Intensive Care Med
https://doi.org/10.1007/s00134-018-5070-7

CONFERENCE REPORTS AND EXPERT PANEL

Second consensus on the assessment of sublingual microcirculation in critically ill patients: results from a task force of the European Society of Intensive Care Medicine

Can Ince[1,2*], E. Christiaan Boerma[3], Maurizio Cecconi[4], Daniel De Backer[5], Nathan I. Shapiro[6], Jacques Duranteau[7], Michael R. Pinsky[8], Antonio Artigas[9], Jean-Louis Teboul[10], Irwin K. M. Reiss[11], Cesar Aldecoa[12], Sam D. Hutchings[13], Abele Donati[14], Marco Maggiorini[15], Fabio S. Taccone[16], Glenn Hernandez[17], Didier Payen[18], Dick Tibboel[19], Daniel S. Martin[20,21], Alexander Zarbock[22], Xavier Monnet[10], Arnaldo Dubin[23], Jan Bakker[1,17,24], Jean-Louis Vincent[16] and Thomas W. L. Scheeren[25], On behalf of the Cardiovascular Dynamics Section of the ESICM

Abstract

Purpose: Hand-held vital microscopes (HVMs) were introduced to observe sublingual microcirculatory alterations at the bedside in different shock states in critically ill patients. This consensus aims to provide clinicians with guidelines for practical use and interpretation of the sublingual microcirculation. Furthermore, it aims to promote the integration of routine application of HVM microcirculatory monitoring in conventional hemodynamic monitoring of systemic hemodynamic variables.

Methods: In accordance with the Delphi method we organized three international expert meetings to discuss the various aspects of the technology, physiology, measurements, and clinical utility of HVM sublingual microcirculatory monitoring to formulate this consensus document. A task force from the Cardiovascular Dynamics Section of the European Society of Intensive Care Medicine (with endorsement of its Executive Committee) created this consensus as an update of a previous consensus in 2007. We classified consensus statements as definitions, requirements, and/or recommendations, with a minimum requirement of 80% agreement of all participants.

Results: In this consensus the nature of microcirculatory alterations is described. The nature of variables, which can be extracted from analysis of microcirculatory images, is presented and the needed dataset of variables to identify microcirculatory alterations is defined. Practical aspects of sublingual HVM measurements and the nature of artifacts are described. Eleven statements were formulated that pertained to image acquisitions and quality statements. Fourteen statements addressed the analysis of the images, and 13 statements are related to future developments.

Conclusion: This consensus describes 25 statements regarding the acquisition and interpretation of microcirculatory images needed to guide the assessment of the microcirculation in critically ill patients.

*Correspondence: c.ince@erasmusmc.nl
[1] Department of Intensive Care, Erasmus MC University Medical Center Rotterdam, 's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands
Full author information is available at the end of the article

Springer

Keywords: Hemodynamic monitoring, Microcirculation, Sepsis, Shock, Fluid therapy, Sublingual, Sepsis, Intensive care, OPS imaging, SDF imaging, IDF imaging

Introduction

A large body of evidence on the presence of microcirculatory alterations in critically ill patients has come to light since the clinical introduction of hand-held vital microscopes (HVMs) in the late 1990s. This evidence has led to the need to formulate an update of a consensus on sublingual microcirculatory imaging as applied at the bedside. The first applications of HVM were accomplished by the introduction of the first generation of HVM called orthogonal polarization spectral (OPS) imaging during neurosurgery [1, 2]. Consequently, this led the way to the measurement of microcirculatory alterations in critically ill patients at the bedside where De Backer and co-workers reported that persistent microcirculatory alterations in patients with septic shock were associated with increased mortality, independent of alterations of the systemic macrocirculation [3]. Spronk and co-workers demonstrated that nitroglycerine therapy following pressure-guided resuscitation improved microcirculatory flow in patients with septic shock [4]. Soon after, an increasing number of manuscripts using HVM were published, the majority of which concerned critically ill patients. From the results it became clear that microcirculatory alterations were significantly correlated with the progress of human sepsis and predictive of organ failure and mortality [5–10]. Although most microcirculatory measurements using HVM were carried out in the sublingual area, several investigations of the microcirculation were carried out directly on organ surfaces during surgery [2, 11–15]. Indeed, sublingual microcirculatory alterations have been identified using HVM in a wide range of disease states other than sepsis and shock [16–26] and in different age groups including critically ill newborns [27]. In 2009, an international multicenter observational prevalence study in intensive care patients involving 36 ICUs worldwide enrolling 501 patients identified microcirculatory alterations (microvascular flow index, MFI < 2.6) in combination with tachycardia (heart rate > 90) as an independent risk factor for increased hospital mortality [28]. Various studies have been undertaken to define normal values in adults, newborns, and neonates [29–33], as well as in healthy individuals under extreme environmental conditions [34, 35]. In studies where systemic hemodynamic monitoring was being applied, it became increasingly clear that microcirculatory alterations can occur independently from systemic hemodynamic alterations and that these microcirculatory alterations were independently associated with adverse clinical outcome [6–8]. However, the practical application of microcirculatory measurement using HVM and the interpretation of the images of these measurements remain challenging.

Technical developments of HVM helped in making the measurement of the microcirculation increasingly feasible. The technical issue that had to be overcome in the application of HVM for studying the microcirculation on organ surfaces was the need for an optical system to be developed, where illumination and observation of the microcirculation beneath the surface of an organ are accomplished through the same optical pathway embedded in a light guide with a magnification lens attached to its end.

The first generation of such HVM devices which solved this problem was based on orthogonally polarized spectral (OPS) imaging [1, 36], where an externally filtered light source illuminated the organ surface with linearly polarized light, and reflected light was blocked by an orthogonally polarized analyzer. Although these first-generation HVM devices pioneered the initial studies, they are no longer commercially available. The second generation was based on sidestream dark-field (SDF) imaging, whereby illumination was achieved by surrounding the tip of the light guide with light-emitting diodes creating dark-field illumination [37]. Recently, a third-generation device was introduced which is based on an alternative mode of dark-field microscopy called incident dark-field (IDF) imaging [38] with improved optical resolution which gave better image quality and allowed for the visualization of more capillaries than previous-generation devices [39–42]. The last two techniques are currently commercially available. Massey and Shapiro wrote an excellent comprehensive review of the technical aspects of HVM [43]. Table 1 summarizes the different technical properties of the currently available HVM devices obtained from this publication.

An important reason to evaluate the functional state of the microcirculation in conditions of shock and resuscitation is to verify the expectation that correction of systemic hemodynamic variables results in a parallel improvement in tissue perfusion, a condition referred to as hemodynamic coherence [44]. However, uncertainty remains when correction of systemic hemodynamic variables has been achieved, but downstream variables used as surrogates for tissue hypoperfusion, such as lactate, mottling skin, oliguria, and cold extremities, remain unaltered and abnormal. This can occur as a consequence

Table 1 Comparison between SDF/IDF technical specifications (adapted from Massey and Shapiro [53] with permission)

	Microscan (Microvision Medical, Amsterdam, Netherlands)	Capiscope HVCS (KK technology, Honiton, UK)	Capiscope HVCS-HR[a] (KK technology, Honiton, UK)	Cytocam (Braedius Medical, Huizen, Netherlands)
Type	SDF	SDF	SDF	IDF
Image size (pixels)	NTSC: 720 × 480 PAL: 720 × 576	752 × 480	1280 × 1024	2208 × 1648
Resolution (μm/pixel)	1.45 (horizontal) 1.55 (vertical)[b]	0.92	0.81	0.66[c]
Field of view (μm)	1044 × 758 (NTSC)	692 × 442	1037 × 829	1457 × 1061
Frame rate (frames/s)	NTSC: 30 PAL: 25	Up 87[d]	25[d]	25
Illumination time (ms)	10	0.5–2[d]	0.5–2[d]	2

SDF sidestream dark-field (imaging), *IDF* incident dark-field (imaging), *NTSC* national television system committee, *PAL* phase altering line

[a] Capiscope HVCS-HR uses the same camera, illumination, and optics as the Capiscope HVCS, with a modified sensor and electronics

[b] Measured using an NTSC version and Canopus ADVC110 video digitizer

[c] Measured using a 150 line-pairs per inch Ronchi ruling (Edmund Optics, Barrington, NJ, USA)

[d] Private communication with manufacture

of the nature of the disease or due to an ineffective therapy such fluids [45, 46] or vasopressors not being effective in improving the microcirculation [47–49]. Such a loss of coherence between the macro- and microcirculation has been described in several clinical and experimental studies [8, 23, 44, 48–52] and has been found to be an independent predictor of adverse outcome and organ dysfunction [6–10]. Its manifestation, however, is a dynamic process depending on the interactions between disease, therapy, and time. Figure 1 summarizes four conditions in which this loss of hemodynamic coherence occurs and microcirculatory alterations persist despite optimization of systemic hemodynamic variables [44]. Monitoring the microcirculation during resuscitation procedures can then verify whether they have been effective in restoring tissue perfusion to some minimal value, which is the ultimate expected result of resuscitation procedures. Observation of the type of microcirculatory alterations can unveil information about the mechanisms causing shock and help to determine an appropriate therapeutic response [46].

A consensus was published in 2007 by a group of experts to discuss the various technical, physiological, and clinical aspects of microcirculatory monitoring. The resulting consensus paper described the conditions that needed to be met for image acquisition and consolidated the various scoring methodologies being developed at the time [53]. These methodologies related to the description of functional variables obtained from analysis of the HVM-derived microcirculatory images. Some 10 years later more than 600 articles about clinical and experimental investigations using HVM have been published. Together with current developments in the technology of HVM by introduction of a third-generation computer-controlled image sensor-based HVM with improved optics, it became clear that the definitions in the previous consensus had to be adapted and that it was necessary to organize an updated second consensus on the state of the art of HVM and its application in intensive care medicine. Table 2 summarizes the main differences between the previous *Critical Care* 2007 consensus paper and the current *Intensive Care Medicine* 2018 consensus article, which comprises image quality, analysis, and future perspectives.

Methods

This paper reflects the current state of the art of monitoring of the (sublingual) microcirculation and describes the consensus reached on the requirements for ensuring good quality measurements, as well as the analysis for obtaining variables reflecting the functional status of the microcirculation by the experts listed as authors. Since HVM measurements are predominantly performed in the sublingual area, we will restrict ourselves to the methodology in this particular vascular bed. The present paper was endorsed by the Executive Committee of the European Society of Intensive Care Medicine (ESICM) as an expert opinion guideline for microcirculation monitoring in 2017. The intended format of this paper has been taken from previous expert opinion papers, e.g., the guideline on circulatory shock and hemodynamic monitoring [54] and the position paper on hemodynamic monitoring [55]. We addressed five questions regarding the methodology and interpretation of microcirculatory alterations using HVM: (1) What has been the development of the technology since the last consensus of 2007?

