British Journal of Anaesthesia **108** (6): 973–78 (2012)
Advance Access publication 30 March 2012 · doi:10.1093/bja/aes093

BJA

CRITICAL CARE

Microvascular and macrovascular flow are uncoupled in early polymicrobial sepsis

A. Dyson[1], S. Cone[2], M. Singer[1] and G. L. Ackland[1,2]*

[1] Department of Medicine, Bloomsbury Institute of Intensive Care Medicine and [2] Centre for Anaesthesia, Critical Care and Pain Medicine, University College London, London, UK

* Corresponding author. E-mail: g.ackland@ucl.ac.uk

Editor's key points

- A rat model of sepsis was used.
- Macrovascular flow was measured as stroke volume and cardiac output.
- Microvascular flow was measured in the vastus lateralis muscle using videomicroscopy.
- Microvascular flow was uncoupled from macrovascular function.
- Surgery without sepsis also caused microvascular changes and was not different in septic animals.

Background. Microvascular dysfunction is considered to play an important pathophysiological role in sepsis. We addressed the hypothesis that macrovascular and microvascular flow are uncoupled in early sepsis, using a rodent model with well-characterized haemodynamic and biochemical markers of severity and subsequent mortality.

Methods. Male Wistar rats received either an intraperitoneal injection of faecal slurry (sepsis, $n=14$) or sterile saline (sham, $n=6$). Identical i.v. fluid resuscitation regimens were administered 2 h later through tethered lines while conscious. At 6 h post-sepsis and in sham-operated controls, sidestream dark-field microvascular imaging of the left vastus lateralis muscle and transthoracic echocardiography were undertaken, again under anaesthesia. Non-operated rats (naive; $n=5$) served as negative controls. Mild and severe sepsis were defined *a priori*, based on the established predictive relationship between stroke volume and mortality in this model.

Results. Compared with sham-operated animals, there was a 19 (12–19)% and 62 (54–66)% decline in cardiac output in mild ($n=8$) and severe sepsis ($n=6$), respectively [median (inter-quartile range), $P<0.0001$]. Blinded assessment of microvascular imaging revealed that the microvascular flow index (MFI) was impaired in sepsis and in sham-operated controls ($P<0.01$), regardless of the degree of reduction in stroke volume and cardiac output. The MFI heterogeneity index revealed that only naive rats displayed a normal microvascular flow pattern.

Conclusions. Microvascular flow is impaired during early sepsis and uncoupled from macrovascular function. The severity of macrovascular/cardiovascular compromise in early sepsis is not reflected by microvascular changes. Furthermore, surgery alone causes significant microvascular derangement, highlighting the importance of appropriate control subjects when using this technique.

Keywords: cardiac failure; macrocirculation; microcirculation; sepsis

Accepted for publication: 9 January 2012

Microvascular disturbances are frequently observed in sepsis and are considered to play an important pathophysiological role.[1] These abnormalities are thought to relate to changes in vascular tone, intravascular volume, red blood cell rigidity, and leucocyte and endothelial activation.[2] Microvascular dysfunction may contribute to cellular hypoxia, even though macrovascular physiology either strives to preserve or is manipulated clinically to maintain global oxygen delivery.[3] Clinical studies have shown that sepsis-induced microvascular dysfunction is associated with organ dysfunction and worse clinical outcomes.[4] Furthermore, in patients with a similar microvascular density and number of perfused vessels after the onset of septic shock, small-vessel perfusion only improved with resolution of shock in eventual survivors.[5]

Microvascular perfusion appears to be relatively independent of global haemodynamic variables in sepsis.[3] Additionally, the microvascular response to therapeutic interventions is often dissociated from systemic effects.[3] However, it remains unclear when this dissociation develops and whether the physiological severity of the septic insult influences microvascular dysfunction. We investigated these questions in a well-characterized fluid-resuscitated rodent model of sepsis that exhibits marked heterogeneous macrovascular and metabolic responses.[6 7] Our underlying hypothesis was that the

uncoupling of macrovascular and microvascular flow occurs independently of the severity and onset of the septic process.

