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Reproducibility of GlycoCheck measurements in patients under general anesthesia with muscle relaxants: A prospective observational study

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Abstract

Purpose:

To evaluate the inter- and intraobserver reproducibility of sublingual microcirculatory indices measured using the GlycoCheck system, including the perfused boundary region (PBR), vascular density (VD), and red blood cell filling (RBCF), in patients under general anesthesia without any motion artifacts.

Methods:

Fifty patients who received general anesthesia for laparoscopic gastrointestinal surgery were included in this study. After the induction of general anesthesia, the leading observer and one of the five subobservers took two and one measurements of sublingual microcirculation with the GlycoCheck system, respectively. Inter- and intraobserver reproducibility was assessed using intraclass correlation coefficients (ICC). Interobserver reproducibility was calculated using the first measurements of the leading observer and subobservers, and intraobserver reproducibility was calculated using two consecutive measurements of the leading observer.

Results:

The interobserver reproducibility of a single measurement was poor for all three parameters. The

interobserver ICCs for PBR were 0.13 [95% CI: -0.15, 0.39], for VD was -0.01 [95%CI: -0.29, 0.27], and for RBCF were 0.31 [95%CI: -0.45, 0.78]. The intraobserver ICCs for PBR was 0.32 [95% CI: 0.05, 0.55] for all 50 cases, 0.17 [95% CI: -0.25, 0.53] for the first 25 cases, and 0.46 [95% CI: 0.09, 0.72] for the second 25 cases. The Bland-Altman plots indicated that the measurement errors were random.

Conclusion:

In patients under general anesthesia, single PBR, VD, and RBCF measurements using the GlycoCheck system showed poor interobserver reproducibility. Although the intraobserver reproducibility of PBR measurements was poor, improving measurement proficiency might improve reproducibility. Further research is required to establish measurement methods that achieve better reproducibility and adequate observer training.

Keywords

Sidestream dark-field imaging, measurement reproducibility, glycocalyx, GlycoCheck, under general anesthesia

Introduction

Glycocalyx (GCX) is a glycan-rich structure on the vascular endothelium, anchored to endothelial cells via proteoglycans and glycoproteins [1]. It plays a critical role in maintaining vascular homeostasis by regulating vascular permeability, signal transduction, blood cell–vessel wall interactions, and hemostasis [1-4]. Recently, the disruption of GCX has garnered attention due to its association with both chronic conditions, such as diabetes and atherosclerosis, and acute conditions, including sepsis and trauma. Several tools for assessing GCX integrity in the microcirculation have been developed, demonstrating their clinical utility [1].

Although the importance of GCX evaluation has been considered, research has not progressed, because observing 2–5 μm large structures in the vascular endothelium in vivo is challenging. Sidestream dark-field (SDF) imaging has recently become a popular method for the simple and indirect quantification and evaluation of GCX at the bedside. GlycoCheck (GlycoCheck ICU, GlycoCheck BV, Maastricht, the Netherlands) software automatically analyzed images obtained from SDF imaging. It measures the perfused boundary region (PBR), which is the distance from the vessel wall through which red blood cells (RBC) do not pass, the vessel density (VD), which is the length of identified microvessels expressed in μm per square millimeter, and red blood cell filling (RBCF), which indicates the percentage of RBC in the vascular area. In particular, the PBR is inversely correlated with GCX thickness and is an essential indicator of GCX damage. A growing number of reports have examined the relationship between disease and GCX using

SDF imaging and the GlycoCheck system [5-9]. However, previous studies have reported that test reproducibility is sufficient and insufficient, and no conclusions have been reached [10-12].

Many unknown factors exist regarding the reproducibility of GCX measurements using SDF imaging and the GlycoCheck system. As the measurement is performed by pressing a probe against the sublingual mucosa, tongue motion artifacts may affect the measurement in awake patients. However, no studies have examined this issue. Therefore, in this study, we investigated the interobserver reproducibility of microcirculatory measurements using the GlycoCheck software in patients under general anesthesia and muscle relaxation without any motion artifacts.

Methods and Materials

This study was approved by the Ethics Committee of Hokkaido University Hospital (No. 019-0413). Written informed consent was obtained from all patients. Before patient enrollment, the study was registered with the UMIN Clinical Trials Registry (UMIN 000039877). This manuscript adheres to the STROBE guidelines.

