Shock, Publish Ahead of Print

DOI : 10.1097/SHK.0000000000000777

VASODILATORS IN SEPTIC SHOCK RESUSCITATION: A CLINICAL PERSPECTIVE

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RUNNING HEAD: vasodilators in septic shock resuscitation

FUNDING

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (Grant no. 2014/11120-0), Brasil, awarded to ES.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

ABSTRACT

Microcirculatory abnormalities havebeen shown to be frequent in patients with septic shock despite "normalization"of systemic hemodynamics. Several studies have exploredthe impact of vasodilator therapy (prostacyclin, inhaled nitric oxide, topic acetylcholine and nitroglycerin) on microcirculation and tissue perfusion, with contradictory findings.

In this narrative review, we briefly present the pathophysiologicalaspects of microcirculatory dysfunction,and depict the evidence supporting the use of vasodilators and other therapeutic interventions (fluid administration, blood transfusion, vasopressors and dobutamine) aiming to improve the microcirculatory flowin septic shock patients.

KEY WORDS: sepsis,septic shock, microcirculation, vasodilator agents, nitroglycerin, nitric oxide, resuscitation, multiple organ failure.

INTRODUCTION

Microcirculation is composed of small vessels such as arterioles, capillaries and venules, with a diameter lower than 100 μm (1). In fact, the small vessels (diameter ≤20-25 μm), i.e., capillaries and postcapillary venules, represent the primary site of oxygen exchange between red blood cells (RBC) and tissue cells(1).Microcirculatory blood flow is markedly disturbedin sepsis (2-4) with a clear association between persistent microcirculatory abnormalities, increased morbidity and poor outcomes(5).Therefore, it has been postulated that interventions aiming to recruitmicrocirculation, i.e., open non-perfused or intermittentlyperfusedcapillariesmight improve tissue perfusion, mitigating the progression to organ failure and death (6).

According to Poiseuille's law, blood flow through the capillaries is proportional to the pressure gradient between post-arteriolar and venular pressures (microcirculatory driving pressure) and the fourth power of the radius, and inversely proportional to capillary length and blood viscosity **(Figure 1)**. Therefore, whilst the systemic perfusion pressure is determined by the difference between mean arterial blood pressure (MAP) and central venous pressure (CVP), the perfusion pressure in the microcirculation (microcirculatory driving pressure) is the net result of precapillary inflow pressure (approximately 30 mm Hg) minus venular outflow pressure(approximately 10 mm Hg) (Figure 2) (7-9).

Theoretically, the administration of vasodilating agents, such as acetylcholine (2), nitric oxide (NO) donors (10) and nitroglycerin (11)might improve microcirculatory blood flow by increasing the precapillary inflow pressure while the venular outflow pressure is maintained, i.e., by increasing the microcirculatory driving pressure (Figure 2)(8,9).

The use of vasodilators as adjuvant therapy in the treatment of circulatory shock was introduced into clinical practice in the 1960s (12-14). Nevertheless, the concept supporting vasodilators use to optimize microcirculatory perfusion in critical ill patients was only tested in the 1990s. These studies suggested that different vasodilators, such as prostacyclin (15) and inhaled NO(16) might improve splanchnic perfusion. In the past years, several studies have exploredthe

impact vasodilators(prostacyclin, inhaled nitric oxide, topic acetylcholine and nitroglycerin) on microcirculation and tissue perfusion with contradicting findings.

In this narrative review, we briefly presentthe pathophysiology of microcirculatory dysfunction,and depict the evidence supporting the use of vasodilators and other therapeutic interventions (fluid administration, blood transfusion, vasopressors and dobutamine) aiming to improve the microcirculatory flowinseptic shock patients.

MECHANISMS PROMOTING MICROVASCULAR DYSFUNCTION

Blood flow through thecapillary network is tightly controlledby the arteriolar resistance vessels - the arteriolar network (17,18).Arterioles are surrounded bya smooth muscle layer, which cancontract or relaxin response to tissue oxygen tension and the presence of vasoactive agents, such as acetylcholine, catecholamines, prostaglandins, endothelin, bradykinin, thromboxane and NO(19). Microcirculation perfusion is regulated by an intricate interplay of neuroendocrine, paracrine, and mechanosensory pathways (20)**.** These mechanisms control oxygen delivery to the tissues according to the cellular metabolic demand(17,21).Under physiologicalconditions, NO acts asendogenousvasodilator and the endothelium regulates blood flow to the tissues by "recruiting microvessels"through NOrelease(22). In sepsis, theNO system becomesseverelydisturbeddue to a heterogeneousexpression of the induciblenitric oxidesynthase(iNOS)(23).Moreover, NO can also be consumed by reactive oxygen species (ROS), producing localized areas of relative NO deficiency in the microvascular bed(24).This might explain, at least in part,the pathologic heterogeneity of microvascular blood flow observed in both experimental (25)and human sepsis(3).

The relationship between circulating cells and endothelial surface cells is crucial in sepsis pathophysiology(26). Glycocalyx is a thin layer of glycosaminoglycan that covers the endothelial surface and has the function of facilitating RBC, white blood cells and plateletsflow through the capillaries(27). During sepsis, glycocalyx layer thickness is reduced (27), thus increasing leukocytes and platelets adhesion to the endothelial cells, and promoting endothelial dysfunction(28).Finally,

decreased RBC rheology(29,30), increased blood viscosity (31), increased number of activated neutrophils (32), activation of coagulation and complement systems with fibrin deposition inthe microvascular bed (33), along with derangements ofvascular autoregulatory mechanisms (34),represent mechanisms that further compromise microcirculation and oxygen delivery in septic shock.

