Adrenomedullin in sepsis

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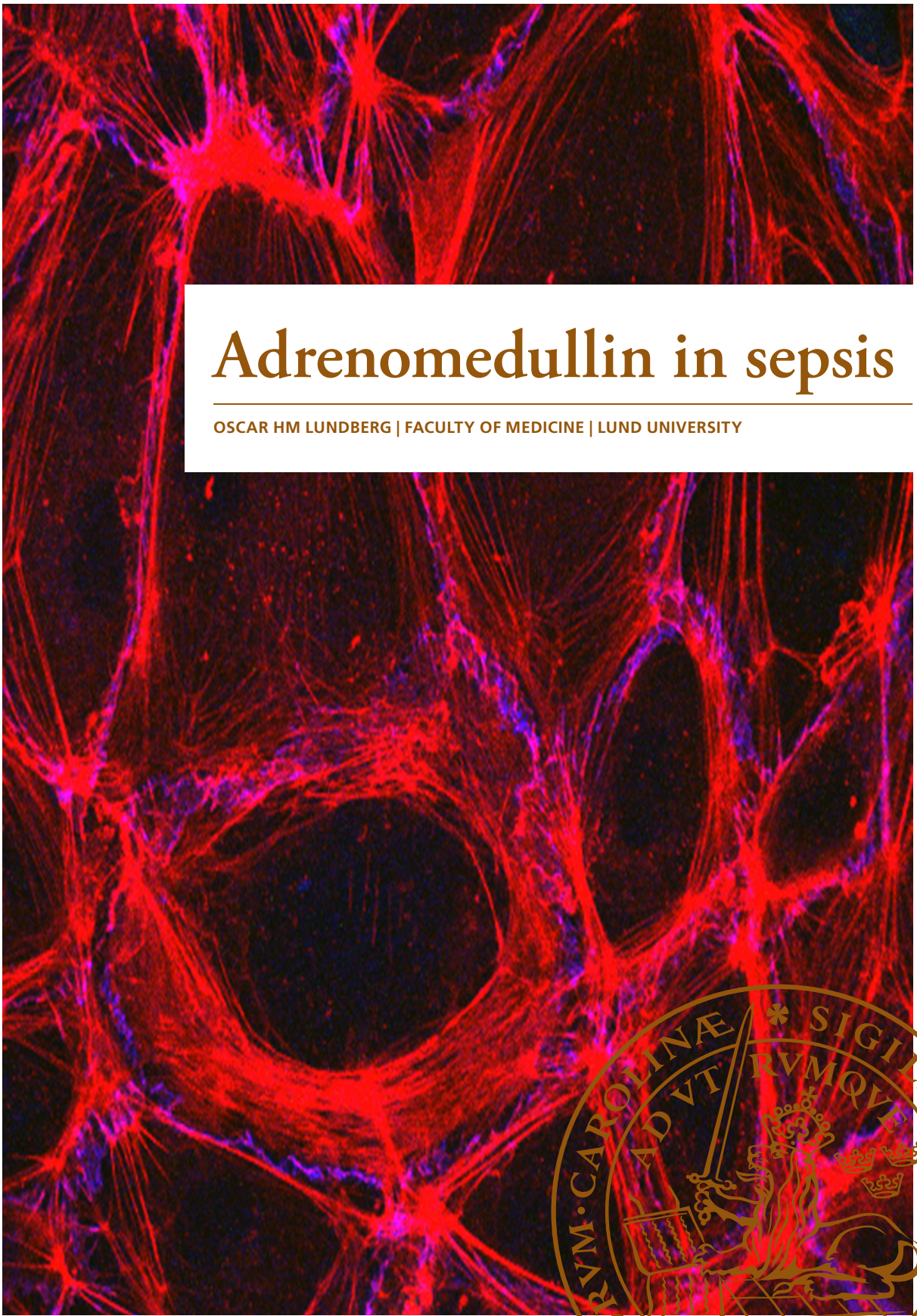

Adrenomedullin in sepsis

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Oscar HM Lundberg

LUND
UNIVERSITY

DOCTORAL DISSERTATION

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Abstract

Background
Sepsis is a syndrome difficult to diagnose and stratify. The epidemiology of sepsis and consistency of criteria fulfillment with diagnosis coding in Swedish intensive care units (ICU) are largely unknown. Biomarkers can be of help to understand pathophysiology, identify clusters within sepsis and to individualize treatment.

Aim
The overarching aim of this thesis was to explore how adrenomedullin (ADM) relates, alone or in combination with other biomarkers, to sepsis in regard to mortality and illness severity among patients in the ICU and emergency department (ED). Due to the suspected underreporting of sepsis, and in order to relate admission ADM levels with sepsis definitions, the epidemiology of sepsis at ICU admission was described.

Methods
The cohorts included in this thesis, formed by sepsis and non-sepsis patients admitted to the ICU as well as sepsis patient in the ED, had their levels of ADM and other biomarkers measured and related to mortality, organ failure, need for organ support, and, when possible, to ICU admission and ED discharge.

Results
The levels of ADM, endothelin-1 (ET-1) and high-sensitivity troponin t (hsTNT) were described during the first 7 days of ICU admission in a septic shock cohort and showed a significant association with mortality and myocardial injury. A positive biomarker panel with all three biomarkers increased the odds for mortality 13 to 20-fold.
Approximately one third of all ICU admissions fulfilled the sepsis-3 criteria, but the consistency with diagnosis coding was poor, as only 31% of these patients had sepsis as main diagnosis.
Among sepsis and non-sepsis ICU patients alike, increasing levels of ADM were associated with mortality and need for organ support. After adjusting for severity of disease an association of ADM with sepsis was seen.
ADM measured among ED sepsis patients showed significant association with mortality, severe organ failure, ICU admission and ED discharge. Further, ADM added information to other known demographic predictors and routine biomarkers.

Conclusions
ADM, alone or in combination with other biomarkers, adds information to known prognostic factors and seems to be of aid in triaging, stratification and prognostication of sepsis patients in the ED and ICU.

Key words sepsis, septic shock, biomarkers, epidemiology, prognostication, stratitification, adrenomedullin, endothelin-1

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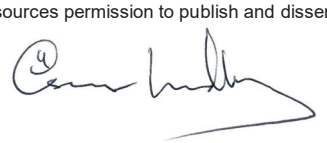
Signature  Date 2022-07-01

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Cover photo “Cells lining the blood vessel walls” by Christopher V. Carman and Roberta Martinelli, Harvard Medical School, Boston, Mass, licensed under CC BY-ND 2.0 (https://creativecommons.org/licenses/by-nd/2.0/).

The structure of the endothelium, the thin layer of cells that line our arteries and veins, is visible here. The endothelium is like a gatekeeper, controlling the movement of materials into and out of the bloodstream. Endothelial cells are held tightly together by specialized proteins that function like strong ropes (red) and others that act like cement (blue).

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To my Mother

Table of Contents

List of publications

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:

I **Lundberg OHM**, Bergenzaun L, Ryden J, Rosenqvist M, Melander O, Chew MS. Adrenomedullin and endothelin-1 are associated with myocardial injury and death in septic shock patients. Crit Care. 2016;20(1):178.

II Lengquist M, **Lundberg OHM**, Spangfors M, Annborn M, Levin H, Friberg H, Frigyesi A. Sepsis is underreported in Swedish intensive care units: A retrospective observational multicentre study. Acta Anaesthesiol Scand. 2020;64(8):1167-76.

III **Lundberg OHM**, Lengquist M, Spangfors M, Annborn M, Bergmann D, Schulte J, Frigyesi A, Friberg H. Circulating bioactive adrenomedullin as a marker of sepsis, septic shock and critical illness. Crit Care. 2020;24(1):636.

IV **Lundberg OHM**, Rosenqvist M, Bronton K, Schulte J, Friberg H, Melander O. Bioactive adrenomedullin in sepsis patients in the emergency department is associated with mortality, organ failure and admission to intensive care. PLoS One. 2022;17(4):e0267497.

Related publications not part of the thesis:

Frigyesi A, Bostrom L, Lengquist M, Johnsson P, **Lundberg OHM**, Spangfors M, et al. Plasma proenkephalin A 119-159 on intensive care unit admission is a predictor of organ failure and 30-day mortality. Intensive Care Med Exp. 2021;9(1):36

List of abbreviations

ADM	adrenomedullin
Aptt	activated partial thromboplastin time
AUROC	area under the receiver operating characteristic curve
bio-ADM	bioactive adrenomedullin
big ET-1	big endothelin-1
bpm	beats per minute
BPS	best practice statement
cDPP3	circulating dipeptidyl peptidase 3
cGMP	cyclic monophosphate
CT-proET-1	c-terminal pro-endothelin-1
DPP3	dipeptidyl peptidase 3
DAMP	damage-associated molecular pattern
ECE	endothelin converting enzyme
ET-1	endothelin-1
eNOS	epithelial nitric oxide synthase
FiO_2	fraction inspired oxygen
hsTNT	high-sensitivity troponin t
ICU	intensive care unit
IL	interleukin
iNOS	inducible nitric oxide synthase
INR	international normalized ratio
LPS	lipopolysaccharide
LVEF	left ventricle ejection fraction
MAP	mean arterial pressure

MODS	multiple organ dysfunction syndrome
MR-proADM	mid-regional pro-adrenomedullin
nNOS	neural nitric oxide synthase
NO	nitric oxide
NOS	nitric oxide synthase
OR	odds ratio
pADMp	proadrenomedullin N-terminal 20 peptide
PAM	peptidyl-glycine α-amidating monooxygenase
PAMP	pathogen-associated molecular pattern
PaO_2	partial pressure of oxygen
PICO	population, intervention, control, outcome
pre-proADM	pre-proadrenomedullin
pre-proET-1	pre-proendothelin-1
PRP	pattern recognition receptor
SBP	systolic blood pressure
SCM	septic cardiomyopathy
SSC	surviving sepsis campaign
SICU	sepsis in the intensive care unit
SIRS	systemic inflammatory response syndrome
SOFA	sequential organ failure assessment
SVR	systemic vascular resistance
TNF-α	tumor necrosis factor-α
WHO	World Health Organisation

Preface

The cure for boredom is curiosity. There is no cure for curiosity. - Ellen Parr

I have always been fascinated by the mysterious ways of the human body. The complex and delicate, yet also by evolution fine-tuned mechanisms which make us humans, unfold to what resembles an inner universe hard to grasp.

This curiosity for the inner un-known was what made me pursue a medical education – I wanted to learn how the human body worked and was, to begin with, not at all appealed by the prospect of "becoming a physician". In a while, however, the curiosity for clinical medicine was also sparked and the mixture of applied physiology and pharmacology in combination with specialized practical skills led me to Anesthesiology and Critical Care - a career choice I have never regretted.

Still, over the years I have met, and also come to envy, enthusiastic scientists who seem truly passionate in their search for new star formations in galaxies from deep within. Enrolling in a doctoral education I saw as an opportunity to gain some of the knowledge to begin optimizing my own telescope.

Context and timeline of this thesis

The patient populations, molecules and modes of action studied in this thesis have been presented to me by clinicians and researchers I have met during my training and employment at Skåne University Hospital, Malmö. The questions raised and ideas sparked are the fruit of collaborations across medical specialties including Internal medicine, Infectious disease and my own, Anaesthesiology and Intensive care. The populations studied in Paper I and IV are both recruited in Malmö. The databases for Paper II and III were created jointly through collaboration across Region Skåne. Paper I, III and IV were only possible due to close collaboration with German laboratory companies.

Figure 1 shows the development of this dissertation.

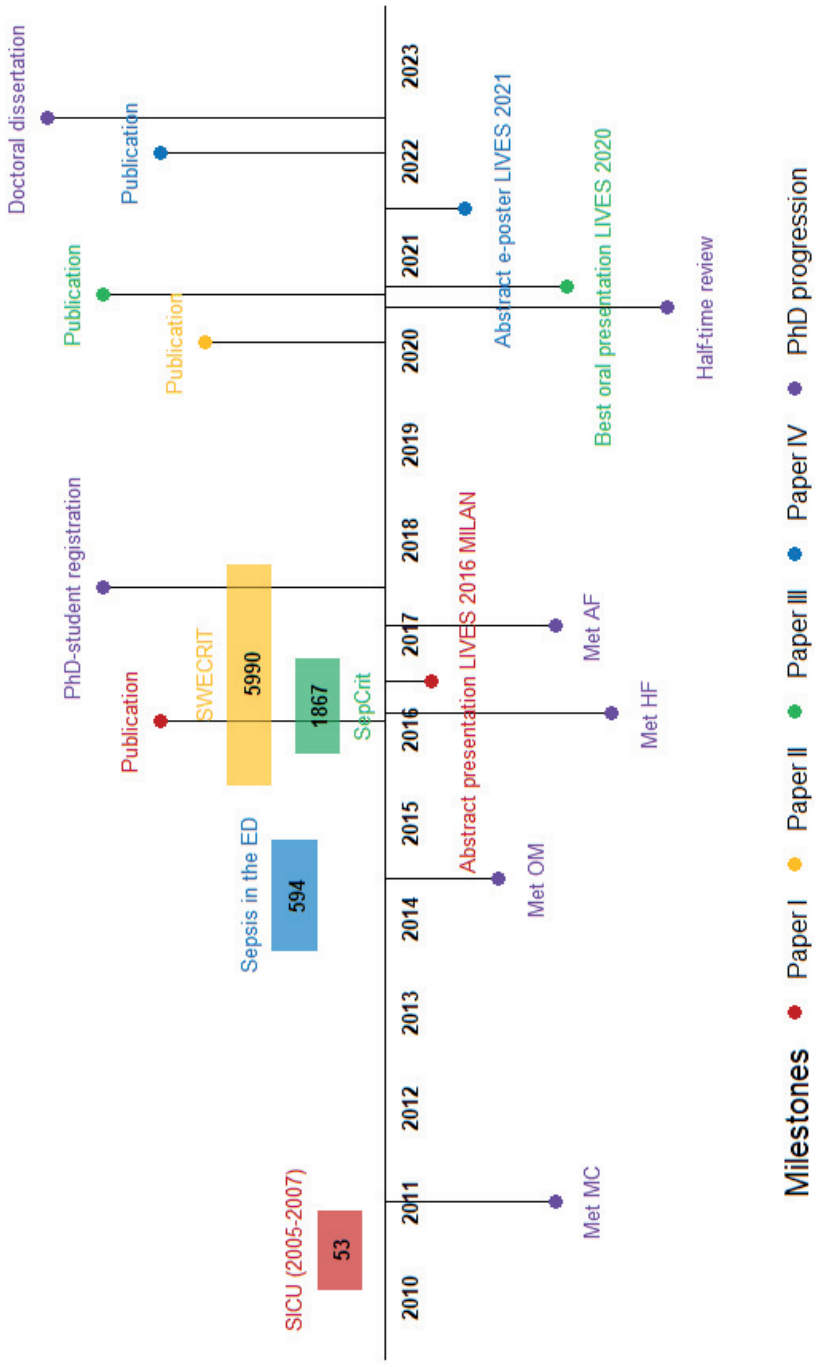

Figure 1. Timeline from first cohort recruitment to dissertation
MC: Michelle Chew; OM: Olle Melander; HF: Hans Friberg; AF Attila Frigyesi

Background

Sepsis

Sepsis definitions

The first record of the term *sepsis* goes back as far as 2700 years ago and is found in Homer's poems as a derivative from the Greek word *sepo* which means "I rot" [1]. The concept of unknown microorganisms invading and disrupting the homeostatic balance evolved over the following centuries and the expression *septicemia* which was defined by the alteration of the blood (aima) with putrid or septic matters, was coined by the French physician Piorry in 1837 [2]. In 1914 the first modern definition of sepsis was presented as Schottmüller wrote "sepsis is present if a focus has developed from which pathogenic bacteria, constantly or periodically, invade the blood stream in such a way that this causes subjective and objective symptoms" [3].

Sepsis-1

The first consensus definition of sepsis was presented after a conference in 1991 and presented concepts like systemic inflammatory response syndrome (SIRS) and multiple organ dysfunction syndrome (MODS) [4]. Sepsis was defined as a systemic response to infection by fulfilment of two or more SIRS criteria as a result of infection. Further, terms as severe sepsis and septic shock were defined as seen in Table 1.

Table 1. Defintions Sepsis-1
Terms presented and defined in Sepsis-1 (1991).

TERMS	DEFINTIONS
Systemic inflammatory response syndrome (SIRS)	The systemic inflammatory response to a variety of severe clinical insults; 1) Temperature >38°C or <36°C 2) Heart rate >90 beats per minute 3) Respiratory rate >20 per minute or $PaCO_2$ <4.3 kPa 4) White blood cell count >12*10^9/L or <4*10^9/L, or >10% immature forms
Sepsis	The systemic response to infection manifested by fulfilment of two or more SIRS criteria as a result of infection
Severe sepsis	When sepsis is associated with organ dysfunction, hypoperfusion or hypotension which may result in lactic acidosis, oliguria or acute alteration in mental status.
Septic shock	Sepsis-induced hypotension, persisting despite adequate fluid rescuitation along with the presence of hypoperfusion abnormalities or organ dysfunction.
Multiple organ dysfunction syndrome (MODS)	Altered organ function in an acutely ill patient such that homeostasis cannot be maintained without intervention.

Sepsis-2

In 2001, an international sepsis definition conference was held [5]. It was recognized that the SIRS criteria were overly sensitive and nonspecific and that the definitions from 10 years earlier not could be used to for prognostication. Further, it was stated that an infection not always could be confirmed by microbiological findings, which led to the inclusion of cases with suspected, not only not confirmed, infection in the sepsis definition. While the definitions for sepsis, severe sepsis and septic shock remained the same, the authors argued that clinicians normally do not use these criteria but instead collect a myriad of symptoms to determine whether a patient “look septic” or not. In relation to this, the authors listed possible signs of infection which experienced clinicians used to raise a suspicion of infection, see Table 2. The authors also showed interest in biomarkers, including adrenomedullin (ADM), but concluded that the application of biomarkers in the definition of sepsis was premature due to lack of sufficient evidence.

Sepsis-3

The weakness of the sepsis definition already pointed out at the conference in 2001 became more and more evident and the call from the scientific community for updated definitions [6] finally led to an update of the sepsis definitions in 2016 [7]. The SIRS criteria were abandoned, and sepsis is since then defined as a life-threatening organ dysfunction due to a dysregulated host response to infection.

Table 2. Diagnostic criteria Sepsis-2
Terms presented and defined in Sepsis-2 (2001).

TERMS	DEFINTIONS
Infection	A pathological process induced by a micro-organism. Documented or suspected infection and some of the following diagnostic for sepsis.
General parameters	Fever (<38.3°C) Hypothermina (<36°C) Heart rate >90 bpm or 2 SD above normal value for age Tachypnea (<30 bpm) Altered mental status Significant edema or positive fluid balance (20ml/kg over 24 h) Hyperglycemia (plasma glucose >7.7 mmol/L) in absence of diabetes
Inflammatory parameters	Leukocytosis (white blood cell count > >12*10^9/L Leukopenia (white blood cell count <4*10^9/L) Normal white blood cell count with >10% immature forms Plasma C reactive protein > 2 SD above normal value Plasma procalcitonin > 2 SD above normal value
Hemodynamic parameters	Arterial hypotension (SBP < 90mmHg, MAP <70mmHg or SBP decrease > 40% in adults or < 2SD below normal for age) Mixed venous oxygen saturation < 70%. Cardiac index >3.5L/min/m^2
Organ dysfunction parameters	Arterial hypoxemia (PaO_2/FiO2 < 40kPa) Acute oliguria (urine output < 0.5mL/kg/h) Creatinine increase ≥0.5mg/dL Coagulation abnormalities (INR > 1.5 or Aptt >60s) Ileus Thrombocytopenia (platelet count < 100*10^9/L) Hyperbilirubinemia (plasma total bilirubin > 4mg/dL or 70 mmol/L)
Tissue perfusion parameters	Hyperlactatemia (> 3 mmol/L) Decreased capillary refill or mottling

Organ dysfunction is classified according the sequential organ failure assessment (SOFA) score already in use as a daily monitoring score system in intensive care unit (ICU) settings [8], see Table 3. An increase in SOFA with two or more (baseline SOFA in a patient with no known pre-existing dysfunction is assumed to be zero) is considered life-threatening and whence a diagnostic criterion for sepsis when infection is present or suspected.

The term suspected infection is operationalized as the administration of antibiotics and the concomitant cultivation of body fluids within 96 hours in relation to the beginning of antibiotic treatment, see Table 4.

The term severe sepsis was abandoned, and septic shock is described as a subset of sepsis in which abnormalities are profound enough to increase mortality. The criteria for septic shock are, in addition to those of sepsis, a level of serum lactate more than 2 mmol/L and, despite adequate fluid resuscitation, a requirement of vasopressors to obtain a mean arterial pressure (MAP) equal or more than 65 mmHg.

Table 3. Sequential organ faliure assessment (SOFA) score
Organ systems and their corresponding score cut-off values.

SYSTEM	SCORE				
	0	1	2	3	4
Respiration PaO2/FiO2, kPa	≥53.3	<53.3	<40	<26.7 with respiratory support	<13.1 with respiratory support
Coagulation Platelets, x 109/L	≥150	<150	<100	<50	<20
Liver Bilirubin, µmol/L	<20	20-32	33-101	102-204	<204
Cardiovascular Catecholamines µg/kg/min	MAP≥70 mmHg	MAP<70 mmHg	Dopamine ≤ 5 or Dobutamine	Dopamine 5-15 or epinephrine ≤0.1 or norepinehprine ≤0.1	Dopamine >15 or epinephrine >0.1 or norepinehprine >0.1
Central nervous system Glascow Coma Scale Score	15	13-14	10-12	6-9	<6
Renal Creatinine, µmol/L	<110	110-170	171-299	300-440	>440
Urine output, mL/day				<500	<200

Table 4. Diagnostic criteria Sepsis-3
Terms presented and defined in Sepsis-3 (2016).

TERMS	DEFINTIONS
Sepsis	A life-threatening organ dysfunction caused by a dysregulated host response to infection.
Organ dysfunction	An acute change in total SOFA score ≥ 2 consequent to infection. The baseline SOFA score can be assumed to be zero in patients with no known pre-existing organ dysfuntion.
Septic shock	A subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality. Identified in sepsis patients with vasopressor requirement to maintain a MAP ≥65 mmHg despite adequate volume resuscitation, and a serum lactate level > 2 mmol/L.
Suspected infection	Administration of antibiotics within 72 hours in relation to culture sampling of body fluids, or if antibiotics given first culture within 24 hours.

Pathophysiology

From the time of ancient Greece up until the 19th century it was believed that diseases could be transmitted by poisonous fumes or gases from putrefying or rotting processes – *miasma [9]*. This Miasma theory is reflected in the name of the disease malaria – a conjunction of the Italian words *mal* and *aria* (which mean "bad" and "air") [10]. The Miasma theory was replaced by the Germ theory during the mid 19th century as scientists like Pasteur and Koch described how microbes could cause disease [2]. Even though triggered by the invasion of a pathogen, the current sepsis definition focuses on the harmful self-inflicted actions taken by the immune system [7]. The hallmarks of sepsis are described below.

Inflammatory response

The body has the ability to respond to threats, both infectious and non-infectious (multiple trauma, burns), by activation of the inflammatory system. The classical signs of *calor, rubor, tumor* and *dolor* are all the result of the actions taken by the inflammatory system [11]. The activators of inflammatory cells like neutrophils, monocytes and lymphocytes can be of both exogenous and endogenous sources [11]. External activators are derived from pathogens, also called pathogen-associated molecular patterns (PAMPs), of which the lipopolysaccharide (LPS) endotoxin, a component of the cell walls of Gram-negative bacteria, is the most classical example. Endogenous damage-associated molecular patterns (DAMPs) are actively released from cells upon inflammasome activation or emerge passively after cell death [12]. Examples of DAMPs are adenosine triphosphate (ATP), histones and DNA [12]. The binding of PAMPs and DAMPs to pattern recognition receptors (PRPs) on innate immune cells leads to a production of cytokines [13]. The cytokines, exemplified by tumor necrosis factor-α (TNF-α), the interleukins (IL) and interferons, are also endogenous activators. Some of the proinflammatory cytokines, mainly IL-6, induce release of acute phase proteins by hepatocytes of which C-reactive protein (CRP) is an example [13].

Vasoplegia

Vasoplegia is a key feature of sepsis [4, 5, 7].

Nitrous oxide (NO) is involved in the regulation of vascular tone and increased levels have been reported in sepsis [14, 15]. NO induces the production of cyclic monophosphate (cGMP), which leads to vascular smooth muscle relaxation [14]. NO synthase (NOS) is responsible for the production of NO and comes in three isoforms [16]. Two of the isoforms, which are dependent on calcium, are mainly expressed in their corresponding tissues - neural NOS (nNOS) in neurological cells, and endothelial NOS (eNOS) in epithelial cells. The third, calcium independent isoform, inducible NOS (iNOS), can be expressed by a variety of

different cell types including immunological cells [11]. Normally, iNOS is not active but is upregulated by both endo- and exogenous inflammatory activators in sepsis [14, 17] resulting in supranormal levels of NO [14, 15]. NO is a gaseous free radical which apart from acting as a second messenger in the regulation of vascular tone, can be directly microbicial, inhibit mitochondrial respiration and also carries regulatory functions within the immunological system itself [14].

Apart from the overproduction of NO, other examples of vasoplegic factors include increased prostacyclin levels, diminished vasopressor levels and downregulation of vasoconstrictive receptors [15, 17].