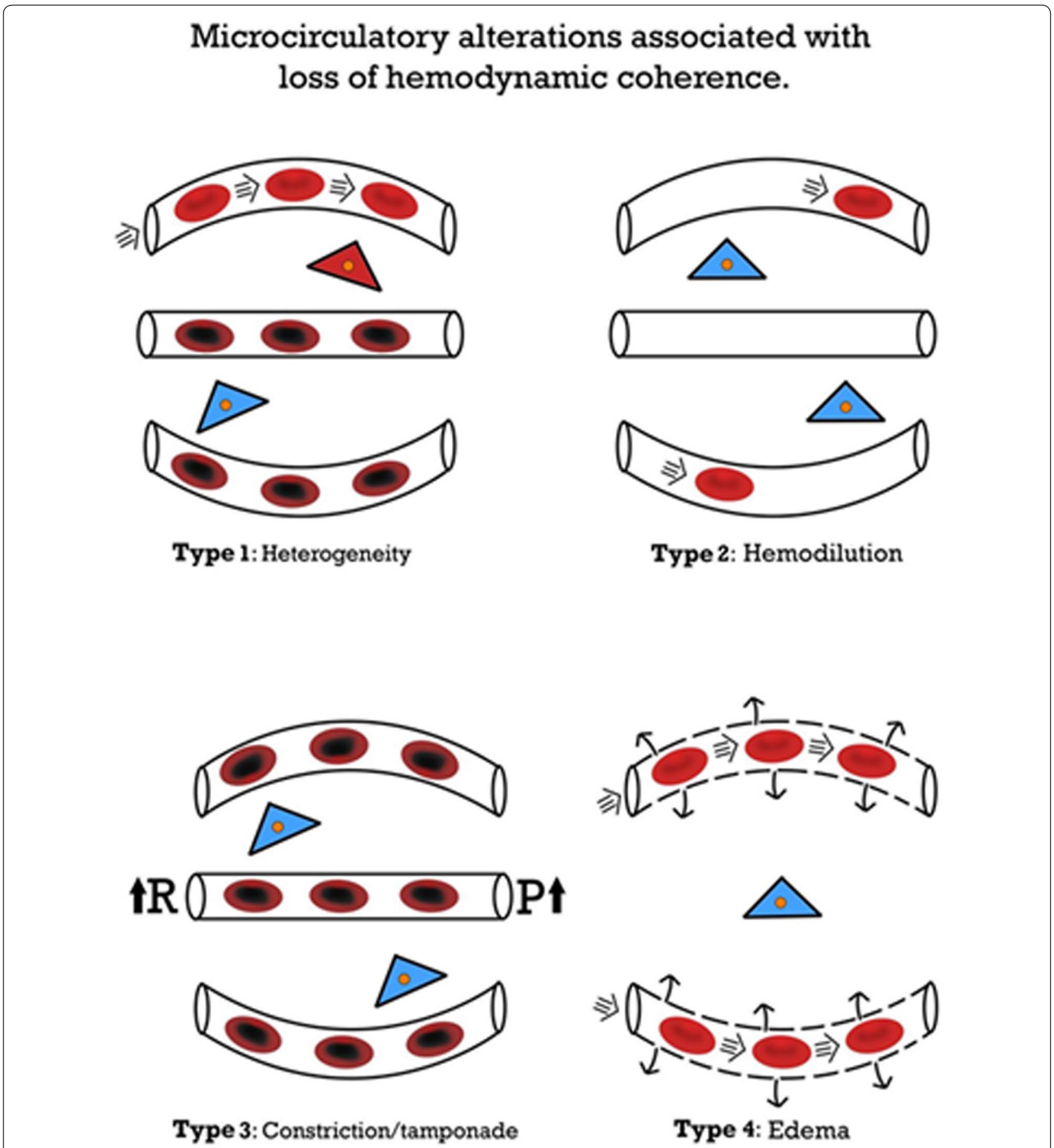

Fig. 1 Microcirculatory alterations associated with loss hemodynamic coherence. States of loss of hemodynamic coherence where macrocirculatory resuscitation does not necessarily cause a parallel improvement in the perfusion of the microcirculation. Type 1: Heterogeneous red blood cell (RBC) flow where flowing RBCs carry oxygen (red RBC) and stagnant RBC (dark red); correspondingly, tissue cells receive oxygen (red tissue cells) or not (blue tissue cells). Type 2: A reduction in the oxygen-carrying capacity of the microcirculation due to hemodilution. Type 3: A stagnation in RBC flow in the microcirculation due to arterial vasoconstriction (increased vascular resistance, R) and/or raised venous pressures (P). Type 4: Increased oxygen diffusion distances due to edema. (Adapted from [44])

Table 2 Main differences between the 2007 and 2018 consensus papers on quantification of microvascular variables

Topic	*Critical Care* De Backer (2007) [53]	*Intensive Care Medicine* Ince 2018
Choice of microvascular variables	We recommended a full description of all available variables present in the non-experimental literature	We recommend a specific set of variables according to the pathophysiologic mechanism (Tables 6 and 7) or type of intervention (Table 8)
Type of microvascular assessment	We recommended a full computer-assisted off-line analysis under all circumstances	We recommend using a grid-based point-of-care tool for the time-limited selection of patients and evaluation of clinical interventions. We recommend performing an (additional) dedicated off-line analysis under all circumstances
Image quality	We recommended to avoid pressure artifacts, elimination of secretions, adequate focus, contrast adjustment, and high-quality recording	We recommend to include a specific scoring sheet (including a cutoff value) that addresses all aspects of image quality
Type of vessel	We recommended to include all vessels with a diameter < 20 µm	We recommend selecting types of vessels (capillaries, venules) for analysis according to the pathophysiology of interest (Tables 6 and 7). Definitions of each vessel type are provided
Number of areas	We recommended a minimum of 3–5 different areas of interest per measurement	We recommend to use single-spot measurements during interventions whenever feasible to enhance discriminative power. Under all other circumstances, a minimum of 3–5 different areas of interest is recommended
Hyperdynamic flow	Recommendations with respect to hyperdynamic flow were not included	We recommend restricting indices of hyperdynamic red blood cell velocity to specific pathophysiologic situations and to limit assessment to space–time-frame-derived exact red blood cell velocity

(2) What are the differences between the current consensus and the previous consensus? (3) What are the current definitions and what quality of measurements and recommendations are needed to perform reliable sublingual measurements? (4) What microcirculatory variables best reflect the functional state of the microcirculation? (5) What recommendations are given for future improvement of the technology for introducing HVM as a standard bedside hemodynamic monitoring tool for clinical decision-making?

To arrive at this consensus we organized three international meetings in accordance with the Delphi method, where an agreement of more than 80% of the authors of the present paper on the statements (definitions, requirements, and recommendations) listed in the tables was considered as being a consensus [54]. During the first meeting (Amsterdam, November 20th 2015) all participants identified the issues including the various aspects of the technology, physiology, measurements, and clinical utility of the current consensus document. During the second meeting (Brussels March 16th 2016) these topics were structured into three categories: image acquisition and quality, image analysis, and future perspectives. In the wake of this meeting a document was composed that summarized the process in a body of statements. All participants were offered the opportunity to respond to the content and formulation of these statement by e-mail. During the final session (Brussels, March 22nd 2017) the aim was to compose the present paper as a consensus document. These three meetings were then considered to be sufficient to meet the Delphi requirements. The body of statements was formulated in its final form and accepted by all participants. The Executive Committee of the ESICM endorsed this consensus by the Cardiovascular Dynamics Section as an update of a previous consensus published in 2007 [53]. We classified the consensus statements as definitions (fact or meaning), requirements (a prerequisite or minimum for sufficient quality), and recommendations (advice that enhances quality).

Results

Quality assessment of acquired HVM microcirculatory images

A prerequisite for assessing the function of the microcirculation using HVM imaging is to ensure that artifact-free measurements are made. This is a requirement of any monitoring device measuring physiological variables, each having its own specific sensitivity to the introduction of artifacts. That is why the use of HVM measurements requires skill and training (Requirement #5, Table 3) similar to other clinical monitoring/diagnostic techniques, such as echocardiography [43]. Although not discussed in the consensus meetings as to the precise

content of a training schedule, it should consist of theoretical and practical knowledge about making and analyzing sublingual microcirculatory imaging as covered in the present consensus. Artifacts can be classified as pressure artifacts, illumination, focus, content, and focus artifacts (uneven focus) (Fig. 2). The main quality requirement for an acceptable HVM measurement is that single red blood cells can be visualized in the capillaries (Requirement #2, Table 3). For this to be accomplished, images should be well focused and obtained free of pressure artifacts (Requirement #3, Table 3). Applying excessive pressure to the tissue surface is the most common of these artifacts and can lead to the incorrect diagnosis of a low flow state. Use of HVM can cause such artifacts because the tip of the probe has to make physical contact with the tissue surface. As was already recognized in the first consensus meeting, pressure artifacts constitute the main technical challenge in performing HVM measurements. The presence of such artifacts can be recognized as flow being impeded in larger venules (> 20 µm), since it is agreed that restricted flow in these large vessels occurs mainly because of excessive pressure being applied. Slight release of the pressure, and noting the restoration of flow, confirms that excessive pressure had been applied during the measurement. Other artifacts that must be avoided consist of content, focus, and illumination, examples of which can be seen in Fig. 2. As a result of the heterogeneity of the microcirculation in the sublingual area, it is recommended to measure in multiple sites, as well as to average the values found during analysis of the different areas. An alteration from the previous recommendation is to take a minimum of three sublingual areas (previous recommendation was five) (Recommendation #6, Table 3). This is needed because the current HVM generation has a significantly larger field of view. Additionally, the possibility to make single-spot measurements for extended periods of time has been added in order to observe changes in single vessels during a therapeutic

Table 3 Summary of the consensus statements: part 1

Image acquisition and quality statements
Definitions
1. Microvessels are defined as vessels with a diameter < 20 µm containing arterioles, capillaries, and venules. Capillaries are defined as vessels < 10 µm in diameter where a single file of red blood cells can be observed. The main characteristics of venules include that they are vessels that are collecting flow from other vessels and have more RBC filling in the lumen than single RBCs as seen in capillaries
Requirements
2. These should be high-quality images where RBC can be clearly seen in the capillaries
3. Images should be evenly illuminated, well focused, possess good contrast, and be free of pressure artifacts. Artifacts are defined as absence of flow in vessels > 20 µm that returns after release of pressure. An additional characteristic of artifacts is alternating bi/directional flow. A quality score should be applied to describe the quality of recording (according to Massey [58]). The quality score should be reported in the article
4. Image sequences should be composed of motion-free images of at least 4 s (100 frames is the minimum needed to generate space–time frames) but ideally should last 20 s in order not to miss intermittent flow. The movement of artifact-free images can be obtained either by hand or by software correction
5. Proper training is required to obtain images of acceptable quality and to perform adequate image analysis. The training and/or experience undergone should be stated when reporting results
Recommendations
6. In the previous consensus, a minimum of 3–5 sites was agreed upon; in this new consensus, we add the possibility of using a single-spot measurement during a procedure for evaluating the response of single vessels
7. Measurements of hyperdynamic flow should not be done routinely but restricted to pathophysiological disease states in which hyperdynamic flow is anticipated. Hyperdynamic flow is defined as a red blood cell velocity above normal flow in healthy volunteers under resting conditions. Measurements of hyperdynamic flow should be done quantitatively by space–time frames and not be added to existing semi-quantitative scores. Measurements of hyperdynamic flow are physically restricted by the frame rate, according to the following equation:
$V_{max} = L \times f/3$ [pm/s], where L is the vessel length in µm, f the frame rate (25 or 30 Hz), and 3 is the number of video frames used for the calculation
8. Unless for specialized application images (specialized locations such as organ surfaces during surgery, cutaneous microcirculation), sublingual measurements should include a mix of capillaries and venules and, if possible, arterioles. Vessel loops should be avoided as they are considered to be from a different anatomical area in the mouth
9. Differences in normal values for vessel density (TVD and PVD) between various optical systems may be up to 30%, depending on resolution, field size, and focus, and therefore are not interchangeable in between. These characteristics do not influence variables of red blood cell velocity
10. In the previous recommendation, the use of 5× objectives with OPS and SDF devices was advised for human sublingual microcirculation and a 10× objectives for small animals. On the basis of the unavailability of 10× objectives and lack of literature supporting this previous recommendation, we abandon this advice and recommend using higher-magnification objectives
11. In the previous consensus, it was stated that "In larger venules, rolling and adherent leukocytes can be observed, but this requires higher magnification and different analytical methods". However, with the current technology, leukocytes can be readily seen in the venules, and higher magnification is not required

RBC red blood cells, *TVD* total vessel density, *PVD* perfused vessel density, *OPS* orthogonal polarization spectral imaging, *SDF* sidestream dark-field imaging

