Sigita Kazūne

ORCID 0000-0002-7010-3238

The role of functional arterial properties in prediction of clinical outcomes of septic patients

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Supervisor of the Doctoral thesis:

Dr. med. Associate Professor **Eva Strīķe**
Dr. med., Dr. habil. med. Professor **Indulis Vanags**

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ANNOTATION

Sepsis is defined as multiple organ damage caused by dysfunctional systemic host response to infection. It is a frequent cause of admission to intensive care unit and carries high mortality. Vascular dysfunction due to reduced nitric oxide bioavailability plays an important role in the pathogenesis of sepsis and multiple organ failure. Conduit arteries, especially the aorta, play a major role in ensuring efficient cardiac function and optimal flow in the periphery due to their viscoelastic properties. Resistance arteries and arterioles adjust peripheral blood flow according to the metabolic needs of the tissues. Laboratory studies on isolated artery models and animal research show that acute systemic inflammation can cause impaired vasoreactivity in peripheral vascular beds and aortic stiffening of conduit arteries which affects hemodynamic efficiency.

This thesis aims to characterize parameters describing functional properties of arteries in intensive care patients with severe sepsis and septic shock and clarify their association with the development of multiple organ dysfunction syndrome and mortality.

This thesis consists of three parts. In part 1, systematic review and meta-analysis of published literature were performed to evaluate the measurement of endothelial function using vasoreactivity tests as a risk stratification tool in intensive care patients with sepsis. From the studies included in this review, there is evidence of moderate strength that vascular reactivity is impaired in septic patients, but not enough evidence has been provided to suggest that it is a consequence of endothelial dysfunction or is convincingly related to clinical outcomes.

In part 2, the author undertook a prospective observational cohort study examining aortic stiffness in patients with sepsis using carotid-femoral pulse wave velocity measurement. Forty-five adult intensive care patients were recruited to the study within 24 hours of admission to intensive care. Carotid-femoral pulse wave velocity was measured once initial resuscitation was completed. Patients were followed up to hospital discharge or death. This study found that patients with severe sepsis and septic shock have higher aortic stiffness than the general population. No convincing association was found between admission pulse wave velocity and progression of multiple organ failure or mortality, although the group with pulse wave velocity > 24.7 m/s had shorter survival time.

Part 3 extends the previous study and examines stiffness of both elastic and muscular arteries at two time points, at admission and after 48 hours of treatment. It also examines confounders associated with changes in carotid-femoral and carotid-radial pulse wave velocity. It found increased aortic and even higher upper limb artery stiffness among the septic population. Higher carotid-femoral pulse wave velocity was associated with higher mean arterial blood pressure and lower C reactive protein concentration. Pulse wave velocity in elastic

and muscular arteries decreased after a 48-hour treatment period in survivors. In non-survivors, carotid-radial pulse wave velocity stayed consistently high.

Overall, this study has shown that altered static and dynamic parameters of artery function are highly prevalent in patients with sepsis and associated with unfavourable outcome. Longitudinal assessment of these parameters has the potential to be used for risk stratification in patients with early sepsis.

ANOTĀCIJA

Sepse ir masīva organisma reakcija uz infekciju, ko raksturo dzīvību apdraudošs orgānu bojājums. Fizioloģiskas nestabilitātes un augstā mirstības riska dēļ sepses pacientus bieži stacionē intensīvās terapijas nodaļās. Asinsvadu disfunkcijai, kas rodas samazinātas NO biopieejamības rezultātā, ir nozīmīga loma sepses un multiplu orgānu mazspējas patoģenēzē. Maģistrālo artēriju elastība nepieciešama, lai nodrošinātu efektīvu asiņu izsviedi no sirds, un to plūsmas optimizāciju perifērijā atbilstoši audu metabolajām vajadzībām. Izmantojot izolētus artēriju segmentus laboratorijas pētījumos un eksperimentos ar dzīvniekiem, pierādīts, ka akūts sistēmisks iekaisums ietekmē gan elastīgās, gan muskuļu tipa artērijas. Elastīgajā artērijās, īpaši aortā, pieaug cietība, bet muskuļu tipa artērijās novēro samazinātu vazoreaktivitāti.

Darba "Artēriju funkcionālo parametru loma multiorgānu disfunkcijas sindroma progresēšanas riska prognozēšanā" mērķis ir raksturot sepses izraisītās artēriju funkcionālo parametru pārmaiņas, lai izveidotu diagnostikas un monitorēšanas paņēmienus, ar mērķi uzlabot multiplu orgānu mazspējas progresēšanas un mirstības riska prognozēšanu.

Pētījums veidots no trim sadaļām. Pirmajā sadaļā sagatavots sistemātisks literatūras apskats un metaanalīze par sepses pacientu endoteliālās funkcijas izvērtēšanu ar vazoreaktivitātes testiem un šo testu rezultātu izmantošanu slimības klīniskās gaitas prognozēšanai. Otrajā sadaļā veikta *a.carotis-a. femoralis* pulsa viļņa izplatīšanās ātruma izpēte intensīvās terapijas pacientiem ar agrīnu sepsi. Pētījumā iesaistīti 45 pieauguši sepses pacienti pirmajās 24 stundās pēc stacionēšanas intensīvās terapijas nodaļā. *A.carotis-a. femoralis* pulsa viļņa izplatīšanās ātrums reģistrēts pēc pacientu stāvokļa sākotnējas stabilizācijas. Pacienti novēroti dinamikā līdz izrakstīšanai no stacionāra vai letālam iznākumam. Pētījumā pierādīts, ka pacientiem ar smagu sepsi un septisku šoku augstu pulsa viļņa izplatīšanās ātrumu, kas liecina par artēriju cietības pieaugumu, novēro biežāk kā vispārējā populācijā. Saistība starp agrīni mērītu pulsa viļņa izplatīšanās ātrumu un multiplas orgānu mazpējas progresēšanu vai mirstību netika atrasta, tomēr pacientiem, kam novēroja pulsa viļņa izplatīšanās ātrumu virs 24,7 m/s, bija īsāks izdzīvošanas laiks.

Trešajā daļā elastīgo un muskuļu tipa artēriju īpašības pētītas dinamikā, izdarot mērījumus divos laika periodos, iestāšanās dienā un pēc 48 stundu intensīvās terapijas. Šajā pētījumā izzināti arī faktori, kuru pārmaiņas saistās ar pārmaiņām *a.carotis-a. femoralis* un *a.carotis-a. radialis* pulsa viļņa izplatīšanās ātrumā. Šajā pētījumā konstatēts, ka augšējās ekstremitātes artēriju cietība sepses pacientiem pārsniedz maģistrālo artēriju cietību abos laika periodos. Augsts *a.carotis-a. radialis* pulsa viļņa izplatīšanās ātrums asociējas augstāku vidējo arteriālo spiedienu un zemāku C reaktīvā proteīna koncentrāciju asinīs. Pacientiem, kas izdzīvo,

pulsa viļņa izplatīšanās ātrums pēc 48 stundu intensīvās terapijas dinamikā mazinās, kurpretim pacientiem, kas nomirst, *a.carotis-a. radialis* pulsa viļņa izplatīšanās ātrums dinamikā saglabājas augsts.

Kopsavilkumā, šajā pētījumā pierādīts, ka sepses pacientiem ir izmainīti artēriju funkcionālie parametri, un šīs pārmaiņas asociējas ar nelabvēlīgu iznākumu. Pētītajiem neinvazīvajiem artērijas raksturojošiem testiem, augsta riska pacientu identifikācijai *a.carotis-a. radialis* pulsa viļņa izplatīšanās ātruma monitorēšanai dinamikā ir vislielākais prognostiskais potenciāls.

CONTENTS

ABBREVIATIONS

ACCP/SCCM – American College of Chest Physicians/Society of Critical Care Medicine

ANOVA – analysis of variance

APACHE II – Acute Physiology, Age, Chronic Health Evaluation II

c-f PWV – carotid-femoral pulse wave velocity

c-r PWV – carotid-radial pulse wave velocity

CRP – C reactive protein

EDVD – endothelium dependent vasodilatation

eNO – endothelial nitric oxide

EPC – endothelium progenitor cells

ET-1 – endothelin-1

FMD – Flow mediated dilatation

ICAM-1 – intracellular adhesion molecule 1

ICD-9, ICD-10 – International Classification of Diseases, Ninth Revision/International Classification of Diseases, Tenth Revision

ICU – intensive care unit

IL-6 – Interleukin-6

IQR – inter-quartile range

L-NMMA – L-NG-Monomethylarginine

LDF – Laser Doppler flowmetry

LED – light-emitting diode

MODS – multiple organ dysfunction syndrome

MRI – magnetic resonance imaging

NO – nitric oxide

NPR – Norwegian Patient registert

PAI – plasminogen activator inhibitor

PAT – peripheral arterial tonometry

PGI2 – prostacyclin

PICO – Participant-Intervention-Comparator-Outcomes

PPG – photoplethysmogram

PROWESS – Protein C Worldwide Evaluation in Severe Sepsis

PWV – Pulse wave velocity

s-Flt-1 – soluble fms-like tyrosine kinase

SHDR – Swedish hospital discharge register

SIRS – systemic inflammatory response syndrome

SMD – standardized mean difference

SOFA – Sequential Organ Failure Assessment Score

sVCAM – soluble vascular adhesion molecule

VEGF – vascular endothelial growth factor

INTRODUCTION

Sepsis, a life-threatening syndrome, is defined by multiple organ damage caused by dysfunctional systemic host response to infection (Singer et al., 2016). Among the critically ill population, patients with sepsis comprise a significant proportion and have a high mortality. Although infection is the necessary trigger for the development of sepsis (Nystrom, 1998), the outcome of the patient is determined mostly by the response of the host (Cohen, 2002). The key component of the host response is endothelial cell dysfunction which leads to vascular dysfunction and/or injury. One of the functions of vascular endothelium is control of arterial tone. Activation of endothelium in sepsis is an adaptive response to lipopolysaccharides and cytokines that leads to altered vasomotor tone and increased blood flow to infected areas (Aird, 2003; Henneke and Golenbock, 2002; McCuskey et al., 1996). Data obtained in experimental studies show that acute systemic inflammation also leads to a temporary increase of large artery stiffness (Vlachopoulos et al., 2005). During generalized endothelial activation, cells can become damaged, which triggers macrocirculatory disturbances and microvascular perfusion abnormalities (Aird, 2003).

Measurement of reactive hyperemia to ischaemic or pharmacologic stimuli, a test of endothelial nitric oxide bioavailability, may be used to quantify endothelial dysfunction (Lekakis et al., 2011). Different stimuli and measurement methodologies have been employed to investigate endothelial function in patients with sepsis with variable results. Several narrative reviews (Aird, 2003; Ince et al., 2016; Opal and van der Poll, 2015) reported the effects of sepsis on endothelial function, and one systematic review (Xing et al., 2012) focused on biomarkers of endothelial activation in sepsis. However, evaluation of endothelial function in sepsis remains a research tool, and the association between altered vascular reactivity and severity of sepsis with respect to the development of multiple organ failure and mortality is not well established. Currently there is not enough research to use these methods as adjuncts to diagnosis and means to direct treatment.

For measurement aortic stiffness, carotid-femoral pulse wave velocity (c-f PWV) is a recognized index. It can be obtained non-invasively and has been shown to be an important predictor of mortality in various chronic diseases (Blacher et al., 1999a; Blacher et al., 1999b; Stefanadis et al., 2000). Another measure of arterial properties, carotid-radial PWV (c-r PWV), represents brachial artery stiffness. It is influenced more by endothelial nitric oxide (eNO) production than aortic PWV, and as vascular dysfunction in sepsis is functionally linked with basal eNO production, it might reflect arterial changes in sepsis better. Investigating effects of sepsis on endothelium and properties of arteries adds to understanding of sepsis pathophysiology and could allow to detect transition of infection to sepsis early in the course

of disease. Data obtained in experimental studies show that acute systemic inflammation leads to a temporary increase of large artery stiffness (Vlachopoulos et al., 2005), but the effect of massive inflammatory response in early sepsis on large artery stiffness has not been previously investigated.

Early sepsis detection and therapy initiation is crucial for survival, long term outcomes of patients and reduction of healthcare expenditure. Current methods of diagnosing sepsis early in the course of the disease are nonspecific and imprecise, resulting in delayed diagnosis. This thesis investigates two non-invasive indices of endothelial dysfunction, impaired vasoreactivity, and increase of arterial stiffness, with the aim to aid early diagnosis of sepsis and patients at risk of death and multiple organ dysfunction syndrome. C-f PWV, c-r PWV, conduit artery and microvascular dysfunction as measured by provocation tests have been assessed in adult patients with early severe sepsis and septic shock, to determine the clinical, inflammatory, and hemodynamic parameters correlated with altered arterial stiffness and vascular reactivity in such patients and their relationship to outcome.

Ethical aspects

The investigation was conducted according to the principles outlined in the Declaration of Helsinki. The study protocol was approved by Rīga Stradiņš University Ethics Committee and informed consent was given by each patient or their next of kin.

Scientific novelty

- Longitudinal changes in properties of elastic and muscular arteries have been characterized in a cohort of intensive care patients with severe sepsis for the first time;
- Based on the relationship between these changes and clinical course of disease, parameters for discrimination of patients with high risk of progression of multiple organ dysfunction and death have been investigated;
- Latvian patent “Method for prediction of the risk of developing inadequate renal vascular perfusion in patients with severe sepsis and septic shock in critical condition: LV 14806 B” obtained.

Aim of the study

The aim of the study was the prediction of progression of multiple organ dysfunction syndrome (MODS) and mortality in patients with severe sepsis based on static and dynamic parameters of arterial function.

Objectives of the study

1. To evaluate the use of vasoreactivity tests for assessment of endothelial function in intensive care patients with sepsis.

2. To quantify and characterize stiffness of elastic and muscular arteries using pulse wave velocity in a cohort of intensive care patients with severe sepsis within 24 hours of admission to intensive care unit.
3. To examine relationship between changes in arterial stiffness, vasoreactivity, severity of disease, markers of inflammation and macrohemodynamic parameters in this cohort of patients.
4. To compare stiffness of elastic and muscular arteries in survivors and non survivors at baseline and longitudinally over first 48 hours of intensive care.
5. To evaluate significance of early changes in artery stiffness for prediction of progression of multiple organ dysfunction syndrome and outcome in patients with severe sepsis.

Working hypothesis

In sepsis patients, the risk of progression of multiple organ dysfunction syndrome and mortality is associated with early changes in stiffness of elastic and muscular arteries and postischaemic reactive hyperaemia, and these parameters can be used to predict clinical outcome.

1 LITERATURE REVIEW

1.1 Sepsis definitions and classification

1.1.1 Biological description of sepsis

Sepsis is an acute, clinically heterogeneous syndrome developed by the host in response to a diverse group of threats. It is a highly conserved biological process across organisms and is not unique to infection. In medicine, "sepsis" is a term used to refer to a population of patients that are thought to have the same underlying pathophysiology and therefore might benefit from the same type of diagnostic procedures and treatment. To be of use in clinical practice, it has to be proven in trials that treatment decisions based on the definition result in improved clinical outcomes. Research on developing a definition of sepsis to support decisions at the bedside is still ongoing.

The key feature of sepsis is that it is a maladaptive response to infection with damage to the host's own tissues and organs. There are no specific clinical signs of sepsis. Clinical and laboratory findings change over the course of disease and may not be present at presentation (Figure 1.1).

Biological effects	**Clinical/biochemical manifestations**	**Individual patient**
Macrovascular abnormalities	Hypotension	Various combinations of clinical signs
Microvascular abnormalities	Oliguria	Do not occur at the same time
Myocardial abnormalities	Altered mentation	
Tissue underperfusion	Skin mottling	
Cellular abnormalities	Increased lactate	
	Coagulopathy	

Figure 1.1 **Schematic overview of sepsis biology**

As sepsis definition is used to facilitate recruitment into trials, vital signs are useful criteria as they are continuously monitored in most hospitalized patients. Non-specific presentation and need for easily obtained criteria make defining sepsis in the context of clinical research challenging. The basis of contemporary sepsis definitions is international consensus rather than understanding of the disease process.

1.1.2 Consensus definitions of sepsis and related disorders 1991–2015

The first international consensus definition of systemic inflammatory response syndrome (SIRS), sepsis and septic shock was developed as inflammatory cascade mediator targeted therapy trials began. These trials needed to recruit patients early in the course of disease before microbiological results were available. It has been used since ACCP/SCCM consensus meeting in 1991 and widely adopted to define inclusion criteria in numerous sepsis trials (Jones et al., 2010; Rivers et al., 2001; Schortgen et al., 2012) and to help early clinical diagnosis by the bedside (Table 1.1).

Table 1.1

ACCP/SCCM consensus sepsis definitions (1991) (Bone, Sibbald, et al. 1992)

Syndrome	Definition
Infection	Microbial phenomenon characterized by an inflammatory response to the presence of microorganisms or the invasion of normally sterile host tissue by those organisms
Systemic inflammatory response syndrome (SIRS)	The systemic inflammatory response to a variety of clinical insults. The response is manifested by two or more of the criteria: (1) temperature > 38 °C or < 36 °C; (2) pulse > 90/min; (3) respiratory rate > 20/min or PaCO2 < 32 mmHg; (4) white blood cell count > 12 000 or < 4 000/m^{-3}, or > 10 % immature band forms
Sepsis	The systemic response to infection; an infection plus two or more SIRS criteria
Severe sepsis	Sepsis associated with organ dysfunction, hypoperfusion or hypotension. Hypoperfusion may include lactic acidosis, oliguria or acutely altered mental status.
Septic shock	Sepsis induced hypotension (systolic blood pressure < 90 mm Hg or reduction of ≥ 40 mm Hg from baseline) despite adequate fluid resuscitation and the presence of perfusion abnormalities

ACCP/SCCM – American College of Chest Physicians/Society of Critical Care Medicine

Using this definition, sepsis is defined as systemic inflammatory response syndrome in the context of infection (Bone et al., 1992). SIRS is defined by changes in at least two of four clinical variables (temperature, pulse rate, respiratory rate and white cell count). SIRS criteria were later found to have low specificity and deemed not adequate to assess patient risk (Kaukonen et al., 2015). The other two categories – severe sepsis and septic shock – have proven more useful in identifying patients at risk of deterioration and death (Shapiro et al., 2006). The 1991 conference also introduced the term “multiple organ dysfunction syndrome (MODS)”. It was introduced to emphasize the importance of sepsis induced organ damage and

described as a process in which organ function cannot be maintained by homeostasis alone. MODS is a continuum with changes over time.

To improve the specificity of SIRS criteria, sepsis definition was modified in 2001 and included a long list of clinical and laboratory criteria (Levy et al., 2003). 2001 definition was complex to use clinically and in research. It did not add specificity compared to the 1991 definition. For trials conducted since then, adaptations of 1991 ACCP/SCCM definition have been developed. The best-known adaptation is Protein C Worldwide Evaluation in Severe Sepsis (PROWESS) study criteria to identify cases of sepsis and severe sepsis (Bernard et al., 2001). It requires three or more SIRS criteria and evidence of at least one acute organ dysfunction in patients with infection to be defined as septic.

The introduction of consensus definitions is considered an important step in clinical sepsis research (Trzeciak et al., 2005). Most clinical sepsis research has adopted the 1991 definition of sepsis-related conditions (Lopez et al., 2004; Stephens et al., 2008). In this thesis, the author has also used this definition with the requirement of two SIRS criteria in the context of infection.

1.1.3 Consensus definition of sepsis related conditions since 2015

Recognizing the limitations of the 1991 definition the European Society of Intensive Care Medicine and Society of Critical Care Medicine set up an expert panel with the purpose of updating definitions of sepsis. In 2016, the expert panel published the third international consensus definitions for sepsis and septic shock (Sepsis-3) (Singer et al., 2016). This definition emphasizes that infection-associated organ dysfunction is one of the determinants of potential mortality. Therefore, sepsis is defined as an acute increase in Sequential Organ Failure Assessment Score (SOFA) of 2 or more points in the context of suspected or documented infection. The term “severe sepsis” is no longer used.

There has been considerable effort to identify validated biomarkers of sepsis, as these are more objective than physiologic variables. Two possible approaches to this task are rapid bacterial nucleinic acid detection in blood using polymerase chain reaction (Dierkes et al., 2009; Westh et al., 2009) and identification of endogenous compounds involved in organism’s response to infection. Many potential biomarkers have been investigated. The best studied are C reactive protein (Lobo et al., 2003; Povoa et al., 2005) and procalcitonin (Becker et al., 2008; Tang et al., 2007). None have proved to be highly sensitive and specific, to distinguish reliably sepsis from non-infectious SIRS. They are used as accessory criteria to aid diagnostic and management decisions in sepsis.

1.1.4 Multiple organ dysfunction syndrome (MODS)

MODS is a syndrome with clinical and biochemical evidence of progressive, but potentially reversible physiologic dysfunction in two or more organs or organ systems that develops as a consequence of acute injury or illness (Bone, Balk, et al., 1992). Host's homeostasis in MODS cannot be maintained without intervention for a period of time, but full recovery is possible. MODS is not a dual entity and represents a spectrum of severity. There is a higher risk of mortality with an increasing number of failed organs (Moreno et al., 1999). MODS can be quantified using scoring systems. The three most popular are Multiple Organs Dysfunction Score (Marshall et al., 1995), Logistic organ Dysfunction System (Le Gall, 1996) and Sequential Organ Failure Assessment Score (SOFA) (Vincent et al., 1996). The use of these scoring systems has been stimulated by the interest of surrogate outcomes in intensive care trials. Of the above mentioned, SOFA score is the most widely used, as it is easy to calculate and has proven predictive value for in-hospital mortality. SOFA score was developed by an expert working group to quantify multiple organ dysfunction using physiological variables.

