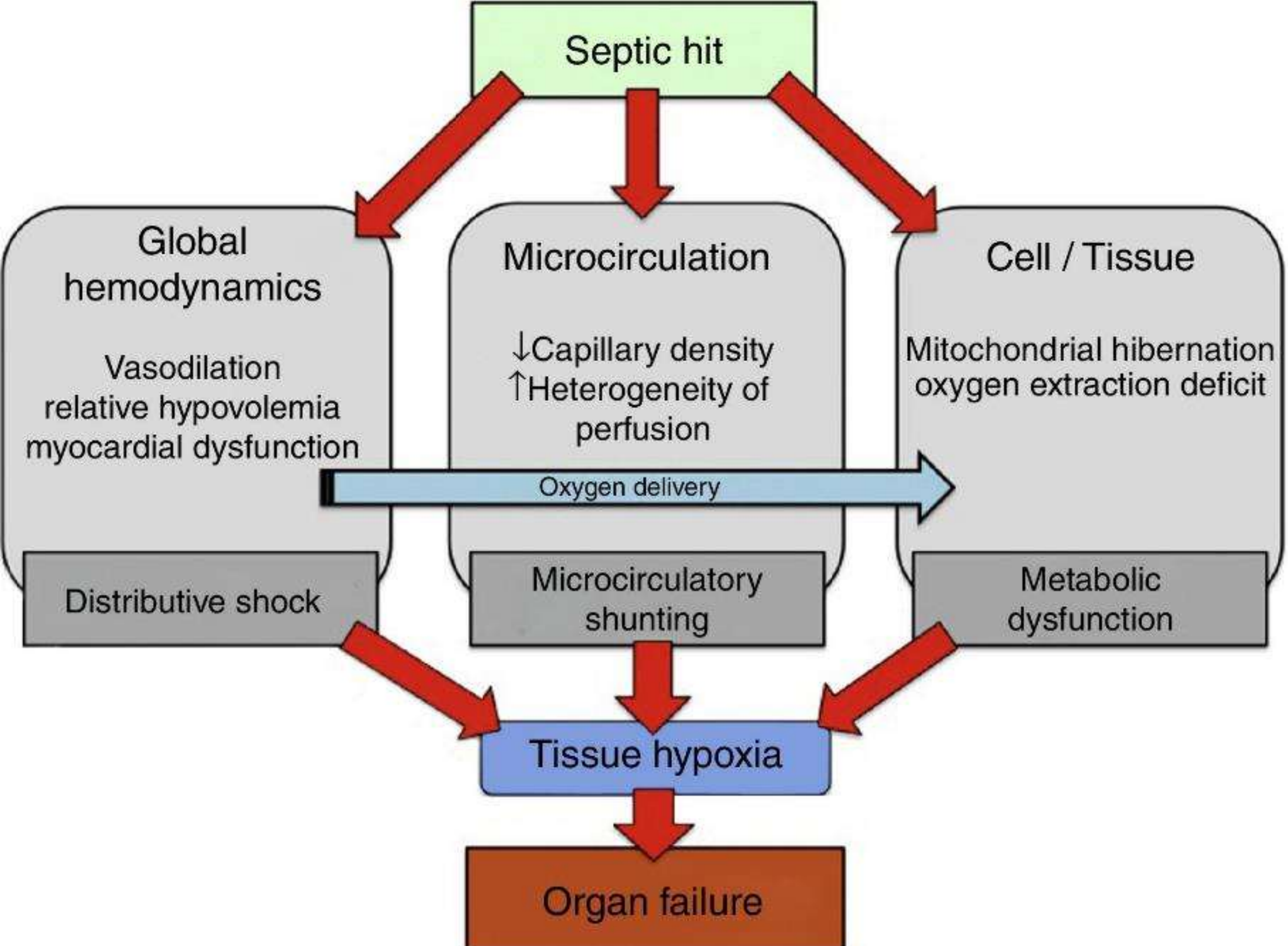
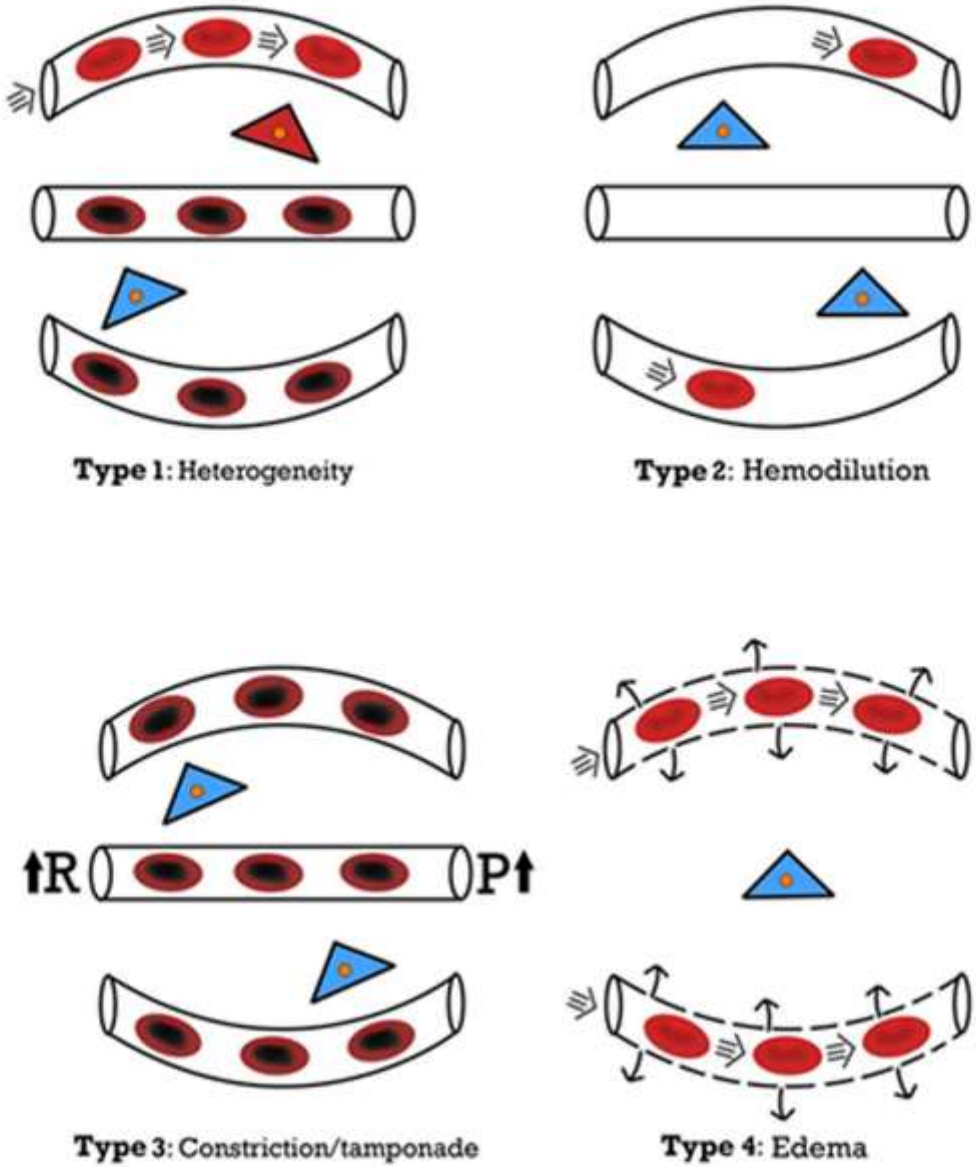
Microcirculation in Sepsis
physiology, targets and strategies

Puneet Saxena


Septic hit
Global hemodynamics
Vasodilation
relative hypovolemia
myocardial dysfunction
Microcirculation
↓Capillary density
↑Heterogeneity of perfusion
Cell / Tissue
Mitochondrial hibernation
oxygen extraction deficit
Oxygen delivery
Distributive shock
Microcirculatory shunting
Metabolic dysfunction
Tissue hypoxia
Organ failure

Loss of Hemodynamic coherence

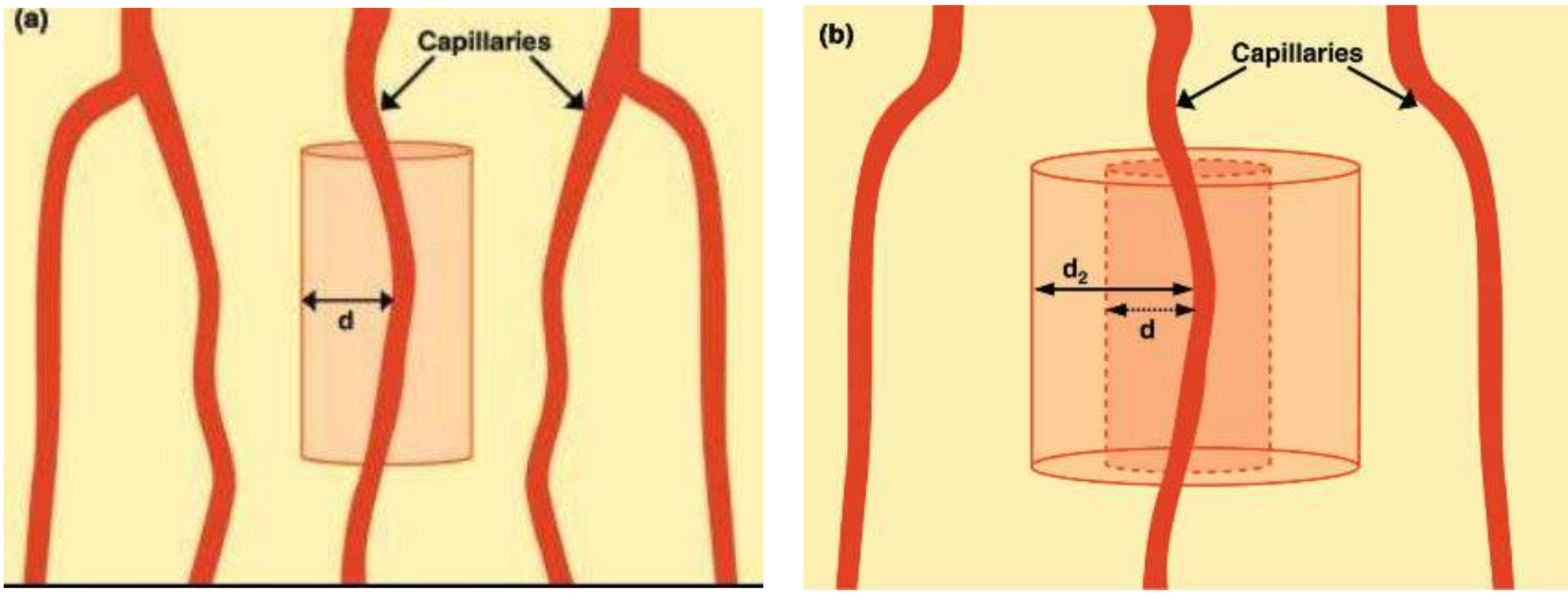


Kara et al. Curr Opin Crit Care 2016

Tissue perfusion

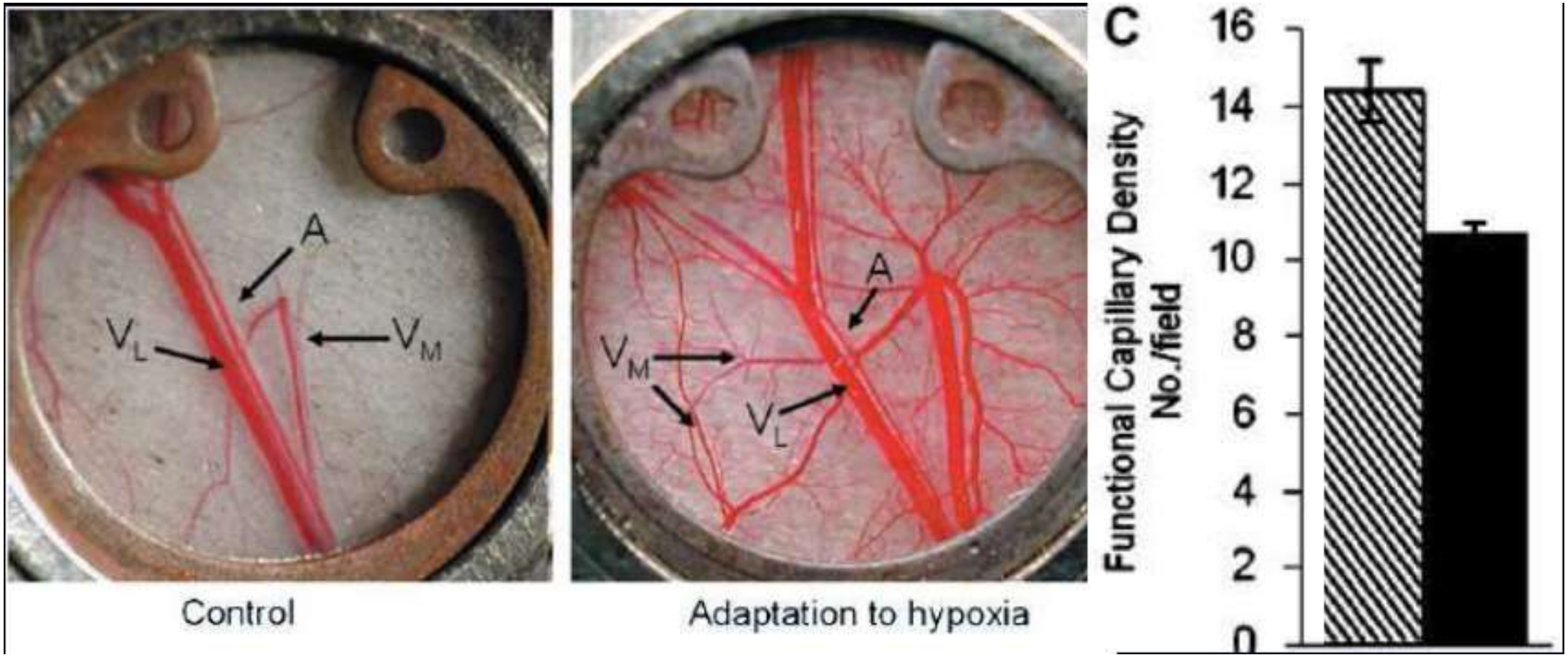
- Microcirculatory circulation is a key determinant of tissue perfusion
- Under control of different mechanisms than systemic haemodynamics
- O2 transport is driven at microcirculatory level by diffusion more than convection

The density of capillaries is primary determinant of tissue oxygenation



Trzeciak et al. Crit Care 2005

Adaptation to chronic hypoxia is characterized by an increased capillary density



= 1 week 10% → Chamber → 1 week 5% → 24 h 21% → Experiment

= 1 week 21% → Chamber → 1 week 21% → 24 h 21% → Experiment

Microcirculatory changes in the window chamber preparation in Syrian golden hamsters, secondary to chronic hypoxia adaptation

Saldivar-E et al. AJP. 2003

Determinants of microvascular blood flow

$$Q = \frac{\pi P r^4}{8 \eta l}$$

- Blood flow is adapted to local metabolic needs through local vasodilation and upstream changes in vasomotor tone

- Diameter of the vessel is more important than the pressure drop

- Viscosity has an important role

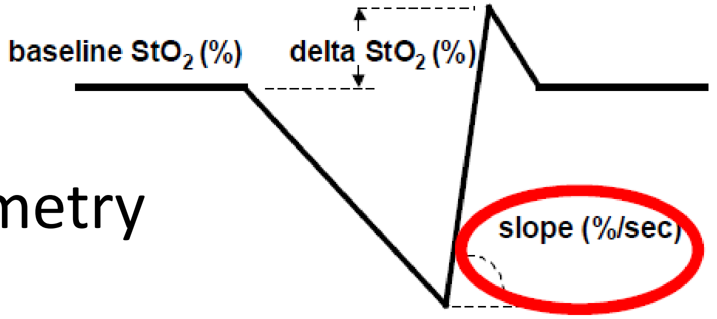
Local regulation of microcirculation

- Neural control –Perivascular sympathetic nerves
- Electrical control - Endothelial cells cross-talk

Hungerford-JE et al. FASEB. 2000

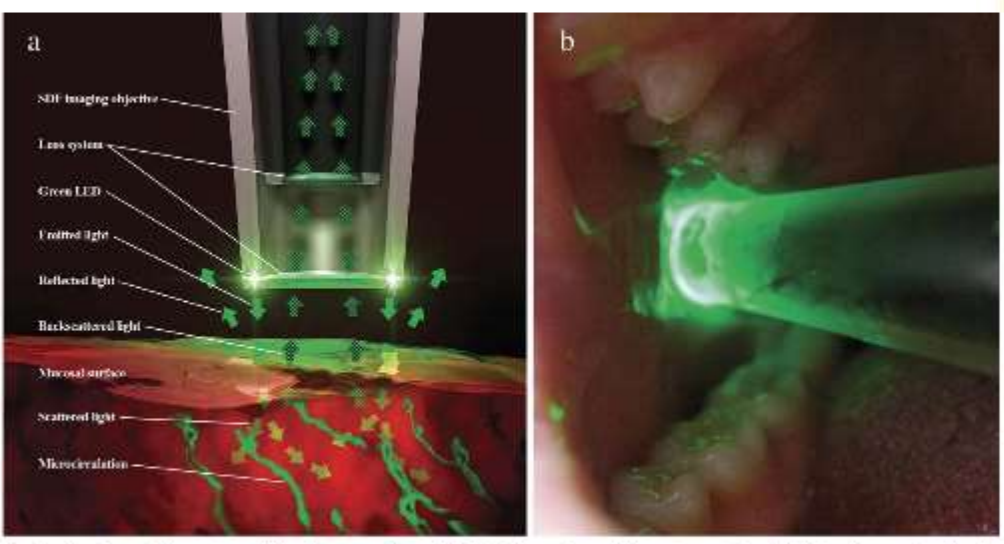
Evaluation of microcirculation

- Videomicroscopy techniques
  - Intravital microscopy
  - Hand-Held Vital Microscopes
- Tissue oxygenation evaluation techniques
  - Nearinfrared spectroscopy (NIRS)
- PCO2-based evaluation techniques
- Lesser-used
  - Laser-Doppler flowmetry
  - Tissue reflectance spectrophotometry



Hand-held vital microscopes (HVMs)

1. Orthogonal polarization spectral (OPS) imaging
2. Sidestream dark-field (SDF) imaging
3. Incident dark-field (IDF) imaging

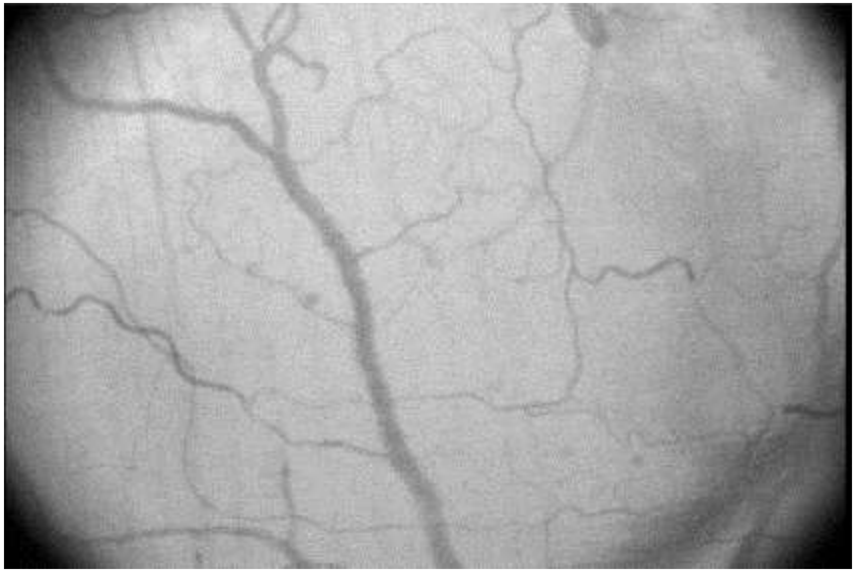


Microcirculatory alterations in sepsis

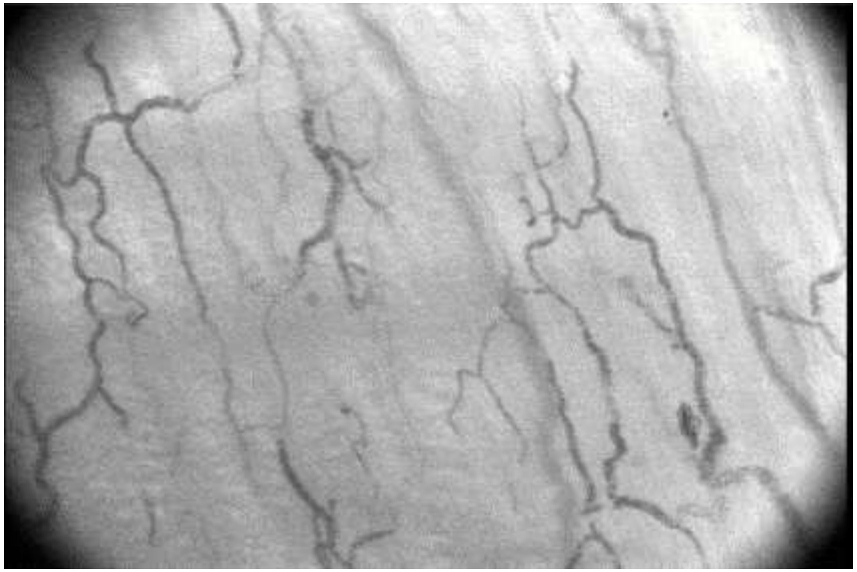
- Reduced capillary density

Gut serosa: Pigs

Normal



Sepsis

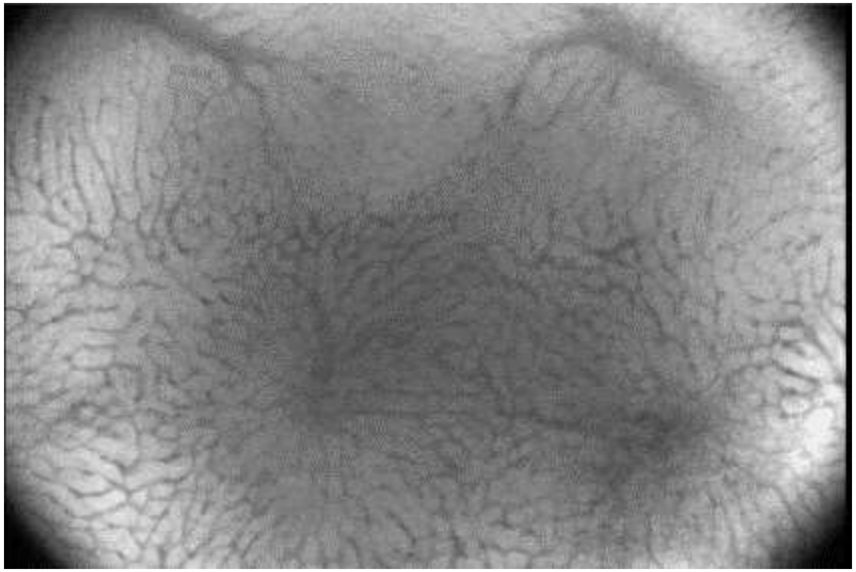


OPS, 5x probe

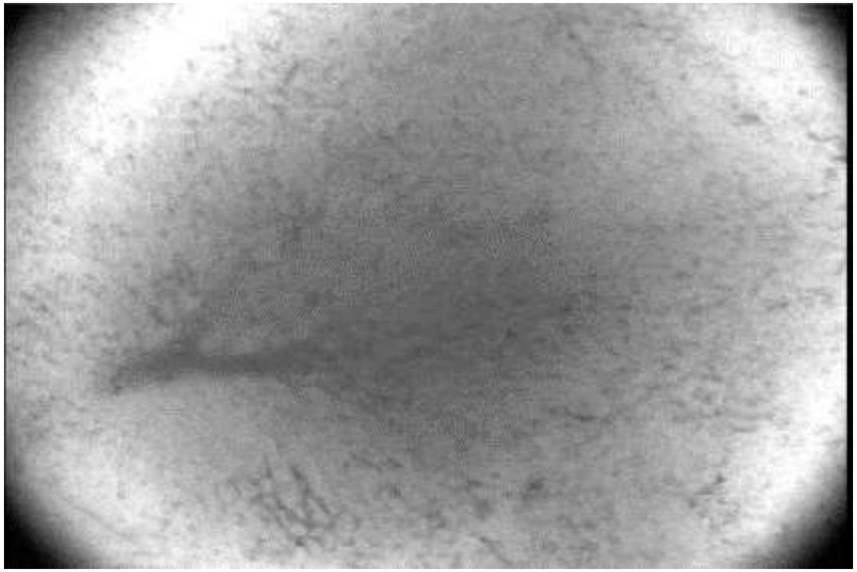
Farquhar I,et al. J Surg Res 1996

Liver : Pigs

Normal



Sepsis

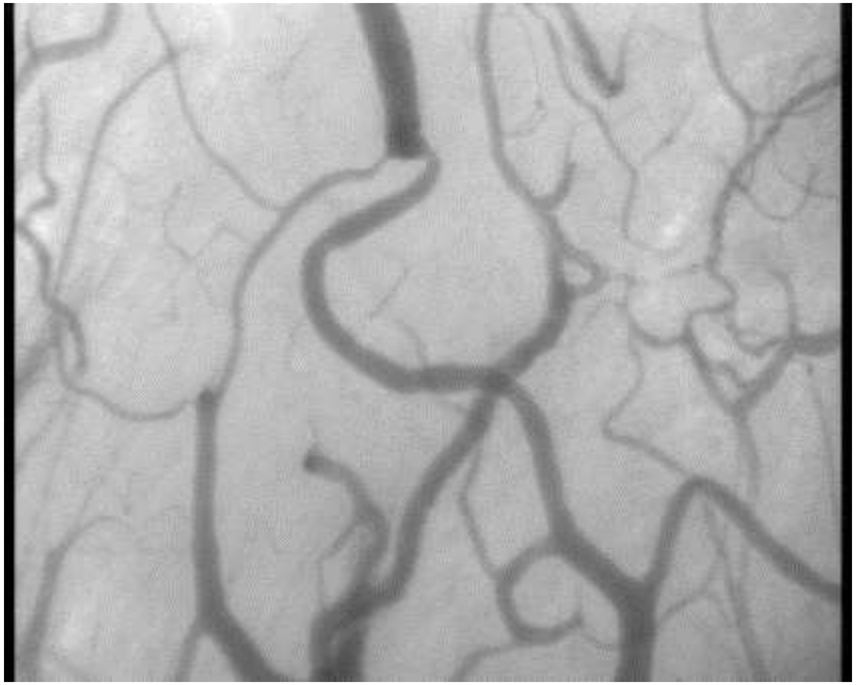


OPS, 5x probe

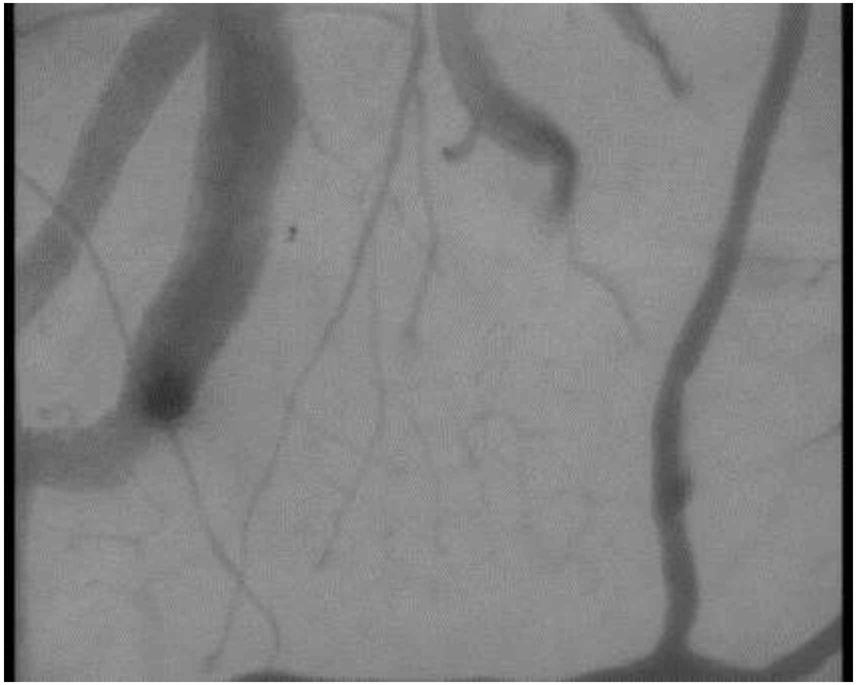
Lam CJ, et al.. J Clin Invest 1994

Brain : Sheep

Normal



Sepsis



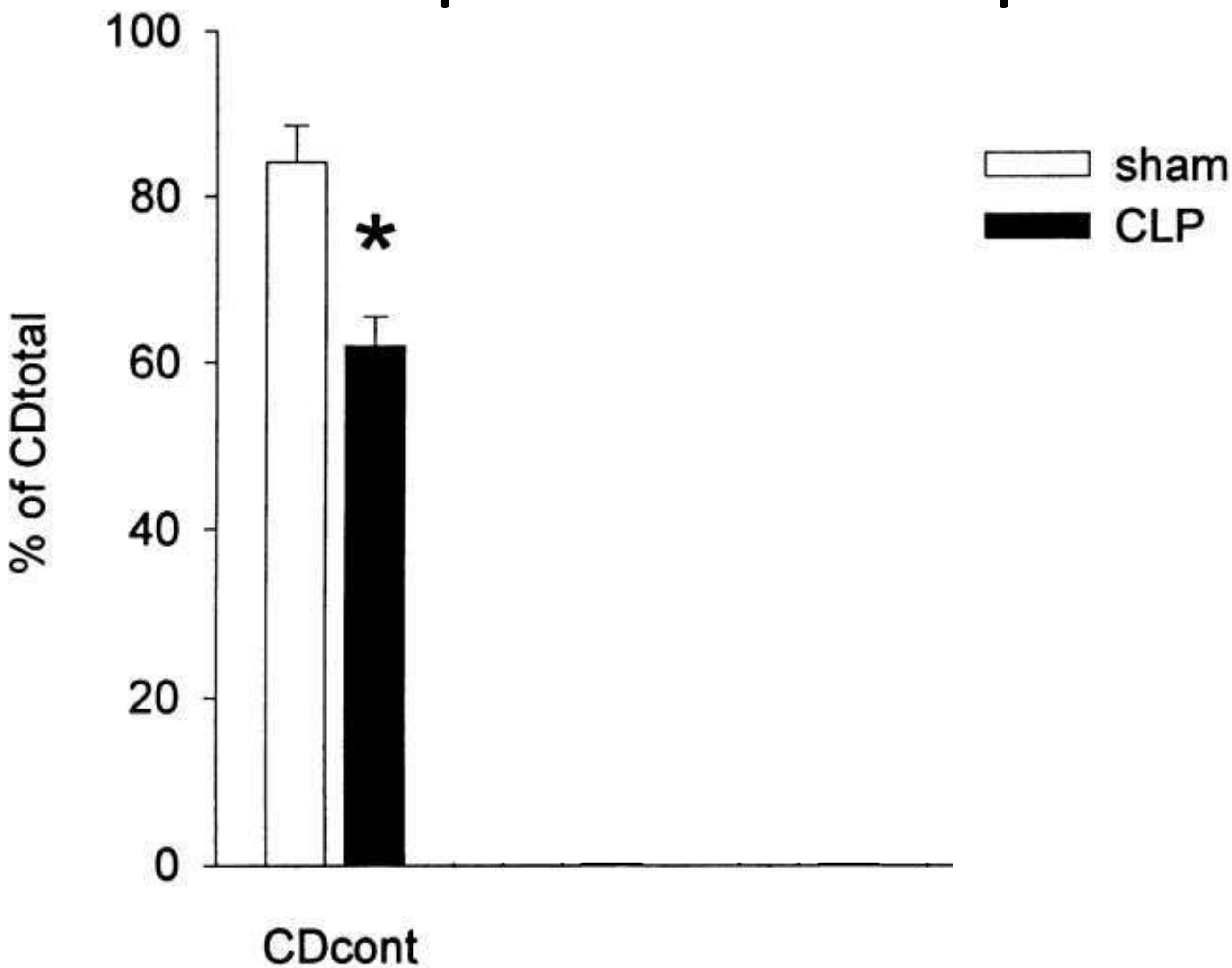
SDF, 5x probe

Taccone FS, et al. Crit Care Med. 2014

Microcirculatory alterations in sepsis

- Reduced capillary density
- Heterogeneity of perfusion

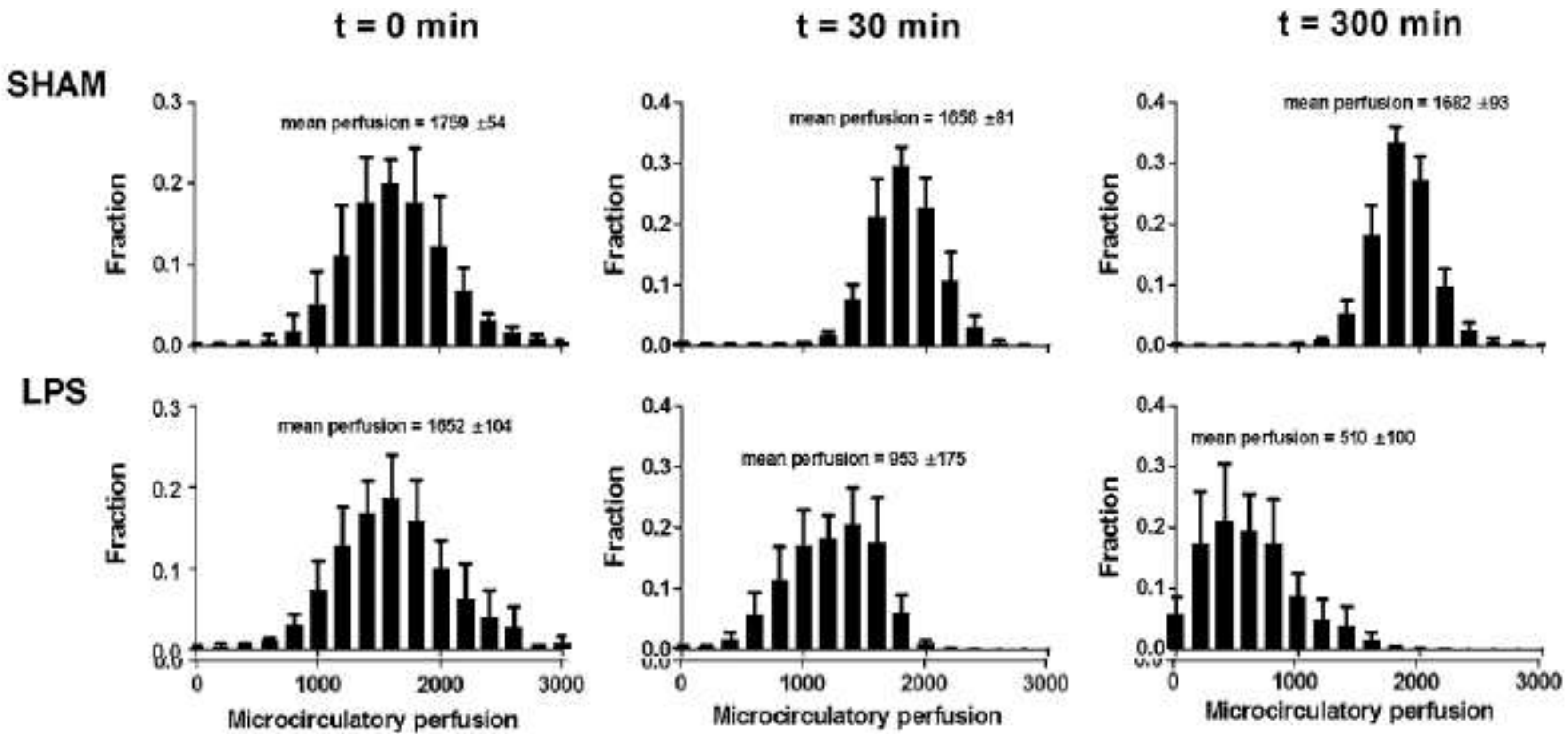
Heterogeneity of capillary perfusion in experimental sepsis



