BRIEF REPORT

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Point-of-care assessment of microvascular blood flow in critically ill patients

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Abstract *Objective:* Sublingual
microvascular videomicroscopy can
assess tissue perfusion in critically ill
patients; however, data analysis is
currently limited to delayed off-line
evaluation. We hypothesized that a
real-time point-of-care (POC) deter-
mination of the microcirculatory flow
index (MFI), an established metric for
assessing microcirculatory perfusion,
agrees well with the conventional off-
line analysis. *Design:* Prospective
observational study. *Setting:* Urban
academic intensive care unit.
Participants: A heterogeneous
population of critically ill patients.
Measurements and results: A single
operator performed side stream
darkfield videomicroscopy of the
sublingual microcirculation and made
a POC determination of MFI in real-
time on a portable bedside monitor by
assigning a score (0 = no flow to
3 = normal) to each quadrant of the
image and averaging the four values.
After image processing, de-identifi-
cation and randomization, the same
operator, blinded to the previous
interpretation, repeated the MFI
assessment by viewing an AVI-
formatted image sequence on a 94 cm
1,080 pixel LCD monitor (reference
standard). There were 205 paired
measurements in 18 subjects. The
POC and reference standard MFI had
good agreement by Bland–Altman
analysis [mean difference of −0.031,
SD = 0.198 (95% CI, −0.43 to
0.37)]. The POC assessment was 94%
sensitive and 92% specific for
detecting impaired microvascular
flow (defined a priori as an MFI < 2.5
based on previously published data).
Conclusions: A POC determination
of MFI had good agreement with
conventional off-line analysis, and
was highly sensitive and specific for
detecting impaired microvascular
flow. This real-time technique may be
useful in future clinical trials target-
ing impaired microcirculatory
perfusion in critically ill patients.

Keywords Microcirculation ·
Shock · Resuscitation · Perfusion ·
Critical care · Emergency medicine

Introduction

Assessment of tissue perfusion is important in critically ill patients. Although the macrocirculation, comprised of the heart and large arteries, distributes blood flow globally throughout the body, it is the microcirculation, the integrated network of arterioles, capillaries and venules, that delivers blood flow to tissues and is the principal site of tissue oxygen transport. Thus, an intact and functional microcirculation is a critical element of the cardiovascular system vital for effective tissue oxygen delivery.

Microcirculatory dysfunction is a key factor in the pathophysiology of a variety of systemic pro-inflammatory states and shock etiologies including septic shock, cardiogenic shock, and ischemia/reperfusion injury [1–4]. New techniques for videomicroscopy of the vascular networks beneath mucosal surfaces have enabled minimally invasive microcirculatory assessment in critically ill patients. One accepted method of quantifying microvascular flow of vessels <20 μm in diameter is the semi-quantitative microcirculatory flow index (MFI) [5, 6]. Clinical studies have shown that MFI measured early in the course of disease can be prognostic in critically ill patients [1, 2, 4, 7, 8] and therefore can yield potentially important information during acute care and resuscitation.

Currently, conventional image analysis techniques for assessment of microcirculatory flow use off-line image processing and delayed evaluation of the image sequences in a laboratory setting. However, real-time assessment of microcirculatory flow at the bedside would be necessary for timely application of clinical interventions targeting improvement in microcirculatory perfusion. No validation studies of a real-time analysis technique for measuring microcirculatory flow at the bedside have yet been reported. This study tested the hypothesis that real-time point-of-care (POC) determination of MFI at the bedside has good agreement with conventional off-line analysis techniques in a heterogeneous patient population across a wide range of flow characteristics.

Materials and methods

This was a prospective observational study within the intensive care unit (ICU) of an urban academic medical center. We obtained 205 paired measurements (POC and reference standard) of MFI obtained and analyzed by a single operator (RCA) in a heterogeneous population of critically ill patients in various stages of disease severity. This investigation was a sub-study of two separate ongoing investigations assessing microcirculatory perfusion [8, 9]. Inclusion criteria were (1) age ≥ 18, (2) admission to the ICU, and (3) co-enrollment in one of the two study protocols at our institution that employs microcirculatory videomicroscopy in the study of circulatory shock (post-cardiac surgery utilizing cardiopulmonary bypass [9] or severe sepsis [8]).

