Ultrasound Monitoring of RBC Aggregation as a Real-Time Marker of the Inflammatory Response in a Cardiopulmonary Bypass Swine Model*

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Objectives: In many pathological conditions, including high-risk surgery, the severity of the inflammatory response is related to the patient outcome. However, determining the patient inflammatory state presents difficulties, as markers are obtained intermittently through blood testing with long delay. RBC aggregation is a surrogate marker of inflammation that can be quantified with the ultrasound Structure Factor Size and Attenuation Estimator. The latter is proposed as a real-time inflammation monitoring technique for patient care.

Design: Ten swine underwent a 90-minute cardiopulmonary bypass, and surveillance was maintained during 120 minutes in the postbypass period. To promote the inflammatory reaction, lipopolysaccharide was administrated two times prior to surgery in six of those swine (lipopolysaccharide group). During the whole procedure, the Structure Factor Size and Attenuation Estimator cellular imaging method displayed a RBC aggregation index (*W*) computed from images acquired within the pump circuit and the femoral vein. Interleukin-6, interleukin-10, C-reactive protein, haptoglobin, immunoglobulin G, and fibrinogen concentrations were measured at specific periods.

Main Results: Compared with controls, the lipopolysaccharide group exhibited higher *W* within the pump circuit ($p < 0.05$). In the femoral vein, *W* was gradually amplified in the lipopolysaccharide group during cardiopulmonary bypass and the postbypass period ($p < 0.05$), whereas interleukin levels were higher in the lipopolysaccharide group but only at the end of cardiopulmonary bypass and beginning of postbypass ($p < 0.05$).

Conclusions: Continuous RBC aggregation monitoring can characterize the evolving inflammatory response during and after cardiopulmonary bypass. The Structure Factor Size and Attenuation Estimator is proposed as a real-time noninvasive monitoring technique to anticipate inflammation-related complications during high-risk surgery or critical care situations. Because RBC aggregation promotes vascular resistance and thrombosis, *W* could also provide early information on vascular disorders in those clinical situations. (*Crit Care Med* 2013; 41:e171–e178)

Key Words: cardiopulmonary bypass; critical care; erythrocyte aggregation; perioperative care; systemic inflammatory response syndrome; ultrasonography

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Cardiopulmonary bypass (CPB) surgery can lead to perioperative endotoxemia and triggers a cascade of events that includes a systemic inflammation sometimes leading to poor patient outcome (1–3). The early diagnosis and treatment when a disproportionate inflammatory reaction is noticed is therefore critically important. However, because of laboratory processing time, cytokine measurements correspond to an inflammatory process that has taken place before the time when the results are obtained. Thus, a noninvasive tool able to monitor real-time inflammation and to recognize it at the earliest state could result in improvement of patient outcome in case of sepsis or high-risk surgery by allowing the most appropriate intervention as early as possible.

Many studies have proposed to use instruments evaluating the microcirculation to detect inflammatory-related ischemic indices in both critically ill and high-risk surgical patients

(4–6). Most of them monitor real-time microvascular perfusion of skin (e.g., with laser Doppler flowmetry and nailfold videocapillaroscopy), perfusion of other superficial tissues (e.g., with orthogonal polarization spectral imaging), or tissue oxygenation (with near-infrared spectroscopy, oxygen/carbon dioxide pressure, cerebral oxygen saturation, etc.), but they do present several theoretical or practical limitations (7–11). They all rely on measures sampled within localized ischemic regions presumably affected by the inflammatory process, and for some of these techniques, microcirculatory abnormalities are not associated with other ischemic indices (12, 13). Therefore, there is a need for other inflammation monitoring methods.

RBC hyperaggregation is a well-known hemorheologic disorder that leads to microvascular dysfunctions (14) and venous thrombosis (15). The two current explicative theories for RBC hyperaggregation emphasize the role of inflammatory plasmatic macromolecules, such as fibrinogen, immunoglobulin G (IgG), haptoglobin, and C-reactive protein (CRP) (16–20). RBC hyperaggregation is a global marker of inflammation during illness or surgery (21–24), and Reggiori et al (12) described a strong association between early alterations of RBC aggregation and sepsis occurrence in critically ill patients. Because abnormal RBC aggregation is a precursor of microvascular disorders and thrombosis, it leads to ischemic events and tissue damages that are further reinforcing the existing inflammatory condition (14).

In the context of postoperative sepsis, monitoring RBC aggregation could be a relevant marker of inflammation and tissue perfusion. To continuously evaluate RBC aggregation, we propose to use the Structure Factor Size and Attenuation Estimator (SFSAE). This new cellular ultrasonic in vivo imaging modality was recently developed and validated by our group (15, 25–27). We propose to use it to assess real-time variations of RBC aggregation as a surrogate marker of inflammation in a CPB swine model.

MATERIAL AND METHODS

The experimental procedures were approved by the institutional animal care committees of the Montreal Heart Institute and of the Research Center of the University of Montreal Hospital in accordance with the recommendations and guidelines on the care and use of laboratory animals issued by the Canadian Council on Animals and the U.S. National Institutes of Health (assurance number A5377-01).

