Neutrophils, Nitric Oxide, and Microvascular Permeability in Severe Sepsis*

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Study objectives: **Alterations in microvascular permeability are prevalent in patients with sepsis; a recent study reported that patients with septic shock had increased capillary filtration coefficient (Kf), a noninvasive index of microvascular permeability. We aimed to determine whether patients with severe sepsis had increased Kf, and whether the magnitude of Kf correlated with indexes of nitric oxide activity and neutrophil activation.**
Design: **Single-center, prospective study.**
Setting: **Twenty-five–bed ICU of a medical college-affiliated teaching hospital.**
Patients: **Fifteen ICU patients with severe sepsis based on the American College of Chest Physicians/Society of Critical Care Medicine consensus criteria of 1992, and 10 nonseptic ICU patients as control subjects.**
Interventions: **Kf was measured by venous congestion plethysmography, plasma nitrate/nitrite (NOx) by chemiluminescence, and neutrophil expression of α_4-integrin (an index of neutrophil activation) by flow cytometry.**
Measurements and results: **Septic patients had higher Kf than nonseptic control subjects. Kf of septic patients was $5.6 \pm 0.6 \times 10^{-3}$ mL·min^{-1}·100 mL tissue^{-1}·mm Hg^{-1} (mean ± SEM, mL·min^{-1}·100 mL tissue^{-1}·mm Hg^{-1} = Kf units [KfU]) as compared to $3.9 \pm 0.5 \times 10^{-3}$ KfU in nonseptic ICU patients ($p < 0.05$). There was no correlation between plasma NOx and Kf, or between neutrophil α_4-integrin expression and Kf in patients with sepsis. Septic patients with clinical evidence of edema had significantly higher Kf ($p < 0.05$) than nonedematous septic patients.**
Conclusions: **ICU patients with severe sepsis have increased Kf, a noninvasive index of microvascular water permeability. The magnitude of hyperpermeability did not correlate with NOx levels or one index of neutrophil activation (α_4-integrin expression). Presence of peripheral edema in these patients was associated with increased Kf, and may represent a simple, clinical indicator of altered microvascular permeability in sepsis.** ***(CHEST 2005; 128:1706–1712)***

Key words: α_4-integrin; capillary permeability; neutrophils; nitric oxide; plethysmography; sepsis

Abbreviations: Jv = rate of fluid filtration; Kf = capillary filtration coefficient; KfU = capillary filtration coefficient unit; NO = nitric oxide; NOx = nitrate/nitrite; Pcuff = cuff pressure of congestion plethysmography device; Pvi = cuff pressure that must be exceeded to generate net fluid filtration; VCP = venous congestion plethysmography

Microvascular permeability refers to the ease of passage or transport of volume and solutes across exchange vessels (*eg*, capillaries and postcapillary venules) and is a major determinant of nutrient delivery to tissues. Pathologic alterations of this process, or hyperpermeability, contribute to the pathogenesis of sepsis,[1–3] a leading cause of death in adult ICUs.[4] Several lines of evidence support a role for nitric oxide (NO) in hemodynamic alterations in sepsis.[5] Experimental sepsis is characterized by enhanced expression and activity of inducible NO synthase,[6,7] and both experimental and clinical sepsis are associated with enhanced plasma markers of NO (nitrate/nitrite [NOx]).[7–11] Further, plasma NOx levels correlate inversely with arterial BP,[11] and NO synthase inhibitors improve some hemodynamic abnormalities in several sepsis models.[8,12] Although work from our laboratory and others[13–15] demon-

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strate that NO can enhance single-vessel permeability coefficients in animal models, it is unclear whether NO contributes to hyperpermeability in human sepsis. Similarly, although an association between neutrophil activation and altered permeability has been described in experimental sepsis,[16,17] the role of activated neutrophils on these abnormalities in septic humans is unknown. Neutrophil α_4-integrin expression has been described as a marker of neutrophil activation in sepsis, elevated in septic patients, but infrequently detected in healthy subjects or those with focal infections.[18] Whether neutrophil α_4-integrin expression correlates with severity of disease or hyperpermeability in sepsis remains to be determined.

The aim of this study was to understand the relationship between neutrophil activation, NO, and microvascular hyperpermeability in septic patients. The global hypothesis was that NO and activated neutrophils contribute causally to the systemic permeability alterations in patients with sepsis. Thus, we anticipated positive correlations between capillary filtration coefficient (Kf) [an index of microvascular permeability], NOx, and neutrophil α_4-integrin expression.