Table 1.2

Sequential Organ Failure Assessment Score (Vincent et al., 1996)

SOFA Score	1	2	3	4
Respiration PaO_2/FiO_2, mm Hg	< 400	< 300	< 200 with respiratory support	< 100
Coagulation Platelets, $x10^{-9}/L$	101–150	51–100	21–50	0–20
Liver Bilirubin, µmol/l	20–32	33–101	102–204	> 204
Central Nervous System Glasgow Coma Score	13–14	10–12	6–9	< 6
Cardiovascular Hypotension	> 70 mm Hg	Dopamine ≤ 5.0 or dobutamine (any dose)[a]	Dopamine 5-14.9 or Adrenaline ≤ 0.1 Noradrenaline ≤ 0.1[a]	Dopamine ≥ 15 or Adrenaline > 0.1 or Noradrenaline > 0.1 [a]
Renal Creatinine, µmol/l	110–170	171–299	300–440	> 440
Or urine output			Or < 500 mL/24h	Or < 200 mL/24h

[a] Adrenergic agents administered for at least 1 hr (doses given are in µg/kg/min)

Several uses of SOFA score are possible in clinical studies:

- Fixed day SOFA – usually reported on the day of inclusion into the trial;
- Delta SOFA, which is the score on a predefined day after inclusion minus baseline score;
- Maximum SOFA score during intensive care stay.

It is known that SOFA trajectory correlates with treatment effect on mortality (de Grooth et al., 2017), so delta SOFA is preferred in clinical trials to compare effects in different arms. All the uses of SOFA have similar predictive power for mortality in observational cohorts (Minne et al., 2008; Moreno et al., 1999).

1.1.5 Sepsis epidemiology

Complexity of estimating population and hospital-level incidence of sepsis is reflected by major differences quoted in literature. Incidence varies depending on the definition of sepsis used, methodology employed and study population. Before 1992, there was no consensus definition of sepsis, so estimates from older studies are difficult to use for comparison. Up until now, administrative discharge databases, which are valuable sources of epidemiological data, use their own definition of sepsis for coding purposes. ICD-10 defines sepsis as the presence of bacteria or their toxins in the blood or tissues. None of expert consensus definitions (1991, 2001 and Sepsis-3) requires infection to be of bacterial origin. Cases of viral and fungal sepsis are therefore missed. The crucial criterion of the host response is also not included in the ICD-10 definition. The data about sepsis incidence in the Baltic states drawn from public health databases are shown in Table 1.3.

Table 1.3

Incidence of sepsis in the Baltic States (administrative data)

Country	Latvia	Estonia	Lithuania
Coding	ICD-10; A40–41	ICD-10; A40–41	ICD-10; A40–41
Source	Centre for Disease Prevention and Control	Health Statistics and Health Research Database	The Institute of Hygiene
Population	306,455	243,134	667,475
Number of cases	331	459	2062
Annual incidence per 1000 hospital discharges	0.2	0.3	0.73
Hospital mortality	13.6 %	8.95 %	30.8 %

ICD – International Classification of Diseases

Special algorithms designed to identify patients according to international consensus criteria in administrative data have been employed. The most often quoted incidence of sepsis using ICD code-based methodology comes from 2001, where Angus et al. estimated that in the United States incidence of sepsis is 3 per 1000 population and hospital mortality of 28.6 % (Angus et al., 2001). Retrospective data from Norwegian Patient Registry show that 140 per 100000 inhabitants are hospitalized with sepsis annually, with 19.4 % dying from the disease (Knoop et al., 2017). Sepsis incidence in ICD code-based studies is shown in Table 1.4.

Table 1.4

Characteristics of studies using ICD codes

	Angus et al., 2001	Flaaten, 2004	Wilhelms et al., 2010	Knoop et al., 2017
Country	USA	Norway	Sweden	Norway
Coding	ICD-9	ICD-10	ICD-9/10	ICD-10
Source	Constructed database	NPR	SHDR	NPR
Study population	6,621,559	700,107	2,024,793	1,198,160
Number of cases	192,980	6,665	37,990, 27,655, 12,512	13,582
Year	1995	1995	1997; 2005	2011–2012
Annual incidence per 1000 hospital dischages	226	149	10; 35 25; 43 3; 13	140
Mortality	28.6 %	n/a	n/a	19.4 %

ICD – International Classification of Diseases
NPR – Norwegian patient register
SHDR – Swedish hospital discharge register

Retrospective and prospective studies that identify sepsis patients according to a consensus definition using patient charts generally find a lower incidence of severe sepsis but a higher incidence of sepsis than diagnostic code-based ones (Mariansdatter et al., 2016). Summary of severe sepsis incidence in Northern Europe is shown in Table 1.5. Available chart-based data from the Baltics is limited. Data from the case file audit conducted by the Estonian Health Insurance Fund in 2013–2014 shows hospital mortality of 42.4 % for hospitalized sepsis patients (Paasma and Starkopf, 2017). Prospective study from Pauls Stradiņš Clinical University Hospital showed that an average of 18 patients with community-acquired severe sepsis were hospitalized in 2016 and hospital mortality was 66.7 % (Žilde, 2017).

Table 1.5

Characteristics of patient record-based studies in Northern Europe

	Karlsson et al., 2007	Vesteinsdottir et al., 2011	Nygard et al., 2014	Henriksen et al., 2015
Country	Finland	Iceland	Norway	Denmark
Population	4500	1524		8358
Number	472	115	220	1071
Year	2005	2009	2008	2011
Severe sepsis incidence per 100 000 person/years	38	48	50	457

Although heterogeneity of studies regarding sepsis epidemiology makes generalizations difficult, meta-analysis of 27 studies by Fleischmann et al., estimated that in the last 10 years incidence of sepsis was 437 and of severe sepsis 270 per 100 000 person-years (Fleischmann et al., 2016).

1.1.6 Cardiovascular dysfunction in sepsis

Endotoxin, a bacterial lipopolysaccharide, plays a major role in pathogenesis of sepsis. Administration of endotoxin to healthy volunteers allows to investigate mechanisms that are activated in the earliest stages of sepsis. Detailed studies using intravenously administered endotoxin have been performed showing that maximum cardiovascular effects occur 3 hours after endotoxin administration and manifest as increased cardiac output, heart rate and decreased total peripheral resistance (Moser et al., 1963). Studies of extremity blood flow using strain gauge plethysmography in volunteers did not show significant changes after exposure to endotoxin (Fong et al., 1990).

Studies of intensive care patients show that in early sepsis cardiovascular function is characterized by decreased peripheral vascular resistance and a normal or high cardiac output. As early sepsis progresses to septic shock, blood pressure falls and the ability of the vasculature to respond to vasopressors can be impaired (Greer, 2015). Recognition of bacteria by immune cells results in activation of nuclear factor kappa B which in turn stimulates tumor necrosis factor-alpha release, as well as, induced NO synthase and cyclooxygenase-2 expression. The result of this cascade is myocardial depression and impaired vasoconstriction due to direct effects on vascular smooth muscle (Danner et al., 1991). Multiple studies have investigated cardiac function in septic shock in patients and animal experiments (Merx and Weber, 2007). Studies regarding the rest of vasculature have mainly concentrated on arteriolar tone which is described by peripheral vascular resistance, a measurement obtained from measurements of cardiac output using thermodilution. In addition to the mechanisms described above, downregulation of adrenergic receptors plays a role in causing low peripheral vascular

resistance. Catecholamines participate in regulation of arterial blood pressure through alpha-1 receptors. Subtypes of these receptors can be found in both large conduit and small resistance arteries. Exposure of blood vessels to inflammatory mediators leads to their downregulation and reduces the capacity of binding to noradrenaline (Burgdorff et al., 2018).

There is limited research on large artery elastic properties in septic patients. These arteries receive the pulsatile component of blood flow generated by the left ventricle. Their elastic properties ensure optimal coupling between the left ventricle and peripheral circulation and can be characterized by properties of pulse wave propagation and reflexion.

1.2 Vascular endothelium

1.2.1 Normal endothelium

Endothelium is a single layer of squamous epithelium which lines the blood and lymphatic vessels. Together with supporting collagenous subendothelial tissue it constitutes tunica intima in the circulatory system. Endothelium has a large surface area and serves to provide an interface between blood and tissues.

Endothelium is both structurally and functionally heterogeneous across the vascular tree, which makes research of endothelial dysfunction challenging (Aird, 2007). Arteries are lined with continuous non-fenestrated endothelium with flat, 1μm thick, elongated cells. Stability of the endothelial layer is provided by tight junctions between cells (*zonula occludens*). Branching points of arteries experience turbulent flow, endothelial cells at these points are prone to activation and expression of inflammatory and procoagulant properties. In veins, the continuous endothelial cells are shorter and wider. When activated, they display powerful inflammatory properties (Eriksson et al., 2005). In microcirculatory vessels, especially postcapillary venules, relative lack of tight junctions allows white blood cells and plasma to extravasate during inflammation. Endothelium at this level is highly organ-specific and can be continuous fenestrated or non-fenestrated and discontinuous.

Endothelial cells have diverse physiological functions to maintain homeostasis. They regulate haemostasis, blood cell adhesion and migration into the tissues, vascular permeability, inflammatory response, microcirculation, blood pressure (via arterial tone) and angiogenesis (Feietou and Vanhoutte, 2006). Large surface area exposes endothelium to circulating substances (cytokines, autacoids, medication) and physical stimuli (shear stress, changes in pressure) and it responds by synthesis and release of a range of factors. Under normal conditions endothelium:

1. inhibits coagulation by expressing thrombomodulin and proteoglycans which bind, accordingly, to thrombin and antithrombin III, and inhibiting platelet adhesion by producing nitric oxide (NO) and prostacyclin (PGI2);
2. does not stimulate leucocyte adhesion, as only a small number of adhesion molecules are expressed;
3. regulates microcirculation efficiently and decreases blood pressure.

The most significant endothelium-derived molecule responsible for regulation of vascular tone is NO which is continuously produced by vascular endothelial cells. Endothelial derived NO relaxes smooth muscle, inhibits platelet function and inflammatory responses. Endothelial cells regulate microvascular smooth muscle tone depending on shear stress imposed by flow. Basal production of NO can be enhanced by specific agonists (e.g., acetylcholine) or shearing forces on the cell surface (occurs with increased blood flow) and cytokines (during inflammation or infection). NO is the most important vasodilator molecule in muscular arteries but in microcirculatory vascular beds, vasodilation can be produced by NO independent mechanisms – PGI2 and a range of substances which hyperpolarize vascular myocyte by acting on voltage channels. PGI2 is a product of arachidonic acid metabolism within endothelial cells. It causes smooth muscle relaxation and inhibition of platelet aggregation and is proven to mediate vasodilatation induced by shear stress (Koller et al., 1993). Its role in vascular response to circulating substances is minimal (Feietou and Vanhoutte, 2009).

In health, endothelium maintains the balance between dilation and constriction of vascular smooth muscle, stimulation and inhibition of thrombus formation, and stimulation and inhibition of angiogenesis. Endothelial dysfunction is a pathological state of the endothelium when the balance of these processes is deranged.

1.2.2 Endothelial activation during inflammation

Endothelium plays an important role in both, physiologic reaction to infection and its progression to systemic pathological host response, sepsis. As part of the innate immune system, endothelial cells become activated when they interact with components of bacterial wall, such as lipopolysaccharide, and cytokines, such as tumour necrosis factor – α, interleukins 1α and 1β, and activated complement. Endothelial dysfunction causes less NO and PGI2 being released, which results in loss of vasodilatory capacity, thrombosis, vascular inflammation, and increased capillary permeability.

Activated endothelium expresses tissue factor with activation of coagulation and suppression of fibrinolysis, resulting in a procoagulant state. The physiologic purpose of local

endothelial activation is to limit the spread of infection by localized inflammation and coagulation.

Interaction between endothelial cells and leucocytes is initiated by the expression of P-selectin, this facilitates leucocyte rolling along the cell surface (Ley et al., 2007). Ongoing endothelial cell activation induces synthesis of adhesion molecules (ICAM 1-5, VCAM-1) from integrin family which are necessary for firm adhesion and migration of neutrophil leucocytes from the bloodstream into interstitium (Langer and Chavakis, 2009) (Figure 1.2).

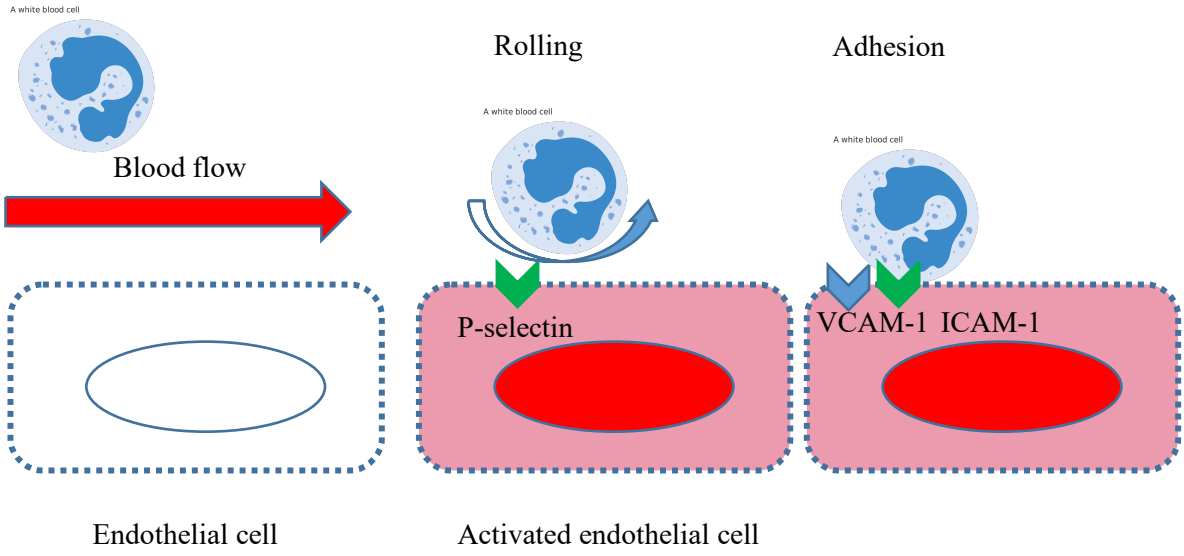

Figure 1.2 **Endothelial response in sepsis**
Expression of P-selectin in activated endothelial cells induces rolling of circulating white blood cells. VCAM-1 and ICAM-1 induce firm leucocyte adhesion

Regulation of arterial tone is significantly altered by inflammatory mediators secondary to changes in synthesis of NO and PGI2. In volunteer studies, exposure to endotoxin impairs endothelium-dependent vasoreactivity for at least 48 hours (Bhagat, Collier, et al., 1996; Bhagat, Moss, et al., 1996). When exposed to tumour necrosis factor – α and interleukin 1, endothelial cells also start producing inducible NO synthase (Vincent et al., 2000). This enzyme produces large amounts of NO and that likely explains hypotension in sepsis (Vincent et al., 2000).

Activated endothelium produces multiple inflammatory mediators, such as platelet activating factor, interleukin-6 and complement factors.

In sepsis, endothelial activation causes generalized proinflammatory and procoagulant state. The actual features (changes in prostaglandin, adhesion molecule production, coagulation, reduced bioavailability of NO) constituting the activated endothelial phenotype differ depending on the nature and concentration of stimulus and vascular bed.

1.2.3 Endothelial dysfunction

Endothelial activation is an important step in the development of dysfunction (Satta, 2004), but the link between inflammation, oxidative stress and endothelial dysfunction is not well understood. Interleukin-6 (IL-6) is an alarm cytokine which can be produced by many types of cells in circumstances when these cells are in danger. In animal models of sepsis IL-6 levels peak after 2–4 hours due to production of this cytokine by monocytes and macrophages. In lethal group, IL-6 levels continue to rise further, suggesting production by endothelial cells injured by inflammation and activation of coagulation (Hack and Zeerleder, 2001). Morphologic destruction caused by lipid peroxidation under oxidative stress and loss of tight junctions between cells results in vacuolization of the nucleus, fragmentation of cytoplasm, and areas of endothelial denudation.

The alarm production of IL-6 by dysfunctional endothelium precedes development of MODS suggesting that endothelial dysfunction is its trigger. Denuded blood vessels lose their vasoreactivity, the result of which is impaired microcirculatory perfusion with organ dysfunction. This is supported by data from animal models of sepsis which shows that prevention of endothelial dysfunction ameliorates capillary leak and MODS (Kumpers et al., 2011; Han et al., 2016).

It can be difficult to distinguish between endothelial activation and dysfunction. Endothelial activation in sepsis is represented by changes that vasculature undergoes to help with clearance of invading pathogens and is characterized by preservation of least some of the physiologic functions of endothelium. In endothelial dysfunction all roles of endothelium are affected, most importantly disruption of barrier function causes capillary leak.

Endothelial dysfunction has been associated with pathogenesis of diverse conditions, such as cardiovascular and inflammatory disease, including vasculitis, infections, sepsis and rheumatoid arthritis. The common pathophysiologic pathway for the development of endothelial dysfunction is thought to be increased oxidative stress (Griendling and FitzGerald, 2003).

1.2.4 Measurement of endothelial dysfunction

Understanding the central role of endothelium, particularly in the development of atherosclerotic disease, has led to the development of a range of methods to test different aspects of endothelial damage and repair (Table 1.6). As endothelium is heterogeneous in terms of structure and function no single marker can be used. A range of functions can be assessed biochemically by measuring markers in the blood, others require functional tests. There is

expert consensus that functional tests are a more reliable measure of endothelial dysfunction than biochemical markers (Lekakis et al., 2011b).

Measurement of vasodilation in response to stimuli that increase bioavailability of NO and other endothelium-derived relaxing factors forms the basis of endothelium function tests. Endothelium dysfunction is a systemic process present across all vascular beds. Therefore, impaired peripheral artery vasodilation can serve as a surrogate of endothelial dysfunction in vascular beds that are more difficult to test, such as coronary circulation (Anderson et al., 1995).

Table 1.6

Summary of biomarkers of endothelial dysfunction

Property	Function	Marker
Selective permeability	Prevention of oedema formation	VEGF, sFlt-1
Non-thrombogenic barrier	Secretion of anticoagulant	Thrombomodulin PAI
Modulation of vascular resistance	Vasodilation in response to acetylcholine/shear stress	Reactive hyperemia
Regulation of immune response	White blood cell adhesion and migration	E-selectin, adhesion molecules (sICAM-1, sVCAM-1)
Maintenance of extracellular matrix	Synthesis of glycocalyx	Syndecan
Mediation of endothelial function	Endothelial cell quiescence	Angiopoietin-1 Angiopoietin-2

VEGF – vascular endothelial growth factor; sFlt-1 – soluble fms-like tyrosine kinase-1; PAI – plasminogen activator inhibitor; sICAM-1 – soluble intercellular adhesion molecule-1; sVCAM-1 – soluble vascular cell adhesion molecule-1

The first report demonstrating impaired vasodilation of coronary arteries in atherosclerosis in response to acetylcholine infusion was published in 1986 (Ludmer et al., 1986). Over the last 30 years, several methods have been developed to test endothelial function in brachial and radial arteries:

A. Venous occlusion plethysmography

This technique measures changes in total forearm blood flow in response to intra-arterial infusion of vasoactive substances. Venous air or mercury strain gauge plethysmography is used to quantify changes in volume of the forearm, and the result is expressed as ratio using pre- and post-infusion values. The contralateral arm can be used as internal control. This technique is invasive but highly reproducible, it can provide valid results even with small sample sizes. The use of acetylcholine and nitroglycerine allows to measure endothelium-dependent and independent vasodilation separately. The results reflect both the conduit artery and microcirculatory endothelial function.

B. Flow mediated dilatation (FMD) of the brachial artery

This technique uses 3 to 5-minute occlusion of the brachial artery with a sphygmomanometer cuff to induce reactive hyperaemia (Celermajer et al., 1992). The increase in shear stress caused by the release of occlusion provokes endothelium-dependent release of NO and other vasodilators. The resulting change in brachial or radial artery diameter is measured by ultrasound. This technique is non-invasive, but precision of results depends upon availability of skilled operators. This highly standardized method evaluates endothelial function in conduit arteries. For results to be reproducible over time and comparable across studies, the procedure of measurement and preparation of patients (fasting, period of rest before measurement) has to be very rigorous (Ghiadoni et al., 2012). This is difficult to achieve in an intensive care environment.

C. Finger plethysmography

Another non-invasive technique which makes use of arterial occlusion induced reactive hyperaemia to test endothelial function is peripheral arterial tonometry (EndoPAT) (Kuvin et al., 2003). EndoPAT utilizes a finger plethysmography probe to track pulsatile blood volume changes. Finger probes are attached to both arms and baseline measurements recorded. After the provocation of reactive hyperaemia in one arm, reactive hyperaemia index is calculated as the ratio between volume changes in the finger with reactive hyperaemia and control finger. Extent of reactive hyperaemia using EndoPAT is easy to measure at the bedside, used standard algorithm and is operator-independent. Reactive hyperaemia index depends on various local, systemic and environmental factors, only one of which is the bioavailability of NO. It does not differentiate between endothelium-dependent and independent vasodilation. This method is not suitable to detect changes in repeated assessment of endothelial function within a short time frame in small groups as is done in intensive care studies (Moerland et al., 2012).

D. Laser Doppler flowmetry (LDF)

Laser Doppler flowmetry measures skin microvascular blood flow. The method is based on the measurement of Doppler shift caused by moving erythrocytes and depends on their velocity. Microvascular blood flow is not measured directly but expressed in perfusion units. It can measure rapid changes in skin blood flow, therefore, is useful for measurement of post occlusive reactive hyperaemia. Acetylcholine iontophoresis can be used to provoke biphasic hyperaemic response and allows to differentiate between endothelium-dependent and independent vasodilation. The protocols for measuring endothelial function using this method are not standardized. Reproducibility of microvascular skin blood flow measurement using this technique is relatively poor (Roustit et al., 2010).

