Endothelial dysfunction assessed by brachial artery ultrasound in severe sepsis and septic shock

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Abstract

Purpose: Noninvasive evaluation of endothelial function may be accomplished by ultrasound assessment of flow-mediated vasodilation (FMD) of the brachial artery. This study aims to investigate the role of FMD analysis on intrahospital prognosis of patients with sepsis.

Methods: Adult patients admitted to the intensive care unit with severe sepsis or septic shock were consecutively included. Brachial artery FMD was measured upon admission, after 24 and 72 hours. A group of apparently healthy subjects paired for sex and age was used as controls. Patients were followed up to discharge or death.

Results: We studied 42 patients (mean age, 51 ± 19 years) with sepsis predominantly of abdominal or respiratory etiology (75%). Acute Physiology And Chronic Health Evaluation II risk score was 23 ± 7, and intrahospital mortality rate was 33%. Flow-mediated vasodilation in septic patients was significantly lower than in healthy controls (1.5 ± 7% vs 6 ± 4%, \(P < .001\)). Most of the nonsurvivors (86%) showed a decline in sequential FMD analyses, whereas only 43% of survivors showed a reduction of FMD \((P = .01\)). In nonsurvivors, FMD was significantly lower 72 hours after sepsis onset \((-3.3% ± 10\% vs 5.2% ± 4\%; \ P < .05;\) time-group interaction \(P\) value = .03).

Conclusions: Brachial FMD is altered in septic patients with hemodynamic instability, and its deterioration may be an early marker of unfavorable prognosis.

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1. Introduction

Sepsis is a clinical syndrome caused by severe infection and is characterized by a systemic inflammatory reaction with varying degrees of organ dysfunction [1]. Organ failure has a cumulative effect on sepsis-related mortality, which may reach 70% in cases with severe hypotension [2]. The endothelium plays a central role in sepsis physiopathology,
producing several biologic mediators of vasomotor function and balancing the release of nitric oxide and endothelin into the bloodstream [3].

Endothelial dysfunction can be indirectly assessed by detection of various inflammatory markers released by the vascular endothelium, measured in plasma or serum [4]. Noninvasive assessment of endothelial vasomotor function can also be achieved by ultrasound measurement of peripheral artery diameters, exploring flow-mediated vasodilation (FMD)—an indicator of endothelial nitric oxide bioavailability [5]. In humans, this technique has been extensively validated in several cardiovascular scenarios, particularly in patients with risk factors or proven atherosclerosis [6-14]. Application of ultrasound-based techniques for endothelial evaluation in septic patients has not been fully explored so far, possibly because this environment is marked by intense vasomotor fluctuations and frequent use of vasoconstrictors. Vaudo et al [15] have recently published a pioneer study that described the presence of significant brachial FMD alterations in early stages of sepsis. In septic patients with endothelial dysfunction, a reduction in the number of leukocytes and high sequential organ failure scores were also observed.

In the present prospective study, we evaluated if ultrasound-based FMD is feasible in severe sepsis and septic shock and might offer prognostic information in this setting.

2. Methods

2.1. Patients and study design

The study included 42 consecutive adult patients admitted to the intensive care unit at Hospital de Clínicas de Porto Alegre within 24 hours of diagnosis of severe sepsis or septic shock according to internationally accepted consensus definitions [1]. Sepsis was defined based on clinical evidence of infection and 2 or more of the following: (1) fever (axial temperature greater than 38°C or hypothermia (axial temperature <36°C), (2) tachycardia (heart rate >90 beats per minute), (3) tachypnea (>20 breaths per minute) or need for mechanical ventilation, (4) leukocytosis (>12 000 cells/mm³) or leukopenia (<4000 cells/mm³), or a ratio of greater than 10% band cells to polymorphonuclear cells. Severe sepsis was defined as clinical signs of sepsis associated with organ dysfunction, alterations in perfusion, or hypotension. Septic shock was defined as sepsis with hypotension even after initial volume expansion. Exclusion criteria were as follows: (1) age older than 80 years, (2) heart failure, (3) liver failure (Child-Pugh class C), (4) bone marrow failure (leukocytes <500/μL) or (5) immunsuppression (acquired human immunodeficiency; use of immunosuppressants, including corticosteroids [prednisone>5 mg/kg/day]; or cancer), and (6) infective endocarditis.

2.2. Controls

Assessment of endothelial-dependent FMD of the brachial artery was carried out in a convenience sample of apparently healthy control subjects (n = 38), matched by age and sex. Most control subjects were health care professionals, with no history of cardiovascular disease and no risk factors for atherosclerotic disease.

