Is the Microcirculation the Key in Understanding the Development of Shock After Cardiothoracic Surgery?

Rowan Toet

February 2023

This page was intentionally left blank.

Is the Microcirculation the Key in Understanding the Development of Shock After Cardiothoracic Surgery?

MSc thesis - Sensing & Stimulation

By

Rowan V. Toet

Student number : 4557131

February 15, 2023

Thesis in partial fulfilment of the requirements for the joint degree of Master of Science in

Technical Medicine

Leiden University ; Delft University of Technology ; Erasmus University Rotterdam

Master thesis project (TM30004 ; 35 ECTS)
Dept. of Intensive Care, Leiden University Medical Centre (LUMC)
February 2022 – February 2023

Supervisors:

Medical supervisor	Dr. M.S. Arbous
Technical supervisor	Dr. R. Tsonaka
Daily supervisor	MSc. F. Brouwer

Thesis committee members:

Dr. M.S. Arbous	LUMC (chair)
Dr. R. Tsonaka	LUMC
Dr. E.G. Mik	Erasmus MC
Prof. Dr. ir. J. Harlaar	Tu Delft

Universiteit Leiden

TU Delft Delft University of Technology

Erasmus Universiteit Rotterdam

This page was intentionally left blank.

Preface and Acknowledgements

This master's thesis ends my journey as a technical physician student. As a little kid, I was already intrigued by medicine and the creative mind to invent and design new innovations. After I discovered, as an unknowing high school student, that the bachelor programs in architecture and medicine did not mix well, TU Delft came up with a different solution. Without any preparation or plan B, I took the plunge and enrolled myself in the bachelor's program in Clinical Technology, from which I graduated in 2019. Then, in September 2019, I started the master's program in Technical Medicine. Until the last day of the education program, six and a half years later, I have never regretted the decision to study Clinical Technology. During my years at university, I have heard many interesting topics pass by, had the opportunity to meet inspiring professors, and during my clinical internships in the second year of the master's program and during my graduate internship, I had the chance to work with and learn a lot from many different physicians.

I was always convinced that after graduation, I was likely to end up as a Technical Physician in the department of neurology or oncology, as these are departments close to my heart, however over the years of my bachelor's in Clinical Technology and first-year master's in Technical Medicine, reality proved otherwise; I had a huge affinity for acute and critical care. Therefore, I decided to conduct my healthcare internship from the bachelor's program in the emergency department and ambulance and a clinical internship in the Intensive Care department during my second master's year. Since then, I knew for sure that this was the department where I wanted to graduate, and so it happened.

I want to express my sincere gratitude to everyone who has supported me and invested their time to guide me in my process of becoming a Technical Physician. The past year was not the easiest for me due to several events, but here I am at the end of my journey as a Technical Physician student. Sesmu, I cannot describe how grateful I am for the guidance, wise words, and warm welcome under your "mother wings" during these days, but especially for all you have taught me during the time you have been my supervisor. Your passion for medicine, the way you interact with your patients, and your loving heart are inspiring and have taught me a lot about who I want to become. Through your wise words and judge of character, I got to know myself in a different way and learned that sometimes I need to make choices with my heart rather than my head. Roula, even though you have not been involved from the beginning of the project, you have shared a lot of your knowledge in the last few months, and I have learned a lot from you. I want to thank you for all your time and enthusiasm in helping me set up the correct statistical analyses and for encouraging me always to report the results carefully. My thanks also go to Fleur; you always made time to help me when I got stuck with a question, you were a sparring partner who always pushed me in the right direction, and on top of that, you were always interested in how I was actually doing. I want to thank Dr. Mik and Professor Harlaar for their contribution to my defense committee. Finally, I am extremely grateful to my family and friends for their support, unconditional love, and limitless trust in me, knowing everything would work out.

I am beyond excited to graduate and can't wait to see what the future holds for me.

R.V. Toet
Zoetermeer, February 2023

This page was intentionally left blank.

Summary

Cardiothoracic surgery is a common treatment for cardiovascular diseases. Patients are admitted to the ICU after cardiothoracic surgery for continuous monitoring to prevent or treat postoperative complications as much and as soon as possible. One of these complications is the development of circulatory shock. It is likely caused by one or a combination of several factors, leading to increased morbidity and mortality in the ICU. The main purpose of the circulation is to transport O_2 and nutrients to the tissues and remove waste products of the tissues via the tissue's microcirculation. Under normal conditions, O_2 supply exceeds O_2 demand. However, during circulatory shock, the circulation cannot meet the perfusion demands of the organs, leading to organ dysfunction and organ failure. Resuscitation procedures for patients with circulatory shock focus on normalizing macrocirculatory parameters, such as CO and SvO_2, by administering fluids and vasopressors to support tissue perfusion. Improvement in macrocirculatory parameters is expected to be paralleled by improvement in microcirculatory perfusion and tissue oxygenation (i.e., hemodynamic coherence), but it appears that these do not always improve simultaneously. Loss of this coherence has been associated with adverse outcomes.

The microcirculation can be imaged sublingually with an HVM. Studies in patients with septic shock have shown that hemodynamic coherence is often lacking. Therefore, it could be valuable to monitor the microcirculation of cardiothoracic surgery patients.

This thesis aimed to investigate the postoperative time course of microcirculatory parameters in patients admitted to the ICU after cardiothoracic surgery with and without circulatory shock, the relationship between macro- and microcirculation, and the usage of leukocyte detection in understanding patient's systemic inflammation.

Chapter 2 provides general background information on cardiothoracic surgery, CPB, the physiology of the microcirculation, the latest generation of HVM, pathophysiological changes in the microcirculation after cardiothoracic surgery, leukocyte-endothelium interactions, the macrocirculation, and hemodynamic coherence. A retrospective study of cardiothoracic surgical patients with shock is described in **Chapter 3** of this thesis. The results showed that the microcirculation might adapt to compensate for the circulatory shock state by decreasing RBCv and increasing FCD, TVD, and cHct compared with normal values of healthy volunteers while maintaining tRBCp. **Chapter 4** describes a prospective study comparing cardiothoracic surgical patients with shock from Chapter 3 with cardiothoracic surgical patients without shock. The comparison between these two groups showed that both groups exhibited different behavior of the microcirculation. However, the underlying mechanism is not understood and requires further research. **Chapter 5** contains an explanatory review of the use of STDs for leukocyte detection. **Chapter 6** provides a general discussion and reviews the future prospects of microcirculation measurements as a tool in the management of critically ill patients. Our findings should be examined in more extensive clinical trials to determine whether microcirculatory changes contribute to the development of shock.

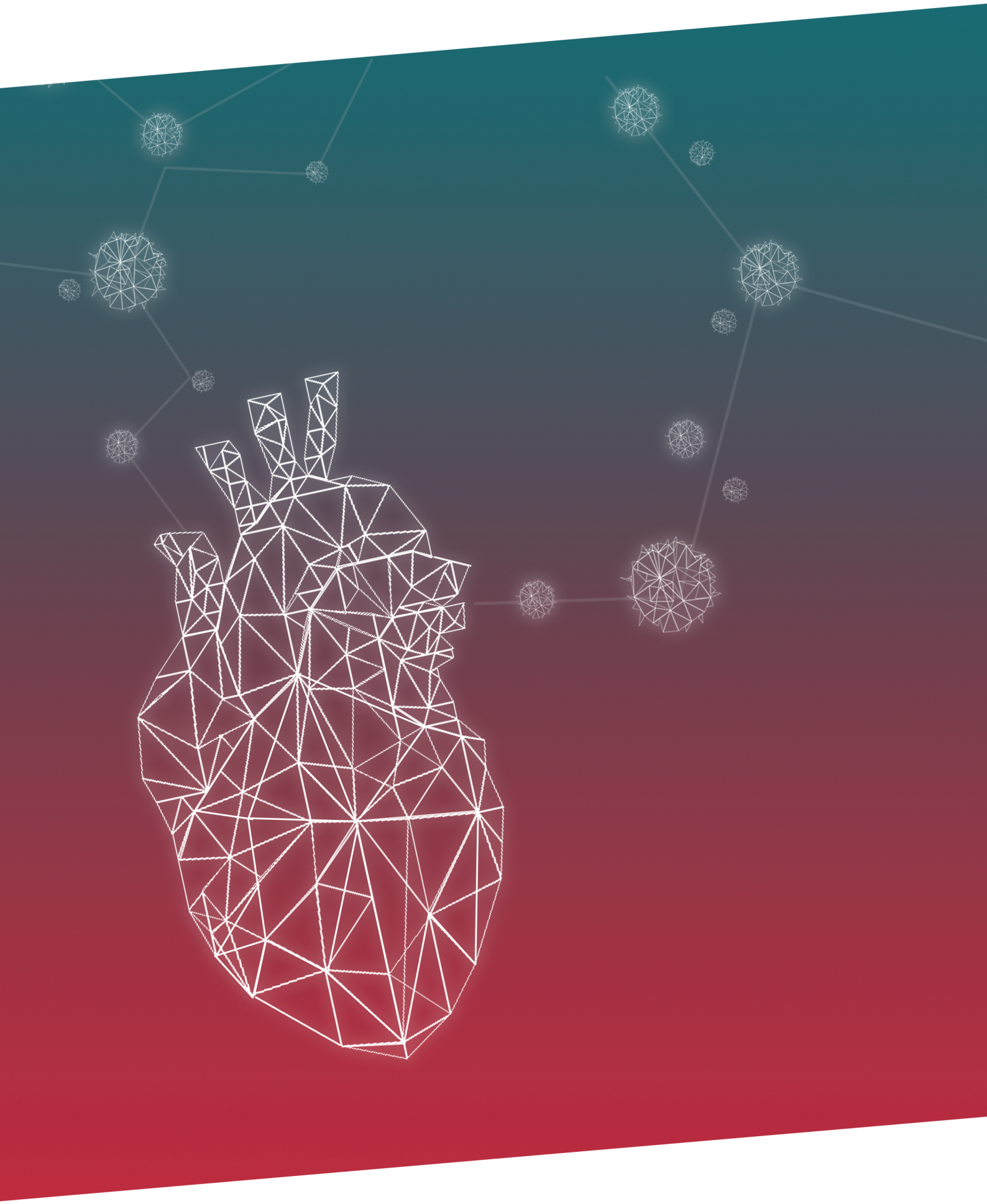
This page was intentionally left blank.

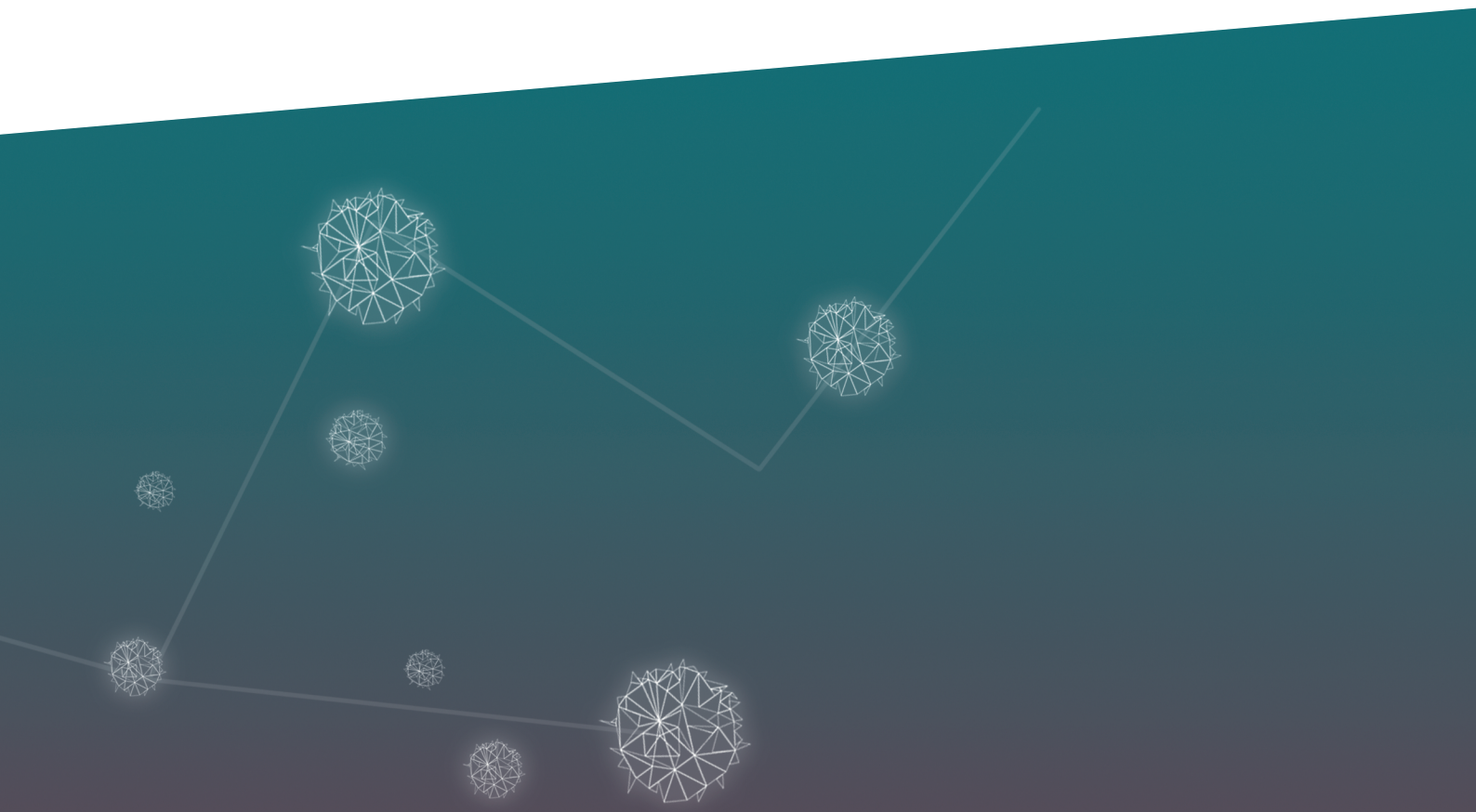
Contents

This page was intentionally left blank.

Nomenclature

Abbreviation	Description
cHct	Capillary hematocrit
CPB	Cardiopulmonary bypass
c-pcv	Capillary-postcapillary venule
ESL	Endothelial surface layer
FCD	Functional Capillary Density
HVM	Hand-held vital microscope
ICU	Intensive Care Unit
IDF	Incident dark field
LMM	Linear Mixed Model
LUMC	Leiden University Medical Centre
MAP	Mean arterial pressure
MFI	Microvascular flow index
NO	Nitric oxide
O_2	Oxygen
OPS	Orthogonal polarization spectral
pcv	Postcapillary venule
PPV	Proportion of Perfused Vessels
PVD	Density of perfused vessels
RBC	Red blood cell
RBCv	Red Blood Cell Velocity
SDF	Sidestream dark field
STD	Space-time diagram
SvO2	Venous oxygen saturation
tRBCp	Tissue red blood cell perfusion
TVD	Total vessel density
VS	Vasoplegic syndrome





1.

The Problem

Cardiothoracic surgery is an effective and standard treatment for cardiovascular diseases. An estimated 1 million patients undergo cardiothoracic surgery throughout the world every year.[1] Innovations in surgical techniques, such as the introduction of the cardiopulmonary bypass (CPB) and an aging population, will lead to an increasing number of older or more critically ill patients undergoing cardiothoracic surgery.[2-4] Although perioperative care, anesthesia, and surgical techniques have greatly improved, cardiothoracic surgery (with or without CPB) remains associated with a high rate of postoperative complications[2, 5] (i.e., the use of blood products (47.3%), atrial fibrillation (32%), renal failure (3.3%) and tamponade (1.1%)[6]) and CPB adverse effects[7] (i.e., the release of pro-inflammatory cytokines, dilution of the clotting factors and platelet dysfunction[8-10]). These complications can lead to prolonged hospital stays, higher healthcare costs, delayed recovery, and poor quality of life after surgery.[11, 12]

Nearly all patients after cardiothoracic surgery are admitted to the Intensive Care Unit (ICU)[13] to ensure adequate continuous hemodynamic monitoring to avoid or treat potential postoperative complications.[14-17] One of the most common complications is the development of circulatory shock (5-50%)[18-21] caused by a combination of many factors, such as the surgery itself, activation of inflammatory and hemostatic systems, anesthesia, hypothermia, hemodilution, micro-emboli formation, and tissue trauma.[19, 22-24] This condition is associated with increased morbidity and mortality in the ICU.[16, 25] The primary function of the circulation is to deliver oxygen (O_2) and nutrients to the tissue cells and remove waste products.[25] This exchange occurs in the tissues' microvasculature, where O_2 passively diffuses from red blood cells into tissue cells. Under normal conditions, O_2 delivery exceeds O_2 demand. In a state of circulatory dysfunction, for example, during circulatory shock, the systemic circulation fails to meet the organs' perfusion requirements (i.e., O_2 requirements).[26] Inadequate delivery of O_2 to tissue cells leads to organ dysfunction and, if it persists, to organ failure.[27, 28] Current resuscitation procedures for patients in circulatory shock are aimed at normalizing macrocirculatory parameters (hereafter "macrocirculation"), such as cardiac output (CO), central venous oxygen saturation (SvO_2), and blood pressure variables, by administering fluids and vasopressors to promote tissue perfusion and therefore O_2 transport to the tissues.[29-31]

It is expected that an improvement in macrocirculation will lead to a parallel improvement in microcirculatory perfusion and restoration of tissue oxygenation.[29] However, sometimes the macrocirculatory parameters are normalized, but we still see a patient in persistent shock without a solid explanation.[28] This discrepancy between macrocirculation and microcirculation is known as a loss of hemodynamic coherence, a condition in which, during resuscitation, microcirculatory parameters improve to average values while the microcirculation remains impaired.[29] This loss of coherence is associated with adverse outcomes.[30, 32, 33]

Microcirculatory perfusion can be monitored sublingually with hand-held vital microscopes (HVMs). The first-generation HVMs were based on orthogonal polarization spectral (OPS) imaging.[34, 35] These devices were improved and replaced by HVMs based on sidestream dark field (SDF) imaging.[28, 36] The third and latest generation HVM is based on incident dark field imaging (IDF)[37]. From studies performed in patients with sepsis and septic shock, where microcirculation was monitored with HVMs, we know that hemodynamic coherence is often lacking, which can result in failure of treatment on the relevant, microcirculatory level, and, ultimately, in increased

mortality.[38-41] Therefore, real-time microcirculation monitoring might be a valuable tool for monitoring alterations in microcirculatory perfusion in patients undergoing cardiac surgery with or without CPB.

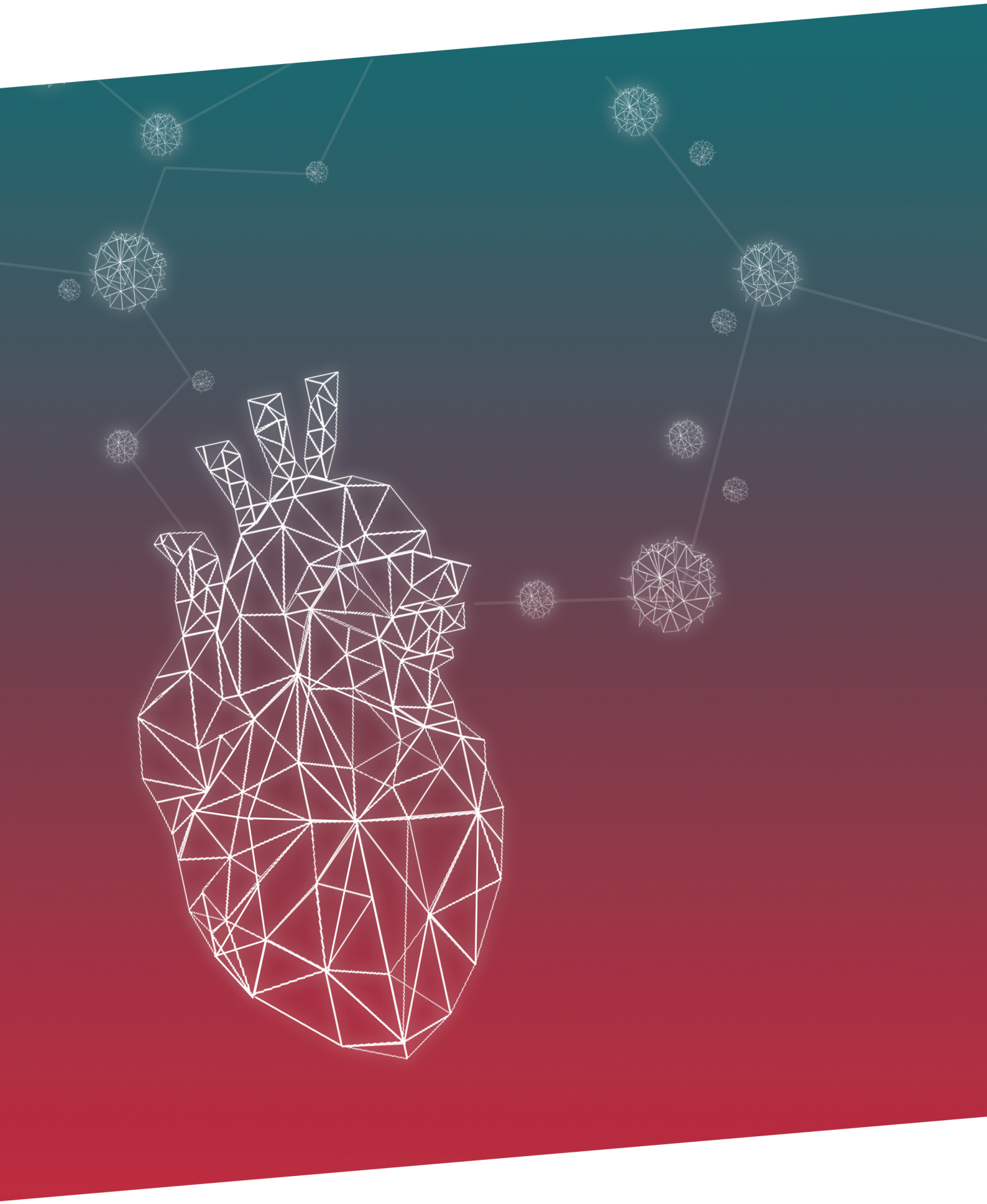
This thesis aimed to investigate the postoperative time course of microcirculatory parameters in patients admitted to the ICU after cardiothoracic surgery with and without circulatory shock. Our secondary objective is to study the relationship between macro- and microcirculation.

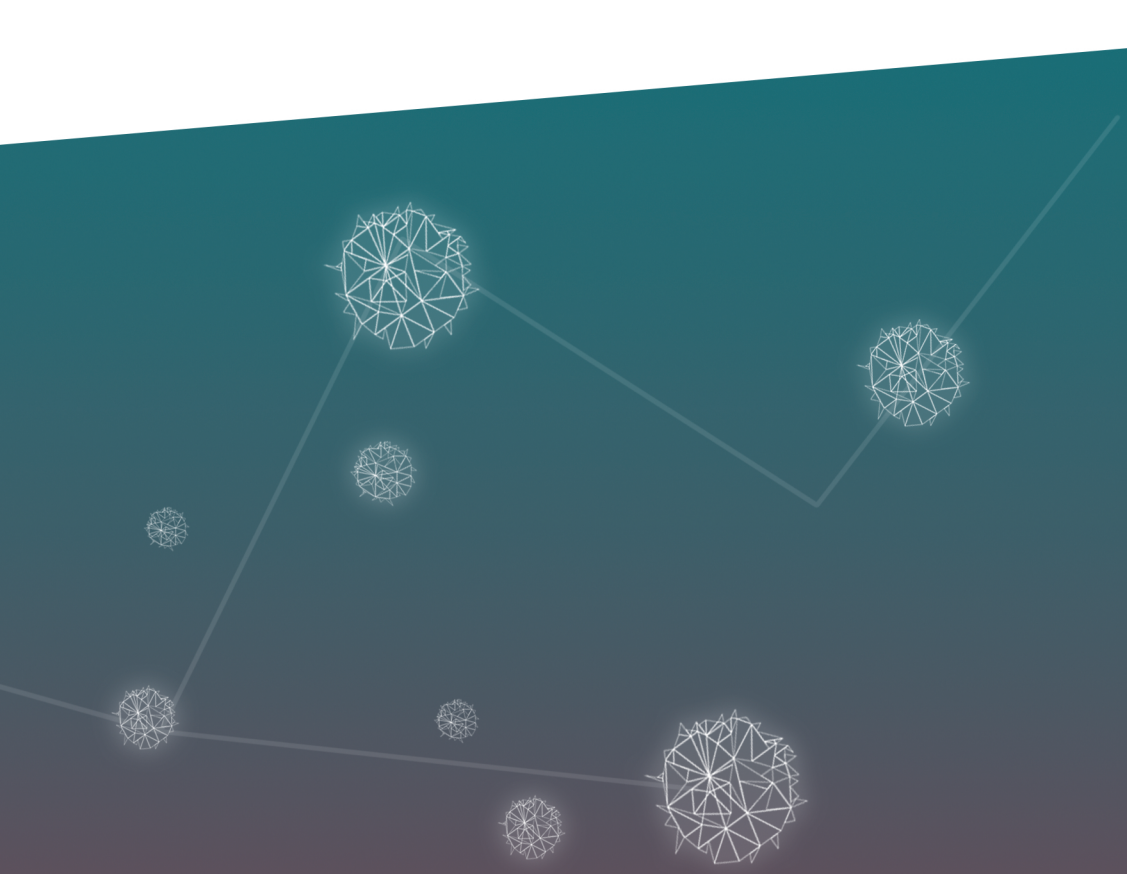
For easy understanding, relevant background information on the subject is given in the next chapter. The experimental design for obtaining and analyzing macro- and microcirculation data is then described. The next chapter contains a draft consensus document for a method of leukocyte analysis as part of microcirculatory measurements. The last chapter includes a general discussion and conclusion on recommendations and future perspectives.

1.1 | References

1. Yeh Y-C, Wang M-J, Chao A, Ko W-J, Chan W-S, Fan S-Z, et al. Correlation between early sublingual small vessel density and late blood lactate level in critically ill surgical patients. journal of surgical research. 2013;180(2):317-21.
2. Mekontso-Dessap A, Houel R, Soustelle C, Kirsch M, Thebert D, Loisance DY. Risk factors for post-cardiopulmonary bypass vasoplegia in patients with preserved left ventricular function. The Annals of thoracic surgery. 2001;71(5):1428-32.
3. Jakobson T, Karjagin J, Vipp L, Padar M, Parik A-H, Starkopf L, et al. Postoperative complications and mortality after major gastrointestinal surgery. Medicina. 2014;50(2):111-7.
4. Laursen LØ, Petersen RH, Hansen HJ, Jensen TK, Ravn J, Konge L. Video-assisted thoracoscopic surgery lobectomy for lung cancer is associated with a lower 30-day morbidity compared with lobectomy by thoracotomy. European Journal of Cardio-Thoracic Surgery. 2016;49(3):870-5.
5. Pahwa S, Bernabei A, Schaff H, Stulak J, Greason K, Pochettino A, et al. Impact of postoperative complications after cardiac surgery on long-term survival. Journal of cardiac surgery. 2021;36(6):2045-52.
6. Downing SW, Edmunds Jr LH. Release of vasoactive substances during cardiopulmonary bypass. The Annals of thoracic surgery. 1992;54(6):1236-43.
7. Boyle EM, Pohlman TH, Johnson MC, Verrier ED. Endothelial cell injury in cardiovascular surgery: the systemic inflammatory response. The Annals of thoracic surgery. 1997;63(1):277-84.
8. Miller BE, Levy JH. The inflammatory response to cardiopulmonary bypass. Journal of cardiothoracic and vascular anesthesia. 1997;11(3):355-66.
9. Handy Jr JR, Asaph JW, Skokan L, Reed CE, Koh S, Brooks G, et al. What happens to patients undergoing lung cancer surgery?: Outcomes and quality of life before and after surgery. Chest. 2002;122(1):21-30.
10. Patel AS, Bergman A, Moore BW, Haglund U. The economic burden of complications occurring in major surgical procedures: a systematic review. Applied health economics and health policy. 2013;11(6):577-92.
11. Stephens RS, Whitman GJ. Postoperative critical care of the adult cardiac surgical patient. Part I: routine postoperative care. Critical care medicine. 2015;43(7):1477-97.
12. Lowenstein E, Hallowell P, Levine FH, Daggett WM, Austen WG, Laver MB. Cardiovascular response to large doses of intravenous morphine in man. New England Journal of Medicine. 1969;281(25):1389-93.
13. Stanley TH, Webster LR. Anesthetic requirements and cardiovascular effects of fentanyl-oxygen and fentanyl-diazepam-oxygen anesthesia in man. Anesthesia & Analgesia. 1978;57(4):411-6.
14. Verrier ED, Wright IH, Cochran RP, Spiess BD. Changes in cardiovascular surgical approaches to achieve early extubation. Journal of cardiothoracic and vascular anesthesia. 1995;9(5):10-5.
15. Sirio CA, Martich GD. Who goes to the ICU postoperatively? Chest. 1999;115(5):125S-9S.
16. Jung C. Assessment of microcirculation in cardiogenic shock. Current Opinion in Critical Care. 2019;25(4):410-6.
17. Leyh RG, Kofidis T, Strüber M, Fischer S, Knobloch K, Wachsmann B, et al. Methylene blue: the drug of choice for catecholamine-refractory vasoplegia after cardiopulmonary bypass. The Journal of thoracic and cardiovascular surgery. 2003;125(6):1426-31.
18. Weis F, Kilger E, Beiras-Fernandez A, Nassau K, Reuter D, Goetz A, et al. Association between vasopressor dependence and early outcome in patients after cardiac surgery. Anaesthesia. 2006;61(10):938-42.
19. Uz Z, Ince C, Guerci P, Ince Y, P Araujo R, Ergin B, et al. Recruitment of sublingual microcirculation using handheld incident dark field imaging as a routine measurement tool during the postoperative de-escalation phase—a pilot study in post ICU cardiac surgery patients. Perioperative Medicine. 2018;7(1):1-8.
20. De Backer D, Dubois M-J, Schmartz D, Koch M, Ducart A, Barvais L, et al. Microcirculatory alterations in cardiac surgery: effects of cardiopulmonary bypass and anesthesia. The Annals of thoracic surgery. 2009;88(5):1396-403.
21. Bauer A, Kofler S, Thiel M, Eifert S, Christ F. Monitoring of the sublingual microcirculation in cardiac surgery using orthogonal polarization spectral imaging: preliminary results. The Journal of the American Society of Anesthesiologists. 2007;107(6):939-45.
22. Murphy G, Angelini G. Side effects of cardiopulmonary bypass: what is the reality? Journal of cardiac surgery. 2004;19(6):481-8.
23. De Backer D, Creteur J, Dubois M-J, Sakr Y, Vincent J-L. Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. American heart journal. 2004;147(1):91-9.
24. De Backer D, Creteur J, Preiser J-C, Dubois M-J, Vincent J-L. Microvascular blood flow is altered in patients with sepsis. American journal of respiratory and critical care medicine. 2002;166(1):98-104.
25. Bakker J, Ince C. Monitoring coherence between the macro and microcirculation in septic shock. Current Opinion in Critical Care. 2020;26(3):267-72.
26. Ince C, De Backer D, Mayeux PR. Microvascular dysfunction in the critically Ill. Critical Care Clinics. 2020;36(2):323-31.
27. Guven G, Hilty MP, Ince C. Microcirculation: physiology, pathophysiology, and clinical application. Blood Purification. 2020;49(1-2):143-50.
28. Ince C, Boerma EC, Cecconi M, De Backer D, Shapiro NI, Duranteau J, et al. 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 medicine. 2018;44(3):281-99.

29. Ince C. Hemodynamic coherence and the rationale for monitoring the microcirculation. Critical care. 2015;19(3):1-13.
30. den Uil CA, Lagrand WK, van der Ent M, Nieman K, Struijs A, Jewbali LS, et al. Conventional hemodynamic resuscitation may fail to optimize tissue perfusion: an observational study on the effects of dobutamine, enoximone, and norepinephrine in patients with acute myocardial infarction complicated by cardiogenic shock. PLoS One. 2014;9(8):e103978.
31. Corstiaan A, Lagrand WK, Spronk PE, van Domburg RT, Hofland J, Lüthen C, et al. Impaired sublingual microvascular perfusion during surgery with cardiopulmonary bypass: a pilot study. The Journal of thoracic and cardiovascular surgery. 2008;136(1):129-34.
32. Černý V, Turek Z, Pařízková R. Orthogonal polarization spectral imaging. Physiol Res. 2007;56:141-7.
33. Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, et al. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. Nature medicine. 1999;5(10):1209-12.
34. Goedhart P, Khalilzada M, Bezemer R, Merza J, Ince C. 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.
35. Ince C. Sidestream dark field imaging: an improved technique to observe sublingual microcirculation. Critical care. 2005;9(1):1-.
36. Lipinska-Gediga M. Sepsis and septic shock-is a microcirculation a main player? Anaesthesiology intensive therapy. 2016;48(4):261-5.
37. 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. Critical care medicine. 2013;41(3):791-9.
38. Hernandez G, Boerma EC, Dubin A, Bruhn A, Koopmans M, Edul VK, et al. Severe abnormalities in microvascular perfused vessel density are associated to organ dysfunctions and mortality and can be predicted by hyperlactatemia and norepinephrine requirements in septic shock patients. Journal of critical care. 2013;28(4):538. e9-. e14.
39. Sakr Y, Dubois M-J, De Backer D, Creteur J, Vincent J-L. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. Critical care medicine. 2004;32(9):1825-31.
40. Potente M, Mäkinen T. Vascular heterogeneity and specialization in development and disease. Nature Reviews Molecular Cell Biology. 2017;18(8):477-94.
41. Ince C. The rationale for microcirculatory guided fluid therapy. Current opinion in critical care. 2014;20(3):301-8.





2.

Background

2.1 | Cardiothoracic surgery and CPB

Cardiothoracic surgery via sternotomy is a common and effective treatment for heart and lung diseases, such as heart valve disease or coronary heart disease.[1] It is an invasive procedure, with a risk for major bleeding[2], postoperative organ dysfunction, due to global hemodynamic alterations, regional blood flow alterations and excess inflammation[3, 4], and the possibility of organ failure[5, 6]. Cardiothoracic surgery is usually performed using CPB to achieve cardioplegic arrest, allowing the surgeon to work without the interference of a beating heart.[7] CPB is a form of extracorporeal circulation that takes over the heart's and lungs' circulatory and respiratory functions during the heart's cardioplegic arrest.[8] The CPB circuit consists of a centrifugal or roller pump, cannulae, connecting tubing, oxygenator, venous blood collection reservoir, arterial line filter, and a heat exchanger.[8] Despite advances in perioperative care, surgical (i.e., CPB), and anesthetic techniques, cardiothoracic surgery is still associated with a high rate of complications[9, 10], and postoperative in-hospital mortality rates of 3-6%.[11-14] Certain intraoperative and postoperative factors, such as hemodilution, CPB duration, blood transfusion, and acute postoperative renal failure, may significantly alter the risk of in-hospital mortality.[15-19] With today's aging population and more chronically ill patients undergoing cardiothoracic surgery, the likelihood of unfavorable postoperative complications is expected to increase even more.[20]

The use of CPB exposes blood to non-biocompatible polymers.[21, 22] The contact between the blood and the artificial surface, ischemia-reperfusion injury, endotoxemia, surgical trauma, and rewarming cause the activation of blood cells (i.e., neutrophils), serum proteins and the initiation of inflammatory cascades such as the complement, extrinsic coagulation, and fibrinolytic pathways.[8, 21] The systemic inflammatory response[23] combined with the non-pulsatile CPB flow[24, 25] contributes to extravascular fluid shift[26], platelet dysfunction[8], bleeding disorders[27], vasoplegia[28], tissue malperfusion[8], and postoperative organ dysfunction, including acute kidney injury (AKI)[29], neurological dysfunction[30], post-perfusion syndrome[31], and acute respiratory distress syndrome (ARDS)[32].

Efforts have been made to improve the biocompatibility of CPB circuits to reduce the systemic inflammatory response. Nevertheless, significant inflammatory activation still occurs after the onset of CPB, regardless of preventive measures, making its use a risk factor for early postoperative morbidity.[22, 33]

2.2 | The microcirculation

After cardiothoracic surgery, patients are admitted to the ICU, where they are monitored and treated to establish and maintain homeostasis.[34] Homeostasis is the tendency of cells, tissues, and the entire living system to maintain and regulate stability and constancy of internal, chemical, and physical conditions in order to function correctly.[35] The goal of achieving homeostasis in the ICU is to restore the patient's physiological state to a medically acceptable state, using interventions, and supporting the body in achieving this medically acceptable state, as the body cannot achieve it on its own.[36] Unfortunately, the development of organ failure cannot always be prevented by achieving a medically acceptable state in terms of quick normalization of vital functions.[37] An organ

can only properly function if it receives adequate O_2 and nutrients. In the case of a healthy state combined with adequate microcirculatory perfusion, the amount of O_2 delivery (DO_2) exceeds the O_2 consumption by the tissues (VO_2).[38]

A factor causing organ dysfunction after cardiothoracic surgery is impaired tissue perfusion due to microcirculatory failure.[39, 40] The microcirculation is the smallest unit of the cardiovascular circulation that has the fundamental task of exchanging respiratory gasses (i.e., O_2 and carbon dioxide (CO_2)), nutrients, water, hormones, heat, and waste products between the circulating blood and the parenchymal cells.[41, 42] Based on the anatomical properties and direction of blood flow, the microcirculation can be divided into arterioles (<100µm), capillaries (<20µm), and venules (<100µm). The microcirculation plays an important role, directly in maintaining organ perfusion

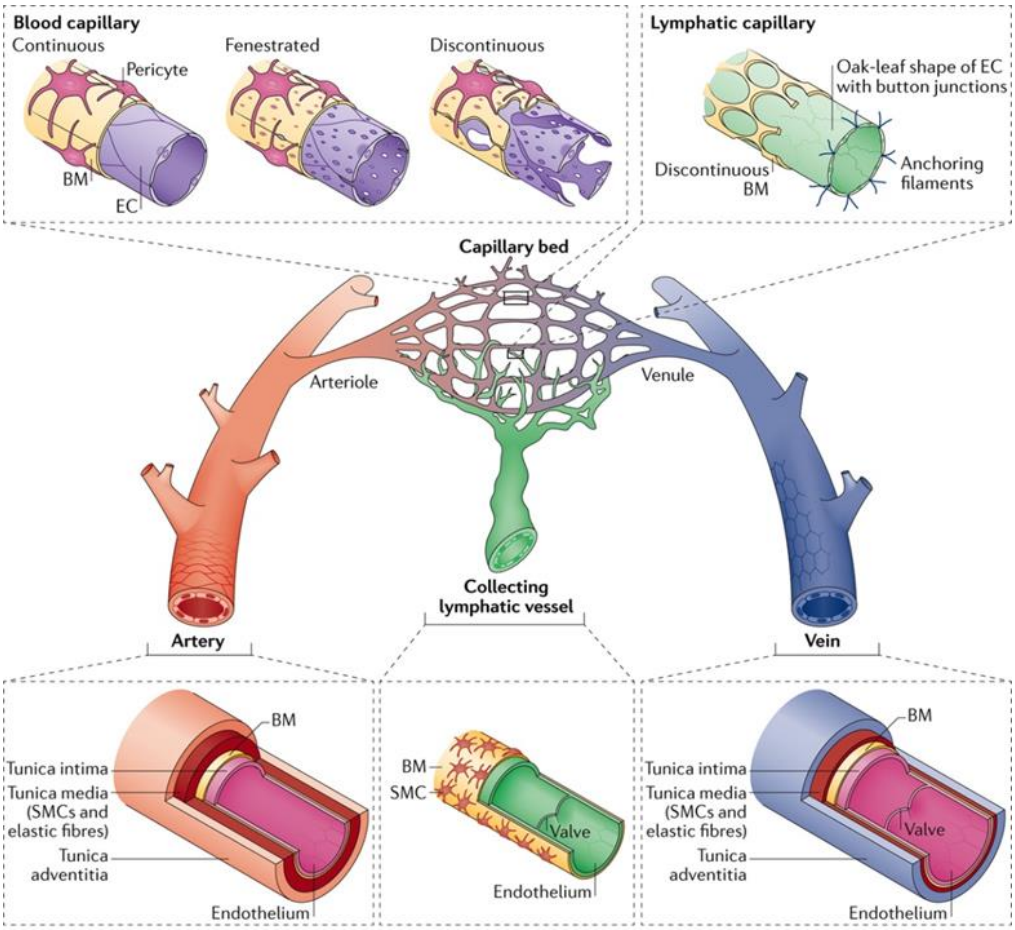


Figure 1

A hierarchical network of arteries (red), veins (blue) and capillaries, as well as lymphatic capillaries and collecting vessels (green) that drain fluid into the venous circulation, form the vasculature of the circulatory system. Most intercellular communication occurs in the microcirculation (i.e., arterioles, capillaries and veins). Arteries and veins are characterized by a continuous lining of ECs, BM and layers of SMCs. The endothelium of capillaries can be continuous, fenestrated or discontinuous, and are covered to varying degrees by BM and pericytes. Lymphatic capillaries consist of ECs that allow fluid to pass through at high interstitial pressure. Collecting lymphatic vessels have little SMC coverage and contain luminal valves that aid in pumping and prevent reflux of lymph. ECs: endothelial cells; BM: basement membrane; SMCs: smooth muscle cells.

and indirectly in maintaining organ function. It must ensure maximum distribution of O_2 and nutrients to mitochondria in support of oxidative phosphorylation by touching almost every parenchymal cell while minimizing the area it occupies to provide space for the parenchymal cells, nerves, structural tissue, inflammatory cells, and other cell types that directly contribute to organ function.[43, 44] In the case of hypoxia, endothelium-mediated vasodilation occurs through the release of vasodilating prostaglandins, nitric oxide (NO), and putative endothelium-derived hyperpolarizing factor.[45-50] In the endothelial response to hypoxia, erythrocyte NO release is also likely to play an active role.[51, 52] The release of metabolites such as lactate, H^+, adenosine, and K^+ by underlying tissues induces a more delayed vasodilatory response.[53]

A capillary consists of a single layer of epithelium and a basement membrane, which allows the capillary to exchange molecules (i.e., O_2 and waste products) between blood and parenchymal cells (**Figure 1**[54]). The vessel walls of capillaries can be morphologically distinct, both within a particular part of the network and between different parts of the network, and are formed by a continuous, fenestrated, or discontinuous monolayer (**Figure 1**). Fenestrated endothelium is found in tissues involved in secretion and filtration, and discontinuous endothelium is characteristic of sinusoidal vascular beds.[54] The main determinants of capillary perfusion are capillary patency and capillary blood flow, consisting of arteriolar tonus, driving pressure, and hemorheology.[55] Two primary mechanisms accomplish oxygen transport by RBC flow: the passive diffusion of O_2 from the RBCs to the mitochondria of tissue cells and the convective transport of the oxygen-carrying RBCs.[56] Fick's diffusion law defines the diffusive movement of O_2 from the RBCs to the mitochondria in tissue cells. The oxygen flux is the product of the difference between

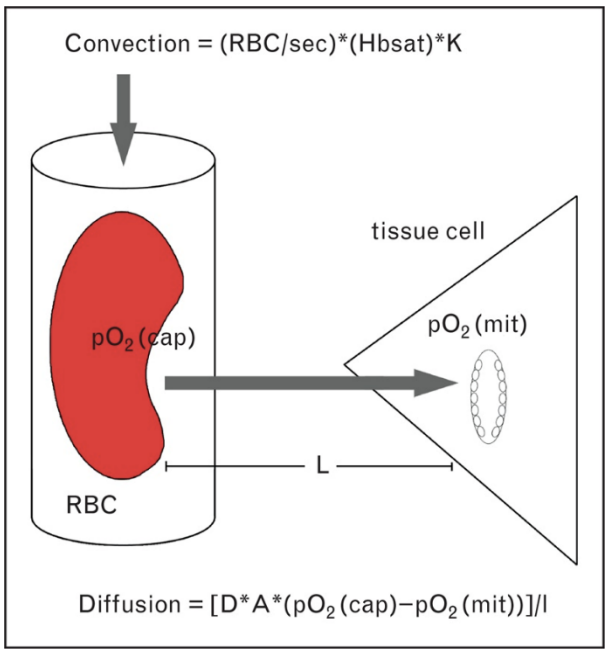


Figure 2

Oxygen transport from the microcirculation to tissue cells by convective and diffusive determinants. Fick's diffusion law defines the diffusive movement of oxygen from the RBCs to the mitochondria in tissue cells. The oxygen flux is the product of the difference between the partial pressure of oxygen at the RBC minus that at the mitochondria and the diffusion distance times the exchange area divided by the diffusion distance from the RBC to the mitochondria. The product of the rate at which the RBCs enter the capillary, the oxygen-carrying saturation of the hemoglobin in RBCs and the oxygen-carrying capacity of an RBC at 100% saturation (0.0362 pl O_2/RBC) define the convective flow of RBCs. A: exchange surface; cap: capillary; D: diffusion distance; Hbsat: oxygen-carrying saturation; K: oxygen-carrying capacity of RBC; l: diffusion distance from the RBC to the mitochondria; mit: mitochondria; RBC: red blood cell; pO_2: partial pressure.

the partial pressure of O_2 at the RBC minus that at the mitochondria and the diffusion distance times the exchange area divided by the diffusion distance from the RBC to the mitochondria, as shown in **Figure 2**.[57, 58] The product of the rate at which the RBCs enter the capillary, the oxygen-carrying saturation of the hemoglobin in RBCs, and the oxygen-carrying capacity of an RBC at 100% saturation (0.0362 pl O_2/RBC) define the convective flow of RBCs (**Figure 2**).[59]

Although O_2 exchange from capillary blood to tissue cells is the primary function of the microcirculation, the arteriolar and venular parts have essential functions in maintaining adequate hemodynamics and tissue perfusion. Arterioles are small branches of arteries that drain via terminal arterioles into capillaries. The vessel walls of arterioles are surrounded by a continuous smooth muscle layer controlled by the sympathetic nervous system (**Figure 1**). In response to changes in local shear stress, vascular endothelial cells can deliver local stimulatory signals to arterioles via cell-to-cell communication.[60, 61] Depending on the local metabolic needs of the tissue cell, independent of changes in blood pressure, central and local control mechanisms (i.e., myogenic, metabolic, and neurohumoral mechanisms) regulate vasoconstriction or vasodilation of arterioles.[62] This process is called autoregulation and serves as a mechanism to maintain an adequate mean arterial pressure (MAP). In an abnormal condition, such as shock, the balance between peripheral tissue flow and vascular resistance to maintain arterial vascular pressure can be disturbed, resulting in insufficient blood flow to less important tissues (i.e., muscle, gastrointestinal tract, skin and subcutaneous) to maintain metabolism in specific vital organs (i.e., brain, heart, kidneys).[63-65] Two or more capillaries join to form postcapillary venules, which then converge to form collecting venules. Venules serve as a large low-pressure reservoir through which blood is returned to the heart and can contain up to 75% of the total blood volume, making the microcirculation a major player in regulating preload and CO.[66] Venules consist of endothelial cells surrounded by a near complete layer of pericytes, instead of smooth muscle cells (**Figure 1**).[67, 68] Pericytes make contact with the endothelium to regulate vascular stability and transendothelial transport.[69] Postcapillary venules and collecting venules are important sites for inflammation-induced changes in macromolecular permeability and inflammatory cell adhesion and transmigration, where leukocyte rolling, sticking, and extravasation can be best observed in the postcapillary venules.[70-72] The quantity of venous blood, cardiac preload, and cardiac output is influenced by active and passive changes in venous vascular tone to maintain the circulating blood pool.[66] To maintain organ perfusion and, consequently, organ function, adequate functioning of the microcirculation is essential.[73]

Microcirculatory perfusion has been studied mainly in patients with severe inflammatory conditions, such as sepsis, in which the microcirculation is significantly impaired.[74] These studies have shown that microcirculatory perfusion disorders contribute substantially to the development of acute organ dysfunction. In cardiothoracic surgery, microcirculatory impairment in perfusion was found to occur after initiation of CPB, of which these changes persisted postoperatively.[39, 75] Nevertheless, the mechanisms behind impaired microcirculatory perfusion during CPB remain to be elucidated.

2.2.1 | Sublingual microcirculation

The sublingual area shares a common embryogenic origin with the splanchnic system.[76] The unique anatomy includes three prominent landmarks: the lingual frenulum in the midline, the sublingual papillae (caruncles), and the bilateral sublingual folds (**Figure 3**[77]). The sublingual artery,

via the lingual artery, which branches off from the external carotid artery, mainly supplies the sublingual area. Venous drainage from the sublingual area passes mainly directly to the internal jugular system via the deep lingual veins (**Figure 4**[78]).[76] Proportionally, more capillaries and veins than arteries are present in the sublingual area.

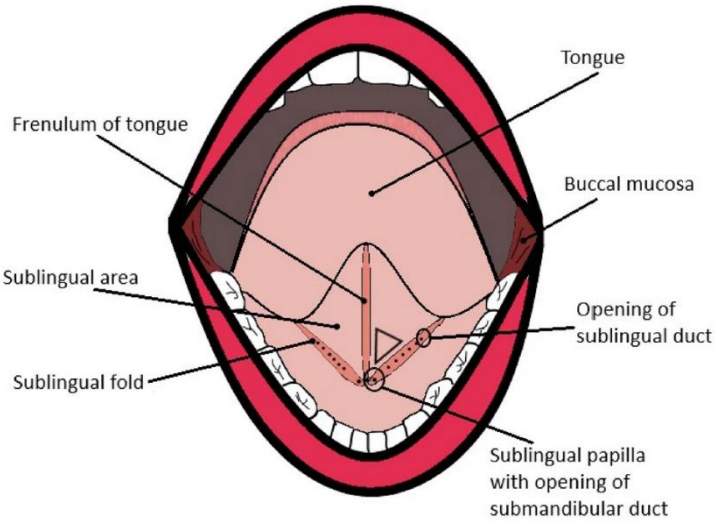


Figure 3

Anatomy of the sublingual space. The microcirculation should be measured in the sublingual triangle (black triangle).

For a long time, it was largely unclear whether microcirculatory changes in the sublingual region were representative of hypoperfusion in other organs, particularly in the splanchnic region. However, recently, a large number of studies have shown that the sublingual microcirculation site is highly clinically relevant, as microcirculatory alterations were found to be highly sensitive and specific, as an indicator for the severity of disease and for predicting morbidity and mortality in critically ill patients, much more than changes in the macrocirculation or oxygen-derived

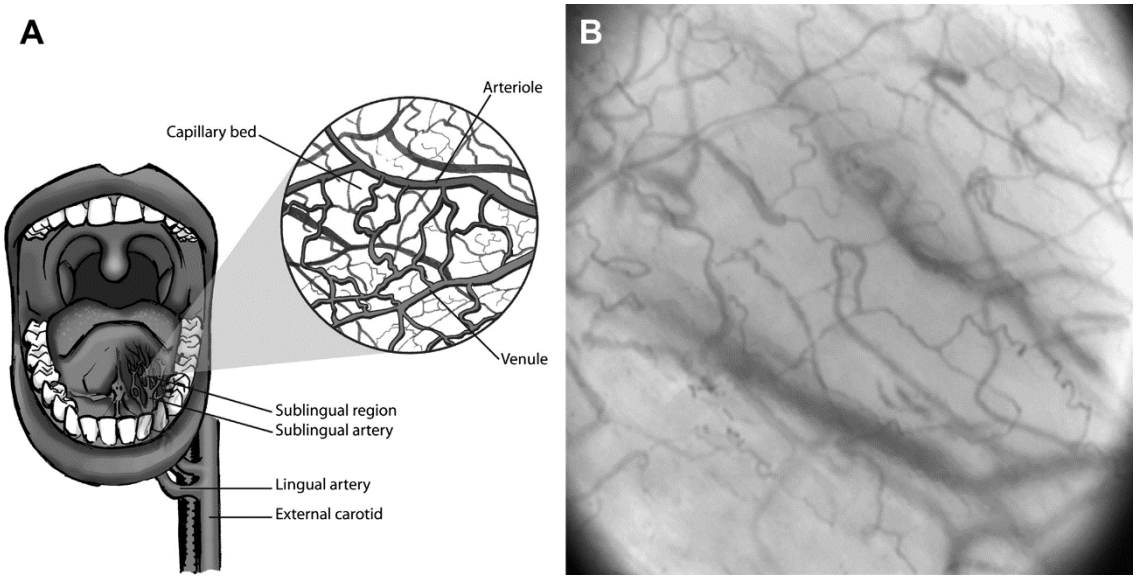


Figure 4

*Overview of the sublingual circulation, including (**A**) the sublingual microcirculation. (**B**) Image of the sublingual microcirculation captured with OPS imaging. OPS; orthogonal polarization spectral imaging.*

variables.[79, 80] Sublingual microcirculatory alterations appear to correlate with microcirculatory alterations in the intestines and kidneys[81-83] and are therefore considered a sensitive indicator of circulatory failure.[81, 82, 84]

2.2.2| Functional microcirculatory measures

Recent developments in microcirculatory measurement techniques in humans have renewed interest in microcirculation by clinicians.[85] For several years, it has been possible to visualize the microcirculation at the bedside of the patient with an HVM using OPS, SDF, or IDF imaging.[86] These techniques use polarized green light, which is only absorbed by the hemoglobin RBCs, enabling visualization of superficial vessels.[87] RBCs are imaged as dark flowing globules against a grayish/white background.[87, 88] The vessel wall is not visible with HVM. In most larger blood vessels, several RBCs flow side by side, making it easy to determine the vessel diameter. In small capillaries, this is more complicated, especially when plasma gaps separate the RBCs. A plasma gap represents fluid that changes shape because it is a rheological medium for blood to flow.[89] To describe the microcirculation's physiological properties, several functional microcirculatory measures have been developed (**Table 1**).[86, 90] Total vessel density (TVD) is the potential capillary density in the current functional state of the microcirculation. The TVD is calculated by dividing the total length of all capillaries filled with RBCs by the total area in the field of view. It is a determinant of the diffusion capacity of the microcirculation.[90]

In a normal healthy state, the microcirculation is characterized by minimal heterogeneity. The visualized capillaries form a dense network of perfused capillaries, although the flow between capillaries may vary according to the metabolic needs of surrounding cells. Capillaries can adapt to the new metabolic needs of surrounding cells by vasodilation, vasoconstriction, and adjusting the velocity of circulating cells within them. Local factors, including direct stimulation of endothelial cells by backward communication and local release of NO by RBCs under hypoxic conditions, primarily regulate the fine-tuning of perfusion in capillaries via modulation of the precapillary sphincter. In severe disease, capillary density may decrease. A variable proportion of capillaries are not perfused or are perfused intermittently, leading to heterogeneous tissue perfusion. Capillary tissue perfusion may change from minute to minute, with non-perfused capillaries suddenly becoming perfused and vice versa.[83, 91]

The functional capillary density (FCD) and proportion of perfused vessels (PPV) describe the number of capillaries contributing to tissue perfusion. FCD stands for capillary patency[92], where FCD is defined as the sum of the length of all capillaries exhibiting normal flow divided by the total surface of the analyzed area. A smaller FCD implies a more heterogeneous capillary perfusion. The FCD is a determinant of the microcirculatory diffusion capacity.[90] The PPV is defined as the number of perfused vessels divided by the total number of vessels. It is a measure of the heterogeneity of capillary perfusion.[90]

Capillary hematocrit (cHct) is also a determinant of microcirculatory diffusion capacity. It is defined as the weighted mean of the whole blood volume to RBC volume ratio in the analyzed area.[90]

The red blood cell velocity (RBCv) is a determinant of the microcirculatory convection capacity. It is described by the weighted mean of the absolute RBC velocities in all capillary segments in the

analyzed area. This measure yields the quantitative movement of each RBC and provides the basis for the quantitative measurement of function heterogeneity of the microcirculation. The RBCv will

Table 1 *Microcirculatory parameters*

Parameter	Abbreviation	Unit	Definition
Determinant of diffusive capacity			
Functional capillary density	FCD	mm/mm^2	Sum of the length of all capillaries exhibiting normal flow divided by the total surface of the analyzed area
Total vessel density	TVD	mm/mm^2	Sum of the length of all capillaries containing RBCs divided by the total surface of the analyzed area
Capillary hematocrit	cHct	%	Weighted mean (by capillary segment length) of the RBC volume divided by the whole blood volume in all capillary segments within the field of view
Determinant of convective capacity			
Red blood cell velocity	RBCv	µm/s	Weighted mean (by capillary segment length) of the absolute RBC velocities in all capillary segments in the analyzed area.
Microvascular flow index	MFI	1	Manual grid-based score per quadrant, no flow (0), intermittent flow (1), slow flow (2), normal flow (3). Mean of subjective, qualitative flow score.
Determinant of diffusive and convective capacity			
Density of perfused vessels	PVD	mm/mm^2	Percentage of Perfused Vessels x TVD
Tissue red blood cell perfusion	tRBCp	µm/min	Weighted mean (by capillary segment length) of the product of the integral over time of the linear displacement of RBCs, capillary segment whole blood volume, and cHct, divided by the total surface of the analyzed area
Aspect of the heterogeneity of capillary perfusion			
Proportion of perfused vessels	PPV	%	Weighted mean (by capillary segment length) of the categorical per-vessel 'non-perfused' property.

RBC: red blood cell

likely replace the manual flow grading via the Microvascular flow index (MFI) due to the subjectivity of the MFI measure.[90]

In 2020, a new functional microcirculation parameter was introduced: tissue red blood cell perfusion (tRBCp). This parameter more precisely describes the concept of tissue perfusion in relation to DO_2. tRBCp is defined as the displacement of RBC volume divided by the tissue volume in the field of view. It is a quantitative parameter focusing on tissue perfusion by RBCs, calculated using algorithm-based analyses on standard microcirculatory image sequences.[90] tRBCp integrates individual TVD, RBCv, and cHct, reflecting a combination of microcirculatory convection and diffusion capacity determinants. It is a promising parameter as a point-of-care target for hemodynamic management in critically ill patients.[90, 93]

2.3| Measurement of the Microcirculation Using an HVM

There are several surrogates for microcirculation and tissue perfusion, which are assessed via blood samples, e.g., lactate or venoarterial CO_2 - gap, or noninvasively by evaluation of skin properties, such as mottling of the skin, capillary refill time, and warmth of the extremities.[94-97] The circulation of the skin is the first vascular system from which blood is diverted during circulatory compromise.[98, 99] A semi-quantitative measurement of oxy- and deoxyhemoglobin saturation in a catalyzed tissue volume can be achieved with near-infrared spectroscopy (NIRS). However, in an ICU population, the derived NIRS parameters were independent of the pathophysiological state of the patient and were not associated with significant changes in the macrocirculation.[100] Although these surrogate-based methods provided some insight into the functioning of the microcirculation, these techniques were unable to visualize the microcirculation. In the early 20th century, direct intravital observation of the microcirculation was limited to the use of bulky capillary microscopes, which were used primarily to determine the microcirculation of the capillary nail bed.[101] This technique was considered the golden standard for in vivo investigation of the microcirculation, but it was not extensively used in clinical practice due to the bulky microscopes.[102] Therefore, HVMs were developed for direct observation and assessment of functional properties of the microcirculation to identify underlying pathology at the bedside.[86]

The easiest way to study the microcirculation in postoperative cardiothoracic surgical patients in the ICU is to assess the sublingual microcirculation with an HVM. It is nowadays the most chosen anatomical site to obtain microcirculatory video images for evaluation because of its easy accessibility.[101, 103]

In this thesis, microcirculation measurements are performed using an innovative non-invasive bedside monitoring system, the CytoCam (Braedius Medical, Huizen, The Netherlands). A CytoCam is a sublingual microcirculation measurement device that consists of a monitor and an HVM (**Figure 5**[104]) and provides high-quality videos of the sublingual microcirculation suitable for data analysis.[101, 105] The technique that is used in the CytoCam relies on the IDF illumination imaging technique.[101]

In 1971, the IDF technique was first described.[106] This method made it possible to observe the microcirculation of an organ surface using epi-illumination without the need to transilluminate the tissue from below. In the late 1990s, OPS imaging, a technique similar to IDF, was added to a

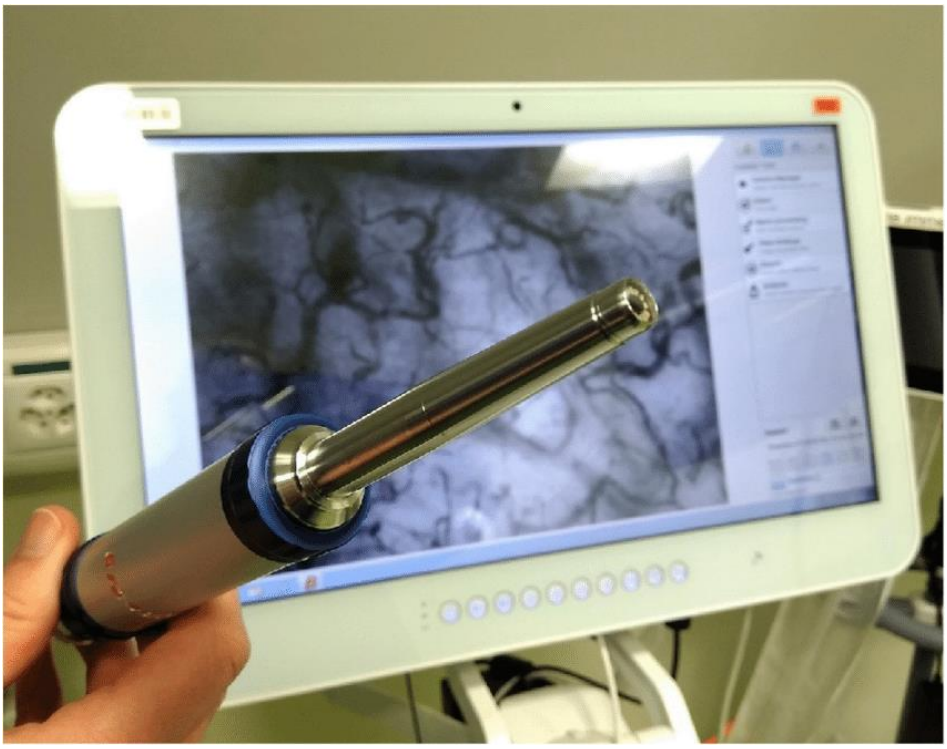


Figure 5
CytoCam device (Braedius Medical, Huizen, The Netherlands) with bedside monitor.

handheld video microscope to capture the organ surface microcirculation of surgical patients.[107-109] This new OPS technique made it possible for the first time to study human microcirculation in tissue and organ surfaces, in real-time, at the bedside, especially in critically ill patients. OPS used green linearly polarized light. Polarized light is projected onto the tissue via a beam splitter. The tissue immediately reflects some of the light. Light penetrating deeper into the tissue first undergoes multiple scattering, becomes depolarized, and will eventually back-scatter. Polarized reflected light originating from surface reflections was blocked by an orthogonally polarized analyzer in order to form an image of the microcirculation below the tissue surface.[86, 101, 107, 110, 111] Next, an HVM with SDF imaging was released. The SDF HVM uses a circularly illuminated tip with light-emitting diodes (LEDs) that create a dark field.[103] The difference between SDF and IDF HVMs lies in the improved optical resolution, making it possible to visualize more capillaries than its OPS and SDF predecessors.[112]

The CytoCam with IDF imaging is a third-generation pen-like handheld microscope. It uses a new hardware platform. This platform includes a high-density pixel-based imaging chip and a short, pulsed illumination source that synchronizes and controls illumination and image acquisition under computer control. The IDF technique is now commercially available and has since been validated as bedside monitoring for the microcirculation.[86, 101]

This technique uses green light of a specific wavelength produced by a ring of 12 circumferential high-brightness LEDs at the end of a light guide, within the middle, a magnifying lens to illuminate the target tissue tangentially. Surface reflections can entirely be avoided when using IDF, as the illumination light is optically isolated from the central column of the microscope. The stroboscopic green light is transmitted to the tissue and absorbed by oxygenated and deoxygenated

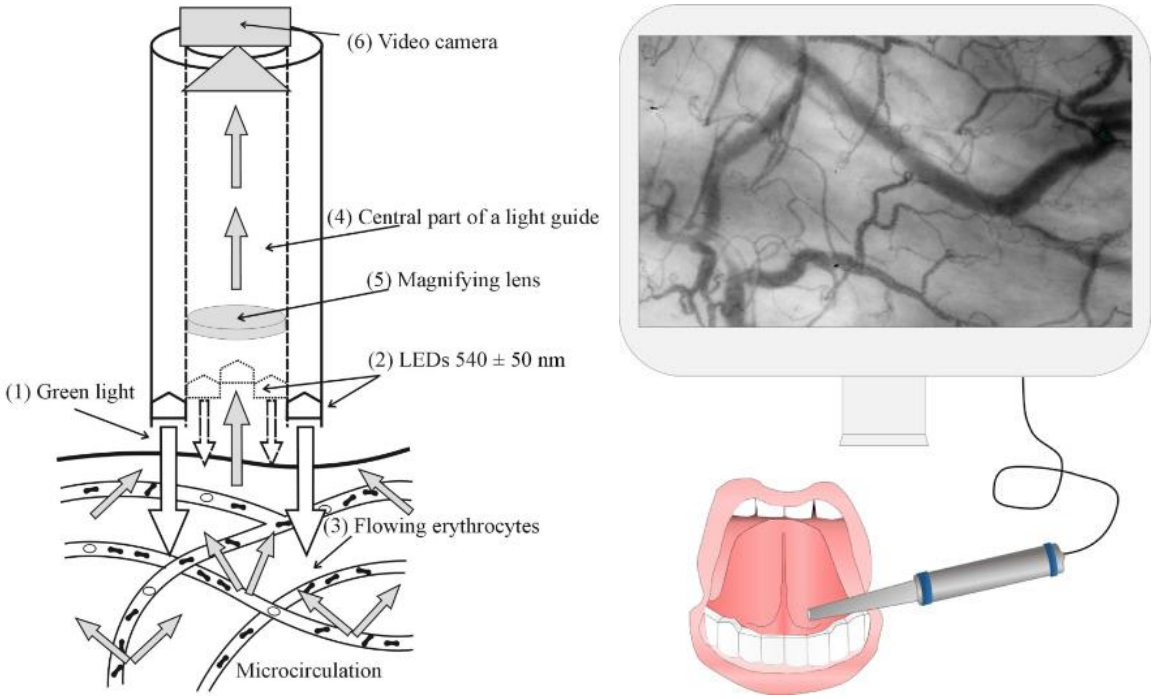


Figure 6

Left) *Schematic representation of SDF imaging. Green polarized light is projected onto the tissue via a beam splitter. Light penetrating deeper into the tissue first undergoes multiple scattering, becomes depolarized, and will eventually back-scatter. The quality of the image sequence is ensured by a focusing mechanism. The data will be transferred to the computer.* ***Right)*** *Representation of the usage of the CytoCam. The probe of the CytoCam is placed at the sublingual triangle in the sublingual area. Direct evaluation of the microcirculation is possible at bedside. LED: light emitting diode; SDF: sidestream dark-field.*

hemoglobin. The back-scattered light is captured through the center of the probe to form a dark-field illumination image, in which RBCs appear as dark globules.[106] The use of stroboscopic light prevents the smearing of flowing RBCs in the recorded images, resulting in sharp contour visualization of the microcirculation, showing flowing RBCs and leukocytes captured by a high-definition image sensor at a depth of approximately 1 mm. Plasma and leukocytes in the blood vessels cause luminous openings in the image.[113] Blood vessels appear as black lines on white/gray background due to the reflecting of the light by surrounding tissues.

The key specifications of the CytoCam can be found in **Table 2**[86].

The CytoCam bedside monitor includes a software package called CytoCam Tools for capturing, playing, and analyzing videos. CytoCam Tools includes a user interface (UI) to operate the CytoCam via a desktop or all-in-one computer. During operation, the user enters patient and study information into the dialog box. Afterward, the user can navigate between the controls for focusing and brightness of the LEDs and recording a video sequence. In addition, the system has a modular design, allowing the user to add modules for image stabilization, video editing, and analysis.[114] The microscope's tip is protected with an unsterile disposable cap perpendicular to the sublingual area of interest. The disposable cap (H & P Moulding Emmen B.V., Emmen, The Netherlands) is based on the use of ALTUGLAS® SG-7 (Arkema, Colombes, France), a hard transparent plastic. Three videos of at least 3 seconds will be recorded from different sublingual areas during each time point. According to international guidelines[86], it is necessary to capture multiple recordings in order to minimize heterogeneity in the microscopic field of view. Videos

Table 2 *Key specifications CytoCam*

Sensor	
Frame size Pixel size	14 megapixel (4416 x 3312 pixels) 1,4 x 1,4 µm
Frame size binning mode Pixel size	3,5 megapixel (2208 x 1648 pixels) 2,8×2,8 µm
Resolution	300 lines/mm
Image area	6227×4653 µm
Output	8-bit RAW, RGB
Image transfer rate	25 frames/s (full resolution)
Time to result	8s (minimum recording time is 3s, max recording time is 16s)
Optics	
Magnification factor lens	4
Field of view	1,55 x 1,16 mm
Focusing	
Focusing	manually controlled motorized focusing system (piezo linear motor)
Step size	4 µm, accuracy <2 µm
Illumination	
Imaging modality	IDF
Illumination source	12 high-brightness LEDs
Color	Green (wavelength 548nm)
Pulse time	2ms

RAW: unprocessed or minimally processed data from the image sensor; RGB: red, green, and blue color system.

will be excluded from further analysis if they do not meet image quality requirements such as illumination, image duration, focus, vessel content, stability, and absence of probe-induced pressure (**Appendix A**).[86, 115] The recorded videos can be analyzed offline with the software programs Automated Vascular Analysis (AVA) and MicroTools to extract relevant functional microcirculatory parameters. AVA software is the current standard for analyzing microcirculatory image sequences. This is a semi-automated software platform, making the analysis time-consuming and requiring considerable precision and skill from the person analyzing the images.

Microtools is a new, fully automated software program that can analyze microcirculation image sequences without human intervention to extract microcirculation parameters objectively. MicroTools calculates the FCD, TVD, cHct, RBCv, tRBCp, and PPV solely. Therefore, only these parameters are used later on in this thesis.

In recent years, many studies have been performed on microcirculation measurements using an HVM. Mainly in the sublingual area in a wide range of disease states and age groups[116-120], but also several studies of microcirculation performed directly on organ surfaces during surgery.[109, 121-123]

2.4| Microcirculatory alterations after cardiothoracic surgery

In cardiothoracic surgery using CPB, the switch from physiological circulation to extracorporeal circulation results in a sudden change in the nature of the systemic circulation. Microcirculatory alterations associated with CPB may have several hemodynamic and metabolic causes, which may lead to enhanced venous flow, functional microcirculatory shunting, and, most importantly, reduced oxygen delivery to tissues.

2.4.1| The surgery

First, the surgery affects blood perfusion in the microcirculation and can be divided into tissue trauma, ischemic injury from cardiopulmonary arrest, and subsequent ischemic reperfusion injury.[124] A circulatory arrest is associated with the immediate shutdown of the sublingual microcirculation, while flow persists in larger microvessels.[125] Additionally, anesthesia potentially interferes directly or indirectly with normal blood flow and vasoreactivity in the microcirculation but is not a significant player.[39, 126, 127]

2.4.2| Blood characteristics

Microcirculatory alterations may be caused by various blood characteristics. Probably the most important one is the relative presence of cells in the blood plasma, referred to as hematocrit (Hct). Hct is essential for tissue oxygenation because of its O_2 transport effect (convection) and its effect on maintaining capillary perfusion (diffusion).[128] Capillary Hct is lower than systemic Hct due to the preferential distribution of RBCs to the high-velocity center of microvessels (known as the Fahraeus effect) and heterogeneous distribution of Hct at microvascular bifurcations (the so-called network Fahraeus effect).[129] In addition to their contribution to tissue oxygenation, RBCs also have a primary function as sensors for blood flow distribution within the microcirculation, which could be disrupted during hemodilution.[32]

Under inflammatory conditions, platelets and leukocytes may adhere to the endothelium, leading to increased vascular leakage and capillary obstruction, which may interfere with microcirculatory perfusion.[130, 131]

2.4.3 | The influence of CPB

2.4.3.1 | Hemodilution

When CPB is used, it is associated with hemodilution. Hemodilution results in increased diffusion distance and a reduced convective flow leading to tissue hypoxia due to the loss of RBC-filled capillaries.[58] It results from mixing circulating blood with 1.5L-2.0L CPB priming solution, causing a 10-20% decrease in Hct values.[132] A sufficiently high RBC content in the blood is essential to maintain the number of perfused capillaries, and conversely, hemodilution leads to a decrease in these perfused capillaries.[128] The reduction of Hct is also associated with reduced blood viscosity, consequently causing altered PVD and FCD.[133]

2.4.3.2 | Non-pulsatile flow conditions

Switching to CPB involves a change from pulsatile to non-pulsatile conditions. Blood flow is necessary for normal endothelial cell function because of its flow sensitivity. Shear stress on the luminal side of the cell leads to increased production of NO by endothelial nitric oxide synthase. NO has anticoagulant and vasodilatory properties.[134] Consequently, reduced microcirculatory perfusion could initiate a vicious cycle of hypoperfusion and endothelial cell dysfunction. Using non-pulsatile flow during CPB reduces endothelial shear stress and NO production.[135] This suggests that endothelial function may deteriorate when switching to non-pulsatile flow during CPB, which is associated with decreased microcirculatory perfusion due to decreased hemodynamic energy resulting in microvascular shunting, capillary collapse, and activation of inflammatory mediators.[25, 136-138] Nevertheless, it is currently unknown whether pulsatile flow during CPB can preserve microcirculatory perfusion.[139]

2.4.3.3 | Hypotension

The systemic inflammatory response, combined with the reduction in blood volume due to the transition to CPB, hemodilution-associated reduction in blood viscosity, and increase in vascular capacitance with rewarming, is associated with reductions in blood pressure and cardiac output and may lead to hypotensive episodes. Whether hypovolemia and/or systemic hypotension affect microcirculatory perfusion is an ongoing debate. Correcting cardiac output and hypotensive episodes via volume or pharmacological interventions may affect microcirculatory perfusion.[132]

2.4.3.4 | Hypothermia

While using CPB, body temperature is reduced to 32-35 degrees Celsius. Hypothermia decreases myocardial and cerebral VO_2, thereby preserving cellular function. The temporary reduced O_2 requirement may conceivably affect microcirculatory perfusion, resulting in a heterogeneous microcirculatory flow.[132]

2.4.3.5 | Hyperoxia

To improve O_2 supply to tissues and compensate for CPB, hyperoxia (20-30kPa) is used during cardiothoracic surgery. However, hyperoxia may adversely affect the microcirculation, including reduced FCD, assuming shunting or vasoconstriction proximal to the microvascular network.[140-142]

2.4.4 | Inflammatory response

The intravascular inflammatory response is partially CPB-related and induced by the non-biocompatible polymers, hypothermia, release of endotoxins, non-pulsatile blood flow, and surgical trauma-related tissue trauma and reperfusion injury.[21, 143] In a state of stress, such as inflammation, the endothelium presents its adhesion molecules to the blood side of the vessel. Subsequently, activated endothelial cells lead to a vasoconstrictive, procoagulant state, in which cellular blood components may adhere to the vessel wall, leading to microvascular obstruction.[144]

In addition to the presentation of adhesion molecules on the endothelial surface with associated leukocyte activation[145], this complex inflammatory response also includes complement activation[146, 147], activation of the contact system of plasma[148], and secondary platelet activation[145], the release of pro-inflammatory cytokines (i.e., tumor necrosis factor (TNF)[149] and Interleukins (IL) 6 and 8[150]) and endotoxins, and production of phenomena such as oxygen free radicals, several arachidonic acid metabolites (i.e., thromboxane (TX) a_2[151]), lipid mediators (i.e., platelet-activating factor (PAF)[152, 153]), NO, and endothelins (**Figure 7**[143]). These pro-inflammatory cytokines are known

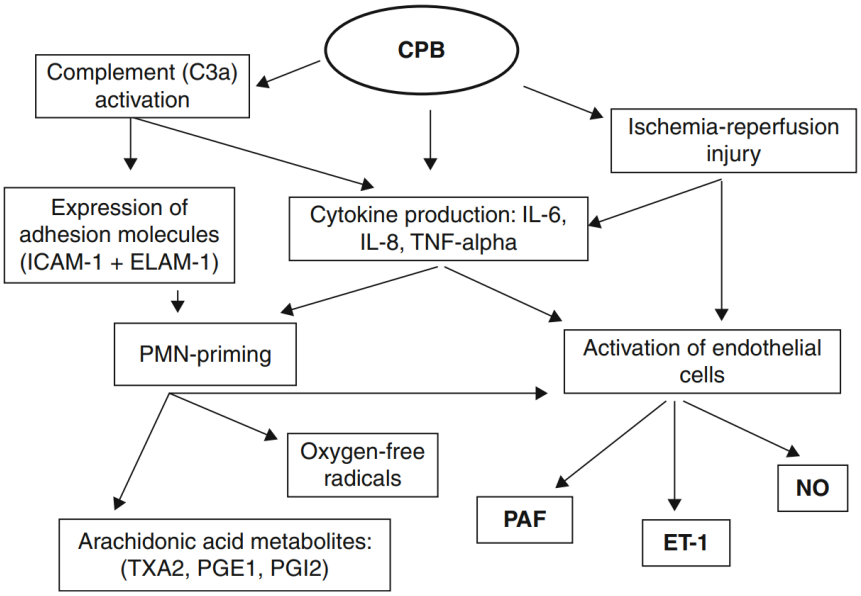


Figure 7

Schematic representation of the inflammatory response to CPB by biological mechanisms. Redundancy and interactions between arms is present. These mechanisms result in tissue injury and organ damage. CPB: cardiopulmonary bypass; ICAM-1: intracellular adhesion molecule-1; ELAM-1: endothelial-leukocyte adhesion molecule-1; IL: interleukin; TNF: tumor necrosis factor; PMN: polymorphonuclear leukocyte; TXA2: thromboxane A2; PGE1: prostaglandin E1; PGI2: prostacyclin; PAF: platelet activating factor; ET-1: endothelin-1; NO: nitric oxide.

to have profound effects on the vasculature, leading to increased vascular permeability, altered vascular morphogenic responses, adhesion, and transmigration of leukocytes, increased procoagulant activities, and increased platelet adhesion and aggregation.[154-157]

Because of the complexity of the inflammatory response in cardiothoracic surgery, the specific contribution of inflammation to derangements in microcirculatory perfusion is unknown. Evidence suggests that a pro-inflammatory state may be associated with microcirculatory dysfunction.[143]

2.4.5 | The occurrence of circulatory shock

2.4.5.1 | Vasoplegic syndrome

Several CPB-side effects known as postperfusion syndrome have been described since the introduction of CPB. Coagulopathy may occur due to the dilution of clotting factors and platelet dysfunction. Systemic inflammation may be induced by releasing pro-inflammatory cytokines[158-160], which may contribute to lung damage, myocardial reperfusion injury, and profound generalized vasodilatation. This generalized vasodilation is also known as vasoplegic syndrome (VS). VS occurs in the early postoperative phase after cardiothoracic surgery with CPB, especially after extended CPB[161], and is characterized by decreased arteriolar reactivity, severe hypotension with decreased systemic vascular resistance (SVR), and an increased requirement for volume filling and vasopressants regardless of a normal or augmented CO, causing organ hypoperfusion.[162, 163]

Physiological contraction of vascular smooth muscle occurs in response to increasing levels of intracellular calcium, which initiates a cascade of events that begins with myosin phosphorylation and leads to myosin-actin filament crossing and vasoconstriction. The influx of cytoplasmic calcium is increased by activation of the alpha-1 adrenergic receptor (a1), vasopressin-1 (V1) receptor, and angiotensin type-1 receptor (AT1).[164] This mechanism is dysregulated during CPB due to the CPB-related inflammatory response with the release of IL-1, IL6, and TNF-α, in particular. These cytokines may stimulate the locus coeruleus and the hypothalamic-pituitary-adrenal axis in the paraventricular nucleus. This will lead to adrenoreceptor desensitization[164], an immediate increase in vasoconstrictive mediators (i.e., norepinephrine (NE), antidiuretic hormone (ADH), arginine vasopressin (AVP) and angiotensin II (ATII)) with subsequent depletion, and the production of NO via inducible nitric oxide synthase (iNOS) (**Figure 8**[165]). NO is vasodilatory, and an excess can cause vasodilatory shock.[166-168] NO leads to an increase in cyclic guanosine monophosphate (cGMP)[169], which inhibits calcium in cells, causing muscle relaxation. NO also activates ATP-sensitive potassium channels (KATP), leading to hyperpolarization and inhibited vasoconstriction, regardless of the activation of G-protein coupled receptors.[167]

In extreme cases of vasoplegia, NE response can be inhibited by some mechanisms. Adrenergic receptors are phosphorylated, inhibiting catecholamines' binding, and increased NO production interferes with adrenergic receptors.[164] Together with acidosis, hyperpolarization of the cell membrane by stimulation of the KATP channel and AVP deficiency contribute to the vasoplegic state.

