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

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The Doctoral thesis is available in RSU library and on RSU website: www.rsu.lv

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CONTENTS

LIST OF ABBREVIATIONS

AC	alternating current
ACCP/SCCM	American College of Chest Physicians/Society of Critical Care Medicine
ANOVA	analysis of variance
APACHE II	Acute Physiology and Chronic Health Evaluation II
c-f PWV	carotid-femoral pulse wave velocity
c-r PWV	carotid-radial pulse wave velocity
CI	confidence interval
CPR	C reactive protein
eNO	endothelial nitric oxide
FMD	flow-mediated vasodilatation
IQR	inter-quartile range
ICU	intensive care unit
MODS	multiple organ dysfunction syndrome
OR	odds ratio
PPG	photoplethysmogram
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
ROC	Receiver Operating Characteristic
RH-PAT	peripheral arterial tonometry
SOFA	Sequential Organ Failure Assessment
SMD	standardised mean difference
TNF-α	tumour necrosis factor α

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 development of sepsis (Nystrom, 1998), 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 association between altered vascular reactivity and severity of sepsis with respect to 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., 1999; Stefanadis et al., 2000). Another measure of arterial properties, carotid-radial pulse wave velocity (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 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 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.

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 of the thesis

- 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 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.

1 AIM, OBJECTIVES AND HYPOTHESES

1.1 Aim of the thesis

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.

1.2 Objectives of the thesis

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 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.

1.3 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.

2 METHODS

The research was conducted in conformity with international recommendations and ethical principles of human research. Research protocol 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 the 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 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).

This meta-analysis addresses the following questions:

- Does endothelial vascular reactivity change in relation to presence or severity of sepsis?
- Does impairment of endothelial vascular reactivity correlate to development or severity of single or multiple organ failure?
- Does 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), definition of sepsis was used as given in the retrieved articles. Primary end-point of interest was changes of endothelial vascular reactivity in relation to presence or severity of sepsis. Secondary endpoints considered were 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.

The following medical databases were searched: MEDLINE (January 1985 onwards), Scopus (January 1985 onwards) and EMBASE (January 1985 onwards).

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

Results of database searches and study selection process are shown in Figure 2.1.

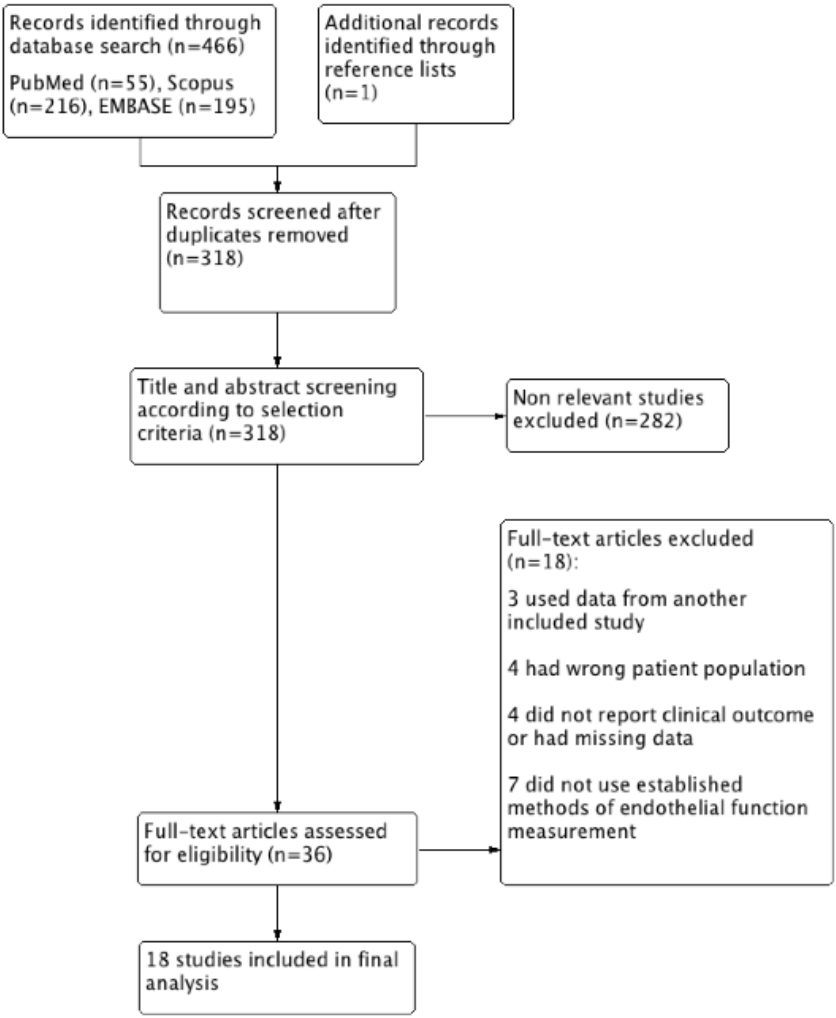

Figure 2.1 **Flow diagram of study selection**

2.2 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).

