The role of venous return in organ perfusion

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THE ROLE OF VENOUS RETURN IN ORGAN PERFUSION

DISSERTATION

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Introduction and outline of the thesis

Shock is a life-threatening clinical state of acute circulatory failure leading to decreased organ perfusion, with inadequate delivery of oxygenated blood to tissues leading to end-organ dysfunction [1]. The causes of circulatory failure are a lack of circulating volume, insufficient pump function, obstruction of blood flow and loss of blood flow regulation [2]. In septic shock, a combination of these causes is seen as a result of a dysregulated host response to infection and results in life-threatening organ dysfunction [3]. Sepsis remains therewith one of the leading causes of death among hospitalized patients and is a frequent cause of admission to intensive care units [4].

Early recognition of impending (septic) shock with circulatory dysfunction and the use of adequate interventions can have a profound beneficial effect on patient outcome [5]. Delaying these interventions will inevitably lead to more severe hypoperfusion, tissue hypoxia, multiple-organ failure and an increased risk of death [6]. Selecting the correct treatment modality can be difficult when a proper pathophysiological diagnosis cannot be made. The quest for adequate monitoring-guided therapies to support organ function in patients with circulatory shock therefore continues. In the following paragraphs, the circulatory system in general, the role of venous return in organ perfusion in particular, and subsequent research questions will be discussed. At the end of this chapter, the outline of this thesis will be presented.

Circulatory system

The goal of the circulatory system is to bring the various components of the blood close to the cells since oxygen, nutrient and metabolic waste exchange takes place by passive diffusion, a transport mechanism which is most efficient over short distances. The circulatory system does so by integrating and regulating the heart, that circulates blood, through sets of vessels [7] (Figure 1).

On the macrocirculatory level, the cardiovascular system uses bulk blood flow (convection) to reduce the distance between the pumping action of the heart and the various organs. Effective pumping of the heart—resulting in sufficient cardiac output—requires both sufficient myocardial contractility and adequate filling. Meanwhile, the ratio between the contractility and the (pulmonary) arterial elastance—as a measure of afterload—should be between 1 and 2.

Next, in order for the cardiovascular system to do its job adequately, there must be sufficient blood flow through the capillaries. This flow of blood

would only be effective if the distance from these capillaries to cells is small. This requires a dense, and homogeneous network of capillaries at the organ level. This main prerequisite for tissue oxygenation and thus organ function is called the microcirculation [8]. Alterations in the microcirculation have been recognized as important in various diseases and can predict outcome of patients [9,10]. The microcirculation is the group of capillaries with a diameter lower than 100 μm . The capillaries have a collective cross-section which is 700 times larger than the cross-sectional area of the aorta [7]. As vessel diameter decreases, the resistance increases and blood flow decreases. This is favorable for the exchange of gases, water, nutrients, and waste products.

Blood pressure is needed to keep blood from the arteries through the high resistance capillaries to the veins. A well operating cardiovascular system requires sufficient bulk pressure of blood (at the level of the macrocirculation) to match tissues' metabolic requirements (at the level of the microcirculation). These requirements fluctuate widely between sleep and wakefulness, between rest and exercise, and between health and disease. Whereby the macrocirculation provides a surplus in hemodynamic energy to meet these variable demands. However, a well operating macrocirculation does not per se mean an adequate microcirculation. The complex disparity between the macro- and microcirculation (e.g. diminished effective capillary blood flow, while blood pressure is high) is one of the most prominent characteristics of (septic) shock [8].

Figure 1

Schematic representation of the cardiovascular system.

Venous return

Very little transmural pressure remains by the time blood leaves the micro-circulation and enters the venous system. Blood flow through the veins is not the direct result of ventricular contraction. Cardiac function is in its turn restricted by the flow of blood entering the heart, venous return [11,12]. Arthur Guyton proposed that characteristics of the venous return were of fundamental importance for blood flow [13]. His concept uses a two-pressure, one-resistance model to provide a framework in order to integrate blood volume, central venous pressure, and cardiac output. Venous return therein is determined by the difference between an ‘upstream pressure’ and the right atrial pressure (i.e. downstream pressure) divided by an overall resistance to venous blood flow. Knowing the upstream pressure of the venous return would potentially provide the clinician with an important indices of a patient’s volume status. This conceptual parameter is confusing—not only because of different naming, but even more because of the methodological issues in measuring it. Measuring this upstream pressure directly is hampered by the simple fact that its (dynamic) location is unknown.

In this thesis, we will use—like Guyton—the term mean circulatory filling pressure when referring to this virtual pressure as the mean pressure that exists in the circulatory system when there is no blood motion. Besides, we use mean systemic filling pressure when acquired in the beating-heart circulation and does not incorporate the pulmonary circulation [14]. Even today, the debate continues whether this virtual pressure bears pivotal information about a patient’s clinical state [15,16]. Therefore, in the first part of this thesis we will focus on the research question: *What is the clinical value of the mean systemic/circulatory filling pressure in patients with circulatory shock?*

Contractility

The use of medication to improve a decreased cardiac contractility—as a result of inflammation of heart disease—is common practice in the treatment of patients in shock [17]. However, heart rate reduction may also support cardiac contractility. Reducing heart rate will decrease myocardial oxygen consumption and will improve diastolic function of the (right) ventricle. Resulting in increased venous return and thereby increasing the cardiac output and ultimately organ blood flow [18–22].

Heart rate control by esmolol, a selective β -blocker, could potentially be a novel therapeutic strategy with substantial impact on clinical management of septic shock in particular. However, excessive β -blockade could reduce cardiac output below the threshold that is needed to maintain adequate organ and tissue perfusion. It is therefore important to answer the clinically important question of how to apply β -blockers safely while maximizing their beneficial effect. In the second part of this thesis we elucidate their use by clarifying *the cardiovascular physiological consequences of β -blocker therapy in septic shock*.

Dissociation between macro- and microcirculation

Perfusion of the microcirculation is regulated to meet the metabolic demands of the tissues, but is—even in health—relatively unaffected by changes of arterial pressure over a considerable range. Septic shock induces marked alterations in vasculature of the microcirculation. Compared with normal conditions in which there is a dense network of capillaries, most of which are perfused, sepsis is associated with endothelial dysfunction, decreased capillary density in association with an increase in heterogeneity of perfusion (Figure 2). This is caused by inappropriate vasodilatation and vasoconstriction, leading to decreased oxygen delivery, tissue hypoxia and organ dysfunction [23]. These microvascular alterations are heterogeneous and are not necessarily predicted by systemic variables. Consequently, a dysfunctional microcirculation will fail to respond to systemic blood flow optimization. The timing of the transition to this non-flow sensitive stage in septic shock is however unknown, nor whether it is organ-specific [24]. When this dissociation is present, resuscitation interventions solely focusing on improving the macrocirculation do not support organ function. Moreover, overly aggressive treatment—with too much too late fluid administration for instance—is harmful and might even

Figure 2

Sublingual microcirculation in a healthy situation (left) and in septic shock (right).

enhance tissue injury and organ dysfunction [25–29]. It is therefore important to determine whether the microcirculation is still flow sensitive or that it will fail to respond to systemic blood flow optimization. In the third and last part of the thesis we investigated *whether optimization of the macrocirculation exerts a beneficial effect on microvascular perfusion in septic shock?*

Outline of this thesis

This thesis focuses on monitoring and supporting patients in circulatory shock in order to optimize tissue perfusion and oxygen delivery. Interventions to improve oxygen delivery and limit oxygen consumption are the cornerstone of resuscitation. Several interventions can be considered, including fluids, vaso-pressor, and inotropic agents [30,31]. Each of these interventions has adverse effects. Or like Paracelsus (1493–1541) has put it: “All things are poison, and nothing is without poison; only the dose permits something not to be poisonous” (Proposition 6 of this thesis). Therefore, we investigated the direct hemodynamic response of both the macro- and microcirculation to these interventions in order to improve their beneficial effects while limiting harm. We will do so by dealing with the previously introduced research questions:

1. What is the clinical value of the mean systemic/circulatory filling pressure in patients with circulatory shock?
2. What are the cardiovascular physiological consequences of β -blocker therapy in septic shock?
3. When does optimization of the macrocirculation exerts a beneficial effect on microvascular perfusion in septic shock?

The first part of this thesis focuses on the Guyton’s model of venous return, of which the mean systemic/circulatory filling pressure is an essential part. This pressure potentially bears information to guide the administration of fluids to a patient, i.e. fluid therapy. In **chapter 2** we tested a method to estimate this virtual pressure in an experimental animal model in the beating-heart situation. This research hinted that there is no single, stable, uniform blood pressure after cardiac arrest. Therefore, in **chapter 3**, we studied this gold standard method—zero-flow blood pressure measurement—under different volumetric conditions, at different vascular sites, and by inducing cardiac arrest in two different ways.

The second part of this thesis addresses the use of a β -blocker in patients with septic shock. Heart rate control using β -blockers is potentially a novel therapeutic strategy in sepsis. A reduced heart rate might improve the cardiac mismatch of oxygen demand and supply in these patients, by decreasing myocardial oxygen consumption [32]. In **chapter 4**, we performed a review of the current literature on the effect of β -blockade in septic shock. We included both animal models as well as clinical studies in critically ill patients. Next, **chapter 5** focuses on the effect of esmolol, a selective β -blocker, on right ventricular function in early experimental endotoxic shock.

Finally, the third and final part of this thesis focuses on the (mis)match between the macro- and microcirculation during septic shock under different therapeutic interventions. In **chapter 6** we evaluated the effect of vasopressors on the macro- and microcirculation during experimental human endotoxemia, a standardized, controlled model of systemic inflammation in humans *in vivo*. In **chapter 7** we studied the effects of HR control with β -blockers on the interaction between the macro- and microcirculation, in particular the kidneys, during early septic shock.

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Effect of inspiratory hold maneuvers with high ventilatory pressures on the assessment of the mean systemic filling pressure

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Submitted for publication

Abstract

Background The upstream pressure for venous return (VR) is considered to be a combined conceptual blood pressure of the systemic vessels—the mean systemic filling pressure (MSFP). The relevance of estimating the MSFP during dynamic changes of the circulation at the bedside is controversial. Herein, we studied the effect of blood volume on the relationship between VR and central venous pressure (CVP) when flow is near zero.

Methods In 9 healthy pigs under anaesthesia and mechanically ventilated, MSFP was estimated from extrapolated CVP versus VR relationships during inspiratory hold maneuvers (IHMS) with different levels of ventilatory pressure (P_{vent}). MSFP was measured 3 times per animal during different volumetric states, i.e. euvoolemia or hypovolemia. Hypovolemia was induced by bleeding with 10 ml/kg. The estimated MSFP values were compared to the arterial blood pressure recording after induced ventricle fibrillation (i.e. mean circulatory pressure).

Results Our results revealed a strong linear correlation between venous return and CVP (R^2 of 0.92 [range: 0.67–0.99]), during IHMS with different levels of P_{vent} . Volume status significantly alters the resulting MSFP, 20 ± 1 mmHg and 16 ± 2 mmHg for euvoolemia and hypovolemia respectively. This estimation of the MSFP was strongly correlated—but not interchangeable—to the blood pressure recording after induced ventricle fibrillation ($R^2 = 0.8$ and $p = 0.045$).

Conclusion In conclusion, we showed a strong linear correlation between VR and CVP—when applying IHMS with different levels of P_{vent} —however the clinical applicability of this method to guide volume therapy in its current form remains unclear.

Background

Reliably estimating the intravascular volume status to guide fluid therapy in critically ill patients is a clinical challenge. Appropriate fluid therapy will increase cardiac output and thereby enhance tissue perfusion and oxygen delivery. However fluid overload results in edema and enhance tissue injury and organ dysfunction [1–5].

Therefore, the search for reliable indicators of both intravascular volume status and fluid responsiveness remains highly relevant [6]. Most available indicators focus on variations of cardiac output in trying to predict fluid responsiveness [6,7]. Cardiac output however is restricted by the flow of blood entering the heart, i.e. venous return (VR) [8,9]. More insight into venous return could prove useful in this respect. Venous return is determined by the difference between an ‘upstream pressure’ and the right atrial pressure divided by the resistance to venous return (RVR). Conceptually the ‘upstream pressure’ is regarded as a combined pressure of the systemic vessels. This pressure reflects a common pressure that drives blood flow from the vascular beds to the right atrium.

There is much confusion about this conceptual parameter, not only because of different naming. Its importance was first recognized by Weber in the 19th century and he introduced the term “statischer Füllungsdruck” (‘static filling pressure’) [10]. Starling [11]—and subsequently Guyton—started using the name ‘mean circulatory filling pressure (MCFP)’ [12]. Mean systemic filling pressure (MSFP), though often confused with MCFP and often similar in value, is considered to be different [13]. Wherein MCFP includes the entire cardiovascular system and is acquired after a cardiac arrest, MSFP represents the pressure generated by the elastic recoil in the systemic circulation only and is acquired while the heart is still beating.

A decade ago, Maas et al. revived the interest in the MSFP [14]. They developed a bedside technique to estimate the MSFP by applying inspiratory hold maneuvers (IHMs) with different levels of static inspiratory ventilatory pressures (P_{vent}). This technique is based on Guyton’s theory of VR, predicting a linear relationship between blood flow and right atrial pressure [15]. Extrapolating this relationship between right atrial pressure and cardiac output to zero flow would provide an estimate of the MSFP, in this case the MSFP_{IH}. Ever since, several studies have used this linear relationship in order to estimate MSFP

using IHMS [14–20]. However, MSFP_IH has never been validated to MCFP and it remains questionable whether the extrapolation to zero flow is accurate.

The aim of the present work is primarily to validate the MSFP_IH by studying two aspects of this method that have not been validated before. The first being the relationship between VR and right atrial pressure near zero flow. The second is the level of agreement between MSFP_IH and MCFP. The secondary aim was to evaluate the influence of different volumetric states on MSFP_IH and MCFP.

Materials and methods

This experiment was performed after approval of the local ethics committee on animal research of the Radboud University Nijmegen Medical Center (RUNMC License number RU-DEC 2014–246) and in full compliance with Dutch and European legal requirements on the use and protection of laboratory animals. A total of 9 domestic piglets (age 3–5 months, mean weight of 36.2 kg [range: 31–45 kg], mean body surface area of 0.8 m² [range: 0.68–0.89 m²]) under general anesthesia were studied. In the context of the principles of replacement, reduction and refinement for the use of animal models, animals used in the study were previously used for medical training (not affecting the cardiovascular system).

Anesthesia and ventilation

Premedication consisted of the intramuscular administration of midazolam (10 mg/kg), ketamine (1 mg/kg), atropine (50 µg/kg) and amoxicilline (20 µg/kg). Induction of anesthesia was performed using IV administration of propofol (2 mg/kg). After endotracheal intubation, general anesthesia was maintained using inhalation of isoflurane (0.5–2 volume %), the continuous IV administration of sufentanil (10 µg/kg/hr) and rocuronium (1 mg/kg/hr) after a loading dose of 1 mg/kg.

The lungs were mechanically ventilated using a volume-controlled mode with tidal volumes of 8–10 mL/kg, 5 cmH₂O PEEP, *F*_{IO₂} 0.4 and an inspiratory-to-expiratory ratio of 1:2. Ventilation was adjusted according to the end tidal CO₂ level (4.5–5.5 kPa).

Surgical preparation

After receiving premedication, a small peripheral intravenous catheter was placed to administer anesthetics. A central venous catheter (5–7 F, 3-lumen,

13 cm; Arrow International, Reading, PA) was inserted via the right jugular vein for administration of fluids/medication and CVP recording. CVP was used as an approximation of right atrial pressure. This central venous catheter was also used for injection of ice-cold fluid in order to calibrate global end-diastolic volume (GEDV) measurement. An indwelling thermistor-tipped arterial catheter (4–5 F, Pulsio cath™, Pulsion Medical Systems SE) was placed in the lower abdominal aorta via the right femoral artery for arterial blood pressure (ABP) recording and GEDV assessment. All intravascular catheters were inserted by a surgical cut-down technique. The pressure transducers were levelled, zeroed and placed on the same transducer bar.

After left thoracotomy, an ultrasound transit-time perivascular flow probe (18 or 22 mm) (PAX series, Transonic Systems, Ithaca, NY) was placed around the main pulmonary artery to measure cardiac output. A flow probe is capable of measuring cardiac output continuously also at blood flows near zero. Two temporary cardiac pacing wires leads were placed on the right ventricular epicardium. Last, the chest was closed using multilayer sutures.

Experimental protocol

At the end of the surgical instrumentation and prior to first intervention, a 30 minutes stabilization period was allowed for all animals. Next, the animals were randomly assigned to start with euvoolemia or hypovolemia (Figure 1). Hypovolemia was induced by blood withdrawal of 10 ml/kg. The shed blood was collected and stored for purpose of re-transfusion. During each volumetric state (T1, T2 and T3) several IHMs were performed by an inspiratory hold of 30 seconds and transpulmonary thermodilution using 3 ice-cold injections for assessment of the GEDV, Figure 1. The duration was the IHM was deliberately

Figure 1

Schematic overview of experimental protocol. In each group, inspiration hold maneuvers (IHM) were performed three times (at T1, T2 and T3) to determine mean systemic filling pressure (MSFP_{IH}) in different volumetric states and mean circulatory filling pressure after stop flow (T4).

longer than the usual 12 second period [14]. A run of five (or less when cardiac output approached zero) IHMs were performed with at pressures of 30, 10, 20, 40 and 50 cmH₂O respectively.

After the last set of IHMs (at T₄) the piglets were euthanized by direct cardiac fibrillation with a 60 Hz alternating current over the cardiac pacing wires mimicking direct cardiac arrest and zero flow without affecting systemic vascular resistance or compliance. In doing so half of the animals died in a state of euvoemia and the other half in a state of hypovolemia. Before inducing ventricular fibrillation (VF), the endotracheal tube was disconnected from the mechanical ventilator. After inducing VF, both CVP and ABP were continuously recorded. The point where CVP and ABP equilibrated was considered the MCFP [21].

Data recording

EKG, hemodynamic/ventilation pressures and flows were continuously recorded on a laptop computer and stored on a hard disk with a sample rate of 200 Hz by an A/D converter (NI USB-6211, National Instrument, Austin, TX, USA). From these signals, hemodynamic parameters were obtained using custom-written MATLAB scripts. Mean ABP, CVP and cardiac output (from the ultrasound flow probe) were acquired by low-pass filtering of the recorded signals (cut-off frequency of 0.5 Hz [22], third order Butterworth filter applied in the forward and reverse direction for a zero-phase response).

Cardiac output was indexed to body surface area (BSA) using formula $BSA = 0.0734 \times \text{body weight}^{0.656}$ [23]. HR was acquired by automatic detection of R-peaks from the EKG-signal. From the recorded pressure and flow signal, the pulse pressure variation (PPV) and stroke volume variation (SVV) were calculated offline as described in our previous work [6]. The results of the thermal indicator dilution curve were used in the calculation of the GEDV, and was indexed to BSA to acquire GEDVi.

Characteristics of VR

During each IHM, CVP and cardiac output were captured by taking the mean over the last 5 seconds of the maneuver. Next, the set of data points from the IHMs was fitted by linear regression to define the VR curve for each volumetric state. MSFP_IH was then defined as the extrapolation of this linear regression to zero flow, RVR was defined as the inverse of the slope of this linear regression, and venous return driving pressure (VRdP) was defined as MSFP_IH minus

CVP (prior to the IHMS) [14]. Alternatively, RVR was calculated as VRAP divided by cardiac output.

Statistics

Statistical analysis was performed using MATLAB (Matlab R2019b, The MathWorks Inc. Massachusetts, USA). Normality was assessed using Shapiro-Wilk tests. Unpaired Student's *t*-tests were done to check for significant impact of a volumetric state on all parameters separately. A paired-samples *t*-test was conducted to compare the consistency of MSFP assessment within animals between moment T1 and T3. Pearson's correlation coefficient was calculated between MSFP_IH and MCFP. Data were compared using the method described by Bland and Altman [24]. The limits of agreement were calculated by multiplying the standard deviation of the bias with 1.96. The percentage error was calculated by dividing the limits of agreement by the mean value of MSFP_IH and MCFP, times 100 % [25]. A *p*-value of < 0.05 was considered to indicate significance.

Results

General

All animals included in the study were considered healthy on physical examination when entering the animals' laboratory. The medical training prior to the experiment did not influence the cardiovascular system. Animals were randomly assigned to be in the euvolemia group ($n = 4$) or the hypovolemia group ($n = 5$).

Volumetric state

Induced hypovolemia led to an unchanged HR and an expected significant reduction in cardiac output and MAP (Table 1). Transfusion, in order to restore hypovolemia, restored both MAP and cardiac output back to baseline. These hemodynamic variables were constant between the volumetric state at T1 and T3 in all animals (Figure 3). The hypovolemic state was highlighted by significantly higher dynamic indices of fluid responsiveness, including PPV and SVV (Table 1).

