Variability of Microcirculatory Measurements in Critically Ill Patients

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VARIABILITY OF MICROCIRCULATORY MEASUREMENTS IN CRITICALLY ILL PATIENTS

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ABSTRACT—**Introduction:** Monitoring the microcirculation may be helpful in guiding resuscitation in patients with circulatory shock. Sublingual side-stream dark field imaging cameras allow for noninvasive, bedside evaluation of the microcirculation, although their use in clinical practice has not yet been validated. The GlycoCheck system automatically analyzes images to determine glycocalyx thickness, red blood cell filling percentage, and vessel density. Although GlycoCheck has been used to study microcirculation in critically ill patients, little is known about the reproducibility of measurements in this population. **Materials and Methods:** A total of 60 critically ill patients were studied. Three consecutive microcirculation measurements were performed with the GlycoCheck system in 40 of these patients by one of two experienced observers. Twenty patients were assessed by both observers. Intra- and interobserver variability were assessed using intraclass correlation coefficients (ICCs). **Results:** ICCs of single measurements were poor for glycocalyx thickness and good for filling percentage and vessel density. Reproducibility could be substantially increased for all parameters when three consecutive measurements were performed and averaged. **Discussion:** GlycoCheck can be used to study microcirculation. However, to obtain reliable results three consecutive measurements should be performed and averaged. The variation of the measurements currently hampers the clinical application in individual patients.

KEYWORDS—Critically ill, glycocalyx, intra- and interobserver variability, microcirculation, side-stream dark field imaging

INTRODUCTION

The cornerstone of resuscitation in circulatory shock is aimed at restoration of macrocirculatory cardiovascular parameters such as mean arterial pressure, cardiac ouput, central venous pressure, and mixed venous oxygen saturation within their desired ranges. Observational studies have shown that abnormal peripheral perfusion during circulatory shock states is associated with organ failure and mortality. Therefore, the Surviving Sepsis Campaign proposes to guide hemodynamic resuscitation by repeated measurements of blood lactate as a measure of tissue hypoperfusion and consequent anaerobic metabolism (1). A recent study however indicates that a resuscitation strategy targeting peripheral perfusion status by the capillary refill time might be associated with decreasing organ damage as compared with a lactate guided strategy (2). Monitoring the microcirculation might therefore be the best tool to guide resuscitation in patients with circulatory shock at this moment.

Microcirculation can be assessed noninvasively and *in vivo* using sublingual side-stream dark field (SDF) and incident dark field video-microscopes (3). The application of SDF is currently limited to preclinical scientific research due to a lack of validated microcirculatory variables that reflect patient status and predict outcome (4, 5). To select robust variables and to move toward the implementation of microcirculatory guided-therapy in clinical practice, a validated, readily applicable, and reliable assessment tool is desirable.

GlycoCheck is an automated software package that simplifies quantitative assessment of the microcirculation as measured with SDF. It selects and analyses SDF images that are of sufficient quality (focus, contrast, and limited movement) and determines glycocalyx thickness, vessel density, and red blood cell filling percentage (6). A measurement takes about 3 min to perform and results are processed within minutes.

Although GlycoCheck has been used to study microcirculation in critically ill patients (7–11), little is known about the reproducibility of measurements in this population. Adequate reproducibility is paramount to warrant its application in research and clinical practice.

The aim of the present study was to assess reproducibility of GlycoCheck measurements in critically ill patients. To that end, we determined both intra- and interobserver agreement and investigated factors that influence reproducibility.

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PATIENTS AND METHODS

Study population

Between April 2016 and February 2018, 60 adult patients who were admitted to the Department of Intensive Care Medicine (IC) of the Maastricht University Medical Center (MUMC+), Maastricht, the Netherlands, were included in this prospective observational study. Inclusion criteria were: age $\geq$ 18 years, stable hemodynamic parameters defined as absence of variations in mean arterial pressure (MAP) $>$ 10 mm Hg in the 10 min prior to measurements, absence of changes in fluid administration, vasoactive medication in the 10 min prior to measurements and availability of the SDF camera. Exclusion criteria were oral bleeding, oral wounds, or oral infections precluding adequate measurement. Patients receiving noninvasive ventilation were also excluded as this prevented access to the mouth. Demographic variables, routine laboratory test results, and certain predetermined physiological parameters were registered. To assure a reasonable temporal relationship between lab values and microcirculation measurements, only routine laboratory variables that were obtained within 2 h of the measurements were included in the analysis.

