The response of the microcirculation during fluid shifts

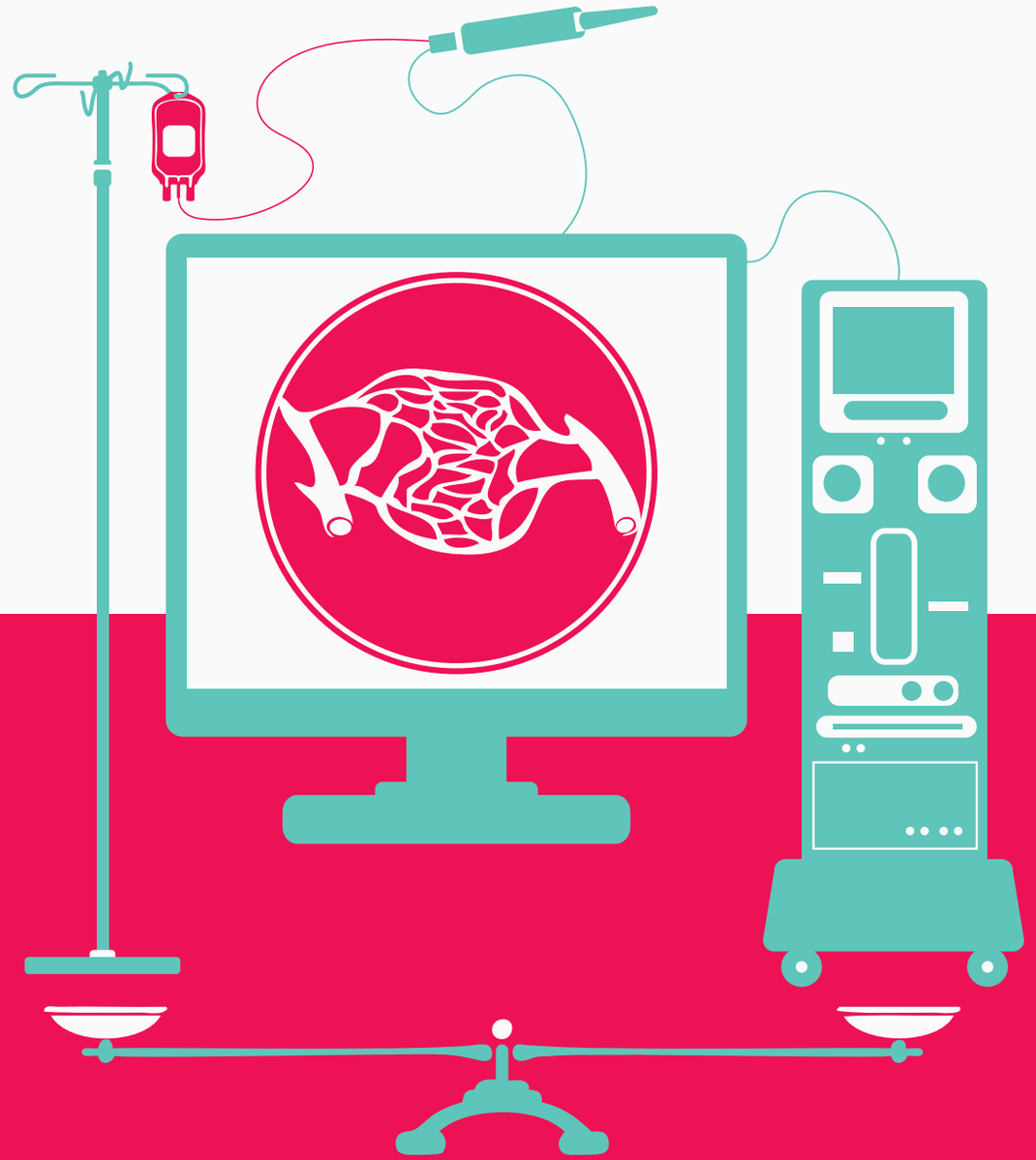
Veenstra, G.

Publication date
2018
Document Version
Final published version
License
Other

The Response of the Microcirculation during Fluid Shifts

Gerke Veenstra

The Response of the Microcirculation during Fluid Shifts



GERKE VEENSTRA

THE RESPONSE OF THE MICROCIRCULATION DURING FLUID SHIFTS

GERKE VEENSTRA

Cover: In Balans, Hasselt.
Layout: G. Veenstra
Paranymfen: mw. M. Veenstra; dr. N.A.R. Vellinga.
ISBN: 978-94-028-1226-8
Printed by: Ipskamp Printing

Financial support for the printing of this thesis has kindly been provided by 'Stichting Intensive Care Onderzoek Friesland'.

THE RESPONSE OF THE MICROCIRCULATION DURING FLUID SHIFTS

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van doctor

aan de Universiteit van Amsterdam op gezag van de Rector Magnificus

prof. dr. ir. K.I.J. Maex

ten overstaan van een door het College voor Promoties ingestelde commissie,

in het openbaar te verdedigen in de Agnietenkapel

op woensdag 21 november 2018, te 10:00 uur

door Gerke Veenstra

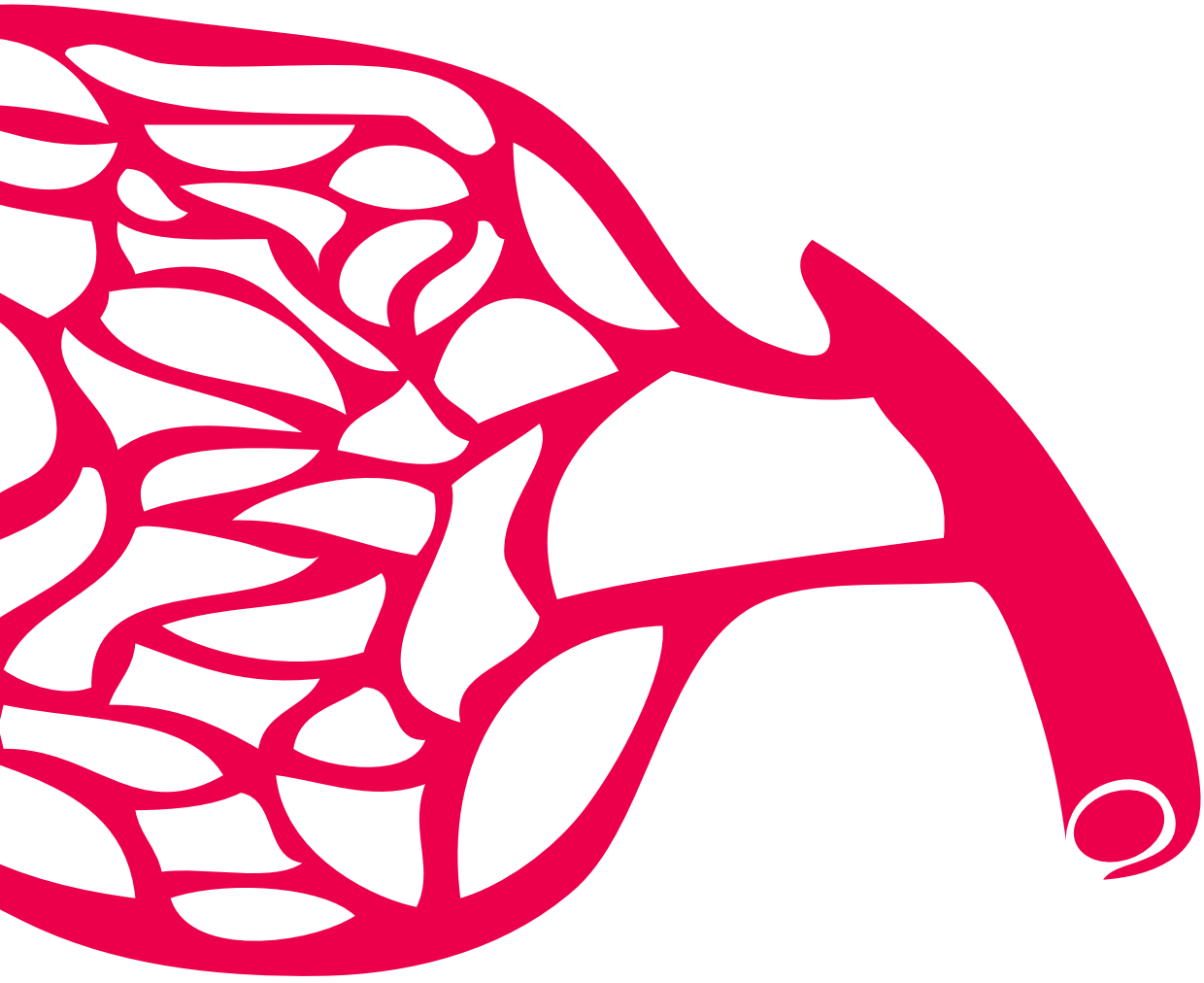
geboren te Smallingerland.

Promotiecommissie:

Promotor(es):	Prof. dr. ir. C. Ince	AMC-UVA
Copromotor(es):	Dr. E.C. Boerma	Medisch Centrum Leeuwarden
Overige leden:	Prof. dr. T.W.L. Scheeren	Rijksuniversiteit Groningen
	Prof. dr. C. Boer	Vrije Universiteit Amsterdam
	Prof. dr. A. Donati	Università Politecnica delle Marche
	Prof. dr. mr. B.A.J.M. de Mol	AMC-UVA
	Prof. dr. T.M. van Gulik	AMC-UVA
	Dr. D.P. Veelo	AMC-UVA

Faculteit der Geneeskunde

CONTENT



CHAPTER 1

INTRODUCTION

1

1

Acute circulatory failure or shock is still a common problem in the modern medical world. Shock is a collective name for the reaction to a certain insult or injury with a distinctive response in tissue perfusion. The circulation fails to deliver sufficient oxygen to the cell to maintain homeostasis and function. Therefore, cells will start to utilise anaerobic metabolism, and they eventually fail to survive. Large amounts of time are spent on understanding its pathophysiology and to intervene on this devastating illness. To recall, the clinical states of shock are defined by four mechanisms: hypovolaemic shock, cardiogenic shock, obstructive shock and distributive shock. The first three are defined by a decrease in cardiac output because of a decreased preload, decreased contractility or increased afterload. The last one is defined by a complex release of cytokines and mediators, resulting in a warm and cold shock type. (1,2) Very different from the other types, distributive or septic shock is distinguished by a loss of autoregulatory mechanisms on the microvascular level. Hereby, disrupting an important determinant of tissue oxygenation results in a mismatch between the oxygen supply and demand. On the microcirculatory level, there are areas with an increased convective flow in close proximity to areas with no flow or an increased diffusion distance. (3-7) Microcirculatory alterations are related to organ failure, and prolonged alterations are correlated with an increased mortality. Therefore, early detection and treatment are important for survival and morbidity. (8-16)

Conventionally, the evaluation of treatment is based on the normalisation of systemic variables of circulation, i.e., heart rate, blood pressure and cardiac output. Such a strategy is based on the assumption that the normalisation of macro-haemodynamic variables will result in a parallel improvement in organ perfusion. However, direct in vivo observation of the microcirculation using handheld microscopes has indicated that this coherence between microcirculation and macrocirculation may not always be present. Well-known conditions in which a loss of coherence has been observed include sepsis and obstructive heart failure, which unveil sustained hypoperfusion despite the correction of systemic variables by fluids and vasoactive medication. Uncoupling of macrocirculation and microcirculation may be the intrinsic result of the disease state. Endothelial dysregulation during sepsis may result in increased permeability, hypercoagulation and loss of vasomotor tone, causing altered microcirculatory blood flow to be not sensed by macrohaemodynamic variables. These effects will result in a decreased perfusion of organs and will have detrimental effects on cell oxygenation. (4, 17-19)

Adequate oxygenation of the cell is based on two key characteristics of perfusion.

1 The first one is convective oxygen transport, which depends on the red blood cell velocity and red blood cell oxygen-carrying capacity. The second determinant of oxygen transport to the cell is diffusion. Given the gas-specific characteristics, oxygen diffusion is related to the pressure gradient and is inversely related to the distance between the capillary and the cell. These two characteristics can be detected by direct in vivo observation of the microcirculation. Convective oxygen transport is expressed by the semiquantitative microvascular flow index (MFI), and diffusion distance is expressed as the capillary density. MFI provides a reproducible and transparent tool for measuring red blood cell velocity, varying between absent (0), intermittent (1), sluggish (2), and normal flow. Using offline analysis, the red blood cell velocity can be estimated. The capillary density is also determined offline and expressed as mm/mm2, and the functional capillary density is measured by combining measurement of the perfused vessels (MFI>2) with the calculated capillary density.

The importance of the visualisation of the microcirculation is hereby explained and has been of interest since the early 20th century. At first, the observation of human microcirculation was hampered by the size of the technical components, which resulted in large microscopes. After the shift to a different light exposure, orthogonal polarisation spectral (OPS) imaging was the first technique that opened up the field of imaging of the human microcirculation in a diversity of organ and tissue surfaces. Cytocam-IDF imaging can be regarded as a third-generation handheld microscope because it uses a completely new hardware platform, a new high-density pixel-based imaging chip with short pulsed illumination source, which is under computer control. Thereby, the illumination and imaging is perfectly synchronised, which results in a strongly improved image quality and detection of the true (small) capillaries.

The introduction of the visualisation techniques has initiated a large volume of studies that characterise the nature of shock at the capillary level and evaluate the potential effects of well-known therapeutic strategies, including fluid administration. In chronic renal failure, rapid changes in volume status during haemodialysis, combined with ultrafiltration and the Trendelenburg position, were traced accurately by swift changes in microcirculatory blood flow. In addition, in septic patients, both fluid expansion and passive leg raising were reflected in the microcirculatory red blood cell velocity.

The use of direct in vivo observation of the microcirculation at the bedside, ideally in an integrative model with conventional systemic haemodynamic variables, has the potential to do the following: 1. select patients potentially eligible for fluid therapy

and support the clinical diagnosis based on abnormal clinical surrogates of organ malperfusion; 2. evaluate the effects of fluid administration at the level of organ perfusion; and 3. stop fluid administration in the absence of beneficial effects, long before detrimental symptoms of fluid overload become eminent.

Thesis

To identify the niche where the visualisation of the microcirculation can be of help, it is important to understand the tools that are already present to guide fluid therapy. Chapter 1 is an extensive review about the pros and cons of a diversity of haemodynamic monitoring options and underlines the importance of visualisation of the microcirculation besides the macrohaemodynamic parameters.

Chapter 2 is a validation study to identify the difference between SDF and IDF imaging, and it gives the operator tools to compare both techniques in microcirculatory values.

Chapters 3 and 4 are designed as a pathophysiological model of induced hypovolaemia. We observed the reaction of the microcirculatory blood flow index during a low-dosage ultrafiltration within stabilised ICU patients in chapter 3. In chapter 4, we observed the microcirculation during swift and large volume changes during classical haemodialysis in patients with chronic renal failure.

Chapters 5 and 6 are designed to evaluate fluid therapy on a microcirculatory level, as this was suggested by others. In chapter 5, we describe the difference in the capillary diffusions between two intensive care patient groups, identifying one that can benefit from fluid therapy and one that will possibly suffer under fluid therapy. Chapter 6 is an observational study in which the direct effects of a fluid bolus were observed by prolonged measurements on one spot to identify the reaction of microcirculatory blood flow, diffusion distance and red blood cell velocity.

1 References

1. M.H. Weil, H. Shubin. Proposed reclassification of shock states with special reference to distributive defects. Adv Exp Med Biol. 1971;23(0):13-23.

2. J.L. Vincent, C. Ince, J. Bakker. Clinical review: Circulatory shock - an update: a tribute to Professor Max Harry Weil. Crit Care. 2012;16(6):239.

3. C. Ince, M. Sinaasappel. Microcirculatory oxygenation and shunting in sepsis and shock. Crit Care Med. 1999;27(7):1369-77.

4. V.S.K. Edul, C. Ince, A.R. Vazquez, P.N. Rubatto, E.D.V. Espinoza, S. Welsh et al. Similar Microcirculatory Alterations in Patients with Normodynamic and Hyperdynamic Septic Shock. Annals of the American Thoracic Society. 2016;13(2):240-7.

5. V.S.K. Edul, C. Enrico, B. Laviolle, A.R. Vazquez, C. Ince, A. Dubin et al. Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. Crit Care Med. 2012;40(5):1443-8.

6. C.G. Ellis, R.M. Bateman, M.D. Sharpe, W.J. Sibbald, R. Gill. Effect of a maldistribution of microvascular blood flow on capillary O(2) extraction in sepsis. Am J Physiol Heart Circ Physiol. 2002;282(1):H156-64.

7. F. Rogers, R. Dunn, J. Barrett, G. Merlotti, C. Sheaff, P. Nolan et al. Alterations of capillary flow during sepsis. Circ Shock. 1985;15(2):105-10.

8. C. Scorcella, E. Damiani, R. Domizi, S. Pierantozzi, S. Tondi, A. Carsetti et al. MicroDAIMON study: Microcirculatory DAIly MONitoring in critically ill patients: a prospective observational study. Annals of Intensive Care. 2018;8(1):S13-.

9. N.A.R. Vellinga, E.C. Boerma, M. Koopmans, A. Donati, A. Dubin, N.I. Shapiro et al. International study on microcirculatory shock occurrence in acutely ill patients. Crit Care Med. 2015;43(1):48-56.

10. A.P.C. Top, C. Ince, N. de Meij, M. van Dijk, D. Tibboel. Persistent low microcirculatory vessel density in nonsurvivors of sepsis in pediatric intensive care. Crit Care Med. 2011;39(1):8-13.

11. D. De Backer, J. Creteur, J.C. Preiser, M.J. Dubois, J.L. Vincent. Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med. 2002;166(1):98-104.

12. E. Rivers, B. Nguyen, S. Havstad, J. Ressler, A. Muzzin, B. Knoblich et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001;345(19):1368-77.

13. S. Trzeciak, E.P. Rivers. Clinical manifestations of disordered microcirculatory perfusion in severe sepsis. Crit Care. 2005;9 Suppl 4:S20-6.

14. K.C. Doerschug, A.S. Delsing, G.A. Schmidt, W.G. Haynes. Impairments in microvascular reactivity are related to organ failure in human sepsis. Am J Physiol Heart Circ Physiol. 2007;293(2):H1065-71.

15. S. Trzeciak, J.V. McCoy, R. Phillip Dellinger, R.C. Arnold, M. Rizzuto, N.L. Abate et al. 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. 2008;34(12):2210-7.

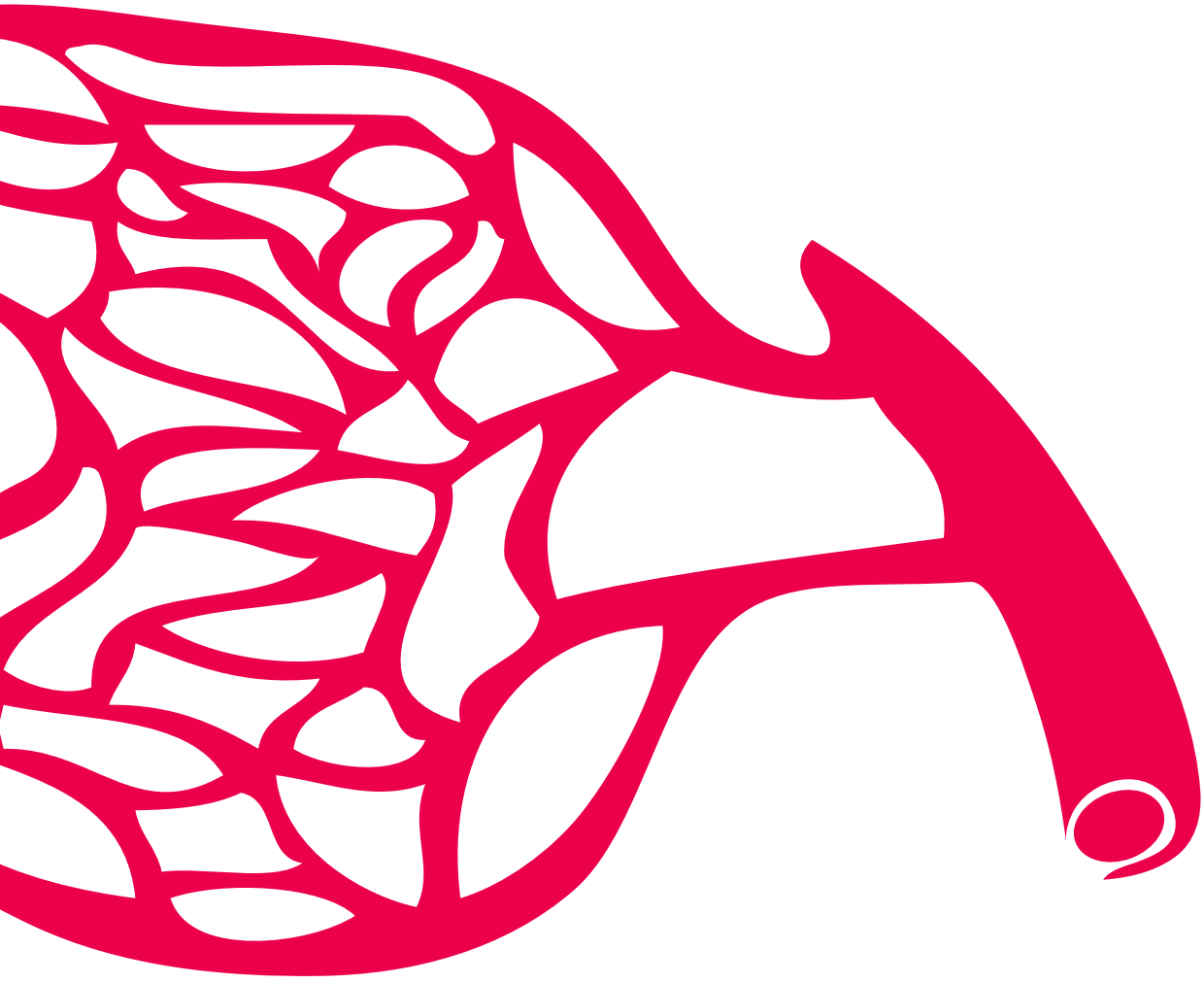
16. S. Trzeciak, R.P. Dellinger, J.E. Parrillo, M. Guglielmi, J. Bajaj, N.L. Abate et al. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Ann Emerg Med. 2007;49(1):88-98, 98.e1-2.

17. G. Ospina-Tascon, A.P. Neves, G. Occhipinti, K. Donadello, G. B, D. Simion et al. Effects of fluids on microvascular perfusion in patients with severe sepsis. Intensive Care Med. 2010;36(6):949-55.

18. A. Dubin, M.O. Pozo, G. Ferrara, G. Murias, E. Martins, C. Canullán et al. Systemic and microcirculatory responses to progressive hemorrhage. Intensive Care Med. 2009;35(3):556-64.

19. C. Ince. Hemodynamic coherence and the rationale for monitoring the microcirculation. Crit Care. 2015;19 Suppl 3:S8.

Bis et faceped molupidunt, sam is aut modicie niendit et aut arunto que doluptasi quia cus magnimus alibust, sequia poreperunt aborehe nesecab oribusda volore sit, int laccum aut aut quo quatquae nostior arum audis sectati antoreh endigendis eos



CHAPTER 2

DIRECT MARKERS OF ORGAN PERFUSION TO GUIDE FLUID THERAPY

when to start, when to stop.

G. Veenstra[1,2], C. Ince[1], E.C. Boerma[2]

1. Department of Intensive Care, Department of Intensive Care,
Erasmus MC University Hospital Rotterdam, 3000 Rotterdam, The Netherlands

2. Medical Centre Leeuwarden ICU, Henri Dunantweg 2, Postbus 888
8901 BR Leeuwarden.

In: Best practice & research: Clinical anaesthesiology.
Volume 28, Issue 3: 217-226, 2014

2 Abstract

Up till now the discussion in the literature as to the choice of fluids is almost completely restricted to the composition, with little to no attention paid to the importance of hemodynamic end points to achieve a desired optimal volume. The determination of fluid volume is left to the discretion of the attending physician with only surrogate markers as guidance the initiation and cessation of fluid therapy. In this review we aim to discuss the available literature on existing clinical and experimental criteria for the initiation and cessation of fluid therapy. Furthermore, we present recent data that have become available after the introduction of direct in-vivo microscopy of the microcirculation at the bedside, and discuss its potential influence on the existing paradigms and controversies in fluid therapy.

Introduction

For many years fluid therapy has been a corner stone in perioperative management and intensive care medicine to maintain organ function in a large variety of disease states. (1,2) Motives for fluid administration are diverse and include supplementation for fluid- or blood loss, compensation for increased resistance to venous return with subconsequent reduction of preload, and maintenance of perfusion pressure under conditions of reduced vasomotor tone. Apart from supplementation of chemical components such as electrolytes and protein (-like) substances, the central idea behind fluid administration has always been the conceived restoration of cell homeostasis, in order to maintain organ function. In this concept the final place of action of fluid therapy is assumed to be in the microcirculation, the vascular compartment where life-conditional processes, such as the exchange of oxygen and waste products, take place. It can be regarded as an organ representing port way to the parenchymal cells.

However, due to practical limitations the microcirculation remains elusive in clinical practice. (3) Instead, almost unrestrained efforts have been exerted to develop and validate surrogate end-points for fluid resuscitation, based on the manipulation of systemic hemodynamic variables. Although such indirect end-points have been successful to some extent, they have not been able to establish definitive start and stop criteria for fluid therapy. (4) This seems to be of utmost importance since both insufficient and excessive fluid administration have been associated with adverse outcome. (5-7) In addition to this view the discussion in the literature as to the choice of fluids used for various categories of patients is almost whole directed at the composition of these fluids, with little to no attention paid to the importance of hemodynamic end points to achieve a desired optimal volume. This issue has especially been a problem in recent large randomized trials where fluids are administered with no clear hemodynamic criteria for fluid administration and the determination of fluid volume is left to the discretion of the attending physician, an approach which is referred to in the literature as a pragmatic. (8) In this review we aim to discuss the available literature on existing clinical and experimental criteria for the initiation and cessation of fluid therapy. Furthermore, we present recent data that have become available after the introduction of direct in-vivo microscopy of the microcirculation at the bedside, and discuss its potential influence on the existing paradigms and controversies in fluid therapy.

2 When do doctors start fluid therapy?

In general there is a large variety of potental triggers that encourage doctors to start fluid administration.

'Clinical signs of impaired organ perfusion'. Traditionally, clinical assessment of volume status is either based on macrocirculatory parameters, such as (orthostatic) hypotension and tachycardia, or parameters of peripheral circulatory perfusion, e.g. capillary refill time, central-to-toe temperature gradient and skin mottling. (9-11) Alternatively, clinicians observe perfusion-related organ function such as altered mental status, oliguria and tachypnoe. However, all of these clinical parameters lack specificity and may be explained by alternative causes, other than hypovolemia-related perfusion abnormalities. For example tachycardia may be a sign of hypovolemia, but also be related to stress or sympathic overdrive.

'Laboratory markers'. Elevated lactate levels are usually associated with an increased anaerobic metabolism and therefore a potential trigger for the restoration of perfusion deficits with fluid therapy. Although increased lactate is repeatedly associated with inverse outcome, many alternative explanations other than hypoperfusion should be considered, e.g. epinephrine-induced stimulation of the Na^+-K^+-ATPase pump, lipopolysaccharide-mediated enhanced lactate production or impaired lactate clearance. (12) Hemoconcentration, reflected by increased hematocrit or protein concentration may also serve as a reason to initiate fluid administration.

'Static filling pressures'. Over the last decades static filling pressures have played a major role in the initiation of fluid therapy. Classically pulmonary artery occlusion pressure and central venous pressure cut-off values have been advocated as a marker of left en right ventricular preload. Although more recent data have clearly demonstrated that this assumption appears to be erroneous, 'optimizing' central venous pressure with fluid therapy is still part of current resuscitation guidelines. (2,13,14)

'Dynamic indices'. The use of dynamic indices is based on the assumption that cardiac function of patients with impaired organ perfusion is on the steeper part of the Frank-Starling curve. Changes in these variables are established after a standardized fluid challenge or passive leg raise test. These indices include stroke volume optimization, reduction in stroke volume- or pulse pressure variability (as a result of circulation-ventilation interaction) and changes in end-tidal carbon dioxide. (15) This is either performed by transpulmonary thermodilution methods, non-invasive pulse-contour analysis of arterial wave forms, or by ultrasound. Although it is the

assumption of the attending clinician that an increase in stroke volume in response to fluid therapy will automatically result in better organ perfusion, this may not necessarily be true at all times. For example in a study by Pottecher et al. (16) passive leg raising as well as fluid challenges were shown to be associated with an increase in microcirculatory perfusion, whereas other studies, especially in conditions of sepsis have demonstrated lack of coherence between raised cardiac output and microcirculatory perfusion. (7,17) Furthermore, the fact that fluid-responsiveness is also generally present in healthy volunteers, challenges the legitimacy of these markers as a trigger to initiate fluid administration.(18)

'Central/mixed venous saturation'. Central or mixed venous oxygen saturation ($S(c)VO_2$) reflects the balance between oxygen supply and consumption. Optimizing $S(c)VO_2$ with a therapeutic protocol that includes fluid therapy has been associated with improved outcome. (4,19) However, using $S(c)VO_2$ as a parameter to start fluid therapy has major limitations. Firstly, although a reduction in $S(c)VO_2$ indicates a reduction in physiological reserve, it is not necessarily a marker for perfusion-related organ failure. Secondly, in case of shunting, as in distributive shock, normal or elevated $S(c)VO_2$ does not rule out hypoperfusion nor the need for fluid administration.

'Bio reactance'. Derived from the original bio impedance technique, bio reactance-based techniques send a high-frequency current with known low amplitude through the thorax and measure the frequency- and phase-modulation, as a result of changes in the thoracic blood volume. Recent data failed to show an adequate correlation with cardiac output and response to passive leg raising. (20)

(When) do doctors stop fluid therapy?

Although fluid overload has been associated with adverse outcome, specific and unequivocal markers to stop fluid administration seem to be missing in the current literature. At best opinion leaders advocate to avoid fluid overload. (21) However, one would not only stop fluid administration before overt fluid overload takes place, but ideally refrain from (further) fluid administration in the absence of improvement of organ perfusion. In the literature several potential clinical and experimental variables for the cessation of fluid therapy can be identified.

'Clinical signs of fluid overload'. Peripheral and pulmonary edema are clearly markers of fluid overload, although they may also indicate misdistribution of fluids in the absence of absolute overload. However, together with (excessive) weight gain these are late markers and unsuitable to guide fluid administration in the perioperative or

 intensive care setting.

'Laboratory markers'. Lactate clearance has been advocated to guide fluid therapy in the resuscitation of shock. Hence, normalization of serum lactate could be used as a marker to stop fluid administration. The fact that even normal lactate levels are associated with adverse outcome, as well as the observation that the reduction in mortality was not associated with the actually achieved lactate levels hamper the clinical application. (22) Hemodilution results in reduction of the oxygen-carrying capacity of blood and counteracts the potential beneficial effects of fluid resuscitation. In cardiac surgery perioperative hematocrit levels lower than approximately 24% are associated with an increased risk to develop postoperative renal failure. In these studies the reduction in hematocrit generally reflects isovolemic hemodilution and cannot be extrapolated to volume overload. (23)

'Static filling pressures'. In the horizontal part of the cardiac volume-pressure-curve static filling pressures may not adequately predict fluid responsiveness. However, in the steep part of this curve a rise in pulmonary artery occlusion pressure or central venous pressure is likely to be a marker of volume overload of the heart. Therefore, in high ranges (a rise in) static filling pressures may serve as an upper safety limit for cardiac fluid overload. (2,24)

'Dynamic indices'. There is general consensus that absence of a significant rise in stroke volume, after fluid challenge or passive leg raising test, is a clear indicator that further fluid administration is no longer beneficial to improve organ perfusion. There is one exception to this rule: volume therapy in the range of so-called unstressed volume is also associated with an absence in increase in cardiac performance. (25)

'Extravascular lungwaterindex (EVLWi)'. Transpulmonary thermodilution-derived EVLWI has been validated for the quantification of pulmonary oedema formation. (26) Noteworthy is that this value can also be high in case of non-hydrostatic lung oedema (by instance ARDS), as reflected by an increase in permeability index. (27) It is conceivable that upper limits of extravascular lung water index, irrespective of its origin, may serve as a stop strategy for fluid administration. Up to date such strategy with clinically relevant endpoints has not been tested.

'Lung ultrasound'. Recently lung ultrasound as a tool asses hemodynamic management of shock has been introduced, and is based on a variety of artefacts. (28) A shift from a so called horizontal A-lines to vertical B-lines has been related

to lung oedema formation. Absence of clinical improvement after fluid therapy, in combination with the formation of B-lines has been proposed as a stop sign for fluid administration. However, the formation of B-lines is not restricted to fluid overload, but also present in case of high-permeability pulmonary oedema and lung ultrasound-based fluid protocols lack clinical validation.

´Bio impedance'. Single frequency bio impedance, based on measurement of body resistance between electrodes, is a method to assess total body water. In combination with multiple frequency spectroscopy extracellular and intracellular water can be differentiated. However, bio impedance (spectroscopy) shows a wide inter-individual variability and is only correlated with substantial weight change, thereby reducing its clinical usefulness to serve as a stop marker for fluid therapy. (29-32). An alternative technique, bio impedance vector analysis (BIVA), combines resistance with reactance to estimate total body water. However BIVA is unable to differentiate between compartmentalized oedema and increased total body water. (33)

'Oxygenation index'. The oxygenation index (OI) (mean air-way pressure × Fio2× 100/ Pao2) may serve as a surrogate marker of pulmonary dysfunction, with higher OI values denoting worsening oxygenation. In children it has been reported that OI increases during the first days of ICU admission until daily fluid balances become negative. (34) However, this association between OI and fluid balances may also be explained by the time course of the disease self. Moreover, pulmonary oedema is a late marker and unsuitable to stop fluid administration in the perioperative or intensive care setting before clinically relevant deterioration takes place.

(How) can the microcirculation be of help to start and stop fluid therapy?

'The microcirculation; a new concept from the past'. The microcirculation is the vascular compartment between arteriolar (resistance) vessels and the venular vasculature. Its observation goes back to the early days of microscopy with Malpighi and van Leeuwenhoek at the end of 17th century. Auto-regulatory mechanism guarantee a local distribution of microcirculatory blood flow that enables adequate oxygen delivery to the tissues in order to maintain cell homeostasis. Two important determinants of oxygen transport need to be acknowledged: convective oxygen transport and diffusion. Convective oxygen transport depends on red blood cell velocity and red blood cell oxygen-carrying capacity. In addition tissue oxygenation is also determined by diffusion. Given the gas-specific characteristics, oxygen diffusion is related to the pressure gradient and inversely related to the distance between the capillary and the cell. In case of inadequate oxygen delivery upstream

2 from the microcirculation during profound reduction of cardiac output, both aspects of microcirculatory oxygen transport are affected. (35) Reduction in systemic blood flow is not only reflected by a decrease in convective oxygen transport, as a result of diminished microcirculatory red blood cell velocity and oxygen saturation. Capillaries are also shut down thus increasing diffusion distance. In distributive shock, such as in sepsis, misdistribution of blood flow also comes in to play. Heterogeneity of blood flow appears a key characteristic of this disease state, even when adequate systemic oxygen carrying capacity is maintained. (36-39)

´Potential effects of fluid therapy in the microcirculatory perfusion´. Fluid administration has the potential to enhance both convective oxygen transport and diffusion. (17,40) However, it must be realized that fluids in themselves are very poor oxygen carriers and can in themselves not be expected to improve tissue oxygenation. (41) In addition one ought to be aware that there is a clear therapeutic window, equivalent to the administration of drugs. Under conditions of reduced systemic blood flow initial fluid administration may increase cardiac output and perfusion pressure, leading to increased red blood cell velocity and opening of previously constricted capillaries. (42,43) However, persistent fluid administration, after normalisation of convective oxygen transport may lead to oedema formation, thus enlarging oxygen diffusion distance with subsequent reduction of diffusion oxygen transport capacity (Figure 1). (42)

'Indirect techniques to monitor the microcirculation'. Tonometry and capnography are techniques based on the local accumulation of tissue CO_2 as a result of an inadequate washout of cellular waste products. The techniques enable detection of perfusion-related imbalances between oxygen supply and consumption. Potentially, these characteristics qualify the technique to provide useful markers for the initiation and cessation of fluid therapy. Devices for gastric and sublingual tonometry/capnography are available for the clinical setting. Microcirculatory shunting during distributive failure complicates the clear distinction between impaired perfusion, altered energy metabolism and anaerobic energy generation. Calculation of a tissue-to-systemic CO_2 gradient, rather than expressing absolute values of CO_2 or pH, has partially overcome this methodological flaw. Although clinical outcome data on tonometry-based fluid resuscitation protocols remain inconclusive (44), intestinal-to-systemic CO_2 gradients appeared to correlate well with variables of convective oxygen transport derived by direct in-vivo microscopy of the microcirculation. (40,45)

PO_2 electrodes maybe used for the measurement of tissue oxygenation. Such

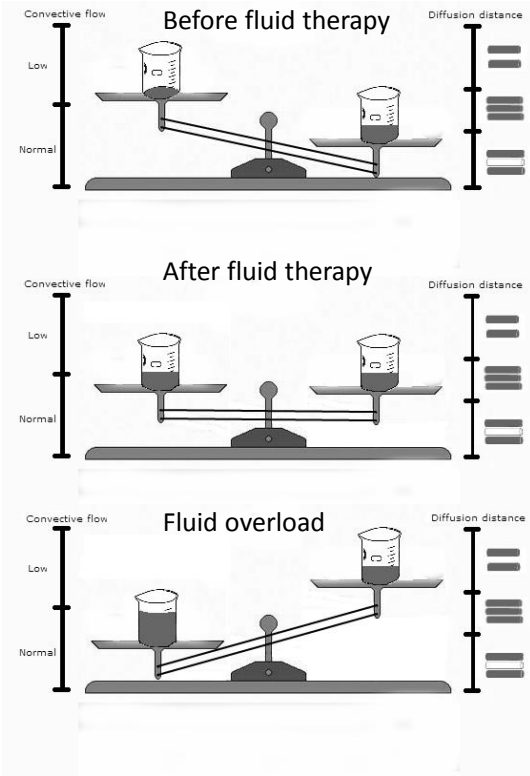


Figure 1: The balance between convective flow, diffusion distance during fluid therapy. Initially convective flow will normalise after the initiation of fluid therapy and diffusion distance will reduce as a result of reflow of previously non-perfused vessels. However, after restoration of convective flow and diffusion distance further fluid administration will remain convective oxygen transport unaffected but diffusion distance will increase as a result of oedema formation.

measurements do not discriminate between hypoxia and dysoxia, i.e. the imbalance between oxygen delivery and consumption. Neither do they provide information on the origin of the hypoxia. Furthermore this technique fails to detect regional hypoxia under conditions of heterogeneity of blood flow. Finally the catchment area of oxygen electrodes for measurement of oxygen pressure are very limited (in the order of 20µm). These characteristics disqualify PO_2-eletrodes to guide fluid therapy. (46)

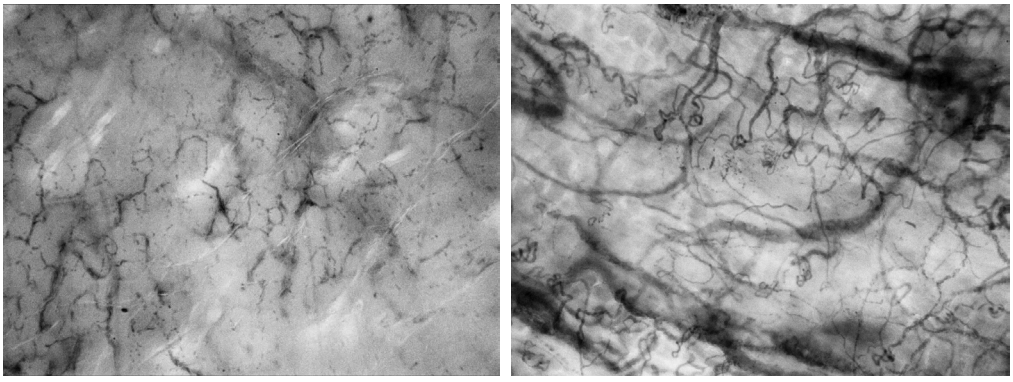
Near infrared spectroscopy (NIRS) is a non-invasive optical measurement of light absorption related to the (de)oxygenation of haemoglobin. It provides accurate measurements of haemoglobin saturation of vessels within the catchment area of the probe, without differentiation between venous or capillary circulation. Combined with a transient vascular occlusion test it can be used for the assessment of

microvascular reactivity on perfusion-mediated hypoxia, thus providing information on the integrity of local regulation of blood flow. (47,48) Although fluid therapy has been associated with changes in the muscle tissue oxygenation recovery slope, despite absence of changes in stroke volume, it remains unclear how this relates to organ perfusion. (49) It must be appreciated that these techniques are primarily related to tissue oxygenation and not perfusion, and they provide limited insight into the reason for abnormal values. This complicates its potential to serve as a marker for guidance of fluid therapy.

