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Highlights

- Organ failure extent in sepsis is linked to gradual transcriptomic changes in blood.
- These changes involve mostly 55 genes of cell cycle, innate and adaptive immunity.
- These changes influence patients' outcome.
- Assessing them could help to evaluate both disease severity and immunity in sepsis.

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Transcriptomic correlates of organ failure extent in sepsis

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KEYWORDS

Sepsis;
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Summary Objectives: Sepsis is characterised by the frequent presence of organ failure and marked immunologic alterations. We studied the association between the extent of organ failure and the transcriptomic response of septic patients.

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Organ failure extent;
SOFA;
Immune response;
Immunosuppression;
Microarrays

Methods: Gene expression profiles in the blood of 74 surgical patients with sepsis were compared with those of 30 surgical patients with no sepsis. Differentially expressed genes were assessed for their correlation with the sequential organ failure (SOFA) score.

Results: The expression levels of a group of genes participating in the cell cycle (HIST1H1C, CKS2, CCNA2, CDK1, CCNB2, CIT, CCNB1, AURKA, RAD51), neutrophil protease activity (ELANE, ADORA3, MPO, MMP8, CTSG), IL-1R and IL-18R response correlated directly with SOFA and mortality. Genes involved in T cell (LCK, CD3G, CD3D, ZAP70, ICOS, CD3E, CD28, IL2RB, CD8B, CD8A, CD40LG, IL23A, CCL5, SH2D1A, ITK, CD247, TBX21, GATA3, CCR7, LEF1, STAT4) and NK cell immunity (CD244, KLRK1, KLRD1) were inversely associated with SOFA and mortality.

Conclusions: The extent of organ failure in sepsis correlates directly with the existence of imbalanced innate and adaptive responses at the transcriptomic level. Quantification of the expression levels of the genes identified here could contribute to the simultaneous assessment of disease severity and immunological alterations in sepsis.

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Introduction

Introduction

Sepsis is defined as a systemic inflammatory response to infection and is a major health problem in Europe and worldwide.¹ Sepsis occurs in approximately 2% of all hospitalised patients in developed countries.² Sepsis is frequently complicated by the presence of organ dysfunction, leading to severe sepsis and, in its most severe form, septic shock, which is characterised by persistent hypotension despite adequate fluid resuscitation.³ In general, more than 50% of severe sepsis patients will require intensive care services (ICU).²

An evaluation of the extent of organ failure is mandatory in the management of septic patients because it provides a good indication of the patient's illness severity. This knowledge influences important clinical decisions, treatment, and prognosis. The extent of organ failure in septic patients is routinely evaluated using the sequential organ failure assessment (SOFA) score, which addresses the respiratory system, central nervous system, cardiovascular system, renal system, liver function and coagulation.^{4,5} The SOFA score presents a good correlation to the ICU outcome, with mortality rates ranging from 3.2% in patients without organ failure to 91.3% in patients with failure of all six organs analysed.⁶

Sepsis is characterised by the presence of marked immunologic alterations that are linked to the existence of an important state of immunosuppression that parallels this severe condition.⁷ The degree of sepsis-associated immunosuppression seems also to deeply influence the prognosis of this disease.⁸ Interestingly, no previous works have evaluated the relationship between the extent of organ failure and the host immune response in sepsis. Gene expression analysis in the blood is a useful tool to study the host immune response to severe infections, including sepsis.^{9–11} In this study, we performed a transcriptomic analysis to identify leucocyte-related expression signatures that are linked to disease severity in sepsis. We have identified a set of 55 genes that participate in the immune response to infection with expression levels that are closely associated to the SOFA score and prognosis in these patients.

Materials and methods

Study design

The EXPRESS study (Gene Expression in Sepsis) was an observational prospective study aimed at evaluating gene expression profiles in patients with sepsis performed at the surgical ICU of Hospital Clínico Universitario de Valladolid, Spain, from April 2012 to April 2013.

Patient selection

During the observation period, 104 patients undergoing surgery were recruited. Seventy-four of these patients presented with sepsis following the definition of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference,³ while 30 patients showed Systemic Inflammatory Response Syndrome (SIRS) with no sepsis (control group). Fifteen healthy volunteers of similar ages to the patients were recruited from the staff of the Hospital Clínico Universitario de Valladolid, Spain for gene expression data normalisation. Approval of the study protocol for both the scientific and ethical aspects was obtained from the Scientific Committee for Clinical Research of Hospital Clínico Universitario, Valladolid, Spain. Informed consent was obtained directly from each patient or legal representative before enrolment.

Clinical data and treatment

A specific standard survey was employed to collect the clinical data, including medical history, physical examination and haematological, biochemical, radiological and microbiological investigations. Treatment decisions were not standardised for all patients but were made by the treating physician.

Microbial diagnosis

When infection was suspected, samples were extracted and sent to the Microbiology Service, where they were routinely Gram stained and cultured on general purpose media (blood agar, chocolate agar, and the differential media McConkey

agar and Chapman agar). Fungal infections were screened by culturing sputum samples on Sabouraud agar containing chloramphenicol.

Sample collection

A sample of 2.5 mL of blood was collected in the first 24 h following clinical diagnosis of SIRS or sepsis using PaxGene venous blood vacuum collection tubes (Becton Dickinson, USA).

