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EDITORIAL

Steve M. Hollenberg

Think locally: evaluation of the microcirculation in sepsis

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S. M. Hollenberg (✉)
Division of Cardiovascular Diseases, Cooper University Hospital, 1 Cooper Plaza, 366 Dorrance, Camden, NJ 08034, USA
e-mail: Hollenberg-Steven@CooperHealth.edu
Tel.: +1-856-9687636
Fax: +1-856-9687420

The ultimate goals of hemodynamic therapy in sepsis are to restore effective tissue perfusion and to maintain cellular metabolism. Fluid resuscitation is the first step in management of septic shock. Fluid administration should be performed vigorously and titrated to clinical endpoints of perfusion such as capillary refill, urine output, and mental status, and also to macrocirculatory parameters of global perfusion, including heart rate, blood pressure, cardiac output, and mixed or central venous oxygen saturation [1]. In sepsis, however, tissue hypoperfusion may result not only from decreased perfusion pressure attributable to hypotension but also from abnormal distribution of blood flow [1, 2]. Thus, defining the adequacy of resuscitation requires attention to both global and regional perfusion. The microcirculation is a critical component of the cardiovascular system that regulates flow to the tissues. Microcirculatory perturbation is a central abnormality in septic shock and represents a logical and promising therapeutic target [3].

In this context, an interesting manuscript in the current issue of *Intensive Care Medicine*, along with one published in the June 2010 issue, provide useful new information. In this issue, Pottecher et al. [4] compare the microcirculatory effects of passive leg raising and volume expansion in septic patients using a sidestream dark field (SDF) imaging device to assess the sublingual circulation. The patients were predicted to be preload-responsive, as assessed by pulse pressure variation greater than 13% [5]. Both passive leg raising and fluids improved microcirculatory parameters, including perfused capillary density, proportion of perfused vessels, microcirculatory flow index, and heterogeneity index [6]. In the paper published in June, Ospina-Tascon et al. [7] used SDF imaging to evaluate the microcirculation in septic patients before and after fluid resuscitation in different patients early (<24 h) or late (>48 h) in their clinical course. Fluid resuscitation increased perfused vessel density early, but not late.

Fluid resuscitation in sepsis starts with identifying patients who are preload-responsive, that is, who are likely to respond to fluids with increased stroke volume [8]. The current studies [4, 7] suggest that fluid resuscitation improves microcirculatory perfusion in ways that are not entirely explained by systemic hemodynamic effects. In the Ospina-Tascon study [7] about half of patients were hemodynamic responders [defined as an increase in mean arterial pressure (MAP) by >5%, or increase in cardiac output (CO) by >15%], but microcirculatory benefits did not correlate with either MAP or CO. In addition, in patients treated late, there was some improvement in MAP and CO, but no change in the microcirculation. In the Pottecher study [4], change in microcirculatory flow index did correlate with change in CO and MAP, and change in proportion of perfused vessels correlated with changes in CO but not MAP. Readers should be cautious about overinterpreting either correlations or lack thereof in relatively small studies such as these. The main point is not that there is no relationship whatsoever between macrocirculatory and microcirculatory hemodynamics, but rather that there are some independent determinants of flow in different circulatory beds. In addition, there is clearly room for individual variability. A recent study examining the effect of varying perfusion pressure with norepinephrine on responses of the sublingual microcirculation found no

effect on the population as a whole, but a fair amount of variation in individual responses, with patients reaching maximal perfusion at different mean arterial pressures [9].

These studies also lend credence to the notion that evaluation of microcirculation perfusion might be worthwhile as a clinical index of the adequacy of fluid resuscitation in individual patients. Direct visualization of the sublingual circulation has shown microcirculatory perturbation in patients with sepsis [10, 11], and changes appear to track the clinical course [12, 13]. The study of Ospina-Tascon et al. suggests that some patients without changes in MAP or CO in response to fluids will still show improvement in the microcirculation. Nonetheless, visualization of the sublingual circulation is not quite ready for routine clinical application at the bedside. As it evolves, this technology needs to become more user-friendly; there is room for improvement in both ease of acquisition and analysis to facilitate generation of reproducible and timely data to guide patient management [6].

Study of the microcirculation might also provide insight into potential mechanisms of disease, something that is hinted at but not fully addressed in the current studies. Local hemodynamics might matter even if global changes in response to therapy are more subtle. The increase in perfused capillary density seen in patients without dramatic changes in cardiac index in the Ospina-Tascon study lends credence to this notion. These authors also found a correlation between changes in the proportion of perfused microvessels and changes in serum lactate, supporting a similar correlation between perfused capillary density and oxygen extraction ratio in sepsis found by Trzeciak et al. [11] and mechanistic data directly relating stopped flow capillaries to oxygen extraction in an animal model of sepsis [14]. This evidence, albeit indirect, supports the notion that perturbation of microcirculatory hemodynamics impairs perfusion at the local level. The studies also provide evidence for increases in perfused capillary density, something that could happen if local pressures were increased to values that exceed their critical closing pressures [15]. Increased cardiac output, which was observed in most of the patients in the Pottecher study and half of the patients in the Ospina-Tascon study, could also potentially mediate local vasodilation by increasing shear stress. Other potential mechanisms include possible influences on blood rheology and plasma viscosity, as well as modulation of the complex humoral and neurologic adaptive responses of microvascular networks to stress.