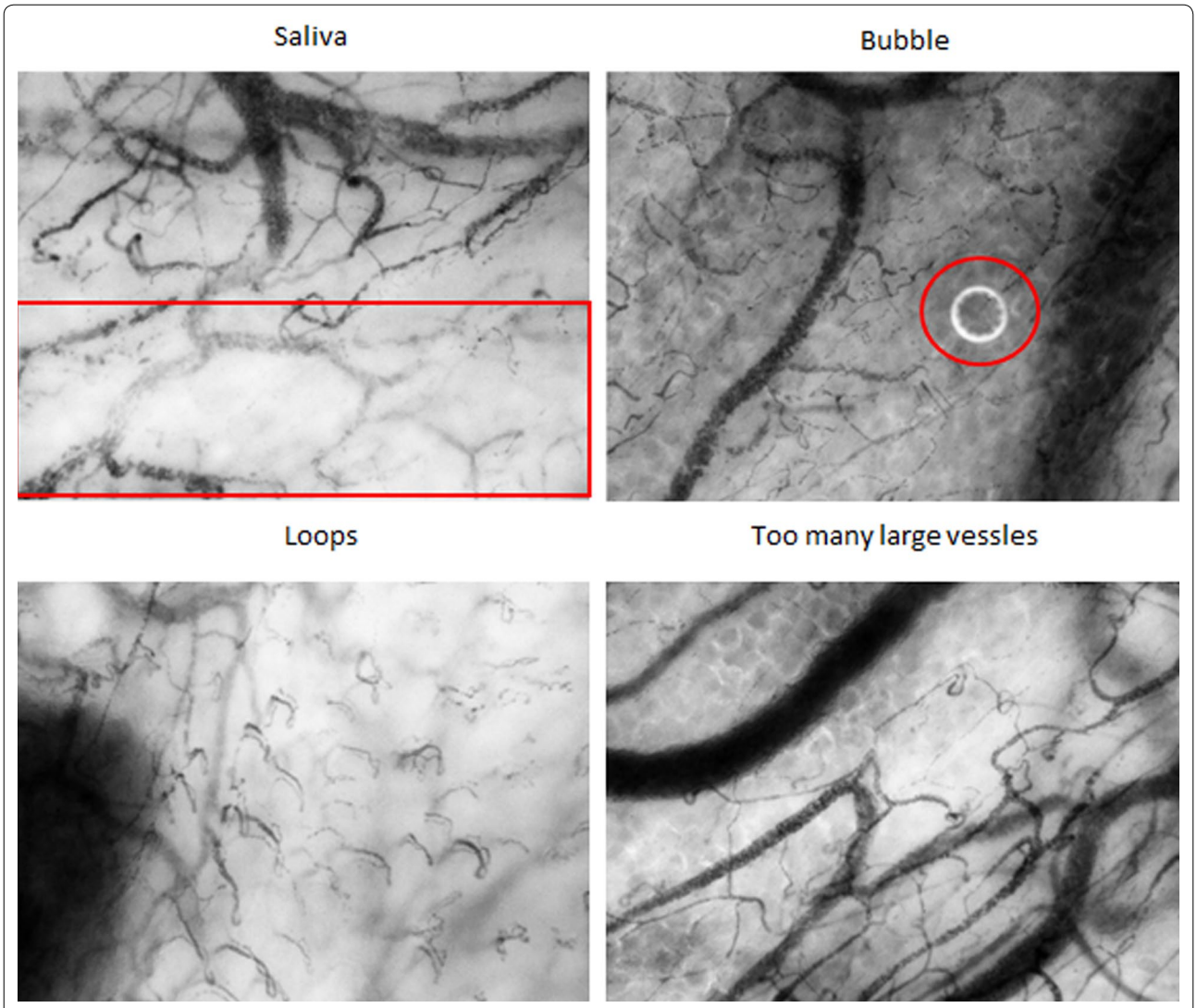

Fig. 2 Conditions of microcirculatory assessment where artifacts can influence the evaluation of the functional state of sublingual microcirculation. **a** Content that can cause an artifact includes saliva and air bubbles obstructing visualization and excessive loops and large venules dominating the mix of capillaries flowing into venules required for a representative sublingual microcirculatory network. **b** Inhomogeneous brightness caused by slanted placement of the tip of the HVM to the tissue or an excessive or inadequate amount of illumination can be a cause of artifact. An even illumination is advised where all vessels, especially the capillaries, are clearly visible

intervention (Recommendation #6, Table 3) [56, 57]. The minimum advised recording is 4 s in the current guidelines (Recommendation #6, Table 3) [43]. However, we suggest to record about 20 s as such time frames can contain extra information about microcirculatory kinetics provided a stable recording can be achieved with no movement or pressure artifacts. Care should be taken when measuring exact red blood cell velocity, especially during hyperdynamic flow, when the limitation of the sampling frequency of the capture card should be taken into account (Recommendation #7, Table 3). Hyperdynamic flow is a source of controversy as several investigators report its presence in critically ill patients, while other do not. Since volunteers at rest do not show such hyperdynamic flow, its presence can be interpreted as a microcirculatory alteration, although its origin and clinical meaning still have to be determined. Even though its presence can be noted, its quantification can be problematic because of limitations of the ability of the image acquisition to capture the images sufficiently rapidly to allow quantification of the velocity of hyperdynamic flow (Recommendation #7, Table 3). Caution is required when comparing normal values of total vessel density, because these can be influenced not only by disease states [26]

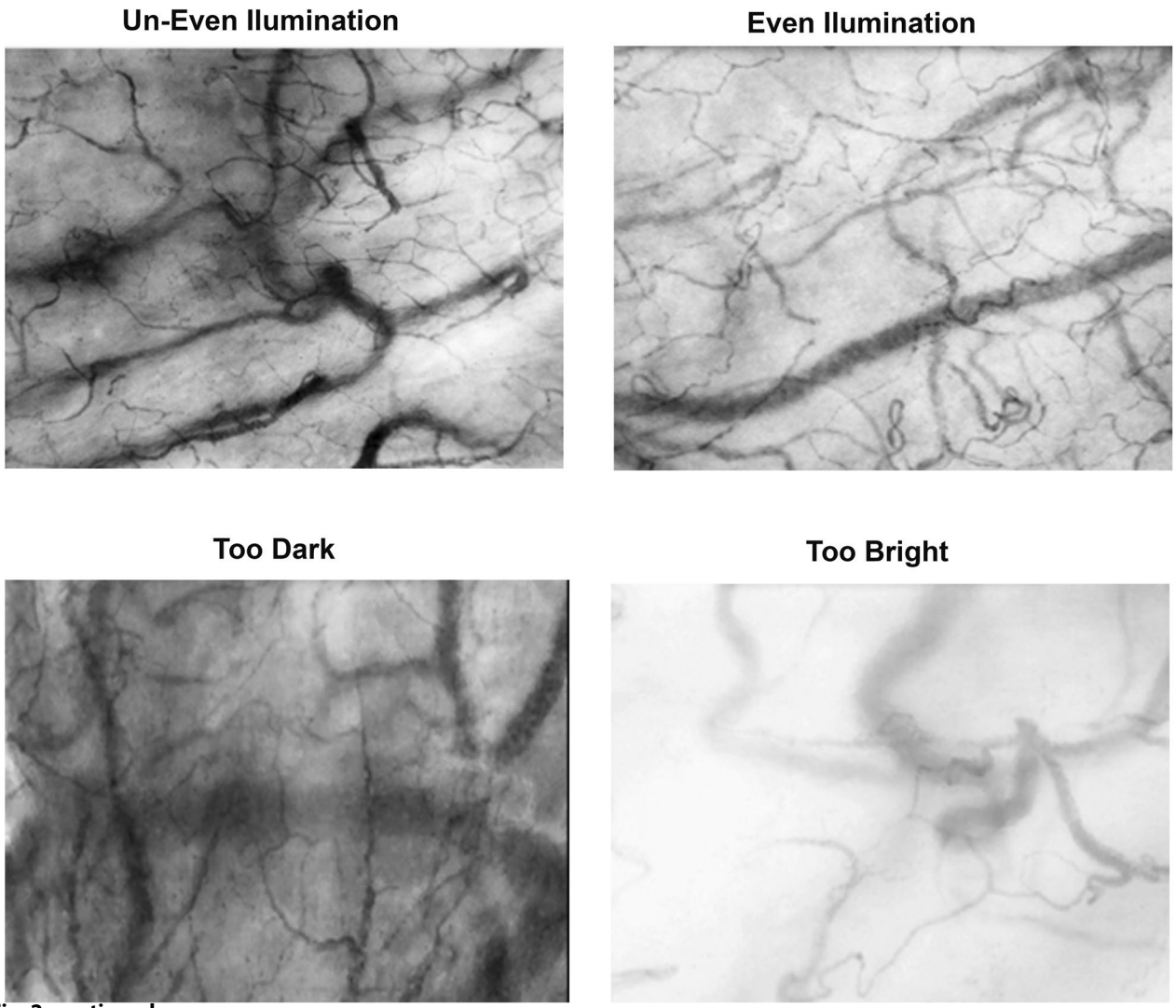

Fig. 2 continued

and ethnicity [33] but, more importantly, by the device used. This is because the third-generation IDF devices have improved optics with which 30% more vessels can be visualized in comparison to the previous-generation devices (Recommendation #9, Table 3) [50, 51]. A representative mix of vessels including capillaries, venules, and possibly arterioles (Recommendation #8, Table 3) should be chosen for a measurement, and care should be taken to avoid too many vessel loops and venules (Fig. 2a). In addition, focus should be adjusted so that single red blood cells in the capillaries are visualized, and the field of view should be free of artifacts such as excessive saliva or bubbles (Fig. 2). This consensus, in contrast to the previous consensus, did not see the urgency to develop higher-magnification devices, since the improved optics currently used are well suited to observe detailed red blood cell and leukocytes presence (Recommendations #10 and #11, Table 3). However, new as yet unexplored subcellular structures such as the glycocalyx or cell-to-cell junctions could be observed with HVM devices with specialized optics. New in this consensus is the requirement to have an objective measure (e.g., score) for the quality of the image based on the above considerations. Such objective quality scores have been developed and used in the literature and open the way for an impartial evaluation of the quality of the image to be embedded in software as a prerequisite for automatic analysis software [58–60]. We recommend that an objective quality score, such as that described by Massey et al., should be reported as part of the methods section in each applicable scientific paper. Its reported interrater agreement (κ) on the individual components ranges between 0.71 and

1.0, with an overall fail–pass agreement (score > 10 AU) of 0.66 [58].

Analysis and interpretation of HVM-recorded sublingual microcirculation

The main functional property of the microcirculation which needs evaluation in the context of shock and resuscitation is its capacity to perfuse the tissues with red blood cells that transport oxygen to the tissue cells. The two main functional components of the microcirculation that describe this physiological function are the flow of red blood cells through the capillaries (convective transport of oxygen) and the density of perfused capillaries (diffusive transport of oxygen; also referred to as functional capillary density) (Definition #12, Table 4). Using these two physiological properties of the microcirculation, plus the microcirculatory alterations which have been observed in states of critical illness (perfusion heterogeneity, specifically in sepsis), several functional microcirculatory measures have been developed, as described in the first consensus meeting [53]. On the basis of the limitations of the technology and software at the time of the first consensus, it was decided that functional measures should be introduced, which could be determined without the need for specialized software. This requirement led to the development of grid-based scoring methods, whereby a grid is drawn on the screen, and the properties of vessels intersecting the lines of the grid can be quantified (De Backer score [53]) and capillary flow evaluated (microvascular flow index, MFI [61]) (Recommendation #13, Table 4). For the assessment of the MFI the screen is divided into four quadrants and a score between 0 and 3 reflecting the average red blood cell velocity is given per quadrant. MFI is then calculated as the mean MFI averaged over the four quadrants. Alternatively, a similar score can be generated by scoring all individual vessels on the screen and averaging their scores [7, 62], although their precise values may deviate from the quadrant-based MFI calculations. Table 4 lists these measurements and their properties.