Microarray processing

Total RNA was extracted from blood samples using the PAXgene Blood RNA System (PreAnalytix, Hombrechtikon, Switzerland). RNA was quantified by spectrometry (NanoDrop ND1000, NanoDrop Technologies, Wilmington, Delaware USA), and RNA quality was confirmed by an RNA Experion Bioanalyser (BioRad, California USA) assay. Up to 1750 ng of each RNA sample was concentrated with the RNeasy MinElute Cleanup kit (QIAGEN, Hilden, Germany). RNA was eluted with 10 μ L of RNase-free H₂O. One hundred nanograms of purified total RNA was used to produce cyanine 3-CTP-labelled cRNA using the Quick Amp Labelling kit (Agilent p/n 5190–0442) according to the manufacturer's instructions. Following the 'One-Colour Microarray-Based Gene Expression Analysis' protocol Version 5.7 (Agilent p/n 4140–90040), 3 μ g of labelled cRNA was hybridised with the Whole Human Genome Oligo Microarray Kit (Agilent p/n G2519F-014850) containing 41,000 + unique human genes and transcripts. Arrays were scanned in an Agilent Microarray Scanner (Agilent G2565BA) according to the manufacturer's protocol, and data were extracted using Agilent Feature Extraction Software 9.5.3 following the Agilent protocol GE1-v5_95_Feb07 and the QC Metric Set GE1_QCMT_Jan08. The resulting microarray data sets were uploaded at the Array Express microarray data repository (E-MTAB-1548). Changes in microarray gene expression for representative genes of our analysis were verified by qPCR using Real-time Ready plates purchased from Roche (Roche Applied Science, Germany), using β -actin as the reference (housekeeping) gene, per the manufacturer's protocol.

Data analysis

Data analysis was performed using GeneSpring GX 12.0 software. The original data were cleaned and normalised in three steps: 1) local background was subtracted from the individual spot intensity; 2) log-transformed signal intensity values were globally normalised using the percentile shift algorithm, shifting to the 75th percentile of each sample, for per chip normalisation; and 3) baseline transformation of the data was performed using the median of the control samples. Before statistical analyses, all microarrays were subjected to quality and filtering criteria. The quality of the microarray data was assessed on Principal Component analysis (PCA) plots. Student's t-tests (GeneSpring GX 12.0) were used to identify genes that were differentially expressed between the sepsis and no sepsis groups at a level of significance $p < 0.05$, with Benjamini-Hochberg multiple

testing corrections and fold changes ≥ 1.5 . Ingenuity pathway analysis (IPA) (Ingenuity Systems-Qiagen, Redwood City, CA) was used to select, annotate and visualize genes by function and pathway.

Statistical analysis

Differences between patients with and without sepsis in clinical variables were assessed using a χ^2 test for categorical variables and t-test for continuous variables when appropriate. Gene expression data did not followed a normal distribution, as assessed by the Shapiro Wilk test. Correlations between the SOFA score and gene expression levels were assessed using the Spearman–Kärber test. Differences in expression levels between survivors and non-survivors were assessed using the Mann Whitney U test. Data analysis was performed using SPSS for WINDOWS version 20.0 software (IBM-SPSS, Chicago, IL, USA). The heat map in Fig. 3 was constructed using the JColorGrid software (University of California San Francisco and University of California Berkeley).¹² This software enables simultaneous representation of multiple correlations, which are otherwise difficult to represent using classical dot plot graphics.

Results

Clinical characteristics (Table 1)

Patients in both groups were comparable in terms of age, sex and comorbidity frequency. The group of patients with sepsis underwent urgent surgery much more frequently than the group of patients with no sepsis. Patients with sepsis stayed longer at the ICU and the hospital. These patients presented with more severe disease, as shown in their APACHE and SOFA scores. Three quarters of these patients had septic shock. While none of the patients without sepsis died, 28% of the patients in the septic group did not survive the disease. Acute phase reaction protein (C reactive protein, procalcitonin) and neutrophil levels in the plasma were higher in patients with sepsis than in those with no sepsis. In the sepsis group, the principal presumed sources of infection were the abdomen (37.8%) and respiratory tract (35.1). Of the septic patients, 86.5% had a confirmed microbial diagnosis. In 29.7% of the cases, the infection was caused by Gram-positive bacteria and 43.7% of the cases were caused by Gram-negative bacteria. Finally, 26.6% of the patients had a polymicrobial infection.

Gene expression profiles

A) **Signatures of septic status: differential profiles between septic and non-septic patients:** Patients with sepsis showed 2396 genes that were differentially expressed compared to patients with no sepsis (1364 genes with relatively higher expression levels and 1032 genes with relatively lower expression levels in patients with sepsis compared to patients with no sepsis) (see [Supplementary Data 1](#)). An IPA analysis