E. Pulse wave velocity (PWV)

Endothelial function is one of the main determinants of arterial stiffness. Decrease in distensibility of a vessel leads to increased PWV. Increased bioavailability of NO has been shown to reduce arterial stiffness in large arteries (Wilkinson et al., 2002), whereas inhibition of NO synthesis increases it (Stewart et al., 2003). Aortic pulse wave velocity is highly predictive of future cardiovascular complications and mortality in the general population, as well as in patients with hypertension, diabetes and kidney disease (Vlachopoulos et al., 2010). The reproducibility of this method is unclear.

1.2.5 Measurement of endothelial dysfunction by vascular reactivity in sepsis

Clinical utility of vascular reactivity tests for diagnosis or prognostication of septic patients in the acute setting has been explored by a small number of studies. The main emphasis in these studies is to clarify if vascular reactivity tests are useful to detect patients at risk of organ hypoperfusion and as a guide to resuscitation.

However, the measurement of reactive hyperaemia in sepsis remains a research tool, and the association between altered vascular reactivity and severity of sepsis with respect to the development of multiple organ failure and mortality is not well established.

One of the objectives of this thesis is to conduct a systematic review and meta-analysis of the currently published literature to provide a summary of existing research and evaluate if the adult population presence/severity of sepsis is associated with the conduit artery and microvascular dysfunction as measured by provocation tests.

1.3 Systemic arteries

1.3.1 Structure and function of arteries

Anatomy of large arteries is depicted in Figure 1.3.

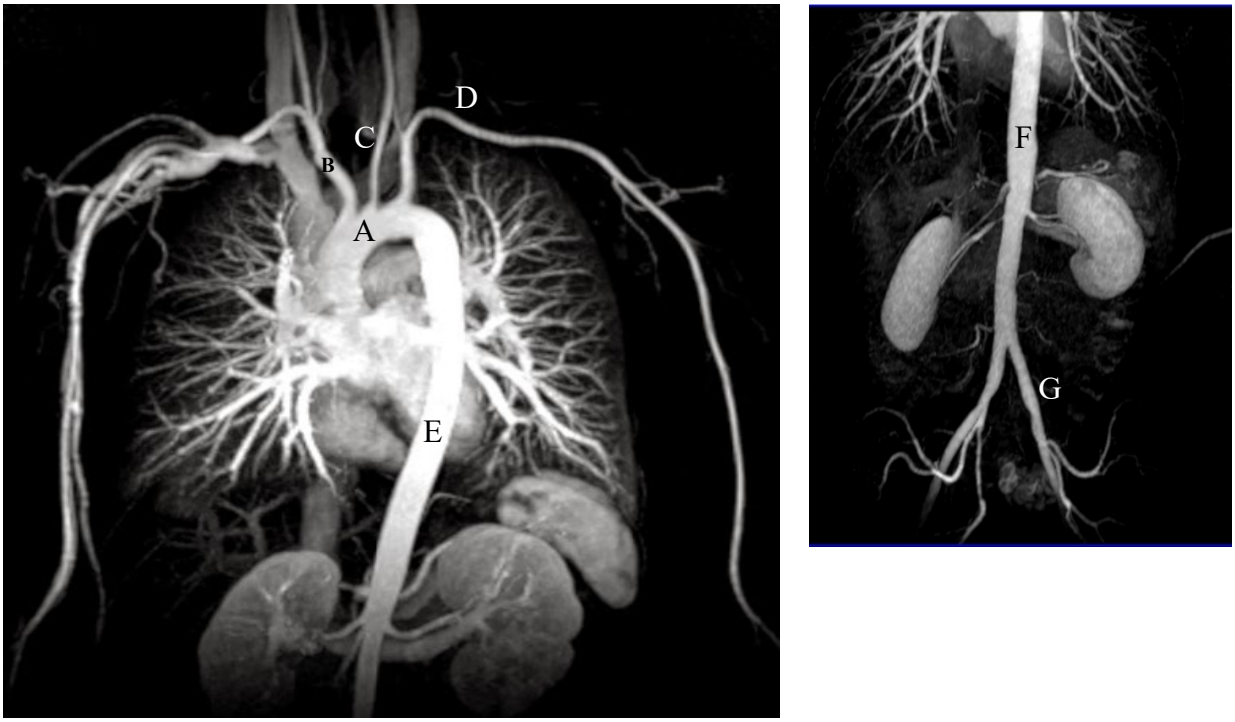

Figure 1.3 **Magnetic resonance imaging of aorta and large vessels**
A – Aortic arch, B – brachiocephalic trunk (further subdivides into right subclavian and right carotid arteries), C – left common carotid artery, D – left subclavian artery, E – thoracic aorta, F – abdominal aorta, G – iliac artery

Aorta originates from the left ventricle of the heart at the aortic valve. Subsequently, it turns at 180° angle forming aortic arch. Aortic arch gives off major branches in the following order: brachiocephalic trunk (further subdivides into right subclavian and right carotid arteries), left common carotid artery and left subclavian artery. Aorta runs downwards through the diaphragm into the abdomen, following a straight course and terminates by dividing into iliac arteries. Aorta tapers along its course and the angle of tapering varies between individuals. Aorta gives off multiple branches along its length in an asymmetrical manner with variable angles of branching and branching ratios. The large vessels closest to the aorta easily accessible for measurements in vivo are the right and left carotid arteries in the neck and the femoral arteries which are the continuations of iliac arteries in the groin.

Arteries have two main functions:

- They serve as conduits to distribute blood coming from the heart to the capillaries in various organs according to metabolic demand;
- They transform pulsatile blood flow into continuous microcirculatory flow.

Blood forced by contraction of the left ventricle causes expansion of the arterial wall during systole. During diastole the walls of the arteries recoil, which helps to maintain pressure in the arteries between beats. Elastic tissue within arterial walls serves to provide the expansion and recoil.

Walls of arteries have a three-layer structure (Figure 1.4). Depending on the amount of elastin and the thickness of the smooth muscle arteries can be classified into:

1. Elastic arteries (e.g., aorta, common carotid arteries, subclavian arteries, pulmonary arteries);
2. Muscular arteries (e.g., radial, femoral, coronary and cerebral arteries).

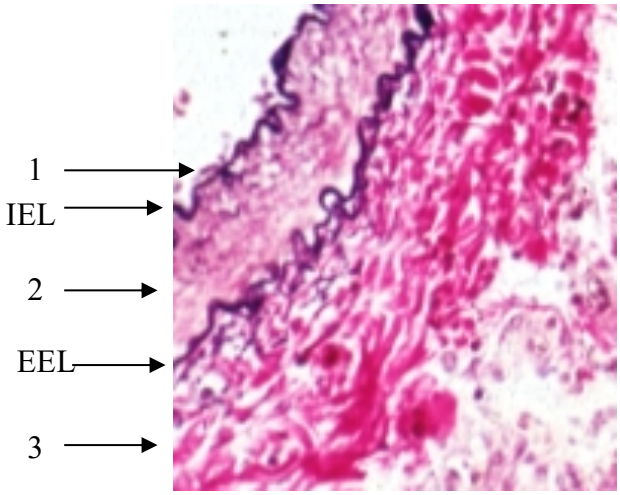

Figure 1.4 **Muscular artery (Verhoeff Van Gieson's elastin; original magnification, x25)**
1 – Tunica intima, not visible at low magnification, 2 – Tunica media composed of smooth muscle fibres with some elastic elements between them, 3 – Adventitia composed of collagen and elastic tissue
In muscular arteries, the elastic tissue is largely concentrated in two elastic sheets: internal (IEL) and external elastic lamina (EEL)

Arteries are lined with endothelial cells. *Lamina propria*, made of connective tissue, provides structural stability to endothelium. This layer contains fibroblasts and myointimal cells that produce collagen. Underneath lies internal elastic lamina made from elastin. Endothelial cells, lamina propria and internal elastic lamina form *tunica intima*.

Under this layer lies *tunica media*. In elastic arteries, it consists of concentric sheets of elastin. In aorta and its primary branches, this layer is extremely elastic to counter the load imposed by cardiac contraction and direct the force of contraction along the vessel rather than increasing its diameter (*Windkessel* effect). In muscular arteries, *tunica media* contains concentric layers of smooth muscle cells. In response to mechanical load and signalling molecules they can change their tone, regulate blood flow to the tissues and blood pressure.

The external layer is called *tunica adventitia*, it is composed of connective tissue. External elastic lamina is much less developed (Young et al., 2013). In large elastic arteries, this layer also contains feeding vessels (*vasa vasorum*).

1.3.2 Propagation of pressure wave in arteries

Pressure wave originates in the aorta as blood is ejected into it from the left ventricle. As a result, pressure in the aorta rises and it expands. This causes stretching of the aortic wall and an increase in wall tension. Towards the end of systole, the rate of ejection starts to fall, leading to a decrease in aortic pressure and return of baseline aortic diameter. Because of inertia, blood in the aorta keeps moving forward even without a pressure difference and causes the inward motion of the aortic wall to overshoot. Thus, the oscillatory movement of the aortic wall is initiated. The same sequence is propagated along the arterial tree as a pressure wave.

During this physical process, balance is kept between force generated by elasticity of arterial wall to restore baseline diameter and inertia of the blood. Inertia can be characterized by blood density and arterial elasticity expressed as effective incremental Young's modulus of the arterial wall. Young's modulus represents the relationship between stress and strain of the vessel in longitudinal direction:

$$E = \frac{Longitudinal\ force\ per\ unit\ area}{Extension\ \ per\ unit\ length} \qquad (1.3.2.1)$$

ρ – blood density,

E – effective incremental Young's modulus of the arterial wall.

Young's elastic modulus can be used to calculate wave propagation speed along an artery, using Moens-Korteweg equation, which was developed to calculate the velocity of a pressure wave in rubber tube filled with water:

$$c = \sqrt{\frac{Eh}{2R\rho}} \qquad (1.3.2.2)$$

c – pulse wave velocity,

E - Young's elastic modulus,

h – wall thickness,

R – artery radius,

ρ – density of blood.

When compared with experimentally measured values of pulse wave velocity, calculated values differ by no more than 15 % (Caro et al., 2012).

To recreate the pressure wave, the arterial tree can be modelled as an elastic tube, representing elastic arteries, which ends at peripheral resistance representing muscular arteries. The elastic properties of the tube allow generation of pressure wave which travels along it, as described above. If the wave is recorded simultaneously at the proximal and distal end of the tube, the delay in the arrival of the foot of the wave can be observed. Peripheral resistance at the distal end of the tube reflects pressure waves and retrograde wave is generated. With normal arterial distensibility, these waves return to the proximal end of the aorta during diastole. On the pressure waveform wave reflections can be seen as secondary waves and they result in pressure difference between central and peripheral arteries. From this model, it can be seen that the decrease in distensibility of conduit arteries will cause not only an increase in pressure wave amplitude (pulse pressure) but also an increased velocity of reflected waves. Reflected waves will return during ventricular systole causing augmentation to late systolic pressure.

1.3.3 Haemodynamic consequences of artery viscoelastic properties

Both processes – wave propagation and reflection – are determined by the mechanical properties of the conduit artery wall. In young healthy individuals, the aorta can accommodate pulsatile flow from the heart and transform it into continuous blood flow. The peak of pressure wave is determined by the forward wave generated by ventricular contraction. In stiffer arteries increase in pulse wave velocity causes significant alteration of pressure waveform and arrival of reflected waves from the periphery during systole.

Studies of pulsatile arterial haemodynamics show that the blood pressure wave can be subdivided into two components:

1. steady, which is quantified by mean arterial pressure;
2. pulsatile, which is quantified by pulse pressure.

Pulse pressure is generated by summation of forward pressure wave resulting from left ventricular contraction and reflected waves returning from resistance vessels. Pulse pressure increases markedly from central to peripheral arteries and systolic pressure can increase significantly along the arterial tree. At the same systolic volume, pulse pressure is influenced by arterial stiffness and the timing of reflected waves. Arterial stiffening increases systolic pressure and widens pulse pressure.

In clinical practice, estimation of complex blood pressure wave is usually reduced to measuring systolic and diastolic arterial pressure by brachial cuff. Only two numeric values are used to make decisions about clinical management and adjustment of treatment and most of the

information from the whole of the blood pressure curve is lost. As hypoperfusion caused by systemic hypotension is a key event in pathogenesis of multiple organ dysfunction in septic patients, defining characteristics of blood pressure wave as a risk factor for impaired organ perfusion in sepsis is a major clinical problem. To better describe the blood pressure curve, intensive care physicians use mean arterial pressure, which is the average pressure throughout the cardiac cycle but there is still scope to utilize information from the blood pressure wave for risk stratification and treatment decisions of septic patients for development of MODS and mortality.

1.3.4 Definitions of arterial elastic property indices

Arterial elastic property indices describe the ability of arteries to accommodate the volume of blood ejected from the heart. There are multiple ways to define the viscoelastic properties of arteries. Some indices, such as arterial compliance, describe arteries as tube-like structures, while others, such as Young's elastic modulus, are independent of vessel geometry. The definitions of most frequently used indices and ways of measuring them under conditions of intact circulation and pulsatile flow are shown in Table 1.7.

Table 1.7

Definitions of arterial elastic property indices (Modified from (from O'Rourke 1995)

Term	Definition	Measurement
Arterial compliance	Absolute change in radius, diameter, flow or cross-sectional area for a given change in pulse pressure	Ultrasonography, MRI
Arterial distensibility	Relative diameter change for a given change in pulse pressure	Ultrasonography, MRI
Incremental elastic (Young) modulus	The pressure difference required per square cm for 100 % stretch from resting length	Ultrasonography, MRI
Pulse wave velocity (PWV)	Speed of travel of pressure wave along a segment of artery	Pressure or volume waveform, ultrasonography, MRI

MRI – magnetic resonance imaging

1.3.5 Mechanisms of arterial stiffness

Arterial stiffness and PWV are determined by structural, functional and genetic factors. Structural factors associated with increased arterial stiffness include anatomic location, aging, vascular calcification and inflammation (Dregan, 2018). Arterial stiffness is not equal along the arterial circulation (Latham et al., 1985), there is a progressive increase of stiffness with increasing distance from the heart. PWV is high and significantly correlates with age, most

likely due to elastin degradation and fatigue (Parikh et al., 2016). Studies show that inflammatory conditions such as lupus erythematoides (Ding et al., 2016), rheumatoid arthritis (Anyfanti et al., 2018) and vasculitis (Booth et al., 2004) are associated with increased PWV. One of the explanations of this phenomenon is accelerated arterial calcification secondary to chronic vascular inflammation, but it has been also shown that anti-inflammatory therapy improves arterial stiffness (Mäki-Petäjä et al., 2006), so it is most likely determined by reversible processes. Even in healthy population. subclinical inflammation leads to changes in arteries as there is a well-described association of arterial stiffness and blood C reactive protein levels (Kullo et al., 2005; Nakhai-Pour et al., 2007).

Functional factors associated with increased PWV are distending pressure, arterial smooth muscle tone, levels of NO and endothelial function. Distending pressure in the arteries affects arterial stiffness in a dynamic way. Elastin is less stiff than collagen and at low distending pressure; elastin is the main fibre responsible for resistance to stretch. At higher pressures, collagen fibres are recruited which results in a non-linear increase in stiffness (Dobrin and Canfield, 1984). Endothelial function exerts its effect on arterial function through its ability to regulate smooth muscle tone by NO-dependent and independent mechanisms. Endothelial effect on stiffness is more pronounced in smaller arteries as they have a well-developed muscular layer. Endothelial dysfunction assessed by brachial artery flow-mediated dilatation has been shown to be associated with increased large artery stiffness in healthy individuals (McEniery et al., 2006).

Chemical signals are important determinants of PWV. Local nitrate administration, which is a donor of NO, decreases stiffness of the iliac artery in vivo (Schmitt et al., 2005). Some substances can have opposing effects on arterial stiffness depending on endothelial health. In healthy individuals, acetylcholine usually causes endothelium-dependent vasodilatation but induces constriction in vessels with endothelial dysfunction or activation (Furchgott and Zawadzki, 1980).

Variations in genes coding for various proteins (collagen, fibrillin) which influence structural and functional determinants of arterial stiffness make it a moderately heritable trait (Brull et al., 2001). Using genome-wide scan in the families involved in Framingham Heart Study, strong evidence was found of association of PWV and a locus on chromosome 14 (D. Levy et al., 2000).

1.3.6 Measurement of vascular stiffness

Systemic arterial stiffness cannot be measured but is calculated using mathematical modelling of circulation. Direct measurement of arterial viscoelastic properties is only possible in vitro and animal research; therefore, indirect methods are used to estimate regional and local artery stiffness in humans.

Arterial distensibility, compliance and incremental (Young's) elastic modulus can be estimated using MRI and ultrasonography as these methods allow to estimate changes in artery diameter during the heart cycle. MRI allows assessment of the aorta, which is inaccessible by other methods, but it is impractical in critically ill patients due to restrictions of using equipment such as infusion devices and ventilators in a strong magnetic field. Ultrasound, although limited to superficial vessels, can be used by the bedside, but has high interobserver variability and requires highly skilled operators.

The most frequently used methods for measuring vascular stiffness utilize artery pressure or volume waveforms to determine PWV. PWV is dependent on vessel distensibility which is inversely related to stiffness, as follows from Moens-Korteweg equation (1.3.2.2). It is measured by timing the arrival of the pressure wave at two different points of the arterial tree at a known distance from each other. The wave speed between carotid and femoral artery is accepted as representative for regional aortic wave velocity. Aortic PWV is considered the gold standard measurement of arterial stiffness by European Network for Non-invasive Investigation of Large Arteries (Laurent et al., 2006). Carotid-radial PWV, which represents mainly brachial artery stiffness, is of interest as it the artery where blood pressure is usually measured. It is more likely to be influenced by endothelial nitric oxide (eNO) production than aortic PWV, and as vascular dysfunction in sepsis is functionally linked with basal eNO production, it is likely to be more influenced by sepsis.

Different methods can be used to record pulse waves in arteries as time-series data to obtain a mathematical and graphical representation of the pulse wave. The methods of recording pulse waves that have been described in the literature include pressure-sensitive transducers, Doppler ultrasound, applanation tonometry, and photoplethysmography. The pulse wave is analysed to identify the landmark points necessary for time delay measurement (Figure 1.5).

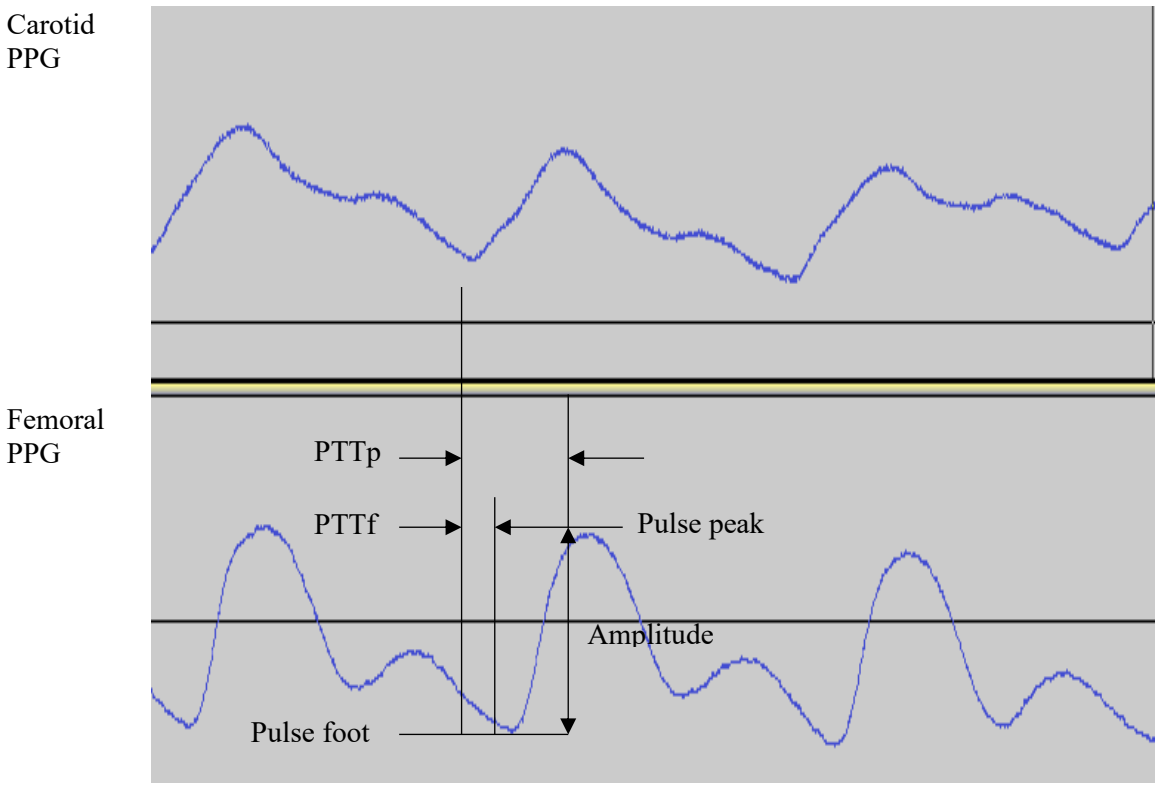

Figure 1.5 **Pulse landmarks for measurement of carotid-femoral PWV with the foot to foot method**

PTTf – beat-to-beat pulse transit time to the foot of the pulse, PTTp – pulse transit time to the peak of the pulse

The time delay of the pulse wave should be calculated from the foot or the up-slope of the wave rather than the systolic part. Estimation of pulse delay from peak of the pulse can lead to overestimation of PWV as arteries are stiffer at higher pressures and augmentation from reflected waves is possible at peak systole. The distance pulse wave travels between the two sites of recording is usually measured from the body surface. PWV is calculated:

$$PWV = \frac{D}{\Delta t} \qquad (1.3.2.3)$$

where D – distance in meters,

Δt – transit time in seconds.