2.3 Methods for prospective cohort and case-control studies of PWV in patients with sepsis

2.3.1 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 ACCP/SCCM Consensus Conference (1992) criteria (Bone et al., 1992). To be included in the study patients had 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. Study involved recording

of pulse waves using originally designed photoplethysmograph, so patients could be recruited only when investigator was available.

Table 2.2

Baseline characteristics of study populations

Parameter	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 and 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. 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.

2.3.2 Pulse wave velocity measurement

Studies 2 and 3 used custom device for recording photoplethysmograms (PPG) 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, 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:

$$PWV = D/\Delta t \qquad (1.3.2.1)$$

where D – inter-probe distance in meters,

Δt – transit time in seconds.

2.3.3 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. C-f 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 right femoral artery.

PPG from both probes was recorded simultaneously and stored for off-line analysis. Path of the pulse wave was measured over the body surface with tape measure in straight line between probe 1 and probe 2. Calculation of c-f PWV was performed as described in Chapter 2.3.2.

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

dysfunction syndrome (defined as increase in SOFA score of at least 1 point) over the first 48 hours of ICU admission.

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

2.3.4 Protocol for Study 3

After inclusion into Study 3 demographic, clinical and hemodynamic parameters were recorded 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 right carotid and right femoral artery and PPG recorded. Then probe 1 was left in position and probe 2 was moved to the 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 the first measurement. Data regarding length of intensive care and hospital stay and hospital mortality were recorded.

2.3.5 Statistical analysis of Studies 2 and 3

Study 2. Demographic, clinical and haemodynamic characteristics of the patient cohort are 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 association of PWV quartile strata with 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 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 (P.J. 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 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; 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; Young and Cameron, 1995). 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. 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.

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.

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 and 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.

The 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, standardised mean difference (SMD) between septic patients and controls and between survivors and non-survivors. Measurements of absolute (peak flow) and relative (reactive hyperaemia) 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; $Z = 5.85$, $p < 0.001$; Figure 3.1).

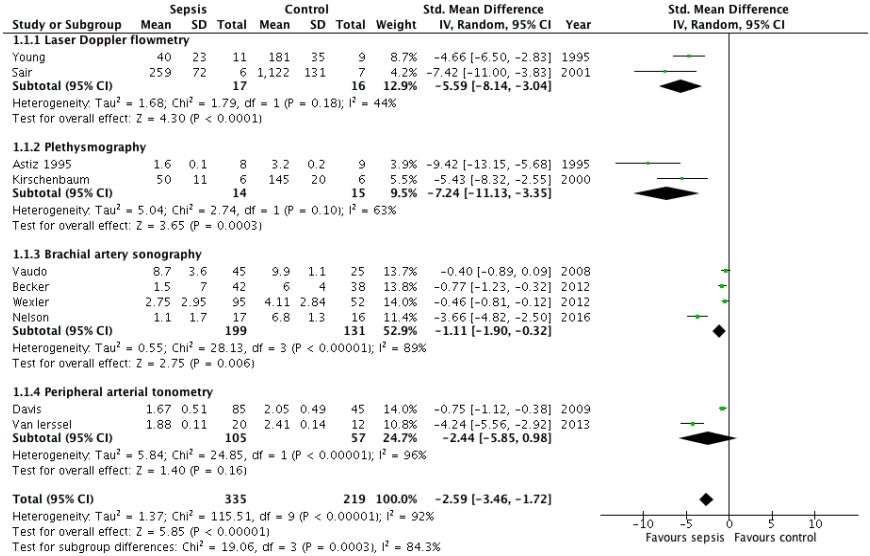

Figure 3.1 **Pooled mean difference in reactive hyperemia between septic patients and controls**

In nine studies with 354 participants peak hyperaemic 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, $p < 0.001$; Figure 3.2). Results of these studies were highly heterogeneous with I^2 values of 92 % ($p < 0.001$) and 85 % ($p < 0.001$), 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.

