Supplemental Digital Content

Low IL-7 receptor mRNA expression is independently associated with day 28 mortality in septic shock patients

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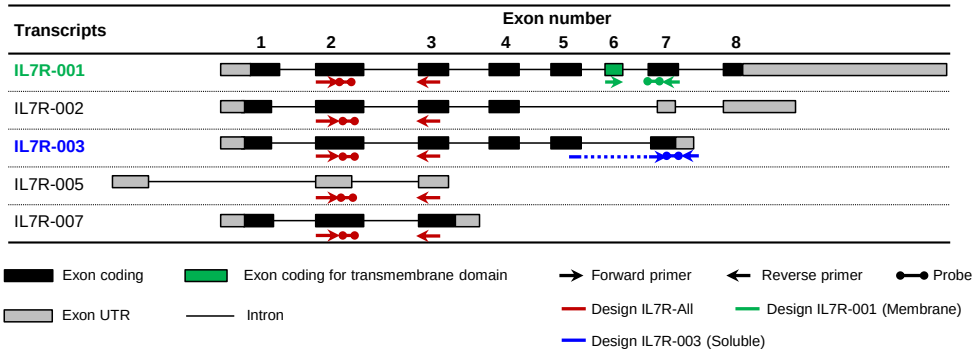
Supplemental Patients Description Discovery and validation cohorts description

Discovery cohort: we included patients from a cohort of adult septic shock patients enrolled from December 2001 to April 2005 in two ICUs from a French university hospital (1). This study is part of a global study on ICU-induced immune dysfunctions and was approved by the local ethics committee (Comité de Protection des Personnes Sud-Est II #IRB 11236). No informed consent was needed, as this study was non-interventional and complementary blood samples were obtained during patients' routine blood sampling after completion of routine follow-up tests. Nevertheless, non-opposition to inclusion in the protocol was recorded from every patient or the patient's next of kin. This study is also registered at the French Ministry of Research and Education (#DC-2008-509). We selected patients with available samples at D1 and D3 to study the variation in IL7R expression levels between these two time-points.

Validation cohort: we included septic shock patients from a cohort of adult ICU patients enrolled from December 2009 to June 2011 in six ICUs from three French university hospitals (2). The study protocol was approved by the local ethics committee (Comité d'Ethique des Centres d'Investigation Clinique de l'Inter-Région Rhône-Alpes Auvergne #IRB 5044). No informed consent was needed, as this study was non-interventional and complementary blood samples were obtained during patients' routine blood sampling after completion of routine follow-up tests. Nevertheless, non-opposition to inclusion in the protocol was recorded from every patient or the patient's next of kin. We included septic shock patients with available peripheral whole blood sample at D3, in order to confirm the results obtained on the discovery cohort.

The Simplified Acute Physiology Score (SAPS) II was recorded at ICU admission (3) and the Sequential Organ Failure Assessment (SOFA) score in the first 24 hours after admission (D1) and at day 3 (D3) (4). The chronic health status was defined using the Charlson comorbidity score (5). Length of stay and survival were measured at ICU discharge, at D28 after admission and at hospital discharge



Supplemental Figure 1 IL7R transcripts detected by the different PCR designs. IL7R transcripts structure is schematically represented from data available in Ensembl database (release 87 - GRCh38.p7). Exons targeted by primers (→ or ←) and probes (●—●) of each PCR design are indicated: IL7R-001, encoding the CD127 membrane form, IL7R-003, encoding a CD127 soluble form, and IL7R-All, specific for all studied transcripts, including the two previous ones.

Supplemental Table1 PCR assays characteristics

PCR design	Primers and probes sequences		Target exon	Detected transcripts	Product length (bp)
IL7R-All	Forward	GGAAGTGAATGGATCGCAGC	2	IL7R-001 (NM_002185) IL7R-002 (ENST00000514217) IL7R-003 (ENST00000506850) IL7R-005 (ENST00000511031) IL7R-007 (ENST00000511982)	110
	Backward	GGCACTTTACCTCCACGAG	3		
	Probe	CTGTGCTTTTGAGGACCCAGAT	2		
IL7R-001	Forward	CTCTGTCGCTCTGTTGGTC	6	IL7R-001 (NM_002185)	99
	Backward	TCCAGAGTCTTCTTATGATCG	7		
	Probe	CTATCGTATGGCCCAGTCTCC	7		
IL7R-003	Forward	GCTCAGGATTAAGCCTATCG	5-7	IL7R-003 (ENST00000506850)	131
	Backward	CACTGGGATGTTGCCAACAC	7		
	Probe	ATCATAAGAAGACTCTGGAACATCT	7		
HPRT1	Forward	CCAAAGATGGTCAAGGTCGC	6	HPRT1-001 (NM_000194)	159
	Backward	GACACAAACATGATTCAAATCC	7-8		
	Probe	CAAGTTTGTTGTAGGATATGCCC	7		