Laser-Doppler spectroscopy uses a Doppler shift in light frequency to calculate blood velocity. Thus, the information provided is by definition restricted to convective oxygen transport. Mostly used on skin, this technique will work on every surface with superficial blood vessels. Flow, detected within the catchment area, will be averaged, and heterogeneity of perfusion will not be detected. Skin is very sensitive to vasoconstriction as a result of temperature changes and the use of vasopressor agents, thereby influencing the measurements. (50,51) In patients after major surgery differences in fluid resuscitation strategies were reflected in both laser-Doppler variables as well as direct markers of tissue perfusion obtained by in-vivo microscopy of the microcirculation. (52)

'Direct techniques to observe the microcirculation'. In-vivo microscopy of the microcirculation has been adapted to the clinical setting since the introduction of different generations of handheld cameras. (53,54) The technique is based upon the visualisation of red blood cells due to the absorption of green light by haemoglobin. Observations have been performed in a large variety of tissues, including gut, liver, and brain, but is for practical reasons mainly restricted to the sublingual area. (53-56) These devices make it possible to assess the two key determinants of oxygen delivery to the cells at the bed side. Convective oxygen transport is expressed by the semi-quantitative microvascular flow index (MFI) and diffusion distance is expressed as capillary density. MFI provides a reproducible and transparent tool for measuring red blood cell velocity, varying from absent (0), intermittent (1), sluggish (2), to normal flow (3). (55,57) An MFI below 2.6 is generally regarded as a cut-off: it reflects the minimum reported lower threshold of the 95 % confidence interval in healthy or non-septic ICU controls and was clinically relevant in the paper by Pranskunas et al. (17,58) Capillary density is expressed as mm/mm^2, functional capillary density will be measured by combing the perfused vessels (MFI ≤ 2) with the capillary density.

Capillary density in healthy controls was reported in the range between 12 to 17



Low MFI, low capillary density High MFI, high capillary density

Figure 2: Cytocam-IDF imaging, left: cardiogenic shock, right: healthy volunteer.

mm/mm^2, but seems more patient dependent. As of now no data are available in terms of different disease states and response to therapy. Furthermore, the technique enables adequate discrimination between capillaries and other (venular) vessels as well as the detection of heterogeneity in blood flow within an area of interest less than 1 mm^2. The introduction of the technique has initiated a large volume of studies that characterize the nature of shock at the capillary level, and evaluate the potential effects of well-known therapeutic strategies, including fluid administration. In the setting of chronic renal failure, rapid changes in volume status during haemodialysis combined with ultrafiltration and Trendelenburg position were traced accurately by swift changes in microcirculatory blood flow. (59) Also in septic patients both fluid expansion and passive leg raising were reflected in microcirculatory red blood cell velocity. (16) Although MFI, as a marker of convective oxygen transport, can be assessed directly at the bed side, variables related to capillary density still need labour intensive software supported off-line analysis. (60) Recently, a third generation hand held microscope incorporating a high resolution computer-controlled image sensor has been introduced, referred to as a Cytocam-IDF (incident dark field imaging) device. (61) In combination with automatic analyse software this device has the potential to provide instant bedside analysis of all vital microcirculatory variables. This microcirculatory variables include MFI, capillary density and functional capillary density. Potentially, these characteristics qualify the technique to provide useful markers for the initiation and cessation of fluid therapy. (62,63)

Efforts to change the paradigm.

Integrating al of the above it becomes clear that there are many triggers to start fluid

therapy in daily clinical practice. Unfortunately, all of these triggers are surrogate markers for potential beneficial effects of fluid therapy, which is the expectation of improving microcirculatory blood flow with the aim of improving oxygen delivery to the parenchymal cells. (42) On top of that, the available potential triggers are restricted to only one aspect of oxygen transport: convection. It is conceivable that the absence of a possibility for the clinician to evaluate the effects of fluid therapy directly at the level of its place of action, i.e. the microcirculation, contributes to persistence of existing controversies. Furthermore, there is a virtual absence in the literature of validated triggers to stop fluid therapy, with the exception of upper-limit safety boundaries to avoid excessive fluid overload. Introduction of direct in-vivo observation of the microcirculation at the bedside, ideally in an integrative model with conventional systemic hemodynamic variables, has the potential to: 1. select patients potentially eligible for fluid therapy and support the clinical diagnosis based on abnormal clinical surrogates of organ malperfusion; 2. evaluate effects of fluid therapy at the level of organ perfusion and 3. stop fluid administration in the absence of beneficial effects, long before detrimental symptoms of fluid overload become eminent. We describe two studies with the purpose to use the microcirculation as the primary endpoint of fluid resuscitation, rather than the existing surrogate endpoints.

Pranskunas et al. evaluated the specificity of so-called 'clinical signs of impaired organ perfusion', such as hypotension, tachycardia, oliguria, increased central-to-toe temperature gradient and hyperlactatemia. (17) At baseline, two third of these mixed ICU patients appeared to have convective oxygen transport abnormalities, predefined as an MFI < 2.6. After a standardized fluid challenge microcirculatory parameters of convective oxygen transport normalized in all patients with an abnormal MFI at baseline. More importantly, in this group, the perceived 'clinical signs of impaired organ perfusion' were significantly attenuated. In contrast in patients with a normal MFI at baseline no significant change in 'clinical signs of impaired organ perfusion' were observed. Furthermore, changes in microcirculatory blood flow were not traced by both static and dynamic indices of systemic blood flow. This study highlights the potential for in-vivo microscopy to select patients potentially eligible for the start of fluid therapy.

In an experimental porcine model of haemorrhagic shock, Xu et al., randomised the animals in two different protocols to guide fluid resuscitation. (64) In one group fluid administration was initiated at a mean arterial pressure (MAP) below 60 mmHg, and continued until a MAP of 90 mmHg was achieved. In the other group

fluid administration was guided by sublingual capnography, aiming for a sublingual-systemic-CO_2 gradient between 50 and 70 mmHg. In the sublingual capnography-guided group the percentage of animals receiving fluid therapy was 40 percent as opposed to 100 percent in the blood pressure-guided group. Also the amount of fluids was significantly lower in the capnography-guided group: 170 ± 239 ml Ringer's lactate versus 955± 381 ml Ringer's lactate in combination with red blood cell transfusion. Interestingly, direct in-vivo observation of the microcirculation revealed similar variables of microcirculatory perfusion. Neurological function and mortality did not differ between groups. This study highlights the potential for microcirculatory monitoring to not only select animals potentially eligible for fluid therapy, but also restrict fluid administration without clinical deterioration.

Conclusion

Up till recently, only surrogate endpoints for fluid resuscitation have been available in clinical setting. Although these surrogate endpoints serve as triggers for the initiation for fluid therapy in daily clinical practice, controversies about the validity as such remain. Clinical markers to stop fluid administration are restricted to upper-limit safety boundaries to avoid excessive fluid overload. Replacement of such surrogate endpoints by either direct observation of the microcirculation or indirect monitoring of microcirculatory perfusion with capnography has the potential to unravel existing controversies and shift the paradigm to an organ-perfusion orientated focus on fluid administration. Fluid therapy will thus become a drug with clear indications, contraindications and a well-defined therapeutic index. Together with a suitable (micro) hemodynamic monitoring platform such strategy can be expected to provide the clinician with a modality for the administration of the optimal type and amount of fluid to the right patient at the right time.

2 Practice Points

• Monitoring of oxygen delivery, consumption and distribution both systemically and regionally is important for every patient.

• Administration of fluids is equivalent to administration of any other drug and should be applied with care and suitable monitoring to avoid harm.

• Systemic parameters pointing to hypovolemia may deceive clinicians and lead to unnecessary administration of fluids.

• In-vivo microscopy is the golden standard for microcirculatory monitoring because it can assess the key points of the circulation at the cellular level: convective transport and capillary density.

• Patients with decreased microcirculatory blood flow may benefit from fluid therapy, and its evaluation must be based on increase in flow and capillary density. Such a patient can be referred to as being a microcirculatory fluid responder.

• Patients with reduced capillary density after fluid therapy are not fluid responsive and show early signs of fluid overload. Such a decreased capillary density should be a trigger for cessation of fluid administration.

Research Agenda

• Further research is needed for validating the newest type of handheld device and analysis system and to test whether fluid administration can be titrated based on the measured microcirculatory functional parameters produced by the Cytocam-IDF device.

• Clinical trials are needed to assess microcirculation guided fluid therapy against the existing resuscitation strategies based on correction of systemic hemodynamic variables.

• Such trial should focus on the selection of patients, the initiation and cessation of fluid therapy with the aim to provide less fluid administration with at least similar clinical outcome in different categories of patients with conceived hypovolemia.

References

Papers of particular interest, have been highlighted (*).

1. D. Chappell, M. Jacob, K. Hofmann-Kiefer et al. A rational approach to perioperative fluid management. Anesthesiology. 2008;109(4):723-40.

2. R.P. Dellinger, J.M. Carlet, H. Masur et al. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. Crit Care Med. 2004;32(3):858-73.

3. C. Ince. The elusive microcirculation. Intensive Care Med. 2008;34(10):1755-6.

4. E. Rivers, B. Nguyen, S. Havstad et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001;345(19):1368-77.

5. D. Payen, A.C. de Pont, Y. Sakr et al. A positive fluid balance is associated with a worse outcome in patients with acute renal failure. Crit Care. 2008;12(3):R74.

6. J.H. Boyd, J. Forbes, T.A. Nakada et al. Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. Crit Care Med. 2011;39(2):259-65.

7. G. Ospina-Tascon, A.P. Neves, G. Occhipinti et al. Effects of fluids on microvascular perfusion in patients with severe sepsis. Intensive Care Med. 2010;36(6):949-55. *

8. C. Ince. The great fluid debate: when will physiology prevail? Anesthesiology. 2013;119(2):248-9.

9. A. Lima, T.C. Jansen, J. van Bommel et al. The prognostic value of the subjective assessment of peripheral perfusion in critically ill patients. Crit Care Med. 2009;37(3):934-8.

10. H.R. Joly, M.H. Weil. Temperature of the great toe as an indication of the severity of shock. Circulation. 1969;39(1):131-8.

11. H. Ait-Oufella, S. Lemoinne, P.Y. Boelle et al. Mottling score predicts survival in septic shock. Intensive Care Med. 2011;37(5):801-7.

12. S. Gibot. On the origins of lactate during sepsis. Crit Care. 2012;16(5):151.

13. A. Kumar, R. Anel, E. Bunnell et al. Pulmonary artery occlusion pressure and central venous pressure fail to predict ventricular filling volume, cardiac performance, or the response to volume infusion in normal subjects. Crit Care Med. 2004;32(3):691-9.

14. D. Osman, C. Ridel, P. Ray et al. Cardiac filling pressures are not appropriate to predict hemodynamic response to volume challenge. Crit Care Med. 2007;35(1):64-8.

15. X. Monnet, A. Bataille, E. Magalhaes et al. End-tidal carbon dioxide is better than arterial pressure for predicting volume responsiveness by the passive leg raising test. Intensive Care Med. 2013;39(1):93-100.

16. J. Pottecher, S. Deruddre, J.L. Teboul et al. Both passive leg raising and intravascular volume expansion improve sublingual microcirculatory perfusion in severe sepsis and septic shock patients. Intensive Care Med. 2010;36(11):1867-74. *

17. A. Pranskunas, M. Koopmans, P.M. Koetsier et al. Microcirculatory blood flow as a tool to select ICU patients eligible for fluid therapy. Intensive Care Med. 2012; *

18. G.E.P. Godfrey, S.W. Dubrey, J.M. Handy. A prospective observational study of stroke volume responsiveness to a passive leg raise manoeuvre in healthy non-starved volunteers as assessed by transthoracic echocardiography. Anaesthesia. 2014;69(4):306-13.

19. R. Pearse, D. Dawson, J. Fawcett et al. Early goal-directed therapy after major surgery reduces complications and duration of hospital stay. A randomised, controlled trial [ISRCTN38797445]. Crit Care. 2005;9(6):R687-93.

20. E. Kupersztych-Hagege, J.L. Teboul, A. Artigas et al. Bioreactance is not reliable for estimating cardiac output and the effects of passive leg raising in critically ill patients. Br J Anaesth. 2013;111(6):961-6.

21. Z. Ricci, C. Ronco. Year in review 2012: Critical Care - nephrology. Crit Care. 2013;17(6):246.

22. T.C. Jansen, J. van Bommel, F.J. Schoonderbeek et al. Early lactate-guided the-

rapy in intensive care unit patients: a multicenter, open-label, randomized controlled trial. Am J Respir Crit Care Med. 2010;182(6):752-61.

23. H. Vermeer, S. Teerenstra, R.G.L. de Sévaux et al. The effect of hemodilution during normothermic cardiac surgery on renal physiology and function: a review. Perfusion. 2008;23(6):329-38.

24. R.J. Trof, I. Danad, M.W.L. Reilingh et al. Cardiac filling volumes versus pressures for predicting fluid responsiveness after cardiovascular surgery: the role of systolic cardiac function. Crit Care. 2011;15(1):R73.

25. J.R.C. Jansen, J.J. Maas, M.R. Pinsky. Bedside assessment of mean systemic filling pressure. Curr Opin Crit Care. 2010;16(3):231-6.

26. F. Michard. Bedside assessment of extravascular lung water by dilution methods: temptations and pitfalls. Crit Care Med. 2007;35(4):1186-92.

27. S. Kushimoto, Y. Taira, Y. Kitazawa et al. The clinical usefulness of extravascular lung water and pulmonary vascular permeability index to diagnose and characterize pulmonary edema: a prospective multicenter study on the quantitative differential diagnostic definition for acute lung injury/acute respiratory distress syndrome. Crit Care. 2012;16(6):R232.

28. D. Lichtenstein. FALLS-protocol: lung ultrasound in hemodynamic assessment of shock. Heart, lung and vessels. 2013;5(3):142-147.

29. A.N. Roos, R.G. Westendorp, M. Frölich, A.E. Meinders. Weight changes in critically ill patients evaluated by fluid balances and impedance measurements. Crit Care Med. 1993;21(6):871-7.

30. R.L. Chioléro, L.J. Gay, J. Cotting et al. Assessment of changes in body water by bioimpedance in acutely ill surgical patients. Intensive Care Med. 1992;18(6):322-6.

31. U.M. Moissl, P. Wabel, P.W. Chamney et al. Body fluid volume determination via body composition spectroscopy in health and disease. Physiol Meas. 2006;27(9):921-33.

32. V. Wizemann, C. Rode, P. Wabel. Whole-body spectroscopy (BCM) in the assessment of normovolemia in hemodialysis patients. Contrib Nephrol. 2008;161:115-8.

33. W.F. Peacock. Use of bioimpedance vector analysis in critically ill and cardiorenal patients. Contrib Nephrol. 2010;165:226-35.

34. A.A. Arikan, M. Zappitelli, S.L. Goldstein et al. Fluid overload is associated with impaired oxygenation and morbidity in critically ill children. Pediatr Crit Care Med. 2012;13(3):253-8. *

35. M.E. van Genderen, E. Klijn, A. Lima et al. Microvascular perfusion as a target for fluid resuscitation in experimental circulatory shock*. Crit Care Med. 2014;42(2):e96-e105. *

36. C. Ince, M. Sinaasappel. Microcirculatory oxygenation and shunting in sepsis and shock. Crit Care Med. 1999;27(7):1369-77.

37. V.S.K. Edul, C. Enrico, B. Laviolle et al. Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. Crit Care Med. 2012;40(5):1443-8.

38. C.G. Ellis, R.M. Bateman, M.D. Sharpe et al. Effect of a maldistribution of microvascular blood flow on capillary O(2) extraction in sepsis. Am J Physiol Heart Circ Physiol. 2002;282(1):H156-64.

39. F. Rogers, R. Dunn, J. Barrett et al. Alterations of capillary flow during sepsis. Circ Shock. 1985;15(2):105-10.

40. A. Dubin, M.O. Pozo, G. Ferrara et al. Systemic and microcirculatory responses to progressive hemorrhage. Intensive Care Med. 2009;35(3):556-64.

41. M. Legrand, E.G. Mik, G.M. Balestra et al. Fluid resuscitation does not improve renal oxygenation during hemorrhagic shock in rats. Anesthesiology. 2010;112(1):119-27. *

42. C. Ince. The rationale for microcirculatory guided fluid therapy. Curr Opin Crit Care. 2014;20(3):301-8. *

43. E.C. Boerma, C. Ince. The role of vasoactive agents in the resuscitation of microvascular perfusion and tissue oxygenation in critically ill patients. Intensive Care Med. 2010;36(12):2004-18.

44. C.D. Gomersall, G.M. Joynt, R.C. Freebairn et al. Resuscitation of critically ill patients based on the results of gastric tonometry: a prospective, randomized, controlled trial. Crit Care Med. 2000;28(3):607-14. *

45. A. Dubin, V.S.K. Edul, M.O. Pozo et al. Persistent villi hypoperfusion explains intramucosal acidosis in sheep endotoxemia. Crit Care Med. 2008;36(2):535-42.

46. D. De Backer, K. Donadello, D.O. Cortes. Monitoring the microcirculation. J Clin Monit Comput. 2012;26(5):361-6.

47. M. Siegemund, J. van Bommel, C. Ince. Assessment of regional tissue oxygenation. (1). Intensive Care Med. 1999;25(10):1044-60.

48. K.C. Doerschug, A.S. Delsing, G.A. Schmidt, W.G. Haynes. Impairments in microvascular reactivity are related to organ failure in human sepsis. Am J Physiol Heart Circ Physiol. 2007;293(2):H1065-71.

49. E. Futier, S. Christophe, E. Robin et al. Use of near-infrared spectroscopy during a vascular occlusion test to assess the microcirculatory response during fluid challenge. Crit Care. 2011;15(5):R214.

50. M.D. Stern, D.L. Lappe, P.D. Bowen et al. Continuous measurement of tissue blood flow by laser-Doppler spectroscopy. Am J Physiol. 1977;232(4):H441-8.

51. A. Humeau, W. Steenbergen, H. Nilsson, T. Strömberg. Laser Doppler perfusion monitoring and imaging: novel approaches. Med Biol Eng Comput. 2007;45(5):421-35.

52. S. Jhanji, A. Vivian-Smith, S. Lucena-Amaro et al. Haemodynamic optimisation improves tissue microvascular flow and oxygenation after major surgery: a randomised controlled trial. Crit Care. 2010;14(4):R151.

53. W. Groner, J.W. Winkelman, A.G. Harris et al. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. Nat Med. 1999;5(10):1209-12.

54. P.T. Goedhart, M. Khalilzada, R. Bezemer et al. Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. Optics express. 2007;15(23):15101-14.

55. E.C. Boerma, K.R. Mathura, P.H.J. van der Voort et al. Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. Crit Care. 2005;9(6):R601-6.

56. D. De Backer, S. Hollenberg, C. Boerma et al. How to evaluate the microcirculation: report of a round table conference. Critical Care. 2007;11(5):R101-.

57. M.O. Pozo, V.S. Kanoore Edul, C. Ince, A. Dubin. Comparison of different methods for the calculation of the microvascular flow index. Critical care research and practice. 2012;2012:102483.

58. S. Trzeciak, R.P. Dellinger, J.E. Parrillo et al. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Ann Emerg Med. 2007;49(1):88-98, 98.e1-2.

59. R.H.H. Bemelmans, E.C. Boerma, J. Barendregt et al. Changes in the volume status of haemodialysis patients are reflected in sublingual microvascular perfusion. Nephrol Dial Transplant. 2009;24(11):3487-92.

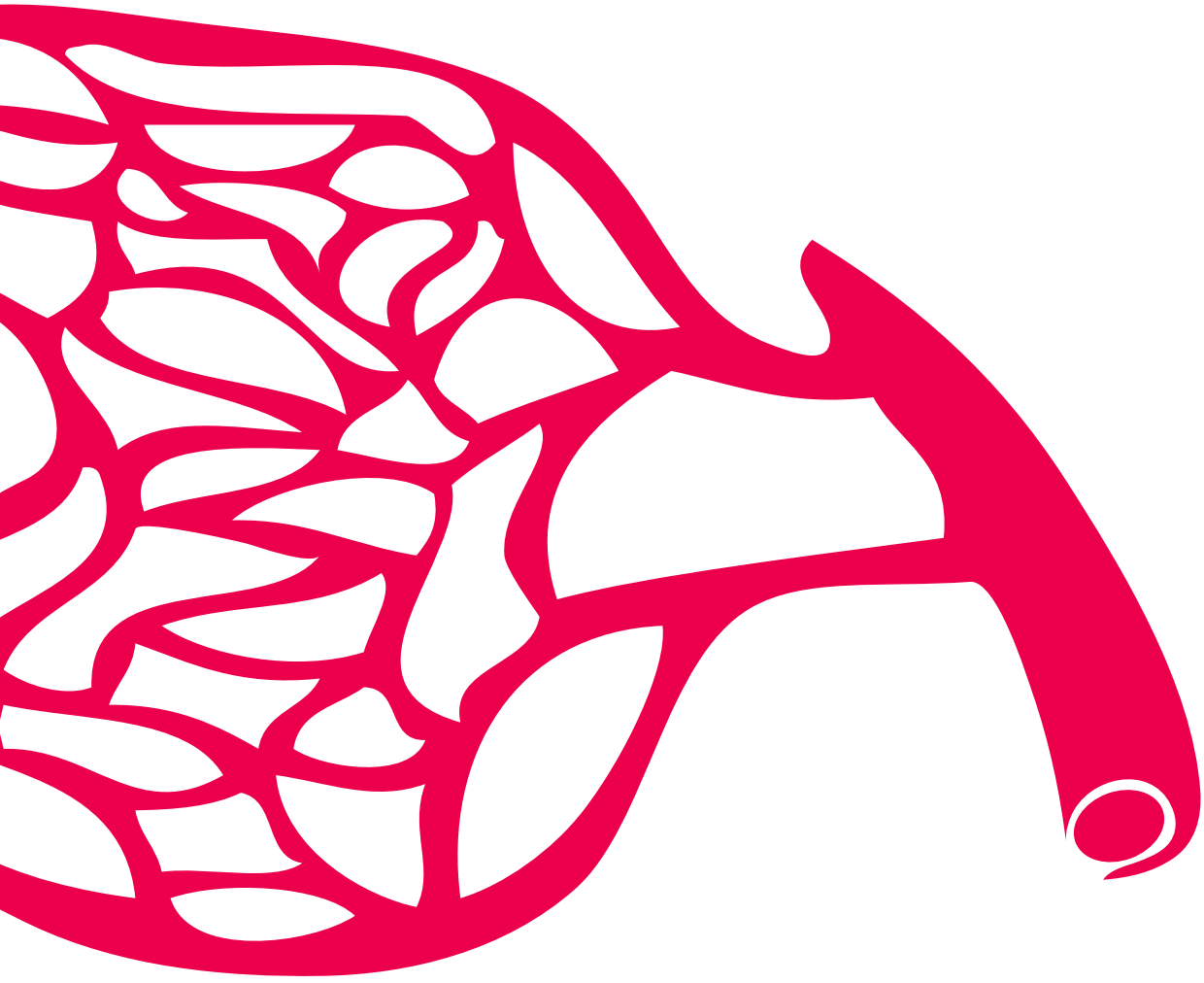
60. R.C. Arnold, J.E. Parrillo, R. Phillip Dellinger et al. Point-of-care assessment of microvascular blood flow in critically ill patients. Intensive Care Med. 2009;35(10):1761-6.

61. H. Sherman, S. Klausner, W.A. Cook. Incident dark-field illumination: a new method for microcirculatory study. Angiology. 1971;22(5):295-303.

62. R. Bezemer, S.A. Bartels, J. Bakker, C. Ince. Clinical review: Clinical imaging of the sublingual microcirculation in the critically ill - where do we stand? Crit Care. 2012;16(3):224.

63. Aykut G., Ince Y., Ince C.. A new generation computer-controlled imaging sensor-based hand-held microscope for quantifying bedside microcirculatory alterations. In: Vincent J.L., editor. Annual update in Intensive Care and Emergency Medicine 2014. Heidelberg: Springer; 2014. p. 367-81.

64. J. Xu, L. Ma, S. Sun et al. Fluid resuscitation guided by sublingual partial pressure of carbon dioxide during hemorrhagic shock in a porcine model. Shock. 2013;39(4):361-5. *



CHAPTER 3

CYTOCAM-IDF (INCIDENT DARK FIELD ILLUMINATION) IMAGING FOR BED-SIDE MONITORING OF THE MICROCIRCULATION.

G. Aykut[1,3], G. Veenstra[1,2], C. Scorcella[2], C. Ince[1], E.C. Boerma[2]

1. Department of Intensive Care, Erasmus MC University Medical Center, Dr. Molewaterplein 50, Rotterdam, 3015 GE, The Netherlands
2. Department of Intensive Care, Medical Centre Leeuwarden, PO Box 888, 8901BR Leeuwarden, The Netherlands
3. Department of Anesthesiology, University Hospital Heidelberg, Im Neuenheimer Feld 110, 69120 Heidelberg, Germany

in: Intensive care medicine experimental. 2015; 3:40.

3 Abstract

Introduction

OPS (orthogonal polarized spectral) and SDF (sidestream dark field) imaging video microscope devices were introduced for observation of the microcirculation, but due to technical limitations have remained as research tools. Recently, a novel hand-held microscope based on incident dark field illumination (IDF) has been introduced for clinical use. The Cytocam-IDF imaging device consists of a pen-like probe incorporating IDF illumination with a set of high resolution lenses projecting images on to a computer controlled image sensor synchronized with very short pulsed illumination light. This study was performed to validate Cytocam-IDF imaging by comparison to SDF imaging in volunteers.

Design

Prospective, observational study

Subjects

25 volunteers

Results

Sublingual microcirculation was evaluated using both techniques. The main result was that Cytocam-IDF imaging provided better quality images and was able to detect 30% more capillaries than SDF imaging (total vessels density Cytocam-IDF: 21.60 ± 4.30 mm/mm2 vs SDF: 16.35 ± 2.78 mm/mm2, p<0,0001). Comparison of the images showed increased contrast, sharpness and image quality of both venules and capillaries.

Conclusion

Cytocam-IDF imaging detected more capillaries and provided better image quality than SDF imaging. It is concluded that Cytocam-IDF imaging may provide a new improved imaging modality for clinical assessment of microcirculatory alterations.

Introduction

Microcirculation is the main means of oxygen delivery to tissue cells and is essential for the maintenance of cellular life and function. Its function relies on the complex interaction of its component cellular systems, including red and white blood cells, endothelial, smooth muscle and parenchymal cells. The function of the organs is directly dependent on the function of their respective microcirculation, and achievement of good microcirculatory function can be considered to be the prime goal of the cardiovascular system and of particular importance to critically ill patients, especially ones who are in shock. (1) Many studies have demonstrated that persistent microcirculation alterations that are unresponsive to therapy are independently associated with adverse outcome, especially in septic patients. (1-5) Additionally, these microcirculatory alterations have been shown in various studies to be independent of systemic hemodynamic variables, making the observation of microcirculation a potentially important extension of the conventional systemic hemodynamic monitoring of critically ill patients. (3,4)

In the early 20th century, direct intravital observation of human microcirculation was limited to the use of bulky capillary microscopes, which were mainly applied to the nailfold capillary bed. In 1964, Krahl made use of incident light directed at an oblique angle to the study tissue surfaces. (6) In 1971, Sherman et al. introduced a new method of microcirculation observations called incident dark field illumination (IDF) microscopy. This method enabled observations of organ surface microcirculation using epi-illumination, without the need for transillumination of the tissue from below. (7) An alternative method to observe microcirculation using epi-illumination was introduced by Slaaf et al., enabling the imaging of subsurfaces using cross polarized light microscopy. (8) In the late 1990's, Groner et al. adapted the Slaaf et al. technique to a hand-held video microscope. (9) This method was called orthogonal polarization spectral (OPS) imaging. We validated and introduced this technique to patients and were able for the first time to produce organ surface microcirculation images in surgical patients. (10,11) This technique opened up the field of studying human microcirculation in organ and tissue surfaces at the bedside especially in critically ill patients.

OPS imaging can be regarded as the first generation hand-held bedside imaging instrument to be applied to critical ill patients, resulting in general recognition that microcirculation is an important physiological process that is compromised during critical illness and needs to be monitored in a clinical environment. (12,13) OPS imaging was improved upon by our development of a second generation hand-held

analogue video microscopes based on side stream dark field (SDF) imaging. (14) Its advancement was that it provided better images than OPS imaging and allowed battery operation, making the device more mobile than its predecessor. A device similar to SDF imaging device fitted with a USB extension called the Capiscope, was also recently introduced. These devices, however, remained research tools mainly due to the technological limitations preventing operator independent reproducible measurements and the inability to achieve automatic microcirculation analysis for quantification needed for clinical decision making. (16-18) Analysis of the images to extract relevant functional microcirculatory parameters required time-consuming off-line analysis (16) precluding their use in bedside clinical decision making and in titrating therapy to reach microcirculatory end-points. (19)

Cytocam-IDF imaging can be regarded as third generation hand held microscope because it employs a completely new hardware platform where a high density pixel based imaging chip and short pulsed illumination source under computer control synchronizes and controls illumination and image acquisition. The device consists of a pen-like probe incorporating IDF illumination with a set of high resolution lenses projecting images on to a computer controlled high-density image sensor synchronized to an illumination unit. The probe is covered by a sterilizable cap. Cytocam-IDF imaging is based on the incident dark field illumination (IDF), a principle originally introduced by Sherman and Cook. (7) It further incorporates a stepping motor for quantitative focusing as well as high resolution optics.

In the first part of this study, Cytocam-IDF imaging is validated by quantitative comparison of microcirculatory parameters to SDF imaging in sublingual tissue using specialized image processing software developed earlier by us. (20) In addition, Cytocam-IDF and SDF images of one and the same sublingual microcirculatory area were obtained to directly compare the image quality to each other in the second part of the study. This feature allows serial measurements to be made without the need to refocus, an important feature with respect to previous generation devices which require time consuming manual adjustment of focus dials.

Subjects

Twenty-five healthy volunteers (8 male and 17 female) between the ages 23 and 55 were recruited. None of the subjects had history or evidence of disease or were taking drugs that are known to affect microcirculatory function.

Methods

SDF imaging:

In SDF imaging (Microscan, MicroVision Medical,Amsterdam The Netherlands), illumination is provided by surrounding a central light guide with concentrically placed light emitting diodes (LEDs) to provide sidestream dark field illumination. (14) The magnification lens in the core of the light guide is optically isolated from the illuminating outer ring, thus preventing tissue surface reflections. Light from the illuminating outer core of the SDF probe penetrates the tissue and illuminates the tissue-embedded microcirculation by scattering. The LEDs use green light (530 nm wavelength) corresponding to an isobestic point in the absorption spectra of oxyhemoglobin and de-oxyhemoglobin. The LEDs provide pulsed illumination to overcome the interlacing of the analogue video cameras used. The SDF device with a total weight of 320 grams, is fitted with a 5x objective lens. It is based on an analogue video camera which allows its output to be directly connected to a television monitor. For analysis of the video sequences, images need to be digitized using an external analogue to digital converting device and then analyzed off-line using specialized image processing software. (20) Illumination intensity and image focus are adjusted manually by a dial on the devices. The probe, covered by a sterile disposable cap, can be placed on organ and tissue surfaces to observe the microcirculation.

Cytocam-IDF imaging:

Cytocam-IDF imaging (Braedius Medical, Huizen, The Netherlands) consists of a combination of IDF illumination with optical and technical features optimized for visualization of the microcirculation on organ surfaces. It uses incident dark field illumination (7) with high-brightness LEDs with a very short illumination pulse time of 2 ms. The image acquisition and sensor are under computer control and electronically synchronized to the illumination pulses. This feature, in combination with a specialized set of lenses, projects images onto a computer controlled image sensor and results in high penetration sharp contour visualization of the microcirculation showing flowing red and white blood cells. The device is constructed of aluminum and titanium, resulting in a lightweight (120 grams) and pen-like instrument (length 220mm diameter 23 mm). The camera is fully digital with a high resolution sensor, which is used in binning mode, resulting in a 3.5 megapixel frame size. The combination of an optical magnification factor of 4 and the large image area of the sensor provides a field of view of 1.55 x 1.16 mm about three times larger than the field of view of previous devices (see Fig 3). The optical system provides an optical resolution of more than 300 lines/mm. The camera is

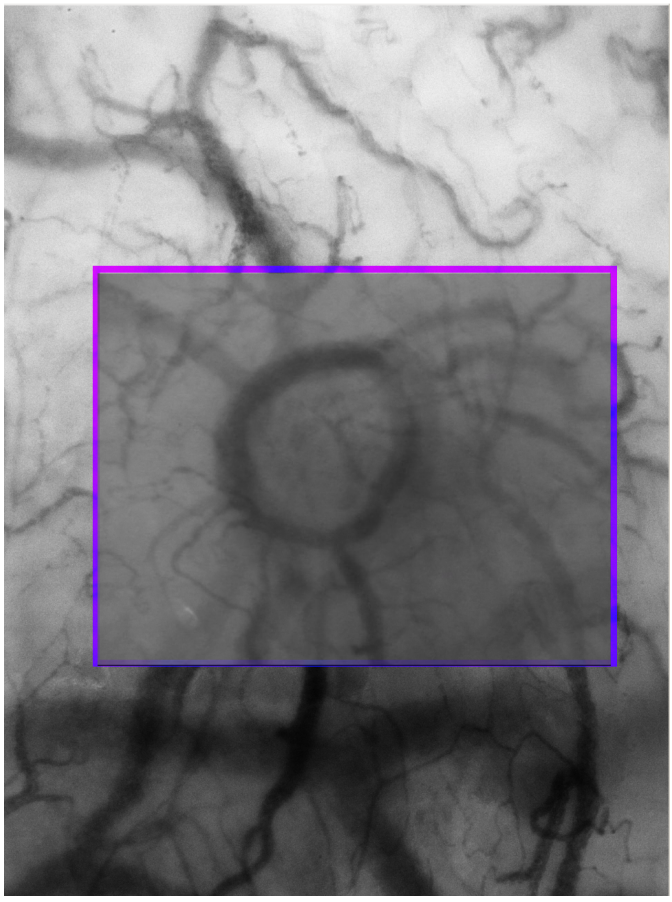


Figure 1: Smaller SDF image in larger Cytocam-IDF image: This figure shows the field of view of SDF and Cytocam-IDF imaging superimposed on each other showing the larger field of view offered by the larger image sensor used by the later technique.

connected to a device controller based on a powerful medical grade computer that is used for image storage and analysis. The device controller includes a camera adapter with a dedicated microprocessor for controlling the camera. Additionally, the camera adapter enables high-speed data transfer between the camera and controller. The Cytocam-IDF imaging device is supplied with an analysis application for quantification of microcirculatory parameters. The digitally recorded images can be analyzed automatically. It is also possible to analyze the recorded files off-line, as we did for this study. A novel feature of the device is its quantitative focusing mechanism, using a piezo linear motor with an integrated distance measuring system to position the sensor within 2 microns. Investigation has shown that each person has a characteristic depth of focus, which allows serial measurements to be made by pre-setting the characteristic focused depth. (21)

Protocol

Comparison of microcirculatory parameters:

The volunteers were evaluated in a supine, 30-degree head-up position. Room temperature was kept between 19 and 220 C. Demographic data (age, gender, weight and length), blood pressure, and heart rate were recorded. Blood pressure was measured non-invasively, heart rate was recorded by plethysmography. The microvascular measurements were obtained as a single measurement in the sublingual mucosa in three different areas with SDF and Cytocam-IDF imaging without special preparation of the mouth. The probe was handheld and adjusted by experienced operators (GA and GV) to obtain optimal image quality. With the SDF technique, after adequate focus and contrast adjustment, steady images of at least 15 seconds were acquired and recorded on a digital videotape (Sony Video Walkman GV-D 1000E; Sony, Tokyo, Japan), which digitizes the analogue SDF images prior to video storage. The images were captured in representative AVI format video clips (SonyDVgate; Sony, Tokyo, Japan) to allow off-line computerized image analysis using specialized software we had previously developed for this purpose. (20)

To use the Cytocam-IDF imaging device, the optimal focus depth and contrast were first adjusted. Steady images of 6 seconds were acquired and computer stored. The image clips were exported for analysis using the same image analysis software used for the SDF images. (20) Analysis took into account the different magnification of the images by the two different techniques (4x magnification in Cytocam-IDF imaging versus 5x magnification in SDF imaging.