MICROCIRCULATION IN SEPTIC SHOCK

De Backer and colleagues published the first study addressing the sublingual microcirculation in septic shock patients with orthogonal polarization spectral imaging (OPS) in 2002(2). Their findings were confirmed by several other authors (4,35), demonstrating that microcirculatory abnormalities in septic shock are more pronounced in small vessels, i.e., capillaries (2), and do not improve over time in non-survivors (5).

Microcirculatory abnormalities in septic shock arecharacterized by a decrease in total vessel density (TVD) and perfused capillary density (PVD) - a surrogate of functional capillary density, as well as a decrease inproportion of perfused capillaries (PPV)(2). At the same time, the proportion of non-perfused and intermittently perfused capillaries increased (2),along with theflow heterogeneity within areas in the same organ separated by few millimeters (3,11,36). Furthermore, capillaries that do not exhibit blood flow at a given time can become perfused few seconds or minutes later, demonstrating the dynamic behavior of microcirculation under septic conditions (6).

Additionally, it was demonstrated in twenty-six severe sepsis and septic shock patients during the first six hours of resuscitation that microvascular blood flow (microvascular flow index; MFI) was slower and more heterogeneous (flow heterogeneity index; FHI) in non-survivals compared to survivals (3). These data are in agreement with findings reported by Trzeciak and colleagues, who demonstrated that septic shock patients who improved their sequential organ failure assessment (SOFA) score during the first 24 hours of resuscitationexhibited a higher improvement inmicrovascular blood flow (MFI) compared to patients who did not improve their

SOFA score (37). Finally, it was demonstrated that PPV was independently associated with ICU survival in severe sepsis and septic shock patients (4).

These observations led to a growing interest in monitoring microcirculation as a valuable adjunct to standard global parameters to predict or diagnose ongoing tissue hypoperfusion(1). Indeed, many studies, particularly on sepsis, have highlighted the importance of clinical evaluation of microcirculation as part of tissue perfusion monitoring in shock(6). As a result, many authors have advocated that therapeutic interventions aiming to improve microcirculation would boost tissue perfusion and, consequently, mitigate the progression towards organ failure and death in septic shock patients (6,21).

VASODILATORS AND THEIR EFFECTS IN MICROCIRCULATION

The concept ofusing pharmacotherapyto optimize the microcirculatory bloodflowin critically ill patientsgained notorietyin the late 1980s, when clinical trials addressing the effects of drugs withvasodilationproperties such as prostacyclin(38) and dobutamine were published(39).Afterwards, several studies have exploredthe impact of vasodilating agents such as prostacyclin, inhaled NO, topic acetylcholine and nitroglycerin in microcirculation, tissue perfusion, organ function and mortality (table 1).

The effect of vasodilator therapy in clinical sepsis was first demonstrated with the use of topical nitroglycerin in postoperative patients complicated by severe sepsisresulting in improvement in cardiac output (CO) and oxygen consumption (40). More recently, it has been demonstrated that administration of vasodilating agents, such as acetylcholine (2) or NO donors (10) was effective in recruiting capillaries of sublingual microcirculation in clinical and experimental sepsis. These observations had important implications, indicating that microvascular derangements could be reversed with drugs (2). Therefore, strategies aiming to open microcirculation through systemic vasodilators became a target for new studies(6) (table 1).

Pittet and colleagues demonstrated in a small crossover study that by increasing systemic oxygen delivery (DO_2) through a 30-min intravenousinfusion of prostacyclin, there was an increase in systemic oxygen consumption (VO_2)and skin microvascular blood flow (41).In another small non-controlled study, continuous intravenously prostacyclin infusion up to 32 days, enhanced gastric mucosal pH (pHi), suggesting animproved splanchnic blood flow (15).Similar findings were demonstrated by Eichelbrönner and colleagues, who reported that aerosolized prostacyclin was effective in increase pHi and reducearterial-gastric mucosal pressure of carbon dioxide difference (PCO_2 gap), while hepatic blood flow, systemic hemodynamics, DO_2 and VO_2 were not affected (42).

After the development of OPS imaging, it was demonstrated that topic acetylcholine applied to the sublingual region, improved microvascular blood flow in septic shock patients (2,43). By proving the "recruitable" aspect of microcirculation, Spronk and colleagues demonstrated in a prospective open label trial that intravenously nitroglycerin (0.5 mg loading dose followed by a continuous infusion of 0.5 to 4.0 mg/h) improved microvascular flow in fluid resuscitated septic shock patients (11).

Following this initial report, Boerma and colleagues randomized 70 fluid resuscitated severe sepsis and septic shock patients to receive either intravenously nitroglycerin (2 mg bolus in 30 min followed by 2 mg /h up to 24 hours) or placebo (0.9%saline) (44). The authors reported a similar improvement in sublingual capillary blood flow during the study period (up to 24 hours) in both groups (44). An interesting finding presented in this trial was the lower number of organ dysfunctions in patients who received nitroglycerin compared to the placebo group (44).

More recently, Lima and colleagues demonstrated that a stepwise increase in nitroglycerin infusion (starting from 2 mg/h up to 16 mg/h) improved peripheral perfusion in circulatory shock patients who persisted with abnormal peripheral perfusion after six hours of resuscitationdespite achieving macrohemodynamic stabilization (45). Finally, Trzeciak and colleagues randomized 49 septic shock patients to receive either inhaled NO (40 ppm) or sham inhaled NO (placebo) for six

hours starting after the achievement of conventional macrohemodynamic goals (MAP ≥65 mmHg, central venous oxygen saturation ($ScvO_2$) ≥70 % or lactate clearance ≥10%) (46). The authors reported no differences between the groups regarding microcirculatory flow or in-hospital mortality (46).