Microcirculatory images can be analyzed by (a) real-time visual evaluation ("eyeballing"), (b) off-line manual analysis (e.g., grid-based or complete screen-based), (c) offline software-aided analysis, or (d) online automatic analysis (Definition #13, Table 4). Several studies have shown that real-time point-of-care (POC) assessment by visual inspection of microcirculatory properties at the bedside shows good agreement with off-line evaluation of the microcirculation afterwards (Recommendation #18, Table 4). These instant evaluations have been described, as used by nurses [63], and scoring systems have been developed and validated for instant evaluation of the severity of microcirculatory dysfunction [63–67]. These analysis methods are focused mainly on alterations in microcirculatory flow by MFI determination, as this value can most easily be evaluated by visual inspection of the moving microcirculatory images. The POC methods for identifying microcirculatory alterations at the bedside can form a crucial bridge to the acceptance of HVM monitoring as a routine monitor in the clinic, pending the development of reliable automatic analysis software, since they can potentially provide instant evaluation of the presence of microcirculatory alterations and potentially support clinical decisions. However, whether such POC use can make a difference to the condition of patients will have to be demonstrated in clinical intervention trials.

From these and other studies, MFI values $\leq$ 2.6 are generally agreed upon to identify states of microcirculatory alterations (Recommendation #23 Table 4). It should be emphasized however that this threshold value of 2.6 requires further validation in intervention trials with clinical outcomes. In analyzing the flow, it is not mandatory to divide the screen into quadrants, as evaluation of the complete screen also yields comparable information to identify the presence of microcirculatory alterations (Recommendation #22, Table 4). For a more detailed analysis and to calculate the measures listed in Table 5, vessel detection is required. This can either be performed manually or can be accomplished by using specialized software such as automated vascular analysis (AVA), which has been developed for this purpose [68]. Although the introduction of AVA software has somewhat eased the methodology of analysis, as grids and vessels previously had to be drawn manually, its application remains time consuming and is a major limitation to the introduction of HVM into routine clinical practice. However, with the advancement of technology, several attempts have been made to develop automatic software capable of analysis for POC use [69–74]. Regardless of how promising these developments may be, their use in the clinical evaluation of microcirculatory status has yet to be validated in clinical practice and cannot be recommended for this purpose presently.

Several measures have been developed and subsequently used in the literature to describe the functional properties of sublingual microcirculation. These measures have included variables describing convective flow of red blood cells (discussed above) and variables related to the diffusion distance of oxygen between the red blood cells and the tissue cells. The latter, quantified by the functional capillary density (FCD), includes total vessel density (TVD) and perfused vessel density (PVD). The minimum dataset required to describe the functional properties of the microcirculation as observed by HVM is listed in Table 6. These measures were developed

Table 4 Summary of the consensus statements: part 2

Analysis statements
Definitions
12. The main functional variables describing the functional state of the microcirculation are related to their oxygen-carrying capacity, defined by a measure of convective flow (RBC flow), diffusive capacity (FCD), and oxygenation
13. Analysis of images can be achieved by (a) real-time visual evaluation, (b) off-line manual analysis (grid-based), (c) off-line software-aided analysis, and (d) on-line automatic analysis. It should be made clear which type of evaluation was used, and reference should be made to validation studies of the chosen analysis methodology
14. Space–time diagrams (STDs) are the gold standard for quantitative measurements of RBC velocity. It should be noted that not all microvessels contain the physical characteristics (clear visualization of particular nature of the RBCs) that make them suitable for STD analysis, creating potential bias in the analysis
15. Alterations in the sublingual microcirculation have previously been used mainly to describe changes observed in patients associated with sepsis. However, because currently other disease states are also being investigated, a differential diagnosis of the type of alterations is required. Such different types of microcirculatory alterations can be classified as follows:
Type 1: complete stagnated capillaries (circulatory arrest, excessive use of vasopressors)
Type 2: reduction in number of flowing capillaries (hemodilution)
Type 3: plugged vessels are seen next to vessels with flowing cells (sepsis, hemorrhage, and hemodilution)
Type 4: hyperdynamic flow within of capillaries (hemodilution, exercise, sepsis)
16. In the previous consensus, reference was made only to video recordings. Now, with advancement in technology, image sensors allow the rapid capture of still images to be made and played back at video speed
Recommendations
17. Surrogates for functional variables can be used in cases where RBC velocity is measured as a semi-quantitative index or preferably quantitative value is used. Previously, grid-based scoring methods have been used for scoring, and these have been shown to discriminate between health and disease states
18. Grid-based scoring systems do not provide quantitative values of velocity but can be used for point-of-care assessment of the presence or absence of microcirculatory alterations. Such assessment is valuable to distinguish patients with a normal microcirculation from patients with an abnormal microcirculation and to characterize a type of shock
19. Space–time frames analysis should be applied when quantitative variation in RBC distinction needs to be made between different levels of velocities (in heart failure or during stepwise flow reduction on cardiopulmonary bypass or assist devices)
20. Previous recommendations stated that "large external monitors should be used instead of the liquid crystal display (LCD) screen of the computer". With the improved high definition (HD) screens currently available, this is no longer required
21. Heterogeneity of blood flow is a hallmark of distributive shock and thus a vital variable to determine the type of shock. Under such circumstances a coefficient of variation between a variable within different areas of interest should be provided to quantify this heterogeneity. Ideally, future software analysis should be able to provide a heterogeneity index based on the red blood cell velocity distribution in between individual vessels
22. In the previous consensus, it was stated that "Separation of the screen into quadrants (or using equidistant lines) is mandatory when analysis is done by eye." However, several studies have demonstrated that microcirculatory alterations can be identified by "eyeballing" without the need to perform a quadrant analysis. This only is applicable to point-of-care assessment and not to quantification
23. As of now, data on the clinical relevance of microvascular alterations are predominantly expressed in PPV and MFI. Although cutoff values for a normal MFI are > 2.9, cutoff values for MFI of 2.6 are suggested as a threshold below which alterations can be considered clinically relevant
24. Clips should be de-identified and randomized before analysis to minimize bias
25. Microcirculatory measurements using intravital microscopy in adult humans are currently also applied to other locations than the sublingual area. For other regions, children- and animal-specific criteria for good measurement and analysis should be developed and validated

RBC red blood cell, *FCD* functional capillary density, *PPV* proportion of perfused vessels, *MFI* microcirculatory flow index

primarily for describing microcirculatory alterations relating to sepsis and therefore biased towards describing the microcirculation under such conditions. The alterations seen in sepsis are often characterized by a highly heterogeneous perfusion, with stopped flow capillaries next to vessels with flowing cells. To better characterize conditions of microcirculatory alteration other than those associated with sepsis, the consensus introduced a classification which describes the four most seen microcirculatory alterations previously observed in various clinical scenarios (Definition #15, Table 4) [75]. These classifications of microcirculatory alterations serve as a methodology to describe the different types of alterations observed by investigators. These types of alterations can occur on their own or mixed together, just as different pathogenic mechanisms can occur at the same time, especially in complex disease states such as sepsis. The types of alterations include: Type 1, complete stagnated capillaries (circulatory arrest, excessive use of vasopressors); Type 2, reduction in number of flowing capillaries (hemodilution); Type 3, stopped flow vessels are seen next to vessels with flowing cells (sepsis, hemorrhage, and hemodilution); Type 4, hyperdynamic flow within capillaries (hemodilution, sepsis).

Table 5 Characteristics of microvascular variables

Variable	Abbreviation	Definition	Characteristics	Units	Strength/weakness
Proportion of perfused vessels	PPV	Grid-based score (3 horizontal and vertical equidistant lines). Percentage of perfused vessels per total number of vessel crossings	Binominal determinant of red blood cell velocity: flow or no-flow	%	Good reproducibility. Based upon tradition of preclinical research. Score is sensitive to isotropy (change in image size during optical magnification)
De Backer score	NA	Grid-based score (3 horizontal and vertical equidistant lines). Total number of vessel crossings per grid length	Proxy of total vessel density. Applicable to different vessel types (capillary density)	n/mm	Together with the percentage of perfused capillaries proxy of functional capillary density
Microvascular flow index	MFI	Grid-based score per quadrant. 0 = stop flow, 1 = intermittent flow, 2 = sluggish flow, 3 = normal flow	Semi-quantitative assessment of the average red blood cell velocity per quadrant	AU	Good reproducibility. Quick and possible by "eyeballing". Non-continuous separation between categories of flow. Potential loss of detail, overcome by similar score per vessel
Total vessel density	TVD	Software supported measurement of total vessel area per surface area	Determinant of capillary distance (diffusive capacity)	mm^2/mm^2	Absolute number, continuous data. Time consuming because of necessary manual correction of software-supported vessel tracing of vessels. Exact measurements of vessel diameter
Perfused vessel density	PVD	Percentage of perfused vessels × TVD	Determinant of capillary distance (diffusive capacity) and red blood cell velocity (convective capacity)	mm^2/mm^2	Equal to functional capillary density = gold standard in preclinical research. Time consuming
Space–time diagram	STD	Measurement of exact red blood cell velocity	Determinant of red blood cell velocity (convective capacity)	mm/s	Absolute number, continuous data. Time consuming, applicability limited to non-tortuous vessels of sufficient length
Heterogeneity index	HI	Coefficient of variation, expressed as (highest − lowest value)/mean	Determinant of heterogeneity of blood flow, characteristic of distributive abnormalities	AU	Provides additional information, missed by absolute numbers. Calculation may be based upon MFI or PPV

NA not applicable, *AU* arbitrary unit

Table 6 Minimum dataset of microvascular variables per type of shock

Type of shock	Variables of convective blood flow		Variables of diffusive capacity		Variables of heterogeneity	Vessel type
Hemorrhagic	1. $MFI_{quadrant}/MFI_{vessel}$	**AND**	1. Total vessel density		NA	Capillaries
	OR					
	2. Percentage of perfused vessels		2. De Backer score		NA	Capillaries
Cardiogenic	1. $MFI_{quadrant}/MFI_{vessel}$	**AND**	1. Total vessel density		NA	Capillaries
	OR					
	2. Percentage of perfused vessels	**AND**	2. De Backer score		NA	Capillaries
Distributive	1. $MFI_{quadrant}/MFI_{vessel}$	**AND**	Total vessel density plus perfused vessel density	**AND**	Heterogeneity index	Capillaries & venules
	OR					
	2. Percentage of perfused vessels	**AND**	De Backer score	**AND**	Heterogeneity index	Capillaries & venules
Obstructive	1. $MFI_{quadrant}/MFI_{vessel}$	**AND**	Total vessel density plus perfused vessel density		NA	Capillaries
	OR					
	2. Percentage of perfused vessels	**AND**	De Backer score		NA	Capillaries

$MFI_{quadrant}$ microvascular flow index per quadrant, MFI_{vessel} microvascular flow index per vessel, *NA* not applicable

The minimum dataset necessary for distinguishing between the various states of microcirculatory alterations is listed in Table 6. Extensive literature has been published related to the effect of various pharmacological therapies on the microcirculation, as seen in states of shock including fluids [45, 46, 76–78], blood [79–82], vasoactive medication [4, 47–49, 83, 84], anesthesia [85, 86], and mechanical support therapies such as extracorporeal membrane oxygenation (ECMO) [6, 87–90], cardiopulmonary bypass (CPB) [91–93], and intra-aortic balloon pulsation (IABP) [94–96]. Table 7 lists which measures need to be assessed in order to demonstrate the efficacy of therapeutic interventions aimed at resuscitating the microcirculation. In addition to such evaluation of measures, intravenous fluids [77] or vasoactive challenge [83, 97] has been used to identify the microvascular reactivity and/or reserve (Table 8).

Although qualitative evaluation has been extensively used in the literature, quantitative evaluation of microcirculatory flow is required for a more precise characterization of other types of flow alterations than those occurring in sepsis, which also allows for a more precise description of the amount of heterogeneity present than the scoring methodology currently used [10]. A practical example is a slower flow associated with heart failure, which can be readily observed but which may be inaptly described by the qualitative flow measures currently available. On the basis of the perception that simply giving numerical values to microcirculatory flow patterns insufficiently describes the complex flow patterns seen in microcirculatory images, the so-called space–time diagrams (STDs) were developed to describe quantitative red blood cell velocity profiles [98] (Fig. 3). These STDs have been used in several HVM studies for quantitative evaluation and identification of abnormal blood flow patterns [7, 93]. The potential to create STDs is embedded in AVA [68] and can also be generated automatically using computer-controlled IDF imaging [72].

