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Reduced red blood cell deformability over time is associated with a poor outcome in septic patients

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ABSTRACT

Background: To investigate changes in red blood cell (RBC) rheology over time in critically ill patients with sepsis and their relationship with outcome.
Methods: In this prospective, non-interventional study, RBC rheology was assessed using the Laser-assisted Optical Rotational Cell Analyzer in a convenience sample of intensive care unit (ICU) patients with (n = 64) and without (n = 160) sepsis. Results were compared to measures in healthy volunteers (n = 20). RBC rheology was also assessed on days 1 and 3 of the ICU stay in 32 of the non-septic and 19 of the septic patients. RBC deformability was determined by the elongation index (EI) in relation to the shear stress (0.3 to 50 Pa) applied to the RBC membrane. An aggregation index (AI) was assessed simultaneously with the same device.
Results: The ICU mortality rate of the septic patients was 31%. RBC deformability was already reduced in septic patients at ICU admission, an effect that persisted during the study period and worsened in the non-survivors for the large majority of shear stresses studied (e.g., EI for 50 Pa of shear stress was 0.527 ± 0.064 in non-survivors vs. 0.566 ± 0.034 in survivors, $p < 0.05$). These changes were not observed in non-septic patients. The AI was more elevated in septic than in non-septic patients at ICU admission, but had no prognostic value.
Conclusions: Alterations in RBC rheology, including reduced deformability and increased aggregation, occur early in septic patients and reductions in RBC deformability over time are associated with a poor outcome.
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Introduction

Sepsis and septic shock are leading causes of death in critically ill patients (Kaukonen et al., 2014; Vincent et al., 2009). Sepsis is characterized by complex pathophysiologic alterations that result in circulatory and cellular alterations. The contribution of microcirculatory alterations to the development of multiple organ dysfunction has been highlighted over the last few decades (De Backer et al., 2002; Edul et al., 2012) and the persistence of these alterations in septic patients is associated with increased morbidity (Trzeciak et al., 2007, 2008) and mortality (Sakr et al., 2004; Top et al., 2011) rates. One factor that can affect the microvasculature (i.e., vessels with a diameter less than 100 μm) is red blood cell (RBC) rheology (Serroukh et al., 2012).

Impaired RBC rheology (reduced deformability and increased aggregation) has been demonstrated in critically ill patients, especially those with sepsis, already at ICU admission (Baskurt et al., 1998a; Kempe et al., 2007; Moutzouri et al., 2007; Piagnerelli et al., 2003; Reggiori et al., 2009), but the time course of these alterations has not been reported. We hypothesized that changes in RBC rheology impairment over time in septic states could be related to mortality. We, therefore, compared RBC deformability and aggregation in septic and non-septic patients and healthy volunteers. We then studied changes in RBC deformability and aggregation in septic and non-septic patients during the first 3 days following ICU admission. Finally, we evaluated the relationship between alterations in RBC rheology and mortality in the septic population.

Patients and methods

Patient selection and study design

This prospective study was conducted in the 34-bed medico-surgical department of intensive care of Erasme University Hospital after approval by the hospital ethics committee. Informed consent was obtained from each patient or their next of kin. We studied 64 septic patients aged ≥18 years who were admitted to the ICU (convenience sample). Exclusion criteria included pregnancy, RBC transfusion in the previous 72 h, acute bleeding that needed RBC transfusion, neutropenia due to

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chemotherapy, transfer from another hospital or ICU readmission, treatment with iron or erythropoietin, brain death or end-of-life (withdrawal process). Sepsis was identified using standard criteria as the presence (probable or documented) of infection together with evidence of a systemic inflammatory response (Levy et al., 2003). We also used data (collected in an overlapping period of time) from 20 healthy volunteers of both sexes and from 160 non-septic patients (with the same exclusion criteria as for the septic patients) (Reggiori et al., 2009).

At ICU admission we recorded age, sex, primary ICU admission diagnosis, source of sepsis, and the Acute Physiology and Chronic Health Evaluation (APACHE) II score (Knaus et al., 1985). The Sequential Organ Failure Assessment (SOFA) score (Vincent et al., 1996) was calculated daily. At admission, and on days 1 and 3 we recorded RBC, white blood cell (WBC) and platelet counts, hematocrit (Hct), hemoglobin concentration (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), coagulation tests (activated partial thromboplastin time [aPTT], prothrombin time [PT], international normalized ratio [INR], D-dimer, fibrinogen), C-reactive protein (CRP), serum sodium, blood urea concentration, creatinine, total bilirubin, lactate dehydrogenase (LDH), lactate, and blood glucose.

Assessment of RBC rheology

On days 0 (ICU admission), 1 and 3 in the ICU, we collected a single blood sample in an EDTA-containing tube (2.5 mL of blood in 0.06 mL EDTA, 0.235 mol/L, Terumo, Venoject) to assess hemorheology.