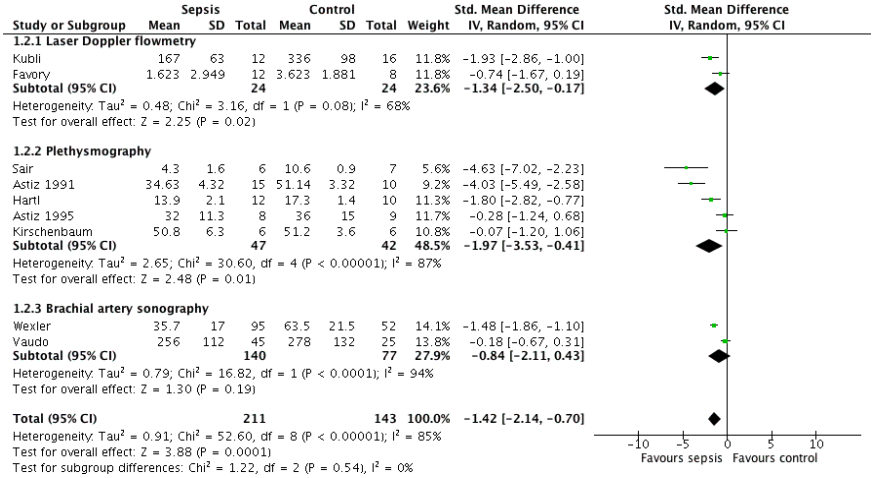

Figure 3.2 **Pooled mean difference in peak reactive hyperemia between septic patients and controls**

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 hyperaemia and –0.70 (95 % CI –1.13 to –0.27; Z=3.23; p = 0.001, Figure 3.4) for peak hyperaemic blood flow. Both reactive hyperaemia and peak hyperaemic flow were lower in non-survivors. Tests for heterogeneity were statistically not significant for both estimates.

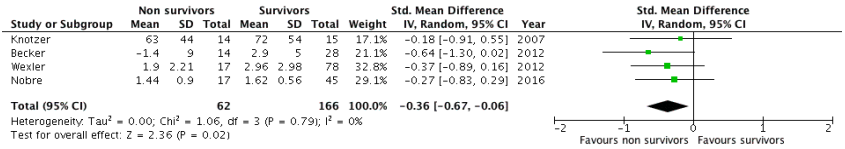

Figure 3.3 **Pooled mean difference in reactive hyperemia between non survivors and survivors**

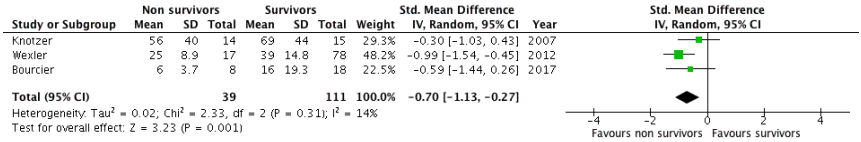

Figure 3.4 **Pooled mean difference in peak hyperemic flow between non-survivors and survivors**

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.

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 2.1.

Table 2.1

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

Parameter	Pulse wave velocity			
	< 8.1 m/s (n = 12)	8.1–14.6 m/s (n = 11)	14.6–24.7 m/s (n = 10)	> 24.7 m/s (n = 12)
Demographic characteristics				
Ag, years	61 (43–73)	57 (49–68)	69 (57–79)	59 (39–78)
Females, n (%)	5 (38 %)	4 (36 %)	5 (45 %)	3 (30 %)
Clinical characteristics				
APACHE II score	21 (14–26)	19 (16–24)	16 (11–19)	19 (15–27)
SOFA score	9 (4–10)	8 (5–9)	4 (3–7)	7 (5–11)
Ventilated, n (%)	6 (46 %)	3 (27 %)	1 (9 %)	8 (80 %)*
Septic shock, n (%)	7 (54 %)	6 (55 %)	4 (36 %)	8 (80 %)*
Hemodynamic characteristics				
Systolic blood pressure, mm Hg	113 (107–120)	101 (95–116)	107 (90–128)	108 (97–121)
Mean blood pressure, mm Hg	80 (77–85)	75 (70–82)	77 (70–92)	72 (70–82)
Pulse pressure, mm Hg	51 (41–53)	48 (34–58)	44 (34–57)	44 (36–56)
Diastolic blood pressure, mm Hg	62 (60–71)	66 (55–67)	65 (60–78)	58 (44–66)*
Dose of noradrenaline, mcg/kg/min	0.03 (0–0.09)	0.03 (0–0.08)	0 (0–0.02)	0.1 (0–0.14)
Heart rate, beats/min	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 and 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.

The association between c-f PWV and 48 hour progression of multiple organ dysfunction (MODS) measured by change in SOFA score from baseline is shown in Table 3.2. 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 3.2

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 dysfunction syndrome, 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 (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).