VS may be present in 5 to 50% of patients undergoing cardiac surgery, with high morbidity and mortality in those patients.[170, 171] VS after cardiovascular surgery accounts for less than 5% of all circulatory shock.[172]

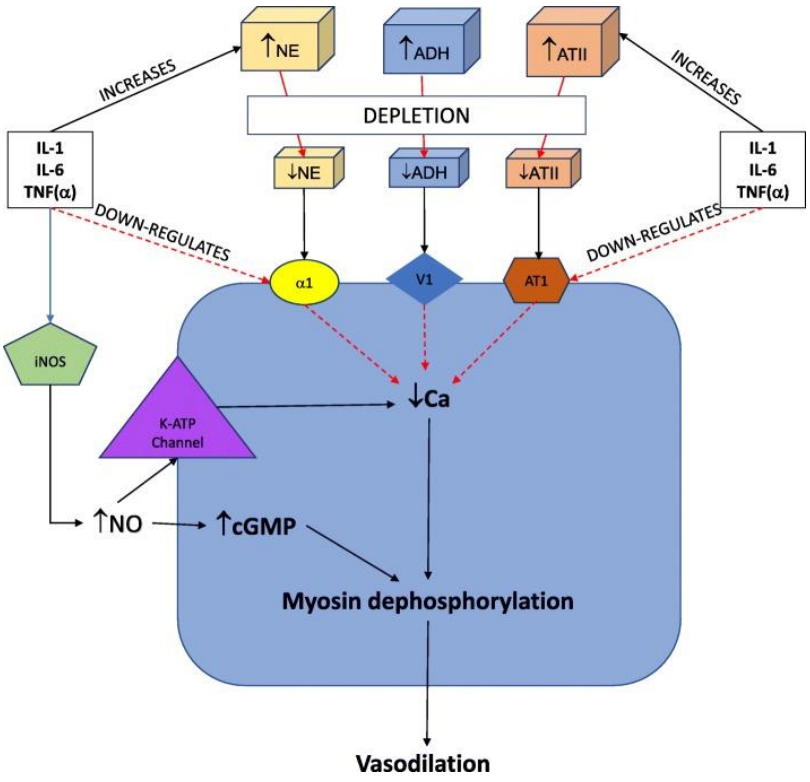


Figure 8

Pathophysiology of vasoplegia. Intracellular calcium causes myosin phosphorylation, leading to myosin-actin filament crossing and vasoconstriction, with physiological contraction of vascular smooth muscle cells in response. α1-, V1- and AT1receptor activation cause increase in cytoplasmic calcium. The release of inflammatory mediators during CPB may lead to a direct increase in vasoconstrictive mediators with subsequent depletion, adrenoreceptor desensitization and production of NO. NO causes an increase in cGMP, which inhibits calcium in cells, resulting in muscle relaxation. NO also causes activation of KATP channels, which causes hyperpolarization and inhibited vasoconstriction. NE: norepinephrine; ADH: antidiuretic hormone ; ATII: Angiotensin II; IL: interleukin; TNF: tumor necrosis factor; α1: Alpha-1 adrenergic receptor; V1: vasopressin-1 receptor; AT1: angiotensin type 1 receptor; iNOS: Inducible nitric oxide synthase; NO: nitric oxyde; KATP: ATP-sensitive potassium channels; cGMP: Cyclic guanosine monophosphate; Ca: calcium, CPB: cardiopulmonary bypass.

2.4.5.2|Circulatory shock

Circulatory shock is defined by the presence of global tissue hypoperfusion and signs of organ dysfunction resulting from severe cardiovascular compromise.[173] There are four main categories of circulatory shock, which can be assigned to four organ systems[174]:

- Hypovolemic shock - blood and fluid compartment

  It is caused by loss of intravascular volume, resulting in a decrease in cardiac preload to a critical level and reduced macro- and microcirculatory parameters. It has adverse effects on tissue metabolism and the elicitation of an inflammatory response.
- Distributive shock - vascular system

  A state of relative hypovolemia caused by a pathological redistribution of intravascular volume. It is caused by a loss of regulation of vascular tone, shifting volume within the cardiovascular system, and/or disordered permeability of the vascular system with a displacement of intravascular volume to the interstitium.

- Cardiogenic shock - heart

  A critical reduction in the heart's pumping capacity due to systolic or diastolic dysfunction leads to reduced ejection fraction or impaired filling of the ventricles.
- Obstructive shock - circulatory system

  It is caused by the obstruction of the great vessels or the heart itself.

Patients after cardiothoracic surgery may suffer from low cardiac preload (i.e., hemorrhage or tamponade) or pump failure (i.e., mechanical complication, myocardial stunning, or infarction), which may lead to one or a combination of types of shock (hypovolemic, obstructive and/or cardiogenic shock). Moreover, pathogen-associated molecular patterns (PAMPs) in case of infection and damage-associated molecular patterns (DAMPs) of injured tissue activate the inflammatory cascade. Rapid identification of the onset of shock and the type of shock is critical for adequate treatment and increased survival. Unfortunately, rapid shock identification in postoperative cardiothoracic surgical patients is challenging because of the coexistence of multiple types of shock, conflicting findings, pending outcomes, and multiple underlying problems.[173] If identified late or untreated, the condition deteriorates into a vicious cycle, leading to distributive shock, multiple organ failure (MOF), and death (**Figure 9**[173]).[175, 176]

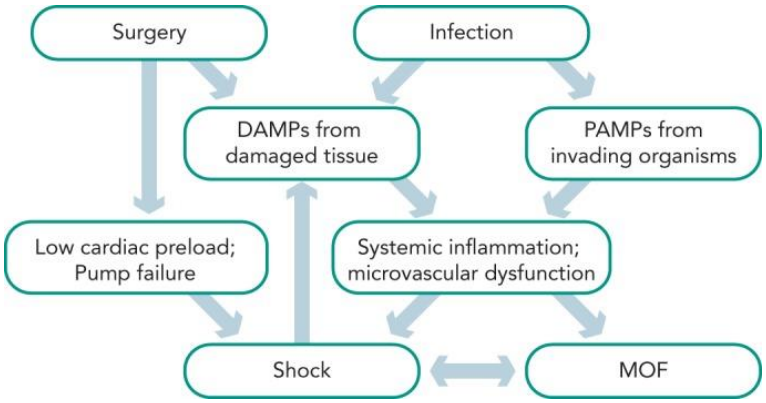


Figure 9

Mechanism of shock onset. After cardiothoracic surgery, patients may suffer from pump failure (e.g., infarction, mechanic complication or myocardial anesthesia) or low preload (e.g., hemorrhage or tamponade). This can cause cardiogenic, obstructive and/or hypovolemic shock. The inflammatory cascade is initiated by PAMPs in the case of infection and DAMPs in the case of injured tissue. Without treatment, this condition worsens into a vicious cycle toward distributive shock, MOF and death. DAMPS: damage-associated molecular patterns; MOF: multiple organ failure; PAMPS: pathogen-associated molecular patterns.

2.4.5.3|Blood lactate levels

Blood lactate level is frequently used as a marker of tissue hypoxia. Hyperlactatemia occurs in 10-20% of patients after cardiothoracic surgery, and a hyperlactatemia of more than two mmol/L at the time of shock diagnosis is associated with increased mortality and morbidity.[177-180] The causes of hyperlactatemia after cardiothoracic surgery include non-hypoxic and hypoxic causes.[178, 181, 182] A possible explanation for lactate acidosis occurring without tissue hypoxia is accelerated glycolysis. Here, stress-induced uptake of glucose by the β_2-receptor of peripheral tissues, due to the release of endogenous epinephrine, causes accelerated glycolysis and pyruvate production.[183, 184] Increased pyruvate production causes increased lactate production due to the mass effect on

the lactate-pyruvate equilibrium. Moreover, increased pyruvate production is associated with significantly increased pyruvate oxidation via the citric acid cycle.[185]

In the case of hypoxemia in a cell, glucose will be metabolized to lactate, resulting in a much lower Adenosine 5'-triphosphate (ATP) yield compared to when glucose is metabolized via pyruvate in the Krebs cycle under oxygenated conditions.[172] Normally, the lactate/pyruvate ratio is 10:1. Tissue hypoxia leads to an increased Adenosine diphosphate (ADP)/ATP and nicotinamide adenine dinucleotide + hydrogen (H^+) (NADH)/ nicotinamide adenine dinucleotide (NAD^+) ratio, which inhibits pyruvate dehydrogenase, blocking pyruvate entry into the citric acid cycle. In the case of hyperlactatemia, due to tissue hypoxia, pyruvate is preferentially converted to lactate, and the lactate/pyruvate ratio goes up.[186] Therefore, hyperlactatemia may reflect inadequate microcirculatory perfusion.[172] Nevertheless, studies highlight the complexity of lactate metabolism and the need to interpret hyperlactatemia with caution.[187]

2.4.6| Microcirculatory alterations to determine the type of shock

Different types of shock can be distinguished based on microcirculation characteristics. Four different types of microcirculatory changes are described (**Figure 10**[188]), which may occur alone or mixed[86, 90]:

- Type 1: complete stagnation of capillaries (e.g., in circulatory arrest, excessive use of vasopressors), reflected in the FCD, PPV, and RBCv
- Type 2: reduction in the number or flowing capillaries (e.g., in hemodilution), reflected in FCD, TVD and cHct
- Type 3: stopped-flow vessels are seen next to vessels with flowing cells (sepsis, hemorrhage, and hemodilution), reflected in FCD, TVD, PPV, RBCv, cHct, and tRBCp
- Type 4: hyperdynamic flow between capillaries (hemodilution, sepsis), reflected in TVD and RBCv

2.4.6.1|Cardiogenic shock

Cardiogenic shock may occur in patients with cardiac or extracardiac filling disorders such as cardiac tamponade.[189, 190] The atria and right ventricle are compressed by the acute rise in pericardial pressure, impeding diastolic filling of the heart[191], leading to decreased CO. As a compensatory response to the low CO, vasoconstriction occurs, restoring the decreasing arterial flow and pressure to previous values. In particular, a type 3 microcirculatory change underlies this type of shock. In cardiogenic shock, the vascular response to metabolic demands appears impaired.[192] Nonuniform arteriolar vasoconstriction due to sympathetic-vagal imbalance may cause a change in the ability of acetylcholine to reverse the observed microcirculatory constrictions and therefore has a vital role in reducing microcirculatory perfusion.[193] Arteriolar vasoconstriction may be exacerbated by increased sensitivity to already increased sympathetic output. Due to the reduced activity of endothelial iNOS, there may also be a systemic reduction in NO production.[194, 195] Rheological changes in cardiogenic shock, in addition to changes in vascular tone, include increased aggregation of RBCs, decreased deformability of RBCs, and early increase in viscosity due to increased fibrinogen and protein concentrations.[196]

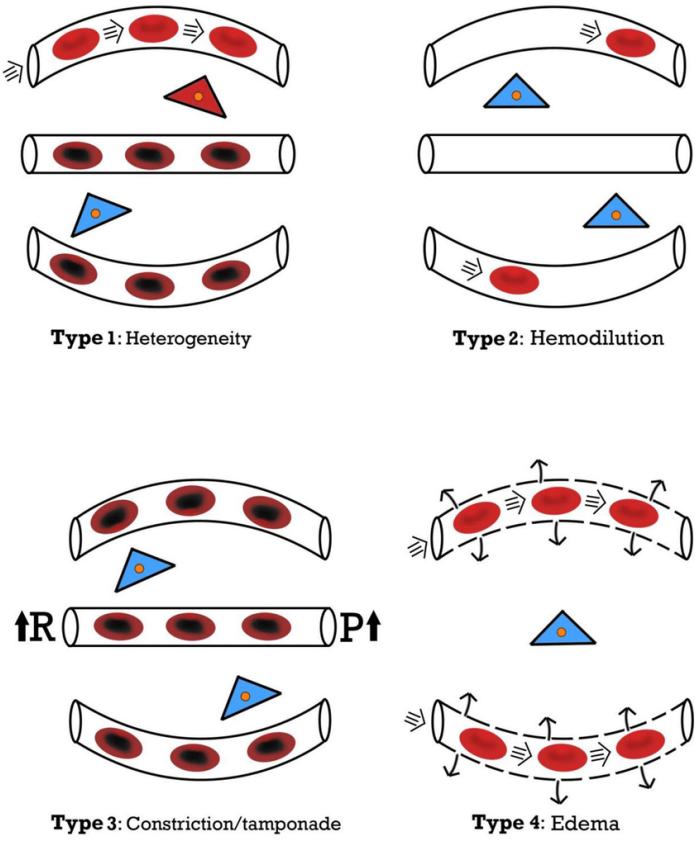


Figure 10

Tissue hypoxia due to loss of hemodynamic coherence between macro- and microcirculation because of microcirculatory changes. Type 1: heterogeneous microcirculatory perfusion with perfused capillaries alongside obstructed capillaries resulting in heterogeneous oxygenation of tissue cells. Type 2: hemodilution with dilution of blood in the microcirculation resulting in loss of RBC-filled capillaries and increased diffusion distance between RBCs in the capillaries and tissue cells. Type 3: Altered vascular variables cause stasis of microcirculatory RBC flow (e.g., tamponade due to increased venous pressure (P) and/or increased arterial vascular resistance (R)). Type 4: Capillary leak syndrome causes edema resulting in increased diffusion distance and decreased ability of oxygen to reach tissue cells. Red: well-oxygenated RBC and tissue cells; purple: RBC with reduced oxygenation; blue: reduced oxygenation of tissue cells. RBC: red blood cell.

The mechanisms for such endothelial and rheological changes may be mediated by a combination of reperfusion injury, a systemic inflammatory response, and increased concentrations of circulating catecholamines.[197]

2.4.6.2 | Obstructive shock

Obstructive shock has much in common with cardiogenic shock, so the two are often conflated.[198]

In obstructive shock, mechanical intra- or extravascular or luminal factors reduce blood flow in the great vessels or cardiac outflow with a critical decrease in CO, blood pressure, global oxygenation, and an increase in SVR.[175] The result is a shock state with tissue hypoxia in all organ systems.[199] Obstructive shock is also an example of type 3 microcirculatory alteration.

2.4.6.3 | Hypovolemic shock

Hypovolemic shock is characterized by increased sympathetic output resulting in altered inotropy and chronotropy. Via aldosterone- and vasopressin-induced salt and water retention and arteriolar vasoconstriction, the central blood volume is defended, making hypovolemic shock particularly prone to type 4 microcirculatory alterations.[200] Endothelial swelling is a consequence of hemorrhage.[201] Tissue damage triggers an inflammatory response that results in endothelial activation, which in part initiates vascular hyporesponsiveness via upregulation of endothelin-1 and iNOS.[202, 203] When activated, the endothelium becomes an adhesive surface to which activated slow-rolling leukocytes can adhere. The leukocytes are consequently exposed to inflammatory cytokines released at the site of injury or ischemia and diaphoretic via the dilating gap junctions of the capillary endothelium.[204] Leukocytes stimulate further endothelial activation[205] and obstruct the capillary lumen, resulting in a positive feedback loop of microcirculatory and cellular dysfunction[206].

Erythrocytes can also affect microcirculation. Erythrocytes have the ability to deform and allow them to pass through capillaries much smaller than their own diameter (~7µm). Under stressful conditions, deformability decreases rapidly due to membrane free radicals, which, combined with endothelial swelling, leads to decreased microcirculatory RBC perfusion[175, 207] and cessation of capillary recruitment. Consequently, FCD decreases, as do oxygenation and effluent removal efficiency.[208]

2.4.6.4 | Distributive shock

Distributive shock is characterized by increased CO with decreased SVR[175] and occurs due to rampant systemic activation of inflammatory pathways (PAMPs). Excessive production of NO and other mediators leads to varying degrees of vasodilation, loss of vascular tone, myocardial depression, and hyporesponsiveness to catecholamines.[209]

Distributive shock is accompanied by profound changes in the microcirculation due to several mechanisms, including a combination of increased blood viscosity[210], glycocalyx degradation[211], neutrophil activation[212], vascular autoregulatory dysfunction[212], and decreased red cell deformability.[210]

A hyperdynamic vasodilatory state characterizes early distributive shock, often accompanied by relative hypovolemia and a concomitant reduction in FCD. Preservation of venous flow[80] supports the presence of arteriovenous shunting[213, 214]. Residual capillary perfusion becomes increasingly heterogeneous.[215] For this reason, distributive shock is generally a combination of type 1 and 4 microcirculatory changes.

2.5| Microvascular endothelium

The microcirculation is the largest endothelial surface of the body.[216] The inner layer of the microcirculatory blood vessels is formed by flow-sensitive endothelial cells, making blood flow necessary for their normal functioning.[134] They are highly multifunctional and include involvement in hemostasis, regulation of local blood flow, host defense, and organ-specific functions. However, as part of the microcirculation, endothelial cells also play a crucial role in the control and activation of coagulation and inflammatory processes.[216, 217]

Under physiological conditions, positive hydrostatic pressure forces blood constituents toward the interstitial space. Colloids, proteins, and other large molecules cannot cross the endothelial barrier in large numbers. This creates an inward-directed oncostatic gradient across the endothelial layer.[218] Inactive endothelial cells form a barrier between blood and tissue (**Figure 11**[62]), with vessels secreting anticoagulants and vasodilators through the endothelium and are responsible for keeping oncotic proteins such as albumin within the circulation space.[219]

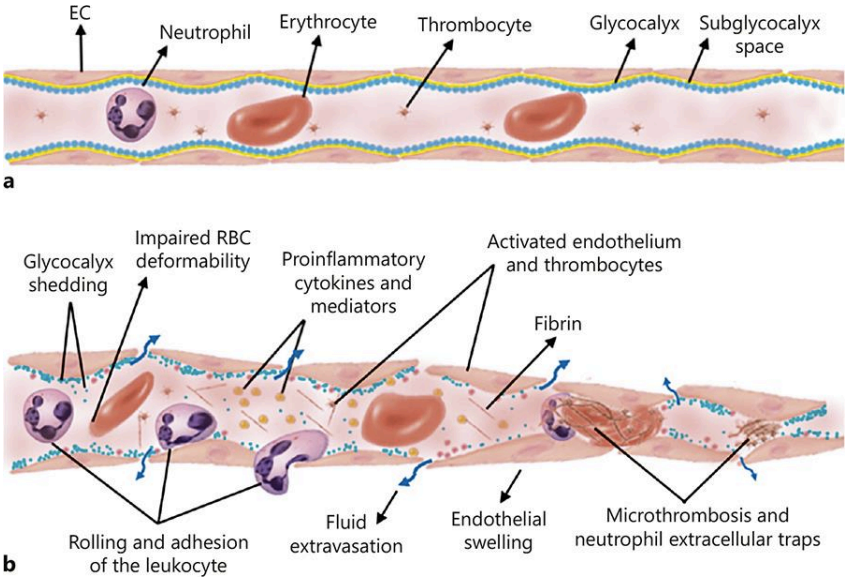


Figure 11

*Schematic representation of microcirculatory dysfunction and endothelial damage. **a)** a healthy microcirculatory vessel. The lumen of the vessel is covered with endothelial cells and glycocalyx. Leukocytes, RBCs and platelets flow with plasma through the microcirculation. **b)** Damage to the microcirculation due to inflammation, ischemia, reperfusion and hypoxia. This causes damage to the endothelium, RBCs and glycocalyx. Inflammation is further accelerated by activation of leukocytes. Vascular leakage and edema formation are caused by decreased vascular permeability. EC: endothelial cell; RBC: red blood cell.*

Albumin is net negatively charged.[220] Maintaining this negative charge is essential for endothelial integrity; loss of this charge on the luminal side of the endothelium leads to extravasation of albumin.[221-223] The amphoteric nature of albumin promotes tight binding to the glycocalyx, resulting in a reduction of hydraulic conductance across the vascular barrier and resistance to glycocalyx breakdown (protection against shedding). Albumin, therefore, contributes to the maintenance of vascular integrity, facilitates shear stress transfer, and maintains normal capillary permeability.[224-227] Under normal conditions, the intravascular concentration of albumin is the main determinant of plasma colloid osmotic pressure according to the Starling hypothesis.[228]

However, under infectious, inflammatory, thrombotic, or shock conditions, endothelial cells become activated and display adhesion molecules (integrins, selectins) to blood components, inducing barrier dysfunction by cellular constriction and allowing pro-thrombotic substances to be secreted.[219] Capillary permeability is increased during shock, leading to the leakage of plasma proteins from the interstitium. Endothelial damage by neutrophil-generated free radicals[229], NO/peroxynitrite generation[230, 231], and release of vasoactive compounds, such as histamine, PAF, bradykinin, TNF-α, and leukotrienes seem to stimulate this pathological process.

Oncotic plasma pressure decreases with the loss of plasma proteins, contributing to the development of interstitial edema, decreased capillary perfusion due to increased extravascular pressure and extravasation of blood plasma, and decreasing circulating volume.[232] In almost all types of shock, there is evidence of intravascular hemagglutination of red and white blood cells and platelets.[233] Intravascular hemagglutination may result from microvascular coagulation leading to microthrombi (**Figure 11**). Clotting may also occur due to endothelial damage secondary to circulating free radicals produced by neutrophils and reperfusion, cytokines, or complement activation. Both cases can lead to further endothelial damage, microcirculatory abnormalities, and insufficient perfusion into tissues.[233]

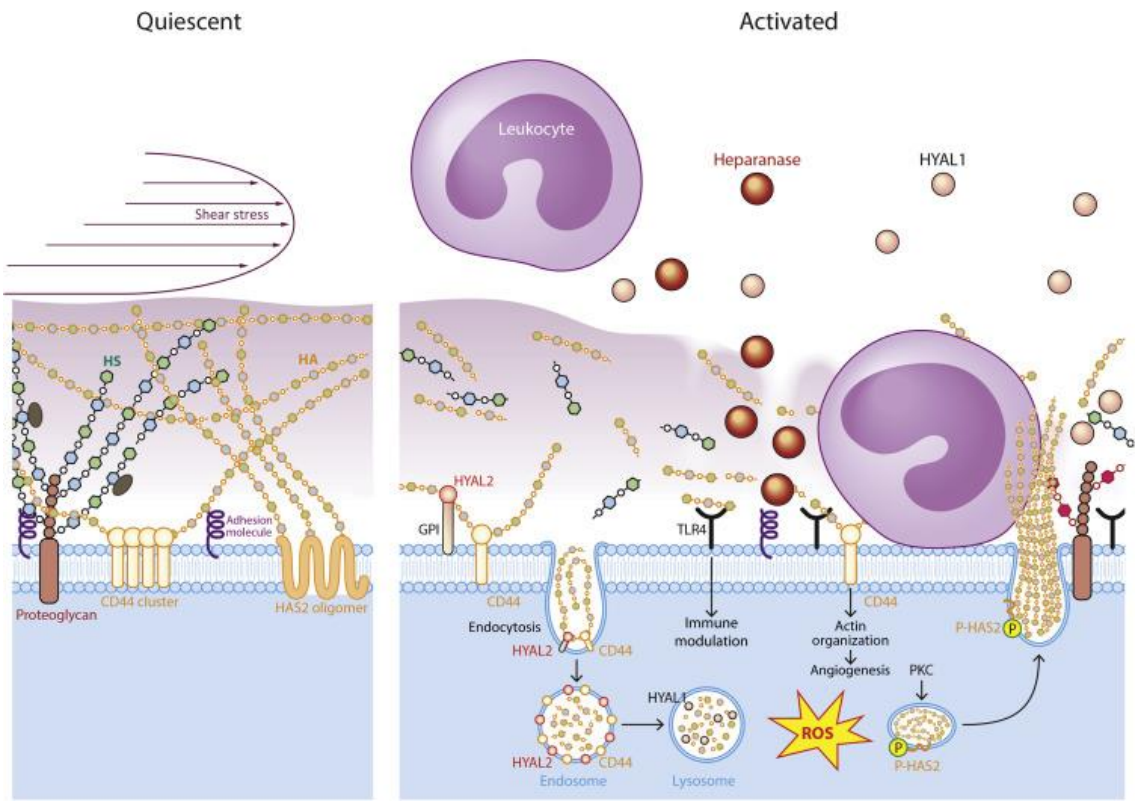


Figure 12

*Schematic overview of glycocalyx modulation under activated conditions. **Left)** Under healthy conditions, dynamic metabolism of hyaluron regulated by laminar shear stress maintains the glycocalyx. The intact endothelial glycocalyx maintains endothelial homeostasis, regulates permeability and plays an anti-inflammation and anti-thrombosis role. **Right)** Upon activation under pathological conditions, such as inflammation, there is increased shedding of the glycocalyx by HYALs. Increased expression of adhesion molecules is also observed under inflammatory conditions. released hyaluron aggresomes on the membrane attract the adhesion of monocytes. CD44: cell-surface glycoprotein; HA: hyaluronan; HS: heparan sulfate; HYALS: heparanase and hyaluronidase; GPI: glycosylphosphatidylinositol; P-HAS2: phosphorylated-HAS2; PKC: protein kinase C; ROS: reactive oxygen species; TLR4: toll-like receptor 4.*

The luminal side of the endothelial cells is lined with transmembrane-bound syndecans and membrane-bound glypicans, which together form the glycocalyx with a thickness of about 1 mm.[234] The membrane-bound part of the glycocalyx consists of glycosaminoglycans (GAGs), proteoglycans, glycoproteins, and glycolipids. The main contribution to the structure and function of the glycocalyx is made by the GAGs, of which hyaluronic acid (HA) and heparan sulfate (HS) make up to 90%.[235, 236] The glycocalyx is a vital structure for the endothelial barrier and cell signaling.[237] It regulates vascular permeability, transfers shear stress forces to endothelial cells, and provides vascular protection via the inhibition of coagulation and leukocyte adhesion.[238] Together with soluble or mobile molecules such as albumin, it forms the endothelial surface layer (ESL). The glycocalyx is very fragile, and the molecules are shed from the endothelial surface with certain stimuli, such as surgery, trauma, hypervolemia, and inflammation (**Figure 12**[239]).[228, 240]

Shedding or modification of the ESL is essential after tissue injury to promote platelet and leukocyte adhesion.[241] Shedding of the ESL leads to increased expression of adhesion molecules, increased cytokine proliferation, and increased leukocyte adhesion.[242-246] Different disease states may have different effects on the ESL, from selective removal of glycocalyx components to complete denudation of the ESL. Recruitment and adhesion of leukocytes are characteristics of systemic inflammation and occur in the postcapillary venules of the microcirculation.[247, 248] It depends on a cascade of events (**Figure 13**[249]) involving selectins, which act as primary molecules that induce and support rolling, and the secondary activation of integrins by chemokines, lipid mediators, and other proinflammatory molecules present on the endothelial surface. The integrins provide strong adhesion to leukocytes. Once attached, leukocytes can transmigrate out of the microvessels. Leukocytes can only roll and adhere to integrins under flow conditions with the presence of selectins.[250]

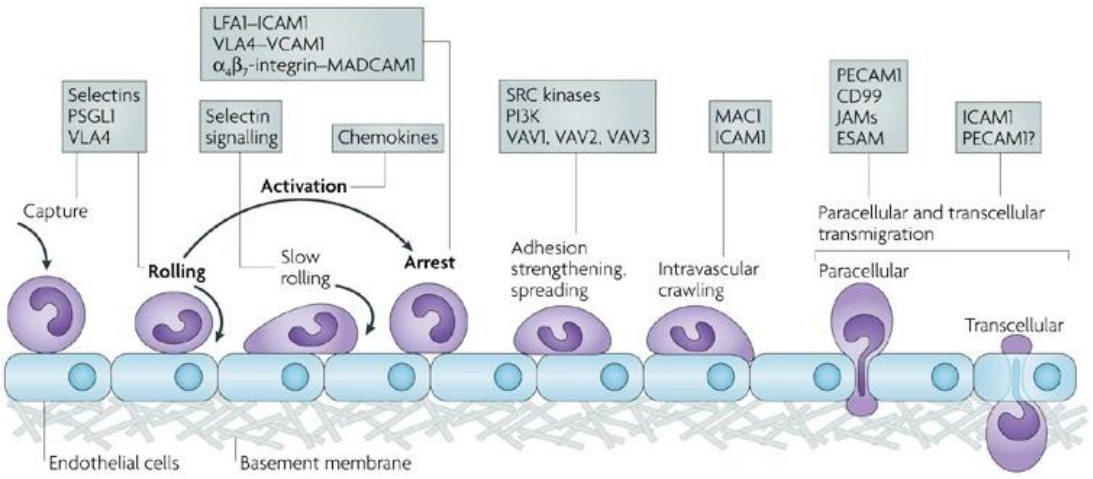


Figure 13

The steps of the adhesion cascade. capture, rolling and slow rolling: selectins play a role; activation: chemokines play a role; arrest: mediated by integrins; adhesion strengthening and spreading: kinases contribute to this; intravascular crawling: mediated by intracellular adhesion molecules; and paracellular and transcellular transmigration: different molecules are involved. ESAM: endothelial cell-selective adhesion molecule; ICAM1: intercellular adhesion molecule 1; JAM: junctional adhesion molecule; LFA1: lymphocyte function-associated antigen 1 (also known as αL62-integrin); MAC1: macrophage antigen 1; MADCAM1: mucosal vascular addressin cell-adhesion molecule 1; PSGL1: P-selectin glycoprotein ligand 1; PECAM1: platelet/endothelial-cell adhesion molecule 1; PI3K: phosphoinositide 3-kinase; VCAM1: vascular cell-adhesion molecule 1; VLA4: very late antigen 4 (also known as α461-integrin).

The release of glycocalyx components into the circulation can have downstream consequences. These components can stimulate inflammation by acting as DAMPs or alarmins. The glycocalyx contains soluble heparan sulfate molecules that play an important role in activating leukocytes, increasing cytokine production and endothelial activation[251, 252], and hyaluronic acid, which stimulates the production of inflammatory mediators[253, 254]. Shed components such as syndecan-1 and -4 ectodomains have an indirect anti-inflammatory effect by promoting neutrophil cytotoxicity.[255] The complexity of glycocalyx components acting as effector molecules in the systemic circulation could be analogous to the systemic inflammatory response, in which the release of specific cytokines promotes the host response. In contrast, a "cytokine storm" may lead to pathological consequences.[256, 257]

Shedding of the ESL alters capillary perfusion, resulting in decreased FCD[258] and increased endothelial permeability[259], leading to tissue hypoxia in certain areas due to decreased microcirculation[260]. The direct tissue damage and inflammatory response associated with the surgery itself, CPB, and ischemia-reperfusion injury lead to shedding of the glycocalyx and altered hydrostatic and oncotic pressure gradients, leading to transcapillary fluid shifts, and increased vascular permeability.[228, 261] Barrier dysfunction of microcirculatory endothelial cells predisposes to fluid excess after cardiothoracic surgery, which is associated with poor clinical outcomes. However, it is not yet known whether reversing barrier dysfunction leads to a reduction in fluid overload, an improvement in microcirculatory perfusion, and a reduction in organ dysfunction.[262]

2.6| Macrocirculation and hemodynamic coherence

The ultimate goal of resuscitation is to avoid or correct tissue hypoxia. Resuscitation of critically ill patients in shock states after cardiothoracic surgery remains challenging in intensive care medicine.[188] These patients require rapid normalization of macrocirculatory parameters, such as blood pressure, cardiac output, venous saturation, and urine output.[37, 263] Currently, resuscitation procedures focus on the administration of fluids, vasopressors, and inotropics to promote tissue perfusion and O_2 transport to the tissues.[188] However, the endpoints (e.g., SvO_2, lactate levels, and MAP) used to assess the efficacy of resuscitation therapy are not sensitive enough to identify regional tissue hypoxia.[264-266]

From a clinical perspective, it is believed that normalization of these macrocirculatory parameters leads to a parallel improvement in microcirculatory perfusion and restoration of tissue oxygenation. Unfortunately, this normalization of macrocirculatory parameters does not always protect the patient from the development of organ failure. This is due to the loss of hemodynamic coherence.[37, 188]

Hemodynamic coherence between macrocirculatory and microcirculatory parameters is the state in which resuscitation procedures aimed at correcting systemic hemodynamic parameters are effective in correcting regional and microcirculatory perfusion and O_2 supply to the parenchymal cells, allowing these cells to perform their functional activities in support of organ function.[188]

For effective hemodynamic coherence, resuscitation based on administering blood and fluids, combined with administered vasoactive drugs, should result in an adequate supply of oxygenated blood that meets the heterogeneous oxygen requirements of the different organs (**Figure 14**[267]).

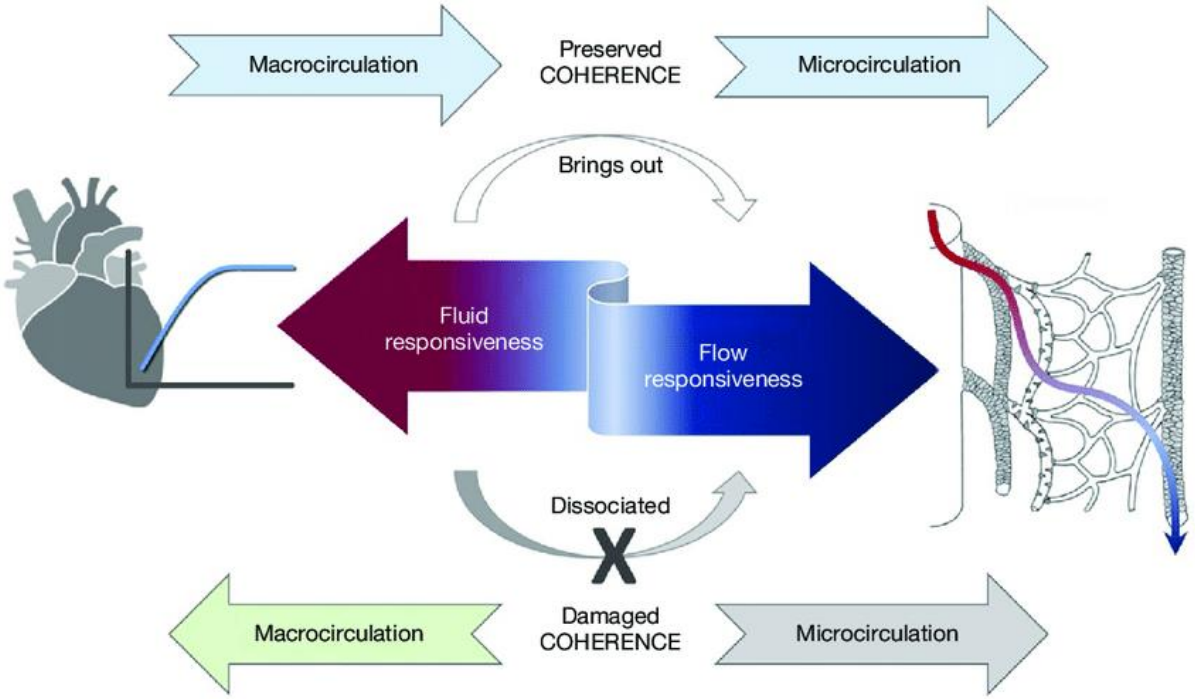


Figure 14

Schematic representation of the relationship between fluid responsiveness, flow responsiveness and hemodynamic coherence. Fluid responsiveness and hemodynamic coherence are markers of macrocirculation and microcirculation. Fluid responsiveness is a dynamic link between the two territories.

Moreover, compensatory mechanisms, including neural, hormonal, biomechanical, and vascular regulatory control systems, must be able to perceive and regulate O_2 transport to the different organs. In a state of shock, infection, inflammation, or reperfusion, these cellular sensing mechanisms to regulate blood flow may no longer work properly. In these cases, simply restoring macrocirculatory parameters is ineffective in restoring microcirculation and correcting tissue hypoperfusion.[188] Resuscitation procedures may even be counterproductive by affecting the ability of the cardiovascular system to distribute oxygen-carrying red blood cells to the various organs effectively.[268]

The four classes of microcirculatory changes (as listed in **Chapter 2.4.6, Figure 10**) associated with different stages of cardiovascular compromise are associated with reduced FCD and the consequent loss of the ability of the microcirculation to transport O_2 to tissues. Functional shunting of O_2 transport to tissues may be facilitated by reduced FCD, which, unless explicitly verified, is not detected by simply monitoring systemic hemodynamics.[214, 269]

For the prevention or treatment of organ failure and for successful hemodynamic monitoring in directing therapy, another target must be pursued: the restoration of microcirculation.[270]

2.7| References

1. foundation TDh. Dutch statistics of cardiovascular diseases in 2018. 2018.
2. Ranucci M, Baryshnikova E, Castelvecchio S, Pelissero G, Surgical, Group COR. Major bleeding, transfusions, and anemia: the deadly triad of cardiac surgery. The Annals of thoracic surgery. 2013;96(2):478-85.
3. Pätilä T, Kukkonen S, Vento A, Pettilä V, Suojaranta-Ylinen R. Relation of the sequential organ failure assessment score to morbidity and mortality after cardiac surgery. The Annals of thoracic surgery. 2006;82(6):2072-8.
4. Sablotzki A, Friedrich I, Mühling J, Dehne MG, Spillner J, Silber RE, et al. The systemic inflammatory response syndrome following cardiac surgery: different expression of proinflammatory cytokines and procalcitonin in patients with and without multiorgan dysfunctions. Perfusion. 2002;17(2):103-9.
5. Atkinson S, Mason R, McColl L, Bihari D, Smithies M, Daly K. Identification of futility in intensive care. The Lancet. 1994;344(8931):1203-6.
6. Holmes L, Loughead K, Treasure T, Gallivan S. Which patients will not benefit from further intensive care after cardiac surgery? The Lancet. 1994;344(8931):1200-2.
7. Bienz M, Drullinsky D, Stevens L-M, Bracco D, Noiseux N. Microcirculatory response during on-pump versus off-pump coronary artery bypass graft surgery. Perfusion. 2016;31(3):207-15.
8. Sarkar M, Prabhu V. Basics of cardiopulmonary bypass. Indian journal of anaesthesia. 2017;61(9):760.
9. Laursen LØ, Petersen RH, Hansen HJ, Jensen TK, Ravn J, Konge L. Video-assisted thoracoscopic surgery lobectomy for lung cancer is associated with a lower 30-day morbidity compared with lobectomy by thoracotomy. European Journal of Cardio-Thoracic Surgery. 2016;49(3):870-5.
10. Jakobson T, Karjagin J, Vipp L, Padar M, Parik A-H, Starkopf L, et al. Postoperative complications and mortality after major gastrointestinal surgery. Medicina. 2014;50(2):111-7.
11. Siregar S, Groenwold RH, de Heer F, Bots ML, van der Graaf Y, van Herwerden LA. Performance of the original EuroSCORE. European journal of cardio-thoracic surgery. 2012;41(4):746-54.
12. Chalmers J, Pullan M, Fabri B, McShane J, Shaw M, Mediratta N, et al. Validation of EuroSCORE II in a modern cohort of patients undergoing cardiac surgery. European Journal of Cardio-Thoracic Surgery. 2013;43(4):688-94.
13. Nashef SA, Roques F, Michel P, Gauducheau E, Lemeshow S, Salamon R, et al. European system for cardiac operative risk evaluation (Euro SCORE). European journal of cardio-thoracic surgery. 1999;16(1):9-13.
14. Le Manach Y, Collins G, Rodseth R, Le Bihan-Benjamin C, Biccard B, Riou B, et al. Preoperative score to predict postoperative mortality (POSPOM) Derivation and validation. Anesthesiology. 2016;124(3):570-9.
15. Salis S, Mazzanti VV, Merli G, Salvi L, Tedesco CC, Veglia F, et al. Cardiopulmonary bypass duration is an independent predictor of morbidity and mortality after cardiac surgery. Journal of cardiothoracic and vascular anesthesia. 2008;22(6):814-22.
16. DeFoe GR, Ross CS, Olmstead EM, Surgenor SD, Fillinger MP, Groom RC, et al. Lowest hematocrit on bypass and adverse outcomes associated with coronary artery bypass grafting. The Annals of thoracic surgery. 2001;71(3):769-76.
17. Murphy GJ, Reeves BC, Rogers CA, Rizvi SI, Culliford L, Angelini GD. Increased mortality, postoperative morbidity, and cost after red blood cell transfusion in patients having cardiac surgery. Circulation. 2007;116(22):2544-52.
18. Hajjar LA, Vincent J-L, Galas FR, Nakamura RE, Silva CM, Santos MH, et al. Transfusion requirements after cardiac surgery: the TRACS randomized controlled trial. Jama. 2010;304(14):1559-67.
19. Pickering JW, James MT, Palmer SC. Acute kidney injury and prognosis after cardiopulmonary bypass: a meta-analysis of cohort studies. American Journal of Kidney Diseases. 2015;65(2):283-93.
20. Yeh Y-C, Wang M-J, Chao A, Ko W-J, Chan W-S, Fan S-Z, et al. Correlation between early sublingual small vessel density and late blood lactate level in critically ill surgical patients. journal of surgical research. 2013;180(2):317-21.
21. Kolackova M, Krejsek J, Svitek V, Kunes P, Mandak J, Holubcova Z, et al. The effect of conventional and mini-invasive cardiopulmonary bypass on neutrophil activation in patients undergoing coronary artery bypass grafting. Mediators of Inflammation. 2012;2012.
22. Warren OJ, Smith AJ, Alexiou C, Rogers PL, Jawad N, Vincent C, et al. The inflammatory response to cardiopulmonary bypass: part 1—mechanisms of pathogenesis. Journal of cardiothoracic and vascular anesthesia. 2009;23(2):223-31.
23. Hill GE. Cardiopulmonary bypass-induced inflammation: is it important? Journal of cardiothoracic and vascular anesthesia. 1998;12(2 Suppl 1):21-5.
24. O'Neil MP, Fleming JC, Badhwar A, Guo LR. Pulsatile versus nonpulsatile flow during cardiopulmonary bypass: microcirculatory and systemic effects. The Annals of thoracic surgery. 2012;94(6):2046-53.
25. Koning NJ, Vonk AB, van Barneveld LJ, Beishuizen A, Atasever B, van den Brom CE, et al. Pulsatile flow during cardiopulmonary bypass preserves postoperative microcirculatory perfusion irrespective of systemic hemodynamics. Journal of Applied Physiology. 2012;112(10):1727-34.
26. Törnudd M, Hahn RG, Zdolsek JH. Fluid distribution kinetics during cardiopulmonary bypass. Clinics. 2014;69:535-41.
27. Levi M, Cromheecke ME, de Jonge E, Prins MH, de Mol BJ, Briët E, et al. Pharmacological strategies to decrease excessive blood loss in cardiac surgery: a meta-analysis of clinically relevant endpoints. The lancet. 1999;354(9194):1940-7.
28. Dayan V, Cal R, Giangrossi F. Risk factors for vasoplegia after cardiac surgery: a meta-analysis. Interactive CardioVascular and Thoracic Surgery. 2019;28(6):838-44.
29. Gaffney AM, Sladen RN. Acute kidney injury in cardiac surgery. Current Opinion in Anesthesiology. 2015;28(1):50-9.
30. Bartels K, McDonagh DL, Newman MF, Mathew JP. Neurocognitive outcomes after cardiac surgery. Current Opinion in Anesthesiology. 2013;26(1):91-7.
31. Boeken U, Feindt P, Mohan E, Zimmermann N, Micek M, Kalweit G, et al. Post-perfusion syndrome and disturbed microcirculation after cardiac surgery: the role of angiotensin-converting-enzyme inhibitors. The Thoracic and cardiovascular surgeon. 1999;47(06):347-51.
32. Verheij J, van Lingen A, Raijmakers PG, Rijnsburger ER, Veerman DP, Wisselink W, et al. Effect of fluid loading with saline or colloids on pulmonary permeability, oedema and lung injury score after cardiac and major vascular surgery. British journal of anaesthesia. 2006;96(1):21-30.
33. Cooley DA, Frazier O. The past 50 years of cardiovascular surgery. Circulation. 2000;102(suppl_4):Iv-87-Iv-93.
34. Stephens RS, Whitman GJ. Postoperative critical care of the adult cardiac surgical patient. Part I: routine postoperative care. Critical care medicine. 2015;43(7):1477-97.

35. Betts JG, Young KA, Wise JA, Johnson E, Poe B, Kruse DH, et al. Anatomy and physiology2013.
36. Carlin CS, Ho LV, Ledbetter DR, Aczon MD, Wetzel RC. Predicting individual physiologically acceptable states at discharge from a pediatric intensive care unit. Journal of the American Medical Informatics Association. 2018;25(12):1600-7.
37. Donati A, Domizi R, Damiani E, Adrario E, Pelaia P, Ince C. From macrohemodynamic to the microcirculation. Critical care research and practice. 2013;2013.
38. Leach R, Treacher D. The pulmonary physician in critical care• 2: Oxygen delivery and consumption in the critically ill. Thorax. 2002;57(2):170-7.
39. De Backer D, Dubois M-J, Schmartz D, Koch M, Ducart A, Barvais L, et al. Microcirculatory alterations in cardiac surgery: effects of cardiopulmonary bypass and anesthesia. The Annals of thoracic surgery. 2009;88(5):1396-403.
40. Bauer A, Kofler S, Thiel M, Eifert S, Christ F. Monitoring of the sublingual microcirculation in cardiac surgery using orthogonal polarization spectral imaging: preliminary results. The Journal of the American Society of Anesthesiologists. 2007;107(6):939-45.
41. Bohlen HG. The microcirculation and the lymphatic system. Medical Phsyiology Boston (Mass): Little, Brown and Company. 1995.
42. Renkin EM. Control of microcirculation and blood-tissue exchange. Handbook of physiology The cardiovascular system, microcirculation. 1984:627-87.
43. Gutterman DD, Chabowski DS, Kadlec AO, Durand MJ, Freed JK, Ait-Aissa K, et al. The human microcirculation: regulation of flow and beyond. Circulation research. 2016;118(1):157-72.
44. Ait-Oufella H, Bourcier S, Lehoux S, Guidet B. Microcirculatory disorders during septic shock. Current Opinion in Critical Care. 2015;21(4):271-5.
45. Michiels C, Arnould T, Knott I, Dieu M, Remacle J. Stimulation of prostaglandin synthesis by human endothelial cells exposed to hypoxia. American Journal of Physiology-Cell Physiology. 1993;264(4):C866-C74.
46. Vallet B. Vascular reactivity and tissue oxygenation. Intensive care medicine. 1998;24(1):3.
47. Jackson WF, Duling BR. The oxygen sensitivity of hamster cheek pouch arterioles. In vitro and in situ studies. Circulation research. 1983;53(4):515-25.
48. Vallet B, Winn MJ, Asante NK, Cain SM. Influence of oxygen on endothelium-derived relaxing factor/nitric oxide and K (+)-dependent regulation of vascular tone. Journal of cardiovascular pharmacology. 1994;24(4):595-602.
49. Vallet B, Wiel E, Rodie-Talbère P-A. Endothelial cell dysfunction and abnormal tissue perfusion. Mechanisms of Organ Dysfunction in Critical Illness. 2002:175-90.
50. Garland CJ, Hiley CR, Dora KA. EDHF: spreading the influence of the endothelium. British journal of pharmacology. 2011;164(3):839-52.
51. Chen K, Popel AS. Theoretical analysis of biochemical pathways of nitric oxide release from vascular endothelial cells. Free Radical Biology and Medicine. 2006;41(4):668-80.
52. Doctor A, Platt R, Sheram ML, Eischeid A, McMahon T, Maxey T, et al. Hemoglobin conformation couples erythrocyte S-nitrosothiol content to O2 gradients. Proceedings of the National Academy of Sciences. 2005;102(16):5709-14.
53. Nakhostine N, Lamontagne D. Adenosine contributes to hypoxia-induced vasodilation through ATP-sensitive K+ channel activation. American Journal of Physiology-Heart and Circulatory Physiology. 1993;265(4):H1289-H93.
54. Potente M, Mäkinen T. Vascular heterogeneity and specialization in development and disease. Nature Reviews Molecular Cell Biology. 2017;18(8):477-94.
55. Ince C. The microcirculation is the motor of sepsis. Critical care. 2005;9(4):1-7.
56. Bateman RM, Sharpe MD, Ellis CG. Bench-to-bedside review: microvascular dysfunction in sepsis–hemodynamics, oxygen transport, and nitric oxide. Critical care. 2003;7(5):1-15.
57. Boerma EC, Ince C. The role of vasoactive agents in the resuscitation of microvascular perfusion and tissue oxygenation in critically ill patients. Intensive care medicine. 2010;36(12):2004-18.
58. Ince C. The rationale for microcirculatory guided fluid therapy. Current opinion in critical care. 2014;20(3):301-8.
59. Ellis CG, Bateman RM, Sharpe MD, Sibbald WJ, Gill R. Effect of a maldistribution of microvascular blood flow on capillary O2 extraction in sepsis. American Journal of Physiology-Heart and Circulatory Physiology. 2002;282(1):H156-H64.
60. Dietrich HH, Tyml K. Capillary as a communicating medium in the microvasculature. Microvascular research. 1992;43(1):87-99.
61. Koller A, Kaley G. Endothelial regulation of wall shear stress and blood flow in skeletal muscle microcirculation. American Journal of Physiology-Heart and Circulatory Physiology. 1991;260(3):H862-H8.
62. Guven G, Hilty MP, Ince C. Microcirculation: physiology, pathophysiology, and clinical application. Blood Purification. 2020;49(1-2):143-50.
63. den Uil C, Klijn E, Lagrand WK, Brugts JJ, Ince C, Spronk PE, et al. The microcirculation in health and critical disease. Progress in cardiovascular diseases. 2008;51(2):161-70.
64. Fiddian-Green RG. Associations between intramucosal acidosis in the gut and organ failure. Critical care medicine. 1993;21(2):S103.
65. Baue AE. The role of the gut in the development of multiple organ dysfunction in cardiothoracic patients. The Annals of thoracic surgery. 1993;55(4):822-9.
66. Peters J, Mack G, Lister G. The importance of the peripheral circulation in critical illnesses. Intensive care medicine. 2001;27(9):1446.
67. Shepro D, Morel NM. Pericyte physiology. The FASEB Journal. 1993;7(11):1031-8.
68. Simionescu M. Ultrastructure of the microvascular wall: functional correlations. Handbook of physiology. 1984;6:41-101.
69. Armulik A, Genové G, Betsholtz C. Pericytes: developmental, physiological, and pathological perspectives, problems, and promises. Developmental cell. 2011;21(2):193-215.
70. Ocak I, Kara A, Ince C. Monitoring microcirculation. Best Practice & Research Clinical Anaesthesiology. 2016;30(4):407-18.
71. Ley K. The microcirculation in inflammation. Microcirculation: Elsevier; 2008. p. 387-448.
72. Kogan AN, von Andrian UH. Lymphocyte trafficking. Microcirculation: Elsevier; 2008. p. 449-82.
73. Moore J, Dyson A, Singer M, Fraser J. Microcirculatory dysfunction and resuscitation: why, when, and how. British journal of anaesthesia. 2015;115(3):366-75.
74. De Backer D, Ospina-Tascon G, Salgado D, Favory R, Creteur J, Vincent J-L. Monitoring the microcirculation in the critically ill patient: current methods and future approaches. Intensive care medicine. 2010;36(11):1813-25.
75. Atasever B, Boer C, Goedhart P, Biervliet J, Seyffert J, Speekenbrink R, et al. Distinct alterations in sublingual microcirculatory blood flow and hemoglobin oxygenation in on-pump and off-pump coronary artery bypass graft surgery. Journal of cardiothoracic and vascular anesthesia. 2011;25(5):784-90.

76. Standring S. Gray's anatomy e-book: the anatomical basis of clinical practice: Elsevier Health Sciences; 2021.
77. Uz Z, Dilken O, Milstein DM, Hilty MP, de Haan D, Ince Y, et al. Identifying a sublingual triangle as the ideal site for assessment of sublingual microcirculation. Journal of Clinical Monitoring and Computing. 2022:1-11.
78. Klijn E, Den Uil C, Bakker J, Ince C. The heterogeneity of the microcirculation in critical illness. Clinics in chest medicine. 2008;29(4):643-54.
79. Scorcella C, Damiani E, Domizi R, Pierantozzi S, Tondi S, Carsetti A, et al. MicroDAIMON study: Microcirculatory DAlly MONitoring in critically ill patients: a prospective observational study. Annals of intensive care. 2018;8(1):1-9.
80. De Backer D, Creteur J, Preiser J-C, Dubois M-J, Vincent J-L. Microvascular blood flow is altered in patients with sepsis. American journal of respiratory and critical care medicine. 2002;166(1):98-104.
81. Sui F, Zheng Y, Li W, Zhou J. Renal circulation and microcirculation during intra-abdominal hypertension in a porcine model. Eur Rev Med Pharmacol Sci. 2016;20(3):452-61.
82. Jacquet-Lagrèze M, Allaouchiche B, Restagno D, Paquet C, Ayoub J-Y, Etienne J, et al. Gut and sublingual microvascular effect of esmolol during septic shock in a porcine model. Critical Care. 2015;19(1):1-12.
83. Verdant CL, De Backer D, Bruhn A, Clausi CM, Su F, Wang Z, et al. Evaluation of sublingual and gut mucosal microcirculation in sepsis: a quantitative analysis. Critical care medicine. 2009;37(11):2875-81.
84. Pranskunas A, Pilvinis V, Dambrauskas Z, Rasimaviciute R, Planciuniene R, Dobozinskas P, et al. Early course of microcirculatory perfusion in eye and digestive tract during hypodynamic sepsis. Critical care. 2012;16(3):1-9.
85. Donati A, Tibboel D, Ince C. Towards integrative physiological monitoring of the critically ill: from cardiovascular to microcirculatory and cellular function monitoring at the bedside. Critical care. 2013;17(1):1-7.
86. Ince C, Boerma EC, Cecconi M, De Backer D, Shapiro NI, Duranteau J, et al. 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 medicine. 2018;44(3):281-99.
87. De Backer D, Hollenberg S, Boerma C, Goedhart P, Büchele G, Ospina-Tascon G, et al. How to evaluate the microcirculation: report of a round table conference. Critical care. 2007;11(5):1-9.
88. Dobbe JG, Streekstra GJ, Atasever B, Van Zijderveld R, Ince C. Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. Medical & biological engineering & computing. 2008;46(7):659-70.
89. Uz Z, Ince C, Shen L, Ergin B, van Gulik T. Real-time observation of microcirculatory leukocytes in patients undergoing major liver resection. Scientific Reports. 2021;11(1):1-15.
90. Hilty MP, Ince C. Automated quantification of tissue red blood cell perfusion as a new resuscitation target. Current Opinion in Critical Care. 2020;26(3):273-80.
91. Secor D, Li F, Ellis CG, Sharpe MD, Gross PL, Wilson JX, et al. Impaired microvascular perfusion in sepsis requires activated coagulation and P-selectin-mediated platelet adhesion in capillaries. Intensive care medicine. 2010;36(11):1928-34.
92. Krogh A. The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. The Journal of physiology. 1919;52(6):409.
93. Hilty MP, Favaron E, Wendel Garcia PD, Ahiska Y, Uz Z, Akin S, et al. Microcirculatory alterations in critically ill COVID-19 patients analyzed using artificial intelligence. Critical Care. 2022;26(1):1-11.
94. Lima A, Jansen TC, van Bommel J, Ince C, Bakker J. The prognostic value of the subjective assessment of peripheral perfusion in critically ill patients. Critical care medicine. 2009;37(3):934-8.
95. Ait-Oufella H, Bige N, Boelle P, Pichereau C, Alves M, Bertinchamp R, et al. Capillary refill time exploration during septic shock. Intensive care medicine. 2014;40(7):958-64.
96. Ait-Oufella H, Bakker J. Understanding clinical signs of poor tissue perfusion during septic shock. Intensive care medicine. 2016;42(12):2070-2.
97. Dubin A, Henriquez E, Hernández G. Monitoring peripheral perfusion and microcirculation. Current opinion in critical care. 2018;24(3):173-80.
98. Lima A, Bakker J. Noninvasive monitoring of peripheral perfusion. . Intensive Care Med. 2005;31:1316-26.
99. Bourcier S, Joffre J, Dubée V, Preda G, Baudel J-L, Bigé N, et al. Marked regional endothelial dysfunction in mottled skin area in patients with severe infections. Critical Care. 2017;21(1):1-8.
100. Lima A, van Bommel J, Sikorska K, van Genderen M, Klijn E, Lesaffre E, et al. The relation of near-infrared spectroscopy with changes in peripheral circulation in critically ill patients. Critical care medicine. 2011;39(7):1649-54.
101. 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):1-10.
102. Struijker-Boudier H, Crijns F, Stolte J, Van Essen H. Assessment of the microcirculation in cardiovascular disease. Clinical Science. 1996;91(2):131-9.
103. Goedhart P, Khalilzada M, Bezemer R, Merza J, Ince C. 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.
104. Scheuzger JD, Zehnder A, Yeginsoy D, Siegemund M. Sublingual microcirculation: a case report. Journal of Medical Case Reports. 2019;13(1):1-6.
105. Gilbert-Kawai E, Coppel J, Bountziouka V, Ince C, Martin D. A comparison of the quality of image acquisition between the incident dark field and sidestream dark field video-microscopes. BMC medical imaging. 2016;16(1):1-5.
106. Sherman H, Klausner S, Cook WA. Incident dark-field illumination: a new method for microcirculatory study. Angiology. 1971;22(5):295-303.
107. Groner W, Winkelman JW, Harris AG, Ince C, Bouma GJ, Messmer K, et al. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. Nature medicine. 1999;5(10):1209-12.
108. Mathura KR, Vollebregt KC, Boer K, De Graaff JC, Ubbink DT, Ince C. Comparison of OPS imaging and conventional capillary microscopy to study the human microcirculation. Journal of Applied Physiology. 2001;91(1):74-8.
109. Mathura KR, Bouma GJ, Ince C. Abnormal microcirculation in brain tumours during surgery. The Lancet. 2001;358(9294):1698-9.
110. Slaaf D, Tangelder G, Reneman R, Jäger K, Bollinger A. A versatile incident illuminator for intravital microscopy. International Journal of Microcirculation, Clinical and Experimental. 1987;6(4):391-7.
111. Černý V, Turek Z, Pařízková R. Orthogonal polarization spectral imaging. Physiol Res. 2007;56:141-7.
112. Van Elteren H, Ince C, Tibboel D, Reiss I, de Jonge R. Cutaneous microcirculation in preterm neonates: comparison between sidestream dark field (SDF) and incident dark field (IDF) imaging. Journal of clinical monitoring and computing. 2015;29(5):543-8.
113. Hutchings S, Watts S, Kirkman E. The Cytocam video microscope. A new method for visualising the microcirculation using Incident Dark Field technology. Clinical Hemorheology and Microcirculation. 2016;62(3):261-71.

114. Massey MJ, Shapiro NI. A guide to human in vivo microcirculatory flow image analysis. Critical Care. 2015;20(1):1-10.
115. 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. Journal of critical care. 2013;28(6):913-7.
116. Djaberi R, Schuijf JD, de Koning EJ, Wijewickrama DC, Pereira AM, Smit JW, et al. Non-invasive assessment of microcirculation by sidestream dark field imaging as a marker of coronary artery disease in diabetes. Diabetes and Vascular Disease Research. 2013;10(2):123-34.
117. Khalilzada M, Dogan K, Ince C, Stam J. Sublingual microvascular changes in patients with cerebral small vessel disease. Stroke. 2011;42(7):2071-3.
118. Dondorp A, Ince C, Charunwatthana P, Hanson J, Kuijen Av, Faiz M, et al. Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. The Journal of infectious diseases. 2008;197(1):79-84.
119. Lindeboom JA, Mathura KR, Aartman IH, Kroon FH, Milstein DM, Ince C. Influence of the application of platelet-enriched plasma in oral mucosal wound healing. Clinical oral implants research. 2007;18(1):133-9.
120. Dababneh L, Cikach F, Alkukhun L, Dweik RA, Tonelli AR. Sublingual microcirculation in pulmonary arterial hypertension. Annals of the American Thoracic Society. 2014;11(4):504-12.
121. Snoeijs MG, Vink H, Voesten N, Christiaans MH, Daemen J-WH, Peppelenbosch AG, et al. Acute ischemic injury to the renal microvasculature in human kidney transplantation. American Journal of Physiology-Renal Physiology. 2010;299(5):F1134-F40.
122. den Uil C, Bezemer R, Miranda DR, Ince C, Lagrand WK, Hartman M, et al. Intra-operative assessment of human pulmonary alveoli in vivo using Sidestream Dark Field imaging: a feasibility study. Medical Science Monitor. 2009;15(10):MT137-MT41.
123. Nilsson J, Eriksson S, Blind P-J, Rissler P, Sturesson C. Microcirculation changes during liver resection—a clinical study. Microvascular research. 2014;94:47-51.
124. Khalil AA, Aziz FA, Hall JC. Reperfusion injury. Plastic and reconstructive surgery. 2006;117(3):1024-33.
125. Elbers PW, Ozdemir A, Heijmen RH, Heeren J, Van Iterson M, Van Dongen EP, et al. Microvascular hemodynamics in human hypothermic circulatory arrest and selective antegrade cerebral perfusion. Critical care medicine. 2010;38(7):1548-53.
126. Bernet C, Desebbe O, Bordon S, Lacroix C, Rosamel P, Farhat F, et al. The impact of induction of general anesthesia and a vascular occlusion test on tissue oxygen saturation derived parameters in high-risk surgical patients. Journal of clinical monitoring and computing. 2011;25(4):237-44.
127. Turek Z, Sykora R, Matejovic M, Cerny V, editors. Anesthesia and the microcirculation. Seminars in cardiothoracic and vascular anesthesia; 2009: SAGE Publications Sage CA: Los Angeles, CA.
128. Cabrales P, Tsai AG, Frangos JA, Briceno JC, Intaglietta M. Oxygen delivery and consumption in the microcirculation after extreme hemodilution with perfluorocarbons. American Journal of Physiology-Heart and Circulatory Physiology. 2004;287(1):H320-H30.
129. Pries A, Ley K, Claassen M, Gaehtgens P. Red cell distribution at microvascular bifurcations. Microvascular research. 1989;38(1):81-101.
130. Goddard CM, Allard MF, Hogg JC, Herbertson MJ, Walley KR. Prolonged leukocyte transit time in coronary microcirculation of endotoxemic pigs. American Journal of Physiology-Heart and Circulatory Physiology. 1995;269(4):H1389-H97.
131. Kurose I, Argenbright LW, Anderson DC, Tolley J, Miyasaka M, Harris N, et al. Reperfusion-induced leukocyte adhesion and vascular protein leakage in normal and hypercholesterolemic rats. American Journal of Physiology-Heart and Circulatory Physiology. 1997;273(2):H854-H60.
132. Koning NJ, Atasever B, Vonk AB, Boer C. Changes in microcirculatory perfusion and oxygenation during cardiac surgery with or without cardiopulmonary bypass. Journal of cardiothoracic and vascular anesthesia. 2014;28(5):1331-40.
133. Atasever B, van der Kuil M, Boer C, Vonk A, Schwarte L, Girbes AR, 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.
134. Noris M, Morigi M, Donadelli R, Aiello S, Foppolo M, Todeschini M, et al. Nitric oxide synthesis by cultured endothelial cells is modulated by flow conditions. Circulation research. 1995;76(4):536-43.
135. Lanzarone E, Gelmini F, Tessari M, Menon T, Suzuki H, Carini M, et al. Preservation of endothelium nitric oxide release by pulsatile flow cardiopulmonary bypass when compared with continuous flow. Artificial organs. 2009;33(11):926-34.
136. Ündar A, Ji B, Lukic B, Zapanta CM, Kunselman AR, Reibson JD, et al. Quantification of perfusion modes in terms of surplus hemodynamic energy levels in a simulated pediatric CPB model. ASAIO journal. 2006;52(6):712-7.
137. Orime Y, Shiono M, Hata H, Yagi S, Tsukamoto S, Okumura H, et al. Cytokine and endothelial damage in pulsatile and nonpulsatile cardiopulmonary bypass. Artificial organs. 1999;23(6):508-12.
138. Takeda J. Experimental study on peripheral circulation during extracorporeal circulation, with a special reference to a comparison of pulsatile flow with non-pulsatile flow. 1960;29(6):1407-30.
139. Murphy GS, Hessel EA, Groom RC. Optimal perfusion during cardiopulmonary bypass: an evidence-based approach. Anesthesia & Analgesia. 2009;108(5):1394-417.
140. Joachimsson P-O, Sjöberg F, Forsman M, Johansson M, Ahn HC, Rutberg H. Adverse effects of hyperoxemia during cardiopulmonary bypass. The Journal of thoracic and cardiovascular surgery. 1996;112(3):812-9.
141. Kamler M, Wendt D, Pizanis N, Milekhin V, Schade U, Jakob H. Deleterious effects of oxygen during extracorporeal circulation for the microcirculation in vivo. European journal of cardio-thoracic surgery. 2004;26(3):564-70.
142. Tsai AG, Cabrales P, Winslow RM, Intaglietta M. Microvascular oxygen distribution in awake hamster window chamber model during hyperoxia. American Journal of Physiology-Heart and Circulatory Physiology. 2003;285(4):H1537-H45.
143. Wan S, LeClerc J-L, Vincent J-L. Inflammatory response to cardiopulmonary bypass: mechanisms involved and possible therapeutic strategies. Chest. 1997;112(3):676-92.
144. Levy BI, Schiffrin EL, Mourad J-J, Agostini D, Vicaut E, Safar ME, et al. Impaired tissue perfusion: a pathology common to hypertension, obesity, and diabetes mellitus. Circulation. 2008;118(9):968-76.
145. Colman RW. Platelet and neutrophil activation in cardiopulmonary bypass. The Annals of thoracic surgery. 1990;49(1):32-4.
146. Bonser R, Dave J, John L, Gademsetty M, Carter P, Davies E, et al. Complement activation before, during and after cardiopulmonary bypass. European journal of cardio-thoracic surgery. 1990;4(6):291-6.
147. Chenoweth DE, Cooper SW, Hugli TE, Stewart RW, Blackstone EH, Kirklin JW. Complement activation during cardiopulmonary bypass: evidence for generation of C3a and C5a anaphylatoxins. New England Journal of Medicine. 1981;304(9):497-503.
148. Wachtfogel YT, Harpel PC, Edmunds LJ, Colman RW. Formation of C1s-C1-inhibitor, kallikrein-C1-inhibitor, and plasmin-alpha 2-plasmin-inhibitor complexes during cardiopulmonary bypass. 1989.

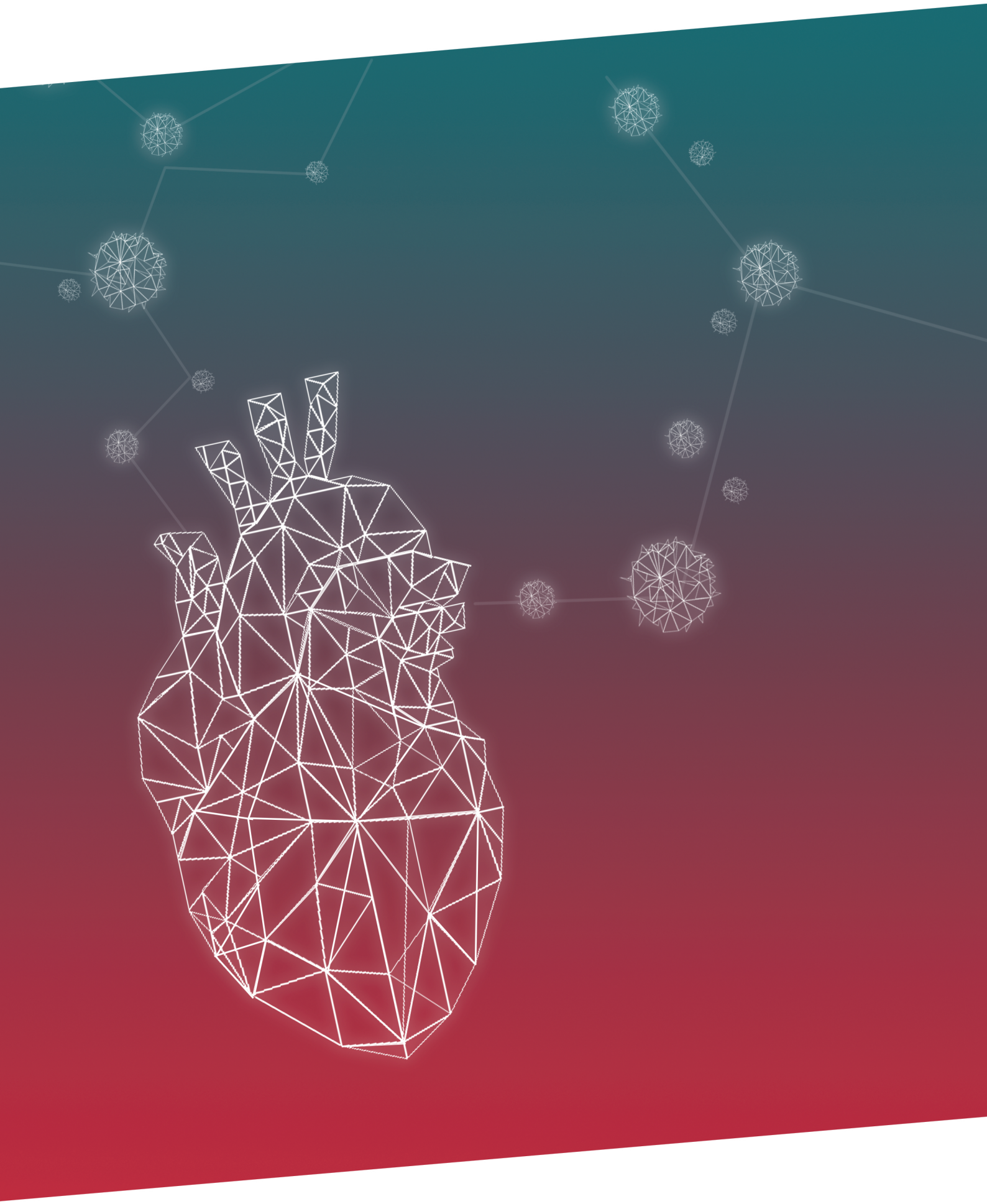
149. Jansen N, Van Oeveren W, Broek L, Oudemans-van Straaten H, Stoutenbeek C, Joen MCN, et al. Inhibition by dexamethasone of the reperfusion phenomena in cardiopulmonary bypass. The Journal of Thoracic and Cardiovascular Surgery. 1991;102(4):515-25.
150. Finn A, Naik S, Klein N, Levinsky RJ, Strobel S, Elliott M. Interleukin-8 release and neutrophil degranulation after pediatric cardiopulmonary bypass. The Journal of Thoracic and Cardiovascular Surgery. 1993;105(2):234-41.
151. Gee MH, Perkowski SZ, Tahamont MV, Flynn JT, Wasserman MA. Thromboxane as a mediator of pulmonary dysfunction during intravascular complement activation in sheep. American Review of Respiratory Disease. 1986;133(2):269-73.
152. Hoshikawa-Fujimura A, JO AJ, Da Rocha T, Brandizzi L, Pascual J, Chamone D, et al. PAF-acether, superoxide anion and beta-glucuronidase as parameters of polymorphonuclear cell activation associated with cardiac surgery and cardiopulmonary bypass. Brazilian Journal of Medical and Biological Research= Revista Brasileira de Pesquisas Medicas e Biologicas. 1989;22(9):1077-82.
153. Donnelly SC, Haslett C. Cellular mechanisms of acute lung injury: implications for future treatment in the adult respiratory distress syndrome. Thorax. 1992;47(4):260.
154. Arroyo AG, Iruela-Arispe ML. Extracellular matrix, inflammation, and the angiogenic response. Cardiovascular research. 2010;86(2):226-35.
155. Mitoma H, Horiuchi T, Tsukamoto H, Ueda N. Molecular mechanisms of action of anti-TNF-α agents–Comparison among therapeutic TNF-α antagonists. Cytokine. 2018;101:56-63.
156. Jackson SP, Darbousset R, Schoenwaelder SM. Thromboinflammation: challenges of therapeutically targeting coagulation and other host defense mechanisms. Blood, The Journal of the American Society of Hematology. 2019;133(9):906-18.
157. Rigor RR, Shen Q, Pivetti CD, Wu MH, Yuan SY. Myosin light chain kinase signaling in endothelial barrier dysfunction. Medicinal research reviews. 2013;33(5):911-33.
158. Boyle EM, Pohlman TH, Johnson MC, Verrier ED. Endothelial cell injury in cardiovascular surgery: the systemic inflammatory response. The Annals of thoracic surgery. 1997;63(1):277-84.
159. Downing SW, Edmunds Jr LH. Release of vasoactive substances during cardiopulmonary bypass. The Annals of thoracic surgery. 1992;54(6):1236-43.
160. Miller BE, Levy JH. The inflammatory response to cardiopulmonary bypass. Journal of cardiothoracic and vascular anesthesia. 1997;11(3):355-66.
161. Kirklin JK. Prospects for understanding and eliminating the deleterious effects of cardiopulmonary bypass. The Annals of thoracic surgery. 1991;51(4):529-31.
162. Omar S, Zedan A, Nugent K. Cardiac vasoplegia syndrome: pathophysiology, risk factors and treatment. The American journal of the medical sciences. 2015;349(1):80-8.
163. Argenziano M, Chen JM, Choudhri AF, Cullinane S, Garfein E, Weinberg AD, et al. Management of vasodilatory shock after cardiac surgery: identification of predisposing factors and use of a novel pressor agent. The Journal of thoracic and cardiovascular surgery. 1998;116(6):973-80.
164. Levy B, Fritz C, Tahon E, Jacquot A, Auchet T, Kimmoun A. Vasoplegia treatments: the past, the present, and the future. Critical care. 2018;22(1):1-11.
165. Busse LW, Barker N, Petersen C. Vasoplegic syndrome following cardiothoracic surgery—review of pathophysiology and update of treatment options. Critical Care. 2020;24(1):1-11.
166. Landry DW, Levin HR, Gallant EM, Ashton RC, Seo S, D'Alessandro D, et al. Vasopressin deficiency contributes to the vasodilation of septic shock. Circulation. 1997;95(5):1122-5.
167. Reid IA. Role of vasopressin deficiency in the vasodilation of septic shock. Circulation. 1997;95(5):1108-10.
168. Jochberger S, Velik-Salchner C, Mayr VD, Luckner G, Wenzel V, Falkensammer G, et al. The vasopressin and copeptin response in patients with vasodilatory shock after cardiac surgery: a prospective, controlled study. Intensive care medicine. 2009;35(3):489-97.
169. Landry DW, Oliver JA. The pathogenesis of vasodilatory shock. New England Journal of Medicine. 2001;345(8):588-95.
170. Weis F, Kilger E, Beiras-Fernandez A, Nassau K, Reuter D, Goetz A, et al. Association between vasopressor dependence and early outcome in patients after cardiac surgery. Anaesthesia. 2006;61(10):938-42.
171. Leyh RG, Kofidis T, Strüber M, Fischer S, Knobloch K, Wachsmann B, et al. Methylene blue: the drug of choice for catecholamine-refractory vasoplegia after cardiopulmonary bypass. The Journal of thoracic and cardiovascular surgery. 2003;125(6):1426-31.
172. Vincent J-L, De Backer D. Circulatory shock. New England Journal of Medicine. 2013;369(18):1726-34.
173. Hauffe T, Krüger B, Bettex D, Rudiger A. Shock management for cardio-surgical ICU patients–the golden hours. Cardiac failure review. 2015;1(2):75.
174. Standl T, Annecke T, Cascorbi I, Heller AR, Sabashnikov A, Teske W. The nomenclature, definition and distinction of types of shock. Deutsches Ärzteblatt International. 2018;115(45):757.
175. Kumar A, Parrillo JE. Shock: classification, pathophysiology, and approach to management. Critical care medicine: principles of diagnosis and management in the adult 3rd ed Philadelphia: Mosby Elsevier. 2008:379-422.
176. Rudiger A, Businger R, Streit M, Schmid ER, Maggiorini M, Follath F. Presentation and outcome of critically ill medical and cardiac-surgery patients with acute heart failure. Swiss medical weekly. 2009;139(7-8):110-6.
177. Ranucci M, De Toffol B, Isgrò G, Romitti F, Conti D, Vicentini M. Hyperlactatemia during cardiopulmonary bypass: determinants and impact on postoperative outcome. Critical Care. 2006;10(6):1-9.
178. O'connor E, Fraser J. The interpretation of perioperative lactate abnormalities in patients undergoing cardiac surgery. Anaesthesia and intensive care. 2012;40(4):598-603.
179. Aduen J, Bernstein WK, Khastgir T, Miller J, Kerzner R, Bhatiani A, et al. The use and clinical importance of a substrate-specific electrode for rapid determination of blood lactate concentrations. Jama. 1994;272(21):1678-85.
180. Oedorf K, Day DE, Lior Y, Novack V, Sanchez LD, Wolfe RE, et al. Serum lactate predicts adverse outcomes in emergency department patients with and without infection. Western Journal of Emergency Medicine. 2017;18(2):258.
181. Gladden L. Lactate metabolism: a new paradigm for the third millennium. The Journal of physiology. 2004;558(1):5-30.
182. Chatham JC. Lactate–the forgotten fuel! The Journal of physiology. 2002;542(Pt 2):333.
183. James JH, Luchette FA, McCarter FD, Fischer JE. Lactate is an unreliable indicator of tissue hypoxia in injury or sepsis. The lancet. 1999;354(9177):505-8.
184. Levy B. Lactate and shock state: the metabolic view. Current opinion in critical care. 2006;12(4):315-21.
185. Gore DC, Jahoor F, Hibbert JM, DeMaria EJ. Lactic acidosis during sepsis is related to increased pyruvate production, not deficits in tissue oxygen availability. Annals of surgery. 1996;224(1):97.
186. Minton J, Sidebotham DA. Hyperlactatemia and cardiac surgery. The journal of extra-corporeal technology. 2017;49(1):7.
187. Kiyatkin ME, Bakker J. Lactate and microcirculation as suitable targets for hemodynamic optimization in resuscitation of circulatory shock. Current opinion in critical care. 2017;23(4):348-54.