Rats; CLP; EDL; intravital microscopy

Ellis C et al. AJP. 2002

Increase heterogeneity of renal perfusion in sepsis



Rats; LPS; laser speckle imaging

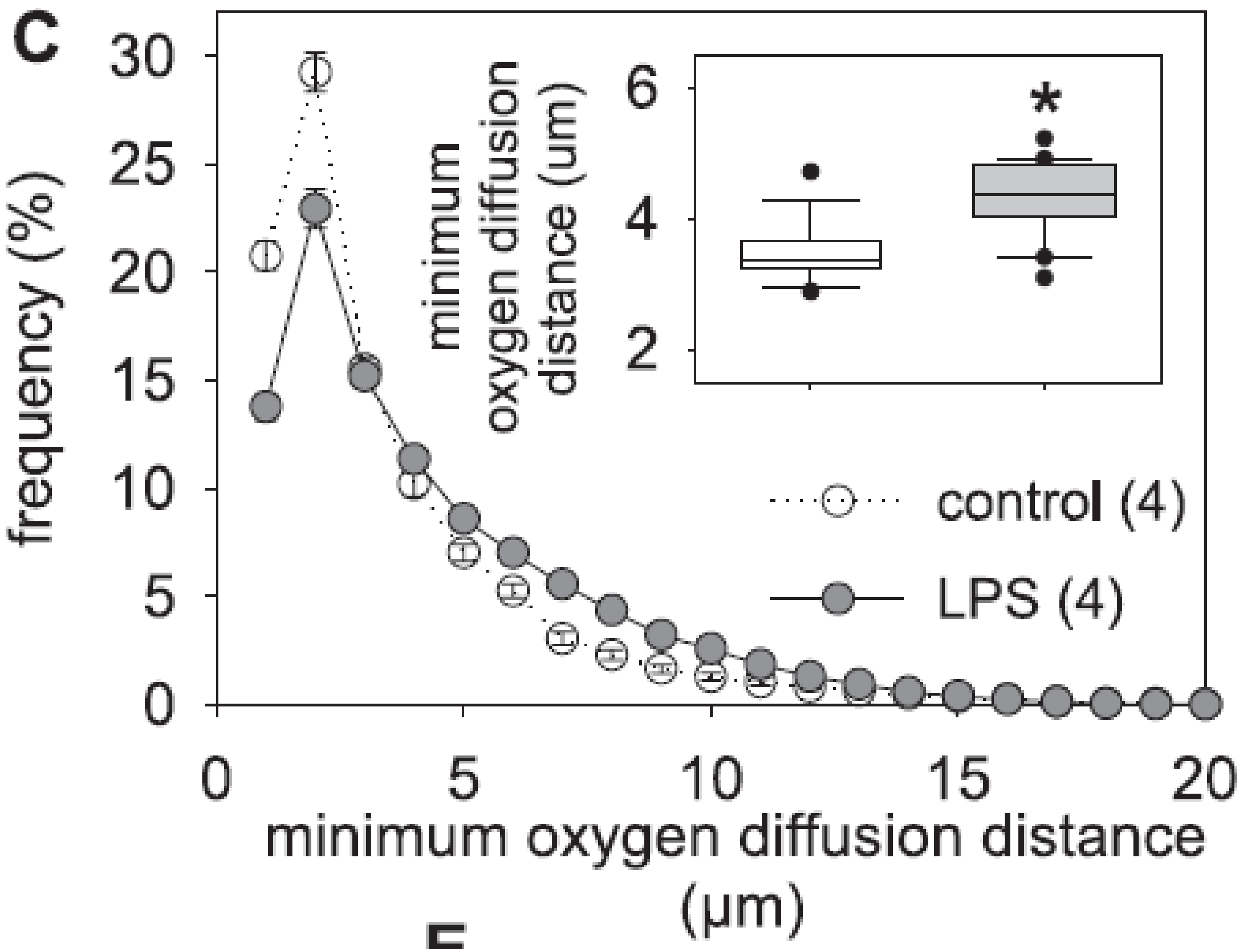
Legrand et al. ICM 2011

Microcirculatory alterations in sepsis

- Reduced capillary density
- Heterogeneity of perfusion
  - Increased areas of “no-flow” and hyperperfusion
  - Demonstrated in all organs
  - Increases when the system is challenged

Consequences?

Tissue hypoxia



Rats, LPS; myocardium; laser-scanning confocal microscopy

Bateman RM et al. Am J Physiol Heart Circ Physiol. 2007

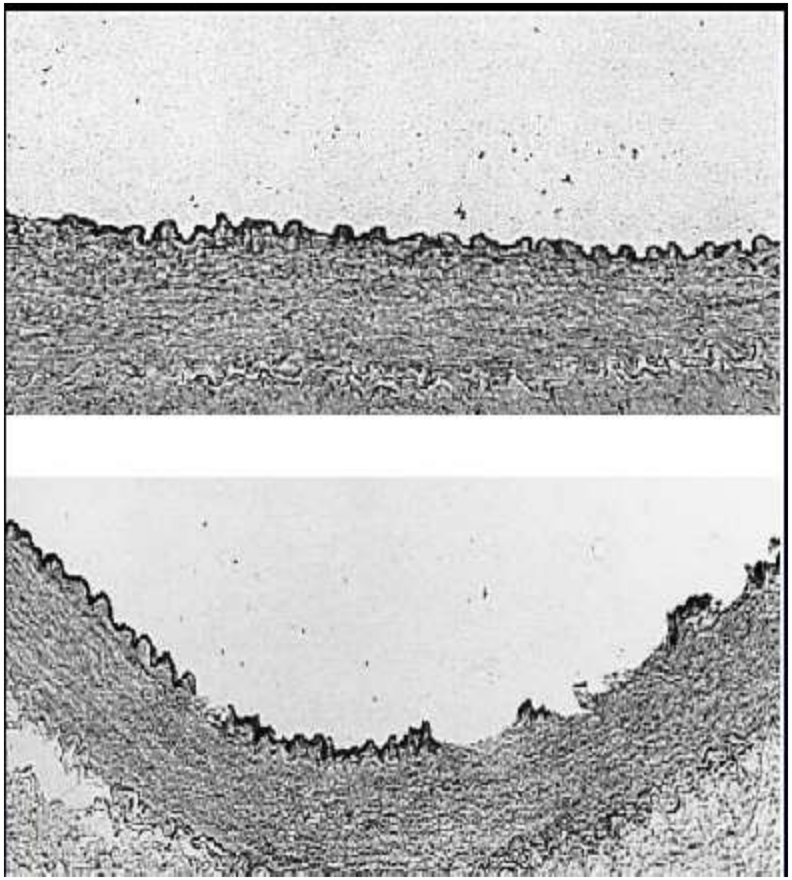
Mechanisms

Mechanisms

- Triggered by inflammatory mediators
  - TNF

Vicaut E JCI 87:1537;1991

ENDOTHELIAL ALTERATIONS

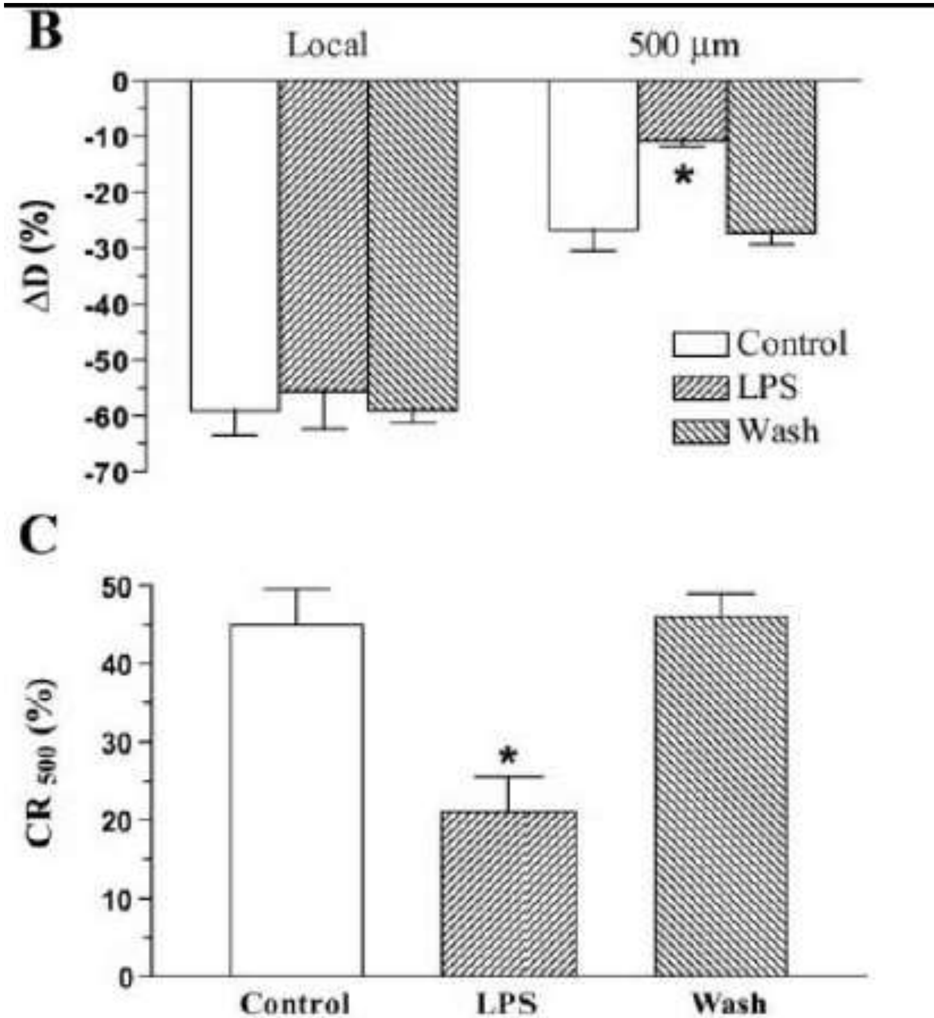


Endothelium of rabbit abdominal aorta stained with PECAM-1 antibodies at
a, control time
b, 5 days after LPS injection (magnification, x40)

Leclerc J et al. CCM. 2000

Loss of neural control

Direct effect of LPS; not inflammatory mediators

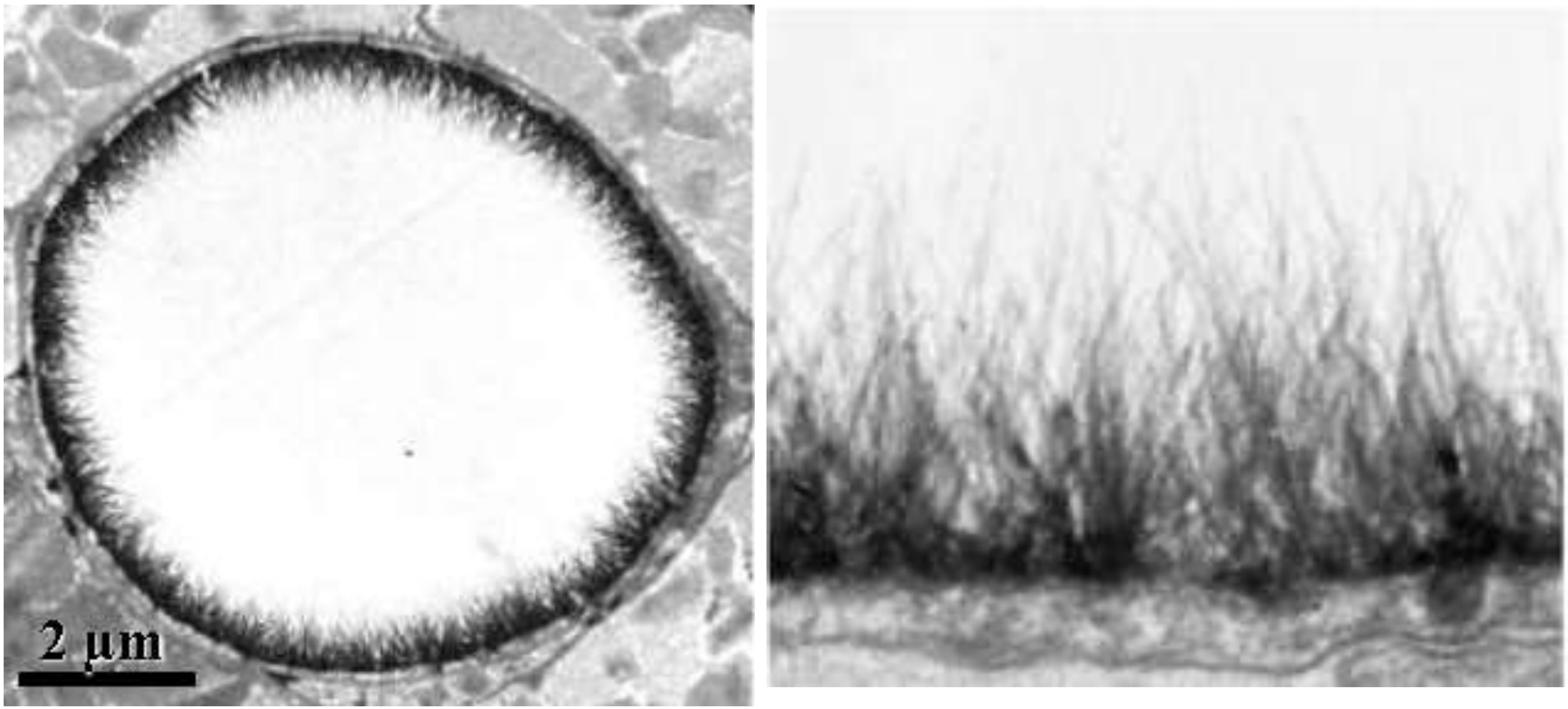


Change in diameter and communication rate (CR500) between 500 µm distant microvessels
(retrograde communication)

Cremaster muscle (mice)

Tyml-K et al. AJP. 2001

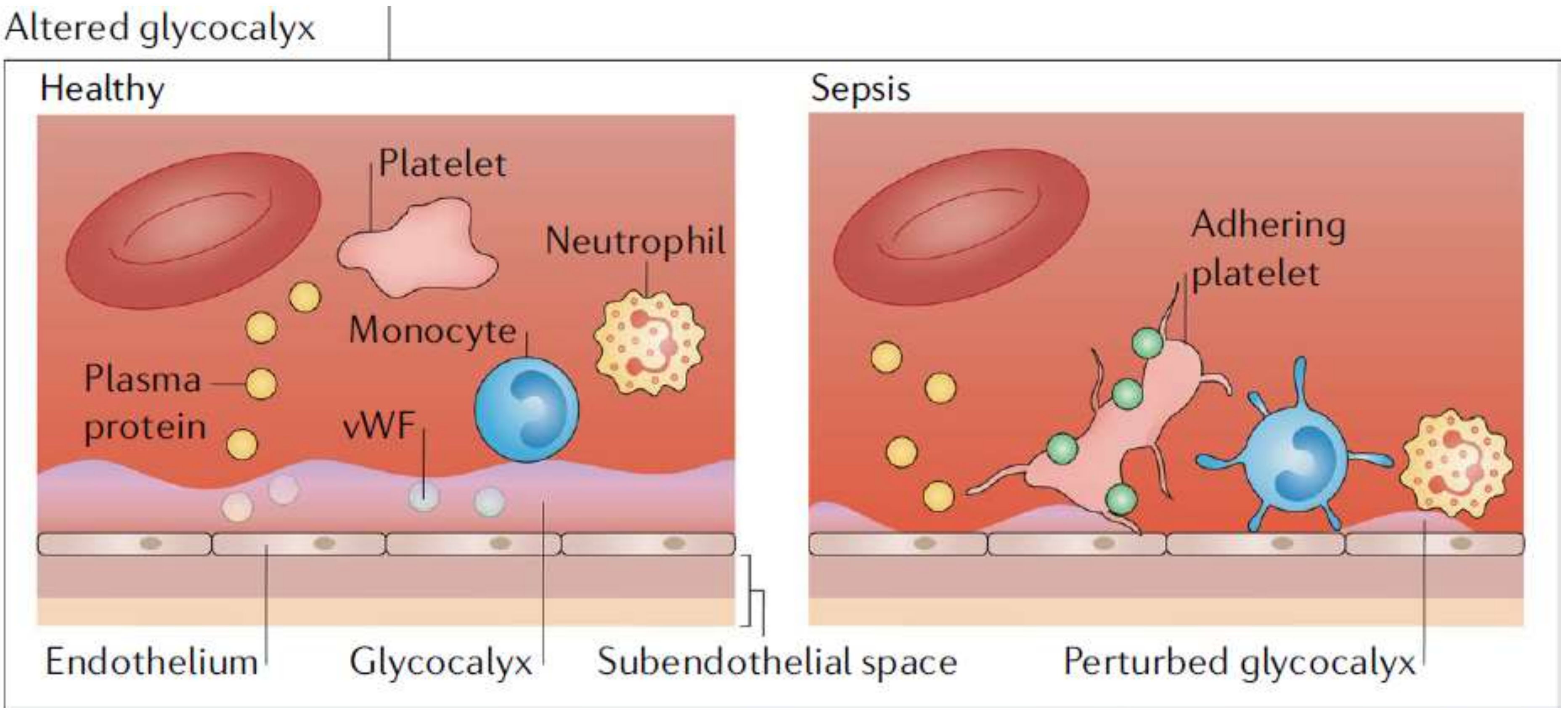
GLYCOCALYX ALTERATIONS



Electron microscopic overview of an Alcian blue 8GX–stained rat left ventricular myocardial capillary
Coat includes glycolipids, glycoproteins, and proteoglycans

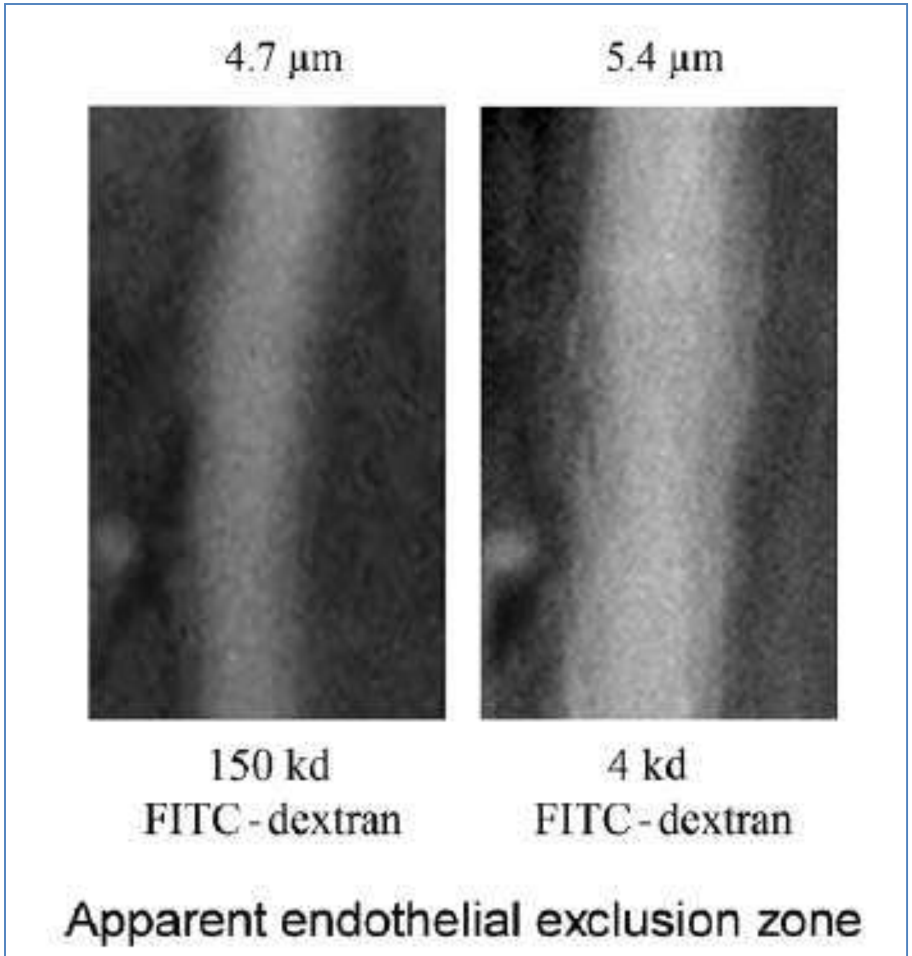
van den Berg et al. Circ Res. 2003

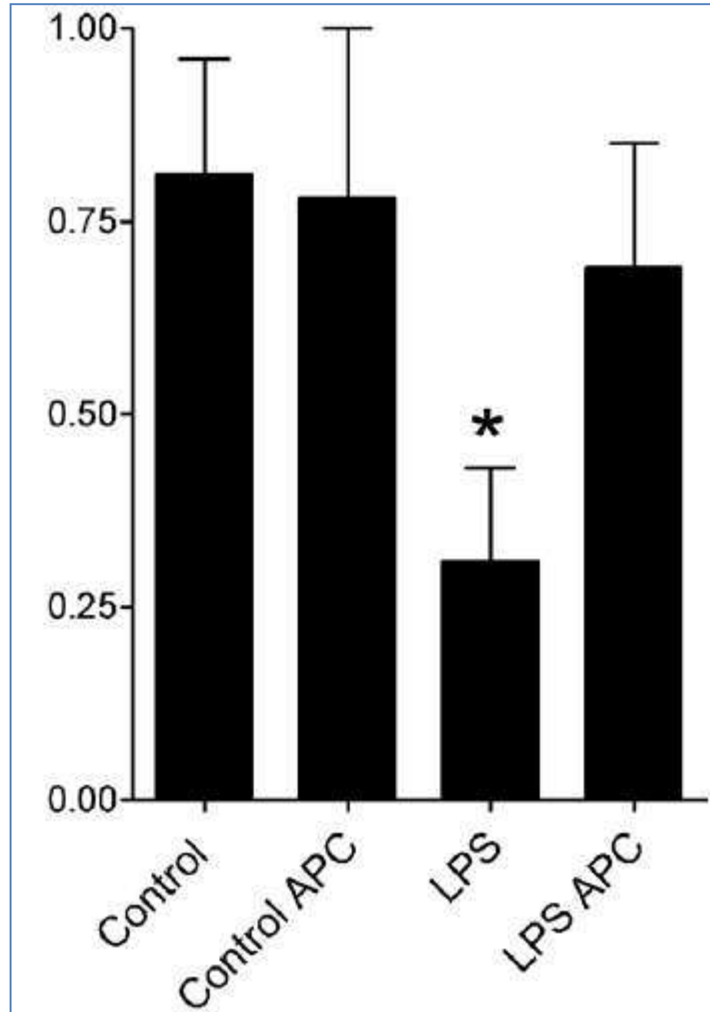
Altered Glycocalyx



Lelubre C et al. Nat Rev Nephrol. 2018

Role of glycocalyx in microvascular alterations

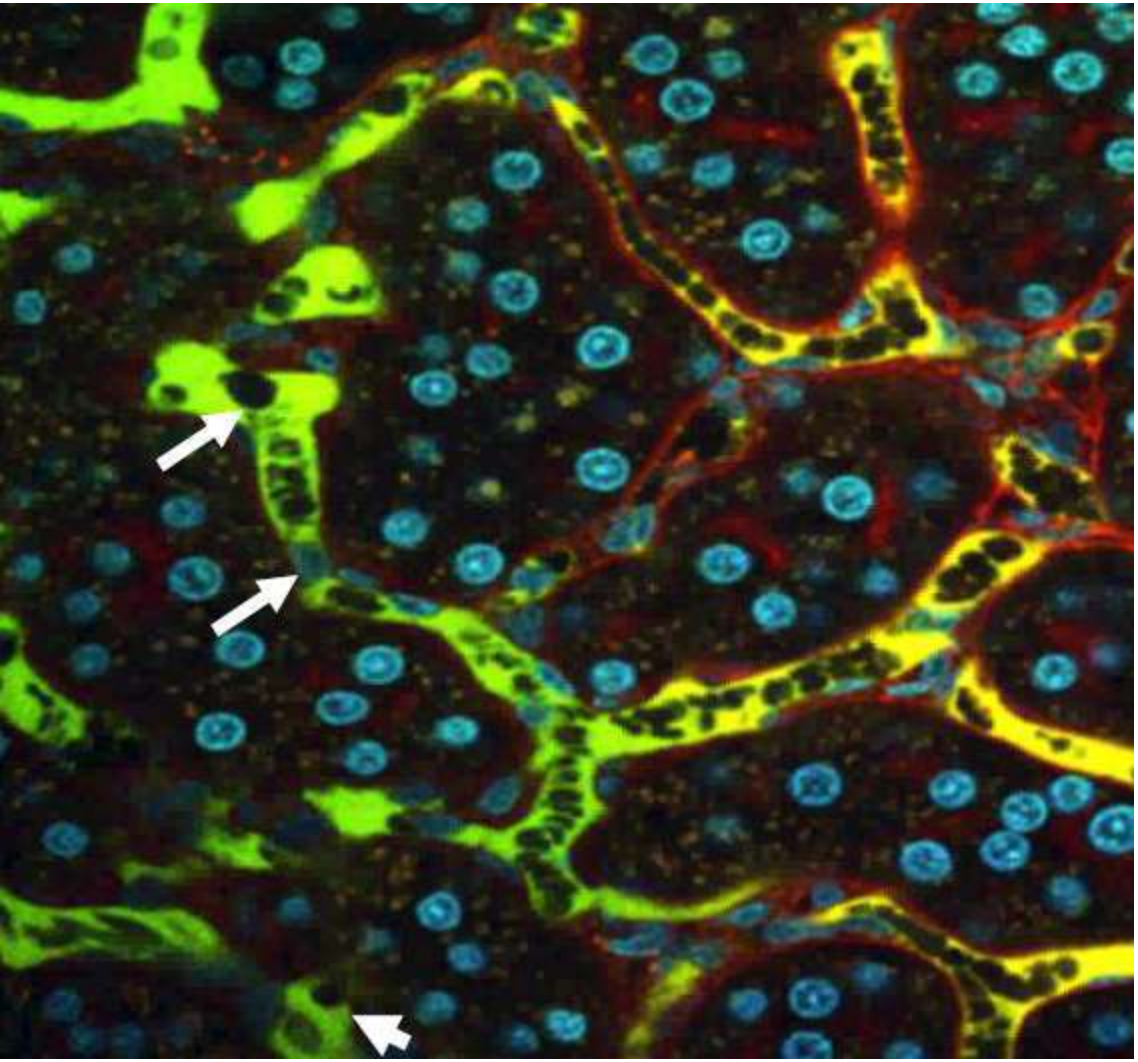


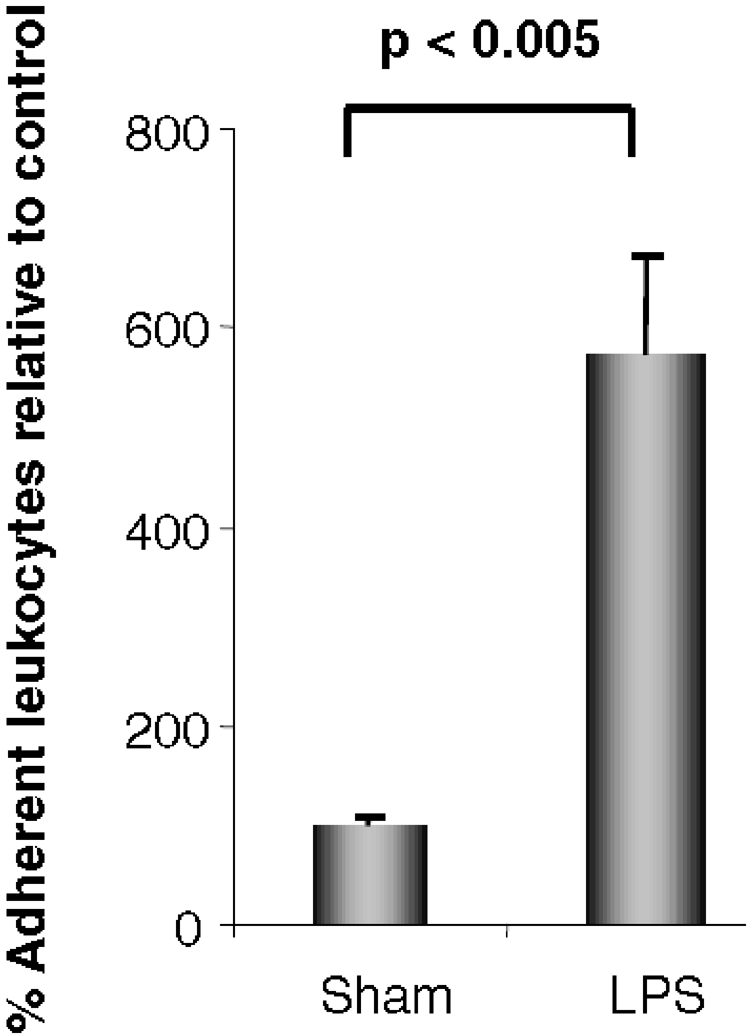


Rats, ileum, in-vitro study; fluorescein isothiocyanate

Marechal X et al. Shock. 2008

LPS promotes rolling and adhesion in renal microcirculation



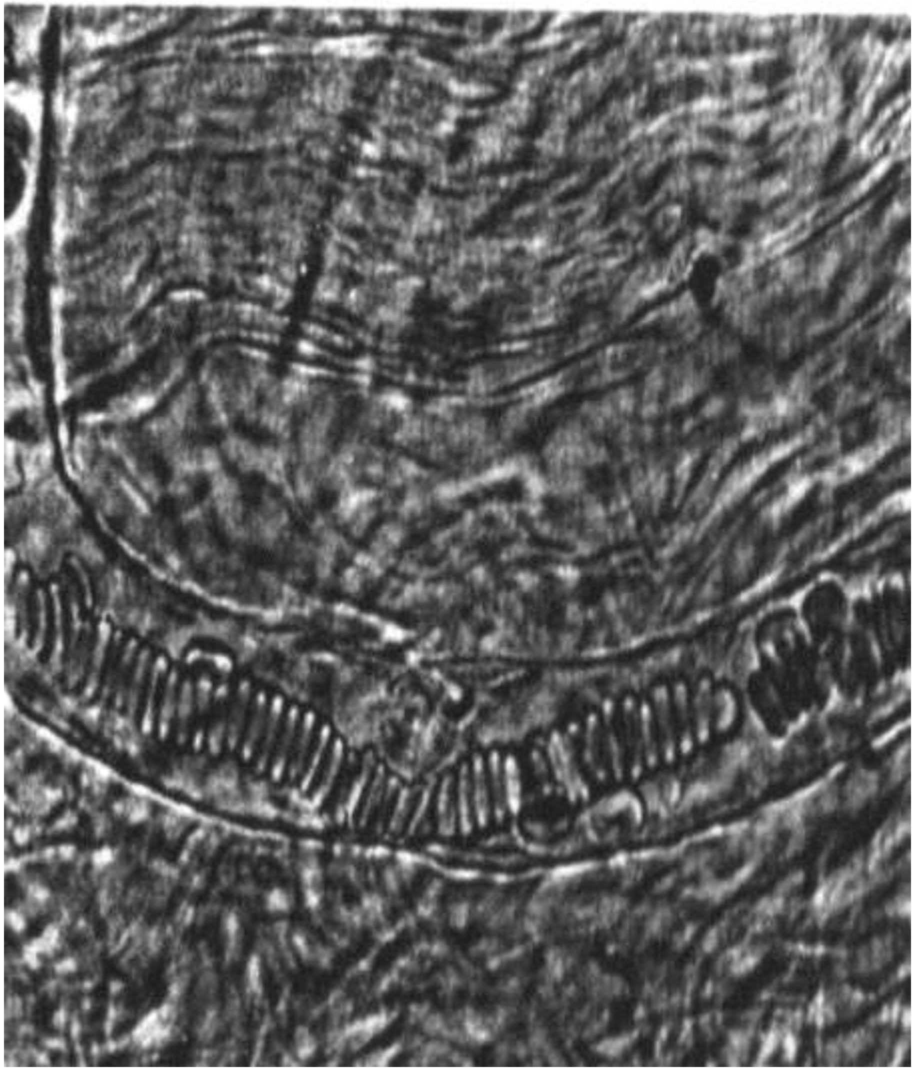


Two photons videomicroscopy, rats

Gupta A et al. AJP. 2007

Altered Red blood cell deformability

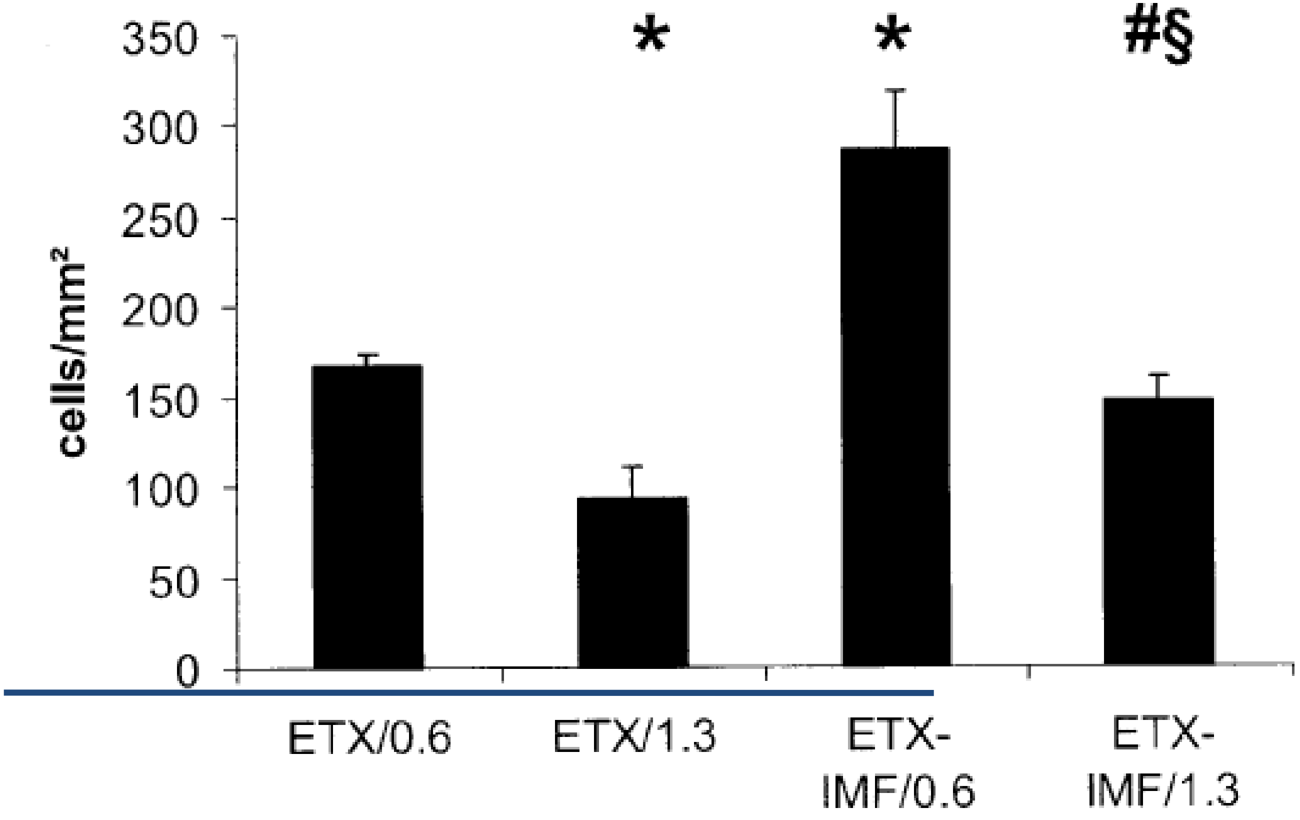
Rouleau formation



Rabbit; LPS, intravital microscopy

McCuskey et al. Cardiovasc Res. 1996

ETX promotes adhesion of RBC to endothelium



Prospective, randomized, controlled in vitro study
Human erythrocytes, vascular endothelial cells

Eichelbronner et al. ICM. 2003

Microthrombi ?