Image acquisition

Subjects were imaged within 3 h of arrival to the ICU at multiple time intervals mandated by the co-enrollment protocols [8, 9]. A single operator performed all of the sublingual imaging with a sidestream dark field (SDF) videomicroscope (Microscan, Microvision Medical, Amsterdam), using acquisition techniques previously described and validated in both animal models and human studies [10–13]. SDF imaging produces a magnified, real-time video sequence of blood flow through submucosal microvascular networks (326× onscreen magnification) through the emission of light within the absorption frequency of hemoglobin (wavelength of 530 nm) producing an image in which red blood cells appear dark against a light background (see the Electronic Supplemental Material). At each imaging time point, video sequences were obtained from three to five separate sublingual sites in 20-s segments.

Image analysis

Flow characteristics of the microvasculature were quantified using the MFI, a semi-quantitative technique originally described by Boerma et al. [5] and consistent with recommendations from the recently published consensus conference on microcirculatory image analysis in human subjects [6]. The image is divided into 4 quadrants and the vessels <20 μm diameter are assigned a score based on the predominant flow characteristics of the vessels in that quadrant (0 = absent flow; 1 = intermittent; 2 = sluggish; 3 = normal). The values in each quadrant are averaged to give an MFI for each sublingual site at each time point. We have used this method in previous investigations and shown good inter-rater agreement among multiple observers ($\kappa = 0.87$) [8]. The bedside POC determination of the MFI (POC-MFI) was performed through real-time microcirculatory image assessment at the bedside without image processing as viewed on a 33 cm analog monitor attached to a portable stand. Using the technique described above for MFI assessment, the images were scored during acquisition by the operator at each sublingual site. The images obtained at the bedside were recorded onto digital tape (DVCAM Recorder, Sony DSR-11, USA). Video sequences from each sublingual site were processed and transferred into AVI format and digitally stored by code (1–10,000) without patient identifiers. Images were assigned a random number to enable offline analysis blinded to all clinical data and POC-MFI value.

Off-line assessment of MFI served as the reference standard for this study. Each processed video sequence was viewed on a 94 cm LCD monitor with 1,080 pixel resolution in a laboratory. The processed images were scored with the investigator blinded to the POC-MFI value.

Statistical analysis

The agreement between POC-MFI and the reference standard MFI was tested using the Bland–Altman method [14], which plots the mean of the paired measurements (x-axis) against the difference between each measurement (y-axis). We also tested the ability of the POC-MFI to detect impaired microcirculatory flow. A priori, we defined impaired microcirculatory flow as an MFI < 2.5 based on MFI averages for control subjects previously reported by our group (2.66–3.0) [4].

The sensitivity, specificity and likelihood ratio of the POC-MFI assessment's ability to detect impaired microvascular flow were calculated using the off-line MFI as the reference standard. All analyses were carried out using SPSS 14.0 software (SPSS Inc, Chicago, Illinois). The sample size estimate for a Bland–Altman method is based on 95% confidence intervals around the standard deviation (SD) of the difference between the two measurements. We calculated that a minimum sample size of 130 paired measurements would give an acceptably low variance (95% CI ± 0.30 SD) [14, 15], and thus we enrolled consecutive subjects imaged by a single operator until a minimum of 130 paired measurements were accrued. After obtaining 150 measurements, interim analysis revealed the consecutive enrollment resulted in a majority of CABG patients; enrollment then continued targeting only sepsis patients until equivalent numbers were obtained.