188. Ince C. Hemodynamic coherence and the rationale for monitoring the microcirculation. Critical care. 2015;19(3):1-13.
189. Bodson L, Bouferrache K, Vieillard-Baron A. Cardiac tamponade. Current opinion in critical care. 2011;17(5):416-24.
190. Topalian S, Ginsberg F, Parrillo JE. Cardiogenic shock. Critical care medicine. 2008;36(1):S66-S74.
191. Tyson Jr GS, Maier GW, Olsen CO, Davis JW, Rankin JS. Pericardial influences on ventricular filling in the conscious dog. An analysis based on pericardial pressure. Circulation research. 1984;54(2):173-84.
192. Kirschenbaum LA, Astiz ME, Rackow EC, Saha DC, Lin R. Microvascular response in patients with cardiogenic shock. Critical care medicine. 2000;28(5):1290-4.
193. De Backer D, Creteur J, Dubois M-J, Sakr Y, Vincent J-L. Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. American heart journal. 2004;147(1):91-9.
194. Katz SD, Khan T, Zeballos GA, Mathew L, Potharlanka P, Knecht M, et al. Decreased activity of the L-arginine–nitric oxide metabolic pathway in patients with congestive heart failure. Circulation. 1999;99(16):2113-7.
195. Smith CJ, Sun D, Hoegler C, Roth BS, Zhang X, Zhao G, et al. Reduced gene expression of vascular endothelial NO synthase and cyclooxygenase-1 in heart failure. Circulation research. 1996;78(1):58-64.
196. Dormandy J, Ernst E, Matrai A, Flute P. Hemorrheologic changes following acute myocardial infarction. American heart journal. 1982;104(6):1364-7.
197. Kohsaka S, Menon V, Lowe AM, Lange M, Dzavik V, Sleeper LA, et al. Systemic inflammatory response syndrome after acute myocardial infarction complicated by cardiogenic shock. Archives of internal medicine. 2005;165(14):1643-50.
198. Cotran RS, Kumar V, Stanley R. Robbins pathologic basis of disease: WB Saunders CompHny, Philadelphia, USA.; 2004.
199. Pich H, Heller AR. Obstruktiver Schock. Der Anaesthesist. 2015;64(5):403-19.
200. Wan Z, Sun S, Ristagno G, Weil MH, Tang W. The cerebral microcirculation is protected during experimental hemorrhagic shock. Critical care medicine. 2010;38(3):928-32.
201. Mazzoni MC, Intaglietta M, Cragoe Jr EJ, Arfors K-E. Amiloride-sensitive Na+ pathways in capillary endothelial cell swelling during hemorrhagic shock. Journal of Applied Physiology. 1992;73(4):1467-73.
202. Liu L-m, Dubick MA. Hemorrhagic shock-induced vascular hyporeactivity in the rat: relationship to gene expression of nitric oxide synthase, endothelin-1, and select cytokines in corresponding organs. Journal of Surgical Research. 2005;125(2):128-36.
203. van Meurs M, Wulfert FM, Knol AJ, De Haes A, Houwertjes M, Aarts LP, et al. Early organ-specific endothelial activation during hemorrhagic shock and resuscitation. Shock. 2008;29(2):291-9.
204. Czabanka M, Peter C, Martin E, Walther A. Microcirculatory endothelial dysfunction during endotoxemia-insights into pathophysiology, pathologic mechanisms and clinical relevance. Current vascular pharmacology. 2007;5(4):266-75.
205. Alcaide P, Auerbach S, Luscinskas FW. Neutrophil recruitment under shear flow: it's all about endothelial cell rings and gaps. Microcirculation. 2009;16(1):43-57.
206. Ivanov K, Mel'nikova N. Leukocytes as a cause of microcirculatory dysfunction. Bulletin of Experimental Biology and Medicine. 2006;141(6):666.
207. Baskurt OK, Gelmont D, Meiselman HJ. Red blood cell deformability in sepsis. American journal of respiratory and critical care medicine. 1998;157(2):421-7.
208. Szopinski J, Kusza K, Semionow M. Microcirculatory responses to hypovolemic shock. Journal of Trauma and Acute Care Surgery. 2011;71(6):1779-88.
209. Spronk PE, Zandstra DF, Ince C. Bench-to-bedside review: sepsis is a disease of the microcirculation. Critical care. 2004;8(6):1-7.
210. Astiz ME, DeGent GE, Lin RY, Rackow EC. Microvascular function and rheologic changes in hyperdynamic sepsis. Critical care medicine. 1995;23(2):265-71.
211. Lelubre C, Vincent J-L. Mechanisms and treatment of organ failure in sepsis. Nature Reviews Nephrology. 2018;14(7):417-27.
212. Linderkamp O, Ruef P, Brenner B, Gulbins E, Lang F. Passive deformability of mature, immature, and active neutrophils in healthy and septicemic neonates. Pediatric research. 1998;44(6):946-50.
213. Lam C, Tyml K, Martin C, Sibbald W. Microvascular perfusion is impaired in a rat model of normotensive sepsis. The Journal of clinical investigation. 1994;94(5):2077-83.
214. Ince C, Sinaasappel M. Microcirculatory oxygenation and shunting in sepsis and shock. Critical care medicine. 1999;27(7):1369-77.
215. Goldman D, Bateman RM, Ellis CG. Effect of decreased O2 supply on skeletal muscle oxygenation and O2 consumption during sepsis: role of heterogeneous capillary spacing and blood flow. American Journal of Physiology-Heart and Circulatory Physiology. 2006;290(6):H2277-H85.
216. De Backer D, Donadello K, Favory R. Link between coagulation abnormalities and microcirculatory dysfunction in critically ill patients. Current Opinion in Anesthesiology. 2009;22(2):150-4.
217. Aird WC. Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. Circulation research. 2007;100(2):174-90.
218. Starling EH. On the absorption of fluids from the connective tissue spaces. 1896.
219. Aird WC. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. Blood, The Journal of the American Society of Hematology. 2003;101(10):3765-77.
220. Schnitzer J, Carley W, Palade GE. Specific albumin binding to microvascular endothelium in culture. American Journal of Physiology-Heart and Circulatory Physiology. 1988;254(3):H425-H37.
221. Antunes E, Mariano M, Cirino G, Levi S, de Nucci G. Pharmacological characterization of polycation-induced rat hind-paw oedema. British journal of pharmacology. 1990;101(4):986.
222. Vehaskari V, Chang C, Stevens J, Robson A. The effects of polycations on vascular permeability in the rat. A proposed role for charge sites. The Journal of clinical investigation. 1984;73(4):1053-61.
223. Chang S, Westcott J, Henson J, Voelkel NF. Pulmonary vascular injury by polycations in perfused rat lungs. Journal of Applied Physiology. 1987;62(5):1932-43.
224. Alphonsus C, Rodseth R. The endothelial glycocalyx: a review of the vascular barrier. Anaesthesia. 2014;69(7):777-84.
225. Ferrer R, Mateu X, Maseda E, Yébenes JC, Aldecoa C, De Haro C, et al. Non-oncotic properties of albumin. A multidisciplinary vision about the implications for critically ill patients. Expert review of clinical pharmacology. 2018;11(2):125-37.
226. Pillinger N, Kam P. Endothelial glycocalyx: basic science and clinical implications. Anaesthesia and intensive care. 2017;45(3):295-307.
227. Becker BF, Chappell D, Bruegger D, Annecke T, Jacob M. Therapeutic strategies targeting the endothelial glycocalyx: acute deficits, but great potential. Cardiovascular research. 2010;87(2):300-10.

228. Woodcock T, Woodcock TM. Revised Starling equation and the glycocalyx model of transvascular fluid exchange: an improved paradigm for prescribing intravenous fluid therapy. British journal of anaesthesia. 2012;108(3):384-94.
229. Carden DL, Smith JK, Zimmerman BJ, Korthuis RJ, Granger DN. Reperfusion injury following circulatory collapse: the role of reactive oxygen metabolites. Journal of critical care. 1989;4(4):294-307.
230. Kubes P, Granger DN. Nitric oxide modulates microvascular permeability. American Journal of Physiology-Heart and Circulatory Physiology. 1992;262(2):H611-H5.
231. Wang LF, Patel M, Razavi HM, Weicker S, Joseph MG, McCormack DG, et al. Role of inducible nitric oxide synthase in pulmonary microvascular protein leak in murine sepsis. American Journal of Respiratory and Critical Care Medicine. 2002;165(12):1634-9.
232. Jerome SN, Akimitsu T, Korthuis RJ. Leukocyte adhesion, edema, and development of postischemic capillary no-reflow. American Journal of Physiology-Heart and Circulatory Physiology. 1994;267(4):H1329-H36.
233. Shah D. Vascular autoregulatory failure following trauma and shock. Plastic and Reconstructive Surgery. 1979;63(5):752.
234. Chappell D, Jacob M, Becker B, Hofmann-Kiefer K, Conzen P, Rehm M. Expedition glykokalyx. Der Anaesthesist. 2008;57(10):959-69.
235. Rix D, Douglas M, Talbot D, Dark J, Kirby J. Role of glycosaminoglycans (GAGs) in regulation of the immunogenicity of human vascular endothelial cells. Clinical & Experimental Immunology. 1996;104(1):60-5.
236. Reitsma S, Slaaf DW, Vink H, Van Zandvoort MA. The endothelial glycocalyx: composition, functions, and visualization. Pflügers Archiv-European Journal of Physiology. 2007;454(3):345-59.
237. Jacob M, Bruegger D, Rehm M, Stoeckelhuber M, Welsch U, Conzen P, et al. The endothelial glycocalyx affords compatibility of Starling's principle and high cardiac interstitial albumin levels. Cardiovascular research. 2007;73(3):575-86.
238. Schött U, Solomon C, Fries D, Bentzer P. The endothelial glycocalyx and its disruption, protection and regeneration: a narrative review. Scandinavian journal of trauma, resuscitation and emergency medicine. 2016;24(1):1-8.
239. Wang G, Tiemeier GL, van den Berg BM, Rabelink TJ. Endothelial glycocalyx hyaluronan: Regulation and role in prevention of diabetic complications. The American journal of pathology. 2020;190(4):781-90.
240. Bruegger D, Schwartz L, Chappell D, Jacob M, Rehm M, Vogeser M, et al. Release of atrial natriuretic peptide precedes shedding of the endothelial glycocalyx equally in patients undergoing on-and off-pump coronary artery bypass surgery. Basic research in cardiology. 2011;106(6):1111-21.
241. Lipowsky HH. The endothelial glycocalyx as a barrier to leukocyte adhesion and its mediation by extracellular proteases. Annals of biomedical engineering. 2012;40(4):840-8.
242. Mulivor AW, Lipowsky HH. Inflammation-and ischemia-induced shedding of venular glycocalyx. American Journal of Physiology-Heart and Circulatory Physiology. 2004;286(5):H1672-H80.
243. McDonald KK, Cooper S, Danielzak L, Leask RL. Glycocalyx degradation induces a proinflammatory phenotype and increased leukocyte adhesion in cultured endothelial cells under flow. PloS one. 2016;11(12):e0167576.
244. Bitan M, Weiss L, Zeira M, Zcharia E, Slavin S, Nagler A, et al. Heparanase promotes engraftment and prevents graft versus host disease in stem cell transplantation. PLoS One. 2010;5(4):e10135.
245. Lygizos MI, Yang Y, Altmann CJ, Okamura K, Hernando AA, Perez MJ, et al. Heparanase mediates renal dysfunction during early sepsis in mice. Physiological reports. 2013;1(6):e00153.
246. Constantinescu AA, Vink H, Spaan JA. Endothelial cell glycocalyx modulates immobilization of leukocytes at the endothelial surface. Arteriosclerosis, thrombosis, and vascular biology. 2003;23(9):1541-7.
247. Granger DN, Senchenkova E, editors. Inflammation and the Microcirculation. Colloquium series on integrated systems physiology: from molecule to function; 2010: Morgan & Claypool Life Sciences.
248. Kolaczkowska E, Kubes P. Neutrophil recruitment and function in health and inflammation. Nature reviews immunology. 2013;13(3):159-75.
249. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nature Reviews Immunology. 2007;7(9):678-89.
250. Lawrence MB, Springer TA. Leukocytes roll on a selectin at physiologic flow rates: distinction from and prerequisite for adhesion through integrins. Cell. 1991;65(5):859-73.
251. Wrenshall LE, Stevens RB, Cerra FB, Platt JL. Modulation of macrophage and B cell function by glycosaminoglycans. Journal of leukocyte biology. 1999;66(3):391-400.
252. Akbarshahi H, Axelsson JB, Said K, Malmström A, Fischer H, Andersson R. TLR4 dependent heparan sulphate-induced pancreatic inflammatory response is IRF3-mediated. Journal of translational medicine. 2011;9(1):1-8.
253. Scheibner KA, Lutz MA, Boodoo S, Fenton MJ, Powell JD, Horton MR. Hyaluronan fragments act as an endogenous danger signal by engaging TLR2. The Journal of Immunology. 2006;177(2):1272-81.
254. Lenart M, Rutkowska-Zapala M, Baj-Krzyworzeka M, Szatanek R, Węglarczyk K, Smallie T, et al. Hyaluronan carried by tumor-derived microvesicles induces IL-10 production in classical (CD14++ CD16−) monocytes via PI3K/Akt/mTOR-dependent signalling pathway. Immunobiology. 2017;222(1):1-10.
255. Webb L, Ehrengruber MU, Clark-Lewis I, Baggiolini M, Rot A. Binding to heparan sulfate or heparin enhances neutrophil responses to interleukin 8. Proceedings of the National Academy of Sciences. 1993;90(15):7158-62.
256. Chousterman BG, Swirski FK, Weber GF, editors. Cytokine storm and sepsis disease pathogenesis. Seminars in immunopathology; 2017: Springer.
257. Uchimido R, Schmidt EP, Shapiro NI. The glycocalyx: a novel diagnostic and therapeutic target in sepsis. Critical care. 2019;23(1):1-12.
258. Cabrales P, Vazquez B, Tsai A, M I. Microvascular and capillary perfusion following glycocalyx degradation. Journal of Applied Physiology. 2007;102:2251-9.
259. Jacob M, Bruegger D, Rehm M, Welsch U, Conzen P, Becker BF. Contrasting effects of colloid and crystalloid resuscitation fluids on cardiac vascular permeability. The Journal of the American Society of Anesthesiologists. 2006;104(6):1223-31.
260. Rovas A, Seidel L, Vink H, Pohlkötter T, Pavenstädt H, Ertmer C. Association of sublingual microcirculation parameters and endothelial glycocalyx dimensions in resuscitated sepsis. Crit Care. 2019;23(1):260.
261. Pesonen E, Passov A, Andersson S, Suojaranta R, Niemi T, Raivio P, et al. Glycocalyx degradation and inflammation in cardiac surgery. Journal of Cardiothoracic and Vascular Anesthesia. 2019;33(2):341-5.
262. Stein A, de Souza LV, Belettini CR, Menegazzo WR, Viégas JR, Costa Pereira EM, et al. Fluid overload and changes in serum creatinine after cardiac surgery: predictors of mortality and longer intensive care stay. A prospective cohort study. Critical Care. 2012;16(3):1-9.

263. Mouncey PR, Osborn TM, Power GS, Harrison DA, Sadique MZ, Grieve RD, et al. Trial of early, goal-directed resuscitation for septic shock. New England Journal of Medicine. 2015;372(14):1301-11.
264. 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. Annals of emergency medicine. 2007;49(1):88-98. e2.
265. Bellomo R, Marik P, Kellum JA, Salon J, Igah I, Ihediwa U, et al. Lactic acidosis. N Engl J Med. 2015;372(11):1076.
266. Bloos F, Reinhart K. Venous oximetry. Intensive care medicine. 2005;31(7):911-3.
267. Kattan E, Castro R, Vera M, Hernández G. Optimal target in septic shock resuscitation. Annals of Translational Medicine. 2020;8(12).
268. Cabrales P, Martini J, Intaglietta M, Tsai AG. Blood viscosity maintains microvascular conditions during normovolemic anemia independent of blood oxygen-carrying capacity. American Journal of Physiology-Heart and Circulatory Physiology. 2006;291(2):H581-H90.
269. Elbers PW, Ince C. Bench-to-bedside review: Mechanisms of critical illness–classifying microcirculatory flow abnormalities in distributive shock. Critical care. 2006;10(4):1-8.
270. Weil MH, Tang W. Welcoming a new era of hemodynamic monitoring: expanding from the macro to the microcirculation. Critical care medicine. 2007;35(4):1204-5.

This page was intentionally left blank.



3.

The Microcirculation in Patients With Circulatory Shock After Cardiothoracic Surgery and Its Relation to the Macrocirculation

3.1 | Introduction

Nearly all patients after cardiothoracic surgery are admitted to the ICU. Circulatory shock complicates 5-50% of all adult cardiothoracic procedures.[1-4] In these patients, changes in the microcirculation occur frequently. An impaired microcirculation results in decreased tissue oxygenation or organ damage if a situation of low tissue oxygenation persists.[4] Monitoring in the ICU is mainly focused on macrocirculatory parameters, such as CO, blood pressure, SvO_2, and biochemical markers (i.e., lactate). However, increasingly also microcirculation is measured in critically ill patients.[5, 6] Although the macrocirculatory parameters can generally be used as surrogate parameters for systemic hemodynamics since the micro- and macrocirculatory systems are strongly interrelated, several studies in patients with sepsis or septic shock have shown that a loss of hemodynamic coherence can occur. This means that macrocirculatory parameters improve to normal values during resuscitation while microcirculation is still impaired, a condition associated with increased morbidity and mortality.[7]

An abundance of literature discussing macro- and microcirculation in patients with sepsis or septic shock is available. However, the number of studies performed in patients after cardiothoracic surgery is much smaller. During surgery, microcirculatory perfusion may be severely impaired as a result of no pulsatile blood flow through CPB, decreased cardiac output, hemodilution, hypothermia, and CPB- and tissue trauma-induced inflammation.[8] As a result, areas of the microcirculation can become shunted and hypoxemic, eventually leading to organ failure.[9, 10] The number of studies describing circulatory shock after cardiothoracic surgery in relation to the exact role of the microcirculation is still in its premature stages.[11]

Therefore, the aim of this chapter was to identify the clinical time course of microcirculatory parameters of postoperative cardiothoracic surgical patients who are evidently macrocirculatory in circulatory shock. Secondly, we also aimed to investigate the relationship (i.e., coherence) between microcirculatory and macrocirculatory parameters in patients admitted to the ICU after cardiothoracic surgery with circulatory shock.

3.2 | Methods

3.2.1 | Dataset

The data used for this chapter were obtained from a pre-existing database of cardiothoracic surgical patients in circulatory shock at the Leids University Medical Center (LUMC) ICU. In general, circulatory shock is defined as a state of circulatory disturbance with cellular and tissue hypoxia due to either reduced oxygen supply, increased oxygen consumption, insufficient O_2 utilization, or a combination of these processes.[12] The diagnosis of shock is based on clinical, hemodynamic, and biochemical markers (**Table 3**[13]). In the ICU of the LUMC, a patient met the definition of circulatory shock at a lactate level of > 2 mmol/L combined with a norepinephrine level of ≥ 0.2 μg/kg/hr.

Table 3 *Diagnosis of shock based on clinical, hemodynamic, and biochemical markers*

Clinical signs of decreased tissue perfusion and organ dysfunction	Hemodynamic sings	Biochemical signs
Cold, clammy, mottled extremities	Hypotension SBP < 90 mmHg > 30 min or use of vasopressors (≥ 0.2 µg/kg/min norepinephrine and/or 5 µg/kg/min dobutamine > 1 h) to achieve an SBP > 90 mmHg or a MAP > 65 mmHg	Hyperlactatemia (> 2 mmol/L)
Nausea and vomiting		
Urine production < 0.5 ml/kg/hour		
Neurological symptoms (i.e., lethargy and/or confusion)		
SvO2 < 65%		
Liver dysfunction		

MAP: mean arterial pressure; SBP: systolic blood pressure

Hemodynamic monitoring occurred according to LUMC's current clinical Shock protocol when a patient was in circulatory shock after cardiothoracic surgery. This protocol assessed microcirculation at predefined moments as part of standard clinical practice (**Figure 15**).

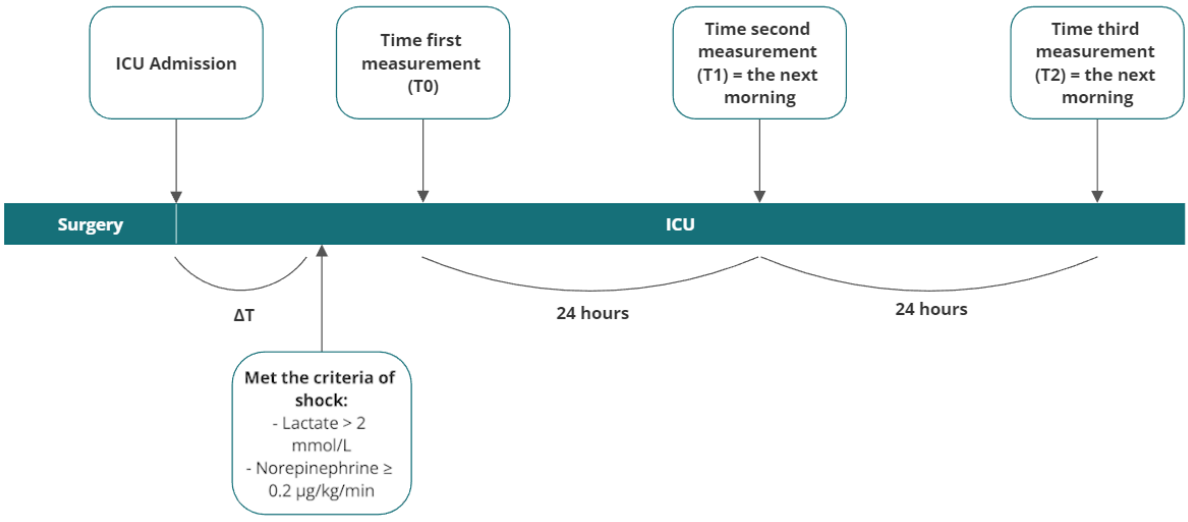


Figure 15

Timeline of microcirculatory measurements in cardiothoracic surgical patients with shock.

In this study, we included data of patients who met the definition of shock, according to the definition of the LUMC, at time point T0 as our study population. Despite the dynamic nature of shock, in which the patient may be resuscitated after T0, all included patients were considered to have shock throughout the study period. No correction was applied if the included patients no longer showed signs of shock after T0.

From the database, we extracted the following data: demographic data (e.g., age, gender), type of surgery, durations (e.g., time to meeting shock criteria, time to the first measurement, and intraoperative times), hemodynamic data (i.e., MAP, lactate, and SvO_2), microcirculation data (i.e., diffusion and convection parameters) and severity of disease score (i.e., Acute Physiology And Chronic Health Evaluation (APACHE) IV). MAP, SvO_2, and lactate were extracted because these are hemodynamic parameters measured by default in all patients.

In each patient, at least three four-second image sequences were recorded during each measurement time point according to the applicable guidelines[14], using the CytoCam. The MicroTools algorithm was used to calculate the microcirculatory hemodynamic variables, such as TVD, FCD, PPV, cHct, RBCv, and tRBCp, from the image sequences of each patient.[15] A pre-existing MATLAB script was used to calculate the average microcirculation parameters of the different image sequences.

3.2.2| Statistical analysis

The statistical analysis has been conducted using the SPSS (Statistical Package for the Social Sciences) statistical package, release 28.0.1 (SPSS Inc., Chicago, IL). Descriptive statistics were used to characterize the study population, with a calculation of mean, standard deviation, median and interquartile range, according to the distribution of variables, for continuous variables, which were assessed with histograms. Categorical variables were stated as numbers and percentages. Normality was tested for each variable; outliers were visualized by creating a 1D single-parameter boxplot. Next, the database was transformed from a wide database to a long database to describe the time course of the micro- and macrocirculation with graphs. Because of the repeated measurements within the same patient, Linear Mixed Models (LMM, **Appendix B**) estimated marginal means has been used to describe the mean values in microcirculatory and macrocirculatory parameters. LMM estimated marginal means is also used to describe the change of micro- and macrocirculatory parameters between the first two measurements (ΔT1 - T0). The hypotheses have been tested across several macro- and microcirculatory parameters. Thus, in order to reduce the chance of a type I error inflation, the Bonferroni multiple testing correction (**Appendix B**) is applied on the p-values of all individual tests.[16] To describe the time course of the microcirculatory variables over time, LMM has also been used.

For all the models in this chapter, the fixed effects included time course. Microcirculatory parameters of healthy volunteers[17] were used as a reference for the microcirculatory parameters of this study population.

Hemodynamic coherence of the macro- and microcirculation is defined as the same direction of change in both categories. It is well known what change for an established MAP at T0 is an improvement (i.e., the MAP increases) and what change is a deterioration. The same applies to lactate (i.e., improvement is a decrease) and SvO_2 (i.e., in this patient population, an improvement is a decrease). However, for the microcirculatory parameters in this specific category of patients, it is not clear, because the behavior of the microcirculation in this category is not well described. Thus, in this thesis, a two-step approach was used: for a patient clearly clinically in shock, the first measurement of a microcirculatory parameter was compared with normal values from healthy volunteers. If this first measurement of a particular microcirculatory parameter in a cardiothoracic surgery patient with shock was higher than the normal value, it was concluded that this had to be

an abnormal value and that a decrease during a second measurement towards normal was an improvement of the microcirculation. Thus followed the qualitative definition of coherence of this thesis: both an improvement in macro- and microcirculation was a signal of coherence. An improvement in the macrocirculation but no improvement in the microcirculation was considered a sign of a lack of coherence. In this thesis, hemodynamic coherence was assessed globally in a qualitative manner per mean group change instead of mean individual changes.

3.3| Results

Initially, a total of 23 patients were included from the database. At T0, after retrospectively reviewing all patients, 20 of 23 patients met the stated criteria for the diagnosis of shock and were included for further analysis. At T1, 6 of 18 patients still met the stated criteria for shock, and at T2, only 4 of 11 patients. Several types of surgeries were performed, with a mean surgery time of 500 ± 201 min and a mean bypass time of 307 ± 160 min. The study population comprised 14 (70,0%) men with a mean age of 67 (IQR 26-76 years). The population had a mean APACHE-IV score of 64 ± 24. The mean time to meet the definition of shock was 4 ± 4 hours. On average, the time between admission to the ICU and the first measurement was 20 ± 12 hours. **Table 4** summarizes the baseline characteristics.

3.3.1 | Microcirculatory time course

The mean values and changes over time of macro- and microcirculatory parameters are shown in **Table 5**. FCD, TVD, and cHct were higher in cardiothoracic surgery patients with shock than in healthy volunteers. Lactate and SvO_2 values showed significant improvement with improvement of shock. Microcirculatory parameters also showed signs of improvement of shock, but not significantly. Only the RBCv and cHct showed no improvement of shock. The number of measured values can be found in **Appendix C**.

Figure 16 a to f show the course in time of the different microcirculation parameters. No significant differences over time were found in these parameters. The Linear Mixed Model (LMM) results are shown in **Appendix D**.

Table 4 *Baseline characteristics of the study population*

General characteristics	**n = 20**
Age (years, median, range)	67 (26 - 76)
Gender (n, %)	
Male	14 (70,0%)
Female	6 (30,0%)
BMI (kg/m^2) (mean ± SD)	28 ± 5
APACHE IV score (mean ± SD)	64 ± 24
Type of surgery (n, %)	
Intracardiac	5 (25,0%)
Coronary	4 (20,0%)
Aortic	2 (10,0%)
Mechanical	1 (5,0%)
Intracardiac and coronary	5 (25,0%)
Intracardiac and Mechanical	-
Intracardiac and aortic	3 (15,0%)
Other	-
Surgery characteristics	
Surgery duration (min) (mean ± SD)	500 ± 201
Anesthesia duration (min) (mean ± SD)	569 ± 206
Bypass duration [a] (min) (mean ± SD)	307 ± 160
Aortic cross-clamp duration [a] (min) (mean ± SD)	165 ± 91
Microcirculatory measurements characteristics	
Time to meet the criteria of shock (hours) (mean ± SD)	4 ± 4
Time to first measurement (T0) [b] (hours) (mean ± SD)	20 ± 12
Time to second measurement [b] (T1) (hours) (mean ± SD)	51 ± 29
Time to third measurement [b] (T2) (hours) (mean ± SD)	69 ± 32
Macrocirculation characteristics	
Noradrenalin at T0 (µg/kg/min) (mean ± SD)	0,54 ± 0,26
Systemic hematocrit at T0 (L/L) (mean ± SD)	0,30 ± 0,04

APACHE IV score: Acute Physiology and Chronic Health Evaluation Version IV Score; BMI: body mass index; SD: standard deviation; a: If a patient had multiple bypass or aortic clamp times, the values were first summed before the group average was calculated; b: difference between the time of admission to the ICU and measurement of the microcirculation.

Table 5 *Mean values and mean change over time of macro- and microcirculatory parameters*

	Normal value	Mean T0 [95% CI]	Mean T1 [95% CI]	Mean ΔT1 - T0 [95% CI]	P-value *	Signs of improvement shock?
Macrocirculatory parameters						
MAP (mmHg)	65 (60-90)[18]	66.850 [62.118, 71.582]	72.067 [67.150, 76.985]	5.217 [-1.164, 11.599]	0,106	Yes
Lactate (mmol/L)	<2[19]	3.750 [3.034, 4.466]	2.259 [1.550, 2.968]	-1.491 [-2.236, -0.746]	**0,000**	Yes
SvO_2 (%)	70 (68-77%)[20]	73.694 [69.624, 77,764]	62.343 [51.973, 72.713]	-11.351 [-21.046, -1.656]	**0,027**	Yes
Microcirculatory parameters						
TVD (mm/mm^2)	18.2 [17.2, 20.1][17]	21.129 [19.600, 22.658]	21.017 [19.166, 22.868]	-0.111 [-2.104, 1.882]	0,910	Yes
FCD (mm/mm^2)	17.3 [16.8, 19.2][17]	19.861 [18.389, 21.333]	19.785 [17.768, 21.803]	-0.075 [-2.043, 1.893]	0,938	Yes
PPV (%)	97.6 [94.3, 98.4][17]	94.109 [91.716, 96.501]	94.262 [89.366, 99.158]	0.153 [-3.221, 3.528]	0,925	Yes
RBCv (µm/s)	340 [302, 357][17]	334.469 [308.427, 360.511]	329.220 [305.209, 353.231]	-5.249 [-33.364, 22.866]	0,707	No
cHct (%)	5.0 [4.7, 5.3][17]	5.720 [5.201, 6.238]	5.813 [5.130, 6.497]	0.094 [-0.586, 0.773]	0,781	No
tRBCp (µm/min)	45.3 [39.5, 52.0][17]	46.322 [41.218, 51.426]	46.402 [40.776, 52.028]	0,080 [-5.858, 6.018]	0,978	Yes

**: Result of the LMM estimated marginal means with Bonferroni multiple testing correction of ΔT1-T0; Δ: change; cHct: capillary hematocrit; FCD: functional capillary density; MAP: mean arterial pressure; PPV: proportion of perfused vessels; RBCv: red blood cell velocity; SvO_2: central venous oxygen saturation; tRBCp: tissue red blood cell perfusion; TVD: total vessel density.*

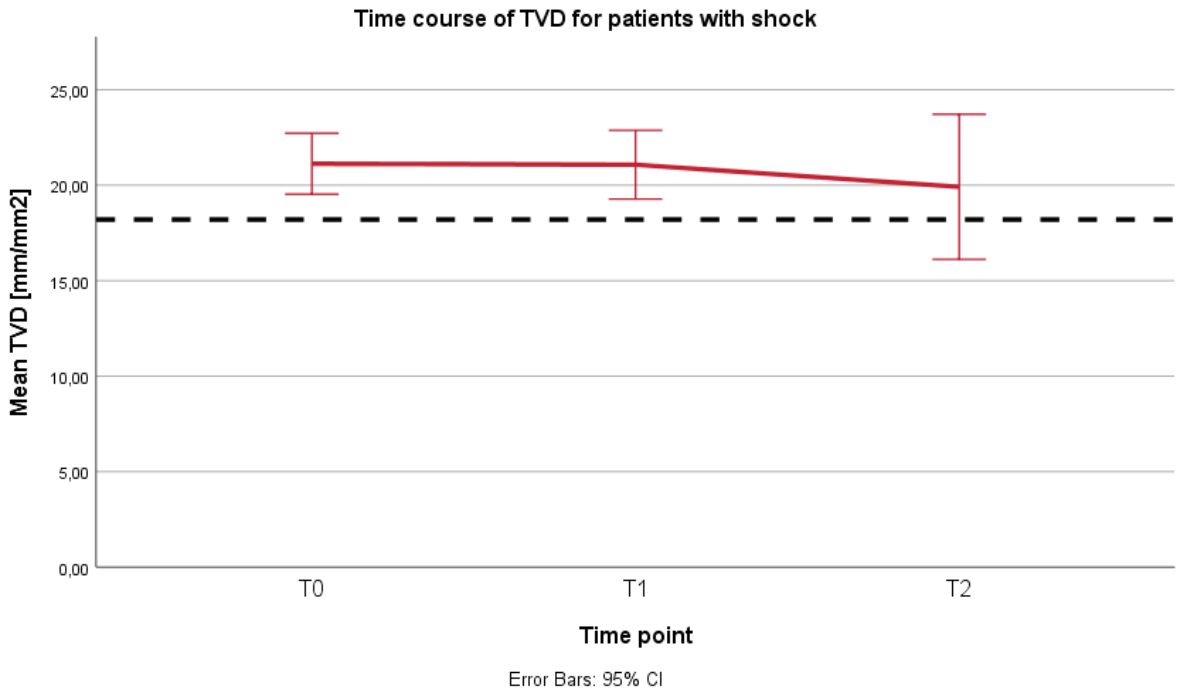


Figure 16a

Time course of the mean total vessel density (TVD) for patients with shock.

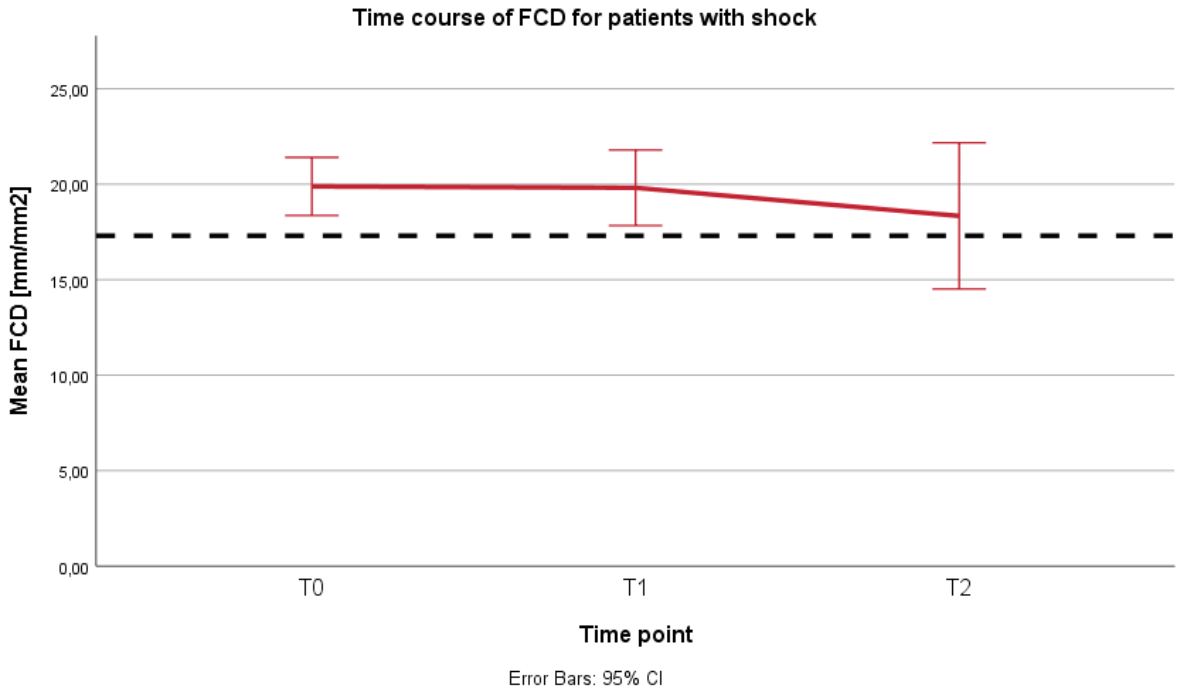


Figure 16b

Time course of the mean functional capillary density (FCD) for patients with shock.

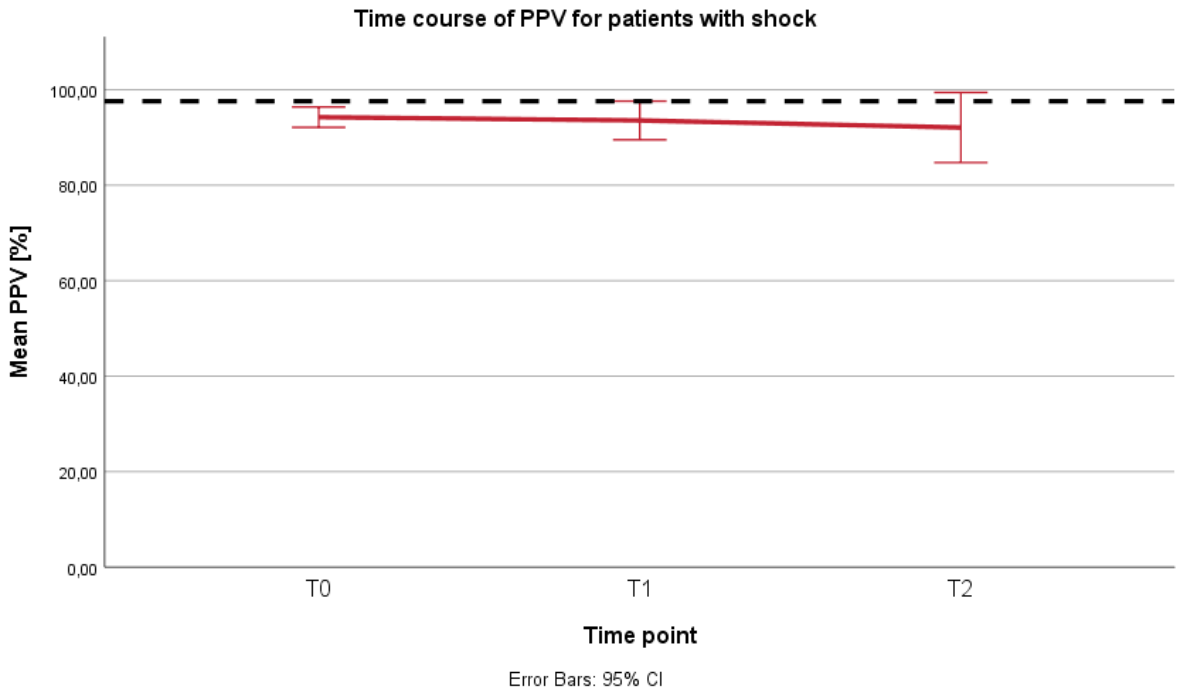


Figure 16c

Time course of the mean proportion of perfused vessels (PPV) for patients with shock.

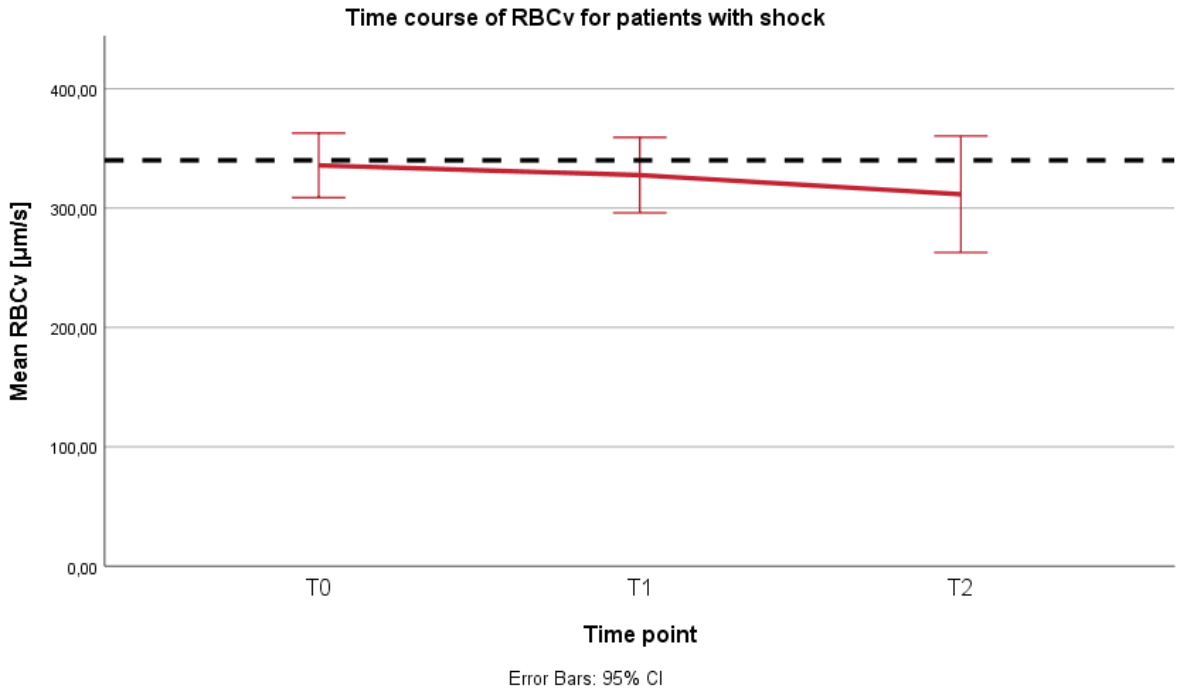


Figure 16d

Time course of the mean red blood cell velocity (RBCv) for patients with shock.

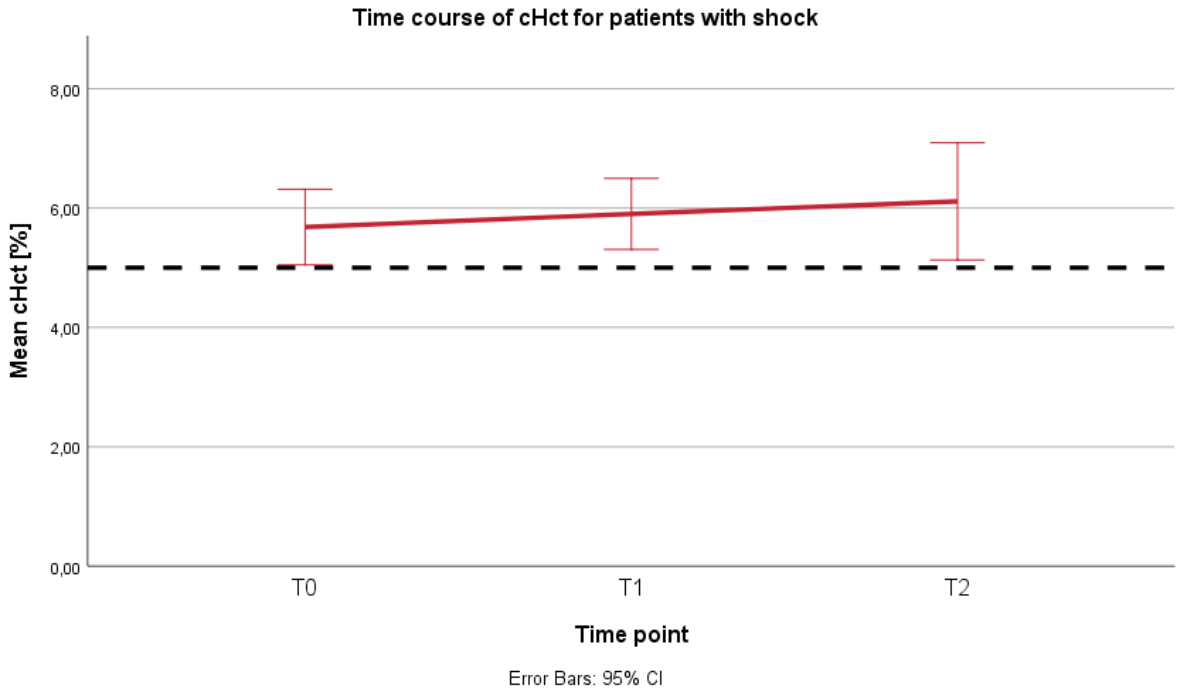


Figure 16e

Time course of the mean capillary hematocrit (cHct) for patients with shock.

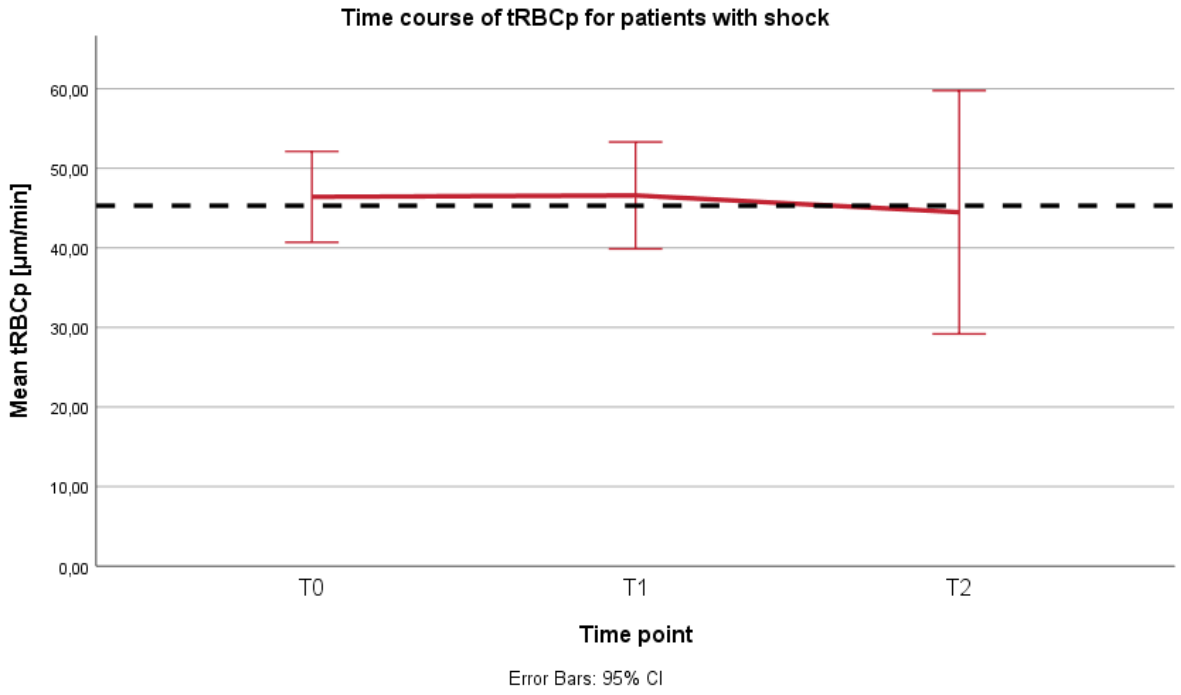


Figure 16f

Time course of the mean tissue red blood cell perfusion (tRBCp) for patients with shock.

3.3.2| Relationship between macro- and microcirculation

The relationship between macro- and microcirculatory parameters can be found in **Table 6**. The mean duration between the measurements of T0 and T1 was 30 ± 21 hours and between T1 and T2 was 27 ± 15 hours. Based on qualitative assessment, hemodynamic coherence was found between all macrocirculatory parameters and the TVD, FCD, and PPV.

Table 6 *Relationship between the macro- and microcirculation*

	ΔMAP (mmHg)	**Δ Lactate (mmol/L)**	**Δ SvO_2 (%)**
Δ TVD (mm/mm^2)	Coherence	Coherence	Coherence
Δ FCD (mm/mm^2)	Coherence	Coherence	Coherence
Δ PPV (%)	Coherence	Coherence	Coherence
Δ RBCv (μm/s)	No coherence	No coherence	No coherence
Δ cHct (%)	No coherence	No coherence	No coherence
Δ tRBCp (μm/min)	No coherence	No coherence	No coherence

Δ: change (no increase or decrease); cHct: capillary hematocrit; FCD: functional capillary density; MAP: mean arterial pressure; PPV: proportion of perfused vessels; RBCv: red blood cell velocity; SvO_2: central venous oxygen saturation; tRBCp: tissue red blood cell perfusion; TVD: total vessel density.

3.4| Discussion

The aim of this study was to describe the time course of the microcirculation in postoperative cardiothoracic surgical patients in shock and to describe the relationship, i.e., hemodynamic coherence, between this microcirculation and the macrocirculation.

First, it is noteworthy that the convective mechanism, reflected in the RBCv, of the microcirculation is impaired in patients with shock. One explanation could be that these patients receive high doses of norepinephrine to achieve adequate MAP to maintain sufficient tissue perfusion.[21] Initially, vasopressors, including norepinephrine, reduce microvascular perfusion by increasing precapillary sphincter tone.[22-24] However, this is inconsistent with the increased diffusion mechanisms, FCD, TVD, and cHct of the microcirculation. Studies in several patients with septic shock have shown that the use of vasopressors can also improve microcirculatory perfusion by restoring tissue perfusion pressure. However, the net effect of norepinephrine on microcirculatory perfusion is highly variable, with alternating neutral, enhancing, or worsening effects.[22-24]

In a capillary, the RBC velocity is always higher than the plasma velocity. The RBCs are mainly located in the middle of the capillaries because that is where the plasma velocity is highest. Consequently, the RBC velocity must exceed the plasma velocity. When the arterial Hct is 50%, plasma and RBCs enter the capillary at the same velocity.[25] However, an RBC moves faster through the capillary than plasma, and since the total RBC flux remains constant across all vessel cross-sections, the Hct in the capillary must be reduced at any instant in time to conserve mass in the microcirculation.[26] The Hct in the blood in a capillary will be reduced below the arterial and venous

Hct by an amount proportional to the ratio of the mean transit time of the RBC to the mean transit time of the whole blood. Because of the Fahraeus effect, the Hct in the capillary will be lower as the RBCs flow faster, resulting in lower transit time.[25] The Fahraeus effect is also reflected in our data, with RBCv being reduced and cHct increasing over time.

In general, macrocirculatory improvement of the shock state is seen in these patients over time. Nevertheless, the microcirculation remains quite stably high compared to normal values. Only RBCv and cHct are contradictory, with a decrease in RBCv and an increase in cHct, which, however, can already be explained by the Fahraeus effect. However, no significant differences were found over time in the course of all microcirculatory parameters, which is logical, considering that many patients were still in shock during the measurement period. It is possible that in patients with shock, the microcirculatory changes are mainly a convective problem (i.e., the reduced RBCv). Increasing the diffusion parameters and decreasing the RBCs' transit time could possibly indicate a compensatory mechanism for the mismatch between O_2 supply and consumption by delivering as much O_2 to the tissues as possible. Since the tRBCp remains stable over time, the microcirculation seems to adapt to the situation in times of shock regardless of changes in the macrocirculation.

The absolute change in RBCv, cHct, and tRBCp showed no hemodynamic coherence with the absolute change in MAP, lactate, and SvO2. In principle, this makes sense, as RBCv continued to decrease and cHct continued to increase from normal, while MAP, lactate, and SvO2 changed in the right direction. The tRBCp remained approximately stable, whereas it was expected to increase with an improvement in macrocirculation. However, if the assumption was made that there is a successful microcirculatory compensation mechanism in the case of shock, the tRBCp should not change with changes in the macrocirculation, which it did in this case.

3.4.1 | Limitations

First, the duration between measurements is not the same for every patient and measurement point. This is mainly due to logistical reasons (e.g., shock development at weekends or nights). These differences in time between T0 and T1 may mean that micro and/or macrocirculation improved or declined more in one patient than in another. Consequently, the qualitative assessment of hemodynamic coherence may yield different results. A change in micro and macrocirculation per hour could correct the difference in time between measurements.

At T0, not all patients no longer met the stated definition of shock of the LUMC. However, all patients, except three, were included in the analyses as patients with shock. Retrospectively, the patient records of seven "non-shock" shock patients were reviewed, after which four patients were still classified as patients with shock. It was assumed that if a patient met one of the two requirements of the shock definition (norepinephrine ≥0.2 μg/kg/min or lactate>2 mmol/L) and was clinically in shock (according to a clinician's expertise), the patient was categorized as being in shock. All patients in the existing shock database were measured only after shock was diagnosed. If a patient was no longer in shock at T0, this could mean that it was a recovering microcirculation, which perhaps should have been included as a different group in the analyses. Not all patients had three measurements. Because of the unpredictable nature of the onset of shock, there was a chance that measurement points T1 or T2 were on weekend days or that no researcher was available to take measurements that day. Only 18 patients were measured at T1 and only 11 at T2.

As a result, the database has many missing values. Because of the completely unsystematic form of missing data, it is very likely that the missing at random mechanism played a role.

Because of the possibility of missing data with repeated measurements, we have used methods that can deal with unbalanced data and give valid results under the missing at random mechanism. As a result, the analysis may lose efficiency, but no bias is introduced.[27, 28]

The sample size consists of only a small group (n = 20). This makes it challenging to define outliers as being outliers, or a possible trend. A small sample size ensures that a missing value is of more significant influence than if the sample size is larger. Unbalanced data in a small sample size potentially suggest certain trends differently than they are. For statistical analyses, a larger sample size is needed to add more covariates, such as surgery, anesthesia, bypass or aortic clamp duration, or inotropic agent values to the model, to test whether this may affect microcirculation.

Another limitation is that our analyses have reduced statistical power to reject an incorrect H0 hypothesis.[29] This is due to the use of the Bonferroni multiple testing correction in combination with the small sample size.

The reference values of the healthy controls from the study of Flick et al.[17] were measured with the same imaging modality (the CytoCam) and analyzed with the same software as in the present study. However, it should be noted that this study is about microcirculatory perfusion and the effect of general anesthesia and non-cardiac surgery. This study included 38 healthy volunteers in their study, to observe any changes in the absence of anesthesia and surgery. In comparison with another study conducted by Hilty et al.[30], there are slight differences in the values of various microcirculation parameters. Studies have shown poor inter- and intraobserver reproducibility of microcirculation measurements, leading to a wide range of reported microcirculatory parameter values. Therefore, the literature recommends using a large sample size in a research setting.[31] More research on normal control values in a large cohort of healthy volunteers is needed to interpret the values of microcirculation parameters.

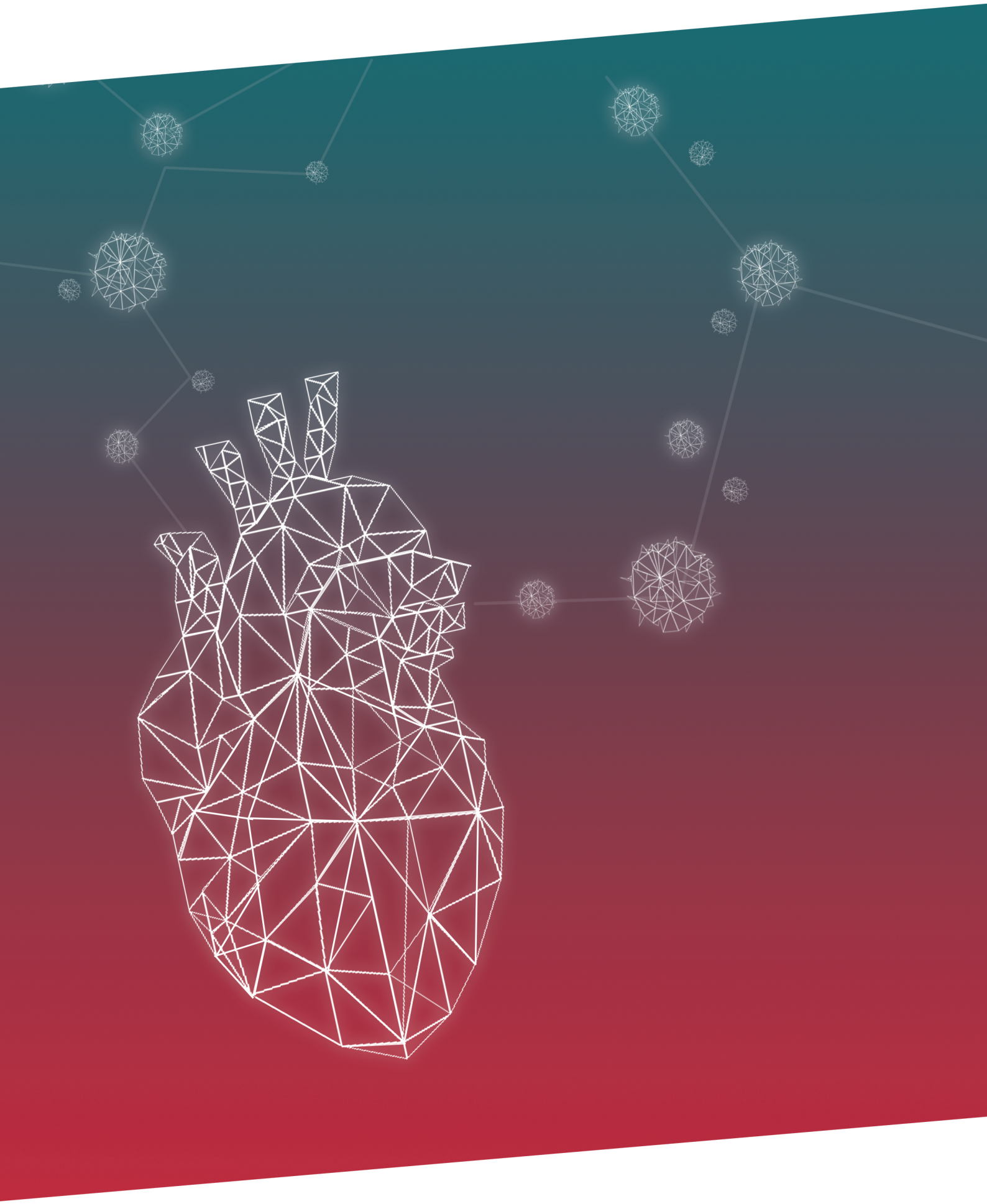
Another limitation, perhaps the most important, is that our study did not measure the patient preoperatively. Consequently, there is no baseline against which to compare the values of the patients. For the shock patients, the microcirculatory parameters seem to be higher than those of the healthy volunteers, but perhaps the microcirculation was already different in these patients before surgery compared to the healthy volunteers. The mean age of the healthy volunteers was 24 years, which is much lower than the mean age of our study population. This could possibly also explain the differences between the microcirculations. Also, the measurement period of the study was too short. No clear turning point could be seen in the microcirculation parameters. Also, the shock patients were measured only after the diagnosis of shock. Therefore, the turning point in the microcirculation between shock and non-shock was missed, and no conclusion can be drawn as to whether the microcirculation contributes to the development of shock. A study in which all cardiothoracic surgical patients are followed before surgery and immediately after surgery until hospital discharge should be performed to draw a conclusion as to whether a difference in microcirculation can be found between the two groups and if the microcirculation contributes to the development of shock.

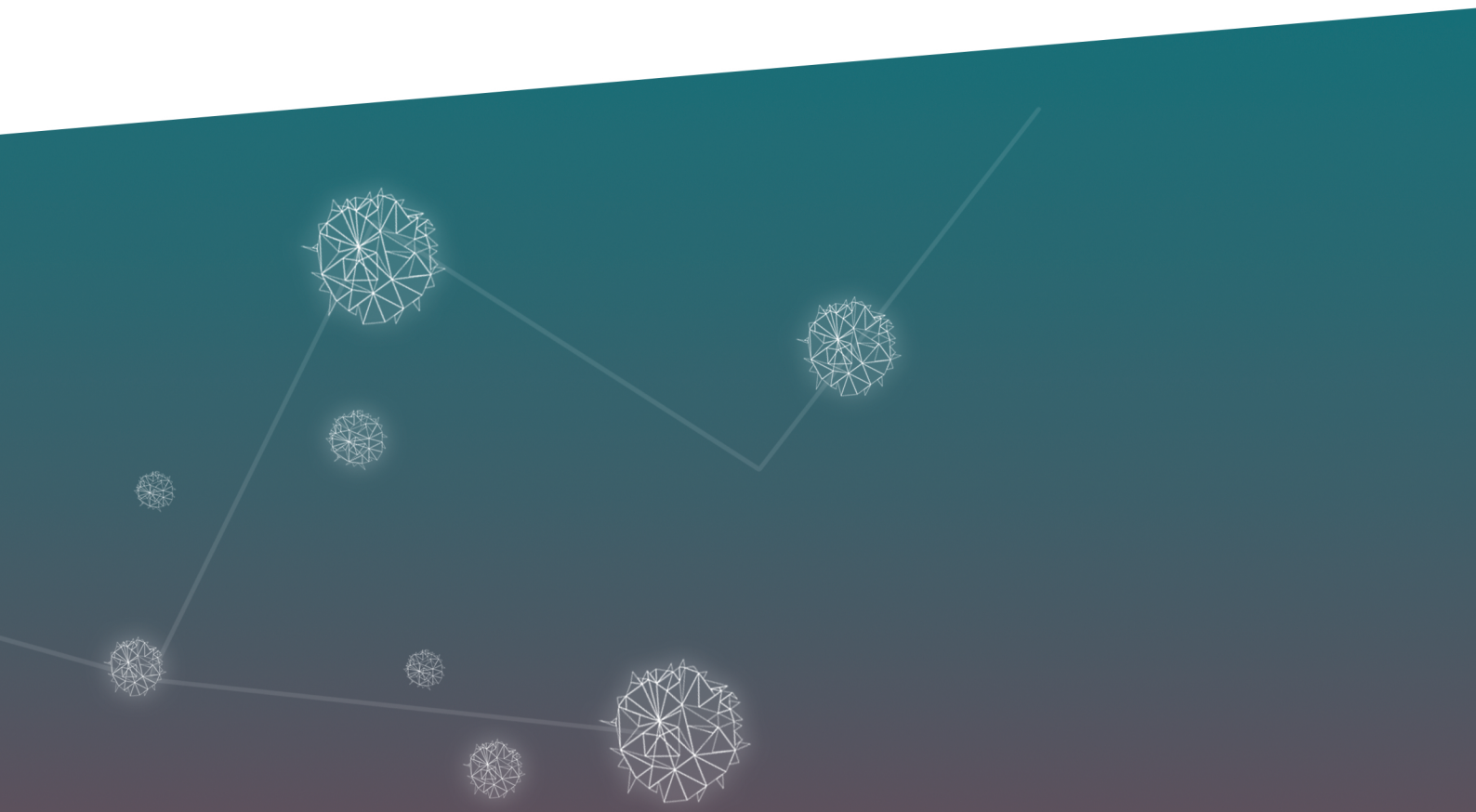
3.4.2| Conclusion

In conclusion, the results imply on a possible diffusive compensatory mechanism in case of shock, which results in an adaptation of the microcirculation to the situation, maintaining the tRBCp at normal values independent of macrocirculatory changes. Nevertheless, more research is needed on the microcirculation of cardiothoracic surgical patients with shock, the possibility of a compensation mechanism, and the contribution of the microcirculation to the development of shock.

3.5| References

1. Jung C. Assessment of microcirculation in cardiogenic shock. Current Opinion in Critical Care. 2019;25(4):410-6.
2. Leyh RG, Kofidis T, Strüber M, Fischer S, Knobloch K, Wachsmann B, et al. Methylene blue: the drug of choice for catecholamine-refractory vasoplegia after cardiopulmonary bypass. The Journal of thoracic and cardiovascular surgery. 2003;125(6):1426-31.
3. Weis F, Kilger E, Beiras-Fernandez A, Nassau K, Reuter D, Goetz A, et al. Association between vasopressor dependence and early outcome in patients after cardiac surgery. Anaesthesia. 2006;61(10):938-42.
4. Uz Z, Ince C, Guerci P, Ince Y, P Araujo R, Ergin B, et al. Recruitment of sublingual microcirculation using handheld incident dark field imaging as a routine measurement tool during the postoperative de-escalation phase—a pilot study in post ICU cardiac surgery patients. Perioperative Medicine. 2018;7(1):1-8.
5. Corstiaan A, Lagrand WK, Spronk PE, van Domburg RT, Hofland J, Lüthen C, et al. Impaired sublingual microvascular perfusion during surgery with cardiopulmonary bypass: a pilot study. The Journal of thoracic and cardiovascular surgery. 2008;136(1):129-34.
6. Tripodaki E-S, Tasoulis A, Vasileiadis I, Vastardis L, Skampas N, Sakellaridis T, et al. Microcirculatory alterations after cardiopulmonary bypass as assessed with near infrared spectroscopy: a pilot study. Canadian Journal of Anesthesia/Journal canadien d'anesthésie. 2012;59(6):620-1.
7. Ince C. Hemodynamic coherence and the rationale for monitoring the microcirculation. Critical care. 2015;19(3):1-13.
8. Haase-Fielitz A, Haase M, Bellomo R, Calzavacca P, Spura A, Baraki H, et al. Perioperative hemodynamic instability and fluid overload are associated with increasing acute kidney injury severity and worse outcome after cardiac surgery. Blood purification. 2017;43(4):298-308.
9. Ince C. The rationale for microcirculatory guided fluid therapy. Current opinion in critical care. 2014;20(3):301-8.
10. Vellinga NA, Ince C, Boerma EC. Microvascular dysfunction in the surgical patient. Current opinion in critical care. 2010;16(4):377-83.
11. den Uil C, Klijn E, Lagrand WK, Brugts JJ, Ince C, Spronk PE, et al. The microcirculation in health and critical disease. Progress in cardiovascular diseases. 2008;51(2):161-70.
12. Hauffe T, Krüger B, Bettex D, Rudiger A. Shock management for cardio-surgical ICU patients–the golden hours. Cardiac failure review. 2015;1(2):75.
13. Kumar A, Parrillo JE. Shock: classification, pathophysiology, and approach to management. Critical care medicine: principles of diagnosis and management in the adult 3rd ed Philadelphia: Mosby Elsevier. 2008:379-422.
14. Ince C, Boerma EC, Cecconi M, De Backer D, Shapiro NI, Duranteau J, et al. 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 medicine. 2018;44(3):281-99.
15. Hilty MP, Guerci P, Ince Y, Toraman F, Ince C. MicroTools enables automated quantification of capillary density and red blood cell velocity in handheld vital microscopy. Communications biology. 2019;2(1):1-15.
16. Armstrong RA. When to use the Bonferroni correction. Ophthalmic and Physiological Optics. 2014;34(5):502-8.
17. Flick M, Schreiber T-H, Montomoli J, Krause L, de Boer HD, Kouz K, et al. Microcirculatory tissue perfusion during general anaesthesia and noncardiac surgery: an observational study using incident dark field imaging with automated video analysis. European Journal of Anaesthesiology| EJA. 2022;39(7):582-90.
18. LeDoux D, Astiz ME, Carpati CM, Rackow EC. Effects of perfusion pressure on tissue perfusion in septic shock. Critical care medicine. 2000;28(8):2729-32.
19. Jansen TC, van Bommel J, Mulder PG, Lima AP, van der Hoven B, Rommes JH, et al. Prognostic value of blood lactate levels: does the clinical diagnosis at admission matter? Journal of Trauma and Acute Care Surgery. 2009;66(2):377-85.
20. Reinhart K, Kuhn H-J, Hartog C, Bredle DL. Continuous central venous and pulmonary artery oxygen saturation monitoring in the critically ill. Intensive care medicine. 2004;30(8):1572-8.
21. Nascente APM, Freitas FGR, Bakker J, Bafi AT, Ladeira RT, Azevedo LCP, et al. Microcirculation improvement after short-term infusion of vasopressin in septic shock is dependent on noradrenaline. Clinics. 2017;72:750-7.
22. Thooft A, Favory R, Salgado DR, Taccone FS, Donadello K, De Backer D, et al. Effects of changes in arterial pressure on organ perfusion during septic shock. Critical Care. 2011;15(5):1-8.
23. Georger J-F, Hamzaoui O, Chaari A, Maizel J, Richard C, Teboul J-L. Restoring arterial pressure with norepinephrine improves muscle tissue oxygenation assessed by near-infrared spectroscopy in severely hypotensive septic patients. Intensive care medicine. 2010;36(11):1882-9.
24. 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. Critical Care. 2009;13(3):1-8.
25. Duling B, Desjardins C. Capillary hematokrit-what does it mean? Physiology. 1987;2(2):66-9.
26. Duling B, Sarelius I, Jackson W. A comparison of microvascular estimates of capillary blood flow with direct measurements of total striated muscle flow. International journal of microcirculation, clinical and experimental. 1982;1(4):409-24.
27. Ibrahim JG, Molenberghs G. Missing data methods in longitudinal studies: a review. Test. 2009;18(1):1-43.
28. Fitzmaurice GM, Laird NM, Ware JH. Applied longitudinal analysis: John Wiley & Sons; 2012.
29. Holm S. A simple sequentially rejective multiple test procedure. Scandinavian journal of statistics. 1979:65-70.
30. Hilty MP, Favaron E, Wendel Garcia PD, Ahiska Y, Uz Z, Akin S, et al. Microcirculatory alterations in critically ill COVID-19 patients analyzed using artificial intelligence. Critical Care. 2022;26(1):1-11.
31. Valerio L, Peters RJ, Zwinderman AH, Pinto-Sietsma S-J. Reproducibility of sublingual microcirculation parameters obtained from sidestream darkfield imaging. PloS one. 2019;14(3):e0213175.





4.

MICCS - Study

The Complex Relation Between the Microcirculation and Macrocirculation in Patients After Cardiothoracic Surgery With and Without Circulatory Shock

4.1| Introduction

The previous chapter described the postoperative time course of the microcirculation and its relation to the macrocirculation in cardiothoracic surgical patients in circulatory shock. It showed that the diffusive microcirculatory parameters were increased in cardiothoracic surgery patients in circulatory shock compared to healthy controls and that the hemodynamic coherence between micro- and macrocirculation only existed between the TVD, FCD, and PPV and MAP, lactate, and SvO_2 of the macrocirculation.

Although it is clear that microcirculatory alterations contribute to the development of postoperative organ dysfunction, shock, and clinical outcome, little is still known about the effects of cardiothoracic surgery on the postoperative microcirculation in patients with circulatory shock and how the microcirculation of these patients compares to the microcirculation of postoperative cardiothoracic surgical patients without circulatory shock. To better understand the microcirculatory alterations of cardiothoracic surgical patients with shock and its role in the development of shock, it is also valuable to routinely measure microcirculation in postoperative cardiothoracic surgical patients without shock.

A comparison of the microcirculation of postoperative cardiothoracic surgery patients who develop shock and those who do not, has scarcely been done, if at all. Therefore, in this chapter of the master thesis, we aimed to investigate the postoperative **clinical time course of microcirculatory** parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock. We also aimed to investigate the relationship (i.e., coherence) between microcirculatory and macrocirculatory parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock.

4.2| Methods

To answer this research question and to conduct human research, permission was needed from the Medical Ethical Research Committees Leiden Hague Delft (MREC LDD). For this reason, a protocol was submitted to the MREC LDD (**Appendix E**). After approval (MREC -number NL81756.058.22), the study was conducted under the acronym: MICCS - study.

4.2.1| Patient population

All patients with a minimum age of 18 years, who were admitted to the ICU of the LUMC after cardiothoracic surgery, were the study population. Patients admitted to the ICU after their cardiothoracic surgery according to the ultra-fast track (UFT) protocol were also included in the study as far as logistically possible. Patients with maxillofacial trauma or known oral or pharyngeal cavity tumors were excluded.

4.2.2| Study design

So far, only a few microcirculation studies have been performed in patients after cardiothoracic surgery. An observational single-center study was performed to get insight in the microcirculation of patients after cardiothoracic surgery in the postoperative period.

The study started at the Intensive Care department of the LUMC. Because of the unpredictable postoperative outcome (shock or non-shock), informed consent was sought preoperatively from each cardiothoracic surgery patient who would be admitted to the ICU of the LUMC. After giving written informed consent to participate in the study, each patient was assigned a unique study number and included in the study if they met the inclusion criteria.

Each patient, whether or not in circulatory shock (as defined in **Chapter 3**), was monitored and treated according to the current clinical guidelines of the LUMC. Macrocirculation was measured continuously and according to current clinical practice. Microcirculation measurement with the CytoCam was performed as soon as possible after ICU admission (within 3 hours (T0)), after 24 hours (T1) after admission to the ICU, and if possible, after 48 hours (T2) after T0 (**Figure 17**). According to applicable guidelines[1], at least three image sequences of at least four seconds were recorded with the CytoCam at different locations in the sublingual space in all patients at each time point. The recorded image sequences were subsequently reviewed for quality according to the guidelines[2] (**Appendix A**). The remaining recordings were loaded into the MicroTools algorithm to calculate hemodynamic variables, such as TVD, FCD, PPV, cHct, RBCv, and tRBCp, based on the individual image sequences of every patient at each time point.[3] A self-written version of the already existing MATLAB script was used to calculate the average microcirculation parameters of the multiple image sequences at each time point (**Appendix F**).