One intriguing finding of the investigation of Ospina-Tascon et al. was the difference between microvascular responses early and late in the course of sepsis despite similar increases in global hemodynamic parameters. It seems clear that measures to support hemodynamics after presentation with sepsis are most effective when applied early [16], and the improvement in microcirculation perfusion has been shown to correlate with improvements in organ function in the early phases of sepsis [13]. Whether patients in whom macrocirculatory goals have been achieved should be resuscitated to microcirculatory endpoints has not been established, and is the subject of ongoing investigation [3]. The later phases of sepsis, when multiple organ failure tends to predominate, however, remain a mystery [17]. It is not at all clear that attempts at hemodynamic optimization late will have the same effect as similar attempts early, and the current data suggest they may not. Defining "early" versus "late" phases in the clinical course of sepsis, however, remains a challenge. Ospina-Tascon et al. used 24 h or less and 48 h or more, which is reasonable for the purposes of study design, but leaves a period of uncertainty in between for the clinician trying to apply their findings to clinical practice. Given the variability in clinical courses in individual patients, further research in this area might try to move beyond time-based definition of phases to more state-based definitions, in which recognizable characteristics are used to define when an early phase has given way to a later one.

These studies [4, 7] have used sophisticated methodology to elucidate microvascular effects of fluid resuscitation, but we still have a lot to learn about the microcirculation in sepsis. In other contexts, we are learning to "think globally, act locally." With respect to our patients with sepsis, we should still be thinking globally (assembling individual pieces of data into a generalized whole to generate a therapeutic plan), but there is a persuasive case to be made that, at least with respect to hemodynamics, it is local actions on the microcirculation that are most important. Learning how to assess the impact of systemic interventions such as fluid resuscitation is only the first step toward developing interventions that target microcirculatory perfusion.

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ORIGINAL

Julien Pottecher
Stéphane Deruddre
Jean-Louis Teboul
Jean-François Georger
Christian Laplace
Dan Benhamou
Eric Vicaut
Jacques Duranteau

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

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J. Pottecher · S. Deruddre · C. Laplace · D. Benhamou · J. Duranteau (✉)
Unité EA 3509, Département d'Anesthésie-Réanimation, Centre Hospitalier Universitaire de Bicêtre, Assistance Publique Hôpitaux de Paris, Université Paris 11, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre Cedex, France
e-mail: jacques.duranteau@bct.aphp.fr
Tel.: +33-1-45213447
Fax: +33-1-45212875

J.-L. Teboul · J.-F. Georger
EA 4046, Service de Réanimation Médicale, Centre Hospitalier Universitaire de Bicêtre, AP-HP, Université Paris 11, Le Kremlin-Bicêtre Cedex, France

E. Vicaut
EA 3509, Laboratoire d'Etude de la Microcirculation, Unité de Recherche Clinique, Centre Hospitalier Universitaire Lariboisière–Fernand Widal, AP-HP, Université Paris 7, Paris, France

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 (ΔPP) 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 ΔPP 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 ΔPP.

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 ± 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 form 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 ± 17
Sex (M/F)	16/9
Weight (kg)	79 ± 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 ($\mu g\ kg^{-1}\ min^{-1}$)	
None	5
Norepinephrine	17; 0.40 (0.24–0.78)
Epinephrine	3; 0.39 (0.20–0.42)
Body temperature (°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_20)	20 (18–25)
PEEP (cmH_2O)	5 (4–6)

Values are given as the mean ± 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	$VE_{\Delta PP\ =\ PLR}$	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 $VE_{\Delta PP\ =\ PLR}$

difference in any of the haemodynamic indices was found between Baseline 1 and Baseline 2. At the first step of VE ($VE_{\Delta PP\ =\ PLR}$), 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, $VE_{\Delta PP\ =\ PLR}$ 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 microcirulatory 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).