2.3. Study protocol

Clinical features and laboratory data of patients were collected at admission, including Acute Physiology And Chronic Health Evaluation II score [16], hemoglobin and leukocytes, C-reactive protein, and serum lactate levels. Brachial artery ultrasound to determine FMD was also carried out at admission. These parameters were reassessed in survivors after 24 hours and 72 hours. Informed consent was signed by all patients or guardians before inclusion in the study. The study was approved by the institution’s research ethics committee.

2.4. Flow-mediated vasodilation evaluation

Flow-mediated vasodilation was assessed in the brachial artery. Measurements were obtained with the patient in the supine position, in an arm without venous or arterial lines. Brachial artery images were obtained using a high-frequency transducer (7.5-10 MHz) and a commercially available ultrasound system (Philips EnVisor; Philips, Andover, Mass). Images were obtained simultaneously with electrocardiographic tracing and digitally recorded. To minimize operational errors, both the transducer and the arm were maintained in the same position during the entire procedure. Baseline images were recorded, and brachial artery posthyperemia diameter was measured in diastole, in 3 adjacent segments, at the best angle of interrogation to determine the intima-media thickness. This procedure was repeated for 3 consecutive beats. A pressure cuff was then placed on the forearm and inflated to 230 to 250 mm Hg for 5 minutes. Brachial artery diameter was measured again 45 to 60 seconds after sudden cuff deflation, following the study protocol [5]. The mean of 9 measurements of baseline and posthyperemia diameters was used for statistical analysis. Flow-mediated vasodilation was expressed as the relative change in brachial artery diameter during hyperemia and defined as 100 × ([posthyperemia diameter – baseline diameter]/baseline diameter). Therefore, positive percentage values indicate vasodilation, whereas negative percentage values indicate constriction. Brachial artery diameter measurements were performed off-line, but immediately after the protocols were finished, on a day-by-day basis. As such, the operator was always blinded to the final clinical outcome. Intraobserver variability was measured by the same investigator (LB) in 9 subjects, with excellent reproducibility between examinations.
(mean variation of baseline diameter of 0.3 mm and mean FMD variation of 0.63%). These values are substantially below the differences observed between survivors and nonsurvivors and are in accordance with the guidelines from the International Brachial Artery Reactivity Task Force [5].

2.5. Blood-derived markers

Quantitative determination of human endothelin 1 (ET-1) in extracted plasma samples from septic patients was performed using commercially available enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, Minn.), according to the manufacturer’s recommendations. The limit of detection for ET-1 was 4.2 to 117 pg/mL. Before the assay, plasma samples underwent solvent extraction (acetone:1 N HCl: water [40:1:5]), were lyophilized in a centrifugal evaporator, and reconstituted into 0.25 mL of sample diluent. Interleukin 6 (IL-6) and soluble vascular cell adhesion molecule 1 (sVCAM-1) concentrations were also quantified in plasma samples using commercially available enzyme-linked immunosorbent assay (R&D Systems), according to the manufacturer’s recommendations. Interleukin 6 detectable levels ranged from 3 to 300 pg/mL and VCAM-1 detectable levels ranged from 6.25 to 200 pg/mL.

2.6. Statistical analysis

Quantitative variables are presented as mean ± standard deviation or median and interquartile range; categoric variables are expressed as absolute numbers and percentages. Variables without normal distribution underwent logarithmic transformation. Student t test, χ², or Fisher exact test was used for comparisons between the groups, as appropriate. To study the behavior of brachial artery FMD over time, 2-way analysis of variance for repeated measures were used using general linear models (SAS Software 8.0; SAS, Cary, NC). Two-tailed P < .05 was considered as statistically significant.

3. Results

3.1. Patients’ clinical characteristics

We enrolled 42 septic patients (62% women; mean age, 51 ± 19 years; 75% of abdominal and respiratory etiology). Mean length of stay in the intensive care unit was 8 days (median, 6 days; 3-13 days). In 79% of cases, vasoactive drug therapy was required. During hospitalization, 14 (33%) patients died because of sepsis. The baseline characteristics of survivors were similar to septic patients who died during follow-up, except for the use of noradrenalin and for baseline lactate levels, which were higher in nonsurvivors (Table 1).

In the control group, we included 38 apparently healthy individuals without risk factors for cardiovascular disease (57% women). Mean age of controls was 47 ± 14 years (P = .18 for comparison with septic patients).

