A holistic approach for perfusion assessment in septic shock: Basic foundations and clinical applications
Hernández Poblete, Glenn

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Severe abnormalities in microvascular perfused vessel density are associated to organ dysfunctions and mortality, and can be predicted by hyperlactatemia and norepinephrine requirements in septic shock patients

Glenn Hernández¹,⁴, E. Christiaan Boerma², Arnaldo Dubin³, Alejandro Bruhn⁴, Matty Koopmans², Vanina Kanoore-Edul³, Carolina Ruiz⁴, Ricardo Castro⁴, Mario Omar Pozo³, Cesar Pedreros⁴, Enrique Veas⁴, Andrea Fuentealba⁴, Eduardo Kattan⁴, Maximiliano Rovegno⁴, Can Ince¹

¹Department of Translational Physiology, Academic Medical Center, University of Amsterdam, The Netherlands
²Departament of Intensive Care, Medical Center Leeuwarden, The Netherlands
³Servicio de Terapia Intensiva, Sanatorio Otamendi y Mirolí, Buenos Aires Argentina
⁴Departamento de Medicina Intensiva, Pontificia Universidad Católica de Chile, Santiago, Chile

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Abstract

Purpose
To determine the general relationship of perfused vessel density (PVD) to mortality and organ dysfunctions, and to explore if patients in the lowest quartile of distribution for this parameter present a higher risk of bad outcome. To identify systemic hemodynamic and perfusion variables that enhances the probability of finding a severe underlying microvascular dysfunction.

Material and methods
Retrospective multicenter study including 122 septic shock patients participating in seven prospective clinical trials on which at least one sublingual microcirculatory assessment was performed during early resuscitation.

Results
PVD was significantly related to organ dysfunctions and mortality but this effect was largely explained by patients in the lowest quartile of distribution for PVD (p=0.037; [OR 8.7, 95% CI 1.14-66.78] for mortality). Hyperlactatemia (p<0.026; [OR 1.23, 95% CI 1.03-1.47]) and high norepinephrine requirements (p<0.019, [OR 7.04 CI 1.38-35.89]) increased the odds of finding a severe microvascular dysfunction.

Conclusions
PVD is significantly related to organ dysfunctions and mortality in septic shock patients, particularly in patients exhibiting more severe abnormalities as represented by the lowest quartile of distribution for this parameter. The presence of hyperlactatemia and high norepinephrine requirements increases the odds of finding a severe underlying microvascular dysfunction during a sublingual microcirculatory assessment.
Introduction

Microcirculatory perfusion has been the subject of extensive research over the last decade, especially in the context of sepsis [1-4]. Several sublingual microcirculatory abnormalities have been described and linked to morbidity and mortality in septic shock patients [1,3,5]. More recent research has been focused on potential therapies to improve microcirculatory flow with some promising results [6-11]. However, there are still fundamental aspects that need to be resolved before launching major controlled clinical trials. First, although several indices to evaluate sublingual microcirculation have been proposed [5], the clinical relevance of individual microcirculatory parameters is far from being well established [1-4]. This is particularly true for perfused vessel density (PVD), a parameter representing functional capillary density. Second, no severity staging has been proposed or validated for any of these microcirculatory indices. Third, the relationship between systemic hemodynamics, global perfusion parameters and microcirculatory derangements is still controversial [6,8,12-14], and as a consequence it is not clear if macrohemodynamic or perfusion parameters can predict the status of microcirculatory perfusion.

As a matter of fact, a significant correlation between systemic hemodynamic parameters and microcirculatory indices was observed in septic shock patients early on after emergency hospital admission [12]. Nevertheless, this finding has not been reproduced in intensive care unit-based studies [1,6]. In the same line, the relationship between microcirculatory derangements and metabolic perfusion-related parameters is controversial. While some studies report parallel changes in microcirculatory flow and lactate during septic shock resuscitation [6,11], others have failed to find any correlation [13]. Therefore, from a bedside perspective, it is hard to identify which septic shock patients are more likely to present a microvascular dysfunction, and thus constitute better candidates for microcirculatory assessment and for potential inclusion in clinical trials.