Supplemental Method IL7R transcripts expression level measurement

Peripheral whole blood was collected in PAXgene™ tubes (PreAnalytix, Hilden, Germany). Total RNA was extracted using PAXgene™ Blood RNA Kit (PreAnalytix). Before RNA elution, the residual genomic DNA was digested using the Rnase-Free Dnase set (Qiagen, Hilden, Germany). RNA integrity was assessed with the RNA 6000 Nano Kit on a Bioanalyzer (Agilent Technologies, Santa Clara, California, USA). Total RNA was reverse transcribed in complementary cDNA using SuperScript® VILO™ cDNA Synthesis Kit (Life Technologies, Chicago, Illinois, USA).

Three specific PCR assays were designed (See **Supplemental Table 1** for oligonucleotides sequences). **Supplemental Figure 1** shows which IL7R exons are hybridized by the different primers and probes. The "IL7R-001" PCR is specific of the IL7R-001 transcript. This transcript, which contains the exon 6 corresponding to the transmembrane domain, encodes the full-length membrane form of CD127. The "IL7R-003" PCR is specific of the IL7R-003 transcript, encoding a CD127 soluble form. The "IL7R-All" PCR quantifies all IL7R transcripts studied, including the two previously described.

PCR reactions were performed on a LightCycler 480 instrument (Roche, Bale, Switzerland), using a LightCycler 480 Probes Master kit (Roche) following manufacturer's instructions. Thermocycling was carried out in a final volume of 20 µL containing 0.5 µM of primers and 0.1 µM of probe, with an initial denaturation step of 10 min at 95°C, followed by 45 cycles of a touchdown PCR protocol (10 sec at 95°C, 29 sec annealing at 68-58°C, and 1 sec extension at 72°C). The Second Derivative Maximum Method was used with the LightCycler software to automatically determine the crossing point for individual samples. Standard curves were generated by using four replicates of cDNA standards and were used to perform efficiency corrected quantification. For comparison between expression levels of the different IL7R transcripts, absolute concentrations (copies/µL) were used. For analysis of association with the outcome, gene expression was normalized using HPRT1 (hypoxanthine phosphoribosyltransferase 1) as reference gene and results were expressed as Calibrated Normalized Relative Quantity (6).

Supplemental Table 2 Association between IL7R transcripts expression levels and outcome at day 28 after septic shock diagnosis in the discovery cohort

IL7R transcripts	**Time-point**	**IQR HR (95% CI)**	***p*-value**
IL7R-All	D1	0.71 (0.32-1.60)	0.407
	D3	0.12 (0.02-0.81)	**0.029**
	D3/D1 ratio	0.14 (0.02-1.09)	0.06
IL7R-001	D1	0.70 (0.32-1.56)	0.385
(Membrane)	D3	0.11 (0.02-0.74)	**0.023**
	D3/D1 ratio	0.27 (0.07-1.00)	0.051
IL7R-003	D1	0.68 (0.29-1.59)	0.370
(Soluble)	D3	0.20 (0.04-0.93)	**0.023**
	D3/D1 ratio	0.33 (0.09-1.28)	0.109

IQR HR refers to a hazard ratio normalized to an increment from 1st to 3rd quartile to allow comparison between models. Values in bold indicate significance at $p<0.05$.

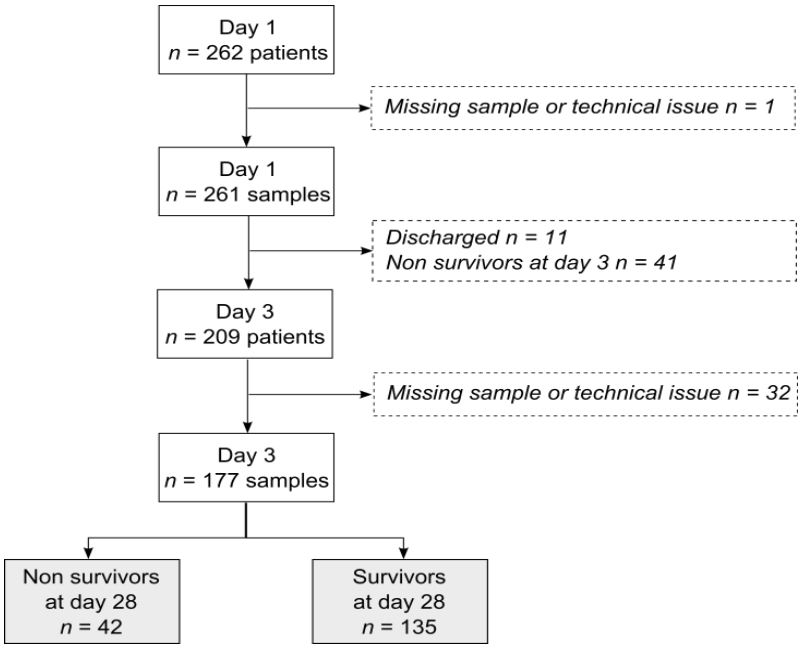
CI: confidence interval.