From these considerations, it is clear that new automatic software analysis is a key requirement for the improvement of HVM. Technological advances including computer-controlled HVM, where illumination and image capturing are synchronized, will allow automatic software to be developed more readily. Within the software, quality analysis of the images, image stabilization, and quantitative recognition analysis will need to be present. Since other applications for HVM analysis of the sublingual microcirculation are being developed, such as in infants [27] and in various organs with multiple morphologies and states of disease [14–16, 99, 100], future software must be flexible to incorporate these. This would open up the possibility to extend its use to other disease states and therapies, where monitoring the hemodynamics, cellular constituents, or morphology of the microcirculation would be relevant for understanding the underlying pathophysiology or the response to therapy (Recommendation #25, Table 4).

Table 7 Minimum dataset per type of microvascular abnormality

Type of microvascular abnormality	Variables of convective blood flow		Variables of diffusive capacity		Variables of heterogeneity	Vessel type
Type 1. Completely stagnated capillaries (circulatory arrest, excessive use of vasopressors)	1. $MFI_{quadrant}/MFI_{vessel}$		NA		NA	Capillaries
	OR					
	2. Percentage of perfused vessels		NA		NA	Capillaries
Type 2. Reduction in number of flowing capillaries (hemodilution)	NA (Optional: space–time frame to quantify hyperdynamic flow)		1. Total vessel density		NA	Capillaries
			OR			
			2. De Backer score		NA	Capillaries
Type 3. Plugged vessels are seen next to vessels with flowing red blood cells (sepsis, hemorrhage)	1. $MFI_{quadrant}/MFI_{vessel}$	**AND**	Total vessel density plus perfused vessel density	**AND**	Heterogeneity index	Capillaries & venules
	OR					
	2. Percentage of perfused vessels	**AND**	De Backer score	**AND**	Heterogeneity index	Capillaries & venules
Type 4. Hyperdynamic flow within of capillaries (hemodilution, exercise, sepsis)	$Space–time\ frames_{vessel}$		NA		NA	Capillaries

$MFI_{quadrant}$ microvascular flow index per quadrant, *MFI_{vessel}* microvascular flow index per vessel, *NA* not applicable

Table 8 Minimum dataset for the evaluation of the change in microvascular variables per intervention

Type of microvascular shock	Variables of convective blood flow		Variables of diffusive capacity		Variables of heterogeneity	Vessel type
Fluid administration	1. $MFI_{quadrant}/MFI_{vessel}$	**AND**	Total vessel density plus perfused vessel density		NA	Capillaries & venules
	OR					
	2. Percentage of perfused vessels	**AND**	De Backer score		NA	Capillaries & venules
Vasopressor administration	1. $MFI_{quadrant}/MFI_{vessel}$	**AND**	Total vessel density plus perfused vessel density	**AND**	Heterogeneity index	Capillaries
	OR					
	2. Percentage of perfused vessels	**AND**	De Backer score	**AND**	Heterogeneity index	Capillaries
Weaning from ECMO or IABP	1. $MFI_{quadrant}/MFI_{vessel}$	**AND**	Total vessel density plus perfused vessel density		NA	Capillaries
	OR					
	2. Percentage of perfused vessels	**AND**	De Backer score		NA	Capillaries

$MFI_{quadrant}$ microvascular flow index per quadrant, *MFI_{vessel}* microvascular flow index per vessel, *NA* not applicable, *ECMO* extracorporeal membrane oxygenation, *IABP* intra-aortic balloon pump

Future developments and horizons

The next phase in the clinical development of HVM for bedside monitoring of the microcirculation is to integrate it into routine clinical practice (Recommendation #29, Table 9) [101]. The mention of microcirculatory monitoring in the consensus on circulatory shock and hemodynamic monitoring of the task force for the ESICM in 2014 [54], as part of the arsenal of hemodynamic monitors which can be used for shock monitoring, indicates an acceptance that HVM has acquired a role in the cardiovascular monitoring for the critically ill. Despite the many publications and the application of microcirculatory monitoring using HVM in numerous clinical and experimental scenarios, along with the recent important technical developments that have been achieved in this field, further developments are needed prior to its integration into routine clinical practice. In this context it will be imperative to conduct trials whereby microcirculatory alterations are identified by HVM and corrected by specific interventions that lead to clinically relevant benefit for the patient (Recommendation #39, Table 9).

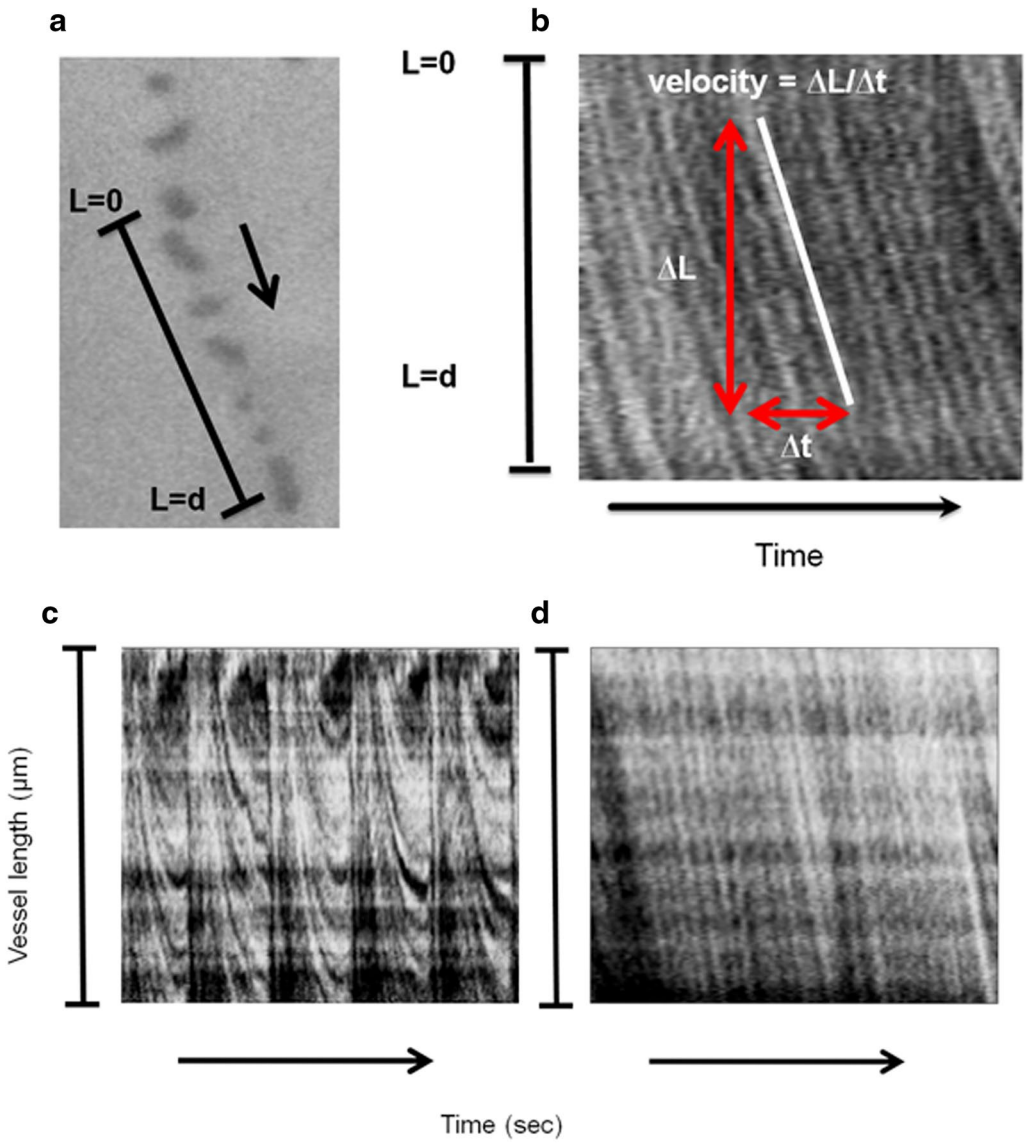

Fig. 3 Space–time diagrams (STD). **a** STD of a specified length of capillary vessel is generated by specialized software. The arrow indicates the direction of red blood cell (RBC) flow. The line *L* is drawn of length *d*, where the entry point to the capillary is defined as $L = 0$. **b** The STD of this vessel in **a** is drawn whereby the *y* axis represents the length of the blood vessel segment *L*, with at the top of the *y* axis the entrance of the vessel ($L = 0$) and at the bottom of the *y* axis the length *d* of the blood vessel. On the *x* axis is time. The STD is then generated by each red blood cell entering the blood vessel at $L = 0$ and exiting the diagram at $L = d$. In this way each moving red blood cell generates a line, the slope of which equals the velocity (velocity $= \Delta L/\Delta t$). **c** Example of an STD with the pulsatile kinetics of capillary flow under normal conditions of a patient prior to cardiac surgery. **d** STD of the same patient as in **c** now with laminar capillary flow during non-pulsatile pump support during cardiac surgery (**c** and **d** reproduced with permission from [93])

The two foremost developments that are required are firstly ease of image acquisition, so that rapid artifact-free images can be obtained promptly, and secondly that the analysis of the images and classifications of the type of alterations present should be done rapidly, reliably, and automatically. Concerning this last point, it is advised that new, more physiologically based microcirculatory variables are introduced because they relate more directly to the function of the microcirculation which is to transport oxygen to the tissues. With the recent development of computer-controlled image sensor-based HVM systems and several automatic microcirculatory analysis software systems in progress, it is envisaged that physiologically based microcirculatory variables will be

Table 9 Summary of the consensus statements: part 3

Future perspectives
Requirements
26. Development of future automatic analysis must be transparent in terms of published algorithms and validated in experimental and clinical settings
27. Automatic algorithms should always allow the user to edit automatically detected vessels and allow analysis of a subset of vessels
28. Automatic algorithms should provide the user information concerning the quality of measurement prior to automatically analyzing the image
Recommendations
29. It is recommended to pursue the objective of inclusion of microcirculatory assessment as part of routine hemodynamic assessment at the bedside
30. Functional measures should be expanded to include the oxygen-transporting capacity of the blood flow in the microcirculation. In doing so, the capillary RBC count should be expanded to include measures related to microcirculatory oxygen-delivering capacity with capillary hematocrit (volume of RBC/volume of capillary) and discharge hematocrit (CapHt/unit time), functional capillary density as well as its oxygen-carrying capacity. To this end, RBC counts will have to be introduced and integrated along with vessel diameters. Velocity measurements should be quantitative and replaced by flow measurements as well as the oxygen-transporting capacity of the microcirculation. In doing so, a measure of heterogeneity, whether physiological or pathophysiological, should be added
31. Reference values should be identified for the various microcirculatory variables below which microcirculatory alterations are defined as persisting. It is expected that such reference values will define microcirculation-guided resuscitation end points
32. For the pediatric age group, development of normalized values from birth until adolescence is essential as background comparative data
33. Microcirculatory measurements should be integrated with macrocirculatory measurements to provide a comprehensive hemodynamic picture and evaluate the presence or absence of regulatory mechanisms responsible for hemodynamic coherence between the different levels of the circulation. Note: This is of significance because the presence of hemodynamic coherence indicates that hemodynamic regulatory mechanisms are intact during resuscitation and that targeting the systemic circulation is an adequate resuscitation strategy
34. The previous consensus stated that "The ideal software, we propose, should automatically recognize all blood vessels and measure their diameters and blood flow in each individual vessel of the investigated field. This is not currently available." Unfortunately, such a solution is not yet available, although several software packages have been released but not yet validated. Analysis software calculating physiological variables related to macrocirculatory oxygen delivery is still wanting
35. Tools should be developed to make pressure-artifact-free measurements and allow single-spot measurements to be made during a therapeutic maneuver
36. Leukocyte tracking and kinetics should be measured and related to activation of inflammatory pathways. Platelet identification is helpful to address the interplay between inflammation and coagulation
37. Technology should be developed to allow stable measurements to be made for longer periods of time to allow continuous measurement during, for example, a therapeutic maneuver allowing observation of the response of single vessels before and after the intervention
38. Optical magnification increases should be possible to identify cellular and subcellular structures (glycocalyx, cell-to-cell connections)
39. It is recommended that trials be undertaken whereby microcirculatory alterations are identified by HVM and corrected by specific interventions that lead to clinically relevant benefit for the patient

RBC red blood cells, *CapHt* capillary hematocrit

introduced in the near future. Analysis of these images would directly relate to the physiological function of the microcirculation, which is to transport oxygen to the tissues (Recommendation #30, Table 9). These measures were defined early on in intravital microscopy investigations as relating to the oxygen-transporting capacity of the microcirculation in muscle by capillary hematocrit [102]. The main functional variables describing the convective capacity of the capillaries of the microcirculation to transport oxygen are the tube hematocrit (the hematocrit of the blood in a capillary at a moment in time) and the discharge hematocrit (the hematocrit flowing through the capillaries per unit of time). The diffusive capacity of the microcirculation is described by the FCD (Recommendation #30, Table 9). When developing the next generation of automatic analysis software, transparency of algorithms and the ability to measure subsets of microcirculatory variables are of vital importance (Requirement #26 and Recommendation #37, Table 9) [103]. When applying such functional analysis software, it will be important to establish normal values in different age populations [24], and specifically in the developmental stages from the neonates on through childhood (Recommendation #32, Table 9) [25]. Following such a description, critical microcirculatory values should be identified, below which pathophysiology is identified (Recommendation #31, Table 9).

