Microcirculation in clinical practice

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Abstract
Introduction

Haemodynamic monitoring is a cornerstone of care in high-risk surgery and critically ill patients. Therapies aimed at optimising haemodynamic targets by means of resuscitation protocols have been demonstrated to improve patient outcomes. Nonetheless, in some specific clinical conditions (e.g. sepsis), due to the loss of vascular tone autoregulation, in spite of cardiac output and mean arterial pressure increase, cellular hypoxia and organ dysfunction may persist. These clinical states are characterised by macrocirculation-microcirculation uncoupling, in which high mortality rates still persist even after macrocirculatory optimisation. Over the last few years, new technologies specifically designed to aid in microcirculatory monitoring have been introduced into the market. This review deals with the most studied and used of these technologies, including videomicroscopic techniques, laser Doppler and near-infrared spectroscopy. The main advantages and limitations of each instrument will be considered.

Conclusion

We believe that the so-called ‘haemodynamic optimisation’ during and after major surgery is a good and efficacious way to improve patient outcomes. Nevertheless, in critically ill patients, microcirculatory status, usually neglected, should be evaluated and monitored to guide therapies based on a more pathophysiological approach.

Introduction

In 1971, Max Herry Weil first classified the mechanisms of shock or ‘acute circulatory failure’ (failure to provide sufficient oxygen and metabolites to cells) into four groups: hypovolemic, cardiogenic, obstructive and distributive. The classification proposed by Dr. Weil, universally adopted worldwide, has improved the diagnostic process and therapeutic approach to high-risk surgery and critically ill patients significantly. Nowadays, physicians have at their disposition a litany of instruments that, based on different technologies, deliver data on macrohaemodynamics. These systems may help caregivers to optimise cardiac output (CO) and oxygen delivery (DO₂), targeting the therapies at specific thresholds. This approach, known as goal-directed therapy (GDT), represents a key component of many protocols of resuscitation in surgery and intensive care units (ICUs). GDT has been shown to be very efficient in the majority of haemodynamically unstable patients. Nevertheless, when there is a loss of vascular tone autoregulation, in spite of CO and mean arterial pressure (MAP) increase, cellular hypoxia with lactic acid and carbon dioxide production remains the main issue. This clinical state, classically observed in septic patients, is then characterised by macrocirculation-microcirculation uncoupling. In these clinical conditions, even after macrocirculatory optimisation (cardiac filling pressure increase/normisation, CO improvement, MAP increase, central or mixed venous oxygen saturation targeted), high mortality rates still persist, because improvement of macrocirculatory haemodynamics does not automatically improve microcirculation. Many experimental studies have demonstrated that during induced sepsis with endotoxin, microvascular blood flow may be dramatically altered, resulting in a reduction of functional capillary density (FCD; see below). The decrease in FCD leads to an increase in the diffusion distance from vessels to cells, resulting in cellular hypoxia. Many studies performed in ICU patients, suffering from septic and cardiogenic shock, have demonstrated that microcirculatory alterations predict mortality. A correlation between microcirculatory failure and postoperative complications was also demonstrated in patients who had undergone major abdominal surgery. Clinical evaluation of microvascular perfusion impairment (mottled skin, increase in central-to-skin temperature gradient) is non-specific and insufficiently sensitive. Flow redistribution in conditions of shock is a pathophysiological response to inadequate DO₂ aimed at direct blood flow to vital organs and cannot be used to quantify microvascular impairment. Technology and research in the field of critical care have evolved in recent years, leading to the development of instruments aimed at studying the microcirculatory state under different clinical conditions. In this article, we review current methods and available technologies able to study microcirculation in experimental and clinical settings, focusing on advantages, limitations, applications and future perspectives.

Videomicroscopic techniques

The direct visualisation of the microcirculatory bed is considered the best way to study the architecture of the capillary bed. It allows a direct and accurate evaluation of the microcirculatory parameters such as capillary density (FCD), blood flow, and vascular calibre. Videomicroscopic techniques have been widely used in experimental studies to study the microcirculation in different pathophysiological conditions, such as sepsis, hypovolemia, and cardiogenic shock. Videomicroscopic imaging has been used to assess the efficacy of resuscitation protocols and to guide therapy. Despite the potential of videomicroscopic techniques, their clinical applications are limited due to the complexity of their use and the need for skilled operators.