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 3.3., including 27 patients without and 32 patients with septic shock.

Table 3.3

Baseline demographic and clinical characteristics of patients included in Study 3

Parameter	All patients	Survivors	Non survivors	p
	n = 59	n = 43	n = 16	
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)	0.001
APACHE II score	19 (14–25)	17 (12–21)	27 (20–32)	< 0.001
SOFA score	7 (4–9)	5 (4–8)	10 (8–12)	< 0.001

APACHE II – Acute Physiology and 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 = non-significant). 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) (Fig. 3.5).

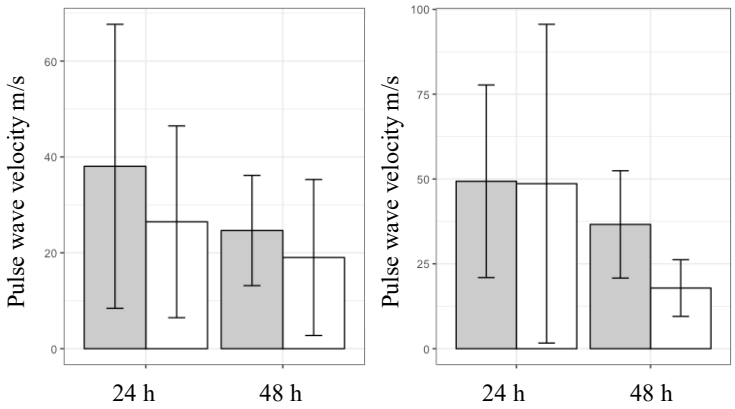

Figure 3.5 **Time course of carotid-femoral (a) and carotid-radial (b) PWV (mean ± CI) in survivors (n = 36; shaded bars) and non-survivors (n=16; white bars) at baseline (24 h) and after 48 hours**

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 3.4).

Table 3.4

Sensitivity and specificity of carotid to femoral and carotid to radial PWV (m/s) in predicting clinical outcomes in septic patients

ROC curve	AUC (95 % CI)	Cut-off	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 3.5.

Table 3.5

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.

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 ($\beta = 0.56$, $p < .001$) and mean blood pressure ($\beta = –0.71$, $p = 0.15$). We observed a significant winsorized correlation between carotid to radial PWV and age ($\rho_w = 0.31$; $p = 0.04$).

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 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 allow 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 except for 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 a 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 a 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.

A minority of studies examined changes in vascular reactivity in the context of the progression of multiple organ dysfunction or mortality.

Association with these critical outcomes was not convincing. The reason for the weakness of 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. An 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 and 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 the acute inflammation model after Salmonella vaccination, a large increase in CRP level

is associated with the 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 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 heterogenous 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 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.

Although the necessary size of the cohort was calculated before starting the study, only robust associations between 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.