An additional variable of interest would be to include the hemoglobin saturation of the red blood cells in the field of view in order to complete the description of the oxygen-transporting capacity of the microcirculation. Such measurements have been used in intravital microscopy to investigate oxygen transport in experimental animals [104]. Indeed, such a variable could quite easily be incorporated in HVM by the inclusion of differently colored LEDs [105]. Such a future HVM spectrophotometry method, with the physiological variables proposed, would indeed be a major development in HVM.

Consequently, it would allow integration in systemic hemodynamic variables for a comprehensive description of the oxygen-transporting capacity of the cardiovascular system at the bedside. In addition, a more comprehensive measure of heterogeneity could be incorporated which is based on the distribution of flow, thereby allowing a better description of the physiological function of the microcirculation to be accomplished in combination with morphometric analysis.

There is also a need for further development of the hardware in order to more readily and reliably perform HVM at the bedside. In this respect, hardware development that allows measurements to be made instantly and without pressure artifacts would greatly facilitate the introduction of HVM into routine use at the bedside. Future developments allowing hands-free measurements to be made, so that measurements can be made in one and the same location for extended periods of time (Recommendation #37, Table 9) during therapeutic maneuvers, would allow a much more precise evaluation of the functional state of the microcirculation. Finally, it is conceivable that developments in the optics of HVM systems could allow for much more detailed observations of cellular and even intracellular structures [106].

The main near-future development for HVM in clinical practice will be its integration with macrohemodynamic measurements, thus providing an integrative and functional evaluation of the cardiovascular system from the heart to the capillaries [107, 108]. In doing so changes in microcirculatory properties in the different phases of life will need to be taken into account alongside the effects of chronic disease states. For the pediatric population longitudinal data are warranted from the "normal" extremely low birth weight infant till adolescence as significant changes have been identified to occur in the first week of life [29]. Furthermore, identifying the presence or absence of hemodynamic coherence between the different compartments of the cardiovascular system may become possible (Recommendation #33, Table 9). Although microcirculatory monitoring has improved, training in its use, both in terms of performing the measurements as well as interpreting the images, is necessary. A comprehensive knowledge of the functional behavior of the microcirculation and its cellular components in health and disease will also be essential.

Finally some shortcomings of this consensus need to be mentioned. The use of the Delphi method can be questioned as an appropriate format to reach a consensus and indeed several other methodologies have been put forward to define a consensus [109, 110]. We chose the Delphi method because a previous similar consensus reached from the Cardiovascular Dynamics Section of the ESICM had used the same methodology [54]. In addition, these other methodologies included criteria which were much better suited for more conventional guidelines and devices such as financial considerations, issues we felt were not relevant for our subject matter. A second shortcoming of our consensus is the absence of the availability of more advanced software for analysis of microcirculatory images. Although some initial versions are currently available they require validation and further development by the field.

Conclusion

In this paper we provided a set of guidelines on microcirculatory imaging, using hand-held microscopy at the bedside. We expect that this consensus paper, created by the Cardiovascular Dynamics Section of the ESICM and endorsed by its Executive Committee, will make an important contribution to the current and future use of HVM in critically ill patients.

Author details

[1] Department of Intensive Care, Erasmus MC University Medical Center Rotterdam, 's-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands. [2] Department of Translational Physiology, Academic Medical Center, Amsterdam, The Netherlands. [3] Department of Intensive Care, Medical Centre Leeuwarden, Leeuwarden, The Netherlands. [4] Anaesthesia and Intensive Care, St George's Hospital and Medical School, London, UK. [5] Department of Intensive Care, CHIREC Hospitals, Université Libre de Bruxelles, Brussels, Belgium. [6] Department of Emergency Medicine and Center for Vascular Biology Research, Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA, USA. [7] Anesthesia and Intensive Care Department, Hôpitaux Universitaires Paris-Sud, Université Paris-Sud, Hôpital De Bicêtre, Assistance Publique Hôpitaux de Paris, Le Kremlin-Bicêtre, France. [8] Department of Critical Care Medicine, University of Pittsburgh, Pittsburgh, PA, USA. [9] Critical Care Center, Hospital de Sabadell, CIBER Enfermedades Respiratorias, Universidad Autónoma de Barcelona, Sabadell, Spain. [10] Service de Réanimation Médicale Hôpital de Bicêtre, Hôpitaux Universitaires Paris-Sud, AP-HP, Le Kremlin-Bicêtre, France. [11] Department of Pediatrics, Division of Neonatology, Erasmus MC-Sophia Children's Hospital, Erasmus Medical Center, Rotterdam, The Netherlands. [12] Department of Anesthesiology and Surgical Critical Care, Hospital Universitario Rio Hortega, Valladolid, Spain. [13] Kings College Hospital, Denmark Hill, London, UK. [14] Anesthesia and Intensive Care Unit, Università Politecnica delle Marche, Ancona, Italy. [15] Medical Intensive Care Unit, University Hospital of Zurich, Zurich, Switzerland. [16] Department of Intensive Care, Hôpital Erasme, Brussels, Belgium. [17] Departamento de Medicina Intensiva, Facultad de Medicina, Pontificia Universidad Católica de Chile, Santiago, Chile. [18] Department of Anesthesiology and Critical Care, Lariboisière Hospital, University of Paris Denis Diderot 7, Paris, France. [19] Intensive Care and Department of Pediatric Surgery, Erasmus Medical Center, Sophia Children's Hospital, Rotterdam, The Netherlands. [20] Intensive Care Unit, University College London, London, UK. [21] Division of Surgery and Interventional Science, Royal Free Hospital, London, UK. [22] Department of Anaesthesiology, Intensive Care Medicine and Pain Medicine, University Hospital Münster, Munster, Germany. [23] Servicio de Terapia Intensiva, Sanatorio Otamendi y Miroli, Buenos Aires, Argentina. [24] Department of Pulmonology and Critical Care Medicine, Columbia University Medical Center, New York, USA. [25] Department of Anesthesiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands.

Acknowledgements

Many have contributed with their expertise to the formation of this paper. The authors wish to specifically thank the following persons: Marly van Assen, Sam Arend, Sam Boerma, Vanina Edul, Hernanado Gomez, Matthias Hilty, Yasin Ince, Michael Massey, Gerke Veenstra, Claudia Scorcella, Sherezade Tovar-Doncel, and ZuhreUz.

Compliance with ethical standards

Conflicts of interest

C. Ince has developed SDF imaging and is listed as inventor on related patents commercialized by MicroVision Medical (MVM) under a license from the Academic Medical Center (AMC). He has been a consultant for MVM in the past but has not been involved with this company for more than 5 years now, and hold no shares. Braedius Medical, a company owned by a relative of Dr. Ince, has developed and designed a hand-held microscope called CytoCam-IDF imaging. Dr. Ince has no financial relation with Braedius Medical of any sort, i.e., he has never owned shares or received consultancy or speaker fees from Braedius Medical. He runs an Internet site https://microcirculationacademy.org which offers services (training, courses, and analysis) related to clinical microcirculation. The other authors have no declared interest with respect to this paper.

Received: 7 November 2017 Accepted: 17 January 2018
Published online: 06 February 2018