Measurements of RBC deformability

RBC deformability was assessed using the Laser-assisted Optical Rotational Cell Analyzer (LORCA, Mechatronics Instruments BV, AN Zwaag, Netherlands). A suspension of RBCs was mixed with polyvinylpyrrolidone 360 solution, an isotonic viscous medium (PVP, 4%; MW 360 kDa; viscosity 30 ± 2 mPa·s), to obtain a final solution with a constant Hct of 0.2%. Using a Couette system composed of a glass cup and a precisely fitting bob, with a gap of 0.36 mm between the cylinders, the liquid solution was sheared and illuminated by a laser beam in order to obtain a diffraction pattern produced by the deformed cells. This pattern, the cup rotational speed and the predetermined shear stresses were analyzed. The elongation index (EI) is calculated as: EI = (L − W) / (L + W), where L and W are, respectively, the length and width of the diffraction pattern. The geometry of the diffraction pattern is elliptical. It has been shown that, for a given shear stress, the greater the RBC deformability, the higher the EI (Baskurt et al., 1998b). At 37 °C, we obtained the shear stress–EI curves for 12 consecutive shear stresses because human RBC deformability reaches a plateau at 50 Pascals (Pa): 0.3, 0.48, 0.76, 1.21, 1.93, 3.07, 4.89, 7.78, 12.3, 19.7, 30 and 50 Pa. Interassay variabilities for each shear stress were: 51, 8.2, 3.9, 2.4, 1.7, 1.6, 1.1, 1.3, 1.7, 1.2, 1.4, and 1.2%, respectively.

Measurements of RBC aggregation

We also used the LORCA ektacytometer to analyze RBC aggregation (Dobbe et al., 2003; Hardeman et al., 2001; Johnson, 1989). One milliliter of whole blood, collected in EDTA-containing tubes, was used for the aggregation analysis: it was placed directly in the LORCA glass and the patient's data were entered into the computerized system. At 37 °C, the aggregation process is reflected by a decrease in laser back-scatter intensity (Isc) after the motor engine is stopped abruptly. The so-called syllectogram is the curve obtained by plotting Isc versus time; it is then possible to calculate the aggregation index (AI), which shows the kinetics and amplitude of aggregation, and the threshold shear rate, which is a measure of the tendency of aggregation (representing the minimal shear rate needed to prevent RBC aggregation) (Dobbe et al., 2003). The kinetics of aggregation are expressed by the aggregation half-time (t½) in seconds, which is the time to half-maximal aggregation. Y at Isc min is the minimum shear rate needed to prevent rouleaux formation and Y at Isc max is the shear rate needed to disrupt the formed rouleaux (Dobbe et al., 2003).

Table 1
Demographic and laboratory characteristics of all patients at ICU admission.

Variable	Normal values	Volunteers (n = 20)	Non-septic (n = 160)	Septic (n = 64)
Age (years)		38 ± 10	56 ± 17[a]	60 ± 14[a]
Sex (M/F)		11/9	96/64	41/23
APACHE II score			11 [8–17]	18 [14–24][b]
SOFA score at ICU admission			3 [2–6]	8 [4–11][b]
WBC (10^3/mm^3)	4.2–11.4	6.0 [5.3–7]	10.7 [8.4–14.4][a]	12.4 [7.8–17.9][a]
Neutrophils (%)	38–73.4	56.7 [50.3–62.3]	81.6 [71.5–87.8][a]	87.8 [81–92.2][ab]
Hct (%)	35.3–52.1	43.3 [39.4–45.2]	36.1 [30.9–40.9][a]	31.9 [26.3–37.1][ab]
Hb (g/dL)	11.8–17.6	14.7 [13.5–15.4]	12.1 [10.7–13.7][a]	10.2 [8.5 – 12.2][ab]
RBC (10^6/mm^3)	3.8–5.9	4.8 [4.2–5.1]	3.8 [3.4–4.4][a]	3.3 [2.7 – 3.8][ab]
Reticulocytes (absolute value)	30–111		64 ± 21	63 ± 47
MCV (μm^3)	80.8–99.2	89.7 [87.7–92.5]	93.7 [91.1–98.6][a]	96.3 [91.8–99.2][a]
MCH (pg)	26.4–34.2	30.6 [30.0–32.1]	32.0 [30.9–33.2][a]	31.4 [30.3–33.1]
MCHC (g/dL)	32–35.4	34.3 [34.1–34.7]	33.9 [33.1– 34.6]	32.9 [32.1–33.7][ab]
Platelets (10^3/mm^3)	150–400	274 [236–313]	253 [178–294]	188 [118–277][ab]
aPTT (s)	24–35	27.7 [25.6–28.6]	30.2 [26.3–40.4][a]	36.7 [31.4–44.7][ab]
PT (%)	70–130	99 ± 7	78 ± 22[a]	56 ± 23 [ab]
INR		1.0 [1.0–1.1]	1.1 [1.0–1.3][a]	1.4 [1.2–1.7][ab]
Fibrinogen (mg/dL)	160–400	285 [243–336]	295 [239–367][a]	562 [338–747][ab]
D-dimer (ng/mL)	<400	110 [85–155]	700 [400–1928][a]	3775 [2340–7590][ab]
CRP (mg/dL)	<0.07		0.4 [0.1–2.0]	17 [11–28][b]
Creatinine (mg/dL)	0.7–1.2		0.8 [0.6–0.9]	1.2 [0.8–2.3][b]
BUN (mmol/L)	5.4–14.3		11.4 [7.5–15.7]	18.6 [9.3–31.1][b]
Total bilirubin (IU/L)	<1.2		0.5 [0.4–0.8]	0.7 [0.5–2.1][b]
LDH (IU/L)	<240		178 [137–229]	227 [164–336][b]
Na (mEq/L)	135–145		140 [138–142]	138 [133–140][b]
Glucose (mg/dL)	70–100		139 [110–172]	136 [105–169]
Lactate (mmol/L)	<1.7		1.3 [0.9–2.5]	2 [1.2–3.4][b]