Analysis

Comparison of microcirculatory parameters:

The perfusion of a tissue depends on the number, distribution and diameters of the capillaries in combination with blood viscosity and driving pressure across the capillaries. There are two main hemodynamic principles governing how oxygen in red blood cells reaches the tissue cells, the first is the convection based on red blood cell flow, and the second is the diffusion distance oxygen must travel from the red blood cells in the capillaries to the parenchymal cells. (19) Convection is quantified by measurement of flow in the microvessels, and diffusion is quantified by the density of the perfused microvessels (functional capillary density).

Subsequent image analysis was performed using microvascular density (total or perfused vessel density) and microvascular perfusion (proportion of perfused

vessels and microcirculatory flow index) parameters in line with international consensus. (22) Software assisted analysis (AVA 3,0; Automated Vascular Analysis, Academic Medical Center, University of Amsterdam) was used on the images. (20) The analysis of the microvascular density was restricted to vessels with a diameter <20 micrometers.

The total vessel density (TVD; mm/mm2) was determined using the AVA software. A semi quantitative analysis previously validated (23) but assisted by the AVA software was performed in individual vessels that distinguished among no flow (0), intermittent flow (1), sluggish flow (2) and continuous flow (3). A value was assigned for each vessel. The overall score, called the microvascular flow index (MFI), is the average of the individual values. (24) The proportion of perfused vessels (PPV) was calculated as the number of vessels with flow values of 2 and 3 divided by the total number of vessels. Perfused vessel density (PVD) was determined as the total vessel density multiplied by the fraction of perfused vessels. (22) Analyses of all images was done offline and blinded to the investigators.

Sublingual microcirculatory image contrast and sharpness analysis:

In the same way we had compared OPS imaging to SDF imaging (14), we evaluated image contrast and sharpness using image analysis software (ImageJ ;developed at the US National Institutes of Health, www.nih.gov). 5 venules and 6 capillaries found in one sublingual location, measured sublingually by the two cameras, the capillary and venular contrast, sharpness and quality were calculated. To determine capillary contrast with respect to the surrounding tissue, cross-sectional grayscale histograms (grayscale value 0 corresponds to black, and 255 corresponds to white) were obtained. The lowest gray value in the capillaries (I min) and the highest gray value in the tissue left (I max, left) and right (I max, right) of the capillaries were measured. The increase of the maximum slope angles α left and α right of the slopes of the gray value at the capillary-tissue interfaces was calculated.

Statistical analysis

Statistical analyses were performed using SPSS statistical software(version 18/21, SPSS Inc., USA). The Kolmogorov-Smirnov test was used to test whether the data were distributed normally. After identifying a normal distribution, the density parameters were compared by the student's t test. As the perfusion parameters did not show a normal distribution, a nonparametric test (Mann-Whitney U) was used to compare these parameters. Data are presented as the mean and standard deviation unless otherwise specified. A p value < 0.05 was considered statistically significant.

Results

Baseline characteristics are presented in Table 1. Tests for normality showed that TVD and PVD had normal distribution. The vascular density parameters TVD and PVD were significantly higher with Cytocam-IDF imaging than with SDF imaging (TVD-Cytocam-IDF: 21.60 mm/mm² ± 4.30 mm/mm² vs TVD-SDF: 16.35 mm/mm² ± 2.78 mm/mm², p<0,0001 and PVD- Cytocam-IDF: 21.50 mm/mm² ± 4,38 mm/mm² vs PVD-SDF: 16.24 mm/mm² ± 2.81 mm/mm², p<0,0001). Boxplots are presented in Figure 3. The perfusion parameters MFI and PPV did not differ significantly between the two techniques (Table 2), and the Bland-Altman plot showed no clinically significant bias. Bland-Altman plots are included in the appendix.

Sublingual microcirculatory image contrast and sharpness analysis.

Cytocam-IDF image quality from the sublingual area is significantly better in the capillaries and venules than the SDF image quality. (Table 3) The quality score was obtained based on contrast and sharpness, both of which are significantly improved with the Cytocam-IDF imaging in both capillaries and venules.

Table 1: baseline caracteristics.

	n=25
Age, years	33 [27.5-46.0]
Gender, male n	8
Systolic blood pressure, mm Hg	130 [119-141]
Diastolic blood pressure, mm Hg	74 [66-87]
Mean arterial blood pressure, mmHg	92 [84-102]
Heartrate, beats per minute	70 [65-77]
Weight, kg	73 [63-78]
Height, cm	173 [170-180]

Table 2: microcirculatory parameters

	Cytocam	SDF	p
MFI small	3.0 [3.0-3.0]	3.0 [2.96-3.00]	0.289
MFI large	3.0 [3.0-3.0]	3.0 [2.96-3.00]	0.494
TVD, mm/mm²	21.60 ± 4.30	16.35 ± 2,78	< 0.0001 *
PPV, %	100 [99-100]	99 [99-100]	0.368
PVD, mm/mm²	21.50 ± 4.38	16.24 ± 2.81	< 0.0001 *

*MFI small: < 20 µm; MFI large: > 20 µm. * p < 0.05 is the cut off value for statistical significance.*

3

Table 3: results from contrast and sharpness analysis.

	Modality	Contrast	Sharpness	Quality
Capillaries	SDF	0.04 ± 0.01	0.78 ± 0.06	0.03 +/- 0.01
	Cytocam	0.99 ± 0.03	0.90 ± 0.05	0.09 +/- 0.03
		p= 0.004 *	p=0.003 *	p=0.005 *
Venules	SDF	0.07 ± 0.03	0.82 ± 0.07	0.06 +/- 0.03
	Cytocam	0.12 ± 0.03	0.88 ± 0.05	0,11 +/- 0.03
		p= 0.037 *	p= 0.144 *	p=0.037 *

** p < 0.05 is the cut off value for statistical significance.*

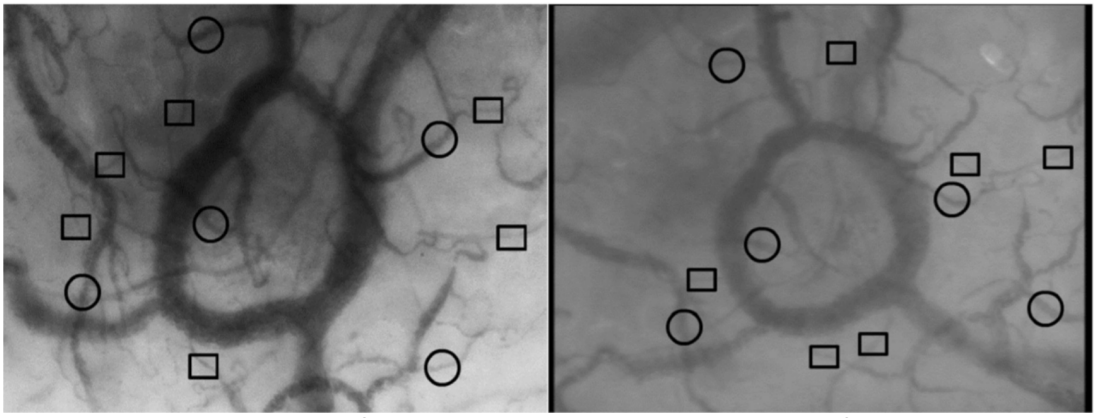


Figure 2: Histogram points taken for analyses; square capillary; circle venule; left Cytocam-IDF imaging; right SDF-imaging.

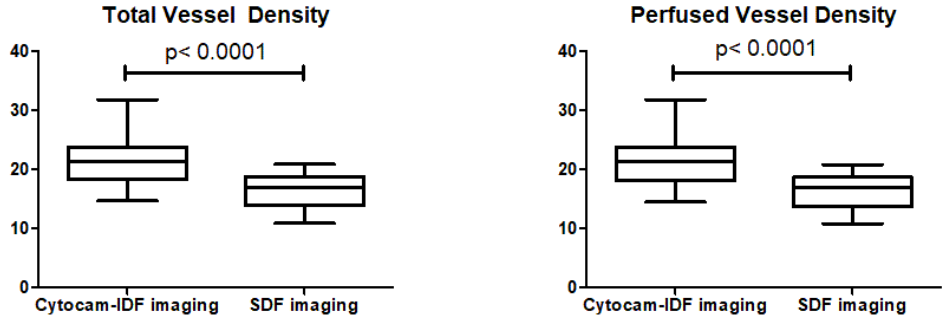


Figure 3: Boxplots of Total Vessel Density and Perfused Vessel Density.

Discussion

In this study, we validated Cytocam-IDF imaging, a third generation novel lightweight computer-controlled imaging sensor-based hand-held microscope, by comparing it to a second generation device, SDF imaging. Our results showed that Cytocam-IDF imaging visualized more (30%) micro vessels as quantified by measurement of total vascular density parameters in the sublingual microcirculation than did SDF imaging. In addition our study showed that Cytocam-IDF imaging provided improved image quality with respect to SDF imaging in terms of contrast and image sharpness, Similar results were found in a recent different preliminary validation study comparing Cytocam-IDF imaging to SDF imaging in neonates. (25)

It is likely that the significantly higher vascular density, as observed with the new Cytocam-IDF technique in comparison to SDF imaging is the direct result of the observed increase in contrast and sharpness, due to the improved magnification lens and high resolution sensor in combination with more precise quantitative focusing. Furthermore, since the new device is fully digitalized there is no loss of image quality in the conversion process from analogue to digital. Analogue cameras have the disadvantage of alternatively scanning odd and even video lines, resulting in a loss of resolution in the time domain as is the case in the SDF-camera. An alternative explanation could be the reduction in pressure artefacts in the lighter Cytocam-IDF device in comparison to the much heavier SDF device resulting in compressed microvessels becoming now more visible. We think, however that this is unlikely since pressure artefacts are mainly reflected in a reduction in red blood cell velocity characteristically in the larger, venular, vessels. Since this study was performed in healthy individuals, such flow abnormalities should be absent, as is the case in the observations in both systems. Therefore, it's seems likely that the observed difference in capillary density is not related to reduction pressure artefacts.

This conclusion is in line with the second important observation of the study, the absence of difference in variables of red blood cell velocity, such as MFI and PPV. Previous publications report a 'normal' MFI in healthy volunteers, equal or close to 3, and a PPV close to 100%. (4,5,26,27) Therefore, the absence of difference in MFI and PPV between the two methods can be considered as an important quality check of the observations found in the present study.

Although the visualisation of vessels with the new device is based on the same physical principal of indirect background illumination applied in all such devices, there are clearly new features with relevance to the development of research in

this field. The high resolution sensor combined with lenses especially made for microcirculatory imaging makes the optical resolution higher than the SDF system. As a result more capillaries become visible (Figure 2), with substantial implications for the observation of the microcirculation in several disease states. This also has the potential to observe smaller structures such as vessel wall abnormalities and possibly the endothelial glycocalyx. The new quantitative focusing mechanism, not only allows more precise focusing, but it also maintains optimal focusing depth throughout a measurement allowing multiple observations to be made over time without the need to refocus each time a measurement is made. Focus of on-going research, i.e. measurements for extended periods of time on one and the same spot, is currently not possible, especially in the non-sedated patient. As such the potential to maintain optimal focusing depth reduces the variability in observations. The potential for development of treatment based on the on-line analysis of the fully digitalized images and increase in frame rate are outside the scope of this article.

Two studies (4,27) have reported sublingual vascular density values in mm/mm^2 in volunteers using SDF imaging. Ours are in exact agreement with the values found by Edul et al who used similar AVA-software. However, SDF-derived TVD in our experiment is lower than in comparison to those found by Hubble et al. but this may be explained by a difference in software analysis (Capiscope, KK Technology, Axminster, UK). Agreement was found however between the three studies on the finding that in volunteers almost all vessels exhibit flow.

Clearly the results of this study are limited to healthy volunteers and further validation is needed in the clinical setting. However, heterogeneity of blood flow within the catchment area of the device has now been recognized as a key characteristic in many disease states. (5) To this end the consensus paper decided to obtain three to five video clips per observation, and report the average of the variables. (28) It was key to our experiment to exclude this heterogeneity.

Secondly, our data do not deal with the potential influence of intra-observer variability. Although a substantial variability has been reported in the jejunal mucosal microcirculation of pigs (29), multiple studies have confirmed the excellent reproducibility in the human sublingual area. (4,5,26)

Conclusion

In this study, we validated a third generation novel lightweight computer-controlled imaging sensor-based hand-held microscope called the Cytocam-IDF imaging by

comparing it to a second generation device, SDF imaging. Our results showed that Cytocam-IDF imaging was able to detect more capillaries in terms of density and provided improved quality image in the sublingual microcirculation. Considering the improved image quality along with its light weight and ability to automatically analyze images, we expect it to contribute to the clinical assessment of microcirculation alterations in various clinical scenarios.

Conflict of interest statement

Dr Ince has developed SDF imaging and is listed as inventor on related patents commercialized by MicroVision Medical (MVM) under a license from the Academic Medical Center (AMC). He has been a consultant for MVM in the past, but has not been involved with this company for more than five years now, except that he still holds shares. Braedius Medical, a company owned by a relative of Dr Ince, has developed and designed a hand-held microscope called CytoCam-IDF imaging. Dr Ince has no financial relation with Braedius Medical of any sort, i.e., never owned shares, or received consultancy or speaker fees from Braedius Medical. The other authors have no conflict of interest.

Authors' contributions

GA and GV coordinated the study, performed the SDF and IDF imaging, performed analysis and participated in the draft of the manuscript. CI and CB participated in the design of the study and the draft of the manuscript.

3 References

1. D. De Backer, K. Donadello, Y. Sakr, G. Ospina-Tascon, D. Salgado, S. Scolletta. Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. Crit Care Med. 2013;41(3):791-9.

2. C. Ince. The microcirculation is the motor of sepsis. Crit Care. 2005;9 Suppl 4:S13-9.

3. A.P.C. Top, C. Ince, N. de Meij, M. van Dijk, D. Tibboel. Persistent low microcirculatory vessel density in nonsurvivors of sepsis in pediatric intensive care. Crit Care Med. 2011;39(1):8-13.

4. V.S.K. Edul, C. Enrico, B. Laviolle, A.R. Vazquez, C. Ince, A. Dubin. Quantitative assessment of the microcirculation in healthy volunteers and in patients with septic shock. Crit Care Med. 2012;40(5):1443-8.

5. S. Trzeciak, R.P. Dellinger, J.E. Parrillo, M. Guglielmi, J. Bajaj, N.L. Abate. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Ann Emerg Med. 2007;49(1):88-98, 98.e1-2.

6. Krahl VE (1962). Observations on the pulmonary alveolus and its capillary circulation in the living rabbit. Anat Rec 142: 350. Anat Rec. 1962;(142):350.

7. H. Sherman, S. Klausner, W.A. Cook. Incident dark-field illumination: a new method for microcirculatory study. Angiology. 1971;22(5):295-303.

8. D.W. Slaaf, G.J. Tangelder, R.S. Reneman, K. Jäger, A. Bollinger. A versatile incident illuminator for intravital microscopy. Int J Microcirc Clin Exp. 1987;6(4):391-7.

9. W. Groner, J.W. Winkelman, A.G. Harris, C. Ince, G.J. Bouma, K. Messmer. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. Nat Med. 1999;5(10):1209-12.

10. K.R. Mathura, K.C. Vollebregt, K. Boer, J.C. De Graaff, D.T. Ubbink, C. Ince. Comparison of OPS imaging and conventional capillary microscopy to study the human microcirculation. J Appl Physiol. 2001;91(1):74-8.

11. K.R. Mathura, G.J. Bouma, C. Ince. Abnormal microcirculation in brain tumours during surgery. Lancet. 2001;358(9294):1698-9.

12. D. De Backer, J. Creteur, J.C. Preiser, M.J. Dubois, J.L. Vincent. Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med. 2002;166(1):98-104.

13. P.E. Spronk, C. Ince, M.J. Gardien, K.R. Mathura, H.M. Oudemans-van Straaten, D.F. Zandstra. Nitroglycerin in septic shock after intravascular volume resuscitation. Lancet. 2002;360(9343):1395-6.

14. P.T. Goedhart, M. Khalilzada, R. Bezemer, J. Merza, C. Ince. Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. Optics express. 2007;15(23):15101-14.

15. L. Dababneh, F. Cikach, L. Alkukhun, R.A. Dweik, A.R. Tonelli. Sublingual microcirculation in pulmonary arterial hypertension. Annals of the American Thoracic Society. 2014;11(4):504-12.

16. E.G. Mik, T. Johannes, M. Fries. Clinical microvascular monitoring: a bright future without a future? Crit Care Med. 2009;37(11):2980-1.

17. M. Sallisalmi, N. Oksala, V. Pettilä, J. Tenhunen. Evaluation of sublingual microcirculatory blood flow in the critically ill. Acta Anaesthesiol Scand. 2012;56(3):298-306.

18. R. Bezemer, S.A. Bartels, J. Bakker, C. Ince. Clinical review: Clinical imaging of the sublingual microcirculation in the critically ill - where do we stand? Crit Care. 2012;16(3):224.

19. C. Ince. The rationale for microcirculatory guided fluid therapy. Curr Opin Crit Care. 2014;20(3):301-8.

20. J.G.G. Dobbe, G.J. Streekstra, B. Atasever, R. van Zijderveld, C. Ince. Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. Med Biol Eng Comput. 2008;46(7):659-70.

21. D. Milstein, E. Romay, C. Ince. A novel computer-controlled high resolution video microscopy imaging system enables measuring mucosal subsurface focal depth for rapid acquisition of oral microcirculation video images. Intensive Care Med. 2012;(38):S271.

22. D. De Backer, S. Hollenberg, C. Boerma, P. Goedhart, G. Büchele, G. Ospina-Tascon. How to evaluate the microcirculation: report of a round table conference. Critical Care. 2007;11(5):R101-.

23. A. Dubin, M.O. Pozo, G. Ferrara, G. Murias, E. Martins, C. Canullán. Systemic and microcirculatory responses to progressive hemorrhage. Intensive Care Med. 2009;35(3):556-64.

24. E.C. Boerma, K.R. Mathura, P.H.J. van der Voort, P.E. Spronk, C. Ince. Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. Crit Care. 2005;9(6):R601-6.

25. H. van Elteren, V. van den Berg, R. de Jonge, C. Ince, I. Reiss. Cutaneous microcirculation in preterm neonates: comparison between sidestream darkfield (SDF) and incident darkfield (IDF) imaging. Pediatr Crit Care Med. 2014;15(4):130-131.

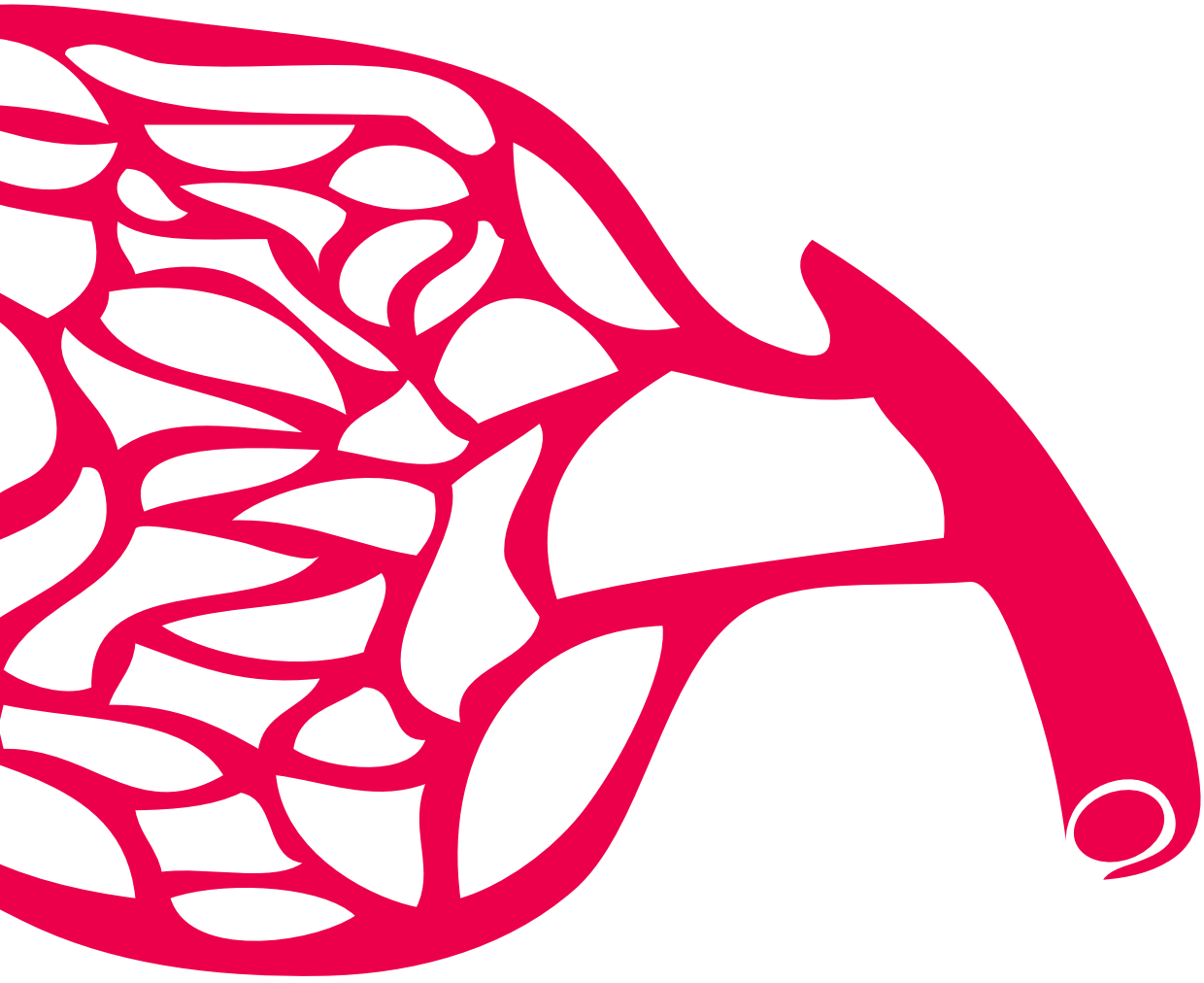
26. D. De Backer, J. Creteur, M.J. Dubois, Y. Sakr, J.L. Vincent. Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. Am Heart J. 2004;147(1):91-9.

27. S.M.A. Hubble, H.L. Kyte, K. Gooding, A.C. Shore. Variability in sublingual microvessel density and flow measurements in healthy volunteers. Microcirculation. 2009;16(2):183-91.

28. D. De Backer, S. Hollenberg, C. Boerma, P. Goedhart, G. Büchele, G. Ospina-Tascon. How to evaluate the microcirculation: report of a round table conference. Crit Care. 2007;11(5):R101.

29. H. Bracht, V. Krejci, L. Hiltebrand, S. Brandt, G. Sigurdsson, S.Z. Ali. Orthogonal polarization spectroscopy to detect mesenteric hypoperfusion. Intensive Care Med. 2008;34(10):1883-90.

3



CHAPTER 4

MICROCIRCULATORY PERFUSION DERANGEMENTS DURING CONTINUOUS HEMOFILTRATION WITH FIXED DOSE OF ULTRAFILTRATION IN STABILIZED ICU PATIENTS.

G. Veenstra, B. Scheenstra, M . Koopmans, W.P. Kingma, H. Buter, E.C. Boerma.

Department of Intensive Care, Medical Centre Leeuwarden,
PO Box 888, 8901BR Leeuwarden, The Netherlands.

In: Journal of Critical Care 29 (2014) 478–481

4 Abstract

Introduction

Acute kidney injury (AKI) is a well-known complication critically-ill patients. Little is known about timing and ultrafiltration dose after initial resuscitation. In-vivo microscopy of the microcirculation has been suggested as alternative for the assessment of volume status. Previous studies contribute to the understanding that intravascular hypovolaemia is reflected by microcirculatory blood flow changes, not detected by conventional methods. The aim of our study was to assess microcirculatory blood flow changes during negative fluid balance ultrafiltration in patients with oliguric AKI.

Materials and methods

Patients with oliguric AKI on renal replacement therapy (RRT) were included after haemodynamic stabilisation. Target was a predefined negative fluid balance; subsequently, a stepwise decrease in amount of substitution fluid was achieved. The data were recorded at baseline and after each change.

Results

15 patients were included in the study. Microcirculatory blood flow index did not change significantly between baseline and endpoint (2.90 [2.87-3.00] vs. 2.90 [2.75-3.00], p=0.57). During treatment, heart rate decreased from 96 bpm [80-111 bpm] to 94 bpm [79-110 bpm], p=0.01, without significant change in mean arterial blood pressure (80 mmHg [68-95 mmHg] vs. 79 mmHg [65-91 mmHg], p=0.5).

Conclusion

Microcirculatory blood flow is not altered by reduced substitution during RRT.

Introduction

Acute kidney injury (AKI) is a recognised complication in patients admitted to the ICU; 6% of ICU patients will develop oliguric kidney failure. The mortality rate increases up to 60% in this group, with an increased risk for chronic kidney disease. (1,2) Oliguric AKI poses several challenges to the ICU physician. Althought still subject to debate, there seems to be consensus to some extent about the initial timing, dose and type of RRT in the acute phase of AKI. However, little is known about the timing and the dose of ultrafiltration dose after the initial sepsis resuscitation. (3-5)

This observation is noteworthy because optimal fluid management appears to be relevant. Although fluid therapy remains an important cornerstone in sepsis treatment, fluid overload is clearly an independent risk factor for morbidity and mortality. More importantly, an independent association exists between negative fluid balance and decreased 90-day mortality. (6-11) However, in the clinical setting, assessing the balance between the ongoing need for fluid therapy and fluid overload is difficult. Practical guidelines concerning when and how to reduce positive fluid balances seem to be lacking. In the last decade, in vivo microscopy of microcirculatory blood flow has been suggested to be an alternative modality for assessing volume status. In an animal model of haemorrhagic shock, the microcirculatory blood flow tracked progressive blood loss, whereas the heart rate and blood pressure changes occurred only in the late phase of shock. (12) In the human setting of fluid resuscitation, both passive leg raising and intravascular volume expansion were associated with increased microcirculatory blood flow during septic shock. (13) In the setting of chronic renal failure, rapid changes in volume status during haemodialysis with ultrafiltration were detected by significant changes in microcirculatory blood flow; however, these rapid volume status changes were not sensed by standard macrohaemodynamic variables. (14) Pranskunas et al not only linked improved microvascular blood flow to fluid therapy but also established its relationship with attenuated clinical signs of impaired organ perfusion.(15) These studies contribute to the understanding that intravascular hypovolaemia can reflect microcirculatory blood flow changes, which are not sensed by conventional methods and that the technique can track rapid changes. (16)

The aim of the present study was to assess potential changes in microcirculatory blood flow during the beginning of negative fluid balance ultrafiltration after the initial stabilisation of shock patients with oliguric AKI. We hypothesised that mild negative fluid balance in this patient group is not associated with impaired microcirculatory blood flow.

4 Materials and Methods

Patients

This single centre, prospective, observational study was approved by the medical ethics committee (Medical Centre Leeuwarden, The Netherlands) and registered with ClinicalTrials.gov number NTC 01362088.

The patients with oliguric kidney failure who were treated with renal replacement therapy after haemodynamic stabilisation were eligible for the study. Haemodynamic stabilisation was defined as the absence of the need for fluid therapy or positive fluid balance, a mean arterial blood pressure (MAP) >60 mmHg, a central/mixed venous oxygen saturation >65%, normal lactate levels, and stable or decreased inotropic dosage.

The inclusion criteria were patients above 18 years of age, informed consent and a clinical status indicative of negative fluid balance. The exclusion criteria included the inability to obtain SDF images, such as maxillofacial surgery.

Protocol

Before the start of the protocol, the attending physician determined the need for and the amount of negative fluid balance for the next 12 hours. Subsequently, a stepwise decrease in the amount of substitution was achieved (Figure 1). Each step lasted 1 hour. SDF imaging of the sublingual area was obtained after each substitution step. At the end of the protocol, the patient was placed in the Trendelenburg position for several minutes, and simultaneous SDF imaging was performed.

Renal Replacement Therapy

All of the patients were treated using continuous venovenous haemofiltration (multiFiltrate, Fresenius Medical Care, Bad Homburg, Germany), equipped with a Nipro UF-205 dialyser (Nipro Corporation, Osaka, Japan). During treatment, the blood flow was standardised at 200 ml/min using an ultrafiltration flow at 3000 ml/h. The total amount of post-filter buffered substitution solution (SH 44 HEP or SH 53, Dirinco, Rosmalen, Netherlands) was adjusted according to the preset net fluid balance. The regional anticoagulation was achieved using trisodium citrate.

Microvascular imaging and Analysis

SDF imaging, which is a stroboscopic light-emitting diode, ring-based imaging modality, is incorporated in a handheld device. SDF imaging has been validated for

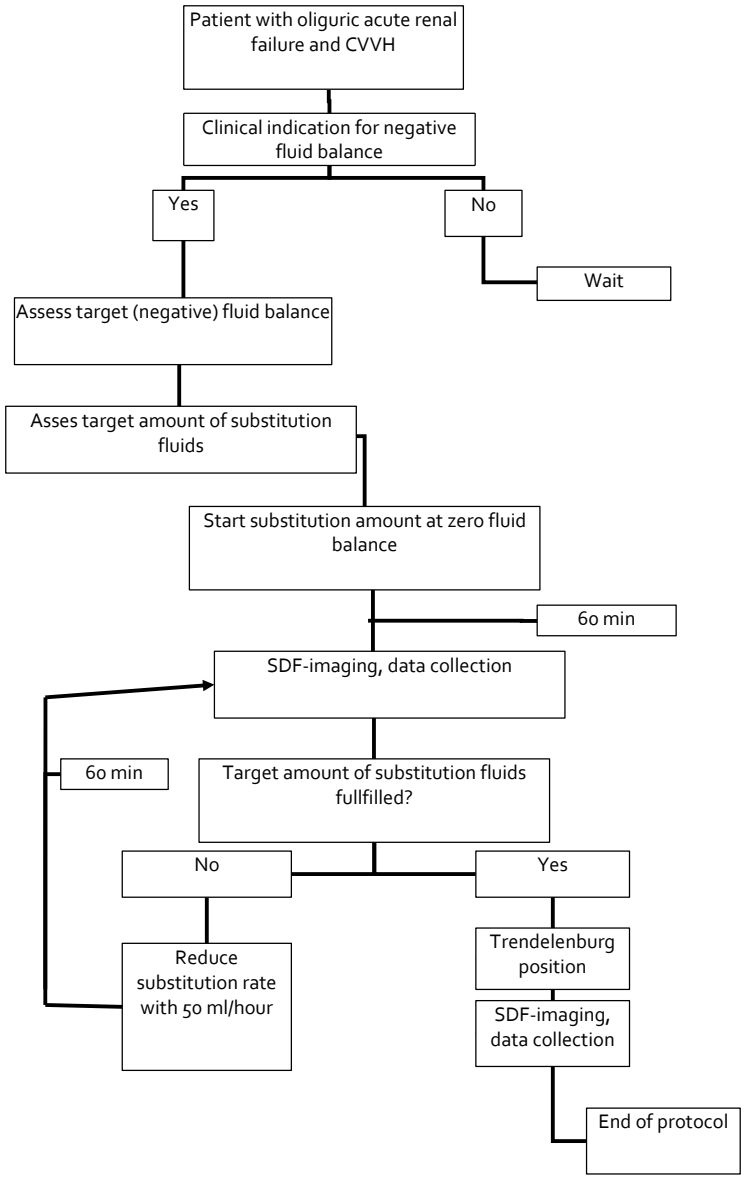


Figure 1: protocol

clinically observing the microcirculation. If a wavelength within the haemoglobin absorptionspectrum(e.g.,530nm)ischosen,redbloodcellswillappeardark.SDFimages are obtained from three different regions of the sublingual microcirculation. (17,18) Clips were acquired and stored using a digital videotape (Video Walkman GV-D 1000E, Sony, Tokyo, Japan). Subsequently, the images were captured in 5 to 10 sec representative AVI format video clips (SonyDVgate, Sony, Tokyo, Japan).

The images were randomly presented to prevent interimage coupling. Off-line analysis was performed using the AVA 3.0 software package (MicroVision Medical, Amsterdam, The Netherlands) in compliance with the recommendations of a roundtable conference. (19)

Data

The following data were recorded at baseline: general characteristics, severity of illness according to Acute Physiology and Chronic Health Evaluation (APACHE) IV and Sequential Organ Failure Assessment (SOFA) scores, calculated over the first 24 h after ICU admission. Macro haemodynamic variables, SDF images, central venous oxygen saturation and arterial blood gases were recorded at baseline and after each substitution step.

The primary outcome included changes in microvascular flow index (MFI) between zero-balance ultrafiltration and maximum negative fluid balance substitution rate. The secondary outcomes included changes in blood pressure, heart rate, delta T (difference between skin temperature and core temperature) and total vessel density (TVD).

Statistics

Based on previous observations, we anticipated a mean MFI at baseline of 3 with a standard deviation (SD) of 0.2. (20) We calculated a sample size of 14 patients to detect an absolute difference of 0.4 in MFI in MFI in a single-sided test with a 0.05 type I error and an 80% probability. SPSS (version 18/21, SPSS Inc., USA) was used for statistical analysis. The data are presented as the median and interquartile range [IQR]. Due to the small sample sizes, comparisons between baseline and endpoint were restricted to non-parametric tests for paired data (Wilcoxon test).

Result

Patients

In a 13-month period, 15 patients were included in the study. The baseline characteristics are presented in Table 1. In one patient, the protocol was stopped prematurely because of hypotension and, because one patient could not endure the Trendelenburg position; both patients are included in the data analysis. During the analysis, one patient was excluded because of poor image quality.

During the protocol, the median targeted fluid balance was -0.5 l, whereas the fluid

balance achieved was -0.9 l (Table 2). Eleven (79%) of fourteen patients completed the protocol after 2 hours (maximum=4 hours).

Primary outcome

The primary outcome of the study was the observed difference between the microcirculatory blood flow before and after target reduction of substitution flow with CVVH. The MFI did not change significantly between the baseline MFI value and the endpoint in the small vessels (2.90 [2.87-3.00] vs. 2.90 [2.75-3.00], respectively, p=0.57) (Figure 2). After being placed in the Trendelenburg position, the patients MFI values did not differ in small vessels (Table 3).

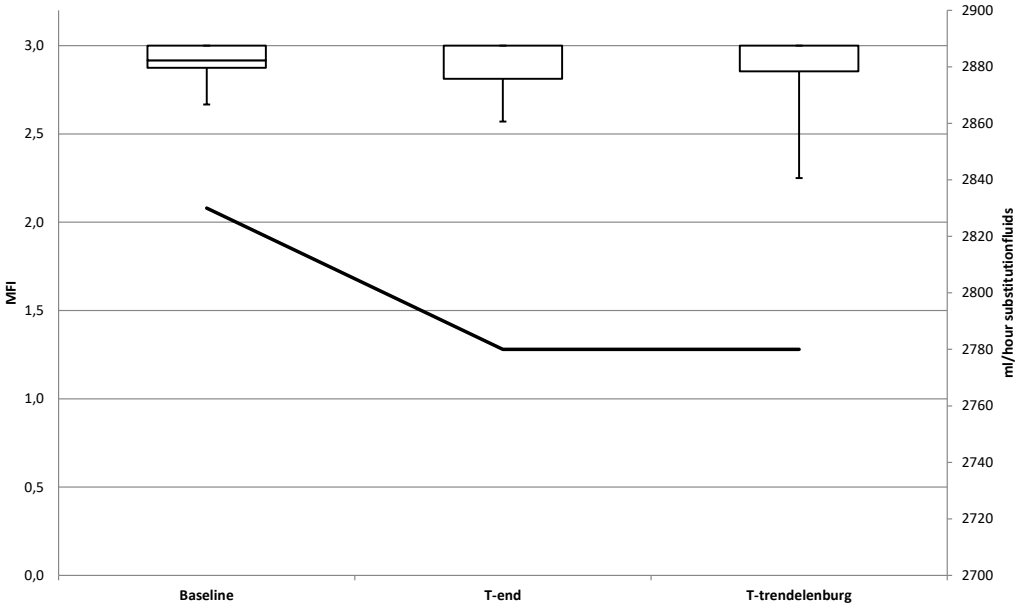


Figure 2: Microcirculation flow index during target reduction of substitution flow.

Secondary outcome

During treatment, the heart rate decreased from 96 bpm [80-111 bpm] to 94 bpm [79-110 bpm], p=0.01, without a significant change in mean arterial blood pressure (MAP) (80 mmHg [68-95 mmHg] vs. 79 mmHg [65-91 mmHg], respectively, p=0.5). Additionally, the central venous saturation was 69% [67-77%] at baseline and did not change over time (70% [66-77%], p=0.95). Interestingly, the paO_2/FiO_2 ratio (P/F) improved during the protocol (Table 3). The haematocrit and lactate levels remained unchanged (Table 4).