Septic cardiomyopathy

Septic cardiomyopathy (SCM) is a condition which lacks a uniform definition [18, 19]. Some variants of SCM diagnostic criteria include echocardiographic findings and some do not [18]. The most common reported criteria are acute and reversible (within 7-10 days), global biventricular systolic or diastolic dysfunction with reduced contraction, left ventricular dilation, absence of coronary syndrome as ethology, diminished response to fluid resuscitation and catecholamines [18, 19]. Even though the left ventricle ejection fraction (LVEF) has a central part in the description of SCM [20], its relevance as a marker of cardiac function has been questioned [20], since the parameter is highly dependent on loading conditions. For example, a depressed LVEF can become pseudo-normalized in presence of low afterload [19] and whence patients with normal LVEF can have worse outcome due to a high degree of vasodilation, than other patients with lower LVEF but maintained vascular tone [19, 20].

It was initially believed that SCM shared the pathophysiology of coronary artery disease of impaired blood supply, but it has been shown that coronary arterial flow in contrary reaches supra-normal levels in septic shock patients [21, 22]. Myocardial function is subject to modification by cytokines and endotoxins. When exposed to PAMPs derived from Staph. aureus and E. coli the activation of the Toll-like innate receptors, a type of PRP, in the heart induces an inflammatory response [23] which can evolve into increased NO levels, mitochondrial dysfunction, myocardial oedema, decreased myocyte calcium influx and disruption of the cellular cytoskeleton [17, 19, 23, 24] all resulting in decreased contractility.

Increased permeability

On the endovascular side of the normal endothelium a matrix of highly hydrated glucosaminoglycans and proteoglycans make up the glycocalyx. This gel-like surface ranges from 0.5 μm to 5 μm in thickness [25] and plays an important role in the permeability homeostasis [25, 26]. In response to PAMPs or DAMPs, for example in sepsis, the glycocalyx is broken down, exposing the endothelium and the until then hidden adhesion molecules making leukocyte interstitial migration

possible [27]. Further, the intercellular adhesion apparatus, made up by tight and adherens junctions, found between endothelial cells are loosed up [26]. These changes lead to increased permeability, capillary leak, interstitial oedema which impairs tissue perfusion and may ultimately lead to organ failure [25-27]. The redistribution of fluid from intra to extravascular compartments is contributing to the state of hypovolemia almost always seen in sepsis [17].

Hypotension

Hypovolemia, caused by either loss of fluid (vomiting, diarrhoea or perspiration) or redistribution of fluid to the extravascular space, in combination with vasoplegia and SCM, results in hypotension once compensating mechanism are exhausted.

Treatment

Since 2004, with updated versions released every four years, the Surviving Sepsis Campaign (SSC) have given guidance regarding the care of sepsis patients [28-32]. The most recent SSC version from 2021 [32] contains 93 statements formulated using the Population, Intervention, Control, Outcome (PICO) questions. Each question resulted in a recommendation, suggestion or no recommendation based on the level of available scientific evidence and if possible graded according to the Grading of Recommendations, Assessment, Development and Evaluation GRADE system [33]. If not suitable to assess with the GRADE methodology, a Best Practice Statement (BPS) could still be given.

Although revised for every new version of SSC, many principles go back way well before the first SSC release [28]. Already in 1964, the vascular surgent Edward Frank advocated the following management of the septic shock patient; constant attendance by well-trained senior physicians taking full responsibility of care, continuous measurement of invasive arterial blood pressure, urinary output and blood volume while biochemical analysis immediately should be available bedside [34]. This almost 60-year-old description of a modern intensive care setting still stands.

Source control

During the transition from the Miasma theory to the Germ theory the change of routines and treatments provided tremendous improvements. Even though not receiving recognition by his peers at the time, Semmelweis managed to reduce the mortality rate of puerpural fever (sepsis in women after childbirth) from 16% to less than 1% by ordering medical students to wash their hands with calcium chloride in between their transition from performing dissection on cadavers and the maternity ward [2]. Inspired by the work of Pasteur, the surgeon Lister, used antiseptic phenol in the treatment of compound open fractures and after

amputations [35] and found the management to be both limb- and lifesaving, reducing the mortality following amputation from 40-50% to 14% [2].

The SSC recommends source control to be achieved as soon as medically and logistically possible following the initial resuscitation, (*BPS*) [32].

Antibiotics

During the latter part of the 19th century scientists, including Lister, observed the inhibition of bacterial growth when exposed to the mold Penicillium [36]. However, the description of the same phenomenon by Fleming in 1929 [37] is often referred to as "the birth of the antibiotic era" [36] which led to the introduction of Penicillin therapy in 1941 [36] and Fleming being awarded the Nobel Prize in 1945.

The use of antibiotics is today a fundamental part of the sepsis treatment. In the SSC 15 of the 93 statements are related to the use of antibacterial, -fungal or viral treatment [32].

Inflammation modulation

Given the hyperactivated immune system being a cornerstone of the sepsis pathophysiology and definition, it is understandable that the exploration of strategies aiming at modulating the inflammation cascade has been extensive. Despite this, to date there has only existed one registered drug for severe sepsis and septic shock – Xigris – recombinant human protein C. Xigris was approved in 2001 after the early termination of a study reporting dramatic reduction of mortality among patients receiving the drug [38]. The promising results could, however, not be reproduced in following studies and the drug was withdrawn in 2011 [39].

The use of steroids in the treatment of sepsis has been investigated during more than four decades with conflicting results [40, 41]. The two most recent randomized control trials (RCT) found a significant trend towards quicker septic shock resolution, less days on mechanical ventilation but no, or little effect on mortality, when patients with septic shock received hydrocortisone [42, 43]. The SSC suggests the use of hydrocortisone in patients with septic shock and ongoing vasopressor requirements [32].

The combination of hydrocortisone with other adjuncts have been a matter of debate. Marik et. al reported a dramatic reduction of mortality from 40% to 9% in a before-after study when septic shock patients received hydrocortisone, vitamin C and thiamine [44]. The findings received massive media attention and the protocol was introduced in several sepsis treatment regimens around the world, but also met a lot of critique. Marik has referred to the scepticism as a "Semmelweis reflex" [45]. Several RCTs have been performed of which only one reported a reduced

mortality as a secondary outcome [46] while others could not demonstrate this effect [47, 48].

The SSC suggests against the use of vitamin C, but also states that future findings may change this statement [32].

Organ support

The developed world´s ICU offers several organ supportive measures. The indications for these interventions are not exclusive for sepsis patients but merely part of the ICU arsenal in the fight for the critically ill patient. Organ systems often supported include respiration, circulation, coagulation, kidney and bowl function.

Circulatory failure is a hallmark of sepsis and can be treated from different angles and perspectives. Out of the 93 statements in SSC [32], 24 are related to circulatory organ support as outlined in Table 5.

Illness trajectory

To diagnose a patient with sepsis can be challenging as typical symptoms not always are present which can lead to under- or overdiagnosis of sepsis [49]. The diagnosing of sepsis has been shown to be subjective and exhibits high degree of interobserver variability [50]. In a study where over 1000 physicians in Europe and United States, of whom half were intensivists, were interviewed, 83% stated that sepsis often could be missed [51]. Early treatment of critically ill sepsis patients is considered crucial for outcome [32, 49]. Further, once a septic patient has been identified, the prognosis and illness trajectory of that individual is often uncertain. Correct triaging and instituting the right level of care is known to be crucial for outcome [32]. Once right type and level of care are offered, there is a need for monitoring and evaluating the patient´s response in order to continuously tailor the treatment.

Negative results

As previously mentioned, there has only existed one registered specific pharmacological treatment for sepsis – during 10 years [39]. The reason for this is not due to a lack of research efforts since over one hundred of phase II and III studies have been undertaken [52]. Novel therapeutics have often been theoretically sound and supported by in vitro and in vivo pre-clinical studies but when evaluated in trials presented disappointing results.

Table 5. Signs of circulatory faliure and statements in Surviving Sepsis Campaign regarding supportive treatment.
Statements from Surviving Sepsis Campaign (2021), referred to by numbers in publication.

SIGN OF CIRCULATORY FAILURE	THERAPY	DETAILS
Hypovolemia	Fluid therapy	• Initial resusitative bolus of 30ml/kg crystalloid (4), preferably balanced instead of saline (32, 33) followed by albumine but not starch or gelatine, if large volumes are required (34-36). • Evalutated and guided by dynamic measures in addition to physical examination, static measures (6) and capillary refill time (8). • Insufficient evidense to advocate restrictive or liberal fluid strategy (45).
Hypotension	Mean arterial pressure (MAP)	• Target a MAP of 65 mmHg (9) • Invasive monitoring of arterial blood pressure as soon as possible (43).
	Vasopressors	• Norepinephrine first-line vasoactive agent (37), preferably via a central line but otherwise peripherally (44). • If inadequate MAP addition of Vasopressin as second agent (38). • If inadequate MAP addition of epinephrine as third agent (39). • Angiotensin II, but not Terlipressin (40), considered plausible adjunctive agents.
	Inotropes	• Dobutamine, but not Levosimendan, added to norepinephrine or epinephrine used alone if hypotensive despite adequate volume status (41, 42).
Metabolic acidosis	Hyperlactatemia	• Lactate should be measured (3). • Resuscitation should aim at decreasing elevated levels of lactate (7).
	Bicarbonate	• Suggested against in cases of hypoperfusion-induced lactic acidosis unless pH $\leq$ 7.2 and acute kidney injury is present (71, 72)
Hyperactive immune system	Immunomodulation	• Intravenous corticosteroids in septic shock patients with ongoing need for vasopressors (58). • Vitamin C is suggested against (70). • Insufficient evidence regarding blood purification techniques apart from polymyxin B hemoperfusion which is suggested against (59, 60).

Heterogeneity

A phenomenon which needs to be addressed is that many theories and modulations of the sepsis pathology are developed in laboratories, typically from rodent models consisting of standardized young healthy animals, while, in the clinical situation, these potential treatments are applied to a very heterogenic group of often elderly humans with significant comorbidities [52]. Mouse models have also been shown to poorly mimic the human inflammatory response [53]. Further, septic patients differ in relation to infectious agent, focus of infection, temporal stage of illness development as well as in their genetic background [52, 54, 55]. This

heterogeneity implies that patients who receive little or no benefit of an intervention dilutes the efficacy. Some patients might even be harmed by the intervention.

Sepsis subgroups

In order to address the problems with heterogeneity, a need for stratification of the sepsis syndrome into subgroups has been identified [39, 52, 56, 57]. Examples of different classification of phenotypes are increasing [58-60], but nor strategies to identify the subgroups or uniform nomenclature are in use [55, 61]. Large quantities of data are often pooled and analysed using advanced machine learning, making the applicability of such classification into clinical practise not yet possible. We are gaining more and more knowledge about the complexity of sepsis but the findings from these studies fall outside of the scope of our current understanding [60]. Hence, we still rely on results from traditional experimental research where complex biological reactions are reduced to, and explained by, arrows [60].

Enrichment and precision medicine

The refinement of a heterogenic group of patients into subgroups where a given treatment has potential to have a positive effect is referred to as *enrichment* [62]. Enrichment can be undertaken in two main ways. The process of identifying patients who are in high risk of unwanted events, such as dying, is *prognostic enrichment*. If the risk of death or suffering is extremely low, there is no need to jeopardize a natural resolution by introducing a potentially harmful therapy – *primum non nocere*. *Predictive enrichment* refers to the identification of subjects more likely to respond to an intervention.

This concept of basing the treatment strategy on the individual patient´s characteristics refers to precision medicine [54]. Precision medicine is most developed in the field of oncology, as genetic and tumour markers are used to tailor chemotherapy [54].

Biomarkers

Definition

Several definitions of the term "biomarker" has been put forward [63]. The World Health Organisation (WHO) stated in a report on environment risk assessment, the biomarker definition to *"include almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical or biological. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction."* [64].

The use of biomarkers goes back as far as clinical medicine itself, and examples thereof range from observations such as skin color or pulse to advanced laboratory tests of blood or other tissues. Biomarkers are objective and reproducible but not necessarily taking into account patients´ experience or sense of well-being [63]. The subjective perspective of how an individual feels, functions or survives are referred to as *clinical endpoints* and are normally the focus for treatments and the outcomes in clinical trials and studies [65]. Sometimes biomarkers are expected, due to solid scientific evidence, to predict clinical outcome to such an extent that it can substitute the clinical endpoint itself and are then referred to as *surrogate endpoints* [63, 65].

Biomarkers in sepsis

The exploration of biomarkers in relation to sepsis has been extensive and is increasing. Pierrakkos et al. have scanned the scientific literature and, up until 2019, identified 258 biomarkers reported to be related to sepsis [66, 67]. So far only three of these biochemical biomarkers have made been mentioned in the SSC; lactate, CRP and pro-calcitonin (PCT) [32].

There are three areas in which biomarkers are evaluated - diagnosis of sepsis, prognosis and treatment evaluation [49, 67].

Diagnosis of sepsis

As earlier mentioned, the diagnosis of sepsis is dependent on two variables - *"Does the patient have a serious organ dysfunction?"* and *"Is the patient infected?"* [7]. The organ dysfunction is defined as an increment of SOFA score with 2 or more, while answering the second question is less straightforward. A sepsis marker should hence be helping to rule in or out an infection [49], which could guide clinicians regarding antibiotics and search for infectious focus.

Prognosis

The severity of disease - *"Is the condition serious?"* - is important in relation to triaging and assigning a patient to the right level of care. Extremely high values of a biomarker could prone towards ICU admission while low values could be reassuring when discharging a patient from hospital.

Treatment evaluation

When multiple testing is possible and thereby trends can be monitored, the treatment can be evaluated - *"Is the patient responding to treatment?"*. A marker trend could suggest whether a treatment is effective or alternative options should be sought. For example, if there is need for antibiotic rotation or (re-)operation.

Adrenomedullin

Almost three decades ago Kitamura and colleagues discovered a new peptide from human pheochromocytoma they named adrenomedullin (ADM) [68]. ADM is a 52 amino acid peptide and part of the calcitonin peptide family [69]. The genetic code for ADM is located in chromosome 11 [70] and the transcription produces the precursor hormone pre-proADM consisting of 185 amino acids [71]. The posttranslational processing of pre-proADM results in four peptides – proadrenomedullin N-terminal 20 peptide (pADMp) (also known as PAMP), midregional pro-ADM (MR-proADM), ADM and adrenotensin [69, 72, 73], as seen in Figure 2. All the peptides, except for MR-proADM, exert physiological effects [73, 74]. However, the initial form of ADM, with a glycinated C-terminal needs to undergo a maturation process, in which the glycine is converted by peptidyl-glycine α-amidating monooxygenase (PAM), to an amide for ADM to become bioactive ADM (bio-ADM) [75].

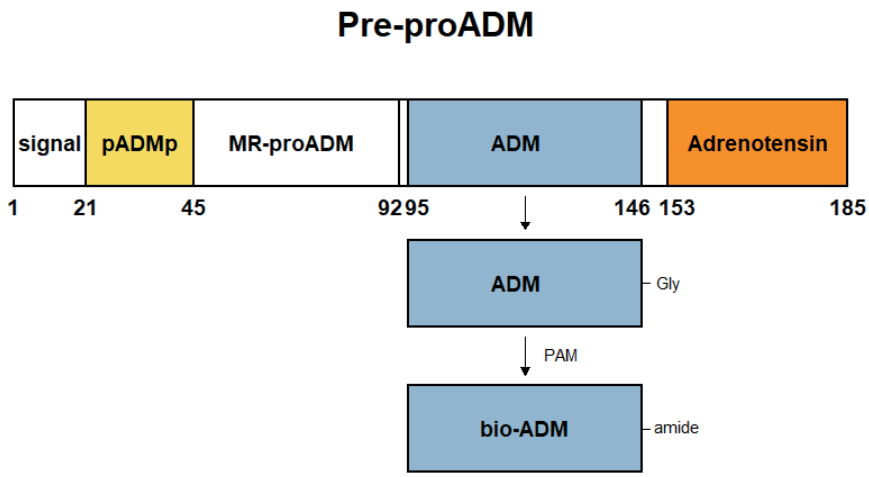

Figure 2. Pre-proadrenomedullin and the peptides resulting from posttranslational processing
Numbers indicate amino acids.

The expression of ADM has been shown in various tissues and organs, including the lungs, kidneys, heart, central nervous system, adipose tissues, intestines and the endothelium [69, 76, 77]. Many cell-types are capable of secreting ADM including macrophages, vascular smooth muscle cells and endothelial cells [69]. ADM has a short half-life of 22 minutes [78] and is cleared by the degradation of proteases mainly in the lung due to the high concentration of ADM-receptors [69, 78].

ADM binds with its C-terminal to heterodimeric receptors AM_1 and AM_2 which are complexes made up by the combination of the structure calcitonin-like receptor (CLR) with one of two different transmembrane structures specific receptor activity-modifying protein (RAMP)2 and RAMP3, respectively [76, 79].

Ever since its discovery, ADM has been postulated to have a regulatory role in circulation control, due to its vasodilatory properties [68]. The effects are however much more diverse than merely vasodilation and are highly dependent on the site of action, and include biological actions like angiogenesis, cell growth, cardiac remodeling, electrolytic and endocrine homeostasis [69, 76, 80, 81].

Adrenomedullin assays

One of two methods to estimate ADM in peripheral blood are predominantly used.

The first, described in 2005, utilizes antibodies against the MR-proADM segment [73] and was offered commercially after the adaptation of the assay in 2009 [82]. MR-proADM was reported to be stable for long period of time in room temperature (at least 72 hours) and not influenced by 4 freeze-thaw cycles [73]. In normal population 90% of MR-proADM measurements were below 0.55 nmol/L [83], a finding which has been confirmed values in the same range by a later publication [84]. MR-proADM has been stated be created stoichiometrically in relation to ADM and pADMp [73] and is more stable than the latter two peptides, possibly because it lacks physiological functions and thereby does not need a regulatory specific protease [73].

The second method, presented in 2014 [85] and refined in 2017 [75], measures bio-ADM directly, using antibodies with affinity of the matured C-terminally amidated ADM moiety. The analyte was stable in room temperature up to 24 h and was unaffected by multiple freeze-thaw cycles [75].

Because of incomplete conversion from ADM-glycine to bio-ADM and doubts regarding the stoichiometric creation of MR-proADM with bio-ADM, it has been argued that the latter method is more precise and give a closer association with biological processes [75]. Further, since the production of other peptides with vasomodulating properties [86] originating from pre-proADM, also renders MR-proADM implies that some of the clinical attributes associated with increased levels of MR-proADM also could be due to the effects of for example pADMp.

Adrenomedullin as a biomarker

Elevated levels of ADM have been observed in a wide variety of disorders including cardiovascular, respiratory, endocrine, renal and inflammatory disorders [69, 87-94]. In the emergency department (ED) or among critically ill patients in

the ICU, ADM has most often been evaluated in specific populations of for example sepsis, heart failure or with dyspnoea as cardinal symptom. A problem with this selection is the lack of controls.

Adrenomedullin in sepsis

The interest of ADM in relation to sepsis was sparked early and increased levels of ADM has been observed in both animals [95, 96] and humans with SIRS and sepsis [97-99]. Also, increasing levels have been associated with mortality, increased severity and need for organ support [99-104].

Two pathological features of sepsis have received special attention in relation to ADM – vasodilation and vascular integrity.

Adrenomedullin and vasodilation

The vasodilatory effect of ADM is believed to be conveyed by two pathways. The binding of ADM to AM receptors on vascular endothelial cells induces the eNOS activity increasing levels of NO, which ultimately leads to relaxation of surrounding vascular smooth muscle cells [69, 76, 105]. When ADM interacts with AM receptors directly on vascular smooth muscle cells, levels of cyclic adenosine monophosphate (cAMP) are increased and, as a result, an endothelial independent, relaxation is induced [76].

Adrenomedullin and permeability

ADM seems to be important for the vascular integrity and normal development, in that knock-out mice with alterations to either ADM, or its important receptor structure CLR, result in fatal embryotic malformations as hydropsis fetalis and cardiovascular abnormalities [106, 107]. In vitro and in vivo studies where animal and human tissues were inflammatory induced with PAMPs and DAMPs, ADM managed to prevent or restore vascular leakage [108, 109]. Epithelial intracellular concentrations in of cAMP increased, which once again is believed to be the intracellular second messenger for the downstream ADM effects [108].

Adrenomedullin as therapeutic target

As the features of ADM have been unveiled, specific therapeutical interventions with the hormonal system have been explored. Two areas of these are specified below.

Adrenomedullin administration

By infusing ADM, modulation of animal models of sepsis has been accomplished [108-111]. In addition to the permeability modulating results already described,

Ertmer et al. reported a prevention and reversion of hypodynamic sepsis in sheep exposed to increasing levels of endotoxemia [110]. Mechanically ventilated mice with pneumonia treated with ADM infusion sustained less lung injuries [111].

Due to the short half-life (22 minutes) [78] requiring infusions, and the avid surface adhesion [112], ADM treatment is challenging.

Adrenomedullin antibody therapy

The use of anti-ADM antibodies has been investigated since long. In 1998, Wang and colleagues demonstrated that the addition of an ADM neutralizing antibody to septic mice, induced with cecal ligation and puncture (CLP), prevented the hyperdynamic sepsis response [96]. Struck et al. demonstrated different responses depending on what epitope of the ADM structure an antibody targeted. Antibodies aiming at the C-terminal part, which also is required to be amidated in order for ADM to become bio-ADM, totally inhibited a cAMP response and did not alternate the mortality among CLP treated mice. However, when the N-terminal part of ADM was targeted, with the antibody later labelled HAM1101, a partial deactivation (25%) was obtained, and improved survival was seen [113]. When HAM1101 was administered to CLP treated mice, they required less vasopressor, showed improved renal function and exhibited less iNOS, but not eNOS, activation [114].

Adrecizumab

These findings have led to the humanization of antibody HAM1101 to HAM8101 which was named Adrecizumab [105]. So far, four papers on Adrecizumab given to humans have been published [115-118]. It is believed that the formation of Adrecizumab-ADM complexes, too big to migrate into the intracellular space, generates elevated intravascular bio-ADM concentrations. Once intravascularly located, ADM is able to exert its endothelium-stabilizing effects, while extravascular effects, including endothelial independent vasodilation, are reduced [87, 105]. The increase of bio-ADM is not accompanied by an elevation of MR-proADM suggesting a redistribution, or decreased metabolism, of ADM rather than an increased synthesis [115, 116]. The phase I and II studies of Adrecizumab concluded that it was safe to give to humans with and without inflammation [115, 116]. A phase III study on sepsis patients is currently in planning [119].

Endothelin-1

In 1988, Yanagisawa and colleagues isolated a new peptide, endothelin (ET), from porcine aortic endothelial cells which was described as the most potent mammalian vasoconstrictor discovered to date [120]. The same group showed that the original ET (ET-1) was part of a greater ET-family with two more peptides, ET-2 and ET-3 [121]. The discovery of ET-1 received great attention resulting in extensive academic and pharmaceutical research. In 1990, the G-protein ET receptor types A and B (ET_A and ET_B) were discovered [122], followed by the development of ET-antagonists few years later [123]. ET-agonists were described already in 1988, when the sarafotoxins, added to the ET-family, and derived from venom of a snake (Atractaspis engaddensis) with its natural habitat in the Middle East, were found [124].

Although cell types as epithelial-, immunological cells and neurons within the central nervous system can produce ET-1, the most prominent producers of the hormone are the vascular endothelial cells [123, 125, 126]. The synthase and release of ET-1 is both continuous and subject to stimulation [123]. The continuous release is believed to maintain vascular tone and is regulated mainly at the level of transcription [123]. Similar to ADM, ET is synthesized from a larger precursor peptide, pre-proET-1. The post-translational processing cleaves the 212 amino acid peptide pre-proET-1 into shorter peptides of which the 39 amino acid big-ET-1 is one [120, 123]. The endothelin converting enzyme (ECE) cleaves big ET-1 and the mature ET-1 of 21 amino acids is formed, see Figure 3.

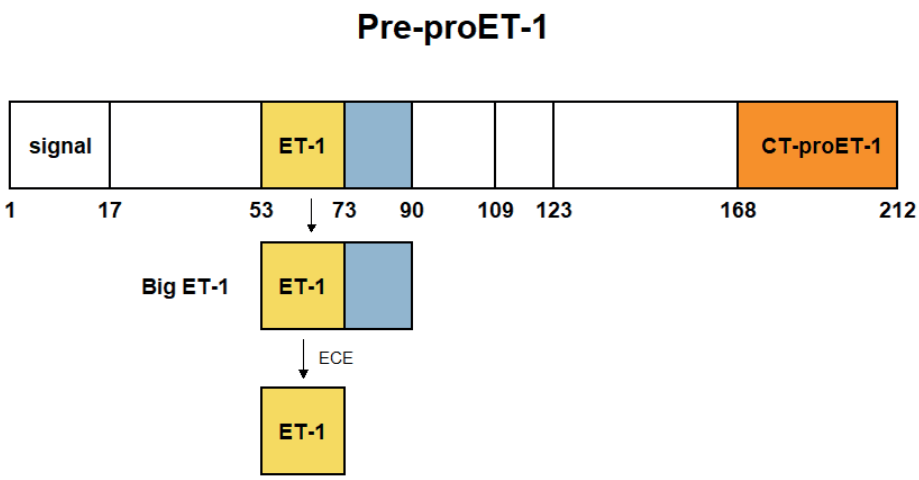

Figure 3. Pre-proendothelin-1 and the posttranslational processing to mature endothelin-1
Numbers indicate amino acids.

