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Both passive leg raising and intravascular volume expansion improve sublingual microcirculatory perfusion in severe sepsis and septic shock patients

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Abstract Purpose: To assess sublingual microcirculatory changes following passive leg raising (PLR) and volume expansion (VE) in septic patients. **Methods:** This prospective study was conducted in two university hospital intensive care units and included 25 mechanically ventilated patients with severe sepsis or septic shock who were eligible for VE in the first 24 h of their admission. Pulse pressure variation (ΔPP), cardiac output (CO) and sublingual microcirculation indices were assessed at five consecutive steps: (1) semi-recumbent position (Baseline 1), (2) during PLR manoeuvre (PLR), (3) after returning to semi-recumbent position (Baseline 2), (4) at the time when VE induced the same degree of preload responsiveness as PLR ($VE_{\Delta PP = PLR}$) and (5) at the end of

VE (VE_{END}). At each step, five sublingual microcirculation sequences were acquired using sidestream darkfield imaging to assess functional capillary density (FCD), microcirculatory flow index (MFI), proportion of perfused vessels (PPV) and flow heterogeneity index (FHI).

Results: The PLR, $VE_{\Delta PP = PLR}$ and VE_{END} induced a significant increase in CO and a significant decrease in ΔPP compared to Baseline 1 and Baseline 2 values. Both PLR and VE induced significant increases in FCD, MFI and PPV and a significant decrease in FHI compared to Baseline 1 and Baseline 2 values. **Conclusions:** In preload responsive severe septic patients examined within the first 24 h of their admission, both PLR and VE improved sublingual microcirculatory perfusion. At the level of volume infusion used in this study, these changes in sublingual microcirculation were not explained by changes in rheologic factors or changes in arterial pressure.

Keywords Microcirculation · Sepsis · Shock · Volume expansion · Fluid responsiveness · Passive leg raising

Introduction

Altered microcirculatory blood flow is a major pathophysiological feature of severe sepsis and septic shock [1]. De Backer et al. [2] showed that microvascular density and microvascular blood flow are both reduced in septic patients compared to healthy volunteers or non-septic intensive care unit (ICU) patients. Moreover, the degree of microvascular impairment has a prognostic value since it worsens in non-surviving septic patients compared to those who ultimately overcome their septic episode [3]. Early systemic haemodynamic resuscitation of septic patients may improve the time-course of microcirculatory dysfunction and eventually the patient's outcome [4, 5]. However, relationships between systemic haemodynamic and microcirculatory changes during resuscitation are complex. In this regard, dobutamine infusion did not induce parallel changes in systemic and sublingual blood flows in septic shock patients [6].

Fluid resuscitation is one of the major therapies aimed at restoring blood pressure and cardiac output (CO) in severe septic patients in the early period as well as in the later phase [7, 8].

Fluid loading may improve microcirculatory blood flow through either systemic effects (such as increased perfusion pressure and/or increased CO), rheologic changes [9] (decreased microvascular blood viscosity) or local vasodilation (shear stress).

The aim of the study reported here was to assess sublingual microcirculatory changes in response to volume expansion (VE) in severe sepsis and septic shock patients eligible for VE within the first 24 h of their admission in the ICU. In order to distinguish between the haemodynamic and rheologic effects of VE on sublingual microvessel perfusion, we also performed passive leg raising (PLR), which is a manoeuvre that mimics VE in terms of preload-related haemodynamic consequences [10, 11] but which is assumed not to exert any rheologic effect. This work has previously been presented in abstract form [12].

Materials and methods

Patients

This observational study was approved by our local Institutional Review Board (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale de Bicêtre), which waived the need for written informed consent. It was conducted in accordance with the ethical standards laid down in the Declaration of Helsinki. Patients were recruited in the surgical and medical ICUs of Bicêtre University Hospital between July 2007 and January 2008. Inclusion criteria were (1) state of severe

sepsis or septic shock, as defined by the 2001 SCCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference [13], within the first 24 h of admission to the ICU, (2) preload-dependency, defined by respiratory variations in arterial pulse pressure (Δ PP) greater than 13% [14] and (3) eligibility for VE according to our local guidelines: mean arterial pressure (MAP) <65 mmHg (or a decrease >30 mmHg in previously hypertensive patients), urine output <0.5 mL/kg/h for 2 h and presence of skin mottling. Exclusion criteria were pregnancy, age <18 years, contraindication either to PLR (unstable spine fracture, orthopaedic transtibial leg traction, increased intracranial pressure) or to VE (suspected or confirmed hydrostatic pulmonary oedema), non-sinus rhythm and spontaneous breathing or ventilator triggering. Patients were sedated (midazolam/sufentanil) and mechanically ventilated in volume-controlled mode with a tidal volume ≥ 7 mL/kg and a 1:2 inspiratory to expiratory ratio.