Methods

All experiments were performed in accordance with the United Kingdom Animals (Scientific Procedures) Act (1986) and with local Ethical Committee approval. Adequate depth of anaesthesia was ensured throughout by assessing the stability of arterial pressure, heart rate, lack of flexor responses to a paw-pinch, and maintaining anaesthesia levels at an appropriate MAC.[8]

Adult male Wistar rats (300 g weight) were instrumented under 2% isoflurane anaesthesia with heparinized carotid arterial and jugular venous catheters tunnelled s.c. to emerge at the nape of the neck. Naive rats were not instrumented, serving as negative controls. Rats were block-randomized to sepsis or sham groups. Sepsis was induced by faecal slurry (1.2 g slurry in 1.8 ml saline) injected intraperitoneally. This results in a spectrum of severity that we characterized *a priori* as mild or severe based on our previous work which has demonstrated that stroke volume <0.14 ml at this time point is highly predictive of subsequent mortality.[7] No antibiotics were administered. Buprenorphine (Vetergesic®, Reckitt Benckiser Healthcare Ltd, Hull, UK) was administered s.c. (0.05 mg kg^{-1}) to all animals undergoing surgery. Anaesthesia was then discontinued; the animals were able to move freely, eat and drink while receiving i.v. fluids, and undergo blood sampling (ABL625 analyzer, Radiometer, Copenhagen, Denmark) and continuous arterial pressure monitoring (P23XL transducer, Viggo-Spectramed, Miami, USA) via the tunnelled lines attached to a mobile tether system.[6 7] Pressure readings were analysed using Chart 5 acquisition/analysis software (AD Instruments, Oxford, UK).

Two hours after injection of slurry and in sham-operated controls, an i.v. fluid infusion consisting of a 1:1 solution of 6% hetastarch (Elohaes®, Fresenius Kabi, Warrington, UK) and 5% glucose (Baxter Healthcare, Thetford, UK) was commenced at 10 ml kg^{-1} h^{-1} and continued throughout. A further 4 h later (6 h post-onset of sepsis), the animals were re-anaesthetized with 1.4% isoflurane, enabling echocardiographic and microvascular imaging.

Echocardiography (Vivid 7, GE Healthcare, Bedford, UK) imaged the left parasternal long- and short-axis views at the papillary muscle level. Stroke volume was calculated from flow measured in the ascending aorta (radius 1.3 mm) by pulsed-wave Doppler. We previously reported that inter- and intra-observer variability is $<10\%$.[7] All microvascular recordings were made before transthoracic echocardiography measurements. The microvascular network of the left vastus lateralis muscle was visualized using a sidestream dark-field videomicroscopy system (MicroScan™, Microvision Medical Inc., Amsterdam, The Netherlands). The lens of the imaging device was covered with a disposable sterile cap and applied without pressure to the muscle. The absence of pressure was defined by preservation of venular perfusion, the presence of which precludes subsequent off-line analysis. At least five recordings from different areas, of minimum duration 20 s, were recorded. The images were then stored under a random number for later analysis using AVA 3.0 (automated vascular analysis) software (Microvision Medical Inc.). An investigator blinded to group allocation and time subsequently analysed these sequences semi-quantitatively according to consensus guidelines,[9] adhering to the five key points for optimal image acquisition. Further verification of these analyses was conducted by an additional blinded investigator. Flow was defined as continuous, intermittent, or absent. To compute the microvascular flow index (MFI),[10] vessels with continuous flow were further divided into normal and sluggish. Vessel size was determined using a micrometer scale and separated into large and small vessels, using a diameter cut-off value of 20 μm. Small-vessel perfusion was defined as the proportion of small perfused vessels (PPV) and calculated as the number of capillaries continuously perfused during the 20 s observation period divided by the total number of vessels of the same type. In addition to the MFI, the De Backer score was also determined by the proportion of perfused vessels [PPV (%)] and perfused vessel density.[11] Vessel density was calculated as the number of vessels crossing defined gridlines divided by the total length of the lines. The heterogeneity index for MFI was also calculated.[9 12] This is determined as the highest site flow velocity minus the lowest site flow velocity divided by the mean of the flow velocities across all measurement sites.[12]