To address these uncertainties, we conducted a clinical study in a large multicenter cohort of patients with septic shock. Our aims were to determine the general relationship of PVD to mortality and organ dysfunctions, and to explore if patients in the lowest quartile of distribution for this parameter presented a higher risk of bad outcome. Additionally, we wanted to identify systemic hemodynamic and perfusion variables that enhance the probability of finding a severe underlying microvascular dysfunction.

Methods

Setting

We conducted a retrospective, cross-sectional study in 3 academic centers (Chile, Argentina and The Netherlands). Septic shock patients [15] enrolled in 7 prospective studies evaluating microcirculation in different settings were considered for inclusion [4,7,9,10,13,16,17]. Although the main objectives of these studies were markedly different, they shared the following aspects: 1) local Institutional Review Boards (IRB) approval with informed
consent requirement (except in study # 17 for which it was waived by the IRB); 2) one or more microcirculatory assessments performed in parallel with macrohemodynamic and perfusion parameters within the first 24 h of septic shock diagnosis; 3) microcirculatory image analysis performed by an experienced investigator; 4) image analysis criteria based on a recent consensus [5]; 5) demographic, severity scores and clinical data registered at baseline, and follow-up until hospital discharge or death; and 6) overall management of septic shock based upon perfusion-driven protocols, as summarized below.

**Background management**

Management protocols shared the following principles: Resuscitation was aimed at restoring macrohemodynamics and global perfusion. Normalization of lactate was the main endpoint in addition to early aggressive source control. Initial fluid resuscitation was directed at correcting basic hemodynamic parameters. Norepinephrine (NE) was used as the sole vasopressor in patients with persistent hypotension after fluid loading. Additional invasive monitoring, vasoactive drugs and mechanical ventilation were decided on an individual basis and center preferences by attending physicians. Cardiac index and related parameters were evaluated with a pulmonary artery catheter. Intravascular volume status was optimized following recent recommendations if suitable [18]. Unresponsive patients received different rescue therapies [10,19] observing center-specific variations.

**Microcirculatory assessment**

Sublingual microcirculation was evaluated as soon as technically feasible during the first 24 h of resuscitation. Whenever microcirculation was evaluated, mean arterial pressure (MAP), cardiac index, lactate, mixed venous O₂ saturation (SvO₂), and NE doses were registered in parallel. The earliest microcirculatory assessment per patient was considered for the present study.

Sublingual microcirculation was assessed with orthogonal polarization spectral imaging [4,13] or side dark stream field videomicroscopy [7,9,10,16,17] with a 5x lens (Microscan®, Microvision Medical, Amsterdam, The Netherlands). At each time-point, at least five, 10-20 seconds, images were recorded. After removing saliva and oral secretions the probe was applied over the mucosa at the base of the tongue. Special care was taken to avoid pressure artifacts, which was verified by checking ongoing flow in larger microvessels (>50 μm).

Microcirculatory analysis Each center followed the recommendations of a recent consensus conference [5], which proposes that image analysis should be at least depicted as measurements of perfusion (proportion of perfused vessels (PPV %), and density (total vessel density (TVD, n/mm) and perfused vessel density (n/mm))). A grid-line consisting of 3 horizontal and 3 vertical equidistant lines was superimposed on the image. TVD was calculated as the number of vessels crossing the lines divided by the total length of the lines. All vessels crossing the lines were counted and classified either as perfused (continuous flow) and non-perfused (no flow or intermittent flow) vessels. PPV was calculated as the number of vessels with continuous flow divided by the total number of vessels, multiplied
by 100. PVD, an estimate of functional capillary density, can be calculated as TVD x PPV. Microcirculatory indices were determined for large and small vessels using a cutoff diameter for small vessels of <20 μm. Only data regarding the small vessels are reported. Considering that centers estimated microcirculatory flow index and heterogeneity with different methodologies, these flow indices were not included in the present analysis. On the other hand, we decided to focus our study mainly in PVD since in our opinion it is the single approach that more comprehensively quantifies microvascular perfusion.

**Statistical analysis**

Due to non-normal distribution of microcirculatory variables, non-parametric statistics were used. Results are expressed as median and interquartile range, or mean when pertinent. Medians were compared by Mann Whitney test and categorical data with Chi square. Association between microcirculatory and hemodynamic and perfusion parameters was assessed with Spearman’s rho.

