On Admission, Microcirculation Abnormality is an Independent Predictor of Sepsis and Sepsis-related Mortality: A Hospital-based Study

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Abstract

Background: Microcirculatory derangement is the primary cause of organ dysfunction in patients with sepsis. Assessment of the microcirculation is usually done by means of indirect parameters (SvO₂, transcutaneous PO₂, serum lactate). The aim of our study is to understand microcirculatory abnormalities in patients with sepsis by directly visualizing the tiny vessels using hand-held video microscopes (HVMs) and determining the role of this modality in the prediction of sepsis-related mortality.

Methods: A longitudinal prospective hospital-based study was carried out in medical ward and ICU of a tertiary care hospital. Patients admitted with the presumed infectious disease were included. Evaluation of sublingual microcirculation was done in these patients from Day 1 to Day 5. Clinical and laboratory variables and microcirculation variables were compared between patients with or without sepsis and between survivors and non-survivors of sepsis. Chi-square test for categorical and Student’s t-test or Wilcoxon rank-sum test for continuous variables were applied. Univariate and multivariate regression analyses were performed using the Cox-proportional hazard model.

Results and discussion: On admission, microcirculation assessment measure, PPV (small), was significantly reduced in those with sepsis, as compared to those without sepsis. Multivariable models indicate the inverse relationship of PPV small with mortality.

Keywords: Critically ill adults, Intensive-care, Microcirculation, Sepsis.

Indian Journal of Critical Care Medicine (2022): 10.5005/jp-journals-10071-24110

Introduction

Sepsis is a life-threatening clinical syndrome caused by dysregulated host response in response to infection.³ Sepsis is treatable, and timely implementation of targeted interventions improves outcomes.² It is estimated by the global burden of disease study that an estimated 48.9 million people develop sepsis annually, 11 million (22%) of whom die. Sepsis-related deaths represent 19.7% of all global deaths, with mortality being highest in low-income countries such as sub-Saharan Africa, Oceania, South Asia, East Asia, and Southeast Asia.³ Sepsis is an overwhelming host response to toxins released by microorganisms, leading to systemic inflammatory response (SIRS) and with organ dysfunction.⁴ Pathophysiology is governed by the release of cytokines that activate the extrinsic coagulation cascade and inhibit fibrinolysis. These overlapping processes result in microvascular thrombosis and poor microcirculatory perfusion, which leads to organ dysfunction.

Microcirculation is defined as the vessels that are devoid of muscular layer, and it commences with arterioles with a diameter of 75 micron and continues through the capillary bed as far as venules with a diameter of 200 micron.³ Microcirculation is altered within the first three hours after a toxin injection and accelerates in the subsequent 5 hours.⁶ It is believed that macrocirculatory effects on peripheral perfusion (such as hypotension and tachycardia) are downstream events in this process.³ Evaluation of microcirculation has been done indirectly, using indicators of tissue perfusion (such as SvO₂, transcutaneous PO₂, tissue CO₂) or tissue metabolism (serum lactate, troponin T). Urine output is also an indirect, inexpensive, and reliable indicator of tissue perfusion.⁶ Direct bedside assessment of microcirculation has now been made possible using hand-held video microscopes (HVMs) based on the first-generation orthogonal polarization spectral or second-generation side-stream dark-field (SDF) imaging.⁹ However, direct microcirculatory abnormalities have been associated with severe sepsis and sepsis-related mortality.¹⁰ It is unclear these are better as compared to traditional predictors. Assessment of microcirculation is still in development and its cutoffs are still undefined.³ Technical quality and intraobserver variations in image quality remains a concern in current microcirculation assessment methods. A training period is necessary before values are considered a reliable.¹¹ Since it is
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an operator-dependent method, recent guidelines have focused on quality of images obtained by HVMs for a reliable assessment. We conducted this study to understand microcirculatory abnormalities in patients with sepsis, and the role of this modality in the prediction of sepsis-related mortality. This is the first reported use of microcirculation assessment from India, where we used this bedside technique among patients with fever, with or without sepsis admitted to medical wards and medical intensive care unit of a tertiary care hospital in Central India.