Table 1: baseline characteristics.

	n=14
Age, years	76 [69-79]
Gender, male/female, n	11/3
Reason for ICU admittance, n (%)	
-Sepsis	8 (57%)
-Cardiogenic shock after cardiac surgery	4 (29%)
-Other	2 (14%)
APACHE II	26 [20-29]
APACHE IV	93 [46-107]
SOFA (Sequential Organ Failure Assessment)	12 [10-13]
Hospital mortality (%)	25%
Mechanical ventilation, n (%)	14 (100%)
Number of patients on vasopressors, n (%)	6 (43%)
Number of patients on inotropics, n (%)	1 (7%)
Creatinine before admittance, mmol/l	86 [76-135]
Diuresis at start protocol, hourly, ml	0.0 [0.0-11.25]
Creatinine at start protocol, mmol/l	125 [71-173]
Urea at start protocol, mmol/l	9.1 [8.0-13.8]
Length of stay ICU, days	15 [10-28]
Length of stay hospital, days	26 [14-32]
ICU-treatment before inclusion, days	5 [3-7]
Renal replacement therapy, days	7 [6-12]
Developed chronic kidney failure, n (%)	2 (14%)

Table 2: fluid balances.

Cumulative fluid balance prior to start protocol, l.	8.5 [6.1-12.1]
Fluid balance 24 hours prior to start protocol, l.	0.4 [-0.2-1.0]
Targeted fluid balance for the first 12 hours of protocol, l.	-0.5 [-0.5- -0.5]
Achieved fluid balance for the first 12 hours of protocol, l.	-0.9 [-1.2- -0.2]

Table 3: macro and microvascular variables.

	Baseline	T-end	p
Heart rate, bpm	96 [80-111]	94 [79-110]	0.01*
Blood pressure syst., mmHg	127 [103-145]	122 [97-145]	0.10
Blood pressure, dia., mmHg	62 [50-71]	59 [49-64]	0.12
Blood pressure, mean, mmHg	80 [68-95]	79 [65-91]	0.60
Norepinephrine, mcg/kg/min	0.08 [0.03-0.12]	0.08 [0.04-0.1]	0.32
ScVO2 %	69 [67-77]	70 [66-77]	0.95
Delta temperature, °C	4.4 [3.2-5.2]	4.4 [2.95-5.6]	0.35
P/F ratio	261 [205-359]	285 [207-370]	0.02*
CVP, mmHg	13 [10-16]	11 [7-14]	0.05
MFI small vessels	2.90 [2.87-3.00]	2.90 [2.75-3.00]	0.57
MFI large vessels	3.00 [3.00-3.00]	2.96 [2.98-3.00]	0.27
TVD small vessels	18.44 [15.76-21.53]	19.46 [16.80-22.39]	0.08
De Backer, small vessels	12.26 [10.21-13.66]	12.92 [11.07-14.26]	0.10
PPV small vessels	0.98 [0.97-1.00]	0.98 [0.98-0.99]	0.50
PVD small vessels	18.10 [15.31-20.86]	19.08 [16.53-21.67]	0.14
Heterogeneity score (%)	8,7 [0.0-13.1]	0 [0.0-20.5]	

*ScVO2: central venous saturation; P/F ratio: PaO2 to FiO2 ratio; CVP: Central Venous Pressure; MFI: Microvasculair Flow Index; TVD: Total Vessel Density; PPV: percentage perfused vessels; PVD: perfused vessel density. * p< 0.05.*

Table 4: laboratory results.

	Baseline	T-end	p
Hemoglobin, mmol/l	5.7 [5.4-6.0]	5.7 [5.5-6.0]	0.76
Hematocrit, %	29 [27-30]	28 [27-29]	1.00
Glucose, mmol/l	7.7 [6.3-9.2]	7.0 [5.7-7.9]	0.44
Sodium, mmol/l	137 [136-138]	137 [135-138]	0.31
Potassium, mmol/l	4.0 [3.7-4.3]	4.0 [3.9-4.2]	0.34
Chloride, mmol/l	108 [107-110]	108 [107-109]	0.11
Lactate, mmol/l	1.6 [1.2-1.9]	1.5 [1.0-2.2]	0.55

4 Discussion

The primary finding of this study was that we observed no change in microcirculatory blood flow, using sublingual in vivo microscopy, during negative fluid balance ultrafiltration in stabilised ICU patients. Moreover, macrohaemodynamic variables, such as blood pressure, heart rate and ScVO2, remained unaltered. These data suggest that after stabilisation of the primary shock insult, a net negative fluid balance may be achieved, without reduced organ perfusion or heart rate acceleration.

At first glance, our result may not be consistent with the findings in the recent literature. During progressive haemorrhage in an animal model, the microcirculatory flow alterations were consistent with the amount of blood loss. (12) Others have observed that in a lower body negative pressure model of hypovolaemia, there was a significant decrease in microvascular flow and capillary density. (21) In stable patients with chronic renal failure, ultrafiltration was associated with profound microcirculatory flow alterations and could be instantaneously reversed in the Trendelenburg position. (14) Moreover, others have demonstrated that swift changes in volume status during septic shock were closely related to sublingual microcirculatory flow alterations. (13,15)

In our study, a step-wise increase in ultrafiltration with a net negative fluid balance was not accompanied by microvasculatory flow changes; this observation may have alternative explanations. Whereas in the previous literature, the different models were indicative of substantial hypovolaemia, our patients may have been hypervolaemic at the start of the study. Because fluid resuscitation was combined with increased vascular permeability, all of the patients had a net positive fluid balance during the days before the study and showed signs of tissue oedema. The fact that ultrafiltration was not associated with flow changes at the capillary level may indicate that extravascular fluids were recruited to the vascular system, thereby preventing hypovolaemia. The reduced heart rate and the increased P/F ratio, as markers of pulmonary oedema, and the absence of change in haematocrit, are consistent with this view. An alternative reason for the absence of microcirculatory flow alterations during ultrafiltration in our study might be hidden in the dosage and in the timing of the amount of ultrafiltrate. It seems plausible that when the targeted fluid balance is more negative, the microvascular flow alterations become present. In the earlier stage of shock, a net fluid exchange from the interstitium to the intravascular space may be more difficult because of endothelial dysfunction. In this case, a negative fluid balance will presumably result in intravascular hypovolaemia.

The amount and timeline of the ultrafiltration are within the generally accepted ICU practice. However, it is conceivable that an earlier start of ultrafiltration or a more rigorous amount may be possible without compromising organ perfusion. Strict control of fluid balance seems important because a positive fluid balance is associated with an increased mortality and incidence of AKI. (10,22) Future studies are needed to determine the optimal timing and dosing of ultrafiltration in acutely ill patients with renal replacement therapy. Direct in vivo observation of the microcirculation may be a valuable tool for assessing organ perfusion at the bedside during such intervention.

We acknowledge several limitations of the study. In this pilot study, the number of patients is limited. Therefore, we cannot exclude smaller changes than 0.4 AU in microvascular blood flow during ultrafiltration, according to the power calculation. Second, the range in the amount of ultrafiltration is limited and the changes in capillary flow may occur outside the scope of this range. Third, the microvascular flow alterations are conceivably present in other vascular beds than in the observed sublingual area. (15,23) The data on the abdominal pressure were not recorded.

Conclusions

The target reduction of substitution flow with CVVH was not associated with a change in microcirculatory blood flow during negative fluid balance ultrafiltration in stabilised ICU patients.

4 References

1. Uchino S, Kellum JA, Bellomo R et al. Acute renal failure in critically ill patients: a multinational, multicenter study. JAMA. 2005;294(7):813-8.

2. Friedrich JO, Wald R, Bagshaw SM et al. Hemofiltration compared to hemodialysis for acute kidney injury: systematic review and meta-analysis. Crit Care. 2012;16(4):R146.

3. Ricci Z, Ronco C. New insights in acute kidney failure in the critically ill. Swiss medical weekly. 2012;142:w13662.

4. Ricci Z, Ronco C. Renal replacement therapy in the critically ill: getting it right. Curr Opin Crit Care. 2012;18(6):607-12.

5. Ricci Z, Polito A, Polito A et al. The implications and management of septic acute kidney injury. Nature reviews. Nephrology. 2011;7(4):218-25.

6. Licari E, Calzavacca P, Ronco C et al. Fluid resuscitation and the septic kidney: the evidence. Contrib Nephrol. 2007;156:167-77.

7. Van Biesen W, Yegenaga I, Vanholder R et al. Relationship between fluid status and its management on acute renal failure (ARF) in intensive care unit (ICU) patients with sepsis: a prospective analysis. J Nephrol. 2005;18(1):54-60.

8. Dellinger RP, Carlet JM, Masur H et al. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. Crit Care Med. 2004;32(3):858-73.

9. Boyd JH, Forbes J, Nakada TA et al. Fluid resuscitation in septic shock: a positive fluid balance and elevated central venous pressure are associated with increased mortality. Crit Care Med. 2011;39(2):259-65.

10. Payen D, de Pont AC, Sakr Y et al. A positive fluid balance is associated with a worse outcome in patients with acute renal failure. Crit Care. 2008;12(3):R74.

11. RENAL Replacement Therapy Study Investigators, Bellomo R, Cass A et al. An observational study fluid balance and patient outcomes in the Randomized Evaluation of Normal vs. Augmented Level of Replacement Therapy trial. Crit Care Med. 2012;40(6):1753-60.

12. Dubin A, Pozo MO, Ferrara G et al. Systemic and microcirculatory responses to progressive hemorrhage. Intensive Care Med. 2009;35(3):556-64.

13. Pottecher J, Deruddre S, Teboul JL et al. Both passive leg raising and intravascular volume expansion improve sublingual microcirculatory perfusion in severe sepsis and septic shock patients. Intensive Care Med. 2010;36(11):1867-74.

14. Bemelmans RHH, Boerma EC, Barendregt J et al. Changes in the volume status of haemodialysis patients are reflected in sublingual microvascular perfusion. Nephrology Dialysis Transplantation. 2009;24(11):3487-92.

15. Pranskunas A, Koopmans M, Koetsier PM et al. Microcirculatory blood flow as a tool to select ICU patients eligible for fluid therapy. Intensive Care Med. 2012;

16. De Backer D, Donadello K, Taccone FS et al. Microcirculatory alterations: potential mechanisms and implications for therapy. Annals of intensive care. 2011;1(1):27.

17. Goedhart PT, Khalilzada M, Bezemer R et al. Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. Optics express. 2007;15(23):15101-14.

18. Boerma EC, Mathura KR, van der Voort PHJ et al. Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. Crit Care. 2005;9(6):R601-6.

19. De Backer D, Hollenberg S, Boerma C et al. How to evaluate the microcirculation: report of a round table conference. Critical Care. 2007;11(5):R101.

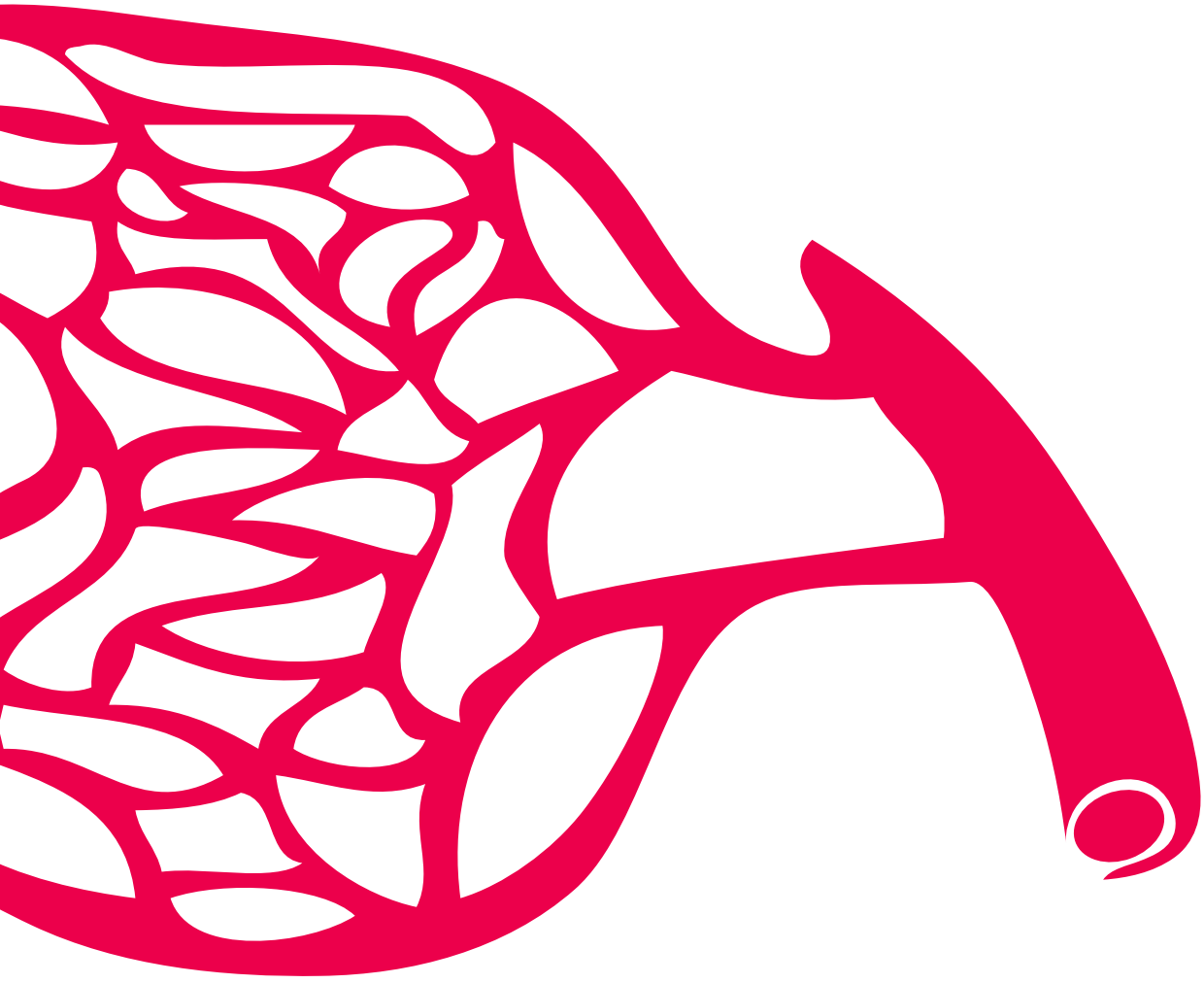
20. Trzeciak S, Dellinger RP, Parrillo JE et al. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Ann Emerg Med. 2007;49(1):88-98, 98.e1.

21. Bartels SA, Bezemer R, Milstein DMJ et al. The microcirculatory response to compensated hypovolemia in a lower body negative pressure model. Microvasc Res. 2011;

22. Teixeira C, Garzotto F, Piccinni P et al. Fluid balance and urine volume are independent predictors of mortality in acute kidney injury. Crit Care. 2013;17(1):R14.

23. Boerma EC, van der Voort PHJ, Spronk PE et al. Relationship between sublingual and intestinal microcirculatory perfusion in patients with abdominal sepsis. Crit Care Med. 2007;35(4):1055-60.

4



CHAPTER 5

ULTRAFILTRATION RATE IS AN IMPORTANT DETERMINANT OF MICROCIRCULATORY ALTERATIONS DURING CHRONIC RENAL REPLACEMENT THERAPY.

G. Veenstra[1,3,4], A. Pranskunas[2], I. Skarupskiene[2], V. Pilvinis[2], M.H. Hemmelder[1], C. Ince[3,4], E.C. Boerma[1].

1: Medical Center Leeuwarden, Leeuwarden, the Netherlands
2: Lithuanian University of Health Sciences, Kaunas, Lithuania.
3: Translational Physiology, Academic Medical Center, Amsterdam, the Netherlands.
4: Erasmus MC University Hospital Rotterdam, Rotterdam, The Netherlands.

In: BMC Nephrology (2017) 18:71

5 Abstract

Background

Hemodialysis (HD) with ultrafiltration (UF) in chronic renal replacement therapy is associated with hemodynamic instability, morbidity and mortality. Sublingual Sidestream Dark Field (SDF) imaging during HD revealed reductions in microcirculatory blood flow (MFI). This study aims to determine underlying mechanisms.

Methods

The study was performed in the Medical Centre Leeuwarden and the Lithuanian University of Health Sciences. Patients underwent 4-hours HD session with linear UF. Nine patients were subject to combinations of HD and UF: 4 hours of HD followed by 1 hour isolated UF and 4 hours HD with blood-volume-monitoring based UF. Primary endpoint: difference in MFI before and after intervention. During all sessions monitoring included blood pressure, heartrate and SDF-imaging. Trial registration number: NCT01396980.

Results

Baseline characteristics were not different between the two centres as within the HD/UF modalities. MFI was not different before and after HD with UF. Total UF did not differ between modalities. Median MFI decreased significantly during isolated UF [2.8(2.5-2.9) to 2.5(2.2-2.8), p=0.03]. Baseline MFI of each UF session was correlated with MFI after the intervention (r_s=0.52, p=0.006).

Conclusion

During HD with UF or isolated HD we observed no changes in MFI. This indicates that non-flow mediated mechanisms are of unimportance. During isolated UF we observed a reduction in MFI in conjunction with a negative intravascular fluid balance. The correlation between MFI before and after intervention suggests that volume status at baseline is a factor in microvascular alterations. In conclusion we observed a significant decrease of sublingual MFI, related to UF rate during chronic renal replacement therapy.

Background

Intermittent hemodialysis (HD) with concomitant ultrafiltration (UF) in chronic renal replacement therapy is associated with hemodynamic instability, usually referred to as 'intradialytic hypotension'. The incidence of this phenomenon ranges between 30-90% depending on the definition on clinically relevant intradialytic hypotension. This unfavourable condition is not only associated with the inability to extract fluids adequately, but also with increased all-cause mortality, hospitalization for heart failure/volume overload and major adverse cardiac events. (1-5) In addition, intradialytic hypotension is likely to represent the tip of the iceberg with respect to consequences of changes in organ perfusion during HD. A striking discordance between hemodialysis-related symptoms or changes in (relative) blood volume and intradialytic hypotension has been reported. (6,7) Intradialytic hypotension is more likely to represent a late symptom of a pre-existing gradual reduction in blood flow during HD, compensated by an increase in vascular resistance and cardiac performance. However, pre-existent cardiac morbidity and concomitant treatment is likely to disturb this compensation mechanisms. More importantly, decreased left ventricular compliance as a result of increased heart mass and a rapidly descending systemic vascular resistance are both risk factors for a decreased cardiac output and potentially hypotension in dialysis patients. (8,9) Apart from non-circulatory effects of HD this discordance also represents a fundamental problem within the current clinical assumption that blood pressure is directly related to organ perfusion. To overcome this problem direct visualisation and quantification of the sublingual microcirculation with a hand-held device has been suggested by Bemelmans and co-workers, as a non-invasive tool to trace 'organ' perfusion during HD. (10) Direct in-vivo microscopy of the sublingual area with sidestream dark field (SDF) imaging during HD revealed marked reductions in microcirculatory blood flow in the absence of intradialytic hypotension in the vast majority of patients. Despite the potential of these observations many questions remain to be answered. The incidence, aetiology and clinical relevance of the microvascular alterations remain to be elucidated. This study has 2 major objectives: 1. Are we able to reproduce previous observations in a comparable subset of patients; and 2. Are the observed microcirculatory alterations the result of UF, HD or a combination?

Methods

Study design and setting.

Phase I consisted of a multi-centre prospective observational study conducted between October 2011 and December 2012. Participating centres were the Medical

Centre Leeuwarden, a tertiary teaching hospital in the Netherlands and The Hospital of Lithuanian University of Health Sciences, an academic medical centre in Kaunas, Lithuania. Local ethical committees of both hospitals approved the study and informed consent was obtained from every patient, according to applicable laws. In phase II (2012) of the study all patients included in the Netherlands were additionally subject to a single-centre prospective interventional study to compare different combinations of HD and UF rates. Study design was registered in advance at Clinicaltrials.gov (NCT01396980).

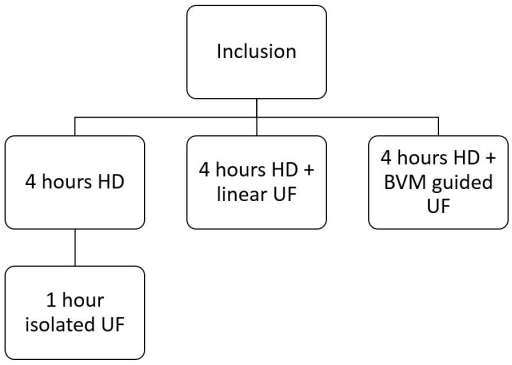


Figure 1: Study design. HD hemodialysis, UF ultrafiltration, BVM blood volume monitoring

Intervention.

In phase I all patients were subject to their routine 4-hours HD session, using a standard bicarbonate dialysate on normal temperature. During this period linear UF was maintained at a constant rate, in order to achieve a quantitative ultrafiltrate target, based upon the registered ideal dry weight of the patient. Primary endpoint is the difference in MFI between baseline and post intervention. In phase II patients from the Netherlands were subject to 2 additional combinations of HD and UF: 4 hours of HD alone followed by 1 hour isolated UF, and 4 hours HD plus UF based on blood-volume-monitoring (BVM)(5008 hemodialysis machine, Fresenius Medical Care) (Figure 1).[11] Sessions were assigned to each patient in random order and performed on the same day of the week; every patient served as his/her own reference. Primary endpoint is the difference in MFI between the HD/UF modalities post intervention.

Measurements.

During all sessions standard hemodynamic monitoring included blood pressure, heartrate and peripheral oxygen saturation using pulse oximetry. Sublingual in-vivo microscopy with sidestream dark field (SDF)-imaging, incorporated in a small hand-held camera, was performed in all patients at baseline and at the end of each session. For each timeframe 3 steady images of at least 10 seconds were obtained and recorded on digital videotape (SONY videowalkman GV-D 1000E®, Sony, Tokyo, Japan). Subsequent analysis was performed off-line and in random order with AVA software (Microvision Medical, Amsterdam, the Netherlands). (12) Quantification

of parameters of red blood cell velocity and capillary density was performed in accordance with an international consensus paper. (13) In short, red blood cell velocity in small vessels (< 20μm) is scored semi-quantitatively for each quadrant between 0 (stand still) and 3 (continuous normal flow).(14) The average score of 3 X 4 quadrants is expressed as microvascular flow index (MFI). Total vessel density (TVD), as a determinant of capillary density, is calculated as the surface area of small vessels per mm^2. Percentage of perfused vessels (PPV) is expressed as the percentage of perfused capillaries (MFI 2 and 3) divided by the total number of capillaries that crosses a grid of three horizontal and vertical equidistant lines. In phase II body composition monitoring (BCM; Fresenius Medical, Bad Homburg, Germany), based upon bioelectrical impedance analysis, and measurements of N-terminal pro b-type natriuretic peptide (NT-proBNP) and Troponine T were additionally performed prior to and after each intervention. (15,16)

Statistics.

All data are expressed as median [IQR]. Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 21, Chicago Illinois, USA). Due to the small sample size non-parametric tests for independent and paired data, as well as for correlation coefficients were applicable. A p-value < 0.05 was considered statistically significant. Based upon previous publications a sample size of 8 patients for phase II was considered adequate to detect a difference of 0.7 arbitrary units in MFI between the different HD/UF modalities. (10)

Results

Phase I.

During a 15-months period in 2011-2012 28 patients were included in the study. Overall, baseline characteristics were not significantly different between the 2 centres (Table 1). The primary endpoint MFI was not different before and after HD in combination with linear UF of 2.3[1.3-3.2]l over a 4 hour period (Table 2). In addition, hemodynamic variables did not change over time, with the exception of a small, but significant increase in peripheral oxygen saturation (Table 2).

Phase II.

In a 2-month period in 2012 all 9 patients from the Netherlands that participated in phase I were included. There was no statistical difference between baseline characteristics of different HD/UF modalities (Table 3). Total UF did not differ between HD/UF modalities, with the exception of HD alone. However, UF rate was significantly

higher in isolated UF ($p < 0.001$) in comparison to combined HD/UF modalities (Table 3). Median MFI decreased significantly during isolated UF [2.8(2.5-2.9) to 2.5(2.2-2.8), p=0.03], but remained unaltered during the other HD/UF modalities. We observed no significant difference between HD + linear UF and HD + BVM-guide UF (Figure 2). With the exception of isolated HD, BCM-derived overhydration and NT-pro-BNP decreased significantly during all HD/UF modalities, indicating a similar trend in volume status. Baseline MFI of each UF session (irrespective of UF modality) was significantly correlated with MFI after the intervention ($rs = 0.52$, $p = 0.006$; figure 3a). The coefficient of correlation for pre- and post-intervention overhydration was also significant ($rs = 0.75$, $p < 0.001$; figure 3b).

Table 1. Baseline characteristics phase I and II.

	All (n=28)	LT (n=19)	NL (n=9)	p-value
Men, %	57	42	89	0.04
Age, years	64[53-74]	60[49-70]	69[55-78]	0.29
Years on HD	3[1-6]	3[1-7]	3[1-5]	0.94
Remaining diuresis, l/24h	0.2[0-0.5]	0.3[0-0.6]	0.2[0-0.4]	0.60
Weight, kg	78[67-87]	73[66-79]	85[88-92]	0.03
BMI, kg/m2	26[24-28]	27[23-30]	26[25-28]	0.94
UF volume, l	2.3[1.3-3.2]	2.6[1.6-3.3]	1.7[1.2-2.1]	0.10
Cause of ESRD, %				
Diabetes	32	21	56	
Hypertension	18	26	0	
ADPKD	7	5	11	0.27
ATN	14	16	11	
Miscellaneous	29	32	22	
Drugs, %				
ß-blocker	61	42	67	1.0
ACE inhibitor	61	74	33	0.1
Calcium antagonist	47	58	22	0.09

LT Lithuania, NL Netherlands, HD hemodialysis, BMI body mass index, UF ultrafiltration, ESRD end stage renal disease, ADKPD autosomal dominant polycystic kidney disease, ATN acute tubular necrosis, ACE angiotensin converting enzyme.

Table 2. Results phase I (n=28). Hemodynamic and microcirculatory variables of small vessels (< 20µm) before and after hemodialysis in combination with linear ultrafiltration.

	Baseline	Post HD/UF	p-value
Mean arterial pressure, mmHg	93[76-111]	96[84-110]	0.39
Heartrate, beats/min	69[62-80]	73[60-84]	0.24
SpO_2, %	97[96-98]	98[98-99]	0.009
MFI, AU	3[2.8-3]	3[2.8-3]	0.55
TVD, mm/mm^2	22.2[18-29.8]	22.7[19.9-29]	0.11
PPV, %	98[96-100]	98[96-99]	0.35

HD hemodialysis, UF ultrafiltration, SpO_2 peripheral oxygen saturation, MFI microvascular flow index, AU arbitrary units, TVD total vessel density, PVD perfused vessel density, PPV, percentage of perfused vessel.

Table 3. Results phase II. Laboratory data, microcirculatory variables of small vessels and bioelectrical impedance analysis before and after intervention.

	Baseline	Post-intervention	p-value
UF isolated (n=9)			
UF	-	1.7[1.2-2]	
UF rate, l/hour	-	1.7[1.2-2]†	
MFI, AU	2.8[2.5-2.9]	2.5[2.2-2.8]	0.03
TVD, mm/mm²	17.7 [16.5-18.4]	18.7[16.1-20.3]	0.26
Hematocrit, %	37[35-39]	39[36-44]	0.11
NT-pro-BNP, pmol/l	599[215-1702]	580[158-1440]	0.01
Troponine T, ng/l	90[60-150]	87[56-130]	0.08
BCM overhydration, l	1.4[0.6-3.2]	0.3[-0.1-0.8]	0.02
HD isolated (n=9)			
UF	-	0[0-0]	
UF rate, l/hour	-	0[0-0]†	
MFI, AU	2.8[2.5-2.9]	2.8[2.7-2.9]	0.61
TVD, mm/mm²	17.7 [16.5-18.4]	18.6[14.6-19.7]	0.86
Hematocrit, %	37[35-39]	NA	
NT-pro-BNP, pmol/l	599[215-1702]	NA	
Troponine T, ng/l	90[60-150]	NA	
BCM overhydration, l	1.4[0.6-3.2]	NA	
HD + linear UF (n=9)			
UF		1.7[1.2-2.1]	
UF rate, l/hour		0.42[0.3-5.1]†	
MFI, AU	2.9[2.5-3]	2.6[2.2-2.9]	0.12
TVD, mm/mm²	17.8[16.6-18.8]	19.8[17.9-21.5]	0.07
Hematocrit, %	38[35-40]	40[35-41]	0.18
NT-pro-BNP, pmol/l	618[279-1926]	536[167-1003]	0.01
Troponine T, ng/l	94[61-179]	84[62-139]	0.02
BCM overhydration, l	1.8[0.5-5.3]	0.3[-0.9-3.2]	0.02
HD + BVM-guided UF (n=9)			
UF		2.0[1.5-2.1]	
UF rate, l/hour		0.5[0.39-0.52]†	
MFI, AU	2.8[2.5-3]	2.8[1.9-2.9]	0.06
TVD, mm/mm²	18.8[16.8-20.6]	18.2[16.9-20.6]	0.77
Hematocrit, %	37[34-39]	39[35-42]	0.12
NT-pro-BNP, pmol/l	574[229-2011]	511[172-1163]	0.01
Troponine T, ng/l	81[54-379]	79[54-387]	0.12
BCM overhydration, l	2.4[1.5-5.1]	0.2[-0.9-4.2]	0.04

UF ultrafiltration, HD hemodialysis, BVM blood volume monitoring, MFI microvascular flow index, TVD total vessel density, PPV percentage of perfused vessels, NT-pro-BNP n-terminal pro b-type natriuretic peptide, BCM body composition monitoring † p< 0.001 across different HD/UF modalities

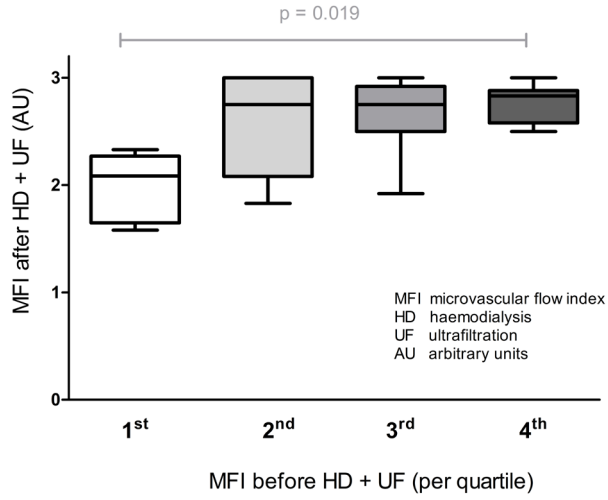


Figure 2: Distribution of post-intervention microvascular alterations per quartile of pre-intervention microvascular blood flow. P-value across groups.

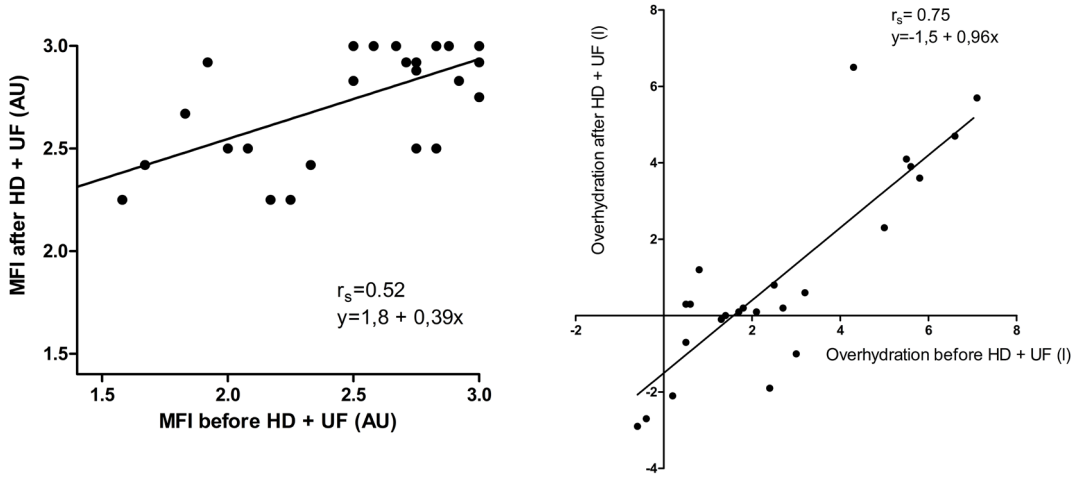


Figure 3: Left: Correlation in microvascular alterations between pre- and post-intervention. Right: Correlation in body-composition-monitoring-derived overhydration between pre- and post-intervention

Discussion

In phase I of the study we did not observe a reduction in sublingual microvascular blood flow or capillary density during HD in combination with linear UF. In an attempt to unravel the aetiology of previously observed alterations in microvascular blood flow during combined HD/UF we changed the ultrafiltration modalities in a subset of patients in phase II. During isolated HD we observed no changes in microvascular blood flow. This indicates that potential non-flow mediated mechanisms for

microvascular derangements, such as hemodialysis-induced inflammation and hypercoagulation, are unlikely to play an important role. (17,18) However, during isolated UF, a modality with the highest UF rate in which the influence of HD itself is absent, we were indeed able to reproduce the reduction in microvascular blood flow, as observed by others. (10,19) This suggests that the observed reduction in microvascular blood flow may be the result of a negative intravascular fluid balance. In case the UF rate exceeds the ability to mobilize interstitial fluids towards the intravascular space, an increase in vascular resistance or viscosity causes a reduction in microvascular blood flow. The fact that Bemelmans et al. observed a marked attenuation of impaired microvascular blood flow during autotransfusion with a Trendelenburg manoeuvre after HD/UF is also in line with this aetiology. (10)

An important issue is the question why we did not observe previously reported microcirculatory alterations during HD in combination with linear UF. At first glance there are important similarities with both articles: UF was 2.5[1.6-3.5]l and 2.5±0.88l respectively, and over a similar period of time.[10,19] But a closer look reveals a marked reduction in MFI at baseline in both studies; 2.8[2.5-5] and 2.7±0.5 versus 3[2.8-3] in our study. Baseline values of these previous publications indicate pre-existing microvascular derangement prior to the start of HD/UF, since they are outside the range of healthy volunteers. (20,21) A secondary analysis of our data in phase II revealed a significant correlation between MFI at baseline and MFI after the intervention, indicating that indeed volume status at baseline is an additional factor in the development of microvascular alterations during UF, irrespective of its modality. This suggest that our patients were less prone for microcirculatory changes, but that a higher rate of isolated ultrafiltration can result in impaired microperfusion in this group. Furthermore, our data suggest that 56% of the post-intervention overhydration is caused by pre-treatment overhydration. (Figure 3B) Further studies are needed to investigate a potental correlation between ultrafiltration-derived changes in microvascular flow and morbidity and/or mortality in hemodialysis patients. The limitations of the study are related to the small sample size in phase II. We anticipated a potential difference in MFI between the UF modalities, based upon previous observations. However, the observed changes in MFI were considerably smaller. As a consequence we may have been unable to detect an existing difference in sublingual microvascular blood flow between HD + linear UF and HD + BVM-guided UF (type I error).

Conclusions

In conclusion we observed a significant decrease of sublingual microvascular blood flow due to rapid isolated ultrafiltration. Additional interventions with different combinations of HD and UF revealed that HD per se is not associated with changes in microvascular flow. During ultrafiltration over a longer period of time, and in combination with hemodialysis, baseline abnormalities were associated abnormal microvascular blood flow at the end of the renal replacement session. By design this study is not suitable to establish the clinical relevance of the observed microvascular alterations.

Abbreviations

HD: Hemodialysis; SDF: Sublingual Sidestream Dark Field; UF: ultrafiltration; MFI: microcirculatory blood flow; TVD: Total vessel density;PPV: Percentage of perfused vessels; NT-proBNP: N-terminal pro b-type natriuretic peptide; Trop-T: Troponine T; BVM: blood-volume-monitoring.

Declarations

Ethics approval and consent to participate.

Local ethical committees (Regionale Toetsingscommissie Patiëntgebonden Onderzoek Leeuwarden and Kauno Regioninis Biomedicininiu Tyrimu Etikos Komitetas) of both hospitals approved the study and written informed consent was obtained from every patient, according to applicable laws.

Consent for publication

Not applicable.

Availability of data and material

Because of patient confidentiality data is not available.

Competing interests

GV, AP, IS, VP, MH and EB have no conflicts of interest. CI is the inventor of SDF technology, which is commercialized by MicroVision Medical. He has been a consultant for this company in the past, but he has broken all contact with this company for more than 2 years now, and he has no competing interests other than his commitment to promote the importance of the microcirculation in the care of patients.

Funding

No funding was used for this study.

Authors' contributions

Study design: GV, AP, IS, VP, MH, CI, EB. Data analysis and interpretation: GV, AP, EB. Drafting of the manuscript: GV, EB. Statistical analysis: GV, EB. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

References

1. Sands JJ, Usvyat LA, Sullivan T, Segal JH, Zabetakis P, Kotanko P, Maddux FW, Diaz-Buxo JA: Intradialytic hypotension: Frequency, sources of variation and correlation with clinical outcome. Hemodialysis International 2014, 18(2):415-422.

2. Chao CT, Huang JW, Yen CJ: Intradialytic hypotension and cardiac remodeling: a vicious cycle. BioMed research international 2015, 2015:724147.

3. McIntyre CW, Goldsmith DJ: Ischemic brain injury in hemodialysis patients: which is more dangerous, hypertension or intradialytic hypotension? Kidney Int 2015, 87(6):1109-1115.

4. Sands JJ, Usvyat LA, Sullivan T, Segal JH, Zabetakis P, Kotanko P, Maddux FW, Diaz-Buxo JA: Intradialytic hypotension: frequency, sources of variation and correlation with clinical outcome. Hemodial Int 2014, 18(2):415-422.

5. Flythe JE, Xue H, Lynch KE, Curhan GC, Brunelli SM: Association of mortality risk with various definitions of intradialytic hypotension. J Am Soc Nephrol 2015, 26(3):724-734.

6. Meredith DJ, Pugh CW, Sutherland S, Tarassenko L, Birks J: The relationship between symptoms and blood pressure during maintenance hemodialysis. Hemodial Int 2015, 19(4):543-552.

7. Booth J, Pinney J, Davenport A: Do changes in relative blood volume monitoring correlate to hemodialysis-associated hypotension? Nephron Clin Pract 2011, 117(3):c179-c183.

8. Straver B, Roggekamp MC, de Vries PM, ter Wee PM: Systemic vascular resistance in intradialytic hypotension determined by means of impedance cardiography. Blood Purif 1998, 16(5):281-289.