References

1. Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, Nadeau R (1999) Orthogonal polarization spectral imaging: a new method for study of the microcirculation. Nat Med 5(10):1209–1212
2. Mathura KR, Bouma GJ, Ince C (2001) Abnormal microcirculation in brain tumours during surgery. Lancet 358(9294):1698–1699
3. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL (2002) Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med 166(1):98–104
4. Spronk PE, Ince C, Gardien MJ, Mathura KR, Oudemans-van Straaten HM, Zandstra DF (2002) Nitroglycerin in septic shock after intravascular volume resuscitation. Lancet 360:1395–1396
5. Ince C (2005) The microcirculation is the motor of sepsis. Crit Care 9(Suppl 4):S13–S19
6. Top APC, Ince C, de Meij N, van Dijk M, Tibboel D (2011) Persistent low microcirculatory vessel density in nonsurvivors of sepsis in pediatric intensive care. Crit Care Med 39(1):8–13
7. Edul VS, Enrico C, Laviolle B, Vazquez AR, Ince C, Dubin A (2012) Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. Crit Care Med 40(5):1443–1448
8. De Backer D, Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S, Vincent JL (2013) Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. Crit Care Med 41(3):791–799
9. Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL (2004) Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. Crit Care Med 32(9):1825–1831
10. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL, Shapiro NI, Parrillo JE, Hollenberg SM (2008) Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. Intensive Care Med 34:2210–2217
11. De Uil CA, Bezemer R, Miranda DR, Ince C, Lagrand WK, Hartman JM, Bogers A, Spronk PE, Somoons ML (2009) Intra-operative assessment of human pulmonary alveoli in vivo using SDF imaging: a feasibility study. Med Sci Monit 15(10):137–140
12. Snoeijs MG, Vink H, Voesten N, Christiaans MH, Daemen JH, Peppelenbosch AG, Tordoir JH, Peutz-Kootstra CJ, Buurman WA, Schurink GWH, van Heurn WE (2010) Acute ischemic injury to the renal microvasculature in human kidney transplantation. Am J Physiol Renal Physiol 299:F1134–F1140
13. Nilsson J, Eriksson S, Blind PJ, Rissler P, Sturesson C (2014) Microcirculation changes during liver resection—a clinical study. Microvasc Res 94:47–55
14. Pérez-Bárcena J, Goedhart P, Ibáñez J, Brell M, García R, Llinás P, Jiménez C, Ince C (2011) Direct observation of human microcirculation during decompressive craniectomy after stroke. Crit Care Med 39(5):1126–1923
15. de Bruin AF, Tavy A, van der Sloot K, Smits A, Van Ramshorst B, Boerma CE, Kars P, Noordzij PG, Boerma D, van Iterson M (2016) Use of an image acquisition stabilizer improves sidestream dark field imaging of the serosa during open gastrointestinal surgery. J Vasc Res 53(3–4):121–127
16. Djaberi R, Schuijf JD, de Koning EJ, Wijewickrama DC, Pereira AM, Smit JW, Kroft LJ, Roos A, Bax JJ, Rabelink TJ, Jukema JW (2013) Non-invasive assessment of microcirculation by sidestream dark field imaging as a marker of coronary artery disease in diabetes. Diab Vasc Dis Res 10(2):123–134
17. Dondorp AM, Ince C, Tipmanee P, Hanson J, van Kuijen A, Faiz MA, Rahman MR, Hasan M, Bin Yunus E, Ghose A, Ruangveerayut R, Limmathurotsakul D, Mathura K, White NJ, Day NPJ (2008) Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. J Infect Dis 197(1):79–84
18. Khalilzada M, Dogan K, Ince C, Stam J (2011) Sublingual microvascular changes in patients with cerebral small vessel disease. Stroke 42(7):2071–2073
19. Dababneh L, Cikach F, Alkukhun L, Dweik RA, Tonelli AR (2014) Sublingual microcirculation in pulmonary arterial hypertension. Ann Am Thoracic Soc 11(4):504–512
20. Lindeboom JAH, Mathura KR, Aartman IH, Kroon F, Ince C (2007) The influence of the application of platelet enriched plasma's in oral mucosal wound healing. Clin Oral Impl Res 18:133–139
21. Meinders AJ, Elbers P (2009) Images in clinical medicine. Leukocytosis and sublingual microvascular blood flow. N Engl J Med 360(7):e9
22. Vollebregt KC, Boer K, Mathura KR, de Graaff JC, Ubbink DT, Ince C (2001) Impaired vascular function in women with pre-eclampsia observed with OPS imaging. Br J Obst Gyn 41:1148–1153
23. Meinders AJ, Nieuwenhuis L, Ince C, Bos WJ, Elbers PW (2015) Haemodialysis impairs the human microcirculation independent from macrohemodynamic parameters. Blood Purif 40(1):38–44
24. De Backer D, Creteur J, Dubois MJ, Sakr Y, Vincent JL (2004) Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. Am Heart J 147(1):91–99
25. Tachon G, Harrois A, Tanaka S, Kato H, Huet O, Pottecher J, Vicaut E, Duranteau J (2014) Microcirculatory alterations in traumatic hemorrhagic shock. Crit Care Med 42(6):1433–1441
26. Edul VSK, Ince C, Estenssoro E, Ferrara G, Arzani Y, Salvatori C, Dubin A (2015) The effects of arterial hypertension and age on the sublingual microcirculation of healthy volunteers and outpatients with cardiovascular risk factors. Microcirculation 22:485–492
27. Kuipers JW, Tiboel D, Ince C (2016) The pediatric microcirculation. Crit Care 20(1):352
28. Vellinga NA, Boerma EC, Koopmans M, Donati A, Dubin A, Shapiro NI, Pearse RM, Machado FR, Fries M, Akarsu-Ayazoglu T, Pranskunas A, Hollenberg S, Balestra G, van Iterson M, van der Voort PH, Sadaka F, Minto G, Aypar U, Hurtado FJ, Martinelli G, Payen D, van Haren F, Holley A, Pattnaik R, Gomez H, Mehta RL, Rodriguez AH, Ruiz C, Canales HS, Duranteau J, Spronk PE, Jhanji S, Hubble S, Chierego M, Jung C, Martin D, Sorbara C, Tijssen JG, Bakker J, Ince C, microSOAP Study Group (2015) International study on microcirculatory shock occurrence in acutely ill patients. Crit Care Med 43(1):48–56
29. Top AP, van Dijk M, van Velzen JE, Ince C, Tibboel D (2011) Functional capillary density decreases after the first week of life in term neonates. Neonatology 99(1):73–77
30. van den Berg VJ, van Elteren HA, Buijs EA, Ince C, Tibboel D, Reiss IK, de Jonge RC (2015) Reproducibility of microvascular vessel density analysis in sidestream dark-field-derived images of healthy term newborns. Microcirculation 22(1):37–43
31. Sheena MA, Hubble A, Hayley L, Kyte A, Gooding K, Shore AC (2009) Variability in sublingual microvessel density and flow measurements in healthy volunteers. Microcirculation 16:183–191
32. Bartels SA, Bezemer R, Milstein DM, Radder M, Lima A, Cherpanath TG, Heger M, Karemaker JM, Ince C (2011) The microcirculatory response to compensated hypovolemia in a lower body negative pressure model. Microvasc Res 82(3):374–380
33. Gu YM, Wang S, Zhang L, Liu YP, Thijs L, Petit T, Zhang Z, Wei FF, Kang YY, Huang QF, Sheng CS, Struijker-Boudier HA, Kuznetsova T, Verhamme P, Li Y, Staessen JA (2005) Characteristics and determinants of the sublingual microcirculation in populations of different ethnicity. Hypertension 65(5):993–1001

34. Gilbert-Kawai E, Coppel J, Court J, van der Kaaij J, Vercueil A, Feelisch M, Levett D, Mythen M, Grocott MP, Martin D, Xtreme Everest 2 Research Group (2017) Sublingual microcirculatory blood flow and vessel density in Sherpas at high altitude. J Appl Physiol 122(4):1011–1018
35. Gassmann NN, van Elteren HA, Goos TG, Morales CR, Rivera-Ch M, Martin DS, Cabala Peralta P, Passano Del Carpio A, Aranibar Machaca S, Huicho L, Reiss IK, Gassmann M, de Jonge RC (2016) Pregnancy at high altitude in the andes leads to increased total vessel density in healthy newborns. J Appl Physiol 121(3):709–715
36. Slaaf DW, Tangelder GJ, Reneman RS, Jäger K, Bollinger A (1987) A versatile incident illuminator for intravital microscopy. Int J Microcirc Clin Exp 6(4):391–397
37. Goedhart PT, Khalilzada M, Bezemer R, Merza J, Ince C (2007) Sidestream dark field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. Opt Express 15(23):15101–15114
38. Sherman H, Klausner S, Cook WA (1971) Incident dark-field illumination a new method for microcirculation study. Angiology 22:295–303
39. Aykut G, Veenstra G, Scorcella C, Ince C, Boerma C (2015) Cytocam-IDF (incident dark field illumination) imaging for bedside monitoring of the microcirculation. Intensive Care Med Exp 3:40
40. van Elteren H, van den Berg V, de Jonge R, Ince C, Reiss I (2014) Cutaneous microcirculation in preterm neonates: comparison between sidestream darkfield (SDF) and incident darkfield (IDF) imaging. Pediatr Crit Care Med 15(4):130–131
41. Gilbert-Kawai E, Coppel J, Bountziouka V, Ince C, Martin D, Caudwell Xtreme Everest 2 Research Group (2016) A comparison of the quality of image acquisition between the incident dark field and sidestream dark field video-microscopes. BMC Med Imaging 16:10
42. Hutchings S, Watts S, Kirkman E (2016) The cytocam video microscope. A new method for visualising the microcirculation using incident dark field technology. Clin Hemorheol Microcirc 62(3):261–271
43. Massey MJ, Shapiro NI (2016) A guide to human in vivo microcirculatory flow image analysis. Crit Care 10(20):35
44. Ince C (2015) Hemodynamic coherence and the rationale for monitoring the microcirculation. Crit Care 19(Suppl 3):S8
45. Pranskunas A, Koopmans M, Koetsier PM, Pilvinis V, Boerma EC (2013) Microcirculatory blood flow as a tool to select ICU patients eligible for fluid therapy. Intensive Care Med 39:612–619
46. Hanson JP, Sophia M, Lam W, Mohanty S, Alam S, Pattnaik R, Mahanta KC, Hasan MU, Charunwatthana P, Mishra SK, Day NPJ, White NJ, Dondorp AM (2013) Fluid resuscitation of adults with severe falciparum malaria: effects on acid-base status, renal function, and extravascular lung water. Crit Care Med 41(4):972–981
47. Jhanji S, Stirling S, Patel N, Hinds CJ, Pearse RM (2009) The effect of increasing doses of norepinephrine on tissue oxygenation and microvascular flow in patients with septic shock. Critl Care Med 37(6):1961–1966
48. Dubin A, Pozo O, Casabell C, Murias G, Mosinco M, Kanoore Edul V, Pálizas F, Estenssoro A, Ince C, Ince EC (2009) Increasing arterial blood pressure with norepinephrine does not improve microcirculatory blood flow: a prospective study. Crit Care 13:R9
49. Buijs EA, Reiss IK, Kraemer U, Andrinopoulou ER, Zwiers AJ, Ince C, Tibboel D (2014) Increasing mean arterial blood pressure and heart rate with catecholaminergic drugs does not improve the microcirculation in children with congenital diaphragmatic hernia: a prospective cohort study. Pediatr Crit Care Med 15(4):343–354
50. Vincent JL, Taccone FS (2016) Microvascular monitoring – Do 'global' markers help? Best Pract Res Clin Anaesth 30(4):399–405
51. Arnold RC, Dellinger RP, Parrillo JE, Chansky ME, Lotano VE, McCoy JV, Jones AE, Shapiro NI, Hollenberg SM, Trzeciak S (2012) Discordance between microcirculatory alterations and arterial pressure in patients with hemodynamic instability. J Crit Care 27(5):5311–5317
52. Ince C, Sinaasappel M (1999) Microcirculatory oxygenation and shunting in sepsis and shock. Crit Care Med 27:1369–1377
53. De Backer D, Hollenberg S, Boerma C, Goedhart P, Buchele G, Ospina-Tascon G, Dobbe I, Ince C (2007) How to evaluate the microcirculation: report of a round table conference. Crit Care 11:R101
54. Cecconi M, De Backer D, Antonelli M, Beale R, Bakker J, Hofer C, Jaeschke R, Mebazaa A, Pinsky MR, Teboul JL, Vincent JL, Rhodes A (2014) Consensus on circulatory shock and hemodynamic monitoring. Task force of the European Society of Intensive Care Medicine. Intensive Care Med 40:1795–1815
55. Teboul JL, Saugel B, Cecconi M, De Backer D, Hofer CK, Monnet X, Perel A, Pinsky MR, Reuter DA, Rhodes A, Squara P, Vincent JL, Scheeren TW (2016) Less invasive hemodynamic monitoring in critically ill patients. Intensive Care Med 42:1350–1956
56. Atasever B, Boer C, van der Kuil M, Lust E, Beishuizen A, Speekenbrink R, Seyffert J, de Mol B, Ince C (2011) Quantitative imaging of microcirculatory response during nitroglycerin-induced hypotension. J Cardiothorac Vasc Anesth. 25(1):140–144
57. Kildal AB, Stenberg TA, Sanden E, Myrmel T, How OJ (2015) Prolonged observation time reveals temporal fluctuations in the sublingual microcirculation in pigs given arginine vasopressin. J Appl Physiol 118(8):965–970
58. Massey MJ, Larochelle E, Najarro G, Karmacharla A, Arnold R, Trzeciak S, Angus DC, Shapiro NI (2013) The microcirculation image quality score: development and preliminary evaluation of a proposed approach to grading quality of image acquisition for bedside videomicroscopy. J Crit Care 28:913–917
59. Damiani E, Ince C, Scorcella C, Domizi R, Carsetti A, Mininno N, Pierantozzi S, Adrario E, Romano R, Pelaia P, Donati A (2017) Impact of microcirculatory video quality on the evaluation of sublingual microcirculation in critically ill patients. J Clin Monit Comput 31:981–988
60. Sallisalmi M, Oksala N, Pettilä V, Tenhunen J (2012) Evaluation of sublingual microcirculatory blood flow in the critically ill. Acta Anaesth Scand 56(3):298–306
61. Boerma EC, Mathura KR, van der Voort PHJ, Spronk PE, Ince C (2005) Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. Crit Care 9:R601–R606
62. Pozo MO, Kanoore Edul VS, Ince C, Dubin A (2012) Comparison of different methods for the calculation of the microvascular flow index. Crit Care Res Pract 2012:102483
63. Tanaka S, Harrois A, Nicolaï C, Flores M, Hamada S, Vicaut E, Duranteau J (2015) Qualitative real-time analysis by nurses of sublingual microcirculation in intensive care unit: the MICRONURSE study. Crit Care 19:388
64. Arnold RC, Parrillo JE, Phillip Dellinger R, Chansky ME, Shapiro NI, Lundy DJ, Trzeciak S, Hollenberg SM (2009) Point-of-care assessment of microvascular blood flow in critically ill patients. Intensive Care Med 35:1761–1766
65. Cerny V, Abdoa I, George RB, Maddisond L, Sharawie N, Lehmann C (2016) Analysis of microcirculation measurements by novice users trained by a standardized interactive tutorial: an inter-observer variability study. Clin Hemorheol Microcirc 62:123–128
66. Naumann DN, Mellis C, Husheer SL, Hopkins P, Bishop J, Midwinter MJ, Hutchings SD (2016) Real-time point of care microcirculatory assessment of shock: design, rationale and application of the point of care microcirculation (POEM) tool. Crit Care 20:310
67. Lima A, Lopez A, van Genderen ME, Hurtado FJ, Angulo M, Grignola C, Shono A, van Bommel J (2015) Interrater reliability and diagnostic performance of subjective evaluation of sublingual microcirculation images by physicians and nurses: a multicenter observational study. Shock 44(3):239–244
68. Dobbe JG, Streekstra GJ, Atasever B, van Zijderveld R, Ince C (2008) Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. Med Biol Eng Comput 46(7):659–670
69. Bezemer R, Dobbe J, Bartels SA, Boerma EC, Elbers PWG, Heger M, Ince C (2011) Rapid automatic assessment of microvascular density in sidestream dark field images. Med Biol Eng Comput 49(11):1269–1278
70. Demir SU, Hakimzadeh R, Hargraves RH, Ward KR, Myer EV, Najarian K (2012) An automated method for analysis of microcirculation videos for accurate assessment of tissue perfusion. BMC Med Imaging 12:37–50
71. Liu C, Gomez H, Narasimhan S, Dubrawski A, Pinsky MR, Zuckerbraun A (2015) Real-time visual analysis of microvascular blood flow for critical care. IEEE Conference on Computer Vision and Pattern Recognition (CVPR), pp 2217–2225. https://doi.org/10.1109/CVPR.2015.7298834
72. Carsetti A, Aya HD, Pierantozzi S, Bazurro S, Donati A, Rhodes A, Cecconi M (2017) Ability and efficiency of an automatic analysis software to measure microvascular parameters. J Clin Monit Comput 31(4):669–676
73. Sorelli M, Ince C, Bocchi L (2017) Particle tracking for the assessment of microcirculatory perfusion. Physiol Meas 38(2):358–373

