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ORIGINAL RESEARCH

Impact of microcirculatory video quality on the evaluation of sublingual microcirculation in critically ill patients

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Abstract We aimed to assess the impact of image quality on microcirculatory evaluation with sidestream dark-field (SDF) videomicroscopy in critically ill patients and explore factors associated with low video quality. This was a retrospective analysis of a single-centre prospective observational study. Videos of the sublingual microcirculation were recorded using SDF videomicroscopy in 100 adult patients within 12 h from admittance to the intensive care unit and every 24 h until discharge/death. Parameters of vessel density and perfusion were calculated offline for small vessels. For all videos, a quality score (-12 = unacceptable, 1 = suboptimal, 2 = optimal) was assigned for brightness, focus, content, stability, pressure and duration. Videos with a total score ≤ 8 were deemed as unacceptable. A total of 2455 videos (853 triplets) was analysed. Quality was acceptable in 56 % of videos. Lower quality was associated with worse microvascular density and perfusion. Unreliable triplets (≥ 1 unacceptable or missing video, 65 % of total) showed lower vessel density, worse perfusion and higher flow heterogeneity as compared to reliable triplets ($p < 0.001$). Quality was higher among triplets collected by an extensively-experienced investigator or in patients receiving sedation or mechanical ventilation. Perfused vessel density was higher in patients with Glasgow Coma Scale (GCS) ≤ 8 (18.9 ± 4.5 vs. 17.0 ± 3.9 mm/mm^2 in those with GCS >8, $p < 0.001$) or requiring mechanical ventilation (18.0 ± 4.5 vs. 17.2 ± 3.8 mm/mm^2 in not mechanically ventilated patients, $p = 0.059$). We concluded that SDF video quality depends on both the operator's experience and patient's cooperation. Low-quality videos may produce spurious data, leading to an overestimation of microvascular alterations.

Keywords Microcirculation · Sidestream dark field imaging · Microcirculatory image quality · Critically ill patients

1 Background

The development of non-invasive videomicroscopy techniques, such as sidestream dark-field (SDF) imaging, has enabled the in vivo assessment of microcirculatory blood flow at the bedside, using the sublingual region as an accessible window to the microcirculation of inner organs [1]. SDF technology is incorporated into a hand-held video microscope system which epi-illuminates the tissue with green (530 nm) light-emitting diodes [2]. As this light is absorbed by haemoglobin, erythrocytes appear as dark globules against a grayish background. Sublingual microcirculatory alterations have been reported in different patient subsets, especially during sepsis [3, 4], and were associated with morbidity and mortality [5–7]. A number of studies have shown a dissociation between macro-hemodynamics and microcirculatory response to several interventions, including fluid infusion [8] and vasopressor administration [9]. These data would encourage the introduction of microcirculatory monitoring in the clinical

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practice as an additional target for therapy. Nonetheless, there are practical challenges to the widespread adoption of this technique in intensive care units (ICUs), mainly the time-consuming offline video analysis and a long learning curve for good-quality image acquisition.

In 2007 a roundtable consensus conference indicated five key-points for optimal image acquisition (sampling 5 sites per organ, avoidance of pressure artefacts, elimination of secretion, adequate focus and contrast adjustment and high quality recording) [10]. However, obtaining good-quality videos may be challenging due to operator's inexperience or poor patient's cooperation. In a study by Sallisalmi et al. [11] excellent technical quality was reported in only 30 % of SDF videos. Inadequate video quality may produce spurious microcirculatory data: blood flow may be artificially obstructed due to excessive pressure applied on the sublingual mucosa; inadequate focus and contrast or occluding artefacts (saliva bubbles or blood) may prevent the visualization of some blood vessels. Massey et al. [12] have proposed a microcirculatory image quality score considering six domains of video quality: illumination, duration, focus, content, stability and pressure. This score has never been used systematically and studies evaluating the microcirculation do not generally report any assessment of image quality. To our knowledge, no study has previously investigated to what extent a low microcirculatory image quality will affect the reliability of microcirculatory assessment.

We hypothesized that SDF video quality depends on both operator- and patient-related factors. Patient's compliance may contribute crucially to high-quality video acquisition: as a result, more severe patients requiring sedation and/or mechanical ventilation could paradoxically show an apparently better microvascular perfusion as compared to less severe patients, merely due to an easier video recording. In this study, we investigated the relationship between SDF video quality and the microcirculatory parameters obtained, and evaluated operator- or patient-related factors potentially influencing microcirculatory video quality.