Supplemental Table 3 Area under ROC curve and performances for day 28 mortality after septic shock diagnosis in the discovery cohort

IL7R transcripts	Time-point	AUC (95% CI)	Threshold	Se (95% CI)	Sp (95% CI)	PPV (95% CI)	NPV (95% CI)
IL7R-All	D3	0.79 (0.63-0.96)	0.22	1 (1-1)	0.62 (0.38-0.81)	0.53 (0.41-0.69)	1 (1-1)
IL7R-001	D3	0.81 (0.65-0.97)	0.20	1 (1-1)	0.67 (0.48-0.86)	0.56 (0.45-0.75)	1 (1-1)
IL7R-003	D3	0.77 (0.61-0.94)	0.21	1 (1-1)	0.52 (0.33-0.71)	0.47 (0.39-0.6)	1 (1-1)

For each IL7R assay, a threshold was determined in the discovery cohort in order to maximize (i) the negative predictive value (NPV) and (ii) the specificity (Sp). Thresholds are expressed as calibrated normalized relative quantity (CNRQ) using HPRT1 as reference gene.

ROC: receiver operating characteristic, AUC: area under ROC curve; CI: confidence interval, Se: sensitivity, PPV: positive predictive value



Supplemental Figure 2 Validation cohort flowchart

Supplemental Table 4 Biological parameters of patients from the validation cohort according to survival status at day 28 after septic shock diagnosis

Parameters	Overall cohort (n=177)	Non-survivors (n=42)	Survivors (n=135)	*p*-value
Biological parameters at ICU admission				
Lactate, mmol/L (*n*=174)	2.45 [1.70-3.77]	3.15 [1.80-5.97]	2.30 [1.70-3.40]	**0.016**
Hemoglobin, g/dL	10.6 [9.30-11.9]	10.2 [9.33-11.3]	10.8 [9.20-12.2]	0.148
Neutrophils, 10^9/L (*n*=140)	11.3 [6.87-17.4]	7.60 [5.84-11.2]	13.4 [7.83-20.2]	**0.001**
Lymphocytes, 10^9/L (*n*=140)	0.76 [0.40-1.16]	0.60 [0.38-0.85]	0.80 [0.40-1.20]	0.122
Monocytes, 10^9/L (*n*=139)	0.43 [0.20-0.83]	0.34 [0.18-0.60]	0.56 [0.20-0.93]	0.079
Platelets, 10^3/mm^3	159 [92-233]	108 [80-187]	171 [113-242]	**0.022**
PaO_2/FIO_2 (*n*=175)	166 [106-239]	172 [116-258]	162 [105-236]	0.549
Creatinine, µmol/L	150 [93-235]	164 [102-229]	141 [81-238]	0.286
Diuresis, mL/day	1250 [650-2250]	800 [263-1825]	1300 [750-2325]	**0.017**
Biological parameters at D3				
Lactate, mmol/L (*n*=145)	1.70 [1.30-2.20]	2.00 [1.65-3.22]	1.60 [1.20-2.00]	**<0.001**
Hemoglobin, g/dL (*n* =174)	10.0 [9.00-11.0]	10.2 [9.10-11.0]	10.0 [9.00-10.9]	0.669
Neutrophils, 10^9/L (*n*=139)	13.0 [8.13-17.7]	14.4 [9.49-17.4]	11.6 [7.91-17.7]	0.287
Lymphocytes, 10^9/L (*n*=139)	0.80 [0.60-1.08]	0.64 [0.40-0.91]	0.84 [0.60-1.11]	**0.005**
Monocytes, 10^9/L (*n*=139)	0.61 [0.35-0.98]	0.55 [0.35-0.82]	0.66 [0.37-0.99]	0.551
Platelets, 10^3/mm^3 (*n*=174)	136 [74-213]	79 [47-167]	147 [81-230]	**0.005**
PaO_2/FIO_2 (*n*=165)	227 [160-295]	222 [147-288]	230 [169-298]	0.568
Creatinine, µmol/L (*n*=175)	107 [66-193]	150 [106-200]	99 [62-186]	**0.020**
Diuresis, mL/day	1400 [850-2300]	1075 [200-1875]	1500 [900-2550]	**0.008**

Parameters are expressed as median [interquartile range]. Comparisons between survivor and non-survivor patients at day 28 were performed with Mann-Whitney or t-tests. Values in bold indicate significance at $p<0.05$.

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