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and transport function of arterioles, capillaries and venules in several conditions (such as certain diseases or in response to drugs or physiological changes), both in experimental and in clinical settings.

In humans, tissue preparations cannot be used, and videomicroscopic technique applications are limited to the nailfold and sublingual areas.

**Nailfold videocapillaroscopy**

It involves a conventional light microscope combined with a video camera and a recording system. It is used to evaluate capillary density, morphological abnormalities and, more recently, blood flow in the nailfold area. Due to the high sensitivity of the nailfold area to temperature and drug-induced and/or pathophysiological vasoconstriction, this technology is unsuitable for critical patients and is mainly limited to studies on chronic diseases such as diabetes, vasculitis and arteritis.

**Sublingual videocapillaroscopy**

When a polarised or incident light is directed at tissue covered by a thin epithelial layer, that light is reflected from the deeper tissue layers, providing illumination of the superficial ones. The 530 nm wavelength is absorbed by haemoglobin (oxygenated and deoxygenated) enclosed in the red blood cells; red blood cells can thus be visualised as black or grey points flowing along the vessels (Figure 1) after a green light illuminates the depth of the tissue (up to 3 mm) and the reflected-scattered light has been absorbed by the red blood cells’ haemoglobin. Since vascular walls cannot be seen, the vessel can be identified only if it contains red blood cells. Based on these physical aspects, orthogonal polarisation spectral (OPS) imaging, introduced by Groner et al. in 1999 (the so-called first generation of videocapillaroscopy), and Sidestream Dark Field (SDF) imaging, introduced by Goedhart et al. (second generation videocapillaroscopy), have been proposed for microcirculatory assessment at the bedside.

The sublingual area is the most commonly investigated area in human studies: relatively easily accessible, this area provides a good spot to visualise a complex network of capillaries and venules with variable diameters, courses and lengths.

Direct visualisation of sublingual vessels must be recorded and analysed in an offline semiquantitative analysis to extrapolate different variables such as the vascular density, the heterogeneity of perfusion and, to some extent, the microvascular blood flow. SDF imaging has replaced OPS in recent years and is now considered the gold standard for the study of sublingual microcirculation in clinical settings, and for research purposes. Although some studies have emphasised the good intra- and interobserver reliability of this method, several limitations must be taken into account. First, the image quality depends mainly on the investigator’s expertise in terms of focus, pressure, hand stability and ‘best spot’. Second, movement artefacts and, third, salivary secretions make the image quality very poor and unlikely to be used. Fourth, the sedation needed to avoid movement can alter microcirculatory properties. Finally, to process an off-line semiquantitative analysis, several videos (5 to 10) must be recorded in order to analyse the best catches and calculate a mean value; while this entire process still largely depends on the investigator, a prolonged crinkle on the sublingual mucosa can also alter microvascular properties in terms of hyperemia.

In order to give a common and interchangeable instrument for evaluation of perfusion with SDF, a scoring system has been proposed (Table 1). Recently, a third generation of sublingual videocapillaroscopy based on the incident dark field principle has been introduced by Braedius Scientific™, in which a manageable...
auto-focusing device with a high-resolution camera is integrated into software that permits real-time analysis of capillary density, perfusion and flow\textsuperscript{15}. Taking into account the above-mentioned limitations of SDF imaging, this advance in technology could provide reliable assessment of microcirculation at the bedside.

**Laser Doppler**

The laser Doppler monitoring system analyses changes in the spectrum of light reflected from living tissues as a response to a beam of monochromatic laser light. When a beam of light enters the tissues and hits moving blood cells, it undergoes changes in wavelength (Doppler shift). On the contrary, if the beam of light hits static tissue structures, it remains unchanged\textsuperscript{16}. Magnitude and frequency distribution of wavelength modifications correlate with the number of moving blood cells, while direction of cell movement does not influence the calculation. The Doppler technique indicates perfusion as ‘perfusion units’, which can be made: the upslope (rate of flow increase), and two different types of vasoreactivity test can be performed. First, after affecting artery occlusion by means of a cuff placed around the arm, the speed of flow measured after the cuff release depends on the capacity of microvasculature to dilate recruiting arterioles. The slope of the ascending part of the flow versus time curve is an indicator of endothelial reactivity\textsuperscript{18}. Second, directly warming the skin causes a vasodilatation that depends on the level and speed of local warming and on the capacity of the microcirculation to change in response to the stimulus\textsuperscript{20}. As with the previously mentioned application to gastrointestinal mucosa, skin evaluation is still limited to experimental settings and studies on diabetic neuropathy\textsuperscript{22}. The limitation of this method for the monitoring of microcirculatory reactivity, either by artery occlusion test or by warming the observed skin, lies in the great variability of dermal vascular tone in response to temperature changes, adrenergic activity or haemodynamic impairment\textsuperscript{21}.