REFERENCES

1. Aird, W. C. 2003. The role of the endothelium in severe sepsis and multiple organ dysfunction syndrome. *Blood*, 101(10), 3765–3777. doi:10.1182/blood-2002-06-1887
2. Astiz, M. E., DeGent, G. E., Lin, R. Y., & Rackow, E. C. (1995). Microvascular function and rheologic changes in hyperdynamic sepsis. *Critical Care Medicine*, *23*(2), 265–271.
3. Astiz, M. E., Tilly, E., Rackow, E. D., & Weil, M. H. (1991). Peripheral vascular tone in sepsis. *Chest*, *99*(5), 1072–1075. https://doi.org/10.1378/chest.99.5.1072
4. Becker, L., Prado, K., Foppa, M., Martinelli, N., Aguiar, C., Furian, T., et al. (2012). Endothelial dysfunction assessed by brachial artery ultrasound in severe sepsis and septic shock. *Journal of critical care*, *27*(3), 316.e9–14. https://doi.org/10.1016/j.jcrc.2011.08.002
5. Bellamkonda, K., Williams, M., Handa, A., & Lee, R. (2017). Flow Mediated Dilatation as a Biomarker in Vascular Surgery Research. *Journal of Atherosclerosis and Thrombosis*, *24*(8), 779–787. https://doi.org/10.5551/jat.40964
6. Ben-Shlomo, Y., Spears, M., Boustred, C., May, M., Anderson, S. G., Benjamin, E. J., et al. (2014). Aortic pulse wave velocity improves cardiovascular event prediction: an individual participant meta-analysis of prospective observational data from 17,635 subjects. *Journal of the American College of Cardiology*, *63*(7), 636–646. https://doi.org/10.1016/j.jacc.2013.09.063
7. Blacher, J., Asmar, R., Djane, S., London, G. M., & Safar, M. E. 1999. Aortic Pulse Wave Velocity as a Marker of Cardiovascular Risk in Hypertensive Patients. *Hypertension*. 33(5), 1111-1117. doi:10.1161/01.HYP.33.5.1111
8. Blacher, J., Guerin, A. P., Pannier, B., Marchais, S. J., Safar, M. E., & London, Gè. M. 1999. Impact of aortic stiffness on survival in end-stage renal disease. *Circulation*. 99(18), 2434-2439. doi:10.1161/01.CIR.99.18.2434
9. Boldt, J., Menges, T., Kuhn, D., Diridis, C., & Hempelmann, G. (1995). Alterations in circulating vasoactive substances in the critically ill--a comparison between survivors and non-survivors. *Intensive Care Medicine*, *21*(3), 218–225.
10. Bone, R. C., Balk, R. A., Cerra, F. B., Dellinger, R. P., Fein, A. M., Knaus, W. A., et al. (1992). American-College of Chest Physicians Society of Critical Care Medicine Consensus Conference - Definitions for Sepsis and Organ Failure and Guidelines for the Use of Innovative Therapies in Sepsis. *Chest*, *101*(6), 1644–55. https://doi.org/10.1378/chest.101.6.1644
11. Bourcier, S., Joffre, J., Dubée, V., Preda, G., Baudel, J.-L., Bigé, N., et al. (2017). Marked regional endothelial dysfunction in mottled skin area in patients with severe infections. *Critical Care*, *21*(1), 155–155. https://doi.org/10.1186/s13054-017-1742-x
12. Boutouyrie, P., Lacolley, P., Girerd, X., Beck, L., Safar, M., & Laurent, S. 1994. Sympathetic activation decreases medium-sized arterial compliance in

humans. *American Journal of Physiology-Heart and Circulatory Physiology*. 267(4), H1368-H1376. doi:10.1152/ajpheart.1994.267.4.H1368
13. Cohen, J. 2002. The immunopathogenesis of sepsis. *Nature*. 420 (6917), 885-91. doi:10.1038/nature01326
14. Davis, J. S., Yeo, T. W., Thomas, J. H., McMillan, M., Darcy, C. J., McNeil, Y. R., et al. (2009). Sepsis-associated microvascular dysfunction measured by peripheral arterial tonometry: an observational study. *Critical care (London, England)*, *13*(5), R155–R155. https://doi.org/10.1186/cc8055
15. Dünser, M. W., & Hasibeder, W. R. (2009). Sympathetic overstimulation during critical illness: adverse effects of adrenergic stress. *Journal of Intensive Care Medicine*, *24*(5), 293–316. https://doi.org/10.1177/0885066609340519
16. Erts, R., Spigulis, J., Kukulis, I., & Ozols, M. 2005. Bilateral photoplethysmography studies of the leg arterial stenosis. *Physiological Measurement*. 26(5):865–74. doi:10.1088/0967- 3334/26/5/022
17. Favory, R., Poissy, J., Alves, I., Guerry, M.-J., Lemyze, M., Parmentier-Decrucq, E., et al. (2013). Activated protein C improves macrovascular and microvascular reactivity in human severe sepsis and septic shock. *Shock (Augusta, Ga.)*, *40*(6), 512–518. https://doi.org/10.1097/SHK.0000000000000060
18. Wells GA, Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp. Accessed 15 September 2018
19. Harrell, F. E., & Davis, C. E. (1982). A new distribution-free quantile estimator. *Biometrika*, *69*(3), 635–640. https://doi.org/10.1093/biomet/69.3.635
20. Hartl, W. H., Günther, B., Inthorn, D., Heberer, G., Hartt, W. H., Günther, B., et al. (1988). Reactive hyperemia in patients with septic conditions. *Surgery*, *103*(4), 440–444.
21. Hatib, F., Jansen, J. R. C., & Pinsky, M. R. (2011). Peripheral vascular decoupling in porcine endotoxic shock. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *111*(3), 853–860. https://doi.org/10.1152/japplphysiol.00066.2011
22. Henneke, P., & Golenbock, D. T. (2002). Innate immune recognition of lipopolysaccharide by endothelial cells. *Critical care medicine*, *30*(5 Suppl), S207-13. https://doi.org/10.1097/00003246-200205001-00006
23. Ince, C., Mayeux, P. R., Nguyen, T., Gomez, H., Kellum, J. A., Ospina-TascÛn, G. A., et al. (2016). The Endothelium in Sepsis. *Shock*, *45*(3), 259–270. https://doi.org/10.1097/SHK.0000000000000473
24. Kienbaum, P., Prante, C., Lehmann, N., Sander, A., Jalowy, A., & Peters, J. (2008). Alterations in forearm vascular reactivity in patients with septic shock. *Anaesthesia*, *63*(2), 121–8. https://doi.org/10.1111/j.1365-2044.2007.05286.x
25. Kirschenbaum, L. A., Astiz, M. E., Rackow, E. C., Saha, D. C., & Lin, R. (2000). Microvascular response in patients with cardiogenic shock. *Critical care medicine*, *28*(5), 1290–4.