Materials and Methods

The study protocol was approved by the Institutional Review Board of Baylor College of Medicine and performed in accordance with the Declaration of Helsinki[19] and Good Clinical Practice Code. Informed consent was obtained from all subjects. Both septic and nonseptic control subjects were recruited prospectively from the 25-bed medical ICU/coronary care unit of Ben Taub General Hospital, a 647-bed, medical college-affiliated teaching hospital, from August 2002 through June 2003. The recruitment was done within the first 48 h of admission to the ICU or within 48 h of clinical onset of sepsis if the subjects were ICU inpatients.

Inclusion Criteria

Septic subjects were recruited based on the American College of Chest Physicians/Society of Critical Care Medicine consensus criteria of 1992 for severe sepsis.[20] Nonseptic control patients were adults (> 18 years of age) recruited from the same ICU who did not fulfill the above definition of sepsis and who did not meet any of the exclusion criteria shown below.

Exclusion Criteria

Patients who met one or more of the following criteria were excluded: (1) confused, agitated, or unable to cooperate for Kf measurements; (2) pregnant or breastfeeding; (3) diastolic BP < 50 mm Hg; (4) acute hemodynamic instability or rapidly escalating doses of vasopressors; (5) receiving oral or IV nitrates; (6) body mass index < 17 kg/m^2 or > 35 kg/m^2; (7) deep venous thrombosis; (8) immediate postoperative period; (9) acute clinical pancreatitis without a proven source of infection; (10) receiving chemotherapeutic agents within the prior 21 days; and (11) having undergone cardiopulmonary resuscitation during the current hospital admission.

Assessment of Microvascular Permeability

We measured Kf, a noninvasive index of microvascular water permeability, using venous congestion plethysmography (VCP), a well-validated method to quantify Kf.[21–24] The assumptions and limitations of this method have been described in detail.[21]

Briefly, the method involves sequential cumulative step increases in limb venous pressure with an occlusive cuff placed around the thigh. It is connected to a built-in air pump, which allows the application a stepwise increase in pressure based on a preset protocol. This results in a change in limb volume, measured with an electromechanical sensor (Filtrass 2001, Software version 2.03 d; DOMED Medizintechnik GmbH; Munich, Germany) placed around the calf. Cuff pressure (Pcuff) and sensor signals are passed via an analog/digital converter card to a personal computer and recorded continuously. The VCP recordings are analyzed off-line.

The application of external pressure results in an increase in limb volume due to changes in both vascular volume and fluid filtration. Pressure steps of ≤ 10 mm Hg are applied to prevent venoarteriolar reflex that can decrease blood flow to the extremity on application of external pressure.[21,22] When Pcuff is elevated above the existing venous pressure, venous outflow ceases while blood continues to flow into the limb. A rapid increase in limb circumference attributable to this altered vascular volume can be seen. This is followed by a slow increase in limb volume, which reflects fluid filtration across the microvasculature (Fig 1). As long as venous pressure is maintained significantly lower than arterial diastolic pressure, limb circumference (*ie*, limb volume) changes as a linear function of the change in venous pressure.[21] The value of limb fluid filtration components (rate of fluid filtration [Jv]) are obtained from a series of pressure steps, as shown in Figure 1. The slope of the relationship (Jv/change in pressure) represents Kf (volume flux/tissue volume/pressure changes), as depicted in Figure 2.[21] The intercept on the x-axis is the Pcuff that must be exceeded to generate net fluid filtration (Pvi). The time constant of the initial rapid vascular volume change is < 15 s, and the total completion time of the change in vascular compliance is assumed at five time constants (75 s) for small pressure steps.[21] As a result, if the slope of the limb circumference record is estimated after this time (we used 2 min as the initial time for slope determination), it will almost exclusively reflect microvascular fluid filtration.

To avoid bias, interpretation of all VCP data and determination of Kf and Pvi was done off-line by an independent investigator, blinded to the patient's group or diagnosis. During this analysis, tracings exhibiting motion or other artifacts, which precluded accurate assessment of Kf were excluded.

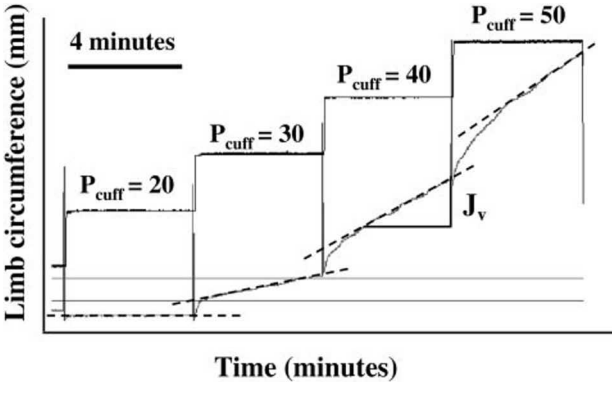

Figure 1. Tracing from VCP at four Pcuffs. The dashed lines reflect the slope used to determine Jv at each pressure.