We recorded the Simplified Acute Physiology Score (SAPS II) [15], the Acute Physiology and Chronic Health Evaluation (APACHE II) score [16] on admission and the Sepsis-related Organ Failure Assessment (SOFA) score [17] at inclusion.

Haemodynamic measurements

The Δ PP was calculated as previously described [14]. As part of routine CO monitoring, patients had either a continuous pulse contour analysis device or an oesophageal Doppler monitor. Details concerning haemodynamic data acquisition are provided in the Electronic Supplementary Material (ESM). At each step of the study, a full set of haemodynamic data was obtained, including heart rate (HR), systolic (SAP), mean (MAP) and diastolic (DAP) arterial pressures, CO, stroke volume (SV) and Δ PP.

Microcirculatory measurements and analysis

Sublingual microcirculation videos were obtained using a side-stream dark field imaging device (SDF; Microscan, MicroVisionMedical, Amsterdam, the Netherlands) derived from the orthogonal polarized spectral imaging technology [18]. Images acquisition and analysis were performed following international recommendations [19] with dedicated software analysis [Automated Vascular Analysis (AVA) ver. 1.0; Academic Medical Center, University of Amsterdam, Amsterdam, the Netherlands] as described in the ESM. All sequences were acquired by the same investigator (JP) and then randomly allocated to an alphanumeric code so that neither the patient's name nor the study step could be identified by a second investigator (SD) that performed the analysis.

Raw quantitative variable assessed with AVA software was functional capillary density (FCD, μm^{-1} or cm cm^{-2}). As small vessels usually account for more than 90% of sublingual microvasculature and are the most altered in sepsis [2], subsequent analyses were restricted to the small vessel category. Semi-quantitative analysis with AVA provided the microcirculatory flow index (MFI), the proportion of perfused vessels (PPV, %) and the flow heterogeneity index.

Study design

Haemodynamic and microcirculatory indices were assessed at five consecutive steps: (1) semi-recumbent position (Baseline 1), (2) during PLR manoeuvre (PLR), (3) after returning to semi-recumbent position (Baseline 2), (4) at the time when VE induced the same degree of preload responsiveness as PLR ($VE_{\Delta PP} = PLR$) and (5) at the end of VE (VE_{END}) (see ESM and ESM Fig. 1 for details). Volume expansion was performed over 30 min using a maximal volume of either 500 mL normal saline or 500 mL hydroxyethyl starch solution 6% (HES 130/0.4; Voluven, Fresenius Kabi, Sèvres, France) according to the attending physician's decision. Haemoglobin concentration was measured at the beginning of the study and immediately after VE_{END} . The ventilator settings, sedative and vasoactive drugs infusion rates were kept constant throughout the study. Patients were followed up for 28-day in-hospital mortality and duration of hospital stay.

Statistical analysis

The distribution of all datasets was checked for normality using the Shapiro–Wilk test and normal chi-square goodness of fit. In the case of non-Gaussian distribution, data were expressed as the median (25th–75th percentiles) and analysed with non-parametric tests: Mann–Whitney test, Spearman correlation ρ , Wilcoxon matched pairs test and Friedman test followed by the Wilcoxon test with Bonferroni correction. When data followed a normal distribution, the results were expressed as mean \pm standard deviation (SD) and analysed using paired Student t test and repeated-measures analysis of variance (ANOVA). We checked that there was no association between the type of solution and the changes in the microcirculatory indices. A P value <0.05 was considered to be statistically significant. Data were analysed using StatEl (adScience, Paris, France; <http://www.adscience.eu>) and Prism4 (GraphPad, San Diego, CA) software. A sample size of $n = 25$ patients was chosen on the basis of feasibility and because for any variable of interest, this sample size allowed a 80% power to detect an effect size (i.e. mean change/standard deviation of change) around

0.65, that was considered as physiologically meaningful, with a alpha risk at 5% adjusted from multiplicity.

Results

Patient characteristics

Twenty-five septic patients (20 with septic shock and 5 with severe sepsis) were included in this study over a 6-month period. Table 1 shows the patient characteristics.