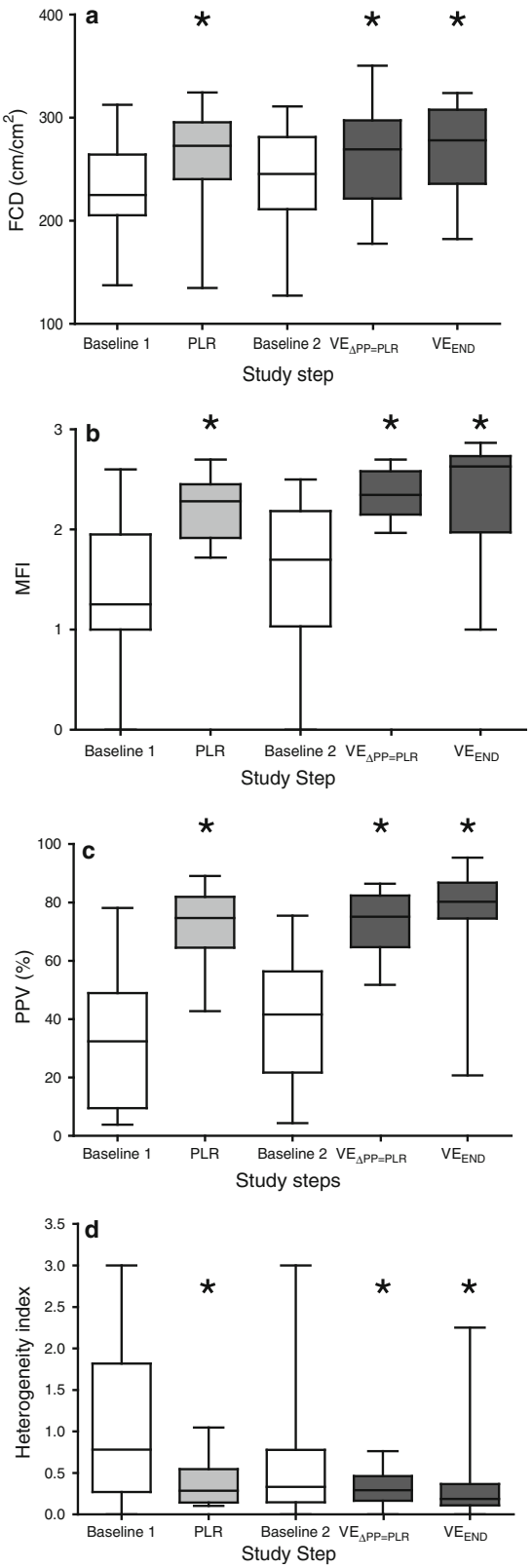
Relationship between microcirculatory perfusion and systemic haemodynamics

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 ($\rho = 0.29$, $P =$ 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 ΔPP (respiratory variations in arterial pulse pressure). *Baseline 2* same as Baseline 1. $VE_{\Delta PP\ =\ PLR}$, the study step obtained when the volume expansion-induced decrease in ΔPP reaches the PLR-induced ΔPP 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 sidestream 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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Gustavo Ospina-Tascon
Ana Paula Neves
Giovanna Occhipinti
Katia Donadello
Gustavo Büchele
Davide Simion
Maria-Luisa Chierego
Tatiana Oliveira Silva
Adriana Fonseca
Jean-Louis Vincent
Daniel De Backer

Effects of fluids on microvascular perfusion in patients with severe sepsis

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G. Ospina-Tascon · A. P. Neves · G. Occhipinti · K. Donadello · G. Büchele · D. Simion · M.-L. Chierego · T. O. Silva · A. Fonseca · J.-L. Vincent · D. De Backer (✉)
Department of Intensive Care, Erasme University Hospital, Université Libre de Bruxelles, Route de Lennik 808, 1070 Brussels, Belgium
e-mail: ddebacke@ulb.ac.be
Tel.: +32-2-5553380
Fax: +32-2-5554698

Abstract *Purpose:* To evaluate the effects of fluid administration on microcirculatory alterations in sepsis. *Methods:* With a Sidestream Dark Field device, we evaluated the effects of fluids on the sublingual microcirculation in 60 patients with severe sepsis. These patients were investigated either within 24 h (early, $n = 37$) or more than 48 h (late, $n = 23$) after a diagnosis of severe sepsis. Hemodynamic and microcirculatory measurements were obtained before and 30 min after administration of 1,000 ml Ringer's lactate ($n = 29$) or 400 ml 4% albumin ($n = 31$) solutions. *Results:* Fluid administration increased perfused small vessel density from 3.5 (2.9–4.3) to 4.4 (3.7–4.9) n/mm ($p < 0.01$), through a combined increase in the proportion of perfused small vessels from 69 (62–76) to 79 (71–83) %, $p < 0.01$) and in small vessel density from 5.3 (4.4–5.9) to 5.6 (4.8–6.3) n/mm ($p < 0.01$). Importantly, microvascular perfusion increased in the early but not in the late phase of sepsis: the proportion of perfused small vessels increased from 65 (60–72) to 80 (75–84) % ($p < 0.01$) in the early phase and from 75 (66–80) to 74 (67–81) ($p =$ ns) in the late phase. These microvascular effects of fluids were not related to changes in cardiac index ($R^2 = 0.05$, $p =$ ns) or mean arterial pressure ($R^2 = 0.04$, $p =$ ns). *Conclusions:* In this non-randomized trial, fluid administration improved microvascular perfusion in the early but not late phase of sepsis. This effect is independent of global hemodynamic effects and of the type of solution.

Keywords Microcirculation · Cardiac output · Colloids · Crystalloids

Introduction

Septic shock is an important cause of death in critically ill patients worldwide [1]. Early, effective fluid resuscitation is a key component in the effective management of patients with septic shock [2, 3] with the goal to improve tissue perfusion. Today, evaluation of the effects of fluids is still only grossly estimated, at best by assessing their effects on cardiac output; however, the impact of fluid resuscitation on tissue perfusion has not been properly evaluated.