26. Knaus, W. A., Draper, E. A., Wagner, D. P., & Zimmerman, J. E. (1985). APACHE II: a severity of disease classification system. *Critical Care Medicine*, *13*(10), 818–29. https://doi.org/10.1097/00003465-198603000-00013
27. Knotzer, H., Maier, S., Dünser, M., Stadlbauer, K. H., Ulmer, H., Pajk, W., & Hasibeder, W. R. (2007). Oscillation frequency of skin microvascular blood flow is associated with mortality in critically ill patients. *Acta anaesthesiologica Scandinavica*, *51*(6), 701–7. https://doi.org/10.1111/j.1399-6576.2007.01336.x
28. Kubli, S., Boëgli, Y., Ave, A. D., Liaudet, L., Revelly, J. P., Golay, S., et al. (2003). Endothelium-dependent vasodilation in the skin microcirculation of patients with septic shock. *Shock (Augusta, Ga.)*, *19*(3), 274–280.
29. Lekakis, J., Abraham, P., Balbarini, A., Blann, A., Boulanger, C. M., Cockcroft, J., et al. (2011). Methods for evaluating endothelial function: a position statement from the European Society of Cardiology Working Group on Peripheral Circulation. *European journal of cardiovascular prevention and rehabilitation : official journal of the European Society of Cardiology, Working Groups on Epidemiology & Prevention and Cardiac Rehabilitation and Exercise Physiology*, *18*(6), 775–89. https://doi.org/10.1177/1741826711398179
30. Liberati, A., Altman, D. G., Tetzlaff, J., Mulrow, C., Gøtzsche, P. C., Ioannidis, J. P. A., et al. (2009). The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. *BMJ*, *339*, b2700. https://doi.org/10.1136/bmj.b2700
31. Mahmud, A., & Feely, J. (2005). Arterial stiffness is related to systemic inflammation in essential hypertension. *Hypertension (Dallas, Tex.: 1979)*, *46*(5), 1118–1122. https://doi.org/10.1161/01.HYP.0000185463.27209.b0
32. Mäki-Petäjä, K. M., Hall, F. C., Booth, A. D., Wallace, S. M. L., Yasmin, null, Bearcroft, P. W. P., et al. (2006). Rheumatoid arthritis is associated with increased aortic pulse-wave velocity, which is reduced by anti-tumor necrosis factor-alpha therapy. *Circulation*, *114*(11), 1185–1192. https://doi.org/10.1161/CIRCULATIONAHA.105.601641
33. Mancia, G., De Backer, G., Dominiczak, A., Cifkova, R., Fagard, R., Germano, G., et al. (2007). 2007 Guidelines for the Management of Arterial Hypertension: The Task Force for the Management of Arterial Hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *Journal of Hypertension*, *25*(6), 1105–1187. https://doi.org/10.1097/HJH.0b013e3281fc975a
34. Mattace-Raso, F. U. S., Hofman, A., Verwoert, G. C., Wittemana, J. C. M., Wilkinson, I., Cockcroft, J., et al. 2010. Determinants of pulse wave velocity in healthy people and in the presence of cardiovascular risk factors: 'Establishing normal and reference values. *European Heart Journal*. 31(19):2338-50doi:10.1093/eurheartj/ehq165
35. McCuskey, R. S., Urbaschek, R., & Urbaschek, B. 1996. The microcirculation during endotoxemia. *Cardiovascular Research*. 32, 752-763. doi:10.1016/0008-6363(96)00113-7