2 Methods

This is a retrospective analysis of data collected in a single-centre prospective observational study, the MICROcirculatory DAIly MONitoring in the ICU (MICRODAIMON-ICU) Study (NCT02649088; www.clinicaltrails.gov). A total of 100 adult (>18-year old) patients admitted to our 12-bed ICU between 1st April and 31st December 2013 were included in the study. Exclusion criteria were factors impeding the sublingual microvascular evaluation (i.e. maxillo-facial trauma or surgery) and enrolment in the same study during a previous admission. The study protocol was approved by the local ethics committee of Azienda Ospedaliera Universitaria "Ospedali Riuniti" of Ancona, Italy. Written informed consent was obtained by the patients or their next of kin.

2.1 Microcirculatory image acquisition

The sublingual microcirculation was assessed using SDF imaging (Microscan, Microvision Medical, Amsterdam, The Netherlands) [2] within the first 12 h of admission to the ICU and every 24 h until discharge or death. In order to minimize variability due to operator's experience with SDF technology, whenever possible video recording sessions were performed by one principal investigator (PI) with extensive (3 years approximately) clinical experience in SDF monitoring. In case of unavailability of the PI, video recording was performed by a group of adequately trained operators (6–12-month experience, on average). No additional sedation was provided during image acquisition. Secretions were gently removed with a gauze. Every effort was made to optimize contrast and focus and avoid pressure artefacts. Stable videos of at least 5 s duration [12] from five different sites of sublingual mucosa were recorded during each session. When adequate stability was difficult to achieve without increasing the pressure on the probe, priority was given to avoidance of pressure artefacts and videos shorter than 5 s were accepted.

2.2 Analysis of microvascular density and perfusion

A random number was assigned to each video sequence through a random number generator. Three videos of the best available quality were selected from each sequence and analysed using the automated vascular analysis software (Microvision Medical, Amsterdam, The Netherlands) by a group of four experienced investigators who had not participated in the video acquisition. The image was divided by three equidistant horizontal lines and three equidistant vertical lines for the calculation of the De Backer score: this resulted from the number of vessels crossing the lines, divided by the total length of the lines [13]. For each vessel crossing the lines, perfusion was categorized as continuous, sluggish, intermittent or absent. The percentage of perfused vessels (PPV) was estimated as follows: $100 \times [(\text{total number of grid crossings} - [\text{no flow} + \text{intermittent flow}])/\text{total number of grid crossings}]$. Total vessel density (TVD) was calculated as the total length of vessels divided by the total area of the image [10]. The perfused vessel density (PVD) was estimated by multiplying TVD by PPV as estimated with the De Backer method [14]. Microvascular flow index (MFI) was calculated semiquantitatively as described elsewhere [15]. For

each video sequence, values obtained from three sites were averaged. The flow heterogeneity index (FHI) was calculated as the highest MFI minus the lowest MFI, divided by the mean MFI of all sublingual sites [10]. For this study, analyses were focused on small vessels (≤20 μ in diameter).

2.3 Analysis of microcirculatory video quality

This was performed by the same investigators before the analysis of microcirculatory parameters. The microcirculatory image quality score used in this study was adapted from the one proposed by Massey et al. [12] (Table 1). For each video, a score of −12 (unacceptable), 1 (suboptimal but acceptable) or 2 (optimal) was given for each category of quality (brightness, focus, content, stability, pressure, duration). A video was defined as unacceptable if it scored any "−12" in any category or "1" in at least four categories (total score ≤8). Among unacceptable videos, quality was categorized as *bad* ("−12" for at least three categories, total score ≤−30) or *poor* ("−12" in less than three categories, total score between −29 and 8). Among acceptable videos, quality was defined as *good* (total score of 9–11) or *excellent* ("2" for all categories, total score of 12). A video triplet was defined as *reliable* if it was of excellent (all videos of excellent quality) or acceptable (three acceptable or excellent videos) quality, *unreliable* if it included at least one video of unacceptable quality or if less than three videos had been suitable for analysis.

2.4 Statistical analysis

This was performed using GraphPad Prism Version 6 (GraphPad Software, La Jolla, CA, USA). Normality of distribution was checked using the Kolmogorov–Smirnov test. Unpaired *t* test or Mann–Whitney U test were used for comparisons of continuous variables, as appropriate. One-way analysis of variance (ANOVA) or Kruskal–Wallis test with Bonferroni or Dunn's post hoc testing were used for comparisons between more than two groups, as appropriate. The Chi square test was used for comparison of proportions. The alpha level of significance was set a priori at 0.05.