Systemic haemodynamic effects of PLR and VE. No adverse event occurred during the study period. The CO was assessed using a continuous pulse contour analysis device and an oesophageal Doppler monitor in 13 and 12 patients, respectively. The VE was performed with normal saline in eight patients and 6% hydroxyethyl starch solution in 17 patients. The time course of HR, MAP, CO, SV and ΔPP throughout the five sequential study steps is presented in Table 2. The HR remained unchanged throughout the protocol. Compared to Baseline 1, PLR simultaneously induced a significant increase in CO and SV and a significant decrease in ΔPP . No significant

Table 1 Baseline characteristics of study subjects ($n = 25$)

Age (years)	57 \pm 17
Sex (M/F)	16/9
Weight (kg)	79 \pm 19
SAPS II score (admission)	45 (37–56)
APACHE II score (admission)	22 (19–27)
SOFA score (inclusion)	12 (9–13)
Time from ICU admission to inclusion (days)	1 (0–1)
Time from sepsis onset to inclusion (days)	1 (1–2)
ICU length of stay (days)	20 (9–24)
In-hospital outcome (S/D)	17/8
Reason for inclusion	
Septic shock	20
Severe sepsis	5
Primary site of infection	
Abdomen	12
Lung	5
Soft tissue	5
Urinary tract	3
Catecholamine, n ; dose ($\mu\text{g kg}^{-1} \text{min}^{-1}$)	
None	5
Norepinephrine	17; 0.40 (0.24–0.78)
Epinephrine	3; 0.39 (0.20–0.42)
Body temperature ($^{\circ}\text{C}$)	37.5 (36.7–38)
Ramsay score	5 (5–6)
Tidal volume (mL kg^{-1})	8 (7.3–8.4)
Plateau airway pressure (cmH_2O)	20 (18–25)
PEEP (cmH_2O)	5 (4–6)

Values are given as the mean \pm standard deviation (SD) or median (25th–75th percentiles) according to data distribution

M Male, *F* female, *SAPS* Simplified Acute Physiology score, *APACHE* Acute Physiology and Chronic Health Evaluation score, *SOFA* Sepsis-related Organ Failure Assessment score, *ICU* intensive care unit, *S* survivor, *D* dead, *PEEP* positive end expiratory pressure

Table 2 Time-course of systemic haemodynamic indices throughout the protocol ($n = 25$)

Parameters	Baseline 1	PLR	Baseline 2	VE _{ΔPP = PLR}	VE _{END}	P ANOVA
HR (min ⁻¹)	100 ± 17	98 ± 18	98 ± 18	97 ± 18	96 ± 19	NS
MAP (mmHg)	72 ± 15	73 ± 16	73 ± 14	80 ± 15*§	81 ± 17*§	<0.001
CO (L min ⁻¹)	5.1 ± 1.5	6.0 ± 1.7*	5.1 ± 1.5	5.9 ± 1.5*	6.5 ± 1.6*§‡	<0.001
SV (mL)	52 ± 16	62 ± 20*	54 ± 19	62 ± 18*	69 ± 20*§‡	<0.001
ΔPP (%)	22 ± 5	15 ± 5*	21 ± 5	15 ± 5*	12 ± 7*§‡	<0.001

Values are given as the mean ± SD. Only those parameters who were significant are given

PLR passive leg raising, VE volume expansion, NS non-significant, HR heart rate, MAP mean arterial pressure, CO cardiac output, SV stroke volume, ΔPP respiratory variation in arterial pulse pressure, ANOVA analysis of variance

* $P < 0.001$ versus Baseline 1 and Baseline 2

§ $P < 0.001$ versus PLR

‡ $P < 0.001$ versus VE_{ΔPP = PLR}

difference in any of the haemodynamic indices was found between Baseline 1 and Baseline 2. At the first step of VE (VE_{ΔPP = PLR}), MAP, CO and SV were significantly increased compared to both Baseline 1 and Baseline 2 values, and these increases (except for MAP) were not significantly different compared to those at the PLR step. At the end of VE (VE_{END}), CO and SV increased yet further, and ΔPP continued to decrease. Neither baseline haemodynamics nor VE-induced changes in haemodynamic indices were statistically different between the two subgroups of patients who received either normal saline or hydroxyethyl starch solution, respectively.