Patients

Patients who underwent elective laparoscopic gastrointestinal surgery under general anesthesia at the Hokkaido University Hospital were included in this study. Those with preoperative dental instability, limited mandibular mobility, a history of lingual surgery, intraoral hemorrhage, and those who sustained dental trauma during tracheal intubation were excluded.

Anesthesia

No preoperative medications were administered. After admission to the operating room, constant electrocardiography, percutaneous oxygen saturation measurements, and periodic noninvasive blood pressure measurements were performed. A thoracic epidural catheter was inserted in all patients without any contraindications. General anesthesia was inducted by propofol (0.8–2.4 mg IV or 3–5 μg/mL target-controlled infusion Terufusion Syringe pump, Terumo, Tokyo Japan), fentanyl (100–500 μg IV),

111 remifentanil (0-0.45 μg/kg/min CIVI), and rocuronium (0.6-1.2 mg/kg IV). After tracheal intubation, the
112 tidal volume was set at 6–8 mL/kg (ideal body weight) and the respiratory rate was adjusted to achieve an
113 end-tidal carbon dioxide partial pressure of 35–40 mmHg. Anesthesia was maintained using sevoflurane,
114 desflurane, or propofol target-controlled infusions.
115
116 *GlycoCheck measurements*
117 We used a Capiscope HVCS (KK Technology, Honiton, UK) for SDF imaging. In SDF imaging,
118 light-emitting diodes with a wavelength (530 nm) absorbed by hemoglobin are arranged in concentric
119 circles around the camera to observe red blood cells in the microcirculation as dark cells [13, 14](Figure.1).
120 PBR was automatically analyzed from the images obtained using the GlycoCheck software. Details of the
121 PBR analysis principle are described in the literature [11, 15-17].
122 All observers participated in the study after receiving an explanation of the operation of the
123 equipment from the manufacturer's representative. Prior to the start of the study, each observer performed
124 multiple practice measurements with the GlycoCheck system within the research group until analyzable
125 data could be obtained in a single attempt. However, no standardized or formalized training protocol was
126 implemented.
127 After induction of general anesthesia and achievement of hemodynamic stability, the leading
128 observer and one of the five subobservers took two and one measurements, respectively. Measurements

were performed at intervals of at least 1 minute. An anesthesiologist (TT) was appointed as the leading observer throughout the study, and five anesthesiologists (KM, YI, MN, YY, and TS) were appointed as subobservers. PBR, VD, and RBCF were evaluated in the capillary range of 5–25 μm.