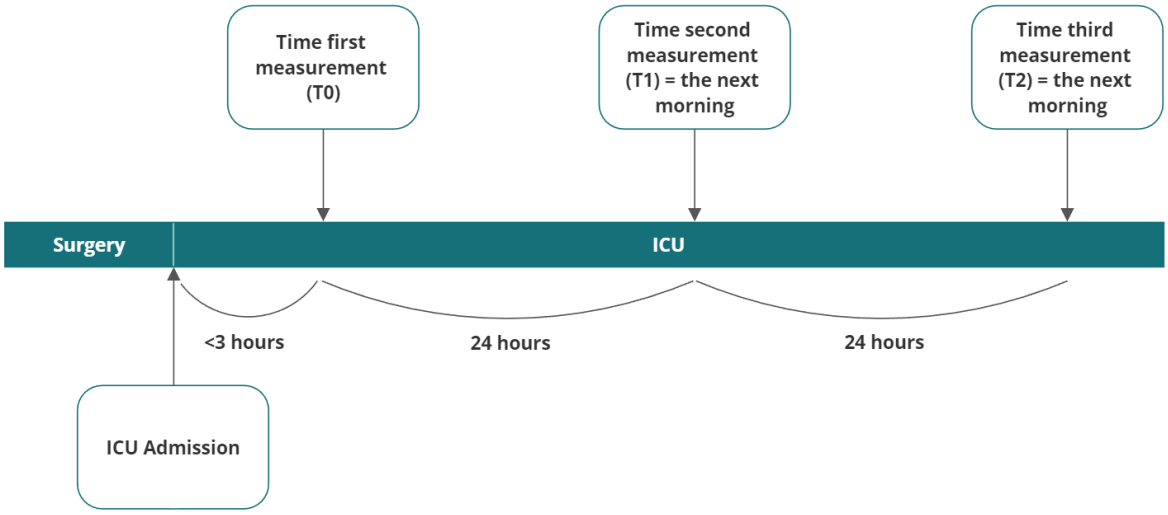


Figure 17

Timeline of microcirculatory measurements during the MICCS - study in cardiothoracic surgical patients with and without shock.

At each microcirculation measurement, data were collected from blood samples, and clinical data were noted as close in time as possible to the microcirculation measurement. Appendix 2 of the attached protocol (**Appendix E**) provides a complete overview of what data were collected. The blood samples from which the data were collected were taken according to standard clinical practice, so no additional blood sampling and/or interventions were performed for this study. Experience has shown that in ICU patients, blood is drawn so regularly that a blood sample was always taken near the times when the microcirculation measurements were done. All parameters

needed for this research were extracted from the hospital's electronic patient dossier system (EPD). Simultaneously with T0, demographic data were collected. The recordings from the microcirculation measurements can only be analyzed offline. The software (AVA or MicroTools) is not available on the computers in the LUMC, so analysis of the recordings was done in the Erasmus MC.

The study population of cardiothoracic surgery patients that were **not in shock** were measured according to the MICCS - study protocol. The study population of cardiothoracic surgery patients with **shock** consisted of two groups: (1) the cardiothoracic surgery patients with shock that were already measured according to the clinical shock protocol that prevails in the ICU of the LUMC and (2) the cardiothoracic surgery patients that gave informed consent to participate in the MICCS - study and happened to be in shock after surgery. If, from a clinical point of view and the shock protocol, they needed additional microcirculatory measurements; these were performed in the light of the clinical shock protocol.

4.2.3| Statistical analysis

To answer the research question of this study, patients were divided into two groups based on the occurrence or non-occurrence of shock. Using the cutoff values for norepinephrine (≥0.2 µg/kg/min) and lactate (>2 mmol/L), the division was made at T0. If a patient met either criterion, a decision was made based on the clinical appearance and the clinician's expertise to which group the patient is assigned. If a patient first belonged to one group and switched to the other group during the measurement period, the patient was still analyzed in the group to which he was first assigned to.

4.2.3.1|Primary study outcome

The primary outcome was the postoperative time course of microcirculatory parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock.

4.2.3.2|Secondary study outcome

The secondary outcome was the coherence between the macrocirculation and microcirculation in patients admitted to the ICU after cardiothoracic surgery with and without circulatory shock. Coherence means whether changes in microcirculatory parameters are congruent with (in the same direction as) expected changes in the microcirculation.

4.2.3.3|Analysis

Descriptive statistics were used to characterize the study population and the subgroups. Data were stated as mean with standard deviations or medians with interquartile ranges for continuous variables, depending on the parametric distribution of the variables, which was assessed with histograms and normal quantile plots. Categorical variables were stated as numbers and percentages. Each variable was tested for normality. A single parameter 1D- boxplot was made to visualize outliers.

After that, data was transformed from a wide format into a long format to describe the time course of the microcirculation and macrocirculation parameters over time with graphs. LMM estimated marginal means has been used to describe the mean values and mean change (ΔT1 - T0) of microcirculatory and macrocirculatory parameters in cardiothoracic surgery patients **with** and **without** circulatory shock. The significance level of the estimated marginal means was adjusted with a Bonferroni multiple testing correction on the p-values of all individual tests in order to reduce the chance of a type I error inflation.[4] The mean change in micro- and macrocirculatory parameters between the first two measurements (ΔT1 - T0) between the patients **with** and **without** circulatory shock is described with LMM. Microcirculatory parameters of healthy volunteers[5] were used as a reference for the microcirculatory parameters of this study population.

LMM for repeated measurement has also been used to analyze the time course of the microcirculation and microcirculatory parameters. The fixed effects of all models in this chapter included time, the occurrence of shock, and the interaction term between the two.

Hemodynamic coherence (as defined in **Chapter 3**) was assessed globally in a qualitative manner per mean group change, with appropriate directional changes towards normal values at T1 in microcirculatory parameters depending on the results found at T0. For congruence with the macrocirculation, microcirculatory changes must be accompanied by an increase in MAP or a decrease in lactate or SvO_2.

The statistical analysis has been conducted using the SPSS statistical package, release 28.0.1 (SPSS Inc., Chicago, IL).

4.3| Results

From 14 November 2022 until 16 December 2022, a total of 27 patients were included in the MICCS - study. The shock group consisted of 20 patients (17 included from the existing database; 3 included from the MICCS - study), and the non-shock group consisted of 29 patients (3 included from the existing shock database; 26 inclusions from the MICCS - study). A total of 402 sublingual HVM measurements were performed in the 29 newly included patients, of which 167 HVM image sequences were assigned a Massey score[2] (**Appendix A**) less than ten and were included for analysis with MicroTools.

Several types of surgeries were performed, with a mean surgery time of 500 ± 201 min in the shock group and 273 ± 128 min in the non-shock group. The mean bypass time in the shock group was 307 ± 160 min, and in the non-shock group, 142 ± 96 min. The mean aortic cross-clamp time was 165 ± 91 min for the shock group and 93 ± 49 min for the non-shock group. The overall study population consisted of 33 (67,3%) men with a mean age of 66 (IQR 26-78 years). The mean APACHE-IV score was 54 ± 21. The mean time to meet the definition of shock was 4 ± 4 hours in the shock group and 13 ± 15 hours in the non-shock group. The time between admission to the ICU and the first measurement was 20 ± 12 hours on average in the shock group and 4 ± 13 hours in the non-shock group. **Table 7** summarizes the baseline characteristics. Average macrocirculatory and microcirculatory parameters can be found in **Table 7**.

Table 7 *Baseline characteristics of the study population*

	All post-cardiothoracic surgery patients	Post Cardiothoracic surgery patients **with shock**	Post Cardiothoracic surgery patients **without shock**
General characteristics	**n = 49**	**n = 20**	**n = 29**
Age (years, median, range)	66 (26-78)	67 (26 - 76)	66 (30 - 78)
Gender (n, %)			
Male	33 (67,3%)	14 (70,0%)	19 (65,5%)
Female	16 (32,7%)	6 (30,0%)	10 (34,5%)
BMI (kg/m^2) (mean ± SD)	28 ± 5	28 ± 5	27 ± 6
APACHE IV score (mean ± SD)	54 ± 21	64 ± 24	46 ± 15
Type of surgery (n, %)			
Intracardiac	14 (28,6%)	5 (25,0%)	9 (31,0%)
Coronary	16 (32,7%)	4 (20,0%)	12 (41,4%)
Aortic	3 (6,1%)	2 (10,0%)	1 (3,4%)
Mechanical	1 (2,0%)	1 (5,0%)	-
Intracardiac and Coronary	9 (18,4%)	5 (25,0%)	4 (13,8%)
Intracardiac and Mechanical	1 (2,0%)	-	1 (3,4%)
Intracardiac and Aortic	4 (8,2%)	3 (15,0%)	1 (3,4%)
Other	1 (2,0%)	-	1 (3,4%)
Surgery characteristics			
Surgery duration (min) (mean ± SD)	370 ± 197	500 ± 201	273 ± 128
Anesthesia duration (min) (mean ± SD)	437 ± 200	569 ± 206	347 ± 138
Bypass duration [a] (min) (mean ± SD)	210 ± 149	307 ± 160	142 ± 96
Aortic cross-clamp duration [a] (min) (mean ± SD)	122 ± 77	165 ± 91	93 ± 49
Microcirculatory measurements characteristics			
Time to meet the criteria of shock (hours) (mean ± SD)	5 ± 7	4 ± 4	13 ± 15
Time to first measurement [b] (T0) (hours) (mean ± SD)	11 ± 15	20 ± 12	4 ± 13
Time to second	35 ± 26	51 ± 29	21 ± 12

measurement [b] (T1) (hours) (mean ± SD)			
Time to third measurement [b] (T2) (hours) (mean ± SD)	66 ± 30	69 ± 32	57 ± 26
Macrocirculation characteristics			
Noradrenalin at T0 (µg/kg/min) (mean ± SD)	0,25 ± 0,31	0,54 ± 0,26	0,03 ± 0,05
Systemic hematocrit at T0 (L/L) (mean ± SD)	0,32 ± 0,04	0,30 ± 0,04	0,33 ± 0,04

APACHE IV score: Acute Physiology and Chronic Health Evaluation Version IV Score; BMI: body mass index; SD: standard deviation; a: If a patient had multiple bypass or aortic clamp times, the values were first summed before the group average was calculated; b: difference between the time of admission to the ICU and measurement of the microcirculation.

4.3.1 | Microcirculatory time course

Table 8 shows the mean values and changes over time of both macro- and microcirculation parameters in the shock and non-shock groups.

The mean change over time of lactate of the shock group and the non-shock group was significant (-1.491, p-value = 0,000, and 0.717, p-value = 0.044, respectively). The mean difference between T1 and T0 of the SvO_2 in the shock group was significant (-11.351, p-value = 0.027). A significant reduction was found in the TVD (-2.476, p-value = 0.009), FCD (-2.351, p-value = 0.013), cHct (0.928, p-value = 0,005), and tRBCp (-6.149, p-value = 0.027) in the non-shock group. A significant difference was found in the changes over time of lactate (0.717 vs. -1.491, p-value = 0,000) and cHct (-0.928 vs. 0.094, p-value = 0,031) between the shock group and the non-shock group. The number of measured values of every parameter on every time point can be found in **Appendix G**

Figure 18 a to f shows the timecourses of the mean of different microcirculation parameters. No significant differences over time were found in these parameters. The RBCv- value was significantly higher in the non-shock group than in the shock group (398.915 vs. 323.520, respectively, p-value = 0,025). The time course between T0 and T2 of RBCv was significantly different for the non-shock group and the shock group (p-value = 0,043). The rest of the parameters showed no significant differences between the two groups and in time course between the two groups. **Appendix H** shows the results of the LMM. The number of measured values of every parameter on every time point can be found in **Appendix G**

		Shock				No shock				
	Normal value	**Mean T0 [95% CI]**	**Mean T1 [95% CI]**	**Mean ΔT1 - T0 [95% CI]**	**P-value ***	**Mean T0 [95% CI]**	**Mean T1 [95% CI]**	**Mean ΔT1 - T0 [95% CI]**	**P-value ***	**P-value ΔT1 - T0 shock vs. no shock#**
Macrocirculatory parameters										
MAP (mmHg)	65 (60-90)[6]	66.850 [62.118, 71.582]	72.067 [67.150, 76.985]	5.217 [-1.164, 11.599]	0,106	79.562 [75.416, 83.708]	79.410 [74.903, 83.917]	-0.152 [-5.925, 5.622]	0,958	0,215
Lactate (mmol/L)	<2[7]	3.750 [3.034, 4.466]	2.259 [1.550, 2.968]	-1.491 [-2.236, -0.746]	**0,000**	1.340 [0.720, 1.959]	2.056 [1.417, 2.696]	0.717 [0.19, 1.415]	**0,044**	**0,000**
SvO_2 (%)	70 (68-77%)[8]	73,694 [69.624, 77,764]	62.343 [51.973, 72.713]	-11.351 [-21.046, -1.656]	**0,027**	71.618 [67.929, 75.306]	72.562 [54.715, 90.410]	0.945 [-16.805, 18.694]	0,908	0,203
Microcirculatory parameters										
TVD (mm/mm^2)	18.2 [17.2, 20.1][5]	21.129 [19.600, 22.658]	21.017 [19.166, 22.868]	-0.111 [-2.104, 1.882]	0,910	18.208 [16.940, 19.477]	15.732 [14.054, 17.411]	-2.476 [-4.287, -0.665]	**0,009**	0,083
FCD (mm/mm^2)	17.3 [16.8, 19.2][5]	19.861 [18.389, 21.333]	19.785 [17.768, 21.803]	-0.075 [-2.043, 1.893]	0,938	17.387 [16.168, 18.605]	15.035 [13.220, 16.851]	-2.351 [-4.164, -0.539]	**0,013**	0,093
PPV (%)	97.6 [94.3, 98.4][5]	94.109 [91.716, 96.501]	94.262 [89.366, 99.158]	0.153 [-3.221, 3.528]	0,925	95.346 [93.395, 97.297]	95.136 [90.993, 99.279]	-0.210 [-3.260, 2.840]	0,887	0,869

RBCv (µm/s)	340 [302, 357][5]	334.469 [308.427, 360.511]	329.220 [305.209, 353.231]	-5.249 [-33.364, 22.866]	0,707	340.530 [318.955, 362.105]	351.086 [329.405, 372.767]	10.556 [-14.731, 35.844]	0,404	0,403
cHct (%)	5.0 [4.7, 5.3][5]	5.720 [5.201, 6.238]	5.813 [5.130, 6.497]	0.094 [-0.586, 0.773]	0,781	7.152 [6.723, 7.581]	6.224 [5.608, 6.841]	-0.928 [-1.553, -0.303]	**0,005**	**0,031**
tRBCp (µm/min)	45.3 [39.5, 52.0][5]	46.322 [41.218, 51.426]	46.402 [40.776, 52.028]	0,080 [-5.858, 6.018]	0,978	42.411 [38.185, 46.638]	36.262 [31.193, 41.331]	-6.149 [-11.562, -0.736]	**0,027**	0,125

**: Result of the LMM estimated marginal means with Bonferroni multiple testing correction of ΔT1-T0; Δ: change; #: p-value results from the interaction term of the LMM between measurement and the occurrence of shock; cHct: capillary hematocrit; FCD: functional capillary density; MAP: mean arterial pressure; PPV: proportion of perfused vessels; RBCv: red blood cell velocity; SvO_2: central venous oxygen saturation; tRBCp: tissue red blood cell perfusion; TVD: total vessel density.*

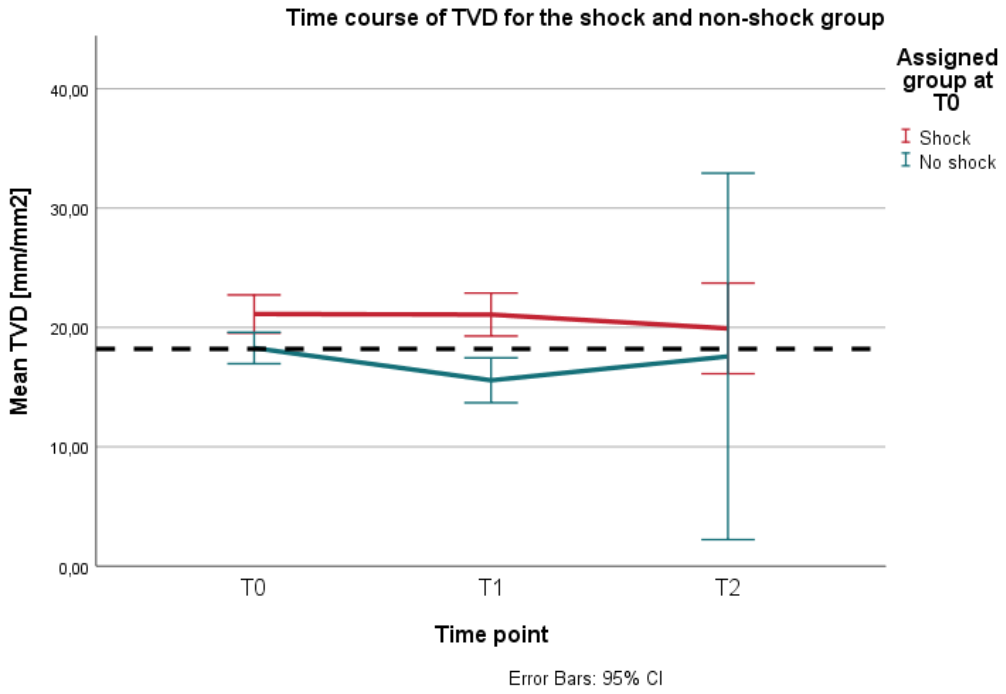


Figure 18a

Time course of the mean total vessel density (TVD) for patients with and without shock.

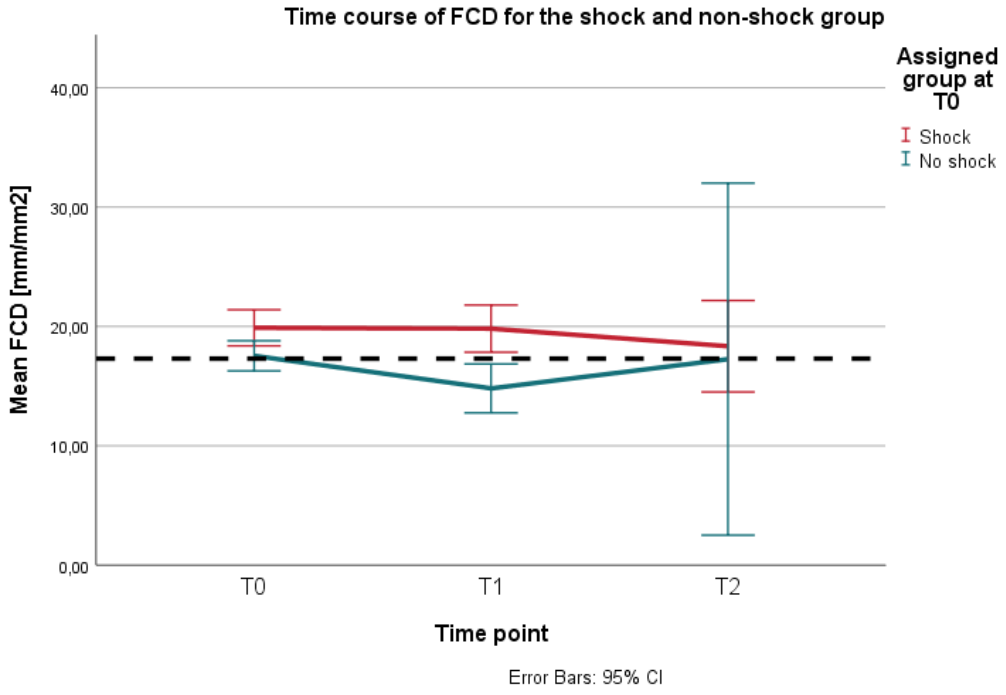


Figure 18b

Time course of the mean functional capillary density (FCD) for patients with and without shock.

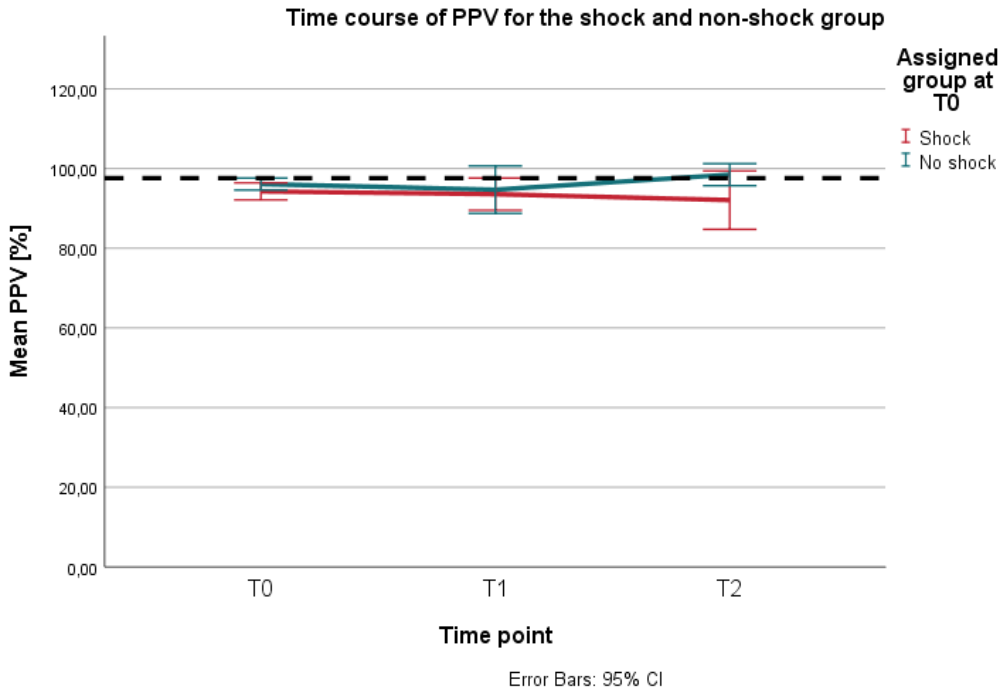


Figure 18c

Time course of the mean proportion of perfused vessels (PPV) for patients with and without shock.

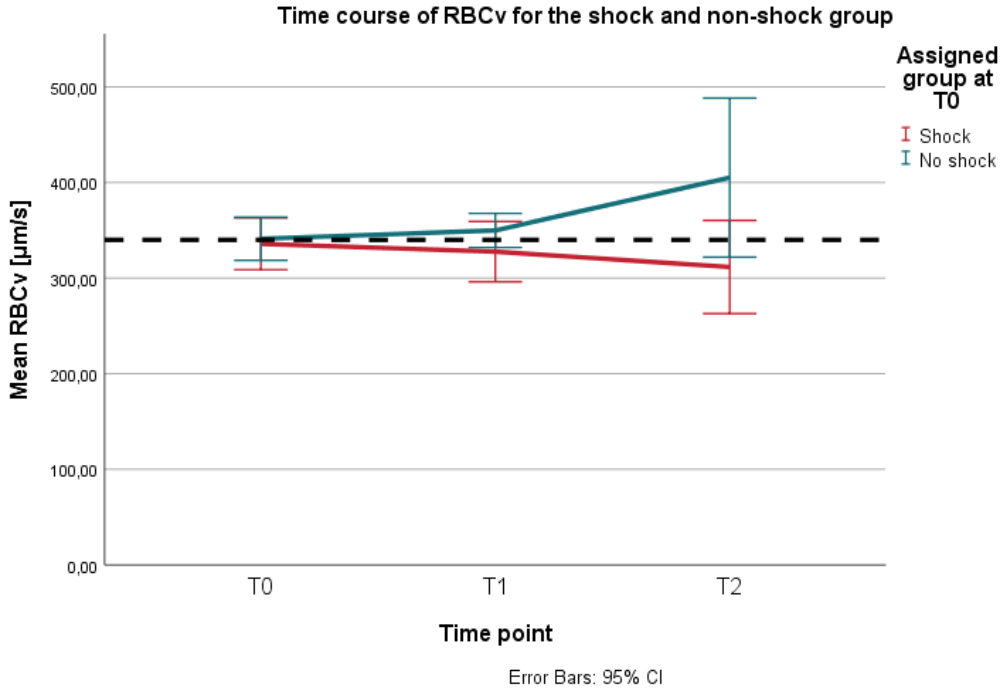


Figure 18d

Time course of the mean red blood cell velocity (RBCv) for patients with and without shock.

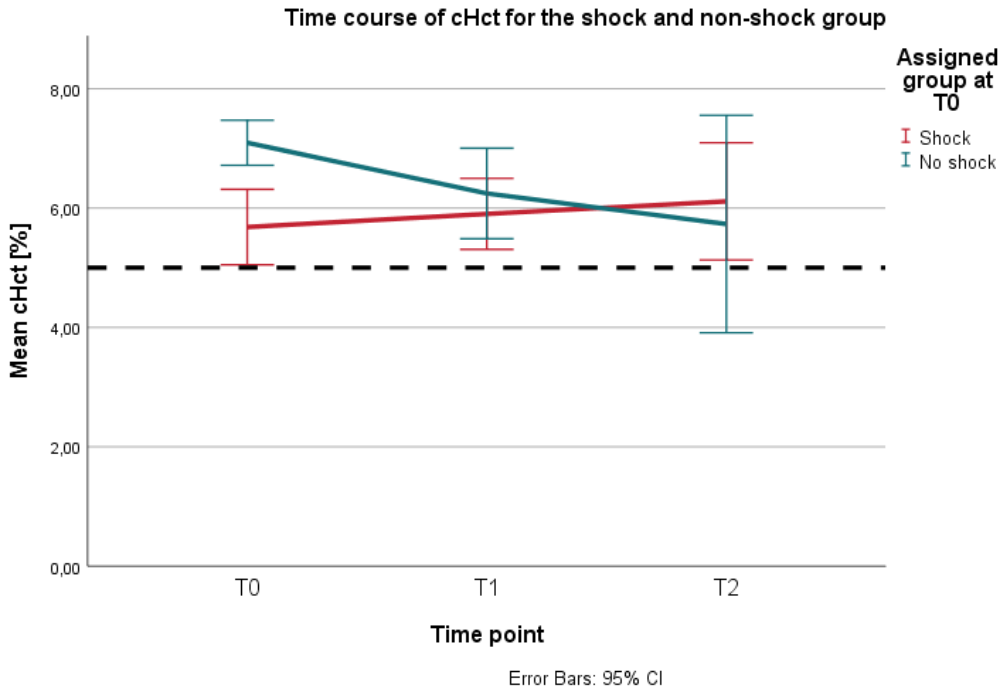


Figure 18e

Time course of the mean capillary hematocrit (cHct) for patients with and without shock.

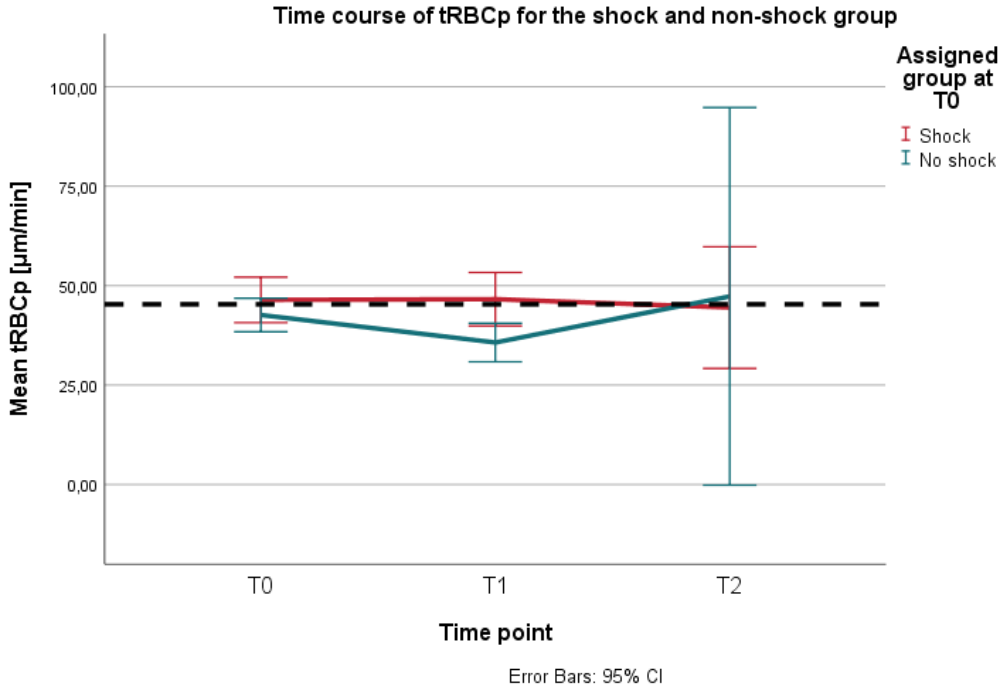


Figure 18f

Time course of the mean tissue red blood cell perfusion (tRBCp) for patients with and without shock.

4.3.2| Relationship between macro- and microcirculation

Table 9 describes the relationship between micro- and macrocirculation. The mean duration between the measurements of T0 and T1 was 20 ± 8 hours for the non-shock group and 30 ± 21 hours for the shock group. The mean duration between T1 and T2 was 16 ± 11 hours for the non-shock group and 27 ± 15 hours for the shock group. Using the qualitative assessment of macro- and microcirculatory parameters, a hemodynamic coherence between the TVD, FCD, PPV, and tRBCp and all macrocirculatory parameters was identified in the non-shock group. In the shock group, hemodynamic coherence was found between all macrocirculatory parameters and the TVD, FCD, and PPV.

Table 9 *Relationship between the macro- and microcirculation*

	Shock			**No shock**		
	ΔMAP (mmHg)	**Δ Lactate (mmol/L)**	**Δ SvO_2 (%)**	**ΔMAP (mmHg)**	**Δ Lactate (mmol/L)**	**Δ SvO_2 (%)**
Δ TVD (mm/mm^2)	Coherence	Coherence	Coherence	Coherence	Coherence	Coherence
Δ FCD (mm/mm^2)	Coherence	Coherence	Coherence	Coherence	Coherence	Coherence
Δ PPV (%)	Coherence	Coherence	Coherence	Coherence	Coherence	Coherence
Δ RBCv (μm/s)	No coherence	No coherence	No coherence	No coherence	No coherence	No Coherence
Δ cHct (%)	No coherence	No coherence	No coherence	No coherence	No coherence	No coherence
Δ tRBCp (μm/min)	No coherence	No coherence	No coherence	Coherence	Coherence	Coherence

Δ: change (no increase or decrease); cHct: capillary hematocrit; FCD: functional capillary density; MAP: mean arterial pressure; PPV: proportion of perfused vessels; RBCv: red blood cell velocity; SvO_2: central venous oxygen saturation; tRBCp: tissue red blood cell perfusion; TVD: total vessel density.

4.4| Discussion

This study aimed to describe the time course of the microcirculation in postoperative cardiothoracic surgical patients with shock and without shock. The second goal was to describe the relationship, i.e., the hemodynamic coherence, between this microcirculation and the macrocirculation. In addition, the aim was to identify possible dissimilarities in microcirculation and hemodynamic coherence between these groups.

First, it must be realized that these are cardiothoracic surgical patients and, therefore, not a completely healthy population. Both groups likely had already other baseline values than the

normal values used in this study, which were measured in the healthy volunteers. The normal values used are only directional and not absolute.

The shock group has already been discussed in detail in Chapter 3 of this thesis. In this chapter, the possible conclusion from the measurements of the microcirculation was that a diffusive compensatory mechanism may have occurred, maintaining tissue perfusion (represented by the tRBCp) as much as possible.

Second, it is noticeable that the mean surgical characteristics and time to measurements are longer for the shock group compared to the non-shock group. One explanation may be that if the surgery takes longer, meaning anesthesia lasts longer, there may be more hemodilution, and there is more time to trigger an inflammatory response due to tissue damage and blood flow along foreign surfaces, and a patient is more likely to develop shock. Besides, the time to measurements was longer for the shock group than for the non-shock group. The difference in measurement time between these two groups was mainly due to logistical reasons. The shock group was first measured after the onset of shock, while the non-shock group was measured within three hours of admission to the ICU. Since it is unpredictable when and if someone develops shock, there was not always someone available to perform the measurements, which caused a large spread in the measurement times of the shock group.

During the measurement period between T0 and T1, TVD and FCD decreased in both the shock and non-shock groups, after which these parameters increased again in the non-shock group and decreased further in the shock group. PPV, cHct, and tRBCp increased in the shock group, whereas they decreased in the non-shock group. The RBCv decreased in the shock group, where it increased in the non-shock group. The lower RBCv in the shock group was possibly an adaptation of the microcirculation, resulting in a longer capillary transit time, with possibly a better O_2 extraction rate. It is unclear why the RBCv continued to decrease over time while the macrocirculation seemed to improve.

On the contrary, the RBCv of the non-shock group increased over time, while the macrocirculation seemed to deteriorate. It is unclear why the non-shock group did not exhibit the possible compensatory mechanism; one explanation could be that they did not need this compensatory mechanism, but if it is true that they do not need the mechanism, it is not yet clear why they do not need it. Given the amount of data and records, it is not possible to explain the discrepancy and directional changes in the RBCv of the shock group and the non-shock group.

A significant difference was found in the mean cHct over time between the two groups. At T0, cHct in the shock group was lower than in the non-shock group and increased over time, but it remained lower than in the non-shock group. In the non-shock group, cHct decreased over time, bringing cHct closer together in the shock and non-shock groups. An increase or decrease in cHct can be explained by filling status. For example, it could be that the shock group was more resuscitated with fluids than the non-shock group, and the increase in cHct could be explained by the onset of the removal of extra fluids. However, several possible explanations for these results were found, all of which were not included in this study.

The tRBCp remained more or less stable in the shock group and decreased significantly in the non-shock group. This is counterintuitive because it is the all-encompassing parameter of diffusion and

convection, and on the contrary, one would expect it to decrease in the shock group. The mechanism and the meaning of that mechanism are, therefore, unclear. The non-shock group showed an increase after a significant decrease in the tRBCp, while the shock group decreased after a stable phase. This may be explained by the possibility that the non-shock group has a different compensatory mechanism that may prevent the onset of shock.

Hemodynamic coherence was found in both groups for the TVD, FCD, and PPV between T0 and T1. For the non-shock group, the tRBCp was also coherent with the macrocirculation between T0 and T1.

4.4.1 | Limitations

First, the duration between measurements is not the same for every patient and measurement point. This is mainly due to the patient groups. The shock patients were drawn from a pre-existing database, in which an initial measurement occurred as soon as possible after the diagnosis of shock. The onset of shock has a different time frame for each patient, which, combined with logistical challenges, resulted in a longer time between admission to the ICU and the first measurement. The group without shock originated mainly from the MICCS - study, in which each patient was measured within 3 hours of arrival in the ICU. However, since the microcirculation changes from minute to minute, the time difference between the T0 measurements of the two groups is negligible because it is more about what the microcirculation looked like at that time (microcirculation of a patient in shock or a patient not in shock). T1 and T2 measurements were taken around 24 and 48 hours for both groups.

Besides, patients were now included in the analyses in the group to which they were assigned at T0. However, during the measurement period, patients recovered from shock or developed shock in a later stadium. This was not included in the current analyses because of time. A sensitivity analysis would have been appropriate to show whether different outcomes would have been observed if patients who had recovered from shock or developed shock at T1 or T2 had switched to the other analysis group.

Furthermore, one patient initially included in the MICCS - study was excluded after a quality assessment of the image sequences based on stability, content, and focus. In addition, one included patient was scheduled for a Video-assisted thoracoscopic surgery (VATS) procedure in which neither sternotomy nor bypass was used.

Another limitation is the poor inter- and intraobserver reproducibility of microcirculatory measurements.[9] Studies have shown poor inter- and intraobserver reproducibility of microcirculation measurements. This is partly due to the fact that the guidelines do not describe anything about an exact anatomic measurement location. In practice, a good location is sought primarily based on what can be seen in the image. However, the image must consist of a mix of the different vessels (arterioles, capillaries, venules, and at least one larger venule to check for pressure artifacts). Therefore, it is necessary to describe an anatomic measurement location in the guidelines, as described by Z. Uz et al.[10], to obtain image consistency and clinical reproducibility.

Also notable is the number of image sequences recorded compared to the number of image sequences included after quality control. Many image sequences were excluded due to pressure artifacts or movement of the probe. In addition to pressure artifacts and movement of the probe, a blurred image due to saliva was another reason for exclusion. It is important to clean the sublingual area before measurement. However, it can be challenging to properly clean the mouth of intubated patients, leading to poor quality measurements. The study population consisted of intubated and non-intubated patients. Performing microcirculation measurements in non-intubated patients is more difficult because the patient holds up the tongue, breathes, tightens and relaxes the muscles, and swallows, displacing the probe. As a result, the number of analyzed image sequences, per patient, per time point was not equal.

To improve the quality of measurements, we know from expert opinions and training courses that observers need extensive training in performing microcirculation measurements to obtain good quality measurements and to perform adequate image analysis.[1, 10] This extensive training is needed to properly handle the HVM and develop the ability to reduce pressure to avoid pressure artifacts without losing the image.[10] However, no guideline determines how trained a person is in these measurements. Therefore, the guideline states that the training or experience of the observer should be mentioned when reporting the results.

Experienced observers performed microcirculation measurements of the shock group. An inexperienced/moderate observer measured the non-shock group. Given the poor inter- and intra-observer reproducibility and the associated wide dispersion in values of the microcirculation parameters, there is a possibility that some form of bias may be introduced when comparing these groups.

The same reference values as in Chapter 3 of the healthy controls from the study by Flick et al. were used as a reference for the microcirculatory parameters in this chapter. However, it should be noted that this study was about microcirculatory perfusion and the effect of general anesthesia and non-cardiac surgery. This study included 38 healthy volunteers in their study, to observe any changes in the absence of anesthesia and surgery. Minor discrepancies were found between this study and another study by Hilty et al. These discrepancies correspond to the ranges in microcirculatory parameters described in the literature due to poor inter- and intraobserver reproducibility. Therefore, a large sample size is recommended in a research setting. For the correct interpretation and clinical applicability of microcirculatory parameters, more research on reference values in healthy volunteers will be needed before microcirculatory measurements can make their appearance at the bedside.

For the statistical analysis of the difference between the group with shock and the group without shock and the time course of the microcirculation of these groups, an LMM was used. In this LMM, an unstructured covariance matrix was used, where the within-patient correlation was captured via an unstructured variance-covariance matrix.

In a longitudinal study, there is a chance of missing data based on the missing at random mechanism. The statistical analysis methods used in this chapter can deal with unbalanced data and give valid results under the missing at random mechanism. As a result, the analysis may lose some efficiency, but no bias is introduced.[11, 12]

The sample size of both groups consisted of only a small group (n = 29 for the non-shock group and n = 20 for the shock group). This made it challenging to define outliers as being outliers, or a possible trend for one of the two groups. A small sample size ensures that a missing value is of more significant influence than if the sample size is larger. Unbalanced data in a small sample size potentially suggest certain trends differently than they are. For statistical analyses, a larger sample size is needed to add more covariates, such as surgery, anesthesia, bypass, duration of the aortic clamp, or inotropic positions to the model, to test whether this may affect microcirculation.

Using the Bonferroni correction also has disadvantages. First, the interpretation of an individual test depends on the number of other tests performed. Therefore, it can be argued that the evidence provided by the data is contained in that particular data set, and therefore the conclusion drawn should not be changed based on the number of other tests performed. In addition, the probability of a type 1 error cannot be reduced without increasing the probability of a type 2 error, such that the real difference may not be detected. In addition, if the number of analyses increases when using the Bonferroni correction, the value of the adjusted p-value that must be exceeded to reach statistical significance decreases markedly, lowering the power of the analysis.[13]

Another limitation, perhaps the most important, is that our study did not measure the patient preoperatively. Consequently, there was no baseline against which to compare the values of the patients. For the shock group, the microcirculation parameters seem to be higher than those of the healthy volunteers and the non-shock group, but perhaps the microcirculation was different in these patients before surgery than in the non-shock group or the healthy volunteers. The mean age of the healthy volunteers was 24 years, which is much lower than the mean age of our study population. This could possibly also explain the differences between the two microcirculations. Also, the measurement period of the study was too short. No clear turning point could be seen in the microcirculation parameters. A study in which all cardiothoracic surgical patients are followed before and immediately after surgery until hospital discharge should be designed to draw a valid conclusion on whether a difference in microcirculation can be found between the two groups.

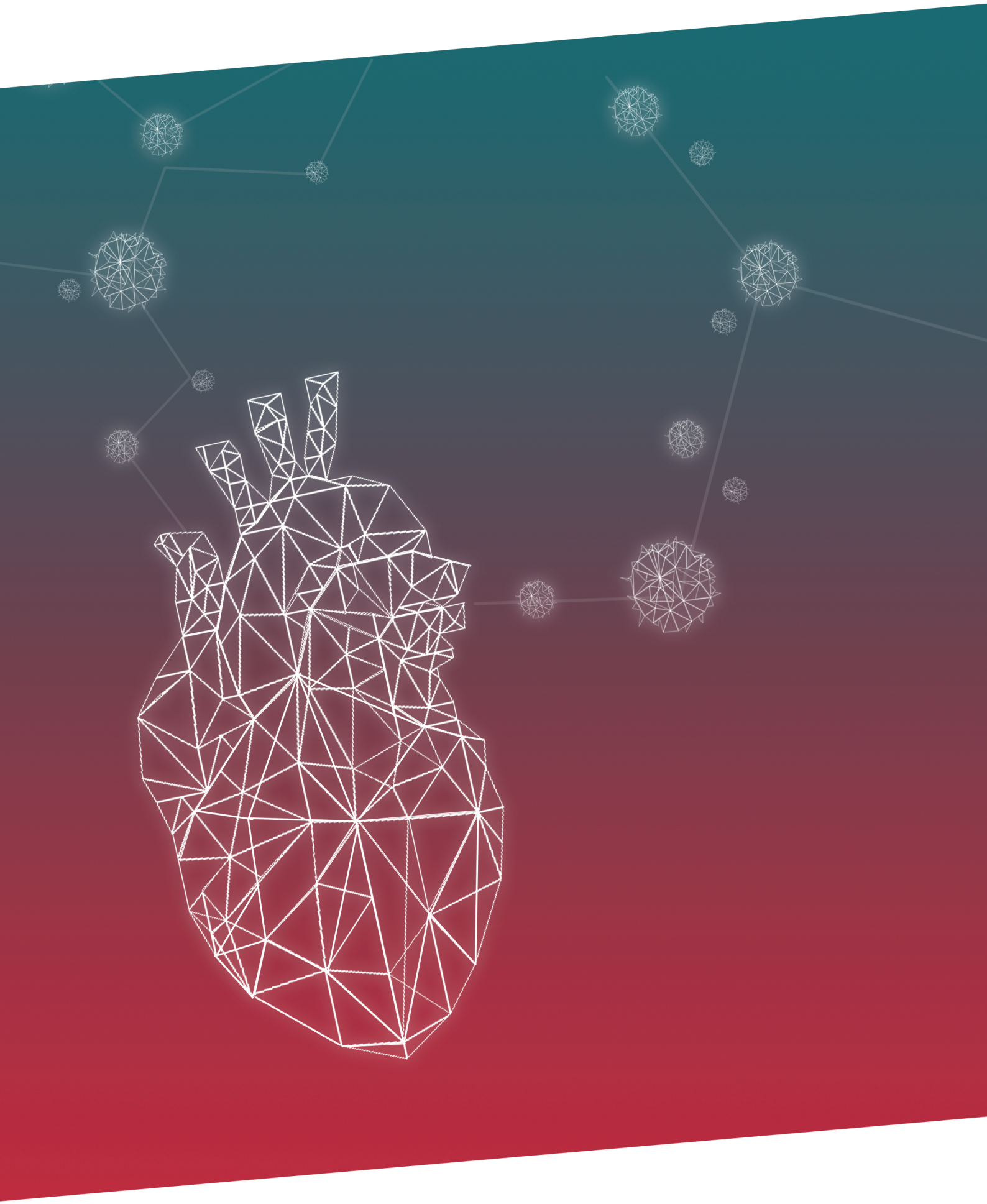
4.4.2| Conclusion

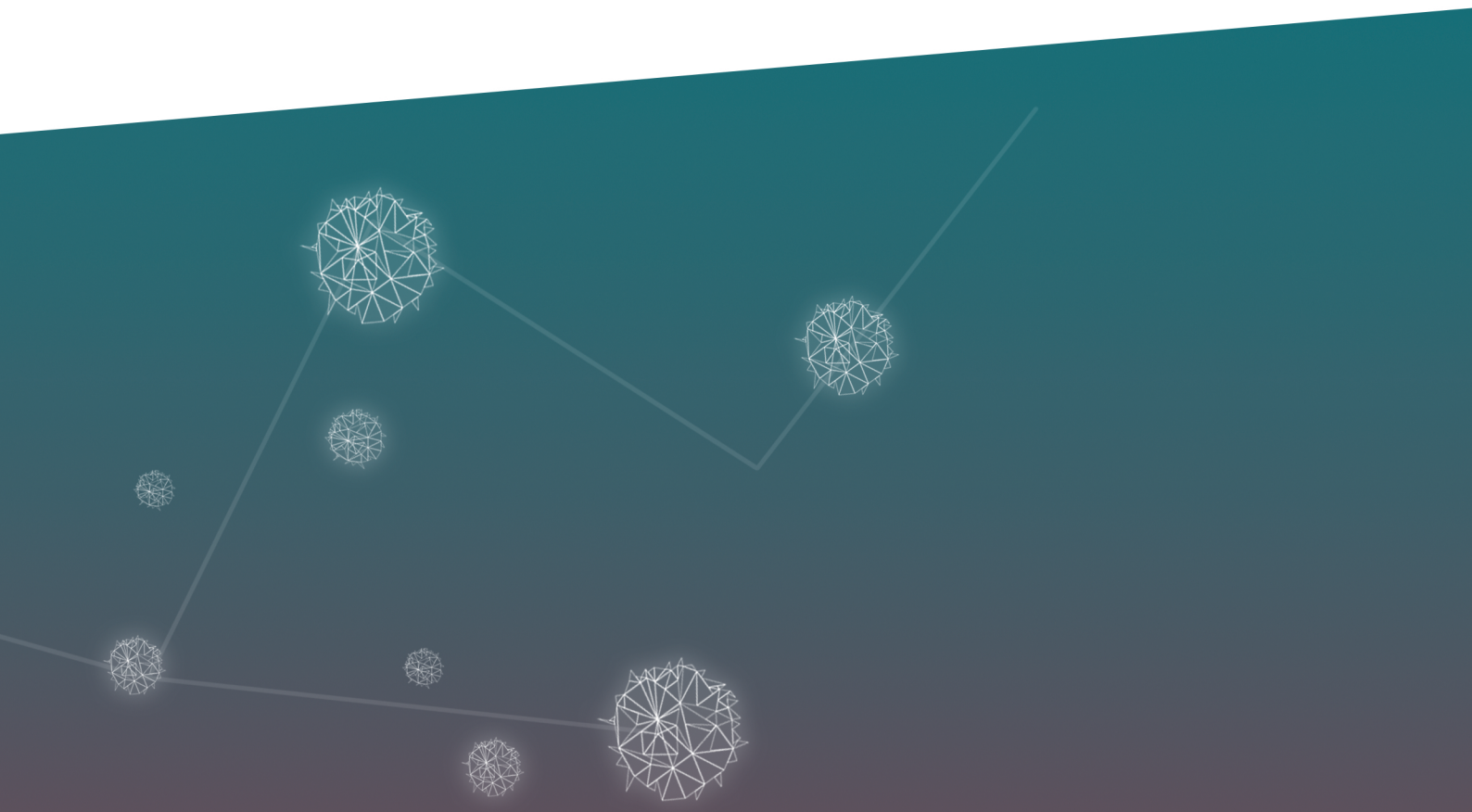
In conclusion, there was a difference in the behavior of the microcirculation of cardiothoracic surgical patients with and without shock, in which patients with shock showed an increase in diffusive parameters (i.e., TVD and FCD) compared to the normal values of healthy volunteers, suggesting that there might be an adaptive mechanism of the microcirculation. This increase in diffusive parameters persisted until the second measurement, most likely because these patients still showed signs of circulatory shock. There is no unambiguous explanation for the difference found or for the underlying mechanisms, for which further research is needed.

4.5| References

1. Ince C, Boerma EC, Cecconi M, De Backer D, Shapiro NI, Duranteau J, et al. 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 medicine. 2018;44(3):281-99.
2. 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. Journal of critical care. 2013;28(6):913-7.
3. Hilty MP, Guerci P, Ince Y, Toraman F, Ince C. MicroTools enables automated quantification of capillary density and red blood cell velocity in handheld vital microscopy. Communications biology. 2019;2(1):1-15.
4. Dunn OJ. Multiple comparisons among means. Journal of the American statistical association. 1961;56(293):52-64.
5. Flick M, Schreiber T-H, Montomoli J, Krause L, de Boer HD, Kouz K, et al. Microcirculatory tissue perfusion during general anaesthesia and noncardiac surgery: an observational study using incident dark field imaging with automated video analysis. European Journal of Anaesthesiology| EJA. 2022;39(7):582-90.
6. LeDoux D, Astiz ME, Carpati CM, Rackow EC. Effects of perfusion pressure on tissue perfusion in septic shock. Critical care medicine. 2000;28(8):2729-32.
7. Jansen TC, van Bommel J, Mulder PG, Lima AP, van der Hoven B, Rommes JH, et al. Prognostic value of blood lactate levels: does the clinical diagnosis at admission matter? Journal of Trauma and Acute Care Surgery. 2009;66(2):377-85.
8. Reinhart K, Kuhn H-J, Hartog C, Bredle DL. Continuous central venous and pulmonary artery oxygen saturation monitoring in the critically ill. Intensive care medicine. 2004;30(8):1572-8.
9. Valerio L, Peters RJ, Zwinderman AH, Pinto-Sietsma S-J. Reproducibility of sublingual microcirculation parameters obtained from sidestream darkfield imaging. PloS one. 2019;14(3):e0213175.
10. Uz Z, Dilken O, Milstein DM, Hilty MP, de Haan D, Ince Y, et al. Identifying a sublingual triangle as the ideal site for assessment of sublingual microcirculation. Journal of Clinical Monitoring and Computing. 2022:1-11.
11. Ibrahim JG, Molenberghs G. Missing data methods in longitudinal studies: a review. Test. 2009;18(1):1-43.
12. Fitzmaurice GM, Laird NM, Ware JH. Applied longitudinal analysis: John Wiley & Sons; 2012.
13. Perneger TV. What's wrong with Bonferroni adjustments. Bmj. 1998;316(7139):1236-8.

This page was intentionally left blank.





5.

Leukocyte Analysis in the Microcirculation - Towards a Consensus

5.1 | Introduction

The microcirculation, defined as blood flow through small blood vessels < 20µm, is the site where oxygen and nutrient exchange with cells occurs in the cardiovascular system.[1] For several years, it has been possible to visualize the microcirculation at the bedside of the patient with an HVM using OPS, SDF, or IDF imaging.[2] These techniques use scattered green light that is only absorbed by the hemoglobin in red blood cells, enabling the visualization of superficial vessels.[3]

Changes in the microcirculation contribute to the pathophysiology of various disorders.[4] During systemic inflammation in various disease states, arterioles are subjected to oxidative stress, leading to disturbed vasomotor function and increased risk of thrombosis. Capillaries may show decreased RBC flow (decreased perfusion) due to capillary obstruction by leukocytes. The venules, mostly postcapillary venules (pcv), are subjected to oxidative stress but may also show an increased number of rolling leukocytes, leukocyte adhesion, and leukocyte transmigration.[5, 6]

By nature, a leukocyte is a spherical cell of 12 to 15 µm in size.[7] However, this diameter is larger than the average diameter of capillaries, requiring some form of deformation to pass through the capillary network. Under normal flow conditions, the deformation time for a leukocyte is 1000 times greater than for an RBC due to its size and high stiffness, leading to temporary obstruction of the capillaries.[8, 9] After the leukocyte enters the capillary, its movement is slower than that of an RBC, causing an accumulation of RBCs behind the leukocyte (i.e., train formation).[10] A non-rolling leukocyte is a cell that moves at a very high speed into the mainstream of blood in the pcv without interacting with the vascular endothelium. Rolling leukocytes are described by the adhesive interaction between the vascular endothelium of the pcv and the leukocyte in which the train formation overtakes the leukocyte, inducing a displacement from the center stream towards the endothelium and a rotational movement of the leukocyte along the vessel wall. The number of rolling leukocytes increases dramatically during inflammatory conditions.[11] In the presence of inflammation, a rolling leukocyte will frequently transition to a stationary state in which the leukocyte remains firmly adhered to the endothelial cell surface of the pcv without the rotational movement.[12] Eventually, the leukocyte may leave the pcv by flattening the cell before leaving the bloodstream and subsequently emerging in the subendothelial space.[13]

Under conditions of reduced perfusion pressure, the leukocytes are not sufficiently deformed to move freely through the capillaries. The cells become stuck at the front or inside the capillary, forming a large surface area with the endothelium, obstructing all flow through such a vessel. Once a leukocyte is stuck in a capillary, it is difficult to remove in many cases. If the leukocyte is activated in the capillary, it is likely to produce oxygen-free radicals and lysosomal enzymes that disrupt the endothelium and cause tissue damage.[14]

Conventional microcirculation parameters, such as the FCD to describe diffusion and PPV to describe convection, are in some cases superior to conventional systemic parameters and have already proven their relevance and valuable role in monitoring disease severity, therapy response, and prognosis.[2, 15, 16] Initial research focused on microvascular alterations in RBC kinetics and has shown that microvascular changes, such as "stop-flow" microvessels, decreased microcirculatory flow rate, decreased capillary blood velocity, low density of perfused capillaries, and elevated heterogeneity of regional perfusion[17-21], can be detected earlier than and independent of changes in systemic hemodynamic parameters, leading to early recognition of a deteriorating clinical

condition and early detection of response to therapy.[4] Additional analysis of leukocyte-endothelial interaction in the microcirculation could provide even more information about the severity of inflammation of an individual patient in order to guide clinical decision-making.[4]

Since the use of HVM, only a small number of articles have been published about the identification of leukocytes and leukocyte interaction using the HVM. Bauer et al.[22] were the first to research rolling leukocytes in cardiac surgery patients in HVM recordings using a manual counting method. However, their method was unsuitable for distinguishing between plasma gaps and leukocytes in the images. In 2018, Uz et al.[23] introduced space-time diagram (STD) analysis for studying microcirculatory leukocytes in HVM recordings. STDs were initially used to determine red blood cell kinetics based on velocity, but they can also be used to differentiate between different types of leukocytes based on their behavior.[24]

In brief, an STD is a diagram where the y-axis corresponds to the length of the segmented blood vessel, and the x-axis corresponds to time according to the frame number. An STD is an image with alternating white and black bands. The flow of red blood cells forms the black bands, and plasma gaps or leukocytes form clear white/grey bands. A distinction between a plasma gap and a leukocyte could be made by a black band directly following the white band of the leukocyte. This black band results from the 'train formation' of red blood cells behind the much larger and slower white blood cell. In an STD, rolling and non-rolling leukocytes can be distinguished based on their speed. A rolling leukocyte is characterized by a linear white line that changes into a parabolic line as soon as the leukocyte is activated. Using an STD to examine leukocyte kinetics in the sublingual microcirculation seems to give promising results.[23]

These new developments have led to the need for a consensus on the use of HVMs and STDs for the analysis of leukocyte kinetics in the microcirculation. In this study, we aim to evaluate the currently used methods for analyzing leukocyte behavior and kinetics with STDs and formulate requirements and guidelines to ensure a reproducible, reliable method for clinical studies. Also, we aim to identify possibilities for improvement with future research. This research is a first step toward a consensus on leukocyte analysis with STDs.

5.2| Methods

For this research project, all studies using space-time diagrams for the analysis of leukocytes in the microcirculation were included. Two researchers thoroughly analyzed the methods of these papers, and uncertainties about the method were identified. Also, a training in leukocyte analysis was attended, and the researchers gained hands-on experience with leukocyte analysis. Next, a microcirculation expert, dr. Z. Uz, was interviewed to clarify uncertainties on, for example, the duration of HVM recording, the number of pcv units required for analysis, the characteristics used to determine what represents a pcv unit, and the minimum length of the segmented blood vessel.

After the expert meeting, the method to assess leukocyte kinetics using HVMs was divided into 1) image acquisition and 2) image analysis. Each part has several subsections: 1.1) device and recording requirements, 1.2) quality assessment, 2.1) selection of capillary - postcapillary venule units, 2.2) leukocyte detection, and 2.3) differentiation between rolling and non-rolling leukocytes. The requirements described in the literature and the wishes after the expert meeting are summarized for each subsection.

Based on the findings, recommendations were given for further development of the method. This research is a first step towards a consensus on leukocyte analysis with STDs and can be used as an outline for a future final consensus paper.

5.3| Results

For this research, three studies by Ince et al. and Uz et al. were included.[23, 25, 26] The methods for leukocyte analysis, as described in these papers, were divided into two main categories (1) image acquisition and 2) image analysis) and five subcategories: 1.1) device and recording requirements, 1.2) quality assessment of the images, 2.1) selection of capillary - postcapillary venule units, 2.2) leukocyte detection and 2.3) differentiation between rolling and non-rolling leukocytes, see **Figure 19**. Every step will be discussed separately.

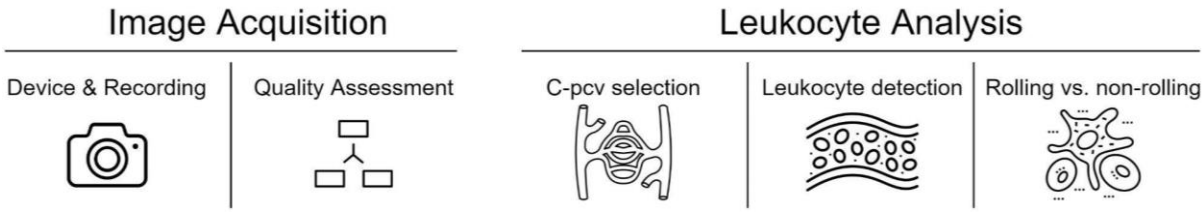


Figure 19

The workflow of leukocyte analysis with space-time diagrams.

5.3.1 | Image acquisition

The first step in leukocyte analysis is focused on image acquisition. There are several prerequisites for the inclusion of images for further analysis. The conclusions are summarized in **Table 10**.

5.3.1.1 | Device and recording requirements

First of all, the device has several requirements. All studies discussing leukocyte analysis use a third-generation HVM device, IDF imaging.[23, 25, 26] This technique has an improved optical resolution, resulting in better image quality and a larger field of view compared to previous-generation devices, leading to the visualization of more capillaries.[2, 27, 28] The high resolution and the large number of capillaries in these images make this technique very suitable for leukocyte identification and is therefore also advised for leukocyte analysis. The rest of this research will focus on the analysis of images made with IDF imaging.

Further requirements are focused on the recording to ensure representative data for analysis. The device used in all papers has a framerate of 25 images/s, allowing for the analysis of hyperperfusion.[2] As described in the second consensus by Ince et al.[2], hyperperfusion should only be analyzed when hyperperfusion is anticipated during pathophysiological disease states and is

Table 10 *Requirements and prerequisites for the image acquisition*

Image acquisition	
Device & recording requirements	
1. HVM device	IDF
2. Nr. of consecutive image sequences	3
3. Framerate (n/s)	25
4. Total nr. of frames	100
Quality assessment	
1. Illumination	Optimal when there is even illumination over the whole field of view, with contrast such that small vessels could be seen against a background. A video clip is acceptable if the borders are too bright or too dark, but the vessels are still identifiable.
2. Duration	150 frames are judged to be optimal, and 100-150 frames are acceptable
3. Focus	Optimal when all vessels have a detailed focus in the entire field of view, where individual plasma gaps or RBCs can be seen. The focus is acceptable if less than half of the field of view is out of focus.
4. Content	The optimal clip is free of occlusions and has a good distribution of small (<20µm) and large vessels (50-100µm). Less than 30% of the vessels are looped upon themselves (may not provide useful information due to difficult flow measurements). A video is acceptable if it contains only a few minor artifacts, small vessels are present (at least some), 30 to 50% are looped vessels, and less than 30% of the video clip shows occlusion due to saliva.
5. Stability	Optimal if the image has no motion blurring and movement is not more than a quarter of the distance to the edge of the field of view. The video clip is scored acceptable if it does not contain motion blurring and only has movement up to half the distance to the edge of the field of view.
6. Pressure	Optimal when flow through the vessels is continuously constant during the whole video, proper flow through the large venules with no clear signs of sluggish or stopped flow due to artifacts. A score of "acceptable" is assigned if intermittent sluggish flow or other pressure artifacts are visible in only one large venule, but the flow around seems unaffected.
7. Leukocyte specific	The images should include at least four c-pcv units.

c-pcv: capillary-postcapillary venule; IDF: incident dark field; RBC: red blood cell

dependent on the framerate. Therefore, a minimum of 25 images/s is needed for leukocyte analysis.

The number of consecutive image sequences is based on recommendation six of the second consensus.[2] In all studies, three consecutive image sequences are recorded at different locations in the sublingual space.[23, 25, 26] Multiple measurements can be averaged to correct for the heterogeneity in the microcirculation. The reasoning behind the reduction from five to three sequences in the second consensus is based on the increased field of view of IDF imaging.[2] However, the software currently used for leukocyte analysis, AVA, crops these images, leading to a smaller field of view and loss of data. Due to the sublingual microcirculation heterogeneity and the image's cropping, three image sequences are insufficient when using AVA. Also, the total number of images needed for a representative quantification of leukocytes currently needs to be researched, see 5.3.2.1. Therefore, we recommend using at least three image sequences for reliable results, but a definite requirement should be investigated and discussed between experts. The total number of frames used is based on recommendation four of the second consensus[2] and the expert opinion of Z. Uz, with a minimum of 100 frames. This is the minimum to research the kinetics of leukocytes while considering practical considerations for the feasibility of recording length. However, it was also stated that the longer the recording, the better the behavior of the leukocytes could be studied. An optimal length should therefore be investigated and determined by an expert group in a consensus meeting to find the optimal balance between feasibility and reliability.

5.3.1.2 | Quality Assessment

After the recording, the image quality should be assessed. All three studies[23, 25, 26] stated that the image quality score developed by Massey et al.[1] was used, see **Appendix A**. However, this score was developed for SDF imaging, and the study stated that further development is needed before applying it to clinical studies. Also, during the expert meeting, Z. Uz stated that the guidelines were always adjusted for the research's specific goals and target group. In the studies, the following exclusion criteria were stated regarding image quality: video clips with inadequate focus, image instability, and disturbed flow of RBCs due to iatrogenic pressure.[23] The full criteria and the corresponding scores of the images were not reported. To ensure reproducibility and reliability, it is important that in future papers, the exact inclusion and exclusion criteria are stated, including how many images were excluded based on the different criteria. This ensures reproducibility and gives new insights into the difficulties experienced with IDF imaging, potentially leading to future improvements.

Another essential criterium specific for the analysis of leukocytes in the sublingual area is the presence of capillary-postcapillary venules (c-pcv) units.[23] A c-pcv unit is defined as a capillary that merges into a pcv, a small venule distal to the capillary, without branching vessels.[23] For a detailed description of these units, see section 5.3.2.1. All included studies stated that a minimum of one c-pcv unit should be detected to include the image for further analysis. However, the studies and the expert meeting showed that a minimum of four c-pcvs is needed for reliable results, making the criterion of one unfunded. Also, the observation that c-pcv units are absent could be interesting for the patient's clinical status. Therefore, we recommend that this criterium be either deleted and the amount of c-pcv units is included as a measurement in the analysis, or the

criterium should be adjusted to the minimum amount of c-pcv units needed for analysis, see also section 5.3.2.1.

5.3.2 | Image analysis

In the sublingual area, the interaction between a leukocyte and the endothelium is best observed in the pcv.[29] This is due to the fact that extravasation of leukocytes mainly occurs in postcapillary vessels due to the low hemodynamic shear stress.[30] Therefore, microcirculatory leukocytes are analyzed based on their behavior in c-pcv units. When research is focused on a different area than the sublingual area, the corresponding physiology should be consulted before selecting vascular units for leukocyte analysis.

The image analysis can be subdivided into three steps: 1) detection of capillary-postcapillary venule units, 2) leukocyte detection, and 3) differentiation between rolling and non-rolling leukocytes. These will be discussed separately. All conclusions are summarized in **Table 12**.

For these steps, the videos are loaded into AVA, a software for the analysis of blood vessels and space-time diagrams.[2] This software is very dated, and the images taken with the IDF imaging method are too large for analysis and therefore need to be cropped. This leads to a loss of data, and future developments should be focused on modern software which is compatible with high-quality images.

5.3.2.1 | Detection capillary – postcapillary venule units

In their validation study, Uz et al.[23] describe a c-pcv unit as a capillary that merges into a pcv, which is a small venule (<20µm) distal to the capillary. This mainly focuses on the blood flow direction and the vessels' size. The blood flow should go from a small capillary to a bigger postcapillary venule. Also, the vessels should not be branched. This definition was further clarified during the expert meeting, including insights about the diameter, the selected length, and the number of c-pcv units.

The diameter can be selected based on anatomical measurements of the different types of vessels, see **Table 11**. In a capillary, only a single RBC passes through for optimal oxygen exchange with the tissues (single file flow).[31] In the pcvs, the diameter increases, and multiple RBCs can pass simultaneously. Important for the detection of leukocytes is the maximum diameter of a vessel. When the diameter of the vessels exceeds the size of a leukocyte, a leukocyte can be blocked from view when sticking to the back of the vessel wall.[23] This leads to a loss of information and underestimation of the number of leukocytes in the microcirculation. Therefore, the diameter of a postcapillary venule should not exceed 20 µm, the size of a leukocyte. The diameter of the vessels is currently not implemented when selecting c-pcv units in the AVA software, where vessels are only selected based on visual inspection (**Figure 20**). The measurements should be used to automatically select the correct vessels based on their diameter and state a maximum to prevent view-blocking of the leukocytes. Also, it became clear that not everyone identifies an equal amount of c-pcv units. Experienced researchers have no problem detecting at least four c-pcv units in one image, while others can only identify two. This shows that visual inspection alone is highly dependent on the investigator, making the analysis less reliable. Therefore, we recommend the

implementation of exact measurements for capillaries and pcvs detection, preferably with automatic detection of the c-pcvs.

Table 11 *Measurements of different types of vessels*

Type vessel	***Diameter***
Capillaries	6um
Postcapillary venules	10-30um

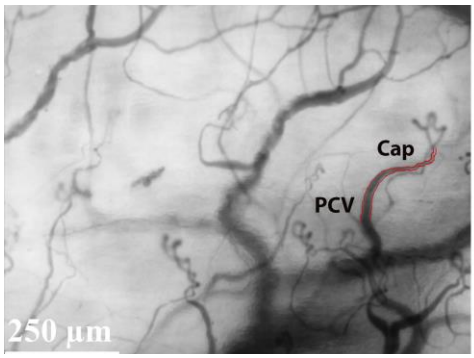


Figure 20
Selection of c-pcv.

No research has been conducted yet for the minimal length of a selected c-pcv unit and the distribution between the capillary and the pcv. During the expert meeting, it was stated that the longer the c-pcv unit, the better the analysis of leukocyte behavior since the change of leukocyte behavior could take place over the entire length of the pcv. This means that the longer the pcv, the higher the chance of detecting a rolling leukocyte. However, it should be noted that the segmented c-pcv does not contain looped blood vessels, as the flow is difficult to measure and may not provide prognostic information. Looped blood vessels are defined in an image as blood vessels with a diameter < 20 µm, which bend backward to form short, tortuous structures[1] (**Figure 21**[2]). Therefore, a consensus should be formulated about the minimal length of a c-pcv unit and how different lengths should be corrected for a representative observation of leukocytes and their behavior.

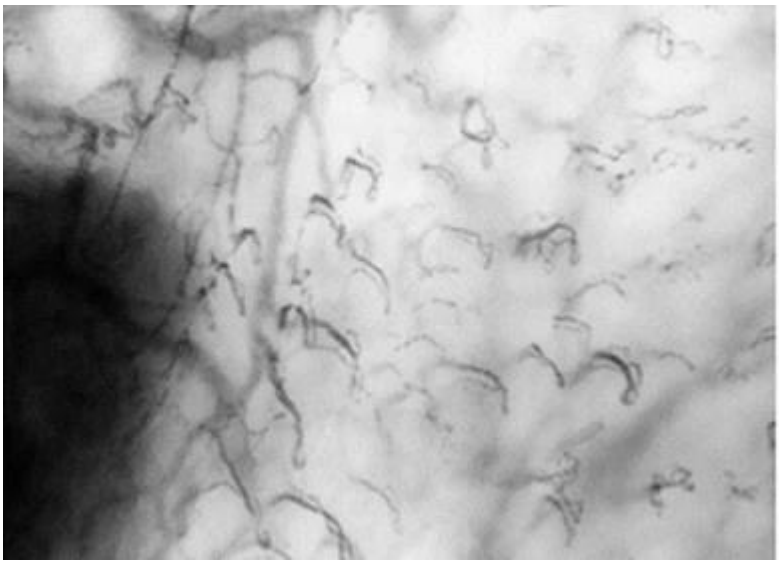


Figure 21
Looped blood vessels.

The number of selected c-pcv units ranges from minimal one[25] to a total of four distributed over four quadrants[26]. Results from previous research by K. Tjepkema (MSc. clinical technology student), the expert meeting, and hands-on practical experience showed a large heterogeneity between different c-pcv units. This shows the importance of selecting multiple c-pcv units and averaging the results of multiple image sequences. Currently, the optimal number of quadrants has not been investigated yet. Therefore, research must determine the optimal number of c-pcvs for generalizability. This could be investigated by collecting multiple consecutive image sequences, for example, ten, and averaging the leukocyte count with different amounts of image sequences and different amounts of quadrants per image. This could lead to an optimization process, with as few videos and c-pcvs per video as needed for practical reasons but enough to ensure reliability.

5.3.2.2 | Leukocyte analysis with space-time diagrams

For this research, we have focused on leukocyte analysis using STD. In an STD, the y-axis corresponds to the length of the segmented blood vessel, and the x-axis corresponds to time according to the frame number. The STD shows alternating white and black bands, where the black bands are formed by red blood cells, and clear white or grey bands are formed by plasma gaps or leukocytes, see **Figure 22**[23].

Leukocyte analysis is based on the different cells' differences in color, physiology, and behavior.[23] Leukocytes present as white globules that maintain their morphology in the venule and are present in multiple consecutive frames. This leads to a white band in the STD. Plasma gaps, on the other hand, continuously change in shape and volume. Another difference between a plasma gap and a leukocyte is the 'train formation' of RBCs, i.e., a number of RBCs behind each other, which can be seen as a black band directly following the white band of the leukocyte. This black band results from the accumulation of red blood cells behind the leukocyte since this cell is much larger and slower than the RBCs.[23] **Figure 23**[23] shows an example of a leukocyte followed by an RBC train, but not all leukocytes are followed by a train formation.

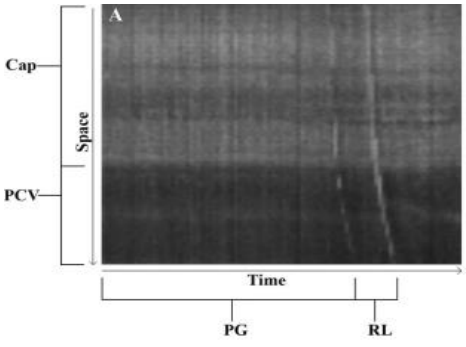


Figure 22
STD showing a PG and a RL. PG: plasma gap; RL: rolling leukocyte; STD: space-time diagram.

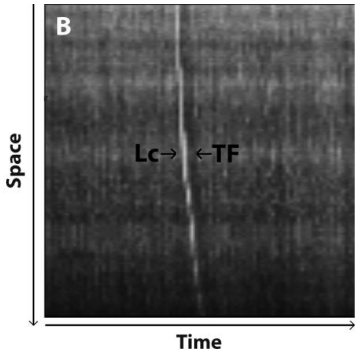


Figure 23
STD showing a Lc and a TF. Lc: leukocyte; STD: space-time diagram; TF: train formation.

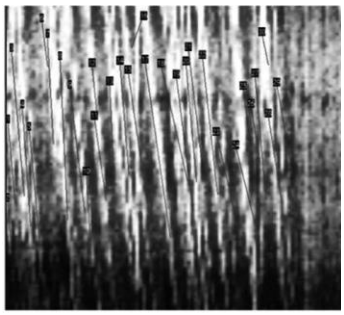


Figure 24
STD showing many leukocytes. STD: space-time diagram.

Currently, the identification of the leukocytes is manually done based on these descriptions. This remains, however, very subjective, and the descriptions leave room for interpretation, especially in complex STDs, see **Figure 24**. Therefore, it is important that objective requirements are formulated and standardized training for researchers is organized. Objective measurements could include 1) the size of a leukocyte, e.g., 15 to 20µm, as the width of the white band, 2) the contrast in color between the RBCs and the leukocyte as pixel intensity, and 3) the minimum of consecutive images that show the leukocyte.

5.3.2.2 | Differentiation between rolling and non-rolling leukocytes

Leukocyte recruitment and adhesion to the endothelial cell wall, followed by extravasation, are signs of systemic inflammation. Therefore, next to the quantification of leukocytes, leukocyte behavior could give insight into a patient's clinical state. Leukocytes are differentiated into two categories: rolling and non-rolling leukocytes. Non-rolling leukocytes stay in the circulation, while rolling leukocytes show interaction with the endothelial wall, leading to an inflammatory reaction.[11-13]

In an STD, rolling and non-rolling leukocytes can be distinguished based on their velocity.[23] A rolling leukocyte is activated and starts to stick to the endothelium, leading to a decrease in velocity. In the STD, this can be observed as a change of the linear white line into a parabolic line as soon as the leukocyte is activated. Non-rolling leukocytes, on the other hand, stay in the circulation and do not show a change in velocity, showing a linear line.[23]

The leukocyte-endothelial interaction occurs primarily in the postcapillary venules. Therefore, the change in velocity should be observed after the transition of the capillary to the pcv. In the STD, this transition can be seen as a horizontal line, with the capillary part having a higher pixel intensity, see **Figure 22**.

The differentiation between rolling and non-rolling is thus based on the linearity of the white line representing the leukocyte. A linear line, e.g., a non-rolling leukocyte, is defined as a line with a slope of 180 degrees, and any deviation from 180 is defined as a change to a parabolic line, e.g., a rolling leukocyte. The slope is currently measured manually with a ruler.

This method, however, is very subjective. First of all, slopes should be calculated automatically by a computer instead of manually with a ruler. Using a ruler on a screen is inaccurate and small differences in slopes cannot be measured with a ruler. Also, a slope of exactly 180 degrees is not investigated. Artifacts, like a curve in the vessels and the increase in diameter of the pcv compared to the capillary, could result in a change in velocity and, thus, in a slight change of the slope of a couple of degrees. Therefore, strict guidelines should be set for slope measurements and cut-off values.

A solution for these problems would be a calculation of acceleration instead of velocity. Since the differentiation is based on a change in velocity, acceleration can give more reliable and precise measurements than velocity. Also, this allows for automatic differentiation based on a threshold.

As stated before, the length of the selected c-pcv greatly influences the possible detection of a rolling leukocyte. Therefore, the quantification of rolling and non-rolling leukocytes should be corrected for the length of the selected c-pcv.

Table 12 *Requirements and definitions of image analysis*

Image analysis	
Capillary-pcv unit	
1. Number of c-pcvs	4, spread over four quadrants
2. Direction blood flow	From capillary to pcv, small to big
3. Diameter	Cap: 6um, pcv: 15-20um
4. Length, min., and max.	As long as possible, no cut-offs
5. Ratio capillary vs. pcv	pcv > capillary
6. Characteristics	No branches
Leukocyte detection	
1. Brightness	White
2. Diameter	15-20um
3. Nr. of consecutive images	Unknown
4. RBC train	Not always present, black band behind white band
Rolling vs. non-rolling	
1. Angle	NR: 180°, R: anything deviating from 180°
2. Location angle change	After transition capillary to pcv
3. Acceleration	Not investigated yet

cap: capillary; c-pcv: capillary-postcapillary venule; NR: non-rolling leukocyte; pcv: postcapillary venule; R: rolling leukocyte; RBC: red blood cell.