Research

Open Access

Hepatic platelet and leukocyte adherence during endotoxemia

Roland S Croner[1], Elfie Hoerer[2], Yakup Kulu[2], Tilo Hackert[2], Martha-Maria Gebhard[3], Christian Herfarth[2] and Ernst Klar[4]

- Rats; CLP model
- Intravital microscopy performed 0, 1, 3, 5, 10 and 20 hrs after CLP
- Recorded
  - Mean erythrocyte velocity
  - Adhesion and rolling
- HR, MAP and portal venous blood

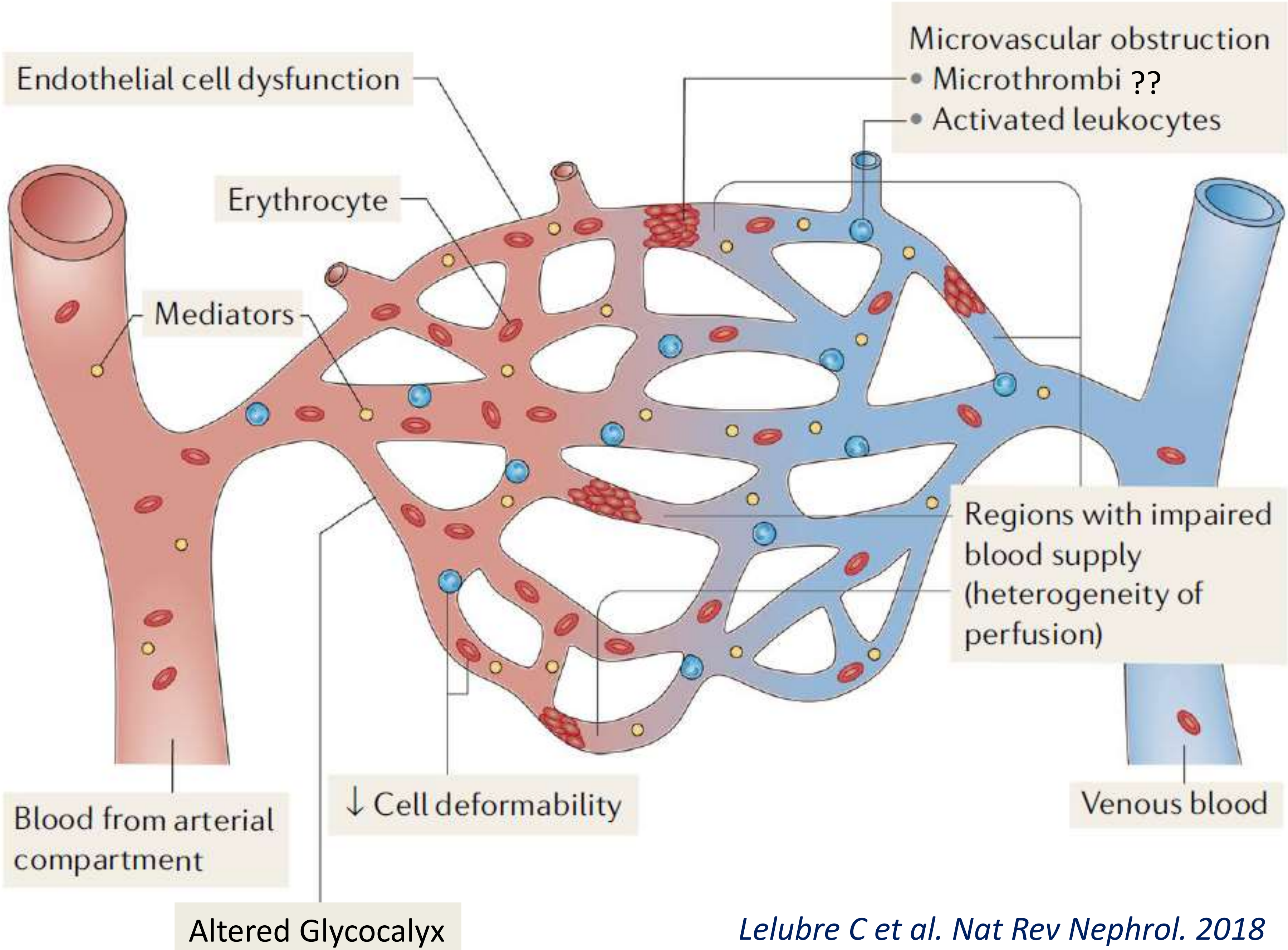
Croner et al. Crit Care. 2006

Key messages

- The hepatic microperfusion damage during endotox-emia follows a time course of ongoing processes.
- Platelet-endothelial adherence during endotoxemia in the liver is an early event.
- Leukocyte-endothelial adherence occurs after the onset of platelet-endothelial adherence.
- Decrease of liver perfusion is the consequence of inflammatory platelet and leukocyte adhesion.
- Hepatocellular damage is a combination of early toxic and late microperfusion related hepatocyte injury.

Microthrombi not frequent

Croner et al. Crit Care. 2006



Lelubre C et al. Nat Rev Nephrol. 2018

Role of Nitric Oxide

- iNOS heterogeneous expression (with deficient areas)
- Decreased production by eNOS

Pool R et al. Crit Care Clin. 2018

Mechanisms

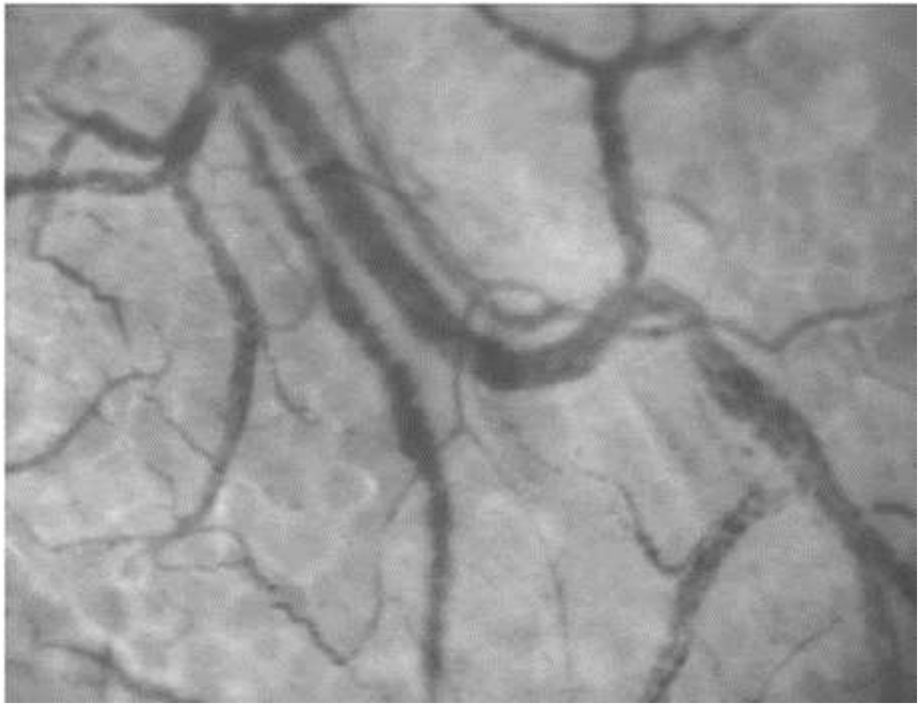
- Endothelial dysfunction
  - Reactivity to vasoactive substances
  - Backward communication
  - Role of e-NOS
- Glycocalyx alteration
- Leukocyte and platelet rolling and adhesion
- Alterations in RBC deformability
- Activation of coagulation

De Backer study

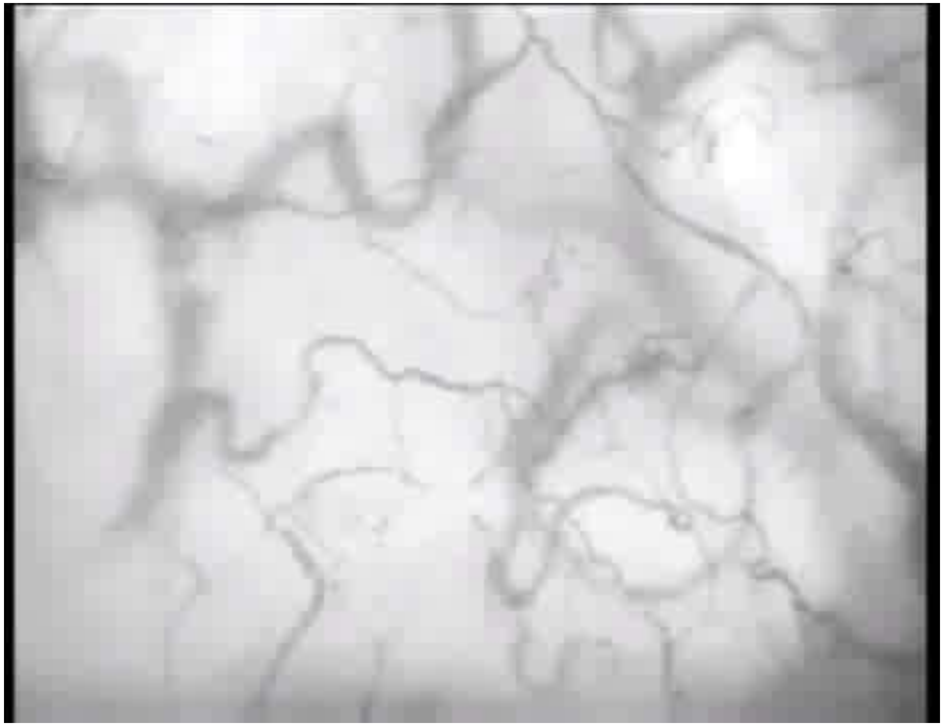
- Hypothesis: alterations of the microcirculation are present in patients with sepsis
- Method: orthogonal polarization spectral imaging technique to investigate the sublingual microcirculation
  - 10 healthy volunteers
  - 16 patients before cardiac surgery
  - 10 acutely ill patients without sepsis
  - 50 patients with severe sepsis
- Effects of topical application of acetylcholine were tested in 11 patients with sepsis
- 5-7 sublingual areas were recorded and analyzed semiquantitatively

De Backer D, et al. Am J Respir Crit Care Med. 2002

Healthy Volunteer

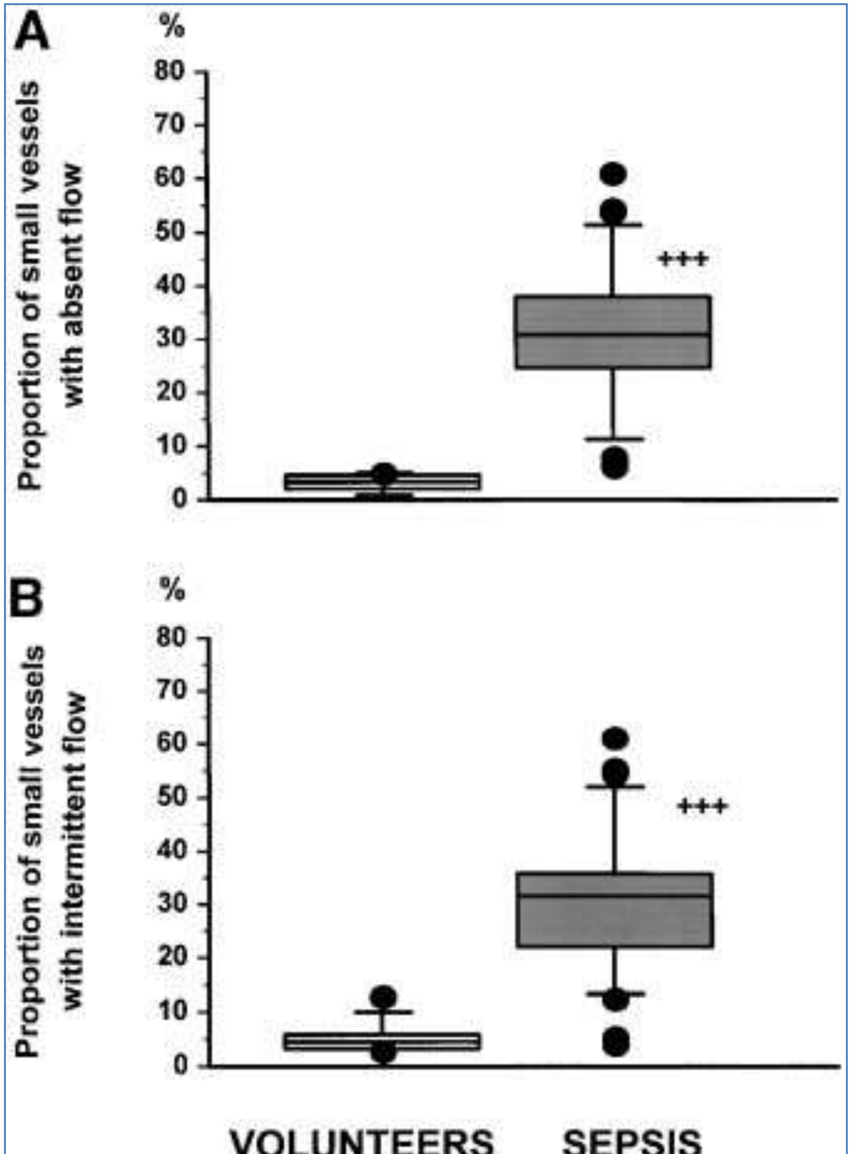


Sepsis Patient

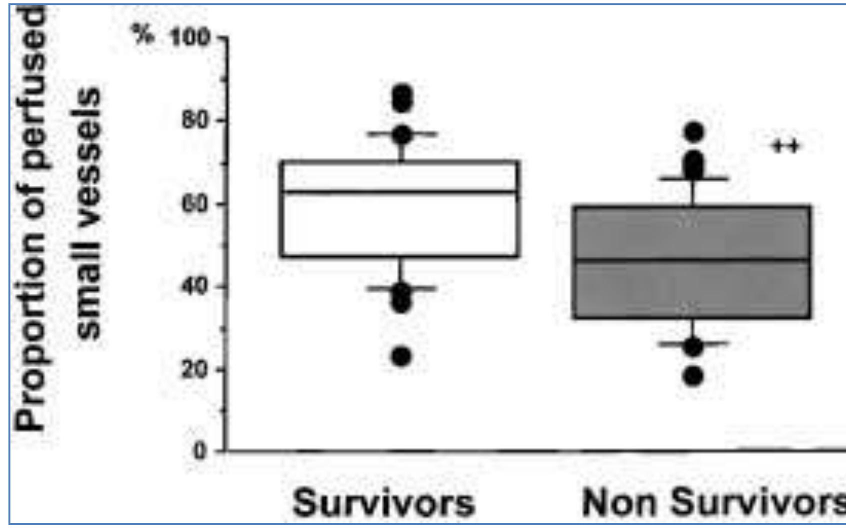


De Backer D, et al. Am J Respir Crit Care Med. 2002

Proportion of small vessels with absent (A) or intermittent (B) perfusion



Proportion of perfused small vessels in survivors (n =22) and nonsurvivors (n =28)



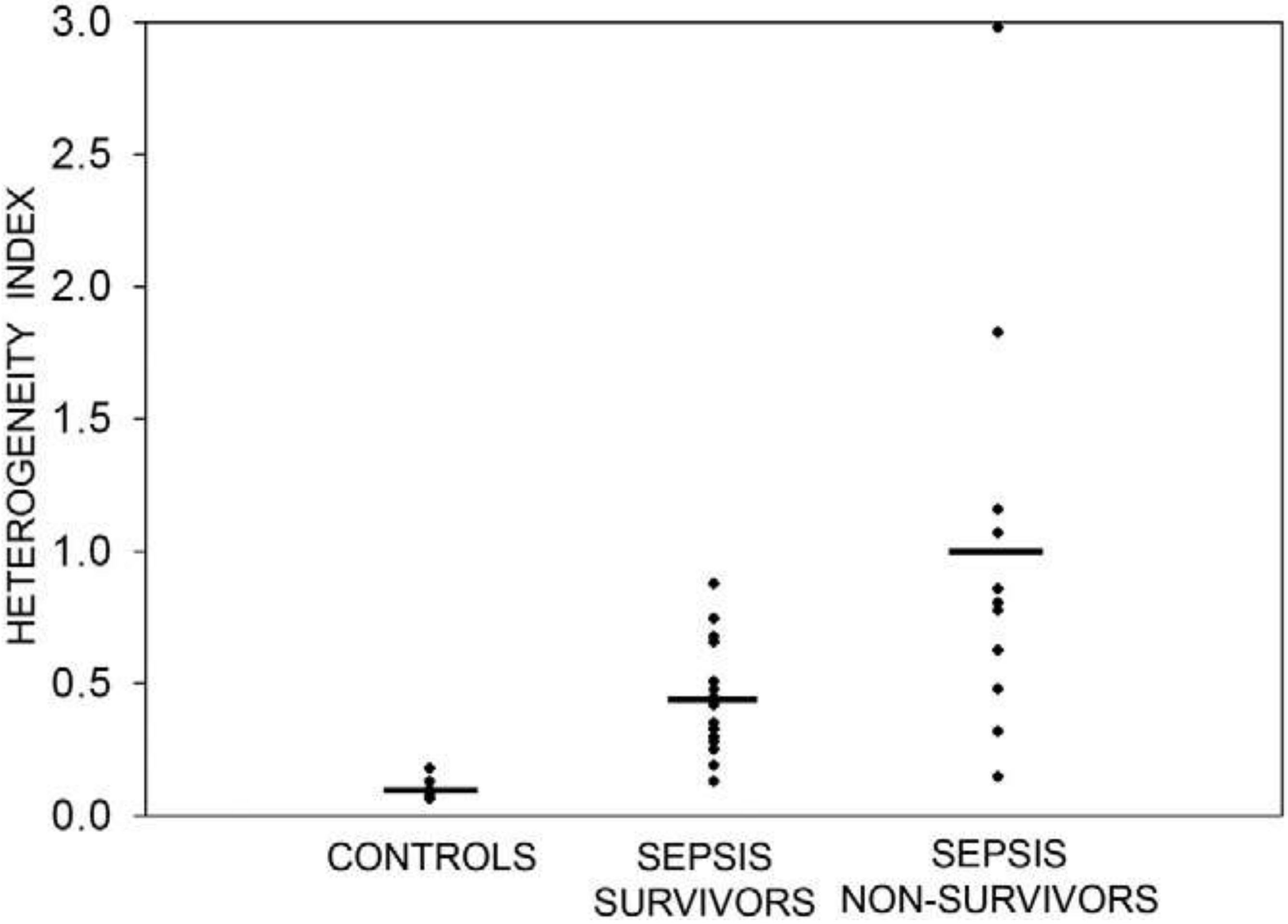
De Backer D, et al. Am J Respir Crit Care Med. 2002

Effect of topical acetylcholine administration in 11 patients with sepsis

	Patients with Sepsis*,† (n = 11)		Volunteers (n = 10)
	Baseline	Acetylcholine (10^{-2} M)	
Total number of vessels, n/mm	4.9 (4.1–5.7)	6.0 (4.7–6.4)‡	5.4 (5.4–6.3)‡
Proportion of vessels perfused, %	83 (77–96)	99 (98–100)‡	98 (97–99)‡
Proportion of venules perfused, %	100 (100–100)	100 (100–100)	100 (100–100)
Proportion of capillaries perfused, %	44 (24–60)	94 (77–96)‡	94 (92–95)‡
Absent flow (capillaries), %	29 (8–44)	1 (0–3)‡	3 (2–5)‡
Intermittent flow (capillaries), %	24 (19–38)	8 (3–19)‡	5 (3–6)‡

De Backer D, et al. Am J Respir Crit Care Med. 2002

Heterogeneity index



- Emergency dept; severe sepsis/septic shock
- Prospective observational study; n=26
- OPS imaging; sublingual

Trzeciak S et al. Ann Emerg Med. 2007

Alterations of sublingual microcirculation in patients with sepsis

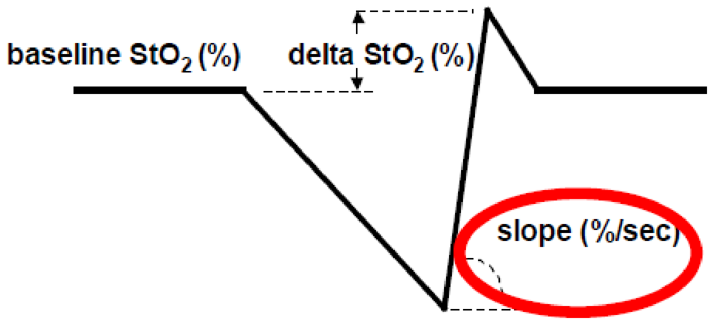
- ↓ total vascular density
- ↓ perfusion of capillaries (no flow or intermittent flow)
- Preserved venular perfusion
- Heterogeneity between areas (close by a few microns)

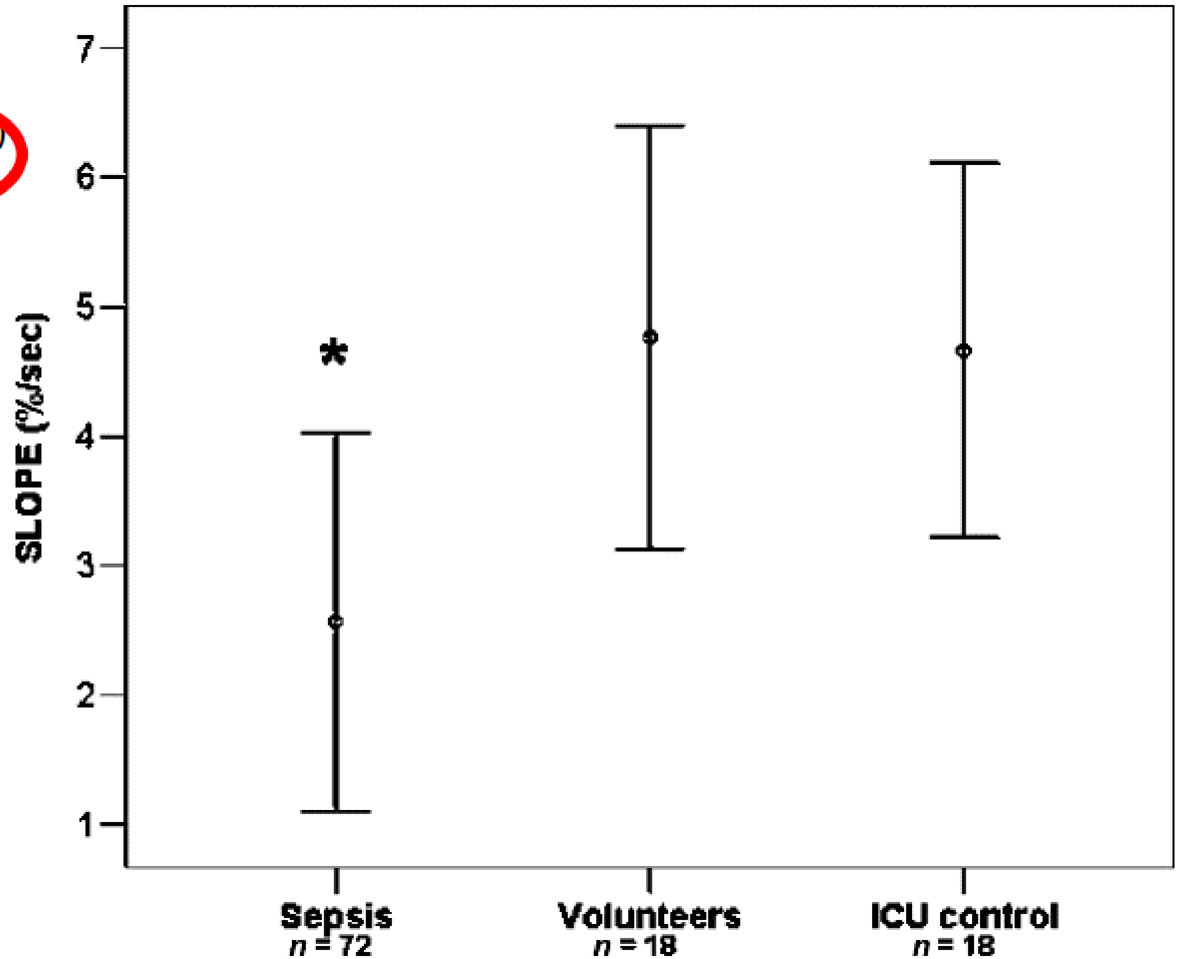
Endothelial reactivity is impaired in sepsis

- Prospective study; ICU
  - 72 patients with severe sepsis or septic shock
  - 18 hemodynamically stable, acutely ill patients without infection
  - 18 healthy volunteers
- Interventions*:* 3-minute occlusion of the brachial artery using a cuff inflated 50 mmHg above systolic BP
- Measurements : Thenar eminence StO2
  - Using NIRS before (StO2baseline), during, and after the 3-min occlusion

Creteur J et al. ICM 2007

Endothelial reactivity is impaired in sepsis





Creteur J et al. ICM 2007

Microcirculatory circulation and outcome

Microcirculatory Alterations in Patients With Severe Sepsis: Impact of Time of Assessment and Relationship With Outcome*

Daniel De Backer, MD, PhD; Katia Donadello, MD; Yasser Sakr, MD, PhD; Gustavo Ospina-Tascon, MD; Diamantino Salgado, MD; Sabino Scolletta, MD; Jean-Louis Vincent, MD, PhD, FCCM

- Design: Analysis of prospectively collected data from previously published studies by the same group.
- 252 patients with severe sepsis

De Backer D, et al. Crit Care Med 2013

Main Hemodynamic and Microcirculatory Variables in ICU Survivors and Nonsurvivors

	ICU Survivor ($n = 122$)	ICU Nonsurvivor ($n = 130$)	p
Heart rate (bpm)	102 [88–117]	105 [94–116]	0.54
Mean arterial pressure (mm Hg)	71 [66–78]	69 [64–75]	0.11
Cardiac index (L/min.m^2)	3.5 [2.8–4.3]	3.2 [2.6–3.8]	0.036
Central venous pressure (mm Hg)	12 [9–14]	13 [10–16]	0.013
Svo_2 (%)	70.0 [64.4–76.7]	67.0 [62.0–72.0]	0.005
Lactate (mEq/L)	1.9 [1.2–2.8]	2.4 [1.4–4.0]	0.004
pH	7.37 [7.32–7.44]	7.35 [7.27–7.40]	0.19
Total vessel density (n/mm)	7.5 [6.2–8.9]	6.8 [5.1–8.3]	0.08
Density of perfused small vessels (n/mm)	3.4 [2.7–4.6]	2.2 [1.6–3. 2]	0.001
Proportion of perfused small vessels (PPV, %)	71 [65–78]	50 [40–66]	0.001
Microvascular flow index	2.35 [1.90–2.52]	1.95 [1.65–2.60]	0.036
Heterogeneity PPV (%)	27 [17–47]	41 [23–60]	0.08
Acute Physiology and Chronic Health Evaluation II score	20 [17–27]	23 [18–28]	0.07
Sequential organ failure assessment score	10 [8–11]	11 [9–14]	0.002
Vasopressor use, *n* (%)	61 (50)	86 (66)	0.016
Vasopressor dose[a] (mcg/kg.min)	0.20 [0.11–0.40]	0.19 [0.11–0.39]	0.18

De Backer D, et al. Crit Care Med 2013

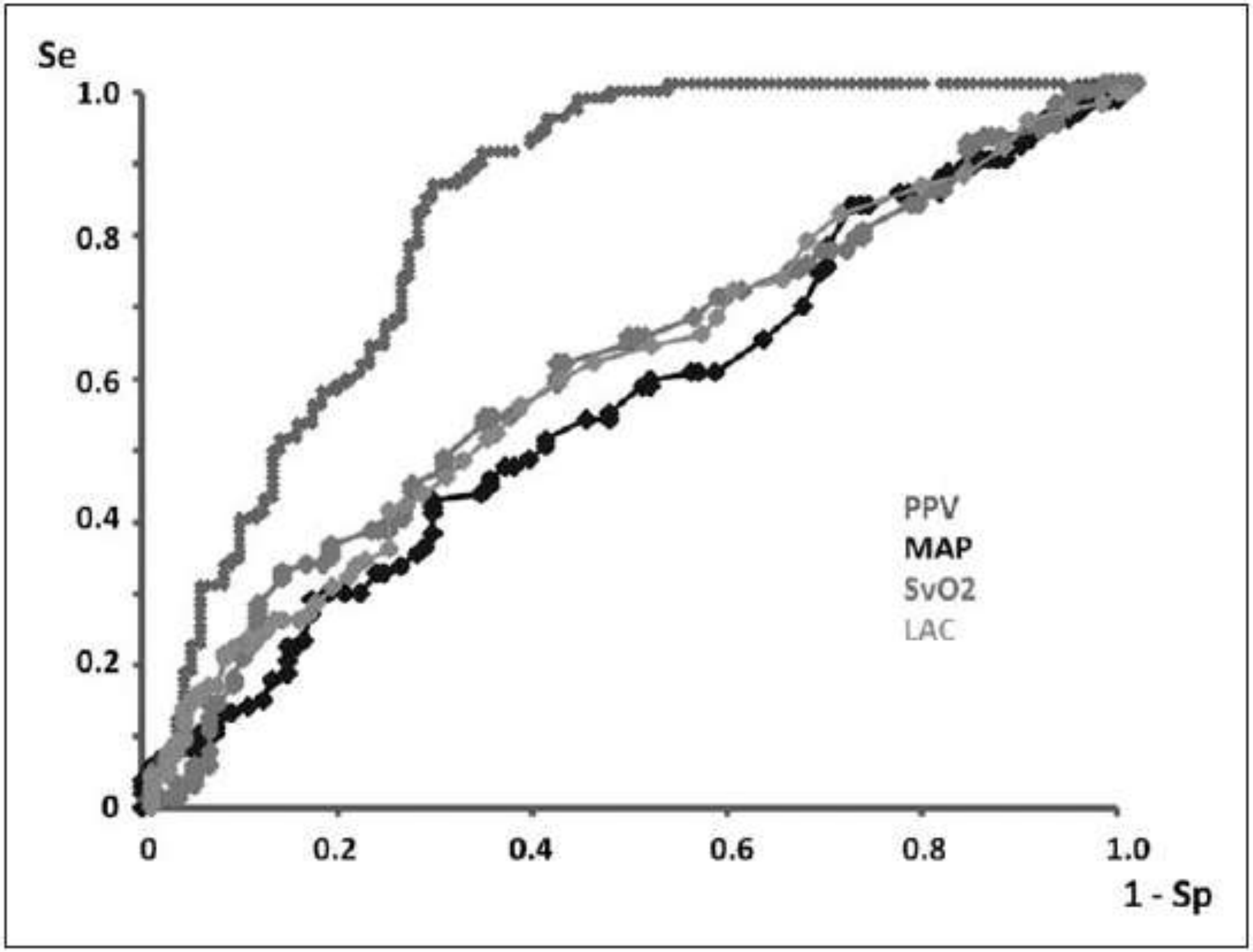


Figure 1. Receiver operating characteristic (ROC) curve for prediction of ICU outcome. The ROC curve areas were 0.818 [0.766–0.871] for proportion of perfused small vessels (PPV), 0.576 [0.505–0.647] for mean arterial pressure (MAP), 0.616 [0.543–0.689] for Svo_2, and 0.612 [0.542–0.681] for lactate (LAC). Svo_2 or $Scvo_2$ = mixed or central venous oxygen saturation.