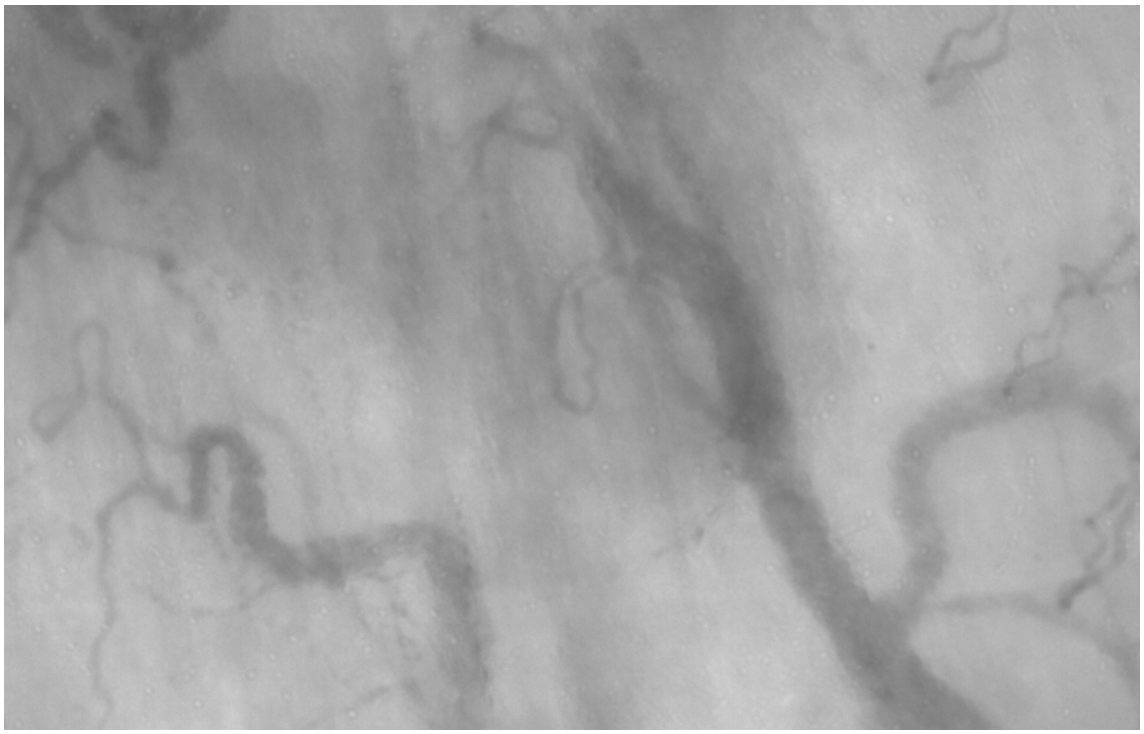


Fig 1. Representative image of sublingual microvessels captured using the GlycoCheck system with sidestream dark field imaging. Red blood cells appear as dark structures due to hemoglobin absorbing light at a wavelength of 530 nm.

Outcomes

The primary outcome of this study was the interobserver and intraobserver reproducibility of the PBR. The secondary outcomes were interobserver reproducibility and intraobserver reproducibility of VD

and RBCF.

Rationale for the Number of Cases

The number of cases in previous studies was 48 [12] for interobserver reproducibility and 30 [10] and 50 [12] for the investigation of intraobserver reproducibility and 40 [10]. Considering feasibility, 50 cases were set up, with 10 patients per subobserver.

Statistical Analysis

Continuous variables are expressed as median values (interquartile ranges [IQR]), and categorical variables are expressed as numbers and percentages. The intraclass correlation coefficient (ICC) (2,1) of the PBR, VD, and RBCF measurements was calculated from the first measurements of the leading observer and five subobservers. In addition, the leading observer calculated the ICC (1,1) of the PBR measurements to investigate the intraobserver reproducibility of the two measurements in all cases. Additionally, considering the potential impact of hypertension on reproducibility, we evaluated the interobserver and intraobserver ICCs separately in patient groups with and without hypertension. We rated the ICC < 0.40 poor, 0.40 to 0.59 fair, 0.60 to 0.74 good, and > 0.74 excellent, according to prior reports [18]. The Wilcoxon signed-rank test was used to assess differences between these measurements for both the interobserver and intraobserver comparisons. Spearman's rank correlation coefficient was applied to

158 evaluate the consistency between these measurements. We also created Bland-Altman plots of inter- and

159 intra-observer measurements and evaluated measurement errors using 95% limits of agreement. R version

160 4.1.0: Language and environment for statistical computing (R Foundation for Statistical Computing, Vienna,

161 Austria at https://www.R-project.org/) was used to create charts and summarize data.

Results

Fifty patients were enrolled in this study between August 2020 and June 2022 (Table 1). The median age was 64 years, and 56% were male. The median body mass index was 22.7 kg/m². Most patients were classified as American Society of Anesthesiologist-physical status 2 (80%). The most common comorbidity was hypertension (36%), followed by smoking (26%) and diabetes mellitus (18%). One patient was on steroid therapy. Preoperative lab data showed a median hemoglobin of 12.4 g/dL and a median hemoglobin A1c of 5.7%. Rectal cancer was the most common diagnosis (54%), and sevoflurane was the most frequently used anesthetic agent (70%).

Table 1. Patients characteristics (n=50)