CPB and Perioperative Procedure

Ten swine from a slaughterhouse (weight 70.3 ± 1.2 kg) experienced a 90-minute CPB procedure, as previously described (28). Twenty-four hours before and at the beginning of the surgical procedure, six swine received lipopolysaccharide (LPS) injection (0.8 µg/kg) to increase the likelihood of a significant inflammatory response, as described elsewhere (29–32). These six swine constituted the LPS group, whereas the four remaining ones formed the control group. Swine were anesthetized with a perfusion of propofol (0.1–0.2 mg/kg/min). A median sternotomy was performed immediately after anesthesia, ventilator connection, heparin injection (400 UI/kg), and the pericardium opening. Both the aorta and right atrium were cannulated and connected to a hollow fiber membrane oxygenator (Monolyth, Sorin, Irvine, CA) and a roller pump (Sarns 7000, Ann Harbor, MI) in a close circuit (**Fig. 1**). The heart was left beating during the whole CPB (90 min). Then, animals were reperfused for 120 minutes. At that time, swine were still ventilated, and anesthesia was maintained with a 2% isoflurane air mix. We tried to maintain the mean systemic arterial pressure between 50 and 70 mm Hg. Animals were euthanized at the end of the postbypass period.

Venous blood samples were drawn from the right leg before the surgery (T_0) and after 30- and 120-minute postbypass (respectively, T_{120} and T_{210}, where subscripts are in min from T_0). During CPB, venous blood was drawn directly from the pump circuit after 30 and 90 minutes (respectively, T_{30} and T_{90}). The mean arterial pressure (MAP), HR (heart rate), and lingual pulse oximetry (Spo_2) were recorded every 15 minutes.

Ultrasound Acquisitions

Ultrasound imaging was performed with a high-frequency scanner (Vevo 770; Visualsonics, Toronto, ON, Canada) equipped with two mono element oscillating probes. During the CPB, the first probe (RMV710; central frequency of 25 MHz) was placed over a tube deviation (length 50 cm, diameter 6 mm) connected to the inlet circuit of the pump, whereas the second probe (RMV707B; central frequency of 30 MHz) was placed over the exposed femoral vein during both CPB and postbypass periods (Fig. 1).

RBC aggregation is a flow shear rate-dependent phenomenon. We therefore momentarily normalized the blood flow for 120 seconds before each measurement (33). In the pump deviation, an electromagnetic flowmeter (Cliniflow II; Carolina Medical Electronics, East Bend, NC) was used to monitor flow that was set at 3 mL/min before ultrasound measurements. In the femoral vein, a vascular occluder (Harvard apparatus, Saint-Laurent, Québec, Canada) was placed downstream of the ultrasound probe to adjust the flow. A Doppler velocity measurement provided feedback for adjusting the degree of occlusion (target velocity 1.5 mm/s for a typical vein diameter of 6 mm). Mean shear rates (γ) were postprocessed, as done by Yu et al (27), in both the pump and

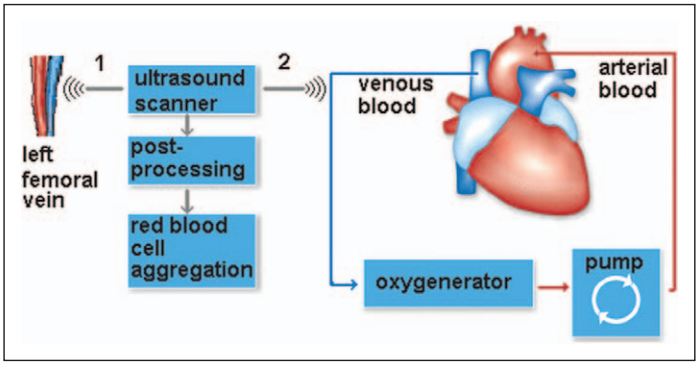

Figure 1. Cardiopulmonary bypass setup and radiofrequency ultrasound acquisition in the swine's left femoral vein (1) and the pump circuit (2), with a high-frequency ultrasound scanner.

femoral vein to control that every aggregation measurement was made under similar shear conditions. Venous instead of arterial monitoring was preferred because low shear rates offer favorable conditions for the formation and maintenance of aggregates.

In Vivo Ultrasonic Radiofrequency Measurements

Ultrasound acquisitions were performed every 15 minutes from T_{15} to T_{90} during the CPB and from T_{105} to T_{210} during the postbypass period. Baseline measurements were therefore performed soon (~15 min) after the beginning of surgery. Radiofrequency (RF) ultrasound echoes from longitudinal views of each vessel were acquired as described elsewhere (15). RF data correspond to the raw ultrasound signal that has not been modified by any signal processing normally used to obtain classical B-mode images. We used the frequency content of RF echoes to compute the proposed tissue characterization variable *W*. The ultrasound RF data were processed with the SFSAE (34) to compute 1) the mean packing factor of RBC aggregates (*W*), which is a dimensionless measure increasing proportionally with RBC aggregation, and 2) parametric images (filtered with the method [35]), as in **Figure 2.** A sequence of 25 vascular images of *W* was spatially averaged to monitor the RBC aggregation process. *W* time-varying curves were smoothed using a moving average filter with a span of three data points.

Blood Sampling Analyses

The hematocrit was measured immediately after every sampling time by microcentrifugation. The plasma was separated from the collected blood by centrifugation and stored at –80°C. Plasma concentrations of interleukin (IL)-6, IL-10, IgG, haptoglobin, and CRP were measured by enzyme-linked immunosorbent assay (Biovet, Sainte-Hyacinthe, Québec, Canada). Fibrinogen plasma concentrations were tested using the heat precipitation method (Biovet). Data were corrected for changes in plasma volume during CPB and postbypass periods using the method of van Beaumont (36).