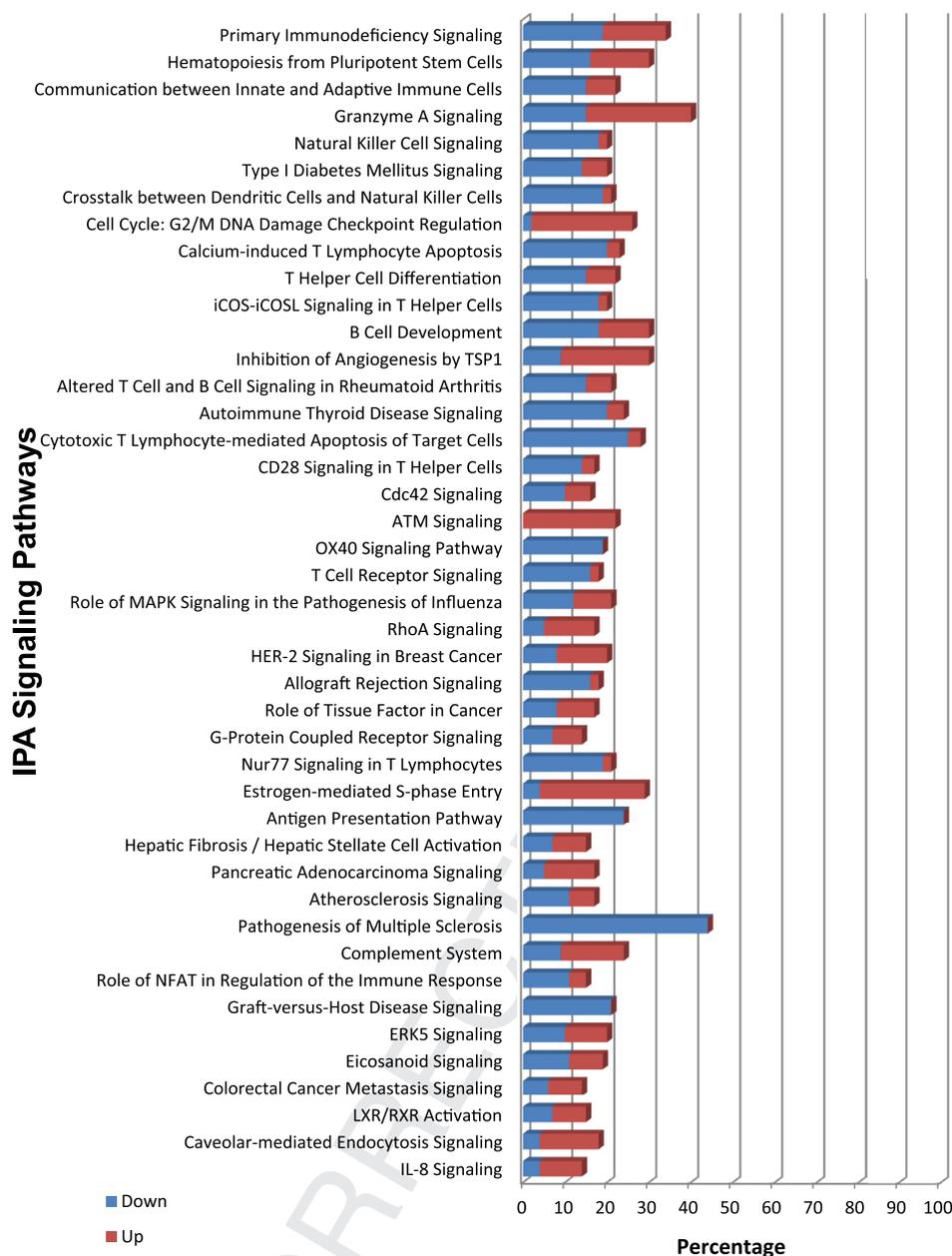


Figure 1 Top intracellular signalling pathways in the comparison of sepsis vs. no sepsis. Histograms represent the percentage of genes with relatively lower expression in the sepsis group (blue) and the percentage of genes with relatively higher expression in the sepsis group (red) over the total number of genes in each pathway.

identified 267 genes participating in 43 major intracellular signalling pathways; most of the genes are involved ontologically in the immune response function (Fig. 1 and Supplementary Data 2). Thus, compared to patients with no sepsis, patients with sepsis showed lower expression levels of a group of genes involved in the development of adaptive immunity that affected the following functions (most representative genes of each function are mentioned).

1) **Antigen presentation**, involving the chemokine receptor CCR7 (−1.5) (gene, fold change sepsis/no sepsis) and the class II HLA molecules (HLA-DRA, −1.9), (HLA-DRB1, −1.7), (HLA-DRB3, −1.5), (HLA-DRB5, −1.7).

2) **T cell biology**, involving the inducible T-cell co-stimulator (ICOS, −1.7); a key signalling molecule in the selection and maturation of developing T cells (LCK, −1.7); a key molecule mediating cytotoxicity by CD8 T lymphocytes, Granzyme A (GZMA, −1.8); a group of genes coding for the TCR/CD3 complex (CD4 (−1.6), CD3D (−1.6), CD3E (−1.9), CD8A (−2.2), CD8B (−2.1), ZAP70 (−1.6); a main co-stimulatory molecule (CD28, −1.8); and finally two major chemokines promoting the mobilisation of mononuclear white blood cells (CCL4, −1.7), (CCL5, −1.6).

3) **Natural killer-mediated response**, involving the v-akt murine thymoma viral oncogene homologue 1

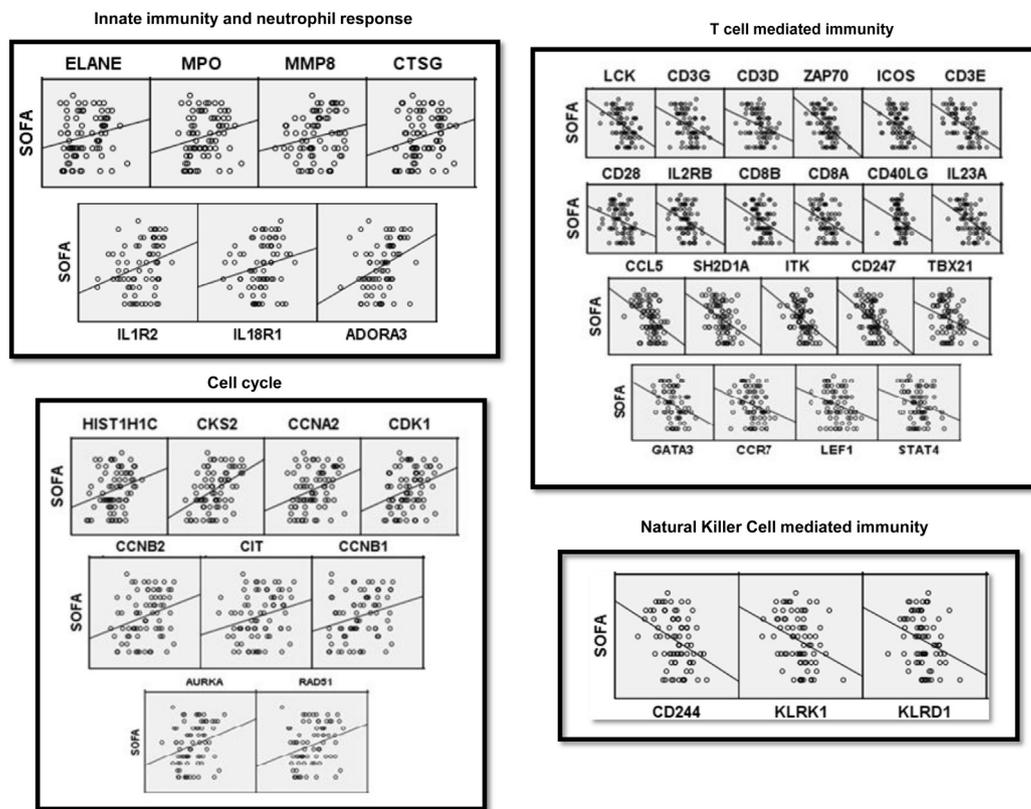


Figure 2 Dot plots showing the correlations between gene expression levels and the SOFA score in the group of septic patients.