Statistical analyses

Data are presented as median and inter-quartile range (IQR), unless stated otherwise. Continuous variable differences between groups were assessed using the Kruskal–Wallis test, followed by *post hoc* Bonferroni–Dunn Z testing for multiple comparisons. Categorical data (comparing qualitative flow characteristics) were analysed by Fisher's exact test. *P*-values <0.05 were considered significant (NCSS 2007, Kaysville, UT, USA).

Results

As demonstrated previously,[7] sepsis resulted in two distinct macrovascular phenotypes, despite a standardized fluid resuscitation regimen and similar mean arterial pressures (Fig. 1A). In mild sepsis, cardiac output was 19 (12–19)% lower than sham-operated values (Fig. 1D) with no difference in heart rate (Fig. 1B). In contrast, severely septic rats exhibited marked reductions in stroke volume [−63 (62–71)% from sham values; $P=0.0003$; Fig. 1C] and cardiac output [−62 (54–66)%; $P=0.0002$; Fig. 1D], accompanied by tachycardia ($P<0.0001$; Fig 1B). Cardiac/macrovascular function was similar between naive and sham-operated rats.

Microvascular imaging, performed before echocardiographic measurements were recorded, revealed that the MFI ($P<0.0001$) was impaired in sepsis and in sham-operated controls (Fig. 2A). Microvascular flow changes in

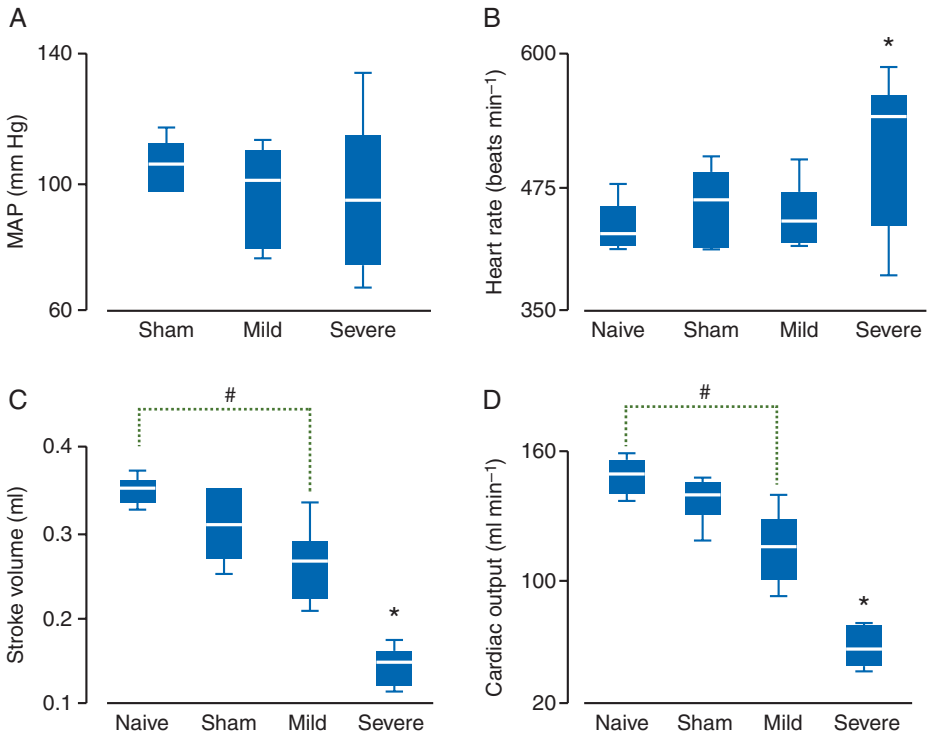

Fig 1 Macrovascular phenotype in naive, sham-operated, and septic rats. (A) Mean arterial pressure. (B) Heart rate. (C) Stroke volume. (D) Cardiac output. All data (measured at 6 h post-surgery) are displayed as median (IQR). *Difference ($P<0.01$; the Kruskal–Wallis test) between severe sepsis and other conditions; #Differences ($P<0.01$; the Kruskal–Wallis test) between naive and mild septic rats.