The binding of ET_A and ET_B receptors in vascular smooth muscle cells results in increased calcium levels rendering in vasoconstriction [123, 125]. In the endothelium, however, ET-1 binding to ET_B receptors leads to the release of vasodilatory agents such as NO and prostacyclin [127]. These two pathways can explain the initial hypotension followed by a later phase of hypertension when ET-1 is given to healthy human volunteers [128].

ET-1 has a short half-life (1-7 minutes) [129] in plasma and is almost totally removed after pulmonary passage. In humans, the lungs, kidneys and liver are rich in ET_B receptors [123], whence the internalization of receptor-ligand complexes in these organs, as well as the activity of endopeptidases, clears ET-1 from the circulation [130].

In order to overcome difficulties to measure ET-1, with the rapid clearance being a factor, a method to quantify a fragment of the prohormone, C terminal proET-1 (CT-proET-1), was described in 2006 [131]. The analyte was reported to be stable up to 4 hours in room temperature whereafter the concentrations decreased. Freezing and thawing up to 3 times had no influence on the measurement. The CT-proET-1 method is an immunoluminometric assay using antibodies and thereby shares many of the aspects of the MR-proADM assay. The patent is held by the same company (BRAMHS, ThermoFisher) as for MR-proADM, but the CT-proET-1 assay does not seem to have been commercially released.

Endothelin-1 as a biomarker

Few years after its discovery, an association of ET-1 and severity of illness among septic patients were reported [132]. Some later studies were able to confirm these findings, reporting association with severity as well as mortality [133], while others were not [134, 135]. When measured in a healthy population, a significant association between CT-proET-1 and age, left atrial size and diastolic blood pressure was seen [136]. Also, in septic patients, CT-proET-1 was associated with systolic left and right ventricle dysfunction [137].

Endothelin-1 as therapeutic target

Today three registered ET-antagonists exist - two non-selective ET_A and ET_B, and one ET_A selective, all of which are approved for pulmonary arterial hypertension [138]. The idea of these medications to be able to alter the immunological and circulatory response in sepsis has been put forward [125, 139] and explored in several animal studies with promising results. In pigs infused with endotoxins, ET-antagonism has been shown to improve hemodynamic parameters [140-143]. In CLP models of pigs and mice, the administration of ET-antagonists improved survival [144, 145].

High-sensitivity Troponin T

The cardiac troponins (cTn) are regulatory peptides in the contractile apparatus in myocytes and leakage of cTn into plasma, exceeding the 99th percentile, is interpreted as the result myocardial injury [146]. Elevated cTns have since 2000 been part of the myocardial infarction definition [147], but are not indicative for the mechanism of injury. Increased levels of cTn can be due to imbalance of oxygen supply and demand, cardiac conditions or systemic conditions [146].

Different generations of biochemical assays have been used over the years [148]. In 2010 a modification of the fourth-generation assay for cTn T, high-sensitivity troponin T (hsTNT) was presented [148]. HsTNT has a lower limit of detection of 5 ng/L and the 99th percentile of hs-TNT was 14 ng/L in healthy volunteers.

Elevated levels of cTn in critically ill patients and their association with poor outcome have been reported [149-151], but the role of these biomarkers in the care of critically ill sepsis or non-sepsis patients have not been fully elucidated [152-154].

Rationale

During the course of this dissertation, the importance of, and need for, better classification of sepsis patients has received increasing global scientific attention. The failure to find new treatments, in spite of theoretical soundness and initial promising basic scientific findings, in the care of heterogenous sepsis patients, warrants new research strategies. Biomarkers can be used in prognostic and predictive enrichment when developing new therapeutical pathways. This is a prerequisite for enhancing sepsis care, moving away from general un-specific broad treatment recommendations into the field of precision medicine.

Some biomarkers can themselves be part of hormonal systems involved in the dysregulated host response of sepsis and may be targeted in specific interventions.

Even though already highlighted as promising biomarkers of endothelial origin, the temporal development of ADM and ET-1 among critically ill patients was prior to Paper I poorly elucidated. Their relation to myocardial injury defined by both echocardiographic and biochemical factors had not been previously explored.

The sepsis definitions have changed and modern epidemiological reports from Swedish ICUs have been lacking. Due to a suspicion of severe underreporting of sepsis, a systematic manual review, presented in Paper II, of medical records was undertaken in order to properly identify and describe patients fulfilling the updated sepsis-3 definitions.

The assay measuring bio-ADM had not been evaluated in a large mixed general ICU population. Also, the sepsis differentiating properties of bio-ADM were completely unknown before Paper III.

The predictive properties of bio-ADM among sepsis patients in the ED had been sparsely reported before Paper IV.

Aims

The overarching aim of this dissertation was to explore the potential role of ADM as a biomarker among sepsis patients treated in the ICU and in a population originating from the ED.

Specific aims

In a cohort of 53 septic shock (sepsis-2) ICU patients:

- test whether MR-proADM and CT-proET-1 are associated with myocardial dysfunction, using transthoracic echocardiography, and myocardial injury, defined as impaired LV systolic function in conjunction with elevated hsTNT.
- describe the dynamics of MR-proADM, CT-proET-1, and hsTNT throughout the ICU stay.
- describe how early measurements of MR-proADM, CT-proET-1, and hsTNT are related to early mortality (day 7) and later mortality (day 28).
- assess whether a positive biomarker panel, consisting of MR-proADM, CT-proET-1, and hsTNT changes the odds for mortality.

In a cohort of 5990 adult ICU admissions:

- describe the fulfilment of sepsis-3 criteria.
- compare the prevalence of sepsis-3 criteria fulfilment with ICU discharge codes.
- describe the sepsis cohort (n=1654) in relation to suspected focus of infection, comorbidities, positive cultures and microbiological tests.

In a cohort of 1867 ICU patients of which 632 with sepsis (sepsis-3):

- assess the association of bio-ADM with 30-day mortality.

- investigate the association of bio-ADM with need for organ support, defined as cardiovascular SOFA ≥ 3 at ICU admission, and/or need for continuous renal replacement treatment (CRRT) during ICU stay.
- describe the ability of bio-ADM to predict sepsis.
- validate a proposed cut-off value of 70 pg/mL bio-ADM.

In a cohort of 597 sepsis patients (sepsis-2) in the ED:

- investigate the association of bio-ADM with 28-day mortality.
- assess whether bio-ADM could improve the prognostic precision of a mortality prediction model.
- compare the prognostic properties of bio-ADM with other commonly used biomarkers.
- investigate the association of bio-ADM with severe MOF, ICU admission (among patients without limitations of care) and ED discharge.

Materials and methods

Register based study methodology

All Papers included in this thesis, present findings from information gathered in databases or registers. The cohorts constituting the databases have been identified either before or after the outcome of interest has occurred. This feature is what differs *prospective* from *retrospective* studies [155].

Prospective vs retrospective registration

In prospective observational studies the participants are followed, and information gathered until the end of the study period, with the occurrence, or not, of the outcome of interest. This enables the recording of important information, such as presence of co-morbitities. The data collectors are often motivated to ensure high quality of data because of the clear purpose – to help in answering a scientific question.

In retrospective data collection, however, information is sought after in databases not created with the purpose of research, which increases the risk of missing values. Also, the databases may not contain warranted information, whence important confounders cannot be accounted for, and different types of biases nestle themselves into the interpretations [156, 157]. An example is the quality of data, which is threatened if the data entering lacks purpose and is felt as irrelevant to the data collector.

List of populations

SICU - Sepsis in the Intensive Care Unit

Data in the SICU cohort were prospectively collected between the years 2005-2007 and included 55 septic shock patients treated at the general mixed surgical and medical ICU at Skåne University Hospital in Malmö, Sweden. Written consent was sought from all participants or their next of kin, but failure to achieve this led to the exclusion of two patients. Other exclusion criteria were pregnancy, inherited abnormalities of coagulation, fibrinolytic therapy, compromised

immunity or a “Do not attempt resuscitation” order. Data collection continued until ICU discharge, death or up to maximum 7 days after admission. Seven and 28-day mortality were recorded. MR-proADM and CT-proET-1 were measured four times day 1, twice day 2 and once daily over the next up to five days. Hs-TNT was measured twice day 1 and once daily until end of study. Echocardiography was performed once daily.

SWECRIT

SWECRIT is a biobank consisting of 7567 admission samples, from 6499 unique individuals, 18 years or older, collected between 2015-2018 in Region Skåne, Sweden. All patients admitted to the four biggest (Malmö, Lund, Helsingborg and Kristianstad) general mixed surgical and medical ICUs, had admission blood samples routinely collected. The blood was centrifuged, aliquoted into 16 vials (8 plasma and 8 serum), frozen and stored in the SWECRIT biobank at Region Skåne (BD-47, SC-1922). The median time and interquartile range (IQR) from admission to sampling was 25 min (15–40). Samples collected later than 6 hours after admission were excluded. Information was given to the patient or next of kin, and information letters were sent home to surviving patients 2–6 months after hospital discharge. Patient consent was on an opt-out basis. For deceased patients, consent was presumed. Data were imported retrospectively into SWECRIT from the Swedish population registry and the patient administrative system for ICUs (PASIVA). PASIVA is the portal by which physicians and nursing staff prospectively submit laboratory and physiological data to the Swedish ICU registry. The biobank also contains blood samples from 120 healthy individuals.

SepCrit

SepCrit is a database containing 2528 primary ICU admissions fulfilling the sepsis-3 criteria. The patients were admitted to the same ICUs and during the same time span (2015-2018) as in SWECRIT.

All patients admitted to general ICUs in Region Skåne are registered in PASIVA, hence chosen as source when the SepCrit database was created. Trained data collectors manually reviewed medical records of all ICU admissions (n=7764) and identified those in which the patients fulfilled the sepsis definitions, as suggested by the sepsis-3 task force [7]. Inclusion criteria were 1) total SOFA $\geq$ 2 (baseline SOFA assumed to be zero) and 2) suspected infection in the time interval 24 h before and after ICU admission. Suspected infection was defined as obtainment of blood culture and concomitant administration of antibiotics (24 h before and 72 h after blood culture). If a patient was eligible for inclusion, the following parameters were collected; suspected site of infection, type of bacteria if positive culture, modified Charlson comorbidity index [158]. Septic shock was defined as

cardiovascular SOFA $\geq$ 3, or identification of vasopressor infusion in the review process and a lactate > 2 mmol/ml in sepsis patients.

Exclusion criteria were transfer from another ICU, cardiac arrest and elective ICU admission.

SepCrit contains both prospectively and retrospectively collected data.

Sepsis in the emergency department

This cohort was formed from patients, 18 year or older, who by screening of trained research nurses during office hours (6 AM to 6 PM, Monday to Friday) 2013-2015 were identified to fulfil inclusion criteria in the ED of Skåne University Hospital in Malmö, Sweden. The hospital has approximately 85000 emergency visits per year. The inclusion criteria were based on sepsis-2 and SIRS criteria; suspicion of infection and two or more of the following: 1) temperature below 36 °C, or higher than 38 °C, or self-reported fever/chills within 24 hours preceding the ED visit, 2) respiratory rate higher than 20 breaths/min, 3) heart rate higher than 90 beats/min. The fourth SIRS criteria white blood cell count was, due to unavailability at the time of screening, not used. Inclusion criteria were met by 647 patients but due to missing values among 53 individuals the final cohort size was 594. Demographics, comorbidities 28-day mortality, site of infection were collected prospectively from medical records and data were reviewed by infectious disease physicians. Blood samples were drawn within 1 h of ED presentation. Both oral and written consent was sought.

List of study cohorts

Paper I

The single-center prospective observational SICU cohort (n=53) was used in this study.

Paper II

This multi-center retrospective observational cohort study comprised the patients identified in SepCrit during the years of 2015-2017. Out of a total of 5990 ICU admissions during that time period, 1654 were identified to fulfil the sepsis-3 criteria.

Paper III

In this multi-center retrospective observational study, the cohort was formed by combining SepCrit and SWECRIT in that all admissions from 2016 (n=2724) in both databases were matched. After merging of data for patients with multiple ICU admissions due to transfers, 1867 primary ICU admissions with matched admission blood samples were identified. Out of these 632 fulfilled the sepsis-3 criteria.

Paper IV

In this single-center prospective observational cohort study the whole Sepsis in the ED population (n=594) was used.

Ethical considerations

All papers in this thesis have been ethically approved prior to their conduction (DNR 2005/187; DNR 2017/802; DNR 2015/267; DNR 2013/635).

Paper II and III have an un-usual consent procedure worth special attention. The study cohorts of paper II and III both consist of more than thousand critically ill patients admitted to the ICU who, by definition, are suffering from a life-threatening condition, affecting the ability to both receive and process information, and give or refuse their consent. Informed consent prior to inclusion would make the conduction of SWECRIT, with the purpose to collect ICU admission blood samples, practically impossible.

Therefore, the opt-out procedure where survivors after their hospital discharge made it possible to withdraw their participation, was put in place. An information letter was sent to participant's home address with contact details to research nurses

and instructions on how to be removed from the records. Individuals who were not reachable due to lack of address were excluded from the study.

One could argue that ICU patients are particularly vulnerable and therefore not should be included in medical research at all. However, since the knowledge from this patient category, would not be acquirable from another category, more capable of giving their informed consent, these types of studies are still justified [159].

Statistics

This thesis relies upon quantitative numerical tests in order to describe differences, associations and predictions among different groups. P-values < 0.05 were considered significant. Examples of tests used in all Papers are Wilcoxon rank-sum test (Mann-Whitney U test) for continuous variables, Pearson's χ^2 test for differences in proportions. For adjustment for multiple testing Holm´s procedure was used. Predictive properties were evaluated with the calculations of area under the receiver operating characteristic curves (AUROC). Sensitivity, specificity, negative and positive predictive values and Youden derived cut-offs were reported when relevant.

In Paper I the statistical software IBM SPSS Statistics version 22 was used, while RStudio was software of choice in Paper II-IV.

Statistical methods specific for the individual Papers are listed below.

Paper I

Spearmans rank correlation, positive and negative likelihood ratios were reported.

Paper II

Differences in standardized mortality ratios were assessed using a permutation test with 5000 permutations.

Paper III & IV

Transformation of skewed parameters with base 2 logarithm. Kruskal-Wallis test for comparison of more than two groups. Kaplan-Meier graphs with rank sum test. Uni- or multivariable logistic regressions. Hosmer-Lemeshow goodness-of-fit test for evaluation of regression models. Differences in AUROCs tested with DeLong’s test.

Results

Sepsis in the ICU

Sepsis was found to be a common condition in Swedish ICUs. The proportions of sepsis-3 fulfilment among ICU admissions were 28% and 34% in Paper II and Paper III, respectively. The incidence of sepsis requiring intensive care was 81 per 100 000 person-years [Paper II].

Of all patients fulfilling the sepsis-3 criteria at admission, 31% had sepsis as main ICU discharge diagnosis [Paper II], see Figure 4.

Sepsis patients were older and sicker than non-sepsis patients with higher severity and organ failure scores at ICU admission [Paper III]. Further, septic patients had higher need for organ support and higher mortality rates than non-septic patients [Paper III].

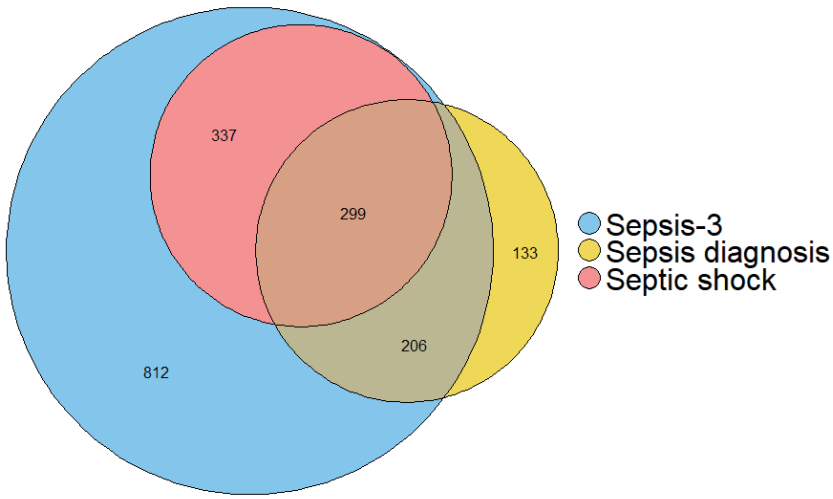

Figure 4. Venn diagramme showing the concurrency between sepsis-3 criteria fulfilment and sepsis diagnosis

Forty-four percent of the sepsis patients tested culture negative. In culture-positive sepsis the three most common pathogens were E. coli, Staph. aureus and Kleibsiella sp [Paper II]. Patients with positive blood cultures presented with higher severity and organ failure scores and had longer lengths of stay, but did not differ in mortality rates compared with culture negative sepsis patients [Paper II].

The observed 30-day mortality rates (24-27.5%) among patients with sepsis at admission were slightly lower than expected [Paper II, III].

Almost half of all sepsis ICU admissions (44-45%) originated from the ED [Paper II, III].

Adrenomedullin in the ICU

The distribution of ADM at admission, measured with the method of bio-ADM, in a general ICU population was highly skewed, see Figure 5.

For both sepsis and non-sepsis patients, bio-ADM exhibited significant association with 30-day mortality and need for organ support, in the form of vasopressor therapy and/or CRRT. Each log-2 increment of bio-ADM conferred age adjusted odds ratios (OR) between 1.22-2.28 for mortality and need for organ support.

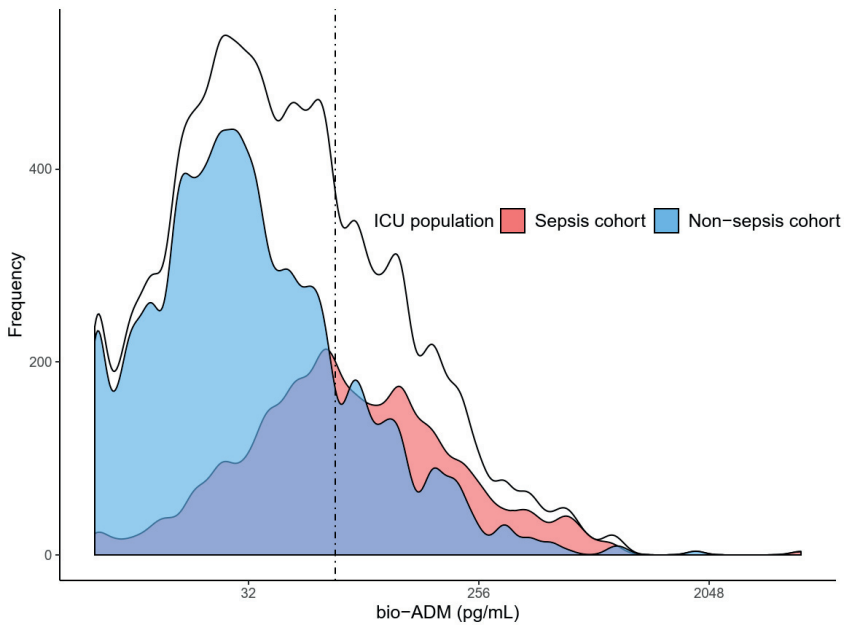

Figure 5. Distribution of bio-ADM in the ICU population, Sepsis cohort and Non-sepsis cohort
X-axis logarithmic with base 2. The dotted line represents the concentration of 70 pg/mL.

When adjusted for the severity of disease, bio-ADM was significantly associated with sepsis and septic shock, both with corresponding ORs of 1.78.

A cut-off value of 70 pg/mL bio-ADM was able to separate survivors from non-survivors, but in the sepsis cohort the Youden´s index derived cut-off was 108 pg/mL bio-ADM, see Figure 6 [Paper III].

In septic shock patients in the ICU, admission levels of ADM, measured indirectly with MR-proADM, were associated with 7- and 28-day mortality and myocardial injury, but less so with myocardial dysfunction. Non-survivors had higher concentrations of MR-proADM during day 1-3 of admission, see Figure 7a [Paper I].

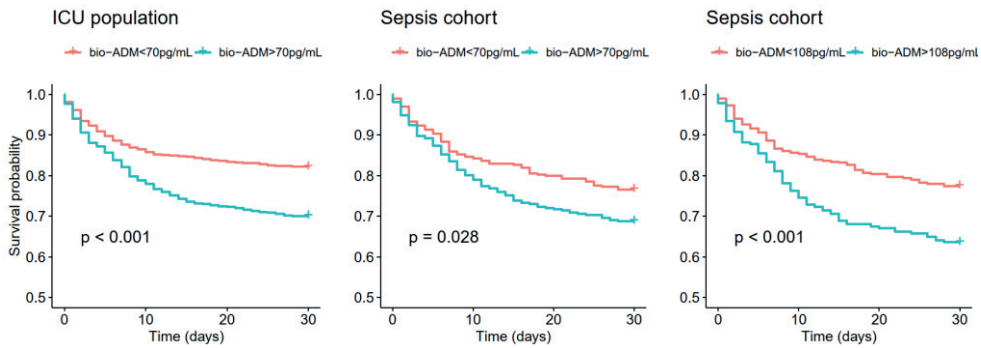

Figure 6. Kaplan-Meier curves for ICU population and the sepsis cohort according to two bio-ADM cut-offs
P-values derived from the log-rank test.

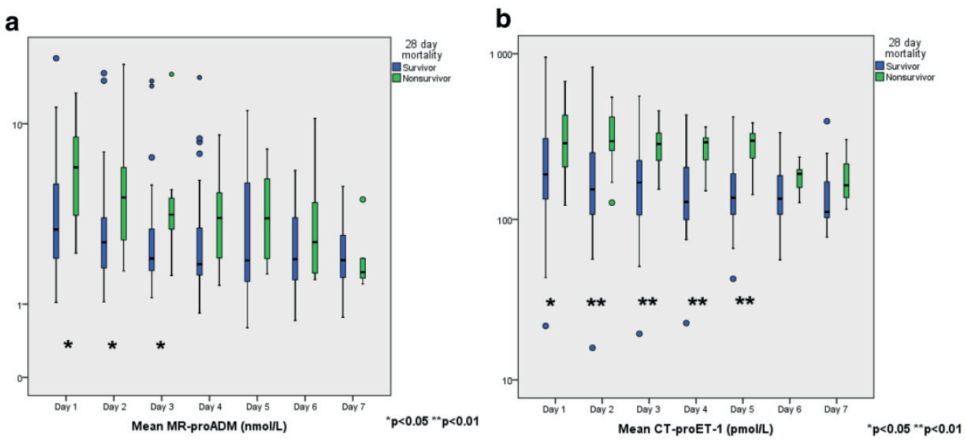

Figure 7. Temporal development of MR-proADM (a) and CT-proET-1 (b) during the first 7 days of ICU admission among septic shock 28-day survivors and non-survivors
P-values derived from the Wilcoxon rank-sum test.

Adrenomedullin in the ED

Bio-ADM was associated with 28-day mortality, severe MOF, ICU admission and ED discharge in a general ED sepsis population, with adjusted ORs of 2.39, 3.30, 1.75 and 0.46, respectively. When bio-ADM was added to a mortality prediction model consisting of age, body mass index, previous cardiovascular disease, sites of infection and the commonly used biomarkers lactate, CRP and creatinine, the prognostic capability improved significantly, see Figure 8 [Paper IV]. Patients admitted to the ICU within the ED population presented similar levels of bio-ADM, as the sepsis ICU patients in Paper III.

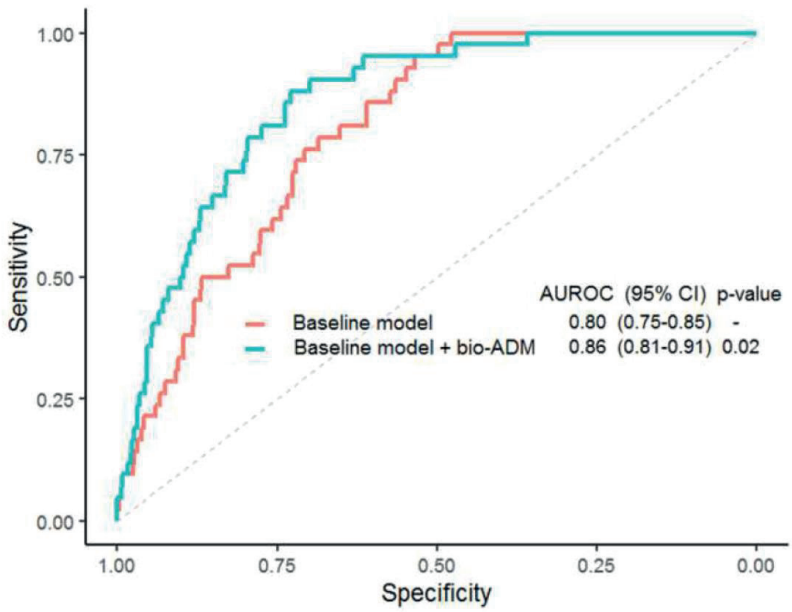

Figure 8. Receiver operating characteristics curves for 28-day mortality predictive models in ED
Baseline model with covariates age, known cardiovascular, body mass index, upper respiratory tract, urinary and pulmonary site of infection, C-reactive protein, lactate and creatinine. The additive value of bio-ADM is shown in Baseline + bio-ADM. The p-value is derived from the DeLong's test for comparison between the two AUROCs.

Adrenomedullin and other biomarkers

Levels of ET-1, measured indirectly with the method of CT-proET-1, were significantly higher among 28-day non-surviving septic shock patients. The elevation of CT-proET-1 remained high longer (1-5 days) than MR-proADM, see Figure 7b. Levels of CT-proET-1 correlated significantly with both myocardial dysfunction and injury [Paper I].

For septic shock patients, a combination of MR-proADM, CT-proET-1 and hsTNT, in a biomarker panel, increased the positive LR for mortality 13 to 20-fold [Paper I].