Methods

Study Design

We conducted a longitudinal hospital-based study among patients presenting with fever and suspected to have sepsis, between January 2018 and August 2019.

Ethics Statement

The study design was approved by the Institutional Human Ethics Committee (IHEC). All participants were included after obtaining a written informed consent.

Setting

This study was conducted at All India Institute of Medical Sciences, Bhopal. Adults with fever usually present either to the medical outpatient department or to emergency services of the hospital. Those who are more seriously ill are admitted to either medical wards or the intensive care unit. At both these locations, treating physicians evaluate patients and record their clinical details, obtain hemodynamic measures, and perform investigations to determine severity. Treating physicians take treatment decisions, which include intravenous fluids, antibiotics, use of inotropes, and other organ-supporting therapies as needed. These details are available in the case-record sheets or monitoring charts of the patients.

Study Definitions

We defined a patient with fever to have a presumed acute infectious disease if symptoms were of a short duration (14 days or less), with or without system-specific features of urinary infection (such as increased frequency or burning micturition), respiratory infection (such as cough or dyspnea), skin or soft tissue infection (such as an abscess or cellulitis). Patient was defined to have sepsis if there were at least two of four SIRS criteria (fever or hypothermia; tachycardia; hypotension; leukocytosis, or leukopenia) with at least one additional evidence of organ dysfunction (acute kidney injury, hypoxia, thrombocytopenia, altered sensorium, or hepatitis).

Participants

We included all adults admitted to the hospital with a presumed acute infectious disease diagnosis in our study. We included individuals above the age of 18 years, who had duration of illness of two weeks or less, and who satisfied definition of a presumed infectious disease. Study procedures were explained to all such participants or their caregivers. We excluded those patients who denied consent, who were admitted to the hospital for longer than 24 hours before microcirculation assessment is done (as treatments will significantly alter their microcirculation assessment results) or in whom microcirculation assessment was not possible (due to poor mouth opening due to previous oral surgery, presence of trismus, or oral submucosal fibrosis). There were no other study exclusions.

Procedures

Before the start of the study, a study investigator (AP) was trained in use of sublingual microcirculation device. Over a period of two months, she obtained sublingual images that were evaluated in about 100 normal individuals, so as to standardize the methodology and improve the quality of obtained images. All eligible and consenting patients were administered a questionnaire to record their demographic details, and their hospital chart and monitoring sheet was reviewed to record variables that determine the presence or severity of sepsis (temperature, heart/pulse rate, blood pressure, oxygen saturation, leukocyte count, serum lactate levels, platelet count, serum creatinine, blood urea, AST/ALT levels, etc). The available information was used to determine if the patient was in sepsis.

All patients underwent a sublingual microcirculation assessment on the day of admission, using a HVM (Micro-scan, Microvision medical, Amsterdam, Netherlands) based on SDF technology. The device probe is a phase-contrast microscope, connected to a lap-top to visualize the images and ensure that properly focused images are obtained. It allows microcirculation assessment at the bedside. This device uses a probe that is placed below the tongue, a process similar to obtaining an oral temperature reading. The probe has a diameter of 8 mm and is covered with a disposable sheath. The device probe is kept below the tongue so as to visualize the capillary network. Once images are acquired, the probe is removed and device software analyzes the images. Automated Vascular Analysis software (AVA 4.3 C Microscan software Microvision medical, Amsterdam, Netherlands) is used for analysis, based on internationally accepted consensus scoring guidelines. The software identifies the small- and large-sized capillaries from the image and assesses if these are perfused or not perfused. Based on this assessment, we obtained the proportion of all perfused vessels (PPV %), proportion of perfused vessels-small (PPV small %), density of vessels (deBacker density) all, and density of vessels (deBacker density) small. DeBacker score is calculated in the following way: The image is divided by three vertical and three horizontal lines; the De Backer score is calculated as the number of vessels crossing the lines divided by the total length of the lines. The DeBacker score was calculated using the same software.

Microcirculation of all participants was evaluated daily for the first three days of hospital admission, to document any changes form baseline. Hospital records were used to determine the complications, mortality, and discharge from the hospital. Among patients with sepsis, the outcome was mortality from sepsis during hospital stay.