9. Straver B, Roggekamp MC, de Vries PM, ter Wee PM: Systemic vascular resistance in intradialytic hypotension determined by means of impedance cardiography. Blood Purif 1998, 16(5):281-289.

10. De Backer D, Ortiz JA, Salgado D: Coupling microcirculation to systemic hemodynamics. Curr Opin Crit Care 2010, 16(3):250-254.

11. Weir MR: Relative plasma volume monitoring and blood pressure control: an overlooked opportunity to achieve dry weight in the hemodialysis patient. Hypertension 2010, 55(2):226-227.

12. Dobbe JGG, Streekstra GJ, Atasever B, van Zijderveld R, Ince C: Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. Med Biol Eng Comput 2008, 46(7):659-670.

13. Ince C: Go with the flow--recruit the microcirculation! Intensive Care Med 2006, 32(4):488-489.

14. Bartels SA, Bezemer R, Milstein DMJ, Radder M, Lima A, Cherpanath TGV, Heger M, Karemaker JM, Ince C: The microcirculatory response to compensated hypovolemia in a lower body negative pressure model. Microvasc Res 2011,

15. Chamney PW, Wabel P, Moissl UM, Müller MJ, Bosy-Westphal A, Korth O, Fuller NJ: A whole-body model to distinguish excess fluid from the hydration of major body tissues. Am J Clin Nutr 2007, 85(1):80-89.

16. Wabel P, Chamney P, Moissl U, Jirka T: Importance of whole-body bioimpedance spectroscopy for the management of fluid balance. Blood Purif 2009, 27(1):75-80.

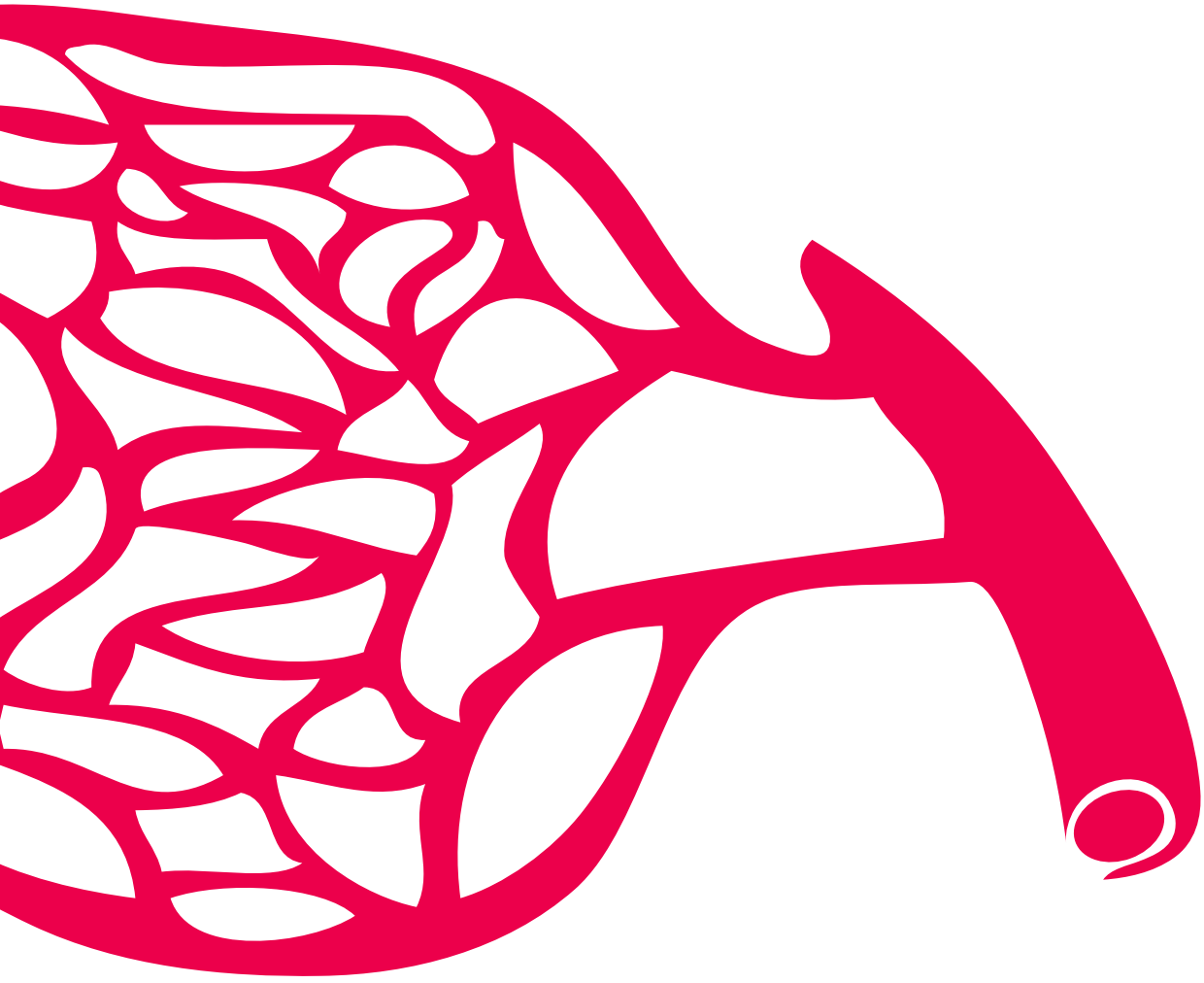
17. Zimmermann J, Herrlinger S, Pruy A, Metzger T, Wanner C: Inflammation enhances cardiovascular risk and mortality in hemodialysis patients. Kidney Int 1999, 55(2):648-658.

18. Topçiu-Shufta V, Haxhibeqiri V, Begolli L, Baruti-Gafurri Z, Veseli S, Haxhibeqiri S, Miftari R, Kurti L, Avdiu D: Correlation of Inflammation and Lipoprotein (a) with Hypercoagulability in Hemodialysis Patients. Medical archives (Sarajevo, Bosnia and Herzegovina) 2015, 69(4):232-235.

19. Meinders AJ, Nieuwenhuis L, Ince C, Bos WJ, Elbers PW: Haemodialysis Impairs the Human Microcirculation Independent from Macrohemodynamic Parameters. Blood Purif 2015, 40(1):38-44.

20. Edul VSK, 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 2012, 40(5):1443-1448.

21. 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 medicine experimental 2015, 3(1):40.



CHAPTER 6

DIFFERENCES IN CAPILLARY RECRUITMENT BETWEEN CARDIAC SURGERY AND SEPTIC PATIENTS AFTER FLUID RESUSCITATION.

G. Veenstra[1,2], C. Ince[2,3], B.W. Barendrecht[1], H.W. Zijlstra[1], E.C. Boerma[1].

1: Department of Intensive Care, Medical Centre Leeuwarden,
PO Box 888, 8901BR Leeuwarden, The Netherlands
2: Department of Translational Physiology, Amsterdam UMC, University of Amsterdam.
3: Department of Intensive Care, Erasmus MC, University Medical Center Rotterdam.

Submitted to Microvascular Research

6 Abstract

Background

Clinical evaluation of the effects of fluid therapy remains cumbersome and strategies are based on the assumption that normalization of macrohemodynamic variables will result in parallel improvement in organ perfusion. Recently, we and others suggested the use of direct in-vivo observation of the microcirculation to evaluate the effects of fluid therapy.

Methods

A single-centre observational study, using in-vivo microscopy to assess total vessel density (TVD) in two subsets of ICU patients.

Results

After fluid resuscitation TVD showed no difference between sepsis patients (N=47) and cardiac surgery patients (N=52): 18.4[16.8-20.8] vs 18.7[16.8-20.9] mm/mm^2, p=0.59. In cardiac surgery patients there was a significant correlation between the amount of fluids administered and TVD, with an optimum in the third quartile. However, such correlation was absent in septic patients.

Conclusions

TVD after fluid administration is not different between 2 subtypes of intensive care patients. However, only in septic patients we observed a lack of coherence between the amount of fluids administered and TVD The observed maximum of TVD may serve as potential endpoint for fluid administration in future studies.

Introduction

Over the last decade the awareness of potential harmful effects of fluid resuscitation is rising. On the one hand the administration of fluids is considered to be the corner stone in treatment of shock, irrespective of aetiology. On the other hand, an association between the administration of fluids and adverse outcome has been observed. Such unwanted side effects may not only be related to the chemical composition of the fluids, but also to the amount of fluids administered. (1-4) Incentives for fluid administration are diverse and include compensation for fluid- or blood loss, attenuation of increased resistance to venous return with subconsequent reduction of preload and maintenance of perfusion pressure under conditions of reduced vasomotor tone. Ultimately, the goal of fluid resuscitation is to optimize the requirements, needed to maintain cell homeostasis.

However, the clinical evaluation of the effects of fluid therapy remains cumbersome. Conventionally, the evaluation is based on normalization of systemic variables of circulation, i.e. heartrate, blood pressure and cardiac output. Such strategy is based on the assumption that normalization of these macrohemodynamic variables will automatically result in a parallel improvement in organ perfusion. However, direct in-vivo observation of the microcirculation by means of hand-held microscopes has revealed that this coherence between macro- and microcirculation may not always be present. Well-known conditions in which loss of coherence has been observed include sepsis and obstructive heart failure, revealing sustained hypoperfusion despite correction of systemic variables by fluids and vasoactive compounds. (1,5) Uncoupling between the macro- and microcirculation may be the intrinsic result of the disease state. Endothelial dysregulation may result in increased permeability, hypercoagulation and loss of vasomotor tone, causing altered microcirculatory blood flow, not sensed by macrohemodynamic variables. In addition, fluid therapy itself can also induce oxidative and nitrosative stress, contributing to reduced vascular regulatory capacity and reduced oxygen-carrying capacity to vulnerable organs such as the kidney. (6) Moreover, it must be acknowledged that adequate oxygenation of the cell is based on two key characteristics of perfusion. The first one is convective oxygen transport, that depends on red blood cell velocity and red blood cell oxygen-carrying capacity. Fluid therapy may increase red blood cell velocity and thereby increase oxygen delivery to the cells. However, fluids intrinsically do not have oxygen-carrying capacity, with the exception of red blood cell transfusion. The second determinant of oxygen transport to the cell is diffusion. Given the gas-specific characteristics, oxygen diffusion is related to the pressure gradient and inversely related to the distance between the capillary and

the cell. Fluid therapy can potentially recruit initially unperfused capillaries, and thus reduce oxygen diffusion distance. (7,8) Conversely, fluid therapy may also promote oedema formation with subsequent reduction of oxygen diffusion capacity. (9) Recently, we and others have suggested the use of direct in-vivo observation of the microcirculation to evaluate the effects fluid therapy. (7,10-13)

The technique enables the quantification of both key characteristics of oxygen transport, needed to determine the line of demarcation between beneficial and detrimental effects of fluid resuscitation. In this study we aim to identify the reaction of microcirculation on fluid resuscitation, by means of diffusion distance, in two subsets of ICU patients.

Methods

Patients

The study was performed as a single centre observational study and conducted between October 2015 and April 2017. Local ethical committee waived the need for informed consent. The study was registered at ClinicicalTrials.gov (NCT02661269). Our aim was to observe the microcirculation under clearly separate circumstances. Patients after cardiothoracic surgery (Group A) were assumed to represent a hypo- or euvolemic condition, whereas patients after septic shock (Group B) were assumed to represent a hypervolemic condition. ICU patients in both categories and above 18 years of age were considered eligible for the study. Exclusion criteria included inability to obtain Incident dark field (IDF) images, such as maxillofacial surgery or oropharyngeal bleeding.

Protocol

Patients in group A were enrolled in the study within 4 hours after admission to the intensive care. Patients in group B were enrolled at the peak of the cumulative fluid balance. Measurements were performed once and included: demographic data, IDF-imaging, bio-impedance measurements, weight, macrohemodynamic parameters, venous saturation, lactate and haemoglobin/ haematocrit. Fluid resuscitation on the department is only done with crystalloids.

Cytocam-IDF imaging

Cytocam-IDF imaging (Braedius Medical, Huizen, The Netherlands) consists of a combination of IDF illumination with optical and technical features optimized for visualization of the microcirculation on organ surfaces. It uses incident dark

field illumination with high-brightness LEDs with a very short illumination pulse time of 2 ms. The image acquisition and sensor are under computer control and electronically synchronized to the illumination pulses. The digitally recorded images where analysed blinded and off-line. (14) Offline analysis was performed using the AVA 3.0 software package (MicroVision Medical, Amsterdam, the Netherlands) in compliance with the recommendations of a roundtable conference. (15)

Bio-impedance

Bio-impedance vector analysis (BIVA) is a non-invasive, quick and inexpensive technique to estimate body composition and showed good correlation between hydration state in ICU survivors and non-survivors with acute kidney injury. (16) This technique measures the resistance of body tissues to the flow of an alternating current of 800 µA at an operating frequency of 50 kHz. Bio-impedance measurements were measured using the BIA-101® (GLNP Medical Devices, Breda, The Netherlands). Reactance (Xc), resistance (R) and phase angle were recorded. Total body impedance can be considered a combination of resistance R (the opposition to the flow of an alternating current through intra- and extracellular electrolyte solutions) and reactance X (the capacity produced by the interfaces of tissues and cell membranes). (17-19)

Statistical analyse

Data are presented as median [inter quartile range] or as average ± standard deviation. Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 21, Chicago Illinois, USA). After identifying type of distribution the designated tests were used. Power calculation was performed to identify a 10% difference in total vessel density (TVD) between groups. Based upon an alpha of 0.05 and a power of 80%, we calculated a sample size of 50 patients per group. A p value < 0,05 was considered statistically significant. Primary outcome was defined as the difference in TVD between both groups.

Results

Primary endpoint

We included 52 patients in group A and 47 patients in group B. Baseline characteristics are presented in Table 1, showing significant differences between groups in a variety in macrohemodynamic variables. There was a higher cumulative fluid balance in sepsis patients in comparison to cardiothoracic surgery patients (7 [5-10] vs 2.3 [1.7-2.8]L, p < 0.0001). Despite reticence to blood transfusion in septic shock, group B

6 received significantly more red blood cell transfusions, but medium haemoglobin and haematocrit values were similar (Table 1). The primary outcome TVD showed no difference between group A and group B (18.4 [16.8-20.8] vs 18.7 [16.8-20.9] mm/mm2, p=0.59). Remaining microcirculatory values are displayed in Table 2. A statistical significant correlation was found between TVD and the cumulative fluid balance within group A (r2=0.31, p 0.02, Figure 1), but not in group B.

Secondary endpoints

Cumulative fluid balances were divided into interquartile ranges. In group A TVD showed a significant difference between interquartile fluid balance ranges, with a maximum of 19.9 [18.3-21.0] mm/mm2 in Q3. Boxplots are included in Figure 2. In group B there was no significant difference in TVD between interquartile fluid balance ranges, with a maximum of 19.8 [18.1-20.9] in Q1.

Bio-impedance-derived values indicative for (over)hydration were significantly higher in group B in comparison to group A. (Table 2). Combining group A and B, showed a significant linear correlation between the cumulative fluid balance and both reactance and resistance (r2=0.33, p < 0.05 and r2=0.29, p<0.05 respectively).

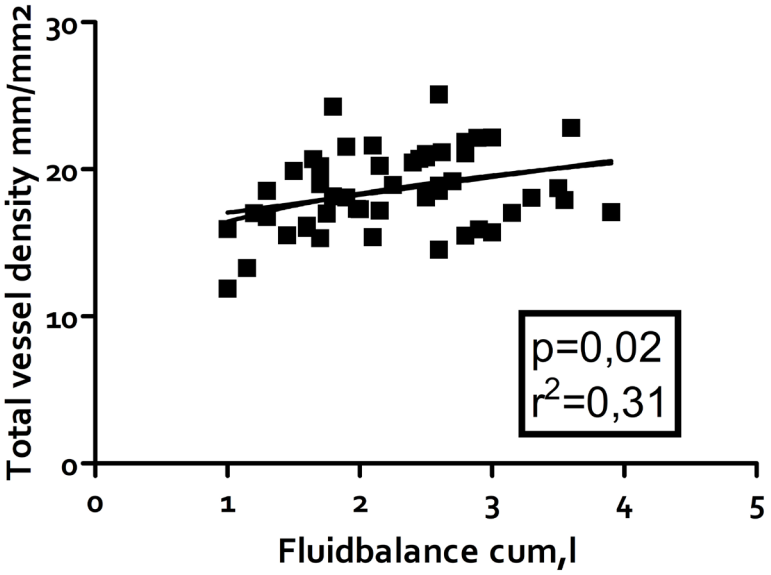


Figure 1: Correlation between cumulative fluidbalance and total vessel density in the cardiothoracic surgery group.

Table 1. Baseline characteristics.

Group	Cardio (N=52)	Sepsis (N=47)	p
Man/Female %	71/29	64/36	0.44
Age, years	67 +/- 11	66 [55-71]	0.19
Length, cm	174 +/- 9	175 [169-180]	0.78
Weigth, kg	82 +/- 14	85 [78-94]	0.15
Heartrate, bpm	90 +/- 7	99 +/- 17	0.05
Systolic bloodpressure, mmHg	105 [97-118]	96 [87-108]	0.007
Diastolic bloodpressure, mmHg	57 [53-65]	52 [44-59]	0.01
Mean arterial bloodpressure, mmHg	72 [66-83]	65 [58-77]	0.01
Central venous pressure	9 +/- 4	9 +/-5	0.49
Cardiac Index	2.4 [2.1-3.0]	3.3 +/-0.9	0.001
S(c)VO2	67 +/- 8	74 [71-77]	0.01
Lactate	1.8 +/- 0.6	1.3 [0.9-1.8]	0.004
Apache IV score	44 +/-13	86 [61-107]	<0.001
Predicted mortality, %	1 [.4-2]	35 [11-58]	<0.001
Inotropic use, %	50	66	0.11
Fluid balance perioperative, l	2.0 [1.5-2.6]	0 [0-4]	0.06
Fluid balance Intensive care, l	0.0 [0.0-0.4]	5.9 [4-7]	<0.001
Fluid balance cumulative, l	2.3 [1.7-2.8]	7 [5-10]	<0.001
Hemoglobine, mmol/l	6.1 [5.6-6.7]	6.5 +/- 1	0.39
Hematocrit, %	30 [28-33]	32 +/-0.05	0.22
Received bloodtransfusion, % (median packages)	9.6 (2)	43 (2)	<0.001

S(c)VO2: central or mixed venous saturation.

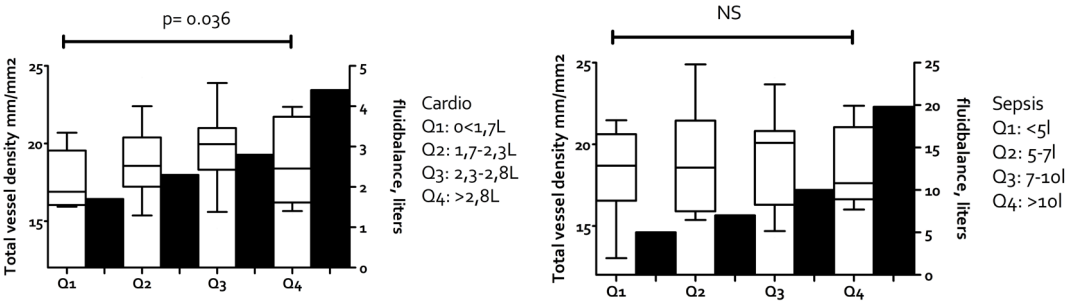


Figure 2: Fluid balance per quartile, sepsis and cardio-group. Fluid balance defined by interquartile ranges.

Table 2. Primary and secondary outcome

Group	Cardio (N=52)	Sepsis (N=47)	p
IDF-imaging			
Microvascular Flow Index (small vessels)	3.0 [2.9-3.0]	3 [2.9-3]	0.74
Microvascular Flow Index (large vessels)	3.0 [3.0-3.0]	3 [2.9-3]	0.04
Total vessel density	18.4 [16.8-20.8]	18.71 [16.8-20.9]	0.59
Total vessel length	13.7 [12.5-15.5]	14.1 [12.5-15.6]	0.52
Percentage of perfused vessel	99 [98-100]	99 [97-99]	0.02
Perfused vessel density	18.2 [16.4-20.7]	18.6 [16.1-20.8]	0.78
De Backer score	11.3 [10.2-13.0]	11.5 [10.4-12.8]	0.56
BIVA			
Reactance, Ω	41.5 [37.5-46.3]	23.2 [18.6-29.2]	<0.001
Resistance, Ω	399 [360-456]	295 [260-350]	<0.001
Phase angle, θ	5.7 [5.1-6.5]	4.1 [3.3-5.1]	<0.001

IDF: Incident Dark-field; BIVA: bio-impedance vector analysis.

Discussion

The main finding of this study is the absence of a significant difference in total vessel density between sepsis en cardiothoracic ICU patients. In both groups the IQR of the maximum TVD was between 18 and 21 mm/mm2. However, despite normalisation of macrohemodynamics variables, TVD did not reach the level as observed in healthy volunteers. (14) The fact that these maximum values showed a significant decline in the highest cumulative fluid balance quartile in cardiac surgery patients (group A) is in line with the present theory. After recruitment of capillaries and restoration of flow, oedema formation further limits and even reduces (functional) capillary density, This finding suggests a ceiling value for the recruitment of capillaries by fluid resuscitation with crystalloids, and carries the

potential to serve as an endpoint for fluid resuscitation. However, in septic patients (group B) we observed an absence of correlation between the amount of fluids administered and capillary density. This observation may either be in line with an intrinsic uncoupling between the macro- and the microcirculation in distributive shock, previously referred to as an example of loss of hemodynamic coherence. (5) Alternatively it may reflect the inability to recruit capillary density in sepsis with crystalloids beyond a certain level, due to enhanced capillary leakage of fluids under such conditions, Although our assumption to use cardiothoracic and sepsis patients as models for different stages of (over)resuscitation may be arbitrary, the observed bio-impedance values suggest indeed a clear separation between groups. These data are difficult to compare with the existing literature. To our knowledge we are the first to report TVD in different categories of patients in relation to resuscitation status. In accordance with previous publications we observed a decrease in capillary density after cardiopulmonary bypass in comparison to healthy controls. (20-22) Similar observations were done in human sepsis. (23-25) However, comparing exact values is virtually impossible, since the number of capillaries visualized during in-vivo microscopy is device dependent. (14,26) The majority of previous data has been obtained by means of sidestream dark-field-imaging. Nevertheless, the observed irresponsiveness of the microcirculation to fluid resuscitation in the late phase of sepsis may alternatively be explained by the same ceiling effect. (27) In other words, in case the maximal number of recruitable capillaries is reached, further crystalloids fluid administration may ad best result in the maintenance of the number of perfused capillaries, or even result in a decrease of perfused capillary density due to oedema formation.

There are certainly limitations to our study. Firstly, we performed a single measurement in two different stages of (over)resuscitation. Multiple observations of the microcirculation over time during fluid resuscitation would clearly further clarify the underlying mechanisms. Secondly, it is of note that we did not observe an anticipated decline in TVD in the extremes of overhydration. This may simply due to inadequate statistical power as a result of small subgroups of patient in each quartile of cumulative fluid balance. Alternatively, Q4 in each patient group did not contain enough extremes to demonstrate an actual decline in TVD. Thirdly, it is clear that macrohemodynamic baseline characteristics were significantly different between groups. As a result it remains unclear in the present study whether microcirculatory abnormalities have been influenced by these differences in macrohemodynamic conditions. Lastly, there is an major difference in the number of patients that received red blood cell transfusion. It is

reported that the microcirculation can be 'resuscitated' by blood transfusions in both cardiac surgery as septic shock patients. However, it is of note that haemoglobin an haematocrit concentrations were equal between groups. (28-33) We acknowledge that the observed maximum in capillary density needs further exploration. Prospective trials are needed to test the clinical relevance of the observed upper limit of capillary density in terms of clinically relevant outcome. Further research is needed to determine whether this cut-off value may serve as a line of demarcation between beneficial and detrimental effects of fluid resuscitation.

Conclusion

After fluid resuscitation there is no significant difference in (functional) capillary density between cardiac surgery and septic patients. In cardiac surgery patients there is a significant correlation between the amount of fluids administered and capillary density, with an optimum in the third quartile. However, such correlation is absent in septic patients, suggesting lack of hemodynamic coherence between the macro- and the microcirculation under these conditions.

Competing interests

Dr. Ince has developed SDF imaging and is listed as inventor on related patents commercialized by MicroVision Medical (MVM) under a license from the Academic Medical Center (AMC). He has been a consultant for MVM in the past but has not been involved with this company for more than 5 years now, holds no shares. Braedius Medical, a company owned by a relative of Dr. Ince, has developed and designed a handheld microscope called CytoCam-IDF imaging. Dr. Ince has no financial relation with Braedius Medical of any sort, i.e., never owned shares or received consultancy or speaker fees from Braedius Medical. The other authors have no competing interests.

List of abbreviations

IDF: incident darkfield; LED: light emitting diode; BIVA: bio-impedance vector analysis; TVD: total vessel density.

References

1. V.S.K. Edul, C. Ince, A.R. Vazquez, P.N. Rubatto, E.D.V. Espinoza, S. Welsh et al. Similar Microcirculatory Alterations in Patients with Normodynamic and Hyperdynamic Septic Shock. Annals of the American Thoracic Society. 2016;13(2):240-7.

2. C. Teixeira, F. Garzotto, P. Piccinni, N. Brienza, M. Iannuzzi, S. Gramaticopolo et al. Fluid balance and urine volume are independent predictors of mortality in acute kidney injury. Crit Care. 2013;17(1):R14.

3. J.R. Prowle, J.E. Echeverri, E.V. Ligabo, C. Ronco, R. Bellomo. Fluid balance and acute kidney injury. Nature Reviews Nephrology. 2009;6(2):107-115.

4. D.J. Kelm, J.T. Perrin, R. Cartin-Ceba, O. Gajic, L. Schenck, C.C. Kennedy et al. Fluid overload in patients with severe sepsis and septic shock treated with early goal-directed therapy is associated with increased acute need for fluid-related medical interventions and hospital death. Shock. 2015;43(1):68-73.

5. C. Ince. Hemodynamic coherence and the rationale for monitoring the microcirculation. Crit Care. 2015;19 Suppl 3:S8.

6. U. Aksu, R. Bezemer, B. Yavuz, A. Kandil, C. Demirci, C. Ince et al. Balanced vs unbalanced crystalloid resuscitation in a near-fatal model of hemorrhagic shock and the effects on renal oxygenation, oxidative stress, and inflammation. Resuscitation. 2012;83(6):767-73.

7. M.E. van Genderen, E. Klijn, A. Lima, J. de Jonge, S. Sleeswijk Visser, J. Voorbeijtel et al. Microvascular perfusion as a target for fluid resuscitation in experimental circulatory shock*. Crit Care Med. 2014;42(2):e96-e105.

8. C.Y. Wu, K.C. Chan, Y.J. Cheng, Y.C. Yeh, C.T. Chien, NTUH Center of Microcirculation Medical Research et al. Effects of different types of fluid resuscitation for hemorrhagic shock on splanchnic organ microcirculation and renal reactive oxygen species formation. Crit Care. 2015;19:434.

9. J.P. Hanson, S.W.K. Lam, S. Mohanty, S. Alam, R. Pattnaik, K.C. Mahanta et al. Fluid resuscitation of adults with severe falciparum malaria: effects on Acid-base status, renal function, and extravascular lung water. Crit Care Med. 2013;41(4):972-81.

10. G. Veenstra, C. Ince, E.C. Boerma. Direct markers of organ perfusion to guide fluid therapy: When to start, when to stop. Best Pract Res Clin Anaesthesiol. 2014;28(3):217-226.

11. J. Xu, L. Ma, S. Sun, X. Lu, X. Wu, Z. Li et al. Fluid resuscitation guided by sublingual partial pressure of carbon dioxide during hemorrhagic shock in a porcine model. Shock. 2013;39(4):361-5.

12. A. Pranskunas, M. Koopmans, P.M. Koetsier, V. Pilvinis, E.C. Boerma. Microcirculatory blood flow as a tool to select ICU patients eligible for fluid therapy. Intensive Care Med. 2013;39(4):612-9.

13. C. Ince. The rationale for microcirculatory guided fluid therapy. Curr Opin Crit Care. 2014;20(3):301-8.

14. G. Aykut, G. Veenstra, C. Scorcella, C. Ince, C. Boerma. Cytocam-IDF (incident dark field illumination) imaging for bedside monitoring of the microcirculation. Intensive care medicine experimental. 2015;3(1):40.

15. D. De Backer, S. Hollenberg, C. Boerma, P. Goedhart, G. Büchele, G. Ospina-Tascon et al. How to evaluate the microcirculation: report of a round table conference. Crit Care. 2007;11(5):R101.

16. A.C.D.R. Hise, M.C. Gonzalez. Assessment of hydration status using bioelectrical impedance vector analysis in critical patients with acute kidney injury. Clin Nutr. 2017;

17. U.G. Kyle, I. Bosaeus, A.D. De Lorenzo, P. Deurenberg, M. Elia, J.M. Gómez et al. Bioelectrical impedance analysis--part I: review of principles and methods. Clin Nutr. 2004;23(5):1226-43.

18. K. Norman, N. Stobäus, M. Pirlich, A. Bosy-Westphal. Bioelectrical phase angle and impedance vector analysis--clinical relevance and applicability of impedance parameters. Clin Nutr. 2012;31(6):854-61.

19. W.F. Peacock. Use of bioimpedance vector analysis in critically ill and cardiorenal patients. Contrib Nephrol. 2010;165:226-35.

20. N.J. Koning, A.B.A. Vonk, L.J. van Barneveld, A. Beishuizen, B. Atasever, C.E. van den Brom et al. Pulsatile flow during cardiopulmonary bypass preserves postoperative microcirculatory perfusion irrespective of systemic hemodynamics. J Appl Physiol. 2012;112(10):1727-34.

21. M. Bienz, D. Drullinsky, L.M. Stevens, D. Bracco, N. Noiseux. Microcirculatory response during on-pump versus off-pump coronary artery bypass graft surgery. Perfusion. 2016;31(3):207-15.

22. D. De Backer, M.J. Dubois, D. Schmartz, M. Koch, A. Ducart, L. Barvais et al. Microcirculatory Alterations in Cardiac Surgery: Effects of Cardiopulmonary Bypass and Anesthesia. Ann Thorac Surg. 2009;88(5):1396-1403.

23. A.P.C. Top, C. Ince, N. de Meij, M. van Dijk, D. Tibboel. Persistent low microcirculatory vessel density in nonsurvivors of sepsis in pediatric intensive care. Crit Care Med. 2011;39(1):8-13.

24. C. Ince, M. Sinaasappel. Microcirculatory oxygenation and shunting in sepsis and shock. Crit Care Med. 1999;27(7):1369-77.

25. A. Spanos, S. Jhanji, A. Vivian-Smith, T. Harris, R.M. Pearse. Early microvascular changes in sepsis and severe sepsis. Shock. 2010;33(4):387-91.

26. Aykut G., Ince Y., Ince C.. A new generation computer-controlled imaging sensor-based hand-held microscope for quantifying bedside microcirculatory alterations. In: Vincent J.L., editor. Annual update in Intensive Care and Emergency Medicine 2014. Heidelberg: Springer; 2014. p. 367-81.

27. G. Ospina-Tascon, A.P. Neves, G. Occhipinti, K. Donadello, G. Büchele, D. Simion et al. Effects of fluids on microvascular perfusion in patients with severe sepsis. Intensive Care Med. 2010;36(6):949-55.

28. K. Yuruk, E. Almac, R. Bezemer, P. Goedhart, B. de Mol, C. Ince et al. Blood transfusions recruit the microcirculation during cardiac surgery. (1). Transfusion. 2011;51(5):961-7.

29. B. Atasever, M. van der Kuil, C. Boer, A. Vonk, L. Schwarte, A.R.J. Girbes et al. Red blood cell transfusion compared with gelatin solution and no infusion after cardiac surgery: effect on microvascular perfusion, vascular density, hemoglobin,

and oxygen saturation. Transfusion. 2012;52(11):2452-8.

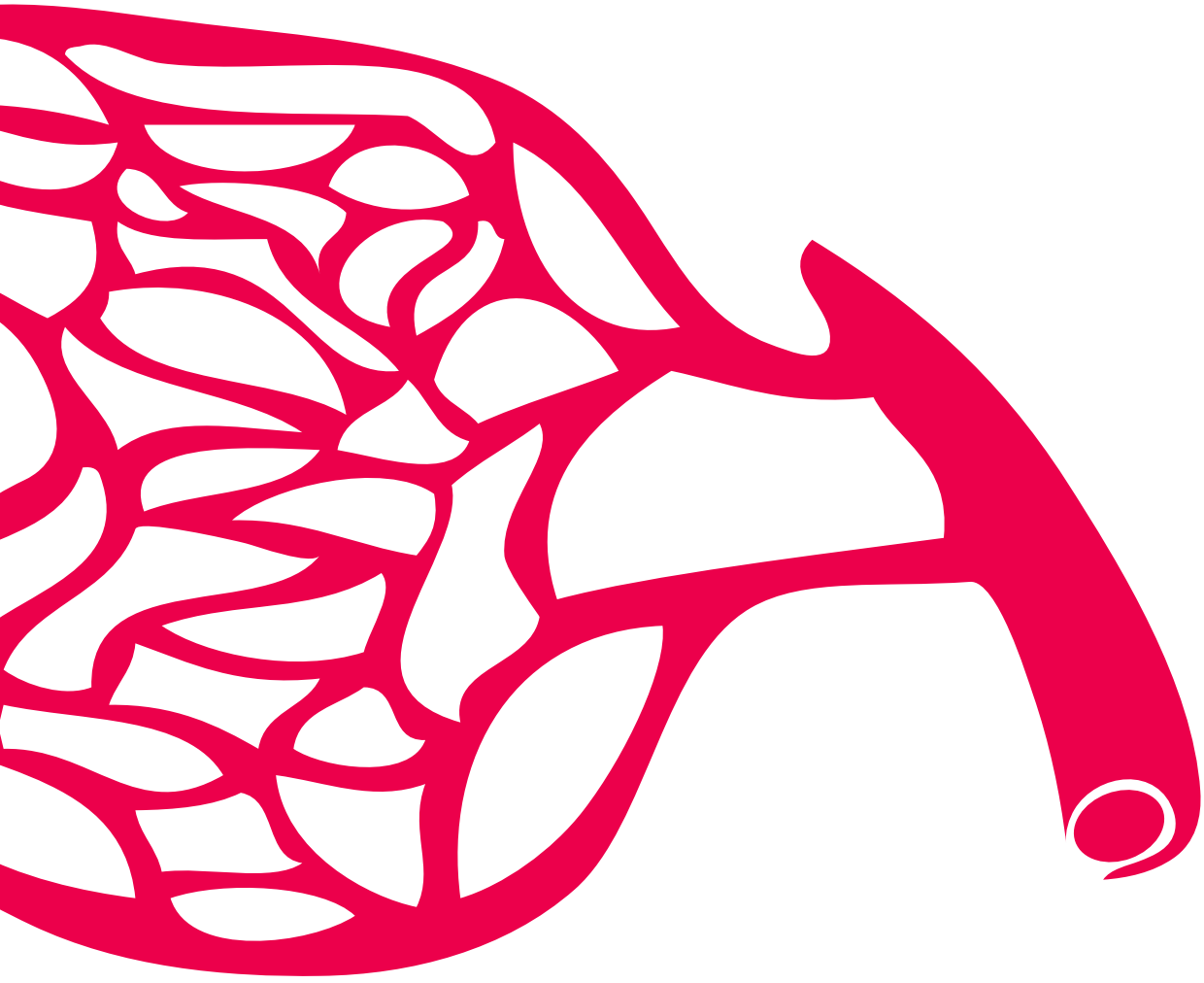
30. C.P. Stowell, G. Whitman, S. Granger, H. Gomez, S.F. Assmann, M.J. Massey et al. The impact of red blood cell storage duration on tissue oxygenation in cardiac surgery. J Thorac Cardiovasc Surg. 2017;153(3):610-619.e2.

31. A. Donati, E. Damiani, M. Luchetti, R. Domizi, C. Scorcella, A. Carsetti et al. Microcirculatory effects of the transfusion of leukodepleted or non-leukodepleted red blood cells in patients with sepsis: a pilot study. Crit Care. 2014;18(1):R33.

32. Y. Sakr, M. Chierego, M. Piagnerelli, C. Verdant, M.J. Dubois, M. Koch et al. Microvascular response to red blood cell transfusion in patients with severe sepsis. Crit Care Med. 2007;35(7):1639-44.

33. N.D. Nielsen, I. Martin-Loeches, C. Wentowski. The Effects of red Blood Cell Transfusion on Tissue Oxygenation and the Microcirculation in the Intensive Care Unit: A Systematic Review. Transfus Med Rev. 2017;31(4):205-222.

6



CHAPTER 7

SURROGATES OF ORGAN PERFUSION LACK SENSITIVITY FOR PREDICTING MICROCIRCULATORY FLUID RESPONSIVENESS.

G. Veenstra[1,2], C. Ince[2,3], B.W. Barendrecht[1], H.W. Zijlstra[1], E.C. Boerma[1].

1: Department of Intensive Care, Medical Centre Leeuwarden,
PO Box 888, 8901BR Leeuwarden, The Netherlands
2: Translational Physiology, Academic Medical Center, Amsterdam, the Netherlands.
3: Erasmus MC University Hospital Rotterdam, Rotterdam, The Netherlands.

In preparation

7 Abstract

Introduction

Fluid administration is the most used treatment for all types of hemodynamic instability. Proper understanding of the indication is key because of potential harmful effects. Clinical decision making around the initiation of fluid therapy is difficult, as reflected by the large amount of potential triggers. The use of surrogates of impaired organ perfusion to identify patients that could benefit from fluid resuscitation is widely used without solid scientific support. Direct observation of the microcirculation has the potential to evaluate all aspects of organ perfusion. We undertook a study to observe the response of the microcirculation to a standardized fluid bolus in a single-spot.

Methods

In this single centre, prospective, observational study postoperative cardiac-surgery patients were included in case the attending physician deemed fluid administration necessary, based on clinical signs of impaired organ perfusion. Primary outcome was an improvement of microcirculatory blood flow (MFI), secondary outcomes were a change in total vessel density, the red blood cell velocity measured using space time diagrams and signs of impaired organ perfusion.