3 Results

A median of six [3–12] video recording sessions per patient was performed. A total of 2455 microcirculatory videos from 100 different patients were analysed. This corresponded to a total of 853 video triplets. Of these, 583 (68 %) had been recorded by the PI. Quality score for each single domain is reported in Table 2. Based on our predefined criteria, only 1365 videos out of 2455 (56 %) were defined as acceptable, 27 % of these being of excellent quality (Fig. 1). If considering video triplets, only 301 triplets out of 853 (35 %) were judged as reliable, with 4 % of total being of excellent quality, while 552 (65 %) were of unacceptable quality. Of these, 72 (8 % of total) were incomplete triplets where less than

Table 1 Microcirculatory image quality score, as adapted from Massey et al. [12]

Category	Quality score		
	−12 (unacceptable)	1 (acceptable)	2 (good)
Brightness	The video is too bright/too dark to visualize all vessels	The video borders on being too bright/too dark but all vessels are still identifiable	Homogeneous illumination and contrast across the entire field of view
Focus	Totally out of focus, no small vessels can be seen	<1/2 field of view is out of focus or edges of vessels are slightly out of focus	Good focus for all vessels (erythrocytes/leukocytes are visible in most of the vessels)
Content	Prevalence of repeating capillary loop motif or saliva bubbles or blood occluding most of the field of view	<50 % looped vessels; a few occluding artefacts (saliva, blood) that do not impede vessel or flow identification	Various size vessel architecture in the entire field of view, with prevalence of capillaries; no saliva bubbles or blood
Stability	Absolute pixel translation on X- or Y-axis >20	Absolute pixel translation on X- or Y-axis between 10 and 20	Absolute pixel translation on X- or Y-axis <10
Pressure	Obvious pressure artefacts associated with probe movements, flow that starts and stops, reversal of flow, obstructed flow in larger venules	Only localized pressure artefacts, flow is unobstructed in most vessels, good flow in larger venules	No signs of pressure artefacts
Duration	Analyzable video segment is <3 s long	Analyzable video segment is 3–5 s long	Analyzable video segment is >5 s long

Table 2 Distribution of microcirculatory videos based on the score assigned for each category of quality

Category	Quality score		
	−12 (unacceptable) (%)	1 (suboptimal) (%)	2 (optimal) (%)
Brightness	23 (1)	548 (22)	1884 (77)
Focus	125 (5)	1112 (45)	1218 (50)
Content	252 (10)	565 (23)	1638 (67)
Stability	770 (31)	759 (31)	926 (38)
Pressure	54 (2)	444 (18)	1957 (80)
Duration	65 (3)	131 (5)	2259 (92)

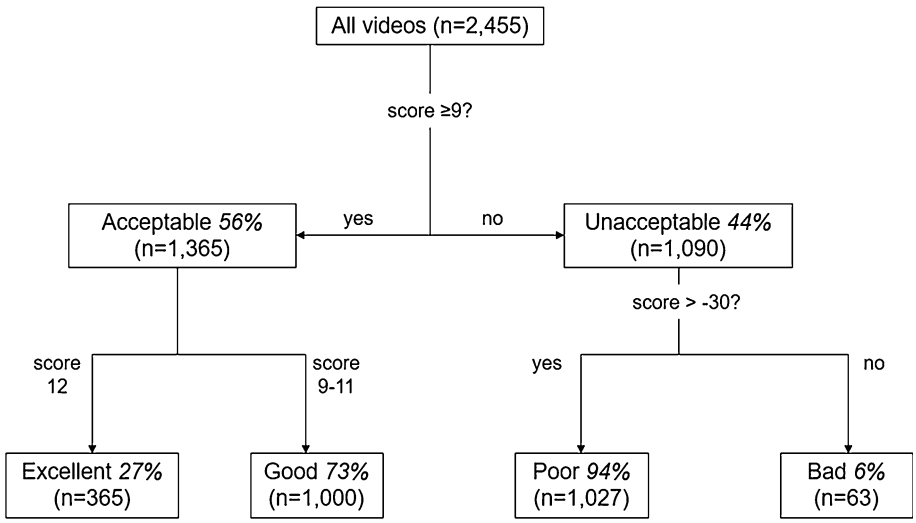

Fig. 1 Quality of all the microcirculatory videos analysed

three videos had been suitable for analysis (104 videos lacking in total). As a result, 67 [44–89] % of microcirculatory assessments performed in each patient were deemed unreliable. Only eight patients out of 100 had reliable microcirculatory data for all the sessions performed, while we were unable to take any reliable video triplets in 20 patients.