Sample Size

Our apriori sample size estimates were based on assumptions that of all patients with an infectious disease condition who are admitted to the hospital, about 15% will develop sepsis. Of all patients with sepsis, about 40–60% may not recover. Further, it is estimated that PPV small % is likely to be greater than 75% in those without sepsis, and less than 50% in those with established sepsis. Given these assumptions, we had planned to obtain a logistically feasible sample size of about 120 individuals without sepsis (so as to have at least 30 individuals with an outcome of sepsis) for the first research question, and a logistically feasible sample size of at least 100 individuals with sepsis. To estimate difference in PPV percentage among patients with sepsis who died and who survived, with power of 80%, Type I error of 5% and large effect size of at least 30 individuals in each group would be required. Therefore, we continued enrollment till a size of 30 was achieved in those who died.
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Statistical Analysis
The study variables were collected on a structured data collection form as either continuous or dichotomous variables. The data was entered on Microsoft Excel, and after data cleaning analyzed using IBM SPSS Version 26 software. We compared demographic, clinical, laboratory, and microcirculation variables of individuals with or without sepsis on admission to the hospital. We used Chi-square test for dichotomous variables and Student’s t-test or Wilcoxon rank-sum test for continuous variables to compare proportions, means, or medians, respectively. We used a p < 0.01 as a significance level for these comparisons. We evaluated the proportion of those with fever, and who developed sepsis during hospital stay as an outcome. In those with sepsis, we compared demographic, clinical, laboratory, and microcirculation variables of individuals who had a mortality, with those who did not have a mortality due to sepsis. We performed a univariate and a multivariate regression using the Cox-proportional hazard (PH) model to determine the role of microcirculation as a predictor of mortality.

Results
Of a total of 258 eligible and consenting patients who have a microcirculation assessment done on admission, we included 249 in the current study, as their follow-up information was available. Of these, 124 (49.7%) had sepsis and the remaining 125 (50.2%) had an infectious disease diagnosis, and did not have sepsis (Flowchart 1, Table 1). Distribution of infectious disease etiology is shown in Supplementary Table 1. Patients who did not have sepsis were younger, were more likely to be admitted in medical ward, had a lower pulse rate, and had higher oxygen saturation. As expected, those with sepsis had higher serum creatinine, higher leukocyte counts, and lower hemoglobin levels (Table 1). Of 124 participants, those who had sepsis on admission 33 (26.6%; 95% CI 19.6–35.0%) died. Non-survivors were more likely to have been treated in an intensive care unit, had higher pulse rate, and had higher on-admission qSOFA scores. These features imply that those who suffer mortality are more seriously ill, but their on-admission biochemical, hematological, and blood-gas values are similar to those who survive (Table 2).

On admission, microcirculation assessment measure proportion of perfused vessels (small) was significantly reduced in those with sepsis, as compared to those without sepsis. This measure was also consistently reduced in those with sepsis who had a mortality, as compared to those who survived. Reduction in this variable was consistent over the first four days of hospital stay (Table 3). Mean deBacker density of large and small vessels, however, was also consistently higher in those with sepsis and in those who had a mortality.

We used the Cox-PH model to identify the predictors of mortality among those having sepsis. In this model, we identified the hazard ratio which is associated with each of the several independent variables to the outcome, which is elapsed time to an event. Here, the event refers to mortality from sepsis and time refers to days since admission to the death/40 days. Results are shown in Table 4. Based on the results of univariate analysis and clinical relevance, we included age, gender, duration of fever, and admission in ICU on Day 1 along with PPV small as independent predictors of mortality. We performed the forward stepwise Cox-PH regression. However, since value of eight persons for PPV small was not available for Day 0, two models were analyzed: One in which independent variable entered was PPV small for Day 0 (115 observations) and other in which independent variable entered was PPV small for Day 1 (123 observations). Multivariable models indicate the inverse relationship of PPV small with mortality independent of admission in ward or ICU, age, gender, and duration of fever (Table 4). It was found that the Cox-PH model 1 has a better diagnostic potential with area under the curve (AUC) of 80.7% as compared to the parameter (100-PPV small 1) with AUC of 72.3%, and the results were statistically significant (p < 0.001) (Fig. 1).13