Data are presented as mean ± SD or median value (25th–75th interquartile ranges) or number (%). SOFA: sequential organ failure assessment; WBC: white blood cell count; Hb: hemoglobin; Hct: hematocrit; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; aPTT: activated partial thromboplastin time; PT: prothrombin time; INR: international normalized ratio; CRP: C-reactive protein; LDH: lactate dehydrogenase; BUN: blood urea nitrogen. Statistically significant difference: [a]non-septic/septic vs. volunteers, [b]septic vs. non-septic.

Statistical analysis. Data were analyzed using SigmaStat v 3.5 software package (Systat Software Inc., San Jose, California, USA) and IBM SPSS version 19 (SPSS Inc., Chicago, IL, US). Data are presented as mean ± SD, median value (25th–75th interquartile ranges) or number (%) as appropriate. Non-parametric tests were used for variables evaluated as not normally distributed. Difference testing between groups was performed using the Kruskal–Wallis one-way analysis of variance, the t-test, the Mann–Whitney test, the chi^2 test and the Fisher exact test, as appropriate. A Bonferroni correction was made for multiple comparisons. To test for a significant difference between two study groups, and within each group during the study period, a linear mixed model procedure was used. For this purpose, we considered group (survivors or non-survivors), time (days: 0, 1 and 3) and group by time interactions as fixed effects and patients as a random effect. Each time point difference between groups was compared in case of a significant group effect and/or of a significant time by group interaction. Time point differences were tested in case of a significant time effect in each group. All tests were two-tailed, and a $p < 0.05$ was considered statistically significant.

Results

Patients

We studied 224 critically ill patients who were admitted to the ICU (64 with and 160 without sepsis, Table 1). Of these patients, 51 (19 septic and 32 non-septic) were analyzed for the full three-day study period (Table 2). Because the group of patients present on the third day was smaller than the whole population present on ICU admission, we analyzed whether these groups were comparable, using the demographic and laboratory data shown in Tables 1–3. There were no significant differences between the groups, except for a higher APACHE II score on admission in the non-septic patients (18 ± 7 vs. 12 ± 7, $p < 0.05$) and a higher hemoglobin concentration in the septic patients (11.6 ± 3.1 g/dL vs. 10.1 ± 2.1 g/dL, $p < 0.05$), suggesting that the patients still present on day 3 were representative of the whole population. Seven patients (22%) died during the ICU stay in the non-septic group, as a result of withdrawal of life-sustaining therapy in all patients except one who was diagnosed with brain death.

Septic patients (n = 19) included in the 3-day analysis were more severely ill on admission than were non-septic patients (n = 32), as reflected by higher severity scores (Table 2). RBC count and Hb concentration on admission were significantly lower in septic and non-septic patients than in healthy volunteers. In septic patients, coagulation, renal and hepatic functions were more markedly altered and CRP concentrations were higher than in non-septic patients (Table 2). As expected, non-surviving septic patients were more severely ill at admission than septic survivors, as demonstrated by higher APACHE II and SOFA scores (Table 3). The coagulation profile (PT and platelet count) was more altered and lactate concentrations were significantly higher in non-survivors compared to survivors throughout the study period (days 0, 1 and 3) (Table 3).