The main sources of error when measuring carotid-femoral PWV are difficulties of recording femoral pulse in patients with obesity and peripheral artery disease, and inaccuracies in distance measurements in patients with abdominal obesity or large breasts. The main challenge in interpreting PWV measurements in vivo under pulsatile flow conditions is that incremental elastic modulus of the arterial wall, and, consequently, PWV is influenced by multiple factors:

1. pressure oscillations with concomitant changes in arterial diameter, wall thickness and degree of distension (Smith et al., 1999);

2. changes of smooth muscle tone, which can be myogenic due to distension (Markos et al., 2012), neurogenic due to sympathetic stimulation, or flow and endothelium mediated (Joannides et al., 2006).

Yet, for clinical and research applications carotid-femoral PWV is the most robust measurement of arterial stiffness and extensive data to support its predictive value in a range of conditions (Vlachopoulos et al., 2010).

1.3.7 Photoplethysmography

Photoplethysmography is a non-invasive optical method based on absorption and reflection of light in biological tissue. Photoplethysmography technology consists of a light source (laser or light-emitting diode (LED)) and a signal detector (photodiode). The amount of light absorbed is proportional to changes in the volume of blood in the illuminated tissue. Fluctuations in blood volume occur with each pulse wave. The signal recorded is called photoplethysmogram (PPG). PPG (Figure 1.6) consists of the pulsatile component (AC) which occurs synchronous with the changes in blood volume and relatively stable baseline component (DC). The AC component corresponds to the pulse wave. It consists of the anacrotic and dicrotic waves, which represent, accordingly, systole and diastolic arrival of reflected waves (Allen, 2007).

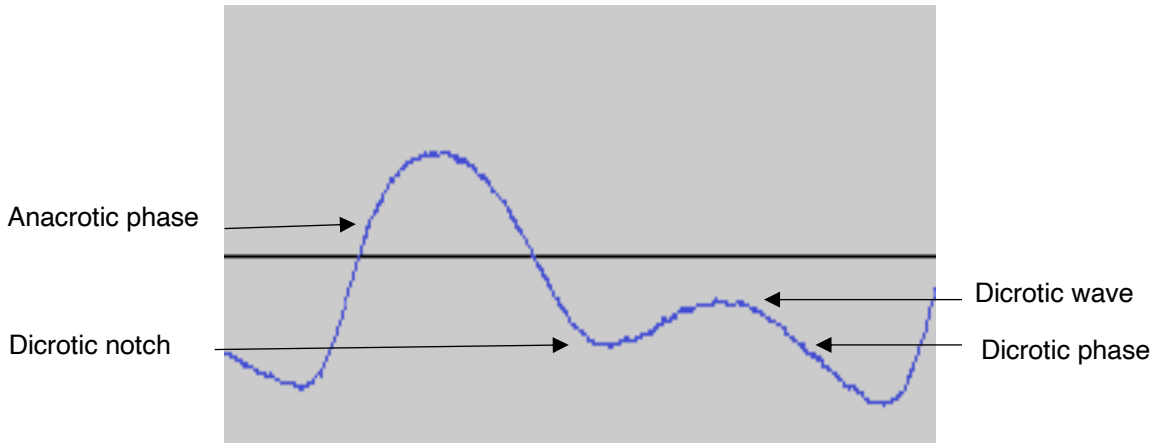

Figure 1.6 **Typical photoplethysmogram**

There are two set-up options for photoplethysmography: transillumination with tissue sample located between the light source and the photodetector, and reflection where both the LED and the photodiode are side-by-side. The transillumination of the fingertip is the set-up

which is most frequently used in everyday clinical practice for monitoring. Transillumination is feasible only on fingertips and ears. To obtain PPG from major arteries reflection set-up is needed. In commercially available medical devices, the electrical signal from the photodetector is conditioned prior to display of PPG waveform. Filtering circuits reduce high-frequency noise from electrical interference and DC component and slow fluctuations, allowing to boost the pulsatile component. The waveform size is also automatically adjusted to be easily displayed on screen. The amount of filtering imposed on the signal results is smoothed and auto-centered waveform which has limited usability for interpretation of PPG morphology (Alian and Shelley, 2014). To perform transcutaneous measurements of pulse wave delay between two arterial sites a two-channel photoplethysmograph with custom-built filtering is needed. Such a technique has been used and validated against invasive blood pressure and ultrasound obtained blood flow waveforms in previous research (Loukogeorgakis et al., 2002).

The following characteristics of the PPG pulse wave useful for investigating properties of arteries can be identified automatically by computer analysis of the signal: pulse transit time to the foot of the pulse, pulse transit time to the peak of the pulse, and foot-to-peak amplitude (Fig. 1.5).

Advantages of photoplethysmography for pressure wave detection include its non-invasiveness, reduction of movement artefacts, minimal interference with physiology and it allows to examine deep arteries which can be important in obese patients.

The factors which affect the reproducibility of PPG include pressure at the probe-tissue interface, the method of fixation of the probe, movement artefacts, posture, wakefulness and breathing of the patient. In healthy adult subjects, changes in pulse transit time along the arterial tree can be detected if they exceed 20–30 ms which proves good individual repeatability (Jago and Murray, 1988).

1.3.8 Standardisation and reference values of arterial stiffness measurement

Several consensus documents have been published by international expert panels to improve uniformity of measurement of arterial stiffness in human populations and allow better comparisons between studies (Laurent et al., 2006; Townsend et al., 2015). According to these documents, measurements:

i. should be done at the right common carotid and common femoral arteries;
ii. should be done at a similar time of day because of diurnal variations;
iii. should be done during at least one respiratory cycle (5–6 secs);
iv. interprobe distance should be measured in a straight line;

v. method to determine the distance between measurement probes should be clearly described;
vi. mean blood pressure should be included in the results;
vii. measurements can be imprecise in patients with arrhythmia.

The reference values for PWV differ by methods that are used to assess the distance between measurement sites and the time delay between pulse waves, as well as the arterial segment studied. 2012 European expert consensus (Van Bortel et al., 2012) suggests a cut-off value of 10 m/s for carotid-femoral PWV.

1.3.9 Arterial stiffness and endothelial function

The classic studies on endothelial function in 1980–1987 were carried out on isolated rings of arteries (Furchgott and Zawadzki, 1980; Moncada et al., 1991). As NO is the main signalling molecule in the arterial wall, a range of substances that stimulate or block NO effects in endothelium-dependent and independent manner were used to study properties of arterial segments. These substances include nitrates to assess endothelium-independent vasodilatation, acetylcholine to stimulate endothelium-dependent NO release and L-NMMA (L-NG-Monomethylarginine) to inhibit production of NO by endothelium.

Using the same substances in experimental animals (Fitch et al., 2001) and human arteries (Joannides et al., 1997; Kinlay et al., 2001), it has been shown that arterial stiffness is reduced by administration of nitrates, whereas inhibition of eNOS by L-NMMA causes increase in arterial stiffness. These findings indicate that endothelium regulates arterial stiffness and arterial distensibility is impaired in cases of endothelial dysfunction (Barenbrock et al., 1999; McEniery et al., 2006).

1.3.10 Arterial stiffness in acute systemic inflammation

Septic shock, the most severe form of sepsis, stems from generalized vasodilatation and maldistribution of blood flow. Vasodilatation is caused by effects of endotoxin on the resistance arterioles, mainly in skeletal musculature. Most present knowledge about the effects of acute systemic inflammation on conduit and resistance arteries comes from laboratory studies on isolated artery models and animal research. The proposed action of endotoxin in these studies has been shown to be indirect, it does not affect the diameter of isolated microcirculatory arterioles but requires a conduit artery to release factors causing dilatation in the arteriolar bed.

On rings of isolated arteries in vitro, the relaxing response is tested by effects of acetylcholine as it is dependent on endothelial function. Leclerc et al. demonstrated that in the rabbit model single injection of lipopolysaccharide caused impaired endothelium-dependent

vasodilatation in rings from abdominal aorta. When examined structurally, after exposure to lipopolysaccharide, vessels had areas of endothelial denudation (Leclerc et al., 2000). Morphologic changes of aortic endothelium after administration of endotoxin have been described by Reidy and Bowyer who found extensive degeneration of cellular organelles in endothelial cells and their subsequent detachment from aortic wall (Reidy and Bowyer, 1977). Even when anatomic injury of conduit arteries is not present, multiple other mechanisms for sepsis-induced abnormalities could be present, such as changes in expression of surface receptors, altered function of NO synthase and impaired degradation of NO (Ma and Danner, 2002).

There have been efforts to reproduce results from in vitro research to human studies. Most populations studied were healthy individuals with low-grade inflammation or patients with chronic inflammatory disease, such as rheumatoid arthritis (Roman et al., 2005), inflammatory bowel disease (Zanoli et al., 2014) and systemic vasculitis (Booth et al., 2004). Acute systemic inflammatory conditions, modelled by the administration of a vaccine (Jae et al., 2013; Vlachopoulos et al., 2005) have shown that acute systemic inflammation significantly increases arterial stiffness as measured by PWV. An increase in PWV has been found to be associated with markers of acute inflammation such as white cell count and levels of C reactive protein (Arnold et al., 2017; Yasmin et al., 2004). Although used as an indicator of vascular inflammation, C reactive protein itself may be one of the active mediators of changes in arteries (Montecucco and Mach, 2008).

Cholley et al. (Cholley et al., 1995) characterized systemic arterial circulation during septic shock in a rabbit model. Regional aortic elastic properties were measured invasively using aortic pressure and diameter relationships and aortic pulse wave propagation described by pulse wave velocity. They showed that PWV in the aorta remained unchanged in unresuscitated shock although there was a progressive fall in distending pressure. In fluid resuscitated shock PWV was reduced below baseline. With low distending pressures PWV should be low, as was shown by authors in haemorrhagic shock model. The unchanged PWV in septic shock with low distending pressures can only be explained by aortic stiffening.

Although refractory hypotension, driven by impaired balance of vasodilation and vasoconstriction mediated through effects of sepsis on conduit and resistance arteries, is the main cause of death in septic shock, there have been no clinical studies investigating effects of sepsis on large artery properties and function.

1.3.11 Practical implications of altered arterial stiffness in critically ill patients

Patients with early sepsis are admitted to intensive care because of the potential for physiologic instability. Hemodynamic monitoring, including measurement of cardiac output, is crucial for this group of patients to clarify the etiology of hypoperfusion and to gauge response to therapy. The traditional method for cardiac output evaluation is thermodilution using a pulmonary artery catheter. Unfortunately, in sepsis the risk of complications with this technique outweighs the benefits, and the use of pulmonary artery catheters has declined over the last two decades (McArthur, 2006). An alternative that is less invasive is the use of pulse wave analysis to estimate stroke volume. This method uses the systolic part of the arterial pulse as an estimate of interaction between ejected blood and central arterial compartment. If properties of the arteries remain constant, the area under the systolic part of the pressure waveform will vary with left ventricular stroke volume (Waal et al., 2009). In practice the pulse waveform used for stroke volume estimation is sampled not from the central compartment but more peripherally, usually from the radial artery, so alterations of peripheral artery tone can affect measurement. Significant difference between central and peripheral arterial pressure waveforms have been described in patients with sepsis (Kim et al., 2013). In porcine model of sepsis, Hatib et al. (Hatib et al., 2011) described central and peripheral vascular tone decoupling. The difference between vascular resistance estimated from the radial artery and aortic impedance increased after fluid resuscitation. In addition to potential prognostic value, detailed knowledge of elastic and muscular elastic properties in patients with early sepsis could be also valuable to improve the precision of arterial waveform based cardiac output measurement as it is a significant part of monitoring armamentarium in intensive care.

2 METHODS

The research was conducted in conformity with international recommendations and ethical principles of human research. Research protocol of Part 2 and 3 of the study was approved by the Ethics Committee of Rīga Stradiņš University, Latvia (10.09.2010). Informed consent was obtained from the patients or their legal representatives.

This thesis consists of three studies and following designs were used:

- Study 1: Systematic review with meta-analysis;
- Study 2: Combined prospective cohort study and case-control study;
- Study 3: Combined prospective cohort and case-control study.

2.1 Methods for systematic review with meta-analysis

The objective of Study 1 was to evaluate the measurement of endothelial function, using vasoreactivity tests, as a risk stratification tool in intensive care patients with sepsis by performing systematic review and meta-analysis of published literature. The meta-analysis was registered at PROSPERO (CRD42018107129) and recommendations of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement were followed (Liberati et al., 2009).

2.1.1 Inclusion and exclusion criteria

PICO (Participant-Intervention-Comparator-Outcomes) framework (Schardt et al., 2007) was used to formulate the inclusion and exclusion criteria.

This meta-analysis addresses the following questions:

- Does endothelial vascular reactivity change in relation to the presence or severity of sepsis?
- Does the impairment of endothelial vascular reactivity correlate to the development or severity of single or multiple organ failure?
- Does the impairment of endothelial vascular reactivity correlate with sepsis outcome?

We included English language publications that met the following inclusion criteria:

- included adult patients (18 years or over) who were diagnosed with sepsis, severe sepsis or septic shock;
- reported measurements of endothelial vascular reactivity by a validated methodology utilizing standardized protocols;
- incorporated clinical end-points.

As the international consensus had changed over the time-period covered by the meta-analysis (January 1985 to November 2018), the definition of sepsis was used as given in the retrieved articles. A preliminary search using the terms “endothelium” or “endothelial function” in combination with “vascular reactivity”, “blood vessel reactivity”, “vascular dysfunction”, “vascular occlusion test” or “reactive hyperaemia/hyperemia” was carried out to identify validated methods for assessment of endothelial function. Final search set of methods included “venous occlusion plethysmography”, “flow-mediated dilation”, “peripheral arterial tonometry”, “laser Doppler flowmetry”, “pulse wave velocity” and “augmentation index”. The study designs considered were randomized controlled clinical trials, cohort studies, case-control studies and case series. The primary end-point of interest was changes of endothelial vascular reactivity in relation to the presence or severity of sepsis. Secondary end-points considered were the development or severity of single or multiple organ failure and intensive care unit, hospital or 28-day mortality (whichever available). Any definition of single or multiple organ failure was accepted.

Studies were excluded if they reported only blood biomarker measurements of endothelial function, included paediatric patients or did not provide quantitative data to calculate effect sizes for either reactive hyperemia or peak hyperemic flow. Single case studies, duplicate publications, previous systematic reviews and meta-analyses were excluded.

2.1.2 Search strategy and publication selection

The following medical databases were searched:

- MEDLINE (January 1985 onwards) is an electronic bibliographic database of articles in life sciences. It has 26 million records from over 5,200 publications.
- Scopus (January 1985 onwards) is a large citation database of 34,346 peer-reviewed journals and conference proceedings covering technology, medicine and social sciences.
- EMBASE (January 1985 onwards) is a biomedical literature database from over 8,500 journals.

The search strategy was constructed by combining key words, as shown in Table 2.1.

Table 2.1

Construction of Search Strategy used for Systematic Literature Search

Framework categories	Search terms
Population of interest	Sepsis OR septic OR septicemia OR “severe sepsis” OR “septic shock” OR “systemic inflammatory response syndrome”
Intervention	“venous occlusion plethysmography” OR “flow mediated dilatation” OR “peripheral arterial tonometry” OR “laser doppler flowmetry” OR “pulse wave velocity” OR “augmentation index”
Search string	1 AND 2
Limitations	English language Human studies

Moreover, to ensure that eligible studies are not missed, references from selected studies were checked to identify any additional articles.

Initial screening was carried out by two researchers independently by reading titles and abstracts of all articles retrieved by search for fulfillment of inclusion criteria. Full text of relevant articles identified by screening was then examined to confirm eligibility. Results of Medline, EMBASE and Scopus searches and study selection process are shown in Figure 2.1.

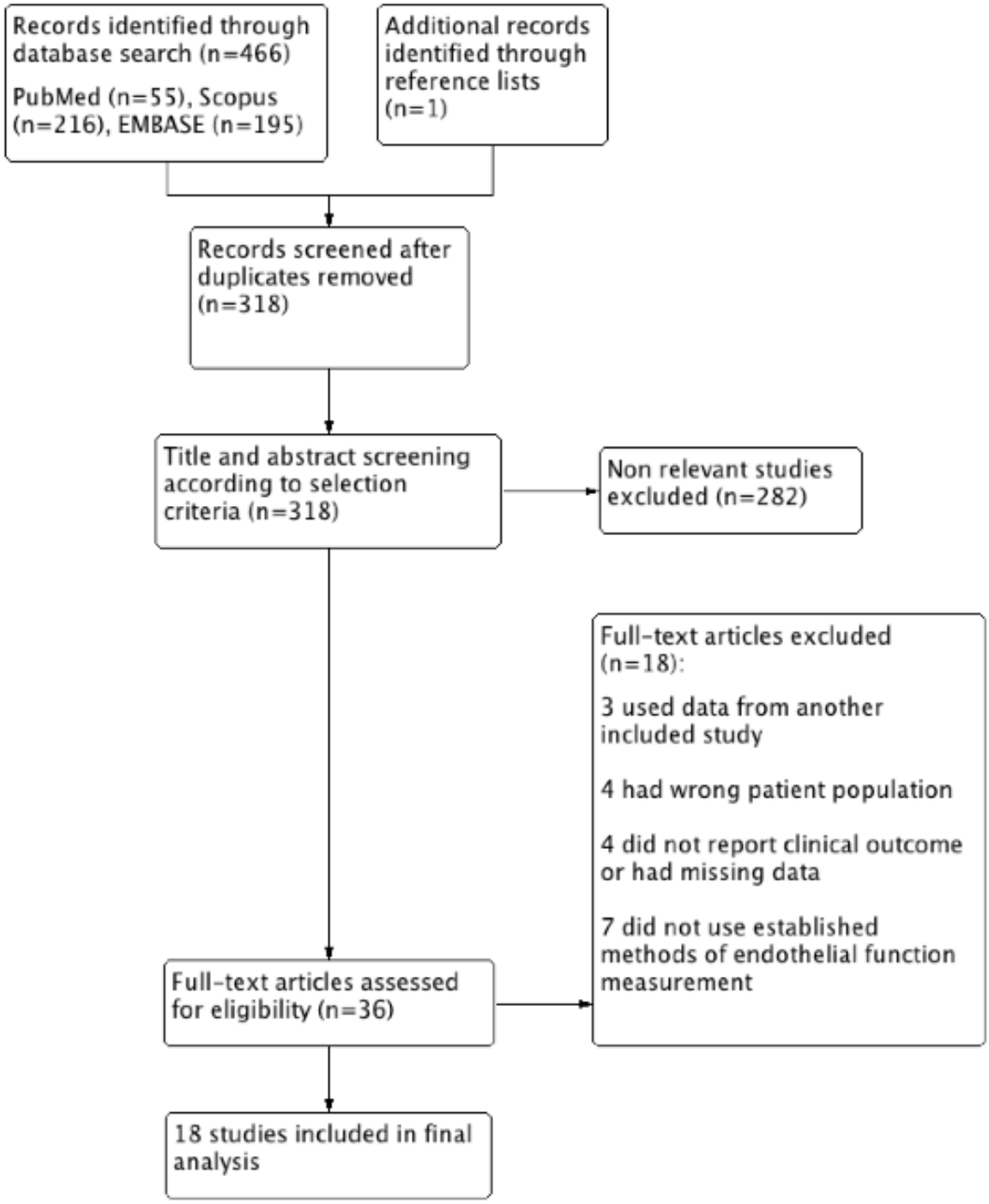

Figure 2.1 **Flow diagram of study selection**

2.1.3 Data extraction and Quality Assessment

The following data were extracted: study setting (emergency department, surgical/medical/mixed intensive care, medical ward), study design, sample size, case mix descriptors (age, comorbidities), sepsis definition used, method, protocol and timing of vasoreactivity evaluation, use of blood-derived markers of endothelial function, group differences in reactive hyperemia and peak hyperemic flow related to any clinical outcome

(presence or absence of sepsis, single or multiple organ dysfunction, severity of sepsis, mortality), possible confounders.

Risk of random error in vasoreactivity measurement was assessed by patient sample size and the reported confidence intervals. Risk of bias assessment was done using customized Newcastle-Ottawa Scale for cross-sectional studies (Wells et al., 2018) by two researchers independently (Appendix 2). This scale evaluates the risk of bias based on group selection, comparability and assessment of outcome. Each of the four methodological domains on the customized Newcastle-Ottawa Scale could be awarded up to two stars with higher scores denoting better methodological quality (Table 2.2). If there were insufficient data to evaluate a domain, it was marked as "?", unknown.

Table 2.2

Summary of quality assessment for the included studies

Study	Sample selection and size	Measurement tool	Comparability	Assessment of outcome	Quality score
Wexler	**	*	**	**	7
Hartl	0	**	?	**	4
Kirschenbaum	0	**	*	**	5
Kubli	0	*	**	**	5
Young	0	*	*	**	4
Vaudo	?*	*	*	**	5
Van Ierssel	*	*	*	**	5
Kienbaum	0	**	0	**	4
Davis	**	*	**	**	7
Astiz, 1995	0	**	0	**	4
Knotzer	*	*	*	**	5
Becker	**	*	*	**	6
Sair	0	**	*	?	3
Payen	*	*	0	**	4
Favory	0	*	?	**	3
Neviere	0	*	*	**	4
Bourcier	**	*	0	**	5
Astiz, 1991	0	*	*	**	4
Nelson	*	*	**	**	6
Nobre	**	*	?	**	5

2.1.4 Statistical analysis

Meta-analyses were performed using Review Manager software (Version 5.3; Cochrane Community). For data reported as median and interquartile range, mean and 95 % confidence interval or provided for two septic subgroups, mean and standard deviation were estimated using methods described in Cochrane Handbook for Systematic Reviews (Higgins, 2011). As vasoreactivity was measured using several different methods, studies were summarized using standardized mean differences (SMD) of the mean of reactive hyperemia/peak hyperemic flow between groups for each relevant clinical outcome. The SMD expresses the size of the difference between two comparison groups relative to the variability observed within the

respective study. Studies included in this meta-analysis were expected to have varied sampling populations with effect sizes that differ from study to study, because of differences in methods used to measure vasoreactivity and study populations and settings. Therefore, pooled SMD and 95 % confidence interval (CI) for each measure of vasoreactivity and outcome was obtained using random effects model for continuous outcomes (DerSimonian and Laird, 1986). Pooled SMD was considered significant if $P < 0.05$. Heterogeneity was assessed using Higgins I^2 test (Higgins et al., 2003). Significant heterogeneity was assumed if I^2 statistic was greater or equal to 75 %.