Measurements

Measurements were performed using an SDF camera (CapiScope HVCS, KK Technology, Honiton, UK) fitted with GlycoCheck software (Microvascular Health Solutions Inc, Salt Lake City, Utah). Two researchers (MEB and TSRD), experienced in performing sublingual measurements with this tool, each performed three consecutive measurements to determine the intra- and interobserver agreement. Thirty subjects were exclusively assessed by observer 1, 10 exclusively by observer 2. Twenty patients were studied consecutively by both observers in random order.

Glycocheck parameters

Perfused boundary region—Glycocalyx thickness was automatically assessed by the GlycoCheck software. In brief, images of approximately 3 $\times$ 5 mm were recorded using a camera that emits a flashing green light (540 nm). Hemoglobin absorbs light of this frequency, while its surroundings do not, allowing the detection of red blood cells. Movies of 1 s (23 frames) were recorded when quality requirements regarding: 1) the lack of motion of the tissue-section being recorded, 2) the intensity, and 3) the focus are fulfilled. The software then automatically detected vessels of 5 to 25 μm in diameter and placed measurement points on these vessels at 10 μm intervals. Lateral movement of red blood cells (RBCs) was determined at these points from which glycocalyx thickness is inferred. An intact glycocalyx is less permeable to RBCs, hence leading to a smaller lateral movement. The lateral movement into the glycocalyx is expressed as the perfused boundary region (PBR). A higher PBR thus indicates a thinner glycocalyx.

Red blood cell filling percentage—Red blood cell filling percentage is a measure of microvascular perfusion. To assess RBC filling percentage, the percentage of time that RBCs are present at a measurement point is calculated during a GlycoCheck recording (6). Overall RBC filling percentage is defined as the median value of RBC filling percentages at each individual measurement point.

Microvascular vessel density—Microvascular density is calculated from the measurement points that are placed every 10 μm by the software. The number of measurements was multiplied by 10 μm to calculate cumulative microvessel length. Cumulative microvessel length was divided by the total area that was recorded to calculate microvascular vessel density.

Statistical analysis

Data presentation—Data are presented as mean $\pm$ SD or medians [interquartile range (IQR)] as appropriate. Data analysis was performed with SPSS version 23 (IBM, Armonk, NY). P values $<$0.05 were considered to be statistically significant.

Intraobserver agreement—Intraobserver agreement was assessed with the intraclass correlation coefficients (ICCs) by means of a two-way random model with absolute agreement and reported as ICC [95% confidence interval; CI]. ICCs were deemed as poor ($<$0.40), fair (0.40–0.60), good (0.60–0.75), or excellent ($>$0.75) according to the guidelines written by Cicchetti (12).

Effect of clinical parameters on intraobserver agreement—The correlation between different clinical and biochemical variables and reproducibility of GlycoCheck measurements was assessed in the 50 patients measured by observer 1. Coefficients of variation were calculated for each GlycoCheck parameter of each individual patient. These were correlated with clinical and biochemical parameters. Correlations were tested using Spearman rank test.

TABLE 1. **Patient characteristics of the 60 patients**

Variable	Value
Age (years), mean $\pm$ std	66.0 $\pm$ 10.5
Female, n (%)	20 (33.3)
APACHE IV, median [IQR]	75.5 [58.3–102.8]
SOFA, mean $\pm$ std	8.67 $\pm$ 2.75
C-reactive protein (mg/L) (n=41)	212.3 $\pm$ 130.1
Length of IC stay (days), median [IQR]	6.5 [2.0–15.5]
Ventilated, n (%)	55 (91.7)
Admission diagnosis	
Sepsis, n (%)	25 (41.7%)
Postsurgical, n (%)	22 (36.7%)
Respiratory insufficiency	5 (8.3%)
Other, n (%)	8 (13.3%)
IC survival, n (%)	43 (72.9%)

Interobserver agreement—The average values of three measurements in patients measured by both observers were used for interobserver agreement analysis. Interobserver agreement was assessed using Bland–Altman plots. The Wilcoxon signed-rank test was used to assess whether there was a systematic difference between both observers.