3.2. FMD response

Endothelium-dependent FMD response was 4 times lower in septic patients compared with apparently healthy controls (P < .001; Fig. 1). In addition, we did not observed a

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Clinical characteristics of studied patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall (n = 42)</td>
<td>Survivors (n = 28)</td>
</tr>
<tr>
<td>Age (y)</td>
<td>51 ± 19</td>
</tr>
<tr>
<td>Male</td>
<td>16 (38)</td>
</tr>
<tr>
<td>Source of sepsis</td>
<td></td>
</tr>
<tr>
<td>Abdominal</td>
<td>19 (45)</td>
</tr>
<tr>
<td>Respiratory</td>
<td>12 (30)</td>
</tr>
<tr>
<td>Urinary</td>
<td>6 (14)</td>
</tr>
<tr>
<td>Other</td>
<td>5 (11)</td>
</tr>
<tr>
<td>Time in shock (h)</td>
<td>16 ± 6</td>
</tr>
<tr>
<td>APACHE score</td>
<td>23 ± 7</td>
</tr>
<tr>
<td>Use of noradrenalin</td>
<td>33 (79)</td>
</tr>
<tr>
<td>Days in ICU</td>
<td>8 ± 7</td>
</tr>
<tr>
<td>Leukocytes</td>
<td>16089 ± 12089</td>
</tr>
<tr>
<td>Log lactate</td>
<td>0.9 ± 0.8</td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td>217 ± 129</td>
</tr>
<tr>
<td>Log ET-1</td>
<td>2.0 ± 0.5</td>
</tr>
<tr>
<td>IL-6</td>
<td>250 ± 109</td>
</tr>
<tr>
<td>sVCAM-1</td>
<td>2243 ± 1603</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± SD, median (25-75 interquartile range), or absolute number (%).

APACHE indicates Acute Physiology And Chronic Health Evaluation; ICU, intensive care unit; Log, natural logarithm; CRP, C-reactive protein.
statistically significant difference in FMD in patients with or without intravenous vasopressors at baseline \((P = .56)\).

Table 2 shows the prognostic value of FMD for intrahospital mortality in patients with severe sepsis or septic shock. Although we observed a trend toward increased mortality rates in patients with baseline FMD less than 7\% (internationally accepted cutoff point) and in patients with baseline FMD less than 5.7\% (study median), statistical significance was not achieved for the comparison between survivors and nonsurvivors. Nevertheless, 86\% of nonsurvivors showed a decrease in endothelial function in FMD sequential analyses (comparison between baseline measurement and measurements after 24 or 72 hours), whereas only 43\% of survivors had a reduction in FMD \((P = .01; \text{Table } 2)\).

Fig. 2 illustrates the temporal evolution of stratified endothelial function in survivors and nonsurvivors. We observed that patients with a favorable outcome depicted gradual FMD improvement, reaching an absolute difference in FMD greater than 8\% 72 hours after the onset of sepsis \((P \text{ for time-group interaction } = .03; \text{comparison at 72 hours: } P < .05)\).

No significant association was observed between FMD and the number of leukocytes \((P = .89)\). However, a comprehensive analysis of all assessments revealed a weak but statistically significant negative correlation between FMD and plasma lactate levels \((r = -0.26, P = .007; n = 104)\). Flow-mediated vasodilation was not significantly correlated to ET-1 levels at any time point. We observed significant but modest negative correlations between FMD and IL-6 \((r = -0.32, P = .05)\) and vascular cell adhesion molecule 1 \((r = -0.43, P = .007)\). Finally, aggregated analysis demonstrated that patients that did not deteriorate endothelial function and had low levels of sVCAM-1 or lactate (below median levels) had the best prognosis; patients that deteriorate endothelial function had an intermediate prognosis; and patients that had deterioration of endothelial function and increased levels of sVCAM-1 or lactate had the worse prognosis (Fig. 3A [sVCAM-1] and Fig. 3B [lactate]; \(P \text{ for trend } \leq .01)\).

### 4. Discussion

In this prospective study, we have shown that septic patients depicted remarkable changes in vasomotor endothelial function evaluated by brachial artery ultrasound in the early phases of severe sepsis or septic shock. We also observed that nonsurvivors had a progressive decline in FMD, a finding that was statistically significant 72 hours after inclusion in the protocol.

