Measuring microcirculation

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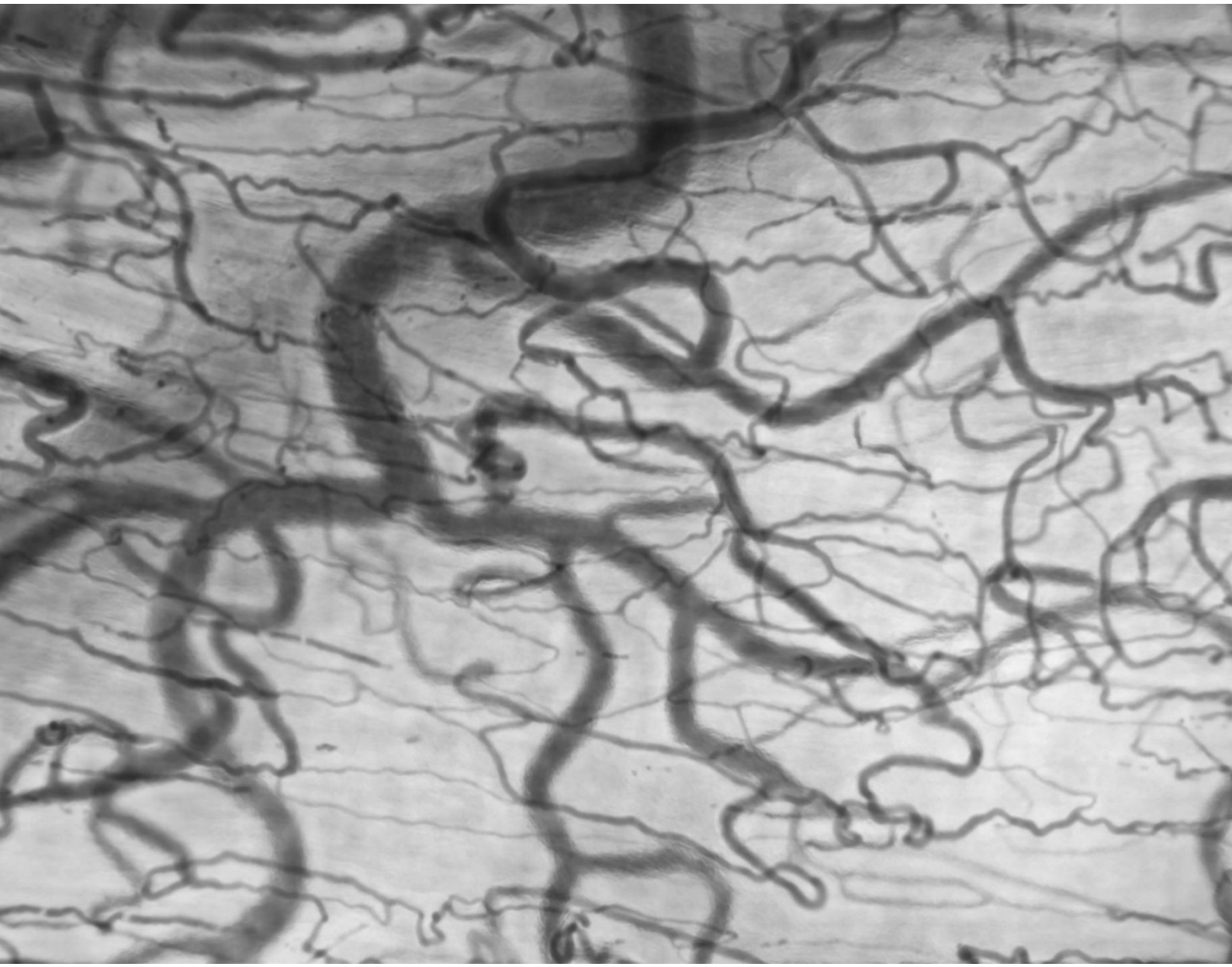

SUBLINGUAL MICROCIRCULATION IN PATIENTS WITH A SEVERE COVID-19 INFECTION AND IN PATIENTS WHO DEVELOP CARDIOGENIC SHOCK AFTER CARDIOTHORACIC SURGERY

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Preface

This document contains the results of my master thesis project that I conducted at the intensive care unit (ICU) of the Leiden University Medical Center (LUMC). The aim of this project was to investigate sublingual microcirculation in patients with cardiogenic shock or heart failure. In order to learn more about the topic of microcirculation, I started with a literature review. This review focusses on (loss of) hemodynamic coherence in patients with cardiogenic shock or heart failure, a phenomenon where a change in macrocirculatory parameters, such as heart rate and blood pressure, does not lead to a parallel change in microcirculatory parameters. Following the literature review, I would execute a clinical research in this patient population to confirm or reject the hypothesis that loss of hemodynamic coherence occurs in patients with cardiogenic shock or heart failure. However, at that moment, the ICU was occupied with patients with a COVID-19 infection. So, instead of measuring the microcirculation in patients with cardiogenic shock or heart failure, I did microcirculation measurements in patients with a COVID-19 infection. The aim of the research in COVID-19 patients was to investigate the changes of the microcirculation and macrocirculation over time and see if this was different in patients who are more or less severely ill.

During my internship on the ICU of the LUMC, the admission of patients with a COVID-19 infection to the ICU gradually decreased. As a result, the 'normal' ICU patient population returned, including patients with cardiogenic shock. So finally, I could start with doing microcirculation measurements in the patient population that I initially intended to investigate. The study on sublingual microcirculation in patients with cardiogenic shock and heart failure is well on its way, but unfortunately, I was not able to report results in this master thesis within the time frame.

Despite the fact that my master thesis project did not go exactly as planned, I have learned a lot and I really enjoyed performing the microcirculation measurements. I want to thank my supervisors Sesmu Arbous and Can Ince for their valuable insights, critical questions and contribution to the final paper. Sesmu, for making time for me in your always full agenda. You always helped me out when I got stuck. Prof. Ince, for your expertise on the topic of microcirculation and your knowledge of how to perform a clinical research and interpret the results. And finally, I want to thank Zehra Uz for teaching me the basics of the microcirculation and performing a microcirculation measurement; and Fleur Brouwer who I worked closely together with and who was always there to answer my questions when Sesmu was not available. I really enjoyed the days that we did microcirculation measurements all day long on the COVID cohort.

Jiska Pols
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Contents

Literature review

A systematic review of the sublingual microcirculation and hemodynamic coherence in patients with cardiogenic shock or heart failure

Abstract

Background and aim: In cardiogenic shock and heart failure, the heart fails to pump a sufficient amount of blood to the organs. This can lead to a decrease in organ tissue perfusion and thus to tissue hypoxia. The resuscitation of patients in shock endeavors normalization of macrohemodynamic parameters. However, during shock resuscitation, improvement in macrohemodynamic parameters does not automatically result in a parallel improvement of the microcirculation. This discrepancy between macrocirculation and microcirculation is known as a loss of hemodynamic coherence. Sublingual microcirculation monitoring may be valuable to assess the existence of coherence between macrocirculation and microcirculation. The aim of this systematic review is to investigate the presence or lack of hemodynamic coherence in patients with cardiogenic shock (CS) or heart failure (HF).

Methods: A search was performed in both the Embase and the MEDLINE database (PubMed US National Library of Medicine) for publications until June 14, 2021. For inclusion, studies needed to (1) involve a patient population with CS or HF, (2) include patients older than 18 years of age (3) perform microcirculation measurements with HVM, (4) evaluate macrocirculation as well as microcirculation and (5) be a study performed in humans. Studies were excluded if (1) it was a study performed in pediatric patients, (2) it was a review article, (3) it was a case-study, (4) it was not written in English or (5) it did not have a full text available.

Results: In total, 17 studies were included of which 12 involved a patient population with CS, two involved HF and three studies investigated both CS and HF. The size of the study population varied from 10 to 68 patients and macrocirculatory and microcirculatory outcome measures varied a lot between studies.

Conclusion: Based on the included studies, it is very difficult to state whether there is loss of hemodynamic coherence in patients with CS or HF or not. Mostly because hemodynamic coherence is often not the primary outcome, or not an outcome measure at all. When we solely look at the outcome of the investigated microcirculatory parameters, variables that say something about vessel density and the proportion of the total amount of vessels that is perfused, are most often reported as interesting results. So in patients with CS or HF, TVD, PVD/PCD and PPV are probably the most interesting and informative parameters to investigate.

Introduction

Patients admitted to the intensive care unit (ICU) often suffer from circulatory dysfunction, a condition that is associated with high morbidity and mortality [1]. The primary function of the circulation is to deliver oxygen and nutrients to the tissue cells and remove waste products [2]. This exchange takes place in the microvasculature of the tissues, which consists of arterioles, post-capillary venules, capillaries and their cellular constituents [3]. Here, oxygen passively diffuses from red blood cells into tissue cells. Under normal conditions, oxygen delivery surpasses oxygen consumption. In a condition of circulatory dysfunction, for example during a state of shock, the systemic circulation fails to meet the perfusion requirements of organs [4, 5]. Inadequate delivery of oxygen-carrying red blood cells to tissue cells leads to organ dysfunction and if it persists, to organ failure [2, 6].
The resuscitation of patients in shock endeavors normalization of macrocirculatory parameters such as heart rate (HR), blood pressure variables, cardiac output and venous oxygen saturation (SvO2) [2, 7]. However, during shock resuscitation, improvement in the macrocirculatory parameters does not automatically result in a parallel improvement of the microcirculation [7, 8]. This discrepancy between macrocirculation and microcirculation is known as a loss of hemodynamic coherence [7].