Ethics—The protocol was reviewed by the institutional Review Board (METC 15-4-212.1/ab). Patients or legal representatives were informed about the study in word and writing. Written consent was obtained from all participants or their representatives. The study was performed in accordance with the Declaration of Helsinki.

RESULTS

Patients

The mean age of our cohort was 66 years and 33% was female. Most subjects (92%) were mechanically ventilated at the time of measurement. The median APACHE IV score was 75.5 [58.3–102.8] and the mean SOFA score 8.7 $\pm$ 2.8, indicating a high disease severity. Patient characteristics and demographic data are presented in Table 1.

Intraobserver agreement

ICC increased when an increasing number of measurements were averaged. For PBR, ICC exceeded 0.6 (indicating good reproducibility) in both observers when three measurements were averaged. For RBC filling percentage and perfused vessel density, ICC exceeded 0.6 in both observers when two measurements were averaged. When three measurements were averaged ICC exceeded 0.75 (indicating excellent reproducibility) in both observers. ICCs of both observers for PBR, RBC filling percentage, and perfused vessel density are presented in Table 2.

Effect of clinical parameters on intraobserver agreement

Coefficients of variation (COV) of PBR, RBC filling percentage, and perfused vessel density were calculated. Neither hemodynamic indices, nor respiratory indices, nor SOFA score correlated with these COVs with a rho$>$0.3. Pearson statistics are shown in Table 3.

Interobserver agreement

The Bland–Altman plots for PBR, RBC filling percentage, and vessel density are shown in Figure 1A–C.

There were no systematic differences between both observers for PBR, RBC filling percentage, and perfused vessel

TABLE 2. **Intraobserver agreement: ICCs [95% CI] of both observers for single measurements and the average of two and three measurements**

	Measurements	Observer 1 (n = 50)	Observer 2 (n = 30)
PBR	Median [IQR]	1.99 [1.87–2.14]	1.97 [1.86–2.22]
	ICC single measurement	0.57 [0.41–0.71]	0.39 [0.16–0.61]
	ICC Average of two measurements	0.75 [0.56–0.86]	0.28 [−0.54 to 0.66]
	ICC Average of three measurements	0.80 [0.68–0.88]	0.66 [0.37–0.83]
RBC filling percentage	Median [IQR]	73.1 [69.5–75.8]	72.4 [69.0–76.0]
	ICC single measurement	0.76 [0.64–0.84]	0.55 [0.34–0.74]
	ICC Average of two measurements	0.85 [0.73–0.91]	0.75 [0.48–0.88]
	ICC average of three measurements	0.90 [0.84–0.94]	0.79 [0.61 −0.89]
Perfused vessel density	Median [IQR]	216.4 [183.9–260.4]	218.7 [176.5–248.5]
	ICC single measurement	0.59 [0.44 – 0.72]	0.65 [0.46– 0.80]
	ICC Average of two measurements	0.71 [0.50 −0.72]	0.80 [0.57 −0.90]
	ICC Average of three measurements	0.81 [0.70–0.89]	0.85 [0.72–0.92]

ICC indicates intraclass correlation coefficient; IQR, interquartile range; PBR, perfused boundary region; RBC, red blood cell.

TABLE 3. **Correlation between the COV of the PBR, RBC filling percentage, and vascular density with several clinical parameters for patients measured by observer 1 (n = 50)**

Clinical parameter	Value	COV of PBR (Rho; *P* value)	COV of RBC filling percentage (Rho; *P* value)	COV of density (Rho; *P* value)
MAP [mm Hg]	73.7 ± 13.6	0.16; 0.28	0.19; 0.19	0.29; 0.04
Heart frequency	91.1 ± 23.0	0.13; 0.38	−0.28; 0.047	0.00; 0.98
Breathing frequency	18.9 ± 6.7	−0.13; 0.38	−0.10; 0.48	−0.03; 0.86
HB [mmol/ml]	6.34 ± 1.4	0.02; 0.89	−0.17; 0.23	0.05; 0.74
SOFA	8.7 ± 2.9	−0.16; 0.28	−0.18; 0.22	0.29; 0.04
RASS	−3.26 ± 1.74	0.26; 0.07	0.09; 0.55	−0.14; 0.33