mild and severe sepsis were similar ($P=0.69$), regardless of the degree of impaired macrovascular function (Fig. 2A). Calculation of the MFI heterogeneity index revealed that only naive rats displayed a normal microvascular flow pattern (Fig. 2B). No differences in perfused small-vessel density ($P=0.36$; Fig. 2C), the proportion of perfused small vessels ($P=0.33$; data not shown), or the De Backer score ($P=0.08$; Fig. 2D), were observed between naive, sham-operated, and septic rats.

Arterial blood-gas analysis revealed marked haemoconcentration, hyperlactataemia, and abnormal acid–base status in severely septic animals only ($P<0.001$; Table 1). Severely septic animals also showed higher Pa_{O_2} and lower Pa_{CO_2} levels compared with sham-operated controls ($P<0.001$; Table 1). Values obtained in mildly septic animals were similar to those observed in sham-operated animals (Table 1).

Discussion

These data show that microvascular alterations are uncoupled from macrovascular function early in a clinically relevant model of sepsis. Importantly, surgical intervention alone causes significant microvascular derangement, highlighting the importance of adequate control subjects to dissect the relative contributions of sepsis/inflammation to microvascular function. These data support previous similar findings using intravital microscopy[13 14] but importantly extend those findings by the concomitant measurement of macrovascular performance, as assessed by transthoracic echocardiography.

The chief purpose of this study was to establish whether microvascular dysfunction was a severity-related feature of early sepsis. The vastus lateralis muscle was chosen for ease of access, stability of recordings, consistent anatomic localization, and a microvascular architecture that lends itself to flow assessment. We do acknowledge that microvascular changes in this muscle bed may not reflect other important vascular beds and also the inherent limitations of the sidestream dark-field imaging technology, as discussed in detail elsewhere.[5] The macrovascular heterogeneity of the faecal slurry model, despite a standardized fluid resuscitation regimen, affords important insights into potential mechanisms underlying microvascular changes during early sepsis. Despite severely septic rats sustaining a profoundly reduced stroke volume, impaired cardiac output, increased capillary leak, and consequently marked haemoconcentration, the microvascular profile was similar to mildly septic rats in which metabolic acidosis and lactataemia were absent.

Clearly, a disconnect exists between the macrocirculation and microcirculation during sepsis. Arterial pressure was preserved in this model, negating a possible contribution of