Over the past decade, there has been great interest in the assessment of endothelial function during sepsis. Most published studies, however, have used serum markers directly or indirectly associated to the infectious process and with endothelial cells’ activation. Reinhart et al [17] have shown that IL-6 plasma levels greater than 1000 pg/mL were highly predictive of increased risk of death by sepsis. Interleukin 6, however, is a nonspecific inflammatory marker, and increased levels of this cytokine may not directly reflect changes on endothelial function. Endothelin 1, a powerful vasoconstrictor produced by endothelial cells and released in response to physical and chemical stimuli, has also been studied in the context of sepsis. In the early stages of infection, ET-1 plasma levels raise considerably, an event that has a potential beneficial effect on arterial pressure and organ perfusion. However, long-lasting increments of ET-1 concentration in the blood trigger a profound vasoconstriction inducing tissue hypoperfusion, alterations that are notably harmful to tissue and hemodynamic homeostasis [3]. We have shown previously that increased levels of ET-1 might be predictive of increased risk of sepsis-related mortality if measured very early (approximately 6 hours) after clinical deterioration [18]. In the present analysis, ET-1 measured during the first 24 hours of severe sepsis was not predictive of increased risk of death, in accordance with our previous findings [18].

Noninvasive point-of-care techniques might allow safe, reproducible, and accurate evaluation of endothelial function at bedside. A noninvasive technique for viewing the microcirculation has been recently developed (video microscope), thus enabling in-depth observation of small blood vessels [19,20]. De Backer et al [21] have used this technique to assess microvascular blood flow in the sublingual region of patients with severe sepsis and healthy controls. Vessel
density was found to be significantly lower in patients with severe sepsis (4.5 vs 5.4 mm, \( P < .01 \)). Endothelium-dependent microvascular reactivity has also been evaluated by peripheral arterial tonometry in septic patients. Bedside microvascular reactivity was impaired in proportion to sepsis severity [22] and is inversely correlated to plasma levels of angiopoietin 2 [23], an angiogenic peptide released by endothelial cells that increases endothelial activation and permeability. As such, this novel and exciting technique may have a role as a user-independent method of monitoring endothelial dysfunction in sepsis, if validated in large-scale multicentric prospective studies.

The use of brachial artery ultrasound as a marker of endothelial dysfunction in sepsis was recently described in an Italian observational study. Vaudo et al [15] have analyzed brachial artery FMD in 45 patients with Gram-negative sepsis. Clinical parameters and FMD were measured at baseline and 3 days after admission. In this study, one third of the included patients had ultrasound-based endothelial dysfunction at admission (FMD <7.5%). This subset of individuals showed a decline in FMD and sequential organ failure score after 3 days. The authors concluded that reduction in brachial artery FMD predicted sepsis-associated organ failure. In our study, we also observed reduced FMD in septic patients when compared with healthy controls, but our mean values were significantly lower than those measured by the Italian group (81% of our sample had FMD <7% at baseline). This finding is probably
explained by differences in sepsis severity between studies. In the Italian report, all patients had sepsis without evidence of organ failure at study entry, whereas severe sepsis and septic shock were both inclusion criteria in our protocol. Furthermore, intrahospital mortality was 33% in our sample, as opposed to only 4% in their report.

Interestingly, ET-1, a renowned marker of endothelial activation, was not correlated to brachial artery FMD in our patients, demonstrating that both parameters might depict different aspects of endothelial dysfunction. The potential role of bedside endothelial function analysis as a prognostic tool in sepsis was demonstrated by the significant differences in FMD 72 hours after baseline evaluation (Fig. 2). In addition, increased sVCAM-1 plasma levels appeared to add prognostic information over FMD analysis.

Some aspects of our study design deserve consideration. Sample size was calculated to detect differences in FMD between septic patients and healthy controls. We acknowledge that this sample size was relatively limited and some of our findings should be interpreted as hypotheses generator. In addition, endothelium-independent vasodilatation was not evaluated by sublingual nitrate administration in the present protocol because of the hemodynamic instability that characterized our patients, thus limiting our conclusions to the endothelial-dependent aspect of the vasomotor function. The relatively low values of brachial FMD observed in the control group might be explained, in part, by the relatively high mean age of this subjects (47 ± 14 years old; range, 31-85 years). In analysis of FMD restricted to the youngest subgroup of controls (lowest quartile), mean FMD was in the expected reference range (8 ± 5%) [24]. Finally, it is difficult to conclusively separate the effect of vasoactive drugs on endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease. J Am Coll Cardiol 2003;41:1769-75. [8] Wu WC, Sharma SC, Choudhary G, et al. Predictive value of reactive hyperemia for cardiovascular events in patients with peripheral arterial disease undergoing vascular surgery. Arterioscler Thromb Vasc Biol 2007;27:2113-9.

In summary, we conclude that changes in FMD occur early on in septic patients with hemodynamic instability, with progressive FMD decline over the first 72 hours representing poor prognosis. However, further prospective studies with larger samples are required to validate the predictive value of endothelial dysfunction assessed by brachial artery ultrasound for risk assessment in severe sepsis and septic shock.

References