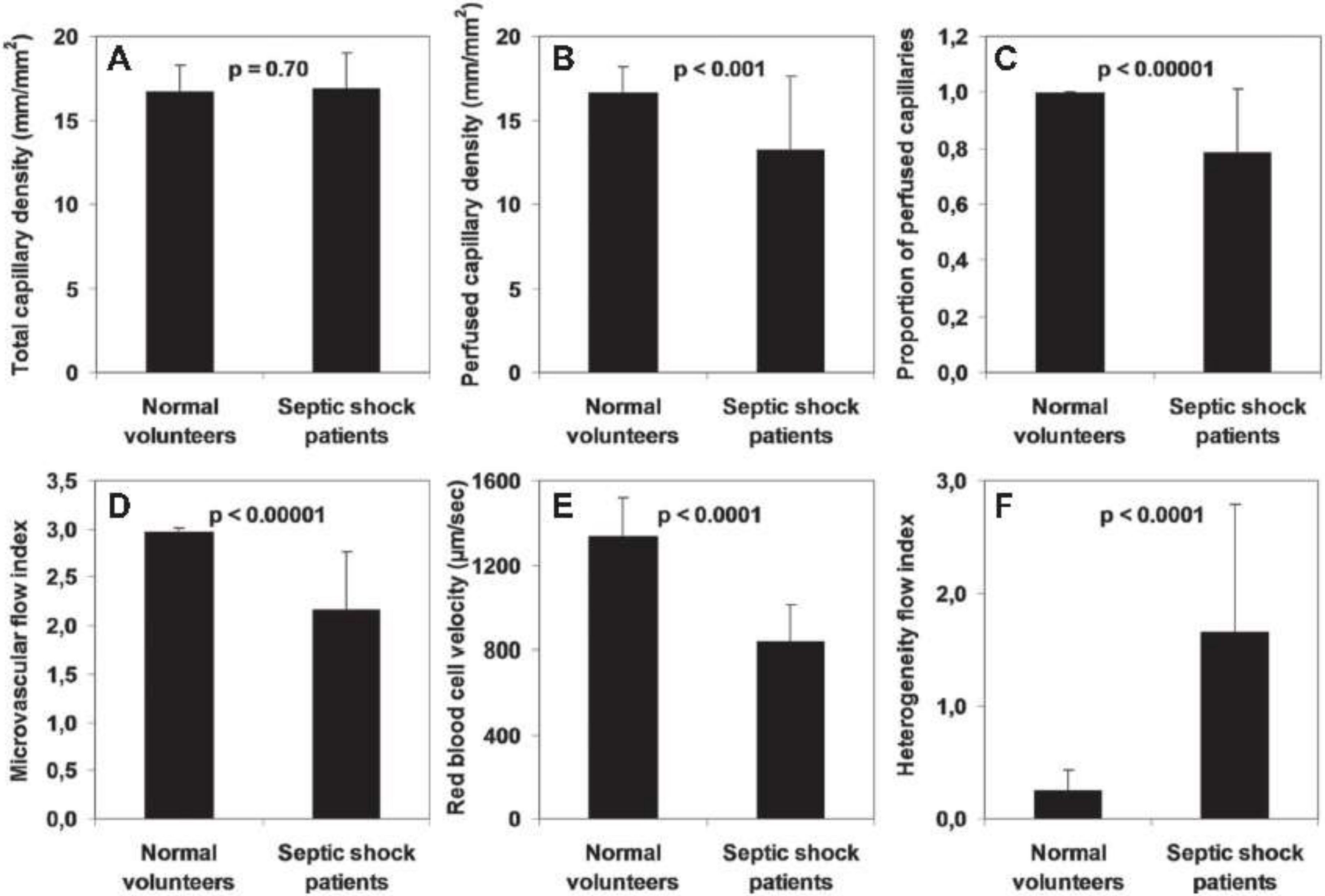
De Backer D, et al. Crit Care Med 2013

Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock*

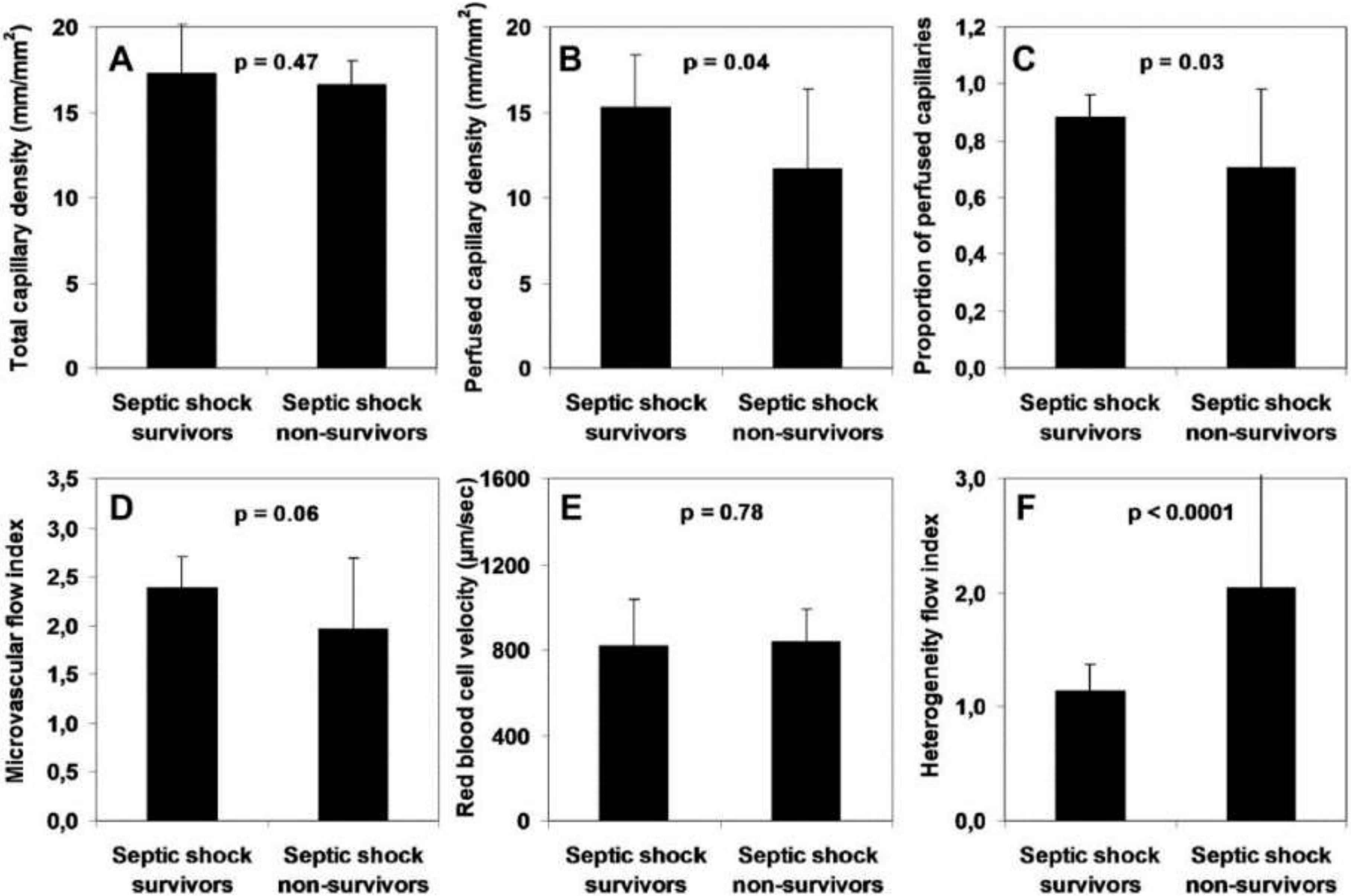
Vanina S. Kanoore Edul, MD; Carolina Enrico, MD; Bruno Laviolle, MD, PhD; Alejandro Risso Vazquez, MD; Can Ince, PhD; Arnaldo Dubin, MD, PhD

- Design: Prospective, observational study.
- Setting: ICU
- Subjects: 25 normal volunteers and 25 patients with septic shock
- Sidestream dark field imaging
- First quantitative characterization of the sublingual microcirculation in normal volunteers and in patients with septic shock

Edul et al. CCM 2012

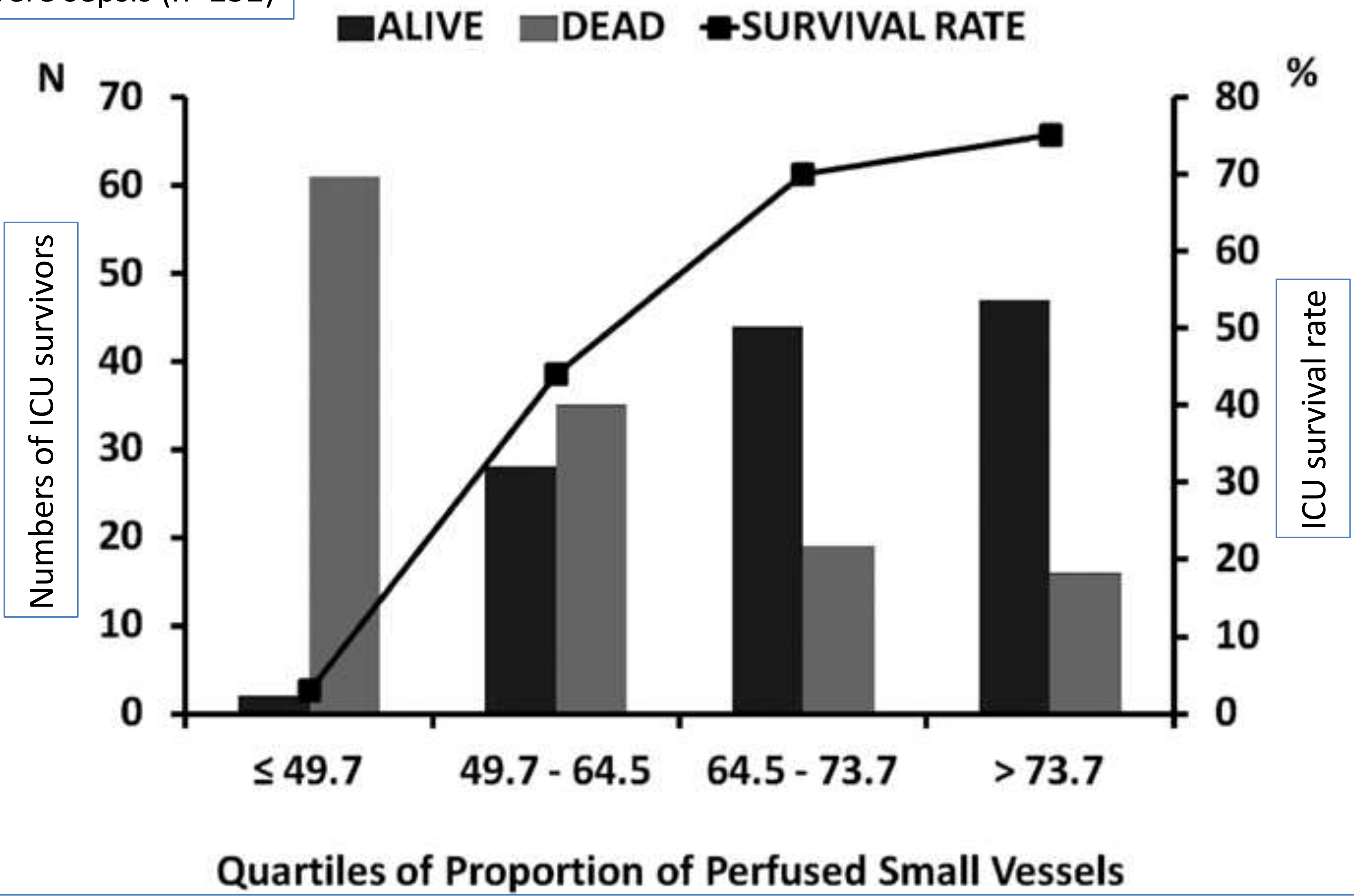


Edul et al. CCM 2012



Edul et al. CCM 2012

Severe sepsis (n=252)



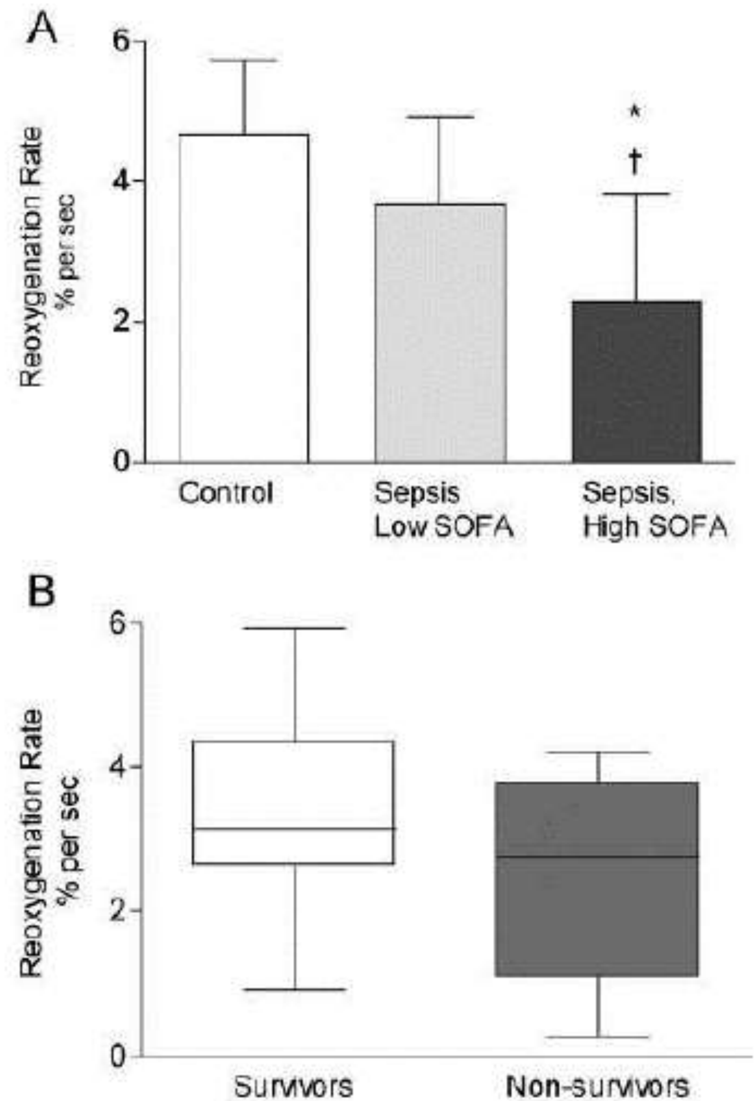
ICU outcome according to quartiles of proportion of perfused small vessels. Each quartile counts 63 patients. Chisquare p < *0.001.*

De Backer et al CCM. ;2013

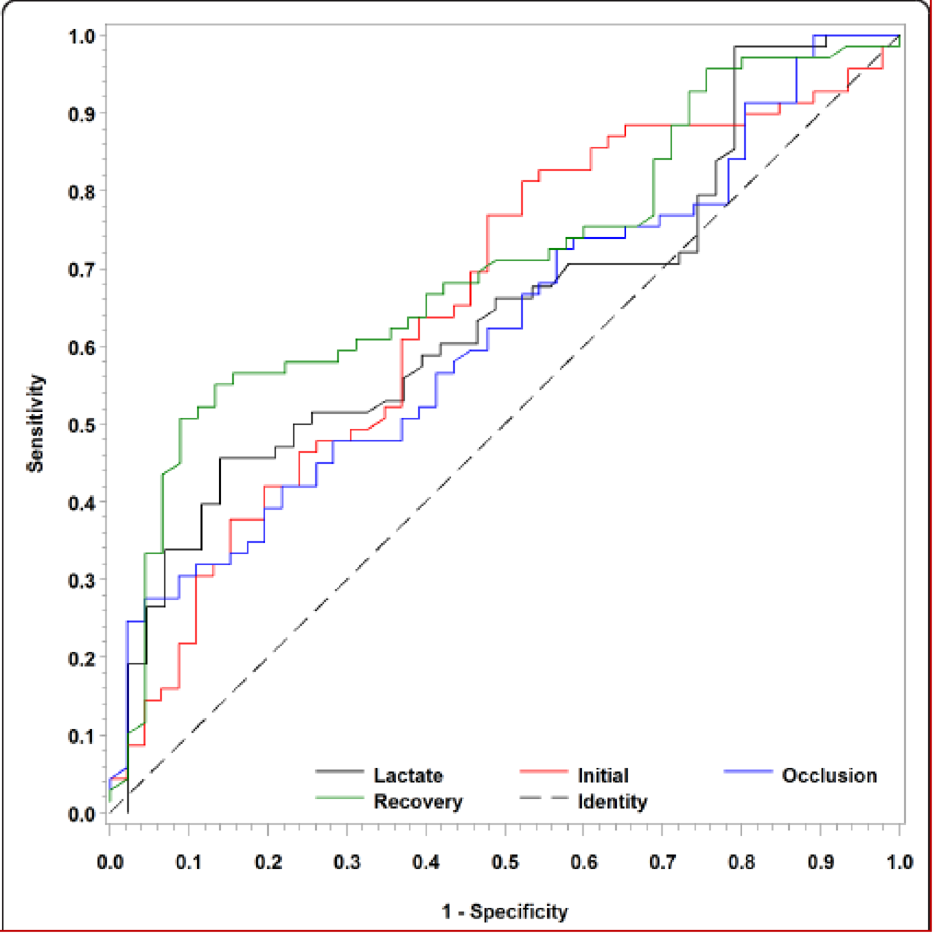
Impairments in microvascular reactivity are related to organ failure in human sepsis

- •24 severe sepsis subjects 24 h after recognition of organ dysfunction
- •Controls: 15 healthy subjects

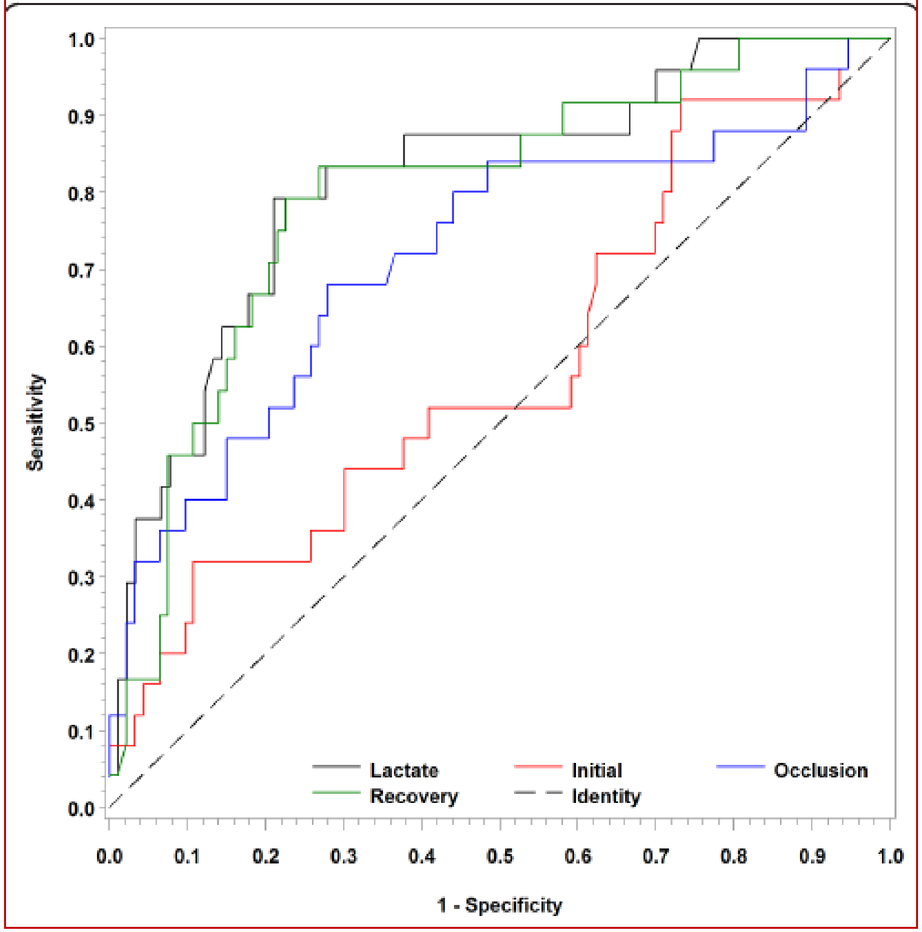
- •NIRS; thenar muscle



Doerschug et al. AJP. 2007



ROC curves for SOFA scores ≥ 2

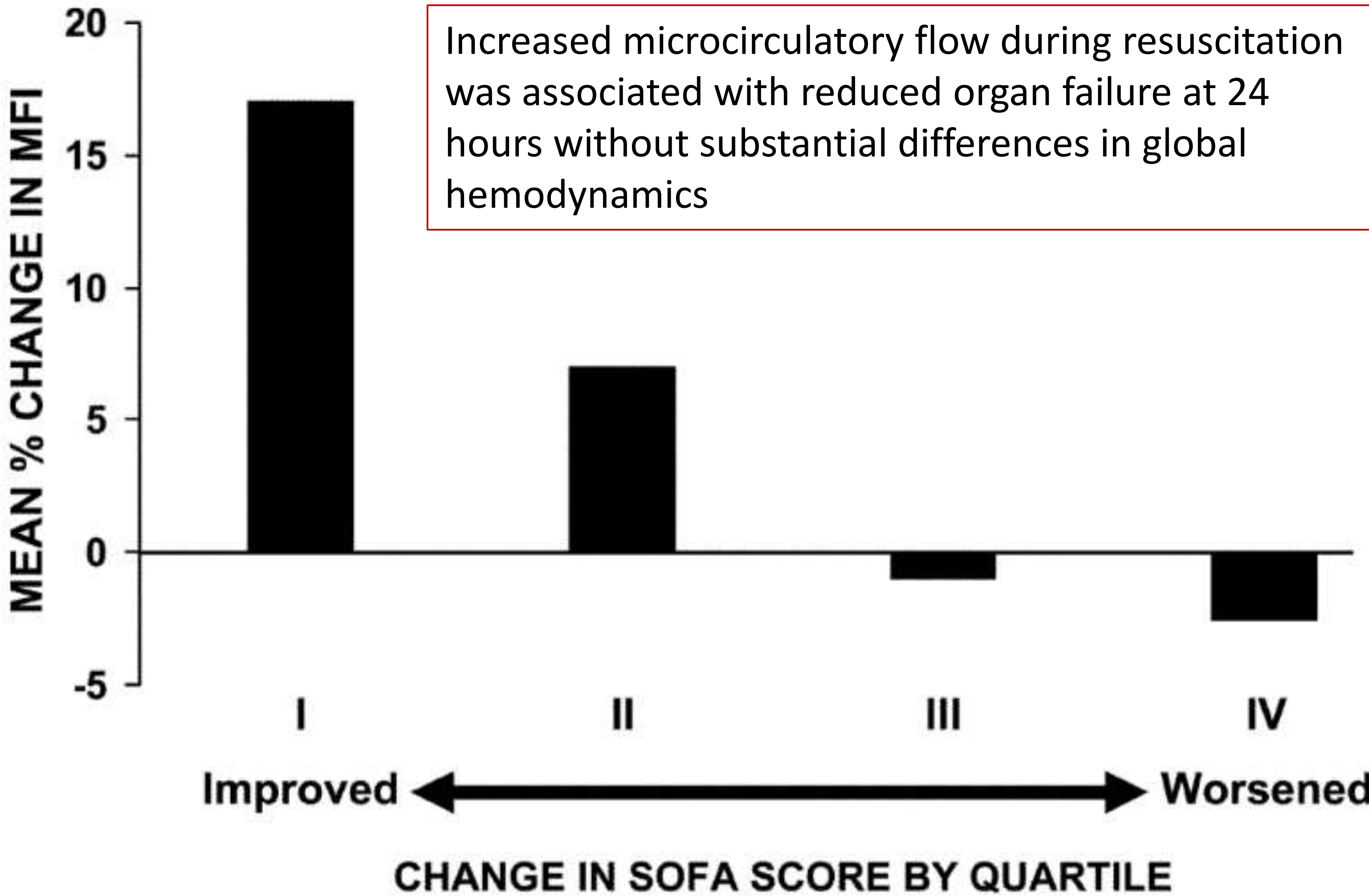


ROC curves for mortality

- Patients: 3 cohorts:
- Septic shock cohort (SBP< 90 after fluid challenge), n = 58
- Sepsis without shock cohort , n = 60
- Emergency department patients without infection, n = 50
- NIRS, StO2; SOFA scores, mortality

Shapiro et al. Crit Care. 2011

SDFI videomicroscopy of the sublingual microcirculation <3 hours from EGDT initiation and again within a 3–6 hour time window after initial

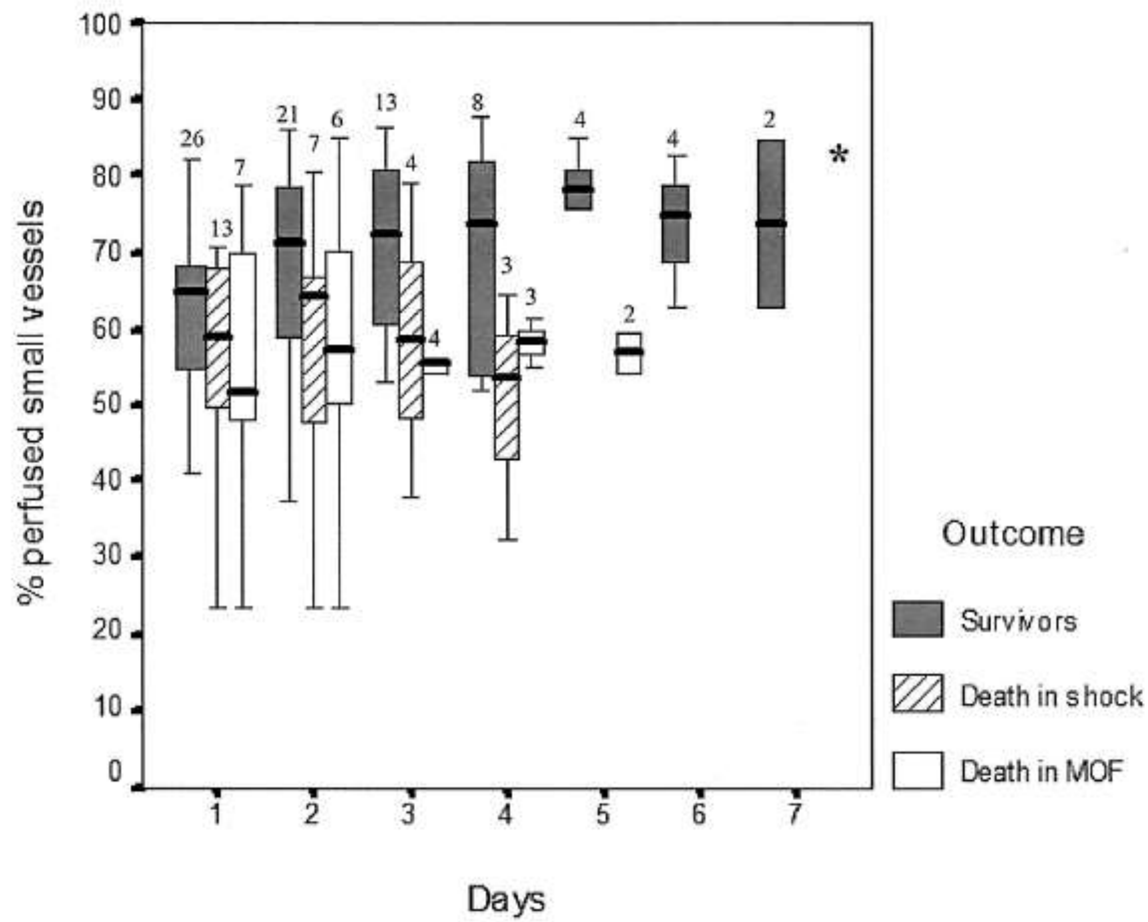


Increased microcirculatory flow during resuscitation was associated with reduced organ failure at 24 hours without substantial differences in global hemodynamics

33 pts with septic shock

Trzeciak et al. ICM. 2008

Evolution of microcirculatory alterations in septic patients



49 patients with septic shock; OPSI

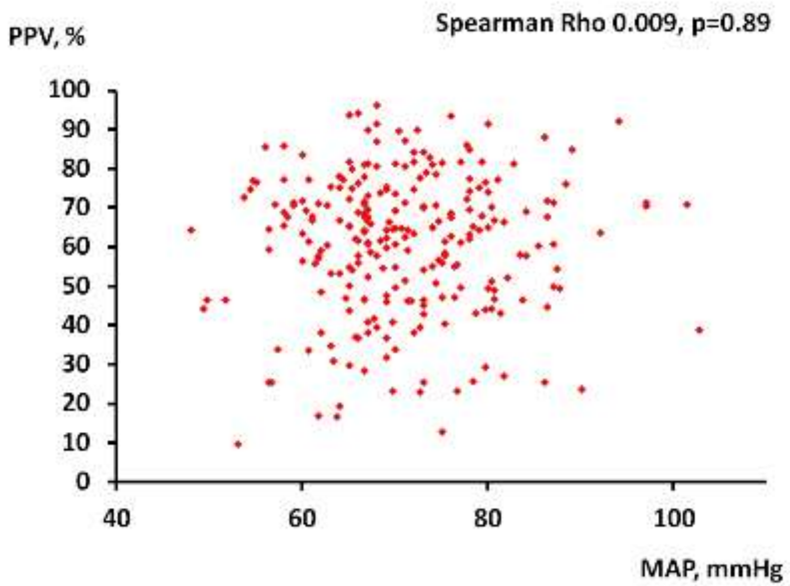
Sakr et al. CCM. 2004

Just the consequence of the altered global hemodynamics ?

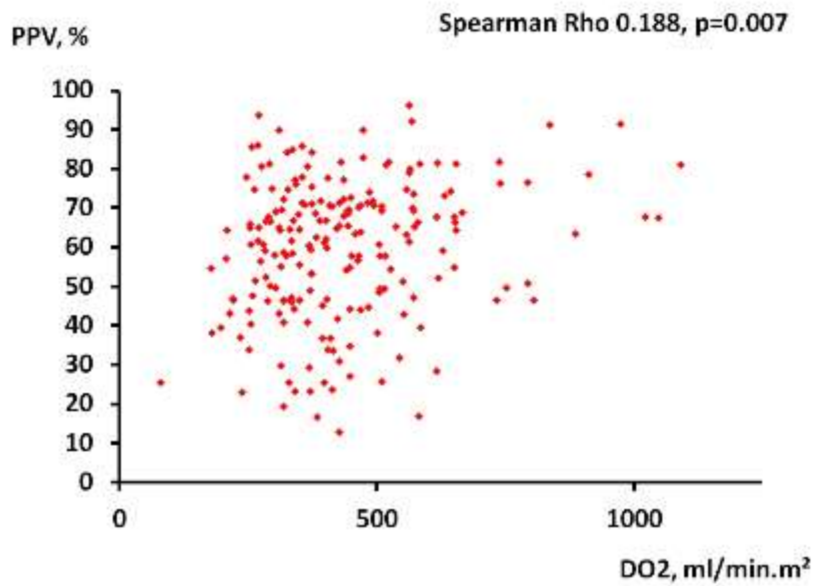
- Can we detect it using hemodynamic measurements, clinical assessment or biomarkers?

Association with systemic variables ?

PPV and MAP



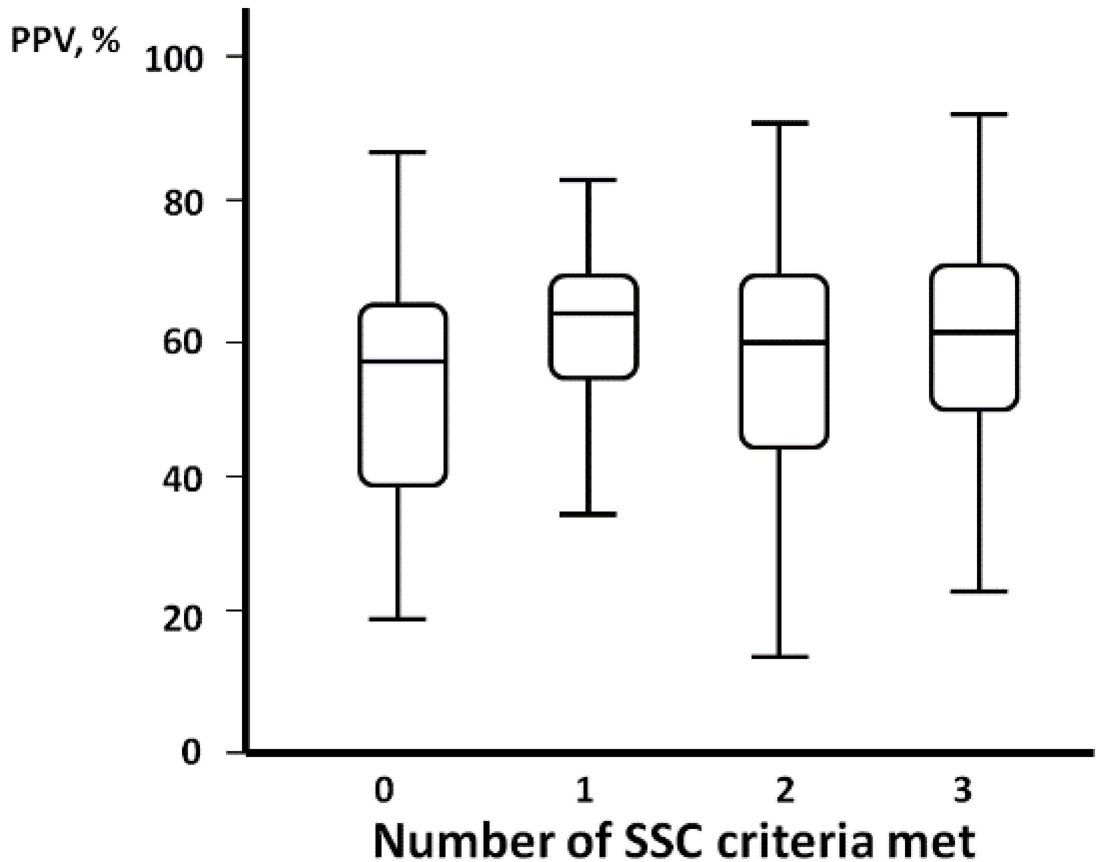
PPV and DO2



Severe sepsis (n=252)

De Backer et al. CCM. 2013

Association with systemic variables ?