3.1 Video quality and microcirculatory parameters

Videos of lower quality for each category were generally associated with lower vessel density, PPVs and MFIs as compared to videos of optimal quality (Electronic Supplementary Material, ESM 1 and 2). This trend was less pronounced for the category "stability", for which unacceptable videos showed slightly lower vessel densities but higher PPVs, without any differences in MFIs depending on video quality. Vessel density, PPVs and MFIs were progressively lower with decreasing overall video quality (Fig. 2). All microcirculatory parameters varied significantly between reliable and unreliable video triplets, with unreliable triplets indicating worse vessel density and perfusion and higher flow heterogeneity (Electronic Supplementary Material, ESM 3).

3.2 Influence of operator and patient-related factors on video quality and microcirculatory assessment

Triplets recorded by the PI were less likely to be of unacceptable quality (60 vs. 74.8 % among triplets collected by other investigators, $p = 0.041$, Electronic Supplementary Material, ESM 4) and yielded higher PVD (18.2 ± 4.1 vs. 17.4 ± 4.8 mm/mm^2, $p = 0.001$) as compared to those recorded by other operators. The vast majority of triplets recorded in non-sedated or not mechanically ventilated patients were of unacceptable quality (81 and 96 % respectively), while the percentage of unreliable video triplets was lower among sedated or mechanically ventilated patients (55 and 63 % respectively; $p < 0.001$; Electronic Supplementary Material, ESM 5). Among sedated patients, a higher video quality was associated with higher doses of propofol being administered at the time of microcirculatory assessment (Fig. 3). Among non-sedated patients, a lower video quality was associated with higher values of Glasgow Coma Scale (GCS) (Fig. 3), and those with GCS ≤ 8 showed higher PVD as compared to those with GCS >8 (18.9 ± 4.5 vs. 17.0 ± 3.9 mm/mm^2, $p < 0.001$). Similarly, mechanically ventilated patients tended to show a

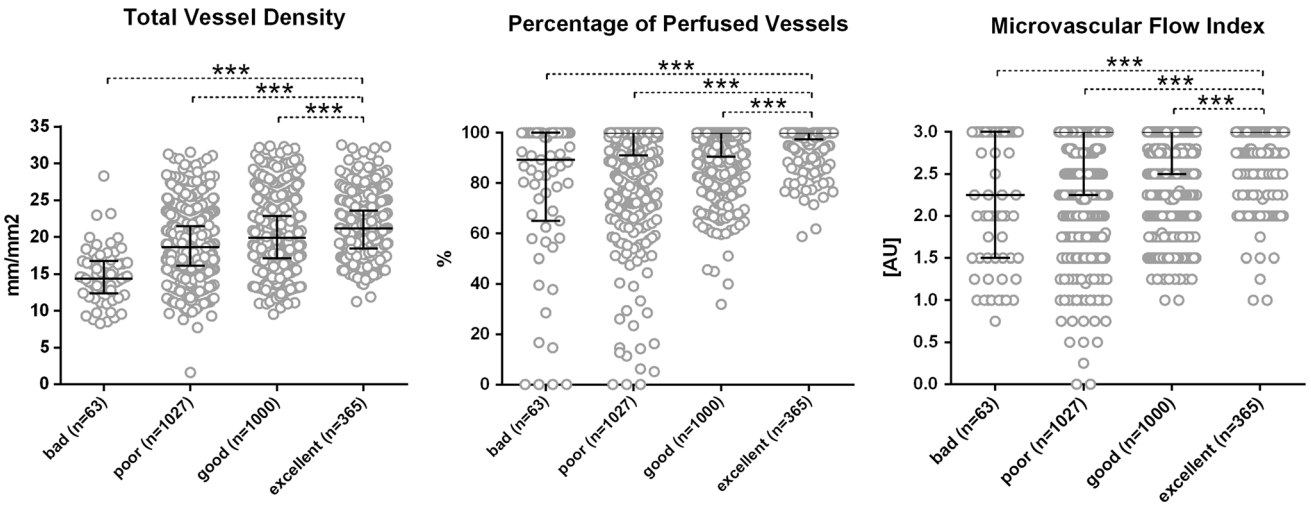

Fig. 2 Total vessel density, percentage of perfused vessels (PPV) and microvascular flow index (MFI) for small vessels in videos stratified based on their quality. ***$p < 0.001$, Kruskal–Wallis test with Dunn's test for multiple comparisons. Data are expressed as median and interquartile range

