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Supplemental information

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Towards personalized immunotherapy in sepsis: the PROVIDE randomized clinical trial

Running title: PERSONALIZED IMMUNOTHERAPY IN SEPSIS

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Table S1 Multivariate Cox regression analysis of risk factors associated with 28-day mortality including colistin treatment as a co-variate Related to Figure 3.

Variable	**Survival N=95**	**Death N=145**	**Univariate analysis HR (95%CIs)**	**p-value**	**Multivariate analysis HR (95%CIs)**	**p-value**
Immune classification, n (%)						
Intermediate state	52 (54.7)	37 (25.5)	1 (Baseline HR)	-	(Baseline HR)	-
Immunoparalysis	34 (35.8)	69 (47.6)	2.08 (1.39-3.10)	<0.0001	1.63 (1.08-2.42)	0.019
MALS	9 (9.5)	39 (26.9)	3.37 (2.14-5.29)	<0.0001	2.25 (1.40-3.59)	<0.0001
APACHE II score ≥ 27*, n (%)	24 (25.3)	69 (47.6)	2.14 (1.54-2.96)	<0.0001	1.55 (1.10-2.20)	0.013
SOFA score ≥ 8*, n (%)	59 (62.1)	129 (89.0)	3.10 (1.67-5.07)	<0.0001	****	
Chronic heart failure, n (%)	16 (16.8)	46 (31.7)	1.64 (1.16-2.33)	0.006	**	
Septic shock, n (%)	54 (56.3)	123 (84.8)	2.65 (1.68-4.18)	<0.0001	2.03 (1.25-3.31)	0.004
Colistin treatment, n (%)	27 (31.0)	57 (40.4)	1.21 (0.87-1.70)	0.256	**	

Abbreviations APACHE acute physiology and chronic health evaluation, CI: confidence interval; HR; hazard ratio; MALS: macrophage activation-like syndrome; n: number of patients; SOFA sequential organ failure assessment,

*Values represent Youden Index of receiver operating characteristics curves for mortality

**not entering the equation after two steps of forward analysis

Table S2 Adverse events captured during the randomized clinical trial. Related to Figure 4

	Placebo (n=21)	**Personalized immunotherapy (n=15)**	**p-value***
Incidence of at least one SAE**, n (%)	4 (19.0)	4 (16.7)	0.694
Venticular tachycardia	0 (0)	2 (13.3)	0.167
Digital ischemia	0 (0)	2 (13.3)	0.167
Acute myocardial infarction	1 (4.8)	0 (0)	1.00
Brain tumor metastasis	1 (4.8)	0 (0)	1.00
Obstructive ileus	1 (4.8)	0 (0)	1.00
Pulmonary embolism	1 (4.8)	0 (0)	1.00
At least one AE, n (%)	2 (9.5)	0 (0)	0.500
Grade I hyperkalemia	1 (4.8)	0 (0)	1.00
Grade III increase of γGT	1 (4.8)	0 (0)	1.00

*this does not include the number of deaths
**by the Fisher exact test
Abbreviation AE: adverse event; γGT: gamma glutmayl transpeptidase; SAE: serious adverse event

Table S3. Definitions of eligible infections. Related to Methods details.

Infection	All the following	At least 2 of the following	At least 1 of the following
Acute cholangitis	• Pain at the right upper quadrant • Fever (tympanic or oral temperature ≥38^0C, rectal ≥38.3^0C)	None	Consistent ultrasound or CT findings
Primary bacteremia	• At least 1 positive blood culture • Failure to identify a primary infection site despite thorough clinical and radiology investigation	None	None
Community-acquired Pneumonia	New or evolving infiltrate on chest X-ray	• New onset or worsening of cough • Dyspnea • Auscultatory findings consistent with pulmonary consolidation	• Procalcitonin ≥0.25 ng/ml • Hypoxemia (pO2≤60mmHg or oxygen saturation ≤90% in room air) • Respiratory rate ≥20 breaths/min
Hospital-acquired pneumonia	• Onset >48 hours from hospital admission • New or evolving infiltrate on chest X-ray	• New onset or worsening of cough or dyspnea • Purulent tracheobronchial secretions • Auscultatory findings consistent with pulmonary consolidation	• Procalcitonin ≥0.25 ng/ml • Hypoxemia (pO2≤60mmHg or oxygen saturation ≤90% in room air) • Respiratory rate ≥20 breaths/min
Ventilator-associate pneumonia	• Onset >48 hours from start of mechanical ventilation • New or evolving infiltrate on chest X-ray	• Purulent tracheobronchial secretions • Auscultatory findings consistent with pulmonary consolidation	• Procalcitonin ≥0.25 ng/ml • Clinical pulmonary infection score (CPIS) ≥6