Microvascular alterations are frequent in patients with septic shock, even when global oxygen delivery seems adequate, and may play an important role in the development of organ failure [4, 5]. Numerous experimental and clinical studies have reported that microvascular blood flow is altered in sepsis. Common findings include a decrease in functional capillary density and

heterogeneity of blood flow with perfused capillaries in close vicinity to non-perfused capillaries [4, 6, 7]. These alterations are more severe in non-survivors than in survivors, and their persistence is associated with organ failure [8, 9] and death [9]. The effects of some therapeutic interventions on the microcirculation have been reported recently [6, 10–13], but the effects of fluids have not been well defined.

Several experimental studies have shown that fluids may improve the microcirculation in sepsis. Hoffman et al. [14] showed that hydroxyethyl starch but not saline solutions improved functional capillary density in hamster skinfold. In the hamster cheek pouch, de Carvalho et al. [15] showed that the administration of Ringer's acetate improved microvascular perfusion to a similar extent as dextrans. Recently, Schaper et al. [16] reported that saline failed to improve functional capillary density in the gut microcirculation, while gelatins preserved it to pre-sepsis values. These data suggest that colloids may be more effective that crystalloids in increasing microvascular perfusion.

A final issue may be the time dependency of the microvascular effects of fluids. One may expect fluids to be more beneficial when given early rather than later on during the course of sepsis. Axler et al. [17] reported that fluid administration hardly increased cardiac output when given after the initial resuscitation phase. However, these results have not been confirmed by others, and fluid challenge is often attempted several days after sepsis has been recognized. For example, fluids were administered up to 21 days after inclusion in a recent study evaluating two different fluid strategies [18].

In this study, we evaluated the effects of fluid administration on the microcirculation, and in particular the influence of the type of fluid and the timing of administration. We also evaluated the relationship between the microvascular and global hemodynamic response to fluids.

Patients and methods

This study was approved by the local ethics' committee, and informed consent was obtained from the patients or their relatives.

Between October 2006 and November 2008, the study included a convenience sample of 60 patients with severe sepsis who required fluid administration, as assessed by the physician in charge, within 24 h (early) or more than 48 h (late) of a diagnosis of severe sepsis. Patients who received fluids between 24 and 48 h were not included to allow clear differentiation between groups. The choice of the fluid solution (Ringer's lactate or 4% albumin solution, the two main solutions used in our department) was left to the physician in charge of the patient. Fluid administration was performed using the fluid challenge method [19]. Indications for fluid administration included signs of hypovolemia in the presence of indices of tissue hypoperfusion such as arterial hypotension (mean arterial pressure <65 mmHg), oliguria (<0.5 ml/kg h) or increased arterial blood lactate levels (>2.0 mEq/l) that could be ascribed to altered tissue perfusion. Sepsis was defined according to the International Sepsis Definition Conference [20]; the onset of sepsis was defined as the time of hospital admission for patients admitted through the Emergency Department and the time of identification of sepsis-related organ dysfunction for patients transferred from the hospital ward. Exclusion criteria were age <18 years, pregnancy, advanced liver cirrhosis, non-invasive mechanical ventilation or use of a facemask with a FiO_2 above 0.5, and previous inclusion in the study.

All patients were equipped with an arterial and central venous catheter. Cardiac output was measured in 42 patients using continuous thermodilution (CCO; Edwards Lifesciences, Irvine, CA) in 38 patients and transpulmonary thermodilution (PiCCO; Pulsion, Munich, Germany) in 4 patients. Cardiac output (mean value displayed on the monitor or average of five pulse contour derived values) was measured after collection of all other variables in order to reflect the mean cardiac output values during the other measurements.

Temperature, heart rate, arterial pressure and cardiac output were obtained before and 60 min after infusion of either 1,000 ml of a Ringer's lactate solution or 400 ml of a 4% albumin solution (Belgian Red Cross) over 30 min. In addition, arterial and mixed-venous or central venous blood samples were obtained for determination of blood gas analysis and determination of arterial lactate and hemoglobin levels (Gem 4000; Instrumentation Laboratory, Lexington, MA). Pulse pressure variations were determined from three successive breaths on digital recording of arterial pressure traces (Drager, Lubeck, Germany).

Microvideoscopic measurements and analysis

Measurements of the sublingual microcirculation were obtained at the time of hemodynamic measurements using a Sidestream Dark Field (Microscan, Microvision medical, Amsterdam, The Netherlands) with a 5× objective. The device was gently applied without pressure to the lateral side of the tongue in an area approximately 1.5–4 cm from the tip of the tongue after gentle removal of secretions with gauze. Five sequences of 20 s each from different adjacent areas were recorded using a computer and a videocard (MicroVideo; Pinnacle System, Mountain Views, CA). These sequences were stored under a random number and later analyzed semi-quantitatively by an investigator blinded to the origin of the sequences [4, 21]. The details of this analysis are reported in the Electronic