Microcirculatory effects of PLR and VE. We performed 125 SDF studies and therefore recorded 625 video sequences in the 25 septic patients. At Baseline 1, the distribution of microvessels in relation to their diameter was as follows: small vessels, 96.3%; medium vessels, 3.6%; large vessels, 0.03% (data not shown). This distribution was unaltered during the experimental protocol. The time-courses of FCD, MFI, PPV and the flow heterogeneity index during the study period are depicted in Fig. 1a–d, respectively. No significant difference was seen between Baseline 1 and Baseline 2 in terms of the following microvascular indices: FCD, MFI, PPV, and the flow heterogeneity index. The PLR, VE_{ΔPP = PLR} and VE_{END} significantly increased FCD, MFI and PPV compared to the values at Baseline 1 and Baseline 2. The heterogeneity index was significantly reduced by both PLR and VE. Baseline 1 and Baseline 2 values of FCD, MFI, PPV and the heterogeneity index were not significantly different between the patients who received normal saline and those who received hydroxyethyl starch solution. VE-induced changes in microcirculatory indices were also not significantly different between patients receiving normal saline and those receiving the hydroxyethyl starch solution. Haemoglobin values at the beginning of the protocol (Baseline 1: 9.8 ± 1.6 g/dL) and immediately after VE (VE_{END}: 9.5 ± 1.5 g/dL) were not significantly different for the whole population. However, hydroxyethyl starch-induced VE was associated with a statistically significant decrease in haemoglobin from

Baseline 1 (10.3 ± 1.3 g/dL) to VE_{END} (9.8 ± 1.4 , $P = 0.007$) that was not evidenced in saline-infused patients ($P = 0.49$). At either baseline or after VE (data not shown), the microcirculatory scores of septic shock patients ($n = 20$) who received norepinephrine or epinephrine were not significantly different from those of severe sepsis patients ($n = 5$) without vasoactive drugs. Examples of sublingual microcirculation videos in the same patient at the five steps of the protocol can be seen in the ESM (Animations 1, 2, 3, 4 and 5).

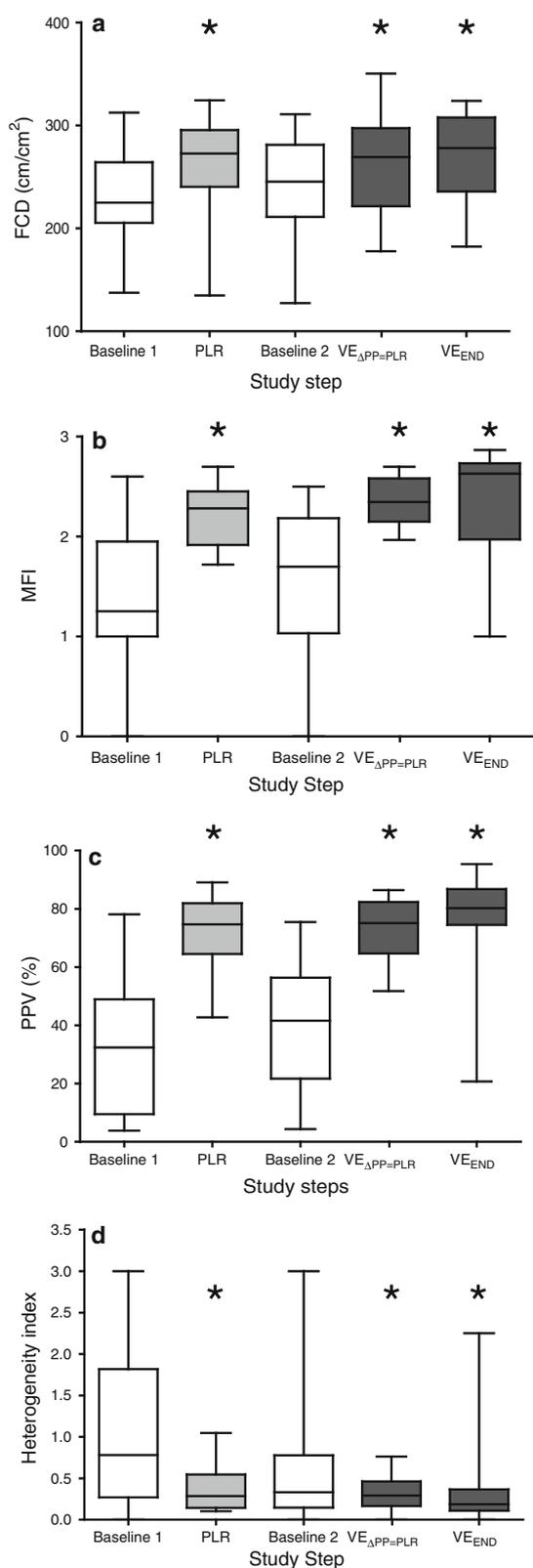
Relationship between microcirculatory perfusion and systemic haemodynamics

There was no statistically significant relationship between PLR-induced changes in macrocirculatory and microcirculatory indices.