The microcirculation can be monitored with hand-held vital microscopes (HVM). The first generation microscopes were based on orthogonal polarization spectral (OPS) imaging [9, 10]. These devices were improved and replaced by HVM based on sidestream dark field (SDF) imaging [11, 12]. The third and latest generation HVM is based on incident darkfield imaging (IDF) [13]. Microcirculation parameters that can be monitored with IDF are the proportion of perfused vessels (PPV), De Backer score, microvascular flow index (MFI), total vessel density (TVD), perfused vessel density (PVD) and the heterogeneity index (HI). A detailed description of these variables can be found elsewhere [13]. 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 results in inadequate treatment and may ultimately contribute to increased mortality [14-17].
An abundance of studies discussing macrocirculation and microcirculation in patients with sepsis or septic shock is available. However, the number of studies performed in patients with cardiogenic shock (CS) or severe heart failure (HF) is much smaller [2]. In CS and HF, the heart fails to pump a sufficient amount of blood to the organs. As a result, organ tissue perfusion decreases, leading to a discrepancy between oxygen delivery and oxygen demand and thus to tissue hypoxia [5]. Comparable to septic patients, loss of hemodynamic coherence may occur in CS and HF as well. This hypothesis is strengthened by the fact that mortality in patients with CS remains high, despite modern treatment strategies [18-20]. Furthermore, it has been demonstrated that a substantial number of patients with CS die with a preserved cardiac index (CI). Additionally, systemic vascular resistance (SVR) is highly variable in patients with CS, as not all patients respond to low arterial pressure by increasing systemic vasoconstriction. So, normalization of macrocirculatory parameters may not be enough for patients to recover [21, 22]. Measuring the microcirculation congruently with the macrocirculation may therefore be valuable to assess the existence of coherence between macrocirculation and microcirculation and with that may contribute to tailoring therapy.
The aim of this systematic review is to establish the presence or lack of hemodynamic coherence in patients with CS or severe HF.

Methods

Data source and search strategy

To evaluate the existence of hemodynamic coherence between macrocirculation and microcirculation in patients with CS or HF, a search was performed in both the Embase and the MEDLINE database

(PubMed US National Library of Medicine) for publications until June 14, 2021. The following query was used to search the MEDLINE database: (("Heart Failure"[Mesh] OR "Heart Failure"[tw] OR "Cardiac Failure"[tw] OR "Myocardial Failure"[tw] OR "Shock, Cardiogenic"[Mesh] OR "Cardiogenic Shock"[tw]) AND ((("Microcirculation"[Mesh] OR "Microcirculation"[tw] OR "Microcirculat*"[tw] OR "Micro circulation"[tw] OR "Microvascular Blood Flow"[tw] OR "Sublingual microcirculation"[tw] OR "Peripheral perfusion"[tw] OR "Peripheral perf*"[tw] OR "Peripheral circulation"[tw] OR "Peripheral circ*"[tw] OR "Tissue perfusion"[tw]) AND ("Macrocirculation"[tw] OR "Macrocirculat*"[tw] OR "Macro circulation"[tw] OR "Macrovascular Blood Flow"[tw] OR "systemic circulation"[tw] OR "systemic hemodynamics"[tw] OR "systemic haemodynamics"[tw] OR "systemic hemodynamic*"[tw] OR "systemic haemodynamic*"[tw])) OR ("Macro"[tw] AND ("Microcirculat*"[tw] OR "micro circulat*"[tw]))) NOT ("Animals"[mesh] NOT "Humans"[mesh]) NOT ("veterinary"[ti] OR "rabbit"[ti] OR "rabbits"[ti] OR "animal"[ti] OR "animals"[ti] OR "mouse"[ti] OR "mice"[ti] OR "rodent"[ti] OR "rodents"[ti] OR "rat"[ti] OR "rats"[ti] OR "pig"[ti] OR "pigs"[ti] OR "porcine"[ti] OR "horse"[ti] OR "horses"[ti] OR "equine"[ti] OR "cow"[ti] OR "cows"[ti] OR "bovine"[ti] OR "goat"[ti] OR "goats"[ti] OR "sheep"[ti] OR "ovine"[ti] OR "canine"[ti] OR "dog"[ti] OR "dogs"[ti] OR "feline"[ti] OR "cat"[ti] OR "cats"[ti]) AND (dutch[la] OR english[la])). The Embase database was subjected to the following search terms: ("Heart failure" or "Cardiogenic shock").ab. and ("Microcirculation" or "Sublingual microcirculation").af. and ("Macrocirculation" or "Systemic hemodynamic*" or "Systemic circulation").af with a filter for human studies.

Eligibility

For inclusion, studies needed to (1) involve a patient population with CS or HF, (2) include patients older than 18 years of age (3) perform microcirculation measurements with HVM, (4) evaluate macrocirculation as well as microcirculation and (5) be a study performed in humans.
Studies were excluded if (1) it was a study performed in pediatric patients, (2) it was a review article, (3) it was a case study, (4) it was not written in English or (5) it did not have a full text available.

Definition of primary and secondary outcomes

The primary outcome is the existence or loss of hemodynamic coherence (if investigated by the study). Secondary outcomes are the measured macrocirculatory and microcirculatory parameters, which of these parameters changed, e.g. as a result of a certain intervention or mechanical circulatory support device, and in which direction they changed.

Study selection

First, the title and abstract of the retrieved studies were read. The aforementioned selection criteria were used to include and exclude the studies. After this first selection, the full text of all the remaining studies was read to determine whether the study could be included or not, which was also based on the selection criteria. Finally, the references of the included studies were examined from which potentially useful studies were evaluated and subsequently included when they met the inclusion criteria.

Data extraction

From the included studies, the following contents were extracted: the author, the journal the study was published in, the country and city where the study was conducted, name of the hospital, start and end date of the study, patient population (CS or HF), if the study reported a definition of CS or HF, number of patients and imaging modality of the HVM device (OPS, SDF or IDF).
Additionally, the microcirculatory and macrocirculatory parameters that were measured in the study were extracted. If hemodynamic coherence was investigated, the result was summarized. If hemodynamic coherence was not an outcome measure of the study, an assumption regarding

existence or loss of hemodynamic coherence was made, based on changes of the measured macrocirculatory and microcirculatory parameters.

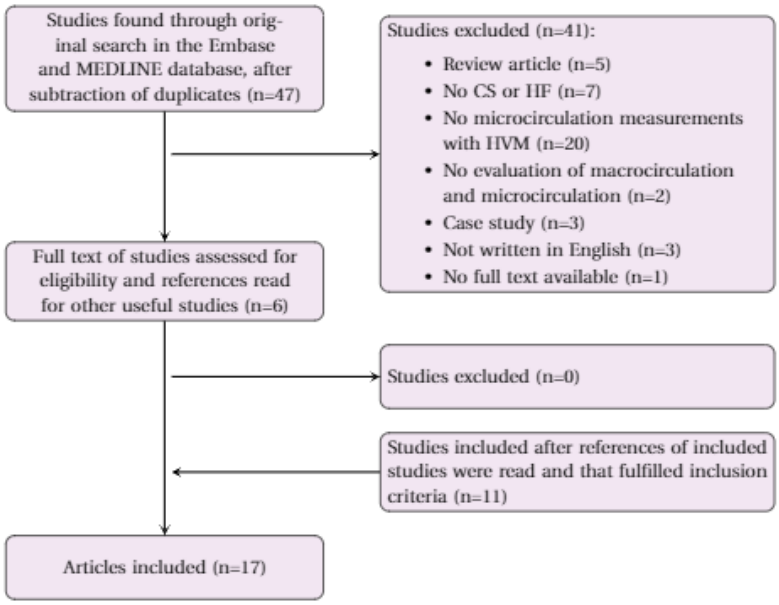
Results

Study selection and study characteristics

The search in the MEDLINE database resulted in 37 studies and with the search in Embase, 26 studies were retrieved of which 16 were duplicates. So in total, 47 studies were found. After reading the title and abstract of the retrieved studies, 41 studies were excluded. Reasons for exclusion were that it concerned a review article (5), did not involve a patient population with CS or HF (7), did not concern microcirculation measurements with HVM (20), did not evaluate the macrocirculation as well as the microcirculation (2), was a case study (3), was not written in English (3) or did not have a full text available (1).

Of the remaining six studies, the full text was read to assess if these studies met the inclusion criteria. All six studies met the inclusion criteria, so the references of these studies were checked on additional eligible studies. This resulted in the inclusion of another 11 studies that fulfilled the inclusion criteria, resulting in a total of 17 studies. The PRISMA flow chart illustrates the search methodology and results (Fig. 1).

Figure 1: Flowchart describing the selection of articles



Extracted results

Of the included studies, 12 involved a patient population with CS, two involved HF and three studies investigated both CS and HF. The size of the study population varied from 12 to 68 patients and the used HVM devices were most often based on SDF imaging**.** Macrocirculatory and microcirculatory outcome measures varied a lot between studies. An overview of the included studies and their outcome measures can be found in tables 1 and 2.

Discussion of studies using SDF

Of the studies using SDF, two were performed in patients who were supported by venoarterial membrane oxygenation (VA-ECMO) [23, 24]. In the study of Chommeloux et al. the impact of VA-ECMO

on macrocirculatory as well as microcirculatory parameters was investigated [23]. In addition, they evaluated and compared the course of these parameters in successfully weaned and non-successfully weaned patients. They found that HR, inotropic score and lactate levels were significantly lowered within 48 hours after VA-ECMO initiation. Perfused small vessel density (PSVD), percentage of perfused vessels (PPV), microvascular flow index (MFI) and heterogeneity index (HI) all improved significantly on VA-ECMO. In the successfully weaned patients, microcirculation parameters were normalized until weaning from VA-ECMO and remained normal six hours after weaning. Unsuccessfully weaned patients also showed improvement of microcirculation on VA-ECMO, but with significantly lower parameter values compared to the successfully weaned patients. Interestingly, values for mean arterial pressure (MAP) during VA-ECMO were similar in these patient groups. So, in the non-successfully weaned patients, there might have been a phase of hemodynamic loss of coherence while on VA-ECMO.

The study by Petroni et al. involved patients with CS supported by VA-ECMO in combination with an intra-aortic balloon pump (IABP) [24]. They investigated the impact of the IABP on the macrocirculation and the microcirculation by interrupting and restarting the IABP. HR and MAP as well as parameters measured with a pulmonary artery catheter significantly changed after interrupting the IABP and returned to baseline values after restarting the IABP. Functional capillary density (FCD), MFI, PPV and HI did not differ significantly with or without the support of the IABP. This might be an indication for loss of hemodynamic coherence, but it can also be assigned to autoregulatory mechanisms of the microcirculation. These mechanisms ensure a constant flow of blood through the capillary network, even during varying arterial blood pressure [25, 26].