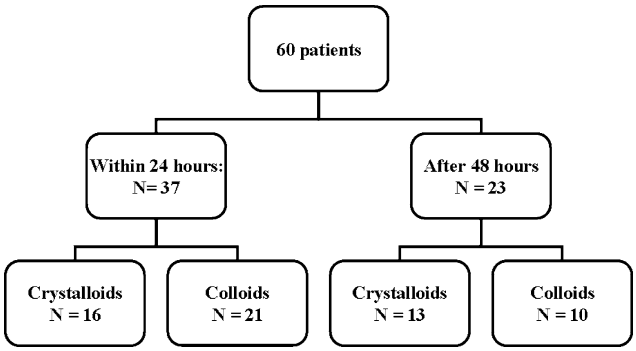

Fig. 1 Flowchart of the study. Patients were investigated once only, either within 24 h of the diagnosis of severe sepsis (early) or more than 48 h after diagnosis (late)

Supplementary Material. According to recent guidelines [21], we measured the vascular density, proportion of perfused vessels and perfused vascular density for all (total) and for small vessels. The proportion of perfused large vessels is reported as quality control, as most of these large vessels should remain perfused. In addition, the microvascular flow index (MFI) and heterogeneity index were calculated. Given the intrinsic variability of measurements [4, 13] and previous data showing delineation between survivors and non-survivors [5], an absolute change of 10% in the proportion of perfused small vessels can be considered as clinically significant.

Statistical analysis

A non-Gaussian distribution was observed for most microcirculatory variables; accordingly non-parametric tests were used whenever available, and data are presented as median (percentiles 25–75). Interactions between the effects of fluid and timing of intervention (early vs. late) or type of fluid (crystalloids vs. albumin) were tested by analysis of variance (ANOVA). Differences from baseline were evaluated using Wilcoxon rank tests and differences between subgroups by Mann-Whitney test.

The relationship between changes in lactate levels and relevant global hemodynamic and microcirculatory variables was evaluated with linear regression.

Results

Sixty patients were enrolled: 37 in the early and 23 in the late phase of severe sepsis. A total of 29 received a crystalloid and 31 an albumin solution (Fig. 1). There were no differences in the proportions of patients receiving crystalloid and albumin in the early and late periods ($p = 0.41$). The principal clinical data are shown in Table 1. Most patients had signs of shock and were treated with mechanical ventilation. There was no difference between the subgroups in therapy, including the doses of vasoactive agents. Twelve patients were treated

Table 1 Patient characteristics

	All patients (60)	Early (37)	Late (23)	Crystalloids (29)	Colloids (31)
Age	71 [63–79]	72 [62–79]	71 [67–78]	75 [63–82]	71 [65–77]
Male, *n* (%)	35 (58)	22 (59)	13 (56)	16 (55)	19 (61)
Medical, *n* (%)	33 (55)	18 (49)	15 (65)	14 (48)	19 (61)
APACHE II score	22 [17–28]	20 [15–27]	23 [19–29]	22 [18–31]	21 [15–27]
SOFA score	10 [7–12]	10 [7–12]	11 [8–14]	11 [8–12]	10 [7–12]
ICU survival, *n* (%)	34 (57)	22 (59)	12 (52)	18 (62)	16 (52)
Alive 28 days, *n* (%)	30 (50)	21 (57)	9 (39)	16 (55)	14 (45)
Source of infection *n* (%)					
Lungs	20 (33)	13 (35)	7 (30)	10 (35)	10 (33)
Abdomen	27 (45)	18 (49)	9 (39)	13 (45)	14 (45)
Urinary tract	1 (2)	0 (0)	1 (4.3)	0 (0)	1 (3)
Catheter	1 (2)	1 (3)	0 (0)	0 (0)	1 (3)
Soft tissue	8 (13)	3 (8)	5 (22)	5 (17)	3 (10)
Endocarditis	1 (2)	1 (3)	0 (0)	0 (0)	1 (3)
Mechanical ventilation, *n* (%)	56 (93)	34 (92)	22 (96)	27 (93)	29 (93)
CVVH, *n* (%)	12 (20)	4 (11)	8 (35)$^{\$}$	6 (21)	6 (19)
Vasoactive agents					
Dopamine, *n*; μg/kg min	19; 20 [13–20]	12; 17 [10–20]	7; 20 [20]	11; 15 [9–20]	8; 20 [19, 20]
Norepinephrine, *n*; μg/kg min	38; 0.29 [0.12–0.48]	21; 0.20 [0.10–0.42]	17; 0.33 [0.20–0.57]	16; 0.35 [0.22–0.65]	22; 0.21 [0.10–0.33]
Dobutamine, *n*; μg/kg min	19; 5 [4–13]	10; 6 [2–15]	9; 5 [5–9]	10; 10 [5–15]	9; 5 [2–5]
Hydrocortisone, *n* (%)	27 (45)	15 (41)	12 (52)	15 (52)	12 (39)
Drotrecogin alfa, *n* (%)	7 (12)	5 (13)	2 (9)	3 (10)	4 (13)

CVVH continuous veno-venous hemofiltration
$^{\$}$ $p < 0.05$ versus other subgroup