Inspirations hold maneuvers

On average, 4 IHMS were executed per volumetric state [range: 2–5]. The IHM with the highest P_{vent} reduced the cardiac output to 0.4 l/min on average [range:

Table 1

Hemodynamic parameters, characteristics of venous return, and dynamic indices per volumetric state.

	Euvoolemia		Hypovolemia		Sig ^a
	Mean	Std	Mean	Std	
Central hemodynamic					
CO (l/min)	3.0	1.2	2.4	0.9	*
CI (L/min/m ²)	3.9	1.5	3.1	1.0	*
MAP (mmHg)	57	10	22	6	***
PP (mmHg)	39	10.2	25	6	***
CVP (mmHg)	11	5.2	8	5	ns
HR (bpm)	147	28	141	26	ns
SV (ml)	42	14	33	9	**
Venous return curve					
MCFP (mmHg)	16	4	14	3	ns
MSFP_IH (mmHg)	20	5	16	4	*
Venous resistance ^b (mmHg/L/min)	3.4	2.0	3.6	1.6	ns
R ²	0.9	0.1	0.9	0.1	ns
VRdP (mmHg)	8.8	2.5	7.3	2.4	ns
Venous resistance ^c (mmHg/L/min)	3.4	1.8	3.3	1.6	ns
Dynamic indices					
PPV (%)	15.4	4.1	22.3	5.7	***
SVV (%)	31.0	5.6	40.2	12.8	*
Static indices					
GEDV (ml)	411	42	333	53	***
GEDVi (ml/m ²)	537	49	432	49	***

CO: Cardiac output, CI: cardiac index, MAP: mean arterial pressure, PP: pulse pressure, CVP: central venous pressure, HR: heart rate, SV: stroke volume, MCFP: mean circulatory filling pressure, MSFP_IH: estimation of the mean systemic filling pressure using the inspiratory hold method, VRdP: driving pressure of venous return, PPV: pulse pressure variation, SVV: stroke volume variation, GEDV: global end-diastolic volume, GEDVi: indexed global end-diastolic volume.

ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

^a Significance between volumetric states (Unpaired Student's t-test)

^b Inverse of the CVP/CO relationship

^c $(MSFP - CVP)/CO$

0–1.5 l/min]. The P_{vent} level at which this was achieved depended on the volumetric state, ranging from 30 to 50 cmH₂O. A steady state was reached within the 30 seconds of the IHM for CVP (increase) and cardiac output (decrease). Although not used for the assessment of the MFSP_{IH}, we showed that the ABP continued to decline and did not reach a plateau during the 30 seconds of the IHM, see example Figure 2A.

Venous Return

Venous resistance

The IHM induced decrease in cardiac output and increase in CVP were linearly correlated with a coefficient of determination (R^2) of 0.92 [range: 0.67–0.99] with no difference between eu- and hypovolemia (see example Figure 2B). The correlation between cardiac output and CVP was not improved nor reduced by excluding the IHM with the highest P_{vent} (at which cardiac output was zero).

Absolute values of RVR (i.e. 1/slope) were not significantly different between eu- and hypovolemia, 3.8 ± 0.76 and 3.5 ± 0.45 respectively. Within the eu-hypo-eu group (Figure 3B), a large standard deviation in RVR was observed. Still, when RVR was normalized to baseline the RVR was significantly higher during

Figure 2

- A** Example of piglet 4 during hypovolemia of four inspiration hold maneuvers with ventilatory plateau pressures of 30, 10, 40 and 50 cmH₂O respectively. Yellow stars indicate points at which both CVP and cardiac output were taken to construct the venous return curve.
- B** Three constructed venous return curves during different volumetric states in order to estimate the mean systemic filling pressure in piglet 4. Yellow stars corresponds to those in panel A.

hypovolemia (Figure 3A and 3B). When RVR was calculated by dividing VRdP by the cardiac output, similar results were obtained (Table 1).

MSFP_{IH}

The MSFP_{IH} showed a significant difference between eu- and hypovolemia, 20 ± 1 mmHg and 16 ± 2 mmHg respectively (Table 1). The MSFP_{IH} within a piglet was never higher during hypovolemia compared to euvoolemia, but no cut-off value could be found to use the MSFP_{IH} in order to distinguish the volumetric states from each other. Paired Student's *t*-test showed a significant correlation between the MSFP_{IH} values from T1 and T3.

Venous return driving pressure

During hypovolemia, the MSFP_{IH} was reduced more than CVP, causing the VRdP to decrease. Bleeding always led to a reduction in VRdP, but no significant difference in absolute VRdP value was found between the two volumetric states (Figure 3).

Mean circulatory filling pressure

VF resulted in an instant drop in ABP, an increase in CVP and no measurable pulmonary flow. The blood pressure signals crossed on average 32 seconds after VF. In contrast to MSFP_{IH}, the MCFP values were not significantly

Figure 3

Hemodynamic parameters (normalized to baseline) during the different volumetric states: Euvoolemia (Eu) and Hypovolemia (Hypo) for the both groups (ending in hypovolemia **A** or euvoolemia **B**).

Estimation of the mean systemic filling pressure using the inspiratory hold method (MSFP_{IH}), cardiac output (CO), mean arterial pressure (MAP), central venous pressure (CVP), venous return driving pressure (VRdP).

Data are expressed as mean and SEM. ★: $p < 0.05$, ★★: $p < 0.01$ (repeated measures one-way ANOVA).

influenced by their volumetric state (Table 1). Paired samples *t*-test showed that MSFP_IH, from the latest volumetric state just before inducing VF (T3), was significantly different from MCFP (T4) (Table 1). However, MSFP_IH and MCFP were significantly correlated ($R^2 = 0.8$ and $p = 0.045$) (Figure 4A). Differences between MSFP_IH and MCFP are shown in the Bland-Altman plots (Figure 4B). The mean bias was 3.5 mmHg (limits of agreement ± 1.8 mmHg) and the coefficient of variation 17 %. The percentage error was 29 %, relatively high knowing the small standard deviation of intravascular blood pressure measurement [25].

Discussion

General

Our results show a strong linear correlation between cardiac output and CVP, during IHMS with various levels of P_{vent} up to an almost zero flow state. Also, this MSFP_IH is strongly correlated—but not interchangeable—to the blood pressure recording after induced VF (i.e. MCFP).

Figure 4

- A** Spearman rank correlation mean systemic filling pressure (MSFP) measurements by inspiration hold maneuvers (MSFP_IH) and after ventricular fibrillation (MCFP) ($p < 0.05$). Continuous line represents linear regression line with regression coefficient R^2 .
- B** Bland-Altman plot comparing MSFP_IH and MCFP. Continuous line represents linear regression line with regression coefficient (R^2). Dashed horizontal lines represent the limits of agreement. The bold continuous horizontal lines represent the bias.

MSFP values

Our MSFP_{IH} values were in the same range as others have obtained in post-operative patients [17]. However, a wide range of MSFP values and many different measurement techniques to estimate this conceptual blood pressure are reported [26]. Under normal volumetric conditions MSFP_{IH} values from as low as 8 mmHg in dogs [27], to 11 mmHg in piglets [28], and all the way up to 33 mmHg in septic patients [29] are reported. This wide variation in MSFP values extends even further when alternative measurement techniques—right atrium occlusion [18], arm occlusion [30,31], a model analogue [32,33] or cardiac arrest [34]—are considered. Comparing these measurement techniques revealed that they are not interchangeable [18,32].

The lack of both normative MSFP values and an undisputed measurement technique limits the applicability of using absolute MSFP values for guiding clinical therapy. In this experiment we indeed showed a significant influence of volume status on the MSFP_{IH}, without finding a cut-off value to discriminate euolemia from hypervolemia. This also applies for GEDV, SVV, and PPV.

Correlation with MCFP

Our observed MSFP_{IH} strongly correlated with the recorded blood pressure after VF (i.e. MCFP), but with a large bias, systematic error and no correlation with the volume status. This suggests that both parameters are connected but do not represent the exact same physiological parameter. Comparing MCFP values between studies is inept, because the induction of cardiac arrest differs greatly [21,34,35]. In addition, it appears incorrect to compare the MSFP_{IH} with the MCFP. MSFP_{IH} reflects a dynamically controlled cardiovascular system while MCFP reflects a static uncontrolled non-physiological state. It is indeed speculated that MCFP is not measurable in beating-heart [21,36].

Reconstructed VR curve

We demonstrated that the linear behavior of the constructed VR curve was maintained even when cardiac output was reduced to almost zero when applying IHMS with high levels of P_{vent} . In clinical practice, only short holds with moderate ventilatory pressures can be applied since prolonged and high pressures may induce a deterioration of the patient's clinical state [37]. Our results validate the method using a limited number of short IHMS with moderate ventilatory pressure in patients [28]. However, the presented linear relationship between cardiac output and CVP is in line with Guyton's theory on

VR [38] and is also observed by multiple studies in both animal models [15,21] and humans [14,17,39–41], while in the past they left room for discussion on the linearity when flow approached zero. However, it remains questionable if these CVP versus cardiac output points are part of one single venous return curve [21].

The increased intra-alveolar pressures and lung expansion with concurrent increase in pleural and pericardial pressure during an IHM might directly modify the characteristics of the VR curve, by shifting the curve or by modifying its slope [18]. This might result in an individual VR curve for each applied IHM. A visual representation of this hypothesis is made in Figure 5 (not supported by our data). However, some evidence does support our hypothesis that P_{vent} changes not only the slope but also shifts the pressure/flow relationship.

First, pressure ventilation in itself has been reported to increase the MSFP in piglets [18] and in critically ill patients [21]. The influence of P_{vent} on VRdP is not straightforward [18,42,43]. Its effect depends on the extent by which P_{vent} causes a redistribution of both pulmonary and systemic blood volumes. The associated increase in thoracic and abdominal pressure – thereby squeezing blood from the pulmonary vessels and liver – can cause an increase in MSFP preventing VRdP to decrease [35], and shifting the VR curve to the right [44]. Second, P_{vent} may decrease the conducting area of veins by constriction or compression [45], via either reflexes or mechanical factors [20]. Multiple studies have confirmed that P_{vent} increases RVR [35,43,46,47], while others showed no interaction [18,42]. Our data confirms the inconsistency in RVR (1/slope of VR curve), RVR was not necessarily higher during hypovolemia compared to euvolemia as one might expect.

Last, the extent to which P_{vent} modifies the VR curve—regardless of the mechanism—is further complicated by the volumetric state. It is hypothesized that the distribution of intrathoracic pressure is related to the patient’s volume status [44]. Berger et al. indeed showed that IHMs shift the cardiac output/CVP relationship rightwards in euvolemia, but did not do so in bleeding or hypervolemia [18].

Altogether, this would imply that a new steady state is achieved rapidly after applying an IHM, resulting in a new (conceptual) MSFP_IH and/or RVR (Figure 5). Interpreting the characteristics of the acquired VR curve may therefore be misleading because of the (unclear) intervening influence of ventilatory pressures [18].

Clinical implication

It seems reasonable to conclude that the validity of $MSFP_{IH}$ in the beating-heart situation using cardiopulmonary interactions in its current form is questionable [21]. Also it does not seem efficient to determine normative $MSFP$ values [26]. Applying perturbations to a closed-loop physiological system for system identification does seem elegant therefore the question should therefore not be; can we construct the VR curve using IHMs, but rather; does applying perturbations using IHMs provide information about a clinically relevant hemodynamic state of the patient.

Here we demonstrated that within a piglet the $MSFP_{IH}$ was consistent within animals and linked to the volumetric state, but the absolute value was unsuitable for guiding clinical therapy. We do see a future when the characteristics of this flow-pressure relationship, acquired by using IHMs, are seen as dynamic indices rather than the VR curve per se. Even when a patient is ventilated with low TV (< 8 ml/kg) [6]. After all, the underlying theory of Guyton's on VR qualitatively predicts the dynamic response from changing right atrial pressure [48]. Using IHMs to detect preload-responsive patients has indeed been done before successfully [49].

Limitations

In addition to the conceptual limitation of estimating the $MSFP$ with the use of IHMs, there are still some experimental limitations. We used an experimental

Figure 5

Concept [not supported by data]: several VR-curves are possibly hidden within the constructed VR curve when applying IHMs with different levels of ventilatory plateau pressure. Solid colored lines indicated possible individual lines whereas the dashed line represented the resulting 'hidden' underlying VR curve constructed from all points.

animal model with only a limited number of animals. Furthermore, anesthetic drugs may have influenced both the assessment of MSFP as well as MCFP. The concept presented in Figure 5 is not supported by data. We did not measure RVR or could not acquire MSFP during an individual IHM to support the concept of Figure 5. Last, ABP did not reach a plateau level within one inspiratory hold. This made it impossible to calculate the critical closing pressure, as proposed by others [16].

Conclusion

In conclusion, despite a strong linear correlation between VR and CVP—when applying IHMs with different levels of P_{vent} —the acquired MSFP_IH was related to but did not discriminate between volumetric states. MSFP_IH was not interchangeable with MCFP. Therefore, the flow-pressure relationship using IHMs reflects a dynamic systemic circulatory characteristic rather than an absolute value of the volume state.

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Zero-flow blood
pressure measurements
do not provide useful
clinical information
regarding circulating
volume status

3

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Abstract

Background The mean circulatory filling pressure is a concept introduced by Arthur Guyton. It plays an important role in physiology teaching and clinical medicine. This virtual pressure is presumed to act as the ‘upstream pressure’ for venous return, and is regarded an important measure of a patient’s volume status. This study aimed to validated the gold standard method for obtaining this upstream pressure to venous return, measuring intravascular blood pressure at zero flow.

Methods In 14 healthy pigs under anaesthesia and mechanically ventilated, we studied the blood pressure for 10 minutes following cardiac arrest. Blood pressure recordings were performed under different volumetric conditions, at different vascular sites (lower abdominal aorta and right atrium), and by inducing arrest in two different ways (i.e. overdose of pentobarbital or by direct cardiac fibrillation).

Results Our results show that a pentobarbital overdose resulted in an instant drop in arterial blood pressure and in a rise in central venous pressure. However, after direct cardiac fibrillation a dynamic course in blood pressures with a lingering retrograde difference between arterial and venous blood pressures was observed.

Conclusion In conclusion, we showed that there is no single, stable, uniform blood pressure after cardiac arrest. We question the clinical relevance of this virtual pressure, as well as its relationship to the beating-heart situation.

Introduction

The mean circulatory filling pressure is considered a key hemodynamic parameter because of its crucial role as the upstream pressure in Guyton's model of venous return. This (virtual) pressure has been defined as "the pressure that would be measured at all points in the entire circulatory system if the heart were stopped suddenly and the blood were redistributed instantaneously in such a manner that all pressures were equal" [1]. Guyton warned that in order to accurately measure the mean circulatory filling pressure the value should be obtained within a few seconds after the heart stops beating. Because intravascular pressure is dependent on external pressure, wall stress and blood volume, the mean circulatory filling pressure is proclaimed to be a unique measure of the intravascular volume and overall level of vasomotor activity. Whereby this pressure is interpreted by some as a indices of volume status and/or fluid responsiveness [2].

In order to acquire the mean circulatory filling pressure, the heart must be stopped to elicit a zero-flow condition. However, studies that have done so share potential methodological issues. They likely affected the mean circulatory filling pressure by (in)directly intervening with blood volume and/or vasomotor activity [3]. This is not only confusing, but potentially undermines Guyton's claim that there is "a mean integrated pressure in the circulatory system all of the time [4]" and with that its clinical and educational applicability.

Using an experimental animal model, we systematically assessed zero-flow blood pressures under different volumetric conditions, at both the arterial and venous side, and by inducing the arrest in different ways. The purpose of this study is to test the hypothesis that stopping the heart imposes an effect on the value of the mean circulatory filling pressure.

Material and methods

This experiment was performed after approval of the local ethics committee on animal research of the Radboud University Nijmegen Medical Center (RUNMC License number RU-DEC 2014-246) and in full compliance with Dutch and European legal requirements on the use and protection of laboratory animals. A total of 14 female domestic piglets (age 3-6 months, mean weight of 41.5 kg [range: 31-55 kg], mean body surface area of 3.1 m² [range: 2.2-3.8 m²]) under general anesthesia were studied. In the context of the principles of

replacement, reduction and refinement for the use of animal models, animals used in the study were previously used for medical training, placing a neural flow diverter (not affecting the cardiovascular system).

The reported results are a follow-up of a previous experiment in which we studied the applicability and validity of a bedside technique for estimating the mean systemic filling pressure. For a detailed description on the anesthesia, ventilation and surgical preparation we refer to the materials and methods section of our previous publication [5].

Anesthesia

In summary, premedication consisted of the intramuscular administration of midazolam (10 mg/kg), ketamine (1 mg/kg), atropine (50 µg/kg) and amoxicillin (20 µg/kg). After endotracheal intubation, anesthesia was induced using iv administration of propofol (2 mg/kg) and maintained using inhalation of isoflurane (0.5–2 volume %), the continuous iv administration of sufentanil (10 µg/kg/hr) and rocuronium (1 (mg/kg)/hr) after a loading dose of 1 mg/kg. Lungs were mechanically ventilated using a volume-controlled mode. Ventilation was adjusted according to the end tidal CO₂ level (4.5–5.5 kPa). All animals received an iv saline fluid bolus of 5 ml/kg at the end of instrumentation to prevent initial hypovolemia.

Experimental protocol

Cardiac arrest was either induced with an overdose of pentobarbital (150 mg/kg iv) ($n = 5$) or by direct cardiac fibrillation with a 60 Hz alternating current over cardiac pacing wires ($n = 9$), Figure 1. The animals in the ventricular fibrillation

Figure 1

Schematic overview of experimental protocol.

Pento: pentobarbital and VF: ventricular fibrillation.

(vF) group were randomly assigned to be euvoletic ($n=4$) or hypovolemic ($n=5$). Hypovolemia was induced by blood withdrawal of 10 ml/kg.

All animals in the barbiturate group were euthanised while being euvoletic, verified by a stable mean arterial blood pressure. Before inducing cardiac arrest, the endotracheal tube was disconnected from the mechanical ventilator. Furthermore, the pressure transducers were levelled, zeroed and placed on the same transducer bar. After established cardiac arrest with no flow, cardiac output (CO), and central venous (CVP) and arterial blood pressures (ABP) were continuously recorded for another 10 minutes.

Data recording and processing

CO was acquired using an ultrasound transit-time perivascular flow probe (18 or 22 mm) (PAX series, Transonic Systems, Ithaca, NY) which was placed around the main pulmonary artery. ABP was acquired using an indwelling thermistor-tipped arterial catheter (4–5 F, PulsioCathTM, Pulsion Medical Systems SE) which was placed in the lower abdominal aorta via the right femoral artery. CVP was acquired using a central venous catheter (5–7 F, 3-lumen, 13 cm; Arrow International, Reading, PA) which was inserted via the right jugular vein for CVP recording. All signals were sampled at a rate of 200 Hz, A/D converted (NI USB-6211, National Instrument, Austin, TX, USA), and stored on a hard disk. Mean ABP, CVP and CO were acquired by low-pass filtering of the recorded signals (cut-off frequency of 0.5 Hz [6], third order Butterworth filter applied in the forward and reverse direction for a zero-phase response) using custom-written MATLAB (Matlab R2019b, The MathWorks Inc. Massachusetts, USA) scripts.

Statistics

Statistical analysis was performed using MATLAB (Matlab R2019b, The MathWorks Inc. Massachusetts, USA). Normality was assessed using the Shapiro-Wilk tests. Four discrete points of the continuous blood pressure curves were taken for statistical analysis. At baseline (T₀, approx. 20 seconds prior to the induction of cardiac arrest), when CVP and ABP equilibrated (T₁, approx. 30 seconds after cardiac arrest), when CVP and ABP were at their maximum (T₂, approx. 300 seconds after cardiac arrest), and 10 minutes after cessation of flow (T₃, approx. 600 seconds after cardiac arrest).

Changes in parameters over time after cardiac arrest were analyzed using repeated measures one-way ANOVA. Differences between the euvoletic

Table 1

Hemodynamic data before and after cardiac arrest.