Gender female / male	22 (44%) / 28 (56%)	**Preoperative laboratory data**	
Age (years)	64 (54, 72)	White blood cell (/μL)	5.35 (4.03, 6.70)
Height (cm)	161.8 (153.9, 167.6)	C-reactive protein (mg/L)	0.04 (0.02, 0.19)
Weight (kg)	59 (52, 68)	Hemoglobin (g/dL)	12.40 (11.33, 13.90)
Body mass index (kg/m^2)	22.7 (20.4, 25.1)	Hematocrit (%)	38.2 (35.1, 41.9)
		Hemoglobin A1c (%)	5.70 (5.32, 6.07)
ASA-PS			
1	5 (10%)	**Diagnosis**	
2	40 (80%)	Rectal cancer	27 (54%)
3	5 (10%)	Crohn's disease	5 (10%)
		Caecum cancer	4 (8.0%)
Complications		Transverse colon cancer	3 (6.0%)
Hypertension	18 (36%)	Descending colon cancer	2 (4.0%)
Diabetes mellitus	9 (18%)	Ascending colon cancer	2 (4.0%)
Dyslipidemia	9 (18%)	Sigmoid colon cancer	1 (2.0%)
Smoking	12 (26%)	Lymphoma	1 (2.0%)
Peripheral vascular disease	3 (6.0%)	Familial adenomatous polyposis	1 (2.0%)
Steroid using	1 (2.0%)	Small intestine tumor	1 (2.0%)
		Appendiceal tumor	1 (2.0%)

Anesthetic agents		Coproma	1 (2.0%)
Sevoflurane	35 (70%)	Rectal carcinoid	1 (2.0%)
Propofol	9 (18%)		
Desflurane	6 (12%)		

All data were presented as median (inter quartile range) or numbers (%).

ASA-PS, American Society of Anesthesiologist-physical status

170

171 The measurement results are presented in Table 2. The median PBR value for the first leading

172 observer measurement was 2.08 μm [IQR 1.86, 2.31 μm], the median for the second measurement was 2.16

173 μm [IQR 1.90, 2.31 μm], and the median for the subobserver measurement was 2.19 μm [IQR 1.85, 2.41

174 μm]. These values are consistent with those in previous reports [10, 12, 19-22]. The median VD value for

175 the first leading observer measurement was 428 μm/mm^2 [IQR 352, 527 μm/mm^2], the median for the

176 second measurement was 466 μm/mm^2 [IQR 390, 586 μm/mm^2], and the median for the subobserver

177 measurement was 424 μm/mm^2 [IQR 349, 498 μm/mm^2]. The median RBCF value for the first leading

178 observer was 71% [IQR: 67%–75%]. The median for the second measurement was 70% [IQR: 64%–75%],

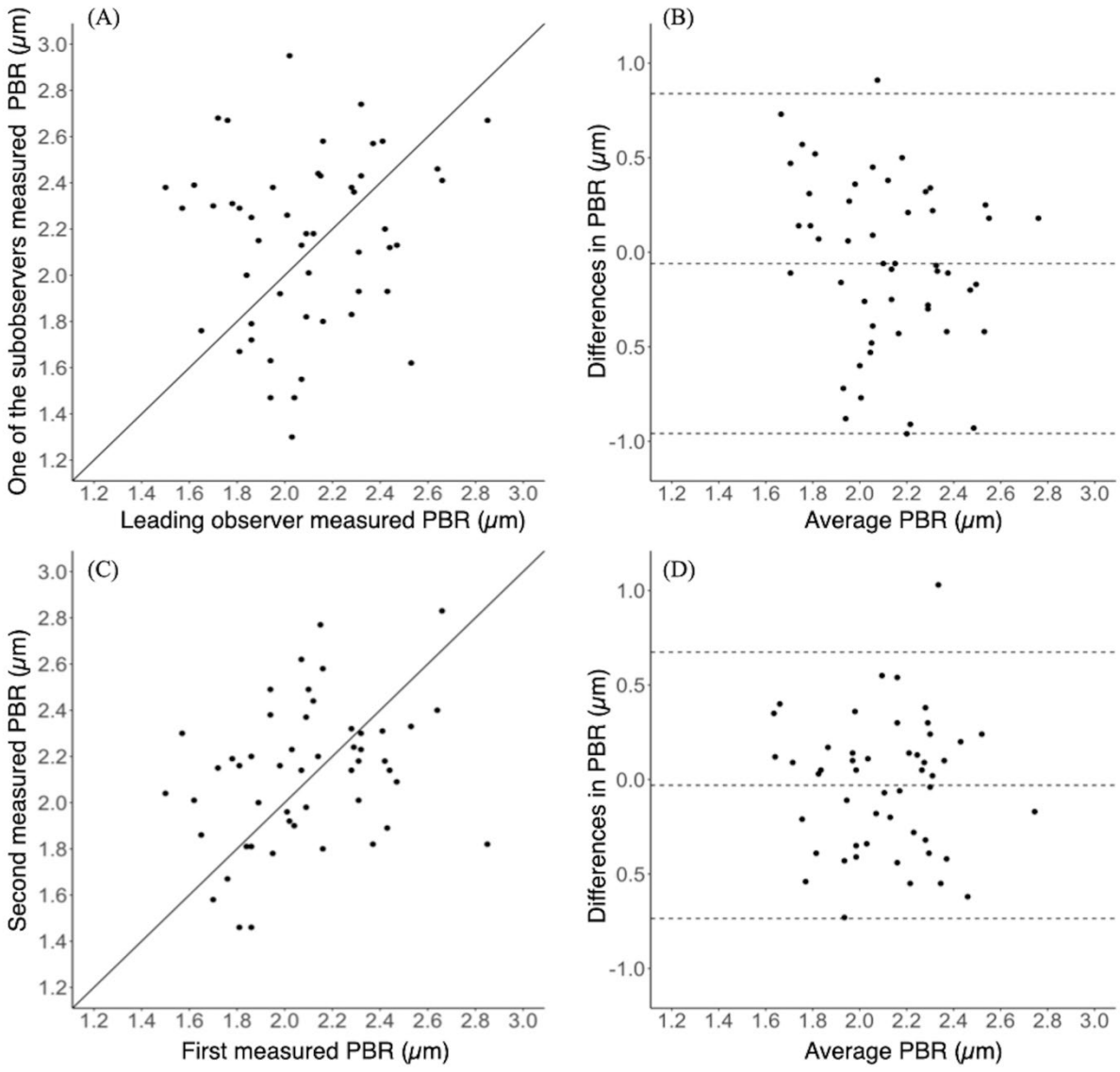
179 and the median for the subobserver measurement was 69% [IQR: 64%–75%].