Table 2 Hemodynamic response to fluids in early and late phases of sepsis

	Early		Late		p Value (ANOVA)[a]
	Baseline	Fluids	Baseline	Fluids	
Global hemodynamic variables					
Temperature, °C	37.0 [36.5–37.6]	37.0 [36.5–37.9]	36.9 [36.5–38.3]	36.9 [36.5–38.7]	NS
Heart rate, bpm	100 [92–113]	102 [88–114]	112 [87–127]	103 [89–119]	NS
Mean arterial pressure, mmHg	73 [67–77]	75 [70–81]**	69 [64–76]	76 [70–80]**	NS
Central venous pressure, mmHg	11 [8–13]	14 [11–17]**	11 [8–13]	12 [11–15]**	NS
Cardiac index[b], l/min M^2	2.9 [2.1–3.6]	3.2 [2.4–3.8]**	3.2 [2.9–3.5]	3.5 [3.2–3.8]*	NS
Mixed- or central venous O_2 saturation, %[c]	69 [62–75]	71 [67–76]*	69 [65–75]	70 [65–74]	NS
Lactate, mmol/l	2.1 [1.2–2.9]	1.9 [1.1–2.6]**	1.8 [1.4–2.4]	1.9 [1.4–2.5]	$p < 0.05$
Pulse pressure variation, %[d]	12 [7–18]	9 [8–12]*	10 [4–15]	9 [7–10]	NS
Microcirculatory variables					
Total vessel density, n/mm	7.8 [7.2–8.5]	8.7 [7.9–9.3]**	8.7 [7.0–9.4]	8.3 [7.4–9.3]	$p < 0.01$
Small vessel density, n/mm	5.1 [4.5–5.8]	5.8 [4.9–6.3]**	5.8 [4.1–6.4]	5.5 [4.5–6.3]	$p < 0.01$
Proportion of perfused large vessels, %	100 [100–100]	100 [100–100]	100 [100–100]	100 [100–100]	NS
Proportion of perfused small vessels, %	65 [60–72]	80 [75–83]**	75 [66–80]$	74 [67–81]$	$p < 0.001$
Perfused small vessel density, n/mm	3.4 [2.9–3.8]	4.5 [4.0–4.9]**	4.1 [2.9–4.8]	4.1 [3.0–4.9]	$p < 0.0001$
Microvascular flow index	1.9 [1.5–2.3]	2.6 [2.3–2.8]**	2.5 [1.9–2.7]$	2.4 [2.0–2.7]	$p < 0.0001$
Heterogeneity index, %	47 [28–66]	32 [23–51]*	36 [25–58]	41 [27–59]	NS

[a] Two-way ANOVA tested the interaction between the response to fluid challenge and the timing of the intervention (early vs. late)
[b] Cardiac index was measured in 42 patients
[c] Mixed venous O_2 saturation was measured in 38 patients and central venous O_2 saturation in 18 patients
[d] Pulse pressure variation was measured in 44 patients
* and ** $p < 0.05$ and $p < 0.01$ fluids versus baseline, $\$p < 0.05$ late versus early

with continuous hemofiltration; as expected, this technique was more frequently used in late than in early phases.

Global hemodynamics

During fluid challenge, mean arterial pressure (MAP) increased from 72 (65–76) to 75 (70–81) mmHg ($p < 0.01$) and the cardiac index from 3.1 (2.5–3.5) to 3.3 (2.7–3.8) l/min M^2 ($p < 0.01$) (ESM Table 1). The proportion of responders was 55%, when defined as an increase in MAP of at least 5, and 45% when defined by an increase in cardiac index of at least 15%. The proportion of responders was similar in the two time periods and irrespective of the type of fluid (ESM Tables 2 and 3) or use of vasopressor agents (ESM Table 4). Arterial pressure, but not cardiac output, was a predictor of response to fluid (a positive response to fluids was more likely in hypotensive patients) (ESM Figs. 1 and 2). The type of fluid did not significantly affect the global hemodynamic response to fluid administration (ESM Table 5).

Interestingly, blood lactate levels decreased with fluid challenge in the early group but not in the late group: −0.2 (−0.4 to −0.1) versus 0.0 (−0.2 to 0.1) mEq/l ($p < 0.01$).

There was no difference at baseline in hemoglobin levels (data not shown), and changes in hemoglobin levels were similar in early and late [0.0 (−0.2 to 0) versus −0.3 (−0.6 to −0.2) g/dl, p = ns] as well in crystalloids and colloids [−0.1 (−0.6 to 0.0) versus −0.1 (−0.5 to 0.0) g/dl, p = ns].

Microcirculatory effects

Globally fluid administration improved microvascular perfusion, as reflected by an increase in total and small vessel density together with an improvement in the proportion in perfused small vessels (ESM Table 5). As a result, the density of perfused small vessels also increased. The heterogeneity index was not affected. The changes in microvascular perfusion were not related to pulse pressure variation (ESM Fig. 3). The microcirculatory response was not related to baseline values of MAP or cardiac index, or with changes in these variables (ESM Figs. 1 and 2). In addition changes in MAP and cardiac index failed to predict microvascular changes (chi-square p value of 0.84 and 0.32 and concordance of 0.49 and 0.47, respectively). ROC curve areas for detection of microvascular responders were 0.51 (0.33–0.69) for MAP and 0.62 (0.44–0.77) for cardiac index; both were not significant.