36. Nelson, A. D., Rossman, M. J., Witman, M. A., Barrett-O'Keefe, Z., Groot, H. J., Garten, R. S., & Richardson, R. S. (2016). Nitric oxide-mediated vascular function in sepsis using passive leg movement as a novel assessment: a cross-sectional study. *Journal of Applied Physiology (Bethesda, Md.: 1985)*, *120*(9), 991–999. https://doi.org/10.1152/japplphysiol.00961.2015
37. Nobre, V., Ataíde, T. B., Brant, L. C., Oliveira, C. R., Rodrigues, L. V., Ribeiro, A. L. P., et al. (2016). Use of reactive hyperemia - peripheral arterial tonometry and circulating biological markers to predict outcomes in sepsis. *Revista Brasileira de Terapia Intensiva*, *28*(4), 387–396. https://doi.org/10.5935/0103-507X.20160072
38. Nyström, P. O. 1998. The systemic inflammatory response syndrome: definitions and aetiology. *The Journal of Antimicrobial Chemotherapy*, 41 Suppl A, 1–7. doi:10.1093/jac/41.suppl_1.1
39. Oliver, J. J., & Webb, D. J. (2003). Noninvasive assessment of arterial stiffness and risk of atherosclerotic events. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *23*(4), 554–566. https://doi.org/10.1161/01.ATV.0000060460.52916.D6
40. Opal, S. M., & van der Poll, T. 2015. Endothelial barrier dysfunction in septic shock. *Journal of Internal Medicine*. 277(3), 277–293. doi:10.1111/joim.12331
41. P.J. Huber. (1981). *Robust statistics*. New York, USA: John Wiley & Sons.
42. Roman, M. J., Devereux, R. B., Schwartz, J. E., Lockshin, M. D., Paget, S. A., Davis, A., et al. (2005). Arterial stiffness in chronic inflammatory diseases. *Hypertension (Dallas, Tex.: 1979)*, *46*(1), 194–199. https://doi.org/10.1161/01.HYP.0000168055.89955.db
43. Sair, M., Etherington, P. J., Peter Winlove, C., & Evans, T. W. (2001). Tissue oxygenation and perfusion in patients with systemic sepsis. *Critical care medicine*, *29*(7), 1343–9.
44. Singer, M, Deutschman, C. S., Seymour, C. W., Shankar-Hari, M., Annane, D., Bauer, M., & Bellomo, R. 2016. The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA*, 315(8), 801–810. doi:10.1001/jama.2016.0287
45. Stefanadis, C., Dernellis, J., Tsiamis, E., Stratos, C., Diamantopoulos, L., Michaelides, A., & Toutouzas, P. 2000. Aortic stiffness as a risk factor for recurrent acute coronary events in patients with ischaemic heart disease. *European Heart Journal.* 21(5), 390–396. doi:10.1053/euhj.1999.1756
46. Stoller, J., Halpin, L., Weis, M., Aplin, B., Qu, W., Georgescu, C., & Nazzal, M. (2016). Epidemiology of severe sepsis: 2008-2012. *Journal of Critical Care*, *31*(1), 58–62. https://doi.org/10.1016/j.jcrc.2015.09.034
47. van Ierssel, S. H., Van Craenenbroeck, E. M., Hoymans, V. Y., Vrints, C. J., Conraads, V. M., & Jorens, P. G. (2013). Endothelium dependent vasomotion and in vitro markers of endothelial repair in patients with severe sepsis: an observational study. *PloS one*, *8*(8), e69499–e69499. https://doi.org/10.1371/journal.pone.0069499
48. Vaudo, G., Marchesi, S., Siepi, D., Brozzetti, M., Lombardini, R., Pirro, M., et al. (2008). Human endothelial impairment in sepsis. *Atherosclerosis*, *197*(2), 747–52. https://doi.org/10.1016/j.atherosclerosis.2007.07.009

49. Vincent, J. L., Moreno, R., Takala, J., Willatts, S., De Mendonça, A., Bruining, H., et al. (1996). The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. *Intensive Care Medicine*, *22*(7), 707–710. https://doi.org/10.1007/s001340050156
50. Vlachopoulos, C., Dima, I., Aznaouridis, K., Vasiliadou, C., Ioakeimidis, N., Aggeli, C., et al. 2005. Acute systemic inflammation increases arterial stiffness and decreases wave reflections in healthy individuals. *Circulation*. 112(14), 2193–2200. doi:10.1161/CIRCULATIONAHA.105.535435
51. Wexler, O., Morgan, M. A. M., Gough, M. S., Steinmetz, S. D., Mack, C. M., Darling, D. C., et al. (2012). Brachial artery reactivity in patients with severe sepsis: an observational study. *Critical care (London, England)*, *16*(2), R38–R38. https://doi.org/10.1186/cc11223
52. Wilcox, R. R., & Keselman, H. J. (2003). Modern robust data analysis methods: measures of central tendency. *Psychological Methods*, *8*(3), 254–274. https://doi.org/10.1037/1082-989X.8.3.254
53. Xing, K., Murthy, S., Liles, W. C., & Singh, J. M. (2012). Clinical utility of biomarkers of endothelial activation in sepsis--a systematic review. *Critical care (London, England)*, *16*(1), R7. https://doi.org/10.1186/cc11145
54. Young, J. D., & Cameron, E. M. (1995). Dynamics of skin blood flow in human sepsis. *Intensive Care Medicine*, *21*(8), 669–74. https://doi.org/10.1007/BF01711546
55. Zieman Susan J., Melenovsky Vojtech, & Kass David A. (2005). Mechanisms, Pathophysiology, and Therapy of Arterial Stiffness. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *25*(5), 932–943. https://doi.org/10.1161/01.ATV.0000160548.78317.29