A logistic regression analysis was performed to determine if PVD was an independent predictor of hospital mortality, incorporating several variables such as center, APACHE II score, NE dose, lactate, and PVD quartile. PPV was excluded from the model to avoid over-adjustment since PVD is calculated as TVD x PPV.

A second logistic regression analysis was performed to determine systemic and perfusion variables independently associated to the probability of presenting severe underlying microcirculatory abnormalities as represented by the lowest PVD quartile. Several variables such as age, sex, APACHE score; basal and 24 h SOFA scores, NE dose, cardiac index, lactate, and SvO₂ values, were included in the model.

SPSS software version 17.0 (Chicago, IL, USA) was used for statistical calculations. A p value of <0.05 was considered as statistically significant. All reported p values are two-sided. For logistic regression analyses, p values of 0.05 and 0.1 were used as entry and retention criteria, respectively.

**Results**

One hundred and twenty-two patients were included (age 65 y [18-84]; APACHE II score 21 [18-25]; basal SOFA score 10 [7-12]; 24 h SOFA score 9 [7-11]; hospital mortality 33%). Main septic sources were abdominal 44%, respiratory 29%, urinary tract 10% and catheter-related 5. Microcirculatory assessment was performed in all cases during the first 12 h after shock onset. Hemodynamic and perfusion parameters during microcirculatory assessment were: MAP 67 [61-72] mmHg, NE dose 0.15 [0.01–0.43] mcg/kg/min, lactate 2.3 [1.3–4.5] mmol/L, cardiac index 3.8 [3.1–4.9] L/min/m², and SvO₂ 71 [66–76] %.

Among microcirculatory indices, non-survivors exhibited significantly lower PVD and PPV values as compared to survivors (PVD 11.3 [7.4-13.8] vs. 13.8 [10.2-16.8] n/mm;

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p<0.01, and PPV 79.8 [70.1-93.5] vs. 91.1 [80-100]%; p<0.05). No difference was found for TVD (14.8 [12.9-19.5] vs. 14.8 [11.3-19.8] n/mm; p=0.55). When dividing PVD into quartile, we found that the lowest PVD quartile was independently associated to mortality (p=0.037; [OR 8.7, 95% CI 1.14-66.78]) (Figure 1), together with lactate (p=0.02; [OR 1.5, 95% CI 1.2-1.9]) and NE dose (p=0.002; [OR 55.3, 95% CI 4.6-666]). The other tested

Figure 5.1 Relation between PVD quartile and mortality (estimate probability).

Table 5.1 Comparison of lowest versus highest PVD quartiles.

<table>
<thead>
<tr>
<th>Quartile</th>
<th>1 (n=30)</th>
<th>2-4 (n=92)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>56 [48-68]</td>
<td>63 [55-76]</td>
<td>0.22*</td>
</tr>
<tr>
<td>APACHE II score</td>
<td>23 [18-27]</td>
<td>20 [16-23]</td>
<td>0.02*</td>
</tr>
<tr>
<td>SOFA score</td>
<td>12 [10-14]</td>
<td>9 [7-11]</td>
<td>0.001*</td>
</tr>
<tr>
<td>SOFA 24 h</td>
<td>12 [8-16]</td>
<td>9 [7-11]</td>
<td>0.002*</td>
</tr>
<tr>
<td>MAP (mmHg)</td>
<td>67 [63-73]</td>
<td>70 [64-77]</td>
<td>0.21*</td>
</tr>
<tr>
<td>NE dose (mcg/kg/min)</td>
<td>0.37 [0.16-0.72]</td>
<td>0.05 [0.0-0.13]</td>
<td>&lt;0.0001*</td>
</tr>
<tr>
<td>Lactate (mmol/L)</td>
<td>5.8 [2.3-9.5]</td>
<td>2.1 [1.2-3.4]</td>
<td>0.002*</td>
</tr>
<tr>
<td>SvO₂ (%)</td>
<td>75 [67-80]</td>
<td>71 [66-75]</td>
<td>0.064*</td>
</tr>
<tr>
<td>CI (L/min/m²)</td>
<td>4.6 [3.2-5.5]</td>
<td>4.0 [3.5-4.9]</td>
<td>0.68*</td>
</tr>
</tbody>
</table>

Values are expressed as median [interquartile range], *Mann-Whitney test, ** Chi-square test. APACHE, Acute Physiology and Chronic health Evaluation; SOFA, Sequential Organ Failure Assessment; MAP, mean arterial pressure; NE, norepinephrine; SvO₂, mixed venous oxygen saturation; CI, cardiac index.
variables including APACHE II score (p=0.66; OR 1.0, 95% CI 0.9-1.1) were not associated to hospital mortality in this study.