At VE_{END}, VE-induced changes in MFI positively and significantly correlated with VE-induced changes in CO ($\rho = 0.53$, $P < 0.006$; ESM Fig. 2a) and MAP ($\rho = 0.47$, $P < 0.018$; ESM Fig. 3a). Changes in PPV induced by VE also correlated with VE-induced changes in CO ($\rho = 0.51$, $P < 0.005$; ESM Fig. 2b) but not with VE-induced changes in MAP ($\rho = 0.29$, $P = \text{NS}$; ESM Fig. 3b). No other significant correlation was found between VE-induced changes in microcirculatory indices and macrocirculatory indices.

Discussion

Our study shows that both PLR and VE induced a significant sublingual microcirculatory improvement in preload-dependent patients with severe sepsis and septic shock. Indeed, both PLR and VE simultaneously increased vessel density (increased FCD) and vessel perfusion (increased MFI and PPV) and reduced microvascular heterogeneity. Among the microcirculatory variables



◀ **Fig. 1** Time-course of sublingual functional capillary density (FCD, cm cm^{-2}) (a), microcirculatory flow index (MFI) (b), proportion of perfused vessels (PPV, %) (c) and flow heterogeneity index (d) throughout the protocol. *Baseline 1* patient lying in the 45° semi-recumbent position. *PLR* Passive leg raising: simultaneous elevation of the lower limbs and lowering of the patient's trunk to the supine position using automatic bed elevation technique. The hip angle remains constant. PLR induces a reduction in ΔAPP (respiratory variations in arterial pulse pressure). *Baseline 2* same as Baseline 1. $\text{VE}_{\Delta\text{PP}=\text{PLR}}$, the study step obtained when the volume expansion-induced decrease in ΔAPP reaches the PLR-induced ΔAPP value. VE_{END} End of volume expansion. The median is shown by the horizontal line within the box. The values between the lower and upper quartiles (25th–75th centiles) are within the box. Whiskers Minimum and maximum values. * $P < 0.05$ vs. Baseline 1 and Baseline 2; Friedman test followed by the Wilcoxon test with Bonferroni correction

investigated, MFI is known to be rather sensitive to flow variations, while FCD and PPV are more directed towards recruitment of the microcirculation. This opposite relationship between changes in MFI or PPV and changes in heterogeneity is in accordance with the results of an experimental study [20] in which animals were bled, thus producing effects in the opposite direction in comparison with those of our study. To the best of our knowledge, these results have not yet been reported in the clinical setting. It has been shown that the application of early goal-directed therapy in septic patients [7] may induce early improvement in sublingual microvascular flow [4] in association with reduced multi-organ failure [5]. Unlike the aforementioned studies, in which microvascular improvement was the result of a global therapeutic approach (including fluid loading, vasopressors, inotropes and blood products), in targeting systemic haemodynamic endpoints, we obtained haemodynamic assessment and SDF images at predefined steps during calibrated manoeuvres of increased preload. The results observed at the different steps of our protocol may enable clinicians to obtain a better understanding of the links between microcirculation and macrocirculation. Indeed, among our patient cohort, microvascular perfusion improvement with VE was not associated with changes in rheologic factors or changes in MAP.

Potential mechanisms of sublingual microcirculatory improvement

The PLR is assumed not to exert any rheologic effect since blood content remains unaltered. A recent study in patients with shock showed that the haemodynamic effects of PLR are only related to increased cardiac preload [21]. Interestingly, in our study, a similar improvement in microcirculatory perfusion was observed after PLR or VE. It is thus unlikely that the changes in