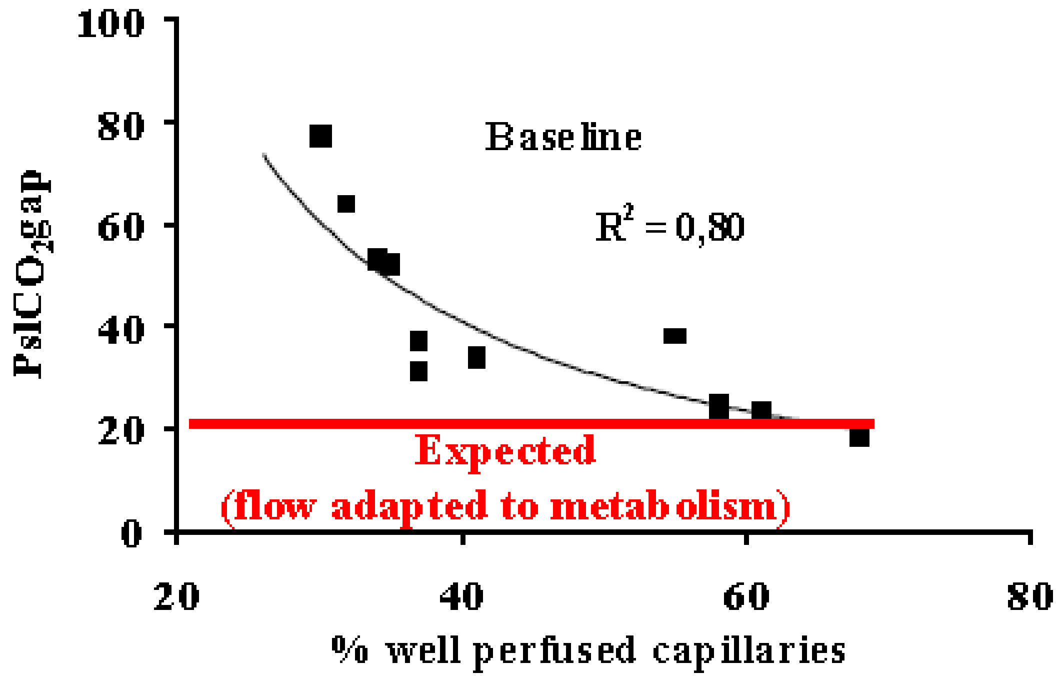


Early severe sepsis (n=204)

De Backer et al. CCM. 2013

Primary event or adaptive phenomenon ?

Stagnation of waste products



Microcirculatory alterations are primary events rather than secondary to altered cellular metabolism.

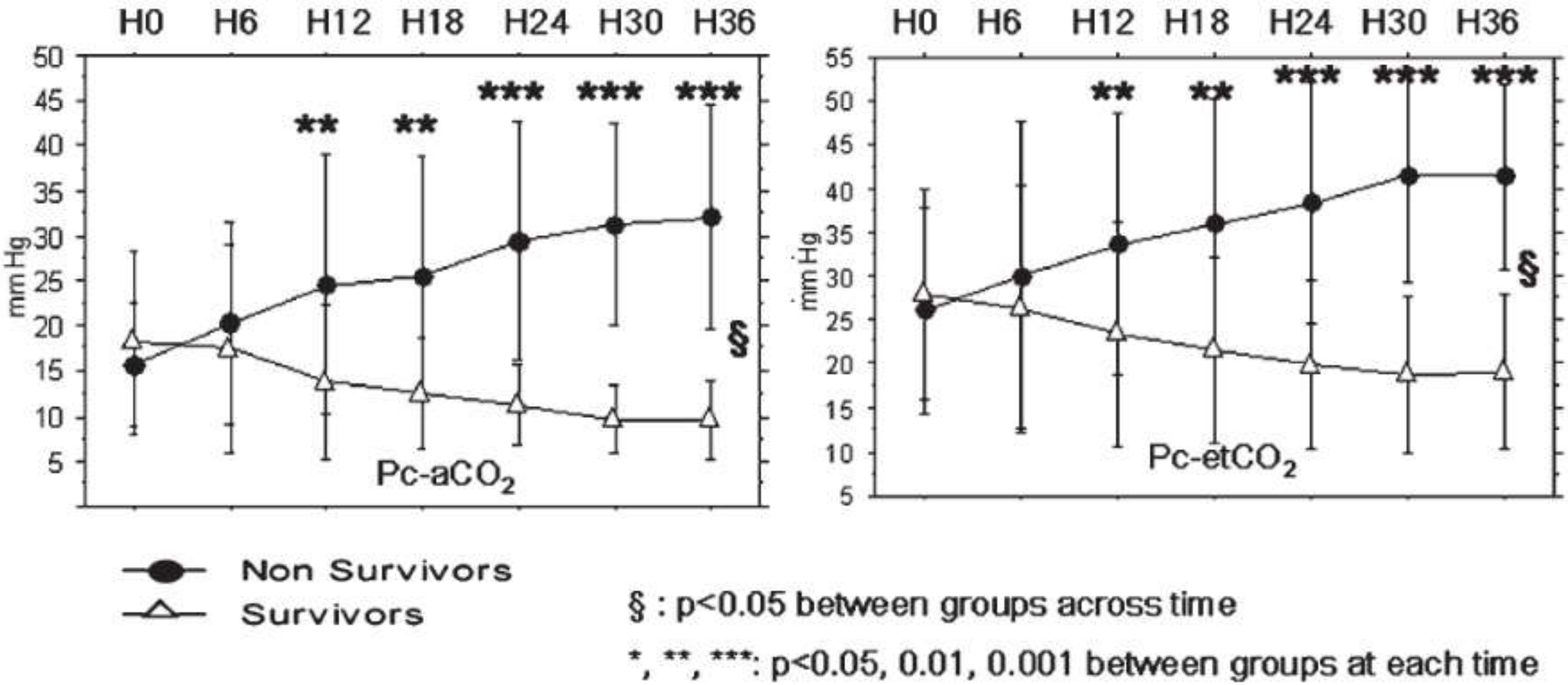
18 consecutive mechanically ventilated patients with septic shock

Creteur et al. ICM 32:516;2006

Cutaneous ear lobe PCO_2 to evaluate microperfusion in patients with septic shock

- Patients: 46 patients with septic shock who were ventilated ; evaluated for 36 h
- Control : 15 stable patients in an ICU
- The difference of the gradients between
  - Pc CO 2 and Pa CO 2 (Pc-a CO 2)
  - Pc CO 2 and end-tidal P CO 2 (Pc-et CO 2)
- Compared with microcirculatory skin blood flow (mBFskin) assessed by laser Doppler flowmetry

Vallee F et al. Chest . 2010



- At 24h, a Pc-aCO_2 > 16mmHg and a Pc-etCO_2>26mmHg were related to poor outcome
- Pc-aCO_2 and Pc-etCO_2 variations during fluid challenge were inversely correlated with changes in mBFskin ($r^2 = 0.7$)

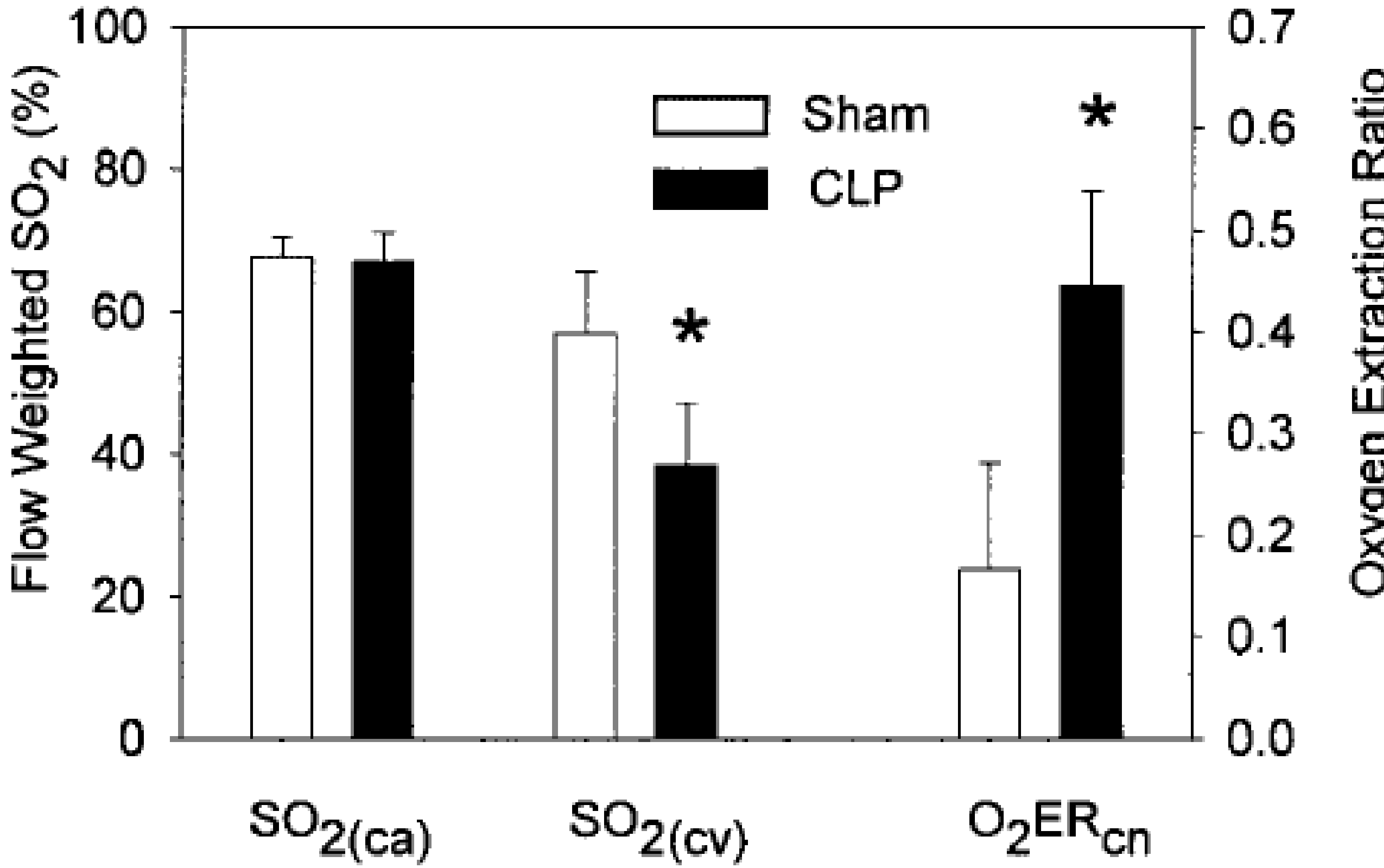
Vallee F et al. Chest . 2010

Effect of a maldistribution of microvascular blood flow on capillary O2 extraction in sepsis

- 24-h rat CLP model
- Studied O2 transport in individual capillaries of the extensor digitorum longus (EDL) skeletal muscle
- Hypothesis : erythrocyte O2 saturation (SO2) levels within normally flowing capillaries would provide evidence of
  - Mitochondrial failure (increased SO2)
  - O2 transport derangement (decreased SO2)
- Spectrophotometric functional imaging system

Ellis C et al. AJP. 2002

In perfused capillaries, O2 extraction is INCREASED in sepsis



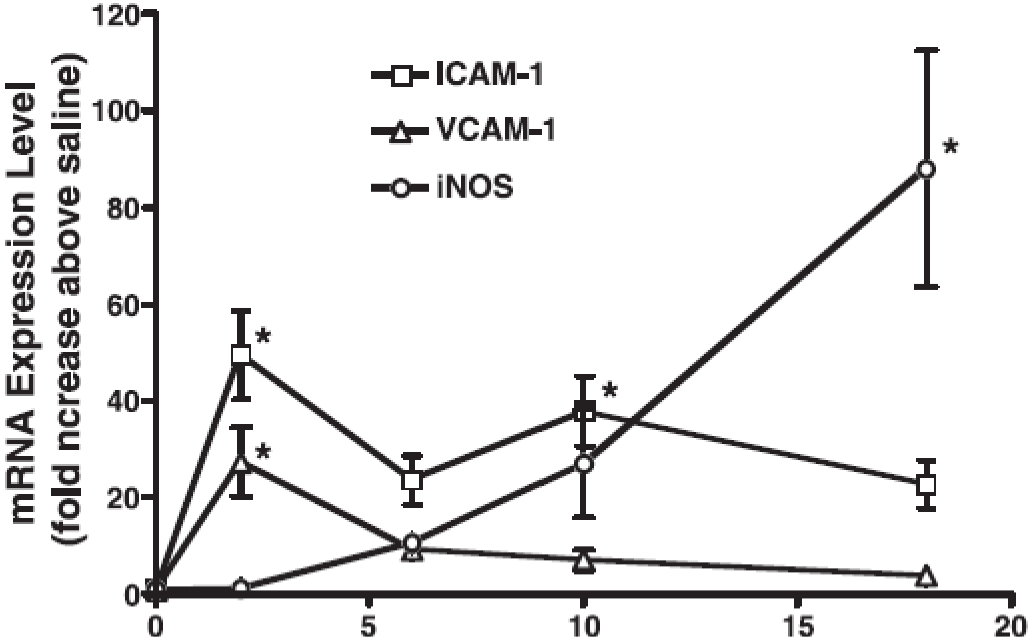
Ellis C et al. AJP 282:H156;2002

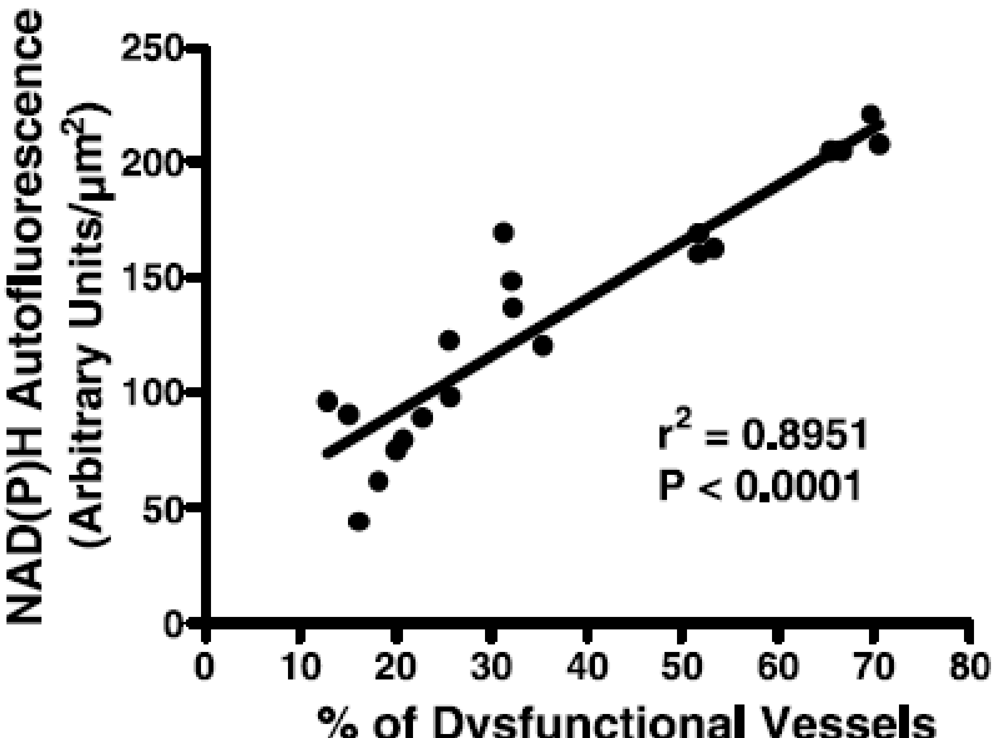
Myocardial hypoxia-inducible HIF-1, VEGF, and GLUT1 gene expression during endotoxemia

- HIF-1, VEGF, and GLUT- 1 were all upregulated
- LPS induces hypoxia in the left ventricle associated with increased microvascular heterogeneity and decreased contractility
- HIF-1 and GLUT1 gene induction are related to a heterogeneous ICAM-1 expression

Cardiomyocytes
Rats, LPS model

Bateman et al. AJP. 2007



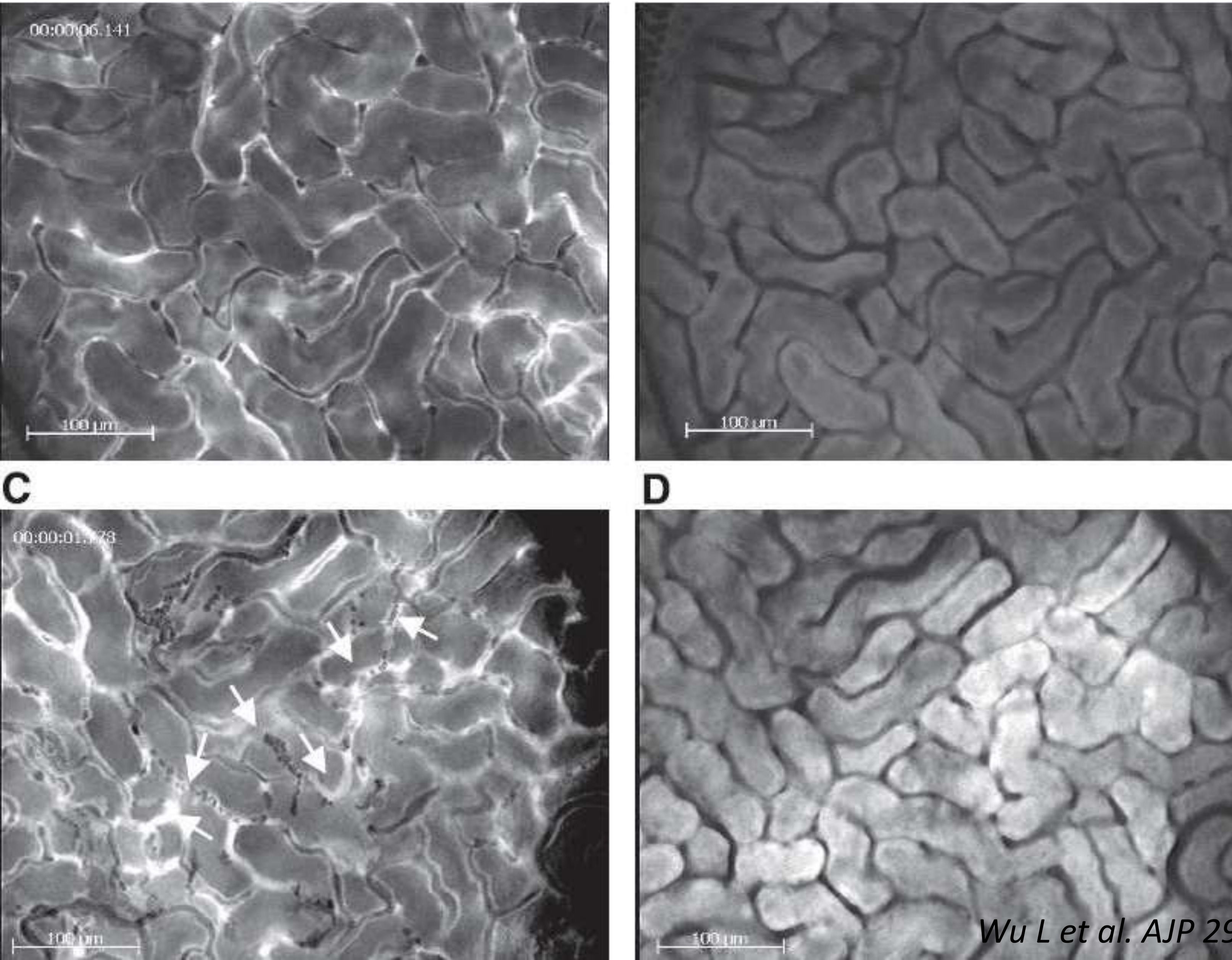


Alteration in redox potential are proportional to microcirculatory alterations

Mice / LPS
Peritubular capillaries
Intravital microscopy

Wu L et al. AJP. 2007

Microcirculatory alterations are associated with renal hypoxia (co-localized with NADH)

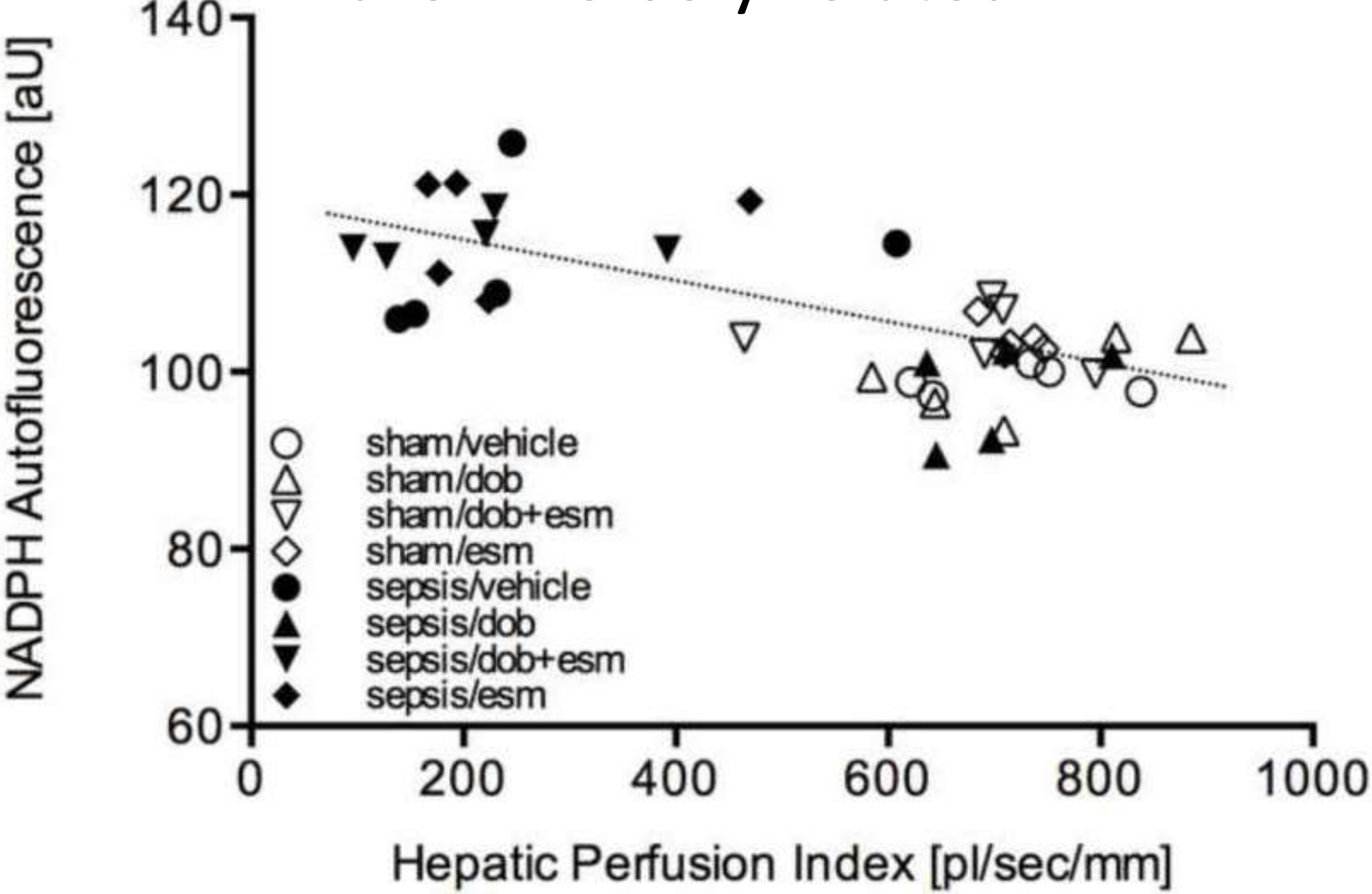


Controls

LPS

Wu L et al. AJP 292:F261; 2007

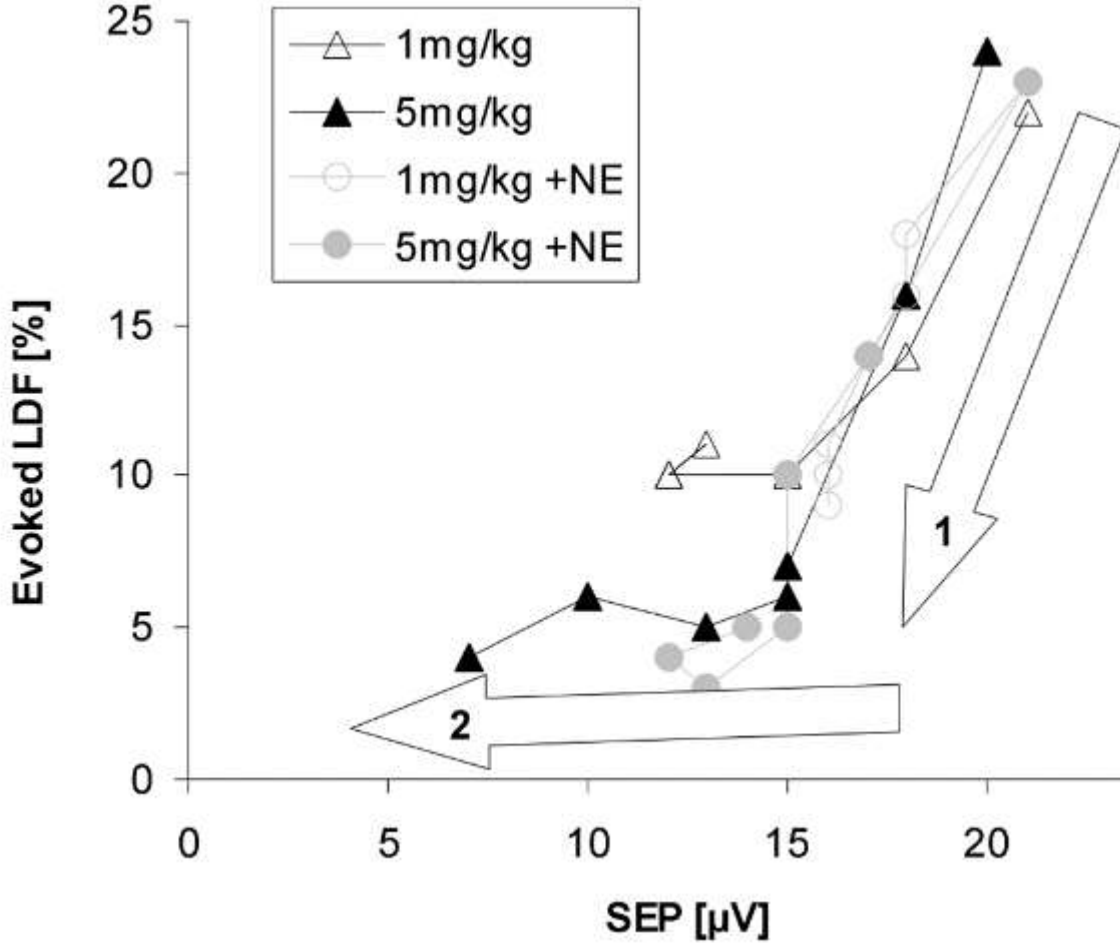
Liver microvascular perfusion and redox state are inversely related



Rats / Fecal peritonitis / pretreatment / absence of shock

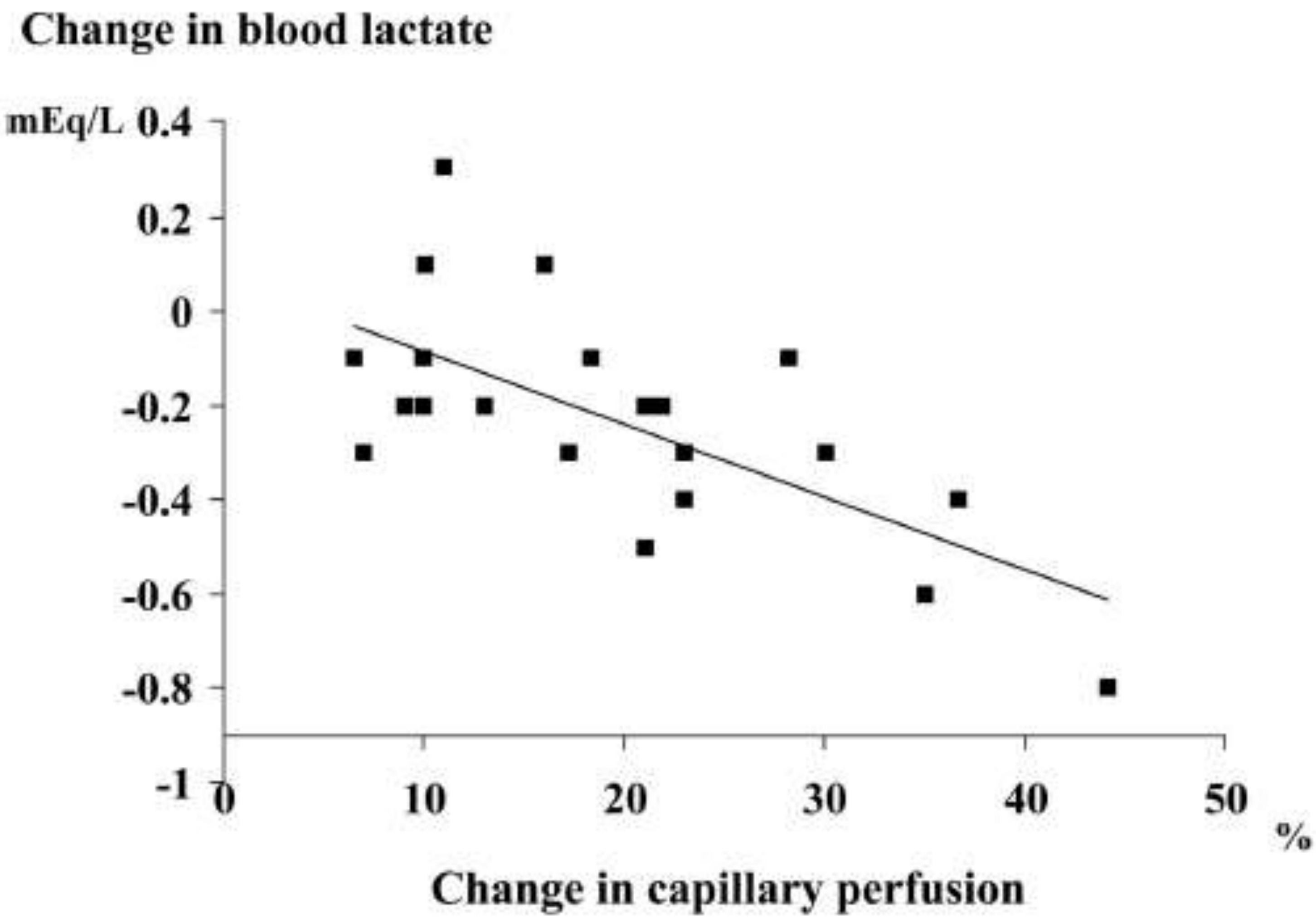
Fink T et al. Shock 2013

Brain microcirculation alteration precedes the loss of function



Rats / LPS; laser-doppler flowmetry

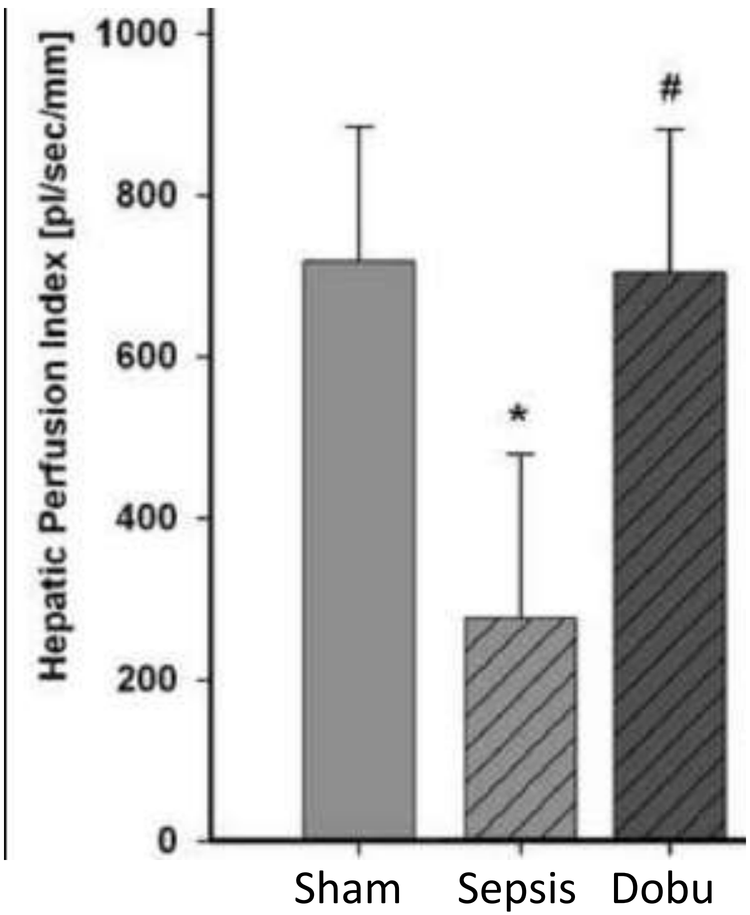
Rosengarten B et al. Crit Care 13:R139;2009

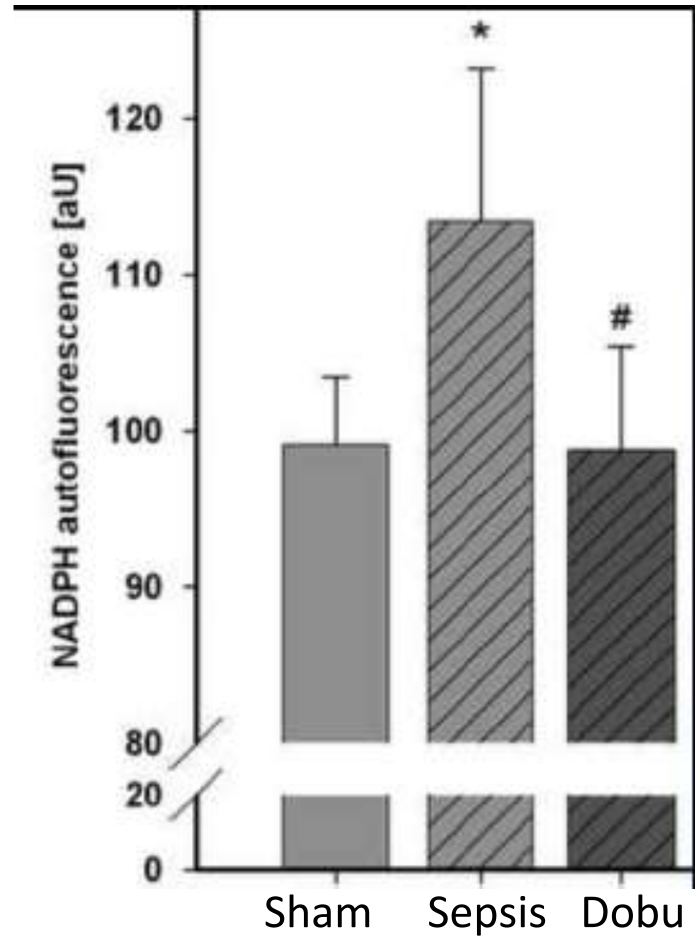


Patients: 22 patients with septic shock.
Interventions: IV dobutamine (5 µg/kg·min) for 2 hrs (n =22) followed by the addition of 10^{-2}M acetylcholine (topically applied, n =10).