Results

20 patients were included who showed clinical signs of impaired organ perfusion. In the total study population MFI did not change during the experiment. Only 20% of the patients had a MFI < 2.6. Patients with a low MFI (< 2.6) at baseline did not show a difference in the number of 'clinical signs of impaired organ perfusion' as compared to patients with a normal MFI at baseline. However, only patients with a low MFI at baseline showed a non-significant trend to improvement of MFI. In the subgroup of 8 patients with hypotension at baseline (mean arterial pressure < 60 mm Hg), there was a significant reduction in hypotension in response to fluid administration (MAP 54 mm Hg [48-57] vs 63 mm Hg [52-65]; p < 0,05) as well as a significant increase in total vessel density (18,5 mm2/mm2 [14,3-23,4] vs 18,9 mm2/mm2 [14,2-23,4], p < 0,05) and red blood cell velocity in two of the five measured capillaries.

Conclusion

In this post-cardiac surgery population, 'clinical signs of impaired organ perfusion' is not associated with the presence of sublingual microvascular alterations, nor

its response to fluid administration. However, in a subgroup of patients with hypotension at baseline, a significant increase in red blood cell velocity in individual capillaries was observed, as well as a significant increase in total vessel density in conjunction with a restoration of blood pressure following fluid administration.

Keywords: microcirculation, blood pressure, capillaries, fluid therapy, hypotension, hemodynamics. Introduction

7 Introduction

Fluid administration is the most frequently used treatment for all types of hemodynamic instability. Proper understanding of the indication is key because of reported potential harmful effects of fluid accumulation. Such unwanted side effects may not only be related to the chemical composition of the fluids, but also to the amount of fluids administered.(1-5) Today clinical decision making around the initiation of fluid therapy is still very difficult, as reflected by the large amount of potential triggers. The use of surrogates of impaired organ perfusion (e.g. hypotension, hyperlactatemia, oliguria, tachycardia) to identify patients that could benefit from fluid resuscitation is widely used in clinical practice, but still lacks proper scientific support. Combination of these markers with the use of (dynamic) macrohemodynamics parameters (e.g. passive leg-raise test, fluid challenge) has contributed to reduction in fluid overload. However, the concept of stroke volume optimisation and fluid responsiveness stills lacks certainty that this will reflect an improvement in organ perfusion. (6) Besides, these 'flow' triggers are restricted to a single aspect of oxygen transport: convection. They fail to reflect another important characteristic of oxygen transport: diffusion distance, i.e. the distance between red blood cells and tissue cells. Direct observation of the microcirculation, using hand held vital microscopes (HVM), has the potential to evaluate all aspects of organ perfusion and maybe regarded as gold standard. Alterations in microvascular blood flow, measured by HVM, are related to organ failure and increased mortality. Therefore, early detection and treatment hold potential for improvement in outcome. (7-15) It is conceivable that the absence of a possibility for the clinician to evaluate the effects of fluid therapy directly at the level of its place of action, the microcirculation, contributes to the persistence of existing controversies. Pranskunas et all. showed in a mixed ICU-population that patients with 'clinical signs of impaired organ perfusion,' accompanied by impaired microcirculatory blood flow, benefit from fluid resuscitation assessed on the basis of a reduction of clinical signs with concomitant increase in microcirculatory blood flow afterwards. At the same time patients with similar 'signs of impaired organ perfusion' and a normal microcirculatory blood flow failed to do so. (16) This study highlights the uncertainty about the value of so called 'clinical signs of impaired organ perfusion' as a surrogate for tissue hypoperfusion and a trigger for fluid administration. To further explore this important issue and address some limitations of the previous observation (e.g. mixed-population, comparison of multiple spots prior to and after fluid administration) we undertook this study to observe the response of the sublingual microcirculation to a standardized fluid bolus in one and the same sublingual location allowing evaluation of the response to the fluid bolus in single capillaries.

Methods

Patients

The study was performed as a single centre observational study and conducted between January 2016 and April 2017 in a mixed ICU of a tertiary teaching hospital. Local ethical committee waived the need for informed consent. The study was registered at ClinicalTrials.gov (NCT02675725).

All sedated on-pump cardiac surgery patients within2 hours after ICU admittance were eligible for inclusion whenever there was indication for a fluid challenge. This indication was determined by the attending physician based upon 'clinical signs of impaired organ perfusion': tachycardia (>100 beats/min), oliguria (<0.5 ml/kg/h), hypotension (mean arterial blood pressure (MAP) <60 mmHg), elevated oxygen extraction (central/mixed venous oxygen saturation (S(c)vO2) <60 %), impaired skin perfusion (> 5 degrees Celsius toe-to-central temperature difference)or hyperlactatemia (>2.2 mmol/L). Exclusion criteria included inability to obtain Incident dark field (IDF) images using HVM, such as maxillofacial surgery or oropharyngeal bleeding. Other reasons for exclusion were the presence of hemodynamic instability, to position the camera or movement of the patients during the measurement.

Protocol

After defining the indication for a fluid challenge by the attending physician the tip of the HVM was placed in the sublingual area of the patient, with the aid of a mechanical arm. After optimising the position, illumination, focus and pressure the fluid challenge was started. At predefined time-points IDF measurements were made (baseline, 5-10-15-20 minutes after start of the fluid challenge) in a single sublingual spot.

Macrohemodynamics measurements during the same time points were performed by continuous arterial blood pressure and a central venous line or a pulmonalis catheter continuous cardiac output (Vigilance; Edwards Lifesciences, Saint-Prex, Switzerland), depending on the type of operation.

The performed fluid challenge was 'standard-care' of the ICU, 250 ml crystalloid solution (Ringer's Lactate), given in 15 minutes (1000 ml/hour).

The following data were recorded at baseline: demographic details, arterial/venous blood gases with arterial lactate, available macrohemodynamic values, dosage inotropic and vasopressors, clinical signs of impaired organ perfusion, IDF-imaging.

7 5/10/15 minutes after starting the fluid challenge: available macrohemodynamic values, dosage inotropic and vasopressors, IDF-imaging and clinical signs of impaired organ perfusion. After 20 minutes: arterial/venous blood gases with arterial lactate, available macrohemodynamic values, dosage inotropic and vasopressors, clinical signs of impaired organ perfusion, IDF-imaging.

Cytocam-IDF imaging

Cytocam-IDF imaging (Braedius Medical, Huizen, The Netherlands) consists of a combination of IDF illumination with optical and technical features optimized for visualization of the microcirculation on organ surfaces. It uses incident dark field illumination with high-brightness LEDs with a very short illumination pulse time of 2 ms. The image acquisition and sensor are under computer control and electronically synchronized to the illumination pulses. (17) The digitally recorded images (6 seconds per movie) where blinded and first analysed on quality as recommended by the recently held international consensus. (18,19) Offline analysis was performed using the AVA 3.0 software package (MicroVision Medical, Amsterdam, the Netherlands) in compliance with the recommendations of the second consensus. (19) In each baseline movie five capillaries and two venules were selected. A pre-requisite for the selection of space-time diagrams (STD), used for quantitative measurement of RBC velocity, included adequate quality in both baseline and post-intervention video's. The vessels were drawn in by hand to optimize the space-time frames, only vessel segments between bifurcations were used. Tracing lines were manually drawn in and controlled for the maximum possible velocity that can be technically measured. (20)

Statistical analysis

Data are presented as median [inter quartile range] because of the small number of patients. Applicable test for paired data were used. Statistical analysis was performed with the Statistical Package for Social Sciences (SPSS 21, Chicago Illinois, USA). Power calculation was performed based on previous observations: an expected incidence of 66% of patients with a microcirculatory blood flow (MFI) < 2.6 AU with an expected increase in MFI of 8%. (16) Together with a one-side alpha (5%) and beta 80% yielded 22 patients. A p value < 0,05 was considered statistically significant.

Results

26 patients were included, 6 patients were excluded because of movement during the observation period. Baseline characteristics are presented in Table 1. At baseline 7 patients had an elevated lactate (> 2,2 mmol/L), 8 patients showed hypotension

Table 1. Baseline characteristics.

	n=20
Man/Female, n (% man)	10/9 (55%)
Age, years	72,5 [61-80]
Length, cm	172 [160-180]
Weight, kg	75 [72-89]
Apache IV score	45 [38-51]
Predicted mortality, %	1% [0.5-2.0]
Aorta-clamp time, min	58 [38-84]
Perfusion time, min	89 [66-115]
Perioperative fluid balance, L	1.9 [1.3-2.8]
Norepinephrine dose, n, µg/kg min	5, 0.04 [0.02-0.12]
Phenylephrine, dose, n, µg/kg min	8, 0.18 [0.15-0.26]
Dobutamine, dose, n, µg/kg min	3, 3.6 [2.2-3.6]
Nicardipine, dose, n, µg/kg min	2, 0.09 [0.05-0.09]

(MAP < 60 mm Hg), 10 patients showed a low ScVO2 (<60%), 13 patients had an high toe to central temperature difference (> 5 degrees Celsius). Oliguria and tachycardia were not present in this study group. In the total population there was no change in the total number of 'clinical signs of impaired organ perfusion' prior to and after fluid administration. (Table 2)

Primary endpoint.

At baseline 20% of the patients had a MFI < 2.6. In the total study population, MFI and other circulatory variables did not change significantly during the observation period. Patients with a low MFI (< 2.6) at baseline showed a non-significant trend to improvement of MFI in response to fluid administration. However, patients with a low MFI at baseline did not show a difference in the number of 'clinical signs of impaired organ perfusion' as compared to patients with a normal MFI at baseline. (Table 3). An increasing number of 'clinical signs of impaired organ perfusion' did not correspond with more microcirculatory alterations. However, in the subgroup of 8 patients with hypotension at baseline, there was a significant increase in blood pressure following fluid administration (mean arterial pressure 54 mm Hg [48-57] vs 63 mm Hg [52-65]; p < 0,05) and significant increase in total vessel density (18,5 mm2/mm2 [14,3-23,4] vs 18,9 mm2/mm2 [14,2-23,4], p < 0,05) and red blood cell velocity in two of the five measured capillaries (Figure 1). In the subgroup of patients with an elevated toe-to-core temperature difference there were no significant changes in microvascular variables.

Table 2. Results: baseline vs after fluid bolus

	Before	After	p
Hemoglobin, mmol/L	6.1 [5.4-6.6]	6.0 [5.5-6.4]	0.81
Hematocrit, %	29 [26-33]	30 [28-32]	0.23
Shock signs, n	2 [1.5-3]	2 [1-3]	0.43
Diuresis, ml per 20 min	40 [28-70]	35 [25-40]	0.11
Lactate, mmol/L	1.8 [1.5-2.4]	1.9 [1.3-2.5]	0.47
Δ Temperature	5.5 [4.4-6.7]	5.4 [4.1-6.3]	0.03
Heart rate, bpm	90 [90-90]	90 [90-90]	0.07
Systolic blood pressure, mmHg	89 [73-104]	89 [83-109]	0.08
Diastolic blood pressure, mmHg	50 [44-55]	51 [47-55]	0.90
Mean arterial blood pressure, mmHg	61 [54-69]	63 [59-69]	0.27
Central venous pressure	9 [6-11]	8 [6-10]	0.74
Cardiac Index, L/m2 (n=10)	1.6 [1.2-1.8]	1.7 [1.4-1.9]	0.14
S(c)VO2, %	61 [50-66]	57 [50-61]	0.05
Norepinephrine dose, n, µg/kg min	5, 0.04 [0.02-0.12]	5, 0.02 [0.01-0.05]	0.18
Phenylephrine, dose, n, µg/kg min	8, 0.18 [0.15-0.26]	7, 0.22 [0.1(8-0.25]	0.66
Dobutamine, dose, n, µg/kg min	3, 3.6 [2.2-3.6]	3, 3.6 [2.2-3.6]	1
Nicardipine, dose, n, µg/kg min	2, 0.09 [0.05-0.09]	2, 0.09 [0.05-0.09]	1
MFI small, AU	3 [2.75-3]	3 [2.75-3]	0.50
MFI large, AU	3 [3-3]	3 [3-3]	0.18
Total vessel density, mm2/mm2	20.2 [17.9-21.6]	20.5 [15.9-22.1]	0.69
Percentage perfused vessel, %	97 [91-100]	98 [95-100]	0.05
Perfused vessel density, mm2/mm2	20.1 [16.6-20.9]	19.6 [14.9-21.6]	0.48
Capillary 1, µm/sec	348 [316-479]	425 [287-563]	0.25
Capillary 2, µm/sec	350 [216-417]	292 [220-359]	0.59
Capillary 3, µm/sec	354 [219-524]	370 [279-486]	0.48
Capillary 4, µm/sec	350 [258-534]	387 [276-615]	0.20
Capillary 5, µm/sec	338 [242-497]	340 [217-412]	0.12
Venule 1, µm/sec	463 [318-521]	321 [220-588]	0.37
Venule 2, µm/sec	408 [338-518]	355 [312-459]	0.59
Massey Score	1 [0 -1]	1 [0.3-1.0]	

Non-parametric, paired data (Wilcoxon.) Δ Temperature: toe to central temperature difference.

Cardiac output was measured in 10 patients. Cardiac-output responders, defined as stroke volume >10% increase (n=3) in response to fluid administration, showed no microcirculatory improvement. Extended tables are provided as supplemental data. Receiver operator curves (ROC) of all surrogates for prediction of microcirculatory alterations showed an area under the curve of less than 0.7, ROC's for the prediction of microcirculatory fluid responsiveness were also non-significant.

Table 3. Results: Low versus normal MFI and the clinical signs of impaired organ perfusion at baseline.

	MFI < 2.6 (n=4)	MFI > 2.6 (n=16)	p
Lactate, mmol/L	1.7 [1.4-2.5]	1.8 [1.5-2.4]	0.62
Mean arterial bloodpressure, mmHg	58 [50-62]	63 [56-71]	0.29
S(c)VO2, %	56 [53-67]	62 [48-66]	0.82
Δ Temperature	5.3 [3.8-7.0]	5.5 [4.5-6.7]	0.82
Diuresis, ml per 20 min	28 [13-40]	43 [33-81]	0.10
MFI small, AU	2.4 [2.1-2.5]	3 [2.8-3]	< 0.05
MFI large, AU	2.9 [2.8-3]	3 [3-3]	0.15
Total vessel density, mm2/mm2	19.8 [12.8-19.8]	20.3 [17.9-22.0]	0.65
Percentage perfused vessel, %	89 [89-89]	99 [95-100]	0.13
Perfused vessel density, mm2/mm2	19.2 [11.4-19.2]	20.2 [16.9-21.1]	0.59
Capillary 1, µm/sec	442 [321-442]	343 [300-535]	0.73
Capillary 2, µm/sec	330 [216-330]	350 [219-433]	0.73
Capillary 3, µm/sec	601 [377-601]	329 [189-495]	0.10
Capillary 4, µm/sec	290 [162-290]	365 [313-590]	0.23
Capillary 5, µm/sec	333 [217-333]	342 [251-642]	0.66
Venule 1, µm/sec	310 [244-310]	474 [430-644]	0.29
Venule 2, µm/sec	437 [336-437]	408 [333-483]	0.91

Non-parametric, Mann-Whitney U., Δ Temperature: toe to central temperature difference.

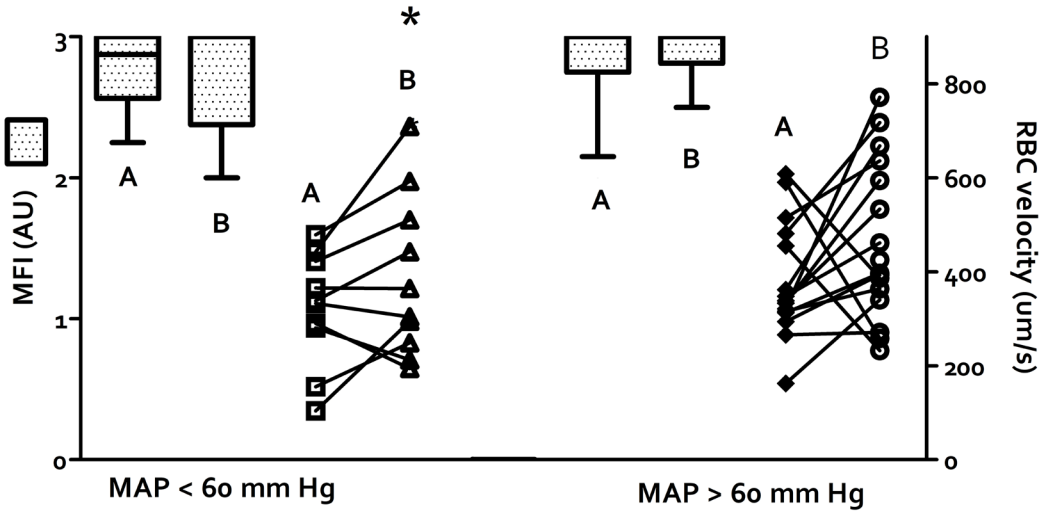


*Figure 1: Convective parameters of the microcirculation, hypotensive versus normotensive. Boxplots represent MFI, single lines represent individual capillaries. A: before fluid bolus, B: after fluid bolus. Microcirculatory blood flow (MFI); Red blood cell (RBC). * = p < 0.05*

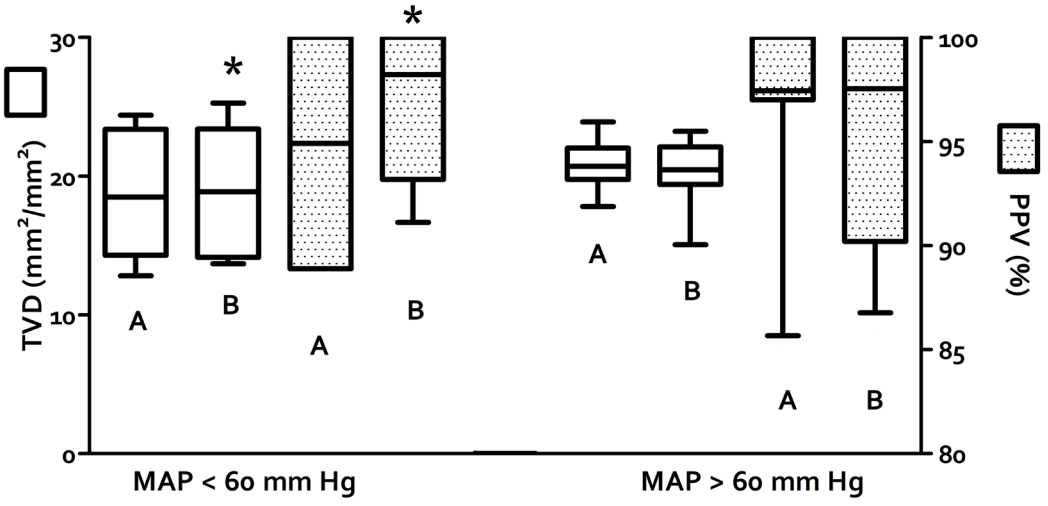


*Figure 2: Diffusion parameters of the microcirculation, hypotensive versus normotensive. Clear boxplots represent total vessel density (TVD), dotted boxplots represent percentage perfused vessels (PPV). A: before fluid bolus, B: after fluid bolus. * = p < 0.05.*

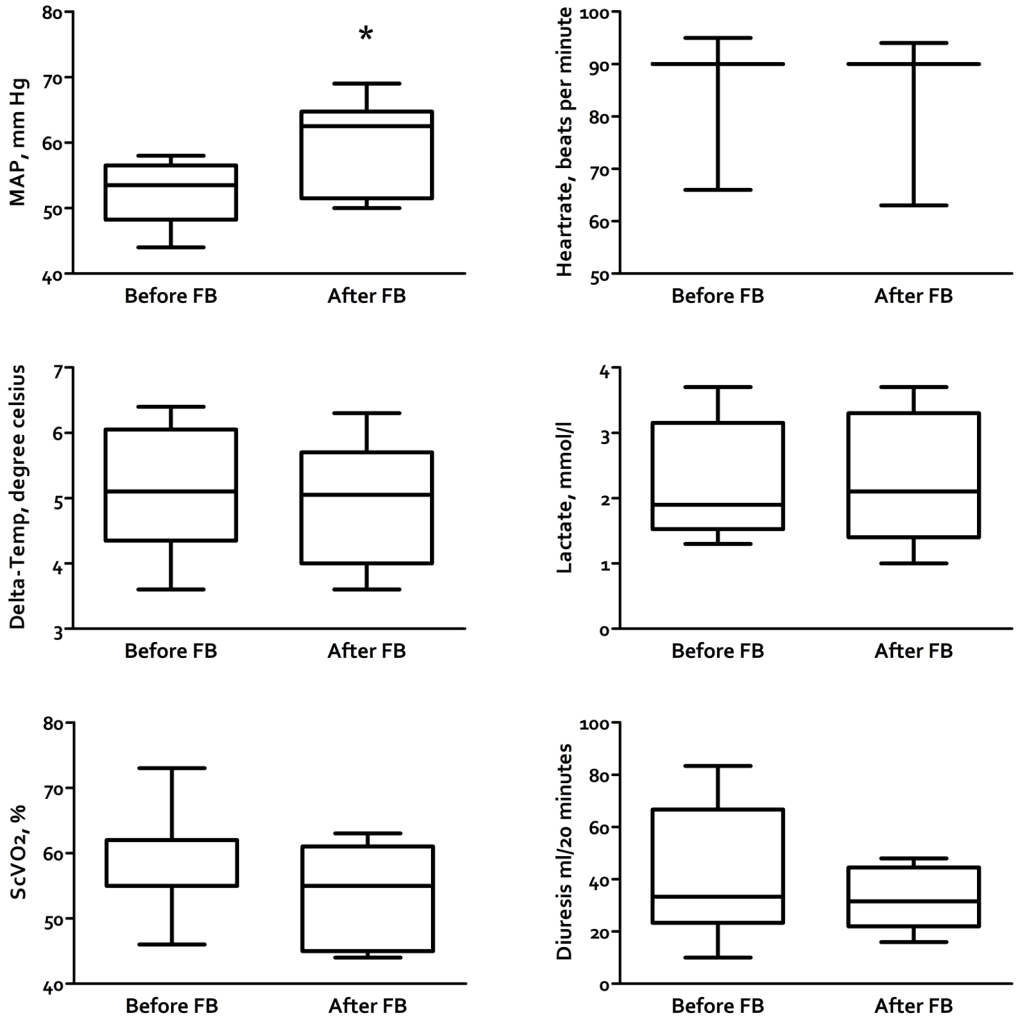


*Figure 3: Reaction of all surrogate markers of impaired organ perfusion in the hypotension group (MAP < 60 mm Hg at baseline).Mean arterial pressure (MAP), Fluid bolus (FB), toe-to-central temperature difference (delta-temp). * = p < 0.05*

Discussion

Main findings of the study include: a. Only 20% of patients had an abnormal MFI at baseline, despite the fact that all patients showed one or more 'signs of impaired organ perfusion'. b. After fluid administration MFI did not improve in the total study population. c. In the subgroup of patients with hypotension at baseline all patients showed a restoration of mean arterial blood pressure in combination with an increase in TVD and red blood cell velocity in individual capillaries after the fluid bolus. d. However, in the subgroup with an elevated toe-to-central temperature difference

or hyperlactatemia the number of 'surrogates of impaired organ perfusion' reduced significantly over time, without changes in the microcirculation. In patients with a low ScVO2 neither the parameter itself nor variables of microvascular perfusion changed in response to a fluid bolus.

To our knowledge there are no data reported on single-spot sublingual microcirculatory imaging in patients during fluid administration. There is also little data on 'surrogates of impaired organ perfusion'. Earlier data already suggested that 'surrogate markers of impaired organ perfusion' were not always reflected by microcirculatory abnormalities. Pranskunas et al. observed that in 66% of patients with 'clinical signs of impaired organ perfusion' an MFI < 2.6 could be detected. Moreover, the presence of such microcirculatory alterations increased the likelihood of a positive response to fluid administration, both in terms of attenuation of the clinical signs and a rise in MFI. (16) In the present study the percentage of patients with microcirculatory alterations at baseline was only 20%, despite the presence of 'signs of impaired organ perfusion' in all patients. Non-significant improvement of microcirculatory blood flow was restricted to patients with hypotension at baseline. The difference between the two studies lies clearly within the selected study population and the volume of the administered bolus. In the Pranskunas paper patients were provided with 500 ml of fluids within a time frame of 30 minutes. In our protocol patients received 250 ml of fluids within a time frame of 15 minutes. Furthermore, the previously published paper represented a mixed group of ICU patients with hemodynamic instability, whereas in our paper routine cardiac surgery patients were selected. However, both papers highlight a potential flaw in the concept of these so-called 'clinical signs of impaired organ perfusion': they potentially lack sensitivity as a trigger for fluid administration. With the exception of hypotension none of the surrogate markers of impaired organ perfusion improved in the course of the fluid challenge in conjunction with changes in microvascular variables, despite the detailed single-spot observation in individual capillaries. The need for solid triggers of fluid administration clearly lacks a gold standard.

Although conceptually the direct observation of the (sublingual) microcirculation is almost equal to the clinical idea of organ perfusion, this assumption is hampered by potential methodological flaws. Nevertheless, in different settings an immediate change in microvascular perfusion was observed in response to a swift (position-induced) fluid shift. (21-24) In the literature a mean arterial pressure of around 65 mm Hg is suggested for sepsis, there are no clear data about the optimal pressure for post-cardiac surgery patients. (25) Common practice is a mean arterial pressure

above 60-65 mm Hg. Data on the response of the microcirculation to an increase in blood pressure and consequent improvement in organ perfusion is limited to septic shock. In patients with a minimum MAP of 65 mmHg, further increment of blood pressure with the use norepinephrine was not reflected in the microcirculation. (26) (27-30) In postoperative cardiac surgery patients the data are limited to cardiopulmonary bypass, in which a further reduction of blood pressure below a MAP of 65 mmHg was not accompanied by changes in microvascular perfusion. However, it must be stressed that under these conditions blood flow is maintained irrespective of pressure. Effects in mortality and morbidity of intraoperative hypotension are well described. (31-37)

Despite the advantage of a prolonged single-spot observation in contrast to previous studies with snap-shot observations in multiple spots our study clearly has limitations. The amount of fluids administered was limited to 250ml in 15 min, in line with our standard of care. It is conceivable that the amount, and composition (crystalloids) contains the potential of underscoring both micro- and macro hemodynamic effects. In addition, effects of the fluid challenge may have occurred outside the scope of the limited observation period. However, the amount of fluid shift during passive leg raising or Trendelenburg position has been estimated to be comparable with our intervention. (38,39) Moreover, it is of note that in a subgroup of 3 patients with an increase in cardiac output of > 10% no changes in microvascular perfusion were observed. The issue of assessment of exact red blood cell velocity in individual capillaries also needs to be addressed. Not all vessels are suitable to construct space-time diagrams, depending on length, direction and focus. Furthermore there is a considerable setting-dependent range in red blood cell velocity reported in the literature. (40-43)

Finally this study raises many questions. Is hypotension, in the specific setting of cardiac surgery and defined as a MAP < 60 mmHg, a better surrogate or indicator of impaired organ perfusion than elevated lactate levels or elevated toe-to-central temperature difference? An additional vital issue is the clinical relevance of our observations. Is the restoration of 'clinical signs of impaired organ perfusion' simply a marker of recovery over time, or does fluid administration alter the course of recovery, and in what direction? Furthermore, the relationship between microvascular alterations and oliguria or tachycardia was not addressed in this study, since none of the patients fulfilled predefined criteria.

7 Conclusion

In a small post-cardiac surgery population, 'clinical signs of impaired organ perfusion' seems to lack sensitivity to adequately predict sublingual microvascular alterations, nor its response to fluid administration. However, in a subgroup of patients with hypotension at baseline, a significant increase in red blood cell velocity in individual capillaries was observed, as well as a significant increase in total vessel density, in conjunction with a restoration of blood pressure indicating that under these conditions fluid therapy is effective.

References

1. Payen D, de Pont AC, Sakr Y, Spies C, Reinhart K, Vincent JL et al. A positive fluid balance is associated with a worse outcome in patients with acute renal failure. Crit Care. 2008;12(3):R74.

2. Zhang L, Chen Z, Diao Y, Yang Y, Fu P. Associations of fluid overload with mortality and kidney recovery in patients with acute kidney injury: A systematic review and meta-analysis. J Crit Care. 2015;30(4):860.e7-13.

3. Teixeira C, Garzotto F, Piccinni P, Brienza N, Iannuzzi M, Gramaticopolo S et al. Fluid balance and urine volume are independent predictors of mortality in acute kidney injury. Crit Care. 2013;17(1):R14.

4. Prowle JR, Echeverri JE, Ligabo EV, Ronco C, Bellomo R. Fluid balance and acute kidney injury. Nature Reviews Nephrology. 2009;6(2):107-15.

5. Kelm DJ, Perrin JT, Cartin-Ceba R, Gajic O, Schenck L, Kennedy CC. Fluid overload in patients with severe sepsis and septic shock treated with early goal-directed therapy is associated with increased acute need for fluid-related medical interventions and hospital death. Shock. 2015;43(1):68-73.

6. Veenstra G, Ince C, Boerma EC. Direct markers of organ perfusion to guide fluid therapy: When to start, when to stop. Best Pract Res Clin Anaesthesiol. 2014;28(3):217-26.

7. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL et al. 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. 2008;34(12):2210-7.

8. Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL et al. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Ann Emerg Med. 2007;49(1):88-98, 98.e1.

9. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med. 2002;166(1):98-104.

10. Doerschug KC, Delsing AS, Schmidt GA, Haynes WG. Impairments in microvascular reactivity are related to organ failure in human sepsis. Am J Physiol Heart Circ Physiol. 2007;293(2):H1065-71.

11. Trzeciak S, Rivers EP. Clinical manifestations of disordered microcirculatory perfusion in severe sepsis. Crit Care. 2005;9 Suppl 4:S20-6.

12. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001;345(19):1368-77.

13. Scorcella C, Damiani E, Domizi R, Pierantozzi S, Tondi S, Carsetti A et al. MicroDAIMON study: Microcirculatory DAIly MONitoring in critically ill patients: a prospective observational study. Annals of Intensive Care. 2018;8(1):S13.

14. Vellinga NAR, Boerma EC, Koopmans M, Donati A, Dubin A, Shapiro NI et al. International study on microcirculatory shock occurrence in acutely ill patients. Crit Care Med. 2015;43(1):48-56.

15. Top APC, Ince C, de Meij N, van Dijk M, Tibboel D. Persistent low microcirculatory vessel density in nonsurvivors of sepsis in pediatric intensive care. Crit Care Med. 2011;39(1):8-13.

16. Pranskunas A, Koopmans M, Koetsier PM, Pilvinis V, Boerma EC. Microcirculatory blood flow as a tool to select ICU patients eligible for fluid therapy. Intensive Care Med. 2013;39(4):612-9.

17. 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 medicine experimental. 2015;3(1):40.

18. Massey MJ, Larochelle E, Najarro G, Karmacharla A, Arnold R, Trzeciak S et al. The microcirculation image quality score: development and preliminary evaluation of a proposed approach to grading quality of image acquisition for bedside videomicroscopy. J Crit Care. 2013;28(6):913-7.

19. Ince C, Boerma E, Cecconi M, De B, Shapiro N. 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. Intensive Care Med. 2018;(10):1.

20. Dobbe JGG, Streekstra GJ, Atasever B, van Zijderveld R, Ince C. Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. Med Biol Eng Comput. 2008;46(7):659-70.

21. Pottecher J, Deruddre S, Teboul JL, Georger JF, Laplace C, Benhamou D et al. Both passive leg raising and intravascular volume expansion improve sublingual microcirculatory perfusion in severe sepsis and septic shock patients. Intensive Care Med. 2010;36(11):1867-74.

22. Bemelmans RHH, Boerma EC, Barendregt J, Ince C, Rommes JH, Spronk PE. Changes in the volume status of haemodialysis patients are reflected in sublingual microvascular perfusion. Nephrol Dial Transplant. 2009;24(11):3487-92.

23. Bartels SA, Bezemer R, Milstein DMJ, Radder M, Lima A, Cherpanath TGV et al. The microcirculatory response to compensated hypovolemia in a lower body negative pressure model. Microvasc Res. 2011;

24. Xu J, Ma L, Sun S, Lu X, Wu X, Li Z et al. Fluid resuscitation guided by sublingual partial pressure of carbon dioxide during hemorrhagic shock in a porcine model. Shock. 2013;39(4):361-5.

25. Dellinger RP, Carlet JM, Masur H, Gerlach H, Calandra T, Cohen J et al. Surviving Sepsis Campaign guidelines for management of severe sepsis and septic shock. Crit Care Med. 2004;32(3):858-73.

26. Klinzing S, Simon M, Reinhart K, Bredle DL, Meier-Hellmann A. High-dose vasopressin is not superior to norepinephrine in septic shock. Crit Care Med. 2003;31(11):2646-50.

27. Dubin A, Pozo MO, Casabella CA, Pálizas F, Murias G, Moseinco MC et al. Increasing arterial blood pressure with norepinephrine does not improve microcirculatory blood flow: a prospective study. Crit Care. 2009;13(3):R92.

28. Jhanji S, Stirling S, Patel N, Hinds CJ, Pearse RM. The effect of increasing doses of norepinephrine on tissue oxygenation and microvascular flow in patients with septic shock. Crit Care Med. 2009;37(6):1961-6.

29. Thooft A, Favory R, Ribeiro Salgado D, Taccone FS, Donadello K, De Backer D et al. Effects of changes in arterial pressure on organ perfusion during septic shock. Crit Care. 2011;15(5):R222.

30. 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. 2010;36(12):2004-1

31. Fritz C, Kimmoun A, Vanhuyse F, Trifan BF, Orlowski S, Falanga A et al. High Versus Low Blood-Pressure Target in Experimental Ischemic Prolonged Cardiac Arrest Treated with Extra Corporeal Life Support. Shock. 2017;47(6):759-64

32. Holmgaard F, Vedel AG, Ravn HB, Nilsson JC, Rasmussen LS. Impact of mean arterial pressure on sublingual microcirculation during cardiopulmonary bypass-Secondary outcome from a randomized clinical trial. Microcirculation. 2018;25(5):e12459.

33. Maheshwari A, McCormick PJ, Sessler DI, Reich DL, You J, Mascha EJ et al. Prolonged concurrent hypotension and low bispectral index ('double low') are associated with mortality, serious complications, and prolonged hospitalization after cardiac surgery. Br J Anaesth. 2017;119(1):40-9.

34. Wesselink EM, Kappen TH, van Klei WA, Dieleman JM, van Dijk D, Slooter AJC. Intraoperative hypotension and delirium after on-pump cardiac surgery. Br J Anaesth. 2015;115(3):427-33.

35. Fischer GW, Levin MA. Vasoplegia during cardiac surgery: current concepts and management. Semin Thorac Cardiovasc Surg. 2010;22(2):140-4.

36. Maas JJ, Pinsky MR, de Wilde RB, de Jonge E, Jansen JR. Cardiac Output Response to Norepinephrine in Postoperative Cardiac Surgery Patients. Crit Care Med. 2013;41(1):143-50.

37. Kalmar AF, Allaert S, Pletinckx P, Maes JW, Heerman J, Vos JJ et al. Phenylephrine increases cardiac output by raising cardiac preload in patients with anesthesia induced hypotension. J Clin Monit Comput. 2018;

38. Jabot J, Teboul JL, Richard C, Monnet X. Passive leg raising for predicting fluid responsiveness: importance of the postural change. Intensive Care Med. 2009;35(1):85-90.

39. Rameau A, de With E, Boerma EC. Passive leg raise testing effectively reduces fluid administration in septic shock after correction of non-compliance to test results. Annals of intensive care. 2017;7(1):2.

40. Edul VSK, Ince C, Vazquez AR, Rubatto PN, Espinoza EDV, Welsh S et al. Similar Microcirculatory Alterations in Patients with Normodynamic and Hyperdynamic Septic Shock. Annals of the American Thoracic Society. 2016;13(2):240-7.

41. Pozo MO, Kanoore Edul VS, Ince C, Dubin A. Comparison of different methods for the calculation of the microvascular flow index. Critical care research and practice. 2012;2012:102483.

42. Edul VSK, 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. 2012;40(5):1443-8.

43. Dubin A, Pozo MO, Ferrara G, Murias G, Martins E, Canullán C et al. Systemic and microcirculatory responses to progressive hemorrhage. Intensive Care Med. 2009;35(3):556-64.