OTHER THERAPIES THAT MAY IMPROVE MICROCIRCULATION IN SEPTIC SHOCK

Fluid therapy

The main determinants of oxygen transport to the tissue cells are convection (convective transport of oxygen carrying RBC through capillaries) and diffusion (diffusion of oxygen from RBC to tissue cells mitochondria) (Figure 3) (47). Convective flow is determined by the product of the rate at which RBC enter the capillaries (RBC/seconds), the RBC oxygen saturation (HbSat) and the oxygen-carrying capacity of a RBC at 100% of saturation (0.0362 pl O_2/RBC)(47). Oxygen diffusion from the capillary RBC to the tissue cells mitochondria is determined by Fick's law of diffusion. Accordingly, oxygen diffusion is the product of the oxygen gradient from RBC to mitochondria (pO_2Grad) and the diffusion coefficient (D) times the exchange surface (S) divided by the diffusion distance (d) from the RBC to the mitochondria(47).

Therefore, fluid administration can improve microcirculatory blood flow by improving the convective transport of oxygen(47). Furthermore, blood flow through capillaries is proportional to the driving pressure across the capillary and by the fourth power of the radius, and inversely proportional to capillary length and blood viscosity. Thus, volume expansion may also improve microvascular blood flow by increasing the driving pressure in microcirculation, decreasing blood viscosity as well as by decreasing the release of endogenous vasoconstrictors (increasing vessels radius) (6).

On the other hand, fluid overload may affect microcirculation by increasing venular outflow pressure (boost venous return) and, therefore, by decreasing microcirculatory driving pressure (convection) and/or by increasing diffusion distance (diffusion) between capillary RBC and tissue mitochondria (Figure 3) (48).

Two observational studies demonstrated that timely fluid administration could improve microvascular perfusion (49,50). Ospina-Tascon and colleagues investigated 60 hypovolemic severe sepsis patients requiring volume expansion within 24 hours (early) or after 48 hours(late) of sepsis recognition (49). The authors demonstrated that volume expansion with Ringer Lactate or 4% albuminimproved capillary blood flow only in early sepsiswhereaslatefluid administration failed to improve microcirculation despite having increasedCO and MAP(49).Fluid administration did not affect flow heterogeneity in either early or late sepsis (49).

Moreover, it was demonstrated that both passive leg raising and volume expansionwith 500 ml of 0.9% saline or 6% hydroxyethyl starch (130/0.4) improved sublingual microcirculatory parametersand decreased flow heterogeneityregardless the type of fluid in preload-responsive severe sepsis and septic shock patients within the first 24 hours of intensive care unit (ICU) admission (50).Interestingly, improvements in microcirculation perfusion after volume expansion either by passive leg raising or volume expansion do not seem to be related to RBC rheologyas hemoglobin levels remained constant during the study procedures (50).

Dobutamine and vasopressors

Dobutamine is primarily beta-adrenergic agent, but it also has mild vasodilatoryeffects(51).Thus, dobutamine administration may improve microvascular blood flow by increasing microcirculatory driving pressure (Figure 2) and by increasing cardiac output (convective transport of oxygen).

Few studies evaluated the effects of dobutamine on the microcirculationof critically ill patients (43,52,53).Initial studies suggested that dobutamine could increase skin microvascular

(52)and gastric mucosal blood flow (53,54).In a prospective open label studywith 22 septic shock patients (<48 hours of onset), De Backer and colleagues demonstrated that dobutamine infusion (5 mcg/kg/h during 2 hours) improve capillary blood flow(43). Interestingly, changes in capillary perfusion did not correlate with changes in systemic hemodynamics (cardiac index, MAP or systemic vascular resistance), but were inverselycorrelated with changes in arterial lactate levels(43).

The effects of different MAP targets on microcirculation and tissue perfusion in septic shock patients were recently revised elsewhere(55). The impact of increasing MAP from 65 mmHg to 85 mmHg on microcirculatory blood flow was prospectively evaluated in twenty fluid resuscitated septic shock patients(56). In this study, increasing MAP with escalating doses of norepinephrinefailed to improvemicrovascular blood flow in small, medium and large vessels (56). Similar findings were reported by Jhanji and colleagues, who demonstrated that escalating doses of norepinephrine aiming to increase MAP from 60 to 70, 80, and 90 mmHg, respectively, had no effects on sublingual microvascular blood flow in septic shock patients (57).

Red blood cells transfusion

It was demonstrated in a prospective observational study with 35 severe sepsis and septic shock patients that anincrease in hemoglobin concentration from 7.1 g/dL to 8.1 g/dL through a leukocyte-reducedRBC transfusion,failed to improvesublingual microcirculation despite improving DO_2(58). Similar findings were reported by Sadaka and colleagues, who demonstrated that an increase on hemoglobin concentration from approximately 7.2 g/dL to 8.8 g/dL through one non-leukoreduced packed RBC transfusion during the first 12 hours of sepsis did not affect sublingual microcirculation and thenar tissue oxygen saturation (StO_2) (59). Finally, whether non-leukodepleted or leukodepleted RBC transfusion affects the microvascular perfusion in septic patients and whether microcirculation analysis is useful to guide RBC transfusion need to be further investigated (60).

FUTURE DIRECTIONS

Microcirculation analysis in critically ill patients is an evolving issue (1).The development of new user-friendly devices to assess microcirculation at the bedside along with a rapid, online, operator independent, and automated image analysis will allow researchers to exploretherapies targeting microcirculation during the early phases of septic shock resuscitation more easily and quickly (61,62).