Three studies were included that investigated CS patients supported by IABP alone [27-29]. In accordance with the study of Petroni, Jung et al. (2015) found no change in microcirculatory parameters before and after interrupting the support of the IABP [26]. They also compared these values with microcirculation measurements performed in patients with CS who were not on IABP and they found no differences at all time points. Additionally, a correlation analysis of microcirculatory parameters with macrocirculatory parameters was done. Perfused capillary density (PCD) and perfused vessel density (PVD) were found to be inversely correlated with noradrenaline dose and had a strong association to MAP. Also body temperature was significantly correlated with PCD and PVD. An inverse correlation was found between total capillary density (TCD) and total vessel density (TVD), and lactate 24 hours after microcirculation measurement. Interestingly, TCD and TVD were not significantly correlated with serum lactate at the moment of microcirculation measurement. From this, we may conclude that changes in serum lactate occur much later than changes in the microcirculation, advocating a loss of hemodynamic coherence in patients with CS.
In a study performed by Jung et al a few years earlier (2009), microcirculation was also investigated with IABP support on and off [28]. However, the only microcirculatory parameter measured was the MFI. In their research, a significant decrease in MFI from 2.86 to 2.03 during IABP on and off respectively was found in small vessels (10–25 µm). In medium vessels (26–50 µm), a significant decrease from 2.93 to 2.00 was found. MFI did not correlate to macrocirculatory parameters.
Other than the research performed by Jung et al, Den Uil et al investigated the change of the microcirculation during different IABP assist-ratios [28]. They found that lowering the IABP assist-ratio from 1:1 to 1:8 decreased the MAP and CI significantly, while PCD and red blood cell velocity (RBCv) did not change. This indicates that IABP support affects the macrocirculation, but not the microcirculation.

In a recent study, Den Uil et al investigated microcirculation in patients with HF or CS supported by any kind of device [30]. They performed microcirculation measurements before and after device implantation and they saw that all devices improved PVD and RBCv. These changes did not correlate to CI.

The above-discussed studies all involved CS supported with a device. So, a conclusion regarding loss of hemodynamic coherence solely due to CS cannot be made. The studies discussed below do not include devices, but focus on CS only.
Of these studies, three involved a patient population of acute myocardial infarction (AMI) complicated by CS [31-33]. In the study performed by Wijntjens et al, macrocirculatory and microcirculatory parameters were investigated directly post percutaneous coronary intervention (PCI) [31]. A significant and independent association between the microcirculatory parameters PCD, percentage of perfused capillaries (PPC) and MFI with the combined clinical endpoint (all-cause death and renal replacement therapy) at 30 days was found. In contrast, no significant relation between macrocirculatory parameters post-PCI and the combined clinical endpoint was found. In addition, they found that patient with a normal blood pressure but with impaired microcirculation had a less favorable outcome than patients with normal blood pressure and normal microcirculation. These results show that the microcirculation rather than the macrocirculation might be important to predict patient outcomes.
These findings are in line with the study performed in 2010 by Den Uil et al [32]. They demonstrated that CS patients with a baseline PCD higher than the median PCD of the total study population showed an improvement in the total SOFA score 24 hours after hospital admission more often than patients with an impaired baseline PCD. The latter patients had a higher risk to die too. Of the macrocirculatory parameters, only MAP was significantly lower in patients with a PCD ≤ median PCD. This could be addressed as hemodynamic coherence, but also as a loss of hemodynamic coherence since all other macrocirculatory parameters did not show any relation to the microcirculation.
In 2014, Den Uil performed another study in patients with AMI complicated by CS, in which they studied the effects of inotropic therapy (dobutamine, enoximone and norepinephrine) on the macrocirculation as well as on the microcirculation [33]. Correlation analysis showed a significant inverse correlation of MAP and central venous pressure (CVP) to PCD, especially in patients receiving noradrenaline. A weak correlation was demonstrated between CI and PCD. These findings suggest that improvement of macrocirculatory parameters does not automatically result in a parallel improvement of the microcirculation.

In a study performed by van den Akker et al. in 2019, microcirculation in patients with CS was evaluated regarding the development of acute kidney injury (AKI) [34]. In patients that developed AKI, CVP was significantly higher compared to patients who did not develop AKI. CI, systolic blood pressure and PCD showed no difference between patients. In addition, PCD was not different in patients who had AKI at the moment of measurement compared to patients who did not, but developed AKI later. No correlation analysis was performed, so conclusions regarding hemodynamic coherence cannot be drawn. However, from this study, we learn that PCD is probably not a good marker predicting kidney failure.

Den Uil et al performed two studies in which they investigated the effects of nitroglycerin (NTG) on the microcirculation [35, 36]. In the study published in February 2009, acute HF patients received a continuous intravenous infusion of low dose NTG [35]. Microcirculation was measured before infusion and 15 minutes after the start of infusion. They found a decrease in CVP and pulmonary capillary wedge pressure (PCWP) after NTG infusion and a significant increase in PCD. Stopping the infusion of NTG caused the PCD values to return to baseline. No correlation analysis was performed.

In the study published in July 2009, Den Uil et al investigated patients with HF as well as CS and was able to reproduce their results [36]. Again, a decrease in CVP and an increase in PCD were found after NTG infusion and these results were consistent in patients with either CS or HF. Interestingly, they demonstrated that significant changes in PCD occurred earlier and at a lower dose of NTG than did changes in macrocirculatory parameters. So, although coherence between macrocirculation and microcirculation was not investigated, it can be concluded from this study that a change in patient hemodynamics (due to medication) is reflected faster in the microcirculation than in the macrocirculation.

Discussion of studies using IDF

The literature search included three studies that used IDF-based microcirculation visualization. All three studies were performed in patients with CS who were supported by VA-ECMO [37-39]. Yeh et al [39] and Kara et al [37] evaluated the course of macrocirculatory and microcirculatory parameters and compared their values for survivors and non-survivors. Both studies demonstrated a loss of coherence in the first hours after VA-ECMO initiation. Kara et al described an alteration in microcirculation (TVD, PVD, and PPV values) within 24 hours after VA-ECMO insertion that was significantly different between survivors and non-survivors, while macrocirculatory parameters were similar between groups [37]. This is in line with the findings of Yeh et al, which showed lower PSVD and PPV values for non-survivors 12 hours after VA-ECMO placement, despite similar values for MAP, inotropic score and lactate levels [39].

In the research performed by Akin et al, the microcirculation was investigated during weaning of VA-ECMO [38]. They found no difference in macrocirculatory parameters during weaning in successfully and not successfully weaned patients. In comparison, the parameters TVD, PVD and PPV in successfully weaned patients showed no change prior to and during a weaning attempt, while those of not successfully weaned patients decreased. So in the non-successfully weaned group, loss of coherence between the macrocirculation and the microcirculation occurred, which is in line with the findings of Chommeloux et al [23].

Discussion of studies using OPS

In 2004, De Backer performed a study in patients with CS and HF and at least one failing organ [40]. They investigated the relationship of the microcirculation with the macrocirculation and found a weak relation of MAP, SvO2 and blood lactate to PPV. CI and SVR were not related to PPV.

Erol-Yilmaz et al investigated the impact on the microcirculation after six months of cardiac resynchronization therapy (CRT) in patients with chronic symptomatic HF [41]. Microcirculation measurements were performed during no pacing, right ventricular (RV) pacing and biventricular pacing. FCD values were lowest during intrinsic rhythm. During biventricular pacing, FCD was significantly increased compared to RV pacing and no pacing. Mean blood pressure was not different during all pacing modalities.

Both studies demonstrate subtle hints for a loss of hemodynamic coherence. In the study of De Backer et al however, some macrocirculatory parameters were related to microcirculatory parameters and some were not [40]. In addition, Erol-Yilmaz only analyzed the relation of the microcirculation to MAP [41]. Thus, no real conclusion regarding the loss of hemodynamic coherence can be drawn.

Conclusion

The aim of this systematic review was to establish the presence or lack of hemodynamic coherence in patients with CS or severe HF. Based on the previously discussed studies, it is very difficult to state whether there is a loss of hemodynamic coherence in patients with CS or HF or not. At first, because

only a small amount of research in which microcirculatory changes are compared with changes in macrocirculation is performed in this patient group. Secondly, hemodynamic coherence is most often not the primary outcome, or not an outcome measure at all. However, if we read between the lines, we can suggest a loss of hemodynamic coherence in some of the discussed studies (Table 2), but no hard conclusions can be drawn.

When we look solely at the outcome of the investigated microcirculatory parameters, variables that say something about vessel density (TVD) and variables that indicate the proportion of the total amount of vessels that are perfused (PVD/PCD, PPV), are most often reported as interesting results. These parameters are important determinants of tissue diffusion capacity. On the contrary, microcirculatory parameters that give information about convection or flow (RBCv, MFI) are less frequently reported as an important result. So in patients with CS or HF, TVD, PVD/PCD and PPV are probably the most interesting and informative parameters to investigate. To learn more about coherence of these parameters with macrocirculatory variables, clinical observational trials in patients with CS and HF need to be done, in which the primary aim is to investigate loss or presence of hemodynamic coherence.