**Near-Infrared spectroscopy**

Near-infrared spectroscopy (NIRS) is probably the most evolved and promising technology available for microcirculatory evaluation. It uses near-infrared light to obtain tissue saturation ($StO_2$), measuring oxyhaemoglobin ($HbO_2$), deoxyhaemoglobin (HHb), total haemoglobin (HbT) and other chromophores (myoglobin, cytochrome aa3) in the tissue\textsuperscript{23}. A brief vascular occlusion test (VAT) can be done in order to obtain a quantitative analysis of a dynamic vascular response to a transient ischemia. The VAT consists of a brief (2–5 min) artery occlusion (pressure cuff placed around the arm cuffed over systolic blood pressure) and rapid release\textsuperscript{24–26}. In order to eliminate some compromising factors such as excessive adipose tissue and oedema, which would limit the accuracy of the measurement, the thenar eminence is usually selected for NIRS measurements\textsuperscript{22}.

During ischemia, the metabolic activity of the muscle leads to a linear decline in $StO_2$ (downslope) that correlates to metabolic activity and oxygen consumption\textsuperscript{22}. After the cuff release, two other measurements can be made: the upslope (rate of increase in $StO_2$) and the hyperemic phase (the area under the curve above the $StO_2$ baseline (Figure 2)). Both upslope and the hyperemic phase have been demonstrated to be indicators of microvascular reactivity\textsuperscript{27}. $StO_2$ upslope represents the removal of HHb from the ischemic skeletal muscle and the influx of $HbO_2$ and is not a measure of blood flow per se; any change in $StO_2$ can be directly proportional to change in flow and inversely to changes in metabolism. Opposite and proportional changes in flow and metabolism may result in unchanged $StO_2$ values. Basic technology, applied by different

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**Table 1** Scoring system for Sidestream Dark Field (SDF) videocapillaroscopy.

<table>
<thead>
<tr>
<th>Microcirculatory perfusion</th>
<th>MFI</th>
<th>No flow</th>
<th>Intermittent flow</th>
<th>Sluggish flow</th>
<th>Continuous flow</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microcirculatory density</td>
<td>TVD</td>
<td>Perfused and non-perfused vessels</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVD</td>
<td>Perfused vessels only</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>PVD/TVD</td>
<td>Proportion of perfused vessels (PPV)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>FCD</td>
<td>Only &lt;20μm vessels are included in the analysis</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MFI, microcirculatory flow index; TVD, total vessel density; PVD, perfused vessel density; FCD, functional capillary density.
tools, may vary in terms of light wavelength, number of wavelengths, optode spacing and proprietary algorithms.

With the development of more complex and precise instruments, different skeletal muscles can be explored with different vascular manoeuvres. For instance, with the NIMO system (Nirox, Italy), much additional information can be obtained by means of a series of venous occlusion tests and an artery occlusion test. De Blasi et al. derived a series of calculated parameters to evaluate microvascular functions by means of a dedicated microvascular regulation analysis (Table 2). They did this by placing the probe on the forearm and using a rapid pneumatic cuff inflator applied around the arm where the NIRS probe was applied and performing progressive venous compression until occlusion (30-40-50 mmHg) and successive ischemia (cuff pressure equivalent to systolic arterial pressure + 50 mmHg) for 5 min. With this approach, De Blasi obtained dynamic measurements of perfusion, blood flow in ml/min per 100 mg of tissue, tissue metabolism-tissue oxygen consumption in ml/min per 100 mg and indications of vascular compliance.

One can argue that the microcirculatory profile in the muscular tissue could be generalised to the entire organism. First, around 40% of the human body is composed of muscular mass; this fact is relevant when one takes into account the metabolic consequences of the hypoperfusion of this district in terms of lactic acidosis and release of inflammatory and apoptotic mediators. Second, skeletal muscular tissue is highly sensitive to hypoperfusion, and its microcirculatory alterations could actually be considered an early signal of macrocirculatory-microcirculatory uncoupling.