In the ICU sepsis cohort, bio-ADM seemed to carry additional information, not captured by lactate, in relation to 30-day mortality [Paper III].

Bio-ADM showed superior predictive properties in relation to 28 day-mortality than lactate, CRP and creatinine in the sepsis ED cohort, see Figure 9 [Paper IV].

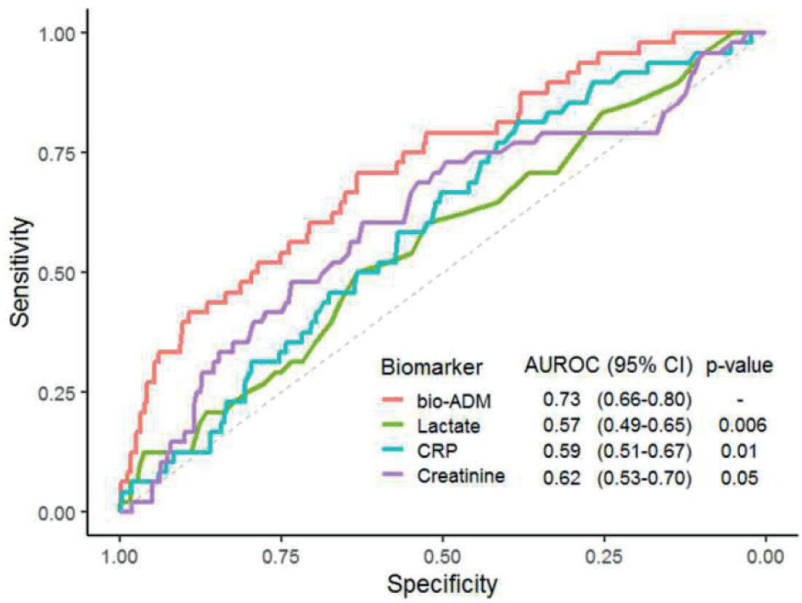

Figure 9. Receiver operating characteristics curves for 28-day bio-ADM and routine biomarkers in ED
Only patients with all four biomarkers analyzed were included. P-values are derived from the DeLong's test for comparison with the AUROC of bio-ADM.

Main results

- Sepsis-3 criteria fulfilment is common in primary ICU admissions.
- Discharge diagnosis codes agree poorly with criteria-based sepsis.
- ADM, measured either with MR-proADM or bio-ADM, is associated with mortality, organ failure and need for organ support in septic shock, sepsis and non-sepsis patients alike.
- Bio-ADM levels in an ED sepsis setting are associated with mortality, organ failure, ICU admission and ED discharge.
- ADM adds prognostic value to commonly used factors as age, comorbidities, site of infection and commonly used biomarkers as lactate, CRP and creatinine as well as other biomarkers as CT-proET-1 and hsTNT.
- Bio-ADM is associated with sepsis at ICU admission, also after adjustment for severity of disease.

Discussion and future directions

Sepsis in the ICU

Our large manual review of medical records in Paper II confirms previous reports on the underestimation of sepsis when relying on diagnosis codes [160, 161]. Less than one third (31%) of the patients who at admission fulfilled the sepsis-3 criteria had a main diagnosis of sepsis at ICU discharge.

It has been pointed out that sepsis in PASIVA could have been registered as a secondary diagnosis [162], but the addition of these secondary sepsis codes only moderately increased coherent coding from 31% to 39% [163].

Even though our strategy strikingly reveals the underreporting of sepsis, it probably still severely underestimates the true incidence of sepsis in the ICU, since we only focused on sepsis at admission. This was a necessary step in order to, in Paper III, be able to link admission blood samples to admissions with sepsis. The development of secondary infections is common (13-15%) among patients in the ICU, regardless if admitted with or without sepsis [164]. Some patient categories seem to be more vulnerable than others. For example, one in five severely injured trauma patients, will after the initial non-infectious insult develop sepsis during the following 30 days of ICU admission [165].

Further, it has been pointed out that sepsis diagnose coding from the ICU often not are transferred in sepsis related hospital discharge codes diminishing reported incidences of sepsis even more [166].

A limitation to our method to estimate sepsis incidence is related to the fact that medical treatment, especially in the care of critically ill patients, often is given with multiple parallel diagnoses in mind. A patient's condition might be so severe that treating physicians do not want to risk a yet un-identified infection untreated, which is reasonable bearing in mind that sepsis diagnosing can be both difficult and subjective [50, 51]. The administration of antibiotics in combination with culture sampling will, in accordance with current recommendations [7], fulfil the criteria for suspected infection and this, in combination with elevated SOFA scores, renders the patient "to have sepsis" even though the treating physician does not immediately considers the patient "to be septic". This discrepancy between fulfilling the sepsis criteria and what by medical staff is considered "being septic" is not new, exemplified in the sepsis-2 publication [5] where a list of diagnostic

criteria is given, shown in Table 2, in spite of a different sepsis definition (infection and 2 $\geq$ SIRS criteria). Since Paper II is retrospective in nature, we do not have information regarding the subjective rated likelihood or suspicion of sepsis at ICU admission.

Adrenomedullin as a biomarker

ADM is one of more than 250 biomarkers so far identified to be related to sepsis [67]. Endothelial dysfunction and vasoplegia, two key areas within the pathology of sepsis, are both moderated partly by ADM [69, 76, 105, 108, 109]. At the same time, both endothelial and vascular smooth muscle cells are known to increase their secretion of ADM upon activation in sepsis [69]. Whence the cardiovascular system is both the producer as well as the target of ADM. Additionally, ADM seem to simultaneously carry both potentially beneficial and harmful properties. This complexity has led to ADM being described as a double-edged sword in relation to sepsis [167]. Exogenous ADM is protective in animal sepsis models, exerting endothelial stabilizing effects, while ADM itself is a potent vasodilator. The question raised by Struck et al. [113], whether the elevated levels of ADM in sepsis should be seen as part of the raging "fire" of the dysregulated immune response, or, on the contrary, as the dispatch of "firemen", is legitimate.

Most studies on bio-ADM have explored its performance and role in selected patients with sepsis [85, 102, 103, 168] or heart failure [88, 90, 169]. Fewer results are available from un-selected populations, as patients with dyspnoea [170] or those admitted to the ICU [171], which are important to be able to interpret the applicability of biomarker in a clinically relevant setting. Paper III belongs to the latter type of studies since we measured bio-ADM in a general un-selected ICU population at admission. Even though bio-ADM after adjustment for severity of disease was associated with sepsis, the correlation to need for organ support and mortality was significant also among non-sepsis patients, opening up for a broader utility of the biomarker.

As almost half of the sepsis admissions in Paper II and III (44-45%) were admitted from the ED, the elaboration on bio-ADM's ability in the ED is important. Rapid identification and stratification of sepsis are recognized as crucial factors for patient outcome [32, 49]. The patients admitted to the ICU in the ED population in Paper IV exhibited similar levels of bio-ADM as the sepsis ICU patients described in Paper III, indicating that bio-ADM can be of use as an ICU admission indicator, for older and updated sepsis definitions alike.

However, *ICU admission* is not a defined and universal entity as this depends on local traditions and factors as availability to intermediate care and ICU beds per capita.

The cut-off 70 pg/mL, presented in the very first paper on bio-ADM [85], deserves to be addressed. Marino and colleagues, reported findings of bio-ADM measured at ED admission and during the four following days in a cohort of 101 patients. In a subgroup (n=40) who had admission levels of bio-ADM > 70pg/mL, none of the patients (n=12) whose levels had dropped to below 70 pg/mL at day 4, died within 28 days. In comparison, the group of patients who at day 4 continued to stay at levels above 70 pg/mL (n=28) had a 28-day mortality of 36% (n=18). Based on the findings above, it might seem reasonable, and in this first presentation of the biomarker also feasible, to use such a cut-off. However, from a statistical and methodological point of view, it lacks support. The authors themselves present the cut-off as an example of *a cut-off* and warrant further validation, which, with few exceptions [103, 171], has been sparsely performed. Paper III does explore this, and in conjunction with others, Youden's index derived cut-offs seem to point towards a higher concentration. Mebaaza et. al reported a Youden index cut-off of 102 pg/mL bio-ADM while we found the corresponding value 108 pg/mL [Paper III]. The 70 pg/mL cut-off does exert prognostic value, also in our material. Overall, depending on different contexts – what kind of population it is applied to, and what consequences a concentration above or below a certain value might have, different cut-offs may be more optimal. Many biomarkers used in clinical practise today are useful not as a single measurement but merely as part of a trend and are always related to other clinical information.

Regardless, if ADM is a friend or a foe, above or below a certain absolute value, the results presented in this thesis add to existing evidence of association with severity of, and mortality in, sepsis and potentially in general populations.

Compound biomarker panel

In Paper I it is shown how biomarkers can be combined to increase predictive precision and risk stratification, as MR-proADM, CT-proET-1 and hsTNT together increased the posttest odds up to 20-fold. Also, Paper IV shows how the addition of a biomarker to other known predictive factors and other biomarkers can increase the AUROC of a predictive model, see Figure 8.

More advanced methods, often incorporating machine learning and large quantitative of clinical and non-clinical data, are increasingly described in order to better understand sepsis. These methods have three main areas of applications [60]. Firstly, to explain different pathways of pathophysiology and thereby being of aid in future treatments. Secondly, to improve diagnostics and personalized care. Thirdly, identify different clusters within the sepsis syndrome.

When the number of measured variables exceeds the number of samples, the data are regarded as high-dimensional [60]. Areas exploring the -omics field

(transcriptomics, proteomics and metabolomics) are examples of when non-clinical data form the platform [58]. Clinical data, as demographics, vital signs and biochemical biomarkers have also been used for the development of different sepsis phenotypes [59].

Still, the application of these findings incorporating, sometimes myriads of, for clinicians often unknown, variables and powerful computer processing, into clinical practise has not yet been possible. The sepsis research issues needs be addressed from multiple angles, but how to apply and use results from high-dimensional data continues to be a great challenge.

Future directions

Adrenomedullin at the bedside

The recently developed point-of-care platform in which a small amount of whole blood (500 µL) without prior preparation, is placed in a CD/DVD/Blue-ray like disc analysed in a fully automated device (Nexus IB10[1]) gives the answers of up to three different biomarkers within 20 minutes, see Figure 10. Bio-ADM is available with this technology making the application of the biomarker possible in close proximity of patient care even without access to a 24/7 laboratory.

The role of a rapidly available bio-ADM concentration at the bedside and the implications this could have on triaging and patient care, could be a future direction.

Figure 10. Medium in which full blood is applied before analysis in the portable Nexus IB10
The point of care platform makes a bio-ADM concentration availible within 20 minutes.

[1] https://sphingotec.com/solutions/nexus-ib10-point-of-care-technology

Adrenomedullin as therapeutic target

As previously mentioned, scientific work has been done on humans modulating the ADM system with the non-neutralizing antibody Adrecizumab [115-117]. A phase III study (with the acrynome *ENCOURAGE-1*) is in planning and the preliminary setup was presented at the ESICIM LIVES conference in 2021 [119].

Precision medicine in sepsis

In order to overcome the lack of specific treatment progress in sepsis, the introduction of predictive and prognostic enrichment can be a promising and necessary step to take [39, 52, 56-59].

An example where different pathological pathways, represented by their corresponding biomarkers, could influence clinical trial design was presented in a recent paper by van Lier et. al. [172]. They suggest the differentiation of septic patients depending on their levels of bio-ADM and another biomarker and enzyme, circulating dipeptidyl peptidase 3 (cDPP3) [173].

Dipeptidyl peptidase 3 (DPP3) is a peptidase normally mainly located intracellularly in the cytoplasm [174], but can also be measured in plasma [173]. DPP3 has a high affinity for, and effectively cleaves, angiotensin-II [175, 176]. Elevated levels of cDPP3 have been shown to be associated with organ dysfunction [177]. The release of DDP3 into the blood stream and the subsequent cleavage of angiotensin-II leading to hypotension, has been suggested to constitute a pathological pathway in sepsis [173].

Further, van Lier and colleagues present results from a cohort (n=583), presented in the ADRENOSS-1 study [103], where levels of bio-ADM and cDDP3 are combined [172]. They report a separation in mortality between sepsis and septic shock patients at ICU admission with different combinations of high/low levels of bio-ADM and cDDP3. Since cDPP3 is available in our cohort of Paper III (n=632), a validation of these findings would be possible.

The phase-III study of Adrecizumab, *ENCOURAGE-1,* already mentioned [119], was presented to incorporate this strategy to differentiate sepsis patients according to bio-ADM and cDDP3. The study design was presented as a randomization of septic shock patients, with bio-ADM levels above 70 pg/mL and cDDP3 below a certain threshold, to either placebo or administration of Adrecizumab. This could be an example of a long wanted tailored treatment of sepsis.

Contact with the investigators of *ENCOURAGE-1* has already been made, offering our participation in the study.

Conclusions

- Discharge diagnosis codes agree poorly with criteria-based sepsis and should not be used to classify sepsis for quality control or for research purposes.
- Increased levels of ADM are associated with increased morbidity and mortality in sepsis both in ICU and ED settings.
- ADM may, in conjunction with other biomarkers, or alone, be used in predictive and prognostic enrichment when developing sepsis therapy into the field of precision medicine.
- Bio-ADM may be a specific sepsis marker and be of clinical importance for triage of sepsis patients in the ED.

Populärvetenskaplig sammanfattning

Om man är sjuk - hur sjuk är man?

När man själv, eller någon man håller nära, är sjuk vill man snabbt avgöra hur allvarligt läget är. Tänk om man med hjälp av en enda undersökning skulle kunna avgöra om man lugnt kan vila ut i hemmet - bara ta igen sig - eller om man på snabbaste sätt ska till sjukhuset och rakt in på intensiven?

Om man är sjuk – hur är man sjuk?

Att tänka på vad det är som orsakar en sjukdom är naturligt. När vi blir sjuka efter att ha blivit smittade eller på något sätt fått ett virus eller en bakterie i oss, brukar man föreställa sig att dessa små organismer har brutit sig igenom kroppens försvar och orsakar stor skada på insidan. I själva verket är det vår egen kropps motreaktion, kroppens immunförsvar som, om det hamnar i obalans, utgör det största hotet!

Om man är sjuk – behöver man behandling och i så fall vilken?

Ibland gör en behandling nytta, ibland gör den varken till eller från och ibland är den tvärt emot farlig. För att kunna utveckla effektiva behandlingar måste man utifrån idéer och rådande kunskapsläge prova sig fram. Genom försök på djur och människor får man, under kontrollerade former, testa om till exempel ett nytt läkemedel gör nytta. Är man dock inte sjuk på ett sätt som gör att en specifik medicin kan göra nytta, är det ologiskt att ens testa den.

~ ֍ ~

Genom att undersöka och beskriva blodprovsnivåer av ett ämne som finns naturligt i kroppen, berör den här avhandlingen alla tre frågor ovan.

Ämnet som studerats i detalj heter adrenomedullin och är ett hormon som hos både friska och sjuka personer är involverat i en mängd olika mekanismer i kroppen. Bland annat påverkar adrenomedullin hur mycket muskellagren i våra blodkärl drar ihop sig. Dessutom medverkar adrenomedullin i regleringen av hur täta blodkärlen är – hur mycket eller lite vätska som sipprar ut från blodet i de minsta blodkärlen.

När en mikroorganism aktiverar immunförsvaret i den grad att kroppen, av försvarsmekanismerna själv tar skada, inträder ett tillstånd som heter sepsis (i

folkmun ”blodförgiftning”). Sepsis har kallats en okänd folksjukdom och fler drabbas av sepsis i Sverige per år än av de tre vanligaste cancerformerna tillsammans. Sepsis kan drabba såväl friska, som sjuka personer, gamla och unga. Dödligheten är hög och tillståndet kan ha världsomfattande konsekvenser vilket COVID-19 pandemin är ett tydligt exempel på.

I den allvarligaste formen av sepsis, septisk chock, har kroppen svårt att behålla ett normalt blodtryck. Blodtrycksfallet orsakas av att blodkärlen blir slappa samtidigt som mycket vätska läcker ut ur blodkärlen. Dessa två sjukdomsmekanismer är därför tätt relaterade till adrenomedullins reglerande egenskaper.

Det kan vara svårt att känna igen sepsis eftersom symptomen kan vara diffusa och likna dem vid andra sjukdomar. Det är också svårt att förutsäga hur det kommer att gå för den enskilda patienten och därmed bestämma lämplig plats för fortsatt vård – i hemmet, på sjukhus och i så fall på vilken slags avdelning. De allra sjukaste patienterna vårdar man på intensivvårdsavdelingar.

Delarbeten I, III och IV i denna avhandling beskriver hur förhöjda halter av adrenomedullin ser ut att hänga ihop med ökad dödlighet och ökad sjuklighet hos patienter som vårdas på intensivvårdsavdelningar såväl som hos dom som söker sig till akutmottagningen. Om adrenomedullinnivåerna är låga, ser risken för att fara illa ut att vara lägre. I delarbete IV beskrivs hur personer med sepsis som sökt till akutmottagningen, men som ansågs inte behöva sjukhusvård och kunde gå hem, hade lägre halter av adrenomedullin. De som däremot behövde vård på intensivvårdsavdelning eller utvecklade grav multiorgansvikt hade högst koncentrationer.

Vår kunskap om sepsis är inte så detaljerad som man hade önskat. Det beror på att sepsis egentligen är många olika sjukdomar. Många olika slags bakterier och virus belastar kroppen på olika sätt beroende på var de finns. Vi människor är också olika då vår genetik och tidigare sjuklighet gör oss mer eller mindre sårbara. Eftersom adrenomedullin har egenskaper som verkar kunna spela stor roll vid sepsis, kan ökad kunskap om hormonet hjälpa oss förstå hur vi blir sjuka.

Om man utifrån mängd av adrenomedullin i blodet kan dela in sepsispatienter i olika grupper, kan man pröva olika specifika behandlingar som denna grupp skulle kunna ha nytta av.

Sammanfattningsvis pekar resultaten i denna avhandling mot att:

- Adrenomedullin kan, ensamt eller i kombination med annan information, användas för att förutspå *hur sjuk man är*.
- Adrenomedullin kan vara delaktig i sjukdomsmekanismer, särskilt de som ses vid sepsis – alltså *hur man är sjuk*.
- Adrenomedullin kan hjälpa till att dela in sjuka personer i grupper som kan *behöva en specifik behandling*.

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My deepest gratitude goes to my supervisor, **Hans Friberg**, whose appearance in Malmö was pivotal and revolutionized both my personal research career as well as the entire clinical research climate within our department. **Hans's** optimistic, including and welcoming research strategy in combination with him being a master of collaboration have shown to be both nationally and internationally fruitful.

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Despite the fact that themselves, during times of no acceptance of dyslexia had no access to any form of higher education, my parents, **Ebbe** and **Tarja**, raised four children - **Erik**, **Pia**, **Carl** and myself - who all, sooner or later, have pursued academic educations. They accomplished this unplanned and seemingly effortless without ever being demanding. This thesis, which is the second among the siblings, I dedicate to my beloved Mother, who is still with us but sadly and slowly is sliding away, dragged by the arms of Alzheimer's disease.

My wife **Frida** for, over a decade now, being in my life offering love, companionship and support in both hard and joyful times.

To be a parent, of **Viking** and **Astrid**, together with **Frida** is the biggest achievement in my professional, educational and personal career.

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Papers I-IV

Paper I

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RESEARCH Open Access

Adrenomedullin and endothelin-1 are associated with myocardial injury and death in septic shock patients

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Abstract

Background: Adrenomedullin and endothelin-1 are hormones with opposing effects on the cardiovascular system. Adrenomedullin acts as a vasodilator and seems to be important for the initiation and continuation of the hyperdynamic circulatory response in sepsis. Endothelin-1 is a vasoconstrictor and has been linked to decreased cardiac performance. Few studies have studied the relationship between adrenomedullin and endothelin-1, and morbidity and mortality in septic shock patients. High-sensitivity troponin T (hsTNT) is normally used to diagnose acute cardiac injury but is also prognostic for outcome in intensive care. We investigated the relationship between mid-regional pro-adrenomedullin (MR-proADM), C-terminal pro-endothelin-1 (CT-proET-1), and myocardial injury, measured using transthoracic echocardiography and hsTNT in septic shock patients. We were also interested in the development of different biomarkers throughout the ICU stay, and how early measurements were related to mortality. Further, we assessed if a positive biomarker panel, consisting of MR-proADM, CT-proET-1, and hsTNT changed the odds for mortality.

Methods: A cohort of 53 consecutive patients with septic shock had their levels of MR-proADM, CT-proET-1, hsTNT, and left ventricular systolic functions prospectively measured over 7 days. The relationship between day 1 levels of MR-proADM/CT-proET-1 and myocardial injury was studied. We also investigated the relationship between biomarkers and early (7-day) and later (28-day) mortality. Likelihood ratios, and pretest and posttest odds for mortality were calculated.

Results: Levels of MR-proADM and CT-proET-1 were significantly higher among patients with myocardial injury and were correlated with left ventricular systolic dysfunction. MR-proADM and hsTNT were significantly higher among 7-day and 28-day non-survivors. CT-proET-1 was also significantly higher among 28-day but not 7-day non-survivors. A positive biomarker panel consisting of the three biomarkers increased the odds for mortality 13-fold to 20-fold.

Conclusions: MR-proADM and CT-proET-1 are associated with myocardial injury. A biomarker panel combining MR-proADM, CT-proET-1, and hsTNT increases the odds ratio for death, and may improve currently available scoring systems in critical care.

Keywords: Sepsis, Shock, Adrenomedullin, Endothelin-1, High-sensitivity troponin, Echocardiography, Myocardial injury, Mortality, Likelihood ratio

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Background

Circulatory failure is one of the most severe manifestations of early sepsis. Whilst numerous studies have investigated novel biomarkers to diagnose and risk-stratify patients with sepsis, none have become universally accepted and few have focused on the circulatory system per se. As septic shock still accounts for an unacceptable number of deaths in the critically ill, we reasoned that a biomarker strategy using a combination of clinical, biochemical, and physiological parameters focusing on the circulatory system may be one way of stratifying very high-risk patients.

Endothelial activation is a hallmark of sepsis and thought to play a key role in the pathophysiology of septic shock. In this regard, three novel biomarkers have been described that may have contributory and/or predictive roles in the development of circulatory failure – mid-regional pro-adrenomedullin (MR-proADM), C-terminal pro-endothelin-1 (CT-proET-1), and high-sensitivity troponin T (hsTNT).

Adrenomedullin (ADM) is a 52-amino acid peptide hormone, which is associated with cardiovascular, endocrine, and renal mechanisms that control fluid and electrolyte homeostasis [1]. ADM acts as a vasodilator, decreases peripheral vascular resistance, and increases cardiac output [2, 3]. ADM also decreases capillary hyperpermeability during septic shock [4, 5]. Because of the instability of the peptide, it has been shown that measurements of the mid-regional portion of the precursor peptide pro-adrenomedullin, is more suitable for clinical practice [6]. Few clinical studies have described ADM in septic shock. In the largest study to date, Guignant et al. [7] showed that increased plasma MR-proADM was associated with 28-day mortality.

Endothelin-1 (ET-1) is a 21-amino acid peptide, which acts as a potent vasoconstrictor and has mitogenic effects on smooth muscle cells. ET-1 has been shown to be involved in multiple physiological functions related to the nervous, renal, cardiovascular, respiratory, gastrointestinal, and endocrine systems [8]. Because of its short half-life (1–7 minutes) [8, 9], and almost total clearance from the blood stream by pulmonary passage, CT-proET-1 has been found to stoichiometrically measure ET-1 [9].

Cardiac troponin (cTn) is the preferred marker of myocardial ischemia and injury [10]. New high-sensitivity troponin assays have, by detecting extremely low levels, been associated with conditions other than myocardial infarction and predict worse outcome in intensive care [10–16]. As both ADM and ET-1 are potent vasoactive factors it is also plausible that they may be associated with myocardial dysfunction in sepsis [17–19]. This has been sparsely investigated in intensive care.

The aim of this study was to test whether MR-proADM and CT-proET-1 are associated with myocardial injury, measured using transthoracic echocardiography and hsTNT in patients with septic shock. We were also interested in the dynamics of MR-proADM, CT-proET-1, and hsTNT throughout the ICU stay, and how early measurements (day 1) were related to early mortality (day 7) and later mortality (day 28). Further, we assessed whether a positive biomarker panel, consisting of MR-proADM, CT-proET-1, and hsTNT changes the odds of mortality.

Methods

The study was approved by the Regional Ethical Review Board, Lund, Sweden (Dnr.187/2005). Informed consent was sought either from the patient or, if not possible, from the patient's next of kin. The study design comprised a single-center, prospective observational cohort of critically ill patients admitted to the mixed-bed ICU of Skåne University Hospital, Malmö, Sweden. Data collection lasted up to a maximum of 7 days, or until ICU discharge, or death if either occurred before 7 days. Early (7-day) and later (28-day) mortality was measured. Fifty-five consecutive patients with septic shock were included between year 2005 and 2007. Septic shock was defined according to the criteria published by Dellinger et al. [20]. Exclusion criteria were pregnancy, inherited abnormalities of coagulation, fibrinolytic therapy, compromised immunity or a "Do not attempt resuscitation" order. Patients could be included only once. All patients were initially treated according to international guidelines for the management of sepsis and septic shock [21]. After the initial resuscitation period, fluids were given at the treating clinician's discretion. Acute physiology and chronic health evaluation (APACHE) II scores were calculated at admission and sequential organ failure assessment (SOFA) scores were calculated daily.