De Backer et al. CCM 34:403;2006

β-adrenoceptor stimulation improved liver microvascular perfusion and redox state





Rats / Fecal peritonitis

Fink T et al. Shock 2013

Primary event and not adaptive phenomena

- Microcirculatory alterations are co-localized with low PO_2, production of HIF or redox potential
- O_2 sat at the capillary end of well-perfused capillaries is low, not elevated
- PCO_2 gap, is increased in sepsis
- Perfusion abnormalities precede alterations in organ function
- Improvement in the sublingual microcirculation in response to initial resuscitation procedures was associated with an improvement of organ function 24 h later
- Decrease in lactate levels is proportional to the improvement of the microcirculation during dobutamine administration

Therapeutic strategies

- More important to recruit the microcirculation than to increase total flow to the organ
  - Heterogeneous nature of the alterations
- Should affect one or several of the mechanisms involved
- Interventions that are currently used for their impact on systemic hemodynamics may also influence the microcirculation to some degree

Effect of fluids?

- Increases perfusion pressure at microcirculatory level
- Decrease in viscosity
- Decrease in WBC adhesion and rolling
- Decrease in endogenous vasoconstrictive substances
- Triggers NO-induced vasodilation at microcirculation

Miranda M et al. Am J Physiol Heart Circ Physiol. 2016

The role of renal hypoperfusion in development of renal microcirculatory dysfunction in endotoxemic rats

- Rats; LPS model
- Randomized into 4 groups
  - Sham group (n = 6)
  - LPS group (n = 6)
  - Early gp: LPS administration followed by immediate fluid resuscitation which prevented the drop of renal blood flow (n = 6)
  - LATE group: LPS administration was followed by delayed fluid resuscitation (n = 6)

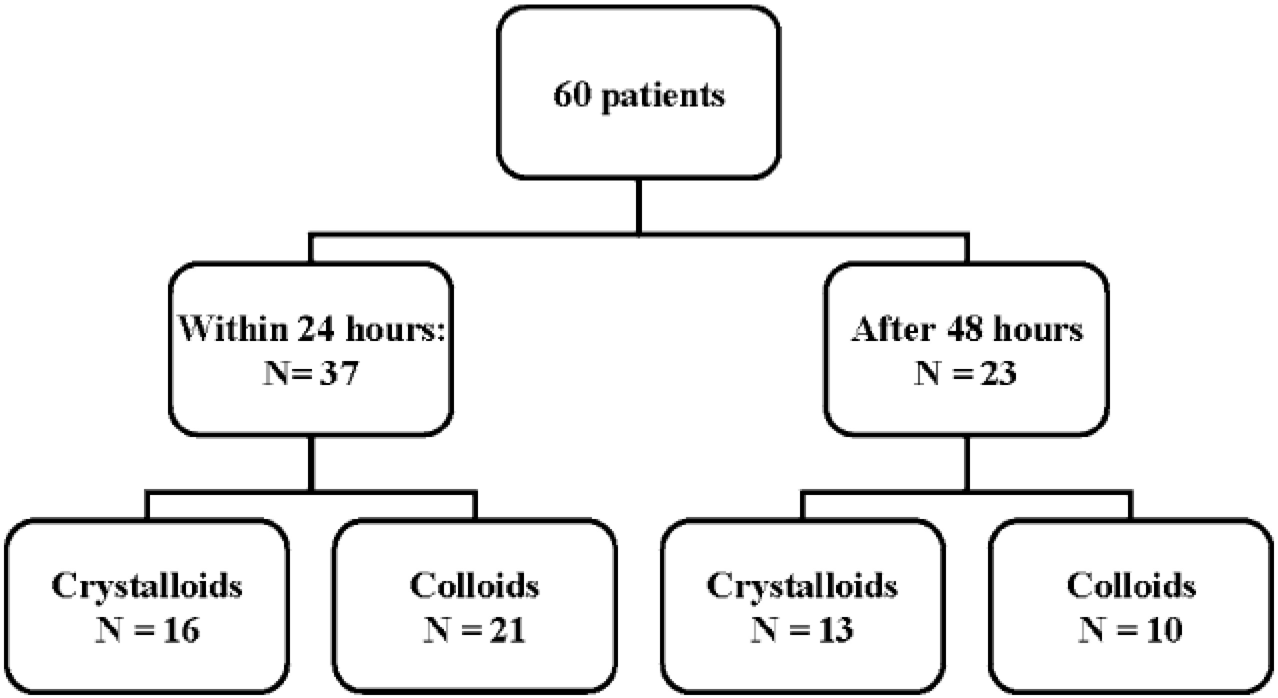
Legrand et al. ICM . 2011

Results

- LPS infusion worsened both microvascular perfusion and oxygenation distributions
- Fluid resuscitation improved perfusion histograms but not oxygenation histograms
- Improvement of microvascular perfusion was more pronounced in the EARLY group compared with the LATE group

Legrand et al. ICM . 2011

Effects of fluids on microvascular perfusion in patients with severe sepsis



Hemodynamic and microcirculatory measurements were obtained before and 30 min after administration of 1L Ringer's lactate (n = 29) or 400 ml 4% albumin (n = 31) solutions

SDFI

Ospina et al. ICM. 2010

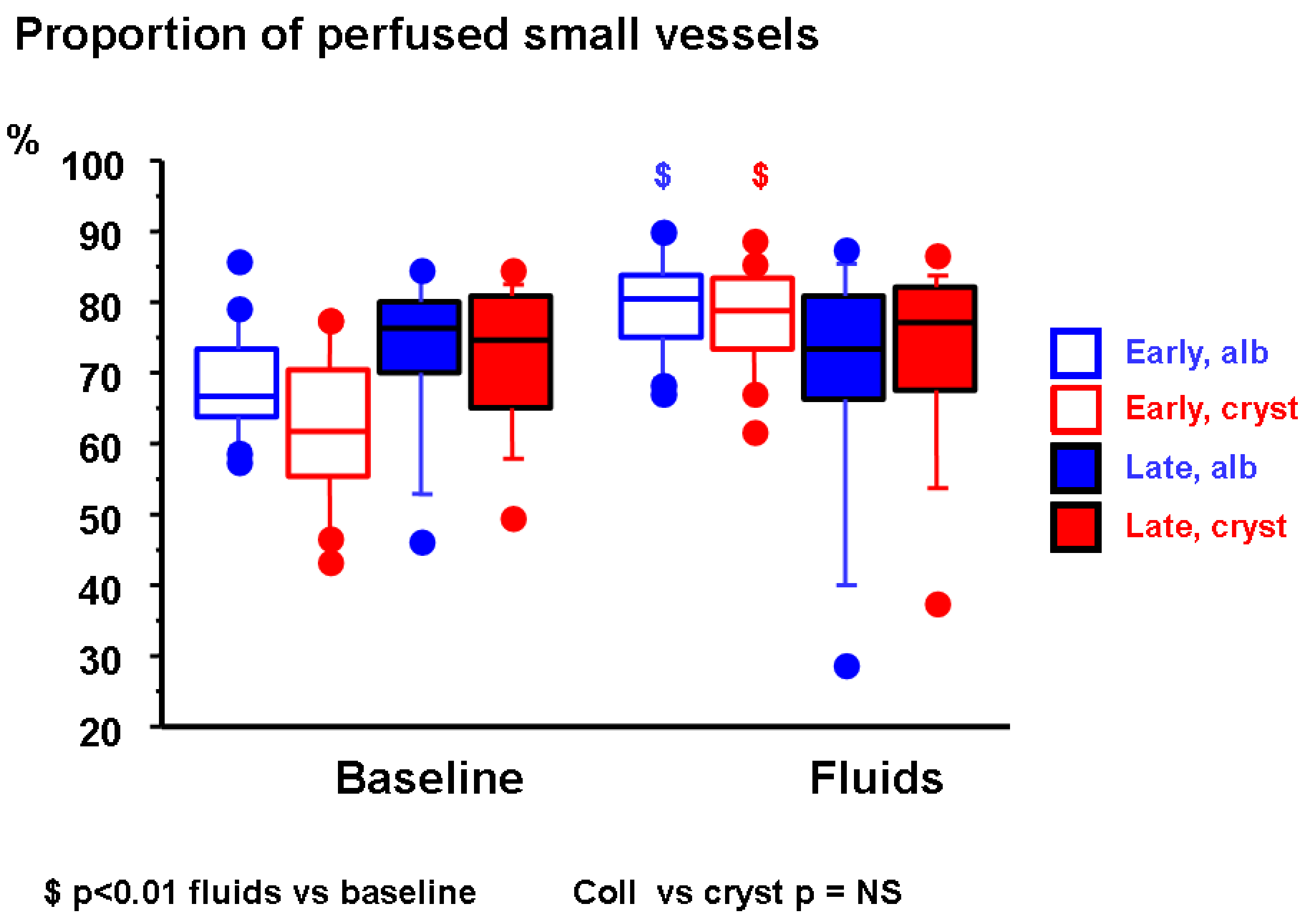
Table 2 Hemodynamic response to fluids in early and late phases of sepsis

	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

- *and ** p<0.05 and p<0.01 fluids versus baseline
- $ p<0.05 late versus early

Ospina et al. ICM. 2010

The time of administration but not the type of fluid influenced the microvascular response

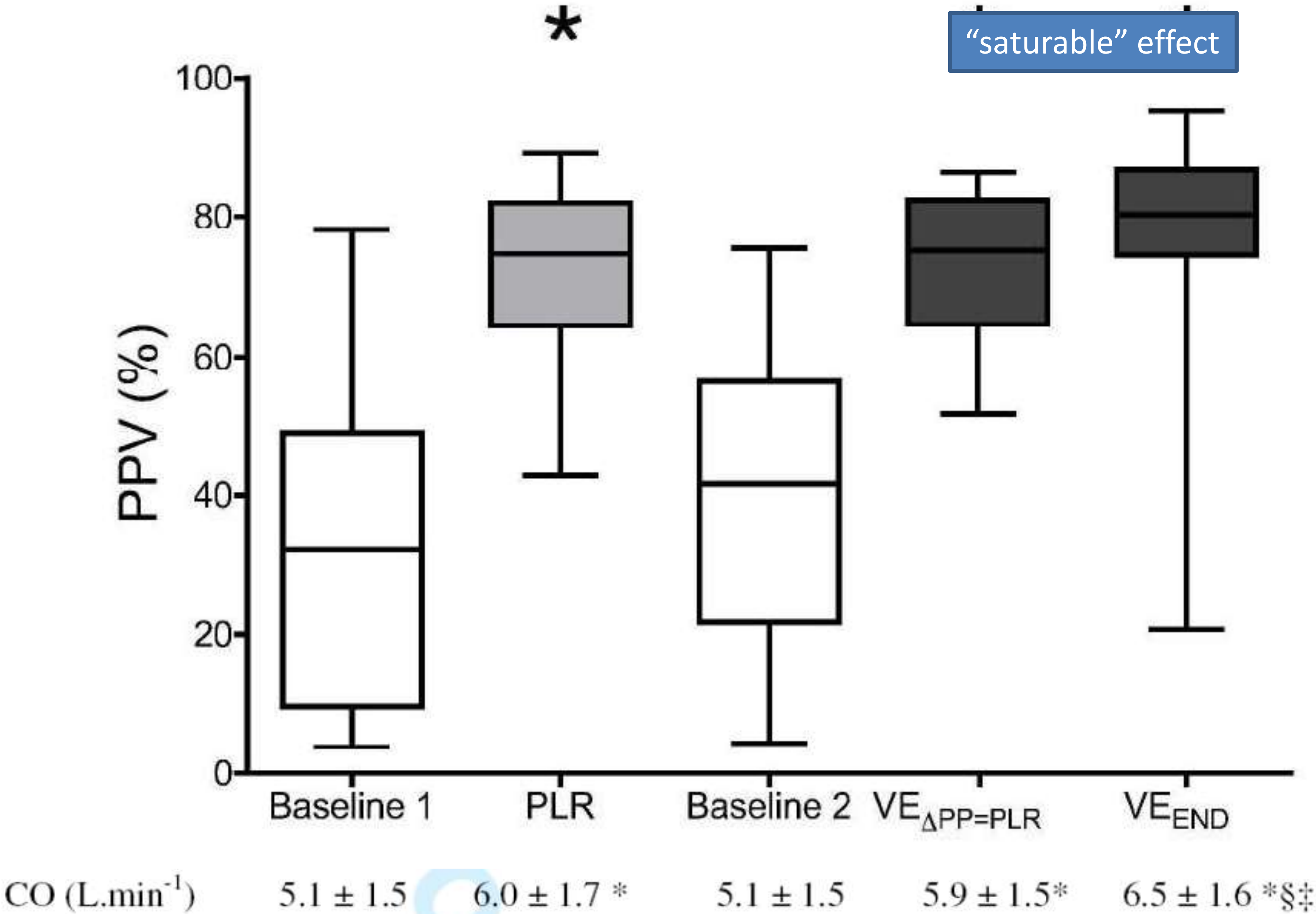


Ospina et al. ICM. 2010

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

- 25 mechanically ventilated patients with severe sepsis or septic shock who were eligible for VE in the first 24 h of their admission
- PPV, CO and sublingual microcirculation indices were assessed at
- 5 consecutive steps:
  - Semirecumbent position (Baseline 1)
  - During PLR manoeuvre (PLR)
  - After returning to semi-recumbent position (Baseline 2)
  - At the time when VE induced the same degree of preload responsiveness as PLR
  - At the end of VE

Pottecher et al. ICM 2010



Pottecher et al. ICM 2010

Effects of RBC transfusions

- Increases functional capillary density by filling RBC-depleted capillaries

Miranda M et al. Am J Physiol Heart Circ Physiol. 2016

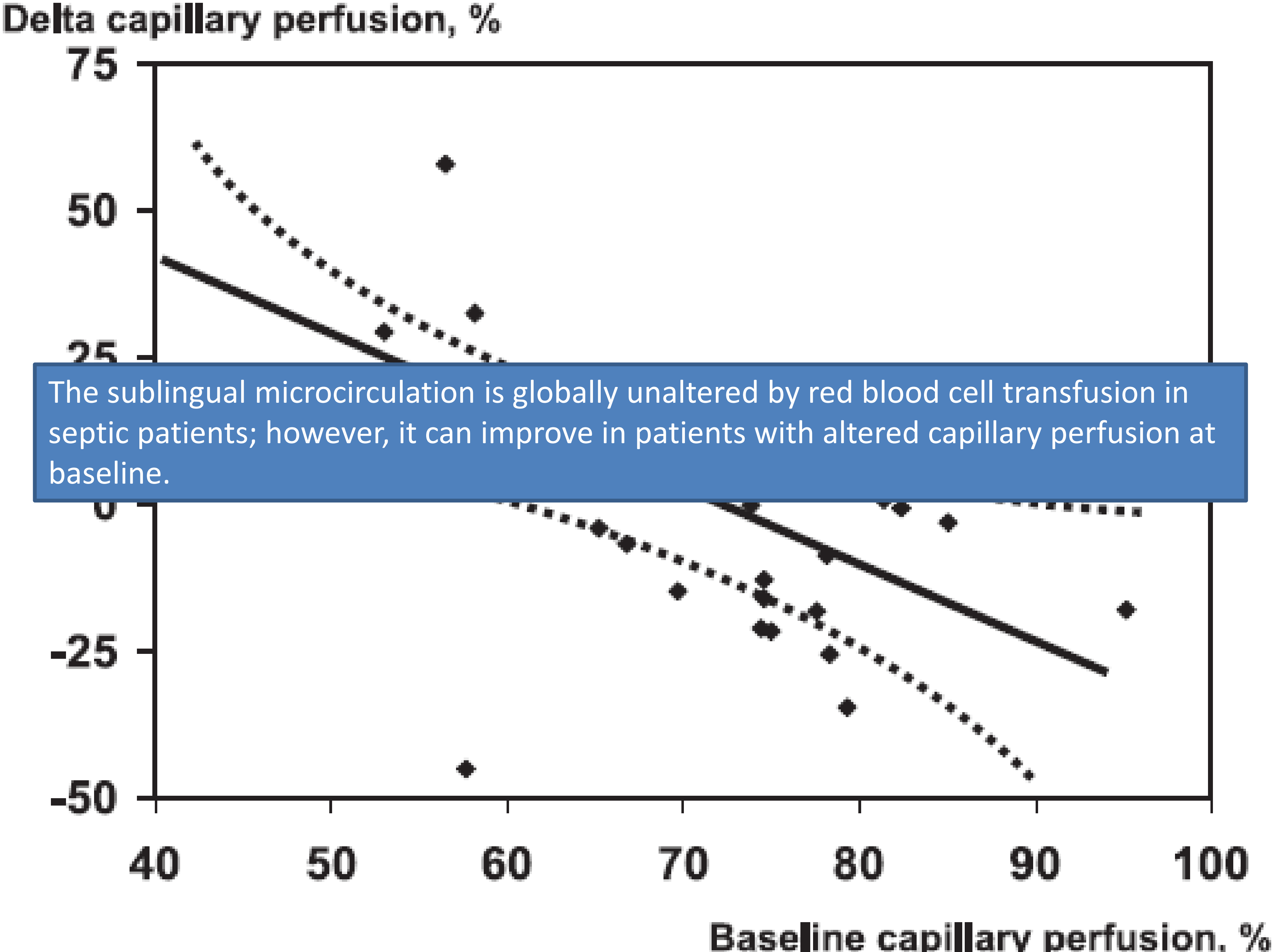
Microvascular response to red blood cell transfusion in patients with severe sepsis

- Design: Prospective, observational study.
- Setting: 31-bed ICU
- Patients: 35 patients with severe sepsis requiring RBC transfusions.
- Interventions: Transfusion of 1-2units of leukocyte reduced RBCs
- Measurements: Sublingual microcirculation was assessed (OPSI) before and 1 hr after txn

Sakr et al. CCM. 2007

	All Patients (n = 35)	
	Baseline	Transfusion
Temperature, °C	36.7 (36.3–37.4)	36.9 (36.4–37.4)
Heart rate, beats per min	99 (90–111)	98 (89–110)
Mean arterial pressure, mm Hg	75 (69–89)	82 (75–90)[a]
Central venous pressure, mm Hg	10 (8–14)	12 (10–16)[a]
Mean pulmonary artery pressure, mm Hg[b]	29 (25–34)	32 (28–35)
Pulmonary artery occlusion pressure, mm Hg[b]	16 (12–18)	17 (13–20)[c]
Cardiac index, L/min·M^2[b]	3.6 (3–4.2)	3.7 (2.6–4.4)
Hemoglobin concentration, g/dL	7.1 (6.7–7.6)	8.1 (7.5–8.6)[a]
$Paco_2$, mm Hg	37 (34–42)	37 (35–42)
Pao_2, mm Hg	100 (77–132)	101 (75–116)
pH	7.40 (7.30–7.43)	7.37 (7.29–7.43)
Sao_2, %	98 (95–100)	99 (97–100)
Lactate, mmol/L	1.3 (0.8–1.8)	1.3 (1.0–1.7)
Mixed venous oxygen saturation, %[b]	64 (59–73)	67 (60–79)
Oxygen delivery, mL/min·M^2[b]	349 (278–392)	391 (273–476)[a]
Oxygen consumption, mL/min·M^2[b]	105 (84–146)	108 (67–159)
Oxygen extraction ratio, %[b]	33 (26–39)	32 (20–38)
Total vascular density, n/mm	5.7 (4.3–8.4)	5.5 (4.4–8.8)
% all vessels perfusion	85 (80–89)	87 (82–93)
Perfused capillary density, n/mm^3	2.4 (1.8–3.2)	2.3 (1.8–2.8)
% capillary perfusion	74 (58–78)	71 (61–80)

Sakr et al. CCM. 2007



The sublingual microcirculation is globally unaltered by red blood cell transfusion in septic patients; however, it can improve in patients with altered capillary perfusion at baseline.

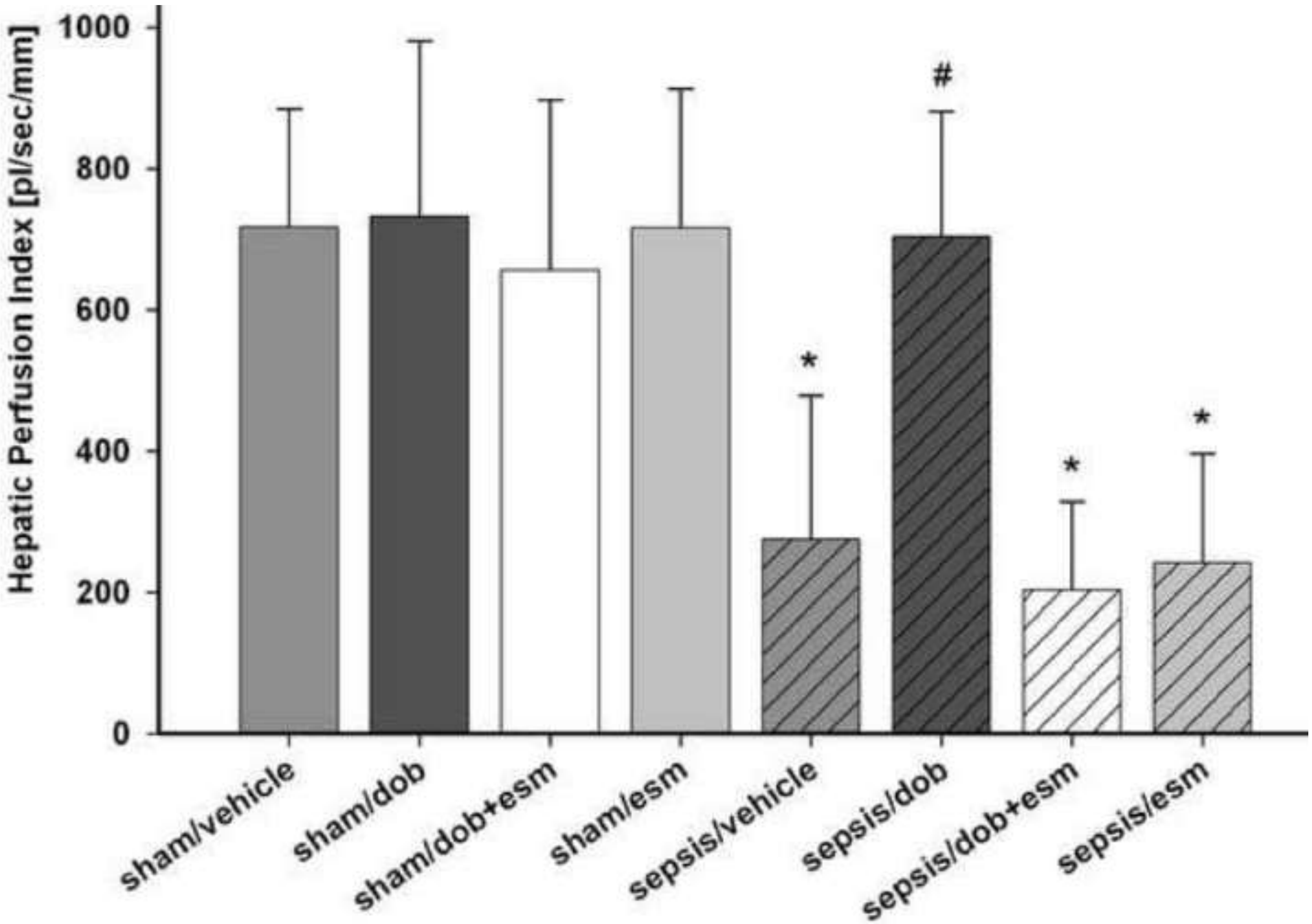
Sakr et al. CCM. 2007

Inotropic agents?

- Dobutamine may decrease leukocytes adhesion
- Milrinone reduces platelet aggregation and exerts protective effects on endothelial barrier function
- Levosimendan may exert anti-inflammatory effects
- All three drugs induce vasodilation at microcirculatory level

Miranda M et al. Am J Physiol Heart Circ Physiol. 2016

Dobutamine pretreatment improves survival, liver function, and hepatic microcirculation after polymicrobial sepsis in rat



Rats; CLP model; pretreatment; intravital microscopy

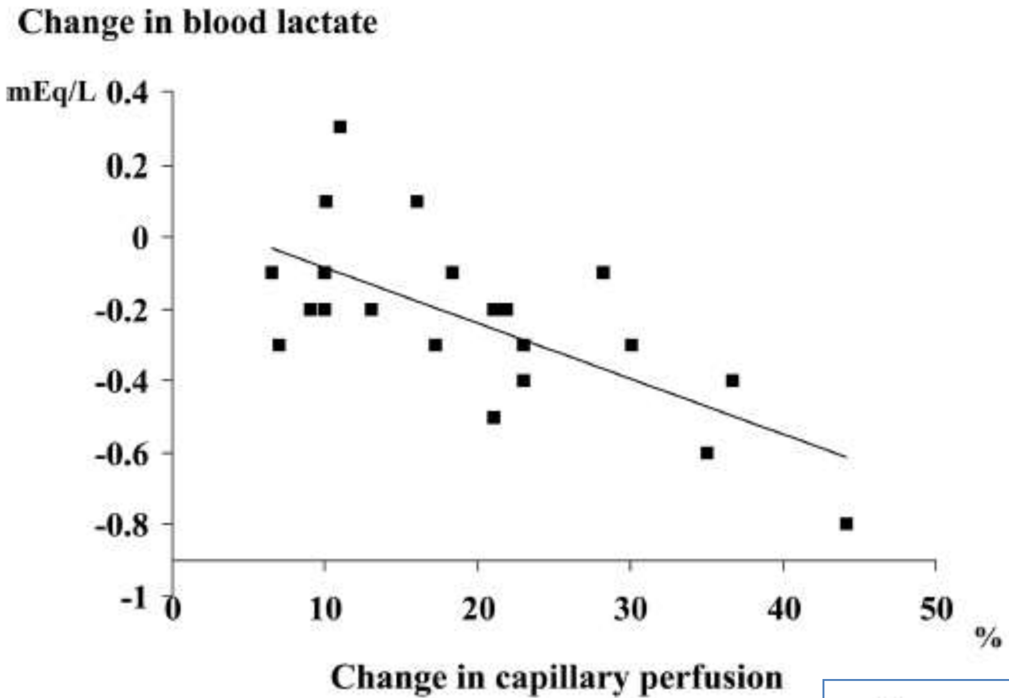
Fink T et al. Shock 2013

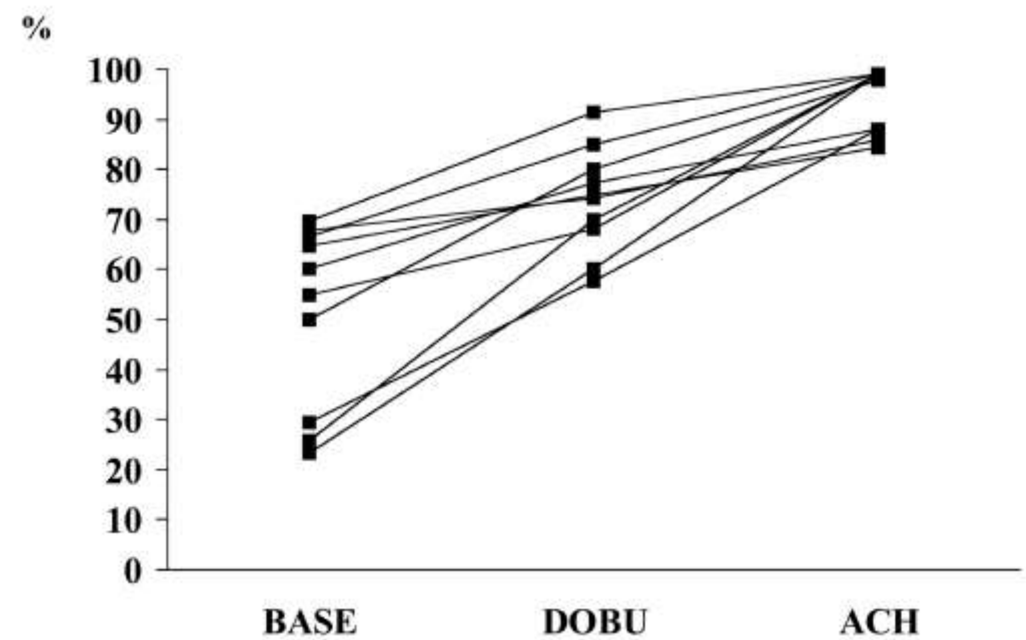
The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects

	Baseline	Dobutamine	*p* Value
Total vascular density, n/mm^2	6.5 ± 1.1	7.4 ± 1.1	.001
Proportion perfused venules, %	99 ± 1	100 ± 0	.50
Proportion perfused capillaries, %	48 ± 16	67 ± 11	.001
Density of perfused capillaries, n/mm^2	5.2 ± 1.3	6.3 ± 1.1	.001
Proportion of nonperfused capillaries, %	19 ± 14	10 ± 8	.004
Proportion of intermittently perfused capillaries	31 ± 8	15 ± 7	.002
Coefficient of variation perfused vessels, %	15 ± 8	12 ± 7	.16

22 patients with septic shock; OPSI sublingual

De Backer et al. CCM. 2006



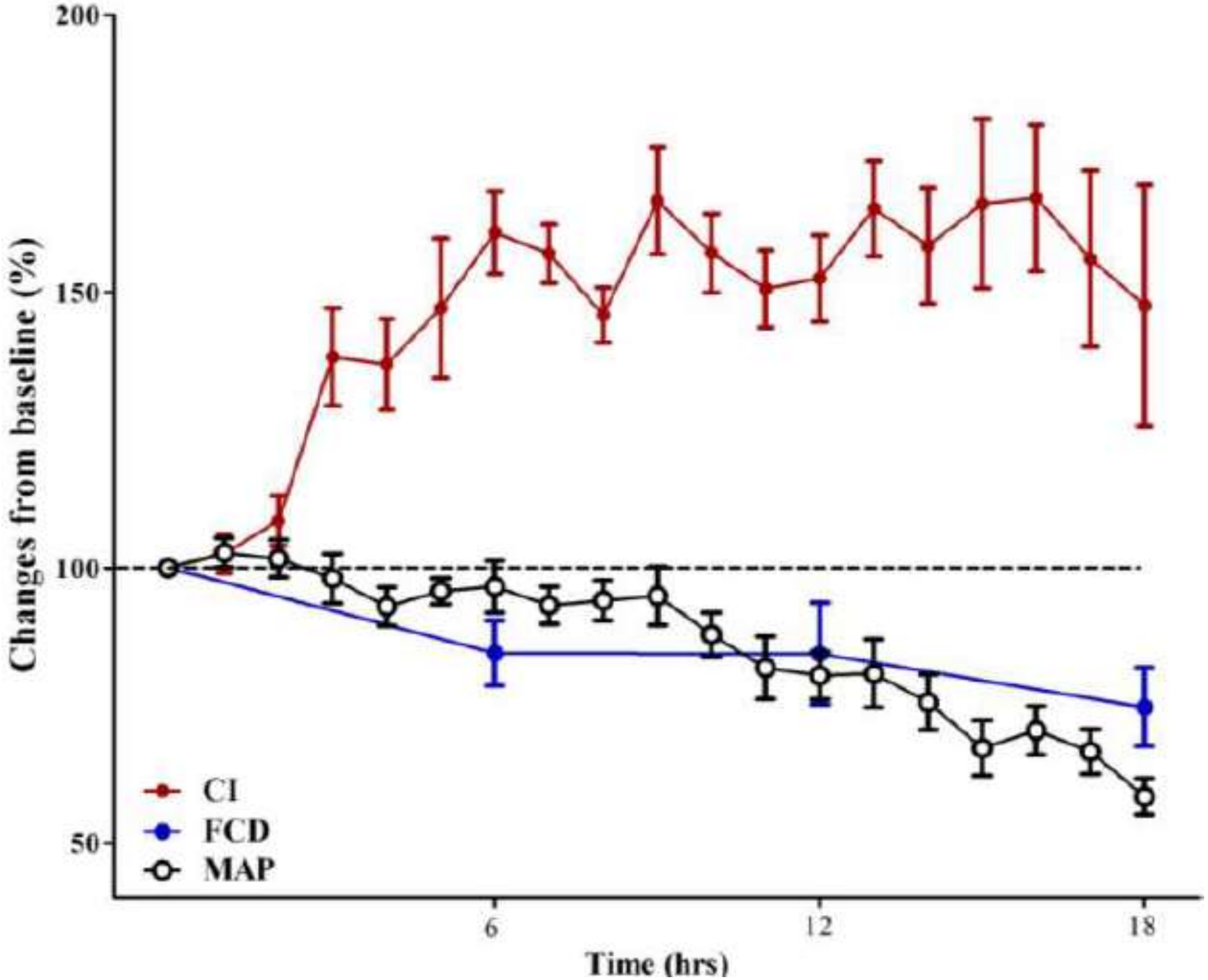


De Backer et al. CCM 34:403;2006

Vasopressor agents ?