	Before VF (-20 sec)		Crossing (+ 30 sec)		Peak (+ 300 sec)		10 min after VF (+ 600 sec)		Significance			
	Mn	Sd	Mn	Sd	Mn	Sd	Mn	Sd	T1-T3 ^a	Over time vs Pento ^b (T1-T3)	vs VF- euvoemia ^c (To)	vs Pento ^c (T3)
VF - euvoemia												
CO (l/min)	3.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	ns	ns	ns	ns
ABP (mmHg)	53	4.7	17	2.2	17.9	1.6	11	0.8	**	**	na	**
CVP (mmHg)	11	6.0	15	4.6	22.9	5.0	16	3.7	**	**	na	**
VF - Hypovolemia												
CO (l/min)	2.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	ns	ns	ns	ns
ABP (mmHg)	45	5.6	14.8	1.1	16.2	1.1	9	1.6	***	***	*	***
CVP (mmHg)	9	3.3	13.3	2.9	19.4	3.1	13	3.1	***	***	ns	***
Pento - euvoemia												
CO (l/min)	4.3	1.2	0.0	0.0	0.0	0.0	0.0	0.0	ns	na	ns	na
ABP (mmHg)	47	3.7	16	2.5	14	2.8	15	2.9	ns	na	ns	na
CVP (mmHg)	12	2.9	15	3.1	14	3.3	14	3.5	ns	na	ns	na

Data are expressed as mean ± SD, ns: not significant, na: not applicable, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

^a Significant changes over time (T₁-T₃) within group to test for dynamic behavior of blood pressure recording (repeated measures one-way ANOVA).

^b Significant changes over time (T₁-T₃) vs. corresponding parameter in the group where cardiac arrest was induced with pentobarbital (repeated measures two-way ANOVA, time × treatment interaction term).

^c Paired Student's *t*-tests versus corresponding parameter in the group where cardiac arrest was induced with pentobarbital.

VF-group and Pentobarbital-group over time were tested using repeated measures two-way ANOVA (interaction term). Differences between venous and arterial blood pressure at 600 seconds after cardiac arrest were analyzed using paired Student's *t*-tests. A two-sided *p*-value of < 0.05 was considered statistically significant.

Results

General

All animals included in the study were considered healthy on physical examination when entering the animals' laboratory and the cardiovascular system was unaffected after being part of medical training. Baseline hemodynamic parameters, 20 seconds prior to the induction of cardiac arrest, are summarized in Table 1.

Cardiac arrest induced by VF

A non-synchronized internal shock induced VF resulted in an instant drop in ABP and complete stoppage of flow, while CVP increased (Figure 2A and Table 1). ABP became lower than CVP within 1 minute after VF (not influenced by volumetric state). ABP and CVP started to increase until reaching a peak (T_2 , ± 300 seconds after VF) before dropping again. ABP and CVP reached a steady-state within 10 minutes after VF (T_3). During this steady-state, the significant negative pressure difference ($ABP - CVP$) remained present. The dynamic

Figure 2

Cardiac output (CO), arterial (ABP) and central venous blood pressure (CVP) recording after cardiac arrest, with euvoolemia (eu) and hypovolemia (hypo).

A Cardiac arrest induced by ventricular fibrillation.

B Cardiac arrest induced by overdosing with pentobarbital.

Data are expressed as mean and SD.

★★: $p < 0.01$ and ★★★: $p < 0.001$ over time (at 30, 300 and 600 seconds) within group to test for dynamic behavior of blood pressure recording (repeated measures one-way ANOVA).

⊙⊙: $p < 0.01$, ⊙⊙⊙: $p < 0.001$ over time (at -30, 30, 300, and 600 seconds) vs. corresponding parameter in the group where cardiac arrest was induced with pentobarbital (repeated measures two-way ANOVA, time $\times$ treatment interaction term).

behavior of ABP and CVP was significant over time in both volumetric states (Table 1 and Figure 2).

Cardiac arrest induced by overdose pentobarbital

As with VF, pentobarbital overdose in euvoletic piglets resulted in an instant drop in CO and ABP, and in a rise in CVP. However, here ABP and CVP came to—and stayed in—an equilibrium 30 seconds from the onset of cardiac arrest (Figure 2B). The course of the blood pressure recordings in this group was significantly different from the course of the blood pressures when cardiac arrest was induced with VF (Figure 2). Arterial and venous blood pressures 10 minutes after cardiac arrest did not significantly differ compared to the euvoletic VF group.

Discussion

Our results show a dynamic course in blood pressures after cardiac arrest induced by ventricular fibrillation with a striking and persistent difference between arterial and venous pressures. Both the dynamic course and the arterial to venous difference are completely blunted by induction of cardiac arrest using high dose barbiturates. This calls into question the interpretation of zero-flow blood pressure as the mean integrated pressure of the circulatory system.

Methodological differences

Zero-flow blood pressure assessments reported in the literature reflects experimental set-ups that vary in the way cardiac arrest was induced (i.e. VF, cessation of therapy, overdosing with acetylcholine or pentobarbitone), time between cardiac arrest and blood pressure measurement, and the presence of externally driving (artificial) shunt between the venous and arterial vessels. Reported zero-flow blood pressure assessments are therefore difficult to compare or to validate. All these methods aimed to measure blood pressure when flow was zero without influencing vasomotor tone. However, all of these methods do influence the obtained mean circulatory filling pressure.

The first measurement of mean circulatory filling pressure in humans was performed by Starr, who inserted a needle into the heart and great vein in patients who had died shortly before [7]. Guyton stopped the heart in his animal model by electrical fibrillation, but—in contrast to us, but similar to others [8]—mechanically pumped blood rapidly (in less than 7 seconds) through

an arterial to venous shunt in order to bring the arterial and venous pressures to an equilibrium [9]. More recently, ventricular fibrillation was induced in patients undergoing surgical implantation of a defibrillator. Arterial and venous pressures were measured, but did not equilibrate during ventricular fibrillation. Matching our results, even after 20 seconds of cardiac arrest, there was no equilibration of pressures [10,11]. The authors attributed the lack of equilibrium to a waterfall mechanism in the arterial system. Gaddis and colleagues already showed potential problems with the measurement of the mean circulatory filling pressure. Very similar to our experiment, they showed that the way cardiac arrest is induced (either acetylcholine or VF) influences this pressure. They also pointed out that central venous pressure did not equal venous portal pressure [3].

In a more physiological setting—similar to Starr’s experiment—Repressé and colleagues reported a broad dispersion in intravascular pressures, recorded shortly after death in patients in the intensive care unit. They showed an influence of norepinephrine, when infused before death, on mean circulatory filling pressure, attributing this effect to post-mortem alterations in vasomotor tone [12]. Our results support their hypothesis that drugs with an effect on vasomotor tone have a significant impact on both arterial and venous pressures after cardiac arrest.

Dynamic course in blood pressure after cardiac arrest

Our finding of a dynamic course in blood pressure recordings after the induction of ventricular fibrillation is consistent with Guyton’s findings. He already pointed out that intense vasoconstriction begins throughout the circulatory system shortly after the heart stops, with significant impact on the mean circulatory filling pressure. Ongoing blood flow after onset of VF is not new and is shown at the microcirculatory level [13].

The observed dynamic course of blood pressures may indeed been caused by time varying contributions of pressure-dependent, shear stress-dependent, and metabolic vasoactive responses (ischemia) to cardiac arrest [14]. Initial vasoconstriction, causing an increase in blood pressures (both venous and arterial), is followed by ischemia induced disruption of the actin cytoskeleton in smooth muscle and reduction in vascular compliance and tone [15]. Vessel wall ischemia—following cardiac arrest—will markedly increase vessel compliance [16]. Since both venous and arterial blood pressures are a function of

intravascular volume and the capacitance of the cardiovascular system, the ischemic induced change in compliance will affect these two compartments differently. Whereat the potent direct vascular effects of barbiturates, for example on vessel caliber, might nullify the previously described slower time varying ischemic effects.

Persistence of a retrograde pressure difference

We are not the first ones to report a pressure difference between vascular beds after cardiac arrest [3,10,11]. However, a retrograde pressure difference has, to our knowledge, not been previously reported. This retrograde pressure can only be maintained when a high resistance or complete blockage exists between the venous and arterial sides of the circulation. This requires the absence of communicating vessels after cardiac arrest, thereby separating the sides. Venous valves are reported to withstand 300 mmHg retrograde pressures without leakage [17], and could therefore easily withstand the reported retrograde pressure difference of 7 mmHg.

Besides intravascular resistance in the form of valves, a reduced transmural pressure does increase resistance as well. Magder showed that a positive perfusion pressure at zero flow can be explained by the presence of an arteriolar Starling resistor mechanism (i.e., vascular waterfall) at the level of the arterioles [18]. He showed that venous pressure needed to be nearly doubled (from 9 to 16 mmHg) before arterial blood pressure was influenced. This could work the other way around as well. In order to overcome this Starling resistor mechanism, arterial blood pressure should at least exceed the intra-abdominal pressure. It is noteworthy that our observed negative pressure difference was below physiological ranges of intra-abdominal pressures in piglets [19].

Limitations

The goal of our study was not to repeat Guyton's experiment, but rather to study methodological issues with the concept and measurement of mean circulatory filling pressure. The absence of a pressure difference as observed by Guyton can easily be attributed to differences in experimental design, his use of an artificial shunt in particular. As measurement errors can never be completely excluded, we took extra care in leveling, zeroing and calibrating the system. Furthermore, a fast-flush test was performed to verify the dynamic response of the pressure system.

Implications

We have showed that linking a beating-heart situation to a presumably steady state following cardiac arrest is at least troublesome. Obtaining the upstream pressure for venous return after cardiac arrest feels like trying to determine the fuel consumption of a car with a stalled engine. Apart from the rare situation where cardiac arrest needs to be induced, and blood mechanically pumped through an arterial to venous shunt, this obtained mean circulatory filling pressure may not provide clinically meaningful information after all. Induction of cardiac arrest as a diagnostic or therapeutic intervention would bring considerable risks with it.

The highly nonlinear pressure-volume properties of the vasculature, its complex parallel structure, transmural influences, differences between the beating-heart and zero-flow situations, and the influence of breathing further cast doubt on the usefulness of obtaining normative MCFP values. Also, intravascular blood pressure is the result of external pressure, volume and spatiotemporal vessel elasticity. It seems therefore incorrect to influence either volume (shunting) or vessel elasticity (drugs) when trying to assess a blood pressure.

Conclusion

All methods to obtain the mean circulatory filling pressure have considerable effects on the cardiovascular system. We question the clinical relevance of this virtual pressure, as well as its relationship to the beating-heart situation.

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Hemodynamic response to β -blockers in severe sepsis and septic shock: A review of current literature

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Abstract

The administration of β -blockers in patients with sepsis is a trending topic in intensive care medicine since the landmark study by Morelli and colleagues, showing a striking decrease in 28 day mortality compared to standard care. While the available evidence suggests that the use of β -blockers in septic shock is safe, the effects on hemodynamics are controversial. In this paper, we review the effect of β -blockade in septic shock on hemodynamics from animal models to critically ill patients.

Introduction

Sepsis is one of the leading causes of death among hospitalized patients and is a frequent cause of admission to intensive care units [1]. Morbidity and mortality caused by sepsis remain high, despite an improved understanding of the pathophysiology [2,3]. Therefore, the search for a more effective therapeutic strategy remains highly relevant [4].

Heart rate control using β -blockers could potentially be such a novel therapeutic strategy. A reduced heart rate might improve the cardiac mismatch of oxygen demand and supply in septic patients, by decreasing myocardial oxygen consumption [5]. Sepsis can cause an adrenergic storm with the release of catecholamines that is associated with cardiac dysfunction. It has been postulated that β -blockers attenuate these ‘toxic’ effects. Despite this theoretical benefit of controlling the adverse effects of sepsis by β -antagonists, it is unclear if this mechanism truly benefits the cardiovascular system [4].

The physiological rationale behind the clinical application of β -blockers in septic shock is not limited to the modulation of the cardiovascular effects, but also to coagulopathy, attenuation of the hypermetabolic state and immune-modulating effects. In this review we will focus on the interaction between β -blockers and the cardiovascular system, in particular the cardiovascular physiological consequences of β -blocker therapy and the concerns that have been expressed regarding the risk of reducing cardiac output and subsequently blood pressure [6–8].

Methods

PubMed was searched by one of the researchers (LvL) for all references through August 2018 for both animal and human studies relating to septic shock and the use of β -blockers. We included candidate articles which were identified using the following criteria: “septic shock” or “severe sepsis” with “beta-blocker” or “beta-blockade”, in combination with the presentation of ≥ 1 of the following cardiovascular parameters: cardiac output, systemic vascular resistance, stroke volume, blood pressure, oxygen consumption, microcirculatory flow. Additional manual search was conducted, exploring the list of references of the relevant articles.

Table 1

Summary of preclinical data evaluating the effect of β -blockade on the cardiovascular system in models of experimental septic shock. Showing that the influence on hemodynamic parameters depends on dose, timing, gender, concomitant therapy, species, the severity of shock, and cardiac function.

Nr	First author	Year	Species	β -blocker *	HR target	Timing	Pressor usage	Stroke volume	Mean arterial pressure	Systemic vascular resistance	Tissue function	O ₂ uptake	Effect of β -blockade on hemodynamics
[9]	Suzuki	2005	Rat	E	Dose response	24 h after sepsis	na	↑	↓	na	=	↑	Improved oxygen utilization of myocardium and preserved myocardial function
[16]	Ackland	2010	Rat	M	20% reduction	6 h after sepsis	na	=	=	na	=	na	Cardioprotective effects
[17]	Aboab	2011	Pig	E	20% reduction	1 h after sepsis	na	↑	=	=	=	=	Preload positive effect
[18]	Calzavacca	2014	Sheep	A	Fixed dose	8 h after sepsis	na	↑	↓	↑	=	=	Increased vascular reactivity
[19]	Kimmoun	2015	Rat	E	No info	4 h after sepsis	=	↑	=	↑	na	na	Enhanced intrinsic cardiac contractility and improved vascular responsiveness
[20]	Jacquet-Lagreze	2015	Pig	E	<90 bpm	After hemodynamic stabilization	↑	=	=	=	=	=	Well tolerated
[21]	Hernandez	2016	Sheep	E	20–30% reduction	After hemodynamic stabilization	=	na	=	na	=	na	Well tolerated and increased exogenous lactate clearance
[13]	Wang	2016	Mice	E	Fixed dose	Direct after septic insult	na	↑	↑	na	na	na	Improved cardiac function
[15]	Wei	2016	Rat	E	Dose response	4 h after sepsis	na	↑	↑	↑	na	na	Inflammatory modulation
[22]	Du	2017	Dog	E	10–15% reduction	After hemodynamic stabilization	na	↑	=	=	↓	na	Restores vascular waterfall
[5]	Hosokawa	2017	Sheep	E	80–100 bpm	2 h after sepsis	na	↑	↓	=	=	↓	Despite earlier onset of hypotension, safe to use.
[23]	Kurita	2017	Pig	L	Dose response	30 min after sepsis	na	↑	↓	na	↓	↓	Depending on dose and stage of shock
[10]	Aboab	2018	Pig	E	10% reduction	1 h after sepsis	na	na	na	na	na	na	Promotion of parasympathetic activity
[11]	Boselli	2018	Pig	E	<90 bpm	After hemodynamic stabilization	na	=	=	↑	na	na	Well tolerated, with no change in parasympathetic activity
[12]	Mathieu	2018	Rat	L	No target	1 h after sepsis	na	↓	↓	na	na	na	Depending on gender by gene expression
[14]	Van Loon	2018	Sheep	E	30% reduction	After hemodynamic stabilization	na	↓	↓	na	=	na	Reduced right ventricular function

* E: Esmolol, M: Metoprolol, A: Atenolol, L: Landiolol

na: not available.

Results

Preclinical data (Table 1)

We identified 16 experimental studies evaluating hemodynamic response to β -blockers (i.e. atenolol, esmolol, landiolol, metoprolol, propranolol) in animal models (i.e. dogs, pigs, rats, mice, sheep) of septic shock induced by endotoxin infusion or cecal ligation and puncture [5,9–23]. The duration of the experimental protocols mainly depended on the size of the animal model. Smaller animal such as rats and mice were studied for 14 h to 24 h, while the large animal models were studied for 4 h on average. The heart rate reduction was targeted using a threshold (range 80–100 beats per minute) or a percentage decrease from baseline (range 10–30 %). Hemodynamics measurements focused on both physiological output (e.g. cardiac output, blood pressure) and dynamic interactions of hemodynamic variables (e.g. left ventricular systolic function, vascular reactivity). Animal studies on the use of β -blockade do not give consistent and predictable results, reporting both hemodynamic improvement and deterioration [24]. These differences in results cannot be attributive to the size of the animal nor to the used β -blocker, but seem related the time of administration (Table 1).

Clinical data (Table 2)

Nonrandomized human studies

Studying the direct effect of β -blockers on hemodynamics during septic shock using nonrandomized studies and case series is challenging, since progression of the actual shock and concomitant use of inotropes and vasopressor agents cannot be eliminated (Table 2). One retrospective study also showed improved hemodynamics from enteral metoprolol in 40 septic shock patients with cardiac depression. However, the effect of concomitant administrated milrinone should not be ignored [25]. Interestingly, all studies showed a remarkable record of clinical safety in patients with septic shock [26–31] without cardiovascular deterioration [28].

Randomized control trials

Clinical data on the use of β -blockers in septic shock is limited [32,33] (Table 2). Up until now, two randomized control trials (RCTs) have been published of which the most relevant one is a single-center, open-label, phase 2 study by Morelli and colleagues [27]. In this study, 154 septic patients were randomized to

Table 2

Summary of clinical trials evaluating the effect of β -blockade on the cardiovascular system in patients with severe sepsis and septic shock. Showing contradicting results for both mean arterial pressure and systemic vascular resistance.

Nr	First author	Year	Design	Pts (n)	β -blocker *	HR target	Timing	Pressor usage	Stroke volume	Mean arterial pressure	Systemic vascular resistance	Tissue function	O ₂ uptake	Mortality	Safety
[65]	Berk	1972	Case series	26	P	No target	In late refractory septic shock	na	na	↑	↑	na	na	40% (no control)	Well tolerated
[25]	Schmittinger	2008	Retro-spective	40	M	<95 bpm	<48 h after septic shock	↓	↑	=	=	=	na	33% (no control)	Feasible
[30]	Balik	2012	Case series	10	E	20% reduction	2 h after sepsis	=	=	=	=	na	=	na	Safe to use
[66]	Morelli	2013	Pilot	25	E	80–94 bpm	After hemodynamic stabilization	↓	=	=	na	=	↓	na	Safe to use
[27]	Morelli	2013	RCT	144	E	80–94 bpm	After hemodynamic stabilization	↓	↑	=	↑	↑	↓	↓	No addition adverse events
[38]	Yang	2014	RCT	41	E	<100 bpm	6 h after sepsis	na	=	=	↑	=	na	na	Well tolerated
[53]	Wang	2015	Prospective	90	E	<95 bpm	Direct after septic shock	=	=	=	na	=	na	↓	Well tolerated
[29]	Wei du	2016	Prospective	63	E	15% reduction	<48 h after septic shock + hemodynamic stabilization	na	↑	=	na	=	na	6.3% (no control)	Safe to use
[28]	Morelli	2016	Pilot	45	E	80–94 bpm	After hemodynamic stabilization	↓	↑	↓	↓	na	na	na	Well tolerated

* P: Propranolol, M: Metoprolol, E: Esmolol
na: not available

receive esmolol infusion after 24 h of hemodynamic stabilization. Prerequisite was that patients were on noradrenaline infusion and heart rate was > 95 beats per minute. β -blockade successfully achieved the primary outcome (reduced heart rate) in all patients and was associated with an increased stroke volume and systemic vascular resistance while leaving mean arterial pressure and oxygen consumption unaffected. This study by Morelli et al. has increased the

interest for β -blocker administration in patients with septic shock, although it is not without debate [34–37]. As there is no structured hemodynamic assessment, the suggested positive effect of β -blockade on the cardiovascular system remains highly speculative. Despite the criticism concerning this trial, the findings are tantalizing and suggest that β -blockers could play a key role in the treatment of patients with septic shock.

The only other RCT was published in the Chinese language and compared two small populations receiving standard treatment and esmolol infusion, with the goal to titrate heart rate below 100 bpm within 2 h [38]. From the English abstract and translated manuscript, the esmolol group showed no changes in blood pressure, stroke volume, and lactate compared to control. No information regarding mortality was provided. The incomplete appreciation of this study strongly limits its utility.

Discussion

Clinical data suggests that the use of β -blockers for heart rate control in septic shock is safe when started at a low dose. However, data are insufficient in unravelling the possible mechanisms driving the favorable effects in septic shock. In particular, the conflicting results on the influence of β -blockers on cardiac output, stroke volume and arterial pressure distracts the debate on the effectiveness of this therapy [32,33] (Table 2).

Preclinical studies show a wide variety in used models, targets and protocols. Furthermore, they show inconsistent and unpredictable results of the effects of β -blockade on hemodynamic output parameters. The duration of large animal models is relatively short, making their design inapplicable to study organ dysfunction and outcome. Similar to clinical data, preclinical data show both unaffected and improved cardiac output, stroke volume and both a decreased and unaffected arterial blood pressure. This inconsistency in data emphasizes that the effect of β -blockers on hemodynamics is multifactorial. Effects are determined by dose [15,23], timing [23], gender [12], concomitant therapy [21,30], species [39], the severity of shock [23] and cardiac function [19]. It is therefore more relevant to understand the functional physiological consequences of β -blockers in septic shock, rather than focusing on central hemodynamic output parameters. Experimental models of septic shock have

addressed these different hemodynamic effects of β -blockers on vascular reactivity, cardiac function and/or their interaction (Table 1).