Biochemical analyses

Blood samples were collected from an indwelling arterial line. MR-proADM and CT-proET-1 were measured four times on day 1 (first sample within 6 hours of arrival to the ICU), twice on day 2, and thereafter once daily until ICU discharge, death or end of study. HsTNT was measured twice on day 1 (first sample within 12 hours of arrival to the ICU) and thereafter once daily until ICU discharge, death or end of study. The daily values of all biomarkers were averaged to give a single representative value for that day. The blood samples were sent to the local clinical chemistry laboratory, Skåne University Hospital, Malmö, Sweden, where they were centrifuged, frozen at −80 °C, and stored.

MR-proADM and CT-proET-1 were batch-analyzed using a sandwich immunoassay (BRAHMS GmbH/ThermoFischer Scientific, Henningsdorf, Germany). In the general population, 90 % of measurements of MR-proADM

are below 0.55 nmol/L [22] and the 99th percentile of CT-proET-1 in a healthy population is 72.9 pmol/L [9]. The analytical detection limits of MR-proADM and CT-proET-1 were 0.08 nmol/L and 4.3 pmol/L. HsTNT was measured using an immunoassay (Cobas e601, Roche Diagnostics GmbH, Penzberg, Germany) [23]. The measurement range is 3–10,000 ng/L and the upper reference limit (99th percentile) is 14 ng/L in healthy volunteers.

Echocardiography

TTE examinations were performed within 12 hours of inclusion for the evaluation of left ventricular (LV) systolic function. Images were acquired using a Hewlett- Packard Sonos 5500 (Andover, MA, USA) scanner and a 3 MHz transducer. Two-dimensional (2D) imaging examinations were performed in the standard apical four-chamber and two-chamber views. Tissue harmonic imaging was used to enhance 2D image quality. Parameters of LV systolic function (left ventricular ejection fraction (LVEF), mitral annular plane systolic excursion (MAPSE), peak systolic tissue Doppler velocity imaging (TDIs) and velocity time integral in the left ventricular outflow tract (LVOT VTI)) were acquired as described previously [24].

Myocardial injury

Myocardial injury was defined as an hsTNT value ≥15 ng on day 1 and at least two of the following echocardiographic parameters on day 1: LVEF ≤50 %, MAPSE ≤12 mm, or TDIs ≤7.5 cm/sec.

Statistics

A sample size of 46 patients was required to detect a posttest myocardial injury risk of 0.75, assuming a baseline risk of 0.3. This was calculated as a test of proportions with a two-tailed α value of 0.05 and β of 0.8, with a continuity correction applied. As we expected dropouts we arbitrarily chose to increase the sample size to a convenience sample of 55 patients.

Data are presented as median (interquartile range), percentages or absolute values. IBM SPSS Statistics version 22 was used for statistical calculations. For non-normally distributed variables we used non-parametric tests. Missing values were considered as randomly missing and were not adjusted for. Spearman's rank correlation was calculated to test correlation between two variables, and for differences between two groups we used the Mann-Whitney *U* test. Categorical data were analyzed with Fisher's exact test. We used Holm's procedure to adjust for multiple testing. Receiver operating characteristic (ROC) curve analysis was performed with calculation of maximal area under the curve (AUC). Youden's index was used to define optimal cutoff values. The positive predictive value (PPV) and negative predictive value (NVP) were calculated. For the evaluation of the diagnostic accuracy of each biomarker, we calculated the positive likelihood ratio (LR+) and negative likelihood ratio (LR-), where LR+ is the sensitivity/(1 – specificity) and LR- is (1 – sensitivity)/specificity. Confidence intervals (CI) were calculated for each likelihood ratio. The pretest odds of mortality is given by $P/(1 - P)$, where P is the probability of the mortality in the current study cohort. The posttest odds, given a positive test, are the product of the LR+ and pretest odds, whereas the posttest odds, given a negative test, are the product of the LR- and the pretest odds.

Results

Two patients were excluded due to lack of written consent leaving 53 patients included in the study. Three patients had missing hsTNT and six patients had missing echocardiographic data. The patients' medical histories divided them into surgical (n = 16) and medical (n = 37) cases. The 7-day and 28-day mortality was 19 % and 28 %, respectively. Survivors tended to be younger, and had lower APACHE II and SOFA scores at admission as shown in Table 1.

Temporal development of biomarkers

Figure 1 (a-c) shows the temporal development of MR-proADM, CT-proET-1, and hsTNT according to short (7-day) and longer-term (28-day) mortality. Non-

Table 1 Baseline characteristics of all patients and according to survival

		Mortality at 28 days			Mortality at 7 days		
	All	Survivors	Non-survivors	*P* value	Survivors	Non-survivors	*P* value
	(n = 53)	(n = 38)	(n = 15)		(n = 43)	(n = 10)	
Age, years	65 (20)	60 (22)	72 (8)	0.007	61 (19)	76 (8)	0.026
APACHE II, score	24 (10)	23 (11)	28 (14)	0.026	24 (11)	29 (10)	0.015
SOFA score, admission	12 (5)	11 (4)	14 (2)	0.002	11 (4)	14 (3)	0.002
Body mass index, kg/m^2	26 (5)	27 (7)	24 (4)	0.008	26 (7)	24 (4)	0.094
Gender (male/female), *n*	37/16	26/12	11/4	1	30/13	7/3	1
Medical/surgical, *n*	37/16	26/12	11/4	1	30/13	7/3	1

APACHE acute physiology and chronic health evaluation, *SOFA* sequential organ failure assessment

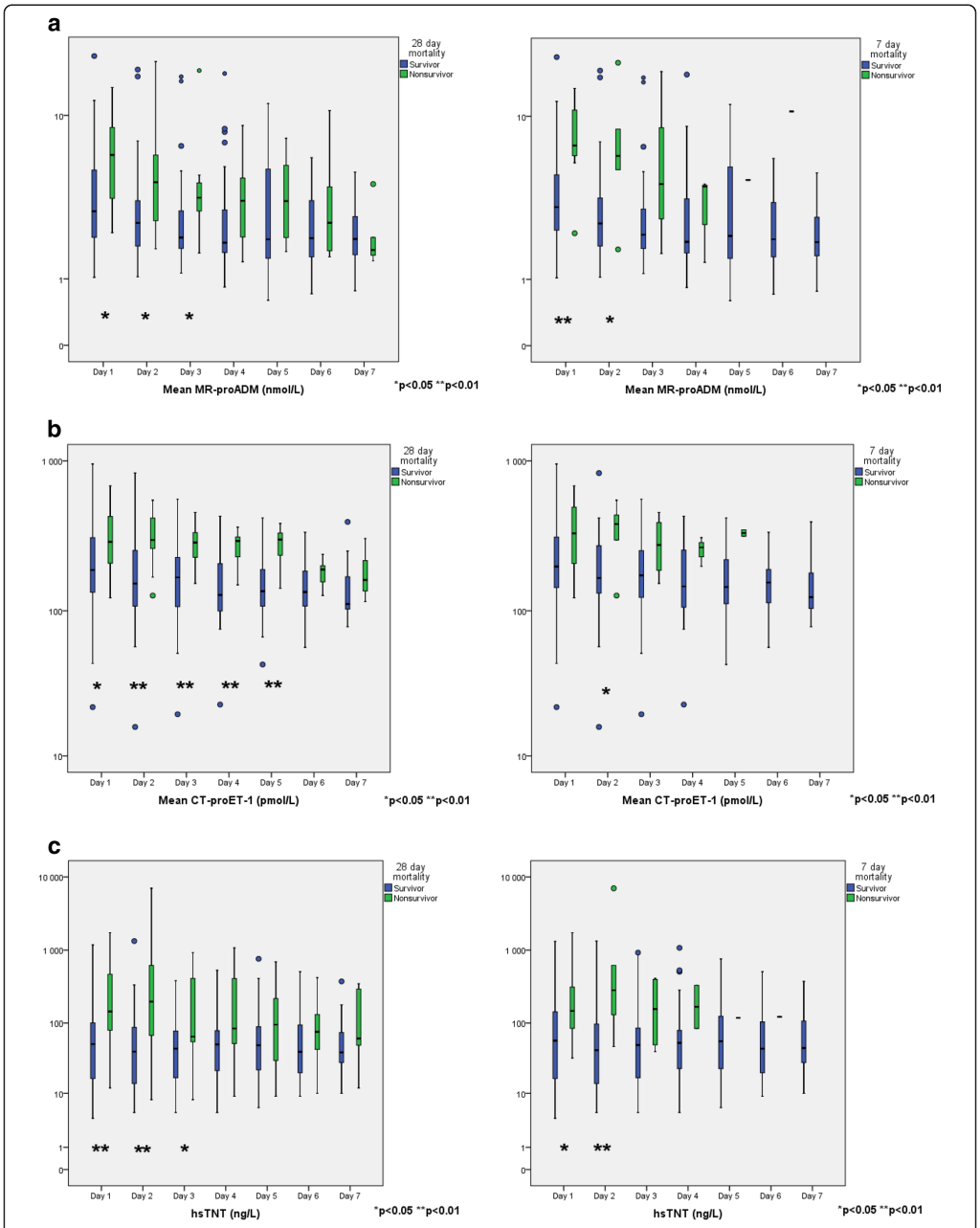

Fig. 1 Temporal development of MR-proADM, CT-proET-1 and hsTNT

survivors generally had higher values of all biomarkers over the 7-day period.

Relationship between MR-proADM, CT-proET-1, and myocardial injury

There was statistically significant inverse correlation between MR-proADM measured on day 1 and two of the four echocardiographic markers of LV systolic dysfunction, MAPSE and LVOT VTI. Day 1 CT-proET-1 concentrations were inversely correlated to all LV systolic function parameters ($\rho = -0.43$ to -0.48, $p = 0.001–0.003$). Both MR-proADM and CT-proET-1 were also correlated with hsTNT ($\rho = 0.38$, $p = 0.007$ and $\rho = 0.40$, $p = 0.004$, respectively). Both biomarkers were significantly correlated with each other ($\rho = 0.68$, $p \leq 0.001$), age, and creatinine (see Table 2).

Twenty-six patients had myocardial injury defined as above, and these patients had significantly higher levels of MR-proADM and CT-proET-1 ($p = 0.007$ and $p < 0.001$, respectively) (see Table 3).

Relationship between biomarker concentrations on day 1 and mortality

The day-1 mean plasma levels according to 7-day and 28-day mortality are displayed in Table 3. MR-proADM, CT-proET-1 and hsTNT were significantly higher among patients who did not survive 28 days. MR-proADM and hsTNT but not CT-proET-1 were higher in patients who did not survive 7 days.

Odds and predictive values for single and combined biomarkers

Table 4 shows the AUC and cutoff values from the ROC curves, and the corresponding PPVs and NVPs. The cutoff values were used when calculating the LR and odds shown in Table 5. The LR+ for MR-proADM was 4.3 when calculated for 7-day mortality. When MR-proADM and CT-proET-1 were combined the LR+ increased. The highest values for the LR+ were obtained when combining all three biomarkers – the difference between the pretest and posttest odds was up to 20-fold (0.35–6.97) for 28-day mortality and 13-fold (0.19–2.49) for 7-day mortality. When MR-proADM and CT-proET-1 were combined the difference between the pretest and posttest odds was 12-fold (1.24–14.9) for myocardial injury.

Discussion

Biomarkers and myocardial injury

In this exploratory study we demonstrated significant relationships between MR-proADM/CT-proET-1 and myocardial injury. The relationship was strongest and most consistent with CT-proET-1. This finding supports a biologically plausible relationship as both pro-hormones are strongly vasoactive and may play key roles in sepsis-associated myocardial injury. Indeed, we demonstrated significant associations between both pro-hormones and hsTNT and echocardiographic markers of LV systolic dysfunction.

In epidemiological studies, increased MR-proADM has been associated with poor cardiovascular outcomes [22, 25, 26]. In sepsis there is upregulation of ADM expression [27, 28] and ADM seems to be important for the initiation and continuation of hyperdynamic shock in animal models [4, 5, 29–31]. Importantly, the administration of anti-ADM antibodies prevents the hyperdynamic response [27] and seems beneficial to survival [32, 33], while exogenous ADM prevents and reverses hypodynamic circulation and pulmonary hypertension, and reduces endothelial hyperpermeability in experimental models of septic shock [4, 5, 30, 34], suggesting possibilities for therapeutic intervention. In this study we found only moderate correlation between MR-proADM and two of four echocardiographic markers of reduced LV systolic function. Despite this there was strongly significant correlation between proADM and hsTNT concentrations, which could suggest a role of this pro-hormone in cardiac injury.

Experimental and clinical studies link increased ET-1 levels to decreased cardiac performance [17, 19, 35–38]. This is supported by our findings of highly significant correlation between CT-proET-1 levels and all echocardiographic markers of reduced LV systolic function, and hsTNT. The results of these studies appear paradoxical to earlier experimental data showing positive inotropic effects of ET-1 [39, 40]. Thus, the role of ET-1 is still

Table 2 Correlation between MR-proADM/CT-proET-1 and echocardiographic markers of left ventricular systolic function, hsTNT, age, and creatinine

		LVEF	MAPSE	TDIs	LVOT VTI	hsTNT	Age	Creatinine
MR-proADM	Correlation coefficient ρ	-0.139	-0.320	-0.142	-0.310	0.376	0.342	0.741
	p value	0.351	0.029	0.342	0.036	0.007*	0.012	>0.001*
CT-proET-1	Correlation coefficient ρ	-0.439	-0.479	-0.430	-0.437	0.396	0.385	0.524
	p value	0.002*	0.001*	0.003*	0.002*	0.004*	0.004*	>0.001*

MR-proADM mid-regional pro-adrenomedullin, *CT-proET-1* C-terminal pro-endothelin-1, *LVEF* left ventricular ejection fraction, *MAPSE* mitral annular plane systolic excursion, *TDIs* peak systolic tissue Doppler velocity imaging, LVOT VTI velocity time integral in the left ventricle outflow tract, *hsTNT* high-sensitivity troponin T.
**P* value lower than adjusted alpha after Holm's procedure for multiple testing

Table 3 Biomarkers related to myocardial injury and mortality

	Myocardial injury			Mortality at 28 days			Mortality at 7 days		
	No (n = 21)	Yes (n = 26)	*P* value	Survivors	Non-survivors	*P* value	Survivors	Non-survivors	*P* value
MR-proADM	2.5 (2.4)	5.2 (5.8)	0.007	3.0 (3.4)	6.3 (6.7)	0.010	3.3 (2.9)	7.1 (5.2)	0.002*
CT-proET-1	153 (111)	324 (238)	<0.001*	188 (183)	289 (247)	0.027	198 (172)	332 (319)	0.088
hsTNT	-	-	-	51 (85)	143 (444)	0.007	57 (126)	146 (388)	0.033
Creatinine	-	-	-	138 (150)	182 (131)	0.211	122 (129)	200 (132)	0.048

MR-proADM mid-regional pro-adrenomedullin, *CT-proET-1* C-terminal pro-endothelin-1, *hsTNT* high-sensitivity troponin T. **P* value lower than adjusted alpha after Holm's procedure for multiple testing

unclear and seems related to the balance between receptor types.

Antagonism of endothelin pathways has been explored in a number of experimental settings, and its effects during septic shock are areas worth exploring [35–37, 41–43]. To our knowledge, there is only one other study investigating the relationship between cardiac function and CT-proET-1 in patients with septic shock. Furian et al. [17] demonstrated significant association between CT-proET-1 and echocardiographic markers of left and right ventricular dysfunction, but did not describe biochemical markers of myocardial injury. Our findings highlight the importance of CT-proET-1 in cardiac dysfunction measured using echocardiography and cardiac troponins, and in mortality. Importantly, the LR- of 0.25 indicates that CT-proET-1 is useful for ruling out myocardial injury. Taken together, our results indicate that the combination of MR-proADM and CT-proET-1 might be a useful supplement for the diagnosis of myocardial injury, as shown by a LR+ of 12.

Biomarkers and mortality

We have shown that increased concentrations of MR-proADM, CT-proET-1, and hsTNT are increased in non-survivors of septic shock, supporting the results of earlier studies [7, 11, 16, 19, 44–46]. MR-proADM and hsTNT seem to be more important determinants of both short-term and longer-term outcome, whereas CT-proET-1 seems to be most significant for longer-term mortality with higher concentrations detected in non-survivors on days 2–5 (Fig. 1b). When considered as a pair, CT-proET-1 and MR-proADM increased the odds for mortality twofold to fivefold. When a combined panel of all three biomarkers were positive, the posttest odds for mortality increased 13-fold to 20-fold.

ProADM and proET-1 are especially attractive biomarkers in septic shock because they are both endothelium-derived pro-hormones and their end products have important vasoregulatory opposing effects. As suggested by Scheutz and colleagues [45] it is plausible that the net balance between the hormones is of significance for clinical outcome. Increased concentrations of ADM and ET-1 have been described in patients with systemic inflammatory response syndrome (SIRS) [47] and septic shock [6, 7, 17, 29, 44–46, 48], and appear to be related to severity and mortality, but dynamic evaluations and their significance for short-term and long-term mortality in patients with shock are poorly investigated. Herein we demonstrated that concentrations of both pro-hormones are higher in non-survivors, particularly during the first 3 days of ICU admission (see Fig. 1).

In line with our results, Guignant et al. reported higher initial levels of proADM among non-survivors of septic shock. Further, the combination of proADM with a vasoconstrictor biomarker, pro-vasopressin, was better for prediction of 28-day mortality when assessed at day 1–2 than the SOFA score and simplified acute physiology score (SAPS) II [7]. Similarly, in a cohort of critically ill patients with sepsis, Christ-Crain et al. found a significantly higher level of proADM among intensive care unit (ICU) non-survivors [46]. They reported an optimal cutoff value of 3.9 nmol/L for MR-proADM, resembling the optimal cutoff of 3.5 nmol/L identified in this study for 28-day mortality. The optimal cutoff identified by Guignant et al. was also in this range (5 nmol/L) [7]. Taken together, these findings support proADM as a useful predictor of mortality.

Our results for ET-1 are different to those reported previously, where no differences between survivors and non-survivors were shown [45, 49]. There may be several explanations for this. First, our patients were severely ill with higher illness severity scores than in previous studies. The median day 1 SOFA and APACHE II scores were 12 and 24, respectively, and all 53 patients were in shock despite fluid resuscitation. Second, we used 7-day and 28-day mortality as outcome parameters, in contrast to in-hospital mortality as used in some of the other studies. Third, we collected blood 6-hourly in the first 24 hours, and used average daily values in an attempt to capture average values for each patient every day. In comparison, Scheutz et al. collected a single sample within 24 hours of ICU admission. Guignant et al. collected a single sample within 48 hours of ICU admission and had a substantial number of missing values. These reflect difficulties in the conduct of clinical studies but may be of significance, as measuring biomarker levels at an early stage, i.e., when the

Table 4 Area under the curve (AUC), cutoff and positive predictive value/negative predictive value (PPV/NPV)

	Myocardial injury					Mortality at 28 days					Mortality at 7 days				
	Cutoff	AUC	Sensitivity	Specificity	PPV/NPV	Cutoff	AUC	Sensitivity	Specificity	PPV/NPV	Cutoff	AUC	Sensitivity	Specificity	PPV/NPV
MR-proADM	4.6 nmol/L	0.729	0.577	0.810	0.79/0.61	3.5 nmol/L	0.730	0.8	0.605	0.44/0.88	5.5 nmol/L	0.823	0.9	0.791	0.5/0.97
CT-proET-1	209 pmol/L	0.855	0.808	0.810	0.81/0.76	206 pmol/L	0.696	0.8	0.579	0.41/0.88	269 pmol/L	0.674	0.7	0.651	0.32/0.90
hsTNT	-	-	-	-	-	114 ng/L	0.752	0.692	0.784	0.53/0.88	114 ng/L	0.74	0.75	0.738	0.35/0.94
APACHE II	-	-	-	-	-	27	0.696	0.667	0.737	0.5/0.88	27	0.744	0.8	0.721	0.4/0.94

MR-proADM mid-regional pro-adrenomedullin, *CT-proET-1* C-terminal pro-endothelin-1, *hsTNT* high-sensitivity troponin T, *APACHE* acute physiology and chronic health evaluation

Table 5 Likelihood ratios (LR) (95 % CI) and odds

	Myocardial injury				Mortality at 28 days				Mortality at 7 days			
	LR+	Given positive test pre/posttest odds	LR-	Given negative test pre/posttest odds	LR+	Given positive test pre/posttest odds	LR-	Given negative test pre/posttest odds	LR+	Given positive test pre/posttest odds	LR-	Given negative test pre/posttest odds
MR-proADM	3.03	1.24/3.76	0.52	1.24/0.64	2.03	0.39/0.79	0.33	0.39/0.13	4.3	0.23/0.99	0.13	0.23/0.03
	(1.18, 7.76)		(0.32, 0.86)		(1.269, 3.236)		(0.116, 0.939)		(2.32, 7.97)		(0.02, 0.82)	
CT-proET-1	3.39	1.24/4.20	0.25	1.24/0.31	1.79	0.39/0.70	0.46	0.39/0.14	2.01	0.23/0.46	0.46	0.23/0.11
	(1.54, -7.46)		(0.11, 0.57)		(1.158, 2.762)		(0.233, 0.987)		(1.13, 3.57)		(0.17, 1.22)	
hsTNT	-	-	-	-	3.20	0.35/1.12	0.39	0.35/0.14	2.86	0.19/0.54	0.34	0.19/0.07
					(1.570, 6.529)		(0.171, 0.903)		(1.50, 5.47)		(0.10, 1.14)	
MR-proADM and CT-proET-1	12	1.24/14.9	0.44	1.24/0.55	2.14	0.39/0.84	0.27	0.39/0.10	5.02	0.23/1.15	0.17	0.23/0.04
	(1.74, 84)		(0.28, 0.70)		(1.25, 3.67)		(0.07, 1.01)		(1.95, 9.47)		(0.02, 1.04)	
MR-proADM, CT-proET-1 and hsTNT	-	-	-	-	19.92	0.35/6.97	NA*	NA*	13.13	0.19/2.49	NA*	NA*
					(2.70, 146.88)				(3.06, 56.24)			

MR-proADM mid-regional pro-adrenomedullin, *CT-proET-1* C-terminal pro-endothelin-1, *hsTNT* high-sensitivity troponin T. NA, not analyzed. *It was not possible to calculate the LR- for all three biomarkers combined, because none of the patients with low values died

patient is most unstable, may reveal important information about the state of the cardiovascular system. It also allows the possibility of early intervention and disease staging.

Although elevated cTn is most commonly used for the diagnosis of MI [50], increased cTns are commonly seen in patients with septic shock without MI and are independent predictors of mortality [11–15]. Recent studies suggest that high-sensitivity assays may add to risk assessment and prediction models [11, 16]. Our study confirms the importance of hsTNT for the outcome of patients with septic shock. When used as an indicator of injury along with echocardiographic parameters, it may potentially be used to stratify risk and monitor treatment. Both alone, but especially when used in a biomarker panel with MR-proADM and CT-proET-1, hsTNT increased the posttest odds ratio of mortality by 13-fold to 20-fold.

It remains to be seen whether this biomarker panel ultimately improves current risk prediction models in critical care. Another potential area of investigation is the use of these biomarkers as a basis for selection of patients for interventional studies, or as pharmacodynamic markers for cardiac dysfunction.

Limitations

This paper has several limitations. This study was designed to be exploratory in nature and the findings here confirm associations between biomarkers and outcome, and refrains from any conclusions on causality. The limited number of outcome events does not allow adequate power for multivariate analysis. As a rule-of-thumb 10 outcome events would be required for each multivariate variable [51], thus, future studies investigating the prognostic potential of these biomarkers should be planned with this in mind. While we realize the limitations of this type of monocenter investigation, in particular the risk of overestimation of effect size, we believe that our study contributes new information to a hitherto under-investigated area. Second, although we defined ICU admission as a starting point for this study, patients have had variable times to presentation, different degrees of shock and variable responses to fluid resuscitation, making the material potentially heterogeneous. As dynamic changes in biomarker levels may be important, particularly early in the course of septic shock, we attempted to capture these changes by measuring up to four times during the first 24 hours, and twice daily during ensuing days. Closer sampling times may have revealed different results. We have no data on right ventricular echocardiographic parameters. As almost all components of the endothelin system are upregulated in pulmonary hypertension [8], and right ventricular dysfunction is common in septic shock, it is plausible that high levels of CT-proET-1 could correlate with right ventricular dysfunction. Because of the lack of a universal definition of myocardial injury, our definition was arbitrary but chosen on the basis of previous studies [23, 52–56]. As premorbid echocardiographic data were not available, we cannot exclude that some patients suffered from co-existing myocardial dysfunction that was unrelated to sepsis.

Conclusion

Our study shows that MR-proADM and CT-proET-1 are associated with myocardial injury and dysfunction. It also supports the concept of a composite biomarker panel for adverse outcome prediction or risk stratification as proposed in earlier studies in patients with sepsis. We found that this particular combination of MR-proADM, CT-proET-1 and hsTNT markedly increased the posttest odds of death in a population of severely ill patients.