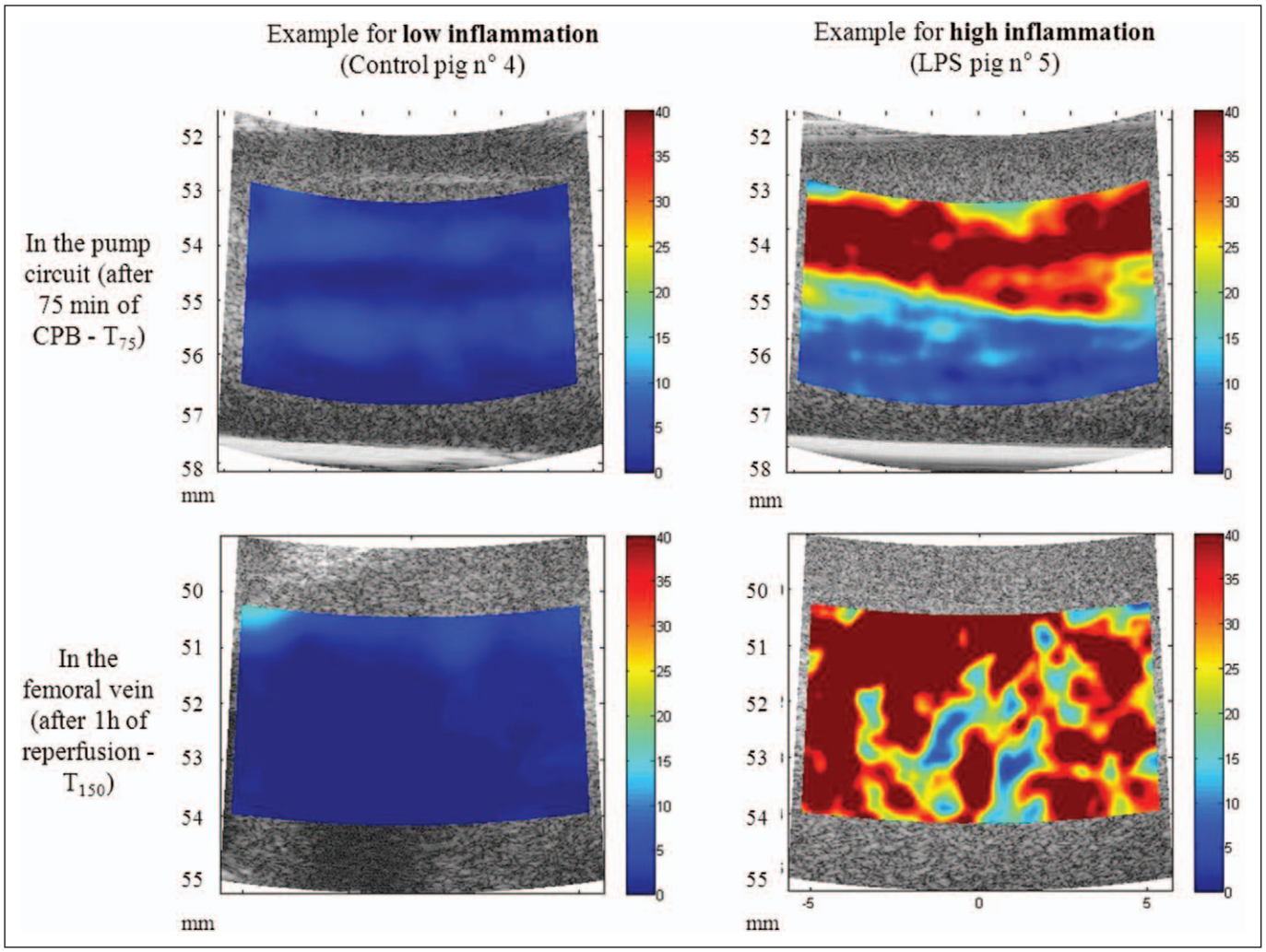

Figure 2. Examples of Structure Factor Size and Attenuation Estimator parametric images of red blood cell aggregation (*W*, longitudinal views) in case of low (*left*) and high inflammation (*right*). Data were acquired within the pump circuit deviation (*top*) and the femoral vein (*bottom*). The color maps vary from 0 (*blue*) to 40 (*red*). Variable *W* has no unit. LPS = lipopolysaccharide; CPB = cardiopulmonary bypass.

Statistical Analyses

Results are presented as mean ± SEM. The effects of LPS and CPB on RBC aggregation, IL-6, IL-10, fibrinogen, IgG, haptoglobin, CRP, and hemodynamic and oximetric changes were investigated separately using two-way analyses of variance (ANOVA) for repeated measures, using logarithmic transformations whenever the dependent variable was not normally distributed. The factors included in the ANOVA were the group, the time (considered a categorical variable), and their interaction. The interaction terms provided time-specific group differences. The Holm-Sidak method was used to correct the *p* values of these tests for multiple comparisons. Further investigations of the effect of CPB on RBC aggregation were conducted using linear mixed models with a group and time interaction to test for a group-specific time trend. Analyses were conducted using R (v.1.4.0, R Foundation for Statistical Computing, Vienna, Austria) and Sigma Stat (v. 3.1, San Jose, CA) packages. In figures and tables, statistical significance was defined as a *p* value of less than 0.05.

RESULTS

No Differences in Most Hemodynamic and Oximetric Variables Between Groups

As summarized in **Table 1**, MAP remained stable throughout CPB and the postbypass period up to T_{210}, where lower values were noticed in both groups (69 ± 7 mm Hg at T_0 vs 36 ± 3 mm Hg at T_{210}; $p < 0.001$). There was no difference between groups except at T_{90} where the control group presented higher MAPs ($p = 0.013$). HR remained constant during all the procedure (even at T_{210}) and did not exhibit any difference between groups. Spo_2 did not present any time or group effect. Values typically remained close to 100% with some nonsignificant variations at the end of the CPB. Shear rates did not show any group or time effect in the femoral vein or in the pump circuit deviation, indicating no confounding influence of hemodynamic variables on RBC aggregation measurements. Following the initial perfusion, the hematocrit increased slightly in both groups, exhibiting higher values compared with baseline at T_{150} and T_{180} (minimum value of 26% ± 1% after the initial perfusion and maximum value of 29% ± 2% at T_{180}; $p < 0.001$), but there were no differences between groups (data not shown). The native hematocrit of pigs before CPB was 30% ± 1%.