Red blood cell deformability

At admission to the ICU, septic patients had marked alterations in RBC deformability compared to non-septic patients and to volunteers (Fig. 1). The differences between septic and non-septic patients persisted during the 3 days of the study (Fig. 2). Among the septic patients, RBC deformability was reduced early in survivors and non-survivors; however, it worsened over time more in the non-survivors than in the survivors (Fig. 3). The differences between survivors and non-survivors were observed for the large majority of shear stresses studied (Fig. 4): for example, the EI for shear stress of 50 Pa on day 3 was less in non-survivors than in survivors (0.527 ± 0.064 vs. 0.566 ± 0.034, $p < 0.05$); the decrease in EI reached 10% for several shear stresses. Such alterations were not observed in non-septic patients (data not shown). At day 0, an EI of 0.3 was correlated with fibrinogen concentration in the patients with sepsis ($r = 0.67$, $p < 0.05$).

Table 2
Demographic and laboratory data at admission for the volunteers and for the patients who were studied for 3 days.

	Volunteers (n = 20)	Non-septic (n = 32)	Septic (n = 19)
Age, years	38 ± 9	57 ± 17	64 ± 11
Male/female	11/9	24/8	13/6
APACHE II score		18 ± 7	21 ± 8
SOFA score		6 ± 3	8 ± 3[$]
Admission diagnosis			
Surgery		12	
Neurological		6	
Post-anoxic coma		3	
Trauma		7	
Respiratory distress		3	
Intoxication		1	
Sepsis			7
Severe sepsis			9
Septic shock			3
Source of infection			
Abdominal			5
Pulmonary			5
Soft tissues			4
Blood			2
Urinary tract			1
Unknown			2
Biological data			
RBC (10^6/mm^3)	4.7 ± 0.5	3.9 ± 0.7[a]	3.5 ± 0.6[a]
Hb (g/dL)	14.5 ± 1.4	12.3 ± 2.1[a]	11.6 ± 3.1[a]
Hct (%)	42.3 ± 3.9	40.0 ± 7.0[a]	33.5 ± 6.0[a]
MCV (μm^3)	90.0 ± 3.0	96.1 ± 5.8[a]	95.2 ± 4.5[a]
MCH (pg)	30.8 ± 1.3	32.3 ± 2.1	31.3 ± 1.6[a]
MCHC (g/dL)	34.3 ± 0.6	33.7 ± 1.2	32.8 ± 0.3[a]
RDW (%)	11.6 ± 0.6	12.5 ± 1.2	14.0 ± 0[a]
Platelets (10^3/mm^3)	289 ± 80	252 ± 97	232 ± 124
APTT (s)	28.1 ± 3.2	34.2 ± 12.7	38.5 ± 13.8[a]
PTT (s)	99 ± 7	78 ± 20[a]	53 ± 24[a]
INR	1.0 ± 0.04	1.2 ± 0.2	1.7 ± 1.0[a]
Fibrinogen (mg/dL)	304 ± 86	382 ± 147	600 ± 269[a]
WBC (10^3/mm^3)	6.1 ± 1.6	12.1 ± 4.7[a]	12.9 ± 5.7[a]
BUN (mmol/L)	NA	20.37 ± 13.78	39.95 ± 27[b]
Creatinine (mg/dL)	NA	1.1 ± 1.0	2.1 ± 1.6[b]
Bilirubin (IU/L)	NA	0.7 ± 0.5	2.1 ± 2.5[b]
Glucose (mg/dL)	NA	157 ± 58	134 ± 39
CRP (mg/dL)	NA	3.5 ± 4.7	20.6 ± 12.5[b]
Lactate (mmol/L)	NA	2.6 ± 2.7	3.0 ± 2.5

Statistically significant difference, [a]versus volunteers, [b]versus non-septic.
Data are presented as mean ± SD or median value (25th–75th interquartile ranges) or number (%). SOFA: sequential organ failure assessment; WBC: white blood cell count; Hb: hemoglobin; Hct: hematocrit; RBC: red blood cell; RDW: red cell distribution width; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; aPTT: activated partial thromboplastin time; PT: prothrombin time; INR: international normalized ratio; CRP: C-reactive protein; LDH: lactate dehydrogenase; BUN: blood urea nitrogen.

Red blood cell aggregation

RBC aggregation, as reflected by AI values, was greater in septic than in non-septic patients (Fig. 5). There were no significant differences in AI in survivors and non-survivors over the study period (Table 4). However, Y values at Isc min and max were lower in non-survivors compared to survivors and this difference was statistically significant on day 1 for Y at Isc min and on day 3 for Y at Isc max.

Discussion

Our study confirms the presence of early decreases in RBC deformability and increases in aggregation in septic patients. We report, for the first time, a continuous worsening of RBC deformability over time, with a decrease in EI of up to 10% by day 3 in septic non-

Table 3
Demographic and laboratory characteristics of the septic patients during the study period.