(AKT1, -1.7), (CD244, -1.6), (CD247, -1.9), the FYN proto-oncogene (FYN, -1.7), two killer cell immunoglobulin-like receptors (KIR2DL4, -1.7) (KIR3DL1, -1.5) and a group of killer cell lectin-like receptors (KLRB1, -1.7), (KLRC1, -1.8), (KLRC3, -1.6), (KLRC4-KLRK1/KLRK1, -1.8), (KLRD1, -2.2).

4) **Interferon response**, involving key interferon response genes (IFIT1, -3.8), (IFIT2, -2.5) and (IFI44L, -2.4). On the other hand, IPA along with examination of the top genes up-regulated in our analysis revealed that sepsis induced the expression of a number of genes participating in the following functions.

1) **Synthesis of immunoglobulins**, involving the heavy constant chains (IGHG3, 4.9), (IGHG1, 4.4), (IGHA1, 3.6), (IGHA2, 3.6). In addition, 57 genes coding for variable chains of immunoglobulins were upregulated in the group of patients with sepsis (Supplementary Data 1).

2) **The neutrophil-mediated response**, involving proteins contained in the granules of neutrophils (MMP8, 13.9), (LTF, 7.7), (DEFA4, 5.9), (PRTN3, 4.7), (ELANE, 4.5), (BPI, 4.4), (DEFA3, 4.2), (CTSG, 4.1), (MPO, 4.0), (BPI, 3.9), (AZU1, 3.3); a cell surface glycoprotein that plays a role in neutrophil activation, (CD177, 4.7); and a protein promoting inhibition of neutrophil degranulation (ADORA3, 2.3).

3) **The innate pro-inflammatory response**: (IL1R2, 5.0), (IL18R1, 3.6). When gene expression levels

were compared between patients with Gram + and Gram - infections, no significant differences were found between both groups of patients. Real-time PCR validation supported the results from the microarray analysis (see Supplementary Data 3).

B) **Signatures of disease severity in septic patients**: Of the 267 genes selected by IPA, 72 genes showed a significant correlation between their expression levels and the SOFA score ($p < 0.05$) (see Supplementary Data 4) in the group of septic patients. Fifty-five of these genes had significant differences in their expression levels between survivors and non-survivors in this group (Tables 2 and 3). Two correlation patterns were identified for these 55 genes; the expression level of 22 genes directly correlated with the SOFA score, while 33 genes had expression levels that correlated in an inverse manner with this score (Figs. 2 and 3). In turn, expression levels of those genes participating of innate immunity, neutrophil response and cell cycle correlated inversely with expression levels of genes involved in T cell and NK cell mediated immunity (Fig 3). A description of the entire analysis process is depicted in Fig. 4.

Discussion

This study aimed to identify the transcriptomic impact of organ failure in sepsis. For this analysis, we compared the transcriptomic signatures in the blood of a group of individuals with sepsis against that of a group of patients who