A Higher Blood-Marker-Sensitive Inflammatory Response in the LPS Group

As illustrated in **Figure 3*A***, the plasma concentration of IL-6 increased during the postbypass period (T_{120} and T_{210}) in both groups ($p < 0.0001$). This increase occurred earlier (at T_{90}) in the LPS group ($p < 0.001$). Differences in IL-6 values between groups were only noted early in the postbypass period (T_{120}, $p = 0.007$). IL-10 displayed in **Figure 3*B*** increased for both groups at T_{90} and T_{120} ($p < 0.001$) before returning to baseline at T_{210}. This increase was more important in the LPS group compared with controls ($p = 0.019$ at T_{90} and $p < 0.001$ at T_{120}). To summarize, the cytokine-sensitive inflammatory state was found higher in the LPS group but only at the end of CPB and early in the postbypass period.

As summarized in **Table 2**, fibrinogen also increased compared with baseline in the LPS group, early in the postbypass period (T_{120}, $p = 0.03$), and differences between groups were only noticed at this time point ($p < 0.001$). IgG and haptoglobin did not show any time or group effect. CRP did not vary over time, but the LPS group presented with higher values throughout all time points during CPB and postbypass periods ($p = 0.014$). It can thus be noticed that fibrinogen and CRP

TABLE 1. Hemodynamic and Oximetric Changes

Variable	Group	T_0 (or T_{15})	T_{30}	T_{90}	T_{120}	T_{210}
Mean arterial pressure (mm Hg)	Control	73 ± 13	56 ± 4	68 ± 7	47 ± 3	33 ± 3[a]
	LPS	61 ± 5	55 ± 4	44 ± 3[b]	44 ± 3	38 ± 4[a]
Heart rate (beats/min)	Control	82 ± 11	64 ± 3	126 ± 14	95 ± 11	124 ± 18
	LPS	84 ± 6	90 ± 6	99 ± 8	88 ± 4	99 ± 12
Spo_2 (%)	Control	100 ± 0	99 ± 1	95 ± 4	100 ± 0	100 ± 0 ($n = 1$)
	LPS	100 ± 0	94 ± 2	92 ± 4	100 ± 0	100 ± 0
γ in the vein (s^{-1})	Control	0.4 ± 0.1	0.3 ± 0.1	0.5 ± 0.2	0.5 ± 0.2	0.3 ± 0.1
	LPS	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.2	0.5 ± 0.2	0.4 ± 0.1
γ in the circuit (s^{-1})	Control	0.9 ± 0.2	1.4 ± 0.4	0.9 ± 0.2	–	–
	LPS	1.1 ± 0.0	1.0 ± 0.1	1.3 ± 0.1	–	–

LPS = lipopolysaccharide, Spo_2 = lingual pulse oxygen saturation, γ = mean shear rates within the vessel; – = no measurement performed in the cardiopulmonary bypass circuit during the postbypass period.

[a]Different from T_0 ($p < 0.05$).

[b]Difference between the two groups ($p < 0.05$).

Values are means ± SEM.