Vascular reactivity

Despite the absence of β_1 -receptors on vessels, selective β -blocker infusion improved sepsis-induced hypo responsiveness to vasopressor treatment [15,18,19,22]. It is postulated that β -blockers increase pressor responsiveness, like clonidine [40], by restoring the sepsis associated down-regulation of α_1 -receptors [19]. Similar to preclinical data, Morelli et al. found an improvement in vascular tone related to a decrease in arterial elastance when septic patients were treated with esmolol [28]. This improvement of vascular reactivity may explain why the use of selective β -blockers in septic shock is associated with unchanged or even decreased vasopressor requirements, despite a reduced cardiac output [25,27]. Additionally, many septic patients are in need for vaso-active support but catecholaminergic drugs can, at the same time, deteriorate the cardiac function [41,42]. Therefore, reducing these exogenous catecholamine load could be a beneficial effect of β -blocker.

Cardiac

Although many studies have demonstrated that preventing cardiac injury is crucial to improving prognosis of septic patients [43], it is unclear if β -blockade is an effective treatment to attenuate this cardiac dysfunction. Preclinical data show attenuation of sepsis induced myocardial dysfunction, including improved myocardial contractility and relaxation of the left ventricle [13].

Both *ex vivo* [9] and *in vivo* [19] intraventricular measurements show an improvement in left ventricular intrinsic myocardial function. Strikingly, in humans the systolic function is reported to decrease after esmolol therapy assessed by transthoracic echocardiography [29]. Although it is not fully understood how the blockade of β -adrenoceptor pathway increases contractility of the septic heart, some investigators have attributed these effects to the suppression of cytokine production such as TNF- α [44].

Others suggest that β -receptor expression is increased after β -blocker therapy, resulting in an improved inotropic response to β -agonists [45]. Observational data shows that continuation of previous β -blocker therapy in septic shock is associated with an unchanged need of inotropic support and possibly an improved outcome [46]. However, too aggressive use of β -blockers could decrease the myocardial contractility by prevailing the chronotropic

effects of β -receptor blockade. For this reason, clinical diligence and caution are necessary when treating septic shock with esmolol in the acute phase. A decreased heart rate could also improve diastolic perfusion time and consequently myocardial perfusion. Some studies argue in favour of this preload positive effect by showing an improved stroke volume, despite a decreased contractility [29]. The influence of β -blockers on the diastolic function of the right ventricle is still an unexplored area of interest [4,14,27]. While systolic dysfunction is not associated with mortality [47], diastolic dysfunction is a predictor of mortality in septic shock [48]. Nonetheless, the role of β -blockers on the diastolic function remains highly speculative as this concept is not supported by structured echocardiography or by *in vivo* load-independent markers.

Cardio-vascular interaction

Assuming that β -blockers leave the intrinsic myocardial function unaffected, they might still benefit the heart by reducing its afterload thereby allowing the heart to operate more economically. Attenuation of cardiac depression by improving its efficiency might support the physiological rationale of using β -blockers during septic shock [27]. Increased cardiac efficiency is defined by an improved ratio between ventricular to arterial elastance, i.e. the concept of ventricular–arterial coupling.

This physiological concept of improving cardiac efficiency using β -blockers seems to work in selected patients with septic shock [28]. In the presence of an adequate preload, heart rate reduction with esmolol could improve arterial elastance, thereby contributing to an improved cardiovascular efficiency while allowing adequate systemic perfusion in septic shock. The lack of direct measurements, resulting in numerous approximations and omissions, raises doubts about the relevance of these results [49]. Our own intraventricular data support the concept of improved ventricular–arterial coupling in experimental endotoxic shock after β -blockade [14]. However, lowering arterial elastance—in order to increase cardiac efficiency—is only beneficial when an adequate perfusion pressure is maintained.

Future perspectives

Taking both preclinical and clinical data into consideration, it makes sense to reduce myocardial oxygen consumption when its need outstrips the capacity. Still, excessive β -blockade with negative inotropic effects could put the cardiac output below the threshold that is needed to maintain organ and

tissue perfusion. Answering the clinically important question of how to apply β -blockers safely while maximizing its beneficial effect depends on; timing, type of β -blocker, resuscitation targets and patient characteristics [50].

Timing of intervention

Just like any pharmacologic intervention, onset time has a major impact in determining its effect on the inflammatory response. Some experimental studies documented survival benefits particularly when β -blockers were administered before the septic insult [16]. Preclinical data show that starting β -blocker therapy right after hemodynamic stabilization [27] is probably not the best moment. However, progressive sepsis-induced cardiac systolic and diastolic dysfunction develops during the early stages of sepsis [43]. Early (low-dose) β -blockade might therefore be associated with a greater number of survivors with septic shock [51]. Even alternate sequences of ‘on and off’ β -blocker administration have been proposed [52]. However, current evidence does not support this.

Type of β -blocker

Currently, esmolol is the only β -blocker that has been tested in a RCT. The pharmacological characteristics of esmolol, an ultrashort acting β_1 -adrenoceptor antagonist, allows titration of the dosage to specific hemodynamic endpoints. However, there is not enough evidence to propose the use of a specific agent in each specific critical condition. Clinical studies have investigated the concomitant use with inotropes in order to counteract the negative inotropic action of β -blockers, and suggested that the combination had positive effects on improving cardiac performance and mortality risk [25,53]. Here too, while combining β -blocker therapy with phosphodiesterase inhibitors seem feasible in patients with septic myocardial depression [25], there is not enough evidence to propose the use of a specific inotropic agent.

Resuscitation targets

For beneficial effects, β -blockers should be administered at limited doses, wherein a fixed dosage and even titration to heart rate is not appropriate [23]. Granting the septic heart more time to recover—by putting the cardiomyocytes in a hibernation-like state by β -blockade—requires resuscitation goals focusing on tissue perfusion rather than central hemodynamics. Previous findings even suggest that β -blockers may cause an increased tolerance to hypotension [5], emphasizing to leave blood pressure ‘cosmetics’ behind and to move

forward to permissive hypotension and a perfusion-based approach [54]. This approach would allow for reducing the possible adverse effects of fluid therapy and vasopressor agents in septic shock [42,55].

It would therefore be essential to monitor the response of the microvascular bed to β -blockers. Current data shows marginal effects on the sublingual and gut microcirculation, which remains depressed from septic shock [20]. A persistent depressed microcirculation is a well-known indicator of adverse outcome [4,56]. The impaired microcirculatory flow during septic shock triggers the generation of anaerobic lactate and potentially impairs hepatic lactate clearance [57,58]. Preclinical studies show that β -blockers are associated with lower arterial and portal lactate levels, and less impairment of exogenous lactate clearance with beneficial effects on gut lactate generation [21]. However, monitoring the response of lactate as a goal of early sepsis therapy should be done with extra care in patients receiving β -blockers since production of lactate might be masked by a reduced adrenaline production [59,60].

Still, titration to microcirculatory parameters, lactate and oxygen delivery in relation to oxygen demand seems advisable. We could allow for reduced cardiac output and arterial blood pressure, thereby protecting—not necessarily improving—the cardiovascular system.

Patient characteristics

Personalized treatment based on cardiac function, adequate preload, the presence of comorbidities and the degree of sympathetic activation may provide better results in terms of outcome [50]. Patients with good cardiac contractility before treatment and those who are still in their early stage of septic shock are more prone to benefited from β -blocker therapy [29].

In pediatrics, the role of β -antagonists is not clear. In a non-septic setting, Jeschke and colleagues successfully used propranolol in severely burned children and demonstrated attenuated hypermetabolism and without complications [61]. However young children are characterized by a physiological higher resting heart rate, higher resting contractility state, lower contractility reserve and higher afterload dependence. Combined with the myocardial depressant effects of sepsis makes the use of β -antagonists in these patients possibly less favorable [62–66]. The use of β -antagonists in young children in septic shock should first be studied carefully under controlled conditions.

Conclusion

Data are inconsistent in how β -blockers change hemodynamics in septic shock. Nevertheless, there is sufficient preclinical and clinical data to suggest that β -blockade is safe when started at low dose. They must be used carefully while focusing on optimizing hemodynamics with resuscitation goals focusing on tissue perfusion rather than central hemodynamics.

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The influence of esmolol on right ventricular function in early experimental endotoxic shock

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Abstract

Background The mechanism by which heart rate (HR) control with esmolol improves hemodynamics during septic shock remains unclear. Improved right ventricular (RV) function, thereby reducing venous congestion, may play a role. We assessed the effect of HR control with esmolol during sepsis on RV function, macrocirculation, microcirculation, end-organ-perfusion, and ventricular–arterial coupling.

Methods Sepsis was induced in 10 healthy anesthetized and mechanically ventilated sheep by continuous IV administration of lipopolysaccharide (LPS). Esmolol was infused after successful resuscitation of the septic shock, to reduce HR and stopped 30 min after reaching targeted HR reduction of 30 %. Venous and arterial blood gases were sampled and the small intestines' microcirculation was assessed by using a hand-held video microscope (CytoCam-IDF). Arterial and venous pressures, and cardiac output were recorded continuously. An intraventricular micromanometer was used to assess the RV function. Ventricular–arterial coupling ratio (VACR) was estimated by catheterization-derived single beat estimation.

Results The targeted HR reduction of >30 % by esmolol infusion, after controlled resuscitation of the LPS induced septic shock, led to a deteriorated RV-function and macrocirculation, while the microcirculation remained depressed. Esmolol improved VACR by decreasing the RV end-systolic pressure. Stopping esmolol showed the reversibility of these effects on the RV and the macrocirculation.

Conclusion In this animal model of acute severe endotoxic septic shock, early administration of esmolol decreased RV-function resulting in venous congestion and an unimproved poor microcirculation despite improved cardiac mechanical efficiency.

Introduction

Sepsis is one of the leading causes of death among hospitalized patients and a frequent cause of admission to intensive care units (ICUs) [1]. Morbidity and mortality from sepsis in ICU patients remain high, despite an improved understanding of the pathophysiology of sepsis [2,3]. Therefore, the search for effective therapeutic strategies remains relevant [4].

Heart rate (HR) control by esmolol, a an ultrashort acting β_1 -adrenoceptor antagonist could be such a novel therapeutic strategy with substantial impact on the clinical management of septic shock. Reduced HR could improve the cardiac mismatch of oxygen demand and supply in septic patients, by decreasing myocardial oxygen consumption [5]. The study by Morelli et al. shows that esmolol is able to control noncompensatory tachycardia during established septic shock after 24 h of hemodynamic optimization and reduce mortality from 81 % to 49 % . Together with a reduction in HR, they showed that esmolol was able to improve macrocirculatory variables significantly, while the microvascular blood flow was preserved and the frequency of adverse events did not increase [6].

Although these results look promising, the optimal dosage, initiation and the mechanism by which esmolol might improve hemodynamic and organ function and reduce mortality remain to be elucidated [7,8]. It is hypothesized that HR reduction during septic shock may improve right ventricular (RV) diastolic function, leading to increased cardiac preload by reducing venous congestion and thereby increasing effective organ perfusion [4,9–12]. These possible positive effects of esmolol on RV function have only been described using echocardiography [13]. However, to obtain more mechanistic insights, a comprehensive approach using intraventricular measurements should be performed. Another supposed beneficial effect of esmolol in septic patients may be related, at least in part, to reduced afterload by better ventricular–arterial (V–A) coupling. V–A coupling is a key determinant of cardiovascular function by describing the interaction between the ventricle (contractility) and the arterial system (afterload) [14,15,16]. Decoupling of contractility and afterload is clearly present in patients with septic shock and may persist after resuscitation to recommended hemodynamic target [17,18].

In this study, we aimed to elucidate the effect of early initiation of HR control by esmolol on RV function in the acute stage of resuscitated sepsis (< 24 h). Using a lamb model of resuscitated severe septic shock, we investigated both

the RV function and v–A coupling, the macrocirculation, microcirculation and end-organ-perfusion as a result of esmolol infusion.

Materials and Methods

This experiment was performed after approval of the local ethics committee on animal research of the Radboud University Medical Center (RUMC License number RU-DEC 2014–10) and in full compliance with Dutch and European legal requirements on the use and protection of laboratory animals. Ten conventionally reared female lambs (crossbred Texelaar–Flevolanders) under general anesthesia were studied. No control group was considered necessary since the animals acted as their own control.

Anesthesia and ventilation

Premedication consisted of midazolam (0.5 mg/kg) and ketamine (4 mg/kg), anesthesia was performed using IV administration of propofol (2 mg/kg). After endotracheal intubation, general anesthesia was maintained using inhalation of isoflurane (0.5–2 volume %), the continuous IV administration of sufentanil (2 µg/kg per h) and rocuronium (1 mg/kg per h) after a loading dose of 1 mg/kg. The lungs were mechanically ventilated using a volume-controlled mode with tidal volumes of 8–10 mL/kg, 5 cmH₂O PEEP, *F*O₂ 0.4, and an inspiratory-to-expiratory ratio of 1:2. Ventilation was adjusted according to the end tidal CO₂ level. Impaired oxygenation was treated by increasing PEEP and or increasing the *F*O₂ to maintain an oxygen saturation > 95 %. During the experiment, continuous IV dextrose 10 % 2.4 mL/kg per h (240 µg/kg per min dextrose) was administered supplemented with IV 0.9 % saline 2–4 mL/kg per h in order to maintain fluid balance and to prevent hypoglycemia. At the end of the experiment, the animals were euthanized with an overdose of pentobarbital (150 mg/kg IV) (T3).

Surgical preparation

All lambs were positioned in dorsal position for inserting the intravascular catheters using surgical cut down procedures. A 7.5 F × 20 cm triple-lumen central venous catheter was placed in the left internal jugular vein (Multicath, Vygon Nederland BV) for central venous blood pressure monitoring, venous blood sampling, and for the administration of fluid and drugs. A 18 G × 10 cm single-lumen catheter (Leaderflex, Vygon Nederland BV) was introduced in

the right femoral artery for arterial blood pressure monitoring and blood sampling.

A 5 F × 8 cm introducer sheath (Cordis Europe, Waterloo, Belgium) was introduced followed by a 5 F guiding catheter (Impulse™, Boston scientific, Kerkrade, The Netherlands) into the right internal jugular vein. The guiding catheter was advanced using fluoroscopy until rv blood pressure was recorded at the tip. The ComboWire® XT (Volcano Corporation, Rancho Cordova, CA), a 0.014" dual-sensor (pressure and Doppler velocity)-equipped guide wire, was advanced into the guiding catheter. The guiding catheter was pulled back in order to locate its tip in the right atrium and leave the pressure wire in the rv. The ComboWire® XT pressure wire was used to perform rv pressure measurements continuously.

Next, the lambs were repositioned for the duration of the experiment in the right lateral position. An ultrasound transit-time perivascular flow probe (14 or 16 mm) (PAX series, Transonic Systems, Ithaca, NY) was placed around the main pulmonary artery to measure cardiac output after left thoracotomy. Laparotomy created a window for Incident Dark Field (IDF) imaging the of the small intestines' microcirculation. Gastrostomy was performed in order to place a 30 cm tube for gastric decompression. Last, an 8 F silicon Foley catheter (Covidien, Mansfield, MA 02048, USA) was introduced into the bladder.

Resuscitated endotoxic shock

After closing all the incisions, the chest and the abdomen, and prior to creating a situation of resuscitated endotoxic shock, we gave a fluid bolus (5–10 mL/kg) to treat potential initial hypovolemia related to induction of anesthesia and surgical manipulation. Next, a stabilization period of 30 min (T_0) was followed by continuous iv administration of lipopolysaccharide (3 µg/kg per h) (LPS, US Standard Reference Endotoxin *Escherichia coli* O:113) after a loading dose of 3 µg/kg, see Figure 1. The LPS dose was defined from previous pilot

Figure 1

Schematic overview of the experimental design.

experiments. Resuscitation started 30 min after LPS induced a 50 % reduction in cardiac output or a 25 % reduction in ABP (T₁). Resuscitation objectives were to return ABP and cardiac output to their baseline values. A fluid load of 50 mL of saline (or whole blood if hemoglobin level < 5 mmol/L) was administered initially to evaluate fluid responsiveness guided by flow probe cardiac output; in case of absence of fluid response, nor-epinephrine and/or dobutamine were used. The initial dose of nor-epinephrine and dobutamine was 0.1 µg/min per kg and 0.5 µg/min per kg, respectively, and could be increased to up to 1.5 µg/min per kg and 2.5 µg/min per kg, respectively to achieve resuscitation goals. In all animals this protocol leads to restoration of blood pressure and cardiac output.

Experimental protocol

Thirty minutes after creating a situation of resuscitated endotoxic shock (T₁), esmolol (Baxter, Maurepas, France) was started at 50 µg/kg per minute and progressively increased to reduce in the HR by 30 %. A percentage change—in the same magnitude as Morelli et al. (2013a)—was used to allow for differences in resting HR. Resuscitation maneuvers were maintained or increased in order to maintain ABP and cardiac output at baseline values; except for dobutamine dosage, which was kept constant in order not to intervene with the esmolol treatment. Esmolol infusion was stopped 30 min after the HR reached the targeted reduction of 30 % (T₂), see Figure 1.

Data collection

Electrocardiography (EKG), blood pressures, and pulmonary flow were simultaneously and continuously recorded on a laptop computer and stored on a hard disk with a sample rate of 200 Hz by an A/D converter (NI USB-6211, National Instrument, Austin, TX, USA). Before, during, and after the esmolol infusion, five sequences of 10 seconds clips of the small intestines' microcirculation were obtained with the Cytocam-IDF video microscope (Braedius Medical, Huizen, the Netherlands). Image acquisition was performed according to published consensus criteria [19]. A hand-held iSTAT® point-of-care analyzer (Abbott Laboratories, IL, USA) was used to obtain arterial hematocrit, hemoglobin, partial pressure of CO₂ and Urea.

Data analysis

A blinded investigator (LvL) scored the captured IDF clips according to Massey et al. In short, images were scored on six categories: illumination, duration, focus, content, stability, and pressure. Videos are assigned a score of 0 = good, 1 = acceptable, or 10 = unacceptable for each category. Any video with composed scored ≥ 10 was discarded from future analysis. Automated Vascular Analysis (AVA 5; Microvision Medical B.V.) was used for off-line analysis on the images of the sublingual region that met the quality requirements. The continuously recorded EKG, pressure and flow signals were analyzed using custom-written MATLAB scripts (Matlab R2017b, The MathWorks Inc. Massachusetts, USA). Mean ABP, CVP and cardiac output were acquired by taking a fourth order Butterworth low-pass filter with a cut-off frequency of 0.02 Hz from their raw signal. HR was acquired by automatic detection of R-peaks from the EKG-signal.

RV contractile function ($dP/dt_{\max}/P$) was acquired by taking the peak-first-derivative of RV pressure waveform divided by the pressure at that point. The maximum isovolumetric pressure ($P_{\max}$) provides an estimate of the maximum pressure that could be generated during an isovolumetric contraction and was determined by fitting a sinusoid to the isovolumetric regions of the pressure tracing, the so-called 'single beat method' [20,21,22]. Tau was calculated by the method of Weiss et. al, assuming a zero asymptote [23]. Ventricular-arterial coupling ratio (VACR) of the RV was approximated by dividing end-systolic pressure (P_{es}) by the difference between $P_{\max}$ and P_{es} [24].

Statistical analysis

Prism Statistical Software was used for statistical analysis (Graph-Pad Prism 5, GraphPad Software Inc., San Diego, CA, USA). RV systolic and diastolic parameters were normalized to baseline. One-way analysis of variance (ANOVA) and the Bonferroni test was used for multiple post-hoc comparisons of the different time points. A p -value of < 0.05 was considered to indicate significance.

Results

A total of 10 female lambs were studied. They were 6–8 month-old, weighing a mean of 20.6 kg [range: 13–24.5 kg] and with a mean body surface area of 0.91 m² [range: 0.67–1.0 m²]. All animals included in the study were considered healthy on physical examination when entering the animals' laboratory. In all animals, LPS infusion plus resuscitation maneuvers caused a septic shock

with increased cardiac output, tachycardia, and significantly decreased microcirculatory parameters. Lactate increased significantly over the course of the experiment and was produced aerobic ($P(v-a)CO_2/C(a-v)O_2 < 1.4$ and $ScvO_2 > 70\%$) (Table 1). Hematocrit and hemoglobin remained within physiological (baseline) ranges.