Discussion
In the current study, we found that individuals who had sepsis were older, had higher pulse rates, elevated serum creatinine, higher leukocyte counts, lower hemoglobin levels, and lower oxygen saturation as compared to those without sepsis. These findings are anticipated as these are defining features of sepsis. Those who died also had a higher pulse rate, higher serum lactate levels, and elevated PaCO₂ levels as compared to those who did not survive. Of microcirculation-related variables, PPV small was significantly lower in those with sepsis (as compared to those without sepsis) and in those with sepsis-related mortality (as compared to survivors). It was a significant independent predictor of mortality. In contrast, dBD overall and for small vessels was higher in those with sepsis and those with mortality, but the proportion of perfused vessels was lower. This implies that this density was largely contributed by non-perfused vessels. However, previous studies conducted in Europe and North America have suggested that poor sublingual microcirculation measured by HVMs is an independent predictor of mortality and is associated with a poor organ perfusion.12 Our

Flowchart 1: Study flow

Participant screened (n = 300)
- Refused consent (n = 26)
- Participants included (n = 274)
- Insufficient data (16 technical issues, 4 Deaths, 5 withdrawal of consent)
- Participants analyzed (n = 249)

No sepsis on admission (n = 125)
- Sepsis on admission (n = 124)
Table 1: Distribution of variables in those with sepsis and those without sepsis at baseline (n = 249)

| Variable                        | Sepsis                  | No sepsis                | p value |
|---------------------------------|-------------------------|--------------------------|---------|
| Number                          | n = 124                 | n = 125                  |         |
| Mean age* (SD)                  | 47.92 (18.6)            | 39.01 (18.12)            | <0.001  |
| Female gender§ n (%)            | 60 (53.1)               | 53 (46.9)                | 0.343   |
| Mean duration of fever in days* (SD) | 8.4 (4.64)            | 8.6 (5.14)               | 0.805   |
| **Initial admission location**% |                         |                          |         |
| Ward n (%)                      | 23 (16.8)               | 114 (83.2)               | <0.001  |
| High dependency unit n (%)      | 32 (97)                 | 10 (30)                  |         |
| Intensive care unit n (%)       | 69 (98.6)               | 1 (1.4)                  |         |
| **On admission clinical variables*** |                       |                          |         |
| Mean SBP in mm Hg (SD)          | 108.73 (20.81)          | 111.5 (15.2)             | 0.235   |
| Mean DBP in mm Hg (SD)          | 65.22 (13.15)           | 70.34 (12.66)            | 0.002   |
| Mean pulse rate (SD)            | 102.35 (18.32)          | 90.55 (16.33)            | <0.001  |
| Mean respiratory rate (SD)      | 23.9 (6.30)             | 20.01 (5.07)             | 0.004   |
| Mean oxygen saturation (SD)     | 95.31 (5.13)            | 96.8 (2.37)              | <0.001  |
| **On admission biochemical variables** |                       |                          |         |
| Median serum creatinine mg/dL (SD) | 1.26 (0.87–2.43)      | 0.90 (0.74–1.04)         | <0.001  |
| Median urea mg/dL (SD)          | 56 (34–100.75)          | 26 (20.5–37.0)           | <0.001  |
| Median ALT level (SD)           | 56 (35–120)             | 54 (22.6–90.4)           | 0.067   |
| Median AST level (SD)           | 72 (38.5–132.5)         | 63 (33.2–108.5)          | 0.087   |
| Mean Na level (SD)              | 132.42 (8.40)           | 131.59 (4.90)            | 0.34    |
| Mean K level (SD)               | 4.08 (0.69)             | 4.04 (0.58)              | 0.607   |
| Median Hb level (SD)            | 10.65 (9.05–12.6)       | 11.8 (10.6–13.45)        | <0.001  |
| Median WBC count (SD)           | 15,615 (11,942–21,910)  | 9,330 (4,870–15,425)     | <0.001  |
| Median platelet count (SD)      | 188,000 (80,750–332,500)| 211,006 (92,500–328,000)| 0.996   |