Key messages

- MR-proADM and CT-proET-1 are correlated with myocardial injury in patients with septic shock
- A positive biomarker panel consisting of MR-proADM, CT-proET-1, and hsTNT increases the odds of both short-term and longer-term mortality

Abbreviations

2D, two-dimensional; ADM, adrenomedullin; APACHE II, acute physiology and chronic health evaluation II; AUC, area under the curve; BMI, body mass index; CI, confidence interval; cTn, cardiac troponin; CT-proET-1, C-terminal pro-endothelin-1; ET-1, endothelin-1; hsTNT, high-sensitivity troponin T; ICU, intensive care unit; LR-, negative likelihood ratio; LR+, positive likelihood ratio; LV, left ventricle; LVOT VTI, velocity time integral in the left ventricle outflow tract; MAPSE, mitral annular plane systolic excursion; MI, myocardial infarction; MR-proADM, mid-regional pro-adrenomedullin; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristics curve; SAPS II, simplified acute physiology score II; SIRS, systemic inflammatory response syndrome; SOFA, sequential organ failure assessment ; TDIs, peak systolic tissue Doppler velocity imaging

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Authors' contributions

OL analyzed the data, performed the statistical analysis, and drafted the manuscript. LB contributed to the data acquisition and statistical analysis, and revised the manuscript. JR contributed to study design and data acquisition, and helped to revise the manuscript. MR contributed to analyzing the data and revising the manuscript. OM contributed to the statistical analysis and drafting of the manuscript. MC conceived the study, participated in the data acquisition, and contributed to the statistical analysis and drafting the manuscript. All authors read and approved the final manuscript for publication.

Competing interests

The authors declare that they have no competing interests.

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Paper II

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ORIGINAL ARTICLE

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Sepsis is underreported in Swedish intensive care units: A retrospective observational multicentre study

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Background: Sepsis is a common indication for admission to the intensive care unit (ICU). Since definitions vary across studies, comparisons of prevalence and outcomes have been challenging. We aimed to compare sepsis according to ICU discharge codes with sepsis according to Sepsis-3 criteria and to investigate the epidemiology of sepsis in the ICU. We hypothesized that sepsis using discharge codes is underreported.

Methods: Adult ICU admissions to four ICUs in Sweden between 2015 and 2017 were screened for sepsis according to the Sepsis-3 criteria. Medical records were reviewed and data extracted from the Swedish Intensive Care Registry.

Results: Of 5990 adult ICU patients, 28% fulfilled the Sepsis-3 criteria on admission, but only 31% of them had sepsis as the registered main diagnosis at ICU discharge. Of the 1654 Sepsis-3 patients, 38% met the septic shock criteria. The Sepsis-3 in-hospital mortality was 26% compared to 33% in patients with septic shock. The incidence rate for ICU-treated sepsis was 81 cases per 100 000 person-years. One in four had a positive blood culture, and 44% were culture negative.

Conclusion: This large Swedish multicentre study showed that 28% of adult ICU patients fulfilled the Sepsis-3 criteria, but only one third of them had sepsis according to ICU discharge codes. We could confirm our hypothesis, that sepsis is severely underreported in Swedish ICUs, and we conclude that discharge codes should not be used for quality control or research purposes.

1 | INTRODUCTION

1.1 | Background

Sepsis is a leading cause of morbidity and mortality in intensive care units (ICUs) worldwide, with a prevalence of 25%-30% and in-hospital mortality rate of 19%-47%.[1]

The criteria for sepsis identification have varied over time and across studies, leading to inconsistent results regarding the incidence and outcomes. Older studies often used hospital discharge codes to identify sepsis, which is known to lead to underreporting compared with sepsis identified using clinical criteria.[1-4] In 2016, the third version of the sepsis criteria (Sepsis-3) was introduced based on the Sequential Organ Failure Assessment (SOFA) score.[5]

The current recommendation in Sweden is that the Sepsis-3 criteria should be used.[6] To our knowledge, there is no update on the characteristics or prevalence of septic patients using the Sepsis-3 criteria in Swedish ICUs. The aim of this study was (1) to compare the ICU prevalence of sepsis according to the Sepsis-3 criteria with

sepsis according to ICU discharge codes, and (2) to provide an updated description of the sepsis population in Swedish ICUs.We hypothesized that the estimated sepsis prevalence in the ICU according to ICU discharge codes is underreported.

2 | METHODS

2.1 | Study design, setting, and data source

The present study was a retrospective multicentre observational study of patients who fulfilled the Sepsis-3 criteria and were admitted at four mixed surgical and medical ICUs in Region Skåne (Scania county), Sweden, between 2015 and 2017. Specialized ICUs, such as thoracic, neurosurgical, or pediatric ICUs, did not participate in the study. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed.[7]

2.2 | Participants

The Sepsis-3 criteria were used to define and include sepsis patients.[8] The inclusion criteria were as follows: ICU patients ≥ 18 years with a SOFA score ≥ 2 on ICU admission and a suspicion of infection within 24 hours before until 24 hours after ICU admission. The baseline SOFA score was assumed to be 0. Suspected infection was defined by blood culture sampling and concomitant administration of oral or intravenous antibiotics (24 hours before until 72 hours after blood culture sampling), as suggested by the Sepsis-3 task force.[8] The exclusion criteria were as follows: (1) direct transfer from another ICU; (2) planned ICU admission after elective surgery; and (3) cardiac arrest 6 hours before or 1 hours after ICU admission, due to difficulty in assessing organ dysfunction and sepsis criteria in the peri-arrest period. Septic shock was defined as the use of a vasopressor, identified by either a cardiovascular SOFA score ≥ 3 or by medical record review, in combination with a lactate level of > 2 mmol/L.

2.3 | Variables

The main diagnosis at ICU discharge was classified according to the tenth revision of the International Statistical Classification of Diseases and Related Health Problems (ICD-10) codes. The sepsis-2 criteria were recommended for sepsis diagnosis during the period of our study(1). In assessing the Simplified Acute Physiology Score 3 (SAPS 3 score), the Swedish 2016 calibration to 30-day expected mortality rate (EMR) was used.[9,10] The suspected focus of infection on ICU admission (only one) was categorized based on the clinical suspicion documented in the medical record.

[1]In Swedish: https://www.icuregswe.org/globalassets/riktlinjer/diagnossattning.pdf

Editorial Comment

For research databases which are dependent on hospital coding, the quality of the coding will influence the reliability of the data. In this retrospective study from the Swedish Intensive Care Registry, only 1 in 3 of patients with sepsis according to the Sepsis-3 criteria was discharged from the hospital with a code for sepsis.

Comorbidities were registered if they had a functional or physiological impact at the time of ICU admission and a modified Charlson comorbidity index was calculated.[11] The classification of comorbidities is shown in Table S1.

Positive culture and microbiological test results obtained between 24 hours before and 24 hours after ICU admission were recorded. Cultures such as *coagulase-negative Staphylococci* in fewer than two blood cultures or moderate growth of *Candida sp* in airway cultures were disregarded, if they were considered clinically insignificant according to the medical records. Serologic and antigen tests were excluded due to the difficulty in assessing their clinical relevance.

2.4 | Data sources/measurement

Data were extracted from two different sources for each patient: 1) the Swedish ICU Registry (SIR), which contains data entered by the treating physician and nursing staff and 2) a systematic retrospective review of medical records by trained data collectors. See Table S2 for details.

Uncertainties in the classification of comorbidities, suspected foci of infection or the relevance of certain culture findings were decided jointly by the group of data collectors.

The incidence rate was calculated for the years 2016 and 2017. Population figures for Skåne were obtained from population reports.[12,13]

2.5 | Bias

The criterion for suspected infection (blood culture and antibiotic administration) was chosen in an attempt to minimize the risk of selection bias, which would arise if the data collectors had determined subjectively whether the patients had suspected infection on ICU admission.

2.6 | Quantitative variables

Mean values and standard deviations (SDs) were reported for variables with normal distribution and median values and

interquartile ranges (IQRs) for variables with a non-normal distribution.

2.7 | Statistical analysis

Standardized mortality ratios (SMRs) were calculated at group levels by dividing the mean observed 30-day mortality rate by the mean SAPS 3 EMR. For all hypotheses tests, *P*-values <.05 were considered significant. To assess for a difference in the location of two independent variables, the Wilcoxon rank-sum test (Mann-Whitney U test) was used. Differences in proportions were assessed using Pearson's χ^2 test. Differences in SMR were assessed using an approximate permutation test with 5000 permutations.[14]

Missing data were excluded for mean and median calculations; for calculations of proportions, the value of the variable was assumed to be zero. Loss to follow-up in the Swedish population register affected long-term mortality and for proportion and SMR calculations, patients were assumed to be alive at day 31 and 1 year if they were lost to follow-up.

When performing subgroup analyses, each admission was only described in one subgroup, making comparisons between the groups possible.

2.8 | Ethical considerations

The study protocol was approved by the Regional Ethical Review Board of Lund, Sweden (registration no. 2017/802, approved on November 9, 2017). The study was conducted in accordance with the tenets of the Declaration of Helsinki.

3 | RESULTS

3.1 | Participants

We evaluated 5990 adult ICU admissions between September 2015 and December 2017. Of these, 1901 fulfilled the inclusion criteria and, after applying the exclusion criteria, 1654 (28%) admissions remained and formed the Sepsis-3 cohort. See Figure 1. Six percent were ICU readmissions; thus, the cohort consisted of 1547 unique patients.

3.2 | Descriptive data

3.2.1 | Sepsis-3 cohort

The Sepsis-3 cohort is presented in Table 1. There were more men than women in the sepsis cohort, and the median age was 69 years. The most common comorbidities were cardiovascular disease, diabetes, and respiratory disease. A respiratory focus of infection was suspected in almost half of the Sepsis-3 cohort and in one of five, the suspected focus of infection was unknown.

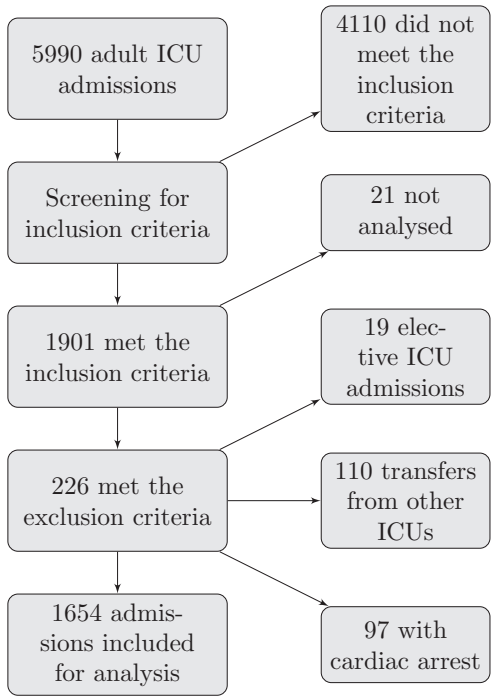

FIGURE 1 Flow chart of case inclusion and exclusion among intensive care unit (ICU) admissions. The inclusion criteria were 1) suspected infection (blood cultures taken and oral or intravenous antibiotics) within 24 h before to 24 h after ICU admission and 2) an admission SOFA score of 2 or more. Fulfillment of inclusion criteria was discovered post analysis in 21 admissions, which were not included in the analyses. The exclusion criteria were 1) direct transfer from other ICUs, 2) elective ICU admission after elective surgery, and 3) cardiac arrest within 6 h prior to and 1 hour after ICU admission. SOFA, Sequential Organ Failure Assessment

3.2.2 | Missing values

The proportion of missing or incomplete values was low (1%-12%) (Table S3).

3.3 | Main results

3.3.1 | Main diagnosis at ICU discharge

Among all adult ICU admissions, 11% had sepsis as a main diagnosis at ICU discharge. In contrast, 28% of the adult ICU

TABLE 1 Demographics of the Sepsis-3 cohort and a comparison between the septic shock and non-shock subgroups

Sepsis-3		Septic shock	Non-shock	*P*-value
Number, n (% of Sepsis-3 cohort)	1654 (100%)	636 (38%)	1018 (62%)	<.001
Age in years, median (IQR)	69 (59-76)	70 (61-77)	68 (58-75)	<.001
Female sex	695 (42%)	262 (41%)	433 (43%)	.63
Department of origin				
Emergency department/out of hospital	742 (45%)	295 (46%)	447(44%)	.35
Hospital ward	720 (44%)	248 (39%)	472 (46%)	.0040
Operating room/post-operative ward	176 (11%)	86 (14%)	90 (9%)	.0035
Diagnostic classification at ICU discharge				
Main diagnosis sepsis	505 (31%)	299 (47%)	206 (20%)	<.001
Main diagnosis: infection related (non-sepsis)	256 (15%)	59 (9%)	197 (19%)	<.001
Comorbidities				
None of those listed below	358 (22%)	127 (20%)	231 (23%)	.21
Cardiovascular disease	775 (47%)	316 (50%)	459 (45%)	.080
Respiratory disease	404 (24%)	122 (19%)	282 (28%)	<.001
Hepatic disease	94 (6%)	38 (6%)	56 (6%)	.77
Renal disease	183 (11%)	71 (11%)	112 (11%)	.98
Cancer	216 (13%)	79 (12%)	137 (13%)	.59
Hematological disease	113 (7%)	48 (8%)	65 (6%)	.42
Immunosuppression	302 (18%)	120 (19%)	182 (18%)	.66
Diabetes	404 (24%)	181 (28%)	223 (22%)	.031
Modified Charlson comorbidity index, mean (SD)	1.4 (1.3)	1.4 (1.4)	1.4 (1.3)	.24
Outcomes				
In-hospital mortality	436 (26%)	208 (33%)	228 (22%)	<.001
30-day mortality	398 (24%)	188 (30%)	210 (21%)	<.001
1-year mortality	631 (38%)	270 (42%)	361 (35%)	.0052
$SMR_{30\text{-}day}$ (95% CI)	0.74 (0.68-0.80)	0.74 (0.66-0.83)	0.74 (0.66-0.82)	.47
ICU LOS in days, median (IQR)	2.1 (1.0-4.4)	2.6 (1.2-5.6)	1.8 (0.9-3.8)	<.001
Hospital LOS in days, median (IQR)	14 (7-28)	14 (7-28)	14 (7-27)	.48
CRRT use during ICU stay	236 (14%)	131 (21%)	105 (10%)	<.001
Organ dysfunction and illness severity on ICU admission				
SAPS 3 score, median (IQR)	65 (57-75)	70 (61-80)	62 (55-71)	<.001
SAPS 3 $EMR_{30\text{-}day}$, median (IQR)	28% (15%-49%)	38% (21%-59%)	23% (12%-40%)	<.001
SOFA score, median (IQR)	7.0 (5-10)	10 (8-12)	6 (4-8)	<.001
Respiratory support	868 (52%)	344 (54%)	524 (51%)	.32
Serum lactate level in mmol/L, median (IQR)	2.8 (1.4-5)	4.5 (3.1-6.3)	1.7 (1.1-3.1)	<.001
Suspected focus of infection on ICU admission				
Respiratory	789 (48%)	246 (39%)	543 (53%)	<.001
Gastrointestinal	225 (14%)	113 (18%)	112 (11%)	<.001
Cardiovascular	18 (1%)	7 (1%)	11 (1%)	1.0
Genitourinary	125 (8%)	71 (11%)	54 (5%)	<.001
Musculo-dermato-hematological	101 (6%)	54 (8%)	47 (5%)	.0020
Neurological	63 (4%)	11 (2%)	52 (5%)	<.001

Note: Data regarding general characteristics, outcomes, organ dysfunction, and illness severity are presented below. Admissions that fulfilled the septic shock criteria were compared to admissions without septic shock, and the p-values refer to that comparison. Proportions (%) are within their subgroups unless otherwise specified. IQR, interquartile range; SD, standard deviation; LOS, length of stay; ICU, intensive care unit; SMR, standardized mortality ratio; CI, confidence interval; CRRT, continuous renal replacement therapy; SAPS 3, Simplified Acute Physiology Score 3; EMR, estimated mortality ratio; SOFA, Sequential Organ Failure Assessment.

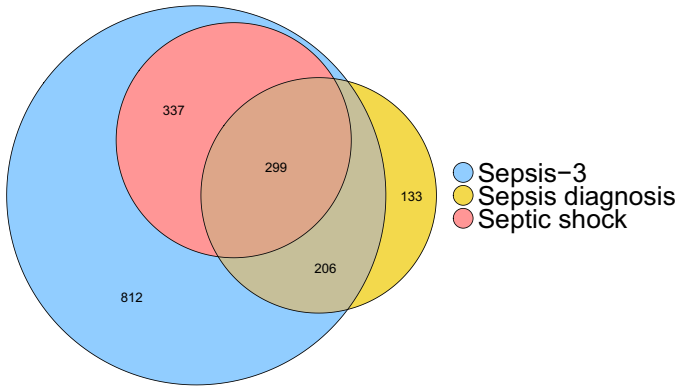

FIGURE 2 Sepsis-3/Septic shock/Sepsis diagnosis. Euler diagram of all intensive care unit (ICU) patients fulfilling the Sepsis-3 and septic shock criteria on admission and those who had sepsis as the main diagnosis at ICU discharge [Colour figure can be viewed at wileyonlinelibrary.com]

admissions fulfilled the Sepsis-3 criteria. Of these, 31% had a main diagnosis of sepsis, while the corresponding figure for the septic shock subgroup was 47% (see Figure 2). Thus, the sensitivity of a sepsis discharge code was 31% (97.5% confidence interval (CI) 28%-33%) in relation to clinical criteria, the specificity was 97% (97.5% CI 96%-97%), and the positive predictive value was 79% (97.5% CI 76%-82%). Another 15% in the Sepsis-3 cohort had an infection-related main diagnosis other than sepsis and, thus, a majority of patients had a non-infectious main diagnosis (see Table S4).

3.3.2 | Incidence rate of ICU-treated sepsis

The incidence rate of sepsis and septic shock requiring intensive care was 81 and 31 cases per 100 000 person-years, respectively[2].

3.4 | Mortality

The 30-day mortality in the Sepsis-3 cohort was 24% and the in-hospital mortality was 26%. The 1-year mortality rate was 38% (see Table 1).

3.5 | Other analyses

3.5.1 | Septic shock subgroup

The Sepsis-3 septic shock criteria were met in 38% of the Sepsis-3 cohort (septic shock subgroup). The 30-day and in-hospital mortality rates in the septic shock subgroup were 30% and 33%, respectively. The 1-year mortality rate was 42%. See Table 1 and Figure 3 for more results.

Positive blood cultures were more common in the septic shock subgroup than in the non-shock subgroup. *Escherichia coli*, *beta-haemolytic Streptococci*, and *Enterococcus sp* were more commonly isolated in blood in the septic shock subgroup. There were fewer patients with positive airway cultures in the septic shock subgroup than in the non-shock subgroup. See Table S5.

3.5.2 | Culture positivity vs culture negativity

In the Sepsis-3 cohort, 44% of patients tested culture negative, 25% had positive blood cultures (bacteremic subgroup), and 30% had other positive cultures, but negative blood cultures (non-blood culture-positive subgroup). The most common bacteremic pathogens were *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella sp* Further descriptions of the positive cultures and microbiological tests are given in Table S5 and Figure 4.

The bacteremic and non-blood culture-positive groups were compared to the culture-negative group, see Table S6.

The burden of pre-existing disease was similar between groups, except for a lower prevalence of respiratory disease, but a higher prevalence of hematological disease and immunosuppression in the bacteremic subgroup.

The bacteremic subgroup had a higher SAPS 3 and SOFA scores, a higher ratio of septic shock, more frequent use of continuous renal replacement therapy (CRRT), but less use of respiratory support.

The mortality measures were similar, but the length of stay (LOS) was longer, for both culture-positive subgroups compared to the culture-negative subgroup.

The suspected focus of infection differed between subgroups, with a lower ratio of respiratory focus, but higher ratios of gastrointestinal, genitourinary, and musculo-dermato-hematological (MDH) foci in the bacteremic subgroup.

[2]Our catchment population was 1 017 902 in 2016 and 1 029 505 in 2017. The number of sepsis cases in Skåne was calculated to be 766 in 2016 and 884 in 2017.

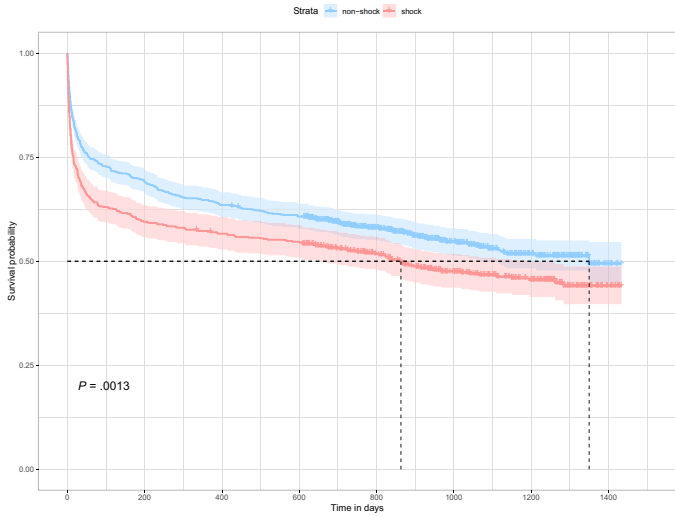

FIGURE 3 Long-term survival among shock/non-shock patients. Kaplan-Meier plot for the shock and nonshock groups. The median survival periods for the non-shock and shock groups were 1350 and 863 days, respectively (log-rank test $P = .0013$) [Colour figure can be viewed at wileyonlinelibrary.com]

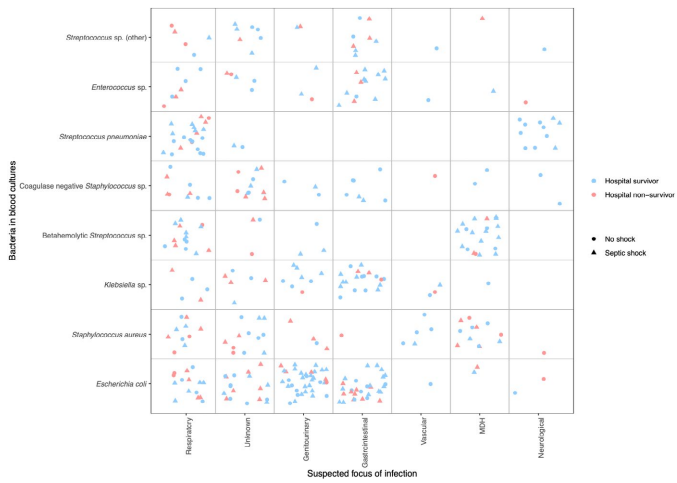

FIGURE 4 ICU admissions with one of the eight most commonly isolated bacteria in blood culture are plotted in relation to the suspected focus of infection. A total of 359 blood culture findings in 320 ICU admissions are described. Some ICU admissions appear multiple times: 32 admissions had two of these bacteria, two admissions had three bacteria, and one admission had four bacteria. MDH, musculo-dermatohaematological [Colour figure can be viewed at wileyonlinelibrary.com]

3.5.3 | In-hospital survivors vs non-survivors

In an analysis between in-hospital survivors and non-survivors, survivors were slightly younger, had lower rates of septic shock, and less comorbidities (Table 2). Suspected focus of infection and positive cultures were similar among survivors and non-survivors.

4 | DISCUSSION

4.1 | Key results

We found that 28% of the adult ICU admissions fulfilled the Sepsis-3 criteria and 11% fulfilled the Sepsis-3 septic shock

TABLE 2 Comparison between the in-hospital survivor and non-survivor subgroups

Hospital survivors		Hospital non-survivors	*p*-value
Number, n (% of Sepsis-3 cohort)	1218 (74%)	436 (26%)	<.001
Age in years, median (IQR)	68 (57-75)	71 (64-78)	
Department of origin			
Emergency department/out of hospital	584 (48%)	158 (36%)	<.001
Hospital ward	501 (41%)	219 (50%)	.0012
Operating room/postoperative ward	123 (10%)	53 (12%)	.27
Comorbidities			
None of those listed below	308 (25%)	50 (11%)	<.001
Cardiovascular disease	542 (44%)	233 (53%)	.0016
Respiratory disease	299 (25%)	105 (24%)	.90
Hepatic disease	58 (5%)	36 (8%)	.010
Renal disease	120 (10%)	63 (14%)	.011
Cancer	133 (11%)	83 (19%)	<.001
Hematological disease	69 (6%)	44 (10%)	.0024
Immunosuppression	196 (16%)	106 (24%)	<.001
Diabetes	301 (25%)	103 (24%)	.70
Modified Charlson comorbidity index, mean (SD)	1.3 (1.3)	1.6 (1.3)	<.001
Outcomes			
ICU LOS in days, median (IQR)	1.9 (1-3.8)	2.8 (1.2-6.2)	<.001
Hospital LOS in days, median (IQR)	15 (8-29)	11 (4-25)	<.001
CRRT use during ICU stay	132 (11%)	104 (24%)	<.001
Organ dysfunction and illness severity on ICU admission			
Septic shock	428 (35%)	207 (47%)	<.001
SAPS 3 score, median (IQR)	62 (55-71)	74 (64-83)	<.001
SAPS 3 $EMR_{30\text{-day}}$, median (IQR)	23% (12%-40%)	47% (26%-65%)	<.001
SOFA score, median (IQR)	7 (5-9)	9 (6-12)	<.001
Respiratory support	618 (51%)	250 (57%)	.021
Serum lactate level in mmol/L, median (IQR)	2.6 (1.3-4.8)	3.3 (1.7-5.7)	<.001

Note: In-hospital survivors were compared to non-survivors. Proportions (%) are within their respective subgroups unless otherwise specified. IQR, interquartile range; ICU, intensive care unit; SD, standard deviation; LOS, length of stay; SAPS 3, the 3rd version of the Simplified Acute Physiology Score; EMR, estimated mortality risk; SOFA, Sequential Organ Failure Assessment.

criteria, with in-hospital mortality rates of 26% and 33%, respectively. Only 31% of the Sepsis-3 patients had sepsis as the main diagnosis on ICU discharge. The calculated incidence of sepsis and septic shock requiring intensive care was 81 and 31 per 100 000 person-years.