Recently, a third generation of handheld microscope, based on Incident Dark Field illumination (IDF) (CytoCam; Braedius Medical BV, Huizen, The Netherlands),along with an automatized analysis software (CytoCamTools V1; Braedius Medical BV, Huizen, The Netherlands)have been launched(61).The CytoCam-IDF imaging provides a better image quality in terms of contrast and image sharpness and has proved effective in detecting 30% more capillaries in health volunteers than Sidestream Dark Field imaging (SDF) due to improved magnification lens and a high-resolution sensor (61). Nevertheless, the accuracyof this new automatized analysis software to detect microvascular abnormalities in septic shock patientsneeds to be addressed (63).

Finally, so far, no study has evaluated if targeting microcirculation during a quantitative resuscitation algorithm would affect outcomes in septic shock patients. Therefore, new and well-designed studies, adequately powered, addressing the impact of such resuscitation strategy on patient centered outcomes are needed.

CONCLUSIONS

While normalization of macrohemodynamic parameters remains a concern in septic shock resuscitation, it wasclearly demonstrated that such parameters poorly reflect the microcirculation condition.Moreover, microcirculatory derangements are frequent in septic shock patients and have been associated with increased morbidity and mortality. Therefore, additional efforts aiming to improve microcirculation,such as intravenousvasodilators administration, have been advocated.

However, the impact of such interventions on patient-centered outcomes need to be further verified throughwell-designed randomized controlled clinical trials.

Acknowledgements

We thank Helena Spalic for proof-reading this manuscript.

REFERENCES

(1) Massey MJ, Shapiro NI: A guide to human in vivo microcirculatory flow image analysis. *Crit Care* 20(1):35, 2016.

(2) 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* 166(1):98-104, 2002.

(3) Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL, Arnold RC, Colilla S, Zanotti S, Hollenberg SM: Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. *Ann Emerg Med* 49(1):88-98, 98, 2007.

(4) De Backer D., Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S, Vincent JL: Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. *Crit Care Med* 41(3):791-799, 2013.

(5) Sakr Y, Dubois MJ, De BD, Creteur J, Vincent JL: Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. *Crit Care Med* 32(9):1825-1831, 2004.

(6) De Backer D., Donadello K, Taccone FS, Ospina-Tascon G, Salgado D, Vincent JL: Microcirculatory alterations: potential mechanisms and implications for therapy. *Ann Intensive Care* 1(1):27, 2011.

(7) Johnson PC: Autoregulation of blood flow. *Circ Res* 59(5):483-495, 1986.

(8) Taylor AE, Moore TM: Capillary fluid exchange. *Am J Physiol* 277(6 Pt 2):S203-S210, 1999.

(9) Boerma EC, Ince C: The role of vasoactive agents in the resuscitation of microvascular perfusion and tissue oxygenation in critically ill patients. *Intensive Care Med* 36(12):2004-2018, 2010.

(10) He X, Su F, Velissaris D, Salgado DR, de Souza BD, Lorent S, Taccone FS, Vincent JL, De BD: Administration of tetrahydrobiopterin improves the microcirculation and outcome in an ovine model of septic shock. *Crit Care Med* 40(10):2833-2840, 2012.

(11) Spronk PE, Ince C, Gardien MJ, Mathura KR, Oudemans-van Straaten HM, Zandstra DF: Nitroglycerin in septic shock after intravascular volume resuscitation. *Lancet* 360(9343):1395-1396, 2002.

(12) Weil MH, Sudrann RB, Shubin H: Treatment of endotoxic shock--the dilemma of vasopressor and vasodilator therapy. *Calif Med* 96:86-88, 1962.

(13) Lillehei RC, Longerbeam JK, Bloch JH, Manax WG: The Nature Of Irreversible Shock: Experimental And Clinical Observations. *Ann Surg* 160:682-710, 1964.

(14) Webb WR: Vasodilators in shock--when? *South Med J* 59(3):257-261, 1966.

(15) Radermacher P, Buhl R, Santak B, Klein M, Kniemeyer HW, Becker H, Tarnow J: The effects of prostacyclin on gastric intramucosal pH in patients with septic shock. *Intensive Care Med* 21(5):414-421, 1995.

(16) Eichelbronner O, Reinelt H, Wiedeck H, Mezody M, Rossaint R, Georgieff M, Radermacher P: Aerosolized prostacyclin and inhaled nitric oxide in septic shock--different effects on splanchnic oxygenation? *Intensive Care Med* 22(9):880-887, 1996.

(17) Ellis CG, Jagger J, Sharpe M: The microcirculation as a functional system. *Crit Care* 9 Suppl 4:S3-S8, 2005.

(18) Bateman RM, Sharpe MD, Ellis CG: Bench-to-bedside review: microvascular dysfunction in sepsis--hemodynamics, oxygen transport, and nitric oxide. *Crit Care* 7(5):359-373, 2003.

(19) Furchgott RF, Zawadzki JV: The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 288(5789):373-376, 1980.

(20) Lehr HA, Bittinger F, Kirkpatrick CJ: Microcirculatory dysfunction in sepsis: a pathogenetic basis for therapy? *J Pathol* 190(3):373-386, 2000.

(21) Ince C: The microcirculation is the motor of sepsis. *Crit Care* 9 Suppl 4:S13-S19, 2005.

(22) Palmer RM, Ferrige AG, Moncada S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327(6122):524-526, 1987.

(23) Vincent JL, Zhang H, Szabo C, Preiser JC: Effects of nitric oxide in septic shock. *Am J Respir Crit Care Med* 161(6):1781-1785, 2000.

(24) Morin MJ, Unno N, Hodin RA, Fink MP: Differential expression of inducible nitric oxide synthase messenger RNA along the longitudinal and crypt-villus axes of the intestine in endotoxemic rats. *Crit Care Med* 26(7):1258-1264, 1998.