Results

We performed 205 paired measurements in 18 subjects (Table 1). Vasopressor medications were used at the time of imaging in 65/205 (32%) of the paired measurements, vasodilators alone in 20/205 (10%), vasodilators, vasopressors, and inotropes in 15/205 (7%), and no vasoactive medications in 105/205 (51%). The reference standard (offline) MFI values ranged from 1.25 to 3.00. When compared using the Bland–Altman method, the POC-MFI demonstrated good agreement with the reference standard. The mean difference between the two tests was −0.031, SD = 0.198 [95% CI, −0.43 to 0.37] (Fig. 1). The Bland–Altman method defines the upper limits of agreement as 2 SDs from the

Table 1 Characteristics of the study sample (n = 18)

Age, years [mean (SD)]	63 (12)
Reason for ICU admission [paired measurements (%)]	
Cardiac surgery	123 (60)
Severe sepsis/Septic shock	82 (40)
MFI (Reference std)[a] [mean (95% CI)]	2.23 [2.16–2.30]
MFI Range	1.25–3.00
MAP, mmHg [mean (95% CI)]	76 [62–90]
Vasoactive Medications [paired measurements (%)]	
Vasopressor	65 (32)
Vasodilator	20 (10)
Vasodilator + vasopressor + inotrope	15 (7)
None	105 (51)
SOFA[b]	
Mean (SD)	3.0 (2.1)

[a] Microcirculatory flow index
[b] Sequential organ failure assessment score [20]

mean (±0.396). When comparing MFI values, 94% of the POC-MFI measurements in this study were ±0.25 from the offline MFI, the smallest measurable increment of disagreement (see the Electronic Supplemental Material).

To control for repeated measures in a data set with an unequal number of replicates, as per Bland and Altman [16], we first checked with the assumption that the within-subject variances for each method were independent of the subjects' mean MFI (see the Electronic Supplemental Material) showing good agreement (perfect agreement = MFI difference of zero) regardless of mean MFI. We then verified this independence with a modified Bland–Altman plot displaying only mean MFI for each subject in the study (Fig. 2) and reported the 95% limits of agreement using the SD of the means and separate limits adjusted for the within-subject variance (SD_v).

Impaired microcirculatory flow (MFI < 2.5) occurred in 120/205 (59%) images by the reference standard. The POC assessment was 94% sensitive (95% CI: 91–97%) and 92% specific for detecting an MFI < 2.5 (95% CI: 87–95%). The overall diagnostic accuracy of the POC assessment was 93% (Table 2). The positive (+) likelihood ratio for impaired microcirculatory flow with a POC-MFI < 2.5 was 11.4 (95% CI: 6.7–19.5). The negative (−) likelihood ratio for impaired microcirculatory flow with a POC-MFI < 2.5 was 0.06 (95% CI: 0.04–0.11). Additionally, the directional change in the average MFI between time points within an individual patient (ΔMFI) as measured by the reference standard was accurately tracked by the POC assessment (see the Electronic Supplemental Material). While the data set includes repeated imaging separated by time in each patient, the operator characteristics for the POC measurement were performed as if each observation were independent of the others.