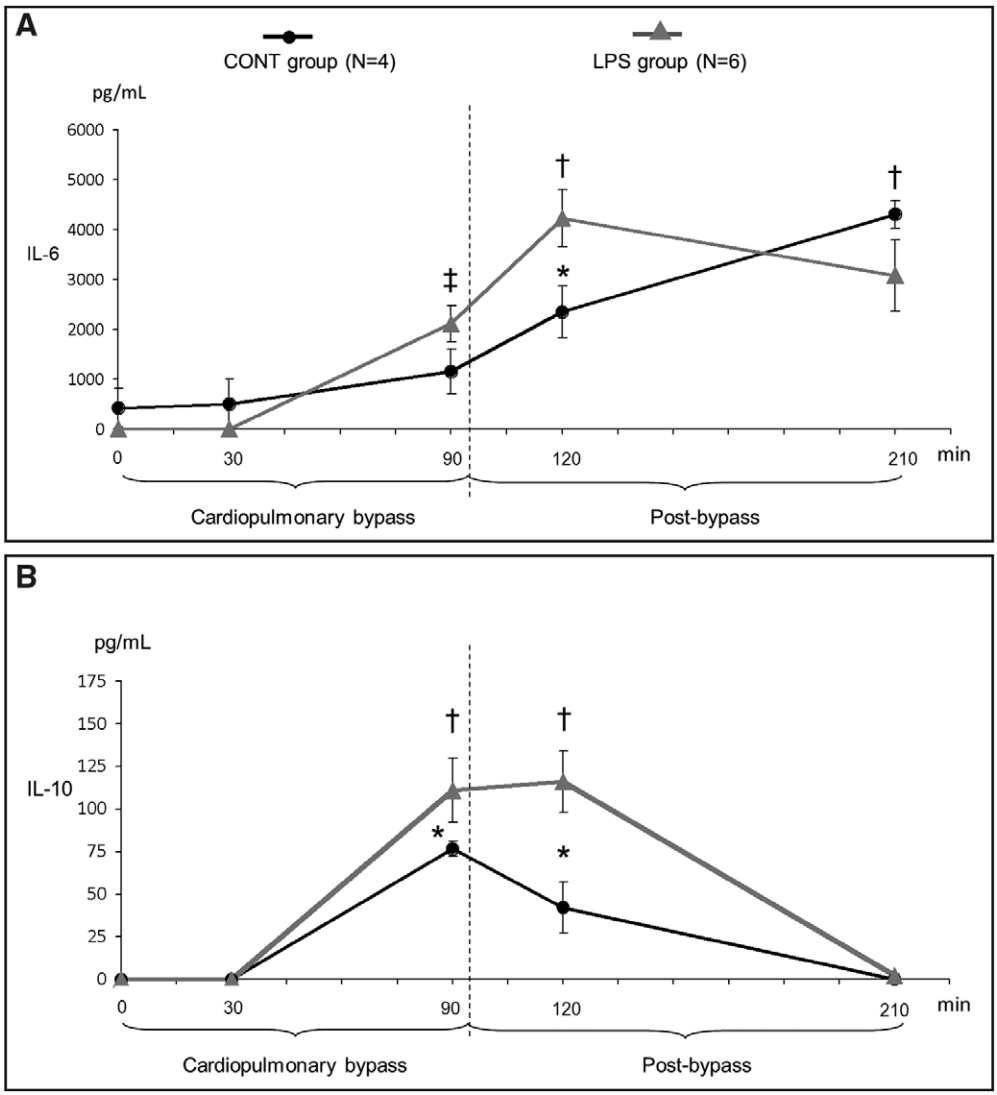

Figure 3. Time course of cytokines during and after the cardiopulmonary bypass surgery. Interleukin (IL)-6 (**A**) and IL-10 (**B**).
*Differences between lipopolysaccharide (LPS) and control (CONT) groups ($p < 0.05$).
†Different from baseline ($p < 0.05$).
‡Different from baseline in the LPS group only ($p < 0.05$).

acute-phase plasma proteins could detect the highest inflammatory condition in the LPS group, but only the fibrinogen changes could identify a temporal evolution early in the post-bypass period.

A Higher Time-Evolving Ultrasound-Sensitive Inflammatory Response in the LPS Group

As seen in **Figure 4*A***, *W* showed higher values in the LPS group at every time point in the pump circuit deviation ($p < 0.05$); however, no time-varying *W* behavior could be noticed for both groups. In the femoral vein, *W* values were higher in the LPS group compared with the control group from T_{45} to T_{210} (Fig. 4*B*; $p < 0.05$ from T_{45} to T_{90} and $p < 0.001$ from T_{105} to the end). Linear mixed models revealed a significant time increase of *W* in the LPS group only ($p < 0.0001$). The ultrasound SFSAE method confirmed the highest inflammatory state in the LPS group and was more sensitive to temporal evolution than classical blood markers.

DISCUSSION

This study is the first to demonstrate the feasibility of in vivo and real-time ultrasound monitoring of RBC aggregation (cf. *W*) during surgery involving experimental CPB. To do so, we used a specific model that has been described as typical of post-pump syndrome in humans (30–32). Enhanced RBC aggregation coincided with both LPS- and CPB-related inflammation, emphasizing the usefulness of SFSAE images to follow the inflammatory response. Ultrasound results revealed to be consistent with cytokine and plasma protein responses but advantageously allowed staging changes in inflammation. RBC aggregation is a reversible process modulated by the membrane surface change density, membrane strain, and plasma proteins (37). The sensitivity of the SFSAE method to the time-varying inflammatory state likely results from the cumulative impact of those mechanisms. This new cellular imaging modality might be applicable to various critical care situations, as discussed below.

RBC Aggregation, Inflammation, and Risk for Thrombosis and Tissue Ischemia

This is the first study to monitor together RBC rheology with a noninvasive method and inflammatory markers during and after surgery. Our results are in accordance with the literature describing an increase of RBC aggregation in response to surgery including or not including CPB (22, 24, 38–40). Baskurt et al (22) proposed two complementary pathways to explain the increase of RBC aggregation concomitantly to inflammation in postoperative sepsis. First, the inflammatory response induces RBC aggregation via the release of plasmatic macromolecules, as observed in our study for CRP. One may also notice a slight time correspondence