7 Supplemental tables:

	MAP	< 60 (n=8)		MAP	> 60 (n=12)	
	Before	After	p	Before	After	p
Hemoglobin, mmol/l	5.7 [5-6.4]	5.9 [4.9-6.4]	0.93	6.1 [5.7-6.7]	6.0 [5.6-6.7]	0.72
Hematocrit, %	29 [25-32]	30 [24-32]	0.68	29 [27-33]	30 [28-33]	0.26
Shock signs, n	3 [2-3]	2 [1-3]	0.10	2 [1-2]	2 [1-2]	0.71
Diuresis, ml per 20 min	33 [23-66]	32 [22-45]	0.61	43 [37-80]	35 [35-40]	0.11
Lactate, mmol/l	1.9 [1.5-3.2]	2.1 [1.4-3.3]	0.53	1.7 [1.5-2.4]	1.7 [1.2-2.4]	0.67
Δ Temperature	5.1 [4.4-6.1]	55 [45-61]	0.23	5.9 [4.4-7.8]	5.9 [4.1-7.9]	0.09
Heartrate, bpm	90 [90-90]	90 [90-90]	0.18	90 [90-90]	90 [89-90]	0.18
Systolic bloodpressure, mmHg	72 [64-83]	83 [79-87]	0.02	100 [90-109]	103 [87-112]	0.84
Diastolic bloodpressure, mmHg	44 [40-46]	50 [40-54]	0.03	54 [50-61]	51 [48-59]	0.10
Mean arterial bloodpressure, mmHg	54 [48-57]	63 [52-65]	0.02	67 [61-74]	66 [60-75]	0.42
Central venous pressure	9.5 [7.3-11]	9 [7-14]	0.16	8 [5-10]	8 [6-10]	0.26
Cardiac Index, l/m2 (n=10)	1.8 [1.5-1.8]	1.8 [1.4-1.9]	0.56	1.4 [1.2-1.9]	1.7 [1.4-2.0]	0.07
S(c)VO2, %	62 [55-62]	55 [45-61]	0.11	59 [48-68]	57 [51-61]	0.27
MFI small, AU	2.9 [2.6-3]	3 [2.4-3]	1	3 [2.75-3	3 [2.8-3]	0.34
MFI large, AU	3 [3-3]	3 [3-3]	1	3 [3-3]	3 [3-3]	0.18
Total vessel density, mm2/mm2	18.5 [14.3-23.4]	18.9 [14.2-23.4]	0.04	21 [20-22]	20 [19-22]	0.29
Percentage perfused vessel, %	95 [89-100]	98 [93-100]	0.11	97 [97-100]	98-100]	0.61
Perfused vessel density, mm2/mm2	17.3 [14.3-20.8]	18.5 [13.8-22.3]	0.04	20 [18-21]	20 [17-22]	0.48
Capillair 1, um/sec	385 [320-499][	510 [413-682]	0.04	338 [287-691]	349 [241-506]	0.89
Capillair 2, um/sec	287 [212-408]	251 [175-615]	1	350 [259-501]	312 [235-354]	0.78
Capillair 3, um/sec	554 [376-700]	452 [366-674]	0.14	325 [199-365]	302 [218-427]	0.78
Capillair 4, um/sec	410 [160-587]	442 [295-677]	0.04	348 [296-534]	376 [267-545]	0.89
Capillair 5, um/sec	314 [214-569]	372 [251-468]	0.24	344 [246-594]	327 [207-387]	0.26
Venuele 1, um/sec	548 [294-770]	262 [188-318]	0.14	459 [348-501]	416 [252-629]	1.00
Venuele 2, um/sec	405 [286-536]	335 [256-394	0.07	408 [360-506]	434 [322-793]	0.35

Mean arterial pressure(MAP). Non-parametric, paired data (Wilcoxon). Δ Temperature: toe to central temperature difference.

	Lactate	< 2.2 (n=13)		Lactate	> 2.2 (n=7)	
	Before	After	p	Before	After	p
Hemoglobin, mmol/l	6.1 [5.45-6.6]	5.9 [5.6-6.5]	0.46	5.9 [5-6.3]	6.0 [5.0-6.4]	0.42
Hematocrit, %	30 [27-33]	30 [28-33]	0.72	28 [25-32]	30 [25-32]	0.10
Shock signs, n	2 [1-3]	2 [1-3]	0.78	2.5 [2-3]	2.0 [1.5-3]	0.16
Diuresis, ml per 20 min	40 [23-67]	37 [22-45]	0.24	37 [30-80]	35 [25-40]	0.31
Lactate, mmol/l	1.6 [1.4-1.8]	1.5 [1.2-2.2]	0.72	2.7 [2.4-3.4]	2.35 [2.1-4.4]	0.04
Δ Temperature	5.6 [4.2-6.6]	5.7 [4.1-6.3]	0.16	5 [4.4-8.4]	4.8 [4.1-8.5]	0.11
Heartrate, bpm	90 [87-90]	90 [86-90]	0.10	90 [90-90]	90 [90-90]	0.32
Systolic bloodpressure, mmHg	90 [81-105]	92 [83-110]	0.18	76 [71-105]	88 [82-106]	0.25
Diastolic bloodpressure, mmHg	50 [44-54]	51 [45-55]	0.69	53 [44-62]	51 [49-55]	0.67
Mean arterial bloodpressure, mmHg	61 [56-67]	64 [56-70]	0.39	61 [53-75]	63 [62-69	0.50
Central venous pressure	8 [5.5-11]	8 [6-11]	0.26	9 [7-11]	9 [7-11]	0.16
Cardiac Index, l/m2 (n=7)	1.8 [1.4-1.8]	1.7 [1.4-1.9]	0.23	1.3 [1.2-1.8]	1.7 [1.3-1.9]	0.41
S(c)VO2, %	59 [54-65]	56 [51-61]	0.15	62 [47-70]	57 [45-63]	0.15
MFI small, AU	3 [2.6-3]	3 [2.6-3]	0.40	3 [2.8-3]	3 [3-3]	1
MFI large, AU	3 [3-3]	3 [2.9-3]	0.18	3 [3-3]	3 [3-3]	1
Total vessel density, mm2/mm2	20.7 [17.8-23.1]	20.2 [14.8-21.7]	0.29	20.2 [19.0-20.9]	20.3 [19.3-22]	0.12
Percentage perfused vessel, %	97 [90-100]	97 [91-100]	0.58	98 [92-99]	98 [94-100]	0.18
Perfused vessel density, mm2/mm2	20.3 [15.7-21.5]	19.8 [14.5-21.7]	0.77	19.6 [16.9-20.5]	19.3 [18.3-22.0]	0.17
Capillair 1, um/sec	455 [327-745]	425 [271-592]	1	332 [291-427]	428 [326-560]	0.08
Capillair 2, um/sec	350 [230-501]	348 [274-485]	0.75	347 [183-408]	251 [163-306]	0.23
Capillair 3, um/sec	354 [269-563]	386 [309-622]	0.87	351 [129-570]	295 [177-452]	0.23
Capillair 4, um/sec	363 [335-604]	387 [340-594]	0.74	301 [142-490]	346 [235-657]	0.12
Capillair 5, um/sec	371 [333-594]	362 [301-417]	0.18	273 [204-527]	225 [209-419]	0.35
Venuele 1, um/sec	459 [340-501]	393 [252-629]	0.61	532 [316-706]	265 [216-390]	0.14
Venuele 2, um/sec	380 [302-537]	434 [371-793]	0.35	452 [382-500]	312 [255-345]	0.07

Non-parametric, paired data (Wilcoxon.) Δ Temperature: toe to central temperature difference.

	Delta-T	< 5 (n=7)		Delta-T	> 5 (n=13)	
	Before	After	p	Before	After	p
Hemoglobin, mmol/l	5.9 [5.5-6.3]	5.0 [5.6-6.4]	0.34	6.2 [5.3-6.7]	6.0 [5.4-6.5]	0.32
Hematocrit, %	28 [26-32]	30 [28-32]	0.14	29 [27-33]	30 [27-33]	0.68
Shock signs, n	2 [1.8-2.2]	2 [0.8-2.3]	.16	2 [1-3]	2 [1-3]	0.78
Diuresis, ml per 20 min	47 [23-80]	35 [22-40]	.24	40 [33-67]	37 [28-45]	0.33
Lactate, mmol/l	2 [1.6-2.7]	2.1 [1.7-2.5]	.5	1.7 [1.5-2.4]	1.6 [1.2-2.4]	0.64
Δ Temperature	4.2 [29-4.4]	3.8 [2.5-4.2]	.03	6.2 [5.5-7.7]	6 [5.4-7.6]	0.47
Heartrate, bpm	90 [90-90]	90 [90-90]	1	90 [87-90]	90 [86-90]	0.07
Systolic bloodpressure, mmHg	88 [75-102]	85 [83-112]	.05	90 [71-106]	90 [82-109]	0.33
Diastolic bloodpressure, mmHg	53 [47-62]	54 [51-63]	.46	49 [44-54]	49 [45-52]	0.83
Mean arterial bloodpressure, mmHg	61 [55-75]	64 [63-79]	.21	61 [51-67]	62 [55-69]	0.51
Central venous pressure	10 [7-11]	9 [6-13]	.71	8 [6-10]	8 [7-9]	1
Cardiac Index, l/m2 (n=4)	1.4 [1.3-1.9]	1.8 [1.4-2.1]	.20	1.8 [1.2-1.8]	1.7 [1.4-1.8]	0.48
S(c)VO2, %	58 [48-62]	57 [54-59]	.5	62 [51-68]	56 [46-61]	0.04
MFI small, AU	3 [2.5-3]	3 [2.3-3]	.59	3 [2.8-3]	3 [2.8-3]	0.20
MFI large, AU	3 [3-3]	3 [3-3]	1	3 [3-3]	3 [3-3]	0.18
Total vessel density, mm2/mm2	20.0 [13.9-21.6]	19.7 [14.4-20.9]	.92	20.6 [18.1-22.1]	20.8 [18.1-22.5]	0.88
Percentage perfused vessel, %	98 [95-100]	97 [97-99]	.79	97 [90-100]	98 [90-100]	0.16
Perfused vessel density, mm2/mm2	19.8 [13.6-21.2]	19.3 [14.2-20.3]	.60	20.1 [17.2-20.9]	20.9 [16.3-22.5]	0.26
Capillair 1, um/sec	377 [314-445]	452 [286-725]	.25	348 [298-745]	425 [271-533]	0.50
Capillair 2, um/sec	347 [212-470]	312 [283-414]	.47	350 [227-411]	251 [175-359]	0.87
Capillair 3, um/sec	365 [331-700]	330 [188-559]	.12	318 [155-516]	378 [305-486]	0.60
Capillair 4, um/sec	301 [160-341]	352 [235-446]	.25	498 [344-604]	442 [294-668]	0.61
Capillair 5, um/sec	344 [243-460]	395 [279-480]	.89	333 [197-596]	301 [215-362]	0.06
Venuele 1, um/sec	452 [308-621]	306 [190-590]	.47	473 [352-672]	321 [252-588]	0.50
Venuele 2, um/sec	400 [353-508]	407 [315-483]	.59	423 [310-518]	345 [291-602]	0.75

Toe to central temperature difference (delta-T). Non-parametric, paired data (Wilcoxon). Δ Temperature: toe to central temperature difference.

	ScVO2 (n=10)	> 60%		ScVO2	< 60%	
	Before	After	p	Before	After	p
Hemoglobin, mmol/l	6.4 [5.7-6.7]	6.2 [5.9-7.0]	.37	5.7 [5.3-6.2]	5.6 [5.1-6.0]	0.10
Hematocrit, %	32 [26-33]	31 [30-35]	.14	29 [26-30]	28 [26-30]	1
Shock signs, n	2 [1-2]	2 [1-3]	.71	3 [2-3]	2 [1-3]	0.10
Diuresis, ml per 20 min	35 [25-40]	39 [30-44]	.61	57 [30-85]	35 [22-42]	0.12
Lactate, mmol/l	1.9 [1.6-2.9]	1.9 [1.4-2.8]	.24	1.7 [1.3-2.3	1.9 [1.2-2.4]	1
Δ Temperature	5.6 [4.7-6.5]	5.4 [4.5-6.1]	.05	5.3 [3.4-7.5]	5.3 [3.3-7.4]	0.37
Heartrate, bpm	90 [86-90]	90 [86-90]	.16	90 [90-90]	90 [90-90]	0.18
Systolic bloodpressure, mmHg	81 [72-96]	87 [82-109]	.08	96 [81-106]	91 [83-110]	0.55
Diastolic bloodpressure, mmHg	49 [44-54]	51 [49-54]	.64	50 [43-60]	50 [42-57]	0.86
Mean arterial bloodpressure, mmHg	59 [55-66]	63 [60-69]	.17	63 [53-73]	64 [53-72]	0.91
Central venous pressure	8 [7-11]	8 [7-13]	.32	10 [5-11]	9 [6-10]	0.48
Cardiac Index, l/m2	1.8 [1.4-3.1] (n=2)	1.9 [1.4-3.4] (n=2)	.32	1.4 [1.3-1.8] (n=10)	1.7[1.3-1.9] (n=10)	0.20
S(c)VO2, %	65 [62-71]	61 [55-66]	.02	51 [47-58]	52 [45-57]	0.76
MFI small, AU	2.9 [2.8-3]	3 [3-3]	.49	3 [2.4-3]	2.9 [2.5-3]	0.68
MFI large, AU	3 [3-3]	3 [3-3]	1	3 [2.9-3]	3 [2.7-3]	0.18
Total vessel density, mm2/mm2	19.8 [17.3-21.6]	20 [15.9 [22.2]	.31	20.3 [19.0-22.8]	20.5 [17.1-21.8]	0.52
Percentage perfused vessel, %	97 [94-99]	99 [98-100]	.03	99 [89-100]	97 [91-99]	0.87
Perfused vessel density, mm2/mm2	19.2 [15.8-21.2]	19.7 [14.6-21.9]	.24	20.2 [17.4-20.7]	19.4 [15.6-21.5]	0.89
Capillair 1, um/sec	385 [295-532]	486 [256-577]	.08	333 [316-479]	393 [304-592]	0.87
Capillair 2, um/sec	350 [210-400]	251 [185-320]	.23	330 [216-438]	334 [263-485]	0.75
Capillair 3, um/sec	417 [189-520]	355 [260-455]	.46	333 [318-602]	370 [255-649]	0.75
Capillair 4, um/sec	362 [155-590]	276 [235-680]	.60	338 [313-515]	397 [365-594]	0.24
Capillair 5, um/sec	251 [164-449]	301 [209-423]	.23	345 [333-642]	353 [225-417]	0.40
Venuele 1, um/sec	613 [444-728]	309 [236-502]	.23	430 [300-474]	357 [206-636]	0.92
Venuele 2, um/sec	398 [260-517	315 [273-719]	.89	408 [369-536]	421 [368-471]	0.47

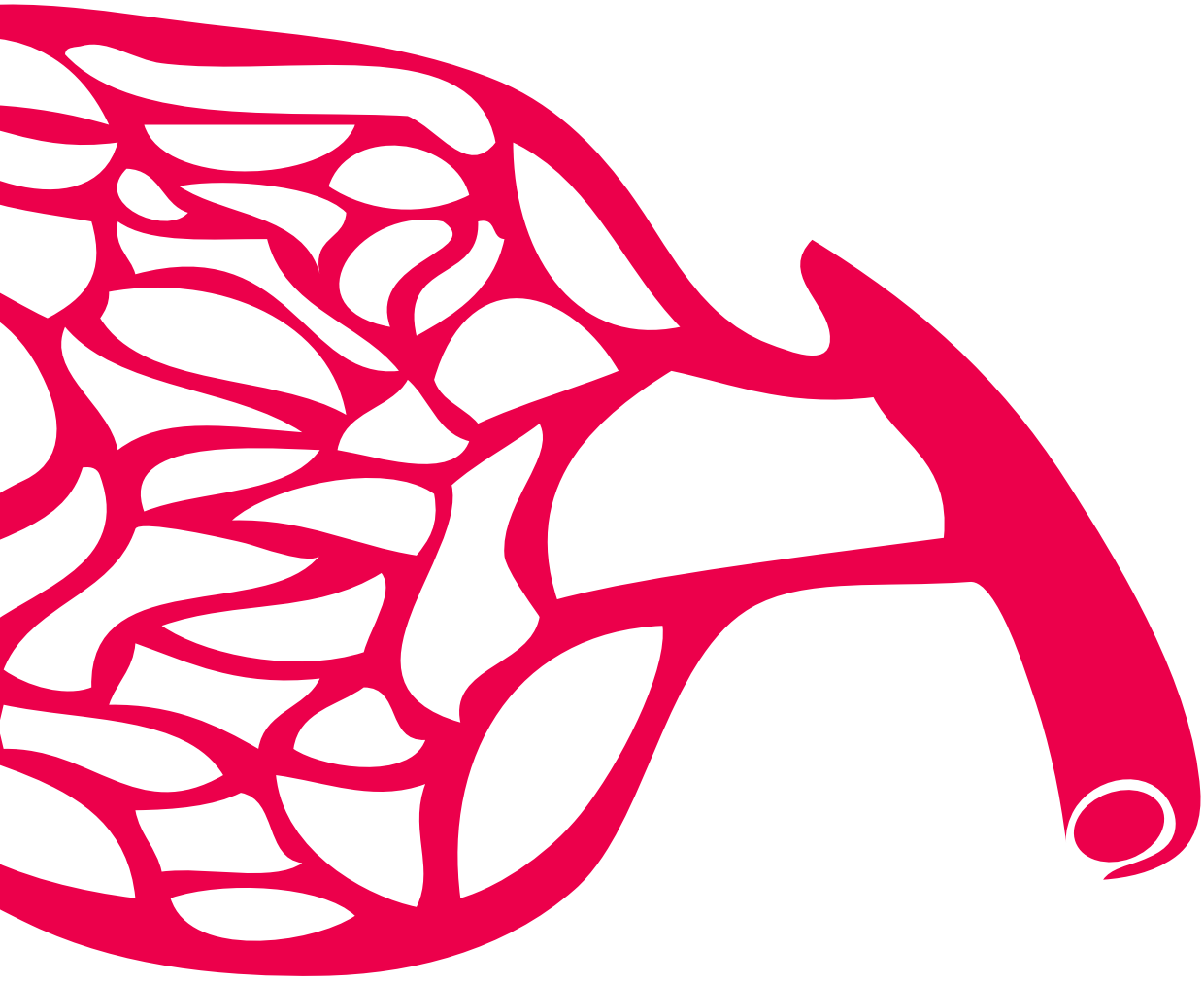
Non-parametric, paired data (Wilcoxon.) Δ Temperature: toe to central temperature difference.

MFI specific.

	MFI < 2.6 (n=4)		
	Baseline	After FC.	p
Lactate, mmol/l	1.7 [1.4-2.5]	2.0 [1.3-2.5]	1
Mean arterial bloodpressure, mmHg	58 [50-62]	64 [56-67]	0.14
S(c)VO2, %	56 [53-67]	55 [46-61]	0.20
Δ Temperature	5.3 [3.8-7.0]	5.3 [3.8-6.9]	0.28
Diuresis, ml per 20 min	28 [13-40]	47 [27-57]	0.07
MFI small, AU	2.4 [2.1-2.5]	2.6 [2.3-3]	0.10
MFI large, AU	2.9 [2.8-3]	2.9 [2.6-3]	0.32
Total vessel density, mm2/mm2	19.8 [12.8-19.8]	20 [13.7-20]	0.11
Percentage perfused vessel, %	89 [89-89]	95 [91-95]	0.11
Perfused vessel density, mm2/mm2	19.2 [11.4-19.2]	19.7 [13.0-19.7]	0.11
Capillair 1, um/sec	442 [321-442]	709 [592-709]	0.11
Capillair 2, um/sec	330 [216-330]	448 [290-448]	0.66
Capillair 3, um/sec	601 [377-601]	354 [156-354]	0.29
Capillair 4, um/sec	290 [162-290]	340 [194-340]	0.29
Capillair 5, um/sec	333 [217-333]	326 [2-4-326]	1
Venuele 1, um/sec	310 [244-310]	220 [180-220]	0.29
Venuele 2, um/sec	437 [336-437]	407 [407-407] (n=1)	
	MFI > 2.6 (n=16)		
	Baseline	After FC	p
Lactate, mmol/l	1.8 [1.5-2.4]	1.9 [1.3-2.5]	0.39
Mean arterial bloodpressure, mmHg	63 [56-71]	63 [59-70]	0.57
S(c)VO2, %	62 [48-66]	57 [50-61]	0.12
Δ Temperature	5.5 [4.5-6.7]	5.4 [4.3-6.3]	0.06
Diuresis, ml per 20 min	43 [33-81]	34 [25-40]	0.01
MFI small, AU	3 [2.8-3]	3 [3-3]	0.73
MFI large, AU	3 [3-3]	3 [3-3]	0.32
Total vessel density, mm2/mm2	20.3 [17.9-22.0]	20.5 [17.4-21.8]	0.65
Percentage perfused vessel, %	99 [95-100]	98 [94-100]	0.51
Perfused vessel density, mm2/mm2	20.2 [16.9-21.1]	19.4 [15.6-21.6]	0.97
Capillair 1, um/sec	343 [300-535]	379 [261-474]	0.80
Capillair 2, um/sec	350 [219-433]	292 [197-348]	0.37
Capillair 3, um/sec	329 [189-495]	386 [284-471]	0.95
Capillair 4, um/sec	365 [313-590]	392 [285-644]	0.29
Capillair 5, um/sec	342 [251-642]	353 [220-440]	0.09
Venuele 1, um/sec	474 [430-644]	405 [267-619]	0.58
Venuele 2, um/sec	408 [333-483]	345 [310-471]	0.89

Non-parametric, paired data (Wilcoxon.) Δ Temperature: toe to central temperature difference.

7



CHAPTER 8

FUTURE PERSPECTIVES

8 This chapter integrates all of the earlier described main conclusions and gives suggestions for further research in the (hopefully) near future.

The introduction summarises the importance of the doctor s attention to the circulation of a patient in shock. Circulation is more than the pressure and volume per minute. It depends on adequate management of flow on the level of the true capillaries, the smallest blood vessels in the human (and animal) body. (1-4) Microvascular alterations are impeccably associated with disease outcome and morbidity, with or without macrohaemodynamic attenuations. (5-7) Persistent or recurring abnormalities are present and of importance. Fast recovery of these alterations will prevent organ failure. (8-10) We therefore must address our attention from the macrocirculation to the microcirculation.

Fluid administration is the cornerstone of shock therapy. The early treatment of the shock has been shown to improve outcomes. (11) Even so, fluid administration reduces complications after major surgery. (12) However, doctors are neglecting the shadowed side of fluid therapy and are constantly seduced to give more because of the large amount of indirect surrogate markers of organ perfusion. In chapter two, we outline the different surrogate markers and the pros and cons of each trigger. We explain that several of these surrogate markers give limited information about the potential beneficial effects of fluid therapy, which is the expectation of improving microcirculatory blood flow with the aim of improving oxygen delivery to the parenchymal cells. (13) Adequate fluid management depends on not only clear indications but also the evaluation and timing of cessation of fluid therapy. This review article was the start of the journey to gain more knowledge about the possibility of microcirculatory-guided fluid therapy, improved by measuring convective flow and diffusion abnormalities.

Improving the technical possibilities of the imaging technique, the evolution to a so-called third-generation camera, is described in chapter three and is based upon an earlier technique called incident dark-field imaging: surrounding the tip of the light guide with light-emitting diodes to create dark-field illumination. (14) This technique uses a set of high-resolution lenses and computer-controlled illumination and imaging, resulting in a much better image quality and visualisation of more capillaries than that of previous devices. Together with the better handling of the probe, these features will allow the technique to become a better bedside tool. However, the ideal measurement must provide information about the indication, evaluation and cessation of fluid therapy.

Chapter three originated from the clinical perspective that there is an independent association between a negative fluid balance and decreased 90-day mortality. (15) Therefore, optimal fluid management is key in the treatment of ICU-patients. Unfortunately, in daily practice, the assessment of the volume status of an ICU-patient is difficult, and the determination of the tipping point from beneficial to detrimental fluid administration lacks guidelines. Previous studies suggested that intravascular hypovolaemia is reflected by a decrease in microcirculatory blood flow, and this was not detected by conventional methods. (16,17) The aim of our study was to assess microcirculatory blood flow changes during negative fluid balance ultrafiltration in patients with oliguric acute kidney injury and treatment with renal replacement therapy (RRT). The result was that, despite a negative fluid balance and vasopressor use, the microcirculatory blood flow remained normal. Interestingly, the heart rate decreased with a preservation of blood pressure and an improvement in the PaO_2/FIO_2 ratio, suggesting that the low rate of ultrafiltration used does not compromise the microcirculation and leads to improvement of the patient. An alternative reason is that the method does not track the changes adequately.

Different from the ICU patients are the patients on haemodialysis. Treatment a few times per week in a short period of time implicates more rapid fluid shifts, and one of the complications from this is so-called intradialytic hypotension. Earlier data suggested a significant decrease in microcirculatory blood flow during haemodialysis with linear ultrafiltration and without signs of hypotension. These microcirculatory alterations recovered during a Trendelenburg position (auto-transfusion). (16) We performed a comparable study with two extra ultrafiltration methods. The results showed that isolated ultrafiltration (1.7 L/hour) could induce a significant decrease in the microcirculatory blood flow. Both linear and haematocrit-controlled ultrafiltration did not show a decrease. More importantly, a great influence of non-flow-mediated mechanisms for microvascular derangements, such as haemodialysis-induced inflammation and hypercoagulation, were ruled out. In addition, the technique appears to be able to detect rapid (negative) fluid shifts and is possibly suited for the evaluation of fluid administration or removal.

The topic of fluid administration was bundled together in chapters five and six. At the same time, there was a theoretical model for microcirculatory guided fluid therapy. (7,13,18) Previous literature suggested that low microcirculatory blood flow (defined as < 2.6 AU) can benefit from fluid administration. (19) This specific study demonstrates the uncertainty about the value of so-called clinical signs of impaired organ perfusion. Furthermore, fluid therapy, when given without the improvement

8 of microcirculatory blood flow, can potentially promote oedema formation with subsequent reduction in oxygen diffusion. (20) These efforts resulted in two studies; in chapter six, we compared the diffusion distance of two separate groups of ICU patients, mimicking hypovolaemia by using a post-cardiac surgery group and a stabilised (hypervolaemic) septic shock group. Bio-impedance data showed a clear separation in fluid status between the two groups. There appeared to be a significant correlation between the amount of fluids administered and the capillary density in the cardiac surgery patients, with an optimum in the third quartile. However, this correlation was absent in the septic patients, suggesting a lack of haemodynamic coherence between the macrocirculation and the microcirculation under these conditions. More importantly, despite a normalisation of macrohaemodynamic variables, the diffusion distance did not reach the level as observed in the healthy volunteers. The fact that these maximum values showed a significant decline in the highest cumulative fluid balance quartile in cardiac surgery patients is in line with the present theory. After the recruitment of capillaries and restoration of flow, oedema formation further limits and even reduces the capillary density. This finding suggests a ceiling value for the recruitment of capillaries by fluid resuscitation with crystalloids and carries the potential to serve as an endpoint for fluid resuscitation.

In chapter six, we replicated previous observations and addressed some limitations: a mixed population and multiple measuring spots. We measured the response of the sublingual microcirculationto a fluid bolus at one spot for 20 minutes. This study demonstrated 20% microvascular alterations at baseline, despite the fact that all patients showed signs of impaired organ perfusion pre-intervention. In the total study population, there was no improvement in the microcirculatory blood flow after the fluid challenge. Surprisingly, patients with hypotension at baseline showed a restoration of mean arterial blood pressure in combination with an increase in total vessel density and red blood cell velocity in individual capillaries. This study confirmed the limited information the signs of impaired organ perfusion holds and that the technique can track small amounts of fluid administration. The clinical value of this study is more extensive, as it potentially narrows the indications for fluid therapy in patients after cardiac surgery. Potentially, patients will have an earlier negative fluid balance with better outcome. (15) Together, these studies highlight the benefits of using sublingual microcirculatory imaging for fluid administration: only a low microcirculatory blood flow improves with fluid administration, simultaneously with a possible recruitment of capillaries to optimise diffusion distance. However, after the restoration of flow, oedema formation further limits and possibly reduces the (functional) capillary density. Imaging of the microvascular perfusion can predict

whether fluid administration is indicated, give a direct evaluation of the effect and provide an early warning of the side-effects of fluid therapy.

Future perspectives

Unfortunately, as it can be deduced, the values that hold information about the perfusion are variable per underlying shock type, and sublingual observations will not be followed in other tissue regions. Therefore, the absence of sublingual alterations does not rule out alterations in other regions. There are still a few flaws in bedside microcirculation measurements and offline analysis, and there are no real-time oxygen supply and demand measurements.

The real-time measurements of microcirculatory values are still the missing holy grail for critics. Until now, the microcirculatory blood flow index has only been an eye-balling method that gives information about the convective flow in the microcirculation.(21) Diffusion capacity can be (validated) calculated only by offline analysis, despite some studies suggesting otherwise. The use of invalidated programs in different versions is undesirable. (22-24) However, these offline data are required to eventually measure the real oxygen supply and demand. For this calculation, red blood cell velocity, oxygen saturation and haematocrit measurements are necessary. The evolution of hardware and software takes time, but it will be a major leap for this technique and is required to move beyond the research field. (25-27)

The number of potential interventions to improve the microcirculation during shock is limited. One intervention is the extensively described fluid therapy. This thesis has mainly focused on the use of crystalloids. The use of semisynthetic colloids has been very restricted over the last few years because of the potential harmful effects. (28) Albumin may improve the survival in septic patients, but it is possibly associated with an increased risk for acute kidney injury in cardiac surgery. (28-32) Unfortunately, most studies lack some form of infusion protocol or proper haemodynamic endpoint. It is not unimaginable that fluids were given without a microcirculatory problem, thereby increasing the chance for harmful effects, particularly an increased diffusion distance by haemodilution and tissue oedema escalating to kidney failure. It is of notice that colloids are more effective in fluid expansion and that they have a low capillary leakage (even with an impaired barrier function) combined with a higher viscosity. Thereby, colloids are intrinsically better for microcirculatory recruitment. (33) Further research should focus on choice of the fluid combined with the right indication, together with the determination of microcirculatory physiological reserve. (34-36).

8 Lastly, the improvement of chemical interventions for the diffusion distance by improving the shunting and thereby recruitment of weak microcirculatory units should be mentioned. (37) Potassium-channel inhibitors may increase the systemic vascular resistance in vasoplegic shock, thereby saving the exogenous vasoconstrictor dosage. High-dosage exogenous vasoconstrictors are known for provoking hypoperfusion of the intestinal microcirculation. This hypoperfusion contributes to the translocation of bacteria and toxins to the circulation via breakdown of the gut barrier function. (38) Venous vasodilators, as a counter-mechanism, may dilate the post-capillary venules, thereby increasing the microcirculatory capillary flow and decreasing the trans-capillary pressure gradient. It is this pressure gradient that causes tissue oedema and an increased diffusion distance. Additionally, it is possible that dilatators improves the heterogeneity of flow in septic shock. (39) Amongst them is ketanserin, which increases the microcirculatory blood flow in septic shock patients with a low microcirculatory blood flow after resuscitation. (40) This thesis adds to the understanding of the applicability of microcirculatory-guided fluid therapy in ICU patients and how it can be additive to the contemporary methods. Further research is required to discover whether this view of circulation can be beneficial for patients, and further technological development is necessary to overcome certain limitations.

References

1. Krogh A. The supply of oxygen to the tissues and the regulation of the capillary circulation. J Physiol. 1919;52(6):457-74.

2. Krogh A. Studies on the physiology of capillaries: II. The reactions to local stimuli of the blood-vessels in the skin and web of the frog. J Physiol. 1921;55(5-6):412-22.

3. Rogers F, Dunn R, Barrett J, Merlotti G, Sheaff C, Nolan P. Alterations of capillary flow during sepsis. Circ Shock. 1985;15(2):105-10.

4. Walley KR. Heterogeneity of oxygen delivery impairs oxygen extraction by peripheral tissues: theory. J Appl Physiol. 1996;81(2):885-94.

5. Vellinga NAR, Boerma EC, Koopmans M, Donati A, Dubin A, Shapiro NI et al. Mildly elevated lactate levels are associated with microcirculatory flow abnormalities and increased mortality: a microSOAP post hoc analysis. Crit Care. 2017;21(1):255.

6. De Backer D, Creteur J, Preiser JC, Dubois MJ, Vincent JL. Microvascular blood flow is altered in patients with sepsis. Am J Respir Crit Care Med. 2002;166(1):98-104.

7. Ince C. Hemodynamic coherence and the rationale for monitoring the microcirculation. Crit Care. 2015;19 Suppl 3:S8.

8. De Backer D, Donadello K, Sakr Y, Ospina-Tascon G, Salgado D, Scolletta S et al. Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. Crit Care Med. 2013;41(3):791-9.

9. Trzeciak S, Dellinger RP, Parrillo JE, Guglielmi M, Bajaj J, Abate NL et al. Early microcirculatory perfusion derangements in patients with severe sepsis and septic shock: relationship to hemodynamics, oxygen transport, and survival. Ann Emerg Med. 2007;49(1):88-98, 98.e1.

10. Trzeciak S, McCoy JV, Phillip Dellinger R, Arnold RC, Rizzuto M, Abate NL et al. 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. 2008;34(12):2210-7.

11. Rivers E, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B et al. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001;345(19):1368-77.

12. Pearse R, Dawson D, Fawcett J, Rhodes A, Grounds RM, Bennett ED. Early goal-directed therapy after major surgery reduces complications and duration of hospital stay. A randomised, controlled trial [ISRCTN38797445]. Crit Care. 2005;9(6):R687-93.

13. Ince C. The rationale for microcirculatory guided fluid therapy. Curr Opin Crit Care. 2014;20(3):301-8.

14. Sherman H, Klausner S, Cook WA. Incident dark-field illumination: a new method for microcirculatory study. Angiology. 1971;22(5):295-303.

15. RENAL Replacement Therapy Study Investigators, Bellomo R, Cass A, Cole L, Finfer S, Gallagher M et al. An observational study fluid balance and patient outcomes in the Randomized Evaluation of Normal vs. Augmented Level of Replacement Therapy trial. Crit Care Med. 2012;40(6):1753-60.

16. Bemelmans RHH, Boerma EC, Barendregt J, Ince C, Rommes JH, Spronk PE. Changes in the volume status of haemodialysis patients are reflected in sublingual microvascular perfusion. Nephrol Dial Transplant. 2009;24(11):3487-92.

17. Bartels SA, Bezemer R, Milstein DMJ, Radder M, Lima A, Cherpanath TGV et al. The microcirculatory response to compensated hypovolemia in a lower body negative pressure model. Microvasc Res. 2011;

18. Ospina-Tascon G, Neves AP, Occhipinti G, Donadello K, B G, Simion D et al. Effects of fluids on microvascular perfusion in patients with severe sepsis. Intensive Care Med. 2010;36(6):949-55.

19. Pranskunas A, Koopmans M, Koetsier PM, Pilvinis V, Boerma EC. Microcirculatory blood flow as a tool to select ICU patients eligible for fluid therapy. Intensive Care Med. 2013;39(4):612-9.

20. Hanson JP, Lam SWK, Mohanty S, Alam S, Pattnaik R, Mahanta KC et al. Fluid resuscitation of adults with severe falciparum malaria: effects on Acid-base status, renal function, and extravascular lung water. Crit Care Med. 2013;41(4):972-81.

21. Boerma EC, Mathura KR, van der Voort PHJ, Spronk PE, Ince C. Quantifying bedside-derived imaging of microcirculatory abnormalities in septic patients: a prospective validation study. Crit Care. 2005;9(6):R601-6.

22. Carsetti A, Aya HD, Pierantozzi S, Bazurro S, Donati A, Rhodes A et al. Ability and efficiency of an automatic analysis software to measure microvascular parameters. J Clin Monit Comput. 2017;31(4):669-76.

23. Sharawy N, Mukhtar A, Islam S, Mahrous R, Mohamed H, Ali M et al. Preliminary clinical evaluation of automated analysis of the sublingual microcirculation in the assessment of patients with septic shock: Comparison of automated versus semi-automated software. Clin Hemorheol Microcirc. 2017;67(3-4):489-98.

24. Boerma EC, Scheeren TWL. Digging into the microcirculation: the rush for gold may excavate apples and oranges. J Clin Monit Comput. 2017;31(4):665-7.

25. Arend S, Ince C, Van Assen M, Fevzi T, Hilty M. A software tool to quantify capillary hematocrit and microvascular hemodilution in sublingual incident dark field microscopy video clips. 2018.

26. Takala J, Jakob SM. Shedding light on microcirculation? Intensive Care Med. 2009;35(3):394-6.

27. Mik EG, Johannes T, Fries M. Clinical microvascular monitoring: a bright future without a future? Crit Care Med. 2009;37(11):2980-1.

28. Crystalloid versus Hydroxyethyl Starch Trial (CHEST) Management Committee. The Crystalloid versus Hydroxyethyl Starch Trial: protocol for a multi-centre randomised controlled trial of fluid resuscitation with 6% hydroxyethyl starch (130/0.4) compared to 0.9% sodium chloride (saline) in intensive care patients on mortality. Intensive Care Med. 2011;37(5):816-23.

29. Frenette AJ, Bouchard J, Bernier P, Charbonneau A, Nguyen LT, Rioux JP et al. Albumin administration is associated with acute kidney injury in cardiac surgery: a propensity score analysis. Crit Care. 2014;18(6):602.

30. SAFE Study Investigators, Finfer S, McEvoy S, Bellomo R, McArthur C, Myburgh J et al. Impact of albumin compared to saline on organ function and mortality of patients with severe sepsis. Intensive Care Med. 2011;37(1):86-96.

31. Caironi P, Tognoni G, Masson S, Fumagalli R, Pesenti A, Romero M et al. Albumin Replacement in Patients with Severe Sepsis or Septic Shock. New England Journal of Medicine. 2014;370(15):1412-21.

32. Heming N, Elatrous S, Jaber S, Dumenil AS, Cousson J, Forceville X et al. Haemodynamic response to crystalloids or colloids in shock: an exploratory subgroup analysis of a randomised controlled trial. BMJ open. 2017;7(10):e016736.

33. He H, Liu D, Ince C. Colloids and the Microcirculation. Anesthesia & Analgesia. 2018;126(5):1747-54.

34. Hilty MP, Pichler J, Ergin B, Hefti U, Merz TM, Ince C et al. Assessment of endothelial cell function and physiological microcirculatory reserve by video microscopy using a topical acetylcholine and nitroglycerin challenge. Intensive Care Medicine Experimental. 2017;5(1):165.

35. Naumann DN, Mellis C, Husheer SLG, Hopkins P, Bishop J, Midwinter MJ et al. Real-time point of care microcirculatory assessment of shock: design, rationale and application of the point of care microcirculation (POEM) tool. Crit Care. 2016;20(1):310.