Test of significance used *unpaired t-test, §Chi-square test, $Mann–Whitney test

Table 2: Distribution of variables in those with sepsis survivors and non-survivors (n = 124)

| Variable                        | Survivors                  | Non-survivors               | p value |
|---------------------------------|----------------------------|-----------------------------|---------|
| Number                          | 91                         | 33                          |         |
| Mean age* (SD)                  | 49.14 (18.02)              | 44.54 (18.02)               | 0.225   |
| Female gender§ n (%)            | 45 (75)                    | 15 (25)                     | 0.694   |
| Mean duration of fever in days* (SD) | 7 (5–10)                 | 7 (6–14)                    | 0.07    |
| **Initial admission location**% |                           |                             |         |
| Ward n (%)                      | 22 (24.1)                  | 1 (3.23)                    | 0.009   |
| High dependency unit n (%)      | 25 (27.2)                  | 7 (22.5)                    |         |
| Intensive care unit n (%)       | 44 (48.3)                  | 25 (80.65)                  |         |
| **On admission clinical variables*** |                       |                             |         |
| Mean SBP in mm Hg (SD)          | 108.89 (22.34)             | 108.27 (16.16)              | 0.867   |
| Mean DBP in mm Hg (SD)          | 66.03 (13.98)              | 62.96 (10.39)               | 0.192   |
| Mean pulse rate (SD)            | 99.60 (14.59)              | 109.90 (15.42)              | 0.003   |
| Mean respiratory rate (SD)      | 24.05 (6.71)               | 23.78 (5.01)                | 0.19    |
| Mean oxygen saturation (SD)     | 95.60 (5.6)                | 94.5 (3.5)                  | 0.020   |
| **On admission biochemical variables** |                       |                             |         |
| Mean serum creatinine mg/dL (SD) | 1.25 (0.86–2.18)          | 1.5 (0.89–3.08)             | 0.271   |
| Mean urea mg/dL (SD)            | 55 (31–99)                 | 68 (44–112)                 | 0.086   |
| Mean ALT level (SD)             | 54 (35.5–104)              | 81 (31.7–334)               | 0.088   |
| Mean AST level (SD)             | 68 (38.5–107)              | 39.7 (9.5–244.5)            | 0.242   |

(Contd...)
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Table 2: (Contd…)

| Variable | Survivors | Non-survivors | p value |
|----------|-----------|---------------|---------|
| Mean Na level (SD) | 132.14 (8.76) | 133.18 (7.39) | 0.545 |
| Mean K level (SD) | 4.09 (0.72) | 4.08 (0.62) | 0.936 |
| Mean Hb level (SD) | 10.7 (9.2–12.6) | 10.5 (10.5–12.6) | 0.663 |
| Mean WBC count (SD) | 15400 (10970–19870) | 17800 (13250–24740) | 0.108 |
| Mean platelet count (SD) | 208,000 (114,000–345,000) | 123,000 (66,000–292,500) | 0.044 |

On admission blood gas variables

| Variable | Survivors | Non-survivors | p value |
|----------|-----------|---------------|---------|
| Mean HCO₃ (SD) | 20.45 (5.93) | 18.97 (4.62) | 0.252 |
| Mean PaCO₂ (SD) | 34 (29.75–42) | 41 (34.7–118.5) | 0.009 |
| Mean lactate (SD) | 2.4 (1.2–3.5) | 4.5 (3.0–5.2) | 0.038 |
| Mean PaO₂ (SD) | 86.27 (26.44) | 77.68 (19.86) | 0.135 |
| Mean qSOFa score (SD) | 1.77 (0.96) | 2.66 (0.47) | <0.001 |

Primary cause of sepsis

| Cause | Survivors | Non-survivors |
|-------|-----------|---------------|
| Respiratory | 42 (46.15) | 18 (58.06) |
| CRBSI | 21 (23.08) | 6 (19.35) |
| Urosepsis | 19 (20.88) | 2 (6.45) |
| SSTI | 0 (0) | 1 (3.45) |
| Unknown | 4 (9.89) | 6 (19.35) |

Test of significance used: *unpaired t-test, #Chi-square test, ^Mann–Whitney test