5.4| Discussion and conclusion

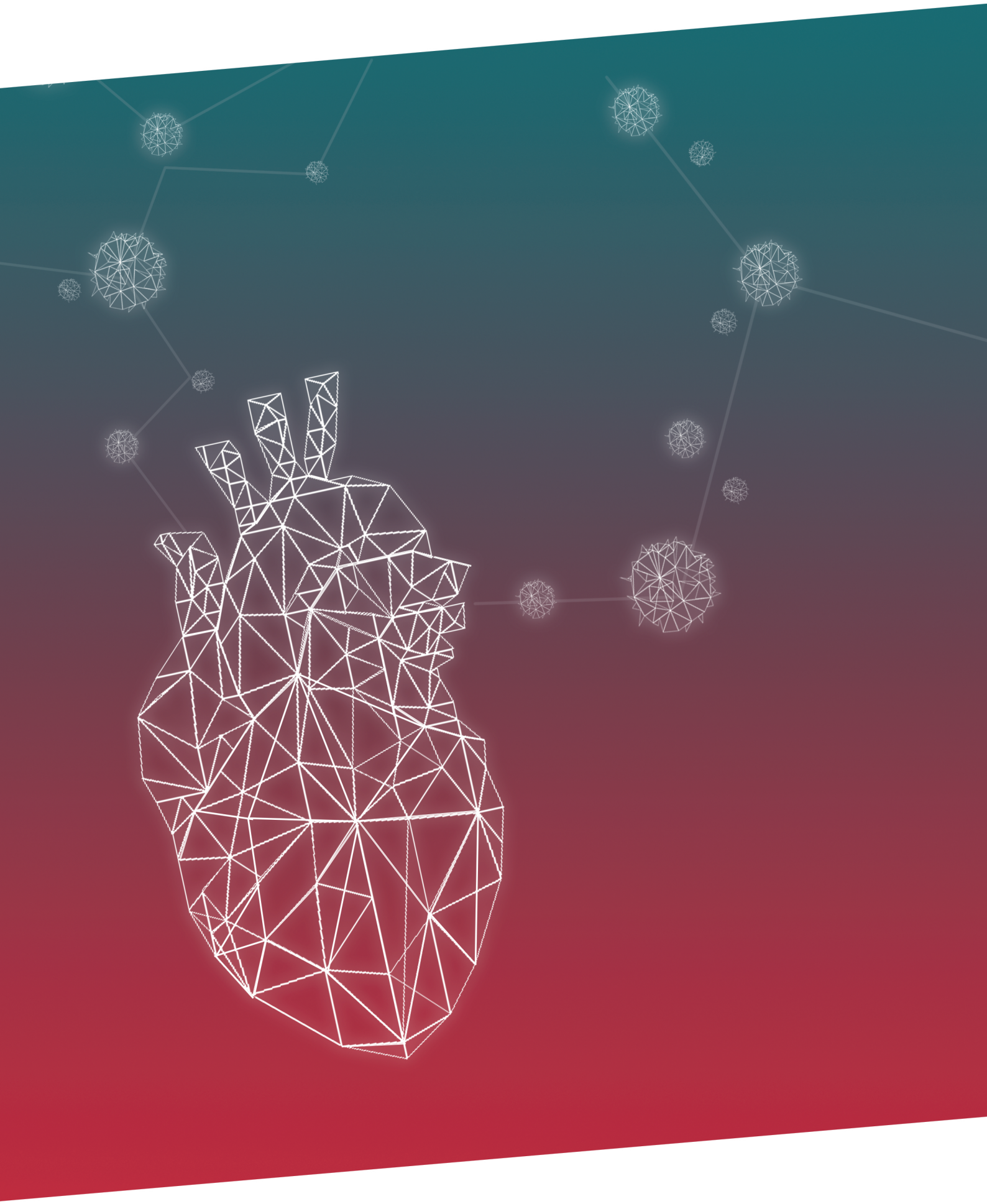
In this study, we have investigated the methods used for leukocyte analysis in the microcirculation with space-time diagrams using incident dark-field imaging. Space-time diagrams enable differentiation between rolling and non-rolling leukocytes based on their behavior as a marker for the inflammatory reaction in the microcirculation. This method shows great potential for bedside monitoring and has been investigated in multiple studies researching the pathophysiological changes in surgical and critically ill patients. However, before this technique can be used in large clinical studies that aim to assist bedside decision-making, the methods should be thoroughly analyzed, and a consensus on the method between experts should be made.

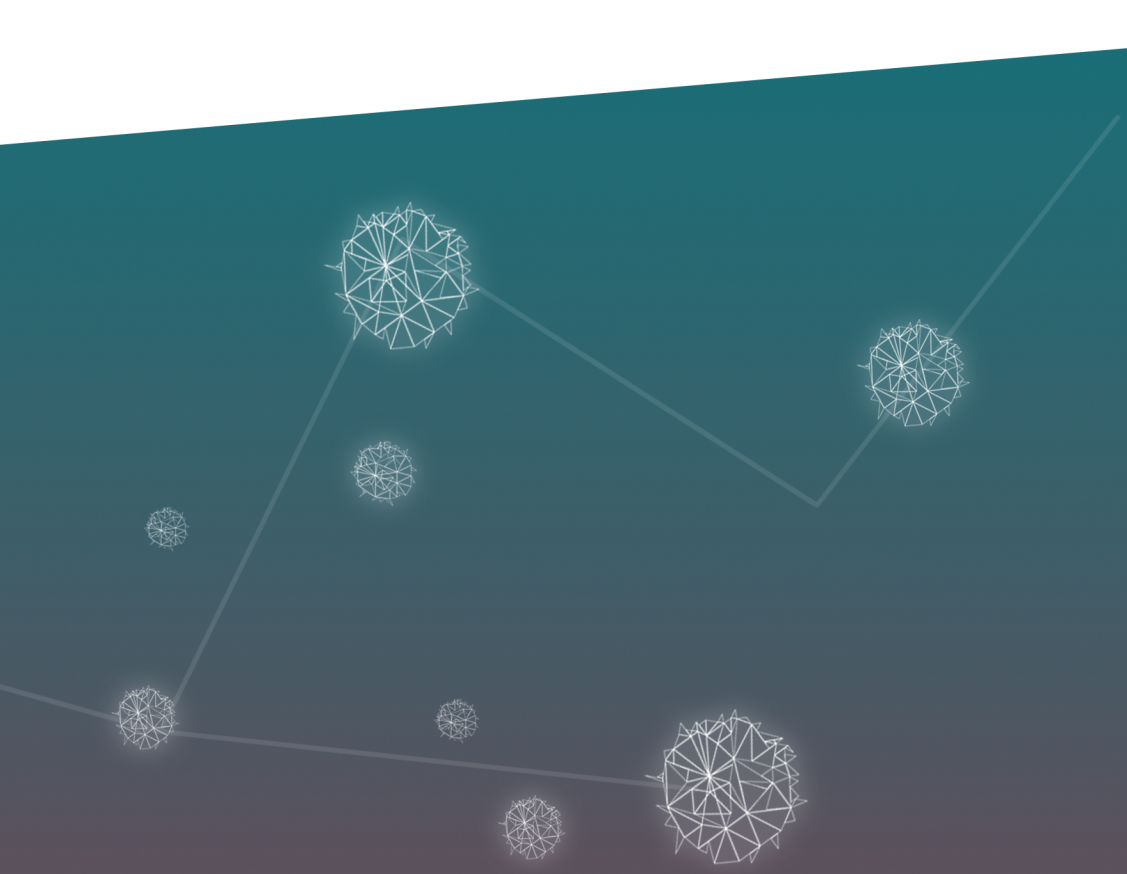
We showed that the method of assessing rolling and non-rolling leukocytes could be divided into two parts: 1) image acquisition and 2) image analysis. These can be further subdivided into

categories, namely 1.1) device and recording requirements, 1.2) quality assessment, 2.1) selection of capillary - postcapillary venule units, 2.2) leukocyte detection, and 2.3) differentiation between rolling and non-rolling leukocytes. For most of these steps, the reasoning behind the decisions is based on previous consensus papers about conventional microcirculatory measurements regarding red blood cell kinetics. This is reliable for several steps in image acquisition and analysis, but more research is needed for steps designed explicitly for leukocyte analysis to guarantee reproducible reliable results. Also, we recommend the development of an automatic software for the leukocyte quantification and differentiation based on behavior, both for reliability and feasibility, since manual analysis is very time-consuming. M.P. Hilty currently takes the first steps toward automatic software. Further research should include their research. This automatization and expert meetings could lead to an optimized method for reproducibility and reliability and has the potential to contribute to bedside patient monitoring and the prognosis and choice in therapy.

5.5| References

1. 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. Journal of critical care. 2013;28(6):913-7.
2. Ince C, Boerma EC, Cecconi M, De Backer D, Shapiro NI, Duranteau J, et al. 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 medicine. 2018;44(3):281-99.
3. De Backer D, Hollenberg S, Boerma C, Goedhart P, Büchele G, Ospina-Tascon G, et al. How to evaluate the microcirculation: report of a round table conference. Critical care. 2007;11(5):1-9.
4. Uz Z, Ince C, Arbous M. Observation of Leukocyte Kinetics Using Handheld Vital Microscopes During Surgery and Critical Illness. Annual Update in Intensive Care and Emergency Medicine 2021: Springer; 2021. p. 111-21.
5. Granger DN, Senchenkova E, editors. Inflammation and the Microcirculation. Colloquium series on integrated systems physiology: from molecule to function; 2010: Morgan & Claypool Life Sciences.
6. Ley K, Laudanna C, Cybulsky MI, Nourshargh S. Getting to the site of inflammation: the leukocyte adhesion cascade updated. Nature Reviews Immunology. 2007;7(9):678-89.
7. Tigner A, Ibrahim SA, Murray I. Histology, white blood cell. 2020.
8. Fenton BM, Wilson DW, Cokelet GR. Analysis of the effects of measured white blood cell entrance times on hemodynamics in a computer model of a microvascular bed. Pfluegers Archiv. 1985;403(4):396-401.
9. Bagge U, PI B. White blood cell rheology. An intravital study in man. 1977.
10. Schmid-Schönbein GW, Usami S, Skalak R, Chien S. The interaction of leukocytes and erythrocytes in capillary and postcapillary vessels. Microvascular research. 1980;19(1):45-70.
11. Zuidema MY, Korthuis RJ. Intravital microscopic methods to evaluate anti-inflammatory effects and signaling mechanisms evoked by hydrogen sulfide. Methods in enzymology. 555: Elsevier; 2015. p. 93-125.
12. Granger DN, Kubes P. The microcirculation and inflammation: modulation of leukocyte-endothelial cell adhesion. Journal of leukocyte biology. 1994;55(5):662-75.
13. Asako H, Korthuis R, Wolf R, Granger D, editors. Phalloidin reduces leukocyte emigration and vascular-permeability in postcapillary venules. Faseb Journal; 1992: FEDERATION AMER SOC EXP BIOL 9650 ROCKVILLE PIKE, BETHESDA, MD 20814-3998 USA.
14. Bagge U, Amundson B, Lauritzen C. White blood cell deformability and plugging of skeletal muscle capillaries in hemorrhagic shock. Acta Physiologica Scandinavica. 1980;108(2):159-63.
15. Dobbe JG, Streekstra GJ, Atasever B, Van Zijderveld R, Ince C. Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. Medical & biological engineering & computing. 2008;46(7):659-70.
16. 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. Critical care medicine. 2013;41(3):791-9.
17. Fries M, Weil MH, Sun S, Huang L, Fang X, Cammarata G, et al. Increases in tissue Pco2 during circulatory shock reflect selective decreases in capillary blood flow. Critical care medicine. 2006;34(2):446-52.
18. Lam C, Tyml K, Martin C, Sibbald W. Microvascular perfusion is impaired in a rat model of normotensive sepsis. The Journal of clinical investigation. 1994;94(5):2077-83.
19. Farquhar I, Martin CM, Lam C, Potter R, Ellis CG, Sibbald WJ. Decreased Capillary Densityin Vivoin Bowel Mucosa of Rats with Normotensive Sepsis. Journal of Surgical Research. 1996;61(1):190-6.
20. Bateman RM, Sharpe MD, Ellis CG. Bench-to-bedside review: microvascular dysfunction in sepsis–hemodynamics, oxygen transport, and nitric oxide. Critical care. 2003;7(5):1-15.
21. Weinberg J, Boyle P, Thomas K, Murphy K, Tooke J, Guz A. Capillary blood cell velocity is reduced in fever without hypotension. International Journal of Microcirculation, Clinical and Experimental. 1991;10(1):13-9.
22. Bauer A, Kofler S, Thiel M, Eifert S, Christ F. Monitoring of the sublingual microcirculation in cardiac surgery using orthogonal polarization spectral imaging: preliminary results. The Journal of the American Society of Anesthesiologists. 2007;107(6):939-45.
23. Uz Z, van Gulik TM, Aydemirli MD, Guerci P, Ince Y, Cuppen D, et al. Identification and quantification of human microcirculatory leukocytes using handheld video microscopes at the bedside. Journal of Applied Physiology. 2018;124(6):1550-7.
24. Ellis CG, Ellsworth ML, Pittman RN, Burgess WL. Application of image analysis for evaluation of red blood cell dynamics in capillaries. Microvascular research. 1992;44(2):214-25.
25. Favaron E, Ince C, Hilty MP, Ergin B, van der Zee P, Uz Z, et al. Capillary leukocytes, microaggregates, and the response to hypoxemia in the microcirculation of coronavirus disease 2019 patients. Critical care medicine. 2021;49(4):661.
26. Uz Z, Ince C, Shen L, Ergin B, van Gulik T. Real-time observation of microcirculatory leukocytes in patients undergoing major liver resection. Scientific Reports. 2021;11(1):1-15.
27. Massey MJ, Shapiro NI. A guide to human in vivo microcirculatory flow image analysis. Critical Care. 2015;20(1):1-10.
28. Van Elteren H, Ince C, Tibboel D, Reiss I, de Jonge R. Cutaneous microcirculation in preterm neonates: comparison between sidestream dark field (SDF) and incident dark field (IDF) imaging. Journal of clinical monitoring and computing. 2015;29(5):543-8.
29. Kubes P, Kerfoot SM. Leukocyte recruitment in the microcirculation: the rolling paradigm revisited. Physiology. 2001;16(2):76-80.
30. Weirather J, Frantz S. Role of the innate immune system in ischemic heart failure. Inflammation in heart failure: Elsevier; 2015. p. 19-38.
31. Secomb T. Red blood cell mechanics and capillary blood rheology. Cell Biophysics. 1991;18(3):231-51.





6.

General Conclusion and Discussion

Microcirculatory alterations contribute significantly to acute organ dysfunction in cardiothoracic surgical patients. Nevertheless, it is only since the last decade that it has been possible to image the microcirculation of patients.

In this master thesis, using the CytoCam, a non-invasive imaging technique, we investigated microcirculatory perfusion in patients after cardiothoracic surgery in a clinical setting. In particular, we focused on the time course of microcirculatory parameters in postoperative cardiothoracic surgical patients with and without shock. Moreover, we compared microcirculatory parameters with macrocirculatory parameters of the same patient to determine the presence or absence of hemodynamic coherence. We also investigated the use of leukocyte detection by STDs to better understand the severity of inflammation in the individual patient.

In **Chapter 3** of this master's thesis, it was found that in cardiothoracic surgical patients with shock, the convective mechanism (reflective in the RBCv) decreased, and the diffusive mechanism (reflective in TVD, FCD, and cHct) increased, possibly due to a compensatory mechanism for the mismatch between O2 supply and O2 consumption. Hemodynamic coherence was found between the TVD, FCD, and PPV and macrocirculation at T0 to T1.

In **Chapter 4**, it was found that TVD, FCD, and tRBCp in the non-shock group seemed comparable to normal values, whereas, in the shock group, these values were increased compared to normal values and remained more or less increased. A hemodynamic correlation was found between the TVD, FCD, and PPV and macrocirculation in both groups. In the shock group, the tRBCp was also congruent with the macrocirculation at T0 to T1.

Chapter 5 of this thesis concluded that the method for leukocyte detection is still suboptimal, and a consensus must be reached before leukocyte detection can be widely used as a diagnostic tool in the ICU.

6.1| Limitations

This study included only a small number of participants, and, in addition, healthy controls were not available to compare these results with. Although a standardized protocol was used to collect macro- and microcirculatory data, given the observational nature of these data, it cannot be ruled out that other factors influenced these variables during ICU admission. Studies showed that very large samples are needed to achieve statistical significance with microcirculatory parameters.[1] The potential for false correlations with large samples is exceptionally high, so follow-up studies should pay particular attention to whether reported correlations with these microcirculatory parameters reflect actual biological differences.[1]

Although postoperative changes were measured, no intraoperative data were obtained to examine the exact time of occurrence of microcirculatory alterations. Moreover, no preoperative data were obtained and analyzed, whereas this could be a baseline measurement to recognize specific patterns in intra- and postoperative microcirculatory alterations. Pre- and intraoperative data should be collected and added to the postoperative data in future research.

Even though much research has been done on various techniques to image microcirculatory perfusion in patients, none has yet found a place at the bedside as a tool for the clinician due to multiple issues.

First, the CytoCam is unsuitable for continuous monitoring because it is an analog technique that produces images rather than numbers. Post-processing is required with AVA software or MicroTools. AVA software is the current standard for analyzing microcirculatory image sequences. This is a semi-automated software platform, making the analysis time-consuming and requiring considerable precision and skill from the person analyzing the images.[2, 3] Microtools is a new, fully automated software program that can analyze microcirculation image sequences without human intervention to extract microcirculation parameters objectively.[3] An automated software tool has advantages and disadvantages. First, saving time during image analysis is the main advantage, which is especially important to guide clinical decision-making based on microcirculation in critically ill patients. A disadvantage could be the lack of precision due to the absence of interaction with the user.

Secondly, IDF as an imaging modality is not comparable to other imaging modalities. IDF imaging visualizes approximately 20% more vessels than SDF imaging, resulting in a higher TVD when using IDF imaging. In addition, image quality is higher due to, for example, shorter pulse duration (more contrast and contour in the images) and lower weight (minimizes pressure artifacts) in IDF than in SDF, resulting in a difference in PPV.[4] Therefore, it is imperative that follow-up studies use the same imaging modality to compare results.

In addition, cardiothoracic surgery is known for its heterogeneous microcirculatory perfusion, making it necessary to evaluate different measurement sites.[5] The degree of heterogeneity may be as important in assessing microcirculatory perfusion as the number of perfused vessels, which are typically measured. Moreover, microcirculation measurements in most patients are often limited to sublingual microcirculation. All vascular beds are affected by systemic inflammation, but differences in endothelial cell architecture and microvascular regulation exist between different organs and tissues.[6] There seems to be a good relationship between the sublingual and gastrointestinal microcirculation and derived microcirculatory parameters[7], but local microcirculatory impairments should not be directly interpreted as impairment of systemic microvascular perfusion.

As mentioned earlier, another limitation of microcirculation measurements with an HVM is that the results are highly user-dependent unless the sufficient experience is gained.[8] There are no concrete guidelines on how much experience is needed to perform the measurements correctly.[9] For example, it takes considerable training to reduce pressure without losing the microcirculation image from view to avoid pressure artifacts at venules.[8] However, in this master thesis, it was still common for many image sequences to be excluded due to poor quality, implying that observers were not adequately trained in performing these measurements. An appropriate site for measurement is often sought based on what can be seen on the image, making it difficult to measure in the same place to measure changes over time, as the probe is easily moved.[9] To date, there are no established guidelines on an anatomical site that should be used for measurements.[8]

Finally, and most importantly, there are currently no therapies for specifically optimizing microcirculatory perfusion. Consequently, if poor microcirculation is observed, macrocirculatory

interventions, including vasopressors, inotropics, vasodilators, and fluid therapy, are suggested to improve microcirculatory perfusion.[10] Studies have shown that vasoactive drugs and fluid therapy have almost no effect on the microcirculation in patients in septic shock with a normal range of macrocirculatory parameters.[11] Until drugs explicitly acting on the microcirculation exist, monitoring the microcirculation at the bedside will remain limited.

6.2| Future perspectives

Microcirculation measurements can provide the clinician with information to better interpret the patient's condition and direct therapy. Yet, in some cases, the underlying pathology cannot be identified with standard microcirculatory measurements alone.[12] The RBCv should be added as a new parameter to the measurements to identify hypovolemia or hypotensive shock in such cases to prevent fluid overload.[13] In addition, inflammatory conditions such as ischemia-reperfusion and cardiac surgery lead to the activation and adhesion of leukocytes to the endothelium.[14] As discussed in **Chapter 5**, the kinetics of activated leukocytes can be quantified using an STD of microcirculatory image sequences. Standardization and automation of leukocyte analysis should be explored in follow-up research because of its added value in understanding the pathogenesis of the development of shock. Hence, microcirculation analysis should always be performed in context and should integrate macrocirculatory parameters for a complete hemodynamic evaluation of the physiological state of the cardiovascular system.[9]

The venoarterial differential partial pressure of carbon dioxide (PCO_2 gap) is increasingly recognized as a reliable macrocirculatory tool to evaluate cardiac output and tissue perfusion. It is considered a marker of poor outcomes during circulatory shock and is currently used to guide treatment.[15, 16] Follow-up studies should identify the relationship between the PCO_2 gap and microcirculatory parameters. However, the design of such a study should consider documenting the amount of fluid and dobutamine a patient has had, as this affects the value of the PCO_2 gap.[17]

Obviously, adequate perfusion is necessary for sufficient tissue oxygenation. However, since current techniques to measure microcirculation are mainly limited to research settings, no reference values for cardiothoracic surgery associated with poor outcomes exist at this point. Follow-up studies should reveal what these cutoff values should be.

Moreover, it is necessary to limit inter- and intra-observer variability for reproducibility and generalizable interpretation of results. To this end, the guidelines should clearly indicate the amount of time and training an observer should have had before taking measurements in patients. It is also important to include an anatomical guided strategy, considering anatomical variation between individuals, in the international guidelines in order to increase imaging consistency associated with clinical HVM.[8] Since larger sample sizes are desirable for the detection of significant differences in microcirculatory parameters, image consistency is also essential. In currently published studies with small sample sizes, there is much variation in the values of reported parameters, and the results do not appear to be reproducible.[1] It is, therefore, imperative for future research to design the guidelines in such a way that microcirculatory measurements become reproducible.

Because of the technical challenges to image acquisition and analysis, the CytoCam and other microcirculation imaging modalities remain primarily research-based tools. As automated image

analysis systems are improved by manufacturers and software developers, these systems may be able to take an active role at the bedside in guiding management decisions for the individual patient.

6.3| Conclusion

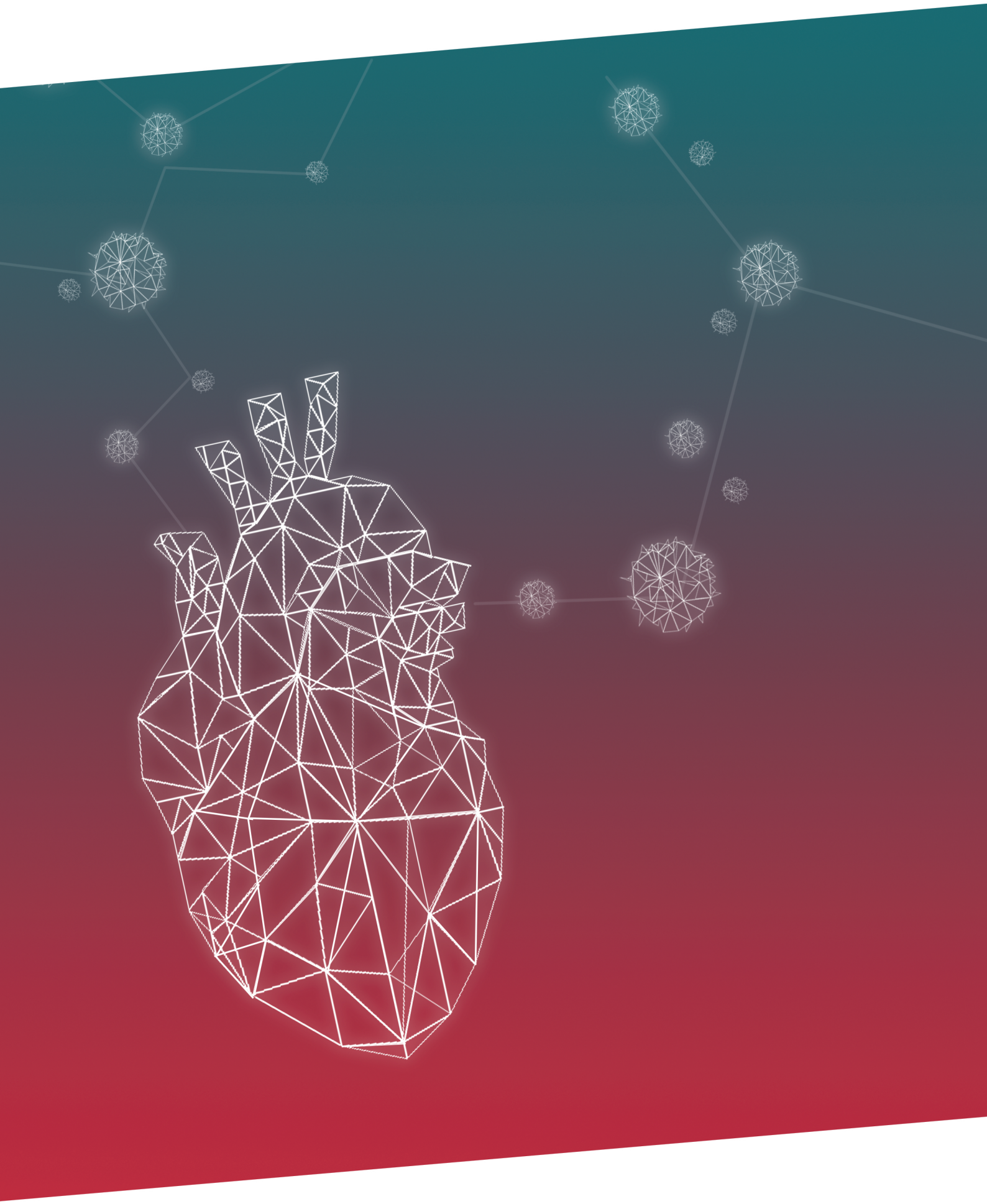
This master thesis shows that microcirculatory perfusion is altered after cardiothoracic surgery. A prospective cohort study was performed assessing sublingual microcirculatory blood flow and systemic hemodynamic parameters after cardiothoracic surgery to examine the time course of the microcirculation and its relationship to the macrocirculation. Impairment of the microcirculation during inflammatory conditions, including cardiac surgery with cardiopulmonary bypass, is a complex problem, often occurring without impaired macrocirculation. Factors contributing to the acute disruption of microcirculatory perfusion in cardiothoracic surgical patients include activation of leukocytes, complements, and platelets, endothelial cell dysfunction, disruption of the endothelial glycocalyx, and consequent vascular leakage, decreased deformability of RBCs and hemodilution.

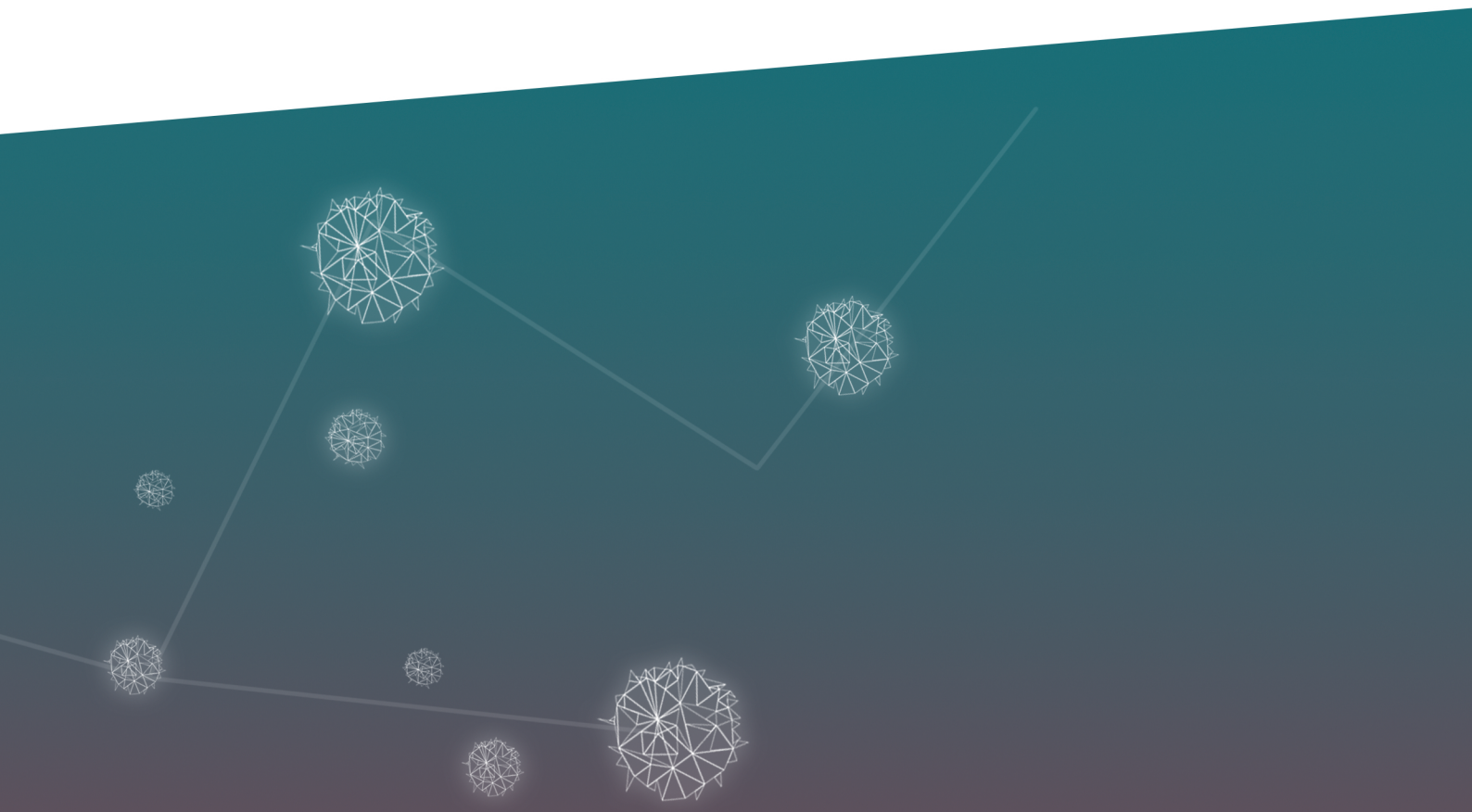
A difference was found in the behavior of the microcirculation of cardiothoracic surgical patients with and without shock. There is no conclusive explanation for the difference found or for the underlying mechanisms. Based on this study, it is not yet possible to give a predictive value to the contribution of the microcirculation to the development of shock. Therefore, further research is needed.

6.4| References

1. Valerio L, Peters RJ, Zwinderman AH, Pinto-Sietsma S-J. Reproducibility of sublingual microcirculation parameters obtained from sidestream darkfield imaging. PloS one. 2019;14(3):e0213175.
2. Dobbe JG, Streekstra GJ, Atasever B, Van Zijderveld R, Ince C. Measurement of functional microcirculatory geometry and velocity distributions using automated image analysis. Medical & biological engineering & computing. 2008;46(7):659-70.
3. Hilty MP, Guerci P, Ince Y, Toraman F, Ince C. MicroTools enables automated quantification of capillary density and red blood cell velocity in handheld vital microscopy. Communications biology. 2019;2(1):1-15.
4. Van Elteren H, Ince C, Tibboel D, Reiss I, de Jonge R. Cutaneous microcirculation in preterm neonates: comparison between sidestream dark field (SDF) and incident dark field (IDF) imaging. Journal of clinical monitoring and computing. 2015;29(5):543-8.
5. Koning NJ, Simon LE, Asfar P, Baufreton C, Boer C. Systemic microvascular shunting through hyperdynamic capillaries after acute physiological disturbances following cardiopulmonary bypass. American Journal of Physiology-Heart and Circulatory Physiology. 2014;307(7):H967-H75.
6. Aird WC. Phenotypic heterogeneity of the endothelium: II. Representative vascular beds. Circulation research. 2007;100(2):174-90.
7. Verdant CL, De Backer D, Bruhn A, Clausi CM, Su F, Wang Z, et al. Evaluation of sublingual and gut mucosal microcirculation in sepsis: a quantitative analysis. Critical care medicine. 2009;37(11):2875-81.
8. Uz Z, Dilken O, Milstein DM, Hilty MP, de Haan D, Ince Y, et al. Identifying a sublingual triangle as the ideal site for assessment of sublingual microcirculation. Journal of Clinical Monitoring and Computing. 2022:1-11.
9. Ince C, Boerma EC, Cecconi M, De Backer D, Shapiro NI, Duranteau J, et al. 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 medicine. 2018;44(3):281-99.
10. van der Voort PH, van Zanten M, Bosman RJ, van Stijn I, Wester JP, van Raalte R, et al. Testing a conceptual model on early opening of the microcirculation in severe sepsis and septic shock: a randomised controlled pilot study. European Journal of Anaesthesiology| EJA. 2015;32(3):189-98.
11. De Backer D, Ortiz JA, Salgado D. Coupling microcirculation to systemic hemodynamics. Current opinion in critical care. 2010;16(3):250-4.
12. Akin S, dos Reis Miranda D, Caliskan K, Soliman OI, Guven G, Struijs A, et al. Functional evaluation of sublingual microcirculation indicates successful weaning from VA-ECMO in cardiogenic shock. Critical Care. 2017;21(1):1-9.
13. Hudetz AG, Wood JD, Biswal BB, Krolo I, Kampine JP. Effect of hemodilution on RBC velocity, supply rate, and hematocrit in the cerebral capillary network. Journal of Applied Physiology. 1999;87(2):505-9.
14. Nakagawa NK, Nogueira RA, Correia CJ, Shiwa SR, Cruz JWMC, de Figueiredo LFP, et al. Leukocyte-endothelium interactions after hemorrhagic shock/reperfusion and cecal ligation/puncture: an intravital microscopic study in rat mesentery. Shock. 2006;26(2):180-6.
15. Ltaief Z, Schneider AG, Liaudet L. Pathophysiology and clinical implications of the veno-arterial PCO2 gap. Critical Care. 2021;25(1):1-9.
16. Huette P, Beyls C, Mallat J, Martineau L, Besserve P, Haye G, et al. Central venous-to-arterial CO2 difference is a poor tool to predict adverse outcomes after cardiac surgery: a retrospective study. Canadian Journal of Anesthesia/Journal canadien d'anesthésie. 2021;68(4):467-76.
17. Waldauf P, Jiroutkova K, Duska F. Using pCO2 Gap in the differential diagnosis of hyperlactatemia outside the context of sepsis: a physiological review and case series. Critical Care Research and Practice. 2019;2019.

This page was intentionally left blank.





7.

Appendix

Appendix A

Quality assessment microcirculatory image sequences

Table A.1 *Quality assessment by Massey et al.*[1]

Criteria	Explanation	Optimal (0 points)	Requirement (1 point)	Exclusion (10 points)
Illumination	Brightness and contrast of the video	Even illumination over the whole field of view, with contrast such that small vessels could be seen against a background of vessels	The video borders are too bright or too dark, but the vessels are still identifiable	The video is oversaturated / too bright or too dark to make out analyzable features. Insufficient contrast to resolve flow rate.
Duration	Number of frames in the video clip	>5 sec (>150 frames)	3-5 sec (90-150 frames)	<3 sec (<90 frames)
Focus	Image sharpness in the region of interest	Good focus for all vessels (small and large) in the entire field of view. Plasma gaps and red blood cells are visible.	<1/2 field of view is out of focus, or edges of the vessels are slightly out of focus.	Video is completely out of focus such that no small vessels can be seen.
Content	Determination of the types of vessels and/or presence of occluding artifacts in the image.	Video is free of occlusions. Good distribution of large and small vessels. Less than 30% of the vessels are looped upon themselves.	Video may have a few artifacts. Acceptable distribution of large and small vessels. About 30% to 50% of the vessels are looped.	Most of the field of view has occluding artifacts such as saliva or bubbles. More than 50% of the vessels are looped upon themselves.
Stability	Frame motion that can be adequately stabilized without motion blur	Movement is within 1/4 of the field of view. No motion blur	Movement is within 1/2 of the field of view. No motion blur	Movement is greater than 1/2 of the field of view and/or motion blur in frame.
Pressure	iatrogenic mechanical pressure causing misrepresentation of flow	Flow is constant throughout the entire movie. No obvious signs of artificially sluggish or stopped flow. Good flow in the largest vessels	Signs of pressure (localized sluggish flow in a specific large vessels), but flow appears to be unimpeded based on good flow in most large vessels	Obvious pressure artifacts associated with probe movement and/or flow that starts and stops, reversal of flow. Poor or changing flow in larger venules

B.7.1 | Bonferroni correction

Researchers who publish medical science research usually set the significance threshold for analyses at 5%. This 5% is based on the probability of falsely rejecting the null hypothesis (α, type I error), where there is a 5% chance of falsely concluding that there is an effect (false positive). In a test with one statistic, a p-value < 0.05 rejects the null hypothesis. A type 2 error (β) means not rejecting the null hypothesis even though there is an effect (false negative).[2] When multiple analyses are performed simultaneously, there has to deal with a so-called family-wise error rate (FWER), which describes the probability that at least one analysis produces a false-positive result.[3] Therefore, the alpha (e.g., 0.05) or the p-values themselves must be adjusted to reduce the likelihood of type 1 errors.[4]

The FWER can be calculated as follows:

$$FWER = 1 - (1 - \alpha)^n \quad (1)$$

where α = significance level for a single analysis (typically 0.05), n = number of tests performed

The simplest way to correct for FWER is the Bonferroni correction, in which the significance threshold is adjusted according to the number of tests performed. Where the corrected threshold is described as[3]:

$$Corrected\ threshold = \alpha / n \quad (2)$$

Where α = significance level for a single analysis (typically 0.05), n = number of tests performed

The Bonferroni correction is applied to the p-values of the individual tests so that the α level for all tests remains equal to 0.05.[5]

B.7.2 | Linear mixed models

LMM analysis is a statistical procedure that provides a flexible approach to static analysis with correlated longitudinal data. Longitudinal data is described as repeatedly measured variables for each patient, with time as the repeated factor. In longitudinal data sets, the number of repeated measurements may not be the same for each patient, nor may the observation time have the same spacing or intervals. Therefore, the characteristics of longitudinal data allow for uneven distribution and observations with missing values.[6] In addition to the flexibility of LMMs concerning unbalanced longitudinal data, LMMs have the ability to account for the covariance among repeated measurements in a relatively parsimonious manner.[6] The LMM is described as the integration of two levels (hierarchical) observations (i.e., between and within a subject) into a single model.[7] The model uses fixed (systemic mean patterns, i.e., covariates of specific scientific

interest) and random (correlation patterns between repeated measurements within patients and heterogeneity between patients or both) effects.[8, 9]

The LMM can be considered as an extension of the standard linear model and is represented as[6, 10]:

$$Y_i = X_i\beta + Z_i b_i + \epsilon_i \quad (3)$$
$$b_i \sim N(0, D)$$
$$\epsilon_i \sim N(0, \Sigma)$$
$$b_1 \dots b_n, \epsilon_1 \dots \epsilon_n \text{ independent}$$

Where:
Y_i = n-dimensional response vector for patient i
β = p-dimensional vector for fixed effects
b_i = q-dimensional vector for random (subject-specific) effects
X_i and Z_i = $(n_i \times p)$ and $(n_i \times q)$ dimensional matrices relating to observations of the fixed and random effects
ϵ_i = n-dimensional vector of residuals
D = a general $(q \times q)$ dimensional covariance matrix with (i, j) element $d_{ij} = d_{ji}$ and $\Sigma_i(n_i \times n_i)$ covariance matrix (usually the same for all i).

From this model, it follows that marginally:

$$Y_i \sim N(X_i\beta, V) \text{ and } V = Z_i D Z'_i + \Sigma_i \quad (4)$$

With repeated measurements, the residuals are not independent of each other by default. In other words, data points from the same patient are more similar to each other than data points from other patients. When using the LMM, correlations between the residuals of repeated measurements in the same patient are taken into account; this can be done with different correlation structures[11], with the unstructured structure being used in the analyses of this thesis. With the unstructured structure as the correlation structure, no assumption is made about the correlations between two time points.[12]

Formula (3) includes random effects. This involves estimating the deviation that measurements of the same patient have from the regression line. It does not matter how many repeated measurements a patient has and at what times (conditions) they are measured. Random effects assume that all measurements from the same patient have a fixed deviation from the "mean" regression line. However, this is beyond the scope of this master's thesis. The statistical analysis in this master's thesis assumes that all patients were measured at the same times and under the same conditions. In that case, a simplified version of the LMM model (**Formula (4)**) can be applied in which the random effects are not explicitly modeled. The random effects are then included as part of the marginal covariance matrix V.[6, 10]

Appendix C

Measured values

Table C.1 *Number of measured values for each parameter of shock patients (n = 20)*

Variable	T0	T1	T2
	n	n	n
MAP	20	16	9
Lactate	20	16	9
SvO_2	14	9	3
TVD	18	16	8
FCD	18	16	8
PPV	18	16	8
RBCv	18	16	8
cHct	18	16	8
tRBCp	18	16	8

cHct: capillary hematocrit; FCD: functional capillary density; MAP: mean arterial pressure; PPV: proportion of perfused vessels; RBCv: red blood cell velocity; SvO_2: central venous oxygen saturation; tRBCp: tissue red blood cell perfusion; TVD: total vessel density.

Appendix D

LMM estimates of fixed effects

Table D.113 *LMM estimates of fixed effects*

						95 % confidence interval	
Parameter	**Estimate**	**std. error**	**df**	**t**	**sig**	**Lower bound**	**Upper bound**
TVD							
Intercept	19.443	1.425	8.750	13.648	0.000	16.207	22.680
T0	1.686	1.651	11.136	1.022	0.329	-1.941	5.314
T1	1.560	1.366	7.967	1.143	0.286	-1.591	4.712
T2	0[b]	0	.	.	.	.	.
FCD							
Intercept	18.084	1.543	7.550	11.719	0.000	14.489	21.680
T0	1.766	1.701	8.976	1.038	0.326	-2.084	5.617
T1	1.696	1.621	8.422	1.046	0.325	-2.009	5.400
T2	0[b]	0	.	.	.	.	.
PPV							
Intercept	92.892	2.915	7.462	31.862	0.000	86.084	99.700
T0	1.214	2.952	7.661	0.411	0.692	-5.645	8.074
T1	1.192	3.077	8.703	0.387	0.708	-5.805	8.188
T2	0[b]	0	.	.	.	.	.

RBCv							
Intercept	323.474	16.883	8.932	19.160	0.000	285.238	361.711
T0	10.448	17.302	9.184	0.604	0.561	-28.576	49.465
T1	7.397	17.175	8.715	0.431	0.677	-31.651	46.444
T2	0[b]	0	.	.	.	.	.
cHct							
Intercept	5.971	0.410	10.254	14.581	0.000	5.062	6.880
T0	-0.225	0.487	12.804	-0.462	0.652	-1.279	0.829
T1	-0.143	0.245	8.831	-0.583	0.574	-0.698	0.412
T2	0[b]	0	.	.	.	.	.
tRBCp							
Intercept	44.751	6.160	7.554	7.264	0.000	30.398	59.103
T0	1.526	6.362	7.951	0.240	0.816	-13.161	16.213
T1	1.532	6.088	7.466	0.252	0.808	-12.684	15.749
T2	0[b]	0	.	.	.	.	.

[b] this parameter was set to zero because it is redundant; cHct: capillary hematocrit; df: degrees of freedom; FCD: functional capillary density; PPV: proportion of perfused vessels; RBCv: red blood cell velocity; sig: significance level; std: standard; t: how far from zero is the estimate; tRBCp: tissue red blood cell perfusion; TVD: total vessel density.

MICCS: The complex relation between the microcirculation and macrocirculation in circulatory shock after cardiothoracic surgery

Coordinating Investigator
- Dr. M.S. Arbous, Department of Intensive Care and Department of Clinical Epidemiology, Leiden University Medical Center

Principal Investigators
- MSc. F. Brouwer, Department of Intensive Care, Leiden University Medical Center
- BSc. R.V. Toet, Department of Intensive Care, Leiden University Medical Center

Participating Investigators
- Prof. Dr. Ir. C. Ince, Department of Intensive Care, Erasmus Medical Center

Independent Physician
- Drs. T. van Hemel, Department of Intensive Care, Leiden University Medical Center

Institute: Department of Intensive Care of the Leiden University Medical Center, Leiden, the Netherlands

Sponsor: Leiden University Medical Center
Funding: -
ABR number: NL81756
Protocol number: NL81756.058.22
Version: 3.0
Date: 18-10-2022

Contact details
Dr. M.S. Arbous
Department of Intensive Care and Department of Clinical Epidemiology
Location J-04-027, Leiden University Medical Center
Albinusdreef 2
2333 ZA Leiden
Telephone:
Email:

PROTOCOL TITLE: MICCS: The complex relation between the microcirculation and macrocirculation in patients after cardiothoracic surgery with and without circulatory shock

Protocol ID	NL81756.058.22
Short title	The MICCS study
Version	3.0
Date	18-10-2022
Coordinating investigator	Msc. F. Brouwer Leiden University Medical Center Department of Intensive Care Albinusdreef 2, 2333 ZA Leiden, the Netherlands
Principal investigator	Dr. M.S. Arbous Leiden University Medical Center Department of Intensive Care and Department of Clinical Epidemiology Albinusdreef 2, 2333 ZA Leiden, the Netherlands
Participating investigators	Prof. Dr. Ir. C. Ince Erasmus Medical Center Department of Intensive Care Dr Molewaterplein 40, 3015 GD Rotterdam, the Netherlands
	Bsc. R.V. Toet Leiden University Medical Center Department of Intensive Care Albinusdreef 2, 2333 ZA Leiden, the Netherlands

Sponsor	Leiden University Medical Center Albinusdreef 2, 2333 ZA Leiden, the Netherlands
Subsidising party	Erasmus Medical Center Dr Molewaterplein 40, 3015 GD Rotterdam, the Netherlands
Independent expert	**drs. T. van Hemel** Leiden University Medical Center Department of Intensive Care Albinusdreef 2, 2333 ZA Leiden, the Netherlands

Protocol signature sheet

Name	**Signature**	**Date**
Principal **investigator**		

Table of Contents

List of abbreviations and relevant definitions

(S)AE	(Serious) Adverse Event
AE	Adverse Event
APACHE	Acute Physiology and Chronic Health Evaluation
AVA	Automated Vascular Analysis
AVG	General Data Protection Regulation
BP	Blood pressure
	but does not commission it is not regarded as the sponsor, but referred to as a
CE	Conformité Européene, declaration that the medical product complies with the essential requirements of the European Directive
CI	Cardiac index
CKCL	Central clinical chemistry laboratory
CKHL	Central clinical hematological laboratory
CO	Cardiac output
CPB	Cardiopulmonary bypass
CRP	C-reactive protein
CRP	C-reactive protein
DSMB	Data Safety Monitoring Board
EPD	Electronic patient dossier system
FCD	Functional capillary density
FiO_2	Fractional inspired oxygen
GDPR	General Data Protection Regulation
HR	Heart rate
HVM	Handheld vital microscope
ICH-GCP	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use - Good Clinical Practice guideline
ICU	Intensive Care Unit
IDF	Incident dark field
LED	Light Emitting Diode
LUMC	Leiden University Medical Center
MAP	Mean arterial pressure
MAP	Mean arterial pressure
METC	Medical research ethics committee (MREC); in Dutch: medisch ethische
NFU	Nederlandse Federatie Universitair Medisch Centra
NICE	National IC Evaluation
OPS	Orthogonal polarised spectrum
OPS	Orthogonal Polarization Spectral
PaO_2	Arterial oxygen tension
PPV	Proportion of perfused vessels
PVV	Proportion of perfused vessels
RBC	Red blood cell
RBCv	Red blood cell velocity
SaO_2	Arterial saturation
SBP	Systemic blood pressure
	scientific organization or investigator. A party that provides funding for a study

SDF	Sidestream dark field
SOFA	Sequential Organ Failure Assessment
Sponsor	The sponsor is the party that commissions the organization or performance of
SPSS	Statistical Package for the Social Sciences subsidizing party.
SUSAR	Suspected unexpected serious adverse reactions
	the research, for example a pharmaceutical company, academic hospital,
	toetsing commissie (METC)
TVD	Total vessel density
UFT	Ultra-fast track
UI	User Interface
UP	Urine production
WMO	Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

Summary

Rationale: Nearly all patients after cardiothoracic surgery are admitted to the ICU. Post cardiotomy circulatory shock complicates 1-2% of all adult cardiothoracic procedures. In these patients changes in the microcirculation occur frequently. Causes of these changes in the microcirculation can be tissue trauma, activation of the inflammatory response and haemostatic system, anaesthesia, hypothermia and formation of micro-embolisms. An impaired microcirculation results in a decreased tissue oxygenation or even organ damage if a situation of low tissue oxygenations persists. [1 2]
Monitoring on the Intensive Care Unit (ICU) is mainly focused on macrocirculatory parameters, such as blood pressure, cardiac output and peripheral oxygen saturation. But increasingly also the microcirculation is measured in critically ill patients. The hemodynamic ***coherence*** between the macrocirculation and the microcirculation is the condition under which resuscitation procedures aimed at correcting macrocirculatory parameters are also effective in correcting microcirculatory perfusion and cellular oxygenation. Different studies in patients with sepsis or septic shock show that a loss of hemodynamic coherence can occur. This means that during resuscitation, macrocirculatory parameters improve to normal values while microcirculation is still impaired, a condition that is associated with increased morbidity and mortality [3]. This loss of coherence may also be present in patients after cardiothoracic surgery. Routinely measuring the microcirculation with the CytoCam may therefore be valuable with respect to better monitoring patients after cardiothoracic surgery, better understanding the specific type of shock and being better able to install therapeutic measures.

Primary Objective:
In this study, we aim to investigate the postoperative **time course of microcirculatory** parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock.

Secondary objectives:

- To study the postoperative **time course of macrocirculatory** parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock.
- To investigate the relationship (i.e. coherence) between microcirculatory parameters and macrocirculatory parameters in patients admitted to the ICU after cardiothoracic surgery with and without circulatory shock.
- To study the relationship between the microcirculation and vital organ (dys)function, particularly the need for vasopressors and/or inotropic therapy or duration of mechanical support.
- To study the relationship between postoperative microcirculatory parameters and clinical outcomes (i.e. acute kidney injury, the need for continuous veno-venous hemofiltration, length of stay ICU and ICU mortality).

Study design: prospective single-centre cohort study.

Study population: patients which are admitted to the ICU after cardiothoracic surgery.

Main study endpoints:

Primary endpoint:

- The time course of microcirculatory parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock.

Secondary endpoints:

- The time course of microcirculatory parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock
- The coherence between the macrocirculation and microcirculation in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock. Coherence meaning whether changes in microcirculatory parameters are congruent with (in the same direction of) expected changes in the microcirculation.
- The relationship between the microcirculation and vital organ (dys)function, particularly the need for vasopressors and/or inotropic therapy or duration of mechanical support.
- The association between microcirculation and clinical outcomes (i.e. acute kidney injury, the need for continuous venovenous hemofiltration, length of stay ICU and ICU mortality

Study procedure: Included patients will undergo treatment after cardiothoracic surgery according to current clinical practice. Microcirculation measurement with the CytoCam will be performed as soon as possible after ICU admission (within 3 hours (T0)), then within 24 hours (T1) after admission to the ICU, and if possible >48 hours (T2) after T0. No delay of treatment will take place. Data collection, including circulatory, respiratory and inflammatory data, as well as data of blood samples and fluid balance, will occur at the same time as the microcirculatory measurements. Simultaneously with T0, demographic data will be collected.

If a patient develops shock (a condition in which there is a lack of effective circulating volume resulting in insufficient tissue perfusion) or the need to receive mechanical support while on the ICU, microcirculation measurements will be measured according to the current Shock protocol (Shock-protocol bij volwassene (medisch beleid IC Volw.) (Versie 2))

Nature and extent of the burden and risks associated with participation, benefit and group relatedness: The risks are negligible in this study with no serious adverse events known. The burden for the patients is minimal since it is a minimal invasive measurement. The normal clinical practice will continue and will not be altered and will be executed according to prevailing practice, protocols and guidelines.

1. Introduction and rationale

Nearly all patients after cardiothoracic surgery are admitted to the ICU [4]. Post cardiotomy circulatory shock complicates 1-2% of all adult cardiothoracic procedures [5]. This condition is associated with increased morbidity and mortality in the ICU.[6,7] The primary function of the circulation is to deliver oxygen and nutrients to the tissue cells and remove waste products[8]. This exchange takes place in the microvasculature of the tissues, where oxygen passively diffuses from red blood cells into tissue cells. Under normal conditions, oxygen delivery exceeds oxygen demand. In a condition of circulatory dysfunction, for example during a state of circulatory shock, the systemic circulation fails to meet the perfusion requirements (i.e. oxygen requirements) of organs[9]. Inadequate delivery of oxygen to tissue cells leads to organ dysfunction and if it persists, to organ failure [8,10].

Microcirculatory disturbances, due to abnormalities in capillary density and heterogeneity in blood flow, and consequent reduction in tissue oxygenation are often described in critically ill, cardiothoracic, patients. [1] These disturbances are caused by many factors such as the surgery itself, activation of inflammatory response and haemostatic systems, anaesthesia, hypothermia, haemodilution, micro-emboli formation and tissue trauma.[1]

One of the main goals is to prevent or treat alterations in macrocirculation during and after cardiothoracic surgery. [11] However, normalization of macrocirculatory parameters, such as blood pressure and venous oxygen saturation, does not always mean a parallel improvement of microcirculatory parameters (e.g., functional capillary density (FCD) and proportion of perfused vessels (PPV), explanation of the microcirculatory parameters can be found in Appendix 1) and therefore no guarantee of adequate microcirculatory perfusion.[12,13] This discrepancy between macrocirculation and microcirculation is known as a loss of hemodynamic coherence, a condition in which during resuscitation, microcirculatory parameters improve to normal values while the microcirculation remains impaired (Microcirculatory alterations associated with loss of hemodynamic coherence can be found in Appendix 1). [3]

The microcirculation can be monitored sublingually with hand-held vital microscopes (HVM). The first generation microscopes were based on orthogonal polarization spectral (OPS) imaging [14,15]. These devices were improved and replaced by HVM based on sidestream dark field (SDF) imaging [16,17]. The third and latest generation HVM is based on incident darkfield imaging (IDF)[18]. From studies performed in patients with sepsis and septic shock, where microcirculation was monitored with HVM, we know that hemodynamic coherence is often lacking, which can result in failure of treatment and ultimately in an increased mortality[19-22].

An abundance of literature discussing macro- and microcirculation in patients with sepsis or septic shock is available. However, the number of studies performed in patients after cardiothoracic surgery is much smaller. During surgery, microcirculatory perfusion may be severely impaired as a result of no pulsatile blood flow through cardiopulmonary bypass (CPB), decreased cardiac output, hemodilution, hypothermia, and CPB- and tissue trauma-induced inflammation.[23] As a result, areas of the microcirculation can become shunted and hypoxemic, eventually leading to organ failure.[24,25] The number of studies describing circulatory shock after cardiothoracic surgery in relation to the microcirculation is again much smaller than the number of studies on microcirculation and cardiothoracic surgery. A comparison of the microcirculation of cardiothoracic surgery patients who develop shock and those who do not, has scarcely been described, if at all. Monitoring the sublingual microcirculation may therefore be valuable to assess the existence of coherence between macro- and

microcirculation and to provide additional information regarding the specific type of shock and provide more leads to individualize care.

2. Objectives

Primary objective:
In this study, we aim to investigate the postoperative time course of microcirculatory parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock.

Secondary objectives:

- To study the postoperative **time course of macrocirculatory** parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock.
- To investigate the relationship (i.e. coherence) between microcirculatory parameters and macrocirculatory parameters in patients admitted to the ICU after cardiothoracic surgery with and without circulatory shock.
- To study the relationship between the microcirculation and vital organ (dys)function, particularly the need for vasopressors and/or inotropic therapy or duration of mechanical support.
- To study the relationship between postoperative microcirculatory parameters and clinical outcomes (i.e. acute kidney injury, the need for continuous veno-venous hemofiltration, length of stay ICU and ICU mortality).

3. Study design

So far, only a few microcirculation studies have been performed in patients after cardiothoracic surgery. To get insight in the changes in the microcirculation of patients after cardiothoracic surgery in the postoperative period, we will perform an observational single-centre study. The study population will consist of patients admitted to the ICU after cardiothoracic surgery.

Each patient, whether or not in circulatory shock, who is admitted to the ICU after cardiothoracic surgery will be monitored according to the current clinical guidelines. Macrocirculation will be measured continuously and according to current clinical practice. Microcirculation measurement with the CytoCam will be performed as soon as possible after ICU admission (within 3 hours (To)), after 24 hours (T1) after admission to the ICU, and if possible after 48 hours (T2) after To. No delay of treatment will take place and blood samples will be taken according to clinical practice, no extra blood will be taken nor extra measurements will be performed for the purpose of this study. Data collection, including circulatory, respiratory and inflammatory data, as well as data of blood samples and fluid balance, will be as much as possible synchronized with the microcirculatory measurements. Simultaneously with To, demographic data will be collected.

Circulatory shock is defined as:

Clinical signs of decreased tissue perfusion):
- Cold, clammy extremities
- Nausea and vomiting
- Urine production < 0.5 ml/kg/hour

- Neurological symptoms, such as confusion and/or decreased consciousness

Hemodynamic signs:
- Hypotension:
Systolic blood pressure (SBP) < 90 mmHg > 30 min or use of vasopressors (≥ 0.2 y/kg/min norepinephrine and/or 5 y/kg/min dobutamine > 1 h) to achieve an SBP > 90 mmHg or a mean blood pressure (MAP) > 65 mmHg.

Biochemical signs:
- Hyperlactatemia (> 2 mmol/l).

When a patient is in circulatory shock, hemodynamic monitoring, will take place according to the current clinical Shock protocol (Shock-protocol bij volwassene (medisch beleid IC Volw.) (Versie 2)). In this protocol the microcirculation is also assessed in patients in shock.

For the purpose of this study the microcirculation will also be assessed in cardiothoracic patients, postoperatively, not in shock.

For the purpose of this study all cardiothoracic patients will be asked informed consent preoperatively.

The follow-up period of included patients will be 2 months. During the study, nurses and clinicians will be blinded for the results of the microcirculation measurements.

Appendix 2 presents study design, measurements and outcomes for the prospective cohort study and figure 1 shows an overview of the study design.

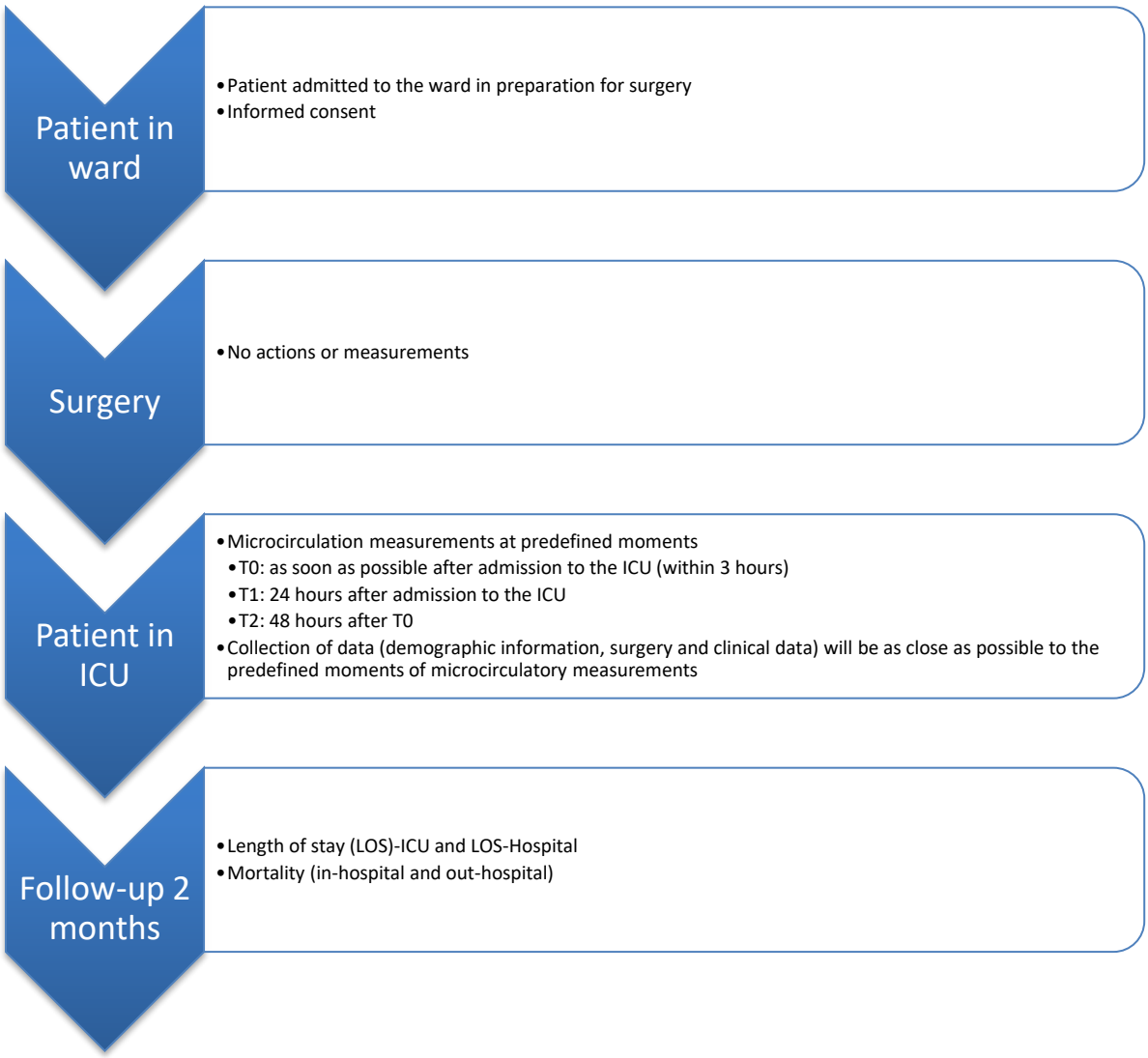


Figure 1 Overview of the study design

4. Study population

4.1 Population (base)

Patients who are admitted to the ICU of the Leiden University Medical Center (LUMC) after cardiothoracic surgery, will be the source population of the study. Patients admitted to the ICU after their cardiothoracic surgery according to the ultra-fast track (UFT) protocol (Ultra-Fast Track (UFT) cardiothoracale chirurgie, zorgpad (Versie 2)) will also be included in the study as far as logistically possible.

4.2 Inclusion criteria

Patients meeting all these criteria will be included in the study:

- Age of patient is at least 18 years
- Patients are admitted to the ICU after cardiothoracic surgery

4.3 Exclusion criteria

Patients meeting one of these criteria will be excluded from the study:

- Patients with maxillofacial trauma
- Patients known with tumor(s) in the mouth or throat area

4.4 Sample size calculation

Many studies reported that resuscitation based on het normalization of microcirculatory parameters does not always lead to parallel improvements in microcirculatory perfusion. When the shock improves at the macrocirculatory level, it does not necessarily mean that there has also been an improvement on the microcirculatory level. Many of these studies focused on the septic patient population. Most of these studies included approximately 100 patients.

Greenwood et al. powered their study on the smallest difference between pre- and postoperative perfused vessel densities (PVD) of cardiac surgery patients and between the highest and lowest quartile of postoperative SOFA scores using microcirculatory data from previous studies. To maintain a power of 80% to detect significant differences in capillary density, flow and SOFA scores, 18 patients had to be included in this study. In the end, they decided to increase the number to 25 patients to account for a variable magnitude of the effect.

Another study by Massey et al. conducted a sample size estimation to determine the impact of resuscitation protocols on microcirculatory perfusion of septic shock patients. This sample size calculation used the estimated mean Microvascular Flow Index (MFI), the 'main' microcirculatory perfusion parameter reported at the time, for standard care, assuming that patients with protocolized goal-directed resuscitation had a 20% higher mean MFI than the mean MFI of standard care. With a power of 0.9, the estimated sample size was 114 participants. To calculate the association between MFI and mortality, it was estimated that the odds ratio (OR) of mortality would increase by 50% for every 0.5 of a standard deviation (SD) decrease in MFI. With a type 1 error of 0.05 and a power of 0.9, the estimated sample size was 115 participants.

Taking into account that for our research, sample size is very difficult, but based on above consideration we decided that to focus on the smallest difference wouldn't be appropriate and thus a number of 25 patients would be too small. Furthermore, as our study is not focused on the relationship between the outcome, our estimated sample size will be 100 patients. With this number of patients, who will have sequential measurements, we hope to see a tend in time and a difference between the microcirculation of patients with and without shock (as this is our main objective and endpoint).

5. Treatment of subjects

Subjects admitted to the ICU will be managed according to current treatment guidelines of the ICU of the LUMC. This includes intravascular access (arterial line, central venous and/or Swan-Ganz catheter, pacemaker wires and thoracic drains), postoperative medications, hemodynamic and ventilation management. The research team will not impose any intervention in the management of the subjects, besides the measurements of the microcirculation as described in Appendix 2.

5.1 Investigational product: CytoCam

Microcirculation measurements are performed using a CytoCam (Braedius Medical, Huizen, The Netherlands). A CytoCam is a sublingual microcirculation measurement device which consists of a monitor and a handheld vital microscope (HVM). The technique which is used in the CytoCam relies on the Incident darkfield illumination (IDF) imaging technique. This technique uses green light of a specific wavelength produced by a ring of circumferential light-emitting diodes at the end of a light guide, with a magnifying lens in the centre. The green light is transmitted to the tissue and absorbed by haemoglobin, causing the red blood cells (RBCs) to appear as dark spheres (Figure 2). The imaging results in a sharp contour visualization of the microcirculation, showing flowing RBCs and leukocytes, captured by a high-definition image sensor. The recorded videos can be analysed offline with the software programs Automated Vascular Analysis (AVA) and MicroTools.

This IDF technique was first published in 1971 [26] and has since been validated as bedside monitoring for the microcirculation.[27] In this study, the CytoCam is only used to monitor the microcirculation for research purposes, any results from the measurements are not used to treat the patient or change treatment based on the information provided by the CytoCam. The device is CE (Conformité Européene) marked as a medical device (TÜVRheinland® certificate number MK 69245350 0001, initially issued on 14 October 2013).

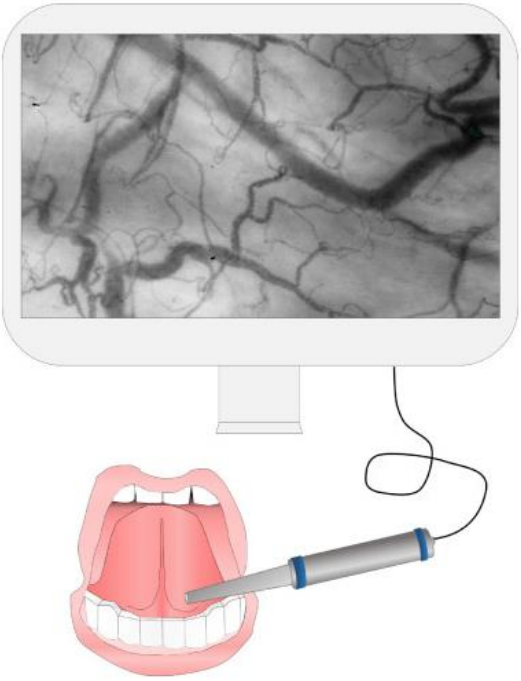


Figure 2 Example of a recording of the sublingual microcirculation with a CytoCam

Reliable measurements can only be performed if the patient's mouth is clean and dry. Therefore, the sublingual area will be carefully cleaned with a suction or gauze swab. No other intervention is required before the measurements with the CytoCam.

The tip of the microscope is protected with an unsterile disposable cap perpendicular to the sublingual are of interest. The disposable cap (H & P Moulding Emmen B.V., Emmen, The Netherlands) is based on the use of ALTUGLAS® SG-7 (Arkema, Colombes, France), a hard transparent plastic (Appendix 3). During each timepoint, three videos of at least 3 seconds will be recorded from different sublingual areas. According to international guidelines[18], it is necessary to capture multiple recordings in order

to minimize heterogeneity in the microscopic field of view. Videos will be excluded from further analysis if they do not meet image quality requirements such as illumination, image duration, focus, vessel content, stability, and absence of probe-induced pressure.[18,28] After every three measurements of the same patient at every timepoint, the disposable cap will be discarded and the microscope itself and the other components of the CytoCam will be cleaned according to the cleaning guidelines for medical devices in the ICU. In this case, the CytoCam will be cleaned with 70% ethanol after each use. In the case of visible dirt, the dirt will be cleaned with soap and water prior to the application of 70% ethanol.

5.2 Use of co-intervention

There are no restrictions on co-medication or co-interventions for the subjects in this study.

5.3 Escape medication

No escape medication is required for this study

6. Investigational Product

6.1 Name and description of medical device

Braedius Medical B.V. has developed an innovative minimal-invasive bedside monitoring system, the CytoCam, to measure sublingual microcirculation. The device provides high quality videos of the sublingual microcirculation suitable for data analysis [27,29]. The CytoCam measurement system consists of a monitor and a handheld microscope. (Figure 3) The CytoCam bedside monitor includes a software package CytoCam Tools for capturing, playing and analysing videos. CytoCam Tools includes a user interface (UI) to operate the CytoCam via a desktop or all-in-one computer. During operation, the user enters patient and study information into the dialog box. Afterward, the user can navigate between the controls for focusing and brightness of the light-emitting diodes (LEDs) and recording a video sequence. In addition, the system has a modular design, allowing the user to add modules for image stabilization, video editing and analysis.[30]

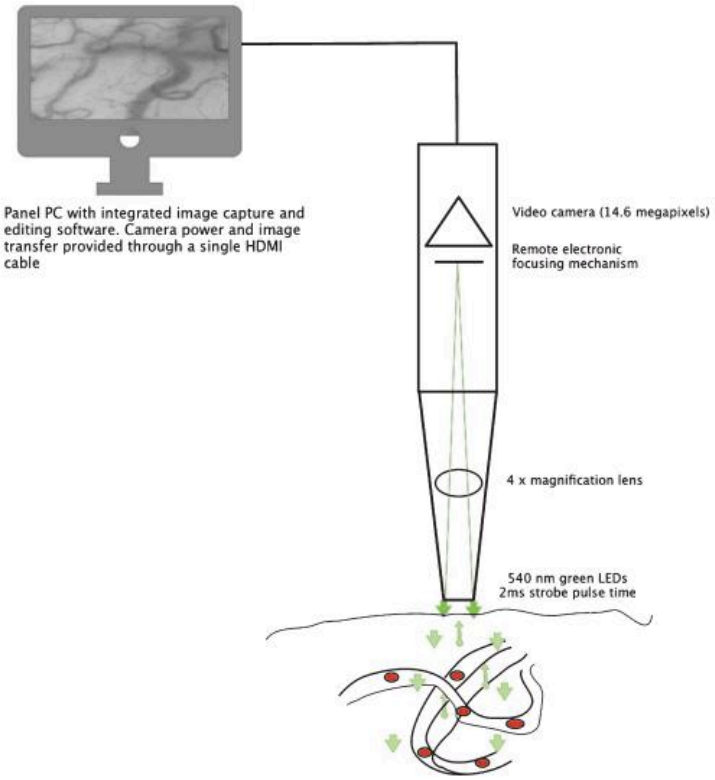


Figure 3 Working mechanism of the CytoCam

6.1.1 The measurement specifications:

The CytoCam measures, among other things, blood flow through the microcirculation. The working mechanism of the microscope is based on IDF microscopy imaging. The IDF imaging technique uses green light of a specific wavelength produced from a ring of circumferential light-emitting diodes at the end of a light guide, with in the middle a magnifying lens to illuminate the target tissue tangentially. The illumination light is excluded from the central column of the microscope. The green light is transmitted to the tissue and absorbed by oxygenated and deoxygenated haemoglobin, which causes RBCs to appear a dark globules. [26] The imaging results in sharp contour visualization of the microcirculation, showing flowing RBCs and leukocytes captured by a high-definition image sensor. Plasma and leukocytes in the blood vessels cause luminous openings in the image[31].
Blood vessels appear as black lines on white/grey background. The recorded videos can be analysed offline with the software programs AVA and MicroTools.

The key specifications of the CytoCam are[18]:

- Illumination unit: based on IDF imaging with a 4x magnification lens.
- Illumination light: emitted with a short pulse time of 2ms and a chosen wavelength of 548nm.[18]

- The imaging probe (microscope) must be connected to the monitor before performing a measurement.
- The IDF handheld vital microscope captures images at a rate of 25 frames/s, of which 100 frames are recorded as a single video clip.
- Time to result: 8 seconds (minimum recording time is 3 seconds, max recording time is 16).
- Simple handling: the use of the CytoCam is not difficult but performing measurements should be practiced to acquire recordings of good quality (after performing 50 measurements, one can be considered as 'experienced'). For the purpose of this study all personnel applying the CytoCam is trained and certified by an experienced trainer of the Microcirculation Academy.

6.1.2 Current stage of development:

The CytoCam has been developed based on all applicable regulations, standards (Medical device directive 93/42EEC, IEC60601-I, ISO 14971) and has been tested in preclinical studies.

Before the development of IDF, in the early 20th century, direct intravital observation of the microcirculation was limited to the use of bulky capillary microscopes, which were used primarily to determine the microcirculation of the capillary nail bed.[27]

In 1971, the IDF technique was first described.[26] This method made it possible to observe the microcirculation of an organ surface using epi-illumination, without the need for transillumination of the tissue from below. In the late 1990s, orthogonal polarization spectral (OPS) imaging, a technique similar to IDF, was added to a handheld video microscope to capture the organ surface microcirculation of surgical patients.[15,32,33] This new OPS technique made it possible for the first time to study human microcirculation in tissue and organ surfaces at the bedside, especially in critically ill patients. OPS used linearly polarized light, where the reflected light was blocked by an orthogonally polarized analyzer.[15,27,34] Next, an HVM with side stream dark-field (SDF) imaging was released. The SDF HVM uses a circularly illuminated tip with light-emitting diodes that create a dark-field.[16] The difference between SDF and IDF HVMs lies in the improved optical resolution, making it possible to visualize more capillaries than its OPS and SDF predecessors.

The CytoCam with IDF imaging is a third-generation handheld microscope. It uses a new hardware platform. This platform includes a high-density pixel-based imaging chip and a short, pulsed illumination source which synchronizes and controls illumination and image acquisition under computer control. The IDF technique is now commercially available.[18,27]

In recent years, many different studies have been performed on microcirculation measurements using an HVM. Mainly in the sublingual area in a wide range of disease states and age groups [35-42], but also several studies of microcirculation performed directly on organ surfaces during surgery. [32,43-45]

6.2 Summary of known and potential risks and benefits

Measuring the microcirculation is minimal-invasive and adverse and serious adverse events are rare. Further information on safety and adverse events can be found in section 9.

6.3 Description and justification of route of administration and dosage

Not applicable as no administration takes place.

6.4 Method of administration

Not applicable as no administration takes place.

6.5 Preparation and labelling of medical device

In accordance with the Dutch 'Kwaliteitswet zorginstellingen', the medical device used for this study has to be controlled for multiple aspects like safety and quality before it can be used in the hospital. This is done by a department in the hospital, called health technology. The CytoCam has already been inspected by this department and received the label of approval for use in the hospital.

7. Non-investigational product

7.1 Name and description of non-investigational product(s)

Name of device	Manufacturer	Model	CE conform 93/42/EEG of EU/2017/745 (Medical devices) OR 98/79/EEG of EU/2047/746 (in-vitro diagnostics)	Conform intended use as described in the instruction manual?
Macrocirculation				
Ventilation	Hamilton	C6	Yes, conform 93/42/EEC	Yes
Cardiac output meter	Edwards Lifesciences	Hemosphere	Yes, conform 93/42/EEC	Yes
Pulse oximeter/ blood pressure/ arterial line/ ECG	Philips	Intellivue MX750 with X2 Multi-Measurement Module (simultaneous monitoring of ECG, respiration, arterial blood oxygen saturation (SpO_2), non-invasive blood pressure (NBP), invasive blood pressure and temperature, or CO_2) and flexible module server FMX4. Pressure module for arterial line (M1006B), Cbl 5-lead Grabber chest IEC ICU (M1978A)	Yes, conform 93/42/EEC	Yes
Arterial/venous blood gas device	Siemens	Rapidpoint 500	Yes, conform 93/42/EEC	Yes
Thermometer	Philips	C400-10HP	Yes, conform 93/42/EEC	Yes
Microcirculation				
CytoCam	Braedius medical	CC01-4MLG	Yes, conform 93/42/EEC	Yes

7.2 Dosages, dosage modifications and method of administration

Not applicable

7.3 Preparation and labelling of Non Investigational Medicinal Product

Not applicable

8. Methods

8.1 Study procedures

The study starts at the Intensive Care department of the LUMC. Each patient who is admitted to the ICU will be monitored according to the current guidelines for post-operative care after cardiothoracic surgery and will be sought for informed consent before surgery. After having given written informed consent to participate in the study, each patient will be assigned a unique study number and included in the study if they meet the study inclusion criteria. Microcirculation measurements will be performed as described in section 3. During this period, patients will receive treatment according to the standard clinical practice in the hospital. There will be no delay of treatment. No extra measurements or blood samples will be taken for the purpose of this study.

At each microcirculation measurement, data will be collected from blood samples and clinical data will be noted, both as close in time as possible to the microcirculation measurement. A complete overview on what data is collected can be found in Appendix 2. The blood samples from which the data is collected are taken according to standard clinical practice, so no additional blood sampling and/or interventions are performed for the purpose of this study. Experience has shown that in ICU patients, blood is drawn so regularly that a blood sample is always taken near the times when the microcirculation measurements are made. Thus, there is absolutely no need for additional blood sampling. All parameters needed for this research will be extracted from the hospital's electronic patient dossier system (EPD).