At baseline, the proportion of perfused small vessels and the MFI were lower in early than in late group patients (Table 2); other microcirculatory variables were similar in the two groups. The microvascular response to fluids differed according to the timing of the intervention. In the early group, total vessel density, small vessel

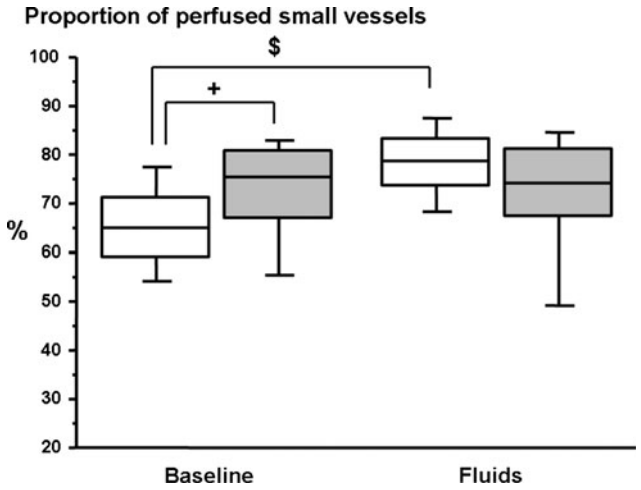

Fig. 2 Evolution of proportion of perfused small vessels in patients investigated early or late after diagnosis of severe sepsis. Patients investigated within 24 h of the diagnosis of severe sepsis (early, $n = 37$) are represented by *white rectangles*; patients investigated more than 48 h after diagnosis (late, $n = 23$) are represented by *gray rectangles*. $^{+}p < 0.01$ between the two groups, $^{\$}p < 0.01$ fluids versus baseline

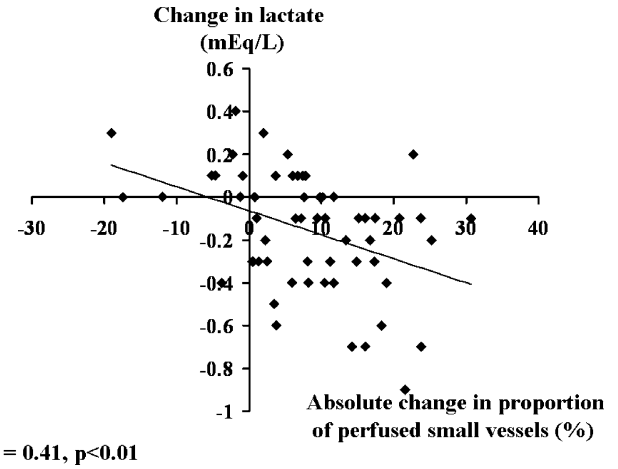

Fig. 3 Relationship between changes in proportion of perfused small vessels and changes in lactate levels. Change in lactate = (−0.011 change in proportion of perfused vessels, 0.064); $R^2 = 0.41$, $p = 0.0012$

density and the proportion of perfused small vessels increased. Accordingly, perfused small vessel density also increased. In the late group, the proportion of perfused small vessels (Fig. 2) and the other microcirculatory variables were not affected. The difference in baseline microvascular perfusion did not explain the difference in response to fluids between early and late groups as the baseline proportion of perfused small vessels extended over the same range in both groups (ESM Fig. 4). In addition, the magnitude of the change in perfusion was inversely related to baseline perfusion in the early group, while no relationship between these factors was observed in the late group.

The evolution of microvascular variables was similar with the two types of fluid (ESM Table 5); in particular, the proportion of perfused small vessels was similar with crystalloids and colloids in the early and late groups (ESM Figs. 5 and 6).

Changes in lactate levels were related to the changes in the proportion of perfused small vessels (Fig. 3) or changes in perfused small vessel density ($R^2 = 0.47$, $p = 0.0002$), but not to changes in cardiac index ($R^2 = 0.05$, $p =$ ns) or in MAP ($R^2 = 0.04$, $p =$ ns).

Discussion

The main finding of this study is that fluids improve the microcirculation in the earlier but not in the later phases of sepsis. These effects are independent of the systemic effects of fluids and are observed with crystalloid as well as with albumin solutions.

What are the mechanisms by which fluids can improve the microcirculation? Microvascular perfusion is directly related to the driving pressure (difference between pressure at the entry and exit of the capillary) and the radius of the vessel (to the fourth power) and inversely related to blood viscosity. In addition, interaction of circulating cells with the endothelial surface may impair microvascular perfusion. In sepsis, multiple factors may contribute to microvascular alterations, including alterations in red blood cell rheology and leukocyte adhesion to endothelial cells, endothelium dysfunction and interstitial edema. Fluids may increase microvascular perfusion by increasing the driving pressure or by decreasing blood viscosity, and also by affecting interactions between the endothelium and circulating cells. In experimental conditions, fluids, and especially colloids, have been shown to decrease adhesion and rolling of white blood cells to the endothelium [14]. We can only speculate on the mechanisms implicated in these patients. Changes in driving pressure (MAP-CVP) are unlikely to play a role as the response to fluids was also observed in patients who did not improve their systemic hemodynamics. Changes in hemoglobin levels were also minimal and unlikely to play a role, but, admittedly, microvascular hematocrit was not measured.