When designing meta-analysis, a number of arbitrary decisions needs to be made regarding study eligibility criteria, which data are extracted and how they are analysed. In the case of this study the following alternative decisions were possible:

1. Different characteristics of comparison group-only age and gender matched healthy individuals versus all studies, regardless of comparison group description;
2. Different characteristics of outcome measures: percentage change in blood flow relative to baseline (reactive hyperemia) or absolute peak hyperemic flow;
3. Analysis using fixed versus random effects model;
4. Analysis as a standardized mean difference across all methods of vasoreactivity measurement or as mean differences individually for each method.

Sensitivity analysis using above mentioned alternatives was repeated to show that findings do not depend on arbitrary design decisions.

To clarify the influence of method of vasoreactivity measurement and the role of baseline endothelial dysfunction in the control group on the effect size and its heterogeneity, subgroup analysis were planned for studies with different methods of vasoreactivity assessment and studies using controls at risk of endothelial dysfunction.

2.2 Methods for prospective cohort study and case-control studies carotid-femoral and carotid-radial PWV in patients with sepsis

The aims of the studies were to characterize carotid-femoral and carotid-radial PWV in patients with sepsis within 24 hours of admission to intensive care and examine the association between admission PWV and progression of multiple organ failure and mortality.

2.2.1 Study population

Consecutive adult (> 18 years old) patients with severe sepsis or septic shock admitted between September 2012 and October 2016 to a mixed 16- bed Toxicology and Sepsis Clinic of Riga East Clinical University Hospital “Gaiļezers” were eligible for inclusion. Severe sepsis

and septic shock were defined according to the ACCP/SCCM Consensus Conference (1992) criteria (Bone et al., 1992). To be included in the study, patients had to be admitted to ICU within the last 24 hours, and readmitted patients were eligible for inclusion only during their first episode of sepsis. Exclusion criteria were pregnancy, arrhythmias, previous aortic surgery and administration of nitrates. The study involved recording of pulse waves using originally designed photoplethysmograph, so patients could be recruited only when the investigator was available.

Table 2.3

Baseline characteristics of study populations

	Study 2 (n = 45)	Study 3 (n = 59)
Age (years)	67 (54–75)	68 (54–75)
Gender (females), n (%)	23 (43 %)	28 (47 %)
Surgical patient	14 (31 %)	26 (44 %)
Site of infection, n (%):		
Pulmonary	15 (33 %)	20 (34 %)
Intraabdominal	16 (36 %)	21 (36 %)
Urinary	4 (9 %)	7 (12 %)
Other	10 (22 %)	11 (19 %)
Microbiology, n (%):		
Gram + bacteria	12 (27 %)	17 (29 %)
Gram - bacteria	9 (20 %)	8 (14 %)
Mixed	6 (13 %)	13 (22 %)
Unknown	18 (40 %)	20 (34 %)
Septic shock	31 (57 %)	38 (64 %)
APACHE II score	19 (15–25)	19 (14–25)
SOFA score	7 (4–10)	7 (4–9)

APACHE II – Acute Physiology, Age, Chronic Health Evaluation II
SOFA – Sequential Organ Failure Assessment Score

Of the 362 sepsis patients assessed for inclusion, pulse waves were recorded in 56 patients in Study 2 and 70 patients in Study 3. Patient recruitment process is presented in Figure 4.1. About 40 % of eligible patients were not included in the study, as it was difficult to obtain consent because of delirium or depressed level of consciousness and refusal of the next of kin.

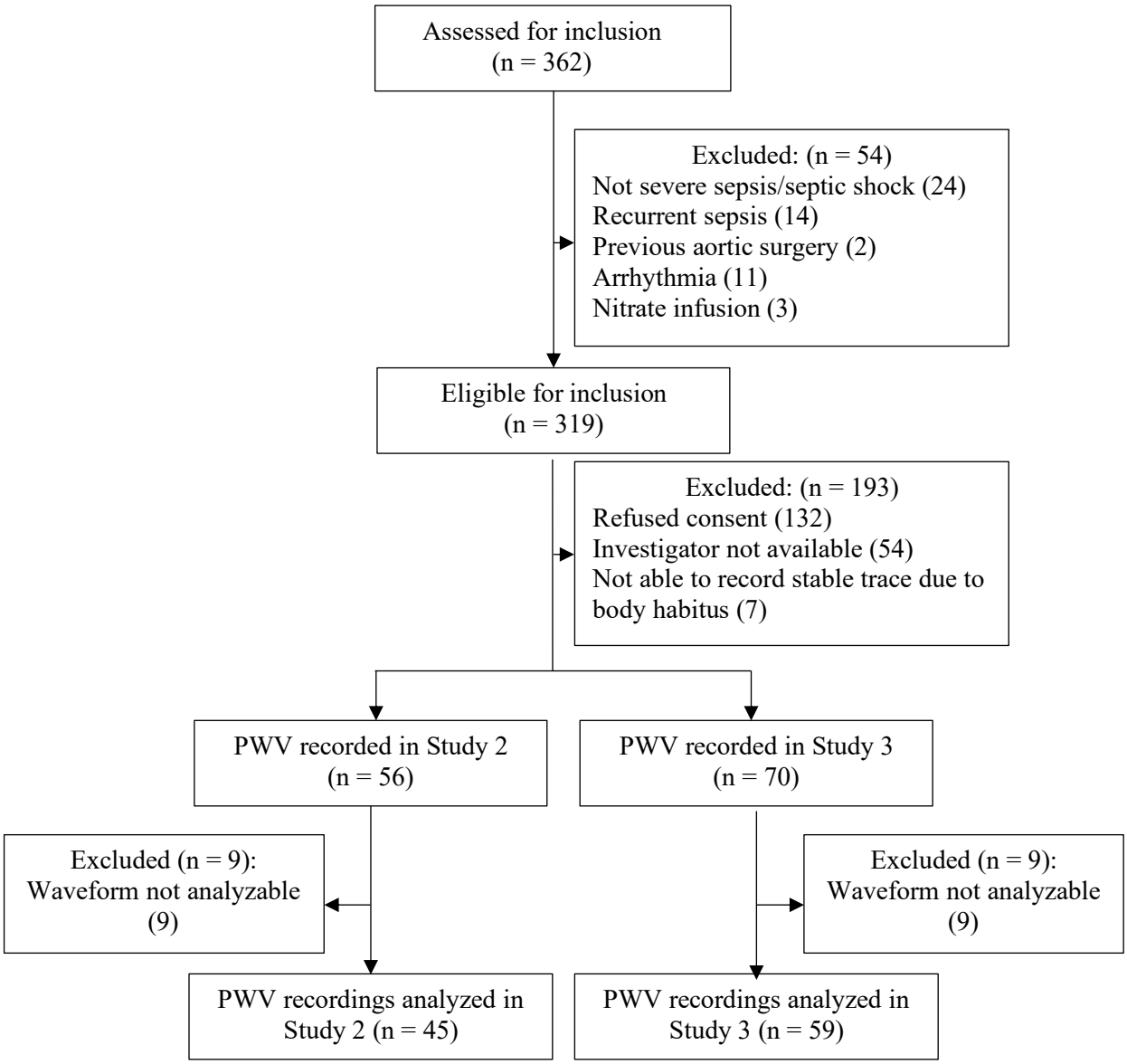

Figure 2.2 **Flow diagram of patient recruitment**

2.2.2 Pulse wave velocity measurement

Studies 2 and 3 used custom device for recording photoplethysmograms from major arteries which has been validated in previous studies (Erts et al., 2005). This photoplethysmograph uses two optical contact probes which comprise a light-emitting diode (peak wavelength 940 nm) and a phototransistor for detection of back-scattered radiation. Infra-red wavelength is chosen to allow good penetration through the tissues and two probes are necessary for simultaneous recordings from two anatomical locations. To obtain pulse wave PPG, sensor probes were fixed with adhesive tape over two blood vessels of interest where maximal arterial pulsation was felt. Once it was confirmed that a stable arterial waveform has been obtained from both sensors, the signal was recorded continuously for 2 minutes. The analogue signal from optical probes was digitalized by an analogue-to-digital converter and

stored on a computer for off-line analysis. The AC component of the recorded signal was separated and reflects arterial pulsations in the volume under the probe. To measure PWV, recordings were assessed visually and segments with movement artefacts discarded. Continuous segments of at least 15 pulse waves in channels from both probes were chosen for further processing. The recordings were smoothed to reduce random noise. The time delay between both pulse sequences was measured using the "foot-to-foot" method as the time interval between upstrokes of two simultaneously recorded arterial waves. PWV in m/s was calculated using the equation:

$$PWV = \frac{D}{\Delta t} \qquad (2.2.2.1)$$

where D – inter-probe distance in meters,

Δt – transit time in seconds.

2.2.3 Protocol for Study 2

After obtaining written informed consent from the patient or the patient's next of kin, demographic and clinical variables including age, sex, date and time of ICU admission, primary site and type of infection and physiologic and treatment variables necessary for calculation of Acute Physiology and Chronic Health Evaluation (APACHE) II (Knaus et al., 1985) and baseline Sequential Organ Failure Assessment (SOFA) scores (Vincent et al., 1996) were collected.

PWV measurement was performed when patients had achieved mean arterial pressure > 65 mm Hg and there had been no change in vasopressor requirements for at least 1 hour. Baseline hemodynamic data (heart rate, systolic, mean and diastolic blood pressure) were obtained from routine monitoring and doses of vasopressor agents recorded. Pulse pressure was calculated as the difference between systolic and diastolic arterial pressure. PWV measurement was performed by the patient's bedside with ambient light dimmed. With the patient in supine position sensor probes were fixed in the following positions for recording of carotid to femoral pulse wave delay:

- probe 1 on the skin over right carotid artery;
- probe 2 over the right femoral artery.

PPG from both probes was recorded simultaneously and stored for off-line analysis. The path of the pulse wave was measured over the body surface with tape measure in a straight line

between probe 1 and probe 2. Calculation of c-f PWV was performed as described in Chapter 2.2.2.

Patients were followed up to hospital discharge or death. Repeated SOFA scores were obtained 48 hours after c-f PWV recording. Results of bacteriological testing were also recorded. The clinical outcomes considered in the analyses were hospital mortality and progression of multiple organ failure (defined as an increase in SOFA score of at least 1 point) over the first 48 hours of ITU admission.

All treatment decisions were made by clinicians not involved in the study according to local protocols that outline the use of antibiotics, fluid resuscitation and vasopressors.

2.2.4 Protocol for Study 3

After inclusion into Study 3 according to criteria outlined in Chapter 2.2.1, demographic, clinical and hemodynamic parameters were recorded at inclusion as described in protocol for Study 2. Initial resuscitation of the patient was considered complete according to the same conditions as in Study 2. Sensor probes for carotid to femoral PWV recording were placed on the skin over the right carotid and right femoral artery and PPG recorded. Then probe 1 was left in position and probe 2 was moved to projection of right radial artery in the wrist, to obtain simultaneous carotid and radial PPG. Both inter-probe distances were measured in a straight line over the body surface. Pulse wave recordings were analysed as described previously.

Hemodynamic, SOFA score and PWV measurements were repeated 48 hours after first measurement. Data regarding length of intensive care and hospital stay and hospital mortality were recorded.

2.2.5 Statistical analysis for Studies 2 and 3

Study 2. Demographic, clinical and haemodynamic characteristics of the patient cohort were described, stratified by quartiles of PWV distribution. Continuous variables are reported as median and inter-quartile range and compared using one-way ANOVA based on quantiles using Harrell-Davis estimator (Harrell and Davis, 1982). Categorical variables are reported as counts and percentages and compared using Fisher's exact test.

Multivariate logistic regression model was used to assess the association of PWV quartile strata with the improvement of multiple organ failure and mortality. Possible co-variates considered included age, sex, APACHE II and baseline SOFA score. Results are reported as adjusted odds ratios and 95 % confidence intervals.

The author used Cox proportional hazards regression to investigate if patients within different PWV quantiles differed in survival time. Relative hazard ratios were calculated by

PWV quartile strata, with and without adjustment for age, APACHE II and baseline SOFA score.

The model with the best fit was determined considering the likelihood ratio test and by removing co-variates with non-significant effects and checking for change in PWV quartile estimate. Results are reported as hazard ratios and 95 % confidence intervals.

Study 3. The demographic and clinical characteristics of the study population were summarized as median and inter-quartile range (IQR) for continuous variables and counts and percentages for categorical variables. Prespecified groups for comparison were patients with progression versus improvement in multiple organ dysfunction syndrome (MODS) and hospital survivors versus non-survivors. As data were not normally distributed and did not have equal variance, robust statistical methods were chosen for group comparisons. To compare continuous variables between groups the author used two-sample test of median and Huber's Ψ estimator differences (Huber, 1981). Categorical variables were compared using Fisher's exact test.

Confounders considered in this analysis were age, gender, admission APACHE II and SOFA score, heart rate, mean arterial and pulse pressure. To assess associations between PWV, potential confounders and outcome variables, first, the 10 % lowest and highest values of the data distribution were replaced by the nearest value above or below the 10 % threshold. The mean value of this data sequence was calculated to obtain winsorized mean at 10 % level (Wilcox and Keselman, 2003). Pearson's correlation was then computed on winsorized data. Variables associated with PWV with p value less than 0.25 were included in multivariate regression to estimate their independent effect. A backward stepwise procedure was applied to obtain the final model. The best model was determined using F test.

Within group differences in different site and time point PWV measurements and between group PWV differences using relative change in PWV were assessed with robust repeated measurement analysis of variance (ANOVA) based on 20 % trimmed means. A p value < 0.05 was considered statistically significant.

All statistical tests were performed in R version 3.3.2 using the nlme, car, survival, WRS2 packages. Graphs were generated using ggplot package.

3 RESULTS

3.1 Results of systematic review with meta-analysis

A total of 18 observational studies met inclusion criteria (Astiz et al., 1991, 1995; L. Becker et al., 2012; Bourcier et al., 2017; Davis et al., 2009; Favory et al., 2013; Hartl et al., 1988; Kirschenbaum et al., 2000; Knotzer et al., 2007; Kubli et al., 2003; Nelson et al., 2016; Nobre et al., 2016; Sair et al. ,2001; van Ierssel et al., 2013; Vaudo et al., 2008; Wexler et al., 2012; J. D. Young and Cameron, 1995). Design and characteristics of included studies are shown in Table 4.1.

Methods of evaluation of vascular reactivity were venous occlusion plethysmography with pharmacological or ischemic provocation, laser Doppler flowmetry using iontophoretically applied or ischemic provocation, flow-mediated brachial artery vasodilatation measured by ultrasound and passive leg movement and peripheral arterial tonometry (RH-PAT) to obtain reactive hyperemia index. One study used more than one method to assess vascular reactivity. The timing of measurement was reported in 10 studies, with seven studies performing initial measurement within 24 hours since ICU admission or sepsis diagnosis. Eight studies used longitudinal measurements of vascular reactivity performed 24 to 48 hours apart.

Table 3.1

Design of included studies

Author	Timing of measurement	Method of measurement Provocation stimulus	Site of measurement	Data provided	Aims of the study
Young, 1995		Laser Doppler flowmetry Occlusion of arterial blood flow until loss of blood flow signal for 2 minutes	Forearm	Red cell flux at baseline Magnitude of reactive hyperemia (%)	Determine if sepsis alters reactive hyperemia in the skin
Neviere, 1996		Laser Doppler flowmetry Occlusion of arterial blood flow with cuff inflated to 200 mm Hg for 3 minutes	Tibialis anterior muscle	Red cell flux at baseline Peak hyperaemic flux	Test if skeletal microvascular blood flow at rest and during reactive hyperemia is impaired in patients with severe sepsis
Sair, 2001		LDF, Strain gauge plethysmography Occlusion of arterial blood flow with cuff inflated to 50 mm Hg over systolic pressure for 20 minutes	Forearm	Red cell flux at baseline Peak hyperaemic flux Magnitude of reactive hyperemia (%) Baseline forearm blood flow	Investigate reactive hyperemia in patients with established sepsis
Kubli, 2003		Laser Doppler Imaging Acetylcholine and SNP electrophoresis	Forearm	Red cell flux at baseline Peak-baseline flux difference during reactive hyperemia	Compare endothelium dependent and independent vasodilation in septic and non septic ICU patients
Knotzer, 2007		Laser Doppler flowmetry Occlusion of arterial blood flow with cuff inflated to 300 mm Hg for 5 minutes	Forearm, skin microvascular response	Red cell flux at baseline Peak hyperaemic flux Magnitude of reactive hyperemia (%) Peak-baseline flux difference	Test association between reactive hyperemia in patients with different degrees of organ dysfunction and outcome

Table 3.1 continues

Author	Timing of measurement	Method of measurement Provocation stimulus	Site of measurement	Data provided	Aims of the study
Payen, 2009	< 24 hours since diagnosis	Laser Doppler flowmetry Occlusion of arterial blood flow with cuff inflated to 300 mm Hg for 3 minutes	Forearm	Red cell flux at baseline Peak hyperemic flux Peak-baseline flux difference Reperfusion slope	Investigate the relationship of NIRS parameters and LDF in homogenous septic shock population controlled for severity under same conditions
Favory, 2013		Laser Doppler flowmetry Occlusion of arterial blood flow with cuff inflated to 30 mm Hg over systolic pressure for 3 minutes	Index fingertip	Red cell flux at baseline Peak hyperemic flux Time to peak (Tmax), time to half recovery (T1/2R)	Assess vascular effects of activated protein C
Bourcier, 2017	< 6 hours since ICU admission	Laser Doppler flowmetry Acetylcholine iontophoresis	Forearm, knee	Red cell flux at baseline Peak hyperemic flux and AUC Magnitude of reactive hyperemia (%)	Investigate relationship between microcirculatory skin response to acetylcholine and 14-day mortality
Hartl, 1988		Strain gauge plethysmography Occlusion of arterial blood flow with cuff inflated to 200 to 220 mm Hg for 3 minutes	Forearm	Forearm blood flow at rest Forearm blood flow during reactive hyperemia	Describe incidence of microcirculatory failure in patients with sepsis and its temporal pattern
Astiz, 1991	Initial presentation	Venous occlusion plethysmography Occlusion of arterial blood flow with cuff inflated to 200 mm Hg for 5 minutes	Forearm	Forearm blood flow at rest Forearm blood flow during reactive hyperemia	Examine relationship of changes in vascular tone to severity of sepsis

Table 3.1 continues

Author	Timing of measurement	Method of measurement Provocation stimulus	Site of measurement	Data provided	Aims of the study
Astiz, 1995	< 24 hours of sepsis	Venous air plethysmography Occlusion of arterial blood flow with cuff inflated to 200 mm Hg for 3 minutes	Forearm	Forearm blood flow at rest Forearm blood flow during reactive hyperemia	Investigate association of decreases in reactive hyperemia with rheologic changes in hyperdynamic sepsis
Kirschenbaum, 2000	< 24 hours of septic shock	Venous air plethysmography Occlusion of arterial blood flow with cuff inflated to 200 mm Hg for 3 minutes	Forearm	Forearm blood flow at rest Absolute change of forearm blood flow from baseline Percentage change in forearm blood flow from baseline	Reactive hyperemia in patients with cardiogenic vs septic shock
Vaudo, 2007		Brachial artery sonography Occlusion of arterial blood flow with cuff inflated to 230 to 250 mm Hg for 4 minutes	Forearm	Flow mediated dilatation Basal and posthyperemic flow	Investigate flow mediated vasodilatation in patients with Gram-negative sepsis
Becker, 2012	< 24 h since diagnosis	Brachial artery sonography Occlusion of arterial blood flow with cuff inflated to 230 to 250 mm Hg for 5 minutes	Forearm	Flow mediated dilatation	Evaluate feasibility and prognostic information of FMD in sepsis
Wexler, 2012	< 48 h since diagnosis	Brachial artery sonography Occlusion of arterial blood flow with cuff inflated to 230 to 250 mm Hg for 5 minutes	Forearm	Flow mediated dilatation Hyperemic velocity	Determine if FMD is associated with severe sepsis and hospital mortality

Table 3.1 continues

Author	Timing of measurement	Method of measurement Provocation stimulus	Site of measurement	Data provided	Aims of the study
Nelson, 2016	< 24 h since ICU admission	Brachial and femoral artery sonography Occlusion of arterial blood flow with cuff inflated to 250 mm Hg for 5 minutes and passive leg movement (PLM)	Forearm Leg	Baseline flow Relative and absolute flow mediated dilatation Relative and absolute PLM induced hyperemia	Compare PLM with FMD as approach to assess NO mediated vascular function in patients with sepsis
Davis, 2009	< 24 h since admission to ICU or < 36 h to the wards	Peripheral arterial tonometry Occlusion of arterial blood flow with cuff inflated to 200 mm Hg or 50 mm Hg above systolic for 5 min	Index finger	Reactive hyperemia index (%)	Examine impairment of microvascular function in proportion with disease severity and relationship to endothelial activation
van Ierssel, 2013	< 72 hours of sepsis	Peripheral arterial tonometry Occlusion of arterial blood flow with cuff inflated to 200 mm Hg or 50 mm Hg above systolic for 5 min	Finger	Reactive hyperemia index (%)	Multiparametric evaluation of endothelial function in patients with severe sepsis compared to healthy subjects
Nobre, 2016	< 48 hours of ITU admission	Peripheral arterial tonometry Occlusion of arterial blood flow with cuff inflated to 200 mm Hg or 60 mm Hg above systolic for 5 min	Finger	Reactive hyperemia index (%)	Investigate association between RH-PAT and 28-day mortality in sepsis

Study population characteristics of the included studies are summarized in Table 3.2. Sepsis criteria used for participant selection depended on the year of the study, with most studies using ACCP/SCCM 1992 Consensus Conference definition (Bone et al., 1992). Four studies recruited patients with the whole spectrum of sepsis, one study collected data from patients who fulfilled sepsis criteria but did not have organ dysfunction at enrolment, four studies enrolled only patients with septic shock and nine studies were performed on a mixed group of patients with severe sepsis and septic shock. The largest number of studies (12 studies) compared septic patients with healthy volunteers. Other comparison groups included intensive care or hospital patients without inflammation (2 studies), patients after cardiac surgery (2 studies) and intensive care patients with cardiogenic shock (1 study). Median patient age in studies varied from 41 to 72 years, with most patients being in their 50s. There was variation in the proportion of male patients from 24 % to 83 %, but gender was not always reported. In eight studies, the comparison group was age and gender matched to the study group.