(25) Lam C, Tyml K, Martin C, Sibbald W: Microvascular perfusion is impaired in a rat model of normotensive sepsis. *J Clin Invest* 94(5):2077-2083, 1994.

(26) Ince C, Mayeux PR, Nguyen T, Gomez H, Kellum JA, Ospina-Tascon GA, Hernandez G, Murray P, De Backer D.: The endothelium in sepsis. *Shock* 45(3):259-270, 2016.

(27) Marechal X, Favory R, Joulin O, Montaigne D, Hassoun S, Decoster B, Zerimech F, Neviere R: Endothelial glycocalyx damage during endotoxemia coincides with microcirculatory dysfunction and vascular oxidative stress. *Shock* 29(5):572-576, 2008.

(28) Secor D, Li F, Ellis CG, Sharpe MD, Gross PL, Wilson JX, Tyml K: Impaired microvascular perfusion in sepsis requires activated coagulation and P-selectin-mediated platelet adhesion in capillaries. *Intensive Care Med* 36(11):1928-1934, 2010.

(29) Condon MR, Kim JE, Deitch EA, Machiedo GW, Spolarics Z: Appearance of an erythrocyte population with decreased deformability and hemoglobin content following sepsis. *Am J Physiol Heart Circ Physiol* 284(6):H2177-H2184, 2003.

(30) Piagnerelli M, Cotton F, Van NM, Vincent JL, Gulbis B: Modifications in erythrocyte membrane protein content are not responsible for the alterations in rheology seen in sepsis. *Shock* 37(1):17-21, 2012.

(31) Astiz ME, DeGent GE, Lin RY, Rackow EC: Microvascular function and rheologic changes in hyperdynamic sepsis. *Crit Care Med* 23(2):265-271, 1995.

(32) Linderkamp O, Ruef P, Brenner B, Gulbins E, Lang F: Passive deformability of mature, immature, and active neutrophils in healthy and septicemic neonates. *Pediatr Res* 44(6):946-950, 1998.

(33) Diaz NL, Finol HJ, Torres SH, Zambrano CI, Adjounian H: Histochemical and ultrastructural study of skeletal muscle in patients with sepsis and multiple organ failure syndrome (MOFS). *Histol Histopathol* 13(1):121-128, 1998.

(34) Avontuur JA, Bruining HA, Ince C: Nitric oxide causes dysfunction of coronary autoregulation in endotoxemic rats. *Cardiovasc Res* 35(2):368-376, 1997.

(35) Edul VS, Enrico C, Laviolle B, Vazquez AR, Ince C, Dubin A: Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. *Crit Care Med* 40(5):1443-1448, 2012.

(36) 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* 9(6):R601-R606, 2005.

(37) Trzeciak S, McCoy JV, Phillip DR, Arnold RC, Rizzuto M, Abate NL, Shapiro NI, Parrillo JE, Hollenberg SM: Early increases in microcirculatory perfusion during protocol-directed resuscitation are associated with reduced multi-organ failure at 24 h in patients with sepsis. *Intensive Care Med* 34(12):2210-2217, 2008.

(38) Bihari D, Smithies M, Gimson A, Tinker J: The effects of vasodilation with prostacyclin on oxygen delivery and uptake in critically ill patients. *N Engl J Med* 317(7):397-403, 1987.

(39) Shoemaker WC, Appel PL, Kram HB, Duarte D, Harrier HD, Ocampo HA: Comparison of hemodynamic and oxygen transport effects of dopamine and dobutamine in critically ill surgical patients. *Chest* 96(1):120-126, 1989.

(40) Cerra FB, Hassett J, Siegel JH: Vasodilator therapy in clinical sepsis with low output syndrome. *J Surg Res* 25(2):180-183, 1978.

(41) Pittet JF, Lacroix JS, Gunning K, Laverriere MC, Morel DR, Suter PM: Prostacyclin but not phentolamine increases oxygen consumption and skin microvascular blood flow in patients with sepsis and respiratory failure. *Chest* 98(6):1467-1472, 1990.

(42) Eichelbronner O, Reinelt H, Wiedeck H, Mezody M, Rossaint R, Georgieff M, Radermacher P: Aerosolized prostacyclin and inhaled nitric oxide in septic shock--different effects on splanchnic oxygenation? *Intensive Care Med* 22(9):880-887, 1996.

(43) De Backer D., Creteur J, Dubois MJ, Sakr Y, Koch M, Verdant C, Vincent JL: The effects of dobutamine on microcirculatory alterations in patients with septic shock are independent of its systemic effects. *Crit Care Med* 34(2):403-408, 2006.

(44) Boerma EC, Koopmans M, Konijn A, Kaiferova K, Bakker AJ, van Roon EN, Buter H, Bruins N, Egbers PH, Gerritsen RT, Koetsier PM, Kingma WP, Kuiper MA, Ince C: 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. *Crit Care Med* 38(1):93-100, 2010.

(45) Lima A, van Genderen ME, van BJ, Klijn E, Jansem T, Bakker J: Nitroglycerin reverts clinical manifestations of poor peripheral perfusion in patients with circulatory shock. *Crit Care* 18(3):R126, 2014.

(46) Trzeciak S, Glaspey LJ, Dellinger RP, Durflinger P, Anderson K, Dezfulian C, Roberts BW, Chansky ME, Parrillo JE, Hollenberg SM: Randomized controlled trial of inhaled nitric oxide for the treatment of microcirculatory dysfunction in patients with sepsis*. *Crit Care Med* 42(12):2482-2492, 2014.

(47) Ince C: The rationale for microcirculatory guided fluid therapy. *Curr Opin Crit Care* 20(3):301-308, 2014.

(48) Vellinga NA, Ince C, Boerma EC: Elevated central venous pressure is associated with impairment of microcirculatory blood flow in sepsis: a hypothesis generating post hoc analysis. *BMC Anesthesiol* 13:17, 2013.