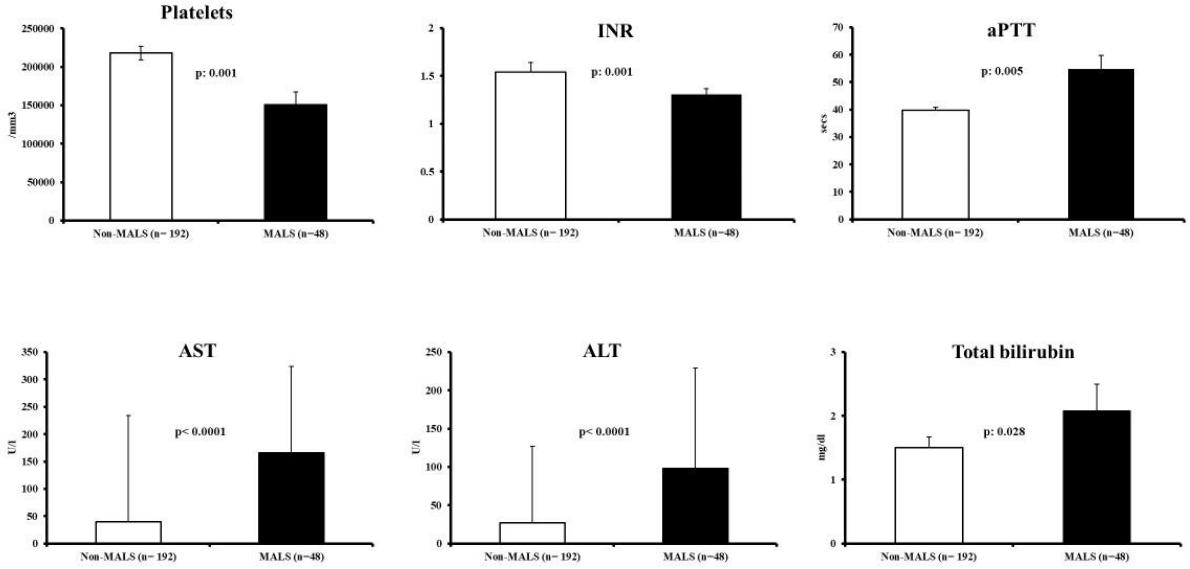

Figure S1 Appropriateness of ferritin for the diagnosis of macrophage activation-like syndrome (MALS). Related to Figure 2

Patients of the entire cohort are split into those with MALS as diagnosed with ferritin >4,420 ng/ml and into those without MALS as diagnosed with ferritin ≤4,420 ng/ml. Absolute platelet count (PLTs), international normalized ratio (INR), activated partial thromboplastin time (aPTT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and total bilirubin are significantly different between patients with and without MALS. The p-value of comparisons are provided.

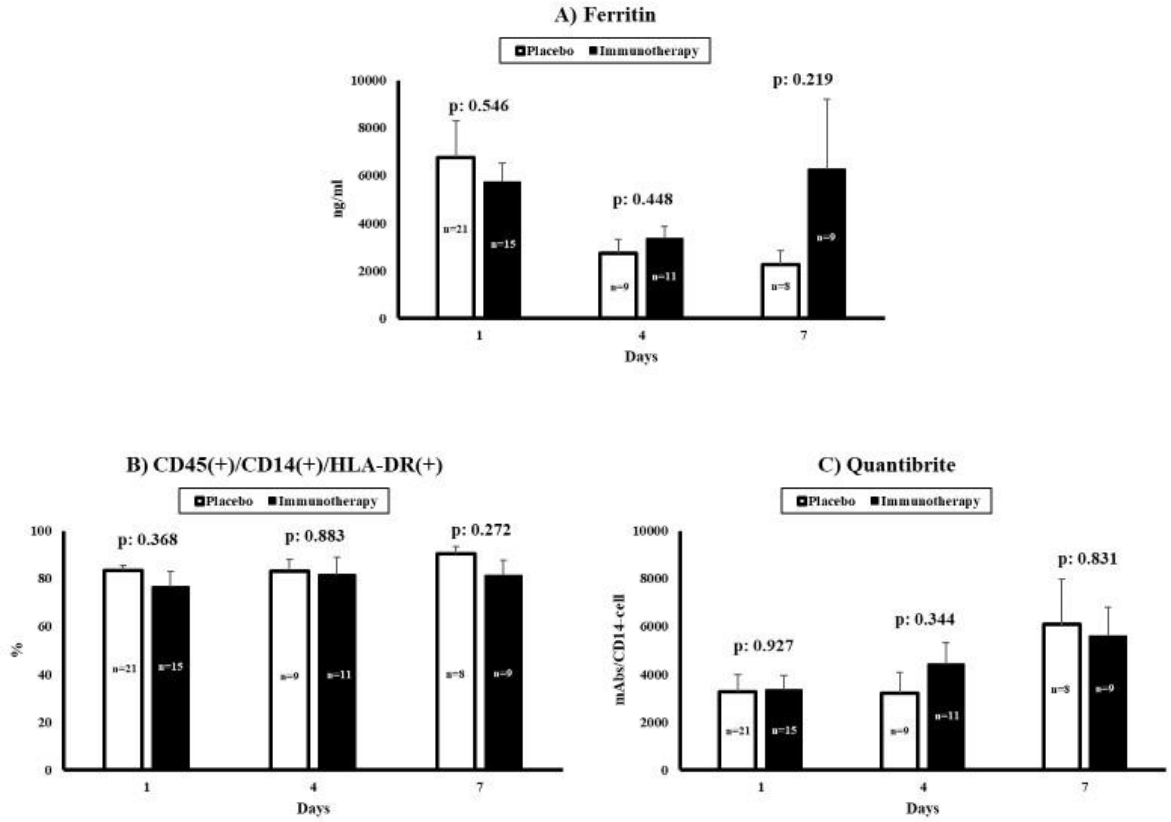

Figure S2 Follow-up measurements of biomarkers for patients enrolled in the interventional trial. Related to Figure 4

Circulating concentrations of ferritin (A), of the % expression of HLA-DR on circulating CD45(+)/CD14(+) monocytes (B) and of the absolute number of HLA-DR receptors on circulating CD45(+)/CD14(+) monocytes (Quantibrite) (C). P values indicate comparisons between patients allocated to the placebo arm and to the personalized immunotherapy arm. The number (n) of patients analyzed on each day of follow-up is also provided.