Effect of esmolol

Esmolol infusion induced on average a HR reduction of 37% [range: 31–41%]. This was accompanied with a significant impairment in MAP, CI, and both RV function parameters (systolic and diastolic), see Figure 2 and Table 1. This impairment led to venous congestion, indicated by a significantly increased CVP. The microcirculation of the small intestines remained depressed ultimately, see Figure 3. Apart from the microcirculation, these effects were reversible by stopping esmolol infusion, showing that the deteriorated hemodynamics was not due to progressive endotoxic effects of LPS.

The measurements of P_{es} , P_{max} , and $VACR$ by single beat estimation are shown in Figure 4B. During esmolol infusion, a trend toward a decreased RV end-systolic pressure and increased estimated RV maximum pressure was

Figure 2

Macrocirculatory variables per study phase.

A Mean arterial pressure (MAP) and central venous pressure (CVP).

B Cardiac output (CO), heart rate (HR) and stroke volume (SV).

Data are expressed as mean $\pm$ SD. Bonferroni's post-hoc test was used to perform pairwise comparisons between phases, $\star: p < 0.05$, $\star\star\star: p < 0.001$

Table 1

Effect of esmolol on hemodynamic and metabolic parameters.

Variable	Baseline (T0) (n = 10)	Resuscitated endotoxemic shock (T1) (n = 10)	Esmolol (T2) (n = 10)	Stop (T3) (n = 8)	T0 vs T1 ^a	T1 vs T2 ^a	T2 vs T3 ^a	T0-T3 ^b
Systolic arterial pressure (mmHg)	76 ± 11	74 ± 11	46 ± 12	67 ± 11	ns	***	**	***
Diastolic arterial pressure (mmHg)	49 ± 8	48 ± 8	32 ± 8	43 ± 9	ns	***	ns	***
RV dP/dt _{min} (mmHg/sec)	-1.3 ± 0.7	-1.8 ± 0.5	-1.4 ± 0.4	-1.6 ± 0.5	ns	ns	ns	ns
Cardiac index (l/min/m ²)	3.5 ± 1.1	4.5 ± 1.5	2.4 ± 0.8	3.3 ± 0.6	ns	**	ns	**
Lactate (g/mol)	1.9 ± 1.3	3.5 ± 0.9	4.3 ± 1.2	4.3 ± 1.2	*	ns	ns	***
Hemoglobin (g/dl)	4.6 ± 0.6	5.5 ± 0.7	5.3 ± 0.8	5.4 ± 0.7	*	ns	ns	*
Hematocrit (l/l)	0.22 ± 0	0.26 ± 0	0.25 ± 0	0.25 ± 0	*	ns	ns	*
Urea (mmol/l)	7.1 ± 1.3	7.5 ± 1.5	8.2 ± 2.3	7.5 ± 1.2	ns	ns	ns	ns
P _a CO ₂ (mmHg)	43 ± 5.8	49 ± 12.6	45 ± 4.8	47 ± 2.3	ns	ns	ns	ns
P _v CO ₂ (mmHg)	46 ± 3.9	53 ± 13.4	51 ± 5.2	51 ± 1.5	ns	ns	ns	ns
S _{cv} O ₂ (%)	86 ± 4	90 ± 4	87 ± 5	89 ± 4	ns	ns	ns	ns
pH	7.4 ± 0.06	7.3 ± 0.09	7.3 ± 0.5	7.3 ± 0.08	*	ns	ns	*
Base excess (mmol/l)	3.4 ± 3.7	-0.4 ± 3.9	0 ± 4.4	0 ± 6.6	ns	ns	ns	ns
HCO ₃ (mmol/l)	27.8 ± 3.1	24.7 ± 2.2	24.5 ± 2.0	24.3 ± 2.0	ns	ns	ns	*

Data are expressed as mean ± SD. ns: not significant *: $p < 0.05$, **: $p < 0.01$ and ***: $p < 0.001$ ^a Significant changes between experimental phase (Paired Student's t-test)^b Significant changes over the course of the experiment (repeated measures one-way ANOVA)

seen. This trend resulted in a significant difference between both, showing that esmolol forced the RV to operate below its maximum capacity. This was accompanied by a significant decrease in the VACR (from 7.6 to 3.5 on average), indicating increased cardiac mechanical efficiency during the infusion of esmolol.

Effect of esmolol on resuscitation requirements

Vasopressor requirements increased during esmolol infusion, while only an additional 2 mL/kg saline [range: 0–5.6 mL/kg] was given in boluses guided by the continuous cardiac output monitoring. This limited amount of fluid showed the reduced fluid responsiveness of the animals during esmolol infusion. Maximum nor-epinephrine dosage increased from 1.4 µg/min per kg at T1 to 1.8 µg/min per kg at T2 [range: 1–2.7 µg/min per kg], and returned to 1.5 µg/min per kg at T3. Dobutamine administration remained unchanged, as stated in the protocol. Two animals became severely bradycardic during esmolol infusion necessitating two boluses of epinephrine and CPR. Therefore, we excluded their data from phase after stopping esmolol.

Discussion

In this experimental model of acute septic shock, beta-blockade with esmolol decreased HR with 37 % and induced a significant worsening in macrohemodynamic parameters without recovery of the microcirculation. We showed that the negative effects of esmolol on the macrocirculation could be attributed to a decreased RV function.

Figure 3

Microcirculatory variables per study phase.

Microcirculatory parameters: total vessel density (TVd), proportion of perfused vessels (PPV), and perfused vessel density (PVD).

Data are expressed as mean $\pm$ SD. Bonferroni's post-hoc test was used to perform pairwise comparisons between phases, *: $p < 0.05$

We created a model of resuscitated septic shock to test the efficacy of beta-blockade using esmolol. The severity of initial shock was characterized by increased lactate levels in combination with a significant reduction in cardiac output, MAP, and sv in all animals. Resuscitation with fluids and vasoactive drugs had a positive effect on all macrohemodynamic parameters and changed a hypodynamic hypotensive ‘cold’ shock, induced by LPS, into a ‘warm’ (or hyperdynamic) septic shock. In doing so, we feel that this model was robust enough to resemble clinical significant severe septic shock in humans.

In contrast to other animal and human studies [13,25,26,27], our results revealed that esmolol had a negative effect on the macrocirculation and failed to improve the affected microcirculation. These negative effects could be explained by a decrease in RV function. First, the observed reduction in RV diastolic function (tau) would have caused a negative effect on preload. This RV dysfunction was confirmed by a markedly elevated CVP, which is associated with reduced microvascular perfusion, increased organ dysfunction and mortality [28]. Increased tau, after esmolol infusion in a nonseptic setting, has been described in other studies in both the right [29] and left ventricle [30]. This is

Figure 4

Right ventricular variables per study phase.

A Right ventricular diastolic relaxation time constant (Tau) and right ventricular systolic function ($dP/dt_{max}/P$).

B Right ventricular maximum isovolumetric pressure (P_{max}), ventricular–arterial coupling ratio (VACR), and right ventricular end-systolic pressure (P_{es}).

Data are expressed as mean $\pm$ SD. Bonferroni's post-hoc test was used to perform pairwise comparisons between phases, $\star$: $p < 0.05$, $\star\star$: $p < 0.01$.

caused by the negative lusitropic effects of esmolol and is reported to be even more pronounced in the RV compared to the LV [31]. Second, the reduced RV $dP/dt/P_{\max}$ reflects an intrinsic myocardial effect and is indicative of an impaired systolic function [32,33]. Esmolol is known for shifting the systolic portion of the pressure-volume downward [34].

The CVP decreased and the reduced RV systolic and diastolic function recovered after stopping the esmolol infusion. This indicates that esmolol forced the RV to underperform, despite severe hypotension, resulting in low cardiac output. The reversible underperformance was supported by measurements using the single beat method. The $P_{\max}$ was unaffected, but the gap between the maximum isovolumetric pressure and end-systolic pressure became wider. We are the first to report these $P_{\max}$ and P_{es} values of the RV in endotoxic shock to our knowledge. Using these pressures, we showed that the ventricular–arterial coupling ratio (VACR)—as a relatively load independent measure of RV chamber performance—recovered during esmolol infusion. Esmolol improving cardiac mechanical efficiency with reduced myocardial oxygen consumption is in agreement with other studies [14,35]. However, in our model, the observed damped RV systolic and diastolic function outweighs this positive effect. The reduced RV function resulted in a declined MAP to critical values and venous congestion during esmolol infusion. Lowering VACR—in order to increase cardiac efficiency—is only beneficial when adequate perfusion pressures are maintained. This advocates for a more subtle use of esmolol during septic shock.

Here, the microcirculation remained compromised while the perfusion pressure was reduced. This may possibly explain why Jacquet-Lagrèze et al. did demonstrate a small improvement of some of the microcirculatory parameters, since they had an increase in perfusion pressure [36]. Our poor microcirculatory state was highlighted by increased lactate levels, microvascular shunting and reduced microcirculatory perfusion parameters. While lactate levels increased significantly, the P_{CO_2} gap ($P_{(\text{v-a})\text{CO}_2}$) remained stable (< 6 mmHg), indicative of microvascular shunting [37]. This is supported by the high levels of ScvO_2 ($> 85\%$).

Our data differ from several clinical studies that show beneficial effects of esmolol in patients with septic shock [6,9,13,14,17,18]. These studies show increased SV, maintained MAP and reduced nor-epinephrine requirements [6,13,14]. Furthermore, they observe an increased microcirculatory blood

flow and improved RV systolic function after esmolol infusion, while the effect in RV diastolic function remained unstudied [9,38]. These discrepancies with our results could be explained by several factors:

First, a nonlinear dose response of esmolol on MAP, SV, and cardiac output has been reported [27]. Titrating esmolol to a relative opposed to an absolute HR reduction may have required a higher esmolol infusion rate and therefore induce a different hemodynamic response. Therefore, while HR reduction was the primary target, one should never stop assessing other hemodynamic parameters while titrating esmolol.

Second, we only studied the short-term effects of esmolol. An immediate esmolol induced depression of LV ejection fraction on day 1 due to the direct negative inotropic effect has been reported, while long-term effects showed an increase in the ejection fraction [39]. The potent myocardial protection of esmolol by reducing myocardial oxygen metabolism could be more relevant beyond our studied-acute setting, since endothelial damage would typically occur only after 48 h in clinical setting [40]. In very early septic shock, reducing HR might counteract the compensatory tachycardia due to a reduced contractility, making this therapy strategy harmful in this condition.

Third, there is no consistency in the (simultaneous) use of fluids and/or vasoactive agents during esmolol therapy, making it hard to compare clinical and experimental results. We kept dobutamine infusion rate constant in all animals but did increase nor-adrenaline in order to sustain the MAP. Esmolol might have blunted the dobutamine effects, further worsening the right ventricular function.

Last, while high doses of esmolol have consistently been associated with nondeleterious effects on cardiac function in small-animal models of sepsis [26,27], the results are the opposite in some large-animal models like ours [36,41]. The applied LPS dose and duration vary significantly between different animal models; hence the induced changes and effect of esmolol also vary [42]. Our loading dose of LPS could have induced a more pronounced cytokine response compared to a continuous infusion only.

Limitations

We used an experimental animal model with a limited number of animals, only females, and no sham treatment. Stopping esmolol infusion before the end of the experiment allowed us not to use a sham group since the animals were

their own controls. However, the effect of the ongoing septic shock and resuscitation maneuvers should be kept in mind. In order to monitor urine production by urethral catheterization, we were limited to using only female animals. Knowing that the ideal model of sepsis does not exist, our sheep model has proven to be convenient, reproducible, representative of the human condition, and met all the criteria of hyperdynamic shock [43,44].

Furthermore, the duration of the experiment was relatively short [range: 7–9 h], making this experimental design inapplicable to study organ dysfunction and outcome. The duration of each phase could also limit the studied changes in microcirculatory parameters. While the blood flow in the microcirculation can fall quickly, recruitment might have taken place if therapy was prolonged. To further study the effect of HR reduction on the VACR, simultaneous recording of intraventricular pressure and volume is advised. This would allow for assessment of end-systolic ventricular elastance in order to quantify the ratio between ventricular and arterial elastance. However, we were unable to generate useful ultrasound images of the RV nor absolute end-diastolic and end-systolic RV volumes using a conductance catheter in our animal model [45].

Conclusion

In conclusion, in this animal model of acute resuscitated severe endotoxic septic shock, esmolol decreased RV function resulting in reduced perfusion pressure, venous congestion, and unimproved microcirculation. For this reason, clinical diligence and caution are necessary when treating septic shock with esmolol in the acute phase.

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Effect of vasopressors on the macro- and microcirculation during systemic inflammation in humans *in vivo*

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Abstract

Background Comparing the effects of different vasopressors in septic shock patients is hampered by high heterogeneity and the fact that current guidelines dictate the use of norepinephrine. Herein, we studied the effects of three vasopressor agents, norepinephrine, phenylephrine and vasopressin, on the macro- and microcirculation during experimental human endotoxemia, a standardized, controlled model of systemic inflammation in humans *in vivo*.

Methods We performed a randomized controlled study in which 40 healthy male volunteers were assigned to a five-hour infusion of either 0.05 µg/kg/min norepinephrine ($n=10$), 0.5 µg/kg/min phenylephrine ($n=10$), 0.04 IU/min vasopressin ($n=10$), or saline ($n=10$), starting one hour before intravenous administration of two ng/kg lipopolysaccharide (LPS). The macrocirculation was monitored using arterial catheter-derived parameters with additional blood pressure waveform contour analysis (PCA) until 4.5 hours following LPS administration. Sublingual microcirculatory density and flow were assessed using a handheld video microscope until 6 hours post-LPS.

Results LPS administration affected all macrocirculatory and microcirculatory parameters. The LPS-induced decrease in blood pressure and systemic vascular resistance (SVR) was refractory to low-dose norepinephrine and phenylephrine, and to a lesser extent, to vasopressin. Only vasopressin exerted effects on PCA parameters compared to placebo, by mitigating the LPS-induced decrease in diastolic blood pressure by stabilizing SVR and cardiac output. The endotoxemia-induced decreased indices of microvascular flow and density were not influenced by vasopressor therapy.

Conclusion In a highly controlled model of systemic inflammation in humans *in vivo*, a five-hour infusion of various vasopressors revealed distinctive effects on macrohemodynamic variables without affecting the sublingual microcirculation.

Introduction

During septic shock, both systemic hemodynamics and the microcirculation are severely affected, and these alterations are associated with organ failure and impaired outcome [1]. Norepinephrine is by far the most widely used vasopressor for septic shock. However, other vasopressors such as vasopressin or phenylephrine might hold an advantage when considering effects on both the microcirculation and systemic hemodynamics [2]. Comparing the effects of different vasopressors in septic shock patients is hampered by the high heterogeneity of the disease and the fact that current guidelines dictate the use of norepinephrine as the first line vasopressor, and only advise the use of other compounds as ‘add-on’ treatment in catecholamine-resistant shock [3]. Experimental human endotoxemia is a controlled, safe and reproducible model of systemic inflammation that mimics several of the microcirculatory and macrocirculatory changes observed in sepsis [4–6]. In the present study, we aimed to study the effects of three vasopressor agents, norepinephrine, phenylephrine and vasopressin, on both the microcirculation and systemic hemodynamics during experimental human endotoxemia.

Materials and methods

Subjects, study design, and ethics

We performed a randomized controlled experimental endotoxemia study in forty healthy male volunteers (18–35 years) at the intensive care department of a tertiary care university hospital in the Netherlands (Clinicaltrials.gov NCT02675868). All subjects provided written informed consent and the study was approved by the local ethics committee (registration no. 2015-2079). Experiments were carried out in accordance with the Declaration of Helsinki, including recent revisions, and Good Clinical Practice guidelines.

Experimental human endotoxemia procedures

All subjects received an intravenous bolus injection with 2 ng/kg lipopolysaccharide (*E. coli*-derived LPS), and were randomized to receive either a five-hour infusion of 0.05 µg/kg/min norepinephrine ($n = 10$), 0.5 µg/kg/min phenylephrine ($n = 10$), 0.04 IU/min vasopressin ($n = 10$) or placebo (NaCl 0.9 %, $n = 10$). Experimental procedures are detailed in our previous work [7]. Infusion was started one hour before LPS administration. Furthermore, the study subjects

received 1500 mL 2.5 % glucose/0.45 % saline during the hour prior to LPS administration, followed by 150 mL/h until six hours after LPS administration, and 75 mL/h for the remaining two hours. Both macro- and microcirculatory measurements were performed at baseline (T₁), 30 minutes after initiation of vasopressor administration but before LPS administration (T₂), 90 [macro-circulation] or 120 [microcirculation] minutes following LPS administration (T₃, the height of the inflammatory response, characterized by peak levels of pro-inflammatory cytokines and flu-like symptoms(5)), 210 minutes post-LPS administration (T₄, maximum hemodynamic effects(5), only macrocirculatory parameters were obtained at this timepoint), and 270 [macrocirculation] or 360 [microcirculation] minutes following LPS administration (T₅, after cessation of vasopressor infusion).

Macrocirculation measurements

All macrocirculation parameters were blood-pressured derived. The radial artery was cannulated using a 20-gauge arterial catheter (Angiocath, Becton Dickinson Pty Ltd, Franklin Lake, NJ, USA) which was connected to an arterial pressure monitoring set (Edwards. Lifesciences LLC, Irvine, California, USA). The arterial blood pressure (ABP) signal was recorded on a laptop computer and stored on a hard disk with a sample rate of 200 Hz by an A/D converter (NI USB-6211, National Instrument, Austin, TX, USA) for offline analysis. The ABP signal was analysed using custom-made MATLAB scripts (Matlab R2017b, The MathWorks Inc. Massachusetts, USA). Mean arterial blood pressure (MAP) was acquired by taking a fourth order Butterworth low-pass filter with a cut-off frequency of 0.02 Hz from the raw ABP signal. Heart rate (HR) was acquired by automatic detection of Rpeaks from the ECG-signal. The used pulse contour analysis (PCA) accounts for the dependence of arterial compliance on arterial pressure by scaling its cardiac output (CO) estimate to pulse pressure, with stroke volume (SV) equalling pulse pressure divided by the sum of systolic (SBP)- and diastolic blood pressure (DBP) as proposed by Liljestrand and Zander [8,9]. SV was subsequently multiplied by HR to calculate cardiac output (CO). Systemic vascular resistance (SVR) was approximated by dividing MAP by CO.

Microcirculation measurements

A minimum of five steady video clips of at least 10 seconds were obtained from the sublingual region using a video microscope (CytoCam-1DF, Braedius

Medical, Huizen, The Netherlands). Video microscopy was performed by a trained investigator (LvL) after removal of saliva while avoiding pressure artefacts. Video scoring was performed according to Massey et al. [10]. Vessel density was calculated as the number of vessels crossing arbitrary lines divided by the total length of these lines (i.e. Number of crossings). Quantification of flow (i.e. microvascular flow index (MFI) was categorized as 0: no flow, 1: intermittent flow, 2: sluggish flow, and 3: continuous flow, as described previously [4].

Statistical analysis

Statistical analysis was performed using GraphPad Prism version 5.0 (GraphPad Software, La Jolla, CA). Normality was assessed using Shapiro-Wilk tests. Effects of vasopressor agents before LPS administration were analysed using paired Student's *t*-tests on T1 and T2. LPS-induced changes over time were analysed using repeated measures one-way ANOVA on T2, T3, T4, and T5 in the placebo group only. Differences between vasopressor and placebo-treated subjects over time during endotoxemia were tested using repeated measures two-way ANOVA (interaction term) on T2, T3 and, for macrocirculatory parameters only, T4. A two-sided *p*-value of < 0.05 was considered statistically significant.

Results

Subjects and symptoms

There were no differences in baseline characteristics between the treatment groups, which are reported elsewhere [7]. All subjects developed typical flu-like symptoms, peaking at 90 minutes following LPS administration, which were completely subsided 7–8 hours after the LPS challenge.

Effects of vasopressors prior to LPS administration

Administration of norepinephrine and phenylephrine caused an immediate increase in blood pressure, but did not affect other macrocirculatory parameters prior to LPS administration (Figure 1). Vasopressin did not affect any of the macrocirculatory parameters, and none of the vasopressors affected microcirculatory parameters before LPS administration (Figure 2).

Figure 1

Macrocirculatory parameters before and after LPS administration.

A Mean arterial pressure

B Pulse Pressure

C Cardiac output (pulse contour analysis)

D Systemic vascular resistance (pulse contour analysis)

E Heart rate (pulse contour analysis)

F Stroke volume (pulse contour analysis)

G Cardiac output (pulse contour analysis)

H Systemic vascular resistance (pulse contour analysis)

LPS was administered at timepoint 0, indicated by the vertical dashed line.

Data are expressed as mean and SEM.

★: $p < 0.05$, ★★: $p < 0.01$ and ★★★: $p < 0.001$

(paired Student's *t*-tests on -90 [T₁] and -30 [T₂] within treatment groups).