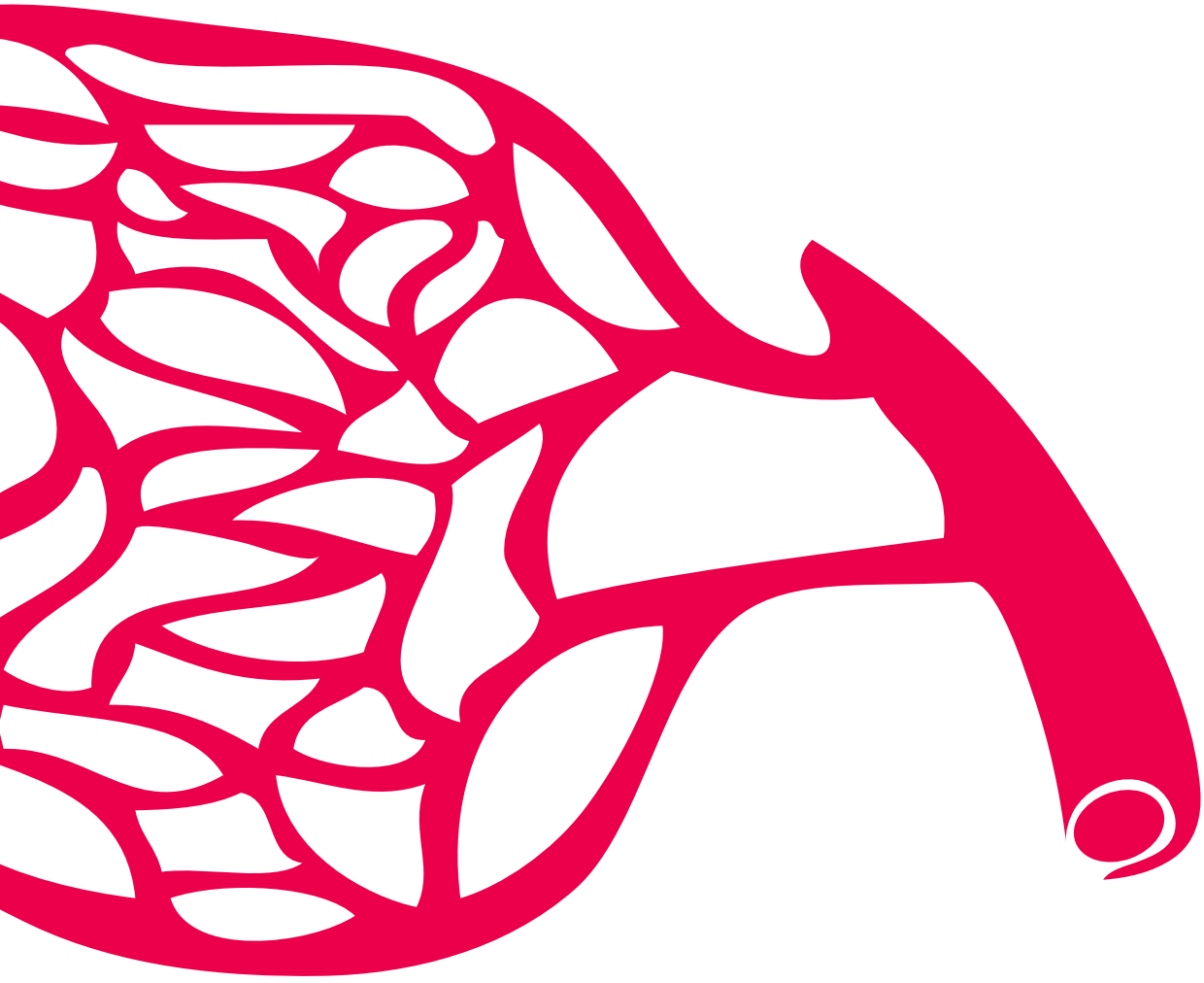
36. Ince C, Boerma E, Cecconi M, De B, Shapiro N. Second consensus on the assessment of sublingual microcirculation in critically ill patients: results from a task force of the European Society of Intensive Care Medicine. Intensive Care Med. 2018;(10):1.

37. Buwalda M, Ince C. Opening the microcirculation: can vasodilators be useful in sepsis? Intensive Care Med. 2002;28(9):1208-17.

38. Hessler M, Pinto BB, Arnemann PH, Kampmeier TG, Seidel L, Morelli A et al. Differential Effects of Selective and Nonselective Potassium Channel Inhibitors in Ovine Endotoxemic Shock (Macrocirculation) and in a Rat Model of Septic Shock (Microcirculation). Shock. 2018;:1.

39. Fuchs C, Ertmer C, Rehberg S. Effects of vasodilators on haemodynamic coherence. Best Pract Res Clin Anaesthesiol. 2016;30(4):479-89.

40. Vellinga NAR, Veenstra G, Scorcella C, Koopmans M, van Roon EN, Ince C et al. Effects of ketanserin on microcirculatory alterations in septic shock: An open-label pilot study. J Crit Care. 2015;30(6):1156-62.



SUMMARY-SAMENVATTING

The response of the microcirculation during fluid shifts

Introduction.

Acute circulatory failure or shock is a common problem in critically ill patients. The circulation fails to deliver sufficient oxygen to the cells and the cells will utilize anaerobic metabolism and eventually fail to survive and organ failure will appear. The clinical state is defined by four mechanisms: hypovolemic, cardiogenic, obstructive and distributive, each with a different underlying problem. The distributive shock is characterized by microcirculatory perfusion alterations, not only resulting in a decreased convective flow but also diffusion heterogeneity. Microcirculatory alterations are clearly correlated with mortality and morbidity. Early detection and response are key. Conventionally, the treatment of shock is based upon normalisation of systemic variables of circulation. Based on the assumption that normalization of macrohemodynamic variables will result in a parallel improvement in organ perfusion. However, direct in-vivo observation of the microcirculation has revealed that this coherence between micro and macro circulation may not always be present. Adequate oxygenation of the cell is based on two key characteristics of perfusion. The first one is convective oxygen transport, the second is diffusion. These two characteristics can be detected by direct in-vivo observation of the microcirculation by handheld microscopes, because of improvement in microscopes the bed side visualisation is closer than ever. Earlier evidence is suggesting that the technique can trace rapid changes in volume status, detects improvement by (auto) transfusion. Thereby potentially being helpful for the indication, evaluation and cessation of fluid therapy.

Direct markers of organ perfusion to guide fluid therapy: When to start, when to stop.

Up until now, the discussion in the literature as to the choice of giving fluids is almost completely restricted to the composition, with little to no attention paid to the importance of hemodynamic end points or indications to achieve a desired optimal volume. The determination of fluid volume is left to the discretion of the attending physician with only surrogate markers as guidance to the initiation and cessation of fluid therapy. In this article, we aim to discuss the available literature on existing clinical and experimental criteria for the initiation and cessation of fluid therapy. Furthermore, we present recent data that have become available after the introduction of direct in vivo microscopy of the microcirculation at the bedside, and discuss its potential influence on the existing paradigms and controversies in fluid therapy.

Cytocam-IDF (incident dark field illumination) imaging for bedside monitoring of the microcirculation.

Orthogonal polarized spectral (OPS) and sidestream dark field (SDF) imaging video microscope devices were introduced for observation of the microcirculation but, due to technical limitations, have remained as research tools. Recently, a novel handheld microscope based on incident dark field illumination (IDF) has been introduced for clinical use. The Cytocam-IDF imaging device consists of a pen-like probe incorporating IDF illumination with a set of high-resolution lenses projecting images on to a computer controlled image sensor synchronized with very short pulsed illumination light. This study was performed to validate Cytocam-IDF imaging by comparison to SDF imaging in volunteers. This study was a prospective, observational study. The subjects consist of 25 volunteers. Cytocam-IDF imaging detected more capillaries and provided better image quality than SDF imaging. It is concluded that Cytocam-IDF imaging may provide a new improved imaging modality for clinical assessment of microcirculatory alterations.

Microcirculatory perfusion derangements during continuous hemofiltration with fixed dose of ultrafiltration in stabilized ICU patients.

Acute kidney injury (AKI) is a well-known complication in critically ill patients. Little is known about the timing and the ultrafiltration dose after initial resuscitation. In vivo microscopy of the microcirculation has been suggested as alternative for the assessment of volume status. Previous studies contribute to the understanding that intravascular hypovolemia is reflected by microcirculatory blood flow changes not detected by conventional methods. The aim of our study was to assess microcirculatory blood flow changes during negative fluid balance ultrafiltration in patients with oliguric AKI. Target was a predefined negative fluid balance; subsequently, a stepwise decrease in amount of substitution fluid was achieved. The microcirculatory blood flow was not altered by reduced substitution during renal replacement therapy.

Ultrafiltration rate is an important determinant of microcirculatory alterations during chronic renal replacement therapy.

Hemodialysis with ultrafiltration in chronic renal replacement therapy is associated with hemodynamic instability, morbidity and mortality. Sublingual Sidestream Dark Field (SDF) imaging during hemodialysis revealed reductions in microcirculatory blood flow. This study aims to determine underlying mechanisms. The study was performed in the Medical Centre Leeuwarden and the Lithuanian University

of Health Sciences. Patients underwent 4-h hemodialysis session with linear ultrafiltration. Nine patients were subject to combinations of hemodialysis and ultrafiltration: 4 h of hemodialysis followed by 1 h isolated ultrafiltration and 4 h hemodialysis with blood-volume-monitoring based ultrafiltration. Primary endpoint: difference in microcirculatory blood flow before and after intervention. During isolated ultrafiltration we observed a reduction in microcirculatory blood flow in conjunction with a negative intravascular fluid balance. The correlation between microcirculatory blood flow before and after intervention suggests that volume status at baseline is a factor in microvascular alterations. In conclusion we observed a significant decrease of sublingual microcirculatory blood flow, related to ultrafiltration rate during chronic renal replacement therapy.

Differences in capillary recruitment between cardiac surgery and septic patients after fluid resuscitation.

Clinical evaluation of the effects of fluid therapy remains cumbersome and strategies are based on the assumption that normalization of macrohemodynamic variables will result in parallel improvement in organ perfusion. Recently, we and others suggested the use of direct in-vivo observation of the microcirculation to evaluate the effects of fluid therapy. We performed a single-centre observational study, using in-vivo microscopy to assess total vessel density in two subsets of intensive care patients. Total vessel density after fluid administration is not different between 2 subtypes of intensive care patients. However, only in septic patients we observed a lack of coherence between the amount of fluids administered and total vessel density . The observed maximum of total vessel density may serve as potential endpoints for fluid administration in future studies.

Surrogates of organ perfusion lack sensitivity for predicting microcirculatory fluid responsiveness.

Fluid administration is the most used treatment for all types of hemodynamic instability. Proper understanding of the indication is key because of potential harmful effects. Clinical decision making around the initiation of fluid therapy is difficult, as reflected by the large amount of potential triggers. The use of surrogates of impaired organ perfusion to identify patients that could benefit from fluid resuscitation is widely used. The concept of stroke volume optimisation and fluid responsiveness stills lacks certainty that this will reflect an improvement in organ perfusion. Besides, these flow triggers are restricted to only one aspect of oxygen transport: convection. We undertook a his study to observe the response of the microcirculation

to a standardized fluid bolus in a single-spot. We performed a single centre, prospective, observational study on postoperative cardiac-surgery patients. Patients were included when fluid administration was considered based on clinical signs of impaired organ perfusion. Systemic hemodynamic variables and sublingual incident dark-field imaging was performed before and during this fluid bolus. Primary outcome was an improvement of microcirculatory blood flow, secondary outcome change in total vessel density, the red blood cell velocity and signs of impaired organ perfusion. In this post-cardiac surgery population, 'clinical signs of impaired organ perfusion' is not associated with the presence of sublingual microvascular alterations, nor its response to fluid administration. However, in a subgroup of patients with hypotension at baseline, a significant increase in red blood cell velocity in individual capillaries was observed, as well as a significant increase in total vessel density in conjunction with a restoration of blood pressure following fluid administration.

Future perspectives.

This chapter summarizes and integrates al chapters of this thesis. It outlines the importance of attention on microcirculatory alterations. And the information it holds for physicians when performing fluid therapy. Eventually resulting in an optimisation of fluid management of critical care patients. It highlights the points of improvement needed to evolve from research method to bed-side monitoring system: the need for fast software and technical improvements on the hardware. Increasing scientific evidence on the choice of fluids to resuscitate the microcirculation, more knowledge about the possible chemical interventions to improve the shunting and thereby recruitment of weak microcirculatory units .

De respons van de microcirculatie tijdens vloeistof shifts.

Introductie.

De term shock omvat vier soorten van circulatoir falen: hypovolemisch, cardiaal, obstructief en distributief. Hierbij wordt er onvoldoende zuurstof getransporteerd naar de cellen en gaan deze over op anaerobe verbranding en kunnen uiteindelijk niet overleven. Door het kapot gaan van cellen zal uiteindelijk orgaanfalen ontstaan. In het geval van een distributieve shock is er een grote heterogeniciteit van flow en diffusie in de microcirculatie. Deze microcirculatoire afwijkingen hangen samen met mortaliteit en morbiditeit. Vroege behandeling en detectie ervan zorgen voor minder orgaanfalen en een betere uitkomst voor de patiënt. Gebruikelijk wordt de behandeling van shock geëvalueerd aan de hand van de normalisatie van systemische variabelen van de circulatie zoals bloedruk en hartfrequentie. Men verwacht dat bij normalisatie van deze macrohemodynamische circulatie er ook een verbetering plaatsvindt in orgaanperfusie. Echter, doormiddel van directe visualisatie van de microcirculatie, met video microscopie, werden er grote afwijkingen gezien in de microcirculatie bij een normale macrocirculatie. Het zuurstof transport in het lichaam is namelijk afhankelijk van twee determinanten: convectieve flow en diffusie afstand. Anders dan macrohemodynamische variabelen, die beperkte informatie geven over de convectieve flow, kan via directe visualisatie van de microcirculatie informatie verkegen worden over beide componenten. En door technische ontwikkeling van de camera's komt het gebruik aan bed dichterbij. Eerder onderzoek suggereert dat de techniek snelle veranderingen in volume status kan detecteren en verbetering kan monitoren door (auto) tranfusie. Potentieel kan het monitoren van de microcirculatie gebruikt worden voor het stellen van de indicatie, evalueren van het effect en het stoppen van vloeistof therapie.

Vloeistof therapie sturen met directe markers van orgaan perfusie: wanneer starten en wanneer er mee stoppen.

In de hedendaagse literatuur is er een discussie gaande over het type vloeistof dat gegeven dient te worden aan een specifieke patiënt. Daarbij vergeet men nog weleens het belang van de indicatie stelling en evaluatie van de therapie doormiddel van te bereiken hemodynamische streefwaardes. In de studies worden de vloeistoffen tegen elkaar getest, maar de indicatie stelling van deze therapie wordt overgelaten aan het oordeel van de behandelende dokter. Die zijn of haar oordeel potentieel baseert op slechte afgeleiden van orgaanperfusie. Dit artikel geeft een overzicht van alle mogelijke triggers waarop een dokter kan besluiten vloeistof te geven en het gebrek aan zowel goede indicatoren als vroege waarschuwingen om

te stoppen met het geven van vloeistof aan de patiënt. We introduceren directe video-microscopie van de microcirculatie als overkoepelende methode om indicatie en evaluatie van vloeistof therapie vast te stellen, hopende hiermee de behandeling van patiënten te optimaliseren.

Het gebruik van de Cytocam-IDF (incident dark field illumination) video microscoop om de microcirculatie te observeren.

De observatie van de microcirculatie aan het bed werd gestart door gebruik te maken van de orthogonal polarized spectral (OPS) video-microscoop, later opgevolgd door de sidestream dark field (SDF) techniek. Ondanks technische verbeteringen aan de camera s is het gebruik nog vooral experimenteel. De nieuwste generatie camera is gebaseerd op de incident dark field illumination (IDF) belichtings-techniek en wordt geintroduceerd als camera voor klinisch gebruik. Dit komt voornamelijk door een verbeterde set van lenzen en een computer gecontroleerde lichtbron met korte belichtingstijden. Wij vergeleken de Cytocam met de SDF-camera en stelden een beter contrast, scherpte en beeldkwaliteit vast. Hierdoor zijn kleinere capillairen beter zichtbaar en is de beoordeling van de microcirculatie verbeterd. Door afname van het gewicht van de camera zijn de beelden makkelijker te maken en is deze geschikter voor klinisch gebruik.

Microcirculatoire perfusie afwijkingen tijdens continue hemofiltratie met een vaste dosis ultrafiltratie bij gestabiliseerde ICU-patiënten.

Acuut nierfalen is een bekende complicatie bij ernstig zieke patiënten. Er is weinig bekend over het moment dat een patiënt voldoende hersteld is om het te veel aan vocht te gaan verwijderen. Er is geen goede methode om de hoeveelheid en snelheid vast te stellen waarmee dit kan. Er is bewijs dat de microcirculatoire flow index, als getal voor rode bloedcel stroomsnelheid, zal verslechteren bij een hypovolemie die veroorzaakt wordt door snelle ultrafiltratie. En dat dit niet door conventionele technieken zoals hartfrequentie en bloeddruk bemerkt wordt. We onderzochten de reactie van de microcirculatoire flow index tijdens een geprotocolleerde toename van ultrafiltratie snelheid in stabiele intensive care patiënten met nierfalen. De microcirculatoire flow index bleek niet te veranderen tijdens deze gestage afname in substitutie vloeistof en hiermee negatief raken van de vochtbalans.

De ultrafiltratie snelheid is een belangrijke oorzaak voor microcirculatoire afwijkingen tijdens chronische nierfunctie vervangende therapie.

Hemodialyse in patiënten met chronisch nierfalen gaat gepaard met hemodynamische instabiliteit en deze is gerelateerd aan verminderde overleving en meer complicaties. Eerder onderzoek toonde afwijkingen aan in de microcirculatoire bloedstroom snelheid tijdens hemodialyse met ultrafiltratie; deze afwijkingen verbeterden door Trendelenburg positie. Daarmee is het waarschijnlijk dat de flow afwijkingen veroorzaakt worden door het onttrekken van vocht. In twee vergelijkbare dialysecentra werden patiënten gevolgd tijdens drie types ultrafiltratie methode bij een hemodialyse behandeling. Alleen tijdens de geisoleerde (snelle) ultrafiltratie methode ontstond er een significante afname in microcirculatoire bloedstroom snelheid. Daarnaast vonden we een correlatie tussen de microcirculatoire bloedstroom snelheid vooraf en na de behandeling, hetgeen suggereert dat de volume status voorafgaande aan de behandeling de afwijkingen voorspelt.

Het verschil in capillaire recruitment door vloeistof therapie tussen cardiothoracale chirurgie en septische shock patiënten.

De klinische evaluatie van vloeistof therapie is lastig en gebruikte strategieën zijn gebaseerd op de verwachting dat normalisatie van de macrohemodynamische variabelen zorgt voor een verbetering in perfusie van organen. Er is echter toenemend bewijs dat dit niet gebeurt. Door gebruik te maken van directe video-microscopie van de microcirculatie kan er beter onderscheid gemaakt worden tussen flow en diffusie verbetering. Doormiddel van de video-microscopie werd geen verschil aangetoond in oppervlakte aan capillairen per vierkante millimeter (total vessel density) tussen patiënten na cardiothoracale chirurgie of na septische shock. Wel was deze afwijkend ten opzichte van gezonde vrijwilligers. Waar de cardiothoracale patiënten potentieel nog kunnen verbeteren op vloeistof therapie, doen de septische shock patiënten dit niet en verslechteren potentieel verder. De gevonden maximale total vessel density zou gebruikt kunnen worden als streefwaarde voor vloeistof therapie.

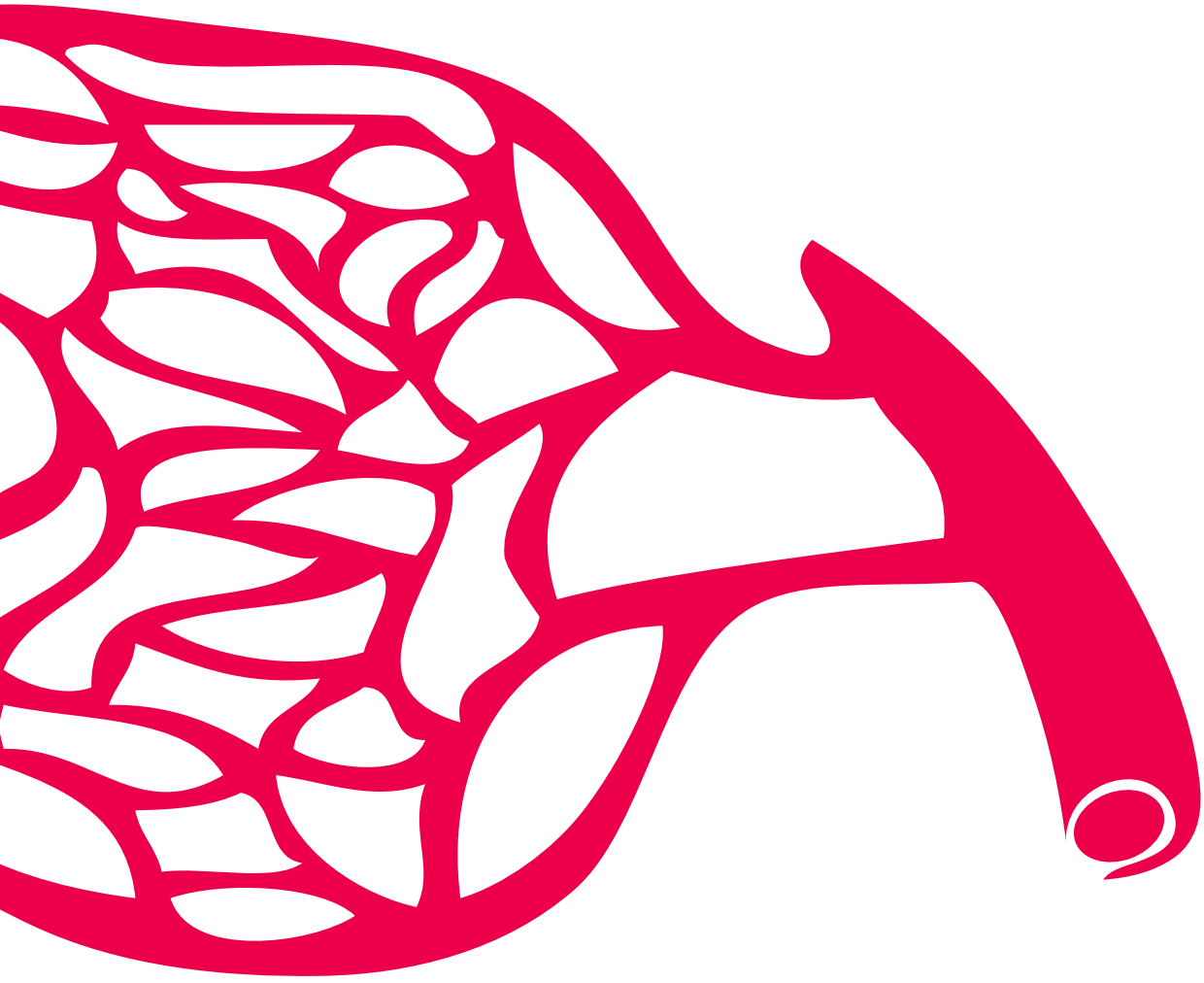
Surrogaat markers van orgaan perfusie missen sensitiviteit voor de voorspelling van de reactie van de microcirculatie op het geven van vocht.

Het geven van vloeistoffen om de circulatie van een kritisch zieke patiënt te optimaliseren is een essentieel onderdeel van de behandeling. Echter, vloeistof therapie wordt niet gegeven om de circulatie te verbeteren maar om het zuurstof transport naar de cellen te optimaliseren. Vloeistof geven wanneer de indicatie er niet is

kan potentieel zorgen voor een verslechtering in dit zuurstof transport. Helaas zijn er veel triggers in de vorm van klinische tekenen van verminderde orgaan perfusie die medici verleiden tot het geven van extra vocht. Onzeker echter is of bij correctie van deze tekenen, bijvoorbeeld door het optimaliseren van slagvolume en cardiac output, er een parallelle verbetering is in het zuurstof transport naar de cel. Daarnaast is de cardiac output maar één determinant van het zuurstoftransport, de convectie. De andere determinant is de diffusie capaciteit en wordt niet gemeten door de conventionele technieken. Eerder onderzoek toonde aan dat intensive care patiënten met klinische tekenen van verminderde orgaanperfusie en een slechte microcirculatory blood flow positief respondeerden op vocht. Niet alleen verbeterde de bloed flow maar ook het aantal klinische tekenen van shock verminderde. Patiënten met alleen de klinische tekenen vertoonden geen verbetering in de microcirculatie, noch in het aantal klinische tekenen. Uitgaande van deze studie werden in ons onderzoek patiënten direct postoperatief na een cardiothoracale ingreep geïncludeerd wanneer er vast gestelde klinische tekenen van verminderde orgaan perfusie waren. Er werd een gestandaardiseerde vloeistofbolus toegediend terwijl er op één en dezelfde plek microcirculatoire video beelden werden gemaakt gedurende het hele experiment. In deze groep konden deze klinische tekenen van shock geen microvasculaire afwijkingen voorspellen en evenmin een voorspelling doen over de respons op de vloeistof therapie. Alleen de subgroep met hypotensie toonde zowel een verbetering in bloeddruk alsok ook een verbetering in gemeten rode bloedcel stroomsnelheid en total vessel density.

Toekomstperspectieven.

Dit hoofdstuk stipt de hoofdpunten aan en integreert alle eerdere hoofdstukken. Het herhaalt het belang voor aandacht op microcirculatoire afwijkingen. En welke informatie dit compartiment bevat voor dokters om vloeistof therapie op te sturen. Daarmee zal het de vloeistof management voor een intensive care patiënten verbeteren. Er is op diverse plekken nog vooruitgang nodig om de techniek te laten evolueren van een research methode naar een monitoring systeem dat aan het bed van de patiënt gebruikt kan worden. Doorontwikkeling van de hardware en met name de software is hiervoor essentieel. Er is meer wetenschappelijk onderzoek nodig naar de mogelijkheid om op de microcirculatie te interveniëren. Niet alleen in keuze van chemische samenstelling van vloeistof, maar ook in de medicatie waarmee shunting en diffusie afwijkingen behandeld kunnen worden. De hoop is dat dit uiteindelijk k zal leiden tot verbeterde behandeling van een kritisch zieke patiënt.



DANKWOORD

Na 5 jaar is 'mijn' boekje voltooid. Ooit begonnen als een kort project en uitgelopen tot een volledig proefschrift. De intellectuele ontwikkeling heb ik heerlijk gevonden en elke tegenslag was een nieuwe uitdaging. Echter, na de intellectuele ontwikkeling volgde de periode waarin het één en ander geschreven diende te worden, een hele kluif! En het is geen geheim dat dit zonder de medewerking van anderen niet was gelukt. Belangrijker nog, zonder de geboden kans om klinisch te werken en wetenschappelijk onderzoek te doen was een PhD-traject voor mij ondenkbaar geweest.

Geachte professor Ince; Beste Can, dank voor alle gesprekken die we hebben gevoerd over niet-fysiologische handelingen die dokters toch uitvoeren. Het was een genoegen met je te filosoferen over de mogelijkheden van 'ons' gebied en het grote enthousiasme waarmee je hierover verteld. Gezien mijn bijzondere positie als 'nomad' vanuit het hoge Noorden zal mijn begeleiding niet altijd makkelijk zijn geweest. Dank voor het vertrouwen en de mogelijkheid voor dit PhD-traject. Dankzij jou kijk ik terug op geweldige congressen, etentjes, meetings en dit proefschrift.

Beste Christiaan, je bent mijn co-promotor maar ook mijn mentor en leermeester. Je kennis en kunde in zowel klinisch als wetenschappelijk oogpunt zijn voor mij van onschatbare waarde geweest. Wanneer ik het even niet zag zitten was jij er altijd met bemoedigende en inspirerende woorden. Om de woorden van je eerste promovendus te herhalen: een betere co-promotor had ik mij niet kunnen wensen.

Ik wil mijn promotiecommissie bedanken voor de evaluatie van mijn proefschrift. Professor Scheeren, Professor Boer, Professor Donati, Professor de Mol, Professor van Gulik en Dr. Veelo: Thank you very much for the investment of your (precious) time and expertise in my thesis.

Matty Koopmans, Claudia Scorcella, Bart Scheenstra, Bart Barendrecht en Rik Zijlstra, bedankt voor de hulp bij de verzameling van data van diverse stukken! Zonder jullie hulp was de data inclusie langzamer verlopen en zeker een stuk minder gezellig geweest. Claudia, de 'rotonde' in je sublinguale microcirculatie hebben we vereeuwigt, een doorbraak in de Cytocam-validatie. Matty, wat moet een 'wetenschap-co' en eigenlijk de hele afdeling wetenschap zonder jou! Je bent een heel belangrijke stuk (koningin?) in het wetenschappelijke schaakspel op de intensive care. Dank voor je ondersteuning in ontelbare manieren en gelijk aantal malen.

De combinatie van wetenschappelijk onderzoek met klinisch werken was voor mij een hele mooie kans, wat zeker niet voor het oprapen ligt. Ik heb mogen genieten van een al goed lopende onderzoekslijn op de intensive care in het Medisch Centrum Leeuwarden. De ontwikkeling van PhD-posities binnen het Medisch Centrum Leeuwarden ondergaat momenteel een fantastische ontwikkeling met de Campus Fryslân en een actieve Wetenschapscommissie. Intensivisten van de afdeling: Christiaan Boerma, Nynke Bruins, Hanneke Buter, Sjieuwke Derksen, Peter Egbers, Rik Gerritsen, Corine de Jager, Peter Kingma, Nadia Koek, Peter Koetsier, Niels Koopmans, Michaël Kuiper, Fellery de Lange en Marco Loos. Dank voor het vertrouwen in mijn kunnen zowel op wetenschappelijk als klinisch vlak. 6 jaar lang heb ik met groot plezier gewerkt onder jullie supervisie en mij kunnen ontwikkelen in een grote diversiteit van onderwerpen. Jullie dragen stuk voor stuk bij aan de ontwikkeling van de arts-assistenten, de patiëntenzorg en het wetenschappelijk onderzoek. Hulde en wat ga ik jullie straks missen.

Tijdens de afgelopen jaren was het een komen en gaan van collega-assistenten. We hadden mijns inziens altijd een goede werksfeer in de groep. Er was in elk geval altijd ruimte voor eigen ontwikkeling. Dank daarvoor. Namkje, mijn voorgangster en paranymph, dank voor de gezelligheid en enthousiasme tijdens onze wetenschappelijk en klinische jaren. Marjolein & Inge, mijn 'wetenschap-collega's, dank voor de luisterende oren, de 'besprekingen' en gezelligheid tijdens 'bureau-dagen'. Het is leuk om enthousiasme voor een onderwerp te kunnen delen en mee te mogen denken met problemen op compleet ander wetenschappelijk terrein.

Verpleegkundigen: 'daar komt hij weer met z'n camera'; 'als je maar niet "push button" gaat roepen'. Vele malen zat ik jullie dwars in de 'normale' gang van zaken, zoals SDD geven, snel vocht willen geven en het verzorgen van de patiënt. Dank voor jullie geduld met mijn onderzoek en de ondersteuning in de registratie van bloedgassen en andere gegevens. Ik heb met éénieder plezierig samengewerkt en ga de serieuze en (vaak) niet serieuze gesprekken missen.

Lieve Henka, dank voor de ruimte die ik heb gekregen om mijn proefschrift af te maken. Ondanks een grote wijziging in onze toekomst door mijn opleidingsplek blijf je me altijd steunen in mijn ontwikkeling. Dankzij jouw waarschuwingen zeg ik ook wel eens 'nee' tegen extra werk, ondanks mijn werkethos en karakter. Schoonouders, bedankt voor de ondersteuning in de vorm van opvang van onze lieve dochter Noa wanneer ik naar een congres of meeting moest.

Matzen, mijn kleine zus, wat bewonder ik je doorzettingsvermogen en leergierigheid. Als MBO verpleegkundige begonnen en zometeen een echte IC-pleeg! Ik ben erg blij met jouw steun en rol als paranymph. Geachte ouders, dank voor jullie geduld en inzet tijdens vrijwel mijn hele opleiding. Zonder jullie inzet had ik het VWO, laat staan de opleiding geneeskunde, nooit volbracht. Ik wil dit proefschrift opdragen aan 'mijn' pake en naamgenoot, Gerke Veenstra, ons dit jaar ontvallen. Een wijs man en geweldige opa. Mijn 'lees-honger' is door jouw altijd gestimuleerd met meer boeken, kritische evaluaties van technieken in het museum Aeolus of het Eise Eisinga Planetarium.

Soms mo-j d'r veur de knokken, um te stoan woar ik now stoa

En heel vaak mos ik buugen, mien grenzen achternoa

Ik heb veel motten leren um te doen wat ik niet kon

Heel völ mos ik heuren as ik weer verkeerd begon

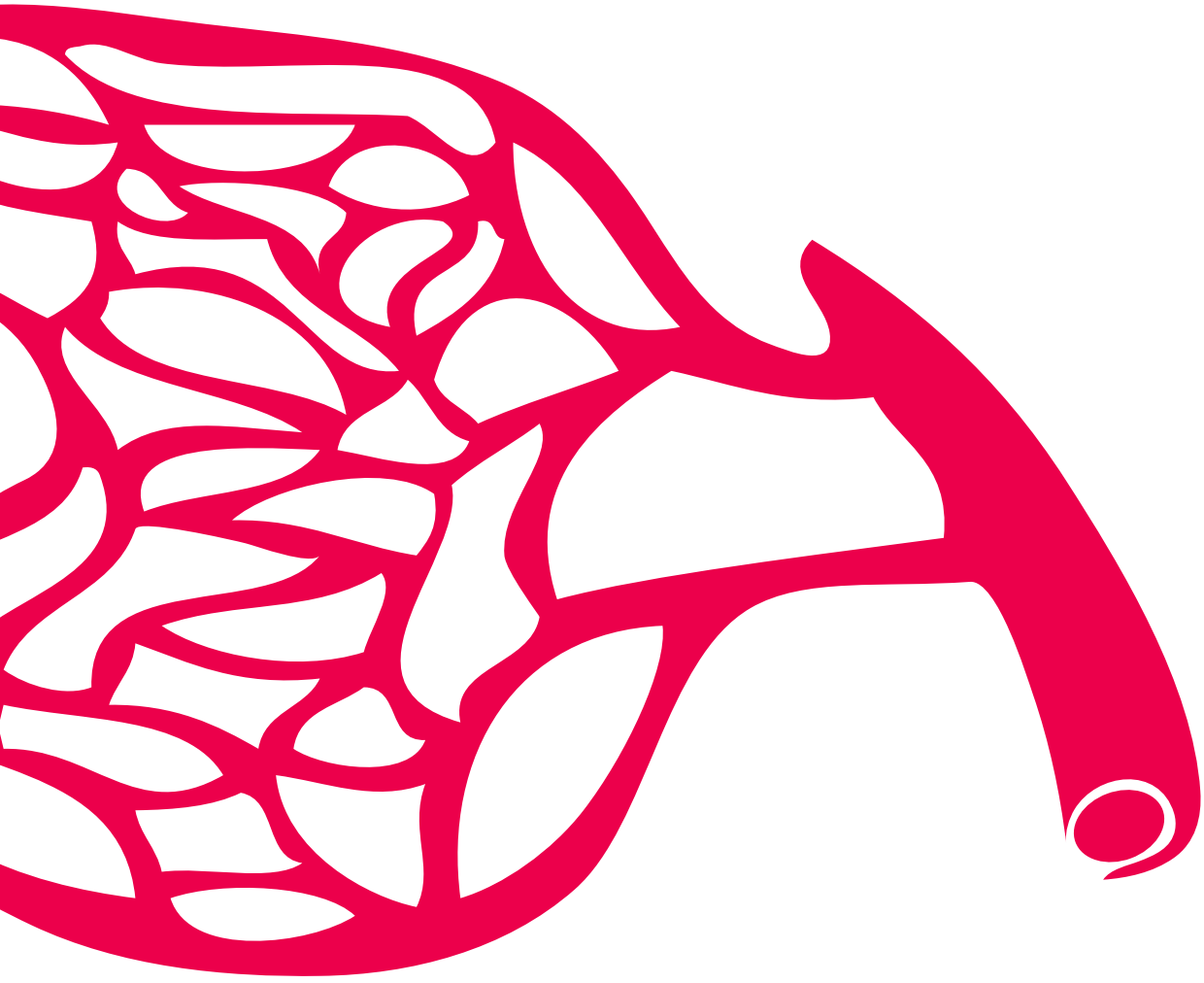
De wind op de kop en de wind in de rug

Mot i-j doarveur knokken, kri-j doar kracht veur terug

Freeweelend heb i-j gin benul van tied

Dan goa i-j onderuut, as de storm begint

© Normaal - Tegenwind



CURRICULUM VITAE & PHD PORTFOLIO

Curriculum vitae

Gerke Veenstra was born on the 3th of May 1987 in Smallingerland, the Netherlands. After completing elementary school in 1999 (CBS. Arendvleugel, Ruinerwold, The Netherlands) he got through high school in 2008 (CSG. Dingstede, Meppel, The Netherlands). After a positive draw in the Dutch lottery system he attended medical school (University of Groningen, Groningen, the Netherlands). From the second year of his internships he could be found in the Medical Center Leeuwarden. During the completion of a final internship in intensive care medicine and anaesthesiology his interest in physiology and critically ill patients expanded. His (scientific) academic internship on both the department of nephrology and intensive care medicine (joint-investigation) resulted in a first poster on the yearly congress of the American Society of Nephrology. His interest for scientific research awakened under inspiring lead of dr. E.C. Boerma, intensivist in the department of intensive care of the MCL. This resulted in a residency in intensive care medicine in the Medical Center Leeuwarden for many years (2012-present). From 2013 he combined his clinical work with a PhD-project under supervision of dr. Christiaan Boerma, intensivist, and professor Can Ince, professor of Translational Physiology, Academic Medical Center, Amsterdam, the Netherlands.
From December 2018 he will start his training in anaesthesiology in the University Medical Center Utrecht, prof. R.G. Hoff.

Portfolio

PhD student: G. Veenstra, MD
Period: 2013- 2018
Supervisor: prof. dr. ir. C. Ince
Co-supervisor: dr. E.C. Boerma

Courses: 4 ECTS

- Fundamental Critical Care Support

Presentations: 7 ECTS

- 2012 Poster on the congress of the American Society of Nephrology, San Diego, USA

- 2014 poster on the ISICEM, Brussels, Belgium

- 2016 poster on the IFSS, Tokio, Japan

- 2016 oral presentation on the Petrus Camper Symposium, Heerenveen, The Netherlands

- Several (>10) research related presentations in the Medical Centre Leeuwarden

- Teaching of nurses in training for intensive care specialisation. (6 times)

Conferences: 3 ECTS

- Future of Critical Care Medicine, Längenfeld, Austria, 2014

- Petrus Camper Symposium, Heerenveen, The Netherlands, 2016

- International Federation of Shock Societies, Tokio, Japan, 2016

- International symposium on intensive care and emergency medicine, Brussels, Belgium, 2018