Interestingly, the microvascular response was dissociated from the macrohemodynamic response to fluids. There was no relation between changes in microvascular perfusion and initial arterial pressure or cardiac index or changes thereof during fluid administration. These observations clearly separate the macro- and

microcirculatory effects of fluid administration and have important implications. As an improvement in tissue perfusion is the main goal for fluid resuscitation, one should not refrain from attempting a fluid challenge in patients with persistent signs of tissue hypoperfusion at early stages, even when indices of fluid responsiveness indicate poor cardiac response to fluids, Conversely, alternatives to fluids should be more seriously considered at later stages. Future trials should test whether microcirculation-guided fluid therapy could better improve organ dysfunction than more conventional guidance by global hemodynamic variables guidance.

Another interesting finding is that the response to fluids varied over time. In contrast to our expectations, the global hemodynamic changes to fluids were similar in the early and late phases of severe sepsis, with a similar proportion of responders as well as a similar magnitude in changes in arterial pressure or cardiac index. Importantly, the microvascular response to fluids was totally blunted in the later phase of sepsis, even in the patients who experienced an increase in cardiac index or arterial pressure. Why was this? A somewhat better preserved microcirculation before fluid administration in the late phase is unlikely, as there was an inverse relation between changes in microvascular perfusion and baseline perfusion in the early phase but not in the late phase. In addition, microvascular perfusion failed to improve in the late phase even in the patients with the worst microvascular perfusion at baseline. As changes in driving pressure (MAP and CVP) as well as changes in systemic hematocrit were similar in early and late phases, it suggests that the mechanisms implicated in the microvascular response to fluids were exhausted. Experimental studies do not provide any explanation, as these were always conducted in the early stages of sepsis.

Interestingly, also the type of fluid did not influence patient response. The controversy between crystalloids (saline) and colloids (albumin) has been active for decades, and microcirculatory studies have not shed any light on whether one is better than the other. Recent experimental studies showed that colloids were more effective than crystalloids in restoring cardiac output, but failed to blunt development of organ failure or to affect outcome [22]. Human studies gave divergent results, as some showed a greater hemodynamic effect with colloids [23], while others failed to demonstrate major differences in macrohemodynamic effects between colloid and crystalloid solutions [18]. Even though our study was not randomized and unmeasured confounding factors may have played a role, our study suggests that short-term macrohemodynamic and microvascular effects of crystalloids and colloids—or at least albumin—are very similar in patients with severe sepsis.

The study has several limitations. First we investigated the microcirculation in the sublingual region, and other microcirculatory beds may have a different response. In a model of endotoxic shock, fluids improved the microcirculation in the sublingual area and in gut serosa, but not in gut mucosa [24]. However, Verdant et al. [25] observed in a fluid resuscitated pig model of septic shock in which abdominal pressure was controlled that sublingual and gut mucosal microvascular perfusion had a similar evolution. Unfortunately, we could not evaluate the gut mucosa. Indirect evidence obtained with gastric tonometry suggests that fluid administration may also improve gut mucosal perfusion in patients with sepsis, especially when it is markedly altered at baseline [26]. The relationship between changes in microcirculatory perfusion and lactate levels suggests that the improvement in microvascular perfusion detected in the sublingual area was also observed in other relevant beds. Second, we investigated only the short-term effects of fluids, and these effects may be transient. Third, we did not randomize either the type of fluids nor timing of interventions. Accordingly, some measured and especially unmeasured factors may have confounded our results. However, we failed to notice any interaction with the measured factors in all the exploratory analyses we performed. Nevertheless, our results should be interpreted with caution and should be confirmed in further trials. Fourth, the volume of fluid infused was predetermined. Of note, colloids and crystalloids resulted in similar global hemodynamic effects (no interaction detected by ANOVA) for a same increase in preload, as roughly estimated by change in CVP. Finally, we did not include a control group, as we felt it would be unethical [27]. In any case, spontaneous changes in the microcirculation during this short period of observation were not likely to be significant.

Our study has important implications. First, it emphasizes the critical role of fluids in early resuscitation, with a marked improvement in microvascular perfusion associated with a decrease in lactate levels. Second, it demonstrates that fluid administration has limited impact on tissue perfusion during the later stages of sepsis, even when cardiac output and arterial pressure may improve. Fluid resuscitation may hence be useless or even detrimental in later stages of sepsis [28].

In conclusion, our data show that fluid administration may improve microvascular perfusion in the early but not in the later phases of severe sepsis and that this effect is independent of the global hemodynamic effects of fluids. The type of solution does not seem to influence the response to fluids. These results should be confirmed in randomized trials.

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