COV indicates coefficient of variation; Hb, hemoglobin; MAP, mean arterial pressure; PBR, perfused boundary region; RASS, Richmond Agitation-Sedation Scale; RBC, red blood cell; SOFA, Sequential Organ Failure Assessment.

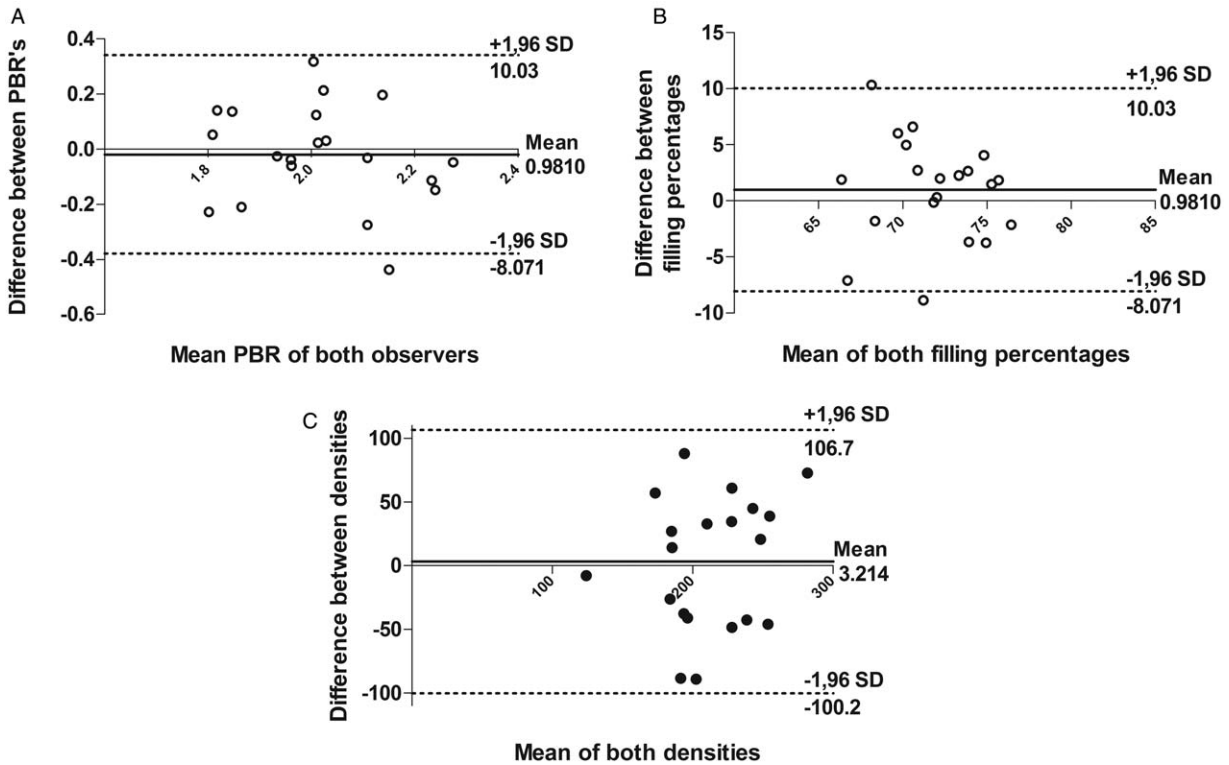

FIG. 1. **Bland–Altman plots of the PBR (A), the RBC filling percentage (B), and the vascular density (C) of observers 1 and 2. PBR indicates perfused boundary region; RBC, red blood cell.**