Table 3.2

Demographic characteristics of patients

Author	Age (years)		Total number (% male)		Severity		Definition of sepsis
	Sepsis	Control	Sepsis	Control	Scoring system	Value	
Young, 1995	45(37–82)	32(23–42)[1] 88(82–89)[2] 67(48–78)[3]	11 (?)	9 (?) 10 (?) 19 (?)			1 B C D
Neviere, 1996	60 ± 11	?[4]	16 (?)	10 (?)	APACHE II	22 ± 7	1 C
Sair, 2001	51 ± 18	?[1] 67±3.4[3]	6 (83 %)	7 (?) 6 (67 %)			1 D
Kubli, 2003	58 ± 12.9	59.8 ± 12.4[5] 58.7 ± 12.4[1]	12 (75 %)	16 (75 %[9]) 12 (75 %[5])	SOFA	13.8 ± 3.6	1 D
Knotzer, 2007	52.3 ± 16[a] 67.6 ± 10.2[b]		15 (73 %)[a] 14 (57 %)[b]		MODS ASA SAPS II	6.5 ± 2.7[a] 9.4 ± 2.0[b] 3.7 ± 0.5[a] 4.0 ± 0.5[b] 11.5 ± 3.4[a] 15.9 ± 2.4[b]	1 A B C D
Favory, 2013	59 (51–69)	?[1]	12 (67 %)	8 (?)	SAPS II	58 (47–64)	1 C D
Bourcier, 2017	59 (49–68)		37 (48 %)		SAPS II	46 (37–72)	3 C D

Table 3.2 continues

Author	Age (years)		Total number (% male)		Severity		Definition of sepsis
	Sepsis	Control	Sepsis	Control	Scoring system	Value	
Hartl, 1988	62.5 ± 2.4	64.3 ± 3.2[6]	12 (?)	10 (100 %)	Sepsis score	16 ± 2	4 D
Astiz, 1991	55 ± 7	32 ± 2[1]	8 (?)	9 (?)	APACHE II	31 ± 8	1 B C D
Astiz, 1995	72 (42–84)	64.3 ± 3.1[6]	23 (?)	10 (?)	Sepsis score	C8.87 ± 0.5 D17.35 ± 2.56	1 C D
Kirschenbaum, 2000	63 ± 3	74 ± 1[7] 76 ± 2[8]	6 (?)	8 (?) 6 (?)	APACHE II	31 ± 2	1 D
Vaudo, 2008	41 ± 8	43 ± 5[5]	45 (24 %)	25 (44 %)	SOFA	4 ± 1	1 B
Becker, 2012	51 ± 19	47 ± 14[5]	42 (38 %)	38 (33 %)	APACHE II	23 ± 7	1 C D
Wexler, 2012	62 (49–74)	60 (53–66)[9]	95 (52 %)	52 (50 %)	APACHE II	23 ± 8	1 C D
Nelson, 2016	59 ± 14	59 ± 15[10]	17 (59 %)	16 (56 %)	APACHE II SOFA	17 ± 7 6 ± 3	1 C D
Davis, 2009	C 52.4 (48.3–56.5) B 50.8 (46.5–55.2)	45.2 (43.1–51.4)[9]	54 (C 61 %) 31 (B 68 %)	45 (67 %)	APACHE II SOFA	C 19.0 (15–23) B 7.5 (5–11) C 6(3-9) B 1 (0-2)	1 B C D
Van Ierssel, 2013	63 ± 2.9	?[10]	30 (67 %)	15 (?[5])	SAPS III SOFA	61.7 ± 1.9 7.8 ± 0.6	1 C D
Nobre, 2016	51.5 ± 18.9		62 (62 %)		APACHE II SOFA	16 (12–20) 7 (5–9)	2 C D

Illness severity indices: APACHE II – Acute Physiology and Chronic Health Evaluation II, SAPS II – Simplified Acute Physiology Score, SOFA – Sequential Organ Failure Assessment score, ASA – American Society of Anesthesiologists Physical Status Classification System, MODS – Multiple Organ Dysfunction score, Sepsis score (35). Sepsis definition: 1 – ACCP/SCCM 1992 Consensus Conference; 2 – ACCP/SCCM Consensus Conference 2001; 3 – Sepsis-3; 4 – custom criteria. Sepsis subgroups: A – Systemic inflammatory response syndrome, B – sepsis, C – severe sepsis, D – septic shock. (?), not described.

1 – healthy volunteers, 2 – elderly women, 3 – patients after cardiac surgery, 4 – patients ventilated for chronic obstructive airway disease, 5 – ICU patients without sepsis, 6 – patients after vascular surgery, 7 – patients with cardiogenic shock, 8 – age matched ventilated subjects, 9 – hospital controls, 10 – age and gender matched volunteers, a – survivors, b – non-survivors

Only in six studies significant comorbidities (cardiovascular disease, diabetes) affecting vascular function were accounted for in the design and analysis of data. In another four studies, patients with cardiovascular comorbidity (cardiogenic shock or after cardiac or vascular surgery)

were used as a comparison group. There was a high risk of confounding as in most studies groups differed significantly regarding the extent of vasopressor use, sedation and mechanical ventilation. Two studies included patients receiving nitrates and activated protein C which are known to influence vascular reactivity measurements.

Number of patients included in individual studies was small and exceeded 30 in only five studies. Confidence intervals of vasoreactivity measurements were reported in four studies. Overall risk of random error was therefore judged as high. In only two studies, both using peripheral arterial tonometry, the precision of the vasoreactivity estimate was high.

There were sufficient data in the included studies for statistical pooling for two effect sizes on vasoreactivity, SMD between septic patients and controls and between survivors and non-survivors. Measurements of absolute (peak flow) and relative (reactive hyperemia) change of blood flow or artery diameter after provocation were pooled separately.

Data from 14 studies were included in the analysis of mean difference in vasoreactivity measurements between septic patients and controls. The pooled mean difference estimate from 10 studies including 554 participants showed that septic patients had less reactive hyperemia than controls (SMD –2.59, 95 % CI –3.46 to –1.72; $Z = 5.85$, $p < 0.00001$; Figure 3.1).

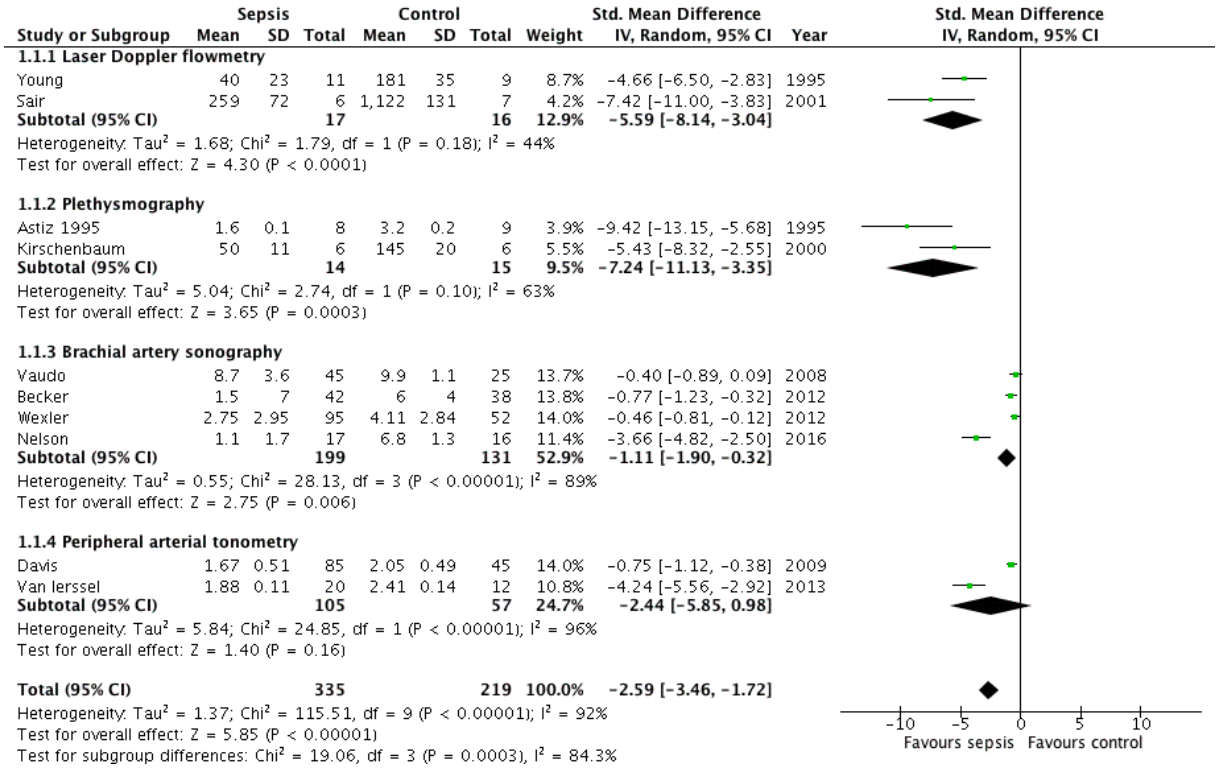

Figure 3.1 **Pooled mean difference in reactive hyperemia between septic patients and controls**

In nine studies with 354 participants, peak hyperemic blood flow was lower in patients with sepsis than in the control group (SMD –1.42, 95 % CI –2.14 to –0.70; Z = 3.88, p = 0.0001; Figure 3.2). Results of these studies were highly heterogeneous with I^2 values of 92 % ($p < 0.00001$) and 85 % ($p < 0.00001$), most likely due to differences in study protocols and populations. After removal of any of the studies in the sensitivity analysis direction of the difference between septic and control group did not change.

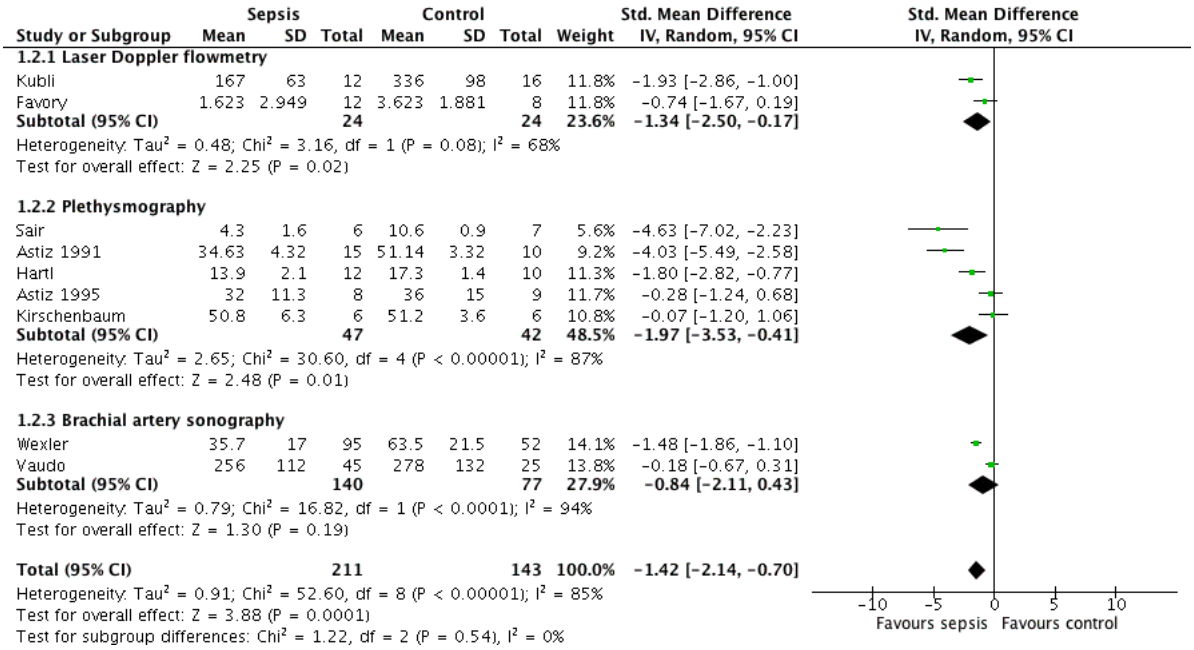

Figure 3.2 **Pooled mean difference in peak reactive hyperemia between septic patients and controls**

Five studies were selected to compare vasoreactivity in survivors and non-survivors of sepsis. The combined SMD between non-survivors and survivors was –0.36 (95 % CI –0.67 to –0.06; Z = 2.36; p = 0.02, Figure 3.3) for reactive hyperemia and –0.70 (95 % CI –1.13 to –0.27; Z = 3.23; p = 0.001, Figure 3.4) for peak hyperemic blood flow. Both reactive hyperemia and peak hyperemic flow were lower in non-survivors. Tests for heterogeneity were statistically not significant for both estimates. By removing the study by Wexler (Wexler et al., 2012), which had the largest number of participants, statistical significance of difference in peak hyperemic flow (SMD –0.42, 95 % CI –0.93 to 0.13; Z = 1.5; p = 0.13) and reactive hyperemia (SMD –0.36, 95 % CI –0.73 to 0.01; Z = 1.92; p = 0.05) between survivors and non-survivors was lost.

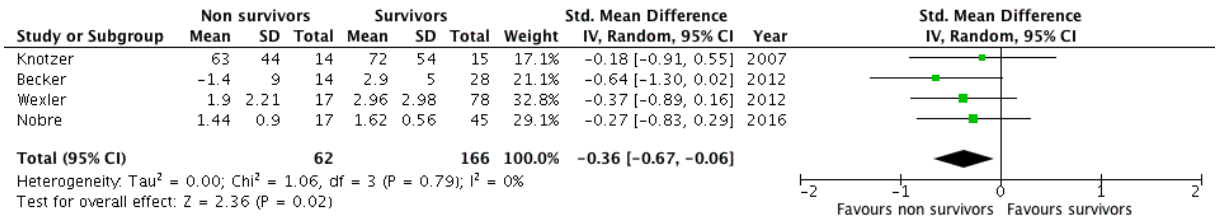

Figure 3.3 **Pooled mean difference in reactive hyperemia between non-survivors and survivors**

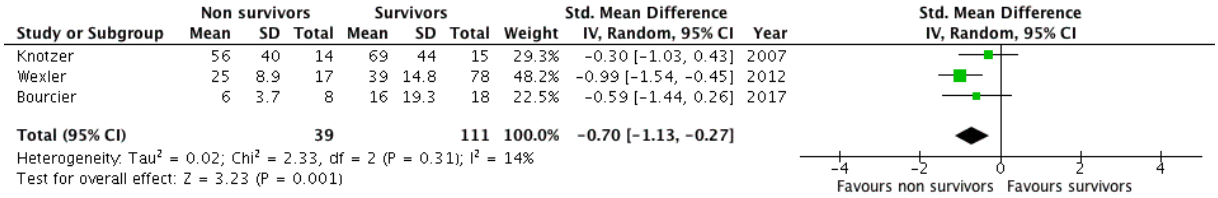

Figure 3.4 **Pooled mean difference in peak hyperemic flow between non-survivors and survivors**

We pooled studies using a control group of patients with severe cardiovascular disease and therefore at high baseline risk of endothelial dysfunction separately. In three studies involving 56 patients, magnitude of reactive hyperemia in the septic and cardiovascular risk groups was similar (SMD –2.23, 95 % CI –4.67 to 0.21; Z = 1.79; p = 0.07; I^2 = 91 %, Figure 3.5).

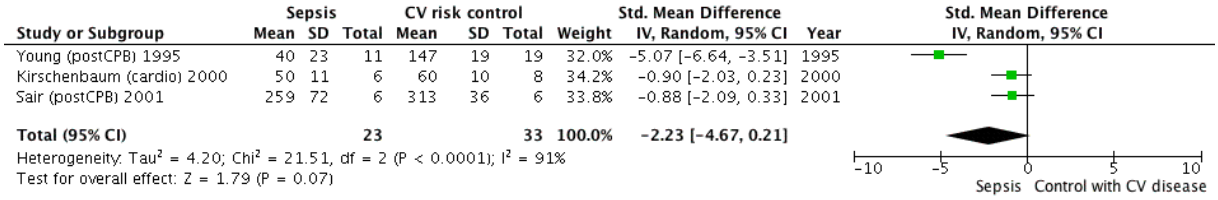

Figure 3.5 **Pooled mean difference in reactive hyperemia between septic patients and control group with severe cardiovascular disease**

One study (Kubli et al., 2003) was able to measure endothelium dependent (EDVD) and independent vasodilatation separately. No difference in EDVD was found between septic and control group.

Conflicting results were reported regarding relationship between vascular reactivity measurements and development of MODS, but the data presented were insufficient to be pooled. Qualitative synthesis of studies is presented in Appendix 3.

Four studies assessed consistency of findings across different methods of measurement or coherence with biochemical markers. Changes in both, macrovascular reactivity measured by venous occlusion plethysmography and microvascular reactivity, measured by laser Doppler flowmetry, were found in a group of severe sepsis patients by Sair et al. (Sair et al., 2001). Three studies assessed coherence of decreased vascular reactivity with biochemical markers of endothelial activation or damage. No correlation was found with levels of endothelin-1 (ET-1) (L. Becker et al., 2012), vascular cell adhesion molecule (sVCAM-1) (L. Becker et al., 2012), intercellular adhesion molecule (ICAM-1) (Davis et al., 2009), E selectin (Davis et al., 2009), endothelial progenitor cells (EPC) (van Ierssel et al., 2013) and endothelial microparticles (van Ierssel et al., 2013).

3.2 Results for Study 2

Simultaneous carotid and femoral pulse waves were recorded in 56 patients with severe sepsis or septic shock. After exclusion of 9 patients because of movement artefacts, which precluded measurement of c-f PWV, data from 45 patients were available for analysis. The patients whose recordings could not be analysed, did not significantly differ in terms of age, gender or disease severity from the analysed patients.

The median ICU length of stay was 8 (5–15) days. The source of sepsis was mostly abdominal (36 %) or respiratory (33 %). Gram-negative bacteria and gram-positive bacteria were involved in 27 % and 33 % of the cases, respectively. At the time of c-f PWV measurement, 31 patients had septic shock and were receiving vasopressors. Overall, 14 (26 %) died in hospital.

The median c-f PWV for the entire sepsis cohort was 14.6 (8.1–24.7) m/s and it exceeded 12 m/s in 25 (55.6 %) of patients. Patient demographic, clinical and hemodynamic characteristics, stratified by c-f PWV quartile (< 8.1 m/s [1st quartile], 8.1–14.6 m/s [2nd quartile], 14.6–24.7 m/s [3rd quartile] and > 24.7 m/s [4th quartile]) are presented in Table 3.3.

Table 3.3

Demographic, clinical and hemodynamic characteristics of septic patient cohort stratified by pulse wave velocity quartile

Parameter	Total	Pulse wave velocity			
		< 8.1 m/s	8.1–14.6 m/s	14.6–24.7 m/s	> 24.7 m/s
	(n = 45)	(n = 12)	(n = 11)	(n = 10)	(n = 12)
Demographic characteristics					
Age (in years)	67 (54–75)	61 (43–73)	57 (49–68)	69 (57–79)	59 (39–78)
Gender (females), n (%)	23 (43 %)	5 (38 %)	4 (36 %)	5 (45 %)	3 (30 %)
Clinical characteristics					
SOFA score	7 (4–10)	9 (4–10)	8 (5–9)	4 (3–7)	7 (5–11)
Ventilated, n (%)	18 (33 %)	6 (46 %)	3 (27 %)	1 (9 %)	8 (80 %)*
Septic shock, n (%)	31 (57 %)	7 (54 %)	6 (55 %)	4 (36 %)	8 (80 %)*
APACHE II score	19 (15–25)	21 (14–26)	19 (16–24)	16 (11–19)	19 (15–27)

Table 3.3 continues

Parameter	Total	Pulse wave velocity			
		< 8.1 m/s	8.1–14.6 m/s	14.6–24.7 m/s	> 24.7 m/s
	(n = 45)	(n = 12)	(n = 11)	(n = 10)	(n = 12)
Hemodynamic characteristics					
Systolic blood pressure (mm Hg)	110 (97–121)	113 (107–120)	101 (95–116)	107 (90–128)	108 (97–121)
Mean blood pressure (mm Hg)	78 (70–87)	80 (77–85)	75 (70–82)	77 (70–92)	72 (70–82)
Pulse pressure (mm Hg)	47 (36–56)	51 (41–53)	48 (34–58)	44 (34–57)	44 (36–56)
Diastolic blood pressure (mm Hg)	62 (56–71)	62 (60–71)	66 (55–67)	65 (60–-78)	58 (44–66)*
Dose of noradrenaline (mcg/kg/min)	0.03 (0–0.1)	0.03 (0–0.09)	0.03 (0–0.08)	0 (0–0.02)	0.1 (0–0.14)
Heart rate (beats/min)	90 (82–98)	86 (82–94)	91 (87–101)	85 (64–93)	95 (82–98)

Values given as median (interquartile range) or number (percentage)
APACHE II – Acute Physiology, Age, Chronic Health Evaluation II
SOFA – Sequential Organ Failure Assessment Score
* Significant at the 0.05 probability level

The second c-f PWV quartile was chosen as the reference group for further analyses as these values fall within the range reported for general population (Mattace-Raso et al., 2010). There was a significantly higher proportion of patients who needed vasopressors and ventilatory support in the group with c-f PWV exceeding 24.7 m/s. Patients in this quartile had lower diastolic pressure than other groups ($p = 0.03$). Other hemodynamic characteristics did not differ between c-f PWV quartiles.