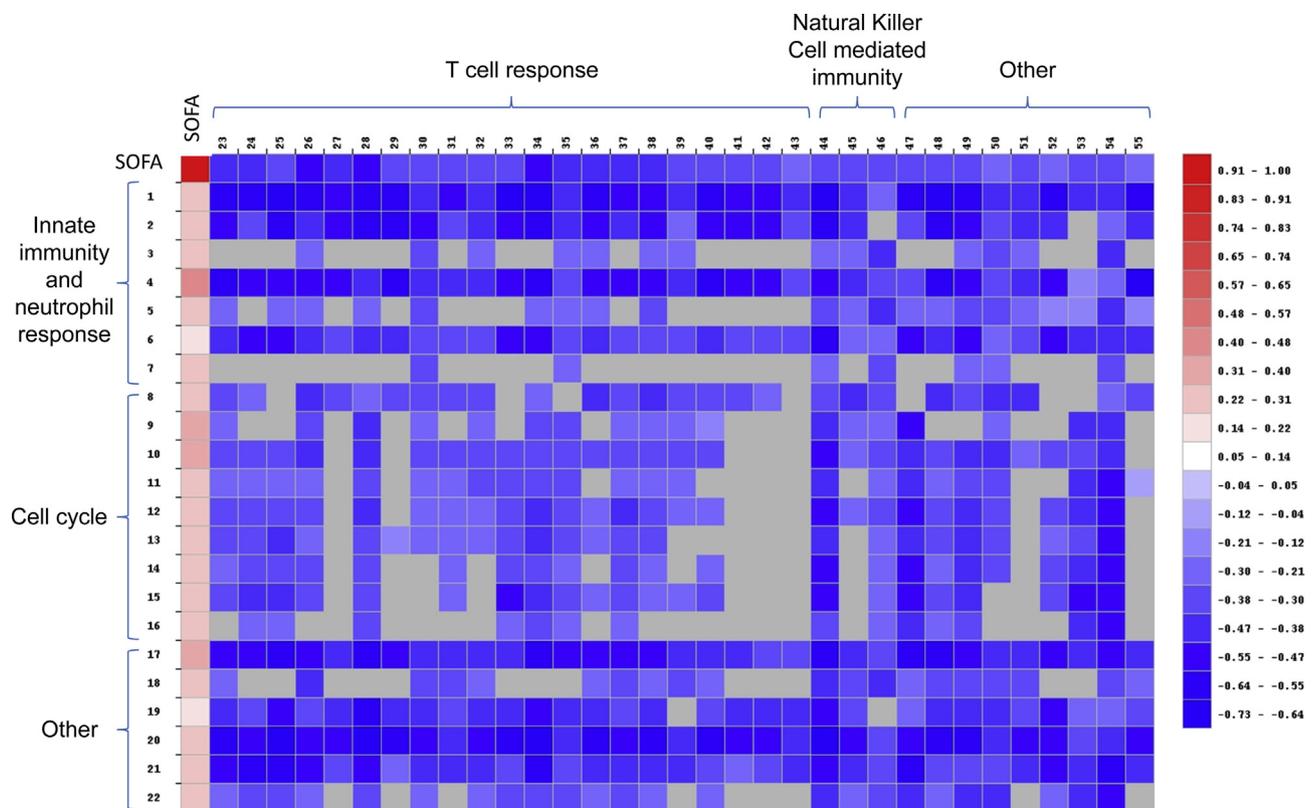


Figure 3 Heat map representing Spearman coefficients for paired correlations involving SOFA score and gene expression levels. Genes included in this figure are those detailed in Tables 2 and 3 (numbered also from 1 to 55). Direct correlations are shown in red, inverse correlation in blue and non-significant correlation in light grey ($p > 0.05$).

did not develop sepsis but who had a non-infectious SIRS at the moment of sample collection in a cohort of patients suffering from major surgery.

Interestingly, we identified a gene expression pattern that could be considered a marker of septic status. This pattern was characterised by the presence of up-regulated expression of a group of genes of innate immunity (neutrophil proteases and the interleukin receptors for IL-1 and IL-18) and up-regulated expression of genes involved in immunoglobulin synthesis. This pattern was complemented by the presence of low expression levels of interferon response genes and genes involved in key events of adaptive immunity, such as antigen presentation, T cell response and NK cell response. The simultaneous coexistence of increased innate and depressed adaptive immunity responses contradicts the classical paradigm of the two consecutive phases in sepsis (an initial exuberant inflammatory response followed by a stage of depressed adaptive immunity).¹³ Our results agree with those of Tang BM et al.¹⁴ In a systematic review of twelve cohorts of patients, these authors did not find any transcriptomic evidence supporting the classic two phase model of sepsis. In addition, the pattern identified in our work seems to represent a common early transcriptomic hallmark of acute organ injury because Xiao W et al. found an almost mimetic signature in white blood cells from critically ill patients suffering from severe blunt trauma.¹⁵

Fifty-five genes in this pattern correlated with the SOFA score in a significant manner and were associated with the

final outcome in septic patients. Although the correlation with SOFA was mild (as evidenced by the Spearman coefficients), the correlation was statistically significant. In fact, with the correlation analysis, we were interested to identify association patterns between the SOFA score and groups of ontologically related genes. The expression levels of genes encoding neutrophil proteases, IL-1 and IL-18 receptors and cell cycle proteins correlated directly with the SOFA score. Parnell GP et al. reported a similar neutrophil signature in patients with sepsis compared to healthy controls.¹¹ Neutrophil proteases contribute to the neutrophil oxygen-independent system-mediated protection of the host against invading pathogens.¹⁶ Although effective in their ability to kill pathogens, they are equally effective at inducing cell and tissue damage.¹⁷ In this sense, Fox ED et al. have recently shown that neutrophils from septic patients mediate a profound loss of endothelial barrier integrity.¹⁸ Moreover, neutrophil serine proteases in concert with externalised nucleosomes promote thrombus formation inside blood vessels,¹⁹ potentially contributing to the pathophysiology of disseminated intravascular coagulation in sepsis (DIC).²⁰ Nonetheless, the increased expression of an inhibitor of neutrophil degranulation, such as ADORA3, could interfere with neutrophil function in patients with sepsis.²¹ In turn, IL-1R and IL-18R are the receptors for two central inflammatory cytokines, IL-1 and IL-18. Johnson Sb et al. also reported a specific increase in the expression of the IL-1/IL-18 receptor family in patients with sepsis.²² Yang H et al. revealed the pro-coagulation

Table 1 Characteristics and outcomes in the 104 surgical patients. APACHE, Acute Physiology and Chronic Health Evaluation; SOFA: Sequential Organ Failure Score; CRP, C reactive protein; INR, international normalized ratio; APTTr, Activated Partial Thromboplastin Time Ratio; ScvO₂, Central venous oxygen saturation; NS, not significant.