The recordings from the microcirculation measurements can only be analysed offline. The software (AVA or MicroTools) is not available on the computers in the LUMC, so analysis of the recordings needs to be done in the Erasmus MC. The microcirculation recordings are stored on a secured hard disk of the LUMC and coded according to the currently applicable privacy guidelines (EU General Data Protection Regulation (GDPR) and the General Data Protection Regulation (AVG). The hard disk can only be accessed with a code. Only two investigators (F. Brouwer and R.V. Toet) know this code. The data are transferred on this secured hard disk to Erasmus MC, where offline analysis of the data takes place on a PC, by one of the researchers involved in the study. On completion of the analysis, the data are uploaded onto the secured hard disk and returned to LUMC. No data will remain on the PC or on the Erasmus MC network. So there is no data exchange with Erasmus MC.

8.1.1 Clinical Care

Patients will be monitored and treated according to current protocols and guidelines of the ICU of the LUMC. It is standard clinical practice that all patients receive a central venous (*vena jugularis*, incidentally *vena subclavia, vena femoralis*) and an arterial (*arteria radialis, arteria brachialis, arteria femoralis*) catheter before start of surgery. Thus, the study population admitted to the ICU directly from the operating room will always have these catheters in situ. Therefore, taking blood samples for standard care will be non-invasive for these patients and is performed according to standard clinical practice. Since all patients admitted to the ICU after cardiothoracic surgery have a central venous catheter in place, $ScvO_2$, PvO_2, and $PvCO_2$ can be assessed. No extra blood samples will be collected for this study. All clinical data on blood counts are from blood samples collected for standard clinical practice.

8.2 Data collection

At admission to the ICU, baseline data will be gathered. Appendix 2 shows an overview of the data collection. First of all, demographic data will be collected: age in years, gender, weight, height, and comorbidities (acute and chronic) based on the National IC Evaluation (NICE). Also, Acute Physiology and Chronic Health Evaluation IV (APACHE-IV) will be assessed. Data from the surgery such as type of surgery, duration of surgery, anaesthesia, CPB, or aortic cross clamp time are also collected. The demographic data can be obtained from the EPD.

In addition to the patient's demographics, clinical data will be assessed using the EPD. For example, *circulatory* (for example heart rate (HR) and mean arterial pressure (MAP)), *respiratory* (for example respiration rate and fraction of inspired oxygen (FiO2)) and *infectious* (for example C-reactive protein (CRP) and temperature) parameters will be collected. Additionally, fluid balance, urine production (UP) and SOFA score will also be collected. These parameters will be assessed at the same times (or as close as possible) as the microcirculation measurements. The measurements are already done, as part of the daily ICU management and will be collected using the EPD. Other data, if applicable, collected from the EPD are mechanical ventilation settings, vasopressor/inotrope pump settings, PaO_2/fractional inspired oxygen (FiO_2) ratio, cardiac output (CO) and cardiac index (CI).

In addition to clinical characteristics and the microcirculation measurements, data of blood samples collected for standard clinical practice are used for this study.

Appendix 2 gives an overview of measurements part of standard care, and which of those measurements are part of the study. It also includes a time frame with associated clinical data for each category.

Information regarding ICU stay, hospital stay, and mortality (ICU and hospital) will be available in and collected from the EPD. The follow-up period will be two months. All data will be entered after validation in a study database for subsequent tabulation and statistical analysis. The data will be handled confidentially and coded.

Patients re-admitted to the ICU and already participating in this study will only be followed according to the study protocol. Thus, re-admitted patients will not be included for the second time.

8.2.1 Sample handling and Measurements

Microcirculation is measured in the sublingual area at the predefined moments, see section 3.
In order to be able to make comparisons between different parts of the body, data of blood samples and other parameters will be collected at the predefined moments. Blood samples for standard clinical practice will be collected through an arterial catheter and a central venous catheter. If these catheters are not present in the patient, no blood will be collected through a venous and/or arterial puncture. As can be seen in appendix 2, all the measurements, except the microcirculatory measurements for patients not in shock, are part of standard care which minimalizes the burden for the patient.

8.2.2 Blood samples

In addition to clinical characteristics, data of blood samples will be gathered (appendix 2). If possible, these measurements will be synchronized with the microcirculatory measurements. If no blood sample is needed for standard clinical practice at the time of microcirculation measurement, the blood

sample that is closest by (in time, maximum of 1 hour before and 2 hours after timepoint) will be used for analysis. The collected blood will be immediately transported via the regular transport tubing system to the central clinical chemistry laboratory (CKCL) and central clinical hematological laboratory (CKHL) department for analyses.

8.2.3 Other measurements

Cardiac Index (CI) is also measured on clinical indication using a minimally invasive method such as FloTrac. This measurement can be performed using the already placed arterial catheter, which is placed according to standard ICU care.

8.3 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so. The investigator can decide to withdraw a subject from the study for urgent medical reasons. These subjects will not be subjected to follow-up.

9. Safety reporting

9.1 Section 10 WMO event

If something occurs in which the disadvantages of participation are significantly greater than foreseen in advance in the research proposal, in accordance with section 10, paragraph 1 of the WMO, the investigator will inform the subjects and the reviewing accredited METC. The study will be suspended pending re-evaluation by the accredited METC, except when suspension of the study causes the health of the subjects to be at risk.

9.2 Possible adverse and serious adverse events

Microcirculation measurements are highly minimal invasive and therefore, adverse and serious adverse events are extremely rare and unreported in the literature.

9.3 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether considered related to the investigational product. All adverse events reported spontaneously by the subject or observed by the investigator, or his staff, will be recorded. The possible adverse effects of this study, such as dental damage, will be monitored during the study by not only the researcher, but also by the physician responsible for the patient, nurse and (representative of the) patient. In case of a possible adverse effect, the study team will be notified and decision of continuation of study will be made by the shared decision of the study team, physician, nurse and (representative of the) patient. In case of discomfort for the patient, the study will be directly stopped. The data collected up to that point will be used for the analysis. No additional data will be collected.

9.4 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- Results in death;
- Is life threatening (at the time of the event);
- Requires hospitalization or prolongation of existing in patients' hospitalization;
- Results in persistent or significant disability or incapacity;

Any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator. An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events. Patients on the ICU are critically ill and therefore more prone to SAEs. ICU mortality in the Netherlands is approximately 8.4%. Thirty-five percent of the patients are in need for vasopressor therapy, and 46% of the patients are mechanically ventilated. Furthermore, the National Intensive Care Evaluation Report of 2015 reports that the mean ICU admittance duration is 3 days with a mean in hospital admittance duration of 13 days. Around 80% of the total ICU patients have a low APACHE IV score, and therefore a mortality chance of less than 30%.[46] Thus, SAEs in relation to underlying disease and expected SAEs during the ICU course will not be reported.

The coordinating investigator will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in

death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.5 Suspected unexpected serious adverse reactions (SUSARs)

There is no administration of a dose of an investigational product in this study. Therefore, no SUSARs are expected during this study.

9.6 Annual safety report

In addition to the expedited reporting of SUSARs, the coordinating investigator will submit, once a year throughout the clinical study, a safety report to the accredited METC. This will be combined with the annual progress report.

This safety report consists of:

- A list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system.
- A report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the CytoCam measurements.

9.7 Follow-up of adverse events

Each adverse event will be followed until it has subsided, or until a stable situation has been reached for the patient. Depending on the adverse event, additional testing or medical intervention may be required and/or referral to the general physician or medical specialist. SAEs will be reported until the end of the study within the Netherlands.

9.8 Data Safety Monitoring Board (DSMB)

No data safety monitoring will take place during the study. Furthermore, the in- and exclusion criteria are optimised to minimize the risk for participants.

10. Statistical analysis

10.1 Primary study outcome (endpoint)

The primary outcome is the postoperative time course of microcirculatory parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock.

10.2 Secondary study outcomes (endpoints)

Secondary study outcomes (endpoints) include:

- The time course of microcirculatory parameters in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock
- The coherence between the macrocirculation and microcirculation in patients admitted to the ICU after cardiothoracic surgery **with** and **without** circulatory shock. Coherence meaning whether changes in microcirculatory parameters are congruent with (in the same direction of) expected changes in the microcirculation.
- The relationship between the microcirculation and vital organ (dys)function, particularly the need for vasopressors and/or inotropic therapy or duration of mechanical support.
- The association between microcirculation and clinical outcomes (i.e. acute kidney injury, the need for continuous venovenous hemofiltration, length of stay ICU and ICU mortality

10.3 Statistical methods to be employed

Descriptive statistics will be used to characterize the study population. Data will be stated as mean with standard deviations or medians with interquartile ranges for continuous variables, depending on the parametric distribution of the variables, which will be assessed with histograms and normal quartile plots. Categorical variables will be stated as numbers and percentages. Independent sample t-test will be used to describe the difference in microcirculatory and macrocirculatory parameters between the cardiothoracic patients **with** and **without** circulatory shock. Thereafter data will be transformed from a wide format into long format to describe the time course of the microcirculation and macrocirculation parameters over time with graphs. GML repeated measurement methods will be used to analyse the time course of the microcirculation and microcirculatory parameters. Confounders that will be put in the model are: age, sex, APACHE IV score. The association between the microcirculation and vital organ (dys)function and clinical outcomes will be assessed using (logistic) linear regression. The statistical analysis will be conducted using the SPSS (Statistical Package for the Social Sciences) statistical package, release 23.0 (SPSS Inc., Chicago, IL).

11. Ethical considerations

11.1 Regulation Statement

The study will be conducted according to the principles of the Declaration of Helsinki (accepted by the 64th WMA General Assembly, Fortaleza, Brazil, October 2013) and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and Consent

Before starting this study, the research protocol will be submitted to the METC of the LUMC. This study will not commence until formal approval has been obtained from the METC.

At admission to the cardiothoracic surgery ward, all patients with a scheduled cardiothoracic surgical intervention and scheduled to be admitted to the ICU will be asked for their consent to participate in the study by a member of the research team of the ICU. The subjects will receive oral and written explanation about the study. If the subject is not able to give his or her consent, the oral and written explanation about the study will be given to his or her legal representative according to the Good Clinical Practice Guideline.

11.3 Objection by minors or incapacitated subjects

Section 4, subsection 2 of the WMO stipulates that a legally incompetent adult cannot be forced to undergo a treatment or behave in a particular manner in the context of non-therapeutic research against his or her will. Subjects younger than 18 years are already excluded from the study. If there are adults, that are incapable of giving informed consent, a legal representative will be asked to give informed consent according to the ICH-GCP. When the subject is able to give informed consent after being included in the study by a legal representative and denies any further participation, the subject will be excluded from the study without any consequences. The data collected up to that point will be used for the analysis. No additional data will be collected.

11.4 Benefits and risk assessment, group relatedness

The proposed study aims to investigate the time course of the microcirculation in cardiothoracic surgical patients over time (1) in relation to the development of shock, (2) in relation to the microcirculation. The hypothesis is that by adding microcirculation measurements to macrocirculatory measurements, we will be able in the future to better understand the different types of shock. And maybe even, it might be possible to detect shock at an earlier stage. Both factors may lead, in the future, to more personalized treatment of cardiothoracic patients.

This monitor is already used in clinical practice on the ICU to monitor circulation in ICU patients in circulatory shock (see Shock protocol of the ICU of the LUMC).

12. Administrative aspects, monitoring and publication

12.1 Handling and storage of data and documents

Clinically available data, such as a patient's medical history and diagnoses, as well as clinically generated data during admission, will be used for this study. This data is generated in the ICU of the LUMC. After ethical approval, all necessary patient data will be collected in a database. Data will be coded and all patients will be addressed with a patient identification code. Hereto a coded datafile and a key file will be made, to be able to trace back each individual patient. Both files will be password protected and the password for both files will be changed every three months. Data stored on the computer will use an alphanumeric code to identify the subject. Access to data is restricted to study personnel and when required to the monitor, accredited METC and IGJ as required by law. Essential study documents and data, as well as a data back-up, will be retained for fifteen years. The data back-up will be stored on the network drive of the principal investigator, within the LUMC. To access this network drive, it is required to know the investigator's password, which is changed every three months. Also, it is required to know the passwords to the coded database as well as the key file, which are also changed every three months. All handling of personal data will comply with the EU GDPR and the Dutch Act on Implementation of the General Data Protection Regulation (in Dutch: Uitvoeringswet AVG). Source data on the CytoCam will be coded. To store CytoCam data, it will be downloaded via a hard disk onto a computer memory conforming to the institution's privacy guidelines and the hard disk memory will be erased.

12.2 Monitoring and Quality assurance

The risks associated with this study are low for the subjects (more information can be found in chapter 12 and in annex 4). Therefore, only on-site monitoring is needed, which will happen in accordance with the Nederlandse Federatie Universitair Medisch Centra (NFU) guideline of monitoring. Being labelled as a low-risk study, the monitoring will be according to the NFU guidelines (once yearly) to ensure the integrity and safety of the study participants. The monitor pool of LUMC, which are qualified for monitoring, will monitor this study. The monitor pool is an independent group of data managers or research nurses, who aren't involved in the study in any way other than monitoring.
The research support team is an independent group of data managers, who aren't involved in the study in any way other than monitoring. They will report to the principal investigators and coordinating investigator. The head of ICU department will be notified by the monitor, when the monitor notices frequent or substantial omissions.

12.3 Amendments

All changes must be communicated to the METC that granted the approval. All substantial amendments are reported to the METC and to the competent authority. Non-substantial amendments are not communicated to the approved METC and the competent authority but are recorded and archived by the sponsor.

12.4 Annual progress report

Once a year, the investigator submits a summary of the progress of the study to the accredited METC. Information is provided on the date of inclusion of the first subject, the number of subjects included and the number of subjects who completed the trial, unexpected problems and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the 90th day after the last patients' admittance to the study. In case the study is ended prematurely, the investigator will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

The study can be stopped preliminary in case the study team notices more than 2 SUSARs in 6 months 'time. In this case the study will be directly terminated. No further inclusions will be performed, and no further measurements will be done in patients already included in the study. The accredited METC will be notified within 15 days. The patients already included in the study will be notified about the premature termination of the study by a letter, which will include the reason for the premature termination.

12.6 Public disclosure and publication policy

The study protocol and analysis plan will be published before start of the study on clinicaltrials.gov. The results of the study will find their way into (inter–) national scientific journals and guidelines.

13. Structured risk analysis

In the following analysis, we distinguish potential issues of concern related to the microcirculation measurements.

a. Level of knowledge about mechanism of action

The mechanism of action of microcirculation measurements has been well studied since 1971 [26], and published in several articles[35-42]. With this technique, it became possible to directly observe the sublingual microcirculation of critically ill patients.[47] Several studies have also been conducted to determine normal values in different age groups[48-52].

The first-generation systems, with the OPS technique, are no longer commercially available and have been replaced by the second generation, the SDF technique, and the third generation, the IDF technique. The third generation has an improved optical resolution allowing for better image quality. In addition, it has allowed more capillaries to be imaged simultaneously than with its predecessors.[15,16,26,27,29]

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

In recent years, many different studies have been performed on microcirculation measurements using an HVM, including the IDF technique. Mainly in the sublingual area, in a wide range of disease states and age groups[35-42], but also several studies of microcirculation performed directly on organ surfaces during surgery.[32,43-45]

Moreover, IDF imaging sublingual microcirculation measurements are the only validated minimal-invasive tool to evaluate microvascular perfusion in critically ill people.[53,54]

c. Can the primary or secondary mechanism be induced in animals and/or in ex-vivo human cell material?

Microcirculation research has been performed in animals prior to this research proposal.[55-61]

d. Selectivity of the mechanism to target tissue in animals and/or human beings

Not applicable.

e. Analysis of potential effect

Microcirculation measurements are highly minimal-invasive and therefore, AEs and SAEs are extremely rare and unreported in the literature.

f. Pharmacokinetic considerations

Not applicable.

g. Study population

Patients who are admitted to the ICU of the LUMC after cardiothoracic surgery, **with** and **without** signs of shock, will be the source population of the study. This is a population that is frequently treated on the ICU, nearly 45% of the ICU population exists of postoperative cardiothoracic patients. According to ICH-GCP, a non-therapeutical study can only take place if it has a likelihood of benefit for group represented by the subject. By studying the difference between cardiothoracic patients

with and **without** shock, this study may contribute to better understanding the type of shock and dynamics of developing shock in this population. Often these patients have a combination of shock which evolves in the postoperative period. Macrocirculatory measurements (such as blood pressure and central venous pressure) are frequently not sufficient to discern between different types of shock or to discern yes or no shock on the level of the tissues. Deepening our knowledge on this subject will contribute to tailoring therapy for this critically ill ICU population. And besides the benefit for critically ill patients, this study entails minimal risk and burden for the subjects since it is a minimal-invasive measurement with no known serious adverse reactions.
If no written informed consent is achieved, subjects will be excluded from the study.

h. Interaction with other products
None expected.

i. Predictability of effect
No effect of the measurements is expected.

j. Can effects be managed?
No effect of the measurements expected, but when there is any indication of an effect, such as weak teeth, the measurements can and will be stopped.

14. References

1. Uz Z, Ince C, Guerci P, et al. Recruitment of sublingual microcirculation using handheld incident dark field imaging as a routine measurement tool during the postoperative de-escalation phase—a pilot study in post ICU cardiac surgery patients. Perioperative Medicine 2018;7(1):1-8.
2. Kara A, Akin S, Ince C. The response of the microcirculation to cardiac surgery. Current Opinion in Anesthesiology 2016;29(1):85-93.
3. Ince C. Hemodynamic coherence and the rationale for monitoring the microcirculation. Critical care 2015;19(3):1-13.
4. Stephens RS, Whitman GJ. Postoperative critical care of the adult cardiac surgical patient. Part I: routine postoperative care. Critical care medicine 2015;43(7):1477-1497.
5. Golstein DJ, Oz MC. Mechanical support for postcardiotomy cardiogenic shock. Seminars in thoracic and cardiovascular surgery: Elsevier; 2000:220-228.
6. De Backer D, Creteur J, Dubois M-J, Sakr Y, Vincent J-L. Microvascular alterations in patients with acute severe heart failure and cardiogenic shock. American heart journal 2004;147(1):91-99.
7. De Backer D, Creteur J, Preiser J-C, Dubois M-J, Vincent J-L. Microvascular blood flow is altered in patients with sepsis. American journal of respiratory and critical care medicine 2002;166(1):98-104.
8. Bakker J, Ince C. Monitoring coherence between the macro and microcirculation in septic shock. Current opinion in critical care 2020;26(3):267-272.
9. Jung C. Assessment of microcirculation in cardiogenic shock. Current Opinion in Critical Care 2019;25(4):410-416.
10. Ince C, De Backer D, Mayeux PR. Microvascular dysfunction in the critically Ill. Critical Care Clinics 2020;36(2):323-331.
11. Ocak I, Kara A, Ince C. Monitoring microcirculation. Best Practice & Research Clinical Anaesthesiology 2016;30(4):407-418.
12. Corstiaan A, Lagrand WK, Spronk PE, et al. Impaired sublingual microvascular perfusion during surgery with cardiopulmonary bypass: a pilot study. The Journal of thoracic and cardiovascular surgery 2008;136(1):129-134.
13. Tripodaki E-S, Tasoulis A, Vasileiadis I, et al. Microcirculatory alterations after cardiopulmonary bypass as assessed with near infrared spectroscopy: a pilot study. Canadian Journal of Anesthesia/Journal canadien d'anesthésie 2012;59(6):620-621.
14. Černý V, Turek Z, Pařízková R. Orthogonal polarization spectral imaging. Physiol Res 2007;56:141-147.
15. Groner W, Winkelman JW, Harris AG, et al. Orthogonal polarization spectral imaging: a new method for study of the microcirculation. Nature medicine 1999;5(10):1209-1212.
16. Goedhart P, Khalilzada M, Bezemer R, Merza J, Ince C. Sidestream Dark Field (SDF) imaging: a novel stroboscopic LED ring-based imaging modality for clinical assessment of the microcirculation. Optics express 2007;15(23):15101-15114.
17. Ince C. Sidestream dark field imaging: an improved technique to observe sublingual microcirculation. Critical care 2005;9(1):1-1.
18. Ince C, Boerma EC, Cecconi M, et al. 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 medicine 2018;44(3):281-299.
19. Lipinska-Gediga M. Sepsis and septic shock-is a microcirculation a main player? Anaesthesiology Intensive Therapy 2016;48(4):261-265.
20. De Backer D, Donadello K, Sakr Y, et al. Microcirculatory alterations in patients with severe sepsis: impact of time of assessment and relationship with outcome. Critical care medicine 2013;41(3):791-799.

21. Hernandez G, Boerma EC, Dubin A, et al. Severe abnormalities in microvascular perfused vessel density are associated to organ dysfunctions and mortality and can be predicted by hyperlactatemia and norepinephrine requirements in septic shock patients. Journal of critical care 2013;28(4):538. e9-538. e14.
22. Sakr Y, Dubois M-J, De Backer D, Creteur J, Vincent J-L. Persistent microcirculatory alterations are associated with organ failure and death in patients with septic shock. Critical care medicine 2004;32(9):1825-1831.
23. Haase-Fielitz A, Haase M, Bellomo R, et al. Perioperative hemodynamic instability and fluid overload are associated with increasing acute kidney injury severity and worse outcome after cardiac surgery. Blood purification 2017;43(4):298-308.
24. Ince C. The rationale for microcirculatory guided fluid therapy. Current opinion in critical care 2014;20(3):301-308.
25. Vellinga NA, Ince C, Boerma EC. Microvascular dysfunction in the surgical patient. Current opinion in critical care 2010;16(4):377-383.
26. Sherman H, Klausner S, Cook WA. Incident dark-field illumination: a new method for microcirculatory study. Angiology 1971;22(5):295-303.
27. 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):1-10.
28. Massey MJ, LaRochelle E, Najarro G, et al. The microcirculation image quality score: development and preliminary evaluation of a proposed approach to grading quality of image acquisition for bedside videomicroscopy. Journal of critical care 2013;28(6):913-917.
29. Gilbert-Kawai E, Coppel J, Bountziouka V, Ince C, Martin D. A comparison of the quality of image acquisition between the incident dark field and sidestream dark field video-microscopes. BMC medical imaging 2016;16(1):1-5.
30. Massey MJ, Shapiro NI. A guide to human in vivo microcirculatory flow image analysis. Critical Care 2015;20(1):1-10.
31. Hutchings S, Watts S, Kirkman E. The Cytocam video microscope. A new method for visualising the microcirculation using Incident Dark Field technology. Clinical Hemorheology and Microcirculation 2016;62(3):261-271.
32. Mathura KR, Bouma GJ, Ince C. Abnormal microcirculation in brain tumours during surgery. The Lancet 2001;358(9294):1698-1699.
33. Mathura KR, Vollebregt KC, Boer K, De Graaff JC, Ubbink DT, Ince C. Comparison of OPS imaging and conventional capillary microscopy to study the human microcirculation. Journal of Applied Physiology 2001;91(1):74-78.
34. Slaaf D, Tangelder G, Reneman R, Jäger K, Bollinger A. A versatile incident illuminator for intravital microscopy. International Journal of Microcirculation, Clinical and Experimental 1987;6(4):391-397.
35. Djaberi R, Schuijf JD, de Koning EJ, et al. Non-invasive assessment of microcirculation by sidestream dark field imaging as a marker of coronary artery disease in diabetes. Diabetes and Vascular Disease Research 2013;10(2):123-134.
36. Dondorp A, Ince C, Charunwatthana P, et al. Direct in vivo assessment of microcirculatory dysfunction in severe falciparum malaria. The Journal of infectious diseases 2008;197(1):79-84.
37. Khalilzada M, Dogan K, Ince C, Stam J. Sublingual microvascular changes in patients with cerebral small vessel disease. Stroke 2011;42(7):2071-2073.
38. Dababneh L, Cikach F, Alkukhun L, Dweik RA, Tonelli AR. Sublingual microcirculation in pulmonary arterial hypertension. Annals of the American Thoracic Society 2014;11(4):504-512.
39. Lindeboom JA, Mathura KR, Aartman IH, Kroon FH, Milstein DM, Ince C. Influence of the application of platelet-enriched plasma in oral mucosal wound healing. Clinical oral implants research 2007;18(1):133-139.

40. Meinders A-J, Elbers P. Leukocytosis and sublingual microvascular blood flow. New England Journal of Medicine 2009;360(7):e9.
41. Vollebregt KC, Boer K, Mathura KR, de Graaff JC, Ubbink DT, Ince C. Impaired vascular function in women with pre-eclampsia observed with orthogonal polarisation spectral imaging. British Journal of Obstetrics and Gynaecology 2001;108(11):1148-1153.
42. Kuipers J, Tiboel D, Ince C. The pediatric microcirculation. Crit Care 2016;20(1):352.
43. den Uil C, Bezemer R, Miranda DR, et al. Intra-operative assessment of human pulmonary alveoli in vivo using Sidestream Dark Field imaging: a feasibility study. Medical Science Monitor 2009;15(10):MT137-MT141.
44. Snoeijs MG, Vink H, Voesten N, et al. Acute ischemic injury to the renal microvasculature in human kidney transplantation. American Journal of Physiology-Renal Physiology 2010;299(5):F1134-F1140.
45. Nilsson J, Eriksson S, Blind P-J, Rissler P, Sturesson C. Microcirculation changes during liver resection—a clinical study. Microvascular research 2014;94:47-51.
46. Evaluation NIC. Jaarboek 20152016 June 2016.
47. Vieira J. Hands free technique: new tool possibility for image capture of sublingual microcirculation with handheld vital microspopy-HVM. Int J Odontostomat 2021;15(1):181-8.
48. Top A, van Dijk M, Van Velzen J, Ince C, Tibboel D. Functional capillary density decreases after the first week of life in term neonates. Neonatology 2011;99(1):73-77.
49. van den Berg VJ, van Elteren HA, Buijs EA, et al. Reproducibility of microvascular vessel density analysis in Sidestream dark-field-derived images of healthy term newborns. Microcirculation 2015;22(1):37-43.
50. Hubble SM, Kyte HL, Gooding K, Shore AC. Variability in sublingual microvessel density and flow measurements in healthy volunteers. Microcirculation 2009;16(2):183-191.
51. Bartels SA, Bezemer R, Milstein DM, et al. The microcirculatory response to compensated hypovolemia in a lower body negative pressure model. Microvascular Research 2011;82(3):374-380.
52. Gu Y-M, Wang S, Zhang L, et al. Characteristics and determinants of the sublingual microcirculation in populations of different ethnicity. Hypertension 2015;65(5):993-1001.
53. Cerny V. Sublingual microcirculation. Appl Cardiopulm Pathophysiol 2012;16:229-48.
54. Sadaka F, Aggu-Sher R, Krause K, O'brien J, Armbrecht ES, Taylor RW. The effect of red blood cell transfusion on tissue oxygenation and microcirculation in severe septic patients. Annals of intensive care 2011;1(1):1-11.
55. Kurose I, Argenbright LW, Anderson DC, et al. Reperfusion-induced leukocyte adhesion and vascular protein leakage in normal and hypercholesterolemic rats. American Journal of Physiology-Heart and Circulatory Physiology 1997;273(2):H854-H860.
56. Ballaux PK, Gourlay T, Ratnatunga CP, Taylor KM. A literature review of cardiopulmonary bypass models for rats. Perfusion 1999;14(6):411-417.
57. Hutchings SD, Naumann DN, Watts S, et al. Microcirculatory perfusion shows wide inter-individual variation and is important in determining shock reversal during resuscitation in a porcine experimental model of complex traumatic hemorrhagic shock. Intensive care medicine experimental 2016;4(1):1-13.
58. Kang C, Cho A-R, Lee HJ, et al. Feasibility study of incident dark-field video microscope for measuring microcirculatory variables in the mouse dorsal skinfold chamber model. Acute and Critical Care 2021;36(1):29.
59. Bársony A, Vida N, Gajda Á, et al. Methane Exhalation Can Monitor the Microcirculatory Changes of the Intestinal Mucosa in a Large Animal Model of Hemorrhage and Fluid Resuscitation. Frontiers in Medicine 2020;7:669.
60. Verdant CL, De Backer D, Bruhn A, et al. Evaluation of sublingual and gut mucosal microcirculation in sepsis: a quantitative analysis. Critical care medicine 2009;37(11):2875-2881.

61. Yin L, Yang Z, Yu H, et al. Changes in sublingual microcirculation is closely related with that of bulbar conjunctival microcirculation in a rat model of cardiac arrest. Shock 2016;45(4):428-433.

15. Appendix 1

Based on the second consensus paper by Ince et al. published in 2018 on the assessment of sublingual microcirculation in critically ill patients, the following parameters are defined as follows:

- Percentage of perfused vessels (PPV).

Percentage of perfused vessels per total number of vessel cross-sections, expressed as a percentage (%) and characterized as a binomial determinant of red blood cell velocity (flow or no flow)

- Microvascular flow index (MFI).

Grid-based score per quadrant, no flow (0), intermittent flow (1), slow flow (2), normal flow (3). MFI is characterized as semi-quantitative assessment of average red blood cell velocity per quadrant.

- Functional capillary density (FCD).

The total length of vessels exhibiting normal flow in relation to image size. FCD is characterized as the diffusion distance between red blood cells and tissue cells.

- Total vessel density (TVD).

Measurement of total vessel area per surface area, is expressed in mm2/mm2 and is characterized as a determinant of capillar distance (diffusive capacity).

- Density of perfused vessels (PVD).

Percentage of perfused vessels x TVD, expressed in mm2/mm2. PVD is characterized as the determinant of capillary distance (diffusive capacity) and red blood cell velocity (convective capacity)

- Red blood cell velocity (RBCv)

Determined by use of a space-time diagram (STD). Each moving RBC generates a line in the STD, the slope of which equals the velocity (velocity = $\Delta L/\Delta t$)

Microcirculatory changes associated with loss of haemodynamic coherence.

Different states of cardiovascular problems are associated with different types of microcirculatory changes (figure 4). These types are classified on the basis of direct observation of the microcirculation. Each of these types is associated with decreased FCD and consequently a loss of the ability of the microcirculation to transport oxygen to the cells. These types can occur separately or in combination. A reduction in FCD may promote a functional shift in oxygen transport to tissues, which cannot be detected by looking at macrocirculatory parameters alone unless explicitly monitored.

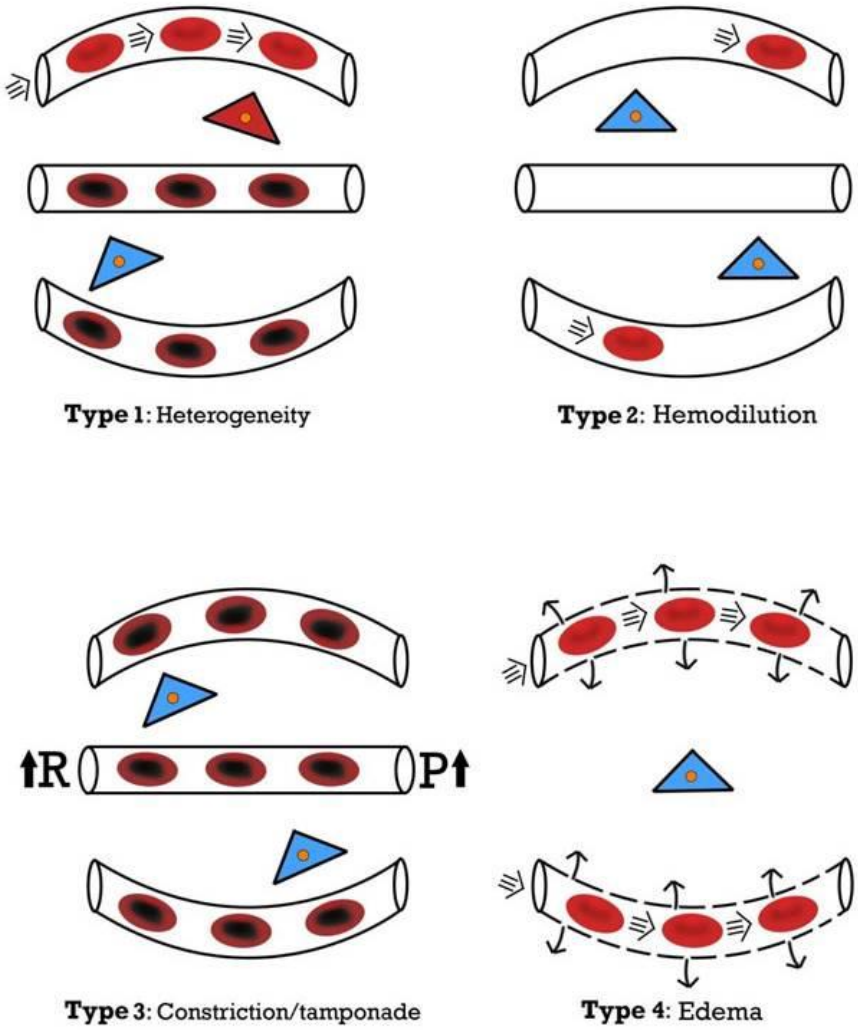


Figure 4 Microcirculatory changes associated with loss of haemodynamic coherence. Red, well-oxygenated red blood cells (RBC) and tissue cells; purple, RBC with reduced oxygenation; blue, reduced tissue cell oxygenation

Four types of microcirculatory changes can be distinguished that underlie the loss of haemodynamic coherence:

- Type 1: Heterogeneous perfusion of the microcirculation, as seen in septic patients, with clogged capillaries adjacent to perfused capillaries resulting in heterogeneous oxygenation of tissue cells.
- Type 2: Haemodilution with dilution of blood in the microcirculation resulting in loss of RBC-filled capillaries and increased diffusion distance between RBCs in the capillaries and tissue cells.
- Type 3: Stasis of microcirculatory RBC flow due to altered systemic variables, e.g. increased arterial vascular resistance or increased venous pressure.
- Type 4: capillary leakage induced edema that results in a large diffusion distance and reduced ability of oxygen to reach tissue cells.

16. Appendix 2

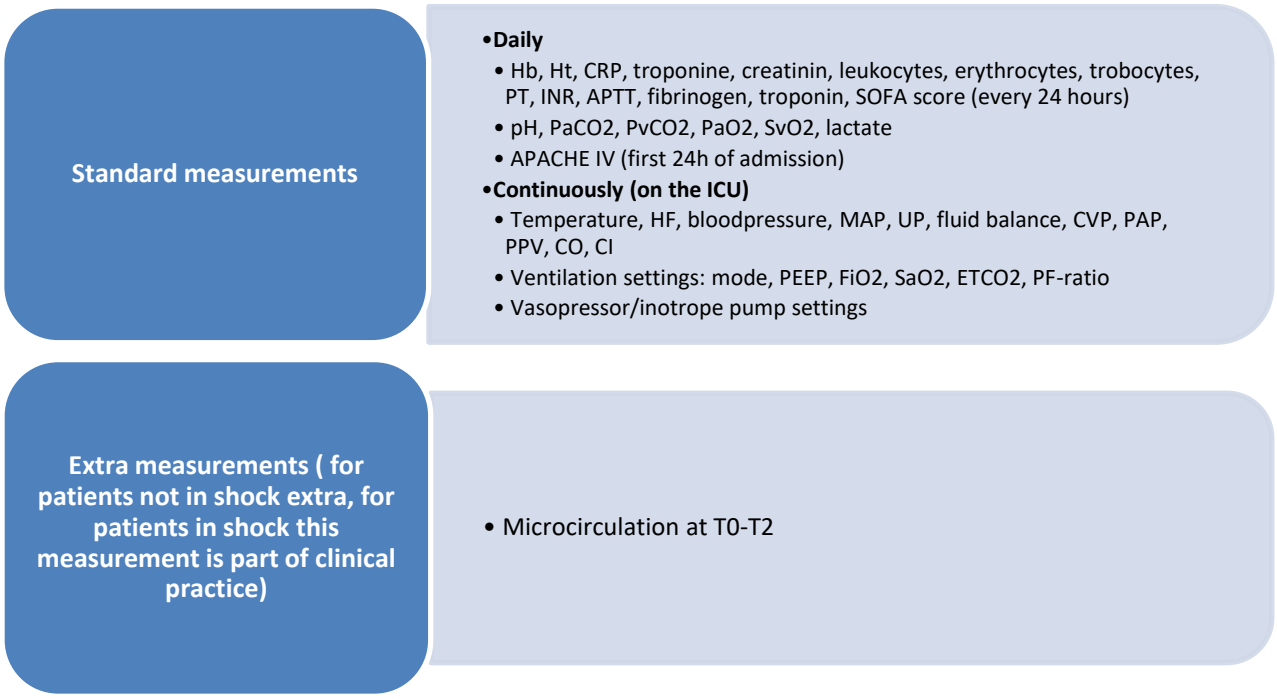


Figure 5 Overview of measurements. Standard measurements are performed as part of standard clinical care. Extra measurements are measurements performed as part of this study.

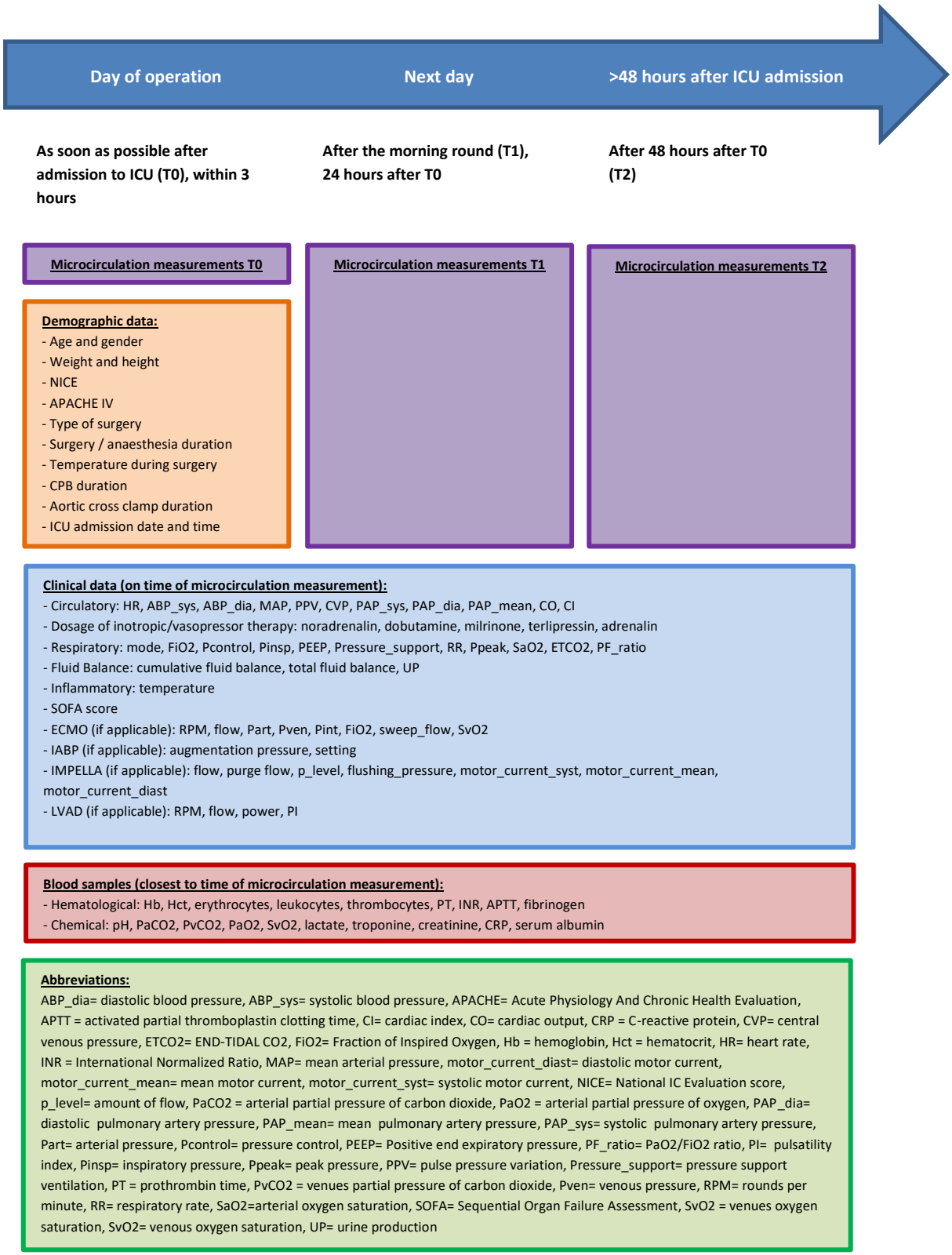


Figure 6 Timeframe of measurements, including all data. Purple: Intervention of the study to obtain data. Orange/Blue/Red: data already gathered as part of standard clinical practice.

17. Appendix 3

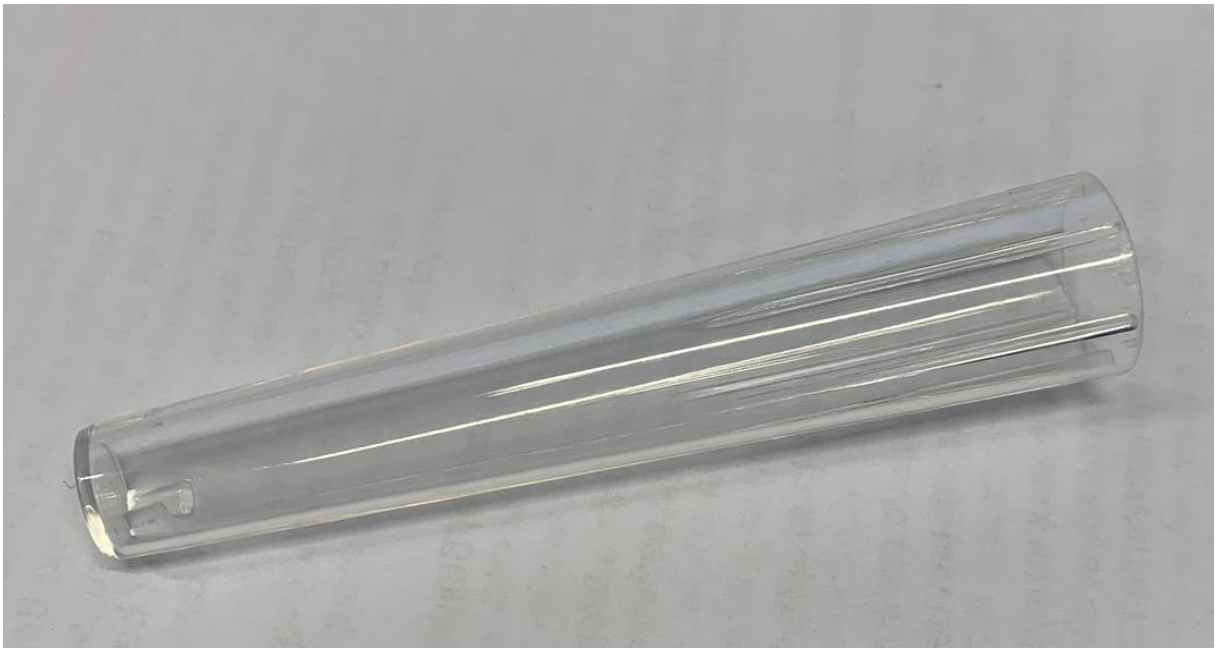


Figure 7 CytoCam protection cap

Appendix F

MATLAB code

```
close all; clear all; clc

%%
data1 = readtable('ZZ_videos.xlsx');
data1.Properties.VariableNames = {'vid_name', 'ver', 'options', 'FOV', 'pxhoriz', 'pxvert', 'fps', 'frames', 'quality_accepted', 'quality_duration',
'quality_illumination', 'quality_stability', 'quality_focus', 'quality_pressure', 'quality_content', 'TVD_small', 'TVD_large', 'CBV_small',
'CBV_large', 'CSA_small', 'CSA_large', 'FCD_small', 'PPV_small', 'RBCv_small', 'RBCv_small_SD', 'RBCv_large', 'RBCv_large_SD', 'tp_small',
'cHct_small', 'cHct_small_SD', 'dHct_small', 'tRBCp_small'};

 %%
data1(:,1) = [];
data1(:,2) = [];
data1 = table2array(data1);
data2 = xlsread('Shock_data_#_t.xlsx');
data = [data2 data1];
xlswrite('Shock_data_microtools_all_quality_scores.xlsx',data)

%%
quality_zero = find(data(:,9)<1);
data(quality_zero,:) = [];
xlswrite('Shock_data_microtools_quality_OK.xlsx',data)

%%
data = array2table(data);
data.Properties.VariableNames = {'Patient','t','options', 'FOV', 'pxhoriz', 'pxvert', 'fps', 'frames', 'quality_accepted', 'quality_duration',
'quality_illumination', 'quality_stability', 'quality_focus', 'quality_pressure', 'quality_content', 'TVD_small', 'TVD_large', 'CBV_small',
'CBV_large', 'CSA_small', 'CSA_large', 'FCD_small', 'PPV_small', 'RBCv_small', 'RBCv_small_SD', 'RBCv_large', 'RBCv_large_SD', 'tp_small',
'cHct_small', 'cHct_small_SD', 'dHct_small', 'tRBCp_small'};

dsa = data(:,{
   'Patient',
   't',
   'TVD_small',
   'TVD_large',
   'CBV_small',
   'CBV_large',
   'CSA_small',
   'CSA_large',
   'FCD_small',
   'PPV_small',
   'RBCv_small',
   'RBCv_small_SD',
   'RBCv_large',
   'RBCv_large_SD',
   'tp_small',
   'cHct_small',
   'cHct_small_SD',
   'dHct_small',
   'tRBCp_small'});

%%
statarray = grpstats(dsa,{'Patient','t'},'mean','DataVars',{'TVD_small','TVD_large',
'CBV_small','CBV_large','CSA_small','CSA_large','FCD_small','PPV_small','RBCv_small','RBCv_small_SD','RBCv_large','RBCv_large_SD','tp_small','
cHct_small','cHct_small_SD','dHct_small','tRBCp_small'});

%%
mean_values = table2array(statarray);

%%
xlswrite('Shock_data_microtools_mean_values.xlsx', mean_values);
```

Appendix G

Measured values

Table G.1 *Number of measured values for each parameter of shock group (n = 20) and non-shock group (n = 29)*

	Shock			No shock		
Variable	**T0**	**T1**	**T2**	**T0**	**T1**	**T2**
	N	**N**	**N**	**N**	**N**	**N**
MAP	20	16	9	26	19	3
Lactate	20	16	9	26	19	3
SvO_2	14	9	3	18	3	0
TVD	18	16	8	26	19	3
FCD	18	16	8	26	19	3
PPV	18	16	8	26	19	3
RBCv	18	16	8	26	19	3
cHct	18	16	8	26	19	3
tRBCp	18	16	8	26	19	3

cHct: capillary hematocrit; FCD: functional capillary density; MAP: mean arterial pressure; PPV: proportion of perfused vessels; RBCv: red blood cell velocity; SvO_2: central venous oxygen saturation; tRBCp: tissue red blood cell perfusion; TVD: total vessel density.

Appendix H

LMM estimates of fixed effects

Table H.1 *LMM estimates of fixed effects*

						95 % confidence interval	
Parameter	**Estimate**	**std. error**	**df**	**t**	**sig**	**Lower bound**	**Upper bound**
TVD							
Intercept	19.439	1.511	12.602	12.867	0.000	16.165	22.714
T0	1.689	1.744	14.920	0.968	0.348	-2.031	5.409
T1	1.578	1.337	9.800	1.180	0.266	-1.410	4.565
T2	0[b]	0	.	.	.	.	.
Shock or no shock at T0*	-3.551	2.675	11.872	-1.327	0.209	-9.387	2.285
T0 * Shock or no shock at T0	0.631	2.910	14.564	0.217	0.831	-5.588	6.849
T1 * Shock or no shock at T0	-1.734	2.492	9.657	-0.696	0.503	-7.313	3.845
T2 * Shock or no shock at T0	0[b]	0	.	.	.	.	.
FCD							
Intercept	18.034	1.605	10.587	11.235	0.000	14.484	21.584
T0	1.827	1.788	11.709	1.021	0.328	-2.081	5.734
T1	1.751	1.573	10.185	1.113	0.291	-1.745	5.248
T2	0[b]	0	.	.	.	.	.
Shock or no shock at T0*	-2.009	2.955	10.244	-0.680	0.512	-8.572	4.554
T0 * Shock or no shock at T0	-0.465	3.131	11.750	-0.148	0.885	-7.303	6.373

T1 * Shock or no shock at T0	-2.741	2.915	9.878	-0.940	0.369	-9.247	3.765
T2 * Shock or no shock at T0	0[b]	0	.	.	.	.	.
PPV							
Intercept	92.960	2.656	9.745	35.006	0.000	87.022	98.898
T0	1.148	2.624	9.935	0.438	0.671	-4.704	7.000
T1	1.302	2.983	11.668	0.436	0.671	-5.218	7.821
T2	0[b]	0	.	.	.	.	.
Shock or no shock at T0*	4.446	4.952	9.650	0.898	0.391	-6.642	15.533
T0 * Shock or no shock at T0	-3.208	4.927	9.602	-0.651	0.530	-14.249	7.832
T1 * Shock or no shock at T0	-3.572	5.260	11.377	-0.679	0.511	-15.102	7.959
T2 * Shock or no shock at T0	0[b]	0	.	.	.	.	.
RBCv							
Intercept	323.520	15.578	11.330	20.768	0.000	289.354	357.685
T0	10.950	17.439	11.021	0.628	0.543	-27.424	49.323
T1	5.700	15.851	10.384	0.360	0.726	-29.442	40.843
T2	0[b]	0	.	.	.	.	.
Shock or no shock at T0*	75.395	28.669	10.313	2.630	**0.025**	11.779	139.011
T0 * Shock or no shock at T0	-69.334	30.467	11.427	-2.276	**0.043**	-136.087	-2.582
T1 * Shock or no shock at T0	-53.529	29.007	10.304	-1.845	0.094	-117.903	10.845

T2 * Shock or no shock at T0	0[b]	0	.	.	.	.	.
cHct							
Intercept	6.064	0.456	8.423	13.294	0.000	5.021	7.107
T0	-0.344	0.520	12.650	-0.663	0.519	-1.470	0.781
T1	-0.251	0.304	11.165	-0.824	0.427	-0.919	0.418
T2	0[b]	0	.	.	.	.	.
Shock or no shock at T0*	0.276	0.721	11.390	0.383	0.709	-1.304	1.856
T0 * Shock or no shock at T0	1.156	0.793	15.389	1.458	0.165	-0.530	2.842
T1 * Shock or no shock at T0	0.135	0.550	10.616	0.245	0.811	-1.082	1.351
T2 * Shock or no shock at T0	0[b]	0	.	.	.	.	.
tRBCp							
Intercept	44.302	6.088	10.291	7.277	0.000	30.790	57.815
T0	2.019	6.655	11.707	0.303	0.767	-12.520	16.559
T1	2.100	5.817	9.635	0.361	0.726	-10.929	15.128
T2	0[b]	0	.	.	.	.	.
Shock or no shock at T0*	-0.030	11.271	10.029	-0.03	0.998	-25.133	25.073
T0 * Shock or no shock at T0	-3.881	11.811	11.361	-0.329	0.748	-29.775	22.014
T1 * Shock or no shock at T0	-10.110	10.988	9.371	-0.920	0.381	-34.818	14.598
T2 * Shock or no shock at T0	0[b]	0	.	.	.	.	.

[b] this parameter was set to zero because it is redundant; cHct: capillary hematocrit; df: degrees of freedom; FCD: functional capillary density; PPV: proportion of perfused vessels; RBCv: red blood cell velocity; sig: significance level; std: standard; t: how far from zero is the estimate; tRBCp: tissue red blood cell perfusion; TVD: total vessel density.

Appendix I

TM30003: Literature review

Microcirculatory Alterations in Cardiothoracic Patients, During and After Surgery
A Systematic Review

BSc. R. Toet*, MSc. F. Brouwer+, Dr. R. Tsonaka#, Dr. S. Arbous^

*: Student MSc Technical Medicine, Erasmus Medical Centre, Rotterdam; Delft University of Technology, Delft; Leiden University Medical Centre, Leiden, The Netherlands
+: Fellow Technical Medicine, department of Intensive Care, Leiden University Medical Centre, Leiden, The Netherlands
#: Assistant Professor, department of Medical Statistics and Bioinformatics, Leiden University Medical Centre, Leiden, The Netherlands
^: Anaesthesiologist- intensivist, department of Intensive Care, Leiden University Medical Centre, Leiden, The Netherlands

Abstract

Background: Cardiothoracic surgery remains associated with a high rate of CPB adverse effects and postoperative complications, such as circulatory shock. Current resuscitation procedures are aimed at normalizing macrocirculatory parameters. It is expected that the correction of macrocirculatory parameters is effective in correcting the microcirculation. This parallel improvement is known as hemodynamic coherence, which is often lost after an episode of circulatory shock. Therefore, real-time monitoring of the microcirculation might be a valuable tool to monitor alterations in microcirculatory perfusion in patients undergoing cardiac surgery with or without CPB. The goal of our systematic review is to determine the effect of CPB on the microcirculation pre-, intra-, and postoperatively, with a specific focus on on/off pump surgery and other interventions, such as perfusion, anesthesia, and medication. The second aim of our review is to determine the relationship between macrocirculation and microcirculation pre-, intra-, and postoperatively.

Methods: A systematic search of PubMed, Embase, Web of Science, Cochrane Library, and PMC PubMed Central was conducted according to PRISMA guidelines for relevant articles from December 1952 to February 2022. Clinical studies were included in this systematic review if they measured sublingual microcirculation pre-, intra-, and postoperatively in adult patients undergoing cardiothoracic surgery with sternotomy. The primary outcomes were the change of the microcirculatory parameters of cardiothoracic patients postoperatively as compared to pre-, and/or intraoperatively. Secondary outcomes were the effect of CPB, the relationship between the microcirculation and macrocirculation, and the relationship between the microcirculation and clinical outcomes.

Results: thirty-six studies were included. Every study reported at least one of the microcirculatory parameters. Nineteen studies found an impaired microcirculation intraoperatively, compared to the postoperative period. Five of them returned to baseline measurements at the end of the CPB period. Nine studies reported significant decrease in postoperatively microcirculation measurements, of which two returned to baseline measurements after the first 24 hours after surgery. 15 Studies found no microcirculatory alterations intraoperatively (n=9) and postoperatively (n=6).

Conclusion: CPB impairs microcirculatory perfusion, which is reflected mostly by diffusive parameters. Uncoupling of hemodynamic coherence is seen in several studies. Microcirculation measurement therefore seems to have added value in cardiothoracic surgical patients. More research is needed on the contribution of microcirculatory changes to a particular clinical outcome.

Trial registration: PROSPERO, CRD42022343137

Keywords: Microcirculation, cardiothoracic surgery, pre-operatively, intra-operatively, post-operatively, sublingual

Introduction

Cardiothoracic surgery is an effective and common treatment for cardiovascular diseases. An estimated 1 million patients undergo cardiothoracic surgery throughout the world every year (1). Innovations in surgical techniques, such as the introduction of the cardiopulmonary bypass (CPB) together with an aging population will lead to an increasing number of older or more critically ill patients undergoing cardiothoracic surgery (2-4). Although perioperative care, anesthesia, and surgical techniques have greatly improved, cardiothoracic surgery (with or without CPB) remains associated with a high rate of postoperative complications (5, 6) (i.e. the use of blood products (47.3%), atrial fibrillation (32%), renal failure (3.3%) and tamponade (1.1%) (7)) and CPB adverse effects (4) (i.e. the release of pro-inflammatory cytokines, dilution of the clotting factors and platelet dysfunction (8-10)). These complications can lead to longer hospital stays, higher health care costs, delayed recovery, and poor quality of life after surgery (11, 12). Therefore, patients are admitted postoperatively to the intensive care unit (ICU) to ensure

adequate continuous hemodynamic monitoring and postoperative stabilization and optimization, to avoid potential postoperative complications (13-16).

One of the most common complications is the development of circulatory shock (5-50%) (17-19) caused by a combination of many factors, such as cardiac stunning or failure, hypovolemia due to blood loss, tamponade, activation of inflammatory and hemostatic systems, anesthesia, hypothermia, hemodilution, micro-emboli formation, and tissue trauma (20-23). Current resuscitation procedures for patients in circulatory shock are aimed at normalizing macrocirculatory parameters (hereafter "macrocirculation"), such as cardiac output, central venous oxygen saturation, and blood pressure, by administering fluids, vasopressors and inotropes to promote tissue perfusion and therefore sustain oxygen transport to the tissues (24-27). However, sometimes the macrocirculatory parameters are normalized, while clinically (or biochemically) a patient is still in persistent shock (28). In recent decades, new techniques have been developed that allow measurements on the level of the microcirculation. The microcirculation is a complex network of arterioles, capillaries, and venules < 20μm and is the final destination of blood flow to tissues for oxygenation. From a clinical point of view, it is expected that the correction of macrocirculatory parameters is effective in correcting regional and microcirculatory perfusion and oxygen delivery to the parenchymal cells such that the cells are able to perform their functional activities. This parallel improvement is known as hemodynamic coherence (26). However, in many resuscitation conditions, with fluids or vasoactive medications, following an episode of circulatory shock, there is a loss of this hemodynamic coherence. The microcirculation and its corresponding tissues can remain hypoperfused despite the correction of macrocirculatory parameters by fluids and vasopressors. This loss of coherence is associated with adverse outcomes (25-27).

Therefore, real-time monitoring of the microcirculation might be a valuable tool to monitor alterations in microcirculatory perfusion in patients undergoing cardiac surgery with or without CPB. Over the past decades, a lot of research has been conducted in the promising field of microcirculation. In these studies, microcirculatory perfusion disorders are being reported in patients undergoing cardiac surgery. (29)

Os et.al. (30) provided a systematic overview of the course of alterations in sublingual microcirculatory perfusion following CPB. To our knowledge, no systematic review has been published that, in addition to the effects of CPB pre-, intra-, and post-operatively, also focuses on off-pump surgery and other CPB variations and therapeutic interventions in cardiothoracic surgical patients.

The goal of this systematic review was to determine the effects of CPB on the microcirculation pre-, intra-, and postoperatively with a specific focus on on/off pump surgery and other interventions, such as perfusion technique, anesthesia, and medication. The second aim of our review was to determine the relationship between macrocirculation and microcirculation pre-, intra-, and postoperatively and the relationship between microcirculation and clinical outcome, such as complications and mortality

Methods

Search strategy

On February 25, 2022, a search was conducted for all publications related to the sublingual microcirculation in adult patients undergoing cardiothoracic surgery (with or without CPB) with sternotomy by performing a systematic search in PubMed, Embase, Web of Science, Cochrane Library, and PMC PubMed Central, from December 1952 to February 25, 2022. The search strategy consisted of a combination of the following terms: "microcirculation", "sublingual" or, "critically ill" and "cardiothoracic surgery." The complete search strategy can be found in Additional File I. Reference lists of all the full texts were screened for further eligible studies.

Protocol and registration

Details of the protocol of this systematic review were pre-registered at the International prospective register of systematic reviews (PROSPERO) with registration number CRD42022343137. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis Statement (PRISMA statement) guidelines were followed to report the systemic review (31).

Eligibility

Clinical studies that measured sublingual microcirculation in patients undergoing

cardiothoracic surgery with sternotomy were included in this study. Studies had to meet the following inclusion criteria:

- Adult patients > 18 years
- Any type of elective cardiothoracic surgery with or without any type of CPB
- Pre- and/or intra- and/or postoperative microcirculatory measurements by orthogonal polarization spectral (OPS) imaging, sidestream dark-field (SDF) imaging or incident darkfield imaging (IDF).

Exclusion criteria were animal studies, in vitro studies, pediatric and neonate subjects, microcirculation measurement technologies other than OPS/SDF or IDF imaging, and non-sublingual microcirculation measurements. All study designs were eligible for inclusion but case reports, letters to the editor, conference abstract, editorials and reviews were excluded. If the full text of an article was not available or if the language of the article was not English or Dutch, the study was excluded as well.

Study selection

After deduplication, all titles and abstracts of the studies were independently screened by two reviewers (FB and RT) for the fulfillment of the inclusion criteria. Subsequently, the full texts of studies of interest were reviewed independently by two reviewers (FB and RT) for eligibility. Disagreements were resolved by discussion with a third independent reviewer (MA).

Data extraction

Data extraction was independently performed by two reviewers (FB and RT). Disagreements were discussed until consensus was reached, if necessary the third reviewer (MA) was asked to participate in the discussion to resolve discrepancies. For each study, general information (i.e. author, year of publication and study period) were extracted in a standardized extraction format. Furthermore, data related to the study design, patient sample size, patient population, CPB protocol, type of surgery, clinical outcomes and macrocirculatory parameters such as blood pressure, lactate, and cardiac output (CO) (Appendix I) and clinical outcomes were extracted. Importantly, microcirculation parameters as well as details regarding the monitoring device of the microcirculation (OPS/SDF or IDF imaging) were also extracted.

Microcirculatory parameters

The microcirculation consists of several functional components, of which the two main ones describe the physiological function and the way they contribute to oxygen transport: (1) the density of perfused capillaries (diffusive oxygen transport) and (2) the flow of red blood cells through the capillaries (convective oxygen transport) (25). The diffusive microcirculation parameters are functional capillary density (FCD), perfused vessel density (PVD) and total vessel density (TVD) and the convective microcirculation parameters are the percentage of perfused vessels (PPV), microvascular flow index (MFI) and red blood cell velocity (RBCv). Additional information about the microcirculatory parameters can be found in Appendix II (25).

Hemodynamic coherence

Hemodynamic coherence between the macrocirculation and the microcirculation is defined as the state in which resuscitation procedures aimed at correcting systemic hemodynamic parameters are effective in correcting regional and microcirculatory perfusion and oxygen supply to the tissues, allowing parenchymal cells to perform their functional activities in support of organ function. (26)

Primary and secondary outcomes

The primary outcome of this systematic review was the change of the microcirculatory parameters (i.e. FCD, PVD, TVD, PPV, MFI and RBCv) of cardiothoracic patients postoperatively as compared to pre- and/or intraoperatively. Baseline microcirculation measurements refer to the first microcirculation measurements of the study. The timing of the baseline measurement may vary among studies.

Secondary outcomes were the effect of CPB on microcirculatory parameters (i.e. by comparing on-CPB versus off-CPB measurements and other interventions, such as perfusion technique, anesthesia, and medication), the relationship between the macrocirculation and microcirculation and the relationship between the microcirculation and clinical outcomes (i.e. length of (hospital) stay (LOS), complications and mortality).

Quality assessment

The quality of each study was independently assessed by two reviewers (FB and RT). The quality of the observational studies was assessed

with the Newcastle Ottawa Scale (NOS) (32,33). The NOS score ranges from 0 (low quality) to 9 (high quality). The quality of the randomized controlled trials (RCTs) was assessed by the Risk of Bias (Rob) 2 tool (34).
Any discrepancies about the scores were resolved by discussion until consensus was reached between the two reviewers (FB and RT). If consensus could not be reached, a third independent reviewer (MA) was requested. The assigned scores are not intended as summary judgements of quality, but help reviewers assess forms of bias.

Data analysis
The sublingual microcirculation was studied during different phases of cardiothoracic surgery: pre-, intra- and postoperatively.
The pre-operative period was defined as all microcirculation measurements prior to the start of surgery (i.e. before the start of anesthesia). The intra-operative period contained the aortic cross-clamp period (i.e. the effect of CPB only), the entire CPB period, during cardiac positioning and after cardiac positioning. The ICU period (after surgery), 24/48, and 72 hours after surgery was defined as the postoperative period. Due to the heterogeneity of the studies, a descriptive synthesis of the effect of cardiothoracic surgery on sublingual microcirculation pre-, intra and postoperatively was performed. If data allowed, we additionally focused on the effect of on-pump vs off-pump studies, different CPB variations and anesthetic or therapeutic interventions.

Data analysis was focused on several sub-analysis:

- Studies with the usage of CPB ("on-pump studies"): postoperative microcirculatory status of the patient was compared with intra-operative microcirculatory status. If data allowed, we additionally focused on the comparison with the pre-operative microcirculatory status and the effect of CPB and/or aortic clamping on microcirculatory status.
- Studies that compared off-pump vs on-pump: Same as the on-pump and the pulsatile vs non-pulsatile studies postoperative microcirculatory status of the patient was compared with intra-operative microcirculatory status for on-pump as well as the off-pump group. If data allowed, we additionally focused on the comparison with the pre-operative microcirculatory status of the patient and the effect of CPB and/or aortic clamping or grafting on intra-operative microcirculatory status.
- Studies that compared pulsatile vs non-pulsatile CPB: postoperative microcirculatory status of the patient was compared with intra-operative microcirculatory status. If data allowed, we additionally focused on the comparison with the pre-operative microcirculatory status and the effect of CPB and/or aortic clamping on intra-operative microcirculatory status
- Studies that described therapeutic interventions to improve microcirculation: post-intervention microcirculatory status was compared with pre-intervention values.

Absolute values of the microcirculatory parameters could not be compared because different microcirculatory monitoring techniques (OPS, SDF and IDF imaging) were used. Therefore, only significant changes in microcirculatory parameters (pre-, intra-, postoperatively) were reported.

Results

Figure 1 presents the study selection. The initial search yielded 1786 studies, of which 1097 were screened. A total of 119 studies were included for full-text analysis.

Eighty-three studies were excluded based on wrong study design (n = 29), no specification for patient population (n = 17), wrong patient population (n = 16), description of an analysis method (n = 6), wrong outcomes (n = 7), wrong intervention (n = 4), no English text available (n = 2) or no full text available (n = 1). Finally, the remaining 36 studies were included that recorded pre- and/or intra- and/or postoperative measurements of the sublingual microcirculatory parameters in patients undergoing cardiothoracic surgery (with or without CPB) with sternotomy (32-67)

Study characteristics
The 36 included studies consisted of 27 observational studies and 9 randomized controlled trials. All studies were published between 2007 and 2021 in 14 different countries (the Netherlands (n = 14), China (n = 2), Turkey (n = 1), Uruguay (n = 1), Canada (n = 2), Egypt (n = 1), France (n = 1), Austria (n = 1), Denmark

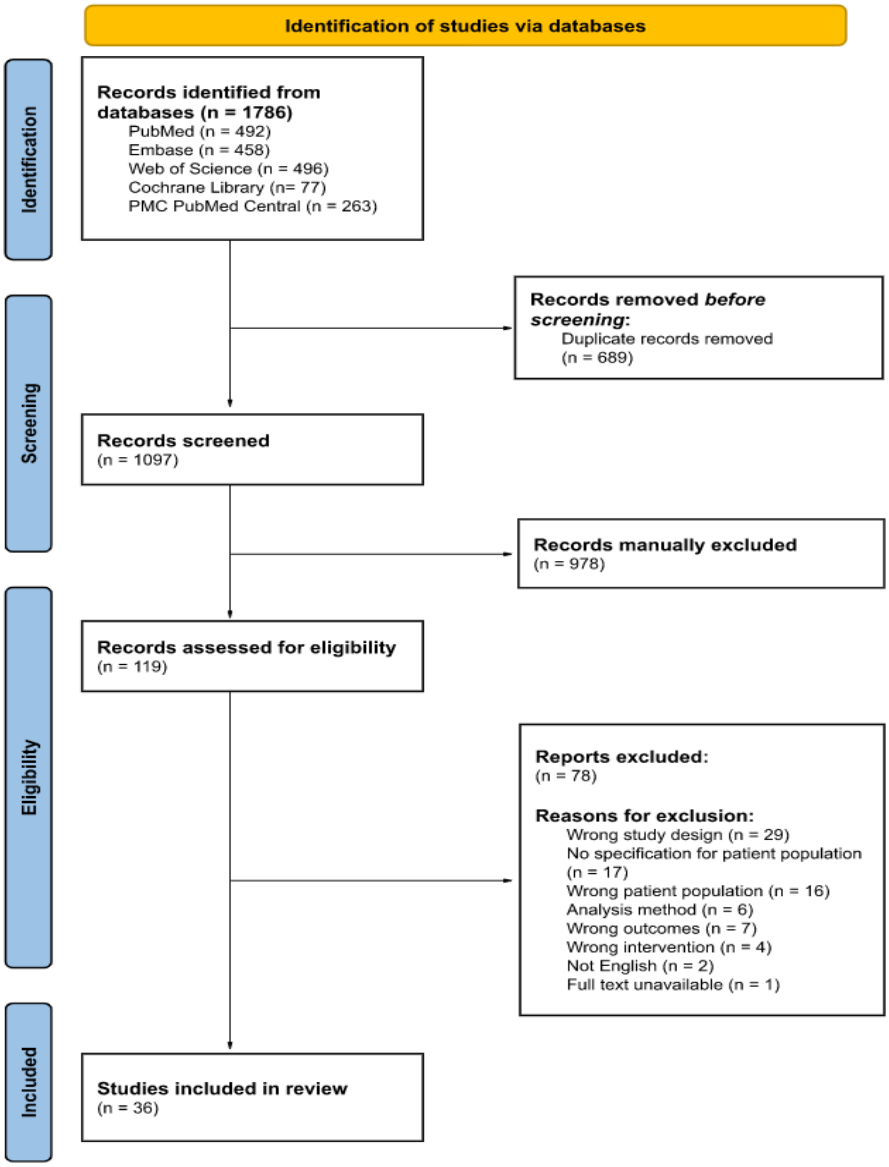


Figure 1 *Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram: study selection*

(n = 1), United States (n = 2), Italy (n = 2), Germany (n = 3) and Belgium (n = 1), multicenter (n = 2)).

Patient characteristics

Patient characteristics are listed in Appendix III. The 36 included studies compromised 1093 patients. Various interventions such as CPB mode, type of anesthesia, blood transfusion, medication administration or fluid administration were studied for their effect on intra- and postoperative microcirculation. No significant differences between patient characteristics were reported at baseline, so the patient characteristics are comparable between the included studies.

Risk of bias

The quality assessment can be found in Appendix IV. All observational studies (n = 27) received an overall score of 5 or higher. This indicates that the included observational studies were of reasonable to good quality. 96% of all 27 observational studies assessed the outcome using independent blind assessment, except Koning et al. (55) did not provide a description about the assessment of the outcome. In 44% of the observational studies there was a control/another intervention group. Only Maier et al. (57) failed in the adequacy of follow-up.

Of the RCTs, 56% reported the sample size calculation. In 22% of RCTs, caregivers were not blinded to the intervention.

Microcirculatory parameters

Effect of CPB (on-pump studies)

Changes in convection and diffusion microcirculatory parameters of the nine on-pump studies are presented in Table 1. A distinction is made between the effect of CPB on the microcirculatory parameters during on-pump cardiothoracic surgery (intra-operative changes) compared to baseline measurements and the effect of CPB on microcirculatory parameters postoperatively at the ICU or at the ward till 72

hours after surgery (postoperative changes) compared to baseline measurements.
Seven out of nine studies (78%) (37, 39, 41, 43, 44, 63, 65) reported the intra-operative changes of the microcirculatory parameters compared to baseline and five out of nine studies (44%) (32, 41, 43, 50, 65) reported the postoperative changes of the microcirculatory parameters compared to baseline.

All seven studies (100%) (37, 39, 41, 43, 44, 63, 65) focusing on the intra-operative change compared to baseline measurements reported diffusion microcirculatory parameters (FCD, PVD and/or TVD). Three of these studies (43%) (37, 41, 65) reported a significant intra-operative change in these parameters compared to baseline measurements.

One out of the seven studies (14%) (37) reported the FCD and this study showed a significant decrease in the FCD compared to baseline measurements during the aortic cross clamp period, which was restored to baseline values at the end of the CPB period (37). Five studies (71 %) (39, 41, 44, 63, 65) reported PVD, of which two studies (29%) (41, 65) showed a significant reduction in PVD during the intraoperative period compared to baseline. The other three studies that reported PVD (60%) (39, 44, 63) reported no significant effect of CPB on PVD. Three of the seven studies (43%) (39, 44, 63) reported the TVD and all three studies showed no effect of CPB on TVD.

The intra-operative change of the convective microcirculatory parameters (PPV, MFI and/or RBCv) compared to the baseline measurements was reported by six out of the nine studies (67%) (37, 39, 41, 43, 44, 63) and three of the six studies (50%) (41, 44, 63) reported a significant change in these parameters compared to baseline measurements.

Four studies (%) (39, 41, 44, 63) reported PPV, of which one study (25%) (41) showed a significant decrease in PPV compared to baseline and the other three studies (75%) (39, 41, 44, 63) showed no effect of CPB on PPV. Four out of the six studies (67%) (39, 43, 44, 63) reported the MFI, two of them with conflicting outcomes. Prestes et al. (63) reported a significant increase in MFI compared to baseline in contrast to di Dedda et al. (44) who reported a significant decrease in MFI compared to baseline.

Only one study (37) reported the RBCv, which did not significantly change during the intraoperative period compared to baseline.
Six of the nine studies (67%) (32, 39, 41, 43, 50, 65) followed patients post-operatively and only three studies (50%) (42, 44, 68) had an additional follow-up of 24 hours (41, 65), 48 hours (65) and 72 hours (39, 41) after surgery. The two studies (41, 65), in which the PVD was significantly reduced intraoperatively, reported a reduction in PVD for at least 24 hours after surgery and only one of these two studies (65) showed return to baseline after 48 hours. Also, Greenwood et al. (50) reported a significantly decreased PVD postoperatively. Two studies (33%) (41, 50) showed a significantly reduced PPV compared to baseline during the post-operative period. The other four studies (67%) (32, 39, 41, 43, 50, 65) showed no effect of CPB on postoperative PPV. Results of the convective microcirculatory parameters showed contradictory results, one study showed a significant decrease in MFI postoperatively compared to baseline (50) and one study showed a significant increase postoperatively compared to baseline (32).

CPB variations
CPB variations such as on-pump vs off-pump and type of blood flow may influence the effect of CPB on the sublingual microcirculation.
Microcirculatory alterations from different CPB variations can be found in Appendix Va Eighteen studies reported microcirculatory parameters in association with a variation of CPB. The studies assessed the difference between on-pump vs off-pump surgery (n = 6) (33, 34, 38, 40, 52, 53), pulsatile CPB versus non-pulsatile CPB (n = 5) (48, 54, 55, 60, 61), a Conventional ExtraCorporeal Circulation (CECC) circuit versus a Minimalized ExtraCorporeal Circulation (MECC) circuit (n = 3) (45, 46, 67), different CPB pump flow rates (n = 1) (49), pursuit of a high Mean Arterial Pressure (MAP) versus a low MAP (n = 1) (51), different types of coating of the CPB circuit (n = 1) (42), and the type of cardioplegia (n = 1) (36).

Four out of six studies (67%) (33, 34, 40, 53) comparing on-pump with off-pump surgery described a significant change intraoperatively compared to baseline measurements and three out of six studies (50%) (40, 52, 53) described a significant postoperative change. Two of the six studies (33%) (33, 34) reported the FCD.