During baseline assessment patients in all c-f PWV quartiles showed similar sepsis severity, measured by APACHE II and SOFA scores with non-significant trend for more severely ill patients to be in the highest PWV quartile (> 24.7 m/s). Within 48 hours five patients had died and sequential 48-hour SOFA scores were available for 40 patients. Baseline and 48-hour SOFA scores stratified by PWV quartile are shown in Figure 3.6.

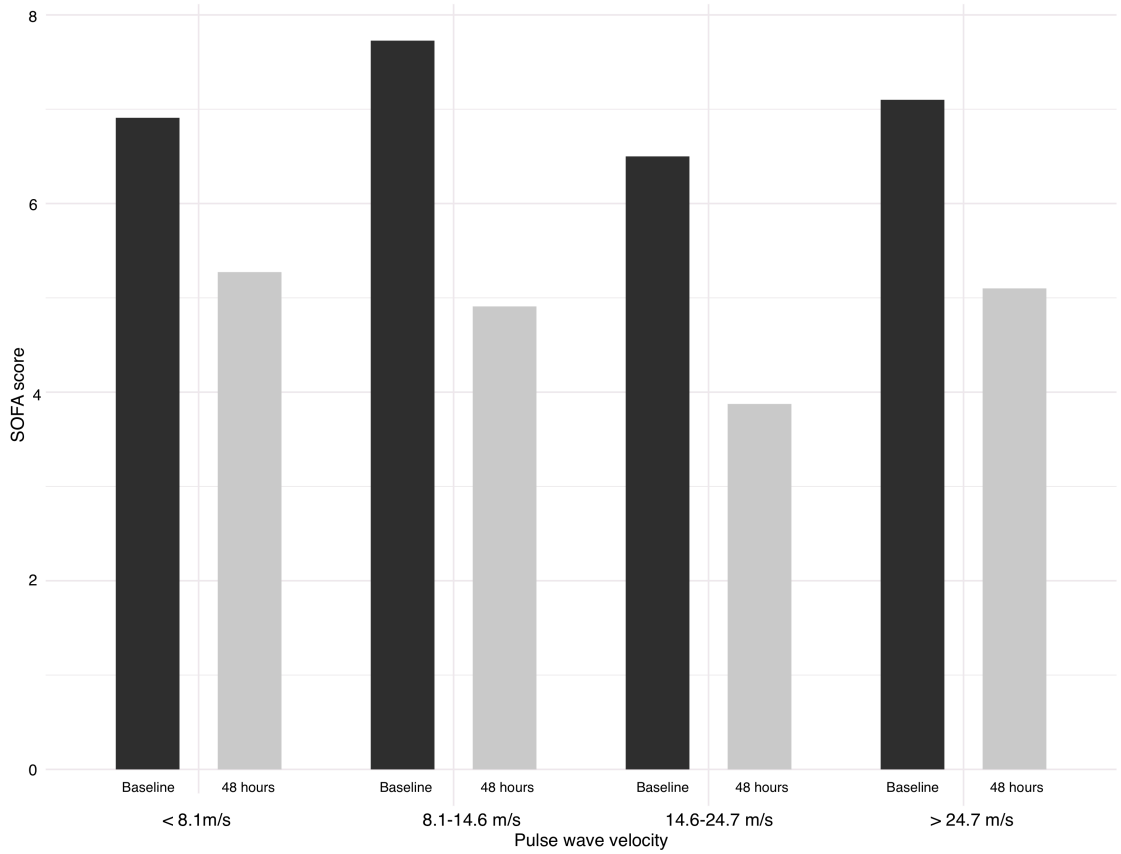

Figure 3.6 **Baseline and 48-hour SOFA score comparison stratified by pulse wave velocity quartiles**

The association between c-f PWV and 48-hour progression of MODS measured by change in SOFA score from baseline is shown in Table 3.4. Compared with the reference c-f PWV quartile (8.1–14.6 m/s), there was no association between PWV and progression of MODS, before or after adjustment for age, APACHE II and admission SOFA scores. Although not statistically significant, a larger proportion of patients (36 % (1st quartile) vs 18, 12 and 20 % (2nd, 3rd, 4th quartiles)) with c-f PWV in the lowest quartile (< 8.1 m/s), had increase of SOFA scores over 48 hours.

Table 3.4

Association of pulse wave velocity with progression of multiple organ failure and mortality

Risk factor	Unadjusted		Adjusted	
	OR (95 % CI)	p value	OR (95 % CI)	p value
Progression of MODS				
Carotid femoral PWV				
< 8.1 m/s	2.57 (0.38–22.76)	0.34	3.64 (0.48–37.79)	0.23
8.1–14.6 m/s	1.0 (reference)	NA	1.00 (reference)	NA
14.6–24.7 m/s	0.64 (0.03–8.13)	0.74	0.63 (0.02–9.68)	0.75
> 24.7 m/s	1.13 (0.11–11.26)	0.92	1.5 (0.14–16.56)	0.73
Age (yrs)			1.01 (0.96–1.06)	0.66
APACHE II Score			0.96 (0.82–1.11)	0.60
Admission SOFA score			1.18 (0.88–1.62)	0.29
Mortality				
Carotid femoral PWV				
< 8.1 m/s	1.33 (0.22–8.71)	0.75	1.92 (0.27–15.97)	0.52
8.1–14.6 m/s	1.0 (reference)	NA	1.0 (reference)	NA
14.6–24.7 m/s	0 (0–375)	0.99	0 (0–490)	0.99
> 24.7 m/s	1.33 (0.22–8.71)	0.75	1.45 (0.19–11.61)	0.72
Age (yrs)			1.04 (0.99–1.1)	0.17
APACHE II score			0.95 (0.80–1.12)	0.56
Admission SOFA score			1.35 (0.96–-2.02)	0.09

MODS: multiple organ failure, APACHE II: Acute Physiology, Age, Chronic Health Evaluation II, SOFA: Sequential Organ Failure Assessment Score, PWV: pulse wave velocity, OR: odds ratio, CI: confidence interval

Overall, 33 %, 27 % and 33 % of patients died in hospital in the 1st, 2nd, and 4th PWV quartile, respectively (non-significant, p = 0.23). All patients in 3rd PWV quartile survived to hospital discharge.

Cox regression and survival analyses with age, APACHE II and baseline SOFA as confounders showed a shorter hospital survival time for patients in the highest PWV quartile (> 24.7 m/s) (hazard ratio = 9.45, confidence interval: 1.24–72.2; p = 0.03), as presented in Table 3.5.

Table 3.5

Results of Cox regression analysis of association between pulse wave velocity and length of survival

	Unadjusted HR (95 % CI)	p value	Adjusted HR (95 % CI)	p value
Carotid femoral PWV				
< 8.1 m/s	1.25 (0.27–5.70)	0.77	1.29 (0.24–6.78)	0.77
8.1–14.6 m/s(reference)	1.0	NA	1.0	NA
14.6–24.7 m/s*	NA	NA	NA	NA
> 24.7 m/s	3.89 (0.71–21.00)	0.11	9.45 (1.24–72.2)	0.03
Age (yrs)			1.03 (0.97–1.09)	0.3
APACHE II score			0.85 (0.71–1.02)	0.08
Admission SOFA score			1.76 (1.14–2.71)	0.01

PWV – pulse wave velocity, HR – hazard ratio, CI – confidence interval, APACHE II – Acute Physiology, Age, Chronic Health Evaluation II, SOFA – Sequential Organ Failure Assessment Score; NA – not applicable; HR – hazard ratio, CI – confidence interval

* No deaths occurred in 3rd PWV quartile

3.3 Results for Study 3

Of 70 patients with severe sepsis and septic shock, 59 had complete recordings of carotid-femoral and carotid-radial PWV available for analysis. Baseline demographic and clinical characteristics are shown in Table 3.6, including 27 patients without and 32 patients with septic shock.

Table 3.6

Baseline demographic and clinical characteristics of patients included in Study 3

	All patients n = 59	Survivors n = 43	Non-survivors n = 16	p
Age (years)	68 (54–75)	61 (44–72)	75 (66–81)	0.003
Male gender	53 %	56 %	44 %	0.59
Height (cm)	175 (167–180)	175 (168-180)	164 (150–164)	0.04
Weight (kg)	82 (70–91)	82 (75–92)	74 (70–77)	0.2
Mean arterial pressure (mm Hg)	78 (70–87)	80 (70–90)	63 (51–69)	0.08
Heart rate (beats/min)	114 (104–129)	111 (104–123)	131 (114–138)	0.017
Temp (°C)	37.6 (37.0–38.4)	37.6 (37.0–38.4)	37.9 (37.0–38.4)	0.98
Surgical patient	26 (44 %)	19 (44 %)	7 (44 %)	1.00
Site of infection				0.30
Pulmonary	20 (34 %)	13 (30 %)	7 (43 %)	
Intraabdominal	21 (36 %)	17 (40 %)	3 (19 %)	
Urinary	7 (12 %)	4 (9 %)	3 (19 %)	
Other	11 (19 %)	9 (21 %)	3 (19 %)	
Microbiology				0.08
Gram + bacteria	17 (29 %)	10 (23 %)	7 (44 %)	
Gram - bacteria	8 (14 %)	5 (12 %)	3 (19 %)	
Mixed	13 (22 %)	9 (21 %)	4 (25 %)	
Unknown	20 (34 %)	18 (42 %)	2 (12 %)	
Vasopressor use	0.03 (0–0.085)	0 (0–0.08)	0.09 (0.04–0.1)	0.0014
APACHE II score	19 (14–25)	17 (12–21)	27 (20–32)	0.00002
SOFA score	7 (4–9)	5 (4–8)	10 (8–12)	0.00003

APACHE II – Acute Physiology, Age, Chronic Health Evaluation II, SOFA – Sequential Organ Failure Assessment Score

Median value for carotid to femoral PWV at baseline was 14.3 (6.8–24.7) m/s and for carotid to radial PWV at baseline – 15.5 (10.6–36.1) m/s. Carotid to radial PWV was significantly higher than carotid to femoral PWV (p = 0.03). Seven patients died within 48 hours of admission, so no follow-up recording was possible. Of the remaining 52 patients, 38 showed improvement in MODS as measured by SOFA score. The median change over the 48-hour period was –1.2 (–12.7; 10.2) m/s in carotid-femoral PWV and –1.5 (–32.6; 62.7) in carotid-radial PWV in improving patients versus 19.1 (7.8–44.5) m/s and 1.6 (–7.5; 10.1) m/s, accordingly, in patients who did not improve (P = NS). There was significant difference in absolute change in carotid-radial PWV between survivor and non-survivor groups (–1.5 (–28.2; 26.5) vs. 70.6 (4.8;196.9) m/s; p = 0.02) (Figure 3.7).

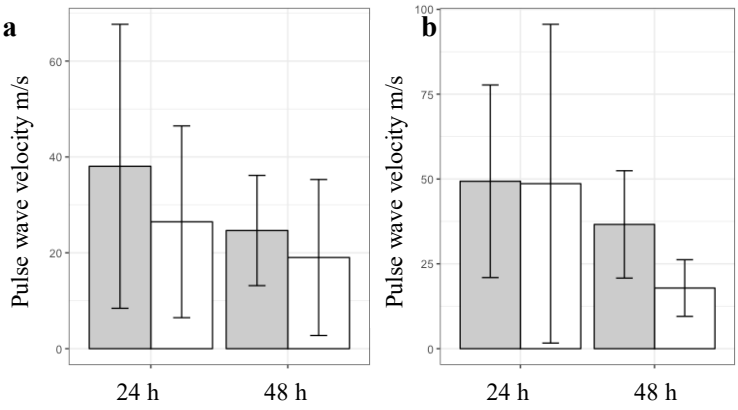

Figure 3.7 **Time course of carotid-femoral (a) and carotid-radial (b) PWV (mean ± CI) in survivors (n = 36; shaded bars) and non-survivors (n = 16; white bars)**

Using logistic regression analysis, the relationship between the baseline carotid to femoral PWV and mortality risk (p = 0.83) or the risk of progression of MODS (p = 0.51) was not statistically significant. Receiver Operating Characteristic (ROC) curve analysis was performed to assess the ability of carotid to femoral PWV and carotid to radial PWV to discriminate between patients with and without progression of MODS and survivors and non-survivors (Table 3.7).

Table 3.7

Sensitivity and specificity of PWV in predicting clinical outcomes in septic patients

ROC curve	AUC (95 % CI)	Cut-off (m/s)	Specificity, %	Sensitivity, %
C-f PWV and progression of MODS	0.70 (0.37–1)	11.7	25	25
C-r PWV and progression of MODS	0.53 (0.27–0.80)	25.2	50	20
C-f PWV and mortality	0.37 (0.02–0.73)	9.8	33	47
C-r PWV and mortality	0.69 (0.44–0.94)	9.7	100	47

ROC – Receiver Operating Characteristic, AUC – area under curve, PWV – pulse wave velocity, MODS – multiple organ dysfunction syndrome

Carotid to femoral and carotid to radial PWV values for patient groups with improvement versus progression of MODS over the first 48-hour period and hospital survivors versus non survivors are shown in Table 3.8.

Table 3.8

Summary statistics for patient groups with improvement versus progression of MODS over the first 48-hour period and hospital survivors versus non survivors

Pulse wave velocity	Improvers n = 38	Nonimprovers n = 14	p	Survivors n = 36	Non survivors n = 16	p
Baseline c-f PWV	15.0 (8.9–29.5)	9.8 (5.9–23.0)	0.16	15.0 (8.1; 25.0)	16.7 (6.0–43.7)	0.39
48 c-f PWV	12.9 (7.7–30.4)	19.1 (7.8-–44.5)	0.49	13.6 (8.0–36.9)	14.1 (7.1–31.8)	0.69
Absolute change in c-f PWV	–1.2 (–12.7; 10.2)	–8.2 (–37.8; 7.3)	0.29	–2.7 (–22.5; 9.0)	5.1 (–2.2; 8.1)	0.06
Relative change in c-f PWV	0.93 (0.48; 2.2)	1.1 (0.4; 1.9)	0.56	0.84 (0.41; 1.9)	1.6 (0.82; 2.48)	0.19
Baseline c-r PWV	21.0 (10.4; 59.4)	17.7 (10.8–41.2)	0.4	16.3 (10.1–41.1)	2.4 (1.2–5.9)	0.36
48-hour c-r PWV	25.0 (15.8–48.3)	25.7 (11.1; 38.2)	0.42	28.2 (15.1; 47.6)	17.8 (15.7; 20.1)	0.04
Absolute change in c-r PWV	–1.5 (–32.6; 62.7)	1.6 (–7.5; 10.1)	0.12	–1.5 (–28.2; 26.5)	70.6 (4.8; 196.9)	0.02
Relative change in c-r PWV	0.91 (0.41; 4.1)	1.2 (0.7–2.4)	0.17	0.90 (0.43; 2.77)	5.8 (1.38; 14.35	0.05

c-f PWV – carotid to femoral pulse wave velocity, c-r PWV – cardotid to radial pulse wave velocity

Based on bivariate analysis results, patient age ($r^2 = 0.04$; $p = 0.21$), admission C reactive protein concentration ($r^2 = 0.06$; $p = 0.12$) and mean blood pressure during PWV measurement ($r^2 = –0.05$; $p = 0.15$) were included in the initial analysis (Figure 3.8).

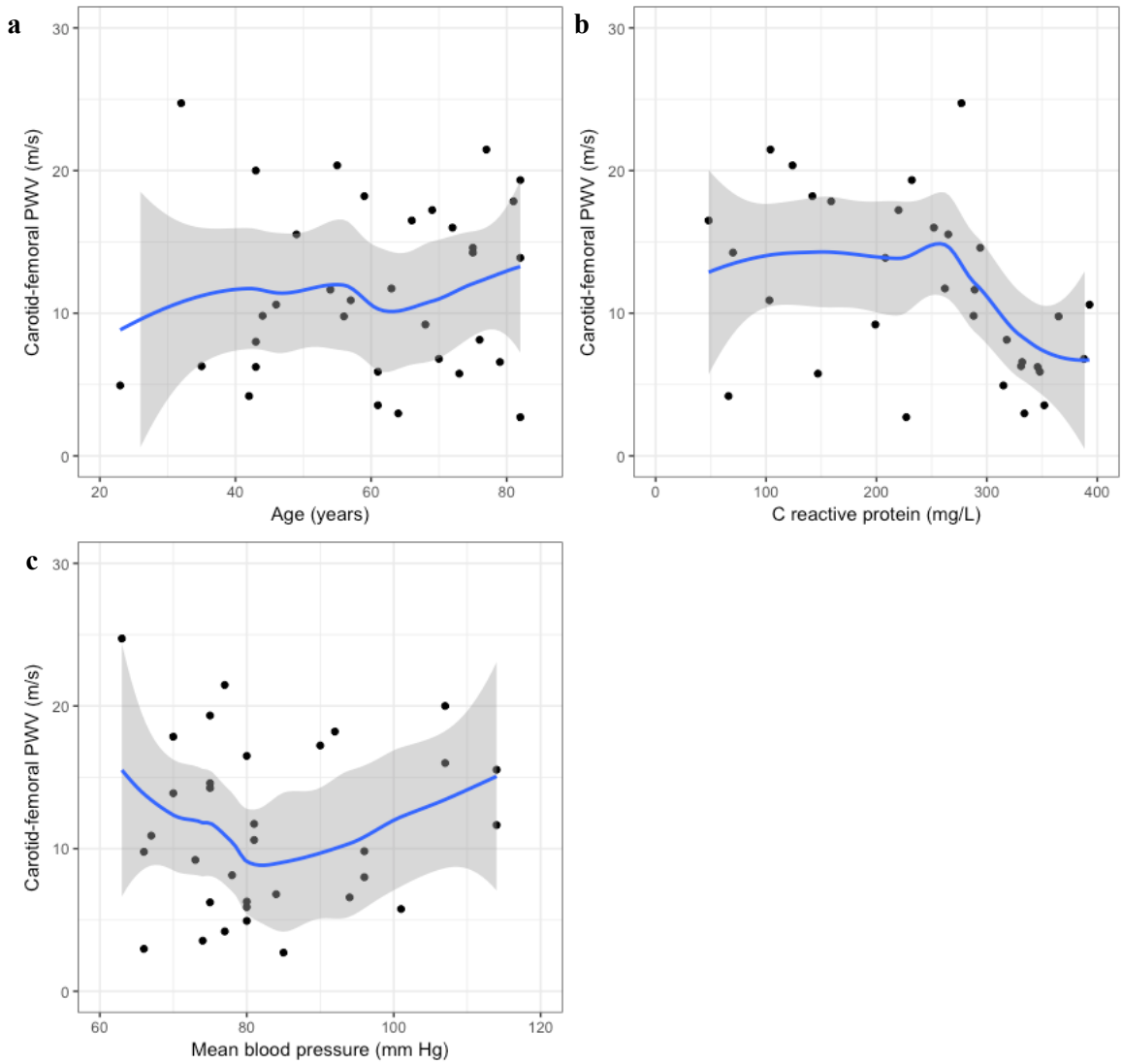

Figure 3.8 **Non-parametric regression fit using locally weighted scatterplot smoothing on carotid to femoral PWV data against age (a), admission C reactive protein concentration (b) and mean arterial pressure (c)**

It showed that age was not independently associated with admission carotid to femoral PWV. The results of final regression model indicated that two predictors explained 9.6 % of the variance ($r^2 = 0.14$, F (2.58) = 3.18, p = 0.05). Carotid to femoral PWV was predicted by admission C reactive protein concentration ($\beta = 0.56$, $p < 0.001$) and mean blood pressure ($\beta = -0.71$, p = 0.15). The final regression model, its coefficients and p-values are shown in Table 3.9. A significant winsorized correlation between carotid to radial PWV and age was observed ($\rho_w = 0.31$; p = 0.04).

Table 3.9

Multivariate regression model of carotid to femoral PWV, beta coefficients with 95 % confidence intervals and p-values

Predictors	Delta r^2	Beta coefficient	Standardized beta	p-value	95 % CI
Step 1	0.08			0.08	
Constant		96.71			15.9–177.5
Mean blood pressure		–0.87			–1.9; 0.11
Step 2	0.14			0.05	
Constant		107.5		0.01	27.6; 187.5
Mean blood pressure		–0.71	–0.22	0.15	–1.69; 0.27
C reactive protein		–0.09	–0.26	0.09	–0.2; 0.02

CI – cofidence interval

4 DISCUSSION

This thesis investigates arterial function in intensive care patients with sepsis as assessed by means of vascular reactivity and pulse wave velocity, including consistency of association between impaired vascular reactivity, arterial stiffness and sepsis as well as the ability of these tests to predict clinically relevant outcomes.

Meta-analysis of 18 studies using established methods of vascular reactivity included in Study 1, found lower vascular reactivity in patients with sepsis compared to controls, but the magnitude of effect was inconsistent across studies. Measures of vascular reactivity in early sepsis were lower in non-survivors but the data were less reliable. There was insufficient data in published literature to quantitatively evaluate the relationship between vascular reactivity and development of MODS.