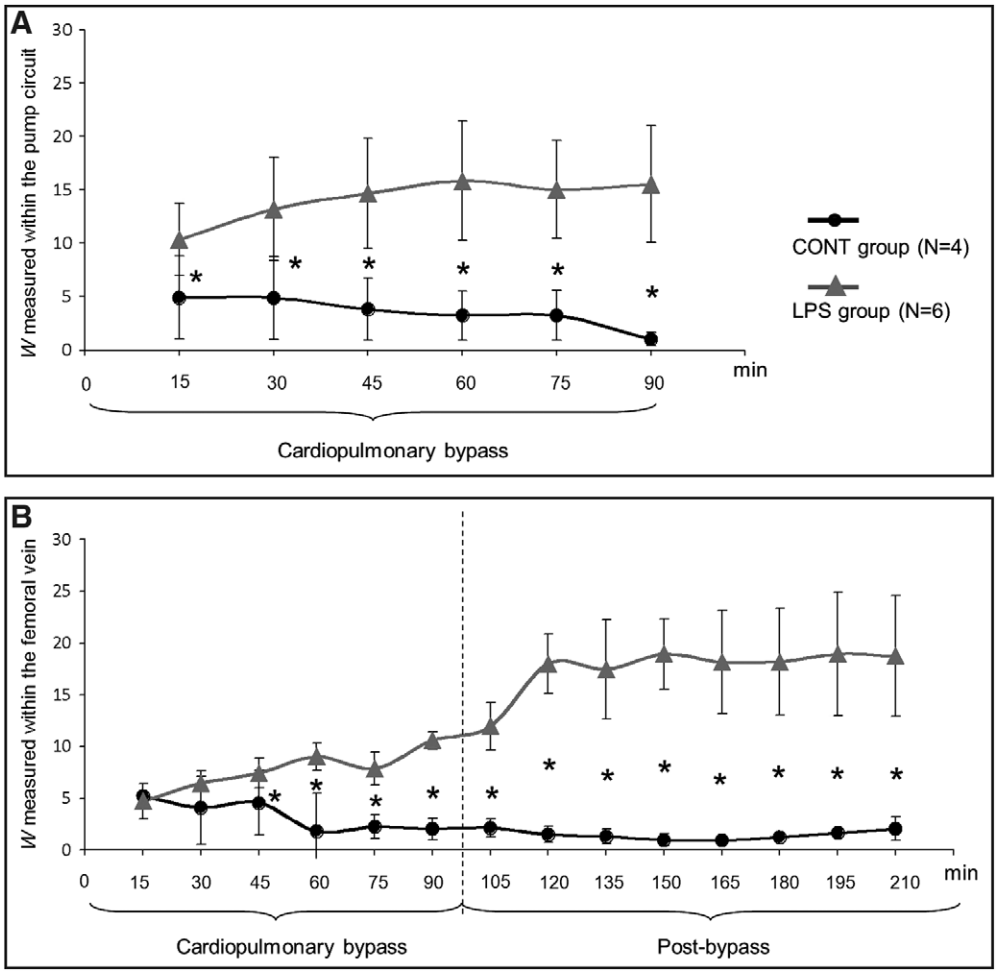

Figure 4. Time course of *W* during and after the cardiopulmonary bypass surgery. Within the pump circuit deviation (**A**) and within the femoral vein (**B**).
*Differences between lipopolysaccharide (LPS) and control (CONT) groups ($p < 0.05$).

between the increase in fibrinogen, usually described as the main plasmatic factor responsible for RBC aggregation and the increase of *W* in the LPS group. Second, oxidative stress, which in this study could have been induced by both LPS and CPB, promotes cellular alterations further enhancing aggregation (41). Both mechanisms were likely involved in the LPS- and CPB-related inflammation, and in the concomitant RBC aggregation response measured with the SFSAE imaging method. Finally, Piagnerelli et al (42) described a reduction of sialic acid content of the RBC membrane in septic patients. This alteration is known to conduct to enhanced RBC aggregation (43) and may have been responsible for the higher *W* observed in the LPS group.

Blood rheology alterations have known impact on venous thrombosis and microcirculatory blood flow. Abnormal RBC aggregation increases blood viscosity and peripheral vascular resistance and has been described to play a significant role in tissue-perfusion disturbances encountered in sepsis (12, 22, 44, 45). A "hemorheologic vicious circle" has been described, emphasizing the fact that enhanced RBC aggregation can further promote the physiopathological process (22, 38). In addition to its relevance for inflammation monitoring, the continuous

TABLE 2. Time Course of Acute-Phase Proteins Corrected for Dilution During and After the Cardiopulmonary Bypass

Variable	Group	T_0	T_{30}	T_{90}	T_{120}	T_{210}
Fibrinogen (g/L)	Control	2.0 ± 0.0	2.4 ± 0.1	2.4 ± 0.7	1.9 ± 0.2	1.7 ± 0.3
	LPS	2.4 ± 0.4	3.7 ± 0.6	3.2 ± 0.5	4.4 ± 0.3[a,b]	2.5 ± 0.6
Immunoglobulin G (g/L)	Control	9.8 ± 1.0	11.3 ± 1.2	9.7 ± 1.1	9.2 ± 0.5	9.1 ± 0.1
	LPS	12.4 ± 1.6	14.6 ± 1.0	11.6 ± 1.1	11.8 ± 0.8	12.5 ± 1.2
Haptoglobin (mg/dL)	Control	23.7 ± 8.1	17.7 ± 6.4	18.4 ± 7.3	16.3 ± 5.4	22.6 ± 14.1
	LPS	32.0 ± 5.7	34.9 ± 5.7	32.1 ± 5.5	27.5 ± 3.6	27.7 ± 4.4
C-reactive protein (μg/mL)	Control	47.8 ± 13.0	53.6 ± 26.8	48.8 ± 17.0	49.0 ± 20.3	56.2 ± 20.5
	LPS	675.7 ± 98.2[b]	621.0 ± 176.4[b]	562.8 ± 168.2[b]	566.4 ± 128.2[b]	670.7 ± 102.0[b]

LPS = lipopolysaccharide.
[a]Different from T_0 ($p < 0.05$).
[b]Difference between the two groups ($p < 0.05$).
Values are plasma concentrations expressed as mean ± SEM.

assessment of RBC aggregation could thus be of interest to predict thrombotic side effects and tissue hypoperfusion in critical care situations.

Clinical Perspectives

This study proposed a preclinical evaluation of the SFSAE imaging method in a swine CPB model. In humans, CPB can trigger a cascade of events that includes systemic inflammatory response syndrome, which is generally well tolerated. In 10% to 15% of cases, multiple organ dysfunctions (MODS) lead to poor patient outcomes and increased mortality (1–3). However, the contribution of the inflammatory response to adverse patient outcomes is potentially remediable. The extent through which the continuous monitoring of the inflammatory response may be useful to recognize early shocks and guide therapy therefore requires further investigation. Therapeutic strategies involving anti-inflammatory (i.e., steroidal or nonsteroidal) and vasoactive drugs could be used more efficiently by considering a proper timing for therapy initiation and a selection of patients who might optimally respond. This would allow a better risk balance between alterations leading to organ failure versus drug-related side effects (46–48). The SFSAE *W* monitoring within the systemic venous circulation may anticipate high-risk surgery-related MODS and concomitant circulatory-related adverse consequences, such as venous thrombosis and tissue-perfusion defects. It thus constitutes a promising avenue complementary to existing methods.