(49) Ospina-Tascon G, Neves AP, Occhipinti G, Donadello K, Buchele G, Simion D, Chierego ML, Silva TO, Fonseca A, Vincent JL, De BD: Effects of fluids on microvascular perfusion in patients with severe sepsis. *Intensive Care Med* 36(6):949-955, 2010.

(50) Pottecher J, Deruddre S, Teboul JL, Georger JF, Laplace C, Benhamou D, Vicaut E, Duranteau J: Both passive leg raising and intravascular volume expansion improve sublingual microcirculatory perfusion in severe sepsis and septic shock patients. *Intensive Care Med* 36(11):1867-1874, 2010.

(51) Secchi A, Ortanderl JM, Schmidt W, Walther A, Gebhard MM, Martin E, Schmidt H: Effects of dobutamine and dopexamine on hepatic micro- and macrocirculation during experimental endotoxemia: an intravital microscopic study in the rat. *Crit Care Med* 29(3):597-600, 2001.

(52) Christ F, Gartside IB, Kox WJ, Gamble J: The assessment of the microcirculatory effects of dobutamine using mercury in silastic strain gauge plethysmography in man. *Postgrad Med J* 67 Suppl 1:S42-S50, 1991.

(53) Duranteau J, Sitbon P, Teboul JL, Vicaut E, Anguel N, Richard C, Samii K: Effects of epinephrine, norepinephrine, or the combination of norepinephrine and dobutamine on gastric mucosa in septic shock. *Crit Care Med* 27(5):893-900, 1999.

(54) Neviere R, Mathieu D, Chagnon JL, Lebleu N, Wattel F: The contrasting effects of dobutamine and dopamine on gastric mucosal perfusion in septic patients. *Am J Respir Crit Care Med* 154(6 Pt 1):1684-1688, 1996.

(55) Leone M, Asfar P, Radermacher P, Vincent JL, Martin C: Optimizing mean arterial pressure in septic shock: a critical reappraisal of the literature. *Crit Care* 19:101, 2015.

(56) Dubin A, Pozo MO, Casabella CA, Palizas F, Jr., Murias G, Moseinco MC, Kanoore Edul VS, Palizas F, Estenssoro E, Ince C: Increasing arterial blood pressure with norepinephrine does not improve microcirculatory blood flow: a prospective study. *Crit Care* 13(3):R92, 2009.

(57) 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* 37(6):1961-1966, 2009.

(58) Sakr Y, Chierego M, Piagnerelli M, Verdant C, Dubois MJ, Koch M, Creteur J, Gullo A, Vincent JL, De BD: Microvascular response to red blood cell transfusion in patients with severe sepsis. *Crit Care Med* 35(7):1639-1644, 2007.

(59) Sadaka F, Aggu-Sher R, Krause K, O'Brien J, Armbrecht ES, Taylor RW: The effect of red blood cell transfusion on tissue oxygenation and microcirculation in severe septic patients. *Ann Intensive Care* 1(1):46, 2011.

(60) Donati A, Damiani E, Luchetti M, Domizi R, Scorcella C, Carsetti A, Gabbanelli V, Carletti P, Bencivenga R, Vink H, Adrario E, Piagnerelli M, Gabrielli A, Pelaia P, Ince C: Microcirculatory effects of the transfusion of leukodepleted or non-leukodepleted red blood cells in patients with sepsis: a pilot study. *Crit Care* 18(1):R33, 2014.

(61) Aykut G, Veenstra G, Scorcella C, Ince C, Boerma C: Cytocam-IDF (incident dark field illumination) imaging for bedside monitoring of the microcirculation. *Intensive Care Med Exp* 3(1):40, 2015.

(62) Tanaka S, Harrois A, Nicolai C, Flores M, Hamada S, Vicaut E, Duranteau J: Qualitative real-time analysis by nurses of sublingual microcirculation in intensive care unit: the MICRONURSE study. *Crit Care* 19:388, 2015.

(63) Carsetti A, Aya HD, Pierantozzi S, Bazurro S, Donati A, Rhodes A, Cecconi M: Ability and efficiency of an automatic analysis software to measure microvascular parameters. *J Clin Monit Comput* , 2016.

Figure 1. Figure 1. The main determinants of the microcirculatory blood flow accordingly to the Poiseuille's law.

Blood flow through the capillaries (Q) is proportional to the pressure gradient between post-arteriolar (P1) and venular pressures (P2), i.e., the microcirculatory driving pressure (P1-P2), and by the fourth power of the capillary radius (r4), and is inversely proportional to capillary length (L) and blood viscosity (μ). The black arrows represent the blood flow direction through microvessels. RBC: red blood cells.