		Total cohort (n = 104)	Sepsis (n = 74)	No sepsis (n = 30)	P
Characteristics	Age (y) mean + -SD	69.53 (12.48)	69.71 (13.13)	69.67 (10.22)	n.s
	Gender (M/F)	71/33	50/24	21/9	n.s
Prior or pre-existing conditions, n (%)	High Blood Pressure	57 (54.80)	39 (52.70)	18 (60.00)	n.s
	Chronic cardiovascular disease	54 (51.90)	37 (50.00)	17 (56.67)	n.s
	Chronic respiratory disease	23 (22.1)	17 (22.97)	6 (20.00)	n.s
	Chronic renal failure	10 (9.61)	8 (10.81)	2 (2.70)	n.s
	Chronic hepatic failure	2 (1.90)	1 (1.35)	1 (3.33)	n.s
	Diabetes mellitus	29 (27.90)	19 (25.68)	10 (33.33)	n.s
	Cancer	31 (29.80)	21 (28.38)	10 (33.33)	n.s
	Immunosuppression	6 (5.80)	4 (5.40)	2 (6.67)	n.s
	Obesity	17 (16.30)	11 (14.86)	6 (20.00)	n.s
	Admission category, n (%)	Urgent surgery	49 (47.11)	46 (62.16)	3 (10.00)
Type surgery, n (%)	Cardiac surgery	47 (45.20)	30 (40.54)	17 (56.67)	n.s
	Abdomen surg	49 (47.11)	36 (48.65)	13 (43.33)	n.s
	Cerebral trauma or brain surgery	1 (1.00)	1 (1.35)	0	n.s
	Thoracic surgery	0	0	0	n.s
	Vascular surgery	3 (2.90)	3 (4.05)	0	n.s
	Other surgery	4 (3.80)	4 (5.40)	0	n.s
	Time course and Outcome	Length of hospital stay (d), mean ± SD	28.16 (18.62)	33.29 (18.62)	15.18 (9.56)
Length of ICU stay (d), mean ± SD		9.40 (11.82)	11.90 (12.32)	2.68 (6.22)	<0.001
Septic Shock, n (%)		56 (53.80)	56 (75.68)	0	<0.001
ICU Mortality, n (%)		21 (20.19)	21 (28.38)	0	<0.001
Measurements mean + -SD	APACHE II points	15.90 (5.39)	17.00 (5.41)	12.93 (3.80)	<0.001
	SOFA points	6.84 (3.70)	8.47 (2.85)	2.47 (1.20)	<0.001
	Creatinine (mg/dl)	1.44 (0.98)	1.68 (1.04)	0.82 (0.21)	<0.001
	Total bilirubin (mg/dl)	1.14 (1.32)	1.29 (1.50)	0.68 (0.34)	0.035
	Glucose (mg/dl)	160.60 (63.92)	163.89 (70.47)	146.13 (40.37)	n.s
	Platelet count (cell/mm ³)	162964.65 (96353.63)	168609.59 (119142.66)	173700.00 (76870.31)	n.s
	INR	1.54 (0.15)	1.58 (0.49)	1.46 (0.26)	n.s
	aPTTr	9.03 (18.48)	10.32 (20.23)	4.87 (11.14)	n.s
	Arterial lactate (mEq/L)	59.67 (33.13)	63.16 (34.32)	53.94 (28.37)	n.s
	CRP (mg/L)	165.37 (132.90)	213.65 (124.34)	44.74 (50.98)	<0.001
	ScvO ₂ (%)	72.49 (6.65)	71.27 (10.05)	75.10 (8.17)	n.s
	Procalcitonin (ng/mL)	13.96 (27.03)	18.78 (30.08)	0.57 (0.66)	0.001
	White Blood cells (cells/mm ³)	12991.72 (7512.80)	13843.29 (8196.02)	10512.67 (4210.38)	0.045
	Lymphocytes (cells/mm ³)	1018.79 (572.25)	944.75 (513.75)	1129.52 (688.45)	n.s
	Monocytes (cells/mm ³)	618.51 (365.36)	615.15 (396.56)	613.16 (263.24)	n.s
	Neutrophils (cells/mm ³)	11256.66 (7147.67)	12178.30 (7826.90)	7826.90 (3585.99)	0.026
	Eosinophils (cells/mm ³)	61.00 (101.87)	62.05 (101.31)	62.06 (112.73)	n.s
	Basophils (cells/mm ³)	27.17 (48.98)	30.10 (55.77)	17.51 (18.17)	n.s

role of the IL-1 pathway²³ during severe bacterial infection, which could contribute to the pathogenesis of DIC in sepsis.²⁰ A recent work from Vanden Berghe T et al. has suggested the therapeutic potential of neutralizing IL-1 and IL-18 simultaneously in this disease.²⁴ In turn, the extent of organ failure was directly associated with the expression levels of genes involved in the cell cycle. Alterations in the expression levels of genes participating in the cell cycle

have been observed in severe influenza²⁵ and may represent an attempt to mediate expansion of circulating neutrophil counts paralleling disease severity because neutrophils constitute the main effector arm of the innate immune response against infection. In fact, in our cohort of patients with sepsis, circulating neutrophil counts correlated directly with the SOFA score (Spearman correlation coefficient $r = 0.30$, $p = 0.012$).

Table 2 List of genes showing significant direct correlations between their expression levels and SOFA score. FC: fold change for each gene in the comparison sepsis vs patients with no sepsis. Expression levels in survivors and non survivors are showed as median [interquartile rank] of their values normalized against healthy control group.

Physiological function	Gene symbol	Description	FC Sepsis/No sepsis	Survivor	Non survivor	p value
Innate immunity and neutrophil function	1 IL1R2	Homo sapiens interleukin 1 receptor, type II	4.99	3.81 (2.61)	5.72 (1.22)	0.000
	2 IL18R1	Homo sapiens interleukin 18 receptor 1	3.97	2.75 (1.55)	3.63 (1.08)	0.003
	3 ELANE	Homo sapiens elastase, neutrophil expressed	4.45	2.45 (4.15)	4.75 (3.18)	0.003
	4 ADORA3	Homo sapiens adenosine A3 receptor	2.29	2.73 (1.70)	3.70 (1.20)	0.004
	5 MPO	Homo sapiens myeloperoxidase	3.95	2.01 (3.23)	3.87 (2.93)	0.006
	6 MMP8	Homo sapiens matrix metalloproteinase 8 (neutrophil collagenase)	13.93	6.03 (4.40)	7.19 (2.82)	0.042
	7 CTSG	Homo sapiens cathepsin G	4.13	1.44 (4.00)	3.48 (3.95)	0.024
Cell cycle	8 HIST1H1C	Homo sapiens histone cluster 1, H1c	2.03	0.90 (1.07)	1.65 (1.36)	0.001
	9 CKS2	Homo sapiens CDC28 protein kinase regulatory subunit 2	1.62	1.17 (1.26)	2.20 (1.72)	0.002
	10 CCNA2	Homo sapiens cyclin A2	2.33	1.12 (1.54)	2.26 (1.82)	0.005
	11 CDK1	Homo sapiens cyclin-dependent kinase 1	3.28	2.22 (1.85)	3.25 (2.14)	0.008
	12 CCNB2	Homo sapiens cyclin B2	3.10	1.62 (1.81)	2.83 (1.75)	0.010
	13 CIT	Homo sapiens citron (rho-interacting, serine/threonine kinase 21)	2.44	0.40 (1.52)	1.55 (1.28)	0.002
	14 CCNB1	Homo sapiens cyclin B1	2.49	0.68 (1.97)	1.56 (1.70)	0.023
	15 AURKA	Homo sapiens aurora kinase A	1.75	0.66 (1.11)	1.43 (1.30)	0.026
	16 RAD51	Homo sapiens RAD51 homologue (S. cerevisiae)	1.95	0.51 (1.55)	1.83 (2.14)	0.029
	Other	17 PDE6H	Homo sapiens phosphodiesterase 6H, cGMP-specific, cone, gamma	1.92	1.90 (1.18)	2.94 (0.96)
18 RGS16		Homo sapiens regulator of G-protein signalling 16	1.88	1.24 (1.35)	2.46 (2.50)	0.003
19 GNAQ		Homo sapiens guanine nucleotide binding protein	1.61	1.74 (0.85)	2.18 (0.71)	0.007
20 IRAK3		Homo sapiens interleukin-1 receptor-associated kinase 3	1.84	2.98 (1.31)	3.55 (0.79)	0.010
21 LDLR		Homo sapiens low density lipoprotein receptor	1.61	0.41 (1.48)	1.38 (1.95)	0.020
22 BIRC5		Homo sapiens baculoviral IAP repeat containing 5	3.40	2.08 (2.11)	3.16 (2.02)	0.029