One limitation of the current protocol is the exposure of the femoral vein to allow imaging. However, the human skin presents less attenuation to ultrasound than in swine, which gives the opportunity to use the SFSAE in clinical practice and benefits from its real-time potential. The noninvasive monitoring of inflammation with a high-frequency probe (e.g., 25 MHz) indeed is feasible on human superficial vessels. It has already been used to monitor RBC aggregation in the cephalic and great saphenous veins of patients with diabetes (49). To control the flow shear rate, the vascular occluder used in this study could be replaced by a cuff inflated intermittently at the time of the measurement.

CONCLUSION

This study used a swine CPB model to demonstrate the feasibility of using the SFSAE ultrasonic cellular imaging method to continuously monitor RBC aggregation as a marker of the inflammatory response and of the risk of tissue hypoperfusion and venous thrombosis. The technique can be viewed as a very promising complementary real-time noninvasive approach to anticipate shock occurrence in surgery with postoperative sepsis risk. Further evidences are nevertheless needed to know if the *W* index could be useful in the surveillance of critically ill patients.

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REFERENCES

1. Laffey JG, Boylan JF, Cheng DC: The systemic inflammatory response to cardiac surgery: Implications for the anesthesiologist. *Anesthesiology* 2002; 97:215–252
2. Levin RL, Degrange MA, Bruno GF, et al: Methylene blue reduces mortality and morbidity in vasoplegic patients after cardiac surgery. *Ann Thorac Surg* 2004; 77:496–499
3. Maharaj C, Laffey JG: New strategies to control the inflammatory response in cardiac surgery. *Curr Opin Anaesthesiol* 2004; 17:35–48
4. Gurgel ST, do Nascimento P Jr: Maintaining tissue perfusion in high-risk surgical patients: A systematic review of randomized clinical trials. *Anesth Analg* 2011; 112:1384–1391
5. Leone M, Blidi S, Antonini F, et al: Oxygen tissue saturation is lower in nonsurvivors than in survivors after early resuscitation of septic shock. *Anesthesiology* 2009; 111:366–371
6. Trzeciak S, Dellinger RP, Parrillo JE, et al; Microcirculatory Alterations in Resuscitation and Shock Investigators: Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: Relationship to hemodynamics, oxygen transport, and survival. *Ann Emerg Med* 2007; 49:88–98, 98.e1
7. Creteur J, Carollo T, Soldati G, et al: The prognostic value of muscle StO2 in septic patients. *Intensive Care Med* 2007; 33: 1549–1556
8. De Backer D, Ospina-Tascon G, Salgado D, et al: Monitoring the microcirculation in the critically ill patient: Current methods and future approaches. *Intensive Care Med* 2010; 36:1813–1825
9. Doerschug KC, Delsing AS, Schmidt GA, et al: Impairments in microvascular reactivity are related to organ failure in human sepsis. *Am J Physiol Heart Circ Physiol* 2007; 293:H1065–H1071
10. Nanas S, Gerovasili V, Renieris P, et al: Non-invasive assessment of the microcirculation in critically ill patients. *Anaesth Intensive Care* 2009; 37:733–739
11. Trzeciak S, Cinel I, Phillip Dellinger R, et al; Microcirculatory Alterations in Resuscitation and Shock (MARS) Investigators: Resuscitating the microcirculation in sepsis: The central role of nitric oxide, emerging concepts for novel therapies, and challenges for clinical trials. *Acad Emerg Med* 2008; 15:399–413
12. Reggiori G, Occhipinti G, De Gasperi A, et al: Early alterations of RBC rheology in critically ill patients. *Crit Care Med* 2009; 37: 3041–3046
13. Sakr Y, Dubois MJ, De Backer D, et al: Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004; 32:1825–1831
14. McHedlishvili G, Maeda N: Blood flow structure related to red cell flow: Determinant of blood fluidity in narrow microvessels. *Jpn J Physiol* 2001; 51:19–30
15. Yu FT, Armstrong JK, Tripette J, et al: A local increase in red blood cell aggregation can trigger deep vein thrombosis: Evidence based on quantitative cellular ultrasound imaging. *J Thromb Haemost* 2011; 9:481–488
16. Imaizumi K, Shiga T: Effect of immunoglobulins and IgG-fragments on the human erythrocyte aggregation, studied by a rheoscope combined with image analyzer. *Biorheology* 1983; 20:569–577
17. Pribush A, Zilberman-Kravits D, Meyerstein N: The mechanism of the dextran-induced red blood cell aggregation. *Eur Biophys J* 2007; 36:85–94
18. Schmid-Schönbein H, Gallasch G, Volger E, et al: Microrheology and protein chemistry of pathological red cell aggregation (blood sludge) studies in vitro. *Biorheology* 1973; 10:213–227
19. Weng X, Cloutier G, Beaulieu R, et al: Influence of acute-phase proteins on erythrocyte aggregation. *Am J Physiol* 1996; 271(6 Pt 2):H2346–H2352
20. Weng X, Roederer GO, Beaulieu R, et al: Contribution of acute-phase proteins and cardiovascular risk factors to erythrocyte aggregation in normolipidemic and hyperlipidemic individuals. *Thromb Haemost* 1998; 80:903–908
21. Assayag EB, Bornstein N, Shapira I, et al: Inflammation-sensitive proteins and erythrocyte aggregation in atherothrombosis. *Int J Cardiol* 2005; 98:271–276