Table 1 *Convection and diffusion parameters of on-pump studies*

					Pre- operative	Surgery			Post-operative			
Author	**Study groups**	**CPB duration (min)**	**Aortic cross-clamp time (min)**		**Pre- operative**	**After anesthesia**	**During aortic cross-clamp**	**End of CPB**	**ICU**	**24h after surgery**	**48 h after surgery**	**72h after surgery**
Arnold, R.C. (32)	Cardiac surgery	112 +/- 40	-	Con	* MFI	-	-	-	↑ MFI	-	-	-
				Dif	-	-	-	-	-	-	-	-
Bauer, A. (37)	Cardiac surgery	126 +/- 40	84 +/- 31	Con	-	*RBCv	~ RBCv	↧ RBCv	-	-	-	-
				Dif	-	*FCD	↓ FCD	~ FCD	-	-	-	-
Boly, C. (39)	Cardiac surgery obese patients	112 +/- 23	75 +/- 18	Con	*PPV/ MFI	~ PPV, ↧ MFI	-	↧ PPV[a], ~ MFI	-	-	-	~ PPV/ MFI
				Dif	*PVD/ TVD	↟ PVD/ TVD	-	↧ PVD/ TVD	-	-	-	↟ PVD/ TVD
	Cardiac surgery lean patients	118 +/- 51	86 +/- 42	Con	*PPV/ MFI	~ PPV, ↧ MFI	-	↧ PPV[a]/ MFI[a]	-	-	-	~ PPV/ MFI
				Dif	*PVD/ TVD	↟ PVD, ↧ TVD	-	↧ PVD, ~ TVD	-	-	-	↧ PVD/ TVD
Dekker, N. (41)	CABG	103 +/- 18	70 +/- 14	Con	*PPV	↟ PPV	-	↓ PPV	↓ PPV	↓ PPV	-	↓ PPV
				Dif	*PVD	↓ PVD	-	-	↓ PVD	↓ PVD		↓ PVD
Uil den, C.A. (43)	CABG	102 (94 - 140)	65 (56 - 84)	Con	*MFI	↟ MFI	-	↟ MFI	↟ MFI	-	-	-
				Dif	-	-	-	-	-	-	-	-
Dedda di, U. (44)	Cardiac surgery	80 (57 - 106)	-	Con	-	*PPV/ MFI	-	~ PPV, ↓ MFI	-	-	-	-
				Dif	-	*PVD/ TVD	-	~ PVD/ TVD	-	-	-	-
Greenwood, J.C. (50)	Cardiac surgery	105 +/- 35	75 +/- 27	Con	*MFI/ PPV	-	-	-	↓ MFI/ PPV	-	-	-
				Dif	*PVD	-	-	-	↓ PVD	-	-	-
Prestes, I.(63)	Cardiac surgery	107 +/- 37.3	71.1 +/- 30.7	Con	-	* PPV/ MFI	-	↟ PPV, ↑ MFI	-	-	-	-
				Dif	-	* TVD/ PVD		↧ TVD/ PVD	-	-	-	-
Wu, Q. (65)	Cardiac surgery	123.57 (92.93 - 154.21)	90.33 (63.57 - 117.09)	Con	-	-	-	-	-	-	-	-
				Dif	*PVD	↓ PVD	↓ PVD	-	↓ PVD	~ PVD	~ PVD	-

End CPB: time from onset of CPB until weaning from CPB. During aortic cross-clamp: time from aortic cross-clamp until aortic cross clamp release. ICU: first 24 hours after surgery. Cardiac surgery: multiple surgeries (i.e. CABG, valve replacement/repair). Min: minutes. Con: convection parameters. Dif: diffusion parameters. MFI: microvascular flow index. PPV: proportion of perfused vessels. RBCv: red bloodcell velocity. FCD: functional capillary density. PVD: perfused vessel density. TVD: total vessel density *: baseline. ↓: significant decrease vs baseline ↑: significant increase vs baseline. ~: no significant change vs baseline. ↟: increase vs baseline (not significant). ↧: decrease vs baseline (not significant), -: not reported, [a]: significantly lower than at timepoint after anesthesia induction

In the study by Atasever et al. (33), a significant reduction in FCD was seen at the end of the CPB period in the on-pump group, while the FCD was not significantly reduced in the off-pump group. PVD was reported in three out of six studies (50%) (38, 40, 53). The study by de Backer et al. (40) reported a significantly reduced PVD at the end of the CPB period in both the on-pump and off-pump group and the PVD recovered to baseline 24 hours after surgery in both groups, in contrast with the study by Koning et al. (53) which only reported a significantly decreased PVD in the on-pump group, which was still significantly decreased after the first 24 hours after surgery. Three out of six studies (50%) reported the TVD (38, 40, 53). Koning et al. (53) reported significantly reduced TVD at the end of the CPB period in the on-pump group and the TVD did not recover within the first 24 hours after surgery. In the off-pump group, no significant change in TVD was found intraoperatively, however, postoperatively the TVD was reduced, but not significantly. The other two studies (67%) (38, 40, 53) found no effect in the on-pump group on TVD. Only one study reported the PPV (40). In this study the PPV was significantly reduced at the end of surgery in both the on-pump and off-pump group, and did not recover to baseline in the first 24 hours after surgery in both groups. The MFI was reported in two out of six studies (33%) (52, 53). The study by Koning et al. (53) reported no difference in MFI during the CPB period of the off-pump surgery group. In the on-pump surgery group, there was a non-significant decrease in MFI intra-operatively. However, two studies of Koning et al. (52, 53) reported a significant postoperative reduction in MFI in the on-pump surgery groups, while the MFI did not change compared to baseline in the off-pump surgery groups. Lastly, RBCv was reported in three of the six studies (50%) (33, 34, 52). Atasever et al. reported a significantly decreased RBCv during off-pump surgery in one study (34), but this significant reduction in RBCv was not reported in his other study in the off-pump surgery group (33). However, in the on-pump study group they reported a significant increase in RBCv intra-operatively (33). Koning et al. (52) described a significantly increased RBCv in the on-pump group in the first 24 hours postoperatively.

CPB generates a non-pulsatile blood flow and this non-physiological blood flow may influence the sublingual microcirculation. Five studies (48, 54, 55, 60, 61) compared a pulsatile blood flow during CPV with a non-pulsatile blood flow. Three of the five studies (60%) (58, 63, 64) described a significant change in microcirculatory parameters intraoperatively compared to baseline measurements and two studies (40%) (60, 61) described a significant postoperative change in microcirculatory parameters compared to baseline measurements. PVD was reported in three of the five studies (60%) (48, 54, 55). Koning et al. (55) reported no change in PVD at the end of the CPB period in the pulsatile CPB group, whereas it was significantly reduced in the non-pulsatile CPB group. The PPV was reported by three out of five studies (60%) (48, 60, 61). The non-pulsatile groups in the two studies by O'neil et al. (60, 61) had a significantly reduced PPV intra-operatively, whereas the PPV in the pulsatile groups did not change from baseline during surgery. The significantly reduced PPV in the non-pulsatile CPB groups was still reduced postoperatively and did not recover at 48 hours postoperatively. In the pulsatile CPB group, the PPV still did not change post-operatively compared to baseline. MFI was described in two of the six studies (33%) (48, 54), both studies did not report a significant change in MFI.

In addition to off-pump surgery, pulsatile CPB and non-pulsatile CPB, there are other variations on the CPB system, such as the use of CECC and MECC circuits (45, 46, 67), a different type of cardioplegia (36) or different coatings of the circuit (42).

The three studies (45, 46, 67) that focused on the influences of CECC and MECC on the microcirculation only described the intraoperative period. Two of the three studies (67%) (45, 46) reported a signification reduction in FCD in both the MECC and CECC group during the aortic cross-clamp period and this reduction in FCD recovered to baseline at the end of the CPB period. Only Yuruk et al. (67) reported the PVD and MFI and found a significantly reduced PVD during the aortic cross clamp period in the CECC group. The MFI was not different compared to baseline in both groups.

Aykut et al. (36) investigated the use of different types of cardioplegia, namely blood cardioplegia and crystalloid cardioplegia. They reported a significantly decreased PVD during the aortic cross clamp period in both the crystalloid cardioplegia and blood cardioplegia groups. PVD was only recovered to baseline the end of the

CPB period in the blood cardioplegia group. TVD showed the same results, TVD was decreased during the aortic cross clamp period in both groups. However, only in the crystalloid cardioplegia group, the TVD was still significant reduced at the end of the CPB period.

Furthermore, Dekker et al. (42) compared two different coatings used for the CPB circuit, namely heparin coated (HC) and phosporylcholine-coated (PC). They reported in both circuits a significantly reduced PVD at the end of the CPB period, that did not recover to baseline measurements within 72 hours after surgery.

Effect of therapeutic interventions

Appendix Vb shows the microcirculatory alterations resulting from different therapeutical interventions. Nine studies reported microcirculatory parameters in association with a therapeutic intervention. The studies assessed the effect of RBC transfusion intra- or postoperatively (n=2) (64, 66), propofol or propofol and dexmedetomidine intra- or postoperatively (n=2) (56, 59), phenylephrine intraoperatively (n=1) (57), different inhalation anesthetics (n=1) (62), different intravenous infusions postoperative (n=1) (35), kentanserin (n=1) (47), methylene blue (n=1) (58). In total, three studies (57-59) found a significant alteration in convective parameters and three (58, 59, 66) studies found significant alterations in diffusive parameters compared to baseline measurements.

A distinction is made between the effect of these therapeutic interventions on the microcirculatory parameters during cardiothoracic surgery (intra-operative changes) compared to baseline measurements and the effect of these therapeutic interventions on microcirculatory parameters postoperatively at the ICU or at the ward till 72 hours after surgery (post-operative changes) compared to baseline measurements.

Four out of nine studies (44%) (57, 59, 62, 66) reported the intra-operative changes of the microcirculatory parameters and six out of nine studies (67%) (35, 47, 56, 58, 62, 64) reported the postoperative changes of the microcirculatory parameters compared to baseline.

In total, three out of the four studies (75%) (59, 62, 66) focused on the intra-operative change compared to baseline measurements and reported diffusion microcirculatory parameters (FCD, PVD and/or TVD). One study of the four studies (33%) (66) reported FCD, which showed a significant increase in FCD after intraoperative RBC transfusion. Two out of four studies (50%) (59, 62) reported the PVD. Özarslan et al. (62) reported no change in PVD compared to baseline in both the sevoflurane and isoflurane groups. A significant intra-operative decrease in TVD was described in one of the two studies (50%) (59, 62) that reported the TVD. This intra-operative decrease in TVD was present in patients who were sedated with dexmedetomidine in combination with propofol (59). The other study, Özarslan et al. (62), concluded that the use of isoflurane as an anesthetic does not contribute to a change in TVD at the end of the CPB period.

The intraoperative change of the convective microcirculatory parameters (PPV, MFI and/or RBCv) compared to the baseline measurements was reported by all four studies (100%) (57, 59, 62, 66). Two of them (57, 59) reported a significant change in these parameters compared to baseline measurements. PPV was reported in two out of four studies (50%) (59, 62) and none of the two studies reported a significant alteration in PPV intra-operatively. All four studies (100%) (57, 59, 62, 66) reported the MFI. MFI was significantly reduced at the end of the CPB period in the study by Maier et al. (57). Mohamed et al. (59) described that the MFI was significantly reduced intraoperatively when using propofol alone as an anesthetic, whereas when using propofol in combination with dexmedetomidine there was no change in the MFI compared to baseline. The study by Özarslan et al. (62) reported no change in MFI during the intraoperative period with the use of sevoflurane. Yuruk et al. (66) did not find any intra-operative alterations in MFI after RBC transfusion.

Six of the nine studies (67%) (35, 47, 56, 58, 62, 64) followed patients post-operatively and only one study had an additional follow-up of 24 hours (62) after surgery.

The postoperative change of the diffusion microcirculatory parameters (FCD, PVD and/or TVD) compared to the baseline measurements was reported by all six studies (100%) (35, 47, 56, 58, 62, 64). PVD was reported in five out of six studies (83%) (47, 56, 58, 62, 64), of which one study (17%) (58) described a significant increase in PVD after a postoperative infusion with methylene blue. The PVD was still unchanged from baseline with the use of sevoflurane and

isoflurane (62). In four out of six studies (56, 58, 62, 64) the TVD was reported postoperatively. Özarslan et al. (62) reported that the use of isoflurane caused no change in TVD compared to baseline measurements. TVD was significantly increased with a postoperative methylene blue infusion (58).

The postoperative change of the convective microcirculatory parameters (PPV and MFI) compared to baseline was reported by all the six studies (100%) (35, 47, 56, 58, 62, 64).
One of them (58) reported a significant change in these parameters compared to baseline measurements. PPV was described in four out of six studies (67%) (47, 56, 62, 64). None of them reported a significant change in PPV compared to baseline measurements. Five out of six studies described the MFI (35, 47, 58, 62, 64). The use of sevoflurane induced no change in MFI in the postoperative period (62). Atasever et al. (35) reported that gelatin infusion caused no change in MFI with respect to the baseline. Methylene blue caused a significant increase in MFI postoperatively (58).

Macrocirculation vs microcirculation
A summary of macrocirculatory parameters can be found in Appendix VIa (Macrocirculatory parameters on-pump studies), VIb (Macrocirculatory parameters of CPB variations) and VIc (Macrocirculatory parameters of therapeutical interventions). Twenty-five out of thirty-six studies (69%) (32-34, 36-45, 47, 48, 50-54, 57, 58, 61, 62, 65) reported the results about the relationship between the macrocirculation and microcirculation (Table 2). Fifteen studies (60%) (36, 38-43, 45, 47, 48, 54, 57, 61, 62) reported no coherence between the macrocirculation and microcirculation. The other studies (40%) reported a coherence between microcirculatory alterations and macrocirculatory alterations.

Of the nine on-pump studies, five studies (56%) (32, 37, 44, 50, 65) reported a coherence between the macrocirculation and microcirculation. Arnold et al. (32) found that the state after CPB was associated with an increase in MFI without a parallel increase in MAP. Bauer et al. (37) reported a weak but significant correlation between FCD and temperature and FCD and hemoglobin (Hb) concentration. Di Dedda et al. (44) showed that the pre-CPB microcirculatory status determined significant changes in platelet count and function postoperatively. Higher values of PVD and TVD corresponded with a better platelet function preservation postoperatively. A negative association was found between TVD values preoperative and the difference between pre-and postoperative platelet count. Greenwood et al. (50) described a correlation between the postoperative lactate level and PVD. Patients in the lowest PVD quartile had higher postoperative lactate levels compared to the patients in the three highest quartiles.

Five out of eighteen studies (28%) (33, 34, 51-53) focusing on CPB variations found a coherence between the macrocirculatory and microcirculatory parameters. Atasever et al. (33) found in the on-pump group a significantly increased CO, which was associated with a significantly increased RBCv, and a reduction in FCD intraoperatively. Off-pump surgery resulted in a significant drop in CO and was associated with a complete stop of capillary blood flow. Another study by Atasever et al. (34) found a coherence between the decrease in CO/ blood pressure and the reduction in microcirculatory RBCv and FCD in off-pump surgery. Holmgaard et al. (51) reported that the microcirculation was affected by CPB to a level where MAP is secondary. The use of vasopressors may have masked the potential benefit of a higher pursued MAP. Koning et al. (52) found an association between a moderately significant increase in hyperdynamic quadrants and a decrease in Hct. Another study by Koning et al. (53) concluded that an early impairment of the PVD was associated with an increased postoperative lactate concentration and duration compared to patients with normal postoperative microcirculatory measurements during the first 6 hours of resuscitation. An early impairment of the MFI was not associated with a postoperatively increased lactate.

Just one of the nine therapeutic intervention studies (11%) (58) found a relationship between macrocirculatory and microcirculatory parameters. This study reported a significant correlation between an increase in MAP and a reduction in PVD.

Table 2 *Main findings included studies*

Author	Year	Main findings for coherence of macrocirculation vs microcirculation	Main findings for the relation between microcirculation and outcome
Arnold, R.C. (32)	2012	Post-CPB state was associated with an increase in MFI without a concomitant rise in MAP	-
Atasever, B. (33)	2011	CPB resulted in a significant increase in CO and was associated with a significant increase in RBCv, and a reduction in FCD, Off-pump resulted in a significant decrease in CO and was associated with a complete halt of capillary blood flow, but no change in FCD.	-
Atasever, B. (34)	2011	A drop in CO and systemic blood pressure during cardiac displacement was associated with a reduction in microcirculatory perfusion and oxygenation	-
Atasever, B. (35)	2012	Not reported	Not reported
Aykut, G. (36)	2021	During this period, no correlations were found between MAP and microvascular parameters	Not reported
Bauer, A. (37)	2007	FCD was significantly reduced at aorta release to 90% of the values observed before CPB, but recovered after weaning from CPB, a weak but significant correlation with temperature and Hb concentration was found.	-
Bienz, M. (38)	2016	There is no significant correlation between vasopressor use, and microcirculatory parameters, blood pressure and temperature. Active warming of patients under CPB could have a positive effect on the microcirculation whereas prolonged exposure to hypothermic temperature would have a negative effect	-
Boly, C. (39)	2021	Sublingual microcirculatory perfusion was equally disturbed in obese and lean patients, independent of the differences in intraoperative blood pressure.	Not reported
Backer de, D. (40)	2009	There was no relationship between microcirculatory and systemic hemodynamic and blood gas variables. There was a significant relationship between the minimum PPV and the peak lactate level.	There was a significant relationship between changes in the SOFA score and maximal change in small vessel perfusion or minimum PPV.
Dekker, N. (41)	2019	Decreased microcirculatory perfusion during bypass may be caused by a combination of systemic inflammation and hemodilution that initiates a vicious circle of glycocalyx damage, endothelial cell dysfunction and altered microcirculatory flow patterns.	Not reported
Dekker, N. (42)	2020	Although changes in glycocalyx dimensions may indeed contribute to alterations in microcirculatory flow, the present study is unable to demonstrate a causal relationship between CPB-induced glycocalyx shedding and microcirculatory perfusion disturbances.	Not reported
Uil den, C.A. (43)	2008	No association was visible between changes in MFI and change in MAP.	Retrospectively, a large decrease in microvascular perfusion during CPB was observed in the patient who died 12 days after surgery.

Dedda di, U. (44)	2018	Pre-CPB microcirculatory status determines significant changes in post-platelet count and function. For PVD and TVD, higher values correspond to a better platelet function preservation. Pre-CPB TVD has a negative association with the pre-post difference in platelet count.	-
Donndorf, P. (45)	2012	There was no significant correlation detectable between the FCD and the respective MAP in the CECC group. The MECC group showed a weak but significant positive correlation between these two parameters	-
Donndorf, P. (46)	2014	Not reported	Not reported
Elbers, P. (47)	2009	Kentanserin lowered ABP. At the same time, a significant increase in mean PVD for large vessels was observed. However, capillary perfusion was unaltered. Changes in global hemodynamics were not reflected in the microcirculation	-
Elbers, P. (48)	2011	Changes in pulse pressure or energy equivalent pressure bear no obvious relationship with microcirculatory parameters	-
Forti, A. (49)	2012	Not reported	-
Greenwood, J.C. (50)	2021	No correlation was found between postoperative MFI and MAP or CI. Postoperative PVD was not correlated with MAP or CI. Patients in the lowest PVD quartile exhibited higher postoperative lactate compared with patients with a PVD in the upper three quartiles	Patients in the lowest PVD quartile exhibited a higher SOFA score at 24 hours compared with patients with a PVD in the upper three quartiles
Holmgaard, F. (51)	2018	It may indicate that the overall response to CPB is affecting the microcirculation to a level where MAP is secondary or the effect of vasopressors may wash out the potential benefit of a higher MAP on a microcirculatory level	-
Koning, N.J. (52)	2014	Reduction in Hct was associated with a moderate significant increase in hyperdynamic quadrants. No correlation with other hemodynamic or blood-gas derived variables or administered vasoactive medication were observed.	Both patients developed AKI showed an increased intra- and postoperative decreased MFI scores compared with preoperative values
Koning, N.J. (53)	2014	Early impairment of microcirculatory PVD and high heterogeneity are associated with increased lactate concentration and duration compared with patients with normal postoperative microcirculation during the first 6h of resuscitation. Early impairment of MFI was not associated with an increased lactate.	-
Koning, N.J. (54)	2012	PP flow during aortic cross-clamp time is associated with preservation of microvascular perfusion in early postoperative period, irrespective of systemic hemodynamics	Despite better recovery of microcirculation in the PP group, no differences in clinical outcome parameters between PP and NP groups were found.
Koning, N.J. (55)	2016	-	-
Liu, X. (56)	2016	Not reported	Not reported

Maier, S. (57)	2009	Initiation of CPB resulted in no alterations in sublingual microcirculation, despite reduction in systemic oxygen delivery. A pressure increase with phenylephrine during CPB and constant blood flow did not alter systemic oxygen transport variables, but resulted in an impairment of microcirculatory blood flow quality.	Not reported
Maurin, C. (58)	2021	An increase in MAP was significantly correlated with a reduction in PVD. The administration of methylene blue could improve microvascular perfusion and reactivity, and partially restore the loss of hemodynamic coherence.	In addition to the microcirculatory alterations, a high mortality rate was found due to high pre-operative surgical risk and significant requirements for vasoactive drugs
Mohamed, H. (59)	2019	Not reported	-
O' Neil, M. (60)	2018	Not reported	-
O'Neil, M. (61)	2012	Patients in the NP group had a greater number of adherent leukocytes than PP group. WBC counts increased after CPB in both groups. The microvascular changes observed occurred independent of systemic hemodynamic variables such as MAP and CI	-
Özarslan, N.G. (62)	2012	There is no relation between the microcirculatory and systemic variables	Not reported
Prestes, I. (63)	2016	Not reported	A significantly higher MFI at start of CPB was observed in patients presenting complications
Stowell, C.P. (64)	2017	-	Not reported
Wu, Q. (65)	2019	A significant and positive correlation between syndecan-1 and lactate concentrations was observed	-
Yuruk, K. (66)	2010	Not reported	-
Yuruk, K. (67)	2012	Not reported	Not reported

-: No description of macrocirculatory parameters/ outcomes in general. Not reported: relationship between the two variables is not reported. ABP: arterial blood pressure. AKI: Acute Kidney Injury. CECC: Conventional ExtraCorporeal Circulation circuit. CI: Cardiac Indec. CPB: cardiopulmonary bypass. CO: Cardiac Output. Hct: hematocrit. MAP: Mean Arterial Pressure. MECC: Minimalized ExtraCorporeal Circulation circuit. NP: Non-pulsatile CPB-flow. PP: pulsatile CPB-flow. SOFA: Sequential Organ Failure Assessment score. WBC: white blood cell. MFI: microvascular flow index. PPV: proportion of perfused vessels. RBCv: red bloodcell velocity. FCD: functional capillary density. PVD: perfused vessel density. TVD: total vessel density.

Relation of microcirculation with clinical outcome

Nineteen out of the 36 studies (53%) provided any information about the clinical outcomes of patients (32, 35, 36, 39-43, 45, 46, 50, 52, 54, 56, 58, 62-64, 67): length of ICU stay (n = 11) (35, 36, 39, 41, 45, 52, 54, 62-64, 67), length of hospital stay (n = 4) (36, 39, 41, 63), mortality (n=9) (32, 39, 43, 46, 50, 52, 54, 58, 64) and postoperative outcomes (i.e. hypotension, AKI and postoperative pulmonary embolism) (n = 6) (32, 42, 45, 52, 54, 56). Furthermore, two studies reported the Sequential Organ Failure Assessment (SOFA) score (40, 50) (Appendix VII). Seven out of the 19 studies (37%) (40, 43, 50, 52, 54, 58, 63) examined the relationship between the microcirculatory parameters and outcomes (Table 2).

In on-pump studies, Den Uil et al. (43) retrospectively found a large reduction in microvascular perfusion during CPB in a patient who had died 12 days after surgery. Greenwood et al. (50) reported that patients in the lowest quartile of PVD had a higher SOFA score at 24 hours after surgery compared to patients with PVD in the highest three quartiles. Moreover, Prestes et al. (63) found a relationship between a significantly higher MFI at the onset of CPB and postoperative complications.

For the studies with the focus on CPB variations, De Backer et al. (40) found a significant relationship between changes in SOFA score and maximum change in small vessel perfusion or minimum PPV. Koning et al. (52) reported the relationship between an AKI after on-pump surgery and increased intraoperatively MFI and postoperatively decreased MFI. Koning et al. (54) found no difference in outcome between pulsatile and non-pulsatile CPB.

Discussion

Cardiothoracic surgery with or without CPB causes alterations in the sublingual microcirculation. The surgery-induced microcirculatory alterations mainly constitute changes in FCD, PVD and PPV and show an impaired microcirculation. These microcirculatory parameters remain disturbed throughout the surgical procedures and sometimes the alterations persist for up to 72 hours after surgery (PVD and PPV). The TVD and MFI appear to be less susceptible to change in the intra- and postoperative periods.

On-pump studies

The overall conclusion for the on-pump studies is in line with the review of den Os et. al. (30). The surgery-induced microcirculatory alterations as represented by a decrease in FCD, PVD or PPV were found in 44% of the on-pump studies. PPV and TVD are the basis for FCD and PVD calculations (25, 68). The results suggest that the observed reductions in FCD and PVD were mainly the result of a reduced PPV since TVD was not significantly altered in most studies. So, CPB mainly reduce the number of perfused vessels which impairs microcirculatory perfusion, but does not affect the number of microvessels. The effect of CPB on MFI showed contrary results (32, 44, 50, 63). This could be partly explained by the modified criteria for the MFI parameter in some studies. An additional category for hyperdynamic flow was used in some studies (62, 63), which could confound the results. MFI currently recognizes only one type of flow as abnormal (sluggish flow), but does not consider another abnormal type of flow (hyperdynamic flow).

CPB variations

The observed microcirculatory alterations are likely a true CPB effect since there was no reduction in FCD, PVD, TVD, PPV and MFI in patients undergoing off-pump surgery (33, 52, 53). Besides the effect of the on-pump and off-pump, other variations of CPB, such as type of blood flow during CPB, usage of a CECC or MECC circuit and the use of different CPB circuit coatings may influence the effect of CPB on the sublingual microcirculation. Furthermore, the traditional non-pulsatile blood flow of CPB may exhibit adverse effects on the sublingual microcirculation. Five studies in this review (48, 54, 55, 60, 61) compared a pulsatile blood flow during CPB with a non-pulsatile blood flow.

However, the question whether pulsatile blood flow during CPB is superior to non-pulsatile blood flow in the preservation of microcirculatory parameters remain unanswered due to contrary results. O'neil et al. (60, 61) concluded that during pulsatile CPB, microcirculatory perfusion (PPV) was preserved, whereas it was not during non-pulsatile CPB. In contrast, Koning et al. (54, 55) reported that microcirculatory perfusion did show alterations during pulsatile CPB, but recovery occurred after weaning from CPB, whereas this was not the case in the non-pulsatile CPB group.

Furthermore, three studies (45, 46, 67) examined the effect of CECC versus MECC and all three studies concluded that MECC has a beneficial effect on the preservation of microvascular blood flow. The use of an HC or a PC circuit (30) does not result in any difference in microcirculatory alterations. Moreover, Forti et al. conclude that changes in CPB flow within 20% of its theoretical value do not alter microcirculation. Also, the pursuit of a high MAP or low MAP (54) does not result in a noticeable difference in the MFI.

Therapeutic interventions

Multiple included studies have specifically measured the effect of various therapeutic interventions on the sublingual microcirculation. An RBC transfusion during surgery (66) would help to correct the anemic conditions and improve the MFI and FCD. Maier et al. (57) studied the administration of phenylephrine during surgery and concluded that the MFI decreased significantly during the intra-operative period due to microvascular blood flow shunting. Both Mohamed et al. (59) and Liu et al. (56) concluded that the combination of dexmedetomidine and propofol improved sublingual microcirculation. Özarslan et al. (62) compared the effect of three inhalation anesthetics on the microcirculatory perfusion and showed that sevoflurane, isoflurane and desflurane have different effects on circulation, but all have a temporary effect.

The administration of methylene blue was studied by Maurin et al. (61) and concluded that methylene blue increased the PVD and TVD. Finally, Stowell et al. (64) demonstrated that there were no differences in microcirculatory parameters in cardiothoracic surgery patients transfused with RBCs stored for less than 10 days or more than 21 days.

Macrocirculation vs microcirculation

Most studies (60%) (33, 37, 44, 50, 53, 58) that reported a relationship, found a relationship between macrocirculatory parameters (i.e., MAP, CO, and platelet function) and diffusion parameters (i.e. FCD and PVD) of the microcirculation. This can be explained by the fact that CPB and variations thereof do not necessarily affect the number of blood vessels, but mainly impairs the microcirculatory blood flow (69-71). Nevertheless, only 15 of 36 included studies found (no) coherence between the macrocirculation and microcirculation. More research is needed to explore the relationship between macrocirculation and microcirculation.

Microcirculation and clinical outcome

The relation between microcirculatory alterations and clinical outcome remains unclear. Of the 19 studies that mentioned clinical outcomes, only seven reported something about the relationship between microcirculation and clinical outcome. Even in these studies, there was limited attention to the extent of microcirculatory changes in relation to clinical outcomes. Only Greenwood et al. presented a detailed description of PVD changes in relation to the SOFA score. They found that patients in the lowest quartile of PVD exhibited a higher SOFA score at 24 hours compared to patients with a PVD in the upper three quartiles. In addition, only a few complications were included in the analyses (i.e. LOS ICU, LOS hospital, some complications and mortality), whereas complications of cardiothoracic surgery such as the development of cardiogenic shock, vasoplegic syndrome, mediastinitis, stroke, and acute kidney injury would be interesting to include in future research.

Limitations

This review includes small (single) center studies of reasonable to good quality. It may be assumed that these studies are representative for the description of microcirculatory changes during and after cardiothoracic surgery. However, the included studies are very heterogeneous. Each study has a different research question and describes different microcirculatory parameters, measured at different time points and intervals with varying baselines. Most studies used a slightly different surgery, anesthesia, or perfusion protocol, which may potentially influence the microcirculatory measurements. The use of different protocols could influence the microcirculation measurements. Therefore, it is possible that this review is subject to different types of bias, such as confirmation bias and selection bias. Because of the heterogeneity, it is difficult to recognize a pattern in the microcirculatory alterations. Also, the studies used multiple units for the microcirculation parameters, making the reported parameters ambiguous.

Another limitation is that only the sublingual microcirculation measurements were included in the studies. Microcirculation measurements are performed with great regularity in the sublingual area. The sublingual space is easily accessible

during surgical procedures and during ICU admission. The sublingual space has the same embryological origin as the intestine. (72) Studies have shown that sublingual microcirculatory changes correlate well with microcirculatory changes of the gut, for example. (73)

Furthermore, many of the studies reported CPB and aortic clamp time, but these variables were usually not included in the analyses. Therefore, it is not possible to draw a conclusion about any association between microcirculatory alterations and shorter or longer CPB and/or aortic clamp time, whereas the aortic cross-clamp time describes exactly the period that gives a visualization of the contribution of the CPB to microcirculatory changes.

Only in nine of 36 studies (36, 37, 45, 46, 49, 54, 57, 65, 67) were sublingual microcirculation measurements taken at the beginning of aortic clamp placement and/or aortic clamp removal. Between these time points, an accurate picture of the influence of CPB on the microcirculation without other additional influences on the microcirculation is provided.

Future perspectives

First, further research should identify which strategies contribute to improving the recovery capacity of the microcirculation after cardiothoracic surgery. Next, future research should identify how microcirculatory alterations after cardiothoracic surgery could be prevented. A small proportion of studies have reported clinical outcomes and even a smaller proportion have attempted to relate the microcirculation to the clinical outcomes. Therefore, the relationship between microcirculation and clinical outcomes in cardiothoracic surgery is still unclear. To prove that microcirculation measurement could contribute to understanding the patient and providing early goal-directed therapy, evidence is needed that microcirculatory changes contribute to a particular clinical outcome. In addition, the influence of the CPB and aorta-cross clamp duration is still unclear. Follow-up studies should show whether and in which ways the duration of this period affects the microcirculation.

Another perspective which needs to be addressed is the MFI as a microcirculatory parameter. MFI is a measure for the global determination of microcirculatory perfusion and secondary to the other parameters for determining microcirculatory perfusion. Yet, MFI remains a popular measure to describe microcirculation. (25) However, MFI has been replaced by a new emerging parameter: RBCv, which describes the absolute velocity (25). RBCv is still hardly used in studies on microcirculation. It is important to include this parameter in future studies, because the RBCv is an objective measurement of the absolute microcirculatory perfusion, instead of a subjective qualitative analysis of the microcirculatory perfusion with the MFI as parameter. (68)

Lastly, the identification of leukocyte-endothelium interaction could contribute to obtaining additional information on inflammation and understanding the pathophysiological status of the patient. (74) Therefore, it is important that more research is initiated in the area of leukocyte-endothelial interaction along with microcirculatory measurements.

Conclusion

In conclusion, CPB impairs microcirculatory perfusion, which is reflected by a reduced FCD, PPV and PVD, whereas TVD remain unaltered. The effect of CPB on MFI differs between studies and remains conflicting. One can imagine that the machine-like laminar CPB flow differs from the pulsatile flow of the heart. Therefore, one can also imagine that it does affect both convection and diffusion of the microcirculation. Uncoupling of hemodynamic coherence is seen in several studies. Microcirculation measurements therefore seems to have added value in cardiothoracic surgical patients. However, not as standard care in all cardiothoracic surgical patients, but as an aid in unexplained postoperative clinical presentation or in high-risk surgical patients. More research is needed on the contribution of microcirculatory changes to a particular clinical outcome.

References

1. Veluz J, Leary M. Cerebrovascular Complications of Cardiac Surgery. Primer on Cerebrovascular Diseases: Elsevier; 2017. p. 650-5.
2. Foundation. TdH. Dutch statistics of cardiovascular diseases in 2018. 2018.
3. Yeh Y-C, Wang M-J, Chao A, Ko W-J, Chan W-S, Fan S-Z, et al. Correlation between early sublingual small vessel density and late blood lactate level in critically ill surgical patients. journal of surgical research. 2013;180(2):317-21.
4. Mekontso-Dessap A, Houel R, Soustelle C, Kirsch M, Thebert D, Loisance DY. Risk factors for post-cardiopulmonary bypass vasoplegia in patients with preserved left ventricular function. The Annals of thoracic surgery. 2001;71(5):1428-32.
5. Jakobson T, Karjagin J, Vipp L, Padar M, Parik A-H, Starkopf L, et al. Postoperative complications and mortality after major gastrointestinal surgery. Medicina. 2014;50(2):111-7.
6. Laursen LØ, Petersen RH, Hansen HJ, Jensen TK, Ravn J, Konge L. Video-assisted thoracoscopic surgery lobectomy for lung cancer is associated with a lower 30-day morbidity compared with lobectomy by thoracotomy. European Journal of Cardio-Thoracic Surgery. 2016;49(3):870-5.
7. Pahwa S, Bernabei A, Schaff H, Stulak J, Greason K, Pochettino A, et al. Impact of postoperative complications after cardiac surgery on long-term survival. Journal of cardiac surgery. 2021;36(6):2045-52.
8. Downing SW, Edmunds Jr LH. Release of vasoactive substances during cardiopulmonary bypass. The Annals of thoracic surgery. 1992;54(6):1236-43.
9. Boyle EM, Pohlman TH, Johnson MC, Verrier ED. Endothelial cell injury in cardiovascular surgery: the systemic inflammatory response. The Annals of thoracic surgery. 1997;63(1):277-84.
10. Miller BE, Levy JH. The inflammatory response to cardiopulmonary bypass. Journal of cardiothoracic and vascular anesthesia. 1997;11(3):355-66.
11. Handy Jr JR, Asaph JW, Skokan L, Reed CE, Koh S, Brooks G, et al. What happens to patients undergoing lung cancer surgery?: Outcomes and quality of life before and after surgery. Chest. 2002;122(1):21-30.
12. Patel AS, Bergman A, Moore BW, Haglund U. The economic burden of complications occurring in major surgical procedures: a systematic review. Applied health economics and health policy. 2013;11(6):577-92.
13. Lowenstein E, Hallowell P, Levine FH, Daggett WM, Austen WG, Laver MB. Cardiovascular response to large doses of intravenous morphine in man. New England Journal of Medicine. 1969;281(25):1389-93.
14. STANLEY TH, Webster LR. Anesthetic requirements and cardiovascular effects of fentanyl-oxygen and fentanyl-diazepam-oxygen anesthesia in man. Anesthesia & Analgesia. 1978;57(4):411-6.
15. Verrier ED, Wright IH, Cochran RP, Spiess BD. Changes in cardiovascular surgical approaches to achieve early extubation. Journal of cardiothoracic and vascular anesthesia. 1995;9(5):10-5.
16. Sirio CA, Martich GD. Who goes to the ICU postoperatively? Chest. 1999;115(5):125S-9S.
17. Jung C. Assessment of microcirculation in cardiogenic shock. Current Opinion in Critical Care. 2019;25(4):410-6.
18. Leyh RG, Kofidis T, Strüber M, Fischer S, Knobloch K, Wachsmann B, et al. Methylene blue: the drug of choice for catecholamine-refractory vasoplegia after cardiopulmonary bypass. The Journal of thoracic and cardiovascular surgery. 2003;125(6):1426-31.
19. Weis F, Kilger E, Beiras-Fernandez A, Nassau K, Reuter D, Goetz A, et al. Association between vasopressor dependence and early outcome in patients after cardiac surgery. Anaesthesia. 2006;61(10):938-42.
20. De Backer D, Dubois M-J, Schmartz D, Koch M, Ducart A, Barvais L, et al. Microcirculatory alterations in cardiac surgery: effects of cardiopulmonary bypass and anesthesia. The Annals of thoracic surgery. 2009;88(5):1396-403.
21. Bauer A, Kofler S, Thiel M, Eifert S, Christ F. Monitoring of the sublingual microcirculation in cardiac surgery using orthogonal polarization spectral imaging: preliminary results. The Journal of the American Society of Anesthesiologists. 2007;107(6):939-45.
22. Uz Z, Ince C, Guerci P, Ince Y, P Araujo R, Ergin B, et al. Recruitment of sublingual microcirculation using handheld incident dark field imaging as a routine measurement tool during the postoperative de-escalation phase—a pilot study in post ICU cardiac surgery patients. Perioperative Medicine. 2018;7(1):1-8.
23. Murphy G, Angelini G. Side effects of cardiopulmonary bypass: what is the reality? Journal of cardiac surgery. 2004;19(6):481-8.
24. Guven G, Hilty MP, Ince C. Microcirculation: physiology, pathophysiology, and clinical application. Blood Purification. 2020;49(1-2):143-50.
25. Ince C, Boerma EC, Cecconi M, De Backer D, Shapiro NI, Duranteau J, et al. 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 medicine. 2018;44(3):281-99.
26. Ince C. Hemodynamic coherence and the rationale for monitoring the microcirculation. Critical care. 2015;19(3):1-13.
27. den Uil CA, Lagrand WK, van der Ent M, Nieman K, Struijs A, Jewbali LS, et al. Conventional hemodynamic resuscitation may fail to optimize tissue perfusion: an observational study on the effects of dobutamine, enoximone, and norepinephrine in patients with acute myocardial infarction complicated by cardiogenic shock. PLoS One. 2014;9(8):e103978.
28. Corstiaan A, Lagrand WK, Spronk PE, van Domburg RT, Hofland J, Lüthen C, et al. Impaired sublingual microvascular perfusion during surgery with cardiopulmonary bypass: a pilot study. The Journal of thoracic and cardiovascular surgery. 2008;136(1):129-34.
29. D'Agostino RS, Jacobs JP, Badhwar V, Fernandez FG, Paone G, Wormuth DW, et al. The Society of Thoracic Surgeons adult cardiac surgery database: 2018 update on outcomes and quality. The Annals of thoracic surgery. 2018;105(1):15-23.
30. den Os MM, van den Brom CE, van Leeuwen AL, Dekker NA. Microcirculatory perfusion disturbances following cardiopulmonary bypass: a systematic review. Critical Care. 2020;24(1):1-12.
31. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. Systematic reviews. 2021;10(1):1-11.
32. Arnold RC, Dellinger RP, Parrillo JE, Chansky ME, Lotano VE, McCoy JV, et al. Discordance between microcirculatory alterations and arterial pressure in patients with hemodynamic instability. J Crit Care. 2012;27(5):531.e1-7.
33. Atasever B, Boer C, Goedhart P, Biervliet J, Seyffert J, Speekenbrink R, et al. Distinct alterations in sublingual microcirculatory blood flow and hemoglobin oxygenation in on-pump and off-pump coronary artery bypass graft surgery. J Cardiothorac Vasc Anesth. 2011;25(5):784-90.
34. Atasever B, Boer C, Speekenbrink R, Seyffert J, Goedhart P, de Mol B, et al. Cardiac displacement during off-pump coronary artery bypass grafting surgery: effect on sublingual microcirculation and cerebral oxygenation. Interact Cardiovasc Thorac Surg. 2011;13(6):573-7.
35. Atasever B, van der Kuil M, Boer C, Vonk A, Schwarte L, Girbes AR, 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.
36. Aykut G, Ulugöl H, Aksu U, Akin S, Karabulut H, Alhan C, et al. Microcirculatory Response to Blood vs. Crystalloid Cardioplegia During Coronary Artery Bypass Grafting With Cardiopulmonary Bypass. Front Med (Lausanne). 2021;8:736214.
37. Bauer A, Kofler S, Thiel M, Eifert S, Christ F. Monitoring of the sublingual microcirculation in cardiac surgery using orthogonal polarization spectral imaging: preliminary results. Anesthesiology. 2007;107(6):939-45.
38. Bienz M, Drullinsky D, Stevens LM, Bracco D, Noiseux N. Microcirculatory response during on-pump versus off-pump coronary artery bypass graft surgery. Perfusion. 2016;31(3):207-15.
39. Boly CA, Venhuizen M, Dekker NAM, Vonk ABA, Boer C, van den Brom CE. Comparison of Microcirculatory

Perfusion in Obese and Non-Obese Patients Undergoing Cardiac Surgery with Cardiopulmonary Bypass. J Clin Med. 2021;10(3).
40. De Backer D, Dubois MJ, Schmartz D, Koch M, Ducart A, Barvais L, et al. Microcirculatory alterations in cardiac surgery: effects of cardiopulmonary bypass and anesthesia. Ann Thorac Surg. 2009;88(5):1396-403.
41. Dekker NAM, Veerhoek D, Koning NJ, van Leeuwen ALI, Elbers PWG, van den Brom CE, et al. Postoperative microcirculatory perfusion and endothelial glycocalyx shedding following cardiac surgery with cardiopulmonary bypass. Anaesthesia. 2019;74(5):609-18.
42. Dekker NAM, Veerhoek D, van Leeuwen ALI, Vonk ABA, van den Brom CE, Boer C. Microvascular Alterations During Cardiac Surgery Using a Heparin or Phosphorylcholine-Coated Circuit. J Cardiothorac Vasc Anesth. 2020;34(4):912-9.
43. den Uil CA, Lagrand WK, Spronk PE, van Domburg RT, Hofland J, Lüthen C, et al. Impaired sublingual microvascular perfusion during surgery with cardiopulmonary bypass: a pilot study. J Thorac Cardiovasc Surg. 2008;136(1):129-34.
44. Di Dedda U, Ranucci M, Porta A, Bari V, Ascari A, Fantinato A, et al. The combined effects of the microcirculatory status and cardiopulmonary bypass on platelet count and function during cardiac surgery. Clin Hemorheol Microcirc. 2018;70(3):327-37.
45. Donndorf P, Kuhn F, Vollmar B, Rosner J, Liebold A, Gierer P, et al. Comparing microvascular alterations during minimal extracorporeal circulation and conventional cardiopulmonary bypass in coronary artery bypass graft surgery: A prospective, randomized study. J Thorac Cardiovasc Surg. 2012;144(3):677-83.
46. Donndorf P, Park H, Vollmar B, Alms A, Gierer P, Steinhoff G, et al. Impact of closed minimal extracorporeal circulation on microvascular tissue perfusion during surgical aortic valve replacement: intravital imaging in a prospective randomized study. Interact Cardiovasc Thorac Surg. 2014;19(2):211-7.
47. Elbers PW, Ozdemir A, van Iterson M, van Dongen EP, Ince C. Microcirculatory imaging in cardiac anesthesia: ketanserin reduces blood pressure but not perfused capillary density. J Cardiothorac Vasc Anesth. 2009;23(1):95-101.
48. Elbers PW, Wijbenga J, Solinger F, Yilmaz A, van Iterson M, van Dongen EP, et al. Direct observation of the human microcirculation during cardiopulmonary bypass: effects of pulsatile perfusion. J Cardiothorac Vasc Anesth. 2011;25(2):250-5.
49. Forti A, Comin A, Lazzarotto N, Battistella G, Salandin V, Sorbara C. Pump flow changes do not impair sublingual microcirculation during cardiopulmonary bypass. J Cardiothorac Vasc Anesth. 2012;26(5):785-90.
50. Greenwood JC, Jang DH, Hallisey SD, Gutsche JT, Horak J, Acker MA, et al. Severe Impairment of Microcirculatory Perfused Vessel Density Is Associated With Postoperative Lactate and Acute Organ Injury After Cardiac Surgery. J Cardiothorac Vasc Anesth. 2021;35(1):106-15.
51. 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.
52. Koning NJ, Simon LE, Asfar P, Baufreton C, Boer C. Systemic microvascular shunting through hyperdynamic capillaries after acute physiological disturbances following cardiopulmonary bypass. Am J Physiol Heart Circ Physiol. 2014;307(7):H967-75.
53. Koning NJ, Vonk AB, Meesters MI, Oomens T, Verkaik M, Jansen EK, et al. Microcirculatory perfusion is preserved during off-pump but not on-pump cardiac surgery. J Cardiothorac Vasc Anesth. 2014;28(2):336-41.
54. Koning NJ, Vonk AB, van Barneveld LJ, Beishuizen A, Atasever B, van den Brom CE, et al. Pulsatile flow during cardiopulmonary bypass preserves postoperative microcirculatory perfusion irrespective of systemic hemodynamics. J Appl Physiol (1985). 2012;112(10):1727-34.
55. Koning NJ, Vonk AB, Vink H, Boer C. Side-by-Side Alterations in Glycocalyx Thickness and Perfused Microvascular Density During Acute Microcirculatory Alterations in Cardiac Surgery. Microcirculation. 2016;23(1):69-74.
56. Liu X, Zhang K, Wang W, Xie G, Cheng B, Wang Y, et al. Dexmedetomidine Versus Propofol Sedation Improves Sublingual Microcirculation After Cardiac Surgery: A Randomized Controlled Trial. J Cardiothorac Vasc Anesth. 2016;30(6):1509-15.
57. Maier S, Hasibeder WR, Hengl C, Pajk W, Schwarz B, Margreiter J, et al. Effects of phenylephrine on the sublingual microcirculation during cardiopulmonary bypass. Br J Anaesth. 2009;102(4):485-91.
58. Maurin C, Portran P, Schweizer R, Allaouchiche B, Junot S, Jacquet-Lagrèze M, et al. Effects of methylene blue on microcirculatory alterations following cardiac surgery: A prospective cohort study. Eur J Anaesthesiol. 2021.
59. Mohamed H, Hosny H, Tawadros Md P, Elayashy Md Desa Fcai M, El-Ashmawi Md H. Effect of Dexmedetomidine Infusion on Sublingual Microcirculation in Patients Undergoing On-Pump Coronary Artery Bypass Graft Surgery: A Prospective Randomized Trial. J Cardiothorac Vasc Anesth. 2019;33(2):334-40.
60. O'Neil MP, Alie R, Guo LR, Myers ML, Murkin JM, Ellis CG. Microvascular Responsiveness to Pulsatile and Nonpulsatile Flow During Cardiopulmonary Bypass. Ann Thorac Surg. 2018;105(6):1745-53.
61. O'Neil MP, Fleming JC, Badhwar A, Guo LR. Pulsatile versus nonpulsatile flow during cardiopulmonary bypass: microcirculatory and systemic effects. Ann Thorac Surg. 2012;94(6):2046-53.
62. Özarslan NG, Ayhan B, Kanbak M, Çelebioğlu B, Demircin M, Ince C, et al. Comparison of the effects of sevoflurane, isoflurane, and desflurane on microcirculation in coronary artery bypass graft surgery. J Cardiothorac Vasc Anesth. 2012;26(5):791-8.
63. Prestes I, Riva J, Bouchacourt J, Kohn E, López A, Hurtado F. Microcirculatory changes during cardiac surgery with cardiopulmonary bypass. Revista Española de Anestesiología y Reanimación (English Edition). 2016;63(9):513-8.
64. Stowell CP, Whitman G, Granger S, Gomez H, Assmann SF, Massey MJ, et al. The impact of red blood cell storage duration on tissue oxygenation in cardiac surgery. J Thorac Cardiovasc Surg. 2017;153(3):610-9.e2.
65. Wu Q, Gao W, Zhou J, He G, Ye J, Fang F, et al. Correlation between acute degradation of the endothelial glycocalyx and microcirculation dysfunction during cardiopulmonary bypass in cardiac surgery. Microvasc Res. 2019;124:37-42.
66. Yuruk K, Almac E, Bezemer R, Goedhart P, de Mol B, Ince C. Blood transfusions recruit the microcirculation during cardiac surgery. Transfusion. 2011;51(5):961-7.
67. Yuruk K, Bezemer R, Euser M, Milstein DM, de Geus HH, Scholten EW, et al. The effects of conventional extracorporeal circulation versus miniaturized extracorporeal circulation on microcirculation during cardiopulmonary bypass-assisted coronary artery bypass graft surgery. Interact Cardiovasc Thorac Surg. 2012;15(3):364-70.
68. Hilty MP, Guerci P, Ince Y, Toraman F, Ince C. MicroTools enables automated quantification of capillary density and red blood cell velocity in handheld vital microscopy. Communications biology. 2019;2(1):1-15.
69. Koning NJ, Atasever B, Vonk AB, Boer C. Changes in microcirculatory perfusion and oxygenation during cardiac surgery with or without cardiopulmonary bypass. Journal of cardiothoracic and vascular anesthesia. 2014;28(5):1331-40.
70. Koning NJ, de Lange F, van Meurs M, Jongman R, Ahmed Y, Schwarte L, et al. Reduction of vascular leakage by imatinib is associated with preserved microcirculatory perfusion and reduced renal injury markers in a rat model of cardiopulmonary bypass. British Journal of Anaesthesia. 2018;120(6):1165-75.
71. Dekker N, van Meurs M, van Leeuwen A, Hofland H, Van Slyke P, Vonk A, et al. Vasculotide, an angiopoietin-1 mimetic, reduces pulmonary vascular leakage and preserves microcirculatory perfusion during cardiopulmonary bypass in rats. British Journal of Anaesthesia. 2018;121(5):1041-51.
72. Edul VSK, Ince C, Navarro N, Previgliano L, Risso-Vazquez A, Rubatto PN, et al. Dissociation between sublingual and gut microcirculation in the response to a fluid challenge in postoperative patients with abdominal sepsis. Annals of intensive care. 2014;4(1):1-9.
73. Verdant CL, De Backer D, Bruhn A, Clausi CM, Su F, Wang Z, et al. Evaluation of sublingual and gut mucosal

microcirculation in sepsis: a quantitative analysis. Critical care medicine. 2009;37(11):2875-81.
74. Uz Z, van Gulik TM, Aydemirli MD, Guerci P, Ince Y, Cuppen D, et al. Identification and quantification of human microcirculatory leukocytes using handheld video microscopes at the bedside. Journal of Applied Physiology. 2018;124(6):1550-7.

Appendix I: Macrocirculatory parameters

Macrocirculatory parameters which are included in this study

- Blood pressure (systolic/diastolic)
- Cardiac index
- Cardiac output
- Hematocrit
- Hemoglobin
- Lactate
- Mean arterial pressure
- pH
- Platelet count
- Red blood cell concentration
- Temperature
- Venous oxygen saturation
- White blood cell count

Appendix II: Definition of microcirculatory perfusion parameters
Based on the second consensus paper by Ince et al. (25) published in 2018 on the assessment of sublingual microcirculation in critically ill patients, the following parameters are defined as follows:

Microcirculatory parameter	**Definition**
Percentage of perfused vessels (PPV)	Percentage of perfused vessels per total number of vessel cross-sections, expressed as a percentage (%) and characterized as a binomial determinant of red blood cell velocity (flow or no flow)
Microvascular flow index (MFI)	Grid-based score per quadrant, no flow (0), intermittent flow (1), slow flow (2), normal flow (3). MFI is characterized as semi-quantitative assessment of average red blood cell velocity per quadrant.
Functional capillary density (FCD)	The total length of vessels exhibiting normal flow in relation to image size. FCD is characterized as the diffusion distance between red blood cells and tissue cells.
Total vessel density (TVD)	Measurement of total vessel area per surface area, is expressed in mm2/mm2 and is characterized as a determinant of capillar distance (diffusive capacity).
Density of perfused vessels (PVD)	Percentage of perfused vessels x TVD, expressed in mm2/mm2. PVD is characterized as the determinant of capillary distance (diffusive capacity) and red blood cell velocity (convective capacity)
Red blood cell velocity (RBCv)	Determined by use of a space-time diagram (STD). Each moving RBC generates a line in the STD, the slope of which equals the velocity (velocity = $\Delta L/\Delta t$)

Appendix III: Information of the included studies

Author	Year	Study period	Patient population	Size (n)	Imaging modality	ECC used?	Time Frame measurements	Which macrocirculatory parameters were measured	Which microcirculatory parameters were measured	Main aim of the study
Arnold, R.C. (32)	2012	-	Scheduled cardiac surgery with CPB with expected and rapidly reversible hemodynamic instability	20	SDF	Yes	Preoperative measurements on the hospital floor until transferred to a general hospital bed	HR, CI, CVP, MAP, SvO2, EF, pulmonary capillary occlusion pressure, SVR	MFI	Microcirculatory alterations and MAP
Atasever, B. (33)	2011	-	Elective on-pump or off-pump CABG	48	SDF	Yes; non-pulsatile	Before CPB and 10 minutes after the switch to CPB or before and during cardiac luxation in off-pump patients	Temp, MAP, Hb, CO	FCD, velocity capillary/ venule, µHbO2	On-pump CABG vs off-pump CABG
Atasever, B. (34)	2011	-	Elective low-risk multivessel OPCAB surgery patients	12	SDF	No	Before cardiac displacement until after cardiac displacement	MAP, CO	FCD, RBCv	Hemodynamic variables vs microcirculatory variables and tissue oxygenation variables
Atasever, B. (35)	2012	-	Cardiac surgery patients admitted to the ICU who are clinically (non-)hypovolemic and (non-) anemic.	39	SDF	Not specified	Before transfusion until 1 hour after the start of transfusion	HR, CVP, temp, MAP, SvO2, Hb, CO, PAOP, DO2, VO2, O2ER, FiO2, Vt, PEEP	MFI, vascular density	RBC group vs gelatin group vs no infusion
Aykut, G. (36)	2021	-	Isolated CABG with CPB	20	IDF	Yes	From the induction of anesthesia to discontinuation of CPB	Lactate, temp, MAP, Hct, pH, partial pressure O2/ CO2	PPV, PVD, TVD	Blood vs crystalloid CPB
Bauer, A. (37)	2007	-	Cardiac surgery with CPB	47	OPS	Yes	After skin incision to 1 h after discontinuation of CPB	HR, lactate, CVP, temp, MAP, leukocyte count, Hb, CD18	FCD, RBCv, microvessel diameter	The microvascular changes during cardiac surgery with the use of CPB
Bienz, M. (38)	2016	-	Isolated elective on-pump/off-pump CABG surgery	32	SDF	Yes	After installation of the arterial line to 4 hours after surgery at the ICU	BP, temp	SVD, PVD, TVD, small vessel count/ perfused length/ total count	On-pump CABG vs off-pump CABG
Boly, C. (39)	2021	June 2016 – March 2018	Obese patients without type II diabetes and lean patients undergoing cardiac surgery with CPB	36	SDF	Yes	The day before surgery until 72h after surgery	BP, lactate, temp, MAP, PO2/PCO2, Hb, Hct, pH, PCO2, base excess, HCO3-	PPV, MFI, PVD, TVD	Obese versus non-obese

Backer de, D. (40)	2009	-	Elective CABG and/or valvular surgery with/without CPB	21	OPS	Yes	The day before surgery to 24 hours after the end of the procedure	HR, lactate, CI, MAP, SOFA, Hb, respiratory rate	PVD, vessel density	With and without CPB
Dekker, N. (41)	2019	-	Elective CABG with bypass	17	SDF	Yes; non-pulsatile	A day before surgery to 72 h following surgery	Lactate, Hb, Hct, heparan sulphate, syndecan-1, base excess	PPV, PVD, PBR, flow, RBC concentration	Microcirculatory perfusion disturbances following CPB in the early postoperative period
Dekker, N. (42)	2020	-	Elective CABG with CPB	26	SDF	Yes; non-pulsatile	After induction of anesthesia to 72h after surgery	Temp, MAP, Hct, heparan suplhate, syndecan-1	PVD, TVD	Heparin of phosphorylcholine coated circuit
Uil den, C.A. (43)	2008	-	Elective CABG with CPB	25	SDF	Yes; non-pulsatile	The day before surgery until postoperatively just after admission to the ICU	BP, lactate, CI, CVP, MAP, Hb, Hct	MFI	Microcirculatory alterations before during and after CABG
Dedda di, U. (44)	2018	October 2017 – January 2018	CABG with/without additional procedures	12	SDF	Yes	After the induction of anesthesia until after weaning from CPB	Hct, serum creatinine, platelet count	PPV, de backer score, MFI, HI, PVD, TVD	Association between microvascular flow pattern and postoperative changes in platelet count and function
Donndorf, P. (45)	2012	June – November 2010	Urgent or elective CABG	40	OPS	Yes	After the induction of anesthesia until 30 minutes after termination of CPB	BP, HR, lactate, SaO2, PO2/PCO2, SvO2, Hb, Hct	FCD, RBCv, vessel diameter	MECC vs CECC
Donndorf, P. (46)	2014	November 2012 – March 2013	Urgent or elective AVR surgery	20	OPS	Yes	After the induction of anesthesia until 30 minutes after termination of CPB	Lactate, SaO2, SvO2, Hb, Hct, EF, troponin, creatine kinase	FCD, RBCv, vessel diameter	MECC vs CECC
Elbers, P. (47)	2009	May – September 2006	Mechanically ventilated patients with elevated arterial blood pressure immediately after ECC	6	SDF	Yes	5 minutes before until 10 minutes after ketanserin administration	BP, HR, CVP, temp, Hb, Hct, SpO2	PPV, MFI, HI, PVD	The effect of ketanserin on macrocirculation and microcirculation
Elbers, P. (48)	2011	-	CABG or AVR with CPB (non-pulsatile vs pulsatile in the same patient)	16	SDF	Yes	10 minutes after the perfusion was started either in the pulsatile or non-pulsatile mode, microvascular recordings were made. The perfusion mode was then switched and after 10 minutes new microvascular recordings were made.		PPV, MFI, PVD, HI-PVD, HI-MFI	Pulsatile vs non-pulsatile
Forti, A. (49)	2012	-	Elective cardiac surgery with CPB	30	SDF	Yes	At 80 % and 100 % CPB pump flow rate	Temp, MAP, SvO2, Hct, flow, PaCO2	PPV, de Backer score, MFI, PVD, TVD	Flow rates

Greenwood, J.C. (50)	2021	-	Elective cardiac surgery requiring CPB	25	IDF	Yes	Before surgery until 4 hours after surgery	HR, lactate, CI, CVP, temp, MAP, SOFA, SvO2, Hb, Hct, CO, SVR, LVEF, PaO2	PPV, MFI, HI, PVD, TVD	Microcirculatory alterations in time
Holmgaard, F. (51)	2018	July 2014 – January 2016	PPCI patients scheduled for CABG, randomized to a MAP at 40-50 mmHg/ 70-80 mmHg during CPB with fixed, equal blood flow	30	SDF	Yes; non-pulsatile	Before onset CPB until skin closure	Temp, MAP, SaO2, Hb, Hct, pH	PPV, MFI, HI, PVD, TVD	To explore the microcirculatory characteristics during cardiac surgery with the use of CPB at 2 different levels of blood pressure
Koning, N.J. (52)	2014	-	Elective CABG with or without CPB	31	SDF	Yes; non-pulsatile	after induction of anesthesia until the first hour after admission to the ICU	CI, CVP, MAP, leukocyte count, SvO2, Hct, SVR, Ca-VO2, DO2, VO2, O2ER	HI, RBCv	With or without CPB
Koning, N.J. (53)	2014	March 2010 – March 2012	CABG with non-pulsatile CPB or OPCAB procedures	26	SDF	Yes; non-pulsatile	Induction of anesthesia until ICU admission	Lactate, CI, temp, MAP, Hct	MFI, PVD, TVD	On-pump vs Off-pump
Koning, N.J. (54)	2012	-	Elective CABG with CPB	33	SDF	Yes; non-pulsatile and pulsatile	Induction of anesthesia until the first hour after ICU admission	BP, CI, temp, MAP, leukocyte count, SaO2, SvO2, Hb, Hct, CRP, creatinine, IL-6, VEGF, TNF-**α**, VO2, DO2, VO2/DO2	MFI, PVD, TVD	Effects of non-pulsatile and pulsatile flow on microcirculatory perfusion during on-pump cardiac surgery
Koning, N.J. (55)	2016	-	Isolated CABG with pulsatile/non-pulsatile CPB or OPCAB	36	SDF	Yes; non-pulsatile and pulsatile	Induction of anesthesia until closure of the sternal wound	-	PVD, PBR	The acute reduction of microcirculatory perfusion during cardiac surgery with CPB and off-pump in the association with alterations in glycocalyx dimensions
Liu, X. (56)	2016	June – August 2015	Elective valve surgery with CPB, which after transferred to the ICU were expected to require sedation for more than 4 hours	61	SDF	Yes	At ICU admission until 24 hours after ICU admission	HR, lactate, temp, MAP, SOFA, APACHE, Hb, NYHA class	PPV, SVD, de Backer score, PSVD	Comparison of the effects of dexmedetomidine and propofol on sublingual microcirculation
Maier, S. (57)	2009	-	Elective CABG with CPB	15	SDF	Yes	Induction of anesthesia until after termination of phenylephrine infusion	HR, CI, CVP, MAP, SaO2, SvO2, stroke volume index, mean PAP, PCWP	MFI	To determine the changes in the sublingual microcirculation produced by phenylephrine during constant blood flow during CPB and to observe the changes in the sublingual microcirculation induced by the CPB itself

Maurin, C. (58)	2021	January 2017 – December 2019	Receiving methylene blue intravenously for refractory vasoplegic syndrome	22	SDF	Yes	Before and 1 hour after Methylene blue	CI, CVP, MAP, SOFA, APACHE	MFI, HI, PVD, TVD	To study microcirculatory abnormalities in refractory vasoplegic syndrome following cardiac surgery with CPB, and assess the effects of Methylene blue
Mohamed, H. (59)	2019	-	Elective on-pump CABG	70	SDF	Yes	Immediately before bypass until 30 minutes after weaning from bypass	Lactate, CO	PPV, MFI, PVD, TVD	The effect of dexmedetomidine vs propofol on sublingual microcirculation
O' Neil, M. (60)	2018	-	High-risk cardiac surgery patients undergoing CPB	20	OPS	Yes; non-pulsatile and pulsatile	After anesthesia induction until 24 hours after surgery	HR, lactate, CI, temp, MAP, Hb, creatine	PPV, blood flow	Microvascular response to pulsatile and non-pulsatile flow
O'Neil, M. (61)	2012	August 2008 – April 2010	High-risk cardiac surgery patients	20	OPS	Yes; non-pulsatile and pulsatile	after anesthesia induction until 48 hours after surgery	HR, CI, temp, MAP, Hb, arterial pulse pressure	PPV, blood flow characteristics	Comparison of the effects of pulsatile vs nonpulsatile perfusion on microvascular blood flow during and after CPB
Özarslan, N.G. (62)	2012	March – September 2010	Elective CABG with CPB	30	OPS	Yes; non-pulsatile	Before anesthesia induction until 24 hours after surgery	BP, CI, CVP, CO, EF, PAP	PPV, FCD, MFi, PVD	Sevoflurane vs Isoflurane vs Desflurane
Prestes, I. (63)	2016	-	Cardiac surgery with CPB	22	SDF	Yes; non-pulsatile	After anesthesia induction until end of surgery	Lactate, MAP	PPV, MFI, PVD, vessel density, HFI	Patients with and without complications
Stowell, C.P. (64)	2017	January 2011 – January 2014	Scheduled for complex cardiac surgery, likely to require RBC transfusion	123	SDF	Yes	Within 6 hours before surgery until 28 hours after end of surgery	Hb, platelet count, creatinine	PPV, MFI, PVD, TVD	The impact of RBC storage duration on tissue oxygenation
Wu, Q. (65)	2019	July 2015 – August 2015	Isolated CABG and elective valve surgery	30	SDF	Yes; non-pulsatile	From preoperative resting state until 48h after CPB	MAP, leukocyte count, Hb, Hct, syndecan-1, heparan sulfate	de Backer score, PVD	Association of glycocalyx shedding during CPB with acute reductions in microcirculatory perfusion
Yuruk, K. (66)	2010	-	On-pump CABG, cardiac valve surgery or both that received allogenic blood transfusions during surgery	12	SDF	Yes; non-pulsatile	Before blood transfusion until after blood transfusion	Temp, MAP, Hb, Hct, CO	FCD, MFI, detected vessel length	The possibility of improvement of microcirculatory density, perfusion and oxygenation with leukoreduced RBC transfusions
Yuruk, K. (67)	2012	-	CABG	20	SDF	Yes	Before the initiation of CPB until the termination of CPB	HR, lactate, MAP, Hb, Hct, whole blood viscosity, plasma NGAL, PAP	MFI, PVD	CECC vs MECC

-: not reported, μHbO_2: Microvascular hemoglobin oxygen saturation, AMC: Academic Medical Center, APACHE: Acute Physiology and Chronic Health Evaluation, AVR: aortic valve repair, BP: blood pressure, CABG: coronary artery bypass grafting, Ca-VO_2: oxygen content difference, CECC: Conventional ExtraCorporeal Circulation circuit, CI: cardiac index, CO: cardiac output, CPB: cardiopulmonary bypass, CRP: C-reactive protein, CVP: central venous pressure, DO_2: oxygen delivery, ECC: extracorporeal circulation circuit, EF: ejection fraction, FCD: functional capillary density, FiO_2: fraction of inspired oxygen, Hb: hemoglobin, HCO_3^-: bicarbonate, Hct: hematocrit, HFI: heterogeneity flow index, HI: heterogeneity index, HR: heart rate, ICU: intensive care unit, IDF: Incident dark field imaging, IL-6: interleukin-6, LVEF: left ventricular ejection fraction, MAP: mean arterial pressure, MECC: Minimalized ExtraCorporeal Circulation circuit, MFI: microvascular flow index, n: number of patients, NGAL: neutrophil gelatinase-associated lipocalin, NYHA: New York Heart Association, O_2ER: oxygen extraction ratio, OLVG: Onze Lieve Vrouwe GasthuisOPCAB: off-pump coronary artery bypass, OPS: orthogonal polarization spectral imaging, $PaCO_2$:partial arterial pressure of carbon dioxide, PaO_2: partial arterial pressure of oxygen, PAOP: pulmonary artery occlusion pressure, PAP: pulmonary artery pressure, PBR: perfused boundary region, PCO_2: partial pressure of carbon dioxide, PCWP: pulmonary capillary wedge pressure, PEEP: Positive End Expiratory Pressure, PO_2: Partial pressure of oxygen, PPV: proportion of perfused vessels, PSVD: perfused small vessel density, PVD: perfused vessel density, RBC: red blood cell, RBCv: red blood cell velocity, SaO_2: arterial oxygen saturation, SDF: Sidestream Dark Field imaging, SOFA: Sequential Organ Failure Assessment, SpO_2: oxygen saturation, SVD: small vessel density, SvO_2: mixed venous oxygen saturation, SVR: systemic vascular resistance, Temp: temperature, TNF-α: tumour necrosis factor α, TVD: total vessel density, VEGF: vascular endothelial growth factor, VO_2: oxygen consumption, Vt: tidal volumeVU: Vrije Universiteit

Appendix IV: Quality assessment Observational studies

Author	Year	Selection				Comparability		Outcome			
		Representative of the exposed cohort	Selection of external control	Ascertainment of exposure	Outcome of interest not present at the start of the study	Main factor	Additional factor	Assessment of outcomes	Sufficient follow-up time	Adequacy of follow-up	Total (9/9)
Arnold, R.C. (32)	2012	*	-	*	*	*	-	*	*	*	7
Atasever, B. (33)	2011	*	*	*	*	-	*	*	-	*	7
Atasever, B. (34)	2011	*	-	*	*	*	-	*	*	*	7
Atasever, B. (35)	2012	*	*	*	*	-	*	*	*	*	8
Aykut, G. (36)	2021	*	*	*	*	-	*	*	-	*	7
Bauer, A. (37)	2007	*	-	*	*	*	-	*	-	*	6
Bienz, M. (38)	2016	*	*	*	*	*	*	*	*	*	9
Boly, C. (39)	2021	*	*	*	*	*	-	*	*	*	8
Backer de, D. (40)	2009	*	*	*	*	*	*	*	*	*	9
Dekker, N.(41)	2019	*	*	*	*	*	-	*	*	*	8
Uil den, C.A. (43)	2008	*	-	*	*	*	-	*	*	*	7
Dedda di, U. (44)	2018	*	-	*	*	*	-	*	*	*	7
Donndorf, P. (45)	2012	*	*	*	*	-	*	*	-	*	7
Donndorf, P. (46)	2014	*	*	*	*	-	*	*	-	*	7
Elbers, P. (47)	2009	*	-	*	*	*	-	*	-	*	6
Elbers, P. (48)	2011	*	-	*	*	-	*	*	-	*	6
Forti, A. (49)	2012	*	-	*	*	-	*	*	-	*	6
Greenwood, J.C. (50)	2021	*	-	*	*	*	-	*	*	*	7
Koning, N.J. (52)	2014	*	*	*	*	*	*	*	*	*	9
Koning, N.J. (53)	2014	*	*	*	*	*	*	*	*	*	9
Koning, N.J. (54)	2012	*	*	*	*	*	*	*	*	*	9
Koning, N.J. (55)	2016	*	*	*	*	*	*	-	-	*	7
Maier, S. (57)	2009	*	-	*	*	*	-	*	-	-	5
Maurin, C. (58)	2021	*	-	*	*	*	-	*	*	*	7
Prestes, I. (63)	2016	*	-	*	*	*	-	*	-	*	6
Wu, Q. (65)	2019	*	-	*	*	*	-	*	*	*	7
Yuruk, K. (66)	2010	*	*	*	*	*	*	*	*	*	9

*: Meets criteria, -: no description/ fails to meet criteria

Appendix IV: Quality assessment RCT's

Author	Year	D1	D2	D3	D4	D5	Overall
Dekker, N. (42)	2020	Low	High	Low	Low	High	High
Holmgaard, F. (51)	2018	Low	High	Low	Low	High	High
Liu, X. (56)	2016	Low	Low	Low	Low	High	Some
Mohamed, H. (59)	2019	Low	Low	Low	Low	Low	Low
O'neil, M. (60)	2018	High	High	Low	Low	Low	High
O'neil, M. (61)	2012	Low	Low	Low	Low	Low	Low
Özarslan, N.G (62)	2012	Low	Low	Low	Low	Low	Low
Stowell, C. P. (64)	2017	Low	High	Low	Low	High	High
Yuruk, K. (67)	2012	High	High	Low	Low	Low	High

Judgement: Low: low risk of bias, some: some risk of bias, high: high risk of bias, ?: no information

Domains:
D1: Bias arising from the randomization process, D2: Bias due to deviations from intended intervention, D3: Bias due to missing outcome data, D4: Bias in measurement of the outcome, D5: Bias in selection of the reported result.