In turn, the expression levels of T and NK cell genes correlated inversely with the extent of organ failure. Depressed expression levels of genes participating in T

and NK cell immunity are a robust signature commonly found in studies addressing gene expression in sepsis in both paediatric and adult patients.^{11,26} These genes are central

Table 3 List of genes showing significant inverse correlations between their expression levels and SOFA score. FC: fold change for each gene in the comparison sepsis vs patients with no sepsis. Expression levels in survivors and non survivors are showed as median [interquartile rank] of their values normalized against healthy control group.

Physiological function	Gene symbol	Description	FC sepsis/No sepsis	Survivor	Non survivor	p value	
T cell function	23	LCK	Homo sapiens lymphocyte-specific protein tyrosine kinase	-1.78	-1.88 (1.48)	-2.76 (0.92)	0.000
	24	CD3G	Homo sapiens CD3g molecule, gamma (CD3-TCR complex)	-1.64	-1.65 (1.70)	-2.71 (1.11)	0.000
	25	CD3D	Homo sapiens CD3d molecule, delta (CD3-TCR complex)	-1.65	-1.14 (1.19)	-1.75 (1.15)	0.000
	26	ZAP70	Homo sapiens zeta-chain (TCR) associated protein kinase 70 kDa	-1.65	-1.84 (1.38)	-2.74 (1.50)	0.000
	27	ICOS	Homo sapiens inducible T-cell co-stimulator	-1.76	-1.56 (1.28)	-2.53 (1.22)	0.001
	28	CD3E	Homo sapiens CD3e molecule, epsilon (CD3-TCR complex)	-1.93	-1.70 (1.28)	-2.85 (0.95)	0.001
	29	CD28	Homo sapiens CD28 molecule	-1.81	-1.73 (1.66)	-2.68 (1.11)	0.003
	30	IL2RB	Homo sapiens interleukin 2 receptor, beta	-2.20	-2.34 (1.23)	-3.05 (1.46)	0.004
	31	CD8B	Homo sapiens CD8b molecule	-2.08	-2.30 (2.21)	-3.45 (1.36)	0.004
	32	CD8A	Homo sapiens CD8a molecule	-2.26	-2.27 (2.19)	-3.16 (1.72)	0.006
	33	CD40LG	Homo sapiens CD40 ligand	-2.23	-2.59 (1.74)	-2.95 (0.49)	0.050
	34	IL23A	interleukin 23, alpha subunit p19	-1.85	-1.68 (1.26)	-2.83 (0.76)	0.000
	35	CCL5	Homo sapiens chemokine (C-C motif) ligand 5	-1.63	-0.83 (1.17)	-1.89 (1.42)	0.001
	36	SH2D1A	Homo sapiens SH2 domain containing 1A	-2.61	-2.40 (1.37)	-3.35 (1.53)	0.002
	37	ITK	Homo sapiens IL2-inducible T-cell kinase	-1.72	-1.97 (1.34)	-2.67 (0.96)	0.002
38	CD247	Homo sapiens CD247 molecule	-1.93	-2.26 (0.90)	-2.81 (1.18)	0.003	
39	TBX21	Homo sapiens T-box 21	-2.27	-2.28 (1.38)	-2.69 (0.94)	0.010	
40	GATA3	Homo sapiens GATA binding protein 3	-1.84	-2.58 (1.49)	-3.25 (0.94)	0.011	
41	CCR7	Homo sapiens chemokine (C-C motif) receptor 7	-1.51	-2.07 (1.33)	-2.65 (0.90)	0.026	
42	LEF1	Homo sapiens lymphoid enhancer-binding factor 1	-1.71	-2.90 (1.76)	-3.46 (1.66)	0.023	
43	STAT4	Homo sapiens signal transducer and activator of transcription 4	-1.69	-1.88 (0.85)	-2.30 (0.93)	0.033	

(continued on next page)

Table 3 (continued)