●●●: $p < 0.01$ and ●●●●: $p < 0.001$ over time (-90 to 270 [T₁ to T₅]) within placebo group (repeated measures one-way ANOVA).

◆: $p < 0.05$ over time (-30 to 210 [T₂ to T₄]) vs. placebo

(repeated measures two-way ANOVA, time × treatment interaction term)

Effects of vasopressors during endotoxemia

Except for SV, LPS administration resulted in significant changes of all macrocirculatory parameters (Figure 1). All blood pressure variables decreased, accompanied by a compensatory increase in HR, increased CO (at constant SV) and decreased SVR. MAP kinetics in the norepinephrine and phenylephrine groups were not significantly different from placebo. Vasopressin mitigated the LPS-induced decrease in DBP by stabilizing SVR and CO. The static blood pressures did not correlate to their corresponding PCA parameters (SVR, CO and SV) in any of the groups (Pearson correlation p -value > 0.10). LPS administration resulted in decreased microvascular density and flow, which were not changed by any of the vasopressors (Figure 2).

Discussion

Our study demonstrates that the decrease in blood pressure and SVR during experimental endotoxemia is refractory to low-dose norepinephrine and phenylephrine therapy, and to a lesser extent, to vasopressin administered at a dosage used in clinical practice for the treatment of septic shock. Vasopressin prevented the endotoxin-induced increase in CO and decrease in SVR. Furthermore, endotoxemia resulted in decreased indices of sublingual microvascular flow, which were not affected by any of the vasopressors.

Expectedly, both norepinephrine and phenylephrine caused an increase in blood pressure prior to LPS administration. While these elevated levels were

Figure 2

Sublingual microcirculatory parameters before and after LPS administration.

A Number of crossings

B Microvascular Flow index

LPS was administered at timepoint 0, indicated by the vertical dashed line.

Data are expressed as mean and SEM.

⊙: $p < 0.05$ over time (-90 to 270 [T1 to T5]) within placebo group (repeated measures one-way ANOVA).

maintained during the peak of the inflammatory response, the LPS-induced decrease in blood pressure was not prevented. Vasopressin did not increase blood pressure prior to LPS administration. Unlike patients with sepsis, this can be explained by the fact that vasoconstrictive effects of vasopressin infusion are antagonized by intrinsic activation of the baroreflex in healthy volunteers under noninflammatory conditions [11]. PCA allowed us to break down blood pressure into flow and resistance. Complementary to our previous findings, we anew showed that experimental human endotoxemia results in a loss of vascular resistance of the arterial bed [7]. Interestingly, vasopressin mitigated the LPS-induced decrease in SVR, a hallmark of sepsis-induced hypotension [12].

Our study underscores that limiting hemodynamic monitoring in critically ill patients to solely blood pressure is insufficient, as it neglects the causative physiological processes (CO and SVR) and its ultimate goal (improving microvascular perfusion). The lack of coherence between blood pressure and these other parameters is a well-known phenomenon in sepsis [13,14]. Accordingly, in our model, there were no correlations between blood pressure and PCA parameters. Furthermore, despite clear effects on the macrocirculation both prior to (norepinephrine and phenylephrine) and after LPS administration (vasopressin), the different vasopressors did not influence sublingual microcirculatory parameters. In accordance with earlier work [4], the sublingual microcirculation was profoundly altered during endotoxemia but remained intact (indicated by high > 2 MFI-values). Previous work in a model of septic shock in pigs revealed that norepinephrine and phenylephrine improved macrocirculatory parameters (e.g. MAP and cardiac index) [2]. However, both pressors only marginally affected microcirculatory flow measured in seven organs: norepinephrine decreased microcirculatory blood flow in the jejunal mucosa, whereas phenylephrine increased microcirculatory jejunal muscularis flow [2]. As such, the sole measurement of blood pressure can be misleading, as it may suggest that vasopressor therapies or resuscitation manoeuvres are adequate, while perfusion at the tissue level is or remains markedly compromised [14].

Several study limitations deserve attention. First, knowing that the ideal model of sepsis does not exist, our model has proven to be highly controlled, reproducible and representative for several hallmarks of sepsis [5]. Nevertheless, since healthy subjects were studied, only low dosages of norepinephrine and phenylephrine could be safely administered. Higher dosages of these agents

may affect the microcirculation. Second, microcirculatory parameters were determined in the sublingual vascular bed. Although the sublingual area is the preferred site for noninvasive microcirculation measurements and this approach is widely accepted as a measure of the systemic microcirculation, we cannot exclude the possibility of heterogeneity between different tissues. Third, because PCA converts pressure measurements into volume parameters using assumptions of the dynamic characteristics of the arterial vasculature, uncalibrated PCA may not yield accurate results upon changes in SVR. Furthermore, PCA remains arduous for implementation in everyday clinical practice, partly because of the use of inscrutable algorithms. We advocate for the use of well-documented, open source, and straightforward formulas, as employed in the present work.

Conclusion

In conclusion, various vasopressors exert distinctive effects on macrohemodynamic variables without affecting the sublingual microcirculation in a highly standardized controlled model of systemic inflammation in humans *in vivo*. Furthermore, our data indicate that blood pressure measurements do not adequately reflect physiological parameters that are of vital importance in the critical care setting, such as CO, SVR, and microvascular perfusion. Uncalibrated PCA could be a helpful, less-invasive tool in monitoring hemodynamic responses to interventions and in disease.

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β -blockade attenuates renal blood flow in experimental endotoxic shock by reducing perfusion pressure

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Abstract

Background Clinical data suggests that heart rate (HR) control with selective β_1 -blockers may improve cardiac function during septic shock. However, it seems counterintuitive to start β -blocker infusion in a shock state when organ blood flow is already low or insufficient. Therefore, we studied the effects of HR control with esmolol—an ultrashort acting β_1 -selective adrenoceptor antagonist—on renal blood flow (RBF) and renal autoregulation during early septic shock.

Methods In 10 healthy sheep, sepsis was induced by continuous IV-administration of lipopolysaccharide, while maintained under anaesthesia and mechanically ventilated. After successful resuscitation of the septic shock with fluids and vasoactive drugs, esmolol was infused to reduce HR with 30 % and was stopped 30-min after reaching this target. Arterial and venous pressures, and RBF were recorded continuously. Renal autoregulation was evaluated by the response in RBF to renal perfusion pressure (RPP) in both the time domain and frequency domain.

Results During septic shock, β -blockade with esmolol significantly increased the pressure-dependency of RBF to renal perfusion pressure (RPP). Stopping esmolol showed the reversibility of the impaired renal autoregulation.

Conclusion Showing that clinical diligence and caution are necessary when treating septic shock with esmolol in the acute phase since esmolol reduced RPP to critical values thereby significantly reducing RBF.

Introduction

In critically ill patients, the mortality rate associated with acute kidney injury (AKI) remains high [1–4]. Sepsis and, in particular, septic shock is an important risk factor for developing AKI [3,4]. There is evidence in septic shock that reducing sympathetic outflow, or blocking the action of catecholamines by administering esmolol (an ultrashort acting β_1 -selective adrenoceptor antagonist), may improve survival [5]. This survival benefit is attributed to improved cardiac function with lower oxygen consumption [6]. However, it is not clear how organ perfusion, and the perfusion of the kidneys in particular, are affected by β -blocker infusion during septic shock. It seems counterintuitive to start β -blocker infusion in a shock state when organ blood flow is already low or insufficient as β -blockers are reported to reduce tissue perfusion pressure by increasing central venous pressure (CVP) [7] and/or by decreasing mean arterial pressure (MAP) [8–12]. Besides renal perfusion pressure (RPP), the influence of β -blockers on renal blood flow (RBF) is further complicated by the unknown effect on renal autoregulation in sepsis or septic shock. Renal autoregulation is mediated by vascular reactivity, unlinking RPP from RBF [13]. In health, renal autoregulation keeps blood flow at a constant level at perfusion pressures greater than approximately 60–100 mmHg, depending on species [14]. Impaired renal autoregulation will expose the kidney to rapid alterations in blood pressure, resulting in hypotensive or hypertensive injury [15].

Assessment and interpretation of renal autoregulation is not trivial [16,17]. Studies have addressed either static or dynamic autoregulation: static refers to RPP and RBF values under steady state conditions that are observed over a time scale of minutes to hours, while dynamic refers to transient RPP and RBF changes that are observed in a time scale of seconds [18]. Even though the mechanisms underlying static and dynamic renal autoregulation may overlap, lack of correlations between the two emphasizes the need to assess both [19].

In the present work, we evaluated the effects of esmolol administration on RBF and the static and dynamic renal autoregulation in an experimental animal model of acute septic shock.

Materials and methods

General

This experiment was performed after approval by the local ethics committee on animal research of the Radboud University Nijmegen Medical Center (RUNMC License number RU-DEC 2014-10) and in full compliance with Dutch and European legal requirements on the use and protection of laboratory animals. Ten conventionally reared female lambs (crossbred Texelaar–Flevolanders) were studied under general anesthesia. In the context of the principles of replacement, reduction and refinement for the use of animal models, no control group was considered necessary to answer our research questions.

The reported results are part of a larger experiment in which we studied the influence of esmolol in experimental endotoxic shock. For a detailed description on the anaesthesia, ventilation, surgical preparation we refer to the materials and methods section of our previous publication [7], in short:

Experimental model

Anesthesia and ventilation

Premedication consisted of midazolam and ketamine i.m., anesthesia was induced with IV administration of Propofol. After endotracheal intubation, general anaesthesia was maintained using inhalation of isoflurane, the continuous IV administration of sufentanil and rocuronium.

Surgical preparation

All lambs were positioned in dorsal position for inserting the intravascular catheters using surgical cut down procedures, into the right femoral artery and the left internal jugular vein. An ultrasound transit-time flow probe (4 mm) (PAX series, Transonic Systems, Ithaca, NY) was placed around the left renal artery after laparotomy for RBF measurement. An ultrasound transit-time perivascular flow probe (14 or 16 mm) (PAX series, Transonic Systems, Ithaca, NY) was placed around the main pulmonary artery to measure CO after left thoracotomy for assessing fluid responsiveness during the resuscitation.

Resuscitation of endotoxic shock

After instrumentation and closing all incisions a stabilization period of 30 min was followed by continuous IV administration of lipopolysaccharide (3 µg/kg/hr) (LPS, US Standard Reference Endotoxin *Escherichia coli* O:113) after a loading dose of 3 µg/kg in order to create a state of endotoxic shock. Only

after LPS had induced a 50 % reduction in cardiac output or a 25 % reduction in ABP, resuscitation was started according to standard clinical protocol [20]. Resuscitation maneuvers consisted of fluid therapy guided by continuous CO measurement and nor-epinephrine. Dobutamine was administered in case of fluid refractory shock. Dosages and timing are detailed in our previous work [7].

Experimental protocol

30 min after creating a situation of resuscitated endotoxic shock with blood pressure and CO equal to baseline, esmolol (Baxter, Maurepas, France) was administrated to reduce of the HR by 30 %. This targeted HR reduction was based on Morelli et al. who reported a similar level of HR reduction by esmolol in patient with septic shock (i.e. from 115 to 85 bpm) [5]. Except for dobutamine, resuscitation maneuvers were maintained or increased in order to maintain ABP and cardiac output at baseline values as far as possible. 30 min after reaching the targeted 30 % reduction in HR, the esmolol infusion was stopped.

Four phases in our experiment were studied; T₀; Baseline (30 min after instrumentation and prior to LPS infusion), T₁; Resuscitation (30 min after successfully restoring MAP and cardiac output to baseline values and prior to esmolol infusion), T₂; Esmolol (30 min after esmolol infusion), T₃; Stop esmolol (30 min after stopping esmolol and prior to euthanasia).

Data recording

Arterial blood pressure (ABP) and CVP were recorded using a 18 G × 10 cm single-lumen catheter (Leaderflex, Vygon India Pvt Ltd) in the right femoral artery and a 7.5 F × 20 cm 3-lumen central venous catheter which was placed in the left internal jugular vein (Multicath, Vygon India Pvt Ltd) respectively. RPP was calculated by subtracting CVP from ABP.

Hemodynamic pressures and flow signals were continuously recorded on a laptop computer and stored on a hard disk with a sample rate of 200 Hz by an A/D converter (NI USB-6211, National Instrument, Austin, TX, USA). Custom-written MATLAB scripts (Matlab R2017b, The MathWorks Inc. Massachusetts, USA) were used to generated mean arterial pressure, mean central venous pressure and mean RBF by low-pass filtering of the recorded signals (cut-off frequency of 0.5 Hz [21], third order Butterworth filter applied in the forward and reverse direction for a zero-phase response). Renal vascular resistance (RVR) was calculated according to Ohm's law, i.e. RPP divided by RBF.

Autoregulation

Static – Time domain

Renal autoregulation in the time domain was analysed by using a moving correlation between slow changes of flow and pressure in the kidney, called the renovascular reactivity index (RVx) [22]. This assessment was performed per study phase by resampling the ABP and RBF waveforms as non-overlapping 10 seconds mean values. The RVx was calculated by performing a continuous moving Pearson correlation between RPP and RBF. The consecutive paired 10-s averaged RPP and RBF values from 300-s analysis periods generated 30 data points for inclusion in each Pearson coefficient used to determine the indices. Positive values of RVx indicate that RBF passively depends on RPP (i.e. impaired autoregulation), and negative values indicate autoregulatory reactivity [22].

Static – Autoregulation curves

Renal autoregulation curves were constructed by plotting RPP versus the simultaneously measured RBF, and RPP versus simultaneously calculated RVx. RPP and RBF measurements were first normalized to a percentage of their baseline, which was determined as their mean over a 5 min period prior to LPS infusion. 20 seconds mean samples of these normalized RBF recordings and RVx values were plotted against their corresponding RPP value. To estimate the lower limit of autoregulation we used the method developed by Turkstra et al. [23] In short, the data of the autoregulation curves were subjected to non-linear regression analysis using a sigmoid function. The lower limit of autoregulation was calculated from the RPP versus RBF curve and was then defined as the perfusion pressure, where the third derivative of the fitted curve was 0, which mathematically defines the shoulder in a sigmoidal curve.

Dynamic – Frequency domain

Transfer function analysis (TFA) was used to assess renal autoregulation in the frequency domain by studying oscillations from the unfiltered pressure to flow signal. These oscillations were visualized in a power spectrum after the time series has been mathematically translated into the frequency domain. The transfer function $H(f)$ between the two signals was calculated according to equation 1:

$$H(f) = \frac{S_{xy}(f)}{S_{xx}(f)} \quad 1.$$

where $S_{xx}(f)$ is the autospectrum of changes in RPP and $S_{xy}(f)$ is the cross-spectrum between the input signal (i.e. RPP) and output signal (i.e. RBF). Using this transfer function (equation 1), the effect of renal autoregulation can be analyzed at a specific frequency of interest [24], i.e.:

1. Tubuloglomerular feedback (TGF), the low-frequency (LF) band [0.02–0.05 Hz].
2. Myogenic response (MR), the high-frequency (HF) band [0.1–0.3 Hz].
3. Baroreflex component (BRC), the very-high-frequency (VHF) band [0.35–0.7 Hz].

For each of these frequency bands of interest; the gain, phase and coherence were calculated. The transfer function gain and phase were derived from the real part of $H(f)$ and the imaginary part of $H(f)$ respectively. The gain quantifies to which extent a change in RBF is caused by a change in RPP. A low transfer gain value implies that oscillations in RPP do not translate into flow fluctuations of similar frequency, i.e. the kidney is effectively autoregulating in the given frequency band [13]. The phase indicates the latency between the RBF and RPP signal. Last, the coherence is a measure of linearity between two signals in a specific frequency range and was acquired by calculating the squared coherence according to equation 2:

$$MSC(f) = \frac{S_{xy}(f)}{\sqrt{S_{xx}(f) \times S_{yy}(f)}} \quad 2.$$

Coherence approaching 1 suggest a linear relationship, while coherence approaching zero suggest no relationship between the signals, severe extraneous noise, or a non-linear relationship. To ensure reliable TFA outcomes, a cut-off value of 0.5 for coherence was used [25].

Statistical analysis

Prism Statistical Software was used for statistical analysis (Graph-Pad Prism 5, GraphPad Software Inc., San Diego, CA, USA). Normality was assessed using Shapiro-Wilk tests. RPP and RBF values were normalized to baseline. Repeated measures one-way analysis of variance (ANOVA) and the Bonferroni test were used for multiple post hoc comparisons of the different time points. Differences in relationship between RPP and RBF or RV_x were tested using repeated measures two-way ANOVA (interaction term) between baseline and

Table 1

Macrohemodynamic parameters and renal autoregulatory parameters per study phase.

Variable	Baseline (To) (n = 8)	Resuscitation (T1) (n = 8)	Esmolol (T2) (n = 8)	Stop (T3) (n = 8)	To vs T1 ^a	T1 vs T2 ^a	T2 vs T3 ^a	To-T3 ^b
Macrocirculation								
RPP (mmHg)	50 ± 7	47 ± 11	29 ± 10	40 ± 10	ns	***	**	***
RBF (ml/min)	137 ± 96	132 ± 96	81 ± 76	114 ± 79	ns	**	ns	***
RVR (mmHg/L/min)	51 ± 10	52 ± 11	46 ± 12	57 ± 11	ns	ns	ns	ns
HR (bpm)	124 ± 23	158 ± 12	100 ± 8	132 ± 11	***	***	***	***
Urea (mmol/L)	7.1 ± 1.3	7.5 ± 1.5	8.2 ± 2.3	7.5 ± 1.2	ns	ns	ns	ns
Autoregulation								
RV _x (unit)	0.21 ± 0.3	0.56 ± 0.5	0.36 ± 0.5	0.7 ± 0.4	*	ns	*	*
Gain – LF (ml/s/mmHg)	0.9 ± 1.4	1.5 ± 1.9	2.3 ± 2.7	2.2 ± 2.1	ns	ns	ns	ns
Gain – HF (ml/s/mmHg)	1.1 ± 1.6	1.5 ± 1.9	2.2 ± 2.8	2.1 ± 2	ns	ns	ns	ns
Gain – VHF (ml/s/mmHg)	2.9 ± 3.1	2.6 ± 2	3.2 ± 3.1	4.1 ± 1.9	ns	ns	ns	ns
Phase – LF (radians)	0.1 ± 0	0.1 ± 0	0.1 ± 0	0.1 ± 0	ns	ns	ns	ns
Phase – HF (radians)	0.3 ± 0	0.3 ± 0	0.3 ± 0.2	0.3 ± 0.1	ns	ns	ns	ns
Phase – VHF (radians)	8 ± 0.6	7.6 ± 0.6	8 ± 0.6	7.8 ± 0.6	ns	ns	ns	ns
Coherence – LF (unit)	0.8 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	ns	ns	ns	**
Coherence – HF (unit)	0.8 ± 0.2	0.8 ± 0.2	0.9 ± 0.1	0.9 ± 0.1	ns	ns	ns	*
Coherence – VHF (unit)	0.8 ± 0.1	0.8 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	ns	ns	ns	ns

Data are expressed as mean ± SD.

Renal perfusion pressure (RPP), Renal blood flow (RBF), renal vascular resistance (RVR), heart rate (HR) Renovascular reactivity index (RV_x), Low frequency (LF), High frequency (HF), and Very high frequency (VHF).ns: not significant, *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.^a Significant changes between experimental phase (Paired Student's *t*-tests),^b Significant changes over time (To-T3) within parameter (Repeated measures one-way ANOVA).

the other experimental phases (To versus T1, To versus T2, and To versus T3) and between esmolol and the previous and prior state (T1 versus T2 and T2 versus T3). A two-sided *p*-value of < 0.05 was considered statistically significant.

Results

General

A total of ten ewe lambs (age 6-8 months, mean weight of 20.9 kg [range: 13-24.5 kg], mean body surface area of 0.94 m² [range: 0.67-1.0 m²] were studied; two lambs were excluded because of insufficient quality of RBF recordings. All animals included in the study were considered healthy on physical examination when entering the animals' laboratory. The resuscitation maneuvers in combination with the continuous LPS infusion resulted in endotoxic shock symptoms, with increased CO, tachycardia and reduced MAP (Figures 1, 2, and 3A). Concurrent esmolol infusion induced on average a HR reduction of 37 % [range: 31-41 %] (Figures 1, 2, and 3A).

Pressure, flow, and resistance

The median RPP at baseline was 48 mmHg [range: 20-55 mmHg]. Resuscitation manoeuvres were able to maintain RPP after LPS infusion, while the esmolol infusion decreased RPP (T2)(Table 1). The RPP recovered after discontinuing the esmolol infusion. RBF followed the same pattern as RPP over the course of the experiment: Resuscitation was able to maintain RBF (T1), esmolol reduced RBF (T2), and RBF recovered after stopping the esmolol infusion (T3) (Figure 1).