LIST OF PUBLICATIONS

Publications in peer reviewed medical journals

1. Kazūne, S., Grabovskis, A., Strīķe, E. and Vanags, I. 2014. Arterial Stiffness Measured by Pulse Wave Velocity in Patients with Early Sepsis / Artēriju Cietība Un Pulsa Viļņa Izplatīšanās Ātrums Pacientiem Ar Agrīnu Sepsi. *Proceedings of the Latvian Academy of Sciences. Section B. Natural, Exact, and Applied Sciences*. 68(5–6), 237–241. doi:10.2478/prolas-2014-0030
2. Kazune S., Grabovskis A., Cescon C., Strike E. and Vanags I. 2019. Association between increased arterial stiffness and clinical outcomes in patients with early sepsis: a prospective observational cohort study. *Intensive Care Med Exp*. 7(1):26. Published 2019 May 16. doi:10.1186/s40635-019-0252-3
3. Kazune, S., Piebalga, A., Strike, E. and Vanags, I. 2019. Impaired vascular reactivity in sepsis - a systematic review with meta-analysis. *Archives of medical sciences. Atherosclerotic diseases*. 4, e151–e161. doi:10.5114/amsad.2019.86754

Latvian patent:

Kazūne, S., Vanags, I. un Grabovskis, A. 2014. Neadekvātas nieru asinsvadu perfūzijas attīstības riska prognozēšanas paņēmiens smagas sepses un septiska šoka pacientiem kritiskā stāvoklī (Eng. Method for predicting risk of inappropriate renal vascular perfusion development in critically ill patients with severe sepsis and septic shock): LV 14806 B: patents

Abstacts and presentations at international meetings

1. Kazune, S., Strīķe, E., Erts, R. and Spīgulis, J. 2011. Photoplethysmographic assessment of microcirculation and vascular reactivity in septic patients: pilot study. Intensive Care Medicine, 37(Suppl 1), S220
2. Kazune, S. and Jagmane, I. 2013. Brachial pulse waveform characteristics predict development of organ failure in septic patients. Critical Care, 17(Suppl 2), P198. doi:10.1186/cc12136
3. Kazune, S., Grabovskis, A., Strīke, E. and Vanags, I. 2014. Aortic stiffness in patients with early sepsis. Critical Care, 18(Suppl 1), P136. doi:10.1186/cc13326

4. Kazūne, S., Grabovskis, A., Strīķe, E. and Vanags, I. 2015. Association between elastic and muscular artery stiffness and organ dysfunction in patients with early severe sepsis. *Intensive Care Medicine Experimental*, 3(Suppl 1), A643. doi:10.1186/2197-425X-3-S1-A643

Abstracts and presentations at local and regional meetings

1. Marcinkevics, Z., Grabovskis, A., Jagmane, I., Rubins, U., Rubenis, O. and Kazune, S. 2012. Monitoring of septic disease altered arterial stiffness. *Biophotonics in Dermatology and Cardiology* (Riga, March 30-31, 2012; stenda referāts)
2. Kazūne, S., Grabovskis, A., Strīķe, E., un Vanags, I. 2014. Artēriju elasticitātes pārmaiņas agrīnas sepses slimniekiem (Eng. Changes in arterial elasticity in patients with early sepsis). 2014.gada Zinātniskās konferences tēzes (Rīga, 2014.g. 10.-11.aprīlī)
3. Kazūne, S., Strīķe, E. un Vanags, I. 2015. Brahiālās artērijas reaktivitāte pacientiem ar smagu sepsi (Eng. Reactivity of the brachial artery in patients with severe sepsis). 2015.gada Zinātniskās konferences tēzes (Rīga, 2015.g. 26.-27.martā)
4. Kazūne, S., Piebalga, A., Strīķe, E., and Vanags, I. 2016. Endothelial vascular reactivity in septic patients: systematic review. 2016.gada Zinātniskās konferences tēzes (Rīga, 2016.g. 17.-18.martā)