Table 3: Microcirculation assessment in those with sepsis and with sepsis mortality (n = 249)

| Variable | No sepsis | Sepsis | Sepsis survivors | Sepsis mortality |
|----------|-----------|--------|-----------------|-----------------|
| Number | n = 125 | n = 124 | n = 91 | n = 33 |
| Mean proportion of perfused vessels | | | | |
| Day 0 | 82.52 (13.12) | 77.98 (14.43) | 80.33 (13.34) | 71.89 (15.58)^ |
| Day 1 | 84.94 (12.28) | 77.72 (14.45)# | 80.03 (13.25) | 71.13 (16.90)^ |
| Day 2 | 84.80 (12.14) | 83.44 (40.92) | 81.70 (12.30) | 88.41 (78.50)^ |
| Day 3 | 89.5 (80–93) | 78.00 (15.50) | 80.67 (14.6) | 71.6 (16.21)^ |
| Mean de-backer density | | | | |
| Day 0 | 3.01 (0.96) | 3.00 (2.34, 3.38)^ | 2.78 (2.09–3.29) | 3.2 (2.42–3.84) |
| Day 1 | 3.17 (0.85) | 3.11 (2.32, 3.66)^ | 2.87 (2.2–3.56) | 3.4 (3.09–4.32)^ |
| Day 2 | 3.08 (0.96) | 2.87 (2.33, 3.46)^ | 2.67 (2.09–3.44) | 3.03 (2.67–4.0)^ |
| Day 3 | 2.99 (0.97) | 3.11 (2.55, 3.97)^ | 3.00 (2.33–3.65) | 3.57 (2.83–4.1) |

These variables in those with sepsis were significantly different as compared to those without sepsis (p < 0.001). These variables in those with sepsis mortality were significantly different as compared to those who survived (p < 0.001)

study, first from a low-middle-income setting also reports that patients with sepsis have a poor perfusion of small vessels in microcirculation. Among those with sepsis, microcirculation is further reduced on admission in non-survivors. Microcirculation assessment technology has evolved. The first-generation devices used orthogonal polarized spectroscopy (OPS). Second-generation devices have used a side-stream dark-field (SDF) imaging. We have used a second-generation device in our study.
Table 4: Cox-PH regression for predictors of mortality

| Variable              | n   | Unadjusted Model coeff. | HR  | 95% CI of HR | p value | Adjusted Model coeff. | HR  | 95% CI of HR | p value |
|-----------------------|-----|-------------------------|-----|--------------|---------|-----------------------|-----|--------------|---------|
| Age                   | 124 | -0.013                  | 0.987 | 0.969–1.006 | 0.180   |                       |     |              |         |
| Gender                | 124 | 0.047                   | 1.048 | 0.744–1.477 | 0.788   |                       |     |              |         |
| Fever duration        | 124 | 0.062                   | 1.064 | 0.999–1.134 | 0.054   |                       |     |              |         |
| ICU vs Ward           | 124 | 1.587                   | 4.888 | 2.016–11.851| <0.001  |                       |     |              |         |
| SBP                   | 124 | -0.001                  | 0.999 | 0.983–1.015 | 0.885   |                       |     |              |         |
| DBP                   | 124 | -0.016                  | 0.984 | 0.958–1.011 | 0.238   |                       |     |              |         |
| RR                    | 124 | -0.004                  | 0.996 | 0.944–1.050 | 0.872   |                       |     |              |         |
| S. Creatinine         | 123 | 0.308                   | 1.360 | 1.047–1.768 | 0.021   |                       |     |              |         |
| SpO2                  | 124 | -0.024                  | 0.977 | 0.936–1.020 | 0.283   |                       |     |              |         |
| ALT                   | 121 | 0.000                   | 1.000 | 1.00–1.00   | 0.060   |                       |     |              |         |
| Hemoglobin            | 124 | -0.030                  | 0.970 | 0.852–1.106 | 0.651   |                       |     |              |         |
| Platelets             | 124 | 0.000                   | 1.000 | 1.00–1.00   | 0.095   |                       |     |              |         |
| HCO3                  | 88  | -0.043                  | 0.958 | 0.890–1.030 | 0.247   |                       |     |              |         |
| PH                    | 88  | -5.137                  | 0.006 | 0.00–0.009  | <0.001  |                       |     |              |         |
| PaCO2                 | 68  | 0.016                   | 1.016 | 1.002–1.030 | 0.230   |                       |     |              |         |
| PaO2                  | 88  | -0.014                  | 0.986 | 0.968–1.005 | 0.140   |                       |     |              |         |
| Blood culture         | 89  | -0.306                  | 0.736 | 0.294–1.845 | 0.514   |                       |     |              |         |
| Chest X-ray           | 115 | -0.199                  | 0.819 | 0.400–1.679 | 0.586   |                       |     |              |         |
| PPV0                  | 115 | -0.026                  | 0.974 | 0.956–0.993 | 0.006   |                       |     |              |         |
| PPV1                  | 123 | -0.030                  | 0.971 | 0.952–0.990 | 0.003   |                       |     |              |         |
| PPV small 0           | 115 | -0.023                  | 0.977 | 0.964–0.990 | <0.001  |                       |     |              |         |
| PPV small 1           | 123 | -0.024                  | 0.976 | 0.963–0.990 | 0.001   |                       |     |              |         |
| PPV 10% fall          | 95  | 0.039                   | 1.040 | 0.487–2.22  | 0.919   |                       |     |              |         |
| PPV 20% fall          | 95  | 0.512                   | 1.660 | 0.676–4.110 | 0.266   |                       |     |              |         |
| deBacker day 0        | 115 | 0.273                   | 1.314 | 0.92–1.878  | 0.134   |                       |     |              |         |
| deBacker day 1        | 123 | 0.692                   | 1.990 | 1.390–2.860 | <0.001  |                       |     |              |         |
| deBacker small day 0  | 115 | 0.228                   | 9.850 | 3.70–26.22  | <0.001  |                       |     |              |         |
| deBacker small day 1  | 122 | 1.840                   | 6.290 | 2.490–15.85 | <0.001  |                       |     |              |         |