Table 1: Included studies

Author	Journal	Year	Country	City	Hospital	Period	Patient population	Definition of CS and/or HF?	Size	Imaging modality
Chommeloux, J.	Critical care medicine	2020	France	Paris	Pitié-Salpêtrière Hospital	6 months	Refractory CS supported by VA-ECMO	No	14	SDF
Wijntjens, G.W.	European heart journal, Acute cardiovascular care	2020	Multicenter	Multicenter	Multicenter	March 2013 – April 2017	CS complicated AMI	Yes	66	SDF, IDF
Akker van den, J.P.C.	Journal of critical care	2019	The Netherlands	Rotterdam	Erasmus University Medical Center	November 2007 – April 2009	CS	Yes	39	SDF
Petroni, T.	Critical care medicine	2014	France	Paris	Pitié-Salpêtrière Hospital	November 2010 – October 2011	Acute refractory CS supported by VA-ECMO and IABP	Yes	12	SDF
Uil den, C.A.	Cardiology	2009	The Netherlands	Rotterdam	Erasmus University Medical Center	-	CS supported by IABP	No	13	SDF
Uil den, C.A.	PLoS One	2014	The Netherlands	Rotterdam	Erasmus University Medical Center	-	CS complicated AMI	Yes	30	SDF
Uil den, C.A.	European heart journal	2010	The Netherlands	Rotterdam	Erasmus University Medical Center	November 2007 – April 2009	CS complicated AMI	Yes	68	SDF
Uil den, C.A.	The journal of heart and lung transplantation	2009	The Netherlands	Rotterdam	Erasmus University Medical Center	May 2008 – April 2009	End-stage HF or CS who received a MCS device	Yes	10	SDF
Jung, C.	Clinical research in cardiology	2015	Germany	Multicenter	Multicenter	June 2009 – March 2012	CS complicated AMI with and without IABP	No	41	SDF
Uil den, C.A.	European journal of heart failure	2009	The Netherlands	Rotterdam	Erasmus University Medical Center	-	Acute HF	No	20	SDF
Uil den, C.A.	Intensive care medicine	2009	The Netherlands	Rotterdam	Erasmus University Medical Center	-	End-stage chronic HF or CS	Yes	17	SDF
Jung, C.	Clinical research in cardiology	2009	Germany	Jena	Friedrich-Schiller-University	-	CS with and without IABP	Yes	13	SDF
Akin, S.	Critical care	2017	The Netherlands	Rotterdam	Erasmus University Medical Center	October 2014 – January 2016	Acute refractory CS supported by VA-ECMO	No	13	IDF
Kara, A.	Critical care	2016	The Netherlands	Rotterdam	Erasmus University Medical Center	September 2014 – October 2015	CS supported by VA-ECMO	No	24	IDF
Yeh, Y.C.	Critical care	2018	Taiwan	Taipei	National Taiwan University Hospital	June 2015 and August 2016	CS supported by VA-ECMO	No	48	IDF
Erol-Yilmaz, A.	Journal of cardiac failure	2007	The Netherlands	Amsterdam	Academic medical center, University of Amsterdam	-	Severe acute HF	Yes	12	OPS

De Backer, D.	American heart journal	2004	Belgium	Brussels	Erasme University Hospital	-	Severe HF and CS	Yes	40	OPS

AMC: academic medical center, AMI: acute myocardial infarction, CS: cardiogenic shock, HF: heart failure, IABP: intra-aortic balloon pump, IDF: incident dark field, MC: medical center, MCS: mechanical circulatory support, OPS: orthogonal polarization spectral imaging, SDF: side-stream dark field, VA-ECMO: veno-arterial extracorporeal membrane oxygenation

Table 2: Outcome of included study regarding microcirculation, macrocirculation and hemodynamic coherence.

Author	**Year**	**Microcirculatory parameters**	**Macrocirculatory parameters**	**Change of microcirculation**	**Change of macrocirculation**	**Coherence?**	
Chommeloux, J.	2020	SVD, De Backer score, PSVD, PPV, MFI, HI	BP, HR, intotropic score, ECMO flow, cardiac echography	PSVD, PPV and MFI had increased significantly after 48 hours on VA-ECMO, HI had significantly decreased	HR and inotropic score had significantly decreased after 48 hours on VA-ECMO	Microcirculatory parameters in non-successfully weaned patients improved less during ECMO compared to successfully weaned patients, despite similar MAPs	No coherence
Wijntjens, G.W.	2020	De Backer score, TCD, PCD, PPC, MFI	BP, HR	PPC and PCD gave the most important results. Change was not reported	Change was not reported	A normal PPC or PCD was generally associated with a favorable outcome, whereas an impaired PPC or PCD was generally associated with an adverse clinical outcome, despite normal SBP or MAP	No coherence
Akker van den, J.P.C.	2019	PCD	BP, PAC-parameters, HR, lactate, CI, CVP	Change was not reported	Change was not reported	PCD was not different between the groups that did and did not develop AKI	Coherence
Petroni, T.	2014	MFI, PPV, FCD, HI	BP, HR, PAC-parameters, cardiac echography	Microcirculatory parameters did not change	BP, pulmonary BP and LVEDD increased when IABP was interrupted	IABP addition to ECMO was associated with reduced LVEDD and decreased pulmonary artery BPs, without modifying microcirculation variables evaluated with thenar eminence and brain NIRS and sublingual SDF imaging	No coherence
Uil den, C.A.	2009	PCD, RBCv	Temp, HR, MAP, CVP, PAC-parameters, CI, lactate	Microcirculatory parameters did not change	MAP and CI decreased when IABP assist ratio was decreased	Lowering the IABP assist ratio decreased the MAP, CI and cardiac power index, but PCD and RBCv did not change significantly. Changes in macrocirculatory parameters did not correlate with changes in PCD or RBCv	No coherence
Uil den, C.A.	2014	PCD	HR, MAP, CVP, PAC-parameters	Dobutamine did not change PCD, Enoximone increased PCD and norepinephrine tended to decrease PCD	HR and MAP increased	Increases in MAP or CVP correlated to deterioration of microcirculatory parameters, whereas an increase in CI weakly correlated to improvement of the microcirculation	No coherence

Uil den, C.A.	2010	PCD	Temp, HR, MAP, CVP, PAC-parameters, CI, lactate	PCD increased	Change was not reported	Baseline PCD was strongly and independently associated with 30-day outcome	Not possible to assess
Uil den, C.A.	2009	PCD, RBCv, tissue perfusion index (PCD*cRBCv)	PAC-parameters, temp, HR, MAP, CI	PCD and RBCv increased one day after device implantation	CVP and lactate decreased one day after device implantation	All devices consistently increased both PCD and cRBCv. No significant correlation between change in CI and change in tissue perfusion index were found.	No coherence
Jung, C.	2015	PCD, PVD, TCD, TVD, PPC, PPV	BP, HR, temp, lactate	Microcirculatory parameters did not change	Change was not reported	TCD and TVD correlated inversely with lactate levels 24h after microcirculation measurement without being significantly correlated with serum lactate levels at the timepoint of the microcirculatory investigation.	Coherence
Uil den, C.A.	2009	PCD	PAC-parameters, CVP, CI, temp, HR, MAP, lactate	PCD increased	CVP and PCWP decreased	CVP and pulmonary capillary wedge pressure decreased after NTG infusion and PCD increased significantly	Coherence
Uil den, C.A.	2009	PCD	PAC-parameters, HR, MAP, CVP, CI	PCD increased	MAP decreased, CI increased	Significant changes in delta-T and PCD occurred at a lower dose of NTG than changes in global hemodynamics	No coherence
Jung, C.	2009	MFI	Temp, BP, CVP, CI, lactate	MFI decreased when the IABP was turned off	Change was not reported	CI, MAP and MFI did not show any correlation with blood lactate levels. Patients with elevated blood lactate levels had lower MFI in small microvessels	No coherence
Akin, S.	2017	TVD, PVD, PPV	Cardiac echography	TVD, PVD and PPV decreased in patients who were non successfully weaned during a weaning attempt	Change was not reported	Microcirculatory parameters showed good correlation with echocardiographic parameter values, especially LVEF	Coherence
Kara, A.	2016	TVD, PVD, PPV	HR, MAP, lactate, cardiac echography	TVD, PVD and PPV did not change while on VA-ECMO	Change was not reported	TVD, PVD, and PPV values within 24 hours after VA-ECMO insertion were significantly different between survivors and non-survivors, while systemic hemodynamic parameters were similar between groups	No coherence
Yeh, Y.C.	2018	TSVD, PSVD, PPV, MFI, HI	HR, MAP, lactate, inotropic score	PSVD and PPV gave the most important results. Change was not reported	Change was not reported	PSVD and PPV values were lower for non-survivors 12 hours after VA-ECMO placement, despite similar values for MAP, inotropic score and lactate levels	No coherence
Erol-Yilmaz, A.	2007	FCD, CV	MAP, cardiac echography	Biventricular pacing increased FCD compared with RV pacing and no pacing	MAP did not change	During biventricular pacing, FCD was significantly increased compared to RV pacing and no pacing. Mean blood pressure was not different during all pacing modalities	No coherence

De Backer, D.	2004	MFI, PPV, TVD, PVD	PAC-parameters, MAP, CI, cardiac echography	PVD and PPV gave the most important results. Change was not reported.	Change was not reported	The PPV was weakly related to MAP, SvO2, and blood lactate, but not to CI and SVR.	Partial coherence

AKI: acute kidney injury, BP: blood pressure, CI: cardiac index, cRBCv: capillary red blood cell velocity, CV: capillary velocity, CVP: central venous pressure, delta-T: delta temperature, FCD: functional capillary density, HI: heterogeneity index, HR: heart rate, IABP: intra-aortic balloon pump, LVEDD: left ventricular end-diastolic dimension, LVEF: left ventricular ejection fraction, MAP: mean arterial pressure, MFI: microvascular flow index, NIRS: near infrared spectroscopy, NTG: nitroglycerin, PAC: pulmonary artery catheter, PCD: perfused capillary density, PCWP: pulmonary capillary wedge pressure, PPC: proportion of perfused capillaries, PPV: proportion of perfused vessels, PSVD: perfused small vessel density, PVD: perfused vessel density, RBCv: red blood cell velocity, SDF: side-stream dark field, SVD: small vessel density, SvO2: venous oxygen saturation, SVR: systemic vascular resistance, TCD: total capillary density, temp: temperature, TSVD: total small vessel density, TVD: total vessel density, VA-ECMO: veno-arterial extracorporeal membrane oxygenation

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Master Thesis project

Time course of sublingual microcirculation in critically ill COVID-19 patients

Abstract

Background and aim: The exact pathophysiology of a COVID-19 infection is still not completely understood, which complicates treatment of the disease. In order to clarify the pathophysiologic mechanisms and optimize treatment methods, monitoring of the sublingual microcirculation may be valuable. From studies in septic patients, we know that microcirculation is impaired and that the microcirculation better describes severity of illness than different macrocirculatory parameters. In this study, we aimed to investigate the changes of the microcirculation and the macrocirculation over time in patients with a COVID-19 infection who were admitted to the ICU and see if this was different in patients who are more or less severely ill based on SOFA score.

Materials and methods: Macrocirculatory and microcirculatory measurements were performed as soon as possible after admission to the ICU (T0) and then at 24 and 48 hours (T1 and T2). Patients were divided into subgroups based on the median SOFA score to make a distinction between more and less severely ill patients. To investigate the development of the macrocirculatory and microcirculatory parameters over time and to compare this in patients with different severity of illness, a linear mixed model analysis was performed.