Table 2. GlycoCheck measurements summary (n=50)

	leading observer		One of subobservers
	1st	2nd	
Perfused boundary region (μm)	2.08 (1.86, 2.31)	2.16 (1.90, 2.31)	2.19 (1.85, 2.41)
Vessel density (μm/mm^2)	428 (352, 527)	466 (390, 586)	424 (349, 498)
Red blood cell filling (%)	71 (67, 75)	70 (64, 75)	69 (64, 75)

All data were presented as median (inter quartile range).

The scatter plots and Bland-Altman plots of the interobserver and intraobserver PBR measurements are shown in Figure 2. No significant differences were observed in either interobserver or intraobserver measurements, as assessed by the Wilcoxon signed-rank test (interobserver: $V = 568$, $p = 0.505$; intraobserver: $V = 662$, $p = 0.491$). The Spearman correlation coefficient was $r = 0.13$ ($p = 0.352$) for the interobserver measurements and $r = 0.30$ ($p = 0.026$) for the intraobserver measurements. All except one of the measurements in both intra-observer and inter-observer analyses were within 95% limits of agreement, suggesting random errors, and no systematic errors were observed.



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189 Fig. 2 Scatter plot and Bland-Altman plot of intraobserver and interobserver measurements.

190 (A) Scatter plot showing two PBRs by the leading observer and one of the subobservers. (B) Bland-Altman

191 plot on variation between two PBRs by the leading observer and one of the subobservers. (C) The scatter

192 plot shows two PBRs by the leading observer. (D) Bland-Altman plot on variation between two PBRs by

193 the leading observer.

194 PBR, perfused boundary region

195

196 Table 3 shows the interobserver ICCs of the leading and subobservers. The ICC (2,1) for the

197 PBR, VD, and RBCF measurements were 0.13 [95% CI: -0.15, 0.39], -0.01 [95%CI: -0.29, 0.27], and 0.31

198 [95%CI: -0.45, 0.78], respectively.

Table 3. ICC of interobserver GlycoCheck measurements (n=50).

	ICC	**95%CI**
PBR (μm)	0.13	-0.15, 0.39
VD (μm)	-0.01	-0.29, 0.27
RBCF (%)	0.31	-0.45, 0.78

ICC, intraclass correlation coefficient; PBR, perfused boundary region; VD, vessel density; RBCF, red blood cell filling; 95%CI, 95% confidence intervals

199

200 Table 4 shows the ICCs for PBR measurements obtained by the leading observer. ICC (1,1) for

201 all 50 cases was 0.32 [95% CI: 0.05, 0.55], ICC (1,1) for the first 25 cases was 0.17 [95% CI: -0.25, 0.53],

202 and ICC (1,1) for the second 25 cases was 0.46 [95% CI, 0.09, 0.72].