Fig. 1 Bland–Altman plot of the comparison between conventional offline and the bedside point-of-care (POC) determinations of microcirculatory flow index (MFI). This graph describes the level of agreement between different methods of measurement. The *solid line* shows the mean deviation (bias) between POC-MFI and reference standard (offline) MFI (-0.031); the *dashed line* represents the limits of agreement (mean $\pm$ 2 SD) with SD = 0.198
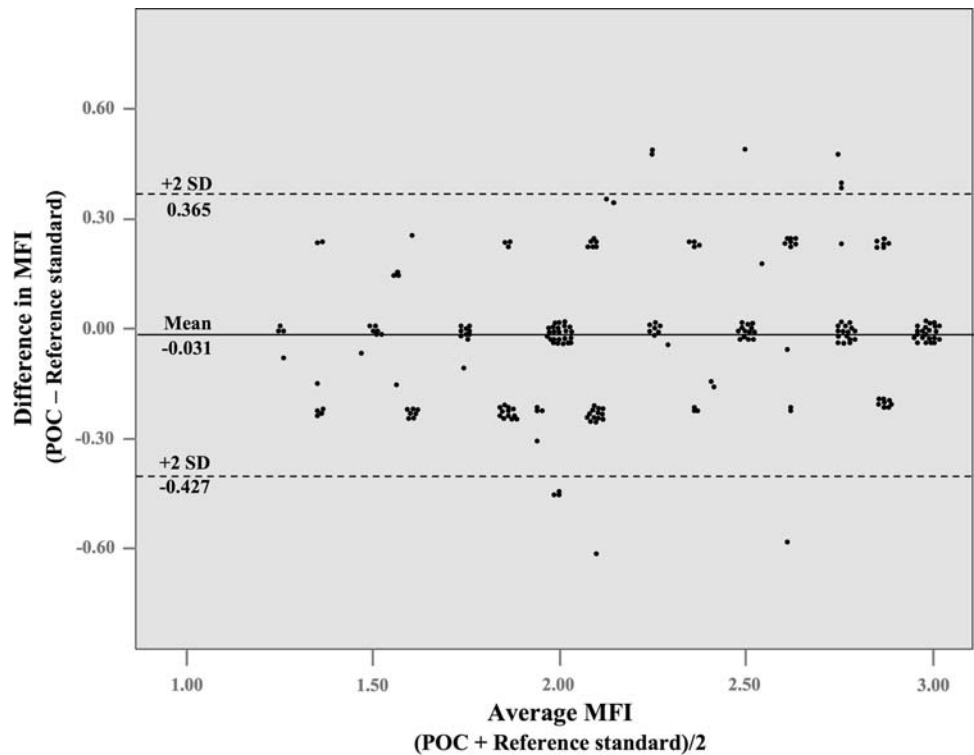
Fig. 2 Bland–Altman plot of the mean differences between conventional offline and point-of-care (POC) determinations of microcirculatory flow index (MFI) for each subject ($n = 18$). The *solid line* shows the mean deviation (bias) between mean POC-MFI and reference standard (offline) MFI (-0.033); the *dashed lines* represent the limits of agreement (mean $\pm$ 2SD) of the means with SD = 0.072. The *dotted lines* represent the 95% limits of agreement adjusted for the within-subject variance of the differences (SD$_v$) between observations by the two methods
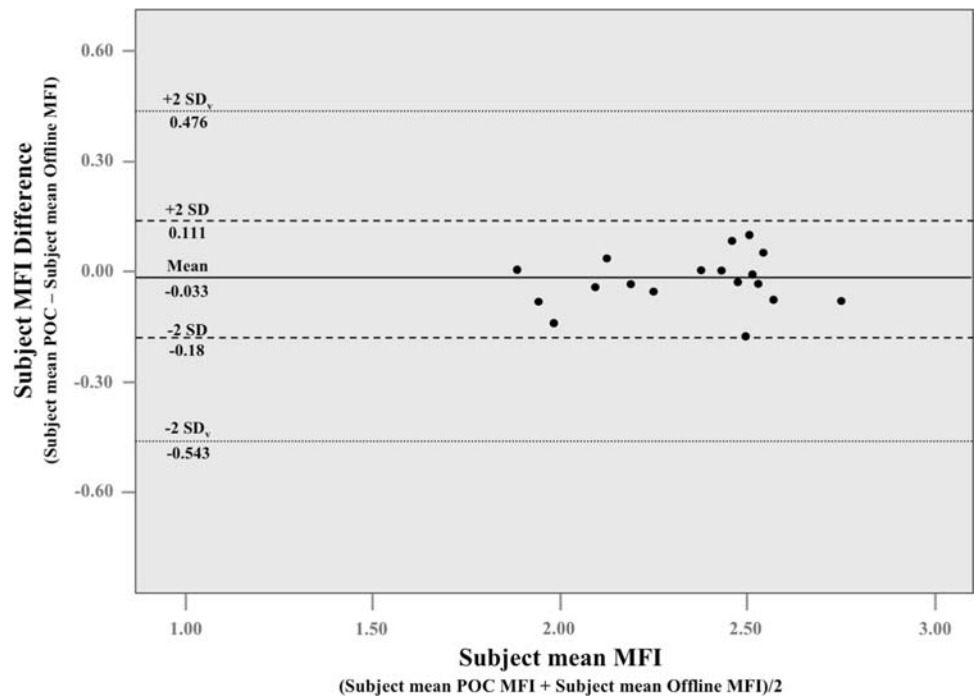
Discussion