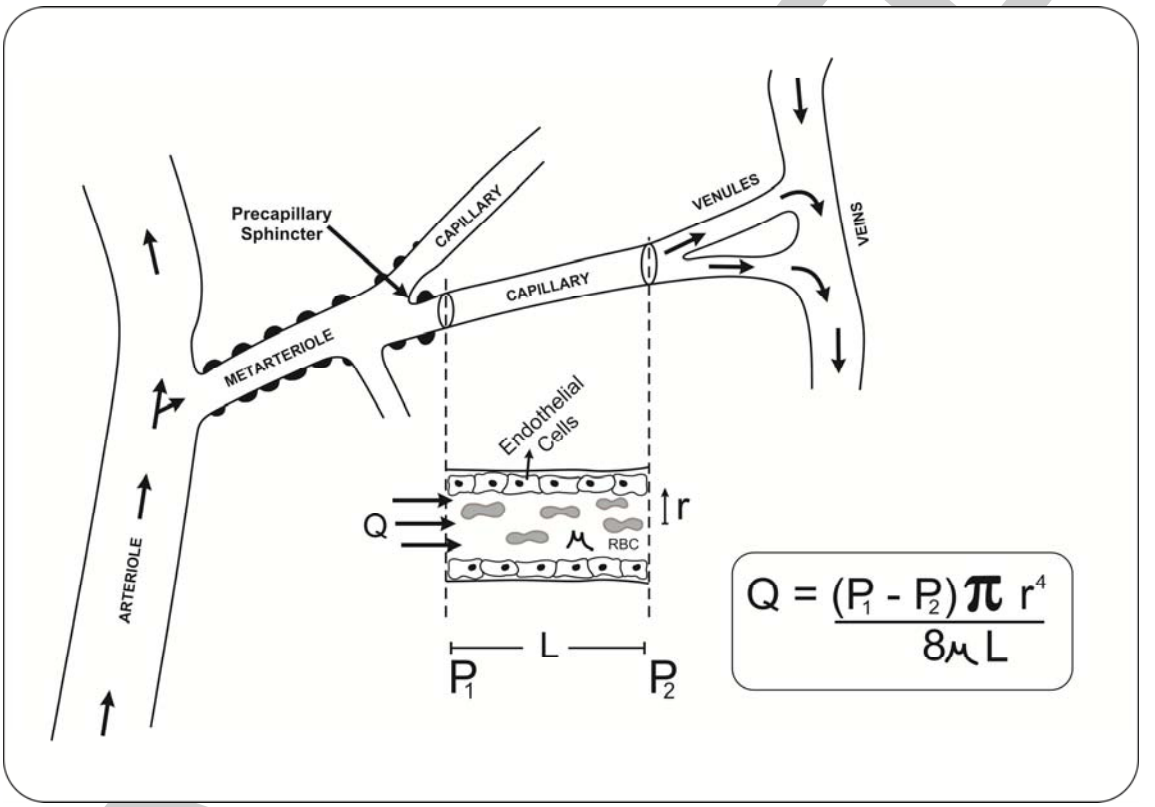

Figure 2. The effect of vasodilators on the microcirculatory driving pressure and on the microcirculatory blood flow.

A: systemic perfusion pressure (difference between mean arterial blood pressure and central venous pressure), B: microcirculatory driving pressure (precapillary inflow pressure minus venular outflow pressure) and C: theoretical effect of a vasodilating administration improving microcirculatory blood flow by increasing the precapillary inflow pressure while the venular outflow pressure is maintained (increased microcirculatory driving pressure). Modified from (23) with permission.

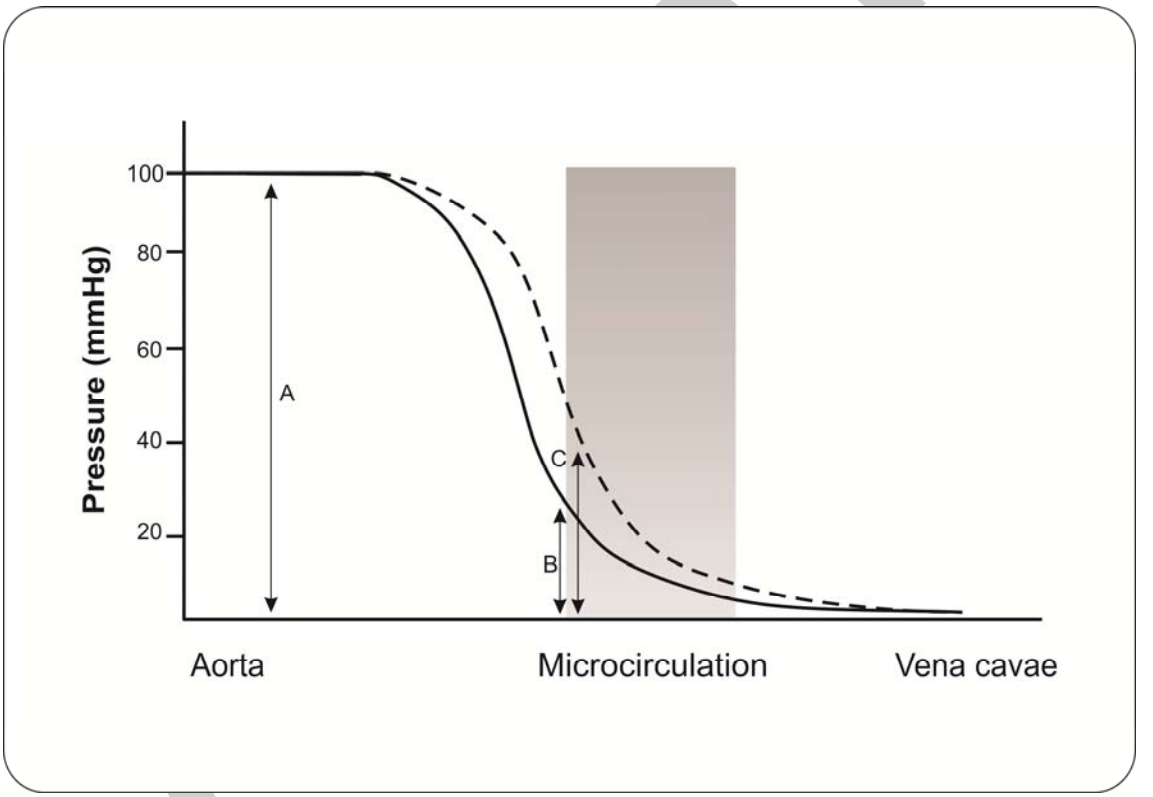

Figure 3. The effects of fluid overload on microcirculatory blood flow.