Table 4. ICC of intraobserver PBR measurements.

		PBR (μm)	
	n	**ICC**	**95%CI**
all	50	0.32	0.05 , 0.55
first half	25	0.17	-0.25 , 0.53
second half	25	0.46	0.09 , 0.72

ICC, intraclass correlation coefficient; 95%CI, 95% confidence intervals; PBR, perfused boundary region

Table 5 shows the ICCs stratified by the presence or absence of comorbid hypertension. Both interobserver and intraobserver ICCs tended to be lower in the group with hypertension.

Table 5. ICCs of interobserver and intraobserver PBR measurements stratified by the presence or absence of hypertension.

		Interobserver		**Intraobserver**	
	n	**ICC**	**95%CI**	**ICC**	**95%CI**
Hypertension(+)	18	-0.05	-0.45 , 0.40	0.11	-0.37 , 0.55
Hypertension(-)	32	0.24	-0.12 , 0.53	0.50	0.18 , 0.72

ICC, intraclass correlation coefficient; 95%CI, 95% confidence intervals; PBR, perfused boundary region

Discussion

The GlycoCheck system provides a noninvasive, real-time evaluation of the microcirculatory environment, including the state of the GCX. The PBR is inversely correlated with GCX thickness and is essential for evaluating GCX damage in vivo. However, several studies have reported poor measurement reproducibility of the PBR, and various factors including motion artifacts have been speculated. In this study, we employed patients under general anesthesia to eliminate the influence of motion artifacts. However, the interobserver reproducibility of GlycoCheck measurements was relatively low. Although the intraobserver reproducibility of the PBR was poor, it was much higher in the following 25 cases than in the first 25 cases.

Causes of low reproducibility

No reports have pointed to a specific cause of the low reproducibility of microcirculatory measurements using the GlycoCheck system. Based on previous reports [12, 21, 23], we believe that two major factors could affect reproducibility: patient and observer. Patient factors include motion artifacts caused by oral manipulation and the patient's general status or illness severity. Observer factors include the effects of various artifacts caused by inadequate measurement techniques, among which pressure artifacts are the most notable.

Patient motion artifacts cannot be ignored as factors that affect the quality of acquired SDF images.

Indeed, poor reproducibility among healthy volunteers in previous studies [12, 21, 23, 24] may indicate the difficulty of measuring in awake subjects. In addition, some reports suggest that better reproducibility in critically ill patients may be due to fewer motion artifacts caused by sedation [23]. However, in the present study, the measurements were not reproducible, even in patients under general anesthesia and muscle relaxation. Therefore, the difference in reproducibility between healthy volunteers and critically ill patients may be due to the patient's condition rather than motion artifacts.

The target vessel diameter analyzed using the GlycoCheck software, ranged from 5 to 25 μm. Because PBR values correlate with vessel diameter [25], the distribution of the diameters of the acquired vessel segments should significantly affect PBR values. Microcirculation changes in critically ill patients due to systemic inflammation [26, 27] and vascular endothelial dysfunction cause a decrease in perfused vessels and vasoconstriction in microcirculation, leading to organ failure [27-29]. We speculated that in critically ill patients, the capillary diameter distribution is more uniform due to capillary constriction, which may contribute to better reproducibility of the GlycoCheck measurements. Additionally, in both inter and intraobserver reproducibility comparisons, ICCs tended to be lower in the presence of hypertension than in the absent hypertension, suggesting a potential decrease in reproducibility. However, the confidence intervals were wide and overlapped substantially, indicating that the observed differences may not be statistically significant. There may be specific patient populations for whom assessment with GlycoCheck is appropriate; however, this remains unclear at present. Further research is warranted.

