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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.

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.