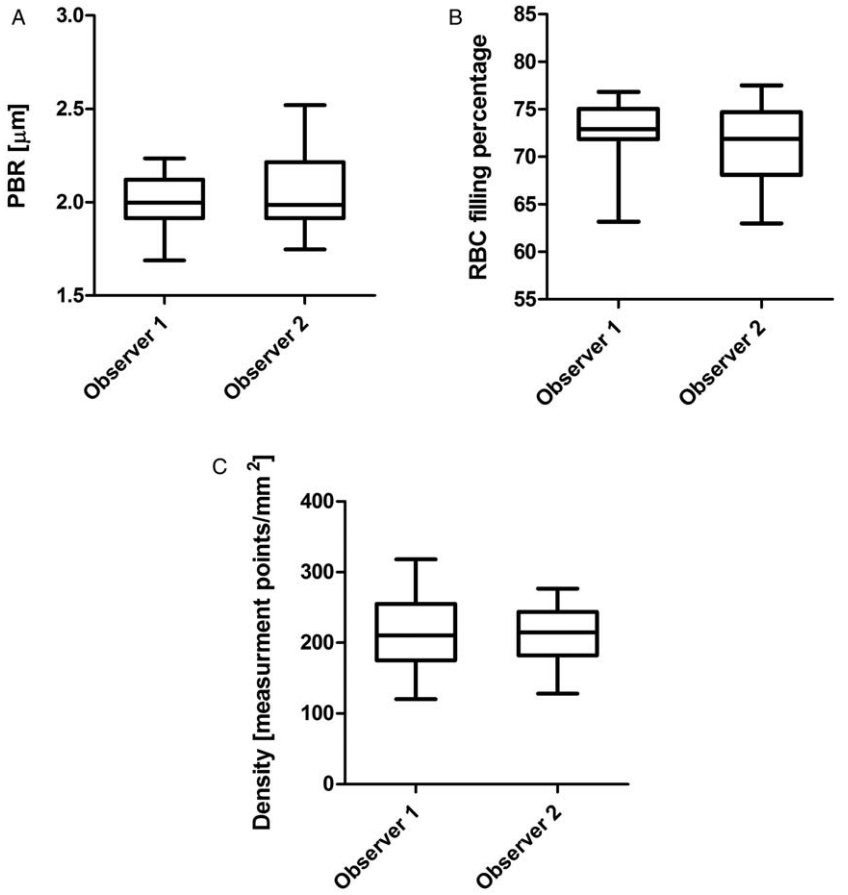

Fig. 2. **Boxplots of the 20 patients measured by both observers of the PBR (A), the RBC filling percentage (B), and the vascular density (C). PBR indicates perfused boundary region; RBC, red blood cell.**

density. Results on these parameters for both observers are presented in Figure 2A–C.

DISCUSSION

Assessment of the microcirculation is considered to be a promising tool to guide hemodynamic treatment in septic shock and other forms of circulatory failure.

This study was performed to determine the reliability and reproducibility of microcirculatory measurements in critically ill patients using SDF imaging with GlycoCheck software. We observed a considerable intra- and interobserver variability between measurements. Intraobserver variability could be reduced to acceptable levels (excellent reproducibility) when three consecutive measurements were performed and averaged.

GlycoCheck software has been widely used in clinical studies (13–19). However, few reports have addressed the reliability of the measurements. Valerio et al. (13) reported intra- and interobserver variability in healthy volunteers and subjects selected from the HELIUS study cohort, which is an urban multiethnic population. They demonstrated poor intra- and interobserver reproducibility for PBR, RBC filling percentage, and vascular density (ICCs of 0.33, 0.51, and 0.28 respectively). In the present study, performed on critically ill patients, we found considerably better ICCs even when single measurements were used (0.57, 0.76, and 0.59 respectively). When three measurements were averaged the ICC increased to >0.75 indicating excellent reproducibility. There are several possible explanations for the observed difference. First, the population studied here is very different from those reported on in the literature: we studied intensive care patients who were mostly sedated, intubated, and mechanically ventilated. Though it is tempting to assume that GlycoCheck studies are more consistent in these patients due to the lack of motion artifacts and a better tolerance of oral manipulation by unconscious subjects, we were unable to find evidence to support these assumptions since factors like breathing frequency and RASS score, within the range that was observed here, did not appear to influence the coefficient of variation of three consecutive measurements. Furthermore, the range of measured values and limits of agreement were comparable between both studies when taking into account the fact that we averaged three consecutive measurements.