Influence of blood pressure on microvascular perfusion ?

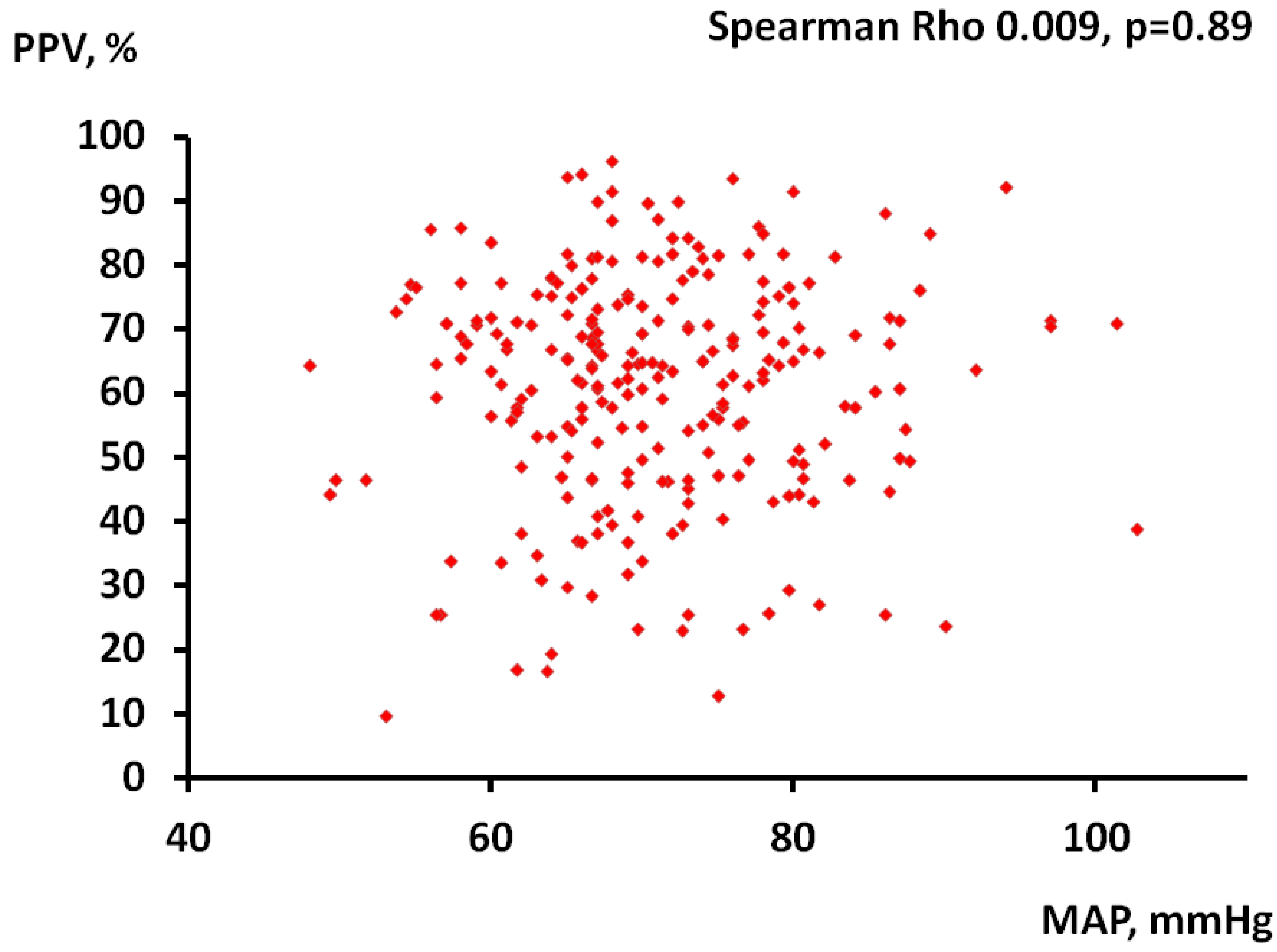
Cerebral microcirculation



Sheep, fecal peritonitis; SDFI

Taccone FS et al. Crit Care. 2010

No clear cut-off



Severe sepsis (n=252)

De Backer et al. CCM 41:791;2013

Blood pressure targets

Counterproductive?

Impact of vasopressor agents

$$Q = \frac{\pi P r^4}{8 \eta l}$$

Impact of vasopressors on the microcirculation (Norepinephrine vs Vasopressine)

	Baseline	Drug infusion	*p* value[a]
MAP (mmHg)			
NE[b]	103 ± 8	129 ± 7	0.221
AVP[b]	98 ± 10	121 ± 8	

Parameter	Drug	Baseline	Drug infusion	Change (%)	*p* value
RBC velocity (mm/s)	NE[a]	1.7 ± 0.3	1.3 ± 0.3	21 ± 14	0.464
	AVP[a]	1.5 ± 0.3	1.1 ± 0.1	27 ± 19	
Arteriolar BF (10^{-4} × mm × μm^2/s)	NE[a]	1.3 ± 1.4	0.4 ± 0.3	63 ± 10	0.837
	AVP[a]	1.2 ± 0.9	0.5 ± 0.3	57 ± 21	

Hamster, control condition

Friesenecker et al. Crit Care. 2006

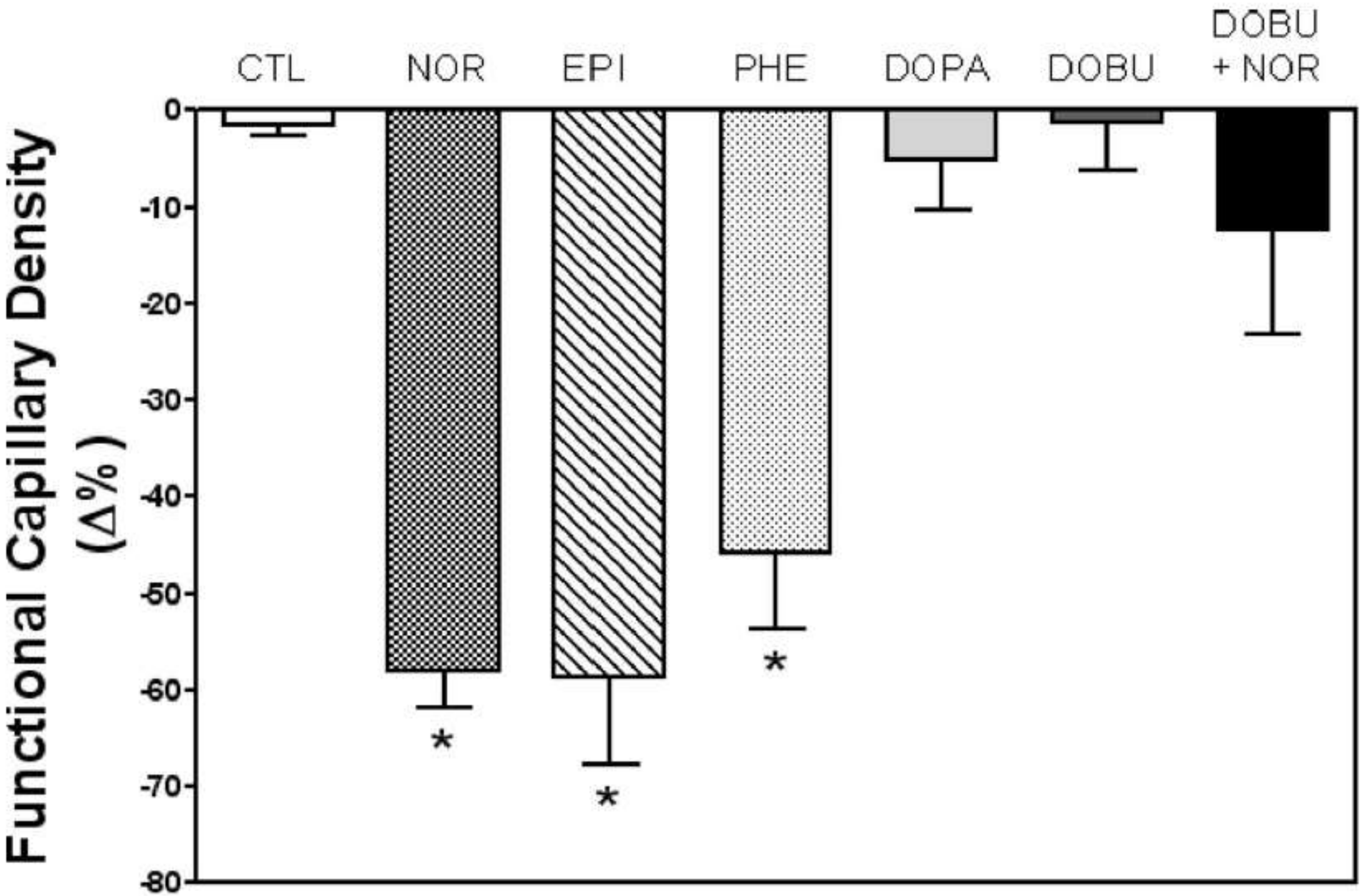
Phenylephrine impairs microvascular perfusion in CPB

	Before CPB	CPB			P-value
		Before phenylephrine	Phenylephrine	After phenylephrine	
Systemic variables					
Perfusion pressure (mm Hg)	72.5 (10.8)	47.0 (8.8)*	68.1 (7.0)†,‡	48.7 (6.0)	<0.001
Syst. flow index (litre m^{-2})	2.6 (0.4)	2.4 (0.0)*	2.4 (0.0)	2.4 (0.0)	<0.001
Temperature (°C)	35.8 (0.4)	33.2 (1.2)*	32.3 (1.4)	32.8 (1.3)	<0.001
Hb (g dl^{-1})	12.1 (1.3)	7.9 (1.1)*	8.2 (0.8)	8.2 (0.7)	<0.001
Hct (%)	35.5 (3.7)	23.3 (3.1)*	24.0 (2.5)	24.3 (2.1)	<0.001
pHa	7.41 (0.04)	7.35 (0.04)*	7.36 (0.04)	7.35 (0.05)	<0.001
HCO_3^- (mmol $litre^{-1}$)	24.6 (2.2)	22.7 (2.2)	23.2 (2.2)	23.0 (2.1)	<0.001
BE	0.0 (2.2)	2.7 (2.5)	2.2 (2.6)	2.6 (2.7)	<0.001
Pa_{O_2} (kPa)	33.3 (16.0)	36.3 (6.0)	30.3 (3.5)	30.6 (5.7)	0.243
Pa_{CO_2} (kPa)	5.2 (0.8)	5.6 (0.5)	5.5 (0.4)	5.5 (0.5)	0.196
Sv_{O_2} (%)	84.2 (2.4)	78.9 (3.8)*	79.3 (4.9)	79.3 (5.1)	0.002
DO_2I (ml m^{-2} min^{-1})	457 (97)	279 (33)*	282 (26)	283 (22)	<0.001
VO_2I (ml m^{-2} min^{-1})	98 (52)	71 (12)	68 (12)	65 (15)	0.006
Microvascular variables					
PU (arbitrary units)	120 (105)	110 (54)	197 (100)†,‡	89 (66)	0.007
Smc_{O_2} (%)	73 (7)	72 (11)	84 (7)†,‡	72 (8)	<0.001
MFI_s (arbitrary units)	2.1 (1.2)	2.5 (2)	1.8 (1.2)†	2.2 (1.2)	0.039
MFI_m (arbitrary units)	3 (2)	3 (2)	2.8 (1)	2.9 (2)	0.281

15 patients undergoing CABG

Maier-S et al. BJA 2009

Impact of vasopressors on the microcirculation



Rats, LPS, gut muscularis, Intravital microscopy

Nacul F et al. Anesth Analg 2010

Would vasopressors benefit?

For

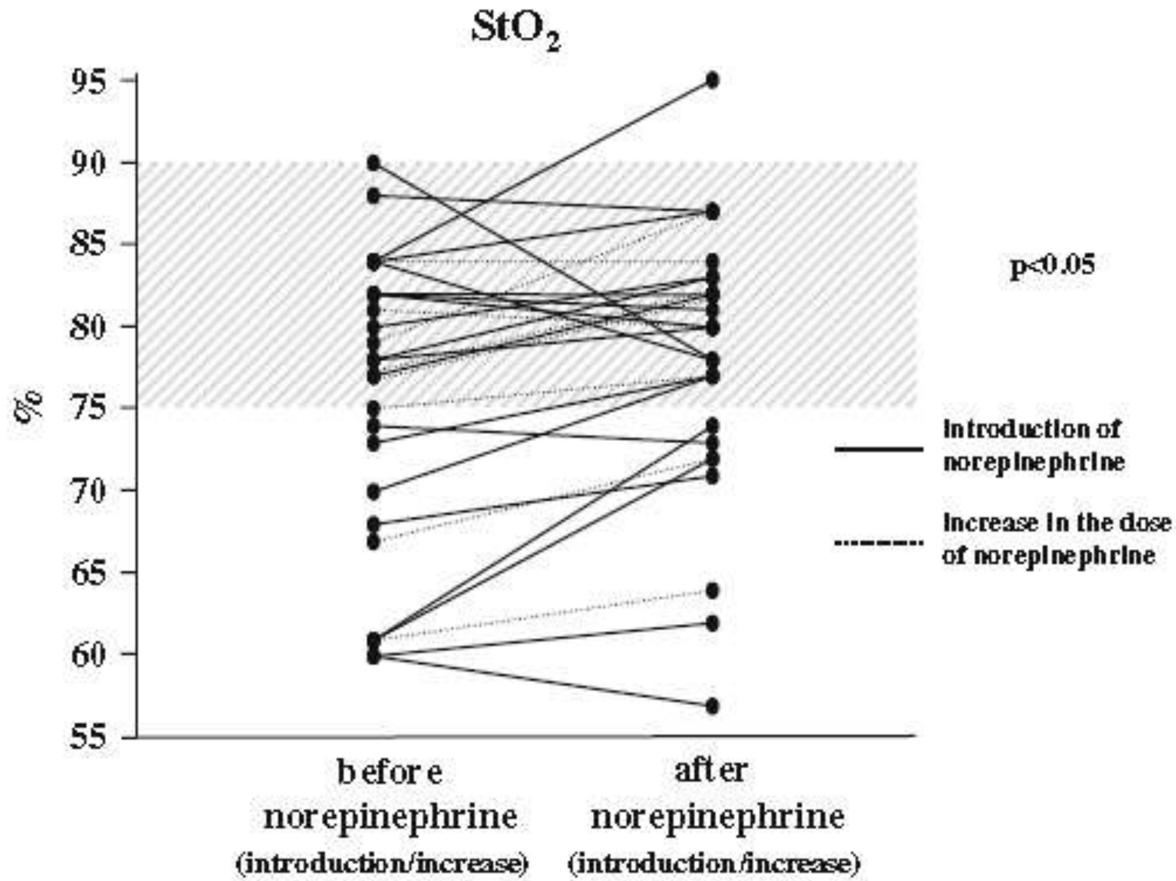
- Maintaining MAP across vascular beds of vital organs
- Beta effect can cause some microvascular dilatation and reduced rolling/adhesion

Against

- Intense vasoconstriction can further reduce the microcirculation

Does correction of hypotension result in an improved tissue perfusion ?

Correction of hypotension improves microvascular reactivity (NIRS)



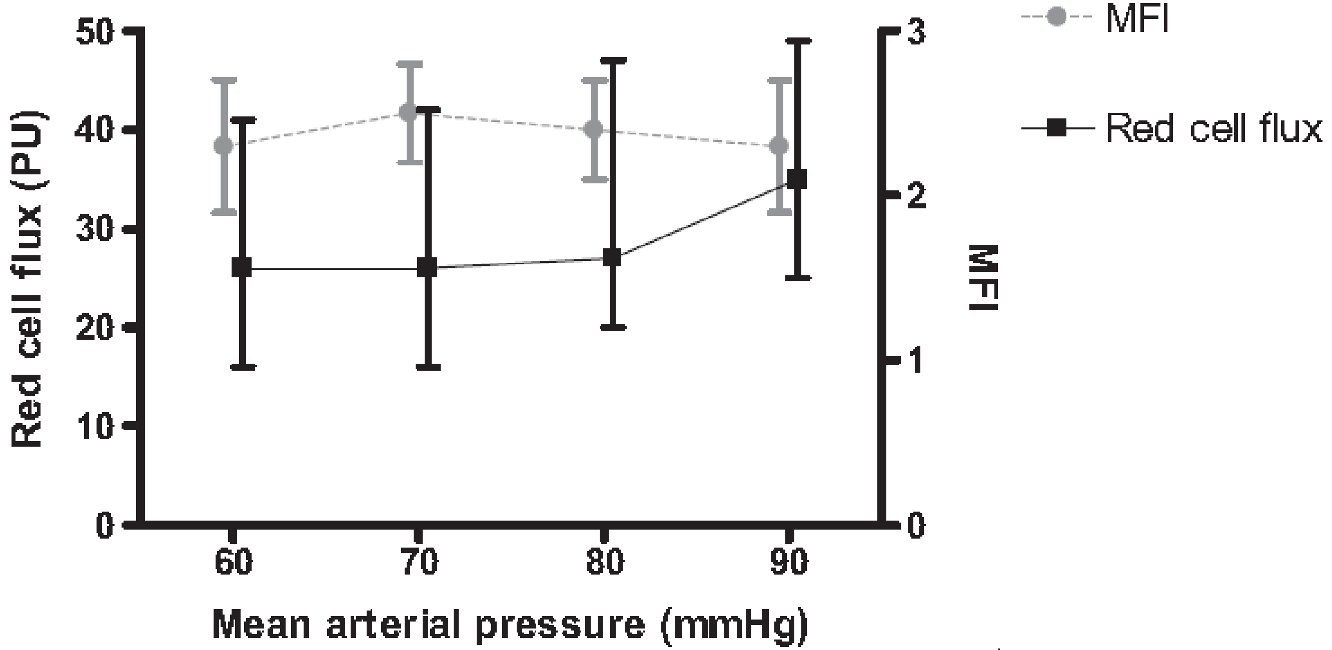
Septic shock; n=28

Georger et al. ICM 36:1882;2010

What is the optimal blood pressure target for the microcirculation ?

Impact of MAP/NE on microvascular perfusion

	60 mm Hg	70 mm Hg	80 mm Hg	90 mm Hg	p
Microvascular flow index	2.3 (0.4)	2.5 (0.3)	2.4 (0.3)	2.3 (0.4)	0.45
Vessel density (mm^{-1})	6.9 (1.5)	7.1 (1.5)	7.1 (1.3)	6.9 (0.9)	0.96
Proportion of perfused vessels (%)	75 (66–87)	84 (74–90)	85 (71–93)	77 (72–84)	0.57
Perfused vessel density (mm^{-1})	5.3 (1.9)	5.9 (1.8)	5.8 (1.5)	5.3 (1.3)	0.75
Heterogeneity index	0.41 (0.28)	0.37 (0.25)	0.32 (0.12)	0.33 (0.22)	0.84
Cutaneous red blood cell flux (PU)	26 (16–42)	27 (18–44)	27 (20–47)	33 (20–47)	0.04
Cutaneous vascular conductance (PU/mm Hg)	0.44 (0.27–0.70)	0.39 (0.25–0.63)	0.34 (0.24–0.59)	0.37 (0.23–0.52)	0.003



Septic shock; n=16; sidestream darkfield imaging

Jhanji et al. CCM ;2009

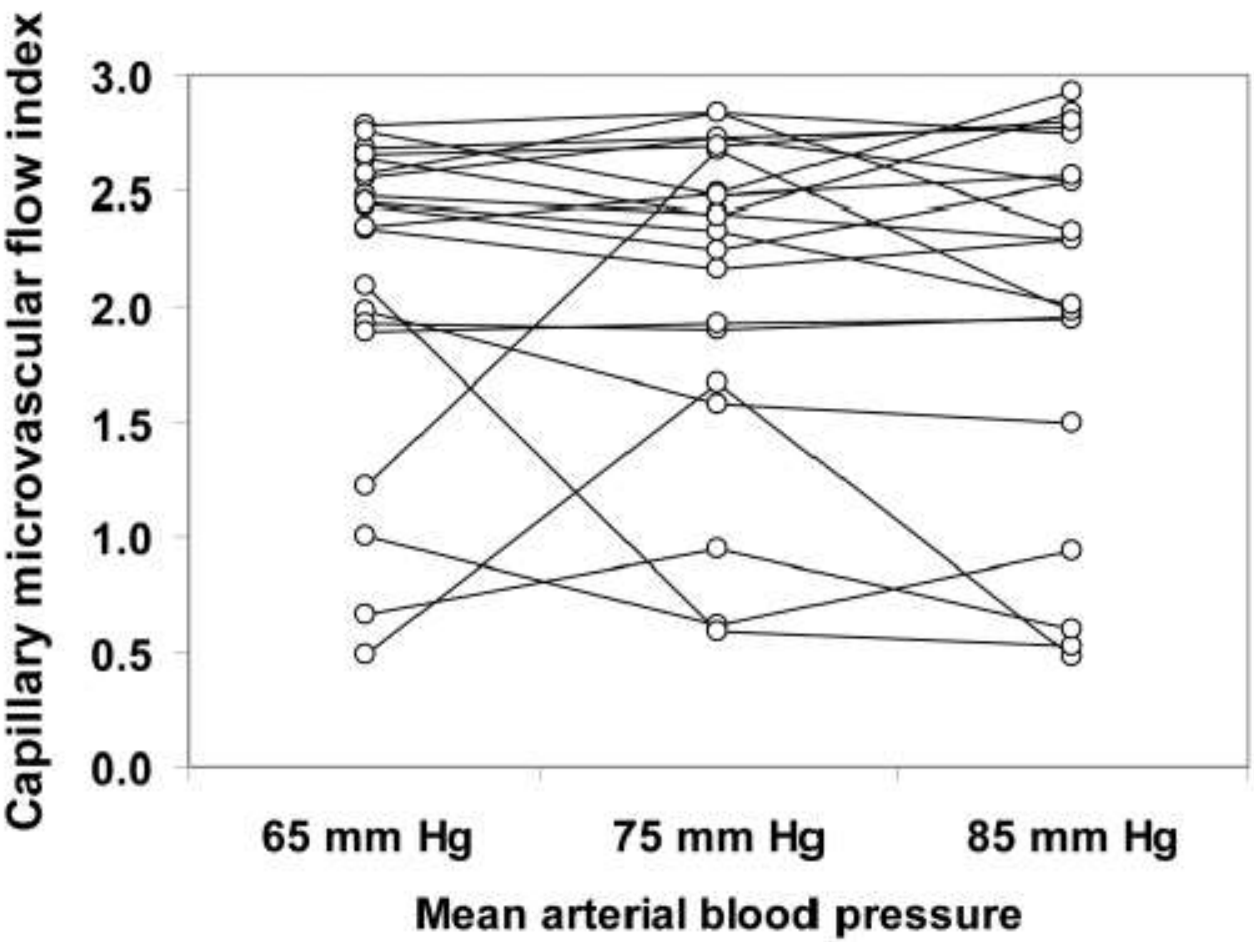
Impact of MAP/NE on microvascular perfusion

Changes in microvascular variables as mean arterial pressure was increased from 65 mmHg to 85 mmHg with norepinephrine

	Mean arterial blood pressure			ANOVA	Linear trend
	65 mmHg	75 mmHg	85 mmHg	*P*	P
Vascular density (vessels/mm²)					
Large diameter vessels	11 ± 1	10 ± 3	10 ± 3	0.81	0.61
Medium diameter vessels	15 ± 3	16 ± 4	16 ± 4	0.82	0.53
Small diameter vessels	24 ± 8	23 ± 8	22 ± 1	0.09	0.03
Microvascular flow index					
Large diameter vessels	2.3 ± 0.6	2.3 ± 0.8	2.2 ± 0.8	0.34	0.16
Medium diameter vessels	2.2 ± 0.7	2.2 ± 0.7	2.1 ± 0.9	0.79	0.52
Small diameter vessels	2.1 ± 0.7	2.2 ± 0.7	2.0 ± 0.8	0.69	0.47
Perfused vessels (%)					
Large diameter vessels	82 ± 21	80 ± 28	87 ± 6	0.46	0.40
Medium diameter vessels	77 ± 27	77 ± 29	77 ± 6	0.98	1.00
Small diameter vessels	72 ± 26	71 ± 27	67 ± 32	0.55	0.38
Total vessels	75 ± 25	75 ± 27	76 ± 4	0.92	0.73
Heterogeneity flow index					
Large diameter vessels	1.0 ± 0.5	1.3 ± 1.2	1.5 ± 1.4	0.07	0.02
Medium diameter vessels	1.6 ± 1.6	1.5 ± 1.4	1.7 ± 1.2	0.86	0.78
Small diameter vessels	1.8 ± 1.3	1.8 ± 1.2	1.7 ± 1.1	0.97	0.80

Septic shock; n=20

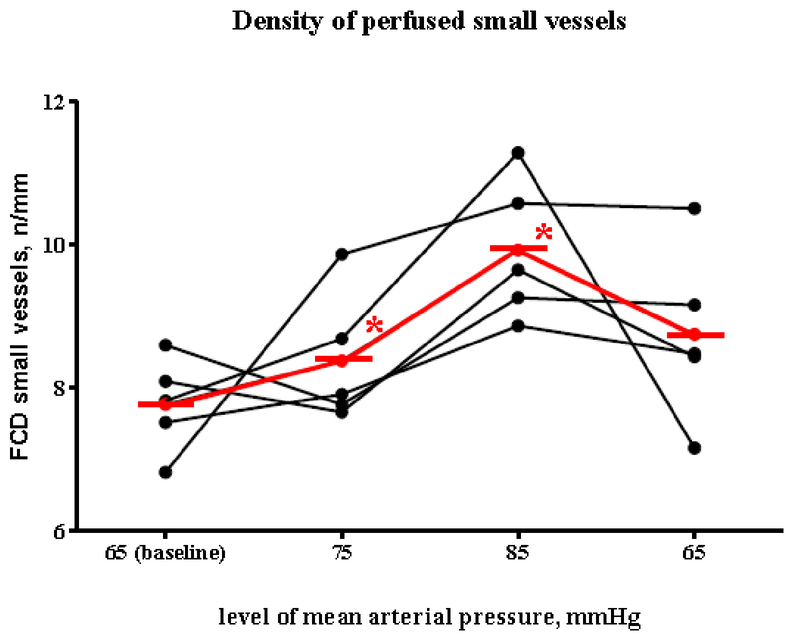
Dubin et al. Crit Care 2009



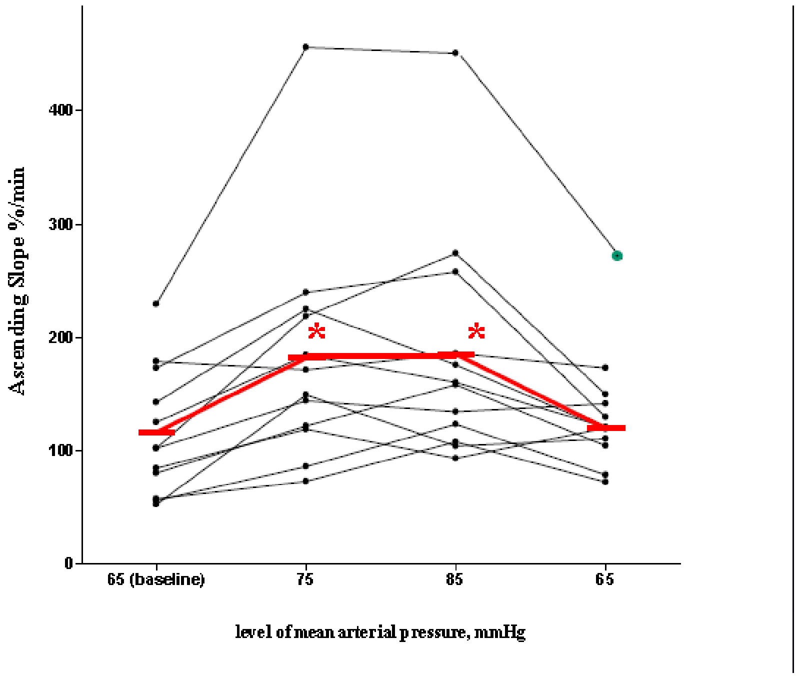
Dubin et al. Crit Care 2009

Impact of MAP/NE on microvascular perfusion

SDFI



NIRS



Septic shock; n=13; NIRS and SDFI

Thooft et al. Crit Care 2011

Vasopressors and the microcirculation

- Vasopressor agents have a dual effect on the microcirculation: on the one hand vasopressors decrease microvascular perfusion by constriction of precapillary sphincters.
- On the other hand, achievement of a minimal perfusion pressure is needed to preserve organ blood flow and microcirculatory perfusion.
- Optimal pressure targets are variable and should be individualized.

Vasodilatory agents ?

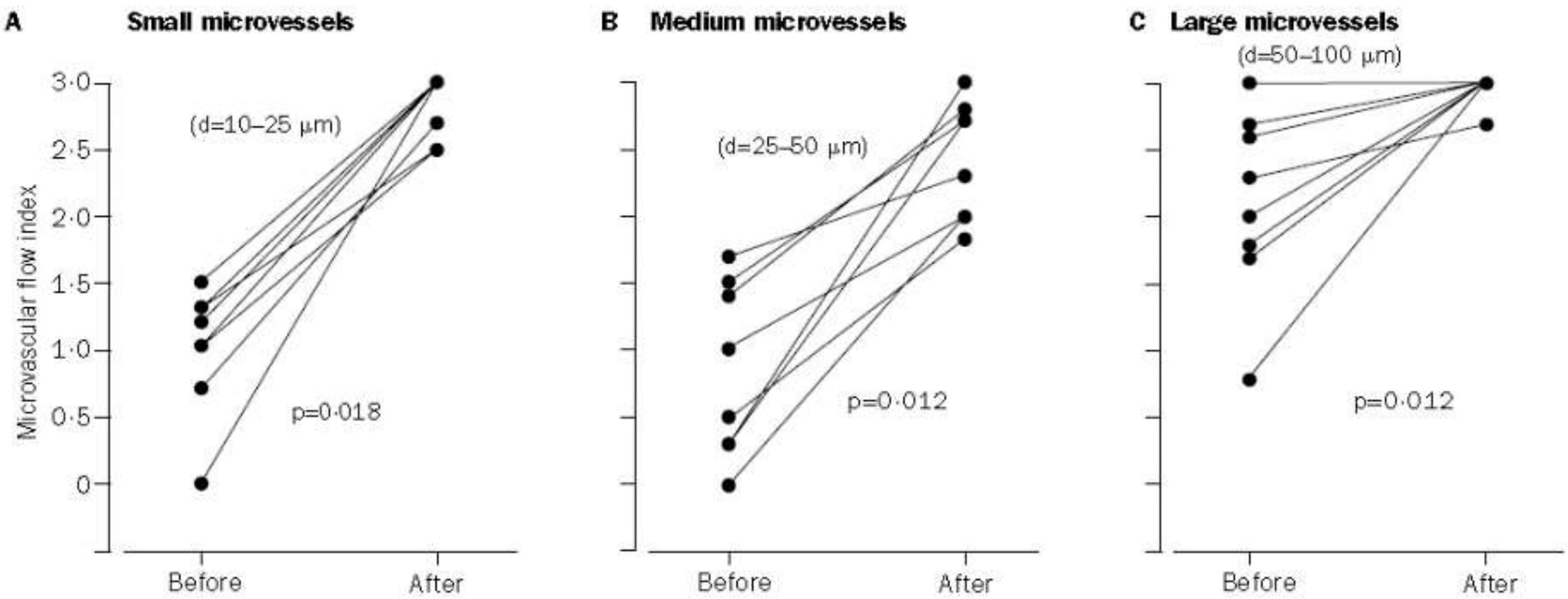
Effect of local Ach

TABLE 4. EFFECT OF TOPICAL ACETYLCHOLINE ADMINISTRATION IN 11 PATIENTS WITH SEPSIS

	Patients with Sepsis*,† (n = 11)		Volunteers (n = 10)
	Baseline	Acetylcholine (10^{-2} M)	
Total number of vessels, n/mm	4.9 (4.1–5.7)	6.0 (4.7–6.4)‡	5.4 (5.4–6.3)‡
Proportion of vessels perfused, %	83 (77–96)	99 (98–100)‡	98 (97–99)‡
Proportion of venules perfused, %	100 (100–100)	100 (100–100)	100 (100–100)
Proportion of capillaries perfused, %	44 (24–60)	94 (77–96)‡	94 (92–95)‡
Absent flow (capillaries), %	29 (8–44)	1 (0–3)‡	3 (2–5)‡
Intermittent flow (capillaries), %	24 (19–38)	8 (3–19)‡	5 (3–6)‡

De Backer et al. AJRCCM. 2002

Nitroglycerin in septic shock after intravascular volume resuscitation

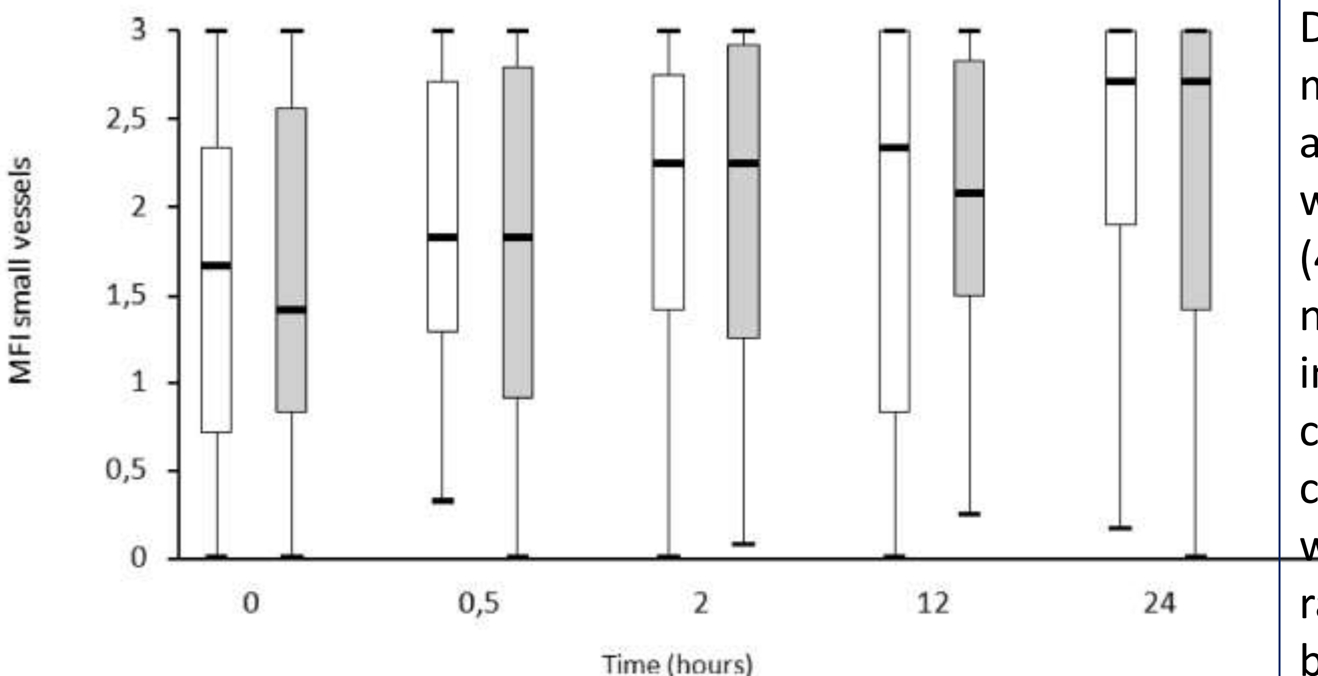


Microvascular flow index before and 2 min after administration of nitroglycerin
d=diameter. 0·5 mg bolus nitroglycerin given intravenously

8 pts with septic shock; OPSI-sublingual

Spronk et al. Lancet 360:1395;2002

Effects of nitroglycerin on sublingual microcirculatory blood flow in patients with severe sepsis/septic shock after a strict resuscitation protocol: a double-blind randomized placebo controlled trial.