Convective flow is determined by the product of the rate at which red blood cells (RBC) enter the capillaries, the RBC oxygen saturation and the oxygen-carrying capacity of a RBC at 100% of saturation. Oxygen diffusion from capillary RBC to tissue cell mitochondria is determined by the product of the oxygen gradient from RBC to mitochondria and the diffusion coefficient, times the exchange surface divided by the diffusion distance (d1 and d2) from RBC to mitochondria. Panel A: Blood flow though microcirculation under normovolemic conditions. Panel B: impaired convection (due to increased venular outflow pressure) and diffusion [increased diffusion distance (d2) between capillary RBC and tissue cells mitochondria].

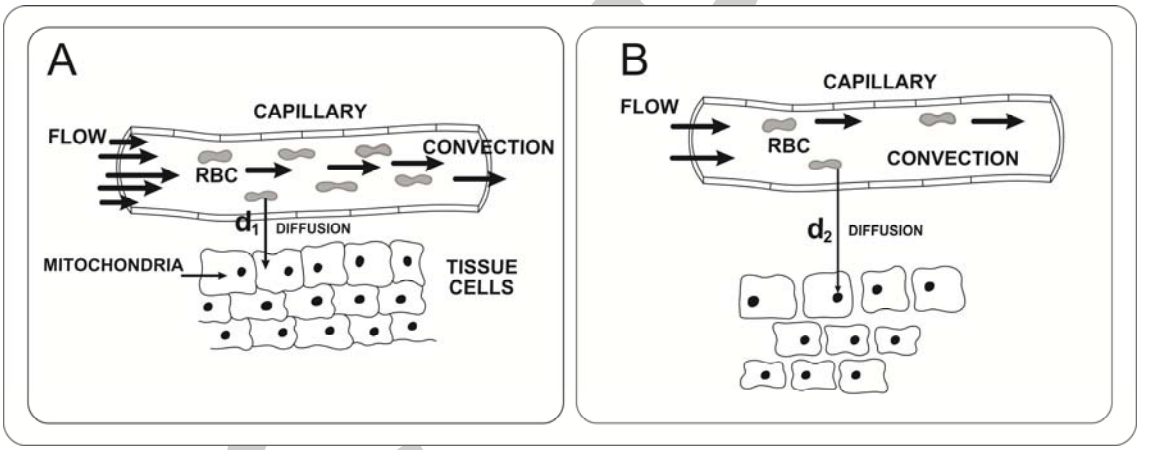

Table 1: Studies addressing the efficacy and safety of vasodilating agents in septic patients.

First Author; year	n	Vasodilator	Site	Device	Main study findings	Type of study
Pittet J-F, 1990 (41)	11	I.V. prostacyclin vs. I.V. phentolamine	Skin (inner thigh)	Laser Doppler flowmeter	Higher improvement in skin microvascular blood flow with prostacyclin infusion than with phentolamine.	Prospective, interventional, randomized
Radermacher P, 1995 (15)	16	I.V. prostacyclin	Splanchnic (Gastric)	Gastric Tonometry	Prostacyclin infusion increased pHi with concomitant increase in DO_2. Oxygen consumption remained unchanged.	Prospective, interventional, non-controlled
Eichelbrönner O, 1996 (42)	16	Aerosolized prostacyclin vs. inhaled NO	Splanchnic (Gastric)	Splanchnic (Gastric)	Only aerosolized prostacyclin increased pHi and reduced PCO_2 gap. Hepatic blood flow (Indocyanine-green plasma disappearance rate) remained unchanged.	Prospective, interventional, randomized
De Backer D, 2002 (2)	11	Topic acetylcholine	Sublingual microcirculation	OPSimaging	Increased TVD and PPV (capillaries)	Prospective, interventional, non-randomized, non-controlled
De Backer D, 2006 (43)	10	Topic acetylcholine + dobutamine (5 mcg/kg/min)	Sublingual microcirculation	OPS imaging	Increased TVD, PPV and PVD (capillaries)	Prospective, interventional, non-randomized, non-controlled
Spronk PE, 2002 (11)	8	I.V. nitroglycerin	Sublingual microcirculation	OPS imaging	Improved capillary MFI	Prospective, interventional, non-controlled, open-label
Boerma EC, 2010 (44)	70	I.V. nitroglycerin vs. placebo	Sublingual microcirculation	SDF imaging	No differences after 24 hours of nitroglycerin or placebo regarding (small vessels) MFI, TVD, PPV, PVD and FHI	Prospective, placebo controlled, double-bind study

Lima A, 2014(45)	15*	I.V. nitroglycerin	Skin	Pulse oximeter, NIRS	Increased CRT, PPI, StO_2 and ReO_2whileTskin-diff decreased.	Prospective, interventional, non-controlled study
Trzeciak S, 2014 (46)	49	Inhaled NO vs. placebo	Sublingual microcirculation	SDF imaging	No differences after 2 hours of Inhaled NO or placebo on MFI and FHI	Prospective, placebo controlled, double-bind study

Legend:OPS: orthogonal polarization spectral, SDF: sidestream dark field imaging, pHi:gastric mucosal pH, PCO_2 gap: arterial-gastricmucosal pressure of carbon dioxidegap difference, TVD:total vessel density, PPV:proportion of perfusedvessels, PVD: perfused vesseldensity, MFI:microvascular flow index, FHI:flow heterogeneity index, CRT: capillary refill time, Tskin-diff:forearm-to-fingertip skin temperature difference, PPI:peripheral perfusion index, StO_2: tissue oxygen saturation, NIRS:near-infrared spectroscopy, ReO_2:peripheral tissue reoxygenation rate after arterial occlusion test. *:Twelve out of 15 patients included had septic shock.