Microcirculatory dysfunction is an important pathophysiologic mechanism in critically ill patients. Observational studies have reported that sublingual microcirculatory blood flow indices are prognostic in patients with overt or impending shock, and these indices may yield distinct physiologic information that macrocirculatory parameters cannot [1, 2, 4, 7]. As this line of research evolves from

Table 2 2×2 table describing the ability of the point-of-care (POC) test to identify impaired microcirculatory flow index (MFI)

	POC-MFI < 2.5	POC-MFI ≥ 2.5	
Offline MFI < 2.5	113	7	120
Offline MFI ≥ 2.5	7	78	85
	120	85	205

Impaired flow was defined a priori based on previously published data as an MFI < 2.5 identified by offline analysis (the reference standard). The POC assessment was 94% sensitive (95% CI: 79–95%) and 92% specific for detecting an MFI < 2.5 (95% CI: 82–96%), with a diagnostic accuracy of 93%

observational studies to interventional trials, microcirculatory flow indices are transforming from markers of disease severity to therapeutic targets [17–19]. Currently, microcirculatory image analysis requires offline processing and scoring, delaying results to the treating clinician, and precluding guidance in clinical decisions. There is a need for a bedside real-time assessment of the microcirculation to enable the timely application of novel therapeutics and resuscitative strategies targeting an improvement in microcirculatory perfusion [19].

In the present study, we aimed to validate a real-time POC measurement of MFI at the bedside as compared to the conventional technique of measuring MFI off-line in a laboratory. We found that real-time POC determination of MFI had good agreement with conventional analysis, and was highly sensitive and specific for detecting impaired microvascular flow. We conclude that the wide range of MFI values in this sample supports the use of the POC-MFI measurement over a wide spectrum of microcirculatory perfusion characteristics. Specifically, the clustering of the points around the mean is greatest at low MFI values (MFI ≤ 2.0) in Figure 1. This supports the conclusion that the POC-MFI technique may prove useful in clinical trials of therapies targeting impaired microcirculatory perfusion in critically ill patients until a more accurate computer-assisted microvascular flow scoring technique is available.

We acknowledge important limitations. First, we examined only the POC method for determining MFI, and did not attempt to validate a real-time determination of perfused vessel density (PVD) [6]. Conventional techniques to assess PVD require a multi-step offline process that may not be as readily translated to the bedside for a rapid POC measurement; and failure to capture PVD could potentially omit important information on the status of the microcirculation [6]. Second, an operator experienced in SDF acquisition obtained the MFI measurements in both the POC and reference standard and these results may be applicable only to similarly experienced operators. Also, the resolution of the bedside monitor was not the same as that of the offline LCD monitor and may have contributed to some of the difference in scoring methods. Third, the analysis to control for within-subject variance widened the 95% confidence intervals for the limits of agreement. While the subject means demonstrated good agreement with a small SD (Fig. 2), there was an increase in the limits of agreement when adjusted for repeated measures (SD$_v$). We felt this increase was partially due to the use of the total variance of each method in the calculation, an expectedly high number in our patient population with a dynamic change in disease severity and wide range of MFI (1.25–3.00).

Conclusion

A POC determination of MFI had good agreement with conventional off-line analysis, and was highly sensitive and specific for detecting impaired microvascular flow. As the microcirculation emerges as a viable resuscitation target for new therapeutic strategies in critically ill patients, this bedside technique can be used to generate real-time data on the status of microvascular perfusion.

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