Variable	Day 0 survivors (n = 44)	Day 0 non-survivors (n = 20)	Day 1 survivors (n = 30)	Day 1 non-survivors (n = 15)	Day 3 survivors (n = 12)	Day 3 non-survivors (n = 7)
Age, years	60 ± 14		60 ± 14		64 ± 11	
Sex (M/F)	41/23		29/16		13/6	
APACHE II score	18 ± 8	24 ± 9*				
SOFA score	6 ± 4	10 ± 4*	6 ± 4	9 ± 4*	6 ± 5	8 ± 3*
WBC (10^3/mm^3)	16.2 ± 14.9	13.5 ± 9.6	11.9 ± 5.5	15.8 ± 9.6	12.9 ± 7.2	17.2 ± 3.8
Neutrophils (%)	83 ± 16	88 ± 7	84 ± 9	83 ± 10	80 ± 9	81 ± 9
Hct (%)	31.6 ± 5.9	31.8 ± 7.2	32.0 ± 5.1	29.4 ± 5.2¿	29.3 ± 5.8§	28.1 ± 4.4µ
Hb (g/dL)	10.4 ± 2.1	10.9 ± 3.3	10.4 ± 1.7	9.5 ± 1.8¿	9.6 ± 1.8	9.2 ± 1.4µ
RBC (10^6/mm^3)	3.33 ± .67	3.21 ± 0.79	3.34 ± 0.53	2.97 ± 0.55¿	3.09 ± 0.58§	2.96 ± 0.47µ
Reticulocytes (absolute value)	19 ± 15	23 ± 5	19 ± 17	28 ± 14	15 ± 10	17 ± 14
MCV (µm^3)	95 ± 6	99 ± 11*	96 ± 5#	99 ± 7*¿	95 ± 6	95 ± 3
MCH (pg)	31.3 ± 2.2	32.7 ± 3.6	31.1 ± 2.1#	32.1 ± 2.2*	31.0 ± 1.9	30.9 ± 1.6
MCHC (g/dL)	32.9 ± 1.4	32.8 ± 1.1	32.4 ± 1.0#	32.4 ± 1.1	32.6 ± 1.0	32.6 ± 1.5
RDW (%)	14 ± 2.3	15.1 ± 3.4	13.4 ± 1.8#	15.2 ± 3.7	13.7 ± 2.0	14.9 ± 2.0µ¶
Platelets (10^3/mm^3)	234 ± 142	161 ± 103*	214 ± 126#	147 ± 132*	186 ± 127§	151 ± 818*
aPTT (s)	39.2 ± 14.3	43.1 ± 15.9	40.4 ± 15.4	54.2 ± 29.7	37.2 ± 9.7	56.4 ± 42.3
PT (%)	61 ± 21	46 ± 24*	68 ± 20	44 ± 21*	74 ± 18	47 ± 23*
INR	1.5 ± 0.9	1.9 ± 1.0	1.28 ± 0.23	2.16 ± 1.48	1.23 ± 0.32	1.95 ± 1.11
Fibrinogen (mg/dL)	619 ± 307	482 ± 275	663 ± 245#	484 ± 270	771 ± 325	372 ± 299
D-dimer (ng/mL)	4444 ± 2806	5373 ± 2091	4278 ± 2440	4308 ± 2779	3796 ± 1984¥	2750 ± 1103
CRP (mg/dL)	19.3 ± 12.5	20.9 ± 15.5	22.2 ± 11.5#	20.4 ± 10.0	16.1 ± 8.7§	17.8 ± 12.3
BUN (mg/dL)	29.4 ± 23.4	40.2 ± 27.6	27.6 ± 25.2	33.2 ± 17.8	32.2 ± 34.6	36.4 ± 20.6
Total bilirubin (mg/L)	1.5 ± 2.2	2.8 ± 2.9	1.2 ± 1.7	2.5 ± 3.0*	1.5 ± 2.4	3.8 ± 4.6*
LDH (IU/L)	343 ± 609	368 ± 208	362 ± 509	676 ± 958*¿	353 ± 315	478 ± 334
Na (mEq/L)	137 ± 5	136 ± 6	139 ± 5#	139 ± 6	137 ± 4	139 ± 3
Glucose (mg/dL)	144 ± 52	130 ± 57	124 ± 41#	116 ± 41	110 ± 17	126 ± 44
Lactate (mmol/L)	2.2 ± 1.5	4.7 ± 4.1*	1.6 ± 1.1	4.4 ± 4.9*	1.4 ± 0.8	3.1 ± 3.2*

Data are presented as mean ± SD or median value (25th–75th interquartile ranges) or number (%). SOFA: sequential organ failure assessment; WBC: white blood cell count; Hb: hemoglobin; Hct: hematocrit; RBC: red blood cell; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; aPTT: activated partial thromboplastin time; PT: prothrombin time; INR: international normalized ratio; CRP: C-reactive protein; LDH: lactate dehydrogenase; BUN: blood urea nitrogen.
Statistically significant difference: * = survivors vs. non-survivors, # = among survivors, day 1 vs. day 0, § = among survivors, day 3 vs. day 0, ¥ = among survivors, day 3 vs. day 1, ¿ = among non-survivors, day 1 vs. day 0, µ = among non-survivors, day 3 vs. day 0, ¶ = among non-survivors, day 3 vs. day 1.

survivors. By contrast, changes in RBC aggregation were not associated with outcome.