Results: 21 COVID-19 patients were included for analysis. The median SOFA score was 8 and no differences were found in clinical and demographic patient characteristics between SOFA groups. Patients in the low SOFA score group had higher PaO2 values and lower lactate levels compared to patients in the high SOFA score group ($p = 0.033$ and $p = 0.007$ respectively). In the low SOFA score group, mean PPV and mean FCD were higher compared to the high SOFA score group ($p = 0.018$ and $p = 0.034$ respectively). TVD showed a trend towards significance, with higher mean TVD values for patients in the low SOFA score group.

Discussion: We found that microcirculation parameters measured at T0 were not significantly different between patient subgroups. Analysis of the course of the microcirculation over 48 hours (T0-T2) however, shows that for less severely ill patients PPV and FCD remain higher than for more severely ill patients. So, there may be a relationship between severity of illness of patients with a COVID-19 infection and the ability to activate microcirculatory compensatory mechanisms in order to draw on reserve capacity in a situation of hypoxemia.

Introduction

The coronavirus disease 2019 (COVID-19) is caused by SARS-CoV-2 and continues to spread worldwide [1]. According to the World Health Organization (WHO), there have been over 237 million confirmed cases of which more than 4.8 million resulted in death [2]. SARS-CoV-2 is a respiratory virus with a wide range of severity of symptoms. Some people develop no symptoms at all, while in severe cases, an infection can lead to acute respiratory distress syndrome (ARDS) induced hypoxemia, requiring admission to the intensive care unit (ICU) [3, 4].

An infection with COVID-19 includes three phases: the early infection phase, the pulmonary phase and the hyperinflammation phase [5]. The exact pathophysiology however, is still not completely understood, which complicates treatment of the disease [6, 7]. In order to clarify the pathophysiologic mechanisms behind a COVID-19 infection and optimize treatment methods, monitoring of the sublingual microcirculation may be valuable. Sublingual microcirculation can be monitored with hand-held vital microscopes (HVM). The first generation microscopes were based on orthogonal polarization spectral (OPS) imaging [8, 9]. These devices were improved and replaced by HVM based on sidestream dark field (SDF) imaging [10, 11]. The third and latest generation HVM is based on incident darkfield imaging (IDF) [12].

From studies in septic patients, we know that microcirculation is impaired and that the microcirculation better describes severity of illness than different macrocirculatory parameters [13]. Furthermore, studies have shown that the severity of impairment of the microcirculation is related to outcome [14, 15].

Just a few studies have investigated the microcirculation in patients with a COVID-19 infection. Edul, V.S.K. et al (2021) found that total vessel density (TVD) and perfused vessel density (PVD) were increased in patients with ARDS following a COVID-19 infection [16]. This result is in accordance with Favaron, E. et al (2021), who found a higher total vessel density (TVD) and functional capillary density (FCD) in patients with a COVID-19 infection compared to healthy volunteers. In addition, patients with a SOFA score higher or equal to 10 showed different microcirculatory alterations than patients with a SOFA score less than 10, indicating that changes in microcirculation are influenced by severity of illness [6].

In these studies, microcirculation measurements were performed at only one timepoint. So no information regarding the time course of the microcirculation in the process of the disease was obtained. In this study, we aimed to investigate the changes of the microcirculation and the macrocirculation over time and see if this was different in patients who are more or less severely ill.

Materials and methods

Ethical approval

This single-center observational cohort study was conducted on the ICU of the Leiden University Medical center (LUMC), Leiden, The Netherlands. The study was approved by the Institutional Review Board of the LUMC for observational COVID-19 studies (protocol number P18.131, NL 64824.058.18).

Inclusion of patients

Patients with a COVID-19 infection who were admitted to the ICU in the period of March 2021 to June 2021, were the source population of the study. Patients could be admitted to the ICU via de emergency department and the hospital ward. Also patient transfers from other hospitals were included. Patients needed to be >18 years of age and have an arterial catheter in situ. Exclusion criteria were maxillofacial trauma or tumor(s) in the mouth or throat area.

Clinical characteristics and hemodynamic monitoring

Demographic data and clinical information were recorded for all patients. Macrocirculatory and microcirculatory measurements were performed as soon as possible after admission to the ICU (T0) and then at 24 and 48 hours (T1 and T2). All patients were monitored with a radial artery catheter. Macrocirculatory parameters included mean arterial pressure (MAP), heart rate (HR), arterial lactate, hemoglobin (Hb), systemic hematocrit (Hct), C-reactive protein (CRP), arterial oxygen tension (PaO2), PF-ratio (PaO2/FiO2), positive end-expiratory pressure (PEEP), fluid balance and the SOFA-score [17].

Sublingual microcirculation

Sublingual microcirculatory measurements were performed with the Cytocam, a hand-held vital microscope based on incident darkfield imaging (Breadius Medical, Huizen, The Netherlands). Detailed information on the working mechanism of this technique can be found elsewhere [18]. At each time-point, the sublingual area was carefully cleaned with suction or a gauze swab. Then, three videos of eight seconds were recorded from three different sites in the sublingual triangle in order to minimize heterogeneity in the microscopic field of view. Videos that that did not meet a 'good' or 'acceptable' quality score in the categories *illumination, duration, focus, content, stability* and *pressure* [19] were excluded for further analysis. Analysis of the videos was performed with the MicroTools automatic software (Active Medical, Leiden, The Netherlands) [20]. From this analysis, the following microcirculatory parameters were retrieved: total vessel density (TVD), proportion of perfused vessesl (PPV), functional capillary density (FCD), red blood cell velocity (RBCv), capillary hematocrit (cHct) and tissue red blood cell perfusion (tRBCp) [12, 21]. A detailed description of these parameters can be found in appendix I.

Statistics

Statistical analyses were performed using SPSS 25.0 for windows (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.). 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. To make a distinction between more and less severely ill patients, subgroups were made based on SOFA score [17]. The median of the SOFA score at admission was taken as a cutoff point to divide patients into low and high SOFA score groups. Differences between SOFA-score groups were tested with the student's t-test or the Mann-Whitney U test, as appropriate.

Data was transformed into long format to describe the course of the microcirculation and traditional parameters. To investigate the development of the microcirculatory parameters over time and to compare this in patients with different severity of illness, a linear mixed model analysis was performed. The SOFA score groups together with the moment of measurement (T0, T1, T2) were entered into the model as fixed effects. The same analysis was done for macrocirculatory parameters. The aim was to measure microcirculation as soon as possible after ICU admission, however, due to logistic matters, this was not always possible. Therefore, the linear mixed model was adjusted for time between ICU admission and T0. P-values were calculated with a likelihood ratio test of the entire model with the effect against a 'null-model' without the effect. A two-sided p-value of 0.05 was considered statistically significant.

Results

Clinical demographics

The cohort of COVID-19 patients in whom microcirculatory measurements were performed consisted of 57 patients. Of these patients, three were excluded due to poor video quality. Another 33 patients were excluded because there was a weekend in between measurements. Thus, 21 patients were included for analysis.

The median SOFA score was 8 and based on this value, two subgroups were made. Patients with a SOFA score ≤ 8 were classified as the low SOFA score group and patients with a score > 9 were classified as the high SOFA score group. Mean age of the entire patient population was 61 ± 11 years and 15 patients were male. Most patients were overweight, with a mean BMI of 30.1 ± 5.8 and 19 of 21 patients were mechanically ventilated. No significant differences were found between patients in the low and high SOFA score groups. Table 1 shows clinical and demographic patient characteristics at the time of enrollment.

Seven patients died while in the hospital, of which six patients on the ICU. In the low SOFA score group, five patients died compared to two patients in the high SOFA group. The patient that died after ICU discharge belonged to the low SOFA score group.

Systemic hemodynamics

At admission, mean HR of low and high SOFA score groups together was 67 ± 19 and mean MAP was 80.2 ± 10.6. Lactate levels were only slightly elevated with a mean value of 1.88 ± 0.57, mean PaO2/FiO2-ratio was 21 ± 7 kPa (moderate ARDS) and mean fluid balance was 1728 ± 2731 ml. No significant differences were found between patients in the low and high SOFA score groups (Table 2).

The time course of PaO2 and lactate differed significantly for the high and low SOFA score group (PaO2: $p = 0.033$, lactate: $p = 0.007$) (Table 3). Patients in the low SOFA score group had higher PaO2 values and lower lactate levels compared to patients in the high SOFA score group. For lactate, time points T0 and T1 also gave a significant result (appendix II).

Table 1. Clinical demographics

	All patients (n = 21)		SOFA ≤ 8 (n = 12)		SOFA > 9 (n = 9)		
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	P-value*
Age	60,81	10,72	62,33	12,67	58,78	7,66	0,436
Weight (kg)	91,62	17,36	91,92	16,00	91,22	20,03	0,933
BMI (kg/m^2)	30,14	5,85	30,49	6,66	29,661	4,90	0,748
SOFA score at admission	8,19	2,42	6,50	1,51	10,44	1,24	<0,0001
Length of stay ICU (days)	15,67	18,59	12,33	9,24	20,11	26,61	0,422
Length of stay hospital (days)	29,85	20,76	26,38	10,31	35,40	32,32	0,574
Duration of ventilation (days)	12,16	12,95	10,40	9,031	14,11	16,66	0,563
Time between ICU admission and T0 (days)	1,71	1,23	1,58	1,241	1,89	1,27	0,589
Sex (male) (n (%))	15		9 (60%)		6 (40%)		1,00**
Transfer from other hospital (n (%))	7		2 (28,6%)		5 (71,4%)		0,159**
Hospital mortality (n (%))	7		5 (71,4%)		2 (28,6%)		0,642**

*Comparison of low and high SOFA score groups with independent samples t-test, p-value 'equal variances not assumed' **Fisher's exact test

BMI: body mass index, ICU: intensive care unit, SOFA: sequential organ failure assessment

Table 2. Hemodynamic parameters at admission

	All patients (n = 21)		SOFA ≤ 8 (n = 12)		SOFA > 9 (n = 9)		
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation	P-value*
Heart rate (beats per minute)	67,0	19,03	69,33	19,17	63,89	19,52	0,532
Mean arterial pressure (mmHg)	80,19	10,61	81,33	13,17	78,67	6,18	0,546
Lactate (mmol/L)	1,88	,57	1,84	,42	1,93	0,74	0,746
Hemoglobin (mmol/L)	8,08	,88	8,16	,77	7,98	1,04	0,668
Hematocrit (L/L)	,39	,04	,40	,04	0,39	0,05	0,576
C-reactive protein (mg/L)	65,50	62,29	62,19	59,42	69,92	69,34	0,792
PaO2/FiO2 ratio (kPa)	20,95	6,76	19,58	6,92	22,78	6,48	0,292
PaO2 (kPa)	10,27	2,96	10,43	3,80	10,06	1,41	0,756
PEEP (cm H2O)	11,76	4,72	11,42	5,72	12,22	3,19	0,687
Fluid balance (ml)	1728,19	2731,11	1677,50	2362,55	1795,78	3311,13	0,929

*Comparison of low and high SOFA score groups with independent samples t-test, p-value 'equal variances not assumed'

PaO2: arterial oxygen tension, FiO2: fraction of inspired oxygen, PEEP: positive end-expiratory pressure

Microcirculation

Two investigators (FB and JP) performed all microcirculation measurements. After quality assessment of the microcirculation videos based on Massey et al. 2013 [19], eight videos were excluded due to poor video quality, but this did not result in loss of timepoints. The main reason for exclusion of these videos was the existence of saliva in the sublingual area that could not be removed with suction or a gauze swab. The acquired image sequences of the videos eligible for analysis were cropped along the time axis to 100 frames and stabilized. After this, videos were analyzed with MicroTools automatic software.