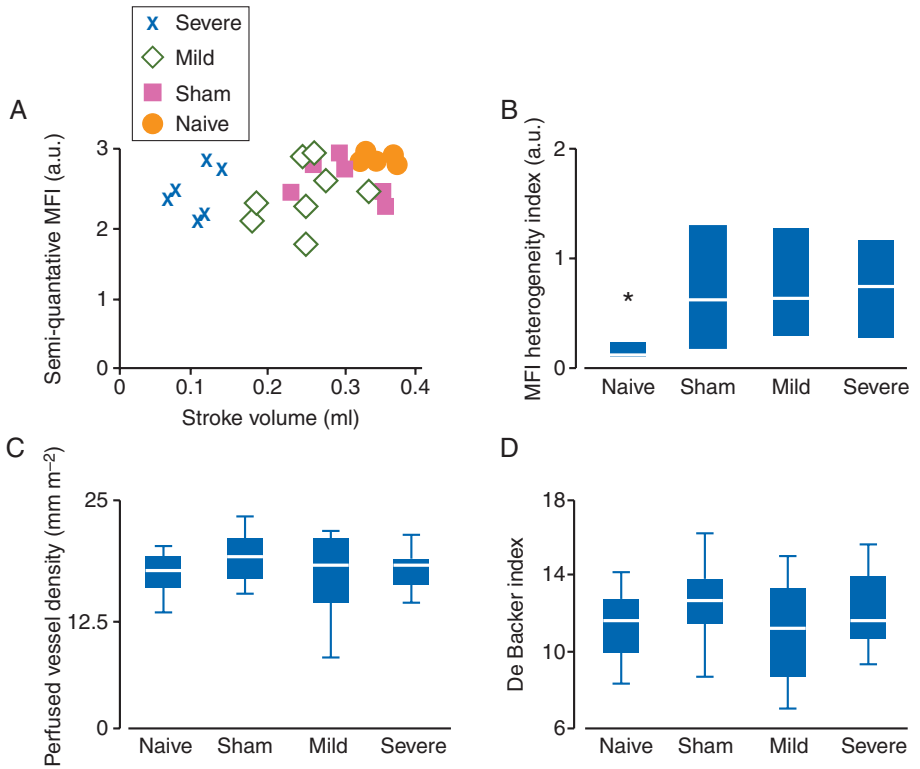

Fig 2 Microvascular phenotype in naive, sham-operated, and septic rats, measured at 6 h post-surgery. (A) Raw MFI scores in relation to stroke volume (R^2=0.13; P=0.08; Spearman's rank correlation test). (B) Microvascular flow heterogeneity index. (C) Perfused small-vessel density. (D) The De Backer score. Data displayed as (A) raw data, (B–D) [median (IQR)]. *Difference ($P<0.05$, the Kruskal–Wallis test) between naive and other treatment groups.

Table 1 Arterial blood gas analysis. All data are displayed as median (IQR). *$P\leq0.001$ (the Kruskal–Wallis test) *vs* sham

	Naive	Sham	Mild sepsis	Severe sepsis
n	3	6	8	6
pH	7.44 (7.38–7.45)	7.44 (7.37–7.47)	7.49 (7.47–7.51)	7.44 (7.35–7.47)
Pa_{CO_2} (kPa)	5.1 (4.9–5.7)	4.7 (4.6–5.6)	4.6 (4.2–4.6)	2.5* (2.4–3.6)
Pa_{O_2} (kPa)	11 (10.6–11.3)	9.8 (9.6–11.1)	11.7 (11.1–12.6)	13.4* (12.6–14.5)
Haemoglobin (g dl^{-1})	13.1 (13.0–13.2)	13.8 (13.2–14.0)	13.8 (13.6–13.9)	19.2* (18.2–20.7)
Glucose (mmol $litre^{-1}$)	11.6 (10.8–11.7)	9.1 (6.8–10.7)	9.1 (8.9–10.0)	6.0* (5.8–7.4)
Arterial base excess (mmol $litre^{-1}$)	1.8 (0.2–1.9)	1.4 (−0.4–2.0)	1.6 (−0.7–3.1)	−10.7* (−12.1 to −7.7)
Arterial lactate (mmol $litre^{-1}$)	1.9 (1.9–2.1)	1.8 (1.5–1.8)	2.2 (2.0–2.2)	3.8* (3.0–4.5)

altered perfusion pressure in driving any of the microvascular changes observed. Previous clinical work has reported that targeting a higher mean arterial pressure through increasing the dose of norepinephrine may result in an increase in global oxygen delivery and tissue oxygenation, but this was accompanied by highly variable inter-individual changes in pre-existing abnormalities of sublingual microvascular flow.[15 16] Administration of dobutamine to septic shock patients resulted in partial restoration of capillary perfusion that was not related to cardiac index or arterial pressure.[17] The authors concluded that the effect of this drug on the microcirculation was independent of its systemic effects. Taken together, these observations strongly suggest that the microvascular phenotype of early sepsis does not reflect either macrovascular or global metabolic derangement.