Alterations in RBC deformability during sepsis have been reported in animals and in humans (Baskurt et al., 1998a; Condon et al., 2007; Piagnerelli et al., 2009; Reggiori et al., 2009; Spolarics et al., 2004). We previously observed, using flow cytometry, that the sphericity index, which was used to assess RBC shape, increased rapidly during sepsis (Piagnerelli et al., 2003), suggesting that during sepsis RBCs have a more spherical shape, which may be associated with a reduced capacity to deform. The flow cytometry technique only provides information on RBC shape, whereas the LORCA used in the present study allows the

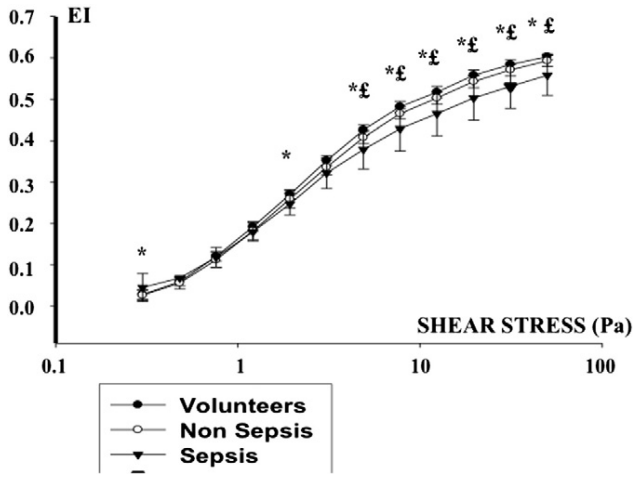

Fig. 1. RBC deformability as assessed by elongation index (EI) at different shear stresses in septic and non-septic patients at admission and in healthy volunteers. *p < 0.05 sepsis vs. volunteers; £: p < 0.05 sepsis vs. non-sepsis.

dynamic study of RBC deformability in relation to the shear stresses applied to the cell membrane. Using LORCA, Baskurt et al. (1998a) demonstrated that EI values were significantly lower in 10 septic patients compared to healthy volunteers at shear stresses <5 Pa. In contrast, using a cell filtration system, they observed no change in RBC deformability in the same cohort of patients, thus demonstrating the high sensitivity of the LORCA device. Furthermore, using the same device, we recently reported that RBC deformability and aggregation were altered early in critically ill patients (Reggiori et al., 2009), with decreased deformability in septic patients compared to non-septic patients and healthy volunteers, and increased aggregation in septic patients compared to healthy volunteers. However, these studies investigated patients at one moment in time, without specific information about the time delay from the onset of sepsis or about the time course of these measurements.

In the present study, we observed a decrease in deformability with time that reached up to 10% for several shear stresses. This decrease in RBC deformability may impact on the microcirculation. In a rat model of isolated perfused leg, Baskurt observed up to 78% increase in blood flow resistance after perfusion of RBCs with reduced deformability following incubation with glutaraldehyde (Baskurt, 2003). Interestingly, the author reported that decreases in blood flow appeared when the percentage reduction in RBC deformability exceeded 7%. Cabrales (2007), also using glutaraldehyde-treated RBCs, demonstrated in an awake hamster model, a significant reduction in functional capillary density, arteriolar diameter and flow, leading to a significant reduction in arteriolar, tissue, and venular oxygenation. Moreover, Simchon et al. (1987) noted that rat RBCs with reduced deformability became entrapped in the microvascular network of the spleen, lung, liver and femur, thus reducing regional blood flow. In a sepsis model using cecal-ligation and puncture in rats, Bateman et al. (2001) reported that reduced RBC deformability was associated with decreased functional capillary density. All these studies, therefore, suggest the