Mean TVD at admission of low and high SOFA score groups together was 22.81 ± 3.26 $mm*mm^{-2}$. Mean FCD was 21.66 ± 3.04 $mm*mm^{-2}$ and mean PPV was 0,95 ± 0,03%. None of the microcirculatory parameters was significantly different in low and high SOFA score groups at admission. Table 4 shows the values of microcirculatory parameters for all patients and the subgroups at admission. For mean microcirculation parameters per timepoint see appendix I.

The analysis of microcirculation parameters over time showed a significant difference of the mean PPV (p = 0.018) and the mean FCD (p = 0.034) between low and high SOFA score groups (Table 3). In patients with a SOFA score equal to eight or lower, mean PPV and mean FCD were higher compared to the high SOFA score group. Figures 1 and 2 show the course of the mean PPV and mean FCD over time for the two SOFA score groups. TVD showed a trend towards significance, with higher mean TVD values for patients in the low SOFA score group (figure 3). There was no significant time course for mean TVD, mean PPV and mean FCD (i.e. factor time in the linear mixed model was not significant, see appendix II)

Table 3. Linear mixed models analysis shows higher values for TVD, PPV, FCD and PaO2 over time and lower values for lactate in the low SOFA score group.

Dependent variable	Estimate	Std. Error	df	t	P-value	95% CI-interval	
						Lower bound	Upper bound
TVD	1,87	,94	21,43	2,0	**,060**	-,085	3,820
PPV	,02	,007	16,56	2,6	**,018**	,004	,032
FCD	2,02	,87	15,75	2,3	**,034**	,172	3,858
RBCv	17,23	9,11	15,08	1,9	,078	-2,181	36,645
cHct	-,0001	,002	25,26	-,1	,952	-,005	,004
tRBCp	4,74	2,75	25,58	1,7	,097	-,927	10,405
PaO2	,91	,38	11,58	2,4	**,033**	,086	1,732
Lactate	-,49	,16	23,48	-3,0	**,007**	-,825	-,147

cHct: capillary hematocrit, FCD: functional capillary density, PaO2: arterial oxygen tension, PPV: proportion of perfused vessels, RBCv: red blood cell velocity, SOFA: sequential organ failure assessment, tRBCp: tissue red blood cell perfusion, TVD: total vessel density

Table 4. Microcirculation parameter values at admission for all patients with a COVID-19 infection and subdivided for severity of illness according to SOFA-score.

Microcirculatory parameters	All patients (n = 21)	SOFA ≤ 8 (n = 12)	SOFA > 9 (n = 9)	P-value*
TVD ($mm*mm^{-2}$)	22,81 ± 3,26	23,61 ± 3,50	21,75 ± 2,74	0,187
PPV (%)	0,95 ± 0,03	0,96 ± 0,02	0,94 ± 0,03	0,091
FCD ($mm*mm^{-2}$)	21,66 ± 3,04	22,61 ± 3,21	20,38 ± 2,39	0,084
RBCv ($\mu m*s^{-1}$)	327,80 ± 30,78	335,91 ± 34,98	316,98 ± 21,32	0,142
CHct (%)	0,05 ± 0,01	0,05 ± 0,01	0,05 ± 0,01	0,940
tRBCp ($\mu m*min^{-1}$)	46,95 ± 8,53	49,43 ± 9,65	43,64 ± 5,68	0,103

*Comparison of low and high SOFA score groups with independent samples t-test, p-value 'equal variances not assumed'. Data are presented as mean ± standard deviation.

cHct: capillary hematocrit, FCD: functional capillary density, PPV: proportion of perfused vessels, RBCv: red blood cell velocity, SOFA: sequential organ failure assessment, tRBCp: tissue red blood cell perfusion, TVD: total vessel density

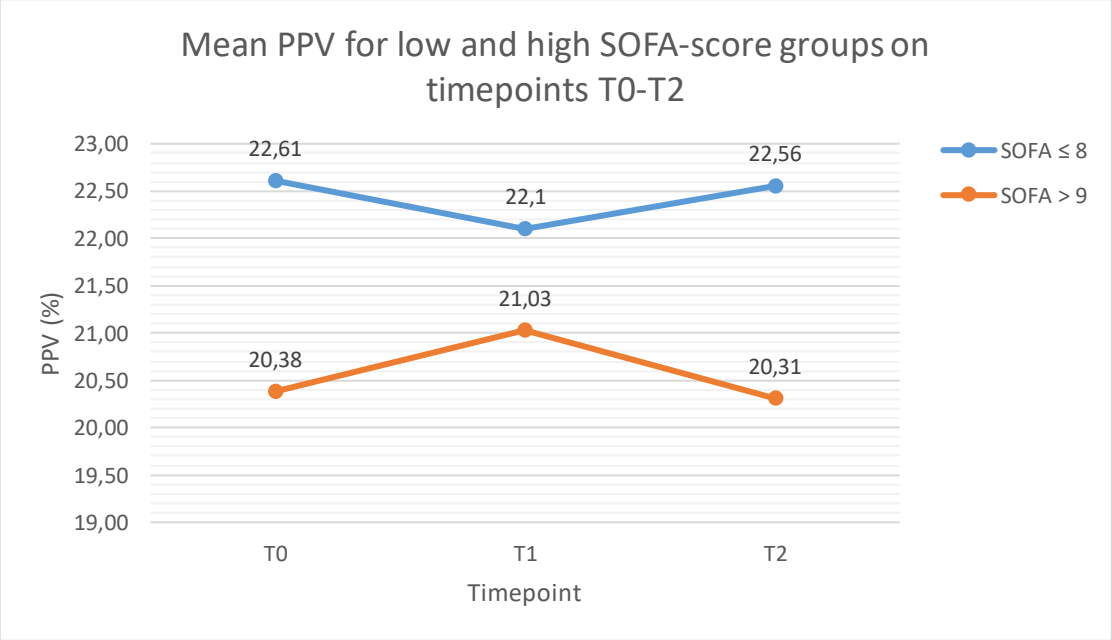

Figure 1. Mean PPV at timepoints T0, T1 and T2 for low and high SOFA score groups.

PPV: proportion of perfused vessels, SOFA: sequential organ failure assessment

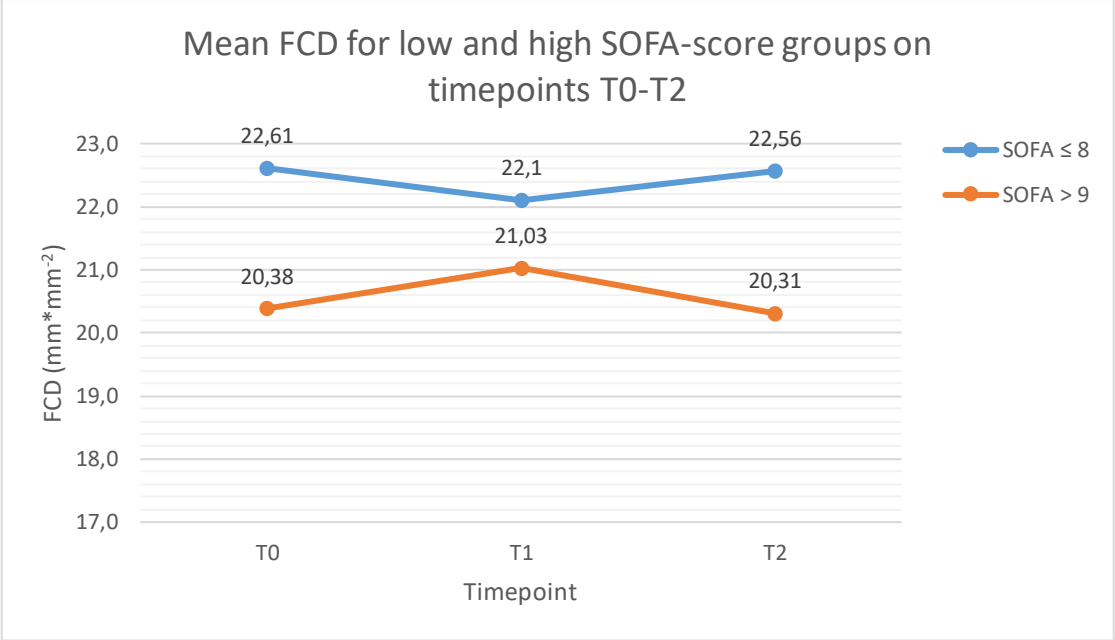

Figure 2. Mean FCD at timepoints T0, T1 and T2 for low and high SOFA score groups.
FCD: functional capillary density, SOFA: sequential organ failure assessment