Figure 1

Example of time series renal perfusion pressure (RPP) and renal blood flow (RBF) from lamb 4. Study phases are indicated on top, dashed line marks the start of LPS infusion.

In contrast, renal vascular resistance (RVR) was not significantly altered over the course of the experiment (Figure 3B and Table 1).

Autoregulation

Static – Time domain

The RV_x increased gradually over the course of the experiment (Figure 4A and Table 1), and showed a significant increase from baseline (0.21 ± 0.36) to resuscitation (0.56 ± 0.26). Showing an increased linear relationship between RPP and RBF during endotoxemia compared to baseline.

Static – Autoregulation curves

Per study phase, both the static autoregulation curves (RPP versus RBF) with their lower limit of autoregulation (Figure 5A and Table 1) and the dynamic autoregulation curves (RPP versus RV_x) are shown for comparison (Figure 5B and Table 1). During all four studied phases, RPP had a significant influence on both RBF and RV_x . Furthermore, this relationship between RPP and RBF was significantly different during each phase (Figure 5A). During the baseline period both RPP and RBF recovered from the surgical instrumentation. A further increase in RPP (above 70 % of its baseline) did not cause a continued rise in RBF, except during esmolol infusion (T2) (Figure 5A). This resulted in the disappearance of the physiological ‘plateau’ as this plateau reappeared after

Figure 2
Example of power spectrum of the RPP and RBF signal from lamb 4 during baseline, with renal autoregulatory operating ranges.

TGF: tubuloglomerular feedback, MR: myogenic response, and BRC: baroreceptor component.

cessation of esmolol infusion, showing the reversibility of these effects. This is further emphasized by the lower limits of autoregulation.

Under baseline conditions, the lower limit was at 30 % of RPP. During infusion of esmolol, the lower limit increased to 120 % and returned to 50 % when esmolol infusion was stopped (Figure 5A). RV_x showed large standard deviation when plotted against simultaneously recorded RPP values (Figure 5B). Fitting sigmoid curves to these data resulted in R^2 values as low as 0.2 (therefore not shown). Nevertheless, mean RV_x increased with reductions in RBF, indicating pressure passivity.

Dynamic – Frequency domain

An example of a power spectra of the RPP and RBF signal during baseline, with the ranges of the different autoregulatory mechanisms is shown (Figure 2). Periodic events can be distinguished in both signals, notably heart rate (around 2 Hz) and respiration rate (around 0.4 Hz), and its harmonics appear as individual peaks in the power spectrum.

Figure 3

- A** Central venous pressure (CVP), mean arterial pressure (MAP), cardiac output (CO) and heart rate (HR) per study phase.
B Renal perfusion pressure (RPP), renal blood flow (RBF), and renal vascular resistance (RVR) per study phase.

Data are expressed as mean $\pm$ SEM.

Repeated measures ANOVA was performed for all parameters. Paired Student's *t*-test was used to perform pairwise comparisons between phases. $\star$: $p < 0.05$, $\star\star$: $p < 0.01$, and $\star\star\star$: $p < 0.001$.

Using these power spectra for TFA showed that the gain and phase were not significantly influenced throughout the experiment in any of the autoregulatory frequency bands (Figure 4B and 4C). Similar to RV_x , coherence—also as a measure for linearity—showed an increase over the course of the experiment for both the MR and TGF mechanism (Figure 4D).

Figure 4

Renal autoregulation analysed in the time domain, Renovascular reactivity index (RV_x) **A** and using transfer function analysis in the frequency domain; **B** Gain, **C** Phase, and **D** Coherence.

Low-frequency (LF) - tubuloglomerular feedback (TGF); High-frequency (HF) - myogenic response (MR); Very-high-frequency (VHF) - Baroreflex component (BRC).

Repeated measures ANOVA was performed for each value and each frequency band, $\odot$: $p < 0.05$ and $\odot\odot$: $p < 0.01$

Paired Student's t-test was used to perform pairwise comparisons between phases. $\star$: $p < 0.05$

Discussion

Main findings

In the present experimental animal model, we found that RBF was preserved during resuscitated septic shock, but was strongly reduced as a result of esmolol infusion. This reduction was reversible and could be contributed to a reduction in RPP.

Renal blood flow

RBF in sepsis has long been a subject of debate given that approximately two-thirds of experimental studies show a decreased RBF while in one-third [26] the RBF was unchanged or even increased. These conflicting results appear to be affected by factors other than the induction of sepsis itself, including the consciousness of the animal, the recovery time after surgery, the hemodynamic pattern and the (non-)use of resuscitation manoeuvres [26]. We observed both phenomena; an initial decrease in RBF after LPS infusion and a

Figure 5

Renal autoregulation plots.

A Renal blood flow (RBF) values are shown versus the simultaneous renal perfusion pressure (RPP).

B Renovascular reactivity index (RVx) values versus the simultaneous RPP (normalized to baseline).

Data are expressed as mean and SEM.

★: $p < 0.05$, ★★: $p < 0.01$, ★★★: $p < 0.001$ influence of RPP on RBF or RVx (repeated measures one-way ANOVA).

⊙⊙: $p < 0.01$ and ⊙⊙⊙: $p < 0.001$ influence of RPP on RBF or RVx vs. baseline (repeated measures two-way ANOVA, time × treatment interaction term).

⊙⊙⊙: $p < 0.001$ influence of RPP on RBF during Esmolol vs. Resuscitation (repeated measures two-way ANOVA, time × treatment interaction term).

⊙⊙⊙: $p < 0.001$ influence of RPP on RBF during Esmolol vs. Stop (repeated measures two-way ANOVA, time × treatment interaction term).

full recovery by the resuscitation manoeuvres (i.e. fluid and vasopressor therapy). Subsequently, esmolol infusion had a significant negative effect on RBF (accompanied with a trend towards RVR reduction), which was eliminated by stopping the esmolol infusion. This suggests that the reduced RBF was caused by the effects of esmolol itself and not by progressive endotoxemia. These acute negative effects of β -blockers have been confirmed by others in both septic [9] and non-septic setting [27].

Renal autoregulation

The large fluctuations in RBF within the experimental phases are clinically undesirable, but allowed us to study renal autoregulation by constructing autoregulation curves per phase. These curves revealed a pronounced protective mechanisms of renal autoregulation at (transient) excessive RPP values [22,28,29]. Protecting the kidney from high blood pressures is indeed the primary function of renal autoregulation in physiological circumstances [30]. The direct effect of esmolol on renal autoregulation is unknown.

Theoretically, esmolol (a selective β_1 -blockers) does not act directly on vascular function since β_1 -receptors are not expressed in the renal vessels. However, some data suggest that β -blockers might positively influence vasoactive mechanisms in acute endotoxemia [31]. A protective renal mechanism from hypertension during β -blocker usage seems however irrelevant. We showed that the initial (T₀ to T₁) increased linearity between RBF and RPP was sustained during esmolol infusion. However, the unaltered dynamic autoregulation parameters indicate that this increased pressure-dependency-impaired autoregulation—can be attributed to a reduced RPP. The observation that acute septic shock did not damage the triple-layered system (i.e. TGF, MR and BRC) is consistent with literature [35]. Apart from the septic shock and esmolol infusion, this transition in blood flow from autoregulated to pressure-dependent would always be seen at those low RPP values (in our experiment below 25 mmHg). Here, the renal autoregulation is operating at the extremes of the physiological autoregulation curve, below the lower limit of renal autoregulation [23].

Both the autoregulation curves and time domain related RV_x values refer to the relationship between average RPP and average RBF under steady-state conditions, ‘dynamic’ autoregulation parameters (i.e. gain, coherence and phase) are of additional values by discriminating the different components of

renal autoregulation (i.e. TGF, MR and BRC). In our experiment, the coherence increased over time and was above 0.5 in all three bands after LPS infusion. The low spontaneous variability in blood pressure during baseline measurements could explain the low coherence between RPP and RBF in the low frequency range. This low coherence increases the chance of bias when interpreting the renal autoregulation during this phase [33]. However, we showed relatively high coherence values in the low frequency band during endotoxic shock (T1 to T3). Admittedly less, but still expressing linearity of the somewhat non-linear behaving TGF mechanism [34].

Reduced renal blood flow

We attributed the impaired autoregulation during esmolol infusion to a reduced RPP rather than to damaged autoregulation mechanisms. These perfusion deficits indeed play an important role in the development of renal dysfunction in sepsis [36]. Herein, this deficit is largely explained by a markedly increased CVP, resulting in a significantly reduced RPP and RBF (Pearson correlation p -value < 0.05). This venous congestion disappeared rapidly after stopping esmolol infusion (also shown in the related paper [7]). While it is unknown to which extent the driving pressure and backpressure are actually experienced by the kidney, it is undisputed that an increased CVP is associated with impaired renal function and independently related to all-cause mortality in a broad spectrum of patients with cardiovascular disease [37].

The renal autoregulatory mechanisms might be better in maintaining RBF with a drop in ABP compared to an increase in CVP. This concept of increased CVP, being transmitted to the renal veins and kidneys leading to renal dysfunction is supported by a substantial amount of literature as early as in the 1930s [38]. A retrospective study of septic patients in the ICU showed associations between a higher CVP and acute kidney injury, but not between MAP or cardiac output and acute kidney injury [39].

Clinical implications

In the acute septic setting, autoregulation is preserved but impaired by esmolol. However, clinical diligence and caution are necessary when treating septic shock with esmolol in the acute phase since esmolol reduced RPP to critical values thereby significantly reducing RBF. In addition, we showed the importance of CVP in regulating RBF. Although controversial and in contrast to others [40], we showed that assessment of CVP can provide clinical information

when guiding intravenous fluids administration considering perfusion of the kidneys.

In the light of our results, future research should focus on studying the impact of the backpressure on RBF and improving non-invasive techniques for assessing RBF during the treatment of sepsis patients. Contrast-enhanced ultrasound could be such a safe and non-invasive imaging technique for assessment of tissue blood flow, although its accuracy has been questioned in recent experiments [41]. Furthermore, with RBF determining oxygen delivery and sodium absorption being the main contributor to oxygen consumption, studying the effect both may help in preventing esmolol induced renal hypoxia.

Limitations

Knowing that the ideal model of sepsis does not exist, our model has proven to be highly controlled, reproducible and representative for several hallmarks of sepsis [42].

In order to monitor urine production by urethral catheterization we were limited to using only female animals. Note, the relatively young lambs had considerable lower perfusion pressure compared to their adult counterparts in other literature [43]. Although clinically irrelevant, the short period between LPS infusion and the start of the resuscitation manoeuvres did not allow for autoregulation analysis of that period. Furthermore, we only assessed the acute septic setting. Burban et al. confirmed the preservation of the renal autoregulation within the first hours of sepsis [35]. Indeed, several experimental data have shown that renal hemodynamics may be different at the initiation, maintenance, and recovery phases of septic AKI [44,45]. The use of the miniature probes to measure RBF require some special consideration. Special care was taken to avoid positions that may have temporarily obstructed flow. Optimal positioning of the probe is not trivial and is critical to determine the maximal flow through the renal artery [46]. We did not use biomarkers from blood samples to assess kidney function, and failed to quantify urine production and glomerular filtration rate (GFR). The relative low urine production made it impossible to correlate diuresis to one of the studied phases. GFR would have helped to separate the effect of afferent and efferent arterioles on RBF.

All renal autoregulation assessment methods have their limitation; First, both RVR and RV_x were measured in the time domain, making them relatively

blunt metrics that treat the separate autoregulatory elements in the kidney as a single unit, detecting overall pressure passivity, but no failure of an individual component of reactivity and they do not take the direction of change into account [22]. Second, the FTA assumes linearity between the input and output signal, but the renal autoregulatory system usually displays some degree of non-linear behaviour [34]. FTA assumes the properties of the system are stationary or have constant mean and variance over time; however, this assumption may not always be valid, particularly when prolonged time series are used in hemodynamically unstable subjects [47]. Finally, the averaged autoregulation curves presented in the Figure 5 underestimate the power of autoregulation of each individual animal, as not all lower limits of autoregulation are exactly similar [23]. Individual curves however bared a too small range in RPP values to calculate these limits per study phase. Despites the limitations of these separate renal autoregulation assessment methods, together they provide a comprehensive view of the kidney's capabilities of managing RBF. Therefore, depending on specific research purposes, the choice and interpretation for renal autoregulation assessment should be weighed carefully.

Conclusion

Using an acute endotoxic septic shock sheep model we showed that renal autoregulation remains unaffected in situations of resuscitated septic shock, but concurrent esmolol infusion significantly increased the pressure-dependency of RBF.

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8

General discussion and future perspectives

Circulatory shock is defined by inadequate tissue blood flow and if untreated, proceeds from organ dysfunction to organ failure and eventually death. Early recognition of patients in (septic) shock with circulatory dysfunction and the use of adequate interventions focused on organ perfusion have a profound beneficial effect on their outcomes [1]. The studies performed in this thesis therefore evaluated monitoring-guided therapies to stabilize—or where possible—improve organ blood flow in patients with (septic) shock.

Blood flow

The flow of blood is the result of a relationship between a pressure difference and a resistance, where the pressure is generated by blood volume and (dynamic) vascular compliance. The flow of blood is in accordance with Ohm's law, which states that a current equals the voltage difference divided by a resistance. In relating Ohm's law to the flow of fluid, the voltage difference is the pressure difference (sometimes called the driving pressure, perfusion pressure or pressure gradient), the resistance is determined by the spatiotemporal resistance of blood and vessels and the current is the flow of blood. This vital hemodynamic relationship can be summarized by equation 1:

$$Flow = \frac{\text{Delta pressure}}{\text{Resistance}} \quad 1.$$

Equation 1 assumes a well-defined upstream and downstream checkpoint, of which the arterial and venous blood pressure seems most logical to use. However, while a low pressure difference between these two points equals insufficient flow at the organ level, a high pressure difference does not automatically guarantee sufficient blood flow [2]. It is the cumulative resistance of many vascular segments that determines the profile of pressure fall between the upstream arterial and downstream venous ends of the circulation. Not only can the resistance of each segment not easily be measured, the resistances are also subject to external influences and some to autoregulation. Thus, we do not know which uniform pressure should be considered the upstream and which one the downstream pressure for perfusion of organs. In this thesis, we investigated the driving pressure of organ blood flow in both healthy subjects and in experimental models with and without septic shock while different therapies were introduced. First, the three research questions—as stated in the introduction of this thesis—will be discussed separately, with focus on the

physiological and clinical implications of our findings. Last, future perspectives will be presented.

What is the clinical value of the mean systemic/circulatory filling pressure in patients with circulatory shock?

The goal of hemodynamic resuscitation is to restore organ blood flow. In healthy individuals, organ blood flow remains constant over a large range of blood pressures by adjusting vascular resistance. This is achieved by autoregulatory mechanism which can be different per organ. Through autoregulation blood is directed to where it is most needed; the heart, kidneys and brain. The 'optimal' blood pressure target may therewith differ from organ to organ and is further complicated by the patient's medical condition [3].

As fundamental as this issue is, there is no clear, high-level evidence for to determine the most effective blood pressure during resuscitation. Much effort is still being put into finding a generic optimal blood pressure target for both the arterial and venous side. Large RCTS targeting a mean arterial pressure of 80 to 85 mmHg, as compared with 65 to 70 mmHg, in a group of patients with septic shock did not result in significant differences in mortality [4–7]. Furthermore, the effects of an increase in mean arterial pressure on the microcirculation are variable [8]. This underlines the need for an individualized assessment where arterial blood pressure should be titrated based on the measures of organ function and tissue perfusion instead of one, non-patient specific, target [9]. The downstream pressure for blood flow, central venous pressure, is despite its limitations still the most frequently used variable to guide fluid resuscitation in critically ill patients [10]. However, the central venous pressure is a very poor predictor of the hemodynamic response to a fluid challenge [11]. An increase in central venous pressure does not reflect changes in cardiac output, nor of organ perfusion. Furthermore, it is not cardiac output that should be targeted with resuscitation maneuvers, but organ blood flow and ultimately organ function. On the other hand, elevated venous pressure does results in a reduced organ blood flow by increasing the interstitial pressure of encapsulated organs such as the kidney and liver [12]. As long as the arterial blood pressure is within autoregulatory range, the venous pressure becomes the major determinant of organ blood flow. Hence, if anything, it may be wise to refrain from administering fluids when the central venous pressure is markedly elevated. This pressure could be used as a stopping rule,

not a target of fluid resuscitation [13]. In **chapter 5** and **7** we indeed showed the importance of the central venous pressure in regulating organ (i.e. renal) blood flow.

However still, finding a more meaningful upstream pressure instead of the arterial pressure makes sense. Arthur Guyton realized this some 70 years ago when he introduced his model of venous return [14]. He also postulated the central venous pressure as the downstream pressure and introduced a new upstream pressure, the mean circulatory filling pressure. Guyton reasoned however that even though the pressures throughout the circulatory system under dynamic conditions are widely variant, there is a mean integrated pressure in the circulatory system all of the time [15]. In this thesis however, we question the clinical relevance of this virtual pressure (**chapter 2** and **3**).

In particular, the link between a beating-heart situation to a presumably static state following cardiac arrest is doubtful. Obtaining this upstream pressure after cardiac arrest, like Guyton did, feels like trying to determine the fuel consumption of parked car with the engine turned off (**chapter 2**). The dynamic course in blood pressure found by us after the induction of ventricular fibrillation would not be invalidated by Guyton. Moreover, Guyton pointed out that intense vasoconstriction begins throughout the circulatory system shortly after the heart stops, with significant impact on the mean circulatory filling pressure. Consequently, in his reasoning, in order to estimate the mean circulatory filling pressure accurately as it exists at any given moment, it is essential that measurement of this value be made within a few seconds after the heart stops beating. In order to forestall the ischemic induced vasoconstriction after cardiac arrest, he proposed to rapidly establish an equilibrium of the circulatory pressures by artificial means. Therefore, Guyton pumped the blood through an arterial to venous shunt while in cardiac arrest. It is doubtful that this postmortem and artificially obtained value will provide useful clinical information. In **chapter 3** we studied an (indirect) bedside method to assess the upstream pressure of venous return. We showed that the absolute values obtained with this alternative method—i.e. constructing venous returns curves using inspiratory hold maneuvers—were unsuitable for guiding clinical therapy. This does not mean that applying perturbations to a closed-loop physiological system for system identification of the heart-lung interactions is useless. We do see a future application when the characteristics of this

flow-pressure relationship, acquired imposing different ventilatory pressures, are seen as dynamic indices rather than absolute values per se.

Last, when teaching cardiovascular physiology, it seems sensible to create awareness among students—like Guyton did—that the upstream pressure for blood returning to the heart is far from equal to the arterial pressure. To suggest that a two-pressure, one-resistance model has some correspondence to the actual dynamics of cardiac vascular system seems a gross oversimplification [16]. We emphasized in **chapter 2** not to sacrifice accuracy and clinical applicability in the interest of accessibility for teaching cardiovascular physiology. This is why, we propose (Proposition 4 of this thesis) to use a disclaimer when using the mean circulatory filling pressure as a single entity driving pressure of venous return in physiological teaching. Disclaiming that there is no single, stable, uniform integrated blood pressure, because of the different (auto)regulatory capacities of individual organs (Figure 1).

What are the cardiovascular physiological consequences of β -blocker therapy in septic shock?

The administration of β -blockers in patients with sepsis is a trending topic in intensive care medicine since the landmark study by Morelli and colleagues, showing a striking decrease in 28-day mortality compared to standard care [17].

Figure 1
Possible better matching model in teaching venous return

In **chapter 4** we took both preclinical and clinical data into consideration regarding the cardiovascular physiological consequences of β -blocker therapy in septic shock. The available evidence suggests that the use of β -blockers in septic shock is safe, the effects on hemodynamics remain however controversial. We showed that it makes sense to reduce myocardial oxygen consumption when demand is not matched by supply. However, excessive β -blockade with negative inotropic effects may decrease cardiac output below the threshold that is needed to maintain organ and tissue perfusion. In order to apply β -blockers safely while maximizing its beneficial effect we advised to individualize the protocol by paying attention to; moment of administration, type of β -blocker, and resuscitation targets [18]. Just like any pharmacologic intervention, onset time has a major impact in determining its effect on the inflammatory response. There is not enough evidence to propose the use of a specific agent in each critical condition. Here too, titration to microcirculatory parameters seems advisable.

Further clarifying the physiological mechanisms of β -blockers during sepsis may help in pinpointing which patient may benefit. In **chapter 5** we studied the effect of esmolol, a selective β -blocker, on right ventricular function in early experimental endotoxic shock. Herein we showed that early administration of esmolol resulted in, among others, a decreased right ventricular function. This illustrates that clinical diligence and caution are necessary when treating septic shock with esmolol in the acute phase, but also emphasizes the significance of adequate right ventricular function assessment.