Appendix Va: Microcirculatory effect of different CPB variations

					Pre-operative	Surgery					Post-operative				Conclusion
Author	**Study groups**	**CPB duration (min)**	**Aortic cross-clamp time (min)**		**Pre-operative**	**After anesthesia**	**During aortic cross-clamp**	**During cardiac positioning**	**End of CPB**	**After cardiac positioning**	**ICU**	**24h after surgery**	**48 h after surgery**	**72h after surgery**	
Atasever, B. (34)	Multivessel off- pump cardiac surgery	/	/	Con	-	* RBCv	-	↓ RBCv	-	↧ RBCv	-	-	-	-	Cardiac displacement is associated with a reduction in CO, which may be associated with a cessation of microcirculatory blood flow and decreases in microcirculatory Hb oxygenation
				Dif	-	* FCD	-	~ FCD	-	↧ FCD	-	-	-	-	
Atasever, B. (33)	CABG on-pump	-	-	Con	-	* RBCv	-	/	↑RBCv	/	-	-	-	-	Both, on-pump and off pump are associated with distinct alterations in sublingual microcirculatory perfusion and Hb oxygenation
				Dif	-	* FCD	-	/	↓ FCD	/	-	-	-	-	
	CABG off-pump	/	/	Con	-	* RBCv	-	↧ RBCv	-	-	-	-	-	-	
				Dif	-	* FCD	/	↧FCD	/	-	-	-	-	-	
Bienz, M. (38)	CABG on-pump	100+/- 6	-	Con	-	-	-	-	-	-	-	-	-	-	No marked preservation of microcirculation during and after off-pump surgery compared to on-pump surgery, they coincide with the fluctuations in temperature during and after surgery. Active warming could impact the microcirculation parameters.
				Dif	* TVD / PVD	↟ TVD/ PVD	-	/	↧ TVD/ PVD	/	↧ TVD/ PVD	-	-	-	
	CABG off-pump	/	/	Con	-	-	-	-	-	-	-	-	-	-	
				Dif	* TVD / PVD	↟ TVD/ PVD	/	-	/	~ TVD, ↟ PVD	↧ TVD/ PVD	-	-	-	
Backer de, D. (40)	Cardiac surgery on-	-	-	Con	* PPV	↓ PPV	-	/	↓ PPV	/	-	↓ PPV	-	-	CPB plays a minor role in microcirculatory alterations after cardiac

	pump			Dif	* PVD / TVD	↓ PVD, ~ TVD	/	-	/	↓ PVD, ~ TVD	-	~ PVD, ↡ TVD	-	-	surgery. Anesthesia partially contributed, but could not by itself explain the severity of the lesions and their persistence up to 24 hours after the surgery.
	Cardiac surgery off-pump	/	/	Con	* PPV	↓ PPV	/	-	/	↓ PPV	-	↓ PPV	-	-	
				Dif	* PVD /TVD	↡ PVD, ↡ TVD	/	-	/	↓ PVD, ↡ TVD	-	~ PVD, ↡ TVD	-	-	
Koning, N.J. (52)	CABG on-pump	113 +/- 29	-	Con	* MFI / RBCv	-	-	/	-	/	↓ MFI, ↑ RBCv	-	-	-	Microcirculatory perfusion remained unaltered throughout off-pump surgery. In contrast, microvascular perfusion declined after initiation of CPB and did not recover in the early postoperative phase.
				Dif	-	-	-	-	-	-	-	-	-	-	
	CABG off-pump	/	/	Con	* MFI / RBCv	-	-	/	-	/	~ MFI, ↡ RBCv[o]	-	-	-	
				Dif	-	-	-	-	-	-	-	-	-	-	
Koning, N.J. (53)	CABG on-pump	106 +/- 24	67 +/- 15	Con	-	* MFI	-	/	↡ MFI	/	↓ MFI	-	-	-	Microcirculatory perfusion remained unaltered throughout off pump surgery. In contrast, microvascular perfusion declined after initiation of CPB and did not recover in the early postoperative phase
				Dif		* TVD/ PVD	-	/	↓ TVD/ PVD	/	↓ TVD/ PVD[p]	-	-	-	
	CABG off-pump	/	/	Con		* MFI	/	~ MFI	/	~ MFI	~ MFI	-	-	-	
				Dif		* TVD/ PVD	/	↡ TVD/ PVD	/	↡ TVD/ PVD	↡ TVD/ PVD	-	-	-	
Elbers, P. (48)	Cardiac surgery PP	-	-	Con	-	-	-	/	* PPV[k]/ MFI[i]	/	-	-	-	-	PP does not alter microvascular perfusion using standard equipment in routine cardiac surgery.
				Dif	-	-	-	/	* PVD[i]	/	-	-	-	-	

	Cardiac surgery NP	-	-	Con	-	-	-	/	* PPV/ MFI	/	-	-	-	-	
				Dif	-	-	-	/	* PVD	/	-	-	-	-	
Koning, N.J. (54)	CABG PP	106 +/- 20	72 +/- 14	Con	-	* MFI	↟ MFI[q]	/	↟ MFI[q]	/	↟ MFI[q]	-	-	-	NP flow during ECC was associated with sustained reduction in microcirculatory density and perfusion upon ICU admission. In contrast, patients exposed to PP flow showed a fast recovery of microcirculatory perfusion after weaning from CPB
				Dif	-	* TVD/ PVD	↡ TVD/ PVD	/	↡ TVD/ PVD	/	↡ TVD, ~ PVD[q]	-	-	-	
	CAPG NP	98 +/- 28	63 +/- 22	Con	-	* MFI	↡ MFI	/	↡ MFI	/	↡ MFI	-	-	-	
				Dif	-	* TVD/ PVD	↡ TVD/ PVD	/	↡ TVD/ PVD	/	↡ TVD/ PVD	-	-	-	
Koning, N.J. (55)	CABG PP	104 (27)	73 (13)	Con	-	–	-	-	-	-	-	-	-	-	Glycocalyx dimensions ae reduced after onset of CPB, in contrast with off-pump. PP during CPB was associated with recovery of glycocalyx dimensions and microvascular perfusion postoperatively, where this recovery was absent after NP CPB.
				Dif	-	* PVD	-	/	~ PVD	/	-	-	-	-	
	CABG NP	94 (24)	60(18)	Con	-	-	-	-	-	-	-	-	-	-	
				Dif	-	* PVD	-	/	↓ PVD	/	-	-	-	-	
	CABG off-pump	/	/	Con	-	-	-	-	-	-	-	-	-	-	
				Dif	-	* PVD	/	↟ PVD	/	-	-	-	-	-	
O'Neil, M.P. (60)	Cardiac surgery PP	128.6 +/- 30.6	97.7 +/- 24.0	Con	-	* PPV	-	/	~ PPV	/	-	~ PPV	-	-	PP during CPB improves microcirculatory blood flow compared with NP, which may reflect attenuation of the systemic inflammatory response and ischemia-reperfusion injury
				Dif	-	-	-	-	-	-	-	-	-	-	
	Cardiac surgery NP	146.7 +/- 26.1	109.3 +/- 16.6	Con	-	* PPV	-	/	↓ PPV	/	-	↓ PPV	-	-	
				Dif	-	-	-	-	-	-	-	-	-	-	
O'Neil, M.P. (61)	Cardiac surgery PP	158.9 +/- 12.1	115.5 +/- 10.9	Con	-	* PPV	-	/	~ PPV	/	~ PPV	~ PPV	-	-	PP during CPB can better preserve the microcirculation and result in less leukocyte activation than conventional NP
				Dif	-	-	-	-	-	-	-	-	-	-	
	Cardiac surgery NP	197.8 +/- 16.6	134.9 +/- 12.0	Con	-	* PPV	-	/	↓ PPV	/	↓ PPV	↓ PPV	-	-	
				Dif	-	-	-	-	-	-	-	-	-	-	

Donndorf, P. (45)	CABG MECC	96 +/- 27	54 +/- 16	Con	-	-	-	-	-	-	-	-	-	-	OPS imaging reveal an impairment of microvascular perfusion during on-pump CABG. Changes in FCD indicate a faster recovery of the microvascular perfusion in MECC during the reperfusion period.
				Dif	-	* FCD	↓ FCD	/	~ FCD	/	-	-	-	-	
	CABG CECC	97 +/- 24	56 +/- 18	Con	-	-	-	-	-	-	-	-	-	-	
				Dif	-	* FCD	↓ FCD	/	~ FCD	/	-	-	-	-	
Donndorf, P. (46)	AVR MECC	96 +/- 19	71 +/- 16	Con	-	-	-	-	-	-	-	-	-	-	The use of MECC resulted in beneficial preservation of microvascular blood flow velocity and significantly reduced haemodilution during CPB.
				Dif	-	* FCD	↓ FCD	/	~ FCD	/	-	-	-	-	
	AVR CECC	87 +/- 19	64 +/- 17	Con	-	-	-	-	-	-	-	-	-	-	
				Dif	-	* FCD	↓ FCD	/	~ FCD	/	-	-	-	-	
Yuruk, K. (67)	CABG CECC	119 +/- 14	81 +/- 22	Con	-	* MFI	~ MFI	/	~ MFI	/	-	-	-	-	The use of MECC system was associated with significant (but clinically insignificant) reduction in haemodilution and microcirculatory hypoperfusion compared with the use of CECC systems.
				Dif	-	* PVD	↓ PVD	/	~ PVD	/	-	-	-	-	
	CABG MECC	71 +/- 16	44 +/- 15	Con	-	* MFI	~ MFI	/	~ MFI	/	-	-	-	-	
				Dif	-	* PVD	↓ PVD	/	~ PVD	/	-	-	-	-	
Aykut, G. (36)	CABG crystaloid cardioplegia	69.0 +/- 3.5	30 (28.5 - 39.5)	Con	-	* PPV	↓ PPV[a]	/	↓ PPV[a]	/	-	-	-	-	Significant difference between the groups in favor of blood cardioplegia. Blood cardioplegia ameliorates CPB-induced microcirculatory alterations better than crystalloid cardioplegia.
				dif		* TVD/ PVD	↓ TVD[a]/ PVD[a]	/	↓ TVD/ PVD[a]	/	-	-	-	-	
	CABG blood cardioplegia	82.8 +/- 6.8	39.5 (33.8 - 51)	Con	-	* PPV	~ PPV	-	~ PPV	-	-	-	-	-	
				Dif		* TVD/ PVD	↓ TVD/ PVD	-	~ TVD/ PVD	-	-	-	-	-	
Dekker, N. (42)	CABG HC circuit	101 +/- 26	72 +/- 22	Con	-	-	-	-	-	-	-	-	-	-	Microcirculatory perfusion was disrupted equally in both groups. CPB induced microcirculatory perfusion disturbances seem to be coating independent.
				Dif	-	* TVD/ PVD	-	/	↓ TVD, ↓ PVD	/	↓ TVD, ↓ PVD	↓ TVD, ↓ PVD	-	↓ TVD, ↓ PVD	
	CABG PC	107 +/-	73 +/- 15	Con	-	-	-	-	-	-	-	-	-	-	

	circuit	17		Dif	-	* TVD/ PVD	-	/	↟ TVD, ↓ PVD	/	↡ TVD, ↓ PVD	↡ TVD, ↓ PVD	-	↡ TVD, ↓ PVD	
Forti, A. (49)	Cardiac surgery CPB flow 80%	-	-	Con	-	-	* MFI^{n}/ PPV^{n}	/	-	/	-	-	-	-	Changes in CPB flow rate within 20% of its theoretic value do not alter the sublingual microcirculation
				Dif	-	-	* TVD^{l}/ PVD^{n}	/	-	/	-	-	-	-	
	Cardiac surgery CPB flow 100%	-	-	Con	-	-	* MFI/ PPV	/	-	/	-	-	-	-	
				Dif	-	-	* TVD/ PVD	/	-	/	-	-	-	-	
Holmgaard, F. (51)	CABG LMAP	83 +/- 32	-	Con	-	* MFI/ PPV	-	/	↡ MFI/ PPV	/	-	-	-	-	No significant difference in sublingual microcirculatory flow expressed as MFI according to 2 different levels of MAP during CPB can be found
				Dif		* TVD/ PVD	-	/	↡ TVD/ PVD	/	-	-	-	-	
	CABG HMAP	73 +/- 21	-	Con	-	* MFI/ PPV	-	/	↟ MFI, ↡ PPV	/	-	-	-	-	
				Dif		* TVD/ PVD	-	/	↡ TVD/ PVD	/	-	-	-	-	
				Dif	-	* PVD	↓ PVD	/	~ PVD	/	-	-	-	-	

/: not applicable, *: baseline, ~: no significant change vs baseline, ↡: decrease vs baseline (not significant), ↟: increase vs baseline (not significant), ↑: significant increase vs baseline, ↓: significant decrease vs baseline, AVR: aortic valve repair, CABG: coronary artery bypass grafting, Cardiac surgery: multiple surgeries (i.e. CABG, valve replacement/repair), CECC: Conventional ExtraCorporeal Circulation circuit, CO: cardiac output, Con: convection parameters, CPB: cardiopulmonary bypass, Dif: diffusion parameters, During aortic cross-clamp: time from aortic cross-clamp until aortic cross clamp release, ECC: extracorporeal circulation circuit, End CPB: time from onset of CPB until weaning from CPB, FCD: functional capillary density, Hb: hemoglobin, HC: heparin coated, HMAP: high mean arterial pressure , ICU: first 24 hours after surgery, ICU: intensive care unit, LMAP: low mean arterial pressure, MAP: mean arterial pressure, MECC: Minimalized ExtraCorporeal Circulation circuit, MFI: microvascular flow index, Min: minutes, NP: Non-pulsatile CPB-flow. OPS: orthogonal polarization spectral imaging, PC: phosporylcholine-coated, PP: pulsatile CPB-flow, PPV: perfused vessel density, PVD: perfused vessel density, RBC: red blood cell, RBCv: red blood cell velocity, TVD: total vessel density, a: significantly lower than blood cardioplegia, i: higher than NP (not significant), k: lower than NP (not significant), o: significantly lower than on-pump, p: significantly lower than off-pump, q: significantly higher than non-pulsatile

Appendix Vb: Microcirculatory effects of therapeutical interventions

					Pre-operative	Surgery					Post-operative				Conclusion
Author	**Study groups**	**CPB duration**	**Aortic cross-clamp time**		**Pre-operative**	**After anesthesia**	**During aortic cross-clamp**	**During cardiac positioning**	**End of CPB**	**After cardiac positioning**	**ICU**	**24h after surgery**	**48 h after surgery**	**72h after surgery**	
Maier, S. (57)m	CABG phenylephrine	-	-	Con	-	* MFI	~ MFI	/	↓ MFI	/	-	-	-	-	Increased perfusion pressure produced by phenylephrine at constant CPB flow may decrease microcirculatory blood flow due to microvascular blood flow shunting
				Dif	-	-	-	-	-	-	-	-	-	-	
Mohamed, H. (59)	CABG propofol	70 (56 - 82)	-	Con	-	* MFI/ PPV	-	/	↓ MFI[t], ↓ PPV	/	-	-	-	-	Dexmedetomidine infusion improved sublingual microcirculation indices in patients undergoing on-pump CABG surgery
				Dif	-	* PVD/ TVD	-	/	↓ PVD[t]/ TVD[t]	/	-	-	-	-	
	CABG dexmedetomidine	75 (62 - 90)	-	Con	-	* MFI/ PPV	-	/	~ MFI, ↟ PPV	/	-	-	-	-	
				Dif	-	* PVD/ TVD	-	/	↟ PVD, ↓ TVD	/	-	-	-	-	
Özarslan, N.G. (62)	CABG sevoflurane	93.9 +/- 34.0	51.3 +/- 16.8	Con	* PPV/ MFI	~ MFI, ↓ PPV	-	/	~ MFI[v], ↟ PPV	/	-	~ MFI, ↟ PPV	-	-	The three inhalation agents affected the microcirculation to different degrees. However, these alterations in the microcirculation were temporary and returned to baseline levels after surgery
				Dif	* TVD/ PVD	~ TVD/ PVD	-	/	~ TVD/ PVD	/	-	↟ TVD, ~ PVD	-	-	
	CABG isoflurane	115.0 +/- 58.8	76.4 +/- 49.1	Con	* PPV/ MFI	~ MFI, ↓ PPV	-	/	↟ MFI, ↓ PPV	/	-	~ MFI, ↓ PPV	-	-	
				Dif	* TVD/ PVD	~ TVD/ PVD	-	/	~ TVD/ PVD	/	–	~ TVD/ PVD	–	–	
	CABG desflurane	79.6 +/- 23.9	49.0 +/- 24.5	Con	* PPV/ MFI	↟ PPV, ↓ MFI	-	/	~ MFI, ↟ PPV	/	-	~ MFI, ↓ PPV	-	-	

				Dif	* TVD/ PVD	~ TVD/ PVD	-	/	~ TVD, ↟ PVD	/	-	↡ TVD[u]/ ~ PVD	-	-	
Yuruk, K. (66)	Cardiac surgery RBC transfusions	92 +/- 19	69 +/- 22	Con	-	* MFI	-	/	~ MFI	/	-	-	-	-	RBC transfusions improve microcirculatory density and oxygenation. It is successful in correcting the anemic conditions caused by blood loss and hemodilution associated with CPB and anesthesiologic procedures.
				Dif	-	* FCD	-	/	↑ FCD	/	-	-	-	-	

/: not applicable, *: baseline, ~: no significant change vs baseline, ↡: decrease vs baseline (not significant), ↟: increase vs baseline (not significant), ↑: significant increase vs baseline, ↓: significant decrease vs baseline, CABG: coronary artery bypass grafting, Cardiac surgery: multiple surgeries (i.e. CABG, valve replacement/repair), Con: convection parameters, CPB: cardiopulmonary bypassDif: diffusion parameters, During aortic cross-clamp: time from aortic cross-clamp until aortic cross clamp release, End CPB: time from onset of CPB until weaning from CPB, FCD: functional capillary density, ICU: first 24 hours after surgery, MFI: microvascular flow index, Min: minutes, PPV: perfused vessel density, PVD: perfused vessel density, RBC: red blood cell, TVD: total vessel density, t: significantly lower than Dexmedetomidine group, v: significant among the three groups

Appendix Vb: Microcirculatory effects of therapeutical interventions

Author	Study groups	CPB duration	Aortic cross-clamp time		Before intervention	After intervention	Conclusion
Atasever, B. (35)	Cardiac surgery: RBC infusion	142 +/- 57	93 +/- 50	Con	* MFI	↟ MFI	Efficacy in favor of RBC transfusion after cardiac surgery.
				Dif	* density	↟ density	
	Cardiac surgery: gelatin infusion	142 +/- 57	93 +/- 50	con	* MFI	~ MFI	
				Dif	* density	↟ density	
	Cardiac surgery: no infusion	142 +/- 57	93 +/- 50	Con	* MFI	↟ MFI	
				Dif	* density	~ density	
Elbers, P. (47)	Patients with elevated ABP after ECC	-	-	Con	* PPV/ MFI	↟ PPV, ↡ MFI	Kentanserin effectively lowers ABP, however capillary perfusion is maintained at steady value. Both effects may be explained by an increase in shunting in the larger vessels of the microcirculation
				Dif	* PVD	↟ PVD	
Liu, X. (56)	Valve surgery Dexmedetomidine	73 (60 - 88)	51 (35 - 64)	Con	* PPV	↟ PPV^{R}	Dexmetedonine may accelerate the recovery of sublingual microcirculatory perfusion compared to propofol
				Dif	* TVD/ PVD	↟ TVD^{R}/ $PVD^{R,S}$	
	Valve surgery Propofol	68 (54 - 80)	46 (34 - 59)	Con	* PPV	↟ PPV^{R}	
				Dif	* TVD/ PVD	↟ TVD^{R}/ PVD^{R}	
Maurin, C. (58)	Cardiac Surgery Methylene blue	130 (75 - 240)	96 (0 - 207)	Con	* MFI	↑ MFI	The administration of methylene blue could improve microvascular perfusion and reactivity, and partially restore the loss of hemodynamic coherence
				Dif	* TVD/ PVD	↑ TVD/ PVD	
Stowell, C.P. (64)	Cardiac surgery RBC < days	150.5 (102.0 - 207.0)	99.5 (68.0 - 126.0)	Con	* MFI/ PPV	↟ MFI, ↡ PPV	There were no differences in microcirculatory blood, in cardiac surgery patients transfused with RBC stored less than 10 days of more than 21 days
				Dif	* PVD/ TVD	↡ PVD, ↟ TVD	
	Cardiac surgery RBC ≥ 21 days	157.0 (105.0 - 212.0)	97.0 (68.0 - 115.0)	Con	* MFI/ PPV	↡ MFI/ PPV	
				Dif	* PVD/ TVD	↡ PVD/ TVD	

*: baseline, ~: no significant change vs baseline, ↡: decrease vs baseline (not significant), ↟: increase vs baseline (not significant), ↑: significant increase vs baseline, ↓: significant decrease vs baseline, ABP: arterial blood pressure, CABG: coronary artery bypass grafting, Cardiac surgery: multiple surgeries (i.e. CABG, valve replacement/repair), Con: convection parameters, CPB: cardiopulmonary bypass, dif: diffusion parameters, ECC: extracorporeal circulation circuit., MFI: microvascular flow index, Min: minutes, PPV: perfused vessel density, PVD: perfused vessel density, RBC: red blood cell, TVD: total vessel density, R: significant over time, S: significant between groups over time

Appendix VIa: Macrocirculatory parameters on-pump studies

				Pre- operative	Surgery			Post-operative			
Author	**Study groups**	**CPB duration (min)**	**Aortic cross-clamp time (min)**	**Pre- operative**	**After anesthesia**	**During aortic cross-clamp**	**End of CPB**	**ICU**	**24h after surgery**	**48 h after surgery**	**72h after surgery**
Arnold, R.C. (32)	Cardiac surgery	112 +/- 40	-	*MAP	-	-	-	↧ MAP	-	-	-
Bauer, A. (37)	Cardiac surgery	126 +/- 40	84 +/- 31	-	*MAP/ temp/ Hb/ lac	↓ MAP[b], temp[b], ↧ Hb, ↟ lac	↧ MAP/ Hb, ↟ temp, ↑ lac	-	-	-	-
Boly, C. (39)	Cardiac surgery obese patients	112 +/- 23	75 +/- 18	-	*MAP/ SBP, DBP/ Hct/ lac[e], Hb/ pH/ temp	-	~ MAP[f]/ SBP[f]/ DBP/ lac/ pH, ↧ Hct/ Hb, ↟ temp	-	-	-	-
	Cardiac surgery lean patients	118 +/- 51	86 +/- 42	-	*MAP/ SBP/ DBP/ Hct/ lac/ Hb/ pH/ temp	-	~ MAP[f]/ SBP[f]/ DBP/ pH/ temp, ↧ Hct/ Hb, ↟ lac	-	-	-	-
Dekker, N. (41)	CABG	103 +/- 18	70 +/- 14	*Hb/ Hct	↓ Hb, ↧ Hct	-	↓ Hb/ Hct	↓ Hb/ Hct	↓ Hb/ Hct	-	↓ Hb/ Hct
Uil den, C.A. (43)	CABG	102 (94 - 140)	65 (56 - 84)	*Temp/ MAP/ Hb/ Hct	*CI, lac, ↧ Temp/ Hb/ Hct, ↟ MAP	-	↧ Temp/ MAP/ Hb/ Hct, ↟ CI, lac	↧ Temp/ MAP/ Hb/ Hct, ↟ CI, lac	-	-	-
Dedda di, U. (44)	Cardiac surgery	80 (57 - 106)	-	*Hct/ platelet count	-	-	↧ Hct/ platelet count	-	-	-	-
Greenwood, J.C. (50)	Cardiac surgery	105 +/- 35	75 +/- 27	*Temp/ MAP/ CI/ Hb/ Hct/ lac	-	-	-	~ Temp/ CI, ↓ MAP, ↧ Hb/ Hct, ↑ lac	-	-	-
Prestes, I. (63)	Cardiac surgery with complications	106.8 +/- 35.0	71.4 +/- 28.1	-	* MAP/ lac	-	↓ MAP, ↑ lac	-	-	-	-
	Cardiac surgery without complications	107.2 +/- 37.0	71.0 +/- 32.2	-	* MAP/ lac	-	↓ MAP, ↑ lac	-	-	-	-
Wu, Q. (65)	Cardiac surgery	123.57 (92.93 - 154.21)	90.33 (63.57 - 117.09)	* Lac	↑ lac	↑ lac	-	↑ lac	↑ lac	↑ lac	-

*: baseline, ~: no significant change vs baseline, ↧: decrease vs baseline (not significant), ↟: increase vs baseline (not significant), ↑: significant increase vs baseline, ↓: significant decrease vs baseline, CABG: coronary artery bypass grafting, Cardiac surgery: multiple surgeries (i.e. CABG, valve replacement/repair), CI: cardiac index, CPB: cardiopulmonary bypass, DBP: diastolic blood pressureDuring aortic cross-clamp: time from aortic cross-clamp until aortic cross clamp release, End CPB: time from onset of CPB until weaning from CPB, Hb: hemoglobin, Hct: hematocrit, ICU: first 24 hours after surgery, lac: lactate, MAP: mean arterial pressure, Min: minutes, SBP: systolic blood pressure, Temp: temperature, b: significantly decreased vs end CPB, e: significantly higher than in lean patients, f: significant CPB effect

Appendix VIb: Macrocirculatory parameters variations of CPB

				Pre-operative	Surgery					Post-operative			
Author	**Study groups**	**CPB duration (min)**	**Aortic cross-clamp time (min)**	**Pre-operative**	**After anesthesia**	**During aortic cross-clamp**	**During cardiac positioning**	**End of CPB**	**After positioning**	**ICU**	**24h after surgery**	**48 h after surgery**	**72h after surgery**
Atasever, B. (34)	Multivessel	/	/	-	*CO/ MAP	/	↓ CO/ MAP	/	↧ CO, ~ MAP	-	-	-	-
Atasever, B. (33)	CABG on-pump	-	-	-	*CO/ MAP/ Hb/ Temp	-	/	↑ CO, ↓ MAP/ Hb/Temp	/	-	-	-	-
	CABG off-pump	/	/	-	*CO/ MAP/ Hb/ Temp	/	↓ CO/ MAP, ~ Hb/ Temp	/	-	-	-	-	-
Bienz, M. (38)	CABG on-pump	100+/- 6	-	*Temp/ blood pressure	~Temp, ↧ SBP/ DBP	-	/	↟ Temp^{c}, ↧ SBP^{c}/ DBP	/	↟ Temp, ↧ SBP, ~ DBP	-	-	-
	CABG off-pump	/	/	-	↧ Temp/ SBP/ DBP	/	-	/	↧ Temp/ SBP/ DBP	↟ Temp, ↧ SBP, ~ DBP	-	-	-
Backer de, D. (40)	Cardiac surgery on-pump	-	-	*MAP	↧ MAP, * CI/ lac	-	/	↧ MAP,↟ CI	/	-	↧ MAP/ CI, ↑ lac	-	-
	Cardiac surgery off-pump	/	/	*MAP	↧ MAP, * CI/ lac	/	-	/	↧ MAP, ~ CI	-	↧ MAP/ CI, ↑ lac^{g}	-	-
Koning, N.J. (52)	CABG on-pump	113 +/- 29	-	-	* MAP/ CI/ Hct/ temp/ lac/ SvO2	↑ SvO2	/	-	/	↓ MAP/ Hct, ↑ CI, ~ temp, ↟ lac, ↧ SvO2	-	-	-
	CABG off-pump	/	/	-	* MAP/ CI/ Hct/ temp/ lac/ SvO2	↧ SvO2	/	-	/	~ MAP/ CI, ↓ Hct/ SvO2, ↑ temp, ↟ lac	-	-	-
Koning, N.J. (53)	CABG on-pump	106 +/- 24	67 +/- 15	-	*Hct/ temp/ CI/ MAP/ lac	-	/	↓ Hct^{p}, ~ temp, ↟ CI, ↧ MAP, ↑ lac	/	↓ Hct, ↧ temp^{p}/ MAP, ↟ CI	-	-	-

	CABG off-pump	/	/	-	*Hct/ temp/ CI/ MAP/ lac	/	↓ Hct, ↟ temp/ CI/ lac, ↡ MAP	/	-	↓ Hct, ↟ temp/ CI, ~ MAP	-	-	-
Elbers, P. (48)	Cardiac surgery PP	-	-	-	-	-	/	*MAP[k]/ pH[j]/ Hct[j]/ temp[j]	/	-	-	-	-
	Cardiac surgery NP	-	-	-	-	-	/	*MAP/ pH/ Hct/ temp	/	-	-	-	-
Koning, N.J. (54)	CABG PP	106 +/- 20	72 +/- 14	-	* Temp/ Hb/ Hct/ MAP/ CI	↓ Temp/ Hb/ Hct, ↡ MAP, ~ CI	/	-	/	↓ Hb/ Hct, ↟ MAP/ CI	-	-	-
	CAPB NP	98 +/- 28	63 +/- 22	-	* Temp/ Hb/ Hct/ MAP/ CI	↓ Temp/ Hb/ Hct, ↡ MAP, ~ CI	/	-	/	↓ Hb/ Hct, ↟ MAP/ CI	-	-	-
O'Neil, M.P. (60)	Cardiac surgery PP	128.6 +/- 30.6	97.7 +/- 24.0		* CI/ MAP/ temp/ Hb/ lac	-	/	↟ CI/ lac, ↡ MAP, temp, ↓ Hb	/	↟ CI/ temp , ↡ MAP, ↓ Hb, ↑ lac	↟ CI/ temp , ↡ MAP/ lac, ↓ Hb		
	Cardiac surgery NP	146.7 +/- 26.1	109.3 +/- 16.6		* CI/ MAP/ temp/ Hb/ lac	-	/	↟ CI, ↡ MAP, temp, ↓ Hb, ↑ lac	/	↟ CI/ MAP/ temp, ↓ Hb, ↑ lac	↟ CI/ temp, ~ MAP, ↓ Hb, ↑ lac	-	-
O'Neil, M.P. (61)	Cardiac surgery PP	158.9 +/- 12.1	115.5 +/- 10.9	-	* CI/ MAP/ temp/ Hb/ lac/ rWBC/ aWBC/ WBC count	-	/	↟ CI/ lac, ↓ MAP/ temp/ Hb, ↡ rWBC/ aWBC	/	↑ CI/ lac, ~ MAP/ temp, ↓ Hb, ↟ rWBC/ WBC count, ↡ a WBC	↑ CI, ~ MAP/ lac, ↟ temp, ↓ Hb, ↡ rWBC/ aWBC, ↑ WBC count	↟ MAP/ temp, ↓ Hb, ↡ lac/ rWBC/ aWBC, ↑ WBC count	-
	Cardiac surgery NP	197.8 +/- 16.6	134.9 +/- 12.0	-	* CI/ MAP/ temp/ Hb/ lac/ rWBC/ aWBC/ WBC count	-	/	↟ CI/ lac/ aWBC, ~ MAP, ↓ temp/ Hb, ↡ rWBC	/	↑ CI/ lac[u]/ WBC count[u], ~ MAP/ temp, ↓ Hb, ↡ rWBC, ↟ aWBC[u]	↑ CI/ lac[u]/ WBC count, ↟ MAP/ temp/ aWBC, ↓ Hb, ~ rWBC	↟ MAP/ temp/ lac, ↓ Hb, ↡ rWBC, ↟ aWBC[u], ↑ WBC count	-
Donndorf, P. (45)	CABG MECC	96 +/- 27	54 +/- 16	-	*MAP/ lac/ Hct	↓ MAP/ Hct, ↑ lac	/	↑ MAP/ lac, ↓ Hct	/	-	-	-	-
	CABG CECC	97 +/- 24	56 +/- 18	-	*MAP/ lac/ Hct	↓ MAP/ Hct[h], ↑ lac	/	~MAP[h], ↑ lac, ↓ Hct	/	-	-	-	-

Donndorf, P. (46)	AVR MECC	96 +/- 19	71 +/- 16	-	*lac/ Hct	↑ lac, ↓ Hct	/	↑ lac, ↓ Hct	/	*CK/ troponin, ↟ lac, ↧ Hct	-	-	-
	AVR CECC	87 +/- 19	64 +/- 17	-	*lac/ Hct	↑ lac, ↓ Hct[h]	/	↑ lac, ↓ Hct	/	*CK/ troponin, ↟ lac, ↧ Hct	-	-	-
Yuruk, K. (67)	CABG CECC	119 +/- 14	81 +/- 22	-	* Hct/ Hb/ lac/ MAP	↧ Hct[y]/ Hb/ MAP[y], ↟ lac	/	↧ Hct/ Hb/ MAP[y], ↟ lac	/	-	-	-	-
	CABG MECC	71 +/- 16	44 +/- 15	-	* Hct/ Hb/ lac/ MAP	↧ Hct/ Hb, MAP, ↟ lac	/	↧ Hct/ Hb, MAP, ↟ lac	/	-	-	-	-
Aykut, G. (36)	CABG crystaloid cardioplegia	69.0 +/- 3.5	30 (28.5 - 39.5)	-	*MAP, Temp, Hct, pH, lac	↓ MAP/ temp[a]/ Hct, ↟ pH/ lac	/	↓ MAP/ Hct, ↟ Temp/ lac, ↧ PH	/	-	-	-	-
	CABG blood cardioplegia	82.8 +/- 6.8	39.5 (33.8 - 51)	-	*MAP, Temp, Hct, pH, lac	↓ MAP/ temp/ Hct, ↟ pH/ lac	/	↓ MAP/ Hct, ↟ temp/ lac, ↧ pH	/	-	-	-	-
Dekker, N. (42)	CABG HC circuit	101 +/- 26	72 +/- 22	-	*Hct/ RBC concentration	-	/	↓ Hct, ↧ RBC concentration	/	↓ Hct, ~ RBC concentration	↓ Hct, ↧ RBC concentration	-	↓ Hct, ↧ RBC concentration
	CABG PC circuit	107 +/- 17	73 +/- 15	-	*Hct/ RBC concentration	-	/	↓ Hct, ↧ RBC concentration	/	↓ Hct, ↧ RBC concentration	↓ Hct, ↧ RBC concentration	-	↓ Hct, ↧ RBC concentration
Forti, A. (49)	Cardiac surgery CPB flow 80%	-	-	-	-	-	/	*MAP[n]/ temp[m]/ SvO2[m]/ Hct[m]	/	-	-	-	-
	Cardiac surgery CPB flow 100%	-	-	-	-	-	/	*MAP/ temp/ SvO2/ Hct	/	-	-	-	–
Holmgaard, F. (51)	CABG LMAP	83 +/- 32	-	-	* Temp/ pH/ Hb/ Hct	-	/	↟ temp, ↧ pH/ Hb/ Hct	/	-	-	-	-
	CABG HMAP	73 +/- 21	-	-	* Temp/ pH/ Hb/ Hct	-	/	↟ temp, ↧ pH/ Hb/ Hct	/	-	-	-	-

/: not applicable, *: baseline, ~: no significant change vs baseline, ↧: decrease vs baseline (not significant), ↟: increase vs baseline (not significant), ↑: significant increase vs baseline, ↓: significant decrease vs baseline, AVR: aortic valve repair, aWBC: adhering white blood cell, CABG: coronary artery bypass grafting, Cardiac surgery: multiple surgeries (i.e. CABG, valve replacement/repair), CECC: Conventional ExtraCorporeal Circulation circuit, CI: cardiac index, CK: creatin-kinase, CO: cardiac output, CPB: cardiopulmonary bypass, DBP: diastolic blood pressure, During aortic cross-clamp: time from aortic cross-clamp until aortic cross clamp release, End CPB: time from onset of CPB until weaning from CPB, Hb: hemoglobin, HC: heparin coated, Hct: hematocrit, HMAP: high mean arterial pressure, ICU: first 24 hours after surgery, lac: lactate, LMAP: low mean arterial pressure, MAP: mean arterial pressure, MECC: Minimalized ExtraCorporeal Circulation circuit, Min: minutes, NP: Non-pulsatile CPB-flow. PC: phosporylcholine-coated, PP: pulsatile CPB-flow, RBC: red blood cell, rWBC: rolling white blood cell, SBP: systolic blood pressure, SvO_2: mixed venous oxygen saturation, Temp: temperature, WBC: white blood cell, a: significantly lower than blood cardioplegia, c: significantly higher than off-pump, h: significantly lower than MECC group, j: no difference with NP (not significant), k: lower than NP (not significant), m: no difference with 100% (not significant), n: lower than 100% (not significant)p: significantly lower than off-pump, u: significantly higher than pulsatile, y: significantly lower than MECC

Appendix VIc: Macrocirculatory parameters therapeutical interventions

				Pre-operative	Surgery					Post-operative			
Author	**Study groups**	**CPB duration (min)**	**Aortic cross-clamp time (min)**	**Pre-operative**	**After anesthesia**	**During aortic cross-clamp**	**During cardiac positioning**	**End of CPB**	**After positioning**	**ICU**	**24h after surgery**	**48 h after surgery**	**72h after surgery**
Maier, S. (57)	CABG phenylephrine	-	-	-	* Temp/ Hb/ Hct/ pH/ SvO2	-	/	↧ Temp/ Hb/ Hct/ pH/ SvO2	/	-	-	-	-
Mohamed, H. (59)	CABG Propofol	70 (56 - 82)	-	-	* Lac/ CO	-	/	↑ Lac, ↓ CO		-	-	-	-
	CABG dexmedetomidine	75 (62 - 90)	-	-	* Lac/ CO/ MAP	-	/	↑ Lac, ↓ CO	/	-	-	-	-
Özarslan, N.G. (62)	CABG sevoflurane	93.9 +/- 34.0	51.3 +/- 16.8	* MAP	↧ MAP, * CO	-	/	↓ MAP, ↑ CO	/	-	~ MAP, ↑ CO	-	-
	CABG isoflurane	115.0 +/- 58.8	76.4 +/- 49.1	* MAP	↧ MAP, * CO	-	/	↓ MAP, ↥ CO	/	-	↧ MAP, ↑ CO	-	-
	CABG desflurane	79.6 +/- 23.9	49.0 +/- 24.5	* MAP	↧ MAP, * CO	-	/	↧ MAP, ↑ CO	/	-	↧ MAP^{u}, ↑ CO	-	-
Yuruk, K. (66)	Cardiac surgery	92 +/- 19	69 +/- 22	-	* MAP	↓ MAP	/	↥ MAP^{x} * Hb^{x}/ Hct^{x}	/	-	-	-	-

/: not applicable, *: baseline, ~: no significant change vs baseline, ↧: decrease vs baseline (not significant), ↥: increase vs baseline (not significant), ↑: significant increase vs baseline, ↓: significant decrease vs baseline, CABG: coronary artery bypass grafting, Cardiac surgery: multiple surgeries (i.e. CABG, valve replacement/repair), CO: cardiac output, CPB: cardiopulmonary bypass, During aortic cross-clamp: time from aortic cross-clamp until aortic cross clamp release, End CPB: time from onset of CPB until weaning from CPB, Hb: hemoglobin, Hct: hematocrit, ICU: first 24 hours after surgery, lac: lactate, MAP: mean arterial pressure, Min: minutes, SvO_2: mixed venous oxygen saturation, Temp: temperature, u: significantly higher than pulsatile, x: significantly higher vs before transfusion

Appendix VIc: Macrocirculatory parameters therapeutical interventions

Author	Study groups	CPB duration (min)	Aortic cross-clamp time (min)	Before intervention	After intervention
Atasever, B. (35)	Cardiac surgery: RBC infusion	142 +/- 57	93 +/- 50	*Temp, MAP, CO, Hb, SvO2	~ Temp, ↟ MAP/SvO2, ↧ CO/Hb^{z}
	Cardiac surgery: gelatin infusion	142 +/- 57	93 +/- 50	*Temp, MAP, CO, Hb, SvO2	↟ Temp/ MAP/ CO^{AB}, ↧ Hb, ~SvO2
	Cardiac surgery: no infusion	142 +/- 57	93 +/- 50	*Temp, MAP, CO, Hb, SvO2	~ Temp/ Hb/ SvO2, ↧ MAP, ↟ CO
Elbers, P. (47)	Patients with elevated ABP after ECC	-	-	*SBP/ MAP/ DBP/ temp	↓ SBP/ MAP/ DBP, ~ temp
Liu, X. (56)	Valve surgery Dexmedetomidine	73 (60 - 88)	51 (35 - 64)	* MAP/ Hb/ lac/ temp	~ MAP, ↧ Hb/ lac, ↟ temp^{R}
	Valve surgery Propofol	68 (54 - 80)	46 (34 - 59)	* MAP/ Hb/ lac/ temp	~ MAP, ↧ Hb/ lac, ↟ temp^{R}
Maurin, C. (58)	Cardiac Surgery Methylene blue	130 (75 - 240)	96 (0 - 207)	* Lac/ temp/ MAP/ CI	↓ lac, ↑ temp/ MAP, ~ CI

*: baseline, ~: no significant change vs baseline, ↧: decrease vs baseline (not significant), ↟: increase vs baseline (not significant), ↑: significant increase vs baseline, ↓: significant decrease vs baseline, Cardiac surgery: multiple surgeries (i.e. CABG, valve replacement/repair), CI: cardiac index, CO: cardiac output, CPB: cardiopulmonary bypass, DBP: diastolic blood pressure, Hb: hemoglobin, lac: lactate, MAP: mean arterial pressure, Min: minutes, SBP: systolic blood pressure, SvO_2: mixed venous oxygen saturation, Temp: temperature, AB: significant increased vs no infusion, R: significant over time, z: significant increased vs gelatin group

Appendix VII: Outcomes

Study	Study groups	LOS ICU	LOS hospital	ICU readmission	Mortality (n)	Complications	Other
Arnold, R.C. (32)	Cardiac surgery	-	-	-	0	No major complications	-
Atasever, B. (35)	Cardiac surgery RBC transfusion	29 +/- 27h	-	-	-	-	-
	Cardiac surgery gelatin infusion	26 +/- 9h	-	-	-	-	-
	Cardiac surgery no transfusion	19 +/- 6h	-	-	-	-	-
Aykut, G. (36)	CABG crystalloid cardioplegia	20.2 +/- 0.7h	6.5 (6-8) days	-	-	-	-
	CABG blood cardioplegia	20.0 +/- 0.4h	6 (6-7.3) days	-	-	-	-
Boly, C. (39)	Cardiac surgery obese patients	1 (1-1) days	5 (4-8) days	-	0	-	-
	Cardiac surgery lean patients	1 (1-1) days	7 (5-9.5) days	-	1	-	-
Backer de, D. (40)	Cardiac surgery on-pump	-	-	-	-	-	SOFA score: 1 (0-1)
	Cardiac surgery off-pump	-	-	-	-	-	SOFA score: 1 (0-1)
Dekker, N. (41)	CABG	1 (1-1) days	6 (5-8) days				-
Dekker, N. (42)	CABG HC circuit	-	-	-	-	Post-operative pulmonary embolism: 1 (8%)	-
	CABG PC circuit	-	-	-	-	Post-operative pulmonary embolism: 0 (0)	-
Uil den, C.A. (43)	CABG	-	-	-	2 (n = 1 during surgery, n = 1 after 12 days)	-	-
Donndorf, P. (45)	CABG MECC	-	-	-	-	None	-
	CABG CECC	-	-	-	1 (8th day post-op)	None	-
Donndorf, P. (46)	AVR MECC	1.56 +/- 0.88 days	-	-	-	-	-
	AVR CECC	1.40 +/- 0.70 days	-	-	-	-	-
Greenwood, J.C. (50)	Cardiac surgery	-	-	-	30 day survival: 25 (100%)	-	SOFA: 24h: 6 (5-9) 48h: 6 (2-8)

Koning, N.J. (52)	CABG on-pump	1 (1-1) days	-	-	1 (3 weeks post-operative)	AKI: 2	-
	CABG off-pump	1 (1-1) days	-	-	0	None	-
Koning, N.J. (54)	CABG PP	< 24 h	-	-	30 day: 0	De novo atrial fibrillation (n = 4 NP and PP)	-
	CAPB NP	< 24 h	-	-	30 day: 0	De novo atrial fibrillation (n = 4 NP and PP)	-
Liu, X. (56)	Valve surgery Dexmedetomidine	-	-	-	-	Hypotension (n = 9), bradycardia (n = 5), nausea/ vomiting (n = 3), AF (n = 17), new-onset AF (n =1/11)	-
	Valve surgery Propofol	-	-	-	-	Hypotension (n = 11), bradycardia (n = 1), nausea/ vomiting (n = 7), delirium (n = 2), AF (n = 19), new-onset AF (n =5/18)	-
Maurin, C. (58)	Cardiac Surgery Methylene blue	-	-	-	72h: 2 (10%) 28 days: 10 (45%)	-	-
Özarslan, N.G. (62)	CABG sevoflurane	56.9 +/- 29.9 h	-	-	-	-	-
	CABG isoflurane	43.1 +/- 21 h	-	-	-	-	-
	CABG desflurane	-	-	-	-	-	-
Prestes, I. (63)	Cardiac surgery	6.3 +/- 7.3 days	10.4 +/- 7.8 days		-	-	-
Stowell, C.P. (64)	Cardiac surgery	2 (1 - 4) days	-	-	2 (2.3%)	-	-
Yuruk, K. (67)	CABG CECC	2.2 +/- 1.8 days	-	-	-	-	-
	CABG MECC	1.5 +/- 0.7 days	-	-	-	-	-

AF: atrial fibrillation, AKI: acute kidney injury, AVR: aortic valve repair, CABG: coronary artery bypass grafting, Cardiac surgery: multiple surgeries (i.e. CABG, valve replacement/repair), CECC: Conventional ExtraCorporeal Circulation circuit, h: hour, HC: heparin coated, ICU: intensive care unit, LOS hospital: length of stay hospital, LOS ICU: length of stay intensive care, MECC: Minimalized ExtraCorporeal Circulation circuit, n: number of patients, NP: Non-pulsatile CPB-flow, PC: phosporylcholine-coated, PP: pulsatile CPB-flow, RBC: red blood cell, SOFA: Sequential Organ Failure Assessment score

Additional file 1: Search strategy

Databases:
PubMed
http://www.ncbi.nlm.nih.gov/pubmed?otool=leiden

((("Microcirculation"[Mesh] OR "microcirculation"[tw] OR "micro circulation"[tw] OR "microcirculat*"[tw] OR "micro circulat*"[tw] OR "microvascular circulation"[tw] OR "microvascular circulat*"[tw] OR "microvascular blood flow"[tw] OR "microvascular blood flow"[tw] OR ("Microvessels"[Mesh] AND "Blood Circulation"[Mesh:NoExp]) OR "Blood Flow Velocity"[mesh] OR "Blood flow velocity"[tw] OR "side stream dark field"[tw] OR "SDF"[tw] OR "incidence dark field"[tw] OR ("incidence"[tw] AND "dark field"[tw]) OR "IDF"[tw] OR "proportion of perfused vessels"[tw] OR "PPV"[tw] OR "perfused vessel density"[tw] OR "PVD"[tw] OR "total vessel density"[tw] OR "TVD"[tw] OR "hand held microscope"[tw] OR "hand held microscopes"[tw] OR "hand held microscop*"[tw] OR "handheld microscope"[tw] OR "handheld microscopes"[tw] OR "handheld microscop*"[tw] OR "OPS"[tw] OR "orthogonal polarization spectral"[tw] OR "microvascular perfusion"[tw] OR "microvascular tissue perfusion"[tw]) AND ("sublingual"[tw] OR "sublingual*"[tw] OR "sub lingual"[tw] OR "sub lingual*"[tw] OR "Critical Illness"[Mesh] OR "Critical Illness"[tw] OR "Critically ill"[tw] OR "Shock"[mesh:noexp] OR "Multiple Organ Failure"[mesh] OR "Shock, Cardiogenic"[mesh] OR "Shock, Surgical"[mesh] OR "multiple Organ Failure"[tw] OR "Cardiogenic shock"[tw] OR "Surgical Shock"[tw] OR "distributive shock"[tw] OR "Leukocytosis"[Mesh] OR "Leukocytosis"[tw] OR "high mortality risk patient"[tw] OR "mortality risk patient"[tw] OR "high mortality risk patients"[tw] OR "mortality risk patients"[tw] OR "high preoperative risk"[tw] OR "high preoperative risks"[tw] OR "preoperative risk"[tw] OR "high preoperative risks"[tw]) AND ("cardiac surgery"[tw] OR "cardiac surg*"[tw] OR "heart surgery"[tw] OR "heart surg*"[tw] OR "cardio thoracic surgery"[tw] OR "cardio thoracic surg*"[tw] OR "cardiothoracic surgery"[tw] OR "cardiothoracic surg*"[tw] OR "thoracic surgery"[tw] OR "thoracic surg*"[tw] OR "Cardiac Surgical Procedures"[Mesh] OR "Arterial Switch"[tw] OR "Arterial Switch Operation"[tw] OR "Cardiac Valve Annuloplast*"[tw] OR "Cardiac Valve Annuloplasty"[tw] OR "Cardiomyoplast*"[tw] OR "Cardiomyoplasty"[tw] OR "Coronary Artery Bypass"[tw] OR "Coronary Artery Bypass*"[tw] OR "CABG"[tw] OR "Coronary Atherectom*"[tw] OR "Coronary Atherectomy"[tw] OR "Coronary Balloon Angioplast*"[tw] OR "Coronary Balloon Angioplasty"[tw] OR "Hypothermia Induced Circulatory Arrest"[tw] OR "Fontan Procedure"[tw] OR "Heart Massage"[tw] OR "Heart Transplant*"[tw] OR "Heart Transplantation"[tw] OR "Heart Valve Prosthesis Implant*"[tw] OR "Heart Valve Prosthesis Implantation"[tw] OR "Heart-Lung Transplant*"[tw] OR "Heart-Lung Transplantation"[tw] OR "Induced Heart Arrest"[tw] OR "Internal Mammary-Coronary Artery Anastomosis"[tw] OR "Maze Procedure"[tw] OR "Mitral Valve Annuloplast*"[tw] OR "Mitral Valve Annuloplasty"[tw] OR "Myocardial Revascularisation"[tw] OR "Myocardial Revascularization"[tw] OR "Norwood Procedure"[tw] OR "Norwood Procedures"[tw] OR "Pericardial Window Technique"[tw] OR "Pericardial Window Techniques"[tw] OR "Pericardiectom*"[tw] OR "Pericardiectomy"[tw] OR "Pericardiocentesis"[tw] OR "Right Heart Bypass"[tw] OR "Right Heart Bypass*"[tw] OR "Transcatheter Aortic Valve Replacement"[tw] OR "Transcatheter Aortic Valve Replacement*"[tw] OR "Transmyocardial Laser Revascularisation"[tw] OR "Transmyocardial Laser Revascularization"[tw] OR "Cardiopulmonary Bypass"[Mesh] OR "Cardiopulmonary Bypass"[tw] OR "Cardio pulmonary Bypass"[tw] OR "Heart Lung Bypass"[tw] OR "Heart Bypass"[tw] OR "Cardio pulmonary Bypass"[tw] OR "Heart Diseases/surgery"[Mesh] OR "Heart/surgery"[Mesh]) NOT ("Animals"[mesh] NOT "Humans"[mesh])) OR
(("Microcirculation"[majr] OR "microcirculation"[ti] OR "micro circulation"[ti] OR "microcirculat*"[ti] OR "micro circulat*"[ti] OR "microvascular circulation"[ti] OR "microvascular circulat*"[ti] OR "microvascular blood flow"[ti] OR "microvascular blood flow"[ti] OR ("Microvessels"[majr] AND "Blood Circulation"[majr:NoExp]) OR "Blood Flow Velocity"[majr] OR "Blood flow velocity"[ti] OR "side stream dark field"[ti] OR "SDF"[ti] OR "incidence dark field"[ti] OR ("incidence"[ti] AND "dark field"[ti]) OR "IDF"[ti] OR "proportion of perfused vessels"[ti] OR "PPV"[ti] OR "perfused vessel density"[ti] OR "PVD"[ti] OR "total vessel density"[ti] OR "TVD"[ti] OR "hand held microscope"[ti] OR "hand held microscopes"[ti] OR "hand held microscop*"[ti] OR "handheld microscope"[ti] OR "handheld microscopes"[ti] OR "handheld microscop*"[ti] OR "OPS"[ti] OR "orthogonal polarization

spectral"[ti] OR "microvascular perfusion"[ti] OR "microvascular tissue perfusion"[ti]) AND ("sublingual"[ti] OR "sublingual*"[ti] OR "sub lingual"[ti] OR "sub lingual*"[ti] OR "Critical Illness"[majr] OR "Critical Illness"[ti] OR "Critically ill"[ti] OR "Shock"[majr:noexp] OR "Multiple Organ Failure"[majr] OR "Shock, Cardiogenic"[majr] OR "Shock, Surgical"[majr] OR "multiple Organ Failure"[ti] OR "Cardiogenic shock"[ti] OR "Surgical Shock"[ti] OR "distributive shock"[ti] OR "Leukocytosis"[majr] OR "Leukocytosis"[ti] OR "high mortality risk patient"[ti] OR "mortality risk patient"[ti] OR "high mortality risk patients"[ti] OR "mortality risk patients"[ti] OR "high preoperative risk"[ti] OR "high preoperative risks"[ti] OR "preoperative risk"[ti] OR "high preoperative risks"[ti]) NOT ("Animals"[mesh] NOT "Humans"[mesh])))

Embase
http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=main&MODE=ovid&D=oemezd

(((*"Microcirculation"/ OR "microcirculation".ti,ab OR "micro circulation".ti,ab OR "microcirculat*".ti,ab OR "micro circulat*".ti,ab OR "microvascular circulation".ti,ab OR "microvascular circulat*".ti,ab OR "microvascular blood flow".ti,ab OR "microvascular blood flow".ti,ab OR *"Blood Flow Velocity"/ OR "Blood flow velocity".ti,ab OR "side stream dark field".ti,ab OR "SDF".ti,ab OR "incidence dark field".ti,ab OR ("incidence".ti,ab AND "dark field".ti,ab) OR "IDF".ti,ab OR "proportion of perfused vessels".ti,ab OR "PPV".ti,ab OR "perfused vessel density".ti,ab OR "PVD".ti,ab OR "total vessel density".ti,ab OR "TVD".ti,ab OR "hand held microscope".ti,ab OR "hand held microscopes".ti,ab OR "hand held microscop*".ti,ab OR "handheld microscope".ti,ab OR "handheld microscopes".ti,ab OR "handheld microscop*".ti,ab OR "OPS".ti,ab OR "orthogonal polarization spectral".ti,ab OR "microvascular perfusion".ti,ab OR "microvascular tissue perfusion".ti,ab) AND ("sublingual".mp OR "sublingual*".mp OR "sub lingual".mp OR "sub lingual*".mp OR *"Critical Illness"/ OR "Critical Illness".ti,ab OR "Critically ill".ti,ab OR *"Shock"/ OR exp *"Multiple Organ Failure"/ OR *"Cardiogenic Shock"/ OR "multiple Organ Failure".ti,ab OR "Cardiogenic shock".ti,ab OR "Surgical Shock".ti,ab OR "distributive shock".ti,ab OR exp *"Leukocytosis"/ OR "Leukocytosis".ti,ab OR "high mortality risk patient".ti,ab OR "mortality risk patient".ti,ab OR "high mortality risk patients".ti,ab OR "mortality risk patients".ti,ab OR "high preoperative risk".ti,ab OR "high preoperative risks".ti,ab OR "preoperative risk".ti,ab OR "high preoperative risks".ti,ab) AND ("cardiac surgery".ti,ab OR "cardiac surg*".ti,ab OR "heart surgery".ti,ab OR "heart surg*".ti,ab OR "cardio thoracic surgery".ti,ab OR "cardio thoracic surg*".ti,ab OR "cardiothoracic surgery".ti,ab OR "cardiothoracic surg*".ti,ab OR "thoracic surgery".ti,ab OR "thoracic surg*".ti,ab OR exp *"Heart Surgery"/ OR exp *"Thorax Surgery"/ OR "Arterial Switch".ti,ab OR "Arterial Switch Operation".ti,ab OR "Cardiac Valve Annuloplast*".ti,ab OR "Cardiac Valve Annuloplasty".ti,ab OR "Cardiomyoplast*".ti,ab OR "Cardiomyoplasty".ti,ab OR "Coronary Artery Bypass".ti,ab OR "Coronary Artery Bypass*".ti,ab OR "CABG".ti,ab OR "Coronary Atherectom*".ti,ab OR "Coronary Atherectomy".ti,ab OR "Coronary Balloon Angioplast*".ti,ab OR "Coronary Balloon Angioplasty".ti,ab OR "Hypothermia Induced Circulatory Arrest".ti,ab OR "Fontan Procedure".ti,ab OR "Heart Massage".ti,ab OR "Heart Transplant*".ti,ab OR "Heart Transplantation".ti,ab OR "Heart Valve Prosthesis Implant*".ti,ab OR "Heart Valve Prosthesis Implantation".ti,ab OR "Heart-Lung Transplant*".ti,ab OR "Heart-Lung Transplantation".ti,ab OR "Induced Heart Arrest".ti,ab OR "Internal Mammary-Coronary Artery Anastomosis".ti,ab OR "Maze Procedure".ti,ab OR "Mitral Valve Annuloplast*".ti,ab OR "Mitral Valve Annuloplasty".ti,ab OR "Myocardial Revascularisation".ti,ab OR "Myocardial Revascularization".ti,ab OR "Norwood Procedure".ti,ab OR "Norwood Procedures".ti,ab OR "Pericardial Window Technique".ti,ab OR "Pericardial Window Techniques".ti,ab OR "Pericardiectom*".ti,ab OR "Pericardiectomy".ti,ab OR "Pericardiocentesis".ti,ab OR "Right Heart Bypass".ti,ab OR "Right Heart Bypass*".ti,ab OR "Transcatheter Aortic Valve Replacement".ti,ab OR "Transcatheter Aortic Valve Replacement*".ti,ab OR "Transmyocardial Laser Revascularisation".ti,ab OR "Transmyocardial Laser Revascularization".ti,ab OR *"Cardiopulmonary Bypass"/ OR "Cardiopulmonary Bypass".ti,ab OR "Cardio pulmonary Bypass".ti,ab OR "Heart Lung Bypass".ti,ab OR "Heart Bypass".ti,ab OR "Cardio pulmonary Bypass".ti,ab OR exp *"Heart Disease"/su OR exp *"Heart"/su) NOT (exp "Animals"/ NOT exp "Humans"/)) OR ((*"Microcirculation"/ OR "microcirculation".ti OR "micro circulation".ti OR "microcirculat*".ti OR "micro circulat*".ti OR "microvascular circulation".ti OR "microvascular circulat*".ti OR "microvascular blood flow".ti OR "microvascular blood flow".ti OR *"Blood Flow Velocity"/ OR "Blood flow

velocity".ti OR "side stream dark field".ti OR "SDF".ti OR "incidence dark field".ti OR ("incidence".ti AND "dark field".ti) OR "IDF".ti OR "proportion of perfused vessels".ti OR "PPV".ti OR "perfused vessel density".ti OR "PVD".ti OR "total vessel density".ti OR "TVD".ti OR "hand held microscope".ti OR "hand held microscopes".ti OR "hand held microscop*".ti OR "handheld microscope".ti OR "handheld microscopes".ti OR "handheld microscop*".ti OR "OPS".ti OR "orthogonal polarization spectral".ti OR "microvascular perfusion".ti OR "microvascular tissue perfusion".ti) AND ("sublingual".ti OR "sublingual*".ti OR "sub lingual".ti OR "sub lingual*".ti OR "Critical Illness"/ OR "Critical Illness".ti OR "Critically ill".ti OR *"Shock"/ OR exp *"Multiple Organ Failure"/ OR *"Cardiogenic Shock"/ OR "multiple Organ Failure".ti OR "Cardiogenic shock".ti OR "Surgical Shock".ti OR "distributive shock".ti OR exp *"Leukocytosis"/ OR "Leukocytosis".ti OR "high mortality risk patient".ti OR "mortality risk patient".ti OR "high mortality risk patients".ti OR "mortality risk patients".ti OR "high preoperative risk".ti OR "high preoperative risks".ti OR "preoperative risk".ti OR "high preoperative risks".ti) NOT (exp "Animals"/ NOT exp "Humans"/)))

Web of Science
http://isiknowledge.com/wos

(((TI=("Microcirculation" OR "microcirculation" OR "micro circulation" OR "microcirculat*" OR "micro circulat*" OR "microvascular circulation" OR "microvascular circulat*" OR "microvascular blood flow" OR "microvascular blood flow" OR "Blood Flow Velocity" OR "Blood flow velocity" OR "side stream dark field" OR "incidence dark field" OR ("incidence" AND "dark field") OR "proportion of perfused vessels" OR "perfused vessel density" OR "total vessel density" OR "hand held microscope" OR "hand held microscopes" OR "hand held microscop*" OR "handheld microscope" OR "handheld microscopes" OR "handheld microscop*" OR "orthogonal polarization spectral" OR "microvascular perfusion" OR "microvascular tissue perfusion") OR AK=("Microcirculation" OR "microcirculation" OR "micro circulation" OR "microcirculat*" OR "micro circulat*" OR "microvascular circulation" OR "microvascular circulat*" OR "microvascular blood flow" OR "microvascular blood flow" OR "Blood Flow Velocity" OR "Blood flow velocity" OR "side stream dark field" OR "incidence dark field" OR ("incidence" AND "dark field") OR "proportion of perfused vessels" OR "perfused vessel density" OR "total vessel density" OR "hand held microscope" OR "hand held microscopes" OR "hand held microscop*" OR "handheld microscope" OR "handheld microscopes" OR "handheld microscop*" OR "orthogonal polarization spectral" OR "microvascular perfusion" OR "microvascular tissue perfusion") OR AB=("Microcirculation" OR "microcirculation" OR "micro circulation" OR "microcirculat*" OR "micro circulat*" OR "microvascular circulation" OR "microvascular circulat*" OR "microvascular blood flow" OR "microvascular blood flow" OR "Blood Flow Velocity" OR "Blood flow velocity" OR "side stream dark field" OR "incidence dark field" OR ("incidence" AND "dark field") OR "proportion of perfused vessels" OR "perfused vessel density" OR "total vessel density" OR "hand held microscope" OR "hand held microscopes" OR "hand held microscop*" OR "handheld microscope" OR "handheld microscopes" OR "handheld microscop*" OR "orthogonal polarization spectral" OR "microvascular perfusion" OR "microvascular tissue perfusion")) AND (TS=("sublingual" OR "sublingual*" OR "sub lingual" OR "sub lingual*" OR "Critical Illness" OR "Critical Illness" OR "Critically ill" OR "Multiple Organ Failure" OR "Cardiogenic Shock" OR "multiple Organ Failure" OR "Cardiogenic shock" OR "Surgical Shock" OR "distributive shock" OR "Leukocytosis" OR "Leukocytosis" OR "high mortality risk patient" OR "mortality risk patient" OR "high mortality risk patients" OR "mortality risk patients" OR "high preoperative risk" OR "high preoperative risks" OR "preoperative risk" OR "high preoperative risks") OR TI=("Shock")) AND (TI=("cardiac surgery" OR "cardiac surg*" OR "heart surgery" OR "heart surg*" OR "cardio thoracic surgery" OR "cardio thoracic surg*" OR "cardiothoracic surgery" OR "cardiothoracic surg*" OR "thoracic surgery" OR "thoracic surg*" OR "Heart Surgery" OR "Thorax Surgery" OR "Arterial Switch" OR "Arterial Switch Operation" OR "Cardiac Valve Annuloplast*" OR "Cardiac Valve Annuloplasty" OR "Cardiomyoplast*" OR "Cardiomyoplasty" OR "Coronary Artery Bypass" OR "Coronary Artery Bypass*" OR "CABG" OR "Coronary Atherectom*" OR "Coronary Atherectomy" OR "Coronary Balloon Angioplast*" OR "Coronary Balloon Angioplasty" OR "Hypothermia Induced Circulatory Arrest" OR "Fontan Procedure" OR "Heart Massage" OR "Heart Transplant*" OR "Heart Transplantation" OR "Heart Valve Prosthesis Implant*" OR "Heart Valve Prosthesis Implantation" OR "Heart-Lung Transplant*" OR "Heart-Lung Transplantation" OR "Induced

Heart Arrest" OR "Internal Mammary-Coronary Artery Anastomosis" OR "Maze Procedure" OR "Mitral Valve Annuloplast*" OR "Mitral Valve Annuloplasty" OR "Myocardial Revascularisation" OR "Myocardial Revascularization" OR "Norwood Procedure" OR "Norwood Procedures" OR "Pericardial Window Technique" OR "Pericardial Window Techniques" OR "Pericardiectom*" OR "Pericardiectomy" OR "Pericardiocentesis" OR "Right Heart Bypass" OR "Right Heart Bypass*" OR "Transcatheter Aortic Valve Replacement" OR "Transcatheter Aortic Valve Replacement*" OR "Transmyocardial Laser Revascularisation" OR "Transmyocardial Laser Revascularization" OR "Cardiopulmonary Bypass" OR "Cardiopulmonary Bypass" OR "Cardio pulmonary Bypass" OR "Heart Lung Bypass" OR "Heart Bypass" OR "Cardio pulmonary Bypass") OR AK=("cardiac surgery" OR "cardiac surg*" OR "heart surgery" OR "heart surg*" OR "cardio thoracic surgery" OR "cardio thoracic surg*" OR "cardiothoracic surgery" OR "cardiothoracic surg*" OR "thoracic surgery" OR "thoracic surg*" OR "Heart Surgery" OR "Thorax Surgery" OR "Arterial Switch" OR "Arterial Switch Operation" OR "Cardiac Valve Annuloplast*" OR "Cardiac Valve Annuloplasty" OR "Cardiomyoplast*" OR "Cardiomyoplasty" OR "Coronary Artery Bypass" OR "Coronary Artery Bypass*" OR "CABG" OR "Coronary Atherectom*" OR "Coronary Atherectomy" OR "Coronary Balloon Angioplast*" OR "Coronary Balloon Angioplasty" OR "Hypothermia Induced Circulatory Arrest" OR "Fontan Procedure" OR "Heart Massage" OR "Heart Transplant*" OR "Heart Transplantation" OR "Heart Valve Prosthesis Implant*" OR "Heart Valve Prosthesis Implantation" OR "Heart-Lung Transplant*" OR "Heart-Lung Transplantation" OR "Induced Heart Arrest" OR "Internal Mammary-Coronary Artery Anastomosis" OR "Maze Procedure" OR "Mitral Valve Annuloplast*" OR "Mitral Valve Annuloplasty" OR "Myocardial Revascularisation" OR "Myocardial Revascularization" OR "Norwood Procedure" OR "Norwood Procedures" OR "Pericardial Window Technique" OR "Pericardial Window Techniques" OR "Pericardiectom*" OR "Pericardiectomy" OR "Pericardiocentesis" OR "Right Heart Bypass" OR "Right Heart Bypass*" OR "Transcatheter Aortic Valve Replacement" OR "Transcatheter Aortic Valve Replacement*" OR "Transmyocardial Laser Revascularisation" OR "Transmyocardial Laser Revascularization" OR "Cardiopulmonary Bypass" OR "Cardiopulmonary Bypass" OR "Cardio pulmonary Bypass" OR "Heart Lung Bypass" OR "Heart Bypass" OR "Cardio pulmonary Bypass") OR AB=("cardiac surgery" OR "cardiac surg*" OR "heart surgery" OR "heart surg*" OR "cardio thoracic surgery" OR "cardio thoracic surg*" OR "cardiothoracic surgery" OR "cardiothoracic surg*" OR "thoracic surgery" OR "thoracic surg*" OR "Heart Surgery" OR "Thorax Surgery" OR "Arterial Switch" OR "Arterial Switch Operation" OR "Cardiac Valve Annuloplast*" OR "Cardiac Valve Annuloplasty" OR "Cardiomyoplast*" OR "Cardiomyoplasty" OR "Coronary Artery Bypass" OR "Coronary Artery Bypass*" OR "CABG" OR "Coronary Atherectom*" OR "Coronary Atherectomy" OR "Coronary Balloon Angioplast*" OR "Coronary Balloon Angioplasty" OR "Hypothermia Induced Circulatory Arrest" OR "Fontan Procedure" OR "Heart Massage" OR "Heart Transplant*" OR "Heart Transplantation" OR "Heart Valve Prosthesis Implant*" OR "Heart Valve Prosthesis Implantation" OR "Heart-Lung Transplant*" OR "Heart-Lung Transplantation" OR "Induced Heart Arrest" OR "Internal Mammary-Coronary Artery Anastomosis" OR "Maze Procedure" OR "Mitral Valve Annuloplast*" OR "Mitral Valve Annuloplasty" OR "Myocardial Revascularisation" OR "Myocardial Revascularization" OR "Norwood Procedure" OR "Norwood Procedures" OR "Pericardial Window Technique" OR "Pericardial Window Techniques" OR "Pericardiectom*" OR "Pericardiectomy" OR "Pericardiocentesis" OR "Right Heart Bypass" OR "Right Heart Bypass*" OR "Transcatheter Aortic Valve Replacement" OR "Transcatheter Aortic Valve Replacement*" OR "Transmyocardial Laser Revascularisation" OR "Transmyocardial Laser Revascularization" OR "Cardiopulmonary Bypass" OR "Cardiopulmonary Bypass" OR "Cardio pulmonary Bypass" OR "Heart Lung Bypass" OR "Heart Bypass" OR "Cardio pulmonary Bypass"))) OR TI=(("Microcirculation" OR "microcirculation" OR "micro circulation" OR "microcirculat*" OR "micro circulat*" OR "microvascular circulation" OR "microvascular circulat*" OR "microvascular blood flow" OR "microvascular blood flow" OR "Blood Flow Velocity" OR "Blood flow velocity" OR "side stream dark field" OR "incidence dark field" OR ("incidence" AND "dark field") OR "proportion of perfused vessels" OR "perfused vessel density" OR "total vessel density" OR "hand held microscope" OR "hand held microscopes" OR "hand held microscop*" OR "handheld microscope" OR "handheld microscopes" OR "handheld microscop*" OR "orthogonal polarization spectral" OR "microvascular perfusion" OR "microvascular tissue perfusion") AND ("sublingual" OR "sublingual*" OR "sub lingual" OR "sub lingual*" OR "Critical Illness" OR "Critical Illness" OR "Critically ill" OR "Shock" OR "Multiple Organ Failure" OR "Cardiogenic Shock" OR "multiple Organ Failure" OR "Cardiogenic shock" OR "Surgical Shock" OR "distributive shock" OR "Leukocytosis" OR "Leukocytosis" OR "high mortality risk patient" OR "mortality risk patient" OR

"high mortality risk patients" OR "mortality risk patients" OR "high preoperative risk" OR "high preoperative risks" OR "preoperative risk" OR "high preoperative risks"))) **NOT (ti=("veterinary" OR "rabbit" OR "rabbits" OR "animal" OR "animals" OR "mouse" OR "mice" OR "rodent" OR "rodents" OR "rat" OR "rats" OR "pig" OR "pigs" OR "porcine" OR "horse" OR "horses" OR "equine" OR "cow" OR "cows" OR "bovine" OR "goat" OR "goats" OR "sheep" OR "ovine" OR "canine" OR "dog" OR "dogs" OR "feline" OR "cat" OR "cats") OR ak=("veterinary" OR "rabbit" OR "rabbits" OR "animal" OR "animals" OR "mouse" OR "mice" OR "rodent" OR "rodents" OR "rat" OR "rats" OR "pig" OR "pigs" OR "porcine" OR "horse" OR "horses" OR "equine" OR "cow" OR "cows" OR "bovine" OR "goat" OR "goats" OR "sheep" OR "ovine" OR "canine" OR "dog" OR "dogs" OR "feline" OR "cat" OR "cats"))**

Cochrane
https://www.cochranelibrary.com/advanced-search/search-manager

((("Microcirculation" OR "microcirculation" OR "micro circulation" OR "microcirculat*" OR "micro circulat*" OR "microvascular circulation" OR "microvascular circulat*" OR "microvascular blood flow" OR "microvascular blood flow" OR "Blood Flow Velocity" OR "Blood flow velocity" OR "side stream dark field" OR "SDF" OR "incidence dark field" OR ("incidence" AND "dark field") OR "IDF" OR "proportion of perfused vessels" OR "PPV" OR "perfused vessel density" OR "PVD" OR "total vessel density" OR "TVD" OR "hand held microscope" OR "hand held microscopes" OR "hand held microscop*" OR "handheld microscope" OR "handheld microscopes" OR "handheld microscop*" OR "OPS" OR "orthogonal polarization spectral" OR "microvascular perfusion" OR "microvascular tissue perfusion") AND ("sublingual" OR "sublingual*" OR "sub lingual" OR "sub lingual*" OR "Critical Illness" OR "Critical Illness" OR "Critically ill" OR "Shock" OR "Multiple Organ Failure" OR "Cardiogenic Shock" OR "multiple Organ Failure" OR "Cardiogenic shock" OR "Surgical Shock" OR "distributive shock" OR "Leukocytosis" OR "Leukocytosis" OR "high mortality risk patient" OR "mortality risk patient" OR "high mortality risk patients" OR "mortality risk patients" OR "high preoperative risk" OR "high preoperative risks" OR "preoperative risk" OR "high preoperative risks") AND ("cardiac surgery" OR "cardiac surg*" OR "heart surgery" OR "heart surg*" OR "cardio thoracic surgery" OR "cardio thoracic surg*" OR "cardiothoracic surgery" OR "cardiothoracic surg*" OR "thoracic surgery" OR "thoracic surg*" OR "Heart Surgery" OR "Thorax Surgery" OR "Arterial Switch" OR "Arterial Switch Operation" OR "Cardiac Valve Annuloplast*" OR "Cardiac Valve Annuloplasty" OR "Cardiomyoplast*" OR "Cardiomyoplasty" OR "Coronary Artery Bypass" OR "Coronary Artery Bypass*" OR "CABG" OR "Coronary Atherectom*" OR "Coronary Atherectomy" OR "Coronary Balloon Angioplast*" OR "Coronary Balloon Angioplasty" OR "Hypothermia Induced Circulatory Arrest" OR "Fontan Procedure" OR "Heart Massage" OR "Heart Transplant*" OR "Heart Transplantation" OR "Heart Valve Prosthesis Implant*" OR "Heart Valve Prosthesis Implantation" OR "Heart Lung Transplant*" OR "Heart Lung Transplantation" OR "Induced Heart Arrest" OR "Internal Mammary Coronary Artery Anastomosis" OR "Maze Procedure" OR "Mitral Valve Annuloplast*" OR "Mitral Valve Annuloplasty" OR "Myocardial Revascularisation" OR "Myocardial Revascularization" OR "Norwood Procedure" OR "Norwood Procedures" OR "Pericardial Window Technique" OR "Pericardial Window Techniques" OR "Pericardiectom*" OR "Pericardiectomy" OR "Pericardiocentesis" OR "Right Heart Bypass" OR "Right Heart Bypass*" OR "Transcatheter Aortic Valve Replacement" OR "Transcatheter Aortic Valve Replacement*" OR "Transmyocardial Laser Revascularisation" OR "Transmyocardial Laser Revascularization" OR "Cardiopulmonary Bypass" OR "Cardiopulmonary Bypass" OR "Cardio pulmonary Bypass" OR "Heart Lung Bypass" OR "Heart Bypass" OR "Cardio pulmonary Bypass")):ti,ab,kw OR (("Microcirculation" OR "microcirculation" OR "micro circulation" OR "microcirculat*" OR "micro circulat*" OR "microvascular circulation" OR "microvascular circulat*" OR "microvascular blood flow" OR "microvascular blood flow" OR "Blood Flow Velocity" OR "Blood flow velocity" OR "side stream dark field" OR "SDF" OR "incidence dark field" OR ("incidence" AND "dark field") OR "IDF" OR "proportion of perfused vessels" OR "PPV" OR "perfused vessel density" OR "PVD" OR "total vessel density" OR "TVD" OR "hand held microscope" OR "hand held microscopes" OR "hand held microscop*" OR "handheld microscope" OR "handheld microscopes" OR "handheld microscop*" OR "OPS" OR "orthogonal polarization spectral" OR "microvascular perfusion" OR "microvascular tissue perfusion") AND ("sublingual" OR "sublingual*" OR "sub lingual" OR "sub lingual*" OR "Critical Illness" OR "Critical Illness" OR "Critically ill" OR "Shock" OR "Multiple Organ Failure" OR "Cardiogenic Shock" OR "multiple Organ Failure" OR "Cardiogenic shock" OR "Surgical

Shock" OR "distributive shock" OR "Leukocytosis" OR "Leukocytosis" OR "high mortality risk patient" OR "mortality risk patient" OR "high mortality risk patients" OR "mortality risk patients" OR "high preoperative risk" OR "high preoperative risks" OR "preoperative risk" OR "high preoperative risks")):ti)

PMC PubMed Central
https://www.ncbi.nlm.nih.gov/pmc/

((("sublingual microcirculation"[all fields] OR "sublingual micro circulation"[all fields] OR "sublingual microcirculat*"[all fields] OR "sublingual micro circulat*"[all fields] OR "sublingual microvascular circulation"[all fields] OR "sublingual microvascular circulat*"[all fields]) AND ("cardiac surgery"[text word] OR "cardiac surg*"[text word] OR "heart surgery"[text word] OR "heart surg*"[text word] OR "cardio thoracic surgery"[text word] OR "cardio thoracic surg*"[text word] OR "cardiothoracic surgery"[text word] OR "cardiothoracic surg*"[text word] OR "thoracic surgery"[text word] OR "thoracic surg*"[text word] OR "Cardiac Surgical Procedures"[Mesh] OR "Arterial Switch"[text word] OR "Arterial Switch Operation"[text word] OR "Cardiac Valve Annuloplast*"[text word] OR "Cardiac Valve Annuloplasty"[text word] OR "Cardiomyoplast*"[text word] OR "Cardiomyoplasty"[text word] OR "Coronary Artery Bypass"[text word] OR "Coronary Artery Bypass*"[text word] OR "CABG"[text word] OR "Coronary Atherectom*"[text word] OR "Coronary Atherectomy"[text word] OR "Coronary Balloon Angioplast*"[text word] OR "Coronary Balloon Angioplasty"[text word] OR "Hypothermia Induced Circulatory Arrest"[text word] OR "Fontan Procedure"[text word] OR "Heart Massage"[text word] OR "Heart Transplant*"[text word] OR "Heart Transplantation"[text word] OR "Heart Valve Prosthesis Implant*"[text word] OR "Heart Valve Prosthesis Implantation"[text word] OR "Heart-Lung Transplant*"[text word] OR "Heart-Lung Transplantation"[text word] OR "Induced Heart Arrest"[text word] OR "Internal Mammary-Coronary Artery Anastomosis"[text word] OR "Maze Procedure"[text word] OR "Mitral Valve Annuloplast*"[text word] OR "Mitral Valve Annuloplasty"[text word] OR "Myocardial Revascularisation"[text word] OR "Myocardial Revascularization"[text word] OR "Norwood Procedure"[text word] OR "Norwood Procedures"[text word] OR "Pericardial Window Technique"[text word] OR "Pericardial Window Techniques"[text word] OR "Pericardiectom*"[text word] OR "Pericardiectomy"[text word] OR "Pericardiocentesis"[text word] OR "Right Heart Bypass"[text word] OR "Right Heart Bypass*"[text word] OR "Transcatheter Aortic Valve Replacement"[text word] OR "Transcatheter Aortic Valve Replacement*"[text word] OR "Transmyocardial Laser Revascularisation"[text word] OR "Transmyocardial Laser Revascularization"[text word] OR "Cardiopulmonary Bypass"[Mesh] OR "Cardiopulmonary Bypass"[text word] OR "Cardio pulmonary Bypass"[text word] OR "Heart Lung Bypass"[text word] OR "Heart Bypass"[text word] OR "Cardio pulmonary Bypass"[text word] OR "Heart Diseases/surgery"[Mesh] OR "Heart/surgery"[Mesh]) NOT ("Animals"[mesh] NOT "Humans"[mesh])) OR (("Microcirculation"[Mesh] OR "microcirculation"[tw] OR "micro circulation"[tw] OR "microcirculat*"[tw] OR "micro circulat*"[tw] OR "microvascular circulation"[tw] OR "microvascular circulat*"[tw] OR "microvascular blood flow"[tw] OR "microvascular blood flow"[tw] OR ("Microvessels"[Mesh] AND "Blood Circulation"[Mesh:NoExp]) OR "Blood Flow Velocity"[mesh] OR "Blood flow velocity"[tw] OR "side stream dark field"[tw] OR "SDF"[tw] OR "incidence dark field"[tw] OR ("incidence"[tw] AND "dark field"[tw]) OR "IDF"[tw] OR "proportion of perfused vessels"[tw] OR "PPV"[tw] OR "perfused vessel density"[tw] OR "PVD"[tw] OR "total vessel density"[tw] OR "TVD"[tw] OR "hand held microscope"[tw] OR "hand held microscopes"[tw] OR "hand held microscop*"[tw] OR "handheld microscope"[tw] OR "handheld microscopes"[tw] OR "handheld microscop*"[tw] OR "OPS"[tw] OR "orthogonal polarization spectral"[tw] OR "microvascular perfusion"[tw] OR "microvascular tissue perfusion"[tw]) AND ("sublingual"[all fields] OR "sublingual*"[all fields] OR "sub lingual"[all fields] OR "sub lingual*"[all fields]) AND ("cardiac surgery"[title word] OR "cardiac surg*"[title word] OR "heart surgery"[title word] OR "heart surg*"[title word] OR "cardio thoracic surgery"[title word] OR "cardio thoracic surg*"[title word] OR "cardiothoracic surgery"[title word] OR "cardiothoracic surg*"[title word] OR "thoracic surgery"[title word] OR "thoracic surg*"[title word] OR "Cardiac Surgical Procedures"[Mesh] OR "Arterial Switch"[title word] OR "Arterial Switch Operation"[title word] OR "Cardiac Valve Annuloplast*"[title word] OR "Cardiac Valve Annuloplasty"[title word] OR "Cardiomyoplast*"[title word] OR "Cardiomyoplasty"[title word] OR "Coronary Artery Bypass"[title word] OR "Coronary Artery Bypass*"[title word] OR "CABG"[title word] OR "Coronary Atherectom*"[title word] OR "Coronary Atherectomy"[title word] OR "Coronary Balloon

Angioplast*"[title word] OR "Coronary Balloon Angioplasty"[title word] OR "Hypothermia Induced Circulatory Arrest"[title word] OR "Fontan Procedure"[title word] OR "Heart Massage"[title word] OR "Heart Transplant*"[title word] OR "Heart Transplantation"[title word] OR "Heart Valve Prosthesis Implant*"[title word] OR "Heart Valve Prosthesis Implantation"[title word] OR "Heart-Lung Transplant*"[title word] OR "Heart-Lung Transplantation"[title word] OR "Induced Heart Arrest"[title word] OR "Internal Mammary-Coronary Artery Anastomosis"[title word] OR "Maze Procedure"[title word] OR "Mitral Valve Annuloplast*"[title word] OR "Mitral Valve Annuloplasty"[title word] OR "Myocardial Revascularisation"[title word] OR "Myocardial Revascularization"[title word] OR "Norwood Procedure"[title word] OR "Norwood Procedures"[title word] OR "Pericardial Window Technique"[title word] OR "Pericardial Window Techniques"[title word] OR "Pericardiectom*"[title word] OR "Pericardiectomy"[title word] OR "Pericardiocentesis"[title word] OR "Right Heart Bypass"[title word] OR "Right Heart Bypass*"[title word] OR "Transcatheter Aortic Valve Replacement"[title word] OR "Transcatheter Aortic Valve Replacement*"[title word] OR "Transmyocardial Laser Revascularisation"[title word] OR "Transmyocardial Laser Revascularization"[title word] OR "Cardiopulmonary Bypass"[Mesh] OR "Cardiopulmonary Bypass"[title word] OR "Cardio pulmonary Bypass"[title word] OR "Heart Lung Bypass"[title word] OR "Heart Bypass"[title word] OR "Cardio pulmonary Bypass"[title word] OR "Heart Diseases/surgery"[Mesh] OR "Heart/surgery"[Mesh]) NOT ("Animals"[mesh] NOT "Humans"[mesh])))

7.1| References

1. 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. Journal of critical care. 2013;28(6):913-7.
2. Sterne JA, Smith GD. Sifting the evidence—what's wrong with significance tests? Physical therapy. 2001;81(8):1464-9.
3. Holland BS, Copenhaver MD. Improved Bonferroni-type multiple testing procedures. Psychological Bulletin. 1988;104(1):145.
4. Feise RJ. Do multiple outcome measures require p-value adjustment? BMC medical research methodology. 2002;2(1):1-4.
5. Perneger TV. What's wrong with Bonferroni adjustments. Bmj. 1998;316(7139):1236-8.
6. Fitzmaurice GM, Laird NM, Ware JH. Applied longitudinal analysis: John Wiley & Sons; 2012.
7. Arnau J, Bono R, Balluerka N, Gorostiaga A. General linear mixed model for analysing longitudinal data in developmental research. Perceptual and motor skills. 2010;110(2):547-66.
8. Xu L, Lee S-Y, Poon W-Y. Deletion measures for generalized linear mixed effects models. Computational Statistics & Data Analysis. 2006;51(2):1131-46.
9. Littell RC, Milliken GA, Stroup WW, Wolfinger RD, Oliver S. SAS for mixed models: SAS publishing; 2006.
10. Laird NM, Ware JH. Random-effects models for longitudinal data. Biometrics. 1982:963-74.
11. Galecki AT. General class of covariance structures for two or more repeated factors in longitudinal data analysis. Communications in Statistics-Theory and Methods. 1994;23(11):3105-19.
12. Ballinger GA. Using generalized estimating equations for longitudinal data analysis. Organizational research methods. 2004;7(2):127-50.

This page was intentionally left blank.