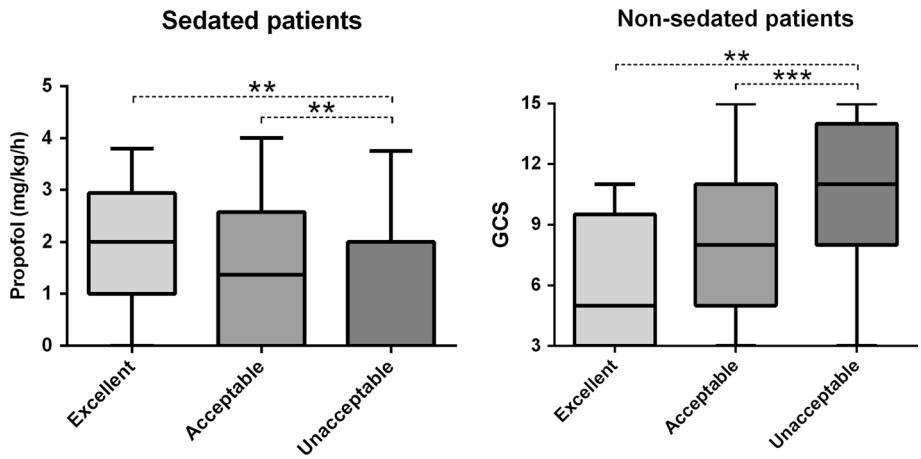

Fig. 3 Relationship between sedation (dose of propofol) or Glasgow Coma Scale and quality of microcirculatory video triplets. Sedation was defined based on the administration of any hypnotic and/or opioid agents at the time of microcirculatory assessment **$p < 0.01$; ***$p < 0.001$, Kruskal–Wallis test with Dunn's test for multiple comparisons. Data are expressed as median (Interquartile range). *Error bars* indicate maximum and minimum values

higher PVD as compared to those not mechanically ventilated (18.0 ± 4.5 vs. 17.2 ± 3.8 mm/mm^2, $p = 0.059$).

4 Discussion

This is the first study that evaluated the impact of microcirculatory image quality on the assessment of sublingual microvascular density and perfusion, using one of the largest existing databases and including a heterogeneous population of 100 critically ill patients monitored in different moments over the course of their acute condition. Our first finding was that almost half of the videos collected were of unacceptable quality, resulting in 65 % of video triplets being unreliable for a clinical assessment of microvascular perfusion. Secondly, parameters of microvascular density and flow varied together with image quality, with videos of lower quality suggesting lower vessel densities and a more altered perfusion. Thirdly, not only the investigator's experience in SDF technology, but also patient-related factors (mainly the neurological state) played a role in determining a good-quality video recording.

Since the first appearance of orthogonal polarization spectral imaging more than 15 years ago [16], an increasing number of studies has explored the sublingual microcirculation and its response to several interventions in different patient populations [17]. Several authors supported the potential role of the microcirculation as a target for therapy [18–20]. Nonetheless, before introducing any monitoring device in the clinical practice, it is necessary to

understand the potential limitations of the technique in order to obtain reliable information. Only a few studies addressed the limitations of sublingual video microscopy [11, 12]. Our study highlights the technical challenges to the widespread adoption of sublingual microcirculation monitoring in ICU patients. A substantial proportion of the videos recorded in each patient was of unacceptable quality. In 20 patients out of 100 we failed to perform any reliable microcirculatory assessment during their stay in the ICU. These data add to the results of Sallisalmi et al. [11], who reported a success rate as low as 8.5 % of video recording sessions in creating video triplets of adequate quality. More importantly, our study demonstrates that low-quality videos can introduce a substantial bias in microcirculatory assessment. The analysis of videos with defects in focus or brightness, as well as artefacts such as secretions or blood, yielded significantly lower vessel densities, PPV and MFI. Similarly, the presence of pressure artefacts was associated with lower vessel density and worse perfusion. Even if they can be recognized and distinguished from real flow alterations by a well-trained investigator during video analysis, pressure artefacts preclude a reliable evaluation of microvascular flow. The first step for the introduction of the microcirculation as a guidance in clinical practice [18–20], but also for its use as a surrogate endpoint in interventional studies, is a comprehensive understanding of factors potentially affecting the reliability of the data obtained. Our results underline the importance of identifying and excluding video triplets of inadequate quality from the analysis of microvascular parameters, as their inclusion can distort our interpretation of the patient's microvascular state and lead to an overestimation of microcirculatory alterations.