To address potential clinical relevance of these results, we compared the lowest with the three upper PVD quartile in several clinical, hemodynamic or perfusion variables. Patients in the lowest PVD quartile (<9.4 n/mm) exhibited higher severity scores, NE requirements and lactate levels (Table 1, figure 2).

When addressing the relationship between hemodynamic and perfusion parameters with this microcirculatory index, we found that PVD was significantly correlated with NE requirements and lactate levels (Spearman’s rho, p<0.0001 for both), but not with MAP, cardiac index or SvO₂ values. Only lactate (p<0.026; OR 1.23, 95% CI 1.03-1.47) and NE requirements (p<0.019, OR 7.04, 95% CI 1.38-35.89) predicted the worst PVD quartile.

**Discussion**

Perfused vessel density was significantly related to organ dysfunctions and mortality in our cohort of septic shock patients, but patients exhibiting more severe microcirculatory abnormalities as represented by the lowest quartile of distribution for PVD, largely explained this effect. The presence of hyperlactatemia and high norepinephrine requirements increased the odds of finding a severe underlying microvascular dysfunction during a sublingual microcirculatory assessment.

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A recent consensus conference proposed a semi-quantitative approach to evaluate microcirculatory abnormalities [5], but very few studies so far have been aimed at establishing their individual specific clinical relevance or hierarchy. Our data suggest that from a clinical perspective PVD is relevant due to its association to major outcomes. This finding makes physiological sense since this parameter takes into account both the diffusional and convective determinants of tissue oxygenation at the microvascular level [5]. However, outcome relationships were mainly evident in patients with more severe derangements, and thus a staging of severity for proposed microcirculatory indices appears as imperative. This is particularly important for future research since microcirculatory-oriented trials should be focused on patients with severe abnormalities, as determined by some staging system, to effectively have the potential to impact morbidity or mortality.

PVD has been only recently highlighted in some small clinical or experimental microcirculatory studies involving cardiogenic and hemorrhagic shock, or septic patients [20-23]. Den Uil et al. performed a prospective study of sublingual microcirculatory assessment in a cohort of patients with cardiogenic shock and found that organ failures recovered more frequently in patients with perfused vessel density > median (10.3 n/mm) and that this parameter was associated to outcome [20]. Peruski et al. demonstrated a good correlation between PVD and macrohemodynamic variables in a canine model of septic shock [21]. Yeh et al. demonstrated a significant correlation between lactate and PVD in a small cohort of patients admitted to the ICU after general or thoracic surgery [22]. Finally, Top et al. found persistent low PVD values in non-survivors of sepsis in pediatric intensive care [23]. Our study involves a large cohort of septic shock patients and provides additional robust data concerning the relevance of PVD as an expression of microvascular dysfunction. Values reported for PVD across the literature are quite variable and in the range of 5.3 to 23 n/mm [10,16,20-25]). Thus, our finding of PVD values < 9.5 n/mm in the lowest distribution quartile is consistent with current literature.

In relation to our second objective, we found a significant association between high norepinephrine requirements and hyperlactatemia, with microcirculatory perfusion as depicted by the PVD parameter. Moreover, the probability of finding a PVD value in the lowest quartile (<9.4 n/mm) was particularly higher in patients with hyperlactatemia > 4 mmol/l and NE requirements > 0.2 mcg/kg/min, representing a more severe septic shock state. Thus, severe septic shock patients could represent a more precise target for interventions.