The GlycoCheck software controls several types of artifacts during image acquisition. Because the software does not start acquiring images unless all three factors of focus, light intensity, and stability are within the acceptable range, the quality of the SDF images used for analysis is considered relatively well preserved. Pressure artifacts occur when the camera tip applies pressure to the sublingual mucosa and can disturb the movement of RBC within microvessels or completely occlude small vessels. A study of SDF imaging procedures in critically ill patients reported that pressure artifacts were observed in 36% of all measurements [24]. This study also reported that artifacts decreased as the observer measured more, suggesting that measurement training may improve reproducibility [24]. In this study, each observer conducted repeated trial measurements prior to patient enrollment in an effort to become proficient with the device. However, individual variability in measurement technique may have persisted. That is, the lack of a standardized training protocol may have affected the reproducibility of the measurements. Furthermore, while the leading observer consistently performed measurements for all 50 cases, the five subobservers each measured only 10 cases. This imbalance in measurement frequency may also have influenced reproducibility. In the intraobserver reproducibility analysis, the ICC improved in the latter half of the measurements compared to the first half, suggesting a learning curve effect. These findings indicate that measurement proficiency may improve over time and can influence reproducibility. Therefore, the implementation of a standardized training protocol may help enhance interobserver

consistency.

How to improve the reproducibility of GlycoCheck measurements

Several reports have shown that averaging the results of multiple PBR measurements using GlycoCheck improves intra-observer reproducibility [20, 21], and ICC increases with an increasing number of measurements averaged [20]. A single measurement would not provide consistent results because the distribution of measurable vessel diameters is biased, and spatial heterogeneity is inevitable. Therefore, it is reasonable to increase the number of vessel segments included in the analysis by measuring them multiple times and improving the reproducibility by averaging the distribution of vessel diameters. Furthermore, because the Bland-Altman plots in this study show that the GlycoCheck measurement errors are likely to be random errors for both intra and interobserver errors, the validity of using the average value of multiple measurements is supported. In contrast, we could not find studies on inter-observer reproducibility using ICC or whether multiple PBR measurements and averaging results improved reproducibility.

Limitation

First, this was a single-center observer-limited study. Second, the results may vary if different cameras are used to acquire SDF images [30]. Third, although the measurements were performed after hemodynamic stabilization, changes in microcirculation may have occurred during the three consecutive

measurements. Because blood pressure data were not collected at the time of measurement, it is unclear whether blood pressure affected the reproducibility of the results. Fourth, the measurement techniques used by the observers, especially the subobservers, may have influenced the results. Although the GlycoCheck software incorporates automatic real-time quality control, no formalized or validated scoring system was used to quantitatively evaluate image quality, such as those assessing pressure artifacts or content quality. As such, subtle variations in image acquisition quality among observers may have contributed to the observed interobserver variability. Moreover, although all observers underwent preliminary training with guidance from the manufacturer, the training was not standardized or assessed using objective proficiency metrics. These limitations highlight the need for future studies to incorporate structured image quality evaluation criteria and formal observer training programs to enhance reproducibility. Fifth, we did not formally randomize the assignment of subobservers to individual patients. Subobserver assignment was determined based on scheduling availability rather than patient characteristics, in an effort to minimize selection bias. Nonetheless, the lack of formal randomization may have introduced potential observer pairing bias. However, given that interobserver ICC values were consistently low across all subobservers, this bias was likely to have had a limited impact on the overall results.

Conclusion

In patients immobilized under general anesthesia, PBR, VD, and RBCF single measurements

297 using the GlycoCheck system had poor inter-observer reproducibility. Although the intra-observer
298 reproducibility of PBR measurements is poor, improving measurement proficiency may improve
299 reproducibility. Further research is required to establish measurement methods that achieve better
300 reproducibility and adequate training for observers.
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Compliance with Ethical Standards

Ethics approval and consent

This study was approved by the Ethics Committee of Hokkaido University Hospital (No. 019-0413). Written informed consent was obtained from all patients.

Competing interest

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Statements and declarations

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Author contributions

Kazuyuki Mizunoya and Takayuki Toki conceived and designed the study; Takayuki Toki acquired, analyzed, and interpreted the data; Takayuki Toki, Kazuyuki Mizunoya., and Yuji Morimoto

411 drafted the manuscript; Ryo Takagi and Isao Yokota advised on statistical analysis; Takayuki Toki wrote
412 the manuscript; Takashi Soejima, Yasunori Yagi, Naoko Nakamine, Yusuke Itosu., Kazuyuki Mizunoya,
413 and Takayuki Toki performed the measurements; all authors critically reviewed the manuscript for
414 important intellectual content and approved the final version for publication.
415
416 Data availability statement
417 The datasets used and/or analyzed during the current study are available from the corresponding
418 author on reasonable request.