4.1.1 | Incidence/Prevalence

The incidence rate in our study is similar to that reported by Shankar-Hari et al, who reported 88-102 ICU sepsis cases and 19 ICU septic shock cases per 100 000 person-years, also using the Sepsis-3 criteria.[15] The most recently reported incidence rate in a Swedish hospital population was provided by Mellhammar et al,[16] who reported 780 hospital-treated patients diagnosed according to the Sepsis-3 criteria per 100 000 person-years. These numbers imply that only about one in ten hospital-treated sepsis patients require intensive care.

In contrast, when discharge codes were used, the incidence rate decreased to 927 per 100 000 inhabitants,[17] which probably is a severe underestimation. This is confirmed by the poor sensitivity of discharge codes to identify sepsis in our study, which is in line with previous studies and underlines that discharge codes should be avoided to identify sepsis for research purposes.[3,4,18]

Our ICU sepsis prevalence of 28% is similar to previous studies conducted in European ICUs.[15,19,20]

4.1.2 | Mortality

Our sepsis cohort had a 26% in-hospital mortality rate, which is slightly lower than that of Shankar-Hari et al, who reported a 32% in-hospital mortality rate.[15] In that study, illness severity scores and comorbidities were reported differently, complicating comparisons regarding the burden of disease, which might explain differences in mortality.

With an SMR of 0.74, mortality was lower than predicted, which is in accordance with Swedish ICUs in general[(3)].

4.1.3 | Culture negativity/positivity

We found that 44% of patients tested culture negative in our cohort, which is similar to previous studies.[21,22] Patterns of culture findings vary geographically, but our findings were consistent with studies conducted in comparable socioeconomic regions, in which *Staphylococcus aureus* and *Escherichia coli* dominate blood culture isolates.[21-23]

We found proven bacteremic patients to have higher morbidity and longer ICU and hospital LOS than culture-negative ones; however, bacteremia was not associated with a higher mortality rate. The results of previous studies comparing culture-negative and culture-positive patients were conflicting, both with regard to illness severity and mortality.[21,23]

4.1.4 | Suspected focus of infection

A suspected respiratory focus of infection was less common in both the septic shock and bacteremic subgroups. The higher ratio of respiratory focus in the culture-negative group was consistent with the results of Heffner et al and Phua et al, who also found culture-positive patients to have a urinary tract focus more often, which is in agreement with our findings.[21,23] One possible interpretation is that some ICU admissions with respiratory failure fulfill sepsis criteria without being infected. Another could be that respiratory sepsis is a single organ disease which seldom leads to neither bacteremia nor septic shock, an explanation which is supported by the higher ratio of positive airway culture/test results in the non-shock subgroup, but needs to be further investigated in a subgroup analysis. Also, Phua et al proposed that the higher ratio of pneumonia in culture-negative sepsis could be partly due to more frequent viral cause and a high threshold for viral testing.[21] In our four ICUs, however, this is less plausible as there is a high degree of vigilance regarding viral pneumonia and a low threshold for viral testing.

In Figure 4 we illustrate the clinically suspected focus of infection at ICU admission. However, this might not be the causative focus of infection, which might explain pathogen/focus combinations that are not normally seen in clinical practice, such as *coagulase-negative Staphylococci* and *Enterococcus sp* in respiratory sepsis.

[3]https://portal.icuregswe.org/utdata/en/home

4.1.5 | Septic shock

In our Sepsis-3 cohort, 38% of patients fulfilled the septic shock criteria, which is almost twice the number reported by Shankar-Hari et al This might be due to our manual review of medical records, in which we found additional patients who received vasopressors, which increased the septic shock subgroup. This underscores the difficulty in relying on big, automatically collected datasets, which may include incorrect registrations. The 33% in-hospital mortality rate in the septic shock group in our study is low in comparison with other studies. A recent meta-analysis found an in-hospital mortality rate of 39%.[24] However, using the Sepsis-3 septic shock criteria alone, mortality rates were significantly higher at 42%-56%.[15,25,26] This was not reproduced in our septic shock cohort, possibly due to our strict inclusion time frame, which excludes patients who develop septic shock later on during ICU care. If this were true, however, a higher mortality rate in the non-shock patients would be expected, which was not the case.

4.2 | Strengths

The strength of this study lies in the large cohort from four centers and the fact that all patients were manually screened for sepsis via a review of medical records, using the most recent Sepsis-3 criteria. Diagnostic coding from the ICU registry, known to be of poor quality, was thus not used. Additionally, the large proportion of clinical data retrieved from medical records, where data are assessed and filtered, minimizes data errors from automatically collected data.

4.3 | Limitations

One weakness of this study is the risk of over-inclusion of patients without infection since the threshold for blood cultures and administration of antibiotics are low in an ICU setting. However, Swedish ICUs have a strict antibiotic policy and antibiotic use is managed in close collaboration with infectious disease specialists, which should minimize that risk.[27] For comparative and pragmatic reasons we chose blood culturing and antibiotic administration as criteria for suspected infection, although a substantially more complex method of classifying infections in the ICU have been suggested by Calandra et al[28] We suspect that these criteria have a high sensitivity but a lower specificity, which is difficult to confirm since there is no gold standard diagnostic tests for infection and sepsis. This weakness reflects the difficulty in identifying infection and sepsis and emphasizes the need for more specific methods to detect sepsis.

Another weakness is that we only assessed admission sepsis, not sepsis which developed during the ICU stay.

Furthermore, the calculated incidence did not include meningitis and endocarditis patients from specialized ICUs since these data were not available. This might have affected the calculated incidence.

4.4 | Interpretation

In this large study of an ICU sepsis population, we used robust and reproducible criteria. We could confirm that the agreement between discharge codes and criteria-based sepsis is poor. However, there is a possibility of discrepancy between criteria-based sepsis and what is clinically considered to be sepsis, a topic which should be further investigated.

We found an ICU sepsis incidence rate and prevalence similar to that reported in previous studies. Although the mortality rate remains high, we found lower mortality rates for both sepsis and septic shock as compared to several other studies. The reason for this difference is unclear and should be investigated further.

We also found that almost half of the ICU sepsis patients had negative cultures, which is in line with previous smaller studies. More research is needed in order to investigate reasons for culture negativity in sepsis patients.

4.5 | Generalizability

Our multi-centre approach, with a large university hospital and regional hospitals, allows for generalizability to most ICU settings in Scandinavia. ICU populations and admission criteria differ geographically, which may limit the generalizability of our results to regions that are very different from Scandinavia.

4.6 | Conclusion

Patients fulfilling the Sepsis-3 criteria represent 28% of the Swedish ICU population; however, less than one third of them received a main diagnosis of sepsis at ICU discharge, which confirms our hypothesis that sepsis is underreported in Swedish ICUs. We conclude that discharge codes should not be used to classify sepsis for quality control or for research purposes.

5 | DECLARATIONS

Ethics approval and consent to participate.

The Regional Ethical Review Board in Lund, Sweden approved the study protocol (registration no. 2017/802, approved November 9, 2017). The study was conducted in accordance with the tenets of the Declaration of Helsinki.

6 | CONSENT FOR PUBLICATION

Not applicable.

7 | AVAILABILITY OF DATA AND MATERIALS

The datasets generated and analyzed during the current study are not publicly available due to limitations in the ethical approval of the study and data management policies of Region Skåne, but are available from the corresponding author on reasonable request.

8 | COMPETING INTERESTS

The authors declare that they have no competing interests.

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AUTHORS' CONTRIBUTIONS

AF and HF designed the study. ML, OL, MA, and MS participated in data collection. ML and AF performed the statistical analyses. ML and OL wrote the first draft of the manuscript. All authors read, provided critical revision of, and approved the final manuscript.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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Supplemental tables

Sepsis is underreported in Swedish intensive care units:
a retrospective observational multicentre study

S 1: **Classification of comorbidities.** Comorbidities were grouped according to the examples below. Note that the examples are non-exhaustive, and other comorbidities than the ones specified in the table could classify the patient as within a comorbid group if the disease was judged clinically relevant at the time of intensive care unit (ICU) admission. Solid tumours were not registered if radically excised without relapse >1 year before ICU admission. Corticosteroid treatment was registered if treatment lasted for >3 days before ICU admission.

Comorbidity	Examples of disease
Cardiovascular	heart failure atrial fibrillation ischaemic heart disease stroke/transient ischaemic attack (TIA)
Respiratory	Chronic obstructive pulmonary disease (COPD) severe asthma restrictive/interstitial lung disease
Hepatic	cirrhosis infectious hepatitis
Renal	chronic kidney failure chronic dialysis
Cancer	solid tumour metastatic cancer
Haematological	haematological malignancy chronic haematological disease
Immunosuppression	chemotherapy neutropenia systemic corticoid steroid
Diabetes	if on medical treatment

S 2: **Variables and time frames according to data sources.** Two different data sources were used to collect data. SIR contains prospectively collected data. Medical records were reviewed retrospectively by trained data collectors. SOFA score and SAPS 3 score were routinely calculated at ICU admission, using laboratory values automatically transferred into the registry, as well as physiological and other parameters manually registered by the admitting ICU physician and nurse. Respiratory support was either CPAP, NIV, or invasive ventilation. *SIR: Swedish Intensive Care Registry, SOFA: Sequential organ failure assessment, SAPS 3: The 3rd version of the Simplified Acute Physiology Score, ICU: intensive care unit, CPAP: continuous positive airway pressure, NIV: non-invasive ventilation, LOS: Length of stay, CRRT: Continuous renal replacement therapy.*

Data source	Time frame	Variables
SIR	1 hour prior, to 1 hour after ICU-admission	Admission SOFA SAPS 3 Respiratory support
	Whole ICU admission	Admission source Date/time of ICU admission ICU LOS ICU main diagnosis Age Sex CRRT use
Medical records	6 hours prior, to 1 hour after ICU-admission	Suspected source of infection Lactate (highest) Vasopressor use (prior to sedation/invasive ventilation)
	24 hours prior, to 24 hours after ICU-admission	Blood culturing Cultures with growth
	24 hours prior, to 72 hours after blood cultivation	Administration of antibiotics
	Whole hospital admission	Comorbidities Survival status at hospital discharge Date/time of hospital discharge
Swedish population register	At least 1 year after ICU admission	Survival data

S 3: **Missing values.** Variables with missing or incomplete values among the Sepsis-3 cohort are presented below. Missing long-term mortality constitutes patients who were lost to follow up in the Swedish population register, which affects 30-day, 6-month, and 1-year mortality figures. *SOFA: Sequential organ failure assessment*

Variable	Proportion of admissions missing
Cardiovascular SOFA	1%
Respiratory SOFA	12%
Renal SOFA	3%
Haematological SOFA	7%
Hepatological SOFA	6%
Serum lactate	4%
Long-term mortality	1%

S 4: **Non-infection main diagnosis.** The five most common non-infectious main diagnoses at intensive care unit (ICU) discharge, among the Sepsis-3 cohort, are presented below. *ICD: International statistical classification of diseases and related health problems.*

Diagnosis, ICD-10 code	Proportion
Respiratory insufficiency, J96.9	9%
Left ventricular failure, I50.1	4%
Chronic obstructive pulmonary disease, J44.9	3%
Pneumonitis due to inhalation of food and vomit, J69.0	3%
Acute kidney failure, N17.9	2%

S 5: **Suspected focus of infection and positive cultures/microbiological tests in the Sepsis-3 cohort, and comparison between septic shock and non-shock subgroups.** Proportions (%) are within each subgroup. P-values refer to comparison between septic shock and non-shock subgroups. *MDH: musculo-dermato-haematological, sp.: species*

	Sepsis-3	Septic shock	Non-shock	p-value
Number, n (% of Sepsis-3 cohort)	1654 (100%)	636 (38%)	1018 (62%)	
Any positive culture/microbiological test	922 (56%)	391 (61%)	531 (52%)	<0.001
Positive blood culture	420 (25%)	233 (37%)	187 (18%)	<0.001
Escherichia coli	104 (6%)	72 (11%)	32 (3%)	<0.001
Staphylococcus aureus	45 (3%)	21 (3%)	24 (2%)	0.32
Klebsiella sp.	42 (3%)	21 (3%)	21 (2%)	0.16
Betahemolytic Streptococcus sp.	41 (2%)	28 (4%)	13 (1%)	<0.001
Coagulase negative Staphylococcus sp.	36 (2%)	15 (2%)	21 (2%)	0.82
Streptococcus pneumoniae	35 (2%)	15 (2%)	20 (2%)	0.71
Enterococcus sp.	32 (2%)	20 (3%)	12 (1%)	0.0083
Streptococcus sp. (other)	26 (2%)	15 (2%)	11 (1%)	0.067
Positive urine culture	256 (15%)	104 (16%)	152 (15%)	0.48
Escherichia Coli	123 (7%)	59 (9%)	64 (6%)	0.031
Enterococcus sp.	51 (3%)	11 (2%)	40 (4%)	0.018
Klebsiella sp.	27 (2%)	12 (2%)	15 (1%)	0.66
Pseudomonas sp.	23 (1%)	5 (1%)	18 (2%)	0.15
Proteus sp.	14 (1%)	6 (1%)	8 (1%)	0.95
Positive airway culture/test	366 (22%)	123 (19%)	243 (24%)	0.036
Haemophilus Influenza	57 (3%)	18 (3%)	39 (4%)	0.34
Staphylococcus Aureus	54 (3%)	17 (3%)	37 (4%)	0.35
Influenza PCR	42 (3%)	14 (2%)	28 (3%)	0.60
Betahemolytic Streptococcus sp.	40 (2%)	19 (3%)	21 (2%)	0.30
Moraxella sp.	38 (2%)	12 (2%)	26 (3%)	0.48
Streptococcus Pneumoniae	36 (2%)	17 (3%)	19 (2%)	0.36
Postive other culture	233 (13%)	97 (15%)	126 (12%)	0.11
Enterococcus sp.	51 (3%)	25 (4%)	26 (3%)	0.15
Staphylococcus Aureus	44 (3%)	20 (3%)	24 (2%)	0.42
Betahemolytic Streptococcus sp.	38 (2%)	21 (3%)	17 (2%)	0.047
Escherichia Coli	32 (2%)	16 (3%)	16 (2%)	0.24
Streptococcus sp. (other)	16 (1%)	6 (1%)	10 (1%)	1.0

S 6: **Comparison between culture-negative and culture-positive subgroups.** Admissions in the culture-negative subgroup had no clinically relevant cultures/microbiological tests within the time frame 24 hours before to 24 hours after intensive care unit (ICU) admission. Admissions in the 'bacteremic' subgroup had a positive blood culture, and the 'non-blood' subgroup had at least one positive culture/microbiological test, but no positive blood cultures, within that time frame. Each admission was included in only one of the subgroups. Note that admissions in the bacteremic subgroup also could have positive cultures other than blood. P-values refer to hypotheses testing between the culture-negative group and the two culture-positive subgroups, respectively. Proportions (%) are shown for respective subgroups unless otherwise specified. *SD: Standard deviation, SMR: Standardised mortality ratio, LOS: Length of stay, IQR: interquartile range, CRRT: Continuous renal replacement therapy, SAPS 3: The 3rd version of the Simplified Acute Physiology Score, EMR: estimated mortality risk, SOFA: Sequential organ failure assessment, MDH: musculo-dermato-hematological.*

	Culture negative	Bacteremic	p-value	Non-blood	p-value
Number, n (% of Sepsis-3 cohort)	731 (44%)	420 (25%)		503 (30%)	
Preexisting comorbidity					
Respiratory disease	209 (29%)	51 (12%)	<0.001	144 (29%)	1
Haematological disease	43 (6%)	40 (10%)	0.029	30 (6%)	1
Immunosuppression	109 (15%)	96 (23%)	<0.001	97 (19%)	0.052
Modified Charlson comorbidity index, mean (SD)	1.5 (1.4)	1.3 (1.2)	0.1	1.3 (1.2)	0.08
Outcomes					
Hospital mortality	196 (27%)	116 (28%)	0.82	124 (25%)	0.43
30-day mortality	176 (24%)	110 (26%)	0.47	112 (22%)	0.50
1-year mortality	277 (38%)	166 (40%)	0.63	188 (37%)	0.90
$SMR_{30\text{-}day}$ (95% CI)	0.79 (0.70-0.88)	0.72 (0.62-0.83)	0.14	0.70 (0.60-0.82)	0.17
ICU LOS in days, median (IQR)	1.9 (0.9-3.8)	2.2 (1-5.8)	0.003	2.4 (1.1-4.8)	<0.001
Hospital LOS in days, median (IQR)	13 (6.5-25)	16 (8-29)	0.005	14 (7-30)	0.017
CRRT use during ICU stay	82 (11%)	91 (22%)	<0.001	63 (13%)	0.54
Status on ICU admission					
Septic shock	244 (33%)	233 (55%)	<0.001	158 (31%)	0.51
SAPS 3 score, median (IQR)	64 (56-74)	68 (59-78)	<0.001	64 (57-75)	0.53
SAPS 3 $EMR_{30\text{-}day}$, median (IQR)	26% (14%-47%)	34% (18%-55%)	<0.001	26 (15-49)	0.53
SOFA score, median (IQR)	7 (5-9)	9 (6-11)	<0.001	7 (5-10)	0.33
Respiratory support	411 (56%)	176 (42%)	<0.001	281 (56%)	0.95
Serum lactate in mmol/L, median (IQR)	2.6 (1.4-5.2)	3.7 (2.1-5.7)	<0.001	2.1 (1.4-5)	<0.001
Antibiotic use prior to blood culturing	308 (42%)	115 (27%)	<0.001	195 (39%)	0.26
Suspected focus of infection on ICU admission					
Respiratory	380 (52%)	102 (24%)	<0.001	307 (61%)	0.0020
Gastrointestinal	88 (12%)	88 (21%)	<0.001	49 (10%)	0.24
Cardiovascular	6 (1%)	10 (2%)	0.056	2 (0.4%)	0.58
Genitourinary	33 (5%)	66 (16%)	<0.001	26 (5%)	0.69
Musculo-dermato-haematological (MDH)	24 (3%)	47 (11%)	<0.001	30 (6%)	0.034
Neurological	24 (3%)	21 (5%)	0.20	18(4%)	0.90
Unknown	176 (24%)	86 (20%)	0.18	71 (14%)	<0.001

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CORRIGENDUM

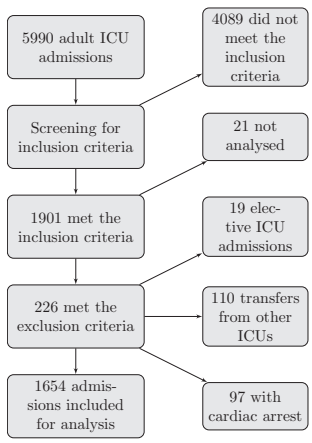
acta Anaesthesiologica Scandinavica

In the Discussion Section 4.1.1 Incidence/prevalence of article entitled "Sepsis is underreported in Swedish intensive care units: A retrospective observational multicentre study," the following sentence is incorrect:

In contrast, when discharge codes were used, the incidence rate decreased to 927 per 100 000 inhabitants,[17] which probably is a severe underestimation.

The correct sentence should read as: In contrast, when discharge codes were used, the incidence rate decreased to **3-43** per 100 000 inhabitants,[17] which probably is a severe underestimation.

In addition, Figure 1 has an incorrect information that "4110 did not meet the inclusion criteria." The correct version of Figure 1 is shown below.



REFERENCE

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Paper III

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Critical Care

RESEARCH Open Access

Circulating bioactive adrenomedullin as a marker of sepsis, septic shock and critical illness

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Abstract

Background: Biomarkers can be of help to understand critical illness and to identify and stratify sepsis. Adrenomedullin is a vasoactive hormone, with reported prognostic and potentially therapeutic value in sepsis. The primary aim of this study was to investigate the association of circulating bioactive adrenomedullin (bio-ADM) levels at intensive care unit (ICU) admission with mortality in sepsis patients and in a general ICU population. Secondary aims included the association of bio-ADM with organ failure and the ability of bio-ADM to identify sepsis.

Methods: In this retrospective observational study, adult patients admitted to one of four ICUs during 2016 had admission bio-ADM levels analysed. Age-adjusted odds ratios (OR) with 95% CI for log-2 transformed bio-ADM, and Youden's index derived cut-offs were calculated. The primary outcome was 30-day mortality, and secondary outcomes included the need for organ support and the ability to identify sepsis.

Results: Bio-ADM in 1867 consecutive patients were analysed; 632 patients fulfilled the sepsis-3 criteria of whom 267 had septic shock. The median bio-ADM in the entire ICU population was 40 pg/mL, 74 pg/mL in sepsis patients, 107 pg/mL in septic shock and 29 pg/mL in non-septic patients. The association of elevated bio-ADM and mortality in sepsis patients and the ICU population resulted in ORs of 1.23 (95% CI 1.07–1.41) and 1.22 (95% CI 1.12–1.32), respectively. The association with mortality remained after additional adjustment for lactate in sepsis patients. Elevated bio-ADM was associated with an increased need for dialysis with ORs of 2.28 (95% CI 2.01–2.59) and 1.97 (95% CI 1.64–2.36) for the ICU population and sepsis patients, respectively, and with increased need of vasopressors, OR 1.33 (95% CI 1.23–1.42) (95% CI 1.17–1.50) for both populations. Sepsis was identified with an OR of 1.78 (95% CI 1.64–1.94) for bio-ADM, after additional adjustment for severity of disease. A bio-ADM cut-off of 70 pg/mL differentiated between survivors and non-survivors in sepsis, but a Youden's index derived threshold of 108 pg/mL performed better.

Conclusions: Admission bio-ADM is associated with 30-day mortality and organ failure in sepsis patients as well as in a general ICU population. Bio-ADM may be a morbidity-independent sepsis biomarker.

Keywords: Critical illness, Sepsis, Septic shock, Adrenomedullin, Bioactive adrenomedullin, Biomarkers, Cut-off

Introduction

Background

Sepsis is a condition with high mortality and suffering, affecting millions of people yearly across all ages and backgrounds [1].

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Since sepsis is a syndrome encompassing a variety of illnesses with multiple pathophysiologies, there is no broadly applicable single efficient treatment pathway.

New methods for stratification and classification of sepsis are warranted in order to better tailor the care of septic patients. The use of biomarkers can potentially help us understand and categorise sepsis into phenotypes [2] and thereby add value to existing risk and severity scoring systems as well as guiding treatment. Further, a better understanding of hormonal systems, which some biomarkers are derived from, can open up for new therapeutical pathways.

Adrenomedullin

Adrenomedullin (ADM) is a 52-amino acid peptide hormone first discovered in human pheochromocytoma cells [3], but is produced by many different cell types [4]. ADM plays a part in the homeostasis of cardiovascular, endocrine, renal and immunological systems and has a role in the electrolyte balance [3–6]. More specifically, ADM has vasodilatory properties [7, 8] by binding to receptors on both endothelial and smooth muscle cells [9]. Further, ADM is capable of modulating the endothelial barrier, where it has a stabilising effect [9].

Adrenomedullin in sepsis

Over the last fifteen years, the role of ADM in sepsis has been investigated. Several studies have reported an association of increased levels of ADM and poor outcomes among patients with sepsis and septic shock [10–16]. The role of ADM in patients with a cardiopulmonary disease has also drawn attention [17–24]. These studies have used two assays measuring different fragments from the ADM precursor, mid regional pro adrenomedullin (MR-proADM) [25] and circulating bioactive adrenomedullin (bio-ADM) [12], making results difficult to compare. A cut-off value of 70 pg/mL bio-ADM has been used, which originates from Marino and colleagues [12]. It is not clear how this threshold was chosen, but the authors reported a 100% 28-day survival rate in a minimal subgroup ($n = 12$) where a reduction of bio-ADM levels to below 70 pg/mL was observed.

In animal models of sepsis, however, exogenous ADM has led to improved outcomes[26–28], why ADM has been referred to as a double-edged sword [29]. Further, modulation of the ADM hormonal system using antibodies against a non-ligand binding site of ADM has been suggested a potential therapy in sepsis [30]. This is currently investigated in a phase II clinical trial [31], where septic patients with initial levels of bio-ADM > 70 pg/mL are randomised to receive either the human ADM antibody adrecizumab or placebo [31]. Since ADM levels in non-septic and non-cardiopulmonary critical care patients are poorly investigated, we decided to perform this exploratory study.

Objectives

The primary aim of this study was to investigate the association of admission bio-ADM with mortality in patients fulfilling the sepsis criteria and in a large mixed general ICU population. Secondary aims were to investigate the association of bio-ADM with organ failure in the ICU, measured as need of circulatory and renal support, and the ability to identify sepsis. Further, we aimed to perform a validation of the proposed cut-off value of 70 pg/mL.

Methods

Study design and setting

The present study was a retrospective multicentre observational study of patients consecutively admitted to one of four general (mixed surgical and medical) ICUs in the Skåne Region (Scania county), Sweden, in 2016. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines were followed [32].

Participants

All adult ICU admissions with valid admission blood samples were included. When direct transfers occurred between the participating ICUs, follow-up data were merged to form cohesive ICU admissions. Transfers from other ICUs were excluded since our aim was to limit our study to primary admissions to intensive care. Information was given to the patient or next of kin, and information letters were sent home to surviving patients 2–6 months after hospital discharge. Patient consent was on an opt-out basis. For deceased patients, consent was presumed.

Variables

The primary outcome was 30-day mortality in sepsis patients and the general ICU population. Secondary outcomes were: (1) need of cardiovascular support, defined as cardiovascular sequential organ failure assessment (SOFA) score ≥ 3, at ICU admission, (2) need for continuous renal replacement therapy (CRRT) during ICU-stay and (3) identification of sepsis at ICU admission.