74. Arnemann PH, Hessler M, Kampmeier T, Morelli A, Van Aken HK, Westphal M, Rehberg S, Ertmer C (2016) Comparison of an automatic analysis and a manual analysis of conjunctival microcirculation in a sheep model of haemorrhagic shock. Intensive Care Med Exp 4(1):37
75. Elbers PE, Ince C (2006) Mechanisms of critical illness: classifying microcirculatory flow abnormalities in distributive shock. Crit Care 10(4):221–299
76. Ospina-Tascon G, Neves AP, Occhipinti G, Donadello K, Büchele G, Simion D, Chierego ML, Silva TO, Fonseca A, Vincent JL, De Backer D (2010) Effects of fluids on microvascular perfusion in patients with severe sepsis. Intensive Care Med 36(6):949–955
77. Pottecher J, Deruddre S, Teboul JL, Georger JF, Laplace C, Benhamou D, Vicaut E, Duranteau J (2010) Both passive leg raising and intravascular volume expansion improve sublingual microcirculatory perfusion in severe sepsis and septic shock patients. Intensive Care Med 36(11):1867–1874
78. Dubin A, Pozo MO, Casabella CA, Murias G, Pálizas F Jr, Moseinco MC, Kanoore Edul VS, Pálizas F, Estenssoro E, Ince C (2010) Comparison of 6% hydroxyethyl starch 130/0.4 and saline solution for resuscitation of the microcirculation during the early goal-directed therapy of septic patients. J Crit Care 25(4):1–8
79. Tanaka S, Escudier E, Hamada S, Harrois A, Leblanc PE, Vicaut E, Duranteau J (2017) Effect of RBC transfusion on sublingual microcirculation in hemorrhagic shock patients: a pilot study. Crit Care Med 45(2):e154–e160
80. Sakr Y, Chierego M, Piagnerelli M, Verdant C, Dubois MJ, Koch M, Creteur J, Gullo A, Vincent JL, De Backer D (2007) Microvascular response to red blood cell transfusion in patients with severe sepsis. Crit Care Med 35(7):1639–1644
81. Yuruk K, Almac E, Bezemer R, Goedhart P, de Mol B, Ince C (2011) Blood transfusions recruit the microcirculation during cardiac surgery. Transfusion 51(5):961–967
82. Atasever B, van der Kuil M, Boer C, Vonk A, Schwarte L, Girbes AR, Ince C, Beishuizen A, Groeneveld AB (2012) Red blood cell transfusion compared with gelatin solution and no infusion after cardiac surgery: effect on microvascular perfusion, vascular density, hemoglobin, and oxygen saturation. Transfusion 52(11):2452–2458
83. De Backer D, Creteur J, Dubois MJ, Sakr Y, Koch M, Verdant C, Vincent JL (2006) The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. Crit Care Med 34(2):403–408
84. Hernandez G, Bruhn A, Luengo C, Regueira T, Kattan E, Fuentealba A, Florez J, Castro R, Aquevedo A, Pairumani R, McNab P, Ince C (2013) Effects of dobutamine on systemic, regional and microcirculatory perfusion parameters in septic shock: a randomized, placebo-controlled, double-blind, crossover study. Intensive Care Med 39(8):1435–1443
85. Koch M, De Backer D, Vincent JL, Barvais L, Hennart D, Schmartz D (2008) Effects of propofol on human microcirculation. Br J Anaesth 101(4):473–478
86. Özarslan NG, Ayhan B, Kanbak M, Çelebioğlu B, Demircin M, Ince C, Aypar Ü (2012) Comparison of the effects of sevoflurane, isoflurane, and desflurane on microcirculation in coronary artery bypass graft surgery. J Cardiothorac Vasc Anesth 26(5):791–798
87. Petroni T, Harrois A, Amour J, Lebreton G, Brechot N, Tanaka S, Luyt CE, Trouillet JL, Chastre J, Leprince P, Duranteau J, Combes A (2014) Intra-aortic balloon pump effects on macrocirculation and microcirculation in cardiogenic shock patients supported by venoarterial extracorporeal membrane oxygenation. Crit Care Med 42(9):2075–2082
88. Akin S, Dos Reis Miranda D, Caliskan K, Soliman OI, Guven G, Struijs A, van Thiel RJ, Jewbali LS, Lima A, Gommers D, Zijlstra F, Ince C (2017) Functional evaluation of sublingual microcirculation indicates successful weaning from VA-ECMO in cardiogenic shock. Crit Care 21(1):265
89. Krupičková P, Huptych M, Mormanová Z, Bouček T, Belza T, Šmíd O, Král A, Skalická H, Linhart A, Bělohlávek J (2017) Effect of pulsatility on microcirculation in patients treated with extracorporeal cardiopulmonary resuscitation: a pilot study. ASAIO J 63(4):386–391
90. Kara A, Akin S, Dos Reis Miranda D, Struijs A, Caliskan K, van Thiel RJ, Dubois EA, de Wilde W, Zijlstra F, Gommers D, Ince C (2016) Microcirculatory assessment of patients under VA-ECMO. Crit Care 20(1):344
91. Koning NJ, Simon LE, Asfar P, Baufreton C, Boer C (2014) Systemic microvascular shunting through hyperdynamic capillaries after acute physiological disturbances following cardiopulmonary bypass. Am J Physiol Heart Circ Physiol 307(7):H967–H975
92. Yuruk K, Bezemer R, Euser M, Milstein DM, de Geus HH, Scholten EW, de Mol BA, Ince C (2012) The effects of conventional extracorporeal circulation versus miniaturized extracorporeal circulation on microcirculation during cardiopulmonary bypass-assisted coronary artery bypass graft surgery. Interact Cardiovasc Thorac Surg 15(3):364–370
93. Atasever B, Boer C, Goedhart P, Biervliet J, Seyffert J, Speekenbrink R, Schwarte L, de Mol B, Ince C (2011) Distinct alterations in sublingual microcirculatory blood flow and hemoglobin oxygenation in on-pump and off-pump coronary artery bypass graft surgery. J Cardiothorac Vasc Anesth 25(5):784–790
94. Munsterman LDH, Elbers PWG, van Dongen EPA, van Iterson M, Ince C (2010) Withdrawing intra-aortic counter pulsation improves microvascular flow. Crit Care 14(4):R16
95. Jung C, Fuernau G, de Waha S, Eitel I, Desch S, Schuler G, Figulla HR, Thiele H (2015) Intraaortic balloon counter-pulsation and microcirculation in cardiogenic shock complicating myocardial infarction: an IABP-SHOCK II substudy. Clin Res Cardiol 104(8):679–687
96. den Uil CA, Lagrand WK, van der Ent M, Jewbali LS, Brugts JJ, Spronk PE, Simoons ML (2009) The effects of intra-aortic balloon pump support on macrocirculation and tissue microcirculation in patients with cardiogenic shock. Cardiology 114(1):42–46
97. Hilty MP, Pichler J, Ergin B, Hefti U, Merz TM, Ince C, Maggiorini M (2017) Assessment of endothelial cell function and physiological microcirculatory reserve by video microscopy using a topical acetylcholine and nitroglycerin challenge. Intensive Care Med Exp 5(1):26
98. Ellis CG, Ellsworth ML, Pittman RN, Burges WL (1992) Application of image analysis for evaluation of red blood cell dynamics in capillaries. Microvasc Res 44:214–225
99. DeGeorge BR, Olenczak JB, Cottler PS, Drake DB, Lin KY, Morgan RF, Campbell CA (2016) Evaluation of sidestream darkfield microscopy for real-time imaging acellular dermal matrix revascularization. Ann Plast Surg 76(Suppl 4):S255–S259
100. Weber MA, Diedrich CM, Ince C, Roovers JP (2016) Focal depth measurements of the vaginal wall: a new method to noninvasively quantify vaginal wall thickness in the diagnosis and treatment of vaginal atrophy. Menopause 23(8):833–838
101. Keenan S, Guyatt G, Sibbald W, Cook D, Heyland D, Jaeschke D (1999) How to use articles about diagnostic technology: gastric tonometry. Crit Care Med 27(9):1726–1731
102. Desjardins C, Duling BR (1987) Microvessel hematocrit: measurement and implications for capillary oxygen transport. Am J Physiol 252:H494–H503
103. Boerma EC, Scheeren TWL (2017) Digging into the microcirculation: the rush for gold may excavate apples and oranges. J Clin Monit Comput 31(4):665–667
104. Ellis CG, Bateman RM, Sharpe MD, Sibbald WJ, Gill R (2002) Effect of a maldistribution of microvascular blood flow on capillary O2 extraction in sepsis. Am J Physiol Heart Circ Physiol 282:H156–H164
105. Kurata T, Li Z, Oda S, Kawahira H, Haneishi H (2015) Impact of vessel diameter and bandwidth of illumination in sidestream dark-field oximetry. Biomed Opt Express 6:1616–1631
106. Marini JJ, Gattinoni L, Ince C, Kozek-Langenecker S, Mehta RL, Pichard C, Westphal M, Wischmeyer P, Vincent JL (2015) A few of our favorite unconfirmed ideas. Crit Care 19(Suppl 3):S1
107. Hernández G, Teboul JL (2016) Is the macrocirculation really dissociated from the microcirculation in septic shock? Intensive Care Med 42(10):1621–1624
108. Donati A, Tibboel D, Ince C (2013) Towards integrative physiological monitoring of the critically ill: from cardiovascular to microcirculatory and cellular function monitoring at the bedside. Crit Care 17(Suppl 1):S5
109. Brouwers MC, Kerkvliet K, Spithoff K (2016) The AGREE Reporting Checklist: a tool to improve reporting of clinical practice guidelines. Brit Med J 352:i1152
110. Vernooij RWM, Alonso-Coello P, Brouwers M, Martínez García L, CheckUp Panel (2017) Reporting items for updated clinical guidelines: checklist for the reporting of updated guidelines (CheckUp). PLoS Med 14(1):e1002207