The association between NE requirements and microcirculatory abnormalities does not necessarily imply a cause/effect relationship. In a recent study by Dubin et al. there was considerable variation in the individual responses to NE that were strongly dependent on the basal condition of the microcirculation [16]. In fact, PVD improved in patients with an altered sublingual perfusion at baseline and decreased in patients with a preserved baseline microvascular perfusion. On the other hand, when MAP decreases below an autoregulatory threshold of 60 to 65 mm Hg, organ perfusion becomes pressure dependent and, consequently, the use of NE to increase MAP could improve tissue perfusion as
was recently confirmed by Georger et al. assessing NIRS-related parameters [25]. High NE requirements could also simply reflect a more profound and widespread circulatory dysfunction involving also the microvascular level. However, regardless of the presence of a cause/effect relationship, an important message for clinicians is that patients with high NE requirements are much more likely to have severe microcirculatory derangements. Therefore, these patients are particularly suitable to microcirculatory assessment and potential inclusion in clinical trials.

Another interesting aspect of our study is the lack of association between systemic parameters such as MAP, cardiac index and SvO₂, and microcirculatory abnormalities in a large series of septic shock patients. The case of SvO₂ is counter-intuitive, since impaired oxygen extraction capacity has been attributed to potential microcirculatory abnormalities during septic shock [3]. Even more, a normal SvO₂ in the presence of hyperlactatemia has been advocated as an indication for the use of vasodilators to improve microcirculatory flow [14]. However, SvO₂ is a poor indicator of microvascular dysfunction: SvO₂ can be high or low for the same degree of microvascular shunting according to a recently proposed theoretical model [26]. Several previous experimental and clinical studies have shown conflicting data about the relationship between SvO₂ and microvascular alterations [1, 27-30].

There are several possible explanations to this fact. Severe mitochondrial dysfunction could theoretically impair tissue O₂ extraction capabilities and this form of cytopathic hypoxia could be associated to high SvO₂ values and a relatively preserved microcirculatory flow. On the other hand, there is a good correlation between systemic, perfusion (including SvO₂) and microcirculatory parameters in the pre-resuscitated septic patient early on after hospital admission [12], and all parameters tend to improve in parallel after a fluid challenge in volume-responsive patients as has been suggested by several studies [31-33]. However, in patients who evolve into a persistent circulatory dysfunction (as is the case of our study population), individual perfusion markers may change in unpredictable or even opposite directions as we discuss in recent review [34]. As an example, sedation and mechanical ventilation may normalize central venous O₂ saturation in most ICU patients (and by the way influencing SvO₂), despite persistent ongoing tissue hypoxia and microcirculatory derangements [35,36]. Several recent papers support the heterogeneity of hemodynamic and perfusion profiles in persistent sepsis-related circulatory dysfunction [13,34,37].

In addition, our study design does not allow us to establish a cause-effect relationship between hyperlactatemia and microcirculatory abnormalities although microcirculatory dysfunction can clearly induce hypoxic hyperlactatemia. However, concurrent non-hypoxic causes for hyperlactatemia may jeopardize a clear-cut interpretation [34]. We also believe that discrepancies between our study and previous reports involving this relationship [4,6,8,13] may be probably explained by different study designs concerning timing and number of microcirculatory assessments or therapeutic interventions.

Our study has several limitations. First, patients were enrolled from clinical trials with different aims and settings. However, all fulfilled fundamental requirements in terms of background clinical management and microcirculatory assessment. Second, the...
retrospective design does not allow us to evaluate dynamic changes in microcirculatory and systemic parameters. Third, we did not include microcirculatory flow index due to inter-center methodological differences in analysis. Thus, we do not know if these results are applicable to that parameter. Fourth, in this analysis microcirculatory assessments were performed at different time-points within the first 24 h of resuscitation. This fact does not invalidate our results since global and microcirculatory parameters were evaluated simultaneously in each case. In contrast, we acknowledge that the relationship between a single assessment of microcirculatory status and outcome is remote, and thus can only be considered as hypotheses generating.

In conclusion, we think that our study provides interesting data for clinical research. Perfused vessel density is significantly related to organ dysfunctions and mortality in septic shock patients, but patients exhibiting more severe microcirculatory abnormalities as represented by the lowest quartile of distribution for PVD, largely explain this effect. The presence of hyperlactatemia and high norepinephrine requirements increases the odds of finding a severe underlying microvascular dysfunction during a sublingual microcirculatory assessment.

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