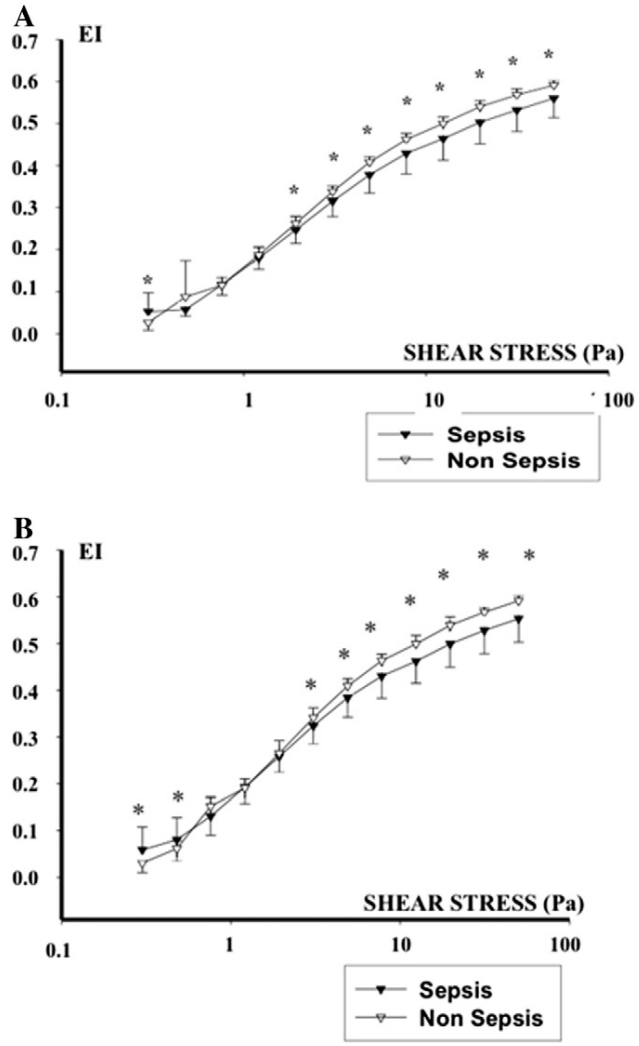

Fig. 2. Elongation index (EI) at different shear stresses in septic and non-septic patients on days 1 (panel A) and 3 (panel B). *p < 0.05.

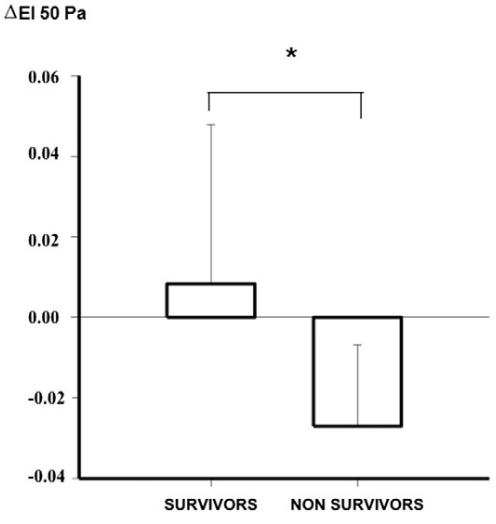

Fig. 3. Change in elongation index (EI) in septic patients on day 3 compared to day 0 (ΔEI) at shear stress of 50 Pa (survivors: 0.008 ± 0.040; non-survivors: −0.027 ± 0.020) *p < 0.05.

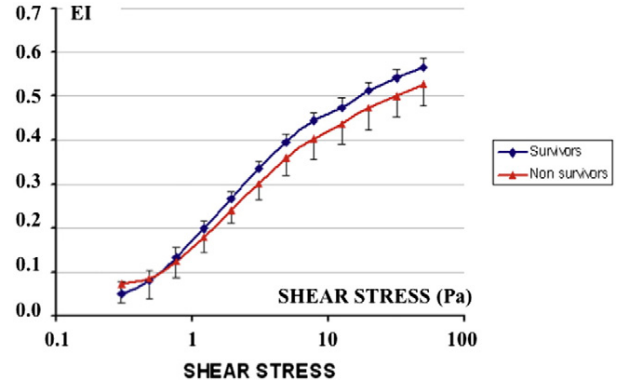

Fig. 4. Elongation index (EI) at different shear stresses in septic survivors and non-survivors on day 3.

existence of a link between reduced RBC deformability and impaired microcirculation. Nevertheless, these data must be interpreted with caution in view of the interspecies differences in RBC rheology (Baskurt et al., 1997).

The alterations in RBC deformability observed during sepsis may also depend on membrane component properties, cell geometry and cytoplasmic viscosity. The cell membrane is fundamental not only for cell integrity, but also for cell function. Indeed, membrane components may be altered during sepsis, as has been reported especially for RBC membrane sialic acid (SA) content (Piagnerelli et al., 2003), a surface membrane carbohydrate, and lipids (Kempe et al., 2007; Poschl et al., 2003). Reduced RBC deformability could be due to a persistent decrease in SA membrane content during the evolution of sepsis. Indeed, in an earlier study, we observed increased activity of neuraminidase, the enzyme that cleaves the glycosidic linkages of neuraminic acids, such as membrane SA, already at ICU admission (Piagnerelli et al., 2009). The decrease in SA content could initially be beneficial, because some studies have shown that RBCs are able to recycle the free SA released by neuraminidase (Bulai et al., 2002, 2003) through a cytosolic sialate pyruvate-lyase that specifically and reversibly catalyzes the cleavage of SA to form N-acetylmannosamine and pyruvate (Bulai et al., 2002, 2003). We have also observed that RBCs with decreased deformability can modify their metabolism to increase 2,3-diphosphoglycerate concentrations (Piagnerelli et al., 2009). This effect induces a change in hemoglobin conformation with increased potential for oxygen downloading to the cells. These adaptations in RBC metabolism could be beneficial at the onset of sepsis. The modification of RBC membrane