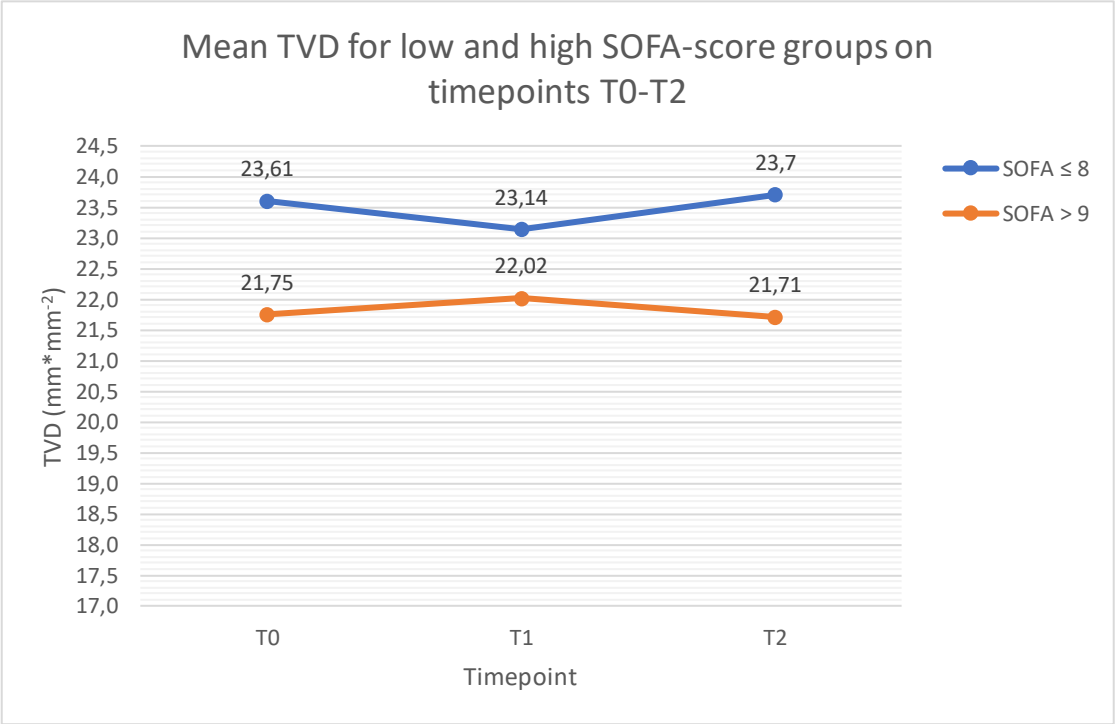

Figure 3. Mean TVD at timepoints T0, T1 and T2 for low and high SOFA score groups.
SOFA: sequential organ failure assessment, TVD: total vessel density

Discussion

The aim of this study was to investigate the change of the microcirculation in patients with a COVID-19 infection over time and to compare this for patients with different severity of illness.

We found that microcirculation parameters measured at T0 were not significantly different between patient in the low SOFA score group compared to patients in the high SOFA score group. Analysis of the course of the microcirculation over 48 hours (T0-T2) however, showed that for less severely ill patients (the low SOFA score group) TVD, PPV and FCD remained higher over time than for severely ill patients (the high SOFA score group). These findings are in line with the results from the studies performed by Favaron, E. et al (2021) and Edul, V.S.K. et al (2021) [6, 16]. They suggested that patients with a COVID-19 infection activate microcirculatory compensatory mechanisms, e.g. capillary recruitment, to increase oxygen extraction as a response to hypoxemia. In the study of Favaron, E. et al, this recruitment of the microcirculation was only seen in patients with a SOFA score less than 10. In our study population, patients with a SOFA score ≤ 8 had similar values for PaO2 at admission as patients with a SOFA score > 9. Furthermore, the PaO2/FiO2 ratio at admission was lower for patients in the low SOFA score group, which is an unexpected result, as a higher PaO2/FiO2 ratio would be expected in this group. However, the analysis of macrocirculatory parameters over time showed that PaO2 values for patients in the low SOFA score group were higher compared to patients in the high SOFA score group. This could be the result of the increase in TVD, PPV and FCD.

According to the analysis of microcirculatory parameters over time, there may be a relationship between severity of illness of patients with a COVID-19 infection and the ability to activate microcirculatory compensatory mechanisms in order to draw on reserve capacity in a situation of hypoxemia. Abou-Arab, O. et al (2021) however, showed results that object this hypothesis [22]. They split their COVID-19 patient population into a severe group (i.e. having a respiratory rate ≥ 30/min or an oxygen saturation of ≤ 90% on room air or signs of severe distress syndrome) and a critical group (i.e. having respiratory failure requiring mechanical ventilation or shock or organ failure that requires ICU care) in which they measured the microcirculation. The critical group included patients that, besides being mechanically ventilated, had a mean SOFA score of 10. They found that in the critical group, values for vessel density - de Backer score (Grid-based score, total number of vessel crossings per grid length) [12] and PVD – were higher compared to the severe group. These differences in outcome might be related to the fact that the PaO2/FiO2 ratio in the patient population of Abou-Arab, O. et al was much lower compared to our patient group (17.46 kPa vs. 20.95 kPa respectively). Pulmonary oxygenation of their patient population was thus lower, which could lead to both capillary recruitment and angiogenesis [23]. As a result, a higher vessel density can be expected despite relatively high SOFA scores.

Our study has some limitations. No power or sample size estimation was performed, so the size of the study population may have been too small. In addition, we did not have a control group to compare our results with. A good control group would have been healthy volunteers or patients with mild and severe ARDS without a COVID-19 infection.

It is possible that our finding of increased PPV and FCD in less severely ill patients had other causes. High metabolic activity for example, could be a stimulus for vascular growth [24] as well as microthrombosis [25]. Both of these mechanisms have been described in patients with a severe COVID-19 infection [23].

Conclusion

This study demonstrated microvascular alterations in patients with a COVID-19 infection who are less severely ill, that may be associated to hypoxemia. As a response to hypoxia, patients with a COVID-19 infection increase their TVD, PPV and FCD, in order to increase the oxygen-extraction capacity.

The next step in the research of microcirculation in patients with a COVID-19 infection could be the response of the microcirculation to certain treatments. It is important to measure the microcirculation before treatment, during and after treatment in order to investigate the effect of treatment on the microcirculation. In addition, it would be interesting to further investigate the relationship between microcirculatory parameters and (long term) follow-up and to investigate the microcirculation of the lung itself.

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Afterword

In the literature review, I investigated the existence or loss of hemodynamic coherence in patients with cardiogenic shock or heart failure. Although it was difficult to draw a conclusion, most studies showed hints for a loss of hemodynamic coherence. In the study that I performed in patients with a COVID-19 infection, identifying existence or loss of hemodynamic coherence was not the main goal. However, the results showed that of all macrocirculatory parameters, only lactate changes congruently with the microcirculation parameters TVD, PPV and FCD. Other macrocirculatory parameters, for example heart rate and blood pressure, did not change significantly.

So, loss of hemodynamic coherence seems to play a role in patients with a COVID-19 infection as well, albeit partially. This is a new insight, as loss of hemodynamic coherence has previously been associated only with critically ill patients in a state of shock. Further research must indicate whether the phenomenon of loss of hemodynamic coherence plays a part in a wider range of medical conditions.

Appendix I

Explanation of microcirculatory parameters

Table 1. Objective parameters of the microcirculation computed with MicroTools automatic software.

Parameter	Unit	Description	Physiological context
TVD	$mm*mm^{-2}$	Sum of the length of all capillaries containing RBCs, divided by FOV	Measure of microcirculatory diffusion capacity
PPV	1	Grid-based score, percentage of perfused vessels per total number of vessel crossings	Aspect of the heterogeneity of capillary perfusion
FCD	$mm*mm^{-2}$	Sum of the length of all capillaries containing moving RBCs, divided by FOV	Density of perfused capillaries as a determinant of microcirculation diffusion capacity
RBCv	$\mu m*s^{-1}$	Weighted mean (by vessel length) of the absolute RBC velocity in all capillaries within the FOV	Absolute blood flow velocity as a measure of microcirculatory convection capacity
cHct	1	Weighted mean (by capillary segment length) of the whole blood volume to RBC volume ratio in all capillary segments within the FOV	Corresponds to the distance of RBCs within the boundaries of the capillaries and represents a determinant of microcirculatory diffusion capacity
tRBCp	$mm^4*min^{-1}*\mu l^{-1} x\ 10^{-3}$	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 FOV	Perfusion of the tissue with RBCs as the most representative measure of tissue perfusion in a clinical and physiological context

cHct: capillary hematocrit, FCD: functional capillary density, FOV: field of view, PPV: proportion of perfused vessels, RBCs: red blood cells, RBCv: red blood cell velocity, tRBCp: tissue red blood cell perfusion, TVD: total vessel density.

Mean values for microcirculatory parameters per timepoint and for low and high SOFA groups

All patients

	T0 (n = 21)		T1 (n = 14)		T2 (n = 12)	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
TVD	22,82	3,26	22,90	2,87	23,04	2,24
PPV	,95	,03	,96	,02	,95	,03
FCD	21,66	3,04	21,87	2,57	21,81	2,45
RBCv	327,80	30,78	330,45	25,25	326,02	44,48
cHct	,05	,01	,05	,01	,05	,01
tRBCp	46,95	8,53	48,21	6,80	47,37	8,48

For patients with SOFA ≤ 8

	T0 (n = 12)		T1 (n = 11)		T2 (n = 8)	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
TVD	23,61	3,50	23,14	3,12	23,70	2,19
PPV	,96	,02	,96	,02	,950	,03
FCD	22,61	3,21	22,10	2,69	22,56	2,40
RBCv	335,91	34,98	334,29	27,08	325,93	23,05
cHct	,05	,01	,05	,01	,05	,01
tRBCp	49,43	9,65	48,89	6,84	49,08	6,84

For patients with SOFA > 9

	T0 (n = 9)		T1 (n = 3)		T2 (n = 4)	
	Mean	Std. Deviation	Mean	Std. Deviation	Mean	Std. Deviation
TVD	21,75	2,74	22,02	1,84	21,71	1,89
PPV	,94	,03	,95	,03	,94	,04
FCD	20,38	2,39	21,03	2,27	20,31	2,02
RBCv	316,98	21,32	316,36	9,97	326,22	77,56
cHct	,05	,01	,05	,01	,05	,01
tRBCp	43,64	5,68	45,71	7,41	43,96	11,45

Appendix II

SPSS output of linear mixed models analysis

Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	14,879	714,016	,000
Measurement	2	9,679	,012	,988
Difference ICU admission and T0 in days	1	13,576	,122	,732
SOFA groups	1	21,429	3,948	,060

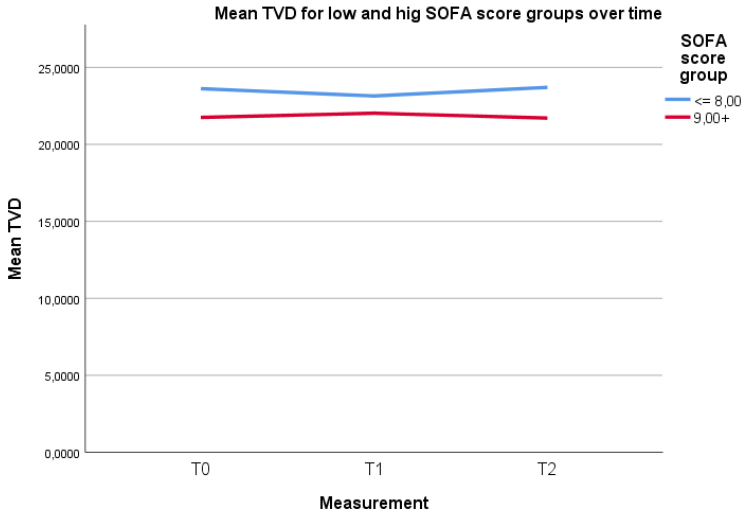
a. Dependent Variable: Total_vessel_density.

