumor necrosis factor. Crit Care Med 36: 1456-1462, e1-6.
41. Orr N, Bekker V, Chanock S (2006) Genetic association studies: marking them well. J Infect Dis 194: 1475-1477.
42. Cooper DN, Nussbaum RL, Krawczak M (2002) Proposed guidelines for papers describing DNA polymorphism-disease associations. Hum Genet 110: 207-208.
43. El-Serag HB, White DL, Mitra N (2008) Genetic association studies: from "searching under the lamppost" to "fishing in the pond". Gastroenterology 134: 662-664.

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