The first clinical study examining the sublingual microcirculation found no difference in microvascular blood flow in patients before cardiac surgery, intensive care unit control subjects, and healthy volunteers.[4] In contrast, patients with severe sepsis exhibited a decrease in vessel density and the proportion of perfused small vessels when compared with healthy volunteers.[4] These microvascular abnormalities

were also found to be severity-related, being more severe in eventual non-survivors. This phenomenon has since been confirmed by others [12 15 18] with small-vessel perfusion only improving with resolution of shock in eventual survivors.[5] While these studies have generated much enthusiasm towards videomicroscopy of the microcirculation during sepsis, they necessarily assume no influence from underlying co-morbidities that are strongly associated with primary microvascular abnormalities, such as diabetes and essential hypertension.[19] In addition, uncertainty regarding the precise time of onset of sepsis also highlights an important contribution of tightly controlled laboratory models towards temporal characterization of videomicroscopy-derived microvascular alterations.

In addition to sepsis, clinical studies have also shown microvascular abnormalities in patients after cardiac arrest,[20 21] those with severe heart failure,[22] and cardiac surgery patients with or without cardiopulmonary bypass.[23] A recent study also identified that microvascular abnormalities are commonplace after elective abdominal surgery, despite adequate fluid resuscitation and normal macrovascular variables.[24] We also found that surgical cannulation of major vessels and subsequent tethering results in notable microvascular changes. These observations strongly suggest that surgical procedures alone influence perfusion at the microvascular level.[25] It is striking that the administration of propofol has prolonged effects on microvascular function well beyond the period of its administration,[26] suggesting a range of potential mechanisms that may account for microvascular derangement independent of inflammatory changes.[27] Our observations may reflect an important contribution of sympathetic-mediated changes in vasomotor tone. Furthermore, the production of the vasodilator nitric oxide[28] via endothelial and inducible nitric oxide synthase resulting from anaesthesia[29] and/or inflammatory mechanisms[30] may be critical.

In summary, our data highlight that dissection of mechanisms underlying microvascular dysfunction in sepsis can be facilitated by utilizing a highly characterized model of sepsis. Notably, differences in microvascular function only become apparent when naive subjects are also assessed, as highlighted before by intravital microscopy studies. This is an important technical observation that highlights the necessity of adequate negative control subjects when using this technique in laboratory and human studies. The uncoupling of microvascular function from both macrovascular and metabolic changes also suggest that interventions beyond the manipulation of cardiac output and oxygen delivery warrant further investigation in both laboratory and clinical settings.

Declaration of interest

None declared.

Funding

Academy Medical Sciences/Health Foundation Clinician Scientist Award (G.L.A.) and Centre for Anaesthesia, Pain Management and Critical Care, University College London (S.C.). This work was undertaken at UCLH/UCL, which received a proportion of funding from the Department of Health's NIHR Biomedical Research Centres funding scheme.

References

1 Ince C. The microcirculation is the motor of sepsis. *Crit Care* 2005; **9**: S13–9
2 Ince C. The microcirculation unveiled. *Am J Respir Crit Care Med* 2002; **166**: 1–2
3 De Backer D, Ortiz JA, Salgado D. Coupling microcirculation to systemic hemodynamics. *Curr Opin Crit Care* 2010; **16**: 250–4
4 De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. *Am J Respir Crit Care Med* 2002; **166**: 98–104
5 Sakr Y, Dubois MJ, De Backer D, Creteur J, Vincent JL. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 2004; **32**: 1825–31
6 Brealey D, Karyampudi S, Jacques TS, *et al.* Mitochondrial dysfunction in a long-term rodent model of sepsis and organ failure. *Am J Physiol Regul Integr Comp Physiol* 2004; **286**: R491–7
7 Dyson A, Rudiger A, Singer M. Temporal changes in tissue cardiorespiratory function during faecal peritonitis. *Intensive Care Med* 2011; **37**: 1192–200
8 de Wolff MH, Leather HA, Wouters PF. Effects of tramadol on minimum alveolar concentration (MAC) of isoflurane in rats. *Br J Anaesth* 1999; **83**: 780–3
9 De Backer D, Hollenberg S, Boerma C, *et al.* How to evaluate the microcirculation: report of a round table conference. *Crit Care* 2007; **11**: R101
10 Boerma EC, Mathura KR, van der Voort PH, Spronk PE, Ince C. Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. *Crit Care* 2005; **9**: R601–6
11 Elbers PW, Ince C. Bench-to-bedside review: mechanisms of critical illness - classifying microcirculatory flow abnormalities in distributive shock. *Crit Care* 2006; **10**: 221
12 Trzeciak S, Dellinger RP, Parrillo JE, *et al.* Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. *Ann Emerg Med* 2007; **49**: 88–98
13 Lam C, Tyml K, Martin C, Sibbald W. Microvascular perfusion is impaired in a rat model of normotensive sepsis. *J Clin Invest* 1994; **94**: 2077–83
14 Piper RD, Pitt-Hyde M, Li F, Sibbald WJ, Potter RF. Microcirculatory changes in rat skeletal muscle in sepsis. *Am J Respir Crit Care Med* 1996; **154**: 931–7
15 Jhanji S, Stirling S, Patel N, Hinds CJ, Pearse RM. The effect of increasing doses of norepinephrine on tissue oxygenation and microvascular flow in patients with septic shock. *Crit Care Med* 2009; **37**: 1961–6
16 Dubin A, Pozo MO, Casabella CA, *et al.* Increasing arterial blood pressure with norepinephrine does not improve microcirculatory blood flow: a prospective study. *Crit Care* 2009; **13**: R92
17 De Backer D, Creteur J, Dubois MJ, *et al.* The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. *Crit Care Med* 2006; **34**: 403–8