Physiological function	Gene symbol	Description	FC sepsis/No sepsis	Survivor	Non survivor	<i>p</i> value	
NK cell function	44	CD244	Homo sapiens CD244 molecule, natural killer cell receptor 2B4	−1.69	−1.38 (1.15)	−1.83 (1.15)	0.008
	45	KLRK1	Homo sapiens killer cell lectin-like receptor subfamily K, member 1	−1.89	−2.41 (1.46)	−3.21 (1.84)	0.009
	46	KLRD1	Homo sapiens killer cell lectin-like receptor subfamily D, member 1	−2.25	−2.06 (1.50)	−2.50 (1.18)	0.019
Other	47	LPA	Homo sapiens lipoprotein, Lp(a)	−1.76	−0.73 (0.83)	−1.28 (0.68)	0.001
	48	FCER1A	Homo sapiens Fc fragment of IgE, high affinity I, receptor for; alpha polypeptide	−6.51	−3.63 (3.22)	−6.16 (2.49)	0.001
	49	LPAR6	Homo sapiens lysophosphatidic acid receptor 6	−1.84	−0.48 (1.30)	−1.14 (1.20)	0.004
	50	PRKCH	Homo sapiens protein kinase C, eta	−1.80	−1.62 (0.68)	−2.31 (1.21)	0.005
	51	FASLG	Homo sapiens Fas ligand (TNF superfamily, member 6)	−2.64	−2.62 (1.66)	−3.33 (1.54)	0.010
	52	CCR3	Homo sapiens chemokine (C–C motif) receptor 3	−2.03	−2.90 (2.38)	−4.09 (2.34)	0.015
	53	ERBB2	Homo sapiens v-erb-b2 erythroblastic leukaemia viral oncogene homologue 2, neuro/glioblastoma derived oncogene homologue (avian)	−1.66	−1.12 (0.84)	−1.50 (0.66)	0.015
	54	PTGDR	Homo sapiens prostaglandin D2 receptor (DP)	−1.71	−1.42 (1.37)	−1.75 (0.83)	0.022
	55	RASGRP1	Homo sapiens RAS guanyl releasing protein 1 (calcium and DAG-regulated)	−1.84	−2.46 (1.13)	−2.92 (0.86)	0.030

to the development of cytotoxic cellular immunity. Patients with sepsis showed depressed expression of IL2RB and IL23A, two major mediators of the Th1 and Th17 cellular immunity, which play central roles in the immune response against bacteria and fungi. Our findings agree with the increasing evidence that considers immune suppression as a major pathophysiological event in sepsis.⁷ Poor expression of the genes forming part of this signature could dramatically impair infection control. In fact, in our study, non-survivors showed lower expression levels of these genes than survivors. In our experience, when an infection is not controlled by adaptive immunity, an exuberant innate response is expected to occur.^{9,10} The inverse correlations found in our study between the innate immunity-related genes and those participating in T and NK cell function support this notion (Fig. 3).

As a limitation of our study, we did not evaluate the evolution of gene expression profiles over time. As evidenced by the study of Xiao W et al., the persistence of transcriptomic alterations in white blood cells characterised patients suffering from complications.¹⁵ Nonetheless, the clinical relevance of the early expression patterns found for the 55 genes identified in our study is supported by the fact that non-survivors showed significant differences in the expression levels of these genes compared with survivors.

While transcriptomic analysis using microarrays offers a semi-quantitative evaluation of gene expression levels, the emergence of next generation technologies, such as droplet digital PCR (ddPCR), allows the quantification of mRNA transcripts with greater precision and reproducibility,²⁷ facilitating its implementation in clinical practice. In this

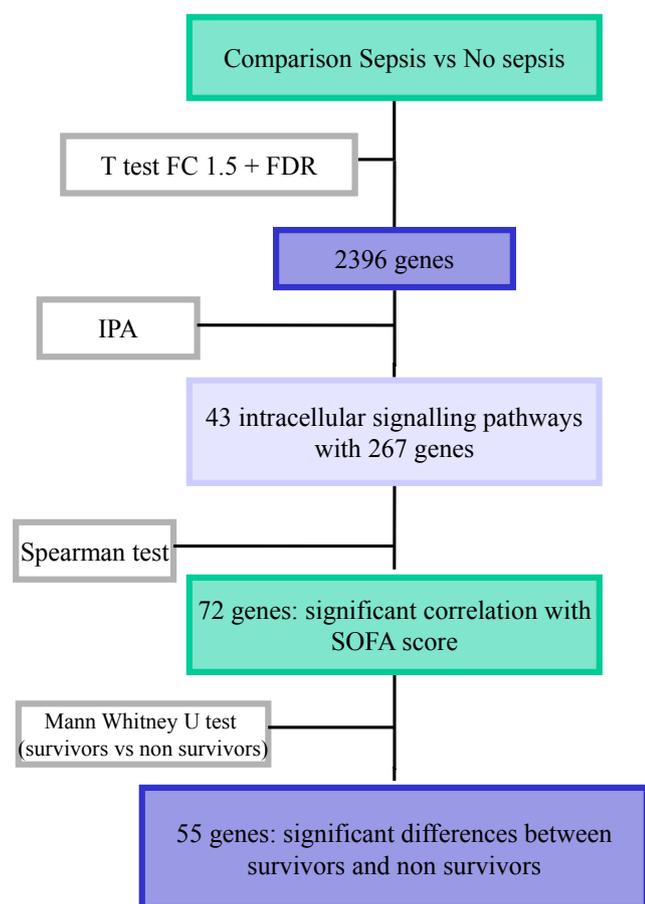


Figure 4 Flow chart showing the workflow and main results.

sense, further studies employing ddPCR could contribute to developing molecular scores based upon the genes identified in this study that can complement/improve current clinical scores for disease severity, such as SOFA.

In conclusion, the extent of organ failure and mortality in sepsis are directly associated with proportional alterations in the expression levels of 55 genes involved in both the innate and adaptive immune response in the blood. Quantification of expression levels of these genes could represent an opportunity to assess simultaneously the disease severity and immunological alterations in sepsis.

Competing interests

The authors declare that they have no competing interests. The authors have no any financial or personal relationship with any organisations that could influence the described research.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.jinf.2014.12.010>.

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