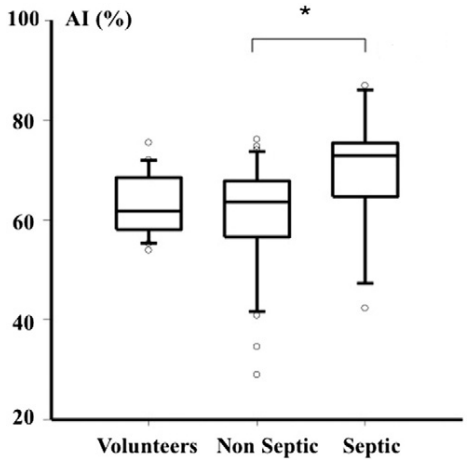

Fig. 5. RBC aggregation, as assessed by the aggregation index (AI), in septic and non-septic patients at admission and in healthy volunteers. *p < 0.05 septic vs. non-septic.

Table 4
Evolution of red blood cell aggregation.

Variable	Day 0 survivors (n = 44)	Day 0 non-survivors (n = 20)	Day 1 survivors (n = 30)	Day 1 non-survivors (n = 15)	Day 3 survivors (n = 12)	Day 3 non-survivors (n = 7)
AI (%)	63.9 ± 16.4	59.9 ± 13.3	67.4 ± 10.6	61.6 ± 10.5	72.0 ± 5.6	59.7 ± 14.6
Y at Isc max (/s)	301 ± 216	240 ± 174	325 ± 194	257 ± 193	422 ± 181	229 ± 86*
Y at Isc min	250 ± 155	203 ± 108	306 ± 185	204 ± 102*	312 ± 78	191 ± 89

* $p < 0.05$.

proteins is more controversial, as the increased integral/peripheral protein ratio in RBCs found in mice (Condon et al., 2007) could not be demonstrated to the same extent in human RBCs (Piagnerelli et al., 2003, 2012). Finally, there is an important relationship between RBC deformability, shear rate and blood viscosity. For shear rates <10 Pa, viscosity of blood with hardened RBCs is higher than for blood with more deformable RBCs (Somer and Meiselman, 1993). More rigid RBCs may encounter difficulties in passing through small capillaries, thus reducing peripheral oxygen delivery and inducing cellular hypoxia.

For aggregation as assessed by the AI, in contrast to the findings for deformability, there were no significant differences between septic survivors and non-survivors. In our study, the threshold shear rate, the force needed to disrupt RBC rouleaux formation, was higher in septic than in non-septic patients and healthy volunteers. This finding confirms the tendency for multiple cell aggregates to form in the blood of septic patients (Reggiori et al., 2009). We could speculate that this may be related to the higher blood fibrinogen concentrations in septic compared to non-septic patients. Indeed, fibrinogen is necessary to form bridges between RBCs and facilitate aggregation. Nevertheless, there was no correlation between aggregation (AI) and fibrinogen concentrations, unlike previous reports (Rogowski et al., 2000), and the significant correlation that we found between deformability (EI) and fibrinogen at day 0 disappeared with time.

The present study has some limitations. First, our results describe the evolution over time of a small cohort of patients. Second, the technique used does not evaluate the relationship with other blood cells (WBCs and platelets), whose role in the microcirculation could also be important. Indeed, Baskurt and Meiselman demonstrated that RBC deformability may be altered by activated WBCs via reactive oxygen species (Baskurt and Meiselman, 1998). Third, we could not isolate erythrocyte subpopulations, because we did not have access to a centrifugal elutriation system (Condon et al., 2003). Fourth, we did not perform concurrent microcirculatory measurements. Finally, we can only speculate on the possible mechanisms underlying our findings and further, mechanistic studies need to be performed to investigate this aspect.

In conclusion, RBC aggregation is increased and deformability decreased early in critically ill septic patients. Reduced deformability over time is associated with increased mortality. As altered RBC rheology may contribute to the impaired microcirculation observed in septic patients, further studies are warranted to investigate the evolution of this determinant of oxygen delivery in sepsis. Indeed, a better understanding of the microcirculation in all its complexity during septic states may lead to new therapeutic strategies to monitor and improve tissue perfusion, so as to decrease the incidence of sepsis-associated multiple organ failure and its associated high mortality rates.

Conflicts of interest

The authors have no conflict of interest to declare.

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