Estimates of Fixed Effects[a]

Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	21,488605	1,179197	19,082	18,223	,000	19,021237	23,955972
T0	,034278	,891966	16,198	,038	,970	-1,854723	1,923280
T1	-,105063	,866451	8,524	-,121	,906	-2,081939	1,871813
T2	0[b]	0	.	.	.	.	.
Difference ICU admission and T0 in days	,131846	,377173	13,576	,350	,732	-,679484	,943177
Low SOFA group	1,867493	,939821	21,429	1,987	,060	-,084590	3,819577
High SOFA group	0[b]	0	.	.	.	.	.

a. Dependent Variable: Total_vessel_density.

b. This parameter is set to zero because it is redundant.



Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	13,342	28935,222	,000
Measurement	2	16,067	,601	,560
Difference ICU admission and T0 in days	1	12,970	,523	,482
SOFA groups	1	16,557	6,947	,018

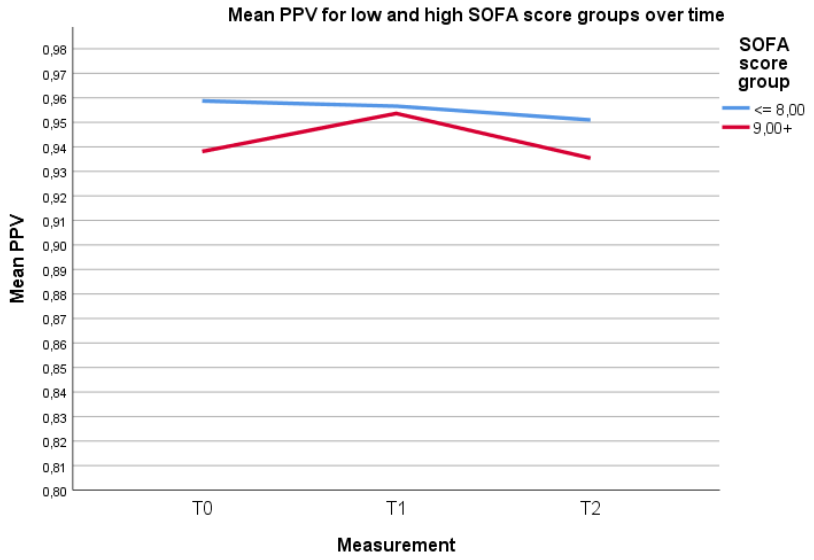
a. Dependent Variable: Proportion perfused vessels.

Estimates of Fixed Effects[a]

Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Intercept	,927281	,010984	19,915	84,422	,000	,904362	,950199
T0	,009383	,011270	15,696	,833	,418	-,014547	,033312
T1	,011850	,010814	13,946	1,096	,292	-,011353	,035052
T2	0[b]	0	.	.	.	.	.
Difference ICU admission and T0 in days	,001769	,002447	12,970	,723	,482	-,003517	,007056
Low SOFA group	,017838	,006768	16,557	2,636	,018	,003530	,032147
High SOFA group	0[b]	0	.	.	.	.	.

a. Dependent Variable: Proportion perfused vessels.

b. This parameter is set to zero because it is redundant.



Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	12,717	773,971	,000
Measurement	2	7,861	,004	,996
Difference ICU admission and T0 in days	1	12,008	,002	,968
SOFA groups	1	15,752	5,387	,034

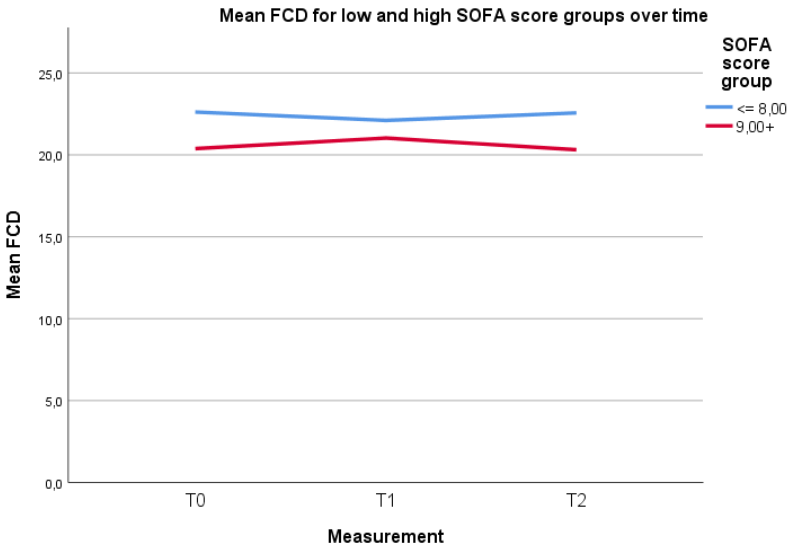
a. Dependent Variable: Functional capillary density.

Estimates of Fixed Effects[a]

Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Intercept	20,532302	1,134284	16,841	18,102	,000	18,137445	22,927158
T0	-,052156	,856904	14,944	-,061	,952	-1,879199	1,774887
T1	,014350	,958539	5,947	,015	,989	-2,336197	2,364897
T2	0[b]	0	.	.	.	.	.
Difference ICU admission and T0 in days	,014612	,353406	12,008	,041	,968	-,755334	,784558
Low SOFA group	2,015287	,868281	15,752	2,321	,034	,172259	3,858314
High SOFA group	0[b]	0	.	.	.	.	.

a. Dependent Variable: Functional capillary density.

b. This parameter is set to zero because it is redundant.



Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	13,069	89,610	,000
Measurement	2	12,211	9,351	,003
Difference ICU admission and T0 in days	1	23,481	8,777	,007
SOFA groups	1	12,669	1,298	,276

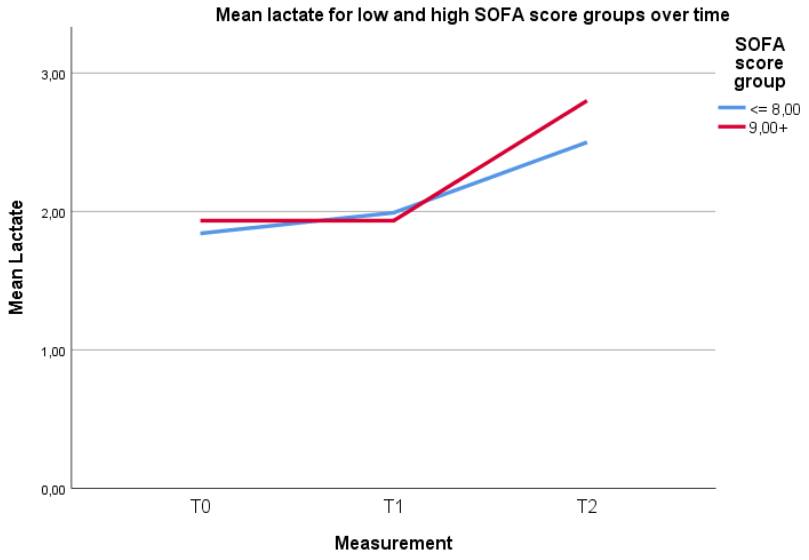
a. Dependent Variable: Lactate.

Estimates of Fixed Effects[a]

Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Intercept	2,676263	,269878	20,664	9,917	,000	2,114463	3,238062
T0	-,713978	,173277	13,885	-4,120	,001	-1,085908	-,342049
T1	-,514180	,179314	10,268	-2,867	,016	-,912304	-,116056
T2	0[b]	0	.	.	.	.	.
Difference ICU admission and T0 in days	,114551	,100539	12,669	1,139	,276	-,103228	,332331
Low SOFA group	-,485985	,164036	23,481	-2,963	,007	-,824934	-,147036
High SOFA group	0[b]	0	.	.	.	.	.

a. Dependent Variable: Lactate.

b. This parameter is set to zero because it is redundant.



Type III Tests of Fixed Effects[a]

Source	Numerator df	Denominator df	F	Sig.
Intercept	1	16,557	822,278	,000
Measurement	2	19,291	,325	,726
Difference ICU admission and T0 in days	1	10,186	,484	,502
SOFA groups	1	11,583	5,836	,033

a. Dependent Variable: Arterial O2 tension.

Estimates of Fixed Effects[a]

Parameter	Estimate	Std. Error	df	t	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
Intercept	9,401893	,441045	12,120	21,317	,000	8,441989	10,361797
T0	,508437	,693926	20,605	,733	,472	-,936347	1,953221
T1	-,303917	,765125	15,477	-,397	,697	-1,930373	1,322540
T2	0[b]	0	.	.	.	.	.
Difference ICU admission and T0 in days	-,092327	,132643	10,186	-,696	,502	-,387144	,202490
Low SOFA group	,908904	,376250	11,583	2,416	,033	,085843	1,731965
High SOFA group	0[b]	0	.	.	.	.	.

a. Dependent Variable: Arterial O2 tension.

b. This parameter is set to zero because it is redundant.