22. Baskurt OK, Temiz A, Meiselman HJ: RBC aggregation in experimental sepsis. *J Lab Clin Med* 1997; 130:183–190
23. Berliner S, Rogowski O, Aharonov S, et al: Erythrocyte adhesiveness/aggregation: A novel biomarker for the detection of low-grade internal inflammation in individuals with atherothrombotic risk factors and proven vascular disease. *Am Heart J* 2005; 149:260–267
24. Caswell M, Corlett MP, Stuart J, et al: Test for monitoring the acute-phase response to surgery. *Clin Hemorheol* 1992; 12:407–413
25. Yu FT, Cloutier G: Experimental ultrasound characterization of red blood cell aggregation using the structure factor size estimator. *J Acoust Soc Am* 2007; 122:645–656
26. Franceschini E, Yu FT, Cloutier G: Simultaneous estimation of attenuation and structure parameters of aggregated RBCs from backscatter measurements. *J Acoust Soc Am* 2008; 123:EL85–EL91
27. Yu FT, Franceschini E, Chayer B, et al: Ultrasonic parametric imaging of erythrocyte aggregation using the structure factor size estimator. *Biorheology* 2009; 46:343–363
28. Aubin MC, Laurendeau S, Mommerot A, et al: Differential effects of inhaled and intravenous sildenafil in the prevention of the pulmonary endothelial dysfunction due to cardiopulmonary bypass. *J Cardiovasc Pharmacol* 2008; 51:11–17
29. El-kebir D, Taha R, Hubert B, et al: The anti-inflammatory effect of inhaled nitric oxide on pulmonary inflammation in a swine model. *Can J Physiol Pharmacol* 2003; 83:252–258
30. Picone AL, Lutz CJ, Finck C, et al: Multiple sequential insults cause post-pump syndrome. *Ann Thorac Surg* 1999; 67:978–985
31. Troncy E, Hubert B, Pang D, et al: Pre-emptive and continuous inhaled NO counteracts the cardiopulmonary consequences of extracorporeal circulation in a pig model. *Nitric Oxide* 2006; 14:261–271
32. Wakayama F, Fukuda I, Suzuki Y, et al: Neutrophil elastase inhibitor, sivelestat, attenuates acute lung injury after cardiopulmonary bypass in the rabbit endotoxemia model. *Ann Thorac Surg* 2007; 83:153–160
33. Kim SY, Miller IF, Sigel B, et al: Ultrasonic evaluation of erythrocyte aggregation dynamics. *Biorheology* 1989; 26:723–736
34. Franceschini E, Yu FT, Destrempes F, et al: Ultrasound characterization of red blood cell aggregation with intervening attenuating tissue-mimicking phantoms. *J Acoust Soc Am* 2010; 127:1104–1115
35. Garcia D: Robust smoothing of gridded data in one and higher dimensions with missing values. *Comput Statist Data Anal* 2010; 54:1167–1178
36. Van Beaumont W: Evaluation of hemoconcentration from hematocrit measurements. *J Appl Physiol* 1972; 32:712–713
37. Baskurt O, Neu B, Meiselman HJ: Red Blood Cell Aggregation. Boca Raton, FL, CRC Press, Taylor & Francis Group, 2012, pp 304
38. Brath E, Nemeth N, Kiss F, et al: Changes of local and systemic hemorheological properties in intestinal ischemia-reperfusion injury in the rat model. *Microsurgery* 2010; 30:321–326
39. Heilmann L, Rath W, Pollow K, et al: The rheological changes after cesarean section: The influence of low molecular weight or unfractionated heparin on the rheological properties of blood. *Clin Hemorheol Microcirc* 2007; 37:211–218
40. Mokken FC, Henny CP, Gelb AW, et al: The effects of propofol compared to high-dose fentanyl anesthesia on rheologic parameters in coronary artery surgery. *J Cardiothorac Vasc Anesth* 1993; 7:10–16
41. Baskurt OK, Temiz A, Meiselman HJ: Effect of superoxide anions on red blood cell rheologic properties. *Free Radic Biol Med* 1998; 24:102–110
42. Piagnerelli M, Boudjeltia KZ, Brohee D, et al: Alterations of red blood cell shape and sialic acid membrane content in septic patients. *Crit Care Med* 2003; 31:2156–2162
43. Hadengue A, Razavian SM, Del-Pino M, et al: Influence of sialic acid on erythrocyte aggregation in hypercholesterolemia. *Thromb Haemost* 1996; 76:944–949
44. Astiz ME, DeGent GE, Lin RY, et al: Microvascular function and rheologic changes in hyperdynamic sepsis. *Crit Care Med* 1995; 23:265–271
45. Lam C, Tyml K, Martin C, et al: Microvascular perfusion is impaired in a rat model of normotensive sepsis. *J Clin Invest* 1994; 94:2077–2083
46. Bourbon A, Vionnet M, Leprince P, et al: The effect of methylprednisolone treatment on the cardiopulmonary bypass-induced systemic inflammatory response. *Eur J Cardiothorac Surg* 2004; 26:932–938
47. Bronicki RA, Backer CL, Baden HP, et al: Dexamethasone reduces the inflammatory response to cardiopulmonary bypass in children. *Ann Thorac Surg* 2000; 69:1490–1495
48. Lamarche Y, Malo O, Thorin E, et al: Inhaled but not intravenous milrinone prevents pulmonary endothelial dysfunction after cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 2005; 130:83–92
49. Nguyen LC, Tripette J, Franceschini E, et al: In-situ characterization of Red blood cell aggregation measured with high frequency ultrasound in type 2 diabetic patients. *IEEE Ultrason Symp* 2010; Oct:612–615