Fig. 1: ROC curve for the Cox-PH model

Currently, third-generation devices using incident dark-field (IDF) technology are also available, which have further improved image quality.12 However, the previous standards of microcirculation assessment were reported as a round-table in 2007.9 These have recently been revised as a 2018 consensus statement. More recent guidelines have emphasized the specific set of measurements that need to be taken, as well as quality indicators in image acquisition.12 However, these guidelines were not available when we planned our study, yet we had incorporated some of the principles in our study. First, training of a person performing microcirculation is important, and the trainee captured images on patients on mechanical ventilation so that hand gets accustomed to capture stable images. First, we captured only those images that have flowing RBCs in the larger vessels. Second, the software we used only interprets artifact-free images. We did not use any image quality scoring, as it was not available in the software we are using. We used PPV and DeBacker density as two indicators of microcirculation perfusion and vessel density in our study. These are two objective measures that are calculated by the software. We did not measure the other two recommended parameters—microvascular flow index (MFI) and the heterogeneity index (HI)—as these guidelines were not available at the time of planning our study. MFI is a subjective assessment of type of flow in each quadrant of the image, and it is anticipated that sluggish flow is a hallmark of poor perfusion. HI is a measure of variability and indicates a ratio of highest and a lowest
value of PPV or MFI. We can measure these additional variables in future studies.

Macrocirculation in large vessels behaves differently as compared to microcirculation in smaller vessels. Various software-based tools are available to interpret sublingual microcirculation vessels. Peripheral perfusion an indicator of microcirculation was compared with microcirculation in a recent study from China. The authors evaluated the change in proportion of perfused small sublingual vessels at 6 hours (δPPV), and peripheral perfusion index (PI) as predictors of poor prognosis in sepsis. Based on this assessment, they divided 74 participants into four groups: (a) high PI and high δPPV, (b) high PI and low δPPV, (c) low PI and high δPPV, and (d) low PI and low δPPV. SOFA scores were highest in the last group.

In a systematic review of pharmacological interventions targeting microcirculation, it was suggested that conventional inotropic drugs such as noradrenaline, or dobutamine, do not improve microvascular flow. Vasodilators such as nitrates also have a modest effect on microcirculation. On the other hand, the addition of vasopressin, or terlipressin, to noradrenaline or the use of levosimendan maintained microcirculatory flow. Passive leg raising does not improve microcirculation among patients with septic shock. This is an emerging area, and beyond initial pilot studies, mortality benefits of such pharmacological alterations in microcirculation have not been demonstrated.