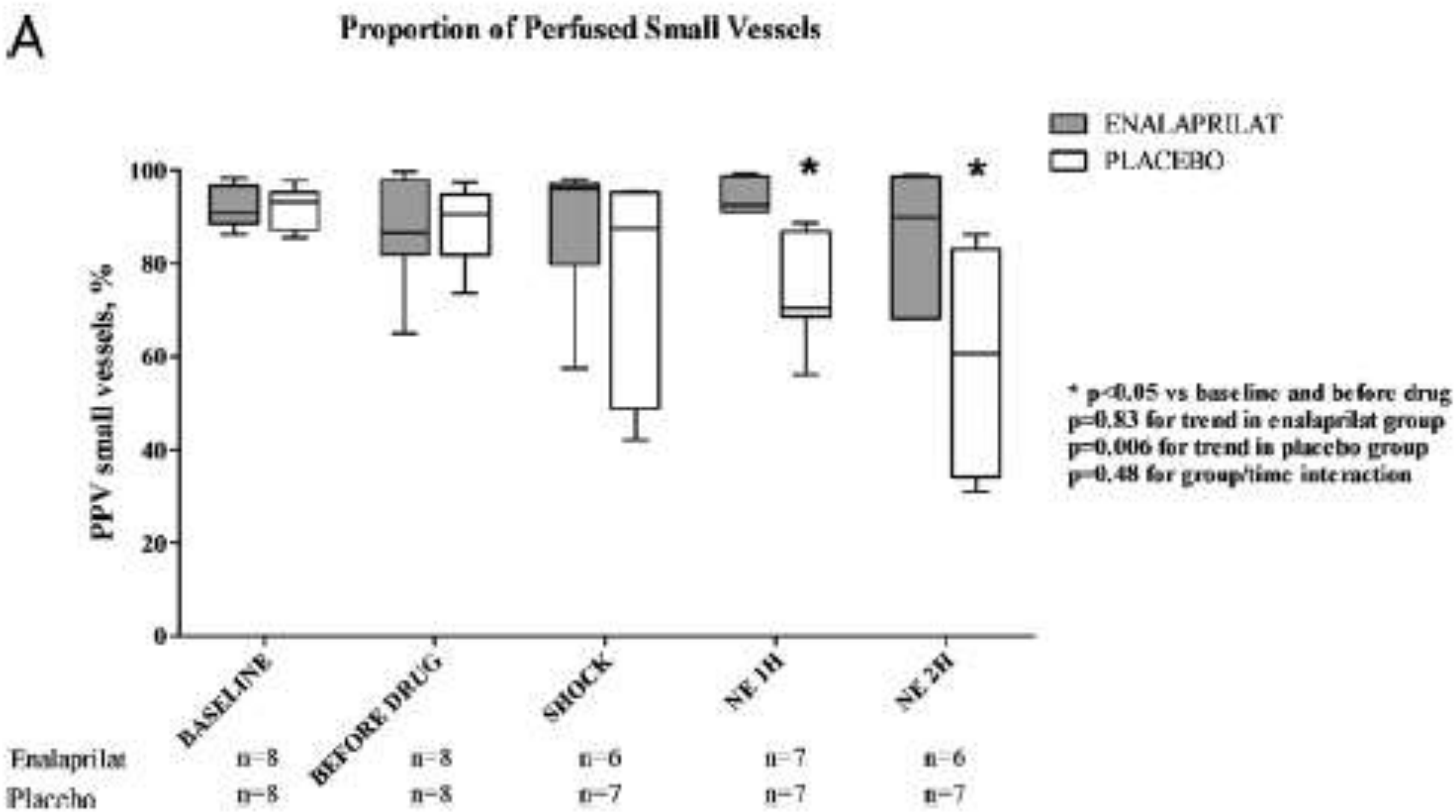
During the first 30 mins of administration, a front load of 2 mL was given continuously (4 mL/hr); during the next 23.5 hrs, the infusion rate was kept constant at 2 mL/hr. In cases of patient body weight 50 kg, infusion rates were reduced by 50%.

NTG (1 mg/mL) or placebo

70 pts with severe sepsis

Boerma E et al. CCM;2010

ACE inhibitors?



Sheep; CLP; sidestream dark-field

Salgado D et al. Shock 2011

Magnesium sulfate?

	Baseline	MgS	*p* Value
Microvascular flow index of small vessels	2.25(1.98-2.69)	2.33(1.96-2.62)	0.65
Microvascular flow index of large vessels	3.00(3.00-3.00)	3.00(3.00-3.00)	0.79
Proportion of perfused small vessels, %	81.5(78.8-89.3)	85.0(79.3-86.3)	0.64
Total vessel density of small vessels, mm/mm^2	26.9(23.2-30.1)	27.8(24.4-29.5)	0.86
Total vessel density of others vessels, mm/mm^2	7.4(6.2-8.9)	6.6(5.8-8.0)	0.26
Perfused vessel density of small vessels, n/mm	13.4(11.8-15.8)	13.6(11.5-15.1)	0.59
Perfused vessel density of other vessels, n/mm	4.2(3.7-4.7)	4.0(3.3-5.0)	0.97
Heterogeneity index	0.30(0.08-0.54)	0.42(0.26-0.50)	0.51

N=14 (12 septic shock, 2 severe sepsis); SDFI

Pranskunas A et al. BMC Anesth. 2011

Verdict – Vasodilatory agents

- At this stage, the use of vasodilating agents cannot be recommended
  - lack of selectivity of agents: steal phenomenon

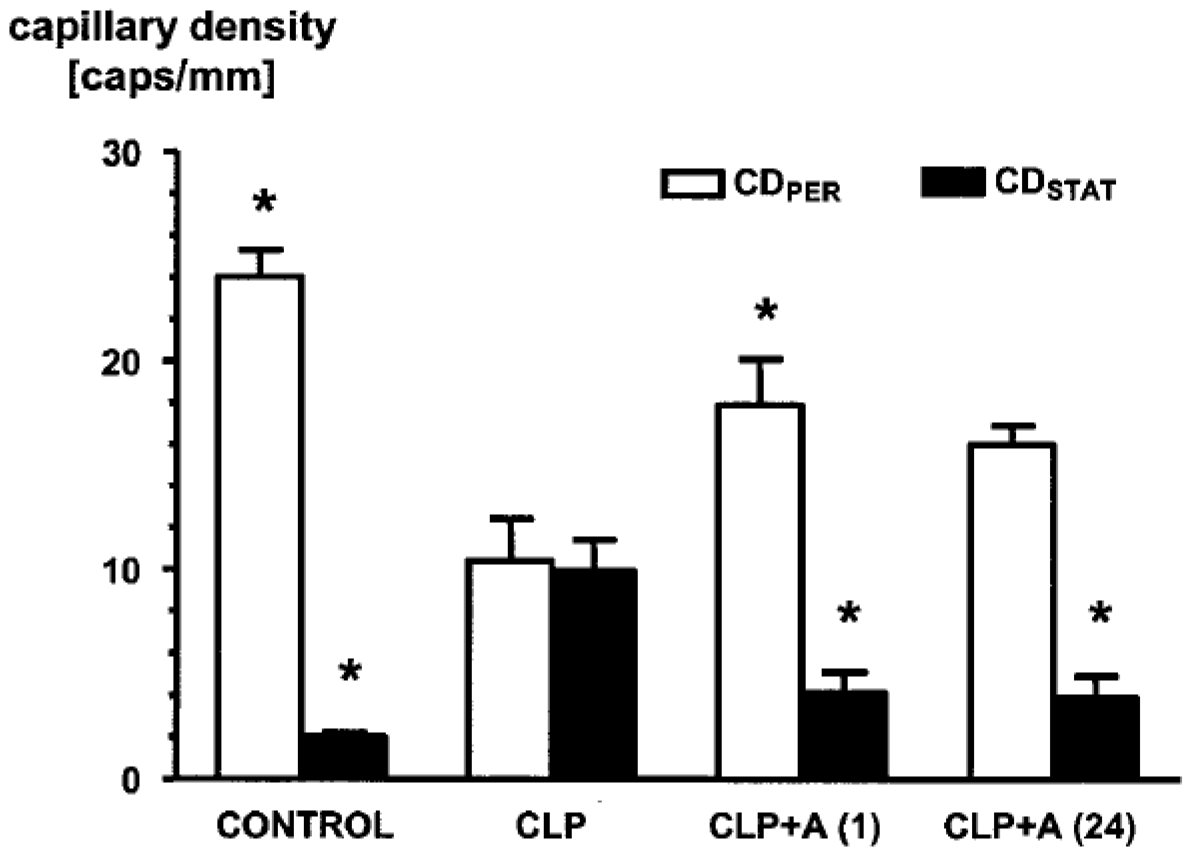
- New pharmacological agents
- More studies to assess optimal dosing, timing and companions (vasopressors/inotropes)

Modulation of endothelial function ?

- eNOS is actively involved in the control of blood flow at the microcirculatory level
- Stimulation leading to an increase in perfusion in the concerned vessels
- In sepsis, eNOS may be dysfunctional
  - impaired perfusion and endothelial reactivity
  - overproduction of ROS, including peroxynitrite
- Modulation of eNOS, enabling NOS to locally produce NO could thus be beneficial

Miranda M et al. Am J Physiol Heart Circ Physiol. 2016

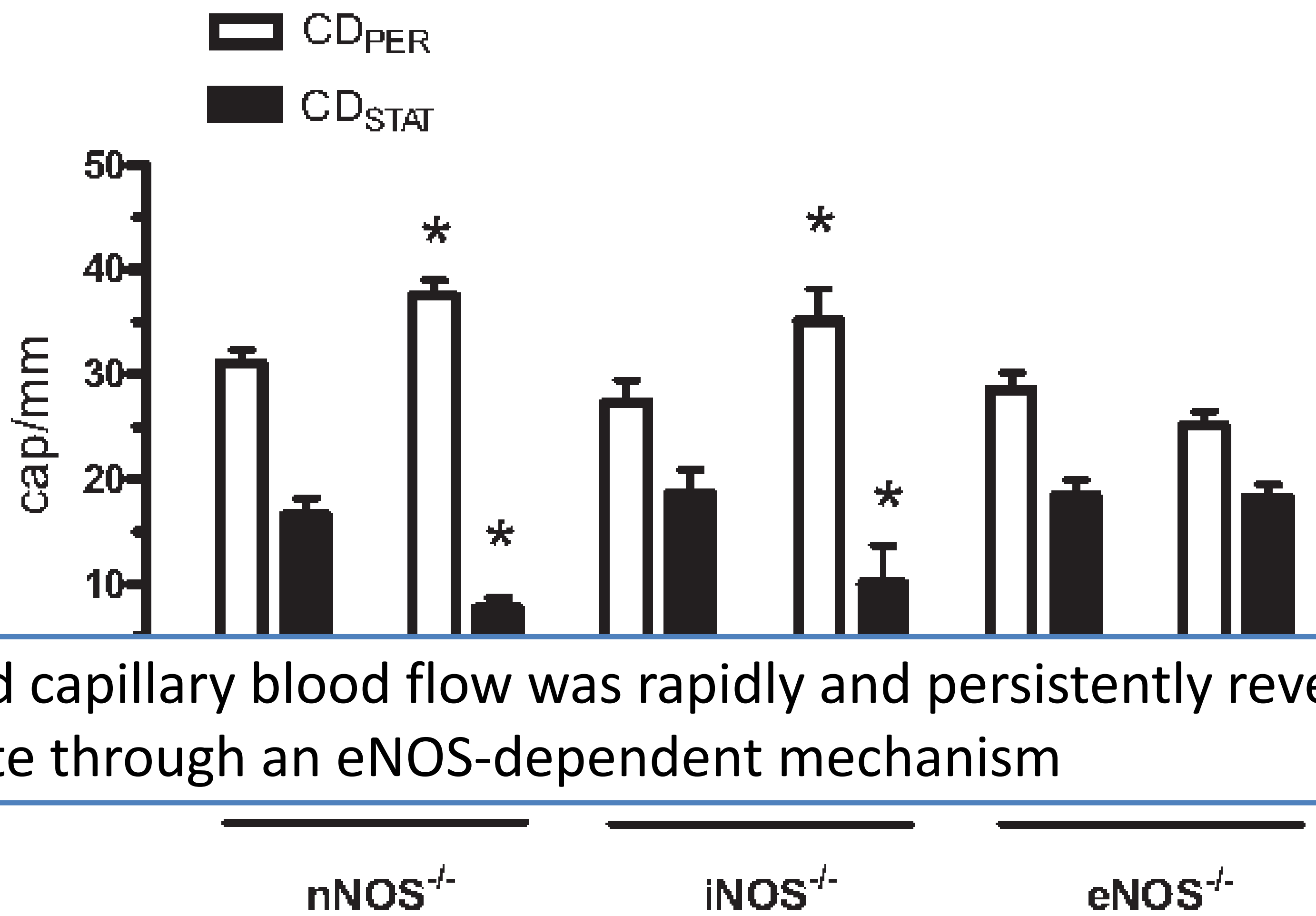
Vitamin C



Rat / muscle; CLP; Intravital microscopy

Tyml K et al. CCM. 2005

Effect of vitamin C

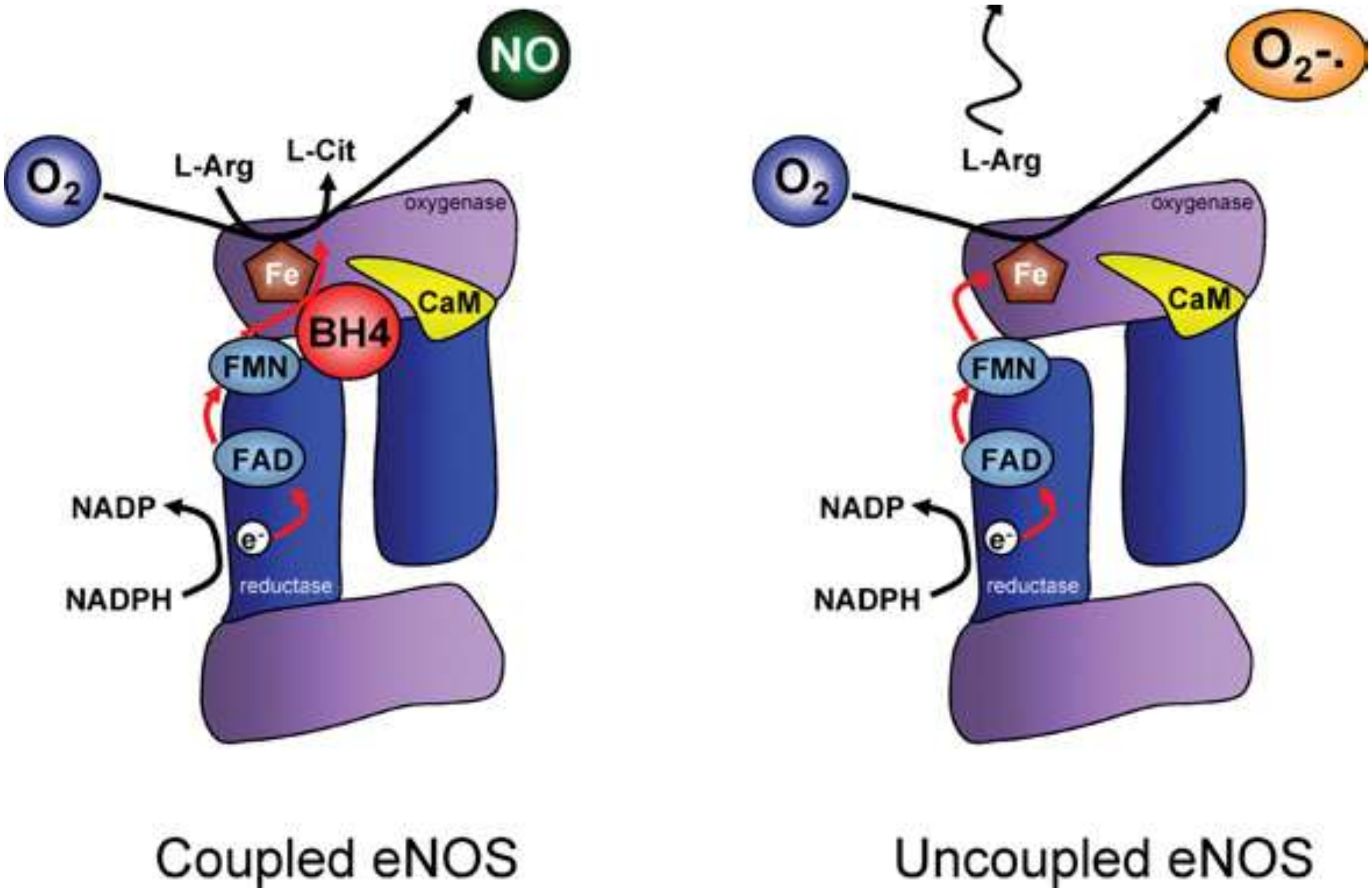


Impaired capillary blood flow was rapidly and persistently reversed by ascorbate through an eNOS-dependent mechanism

Mice / muscle; intravital microscopy
Feces in peritoneum

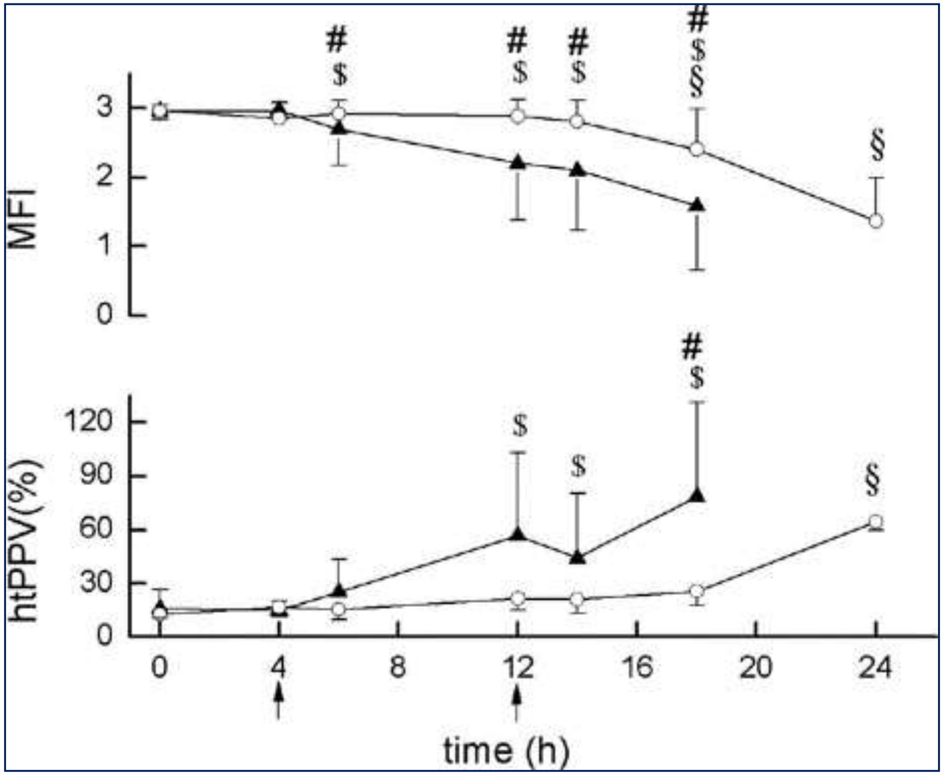
Tyml K et al. CCM 2008

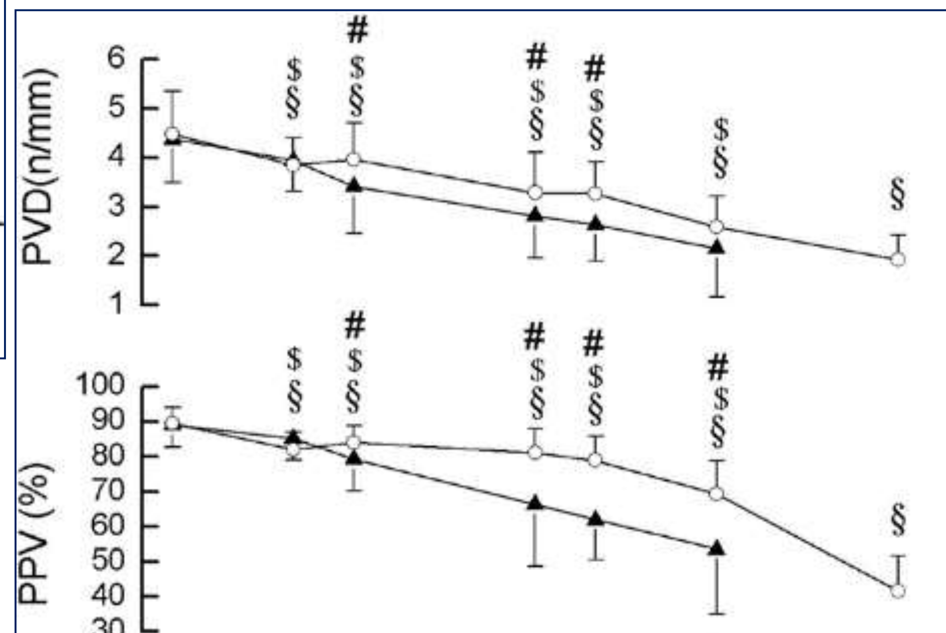
BH4 (tetrahydrobiopterin)



Schmidt S and Alp N. Clinical Science. 1997

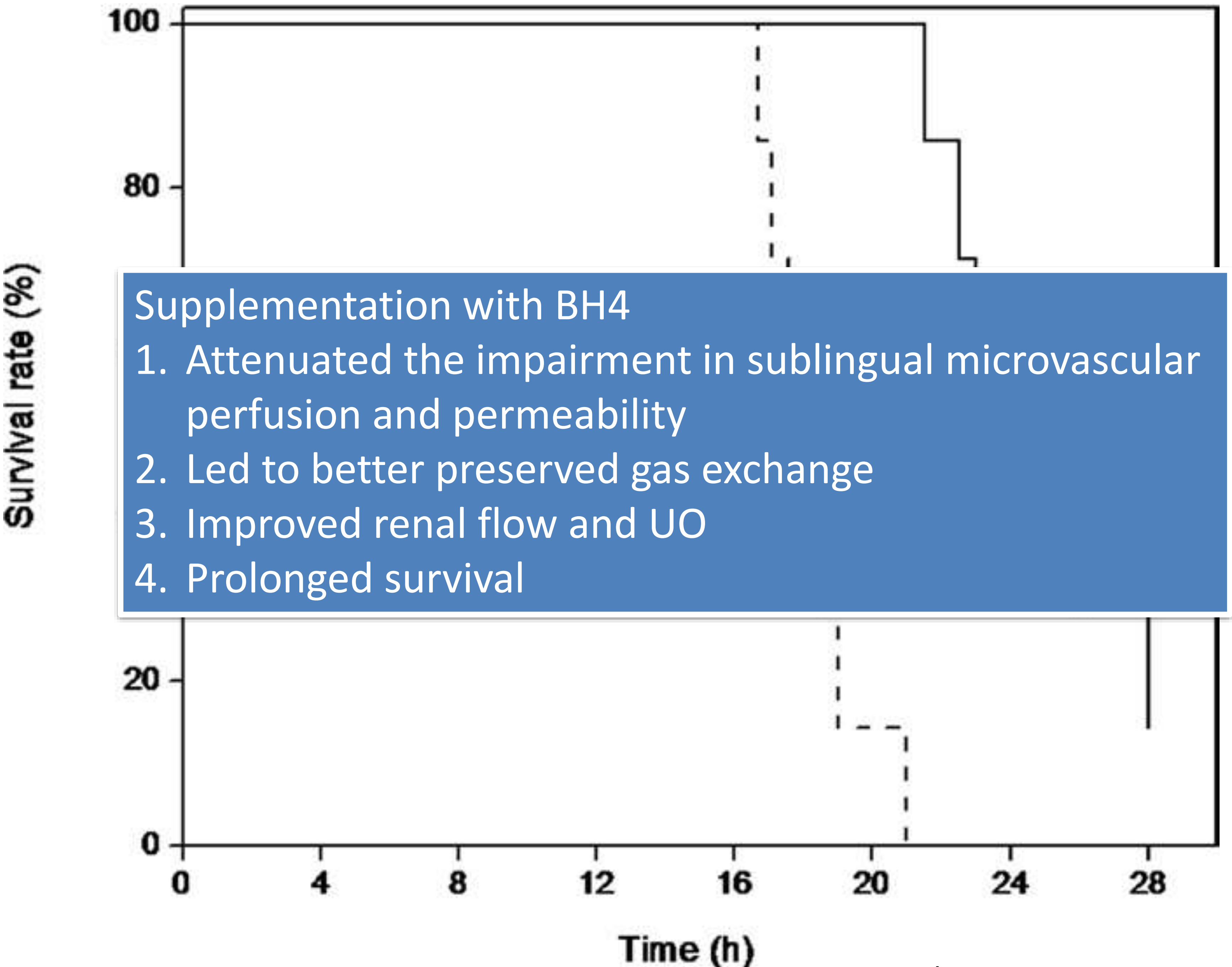
BH4 (tetrahydrobiopterin) in Sepsis





14 adult female sheep; fecal peritonitis; SDFI

He X et al. CCM 20:2833; 2012



Supplementation with BH4

1. Attenuated the impairment in sublingual microvascular perfusion and permeability
2. Led to better preserved gas exchange
3. Improved renal flow and UO
4. Prolonged survival

He X et al. CCM 20:2833; 2012

Controvertial

Sedatives

- Dexmedetomidine increases capillary perfusion by decreasing venular leukocyte-endothelial interactions
- Propofol and midazolam exert negative effects

Anticoagulants

- Decrease leukocyte and platelet rolling and adhesion
- Favor glycocalyx integrity
- Improve endothelial function
- Trigger vasodilation at microcirculatory level

Miranda M et al. Am J Physiol Heart Circ Physiol. 2016

Clinical relevance?

Intensive Care Med (2018) 44:281–299
https://doi.org/10.1007/s00134-018-5070-7

CONFERENCE REPORTS AND EXPERT PANEL

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

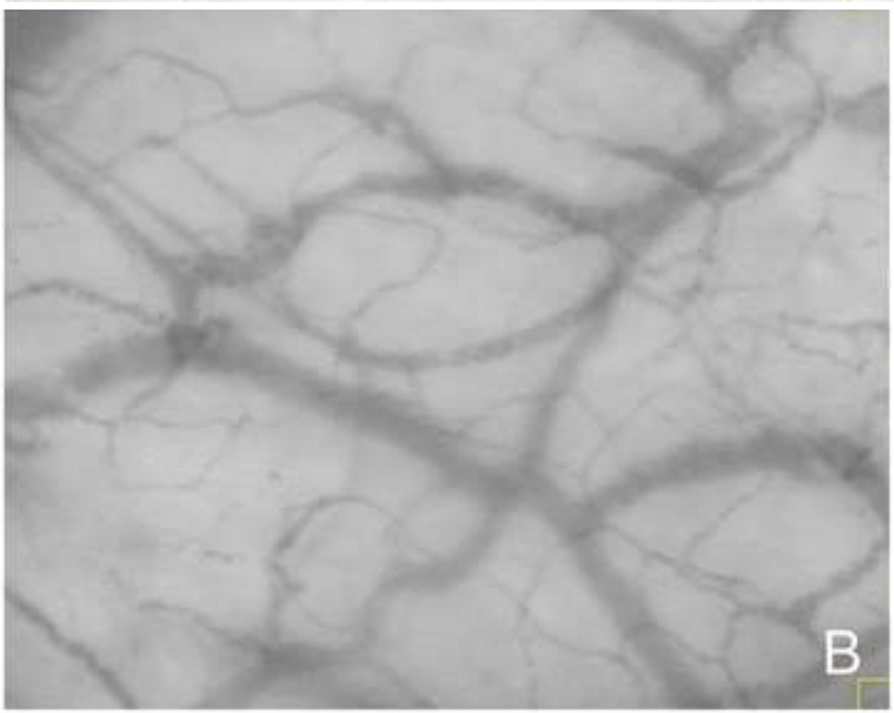
Can Ince[1,2*], E. Christiaan Boerma[3], Maurizio Cecconi[4], Daniel De Backer[5], Nathan I. Shapiro[6], Jacques Duranteau[7], Michael R. Pinsky[8], Antonio Artigas[9], Jean-Louis Teboul[10], Irwin K. M. Reiss[11], Cesar Aldecoa[12], Sam D. Hutchings[13], Abele Donati[14], Marco Maggiorini[15], Fabio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

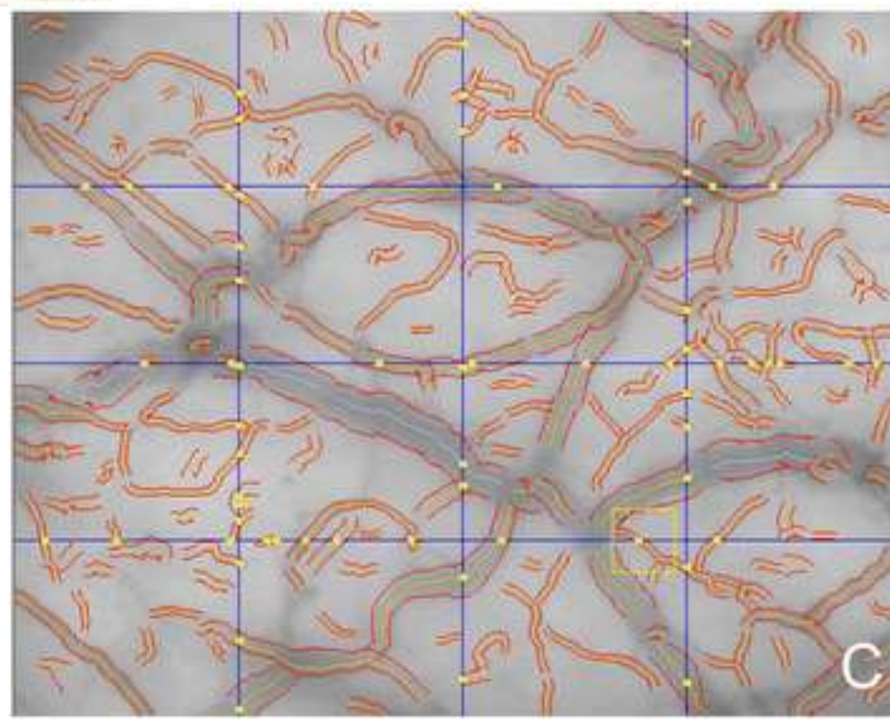
Clinically relevant variables

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

- As of now, data on the clinical relevance of microvascular alterations are predominantly expressed in PPV and MFI.
- Although cut-off values for a normal MFI are > 2.9, cut-off values for MFI of 2.6 are suggested as a threshold below which alterations can be considered clinically relevant
- There's no consensus on targets/goals/cut-offs

Ince, C, et al. Intensive Care Med. 2018





A. Device is applied to the patient on the sublingual area
B. Microcirculatory image acquired by the device
C. Vessels are identified during analysis (in red) allowing calculation of microcirculatory parameters. Crossing points (in yellow) with three equidistant vertical and horizontal lines are marked to calculate De Backer Score

Current recommendations

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

Ince, C, et al. Intensive Care Med. 2018

Comparison between SDF/IDF technical specifications

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

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

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

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

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

[d] Private communication with manufacture

Ince, C, et al. Intensive Care Med. 2018

Take home messages

- Microvascular alterations play a key role in the pathophysiology of sepsis and organ failure.
- Various mechanisms can be involved in the development of these alterations
- Monitoring of the microcirculation is not yet ready for routine clinical practice
  - Endpoints for resuscitation and the impact of many therapeutic interventions have not yet been defined
- Modulation of endothelial NO synthase seems promising.