Our study confirms the operator-dependence of sublingual microvascular monitoring. The involvement of multiple operators was necessary due to the demanding protocol of our study (data collection 7 days a week for 9 months). Notably, our comparison between PI and other investigators must not be merely read as "high versus low experience", as most of those who contributed to video acquisition had previously participated in microcirculatory studies [13, 21, 22] or had undergone an adequate training period. Our results suggest that the quality of microcirculatory data is enhanced if image acquisition is performed by one single and extensively-experienced investigator. Importantly, microvascular parameters may simply vary on the basis of the investigator responsible for video acquisition. The operator-dependence of SDF technique clearly hinders its widespread adoption in the clinical setting. Technological developments of video-microscope devices, such as Cytocam-IDF (Incident Dark Field Illumination) imaging, will hopefully increase their ease of use and limit the bias due to investigator's experience in the video acquisition and analysis [23]. The Cytocam system consists of a lightweight pen-like device with a field of view three times larger than previous devices and improved focus and contrast [24]. This system has also the capability of automatic video analysis, although it still needs validation.

Extensive experience may be not sufficient to ensure high-quality video recording, as indicated by the substantial amount of unacceptable videos among those recorded by the PI. The patient's cooperation during image acquisition is crucial for ensuring reliable microcirculatory data. We were able to demonstrate that the acquisition of reliable video triplets was easier in patients receiving sedation and/or mechanical ventilation. In addition, those among non-sedated patients who received higher-quality microcirculatory videos had significantly lower GCS score. Involuntary movements of the tongue or head can make it difficult to perform adequate image acquisition in fully conscious patients. As a result, microcirculatory assessment may be significantly biased in these patients, since unreliable microcirculatory images may produce an underestimation of microvascular perfusion. In our study, patients not requiring sedation or mechanical ventilation (who were therefore likely to be less severe) paradoxically showed a lower perfused vessel density, thus leading to a spurious dissociation between the microcirculation and the patient's clinical condition.

Our study may have important implications for future research. Image quality should be systematically assessed in studies evaluating the microcirculation. There is an urgent need to define a unified score to assess microcirculatory image quality. The score used in our study was adapted from the one proposed by Massey et al. [12] and takes into account the main domains of video quality. However, it may be limited in the criteria used to define adequateness of stability or duration. The strict criterion used to define stability (absolute pixel translation on X- or Y-axis <20) may explain the high percentage of videos scored as unacceptable for this domain and its relatively minor impact on microvascular parameters. In addition, priority was given to the avoidance of pressure artefacts during image acquisition rather than to stability or duration. An evolution of microcirculatory image quality scoring systems could include a differential weight of different domains in the overall score of quality, based on their impact on the reliability of the analysis. The importance of each component may also vary based on the particular aspect of interest in the study, e.g. more emphasis could be given to the avoidance of pressure artefacts in studies evaluating variations in microvascular convective flow, while aspects such as focus, brightness and content may have major relevance when focussing on vessel density. The exclusion of the confounding factor of video quality will possibly improve microcirculatory data consistency and allow a more reliable identification of even

subtle variations in vessel density or perfusion following interventions.

The problem of quality should be taken in particular consideration when assessing patients with expected low cooperation (awake or not intubated). Video triplets of unacceptable quality should be identified and excluded from the analysis in order to avoid biases in the assessment of microvascular perfusion. Alternatively, the proportion of unreliable video sequences should be reported as a measure of possible methodological bias. In addition, it may be important that measurements are performed by one single investigator with extensive expertise in microcirculatory video acquisition, in order to enhance data quality and consistency.

5 Conclusions

Using a large database of SDF videos from a heterogeneous population of 100 critically ill patients monitored daily during their stay in the ICU, this study showed that microcirculatory image quality is an important problem when assessing the sublingual microcirculation. Recording high-quality images is a challenging task, depending not only on the operator's experience but also on the patient's cooperation. Low-quality videos produce spurious microcirculatory data, leading to an overestimation of microvascular alterations. It is thus important that microcirculatory video quality is assessed systematically in studies evaluating the microcirculation and unreliable triplets must be excluded from the analysis.

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Compliance with ethical standards

Conflict of interest CI is the inventor of sidestream dark field imaging technology and holds shares in MicroVision Medical and was a consultant for this company more than 4 years ago but has had no further contact with the company since then. He has no other competing interests in this field and there are no other relationships or activities that could appear to have influenced the submitted work. The other authors declare that they have no conflict of interest.

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