This meta-analysis was based on a limited number of single-site studies using four different measurement methods, with many of the studies having small sample sizes. There are major differences in the vascular beds explored by the studies – laser Doppler flowmetry evaluates microvascular blood flow, venous air and mercury strain gauge plethysmography allows measurement of total forearm blood flow, flow mediated vasodilatation (FMD) reflects the bioavailability of endothelium derived NO in the brachial artery and RH-PAT measures fingertip reactive hyperemia. An ideal method of testing vascular reactivity should allow to quantify both endothelium dependent and independent vasodilatation. Such methods exist but are invasive and require intraarterial or iontophoretic provocation agent administration which makes them difficult to use in critically ill patients. The only study using gold standard research tool (Kienbaum et al., 2008) – vascular occlusion plethysmography with pharmacological provocation – found no difference in endothelium dependent vasodilation in patients with septic shock compared with volunteers. Unfortunately, this study had insufficient numeric data to be included in meta-analysis. Similar results were obtained in studies (Bourcier et al., 2017; Kubli et al., 2003) using measurement of hyperemia provoked by acetylcholine iontophoresis with laser Doppler flowmetry. Most functional methods in this meta-analysis measure a composite value of different mechanisms producing vasodilatation, only one of which is bioavailability of NO and is related to endothelial function, therefore, it is likely that the consistent decrease of vascular reactivity found in sepsis might be due to mechanisms other than endothelial dysfunction. Methodological heterogeneity evidenced by the variety of measurement protocols and sites used in the studies makes findings difficult to generalize.

When comparing the population from published studies of sepsis epidemiology (Stoller et al., 2016), research in this meta-analysis tended to include patients from the same age group but with considerably greater disease severity. Septic patients with a variety of clinical

characteristics and in a variety of settings exhibited a decrease in vascular reactivity across studies with the exception of studies that used a comparison group consisting of patients with severe cardiovascular disease. Patients after cardiac and vascular surgery used as comparators have factors other than sepsis that can influence vascular reactivity and are known to have particularly high incidence of decreased vascular reactivity (Bellamkonda et al., 2017). On the other hand, patients with sepsis are often elderly, have advanced atherosclerosis, use statins, have hyperlipidemia, hypertension, diabetes, are smokers or obese, and constitute high-risk group for vascular dysfunction. Only one study in this meta-analysis enrolled exclusively young patients and another two corrected for comorbidity using Charlson index. To what extent comorbidities contribute to altered results of vascular reactivity tests in septic patients is yet to be established.

Minority of studies examined changes in vascular reactivity in the context of the progression of multiple organ failure or mortality. Association with these critical outcomes was not convincing. The reason for the weak association could be that the pathophysiological mechanisms tested in vascular beds of the forearm do not directly reflect changes in vasculature involved in splanchnic perfusion. The timing of measurement of endothelial function in the course of sepsis is possibly very relevant. The studies in this meta-analysis evaluated endothelial function early, within the first 24–48 hours from admission and sought to correlate the extent of changes in vascular reactivity to the progression of sepsis. There might be temporal variation in onset of vascular changes as shown by Hartl (Hartl et al., 1988) who found loss of postischemic hyperemia to occur on day 8 to 10 from admission in septic patients with worsening clinical course and poor prognosis. Longitudinal studies documenting endothelial function from admission to discharge or death would be important to clarify the time course of vascular reactivity and its relation to MODS and mortality.

Experimental and animal data show that sepsis causes endothelial dysfunction but finding a surrogate marker of endothelial health for use in patients with sepsis is challenging. Other than measurable physiological responses, circulating biomarkers have been investigated in studies. Previous systematic review addressing clinical utility of biomarkers of endothelial activation in sepsis similarly to our meta-analysis found a correlation between various endothelium-derived molecules and presence of sepsis but correlation with clinically important outcomes was not consistent (Xing et al., 2012). The reason for inconsistency across studies using different markers has been postulated to be lack of method standardization, unclear threshold values and receiver operator characteristics.

Studies 2 and 3, prospective observational studies, concentrated on another aspect of artery function, they explored conduit and muscular artery stiffness in septic patients by

assessing carotid to femoral and carotid to radial PWV. When exploring PWV in patients with early sepsis admitted to ICU, study 2 found that high PWV values are prevalent in this population. In the general population of 60 to 70-year old normotensive individuals, the 90th reference percentile of carotid to femoral PWV is 12.2 m/s (Mattace-Raso et al., 2010) and higher values are considered a marker of significant alterations of arterial function (Mancia et al., 2007). In this sample, 55.6 % of patients (median age 67 years) had PWV of more than 12 m/s. The patient group with the top 25 % PWV values (> 24.7 m/s) was characterised by the presence of septic shock and need for mechanical ventilation. Patients in this group also had shorter survival times when mortality was considered.

Carotid to femoral PWV is a widely measured vascular biomarker and a strong predictor of future cardiovascular events and mortality in hypertensive patients (Ben-Shlomo et al., 2014). It is a composite measure of structural and functional damage in the media of large arteries. Increase in PWV related to structural changes is the result of insults accumulated over time and reflects loss of elastin and calcification in the artery wall, usually secondary to subclinical inflammation. In the cardiovascular risk population, non-invasive identification of those structural changes is the main rationale for PWV measurement. In acute inflammation, including sepsis, changes in PWV happen quickly and, therefore, are most likely due to functional factors, such as arterial smooth muscle tone. Arterial smooth muscle tone depends on sympathetic neural activity via the release of noradrenaline and on endothelial function via NO- dependent and independent mechanisms. PWV in the aorta is also influenced by distending pressure. This factor should have less influence in a cohort of resuscitated sepsis patients. Due to peripheral vasodilatation their arterial pressures and distending pressure in the aorta, are likely to be in the lower range for an individual patient. Indeed, in this cohort of patients median systolic and diastolic pressures were only 110 and 62 mm Hg, accordingly.

To the author's knowledge, this is the first study exploring PWV in a cohort of septic patients in the clinical context but an increase in aortic stiffness has been shown in animal models of sepsis. In acute endotoxic shock the central compartment of vasculature has been shown to become stiffer, but peripheral – more compliant, especially in resuscitated animals (Hatib et al., 2011). Mechanisms for the widespread increase of PWV in intensive care sepsis population are not clear, but functional rather than structural causes appear more likely. Sepsis-induced endothelial dysfunction could account for some of the observed changes, but the magnitude of the effect is better explained by a catecholamine excess state caused by sepsis (Boldt et al., 1995). This is supported by the finding that 80 % of patients with PWV > 24.7 m/s were receiving vasopressors.

Excessively high PWV on admission to intensive care was found to be associated with shorter survival times. If in this group sympathetic overstimulation is, indeed, the main determinant of PWV, detrimental effects of adrenergic stress could explain the finding (Dünser and Hasibeder, 2009). In patients with moderately high PWV, survival times did not differ from the reference group. This may be a reflection of the stress response necessary to maintain adequate perfusion during critical illness with functional adrenergic response.

Study 3 builds upon Study 2 and extends its scope to include characterisation of sepsis-induced alterations both elastic and muscular artery stiffness at two time points, within the first 24 hours of intensive care admission and 48 hours later. Similar to Study 2, increased aortic stiffness was found among the septic population. In addition, the stiffness of muscular arteries measured by carotid to radial PWV was even higher in this cohort. Endothelial and autonomic effects on stiffness are likely to be more pronounced in arteries that have a well-developed muscular layer, such as arteries of the upper limb. Experimental data show that increased sympathetic tone has a profound effect on the stiffness of muscular arteries (Boutouyrie et al., 1994). Extremely high carotid to radial PWV in a subgroup of patients is best explained by high adrenergic activity. Persistently high carotid-radial PWV on repeated measurement was shown to be a significant predictor of mortality. If high adrenergic activity is the reason for persistently high upper limb PWV, it could be potentially useful to identify the subgroup of septic patients to benefit most from adrenergic blockade.

Numerous publications and several reviews (Oliver and Webb, 2003; Zieman et al., 2005) reported the various factors associated with changed, mainly increased, aortic stiffness associated with structural artery wall changes. It is well established that in general population carotid-femoral PWV increases with increasing age whereas carotid to radial PWV does not change. In agreement with previous research, age was one of the candidate factors considered in multivariate analysis of carotid-femoral PWV predictors in Study 3, though was not significant in the final model.

The inflammation process, either acute during Salmonella typhi vaccination (Vlachopoulos et al., 2005) or chronic during rheumatoid arthritis or systemic lupus erythematosus, can cause arterial stiffening through various other mechanisms, including endothelial dysfunction, cell release of inducible matrix metalloproteinases, medial calcifications, modified proteoglycan composition and hydration state, and/or cell infiltration around the vasa vasorum leading to vessel ischemia (Mäki-Petäjä et al., 2006; Roman et al., 2005). The primary proinflammatory cytokines, TNF-α, and interleukin-6, are the main inducers of hepatic CRP synthesis. In untreated patients with essential hypertension, aortic stiffness, assessed with carotid-to-femoral PWV, was significantly associated with CRP and

interleukin-6 (Mahmud & Feely, 2005). Contrary to this finding, in Study 3 high CRP concentrations were associated with lower carotid-to-femoral PWV, a phenomenon most noted with the highest CRP values. The studies describing the association of CRP and PWV have enrolled patients with low grade inflammation and in case of a massive inflammatory response, the impact of CRP concentration on PWV could be different. Indeed, in acute inflammation model after Salmonella vaccination, a large increase in CRP level is associated with return of PWV to lower pre-vaccination values (Vlachopoulos et al., 2005). The current findings can be viewed as an association but cannot be used to draw conclusions that C reactive protein is involved in causing arterial dysfunction.

Because sepsis is a dynamic state and hemodynamic parameters can change quickly, functional determinants of PWV may play a significant role. The functional factors which can cause alterations in arterial distensibility are heart rate, mean and pulse arterial pressure values. In Study 3, correlations of carotid-femoral PWV and mean arterial pressure were significant in sepsis patients, supporting the data indicating that the influence of blood pressure on vascular properties is not modified in septic patients.

There are several limitations because of the included population and study design. In a study involving a single intensive care unit, the number of eligible patients is limited, therefore, we chose to recruit a heterogeneous group of patients with severe sepsis and septic shock. There were major differences between patients in terms of age, use of vasopressors, need for sedation and respiratory support. Measurement in patients with delirium was not possible because of movement artefacts. Baseline cardiovascular risk factors and comorbidities which are important confounders in this context could not be ascertained, as the condition of the patients often precluded effective communication.

PWV is a composite measure, which can be affected by rapid fluctuations in blood pressure. Such fluctuations are likely in the studied sepsis cohort, but we attempted to limit the effects of such fluctuations by including only resuscitated patients with no major cardiovascular instability for 1 hour. In published literature, there are also no reliable reference values for PWV when measured at lower than normal blood pressures.

Considering the small size of the cohort, only robust associations between the progression of MODS and mortality could have been detected. There might be other clinically important associations that this study has missed. Patients who died within the 48-hour window between assessment of SOFA and had the fastest deterioration of organ function were excluded from the analysis looking at the association between PWV and progression of MODS; therefore, the results are applicable only to patients who survive for at least 2 days after intensive care admission.

Overall, this thesis has shown that altered static and dynamic parameters of artery function are highly prevalent in patients with sepsis and can be associated with unfavourable outcome. There is insufficient evidence that decreased arterial vasoreactivity and increased conduit and muscular artery stiffness is the consequence of endothelial dysfunction. The extent of increase in arterial stiffness suggests that adrenergic stimulation might play a major role. Persistently increased carotid to radial PWV is the most promising biomarker to identify patients at risk of death in early sepsis.

CONCLUSIONS

1. There is evidence of moderate strength that vascular reactivity is impaired in septic patients but is not convincingly related to clinical outcomes.
2. Within 24 hours of admission to intensive care, patients with severe sepsis and septic shock have a high prevalence of elevated pulse wave velocity in elastic and muscular arteries.
3. There is no association between admission carotid to femoral and carotid to radial PWV and severity of sepsis, but carotid to femoral pulse wave velocity correlates positively with mean blood pressure and negatively with C reactive protein concentration.
4. There is no association between carotid to femoral pulse wave velocity at baseline or on 48-hour follow-up and improvement of multiple organ failure or mortality, but absolute change in carotid-radial PWV differs significantly between survivor and non-survivor groups.
5. Persistently increased carotid to radial PWV is a promising biomarker to identify patients at risk of death in early sepsis.

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APPENDICES

Ethics Committee Approval

Veidlapa Nr E-9 (2)

RSU ĒTIKAS KOMITEJAS LĒMUMS

Rīga, Dzirciema iela 16, LV-1007
Tel.67409137

Komitejas sastāvs	Kvalifikācija	Nodarbošanās
1. Asoc. prof. Olafs Brūvers	Dr.miss.	teologs
2. Docente Santa Purviņa	Dr.med.	farmakologs
3. Asoc. prof. Voldemārs Arnis	Dr.biol.	rehabilitologs
4. Profesore Regīna Kleina	Dr.med.	patanatoms

Pieteikuma iesniedzējs: Sigita Kazūne
Doktorantūras nodaļa

Pētījuma nosaukums: Mikrocirkulācijas novērtēšana sepses pacientiem

Iesniegšanas datums: 10.09.2010.

Pētījuma protokols:
(X) Pētījuma veids: pētījums kvalificējams kā prospektīvs pētījums.
(X) Pētījuma populācija: 150 Toksikoloģijas un sepses klīnikā stacionēti pacienti
(X) Informācija par pētījumu:
(X) Piekrišana dalībai pētījumā:

Ētiska aspekta ieteikums: Nepieciešams iesniegt atļauju no RAKUS klīnikas „Gaiļezers" vadības par piekrišanu pētījuma veikšanai

Citi dokumenti:
1. Pretendentes CV

Lēmums: piekrist biomedicīniskajam pētījumam, **pēc papildinājumu iesniegšanas**

Komitejas priekšsēdētājs Olafs Brūvers Tituls: Dr.miss., asoc.prof.

Paraksts

Ētikas komitejas sēdes datums: 23.09.2010.

ĒTIKAS KOMITEJA

Papildinājumi apņemti.
/OLAFS BRŪVERS/
09.12.2010.

Newcastle-Ottawa Scale for assessment of study quality (customized)

A study can be awarded a maximum of two stars in each of the four methodological domains below.

Patient selection:
1) Representativeness of the sample:

a) truly representative of the average septic patient population * (all subjects or random sampling);
b) somewhat representative of the average septic patient population * (non-random sampling);
c) selected group of patients;
d) no description of the sampling strategy.

2) Sample size:
a) justified and satisfactory *
b) not justified.

Measurement of vascular reactivity:

a) gold standard measurement method **
b) method is available or described *
c) no description of the measurement tool.

Comparability:

a) The subjects in different outcome groups are comparable, based on the study design or analysis. Confounding factors are controlled. **
b) The subjects in different outcome groups are age and gender matched. *
c) The subjects in different outcome groups are not comparable.

Assessment of outcome (presence, severity of sepsis, MODS, mortality)

a) Independent assessment (i.e. by an investigator) *
b) Record linkage *
c) Method allows possibility for outcome misassignment
d) No description or unclear

Demographic characteristics of patients

Author	Age (years)		Total number (% male)		Severity		Definition of sepsis	Comorbidity exclusions	Mortality	Documented ICU interventions		
	Sepsis	Control	Sepsis	Control	Scoring system	Value				Catecholamines	Ventilation	Sedation
Studies using laser Doppler flowmetry												
Young, 1995	45 (37–82)	32 (23–42)[1] 88 (82–89)[2] 67 (48–78)[3]	11 (?)	9 (?) 10 (?) 19 (?)			1 B C D	Diabetes	?	64 %	55 %	?
Neviere, 1996	60 ± 11	?[4]	16 (?)	10 (?)	APACHE II	22 ± 7	1 C	N	50 %	?	?	?
Sair, 2001	51 ± 18	?[1] 67 ± 3.4[3]	6 (83 %)	7 (?) 6 (67 %)			1 D	N	83 %	100 %	100 %	?
Kubli, 2003	58 ± 12.9	59.8 ± 12.4[5] 58.7 ± 12.4[1]	12 (75 %)	16 (75 %[9]) 12 (75 %[5])	SOFA	13.8 ± 3.6	1 D	N	?	100 %	100 %	75 %
Favory, 2013	59 (51–69)	?[1]	12 (67 %)	8 (?)	SAPS II	58 (47–64)	1 C D	N	50 %	83 %	?	?

Appendix 3 continues

Author	Age (years)		Total number (% male)		Severity		Definition of sepsis	Comorbidity exclusions	Mortality	Documented ICU interventions		
	Sepsis	Control	Sepsis	Control	Scoring system	Value				Catecholamines	Ventilation	Sedation
Knotzer, 2007	52.3 ± 16[a] 67.6 ± 10.2[b]		15 (73 %)[a] 14 (57 %)[b]		MODS ASA SAPS II	6.5 ± 2.7[a] 9.4 ± 2.0[b] 3.7 ± 0.5[a] 4.0 ± 0.5[b] 11.5 ± 3.4[a] 15.9 ± 2.4[b]	1 A B C D	Y	48 %	20 %[a] 29 %[b]	100 %	100 %
Bourcier, 2017	59 (49–68)		37 (48 %)		SAPS II	46 (37–72)	3 C D	N	22 %	70 %	48 %	?
Studies using plethysmography												
Hartl, 1988	62.5 ± 2.4	64.3 ± 3.2[6]	12 (?)	10 (100 %)	Sepsis score	16 ± 2	4 D	N	58 %	100 %	100 %	?
Astiz, 1991	55 ± 7	32 ± 2[1]	8 (?)	9 (?)	APACHE II	31 ± 8	1 B C D	N	63 %	88 %	?	?
Astiz, 1995	72 (42–84)	64.3 ± 3.1[6]	23 (?)	10 (?)	Sepsis score	C8.87 ± 0.5 D17.35 ± 2.56	1 C D	N	27 %	0	?	?
Kirschenbaum, 2000	63 ± 3	74 ± 1[7] 76 ± 2[8]	6 (?)	8 (?) 6 (?)	APACHE II	31 ± 2	1 D	N	50 %	100 %	?	?
Studies using conduit artery sonography												
Vaudo, 2008	41 ± 8	43 ± 5[5]	45 (24 %)	25 (44 %)	SOFA	4 ± 1	1 B	Y	49 %	0	0	0

Appendix 3 continues

Author	Age (years)		Total number (% male)		Severity		Definition of sepsis	Comorbidity exclusions	Mortality	Documented ICU interventions		
	Sepsis	Control	Sepsis	Control	Scoring system	Value				Catecholamines	Ventilation	Sedation
Wexler, 2012	62(49–74)	60 (53–66)[9]	95 (52 %)	52 (50 %)	APACHE II	23 ± 8	1 C D	Y	18 %	28 %	?	?
Nelson, 2016	59 ± 14	59 ± 15[10]	17 (59 %)	16 (56 %)	APACHE II SOFA	17 ± 7 6 ± 3	1 C D	N	0 %	18 %	?	?
Becker, 2012	51 ± 19	47 ± 14[5]	42 (38 %)	38 (33 %)	APACHE II	23 ± 7	1 C D	N	33 %	79 %	?	?
Studies using peripheral arterial tonometry												
Davis, 2009	C 52.4 (48.3–56.5) B 50.8 (46.5–55.2)	45.2 (43.1–51.4)[9]	54 (C 61 %) 31 (B 68 %)	45 (67 %)	APACHE II SOFA	C 19.0 (15–23) B 7.5 (5–11) C 6 (3–9) B 1 (0–2)	1 B C D	N	9 %	32 %	24 %	?
Van Ierssel, 2013	63 ± 2.9	?[10]	30 (67 %)	15 (?[5])	SAPS III SOFA	61.7 ± 1.9 7.8 ± 0.6	1 C D	N	14 %	70 %	57 %	?
Nobre, 2016	51.5 ± 18.9		62 (62 %)		APACHE II SOFA	16 (12–20) 7 (5–9)	2 C D	N	27 %	76 %	?	?

Appendix 4

Qualitative synthesis of included studies

Authors	Presence of sepsis	Severity of disease	Mortality	MODS	Longitudinal change
Studies using laser Doppler flowmetry					
Young, 1995	Sepsis<post CPB/volunteers				
Neviere, 1996	HV sepsis<ICU controls				
Sair, 2001	HV Sepsis=controls (LDF) RH sepsis<controls (LDF) RH sepsis=post CPB(P) RH Sepsis<controls(P) RH sepsis=post CPB(P)				
Kubli, 2003	EDVD sepsis =ICU controls=volunteers			EDVD does not correlate with SOFA	EDVD increases with improvement
Knotzer, 2007			RH Survivors=non-survivors HV survivors=non-survivors		
Favory, 2013	HV sepsis<controls				
Bourcier, 2017		EDVD sepsis>septic shock(knee)	EDVD survivors>non survivors (forearm, knee)		
Hartl, 1988	Sepsis=vascular surgical patients			RH lost in progressive disease	RH lost in progressive disease

Appendix 4

Qualitative synthesis of included studies

Authors	Presence of sepsis	Severity of disease	Mortality	MODS	Longitudinal change
Astiz, 1995	Sepsis<controls				
Astiz, 1991	Sepsis<controls	Septic shock>sepsis	-	-	Vascular profiles of septic shock survivors similar to controls by day 5
Kirschenbaum, 2000	Sepsis<controls Sepsis=cardiogenic shock				
Sair, 2001	HV Sepsis=controls RH sepsis<controls RH sepsis=post CPB RH Sepsis<controls RH sepsis=post CPB				
Studies using conduit artery sonography					
Vaudo, 2008	HV Sepsis<controls			Changes in HV related to changes in SOFA	
Wexler, 2012	FMD sepsis<controls		FMD survivors=non-survivors HV survivors>non-survivors	No relation between FMD and SOFA HV negatively correlated with SOFA	
Becker, 2012	FMD sepsis<controls		FMD survivors=non-survivors		FMD declines over 72 hours in non survivors, improves in survivors

Appendix 4

Qualitative synthesis of included studies

Authors	Presence of sepsis	Severity of disease	Mortality	MODS	Longitudinal change
Nelson, 2016	FMD sepsis<controls LBF sepsis<controls	Negative correlation between FMD and APACHE II			No change in FMD over 72 hours
Studies using peripheral arterial tonometry					
Davis, 2009	Sepsis<controls	Severe sepsis<sepsis<control	Survivors=non-survivors	Low baseline RHI correlates with subsequent SOFA	No change in RHI
van Ierssel, 2013	Sepsis<controls	No correlation with SAPS III		No relation between RHI and SOFA score	
Nobre, 2016		No correlation with APACHE II	Survivors=non-survivors	No relation between RHI and SOFA score	RHI increases in non survivors