The importance of the right ventricle as a predictor of prognosis across a range of hemodynamic conditions has become more accepted [19]. The role of the ‘right side of the heart’ is more than just accepting the blood from the systemic circulation. Its primary role is to sustain an effective cardiac output by optimizing systemic venous return. The right ventricle accomplishes this by keeping right atrial pressure—the downstream pressure for venous return—as low as possible. An increase in right atrial pressure, is therefore an unfavorable sign.

The contribution of the right ventricle to overall hemodynamics is best studied when it is not functioning well [20]. Right ventricular failure cannot be reversed easily and is associated with decreased exercise tolerance, poor lung function, decreased cardiac output, and organ damage [21]. The assessment of the right ventricle remains challenging due to technical difficulties and its

complex geometry and function. Although we used a complex technique with intravascular catheters, less invasive methods like echocardiography are available for quantitative measurements [19]. However, even novel approaches such as 3D echocardiography should be used in conjunction with more established quantitative measures to ensure an accurate assessment of the right ventricle.

The function of the right ventricle is eminently sensitive to external influences. Several, otherwise supportive therapies, including mechanical ventilation and fluid management, can make right ventricular dysfunction even worse [22]. The most common and dominant effects of mechanical ventilation on the right ventricle are reduction in venous return and a raise in afterload. The interaction between the lungs and the right ventricle appears to be a key factor in the ventilation strategy. When assessing its function, the results can therefore only be interpreted while knowing the ventilatory impact and the coupling with the pulmonary arteries.

In **chapter 4** and **5** we showed that β -blockers could play a positive role in reducing right ventricular afterload. Thereby allowing the heart to operate more efficiently. Offering a potential physiological rationale for using β -blockers during septic shock [17]. Increased cardiac efficiency is defined by an improved ratio between ventricular to arterial elastance, i.e. the concept of ventricular–arterial coupling.

Improving cardiac efficiency by using β -blockers may work in selected patients with septic shock [23]. In the presence of an adequate preload, heart rate reduction with a β -blocker could improve arterial elastance, thereby contributing to an improved cardiovascular efficiency while allowing adequate systemic perfusion in septic shock. The lack of direct measurements, resulting in numerous approximations and omissions, raises doubts about the relevance of these results [24]. Our own intraventricular data support the concept of improved ventricular–arterial coupling in experimental endotoxic shock after β -blockade [25]. Still, simultaneously recorded volume and pressure data are highly desired. This would allow for direct assessment of end-systolic ventricular elastance in order to quantify the coupling between ventricle and artery. In conclusion, the available evidence suggests that the use of β -blockers in septic shock is safe, but the effects on hemodynamics remain controversial. Additional quantitative physiological patient data would be helpful in this respect.

When does optimization of the macrocirculation exerts a beneficial effect on microvascular perfusion in septic shock?

Extensive literature exists on the (un)coupling between the macro- and micro-circulation in times of disease [26–29]. The microvascular alterations frequently occur in critically ill patients, and especially during septic shock. The inflammatory response induces numerous pathophysiological changes leading to microcirculatory alterations which may result in organ failure and are associated with outcome [30]. Mechanisms of microcirculatory dysfunction in sepsis shock include arteriolar hypo-responsiveness and degradation of the glycocalyx with endothelial dysfunction, leading to extravasation of fluid protein and neutrophils [31,32].

Under normal conditions, the relation between perfusion pressure and tissue blood flow is minimal, due to intact autoregulatory capacities of individual organs. Confusingly, the pressure-dependency of organ blood flow would also be minimal during resuscitated septic shock. In these (septic) shock states, the relationship between pressure and flow however is disrupted such that organ perfusion may be abnormal despite the recovery of macrocirculatory parameters. This also explains why optimization of arterial blood pressure does not necessarily results in improved organ function during sepsis [33,34].

In **chapter 6** and **7** we evaluated the effects of several drugs (i.e. different vasopressors and a β -blocker) on both the macro- and microcirculatory parameters in sepsis and septic shock. Our results show that the response of the microcirculation to therapeutic interventions is often dissociated from systemic effects. Both chapters clearly show that the sole measurement of blood pressure is misleading, as it may suggest that therapeutic interventions are adequate, while perfusion at the tissue level is or remains markedly compromised. Furthermore, we also found—like others—that besides the dissociation between global hemodynamics and microcirculatory variables, there is also a huge individual variability in the microvascular response to therapeutic interventions [8,35,36].

Unfortunately, it is difficult for clinicians to assess the microcirculation at the bedside. In this thesis we frequently used incident dark-field (IDF) imaging for assessment of the (sublingual) microcirculation. Even as this technology becomes more accessible, image quality and analysis challenges remain significant hurdles. The image quality is highly operator-dependent, whereat the operator can easily find both good and bad microvasculature within one

organ. In addition, automated capture and analysis software and actionable image-based metrics have been disappointing to date [37]. Even if we could obtain high quality and quantitative data of the microcirculation, there is no solid standard for describing the link with the systemic circulation. There is a clear need for signal analysis software programs that describe and analyze systemic and microcirculatory hemodynamics with ‘sense and simplicity’, but still in a reliable and valid manner. For further implementation of hemodynamic analysis in both research and clinical practice, the interface between outcome metrics currently used and clinically recognizable results needs to be improved, standardized and simplified as well [38].

In short, is assessment of microcirculation in critically ill patient relevant? Yes! Are we already able to use it as part of clinical routine? No!

General conclusion

The flow of blood is governed according to Ohm’s law, i.e. perfusion pressure divided by resistance, and depending on dynamic vascular compliance in a controlled setting. The use of Ohm’s law in hemodynamics is ambivalent, since it can be clarifying and confusing at the same time. To consider the cardiovascular system as a two-pressure, one-resistance model seems an oversimplification, since this model does not reflect the actual dynamics of this circular system. In this thesis we showed that blood flow is significantly influenced by pathologies, therapeutic interventions, (right) ventricular function, ventilatory settings and autoregulatory mechanisms. All these factors should be considered when resuscitating a hemodynamically unstable patient in shock.

Future perspective

The administration of intravenous fluids to hemodynamically unstable patients to reach specific blood pressure targets in order to improve organ blood flow is one of the most common, but also one of the most fiercely debated, interventions in intensive care medicine. Even though thousands of patients have been enrolled in large RCTs, consensus remains elusive and practice is widely variable. The indices that are recommended by the guidelines to direct resuscitation maneuvers are limited and not suitable for all patients and may be misleading in many instances [39]. Since critically ill patients are significantly heterogeneous, making a one size fits all approach unlikely to be successful. It seems therefore puzzling why leading journals are still interested in (large) studies that try to find a link between a single index (like systolic blood pressure) and (cardiovascular) outcome [40]. More so, one might not have to battle over static versus dynamic indices, but rather find an optimal combination of many. Future clinical trials—regarding optimization of tissue perfusion—should therefore target personalized macrocirculatory parameters, complemented with functional assessment of the microcirculation. Therewith creating patient-specific therapy, which implies a therapeutic product that is unique and specific for each patient. There is no menu from which to choose. For example, the use of β -blockers is not part of standard resuscitation protocol. However, this thesis shows that there are indeed scenario's where (appropriate) heart rate control with β -blockers could work very well.

Fact remains, that still some of the more fundamental physiology questions that have been raised in this thesis need to be addressed too. For instance, is there an organ specific (dynamic) driving pressure of blood flow and with that not a single mean systemic filling pressure. Combining increased physiological (cardiovascular) understanding, with a suitable combination of indices to guide patient-specific therapy would be something that typically could be done using the widely available, affordable and markedly enhanced computing power and cloud storage. The use of artificial intelligence in medicine, and the deep-learning subtype in particular, has already been enabled by the use of labeled big data [41]. Better prediction of hemodynamically unstable patients in real time could be a new direction of artificial intelligence effort. Colleagues from our group already reported that deep learning of electroencephalogram signals outperforms any previously reported outcome predictor of coma after cardiac arrest [42]. Another deep-learning algorithm has been

used as a data-driven means to automate weaning of patients from mechanical ventilation, which previously has been a difficult and laborious process [43]. The large amount of recorded data at the intensive care unit could make it the prime target for deep-learning algorithms.

All that glitters is not gold with data-driven medicine. This is especially true for sepsis, which remains a difficult story. One retrospective study used data from more than 50,000 patients with documented sepsis, along with their vital signs, electronic medical records, labs, and demographics, to see whether the condition could be detected sooner than before. Unfortunately, the accuracy of the algorithm was not particularly encouraging [44,45]. Furthermore, there is often no clear understanding of why deep-learning driven modes do what they do, and how they might be improved. Using classifying models in clinical medicine should always be done in combination with techniques that gives insight into the function of intermediate feature layers and the operation of the classifier [46].

Nevertheless, we may be able to overcome some aspects of heterogeneity in hemodynamic unstable patient with septic shock. By taking advantage of our increasing understanding of the cardiovascular physiology combined with multiple indicators analyzed by artificial intelligence. It is therefore the clinician's job to integrate (patho)physiological knowledge with large amounts of data. Instead of deferring (a part of) to clinical guidelines, clinical practitioners have epistemological responsibility for their clinical decisions. This means that they are responsible for the collection, critical appraisal, interpretation and fitting together of heterogeneous sources of evidence into a 'picture' of the individual patient [47]. It is and never will be enough to rely on just one index—neither static nor dynamic—in order to predict the effect of therapeutic interventions. For this, interdisciplinary expertise is needed in order to integrate e.g. basic concepts, theories, models, methodologies, technologies, and specific ways of measuring, reasoning and modeling [48]. This requires the integration of diverse expert domains within the interdisciplinary context of healthcare. Hence technical medicine, were the twain shall meet [49].

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Summary | Samenvatting

Summary

In this thesis, the role of venous return in organ perfusion is elucidated. Using experimental studies, we investigated this relationship in order to improve monitoring-guided therapies aimed at supporting organ function in patients with circulatory shock.

To this end, in **chapter 2**, we studied a bedside technique that estimated the mean systemic filling pressure (a potential marker of volume status). We revealed a strong linear correlation between venous return and the central venous pressure with this technique. However, the clinical applicability of this method to guide volume therapy in its current form remains unclear. Moreover, **chapter 3** revealed that the foundation, on which this mean systemic filling pressure is build, rattles. The mean systemic filling pressure is derivate from Guyton's model of venous return. He considered the mean circulatory filling pressure (this pressure includes the pulmonary circulation compared to the mean systemic filling pressure) to be a key hemodynamic parameter being the upstream pressure of venous return. However, all methods to obtain this pressure evoke considerable effects on the cardiovascular system in itself. As a consequence, this exerts a major influence on the measured quantity, and consecutive interpretation and clinical relevance.

In **Chapter 4** and **5** of this thesis we focus on a novel therapeutic strategy for patients with septic shock, namely heart rate control using β -blockers. We took both preclinical and clinical data into consideration regarding the cardiovascular physiological consequences of β -blocker therapy in septic shock. We showed that it is effective to reduce myocardial oxygen consumption when its need outstrips the capacity. However, excessive β -blockade had negative inotropic effects and could put the cardiac output below the threshold that is needed to maintain organ and tissue perfusion. This implies that for safe application of β -blockers with maximum effect, one should focus on; moment of administration, type of β -blocker, resuscitation targets and patient characteristics.

In light of this, the physiological mechanisms that are at play when using β -blockers need to be clarified to improve therapy efficacy. In an animal model with endotoxic shock, we showed negative effects of early administration of esmolol—a selective β -blocker—on right ventricular function. Tis indicates that clinical diligence and caution are necessary when treating septic shock

with esmolol in the acute phase. Also, this emphasizes the significance of adequate right ventricular function assessment.

Another therapy option for patients in septic shock is the use of vasopressors. Of these, norepinephrine is the most widely used. In **chapter 6**, we investigated whether any vasopressor in particular (norepinephrine, phenylephrine, or vasopressin) holds an advantage when considering effects on both the micro- and macrocirculation. Hampered by the use of low dosages of vasopressors in healthy volunteers, we only saw exerted effects on macrocirculatory parameters compared to placebo. These included a mitigation in the decreased diastolic blood pressure, and a stabilization of systemic vascular resistance and cardiac output. Most importantly, this study underscores that limiting hemodynamic monitoring in critically ill patients to solely blood pressure is insufficient. It neglects the causative physiological processes (pressure, flow and resistance) and its ultimate goal: improving organ perfusion.

In the last chapter of this thesis, **chapter 7**, we evaluated renal autoregulation in an animal model focusing on the relationship between micro- and macrocirculatory parameters. By perturbing the cardiovascular system in the following manners; septic shock, resuscitation, and β -blocker administration. These perturbations had profound effects on the macrocirculation, and was accompanied by pressure-dependency of renal blood flow. However, we showed—using extensive analysis of the renal autoregulation—that this dependency of flow to pressure could be attributed to the low perfusion pressures, rather than a damaged autoregulatory system of the kidneys.

Together, in this thesis we revealed that the role of venous return in organ blood flow depends on pathological conditions, therapeutic interventions, (right) ventricular function, ventilatory settings and autoregulatory mechanisms. Moreover, organ blood flow cannot be assessed by solely looking at the macrocirculation.

Samenvatting

In dit proefschrift is de rol van de veneuze terugstroom in orgaanperfusie opgehelderd. Met behulp van experimentele studies hebben we deze relatie onderzocht zodat therapieën beter toegerust zijn op het ondersteunen van orgaanperfusie in patiënten met bloedsomloopshock.

In **hoofdstuk 2** hebben we dit gedaan door onderzoek te doen naar een patiëntvriendelijke techniek die de gemiddelde systemische vullingsdruk meet (een mogelijke maat voor de volume status van een patiënt). Deze techniek liet, tegen onze verwachting in, zelfs bij een erg lage veneuze bloedstroom een sterke relatie zien tussen de veneuze druk en deze veneuze bloedstroom. Desondanks is de klinische toepasbaarheid erg beperkt, omdat er geen relatie was tussen de berekende systemische vullingsdruk en de volume status. Daar komt bij dat we in **hoofdstuk 3** laten zien dat het theoretisch fundament van deze vullingsdruk wankel is. Het blijkt namelijk niet mogelijk om de gouden standaard van deze druk, de bloeddruk na een hartstilstand, te meten met een methode die geen invloed uitoefent op deze druk.

In **hoofdstuk 4** en **5** zijn de mogelijkheden van een nieuwe therapeutische interventie –het gebruik van β -blockers– bij patiënten in septische shock kritisch onder de loep genomen. We hebben eerst in de literatuur gekeken naar experimentele en klinische studies. De huidige literatuur laat zien dat het logisch is om het hart rustiger te laten kloppen als het aanbod van zuurstof beperkt is. Echter zal het gebruik altijd patiënt specifiek gemaakt dienen te worden door te letten op: het moment van toediening, het soort β -blocker, het doel van de behandeling en de patiënteigenschappen.

Daarnaast hebben we experimenten uitgevoerd waarbij we specifiek hebben gekeken naar het effect van deze β -blockers op de functie van het rechter hart. Deze experimenten liet zien dat het gebruik van esmolol –een selectieve β -blocker– in de vroege fase van shock kan leiden tot een lage perfusiedruk. Het is dan ook belangrijk om tijdens de inzet van β -blockers de functie van het (rechter) hart goed in de gaten te houden.

In de laatste twee hoofdstukken van dit proefschrift hebben we gekeken naar de relatie tussen de micro- en macrocirculatie tijdens septische shock. Hierbij is het effect bestudeerd van verschillende therapeutische interventies die veel worden toegepast bij deze groep patiënten op deze parameters. De in **hoofdstuk 6** beschreven vaatvernauwers is zo'n veel gebruikte therapie. We lieten zien dat het verschil tussen verschillende soorten vaatvernauwers

minimaal is, maar wel dat het effect van deze vaatvernauwers op de microcirculatie anders is dan het effect op de macrocirculatie. Ons advies is dan ook om tijdens de behandeling van septische patiënten met vaatvernauwers niet alleen naar de centrale bloeddrukken (de macrocirculatie) te kijken, maar ook naar de doorbloeding van de organen (de microcirculatie).

In het laatste hoofdstuk, **hoofdstuk 7**, is de capaciteit van de nieren om tijdens lage bloeddrukken zijn bloedstroom op peil te houden (autoregulatie) onderzocht. In een diermodel hebben we de centrale bloeddruk beïnvloed door achtereenvolgens: septische shock te induceren, deze shock te behandelen en door het toedienen van β -blockers. Uitgebreide analyse van de nierautoregulatie liet zien dat de stroom van bloed door de nieren sterk vermindert is als de centrale druk onder een bepaalde minimum komt. Het stoppen van de β -blocker infusie liet zien dat er geen permanente schade was aan de autoregulatie van de nier.

Globaal genomen hebben we in dit proefschrift laten zien dat de doorbloeding van de organen afhankelijk is van ziekten, therapeutische ingrepen, (rechter) hartfunctie, beademingsinstellingen en mechanismen van autoregulatie. Daarnaast laten we zien dat de orgaandoorbloeding onvoldoende beoordeeld kan worden als er alleen gekeken wordt naar de bloeddrukken en bloedstroom van de macrocirculatie.

Addenda

10

List of abbreviations
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List of abbreviations

ABP	Arterial blood pressure
AKI	Acute kidney injury
ANOVA	One-way analysis of variance
BRC	Baroreflex component
BSA	Body surface area
$C_{(a-v)O_2}$	Arteriovenous oxygen difference
CO	Cardiac output
CI	Cardiac index
CVP	Central venous pressure
DBP	Diastolic blood pressure
EKG	Electrocardiography
GEDV	Global end-diastolic volume
GEDV_i	Indexed global end-diastolic volume
GFR	Glomerular filtration rate
HF	High frequency
H(f)	Transfer function
HR	Heart rate
ICU	Intensive care unit
IHM	Inspiratory hold maneuver
LF	Low frequency
LPS	Lipopolysaccharide
LV	Left ventricle
MAP	Mean arterial pressure
MCFP	Mean circulatory filling pressure
MFI	Microvascular flow index
MR	Myogenic response
MSFP	Mean systemic filling pressure
P_{aCO_2}	Arterial partial pressure of carbon dioxide
P_{vCO_2}	Venous partial pressure of carbon dioxide
PCA	Pulse contour analysis
PPV	Pulse pressure variation
P_{max}	Maximum isovolumetric pressure
P_{es}	End-systolic pressure
P_{vent}	Ventilatory pressure
RCT	Randomized control trials
RAP	Right atrial pressure
RBF	Renal blood flow
RPP	Renal perfusion pressure
RV	Right ventricle

RVR	Renal vascular resistance
RV_x	Renovascular reactivity index
SBP	Systolic blood pressure
S_{cvO₂}	Central venous oxygen saturation
SV	Stroke volume
SVR	Systemic vascular resistance
SVV	Stroke volume variation
TAU	Diastolic relaxation time constant
TFA	Transfer function analysis
TGF	Tubuloglomerular feedback
VACR	Ventricular-arterial coupling ratio
VF	Ventricular fibrillation
VHF	Very high frequency
V-A	Ventricular-arterial
VR	Venous return
VRdP	Venous return driving pressure

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List of publications

Westerhof BE, **van Loon LM**, Westerhof N. Reflections Revisited, Reinterpretation Required. *Submitted for publication*.

Rood PJT, Zegers M, Ramnarain D, Koopmans M, Klarenbeek T, Ewalds E, van der Steen MS, Oldenbeuving AW, Kuiper M, Teerenstra S, Adang E, **van Loon LM**, Wassenaar A, Vermeulen H, Pickkers P, Boogaard M, the UNDERPIN-ICU study investigators. The impact of nUrsiNg DEliRium Preventive INterventions in the Intensive Care Unit (UNDERPIN-ICU): A multi-center, stepped wedge cluster randomized controlled trial. *Submitted for publication*.

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Curriculum Vitae

Lex Maxim van Loon was born on November 27th, 1988 in Nijmegen. He grew up in Nijmegen and graduated from the gymnasium in 2007 at the Canisius College. The same year he started to study Technical Medicine at the University of Twente, followed by a master's in Medical Signaling. During his clinical internship in the last year of his education, he developed a mathematical model for teaching and simulation of pediatric cardiovascular physiology at the pediatric intensive care unit of the Sophia Children's Hospital in Rotterdam.

In December 2013 he started to work as a teacher and researcher at the Technical Medicine program of the University of Twente, focussing on cardiovascular physiology. His PhD-project was a close cooperation with the Intensive Care of the RadboudUMC, which led to this thesis under supervision of Dr. J. Lemson, Prof. J.G. van der Hoeven and Prof. P.H. Veltink.

Lex is married to Anne Steins and they live with their two sons, Filip (2017) and Oliver (2019) in Utrecht, the Netherlands. In March 2020, Anne and Lex will both start working as a research fellow at the Australian National University in Canberra, contributing to the field of space medicine.