The main limitation of NIRS method, as is the case with other methods, is the need for an off-line analysis of the dynamic responses in both arterial and venous occlusion tests, which is necessary to calculate and obtain values for microvascular perfusion, flow, oxygen consumption and compliance.

The real-time availability of these parameters at the bedside could be of great use in guiding therapies based on the rationale of microcirculatory rather than macrocirculatory optimisation.

### Table 2: Derived series of calculated parameters to evaluate microvascular functions

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>TBV</td>
<td>MI blood/100 ml of tissue. Determined from [HbT] in relation to the endovascular Hb content</td>
</tr>
<tr>
<td>Microvascular compliance</td>
<td>MI/mmHg/l tissue. Calculated during inflation pressure (mmHg).</td>
</tr>
<tr>
<td>CBF and CHF</td>
<td>Calculated by evaluating the linear increase in [HbT] (µmol/s) within the first seconds of venous occlusion CBF = ml blood/100 ml tissue/min</td>
</tr>
<tr>
<td>CVR</td>
<td>Expressed in mm Hg ml/1/min/1 per 100 ml tissue—was calculated as mean arterial pressure/CBF</td>
</tr>
<tr>
<td>VO₂</td>
<td>Measured during venous occlusion as the initial linear increase in [Hb], after subtracting the [HHb] increase from arterial blood (3%)</td>
</tr>
<tr>
<td>StO₂</td>
<td>Obtained from the ratio of [HbO₂] to [HbT]</td>
</tr>
<tr>
<td>RR</td>
<td>After the arterial occlusion was released, the haemoglobin RR was calculated from the haemoglobin resaturation slope during the first 10 s of postischaemic reperfusion</td>
</tr>
</tbody>
</table>

TBV, tissue blood volume; CBF, calf blood flow; CHF, calf Hb flow; CVR, calf vascular resistance; VO₂, muscle oxygen consumption; StO₂, tissue saturation; RR, Hb resaturation rate
Discussion
The study of microcirculation in humans is of great relevance in both experimental and clinical settings to a deeper understanding of the pathophysiology of oxygen delivery to cells in several conditions, such as microvascular diseases, haemodynamic derangements, drug administration and physiological changes. In the critical care area, a growing body of evidence emphasises that depressed microcirculatory function is associated with morbidity and mortality in a wide array of clinical scenarios, especially in septic patients. Microcirculation is the key link between the optimisation of cardiopulmonary functions and cellular life: its inefficiency in terms of oxygen transport and diffusion to mitochondria is the main cause of organ dysfunction, which is the leading cause of death in critically ill patients, keeping in mind that many other mechanisms as well as the architectural and perfusion derangements (interstitial oedema, endothelial dysfunction, alterations in red blood cell aggregability and leukocyte adhesion on endothelial cells) are also involved. The macrocirculatory-microcirculatory uncoupling observed in septic patients seems to be one of the major causes of our inability to decrease mortality rates, casting doubt on the current therapeutic approach aimed at macrocirculatory optimisation. New technologies are now available to facilitate the study of microcirculation in humans. Sublingual videomicroscopy and NIRS seem promising methods for this purpose. The former can give a qualitative and semiquantitative analysis of a directly visualised capillary network; the latter is effective in giving a quantitative analysis of microcirculatory characteristics such as perfusion, flow, oxygen consumption and vascular tone. Recently, Pranskun et al. succeeded in guiding fluid therapy in ICU patients, basing their decisions regarding fluid infusion on microcirculatory alterations by means of microcirculatory monitoring. This is an encouraging result indeed that militates for integration of macrocirculatory and microcirculatory monitoring in the critically ill.

Conclusion
We believe that the so-called ‘haemodynamic optimisation’ during and after major surgery is a good and efficacious way to improve patient outcomes. Nevertheless, in critically ill patients, microcirculatory status, usually neglected, should be evaluated and monitored to guide therapies based on a more pathophysiological approach. An innovation in and upgrading of the available technologies is required in order to allow a real-time, continuous and reliable assessment of microcirculation at the bedside.

Abbreviation list
GDT, goal-directed therapy; ICUs, intensive care units; MAP, mean arterial pressure; FCD, functional capillary density; SDF, Sidestream Dark Field; VAT, vascular occlusion test; NIRS, Near-infrared spectroscopy.

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References