18 Spanos A, Jhanji S, Vivian-Smith A, Harris T, Pearse RM. Early microvascular changes in sepsis and severe sepsis. *Shock* 2010; **33**: 387–91

19 De Boer MP, Meijer RI, Wijnstok NJ, *et al.* Microvascular dysfunction: a potential mechanism in the pathogenesis of obesity-associated insulin resistance and hypertension. *Microcirculation* 2012; **19**: 5–18

20 den Uil CA, Lagrand WK, Spronk PE, *et al.* Impaired sublingual microvascular perfusion during surgery with cardiopulmonary bypass: a pilot study. *J Thorac Cardiovasc Surg* 2008; **136**: 129–34

21 Donadello K, Favory R, Salgado-Ribeiro D, *et al.* Sublingual and muscular microcirculatory alterations after cardiac arrest: a pilot study. *Resuscitation* 2011; **82**: 690–5

22 De Backer D, Creteur J, Dubois MJ, Sakr Y, Vincent JL. Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. *Am Heart J* 2004; **147**: 91–9

23 De Backer D, Dubois MJ, Schmartz D, *et al.* Microcirculatory alterations in cardiac surgery: effects of cardiopulmonary bypass and anesthesia. *Ann Thorac Surg* 2009; **88**: 1396–403

24 Jhanji S, Lee C, Watson D, Hinds C, Pearse RM. Microvascular flow and tissue oxygenation after major abdominal surgery: association with post-operative complications. *Intensive Care Med* 2009; **35**: 671–7

25 Vellinga NA, Ince C, Boerma EC. Microvascular dysfunction in the surgical patient. *Curr Opin Crit Care* 2010; **16**: 377–83

26 Koch M, De Backer D, Vincent JL, Barvais L, Hennart D, Schmartz D. Effects of propofol on human microcirculation. *Br J Anaesth* 2008; **101**: 473–8

27 Segal SS. Regulation of blood flow in the microcirculation. *Microcirculation* 2005; **12**: 33–45

28 Bateman RM, Sharpe MD, Ellis CG. Bench-to-bedside review: microvascular dysfunction in sepsis—hemodynamics, oxygen transport, and nitric oxide. *Crit Care* 2003; **7**: 359–73

29 Brookes ZL, Brown NJ, Reilly CS. Intravenous anaesthesia and the rat microcirculation: the dorsal microcirculatory chamber. *Br J Anaesth* 2000; **85**: 901–3

30 McGown CC, Brookes ZL. Beneficial effects of statins on the microcirculation during sepsis: the role of nitric oxide. *Br J Anaesth* 2007; **98**: 163–75