Rovas et al. (7) show excellent reproducibility of PBR measurements in a population of mildly ill IC- and emergency department patients. Their data show that the range of PBR values of their patients was substantially larger than ours which could explain their higher ICCs for intraobserver variability. This may be due to the difference in population. The limits of agreement they published, however, are very similar to ours. In comparison with their population, we studied an IC population that had a substantially higher disease severity (mean SOFA of

9 as opposed to 1 in the Rovas study) and almost all patients in our cohort were sedated and mechanically ventilated.

In addition, we here also report the variability of both the RBC filling percentage and the vascular density. PBR is indeed an important parameter that indicates glycocalyx thickness. The glycocalyx, a gel-like structure covering the luminal side of the endothelium, serves as an important regulator in the maintenance of a normostasis at the endothelium:blood border and is a crucial contributor to the maintenance of the hemostatic balance. The glycocalyx and hence the PBR does not, however, indicate the functional area of microvessels and the filling within the vessels. Taking all three parameters together provide a more complete view of the status of the microcirculation from both an anatomical and functional points of view. To this extent it is important to also report the intra- and interobserver variability of the RBC filling percentage and microvascular density.

The median PBR in critically ill patients reported by Pranskunas et al. (9) is in line with our results. Donati et al. (8), however, show a median PBR of 2.7 μm in a similar population. Large differences reported by different groups can also be noted in other populations. In healthy volunteers Vlahu et al. (20) report a mean PBR of 3.3 μm whereas Groen et al. (21) report a mean PBR of 1.8 μm. The reason for this variation is unclear. A possible explanation could be differences in population. Yet, the difference in measured results is strikingly large. It is more plausible that the differences arise not only from biological variations but also from the measurements per se due to variation in equipment performance and software settings to transform measured signals into meaningful value. Care must thus be taken when using two different measurement systems within one study. It is imperative to ascertain first that both systems provide the same data in the same settings. Preferably, a standardization of the technical specs of both hardware and software used is implemented, in addition, detailed standard-operating protocols for the performance of measurements should be strived for in order to achieve as small as possible experimental variation.

Our results imply that three consecutive measurements should be performed and averaged to obtain a reliable PBR, whereas two measurements are sufficient when studying the RBC filling percentage and vascular density. Whether averaging three measurements also improves the reproducibility in a healthy population remains to be evaluated.

Two experienced measurers do not yield a systematic difference in our results. Consecutive measurements can thus be performed by different researchers. The absolute difference between their two measurements, however, is rather large. This is important to acknowledge when designing research that uses the GlycoCheck system. Differences in PBR between healthy volunteers and critically ill patients are rather small (<0.45 μm) compared to the variability in the measurements. This suggests that the GlycoCheck system in its current form will not be able to detect gradual recovery or impairment of the glycocalyx in individual patients.

The reliability of measurement results as obtained with the current version of the GlycoCheck system is not sufficient to guide therapy in individual patients. Camera and software improvements may well reduce the variability in the measurements. Improved image quality as achieved by using an IDF camera may be a promising method (22).

A limitation of the current study is the fact that we did not perform a prospective sample size calculation but pragmatically used sample of 50 patients which is equal or similar to the sample size used in similar studies (7, 13). The narrow confidence interval width of the ICC for intraobserver variability of at most 0.3 further underlines that the sample size is adequate. Another limitation is the fact that we only considered critically ill patients. It can be hypothesized that the results also hold for other populations due to the fact that illness severity did not correlate with the COV of the PBR, RBC filling percentage, and the vascular density. Whether this is indeed the case remains to be determined.

CONCLUSION

According to our results the GlycoCheck can be used, when averaging three consecutive measurements to study pathophysiological processes on a population level in the microcirculation as it provides an automated, noninvasive reliable system to assess the microcirculation. However, variation of the individual measurements results with the software and image quality of the GlycoCheck system we used does not guarantee that clinically relevant gradual changes within individual patients are always detected.

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