microcirculatory perfusion induced by the VE were due to changes in rheologic factors. Based on the results of an experimental study involving severe sepsis patients, Castro et al. [22] reported increases in blood viscosity, decreases in erythrocyte deformability and increases in erythrocyte aggregation in patients receiving hydroxyethyl starch in comparison with those receiving saline. By contrast, we did not observe significant differences in microcirculatory perfusion between patients infused with normal saline and hydroxyethyl starch solution. However, it should be stressed that these conclusions should be limited to the range of changes in rheologic factors associated with the amount of fluid we administered (i.e. 500 mL). In our study, VE-induced changes in MFI were positively correlated with VE-induced changes in MAP ($\rho = 0.47$) and CO ($\rho = 0.53$), and VE-induced changes in PPV correlated with VE-induced changes in CO ($\rho = 0.51$). These results are in agreement with those reported by Trzeciak et al. [4] showing correlations between macrocirculatory and microcirculatory variables in patients studied within 6 h of early goal-directed therapy. However, in our study, MAP did not appear to have a major effect on microcirculation. Indeed, microcirculatory changes were similar during PLR, which was associated with unchanged MAP, and after VE, which was associated with increased MAP up to 7 mmHg on average. Lafanechere et al. [23] also reported such different changes in MAP during PLR and VE in preload-responsive patients. Whether the unchanged MAP during PLR is related to changes in vasomotor tone cannot be excluded, although the hypothesis of altered adrenergic tone during PLR has been refuted by previous investigators [21]. Changes in microcirculatory perfusion were associated with increases in CO induced either by PLR or VE. However, this relationship was not linear since the microcirculatory perfusion remained stable despite the additional increase in CO induced by the second step of the VE (VE_{END}), suggesting that a threshold was reached. In addition, it should be stressed that the magnitude of the changes in microvascular variables (+94% for MFI, +205% for PPV) was disproportionate compared to that of the changes in CO (+27%). These two points suggest that different mechanisms are implicated in the regulation of microvascular perfusion and in the changes in CO, respectively. Elucidation of the nature of the relation between changes in CO induced by PLR or VE and changes in microcirculatory variables was beyond the scope of our investigation. We may postulate that the increase in CO with PLR and VE further increased microcirculatory perfusion through shear stress-related vasodilation. Neuro-mediated mechanisms interfering with microvascular flow regulation can also be involved. We cannot exclude that changes in sublingual perfusion during PLR were related, at least in part, to putative changes in vasomotor tone. However, and as reported by others [21, 24], the unchanged HR throughout our study

makes unlikely the possibility of altered sympathetic tone that could have changed the distribution of blood flow within the macro- or microcirculation.

These data support the hypothesis that VE can improve microcirculatory perfusion during the early period of resuscitation in severe sepsis and septic shock patients. At this stage, this microcirculatory improvement is accompanied by systemic haemodynamic changes, although no causal relationship has yet been established between the regulation of microvascular perfusion and changes in CO, suggesting that different mechanisms are involved.

Limitations of the study

Microvascular analysis was conducted in a blinded fashion and performed with the greatest care in order to avoid pressure artefacts; the most recent published recommendations for such studies were followed [19]. However, the following aspects must be acknowledged: (1) the side-stream dark field imaging device only provides a two-dimensional estimate of a three-dimensional network; (2) the suction device used induced negative pressure, which may have changed the microcirculatory blood flow by interfering with driving pressure. A question which remains to be answered is whether the sublingual mucosa is representative of other areas. A recent experimental study performed by Verdant et al. [25] in pigs with cholangitis support the hypothesis that the sublingual region can indeed be used to monitor the microcirculation in sepsis.

Due to its observational design, our study suffers from a non-standardized VE regimen and a non-standardized measure of CO since both were left to the discretion of the attending physician. However, both the pulse contour analysis device and oesophageal Doppler monitor provide real time CO monitoring and are able to estimate rapid changes in SV [26]. By recruiting only preload-responsive patients, our study design may have favoured some correlation between CO and microcirculatory variables since patients were expected to increase CO after VE. It would have been interesting to also have included patients not predicted to be fluid responders in order to evaluate what would have been their microvascular response. This point should be addressed in future studies. As some of the changes in CO were very large, we cannot exclude the possibility that spontaneous changes in the underlying condition also occurred in those early septic shock patients during the data acquisition period. However, a randomized trial including a group without VE would have been unethical. Another limitation is that we investigated patients at an early period of their disease. Therefore, our results cannot be extrapolated to septic patients receiving VE after the first 24 h of their admission in the ICU.

Conclusions

In preload-responsive patients with severe sepsis and septic shock patients studied during the first 24 h of their ICU stay, both PLR and VE improved sublingual microcirculatory perfusion obtained using side-stream dark field imaging. At the level of VE used in our study, changes in microcirculation were not explained by changes in rheologic factors or changes in MAP. We observed a non-linear relationship between changes in CO and changes in a number of microvascular variables. In

addition, the observed changes in microvascular variables were disproportionate compared to changes in CO. These two points suggest that different mechanisms are implicated in the regulation of microvascular perfusion and in the changes in CO.

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