Alternate modalities of microcirculation assessment are thermal challenge tests, biomarkers of glycocalyx degradation, and veno-arterial differences in PaCO₂. Deterioration of the endothelial glycocalyx (eGC), a protective carbohydrate-rich layer lining the luminal surface of the endothelium, plays a key role in vascular barrier dysfunction and eventually organ failure in systemic inflammatory response syndrome and sepsis. It is feasible to measure endothelial glycocalyx using side-stream microcirculation assessment, and it could be a future application of sublingual microcirculation assessment. In severe trauma, many indirect measurements of perfusion do not correlate with microvascular perfusion. However, visualized perfusion deficiencies do reflect a shift toward anaerobic metabolism. More detailed assessments of such a compromise in patients with trauma are currently being evaluated.

Limitations
Use of microcirculation technology is challenging. In terms of technical feasibility, these studies may be performed in non-intubated and intubated patients. It is feasible to obtain measurements in non-intubated patients who are conscious, and cooperate for the procedure. If the non-intubated patient is irritable, or unstable, it becomes difficult to perform the measurement. In a pediatric study, sublingual microcirculation was not feasible in the majority of unstable patients. In contrast, intubated patients are sedated and cooperativeness becomes less of an issue. In a recent pediatric feasibility study, measurements were difficult in unstable intubated patients as well. While in this study only 18 full measurements could be obtained from a total of 102 eligible participants, in our study non-measurement due to feasibility issues was much lower. We have not used MFI and HI, which would have been better indicators of flow measurement as compared to PPV and DeBacker density.

This study itself was a learning curve for us, and it’s just a stepping stone where we were just able to identify a subset of patients in whom microcirculatory changes correlated with organ failures. These microcirculatory changes correlated with clinical outcomes were not in the scope of the study but up next we are given fluid challenges and seeing whether it improves microcirculatory perfusion, or the addition of inotrope in cardiogenic shock improves microcirculatory perfusion. Regarding cost-effectiveness, it is an issue but best thing its non-invasive and just can cater to a large number of patients at single point of time.

Conclusions
Sepsis is a life-threatening condition. It is therefore important to predict its course outcome. Outcome is directly related to the state of perfusion in microcirculation as it is the ultimate source of nutrient and oxygen for end organs. In our study, we tried to visualize the microcirculation directly with hand-held video microscope and found that admission microcirculation assessment measure, PPV (small), was significantly reduced in those with sepsis, as compared to those without sepsis. Multivariable models indicated an inverse relationship of PPV small with mortality. Thus, we concluded from the study that a serial sublingual microcirculation assessment can help in the prediction of sepsis. Although it could not predict mortality earlier or better than the most traditional scoring system like SOFA, qSOFA, APACHE which are combinations of multiple clinical and laboratory parameters, sublingual microcirculation assessment is the single most important and direct indicator of tissue perfusion status if compared head-on with individual parameters.

Abbreviations
OPS—Orthogonal polarization spectral
SDF—Side-stream dark field
SOFA—Sequential Organ Functional Assessment
PPV—Proportion of perfused vessel
PI—Perfusion index
DIC—Disseminated intravascular coagulation
IDF—Incident dark field
LED—Light-emitting diodes
PVD—Perfused vessel density
eGC—Endothelial glycocalyx
SPSS—Statistical Package for Social Sciences
DM—Data missing
dBD—DeBacker density
HVM—Hand-held vital microscopes
MFI—Microvascular flow index
HI—Heterogeneity index

Availability of Data and Materials
The datasets generated during and/or analyzed during the present study are not publicly available, owing to further research based on this dataset, but they are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
The study design was approved by the Institutional Human Ethics Committee (IHEC). All participants were included after obtaining a written informed consent.

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Acknowledgments

The authors would like to thank Department of Medicine and Department of Anaesthesiology AIIMS Bhopal for letting us procuring patients’ data. We also thank all authors for their contribution and patients who consented to participate.

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