Sepsis cohort

The process of identifying the sepsis population, and collection of background data for this cohort, has previously been described in detail [33].

In brief, the sepsis-3 criteria [34] were used to identify patients with sepsis, defined as a SOFA score ≥ 2 on ICU admission with a suspicion of infection within 24 h before or 24 h after ICU admission. A suspected infection

was defined by blood culture sampling and concomitant administration of oral or intravenous antibiotics (24 h before to 72 h after blood culture), as suggested by the sepsis-3 task force [34].

The predefined exclusion criteria for sepsis admissions were: (1) elective ICU admission after elective surgery, and (2) cardiac arrest within 6 h before or 1 h after ICU admission.

Septic shock was defined as the need of a vasopressor, identified by either a cardiovascular SOFA score ≥ 3 or after a medical record review, and a lactate level of ≥ 2 mmol/L among those fulfilling sepsis criteria on ICU admission.

Data sources

Background and survival data were extracted from the patient administrative system for Intensive care units (PASIVA). PASIVA is the portal by which the treating physician and nursing staff submit prospectively collected laboratory and physiological data to the Swedish Intensive Care Registry. PASIVA is synchronised with the Swedish population register, which contains survival data.

Medical records were reviewed retrospectively by trained data collectors to identify sepsis criteria and additional background data [33].

Bio-ADM measurement

Blood samples, used for the analysis of bio-ADM, were collected on ICU admission and then centrifuged, aliquoted, frozen, and stored in the SWECRIT biobank at Region Scania (BD-47, SC-1922). Samples collected later than 6 h after ICU admission were excluded. If the sampling time was missing, samples were included if the time of freezing was within 6 h. Frozen plasma samples were shipped, and batch analysis of bio-ADM was performed on thawed samples in March 2019 at the laboratory of SphingoTec GmbH (Hennigsdorf, Germany). The assay has previously been described elsewhere [35].

Study size

The study size was not predetermined but rather a convenience sample. All adult ICU admissions from 2016, with valid admission blood samples and consent, in the SWECRIT biobank constituted our study material.

Statistics

For all hypothesis tests, we considered p values < 0.05 as significant. To assess a difference in the location of two independent variables, we used the Wilcoxon rank-sum test (Mann–Whitney U test). Differences in proportions were assessed using Pearson's χ^2 test. Medians were reported with their corresponding interquartile ranges (IQR). The Swedish 2016 calibration of the Simplified Acute Physiology Score III (SAPS3) was used to calculate the estimated 30-day mortality risk ($EMR_{30\text{-day}}$) [36, 37]. Multivariable binary logistic regression, adjusted for age, was used to analyse outcomes. The results of the regression analyses are reported as odds ratios (OR) with 95% confidence intervals (CI). The regression models were evaluated with the Hosmer–Lemeshow goodness-of-fit test with ten groups, and models resulting in significant tests were marked [38]. To adjust for severity of disease, SAPS3 was included in the regressions. If a parameter, due to skewness, needed transformation, the base 2 logarithm was used. The difference in Kaplan–Meier curves was evaluated with the log-rank test [39]. Areas under the curve (AUC) were derived from receiver operating characteristic (ROC) curves[40]. Differences in AUCs were tested with the method of DeLong et al. [41]. Youden's index derived thresholds were reported [42]. Admissions with missing data for any variable were excluded for mean and median calculations. If a variable had missing values, the number of observations available was specified.

Results

Participants

There were 2724 adult admissions in 2016. After merging and exclusion, 1867 admissions with valid samples remained, constituting our study population, shown in Fig. 1. The ICU study population was then divided into a sepsis and a non-sepsis cohort, with 632 and 1235 admissions, respectively.

Demographics

Patients in the sepsis cohort were generally older and sicker on admission with higher illness severity scores than patients in the non-sepsis cohort, as seen in Table 1. Septic patients were, to a greater extent, admitted from within the hospital, while non-septic patients more often were admitted from the emergency department and directly after surgery. The suspected focus of infection for the sepsis patients is shown in Additional file 1: Table S1. Positive blood cultures with the most common pathogens are displayed in Additional file 1: Fig. S1.

Outcomes

Mortality rates for the ICU population, sepsis cohort and non-sepsis cohort are shown in Table 1. The sepsis cohort had worse survival data, a greater need for organ support with significantly higher cardiovascular SOFA scores and a higher proportion of CRRT, and a longer ICU stay. A more detailed description of sepsis patients, divided into 30-day survivors and non-survivors, is shown in Table 2. Sepsis patients who did not survive were older and sicker,

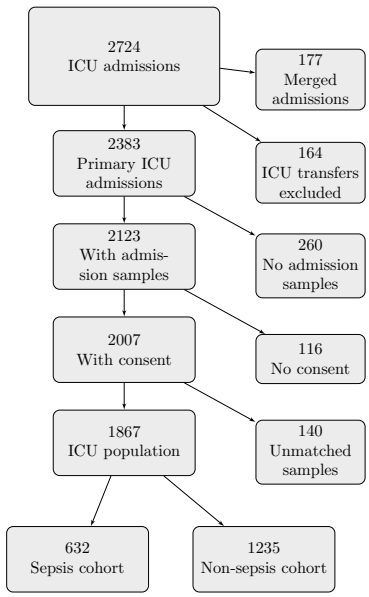

Fig. 1 Flow chart of ICU admissions, admission samples and consent. ICU: Intensive care unit

but with similar pre-existing comorbidities and a similar degree of septic shock on ICU admission, as survivors. Forty-two per cent of sepsis patients fulfilled the septic shock criteria on admission. This subgroup had a 30-day mortality rate of 30.1%, compared to 25.2% in non-shock patients ($p = 0.15$). $EMR_{30\text{-day}}$ among septic shock patients was 40.3% (22.5–58.9), while non-shock patients had an $EMR_{30\text{-day}}$ of 24.2% (12.2–44.5).

Bio-ADM

The range of bio-ADM was 8–4689 pg/mL, and since the distribution was highly skewed, a logarithmic transformation was used, see Fig. 2. The median time from admission to sampling was 25 min (15–40).

Bio-ADM and mortality

Dividing patients by quartiles of bio-ADM resulted in significant survival separation in the sepsis cohort as well as in the entire ICU population, as seen in Fig. 3.

Within the sepsis cohort, non-survivors had significantly higher levels of bio-ADM compared to survivors, shown in Table 2.

The associations of bio-ADM in the regression models for 30-day mortality were almost identical in the sepsis cohort and in the entire ICU population, as in Table 3. A doubling of bio-ADM generated a 22–23% increased OR for death.

In the model where admission lactate among septic patients was added as a covariate, bio-ADM was still significantly associated with 30-day mortality with an OR of 1.20 (1.04–1.38). The OR for lactate in the same model was 1.24 (1.06–1.45), $p = 0.009$. When SAPS3 and bio-ADM were applied in the same model for mortality, the association of bio-ADM and mortality was non-significant (data not shown).

The predictive accuracy for bio-ADM and 30-day mortality in the sepsis cohort, presented as AUC, in addition to c-reactive protein (CRP) and lactate are shown in Additional file 1: Table S2.

Bio-ADM and organ support

The association of bio-ADM with CRRT was strong in the sepsis cohort with OR 1.97 (1.64–2.36) but even stronger in the general ICU population, OR 2.28 (2.01–2.59). The ORs for a cardiovascular SOFA score 3 or 4 were 1.33 for both the septic (1.17–1.50) and the general ICU patients (1.23–1.42), as in Table 3.

Bio-ADM in sepsis and as a sepsis marker

The median bio-ADM in the sepsis cohort was more than twice as high as the median in the non-sepsis group, as in Table 1. The median bio-ADM in the septic shock subgroup was 107 pg/mL (58–188) compared to 62 pg/mL (35–116) in sepsis patients not presenting with shock ($p < 0.001$). In Table 3, the association of increased bio-ADM levels and the risk of having sepsis and septic shock is presented. The OR of having sepsis in the entire ICU population was 1.78 (1.64–1.94) after adjustment for severity of disease.

In the ICU population, the AUC (95% CI) of bio-ADM to identify sepsis was 0.76 (0.73–0.78), see Additional file 1: Table S2. A Youden's index derived threshold of 37 pg/mL for detecting sepsis resulted in a sensitivity and specificity of 61% and 80%, respectively.

Bio-ADM cut-offs

The cut-off of 70 pg/mL separated the ICU population into high and low bio-ADM, as shown in Table 1. The same information is shown graphically in Fig. 2. The sensitivity for 30-day mortality using a cut-off of 70 pg/mL was 42% with a corresponding specificity of 73% in the ICU population. For the sepsis cohort, the sensitivity and specificity were 60% and 50% for 30-day mortality, respectively. Kaplan–Meier curves and results from log-rank tests for bio-ADM levels above or below 70 pg/mL are displayed in Fig. 4. Youden's index identified a threshold for survival prediction of 45 pg/mL in the ICU population and 108 pg/mL in the sepsis cohort. A

Table 1 Demographics and outcomes of the ICU population and a comparison between the sepsis and non-sepsis cohorts

	ICU population	Sepsis cohort	Non-sepsis cohort	*p* value
Number, *n* (% of ICU population)	1867 (100)	632 (33.9)	1235 (66.1)	
Age in years, median (IQR)	67 (54–75)	69 (61–76)	65 (49.5–73)	< 0.001
Female sex, *n* (%)	738 (39.5)	251 (39.7)	487 (39.4)	0.95
Department of origin				
Emergency department/out of hospital, *n* (%)	896 (48)	276 (43.7)	620 (50.2)	0.008
Hospital ward, *n* (%)	604 (32.4)	282 (44.6)	322 (26.1)	< 0.001
Intermediate, *n* (%)	50 (2.7)	32 (5.1)	18 (1.5)	< 0.001
Operating room/postoperative ward, *n* (%)	317 (17)	42 (6.6)	275 (22.3)	< 0.001
Organ dysfunction and illness severity on ICU admission				
SAPS3 score, median (IQR)	59 (47–71)	66 (57–77)	54 (43–67)	< 0.001
SAPS3 $EMR_{30\text{-day}}$, median (IQR)	17.6 (5.2–40.3)	29.9 (14.8–53)	11.1 (3.1–31.9)	< 0.001
SOFA score, median (IQR)	6 (3–9)	7 (5–10)	4 (1–8)	< 0.001
Cardiovascular SOFA score ($n = 1836$), median (IQR)	1 (0–3)	3 (0–4)	1 (0–3)	< 0.001
Outcomes				
ICU mortality, *n* (%)	208 (11.1)	86 (13.6)	122 (9.9)	0.019
30-day mortality, *n* (%)	402 (21.5)	174 (27.5)	228 (18.5)	< 0.001
1-year mortality, *n* (%)	622 (33.3)	261 (41.3)	361 (29.2)	< 0.001
ICU length of stay in days, median (IQR)	1.6 (0.8–3.6)	2.5 (1.1–5.5)	1.1 (0.7–2.7)	< 0.001
CRRT use during ICU stay, *n* (%)	169 (9)	96 (15.2)	73 (5.9)	< 0.001
bio-ADM				
bio-ADM pg/mL, median (IQR)	40 (21–86)	74 (42–145)	29 (18–56)	< 0.001
bio-ADM> 70 pg/mL, *n* (%)	564 (30.2)	333 (52.7)	231 (18.7)	< 0.001

Data regarding general characteristics, outcomes, organ dysfunction and illness severity are presented. The sepsis cohort was compared to the non-sepsis cohort, and the *p* values refer to that comparison. Proportions (%) are within their subgroups unless otherwise specified. ICU: intensive care unit; IQR: interquartile range; SAPS3: Simplified Acute Physiology Score III; $EMR_{30\text{-day}}$: estimated 30-day mortality risk; SOFA: Sequential Organ Failure Assessment; CRRT: continuous renal replacement therapy; bio-ADM: circulating bioactive adrenomedullin

separate Kaplan–Meier curve for the sepsis cohort using the Youden's index-derived cut-off of 108 pg/mL is shown in Fig. 4. Sensitivity, specificity, positive predictive values, negative predictive values, positive and negative likelihood ratios for all cut-offs are displayed in Additional file 1: Table S2.

Discussion

In this study, elevated admission bio-ADM levels were associated with increased 30-day mortality in sepsis and in the general ICU population alike. Increased bio-ADM was also associated with cardiovascular failure and need for dialysis. Furthermore, after adjustment of severity of disease, bio-ADM was strongly associated with sepsis.

Bio-ADM in sepsis

Our sepsis cohort was identified using a structured method where ICU admissions were manually screened for sepsis-3 and septic shock criteria within a narrow time window at ICU admission. Hence, the sepsis diagnosis was not based on discharge diagnose coding, which has been shown to be misleading [33, 43, 44]. We applied predefined exclusion criteria in order to ensure that our sepsis cohort would represent clinically relevant sepsis patients requiring intensive care.

Interestingly, bio-ADM on admission was associated with mortality in sepsis patients and in the general ICU population in a similar fashion. When included in the same regression model for 30-day mortality, lactate and bio-ADM both contributed independently of each other, indicating that bio-ADM carries additional information in sepsis. In line with this, Blet and colleagues, reported added prognostic value of bio-ADM in addition to lactate among septic patients [45].

Bio-ADM has repeatedly been shown to be associated with increased morbidity [15, 16], which also was evident in our study. Sepsis patients were generally sicker and had significantly higher bio-ADM than the general ICU population. Further, patients with septic shock had significantly higher levels of bio-ADM, which is in agreement with previous reports [15, 16, 46].

The association of bio-ADM with sepsis remained after adjusting for severity of disease, implying that

Table 2 Demographics and outcomes of the sepsis cohort and comparisons between 30-day non-survivors and survivors

	Sepsis cohort	Non-survivors	Survivors	*p* value
Number, *n* (% of Sepsis cohort)	632 (100)	174 (27.5)	458 (72.5)	
Age in years, median (IQR)	69 (61–76)	73 (66–79)	68 (59–75)	< 0.001
Female sex, *n* (%)	251 (39.7)	61 (35.1)	190 (41.5)	0.17
Body mass index ($n = 588$), median (IQR)	26.6 (22.9–30.7)	26.7 (23.3–31.2)	26.3 (21.8–30.5)	0.11
Comorbidities				
None of those listed below, *n* (%)	173 (27.4)	46 (26.4)	127 (27.7)	0.74
Cardiovascular disease, *n* (%)	313 (49.5)	87 (50)	226 (49)	0.95
Respiratory disease, *n* (%)	156 (24.7)	47 (27)	109 (23.8)	0.46
Hepatic disease, *n* (%)	32 (5)	12 (6.9)	20 (4.4)	0.27
Renal disease, *n* (%)	63 (10.0)	18 (10.3)	45 (9.8)	0.96
Cancer, *n* (%)	109 (17.3)	37 (21.3)	72 (15.7)	0.13
Haematological disease, *n* (%)	47 (7.4)	17 (9.8)	30 (6.6)	0.23
Immunosuppression, *n* (%)	126 (19.9)	41 (23.6)	85 (18.6)	0.20
Diabetes, *n* (%)	167 (26.4)	40 (23.0)	127 (27.7)	0.27
Modified Charlson comorbidity index, median (IQR)	1 (0–2)	2 (0–2)	1 (0–2)	0.54
Department of origin				
Emergency department/out of hospital, *n* (%)	276 (43.7)	62 (35.6)	214 (46.7)	0.012
Hospital ward, *n* (%)	282 (44.6)	87 (50)	195 (42.6)	0.094
Intermediate, *n* (%)	32 (5.1)	13 (7.5)	19 (4.1)	0.089
Operating room/postoperative ward, *n* (%)	42 (6.6)	12 (6.9)	30 (6.6)	0.88
Organ dysfunction and illness severity on ICU admission				
SAPS3 score, median (IQR)	66 (57–77)	76 (66–82)	63 (56–73)	< 0.001
SAPS3 $EMR_{30\text{-day}}$, median (IQR)	29.9 (14.8–53)	50.9 (29.9–62.7)	24.2 (13.5–44.5)	< 0.001
SOFA score, median (IQR)	7 (5–10)	9 (6–11)	7 (5–9)	< 0.001
Cardiovascular SOFA score ($n = 625$), median (IQR)	3 (0–4)	3 (1–4)	3 (0–4)	0.037
Septic shock, *n* (%)	267 (42.2)	82 (47.1)	185 (40.4)	0.15
Outcomes				
ICU length of stay in days, median (IQR)	2.5 (1.1–5.5)	2.7 (1.2–6.2)	2.4 (1–4.9)	0.16
CRRT use during ICU stay, *n* (%)	96 (15.2)	38 (21.8)	58 (12.7)	0.006
Biomarkers				
bio-ADM pg/mL, median (IQR)	74 (42–145)	93 (51–173)	70 (39–131)	< 0.001
bio-ADM > 70 pg/mL, *n* (%)	333 (52.7)	104 (59.8)	229 (50)	0.035
Lactate ($n = 626$) mmol/L, median (IQR)	2.8 (1.5–4.9)	3.3 (1.7–5.7)	2.5 (1.4–4.6)	0.002
CRP ($n = 600$) mg/L, median (IQR)	113 (35–241)	143 (47–238)	102 (32–242)	0.13

Data regarding general characteristics, outcomes, organ dysfunction and illness severity are presented. Non-survivors were compared to survivors, and the *p* values refer to that comparison. Proportions (%) are within their subgroups unless otherwise specified. IQR: interquartile range; SAPS3: Simplified Acute Physiology Score III; $EMR_{30\text{-day}}$: estimated 30-day mortality risk; SOFA: Sequential Organ Failure Assessment; ICU: intensive care unit; CRRT: continuous renal replacement therapy; bio-ADM: circulating bioactive adrenomedullin

elevated levels of bio-ADM on ICU admission makes it more likely that a patient has sepsis.

To our knowledge, there have been no previous reports on the sepsis discriminating properties of bio-ADM in a general ICU population. The ability of bio-ADM to identify sepsis patients was modest with an AUC of 0.76. A Youden's index derived cut-off of 37 pg/mL generated a sensitivity of 61% and a specificity of 80%, which indicates limited clinical utility of that cut-off.

Bio-ADM in critical care

The finding that bio-ADM could be broadly applicable to critically ill patients has been reported previously [46]. Lemasle and colleagues studied a large population of patients requiring vasopressor or invasive ventilation for more than 24 h and found an association of bio-ADM with mortality and need for organ support. Their patient population was, however, sicker in comparison with ours. In addition, the bio-ADM samples were not admission

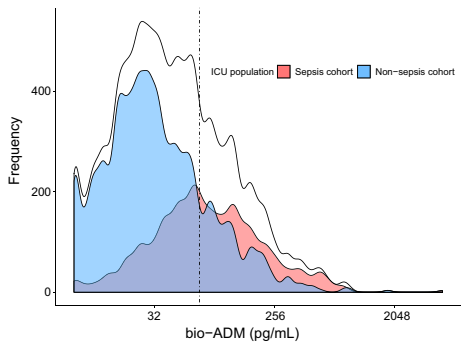

Fig. 2 Distribution of bio-ADM in the ICU population, Sepsis cohort and Non-sepsis cohort. X-axis logarithmic with base 2. The dotted line represents a concentration of 70 pg/mL. ICU: intensive care unit; bio-ADM: circulating bioactive adrenomedullin

samples, which could explain the lower bio-ADM median level in our study (40 pg/mL versus 66 pg/mL).

Bio-ADM cut-offs

In spite of the questionable rationale of using a cut-off of 70 pg/mL for bio-ADM in sepsis, it has been used in several studies since it was first proposed [12].

In the present study, the 70 pg/mL cut-off managed to separate survivors from non-survivors, but a Youden's index derived cut-off of 108 pg/mL performed better in sepsis patients, see Fig. 4. Interestingly, Mebazaa et al. reported a similar Youden's index cut-off of 102 pg/mL from their sepsis cohort in a recent study [15]. For the entire ICU population, the Youden's index identified the cut-off 45 pg/mL, which is a novel finding for bio-ADM.

Limitations

There are several limitations to this study.

The study was designed to focus on bio-ADM levels in sepsis patients. All ICU admissions were initially screened for sepsis-3 criteria, and the aim of our data retrieval was primarily to collect detailed data from this cohort. For the remaining ICU population, collection of data was by necessity limited to the PASIVA database, which resulted in different data availability for the sepsis and non-sepsis cohorts. We did, for example, not collect data on comorbidities systematically nor lactate or c-reactive protein levels in the non-sepsis cohort.

We did not have information on the volume status of the patients, nor whether adequate volume resuscitation measures were taken before vasopressor treatment was commenced, a diagnostic criterion for septic shock. However, this limitation is a common feature of studies aiming at identifying septic shock. Initiation of vasopressor therapy in the ICU would usually imply that adequate fluid resuscitation was done, assuming adherence to the Surviving Sepsis Guidelines [47].

We used a strict time frame in which we identified the sepsis and non-sepsis patients and did not investigate the development of sepsis or septic shock beyond

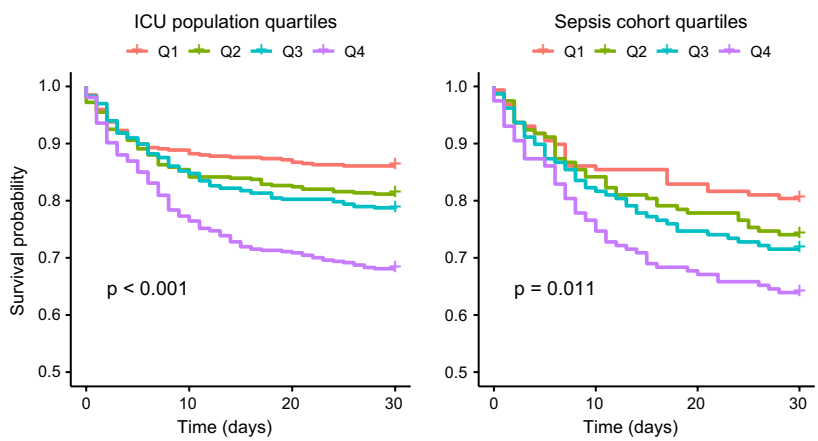

Fig. 3 Kaplan–Meier curves for the ICU population and the sepsis cohort according to quartiles of bio-ADM. The range of bio-ADM (pg/mL) in the quartiles in the ICU populations was < 21; 21–40; 40–86; > 86 and in the sepsis cohort < 42; 42–74; 74–145; > 145. The *p* values were derived from the log-rank test. ICU: intensive care unit; bio-ADM: circulating bioactive adrenomedullin; Q1: quartile 1; Q2: quartile 2; Q3: quartile 3 Q4: quartile 4

Table 3 Odds ratios for bio-ADM from multivariable binary logistic regression analyses for different outcomes

Outcome	ICU population			Sepsis cohort		
	OR	95% CI	*p* value	OR	95% CI	*p* value
30-day mortality	1.22	1.12–1.32	< 0.001	1.23	1.07–1.41	0.003
30-day mortality†	N/A	N/A	N/A	1.20	1.04–1.38	0.010
Cardiovascular SOFA≥ 3	1.33	1.23–1.42	< 0.001	1.33	1.17–1.50	< 0.001
CRRT use during ICU stay	2.28	2.01–2.59	< 0.001	1.97	1.64–2.36	< 0.001
Sepsis	1.91‡	1.76–2.08‡	< 0.001‡‡	N/A	N/A	N/A
Sepsis*	1.78‡	1.64–1.94‡	< 0.001‡	N/A	N/A	N/A
Septic shock	1.95	1.76–2.16	< 0.001	1.45	1.28–1.65	< 0.001
Septic shock*	1.78‡	1.60–1.98‡	< 0.001‡	1.35	1.19–1.54	< 0.001

The odds ratio for bio-ADM was calculated on a base 2 logarithmic scale. Age was included as a covariate in all regressions not including simplified acute physiology score III (SAPS3), as this is already an integral part of SAPS3. An additional covariate for the † model was lactate, and for the * models, the SAPS3 was included. If the Hosmer–Lemeshow test was $p < 0.05$, the model was marked ‡. *ICU: intensive care unit; OR: odds ratio; CI: confidence interval; SOFA: Sequential Organ Failure Assessment; CRRT: continuous renal replacement therapy; N/A: not applicable*

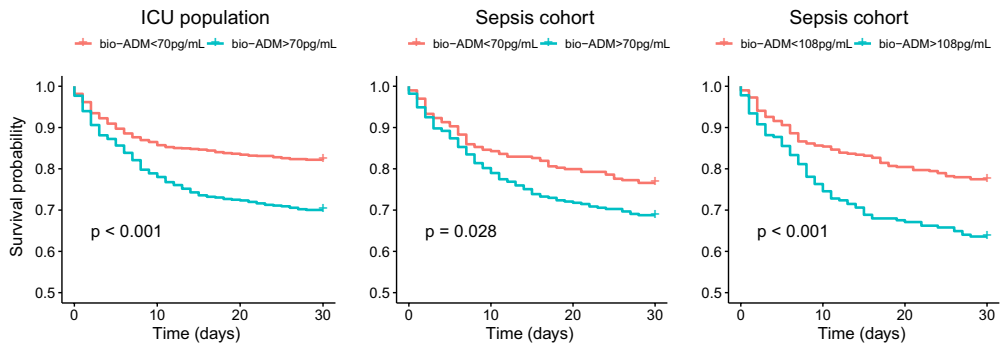

Fig. 4 Kaplan–Meier curves for the ICU population and the sepsis cohort according to bio-ADM admission levels above or below 70 pg/mL and according to an Youden's index derived cut-off of 108 pg/mL for the sepsis cohort. The *p* values were derived from the log-rank test. ICU: intensive care unit; bio-ADM: circulating bioactive adrenomedullin

that time. Our time constraint may have underestimated the diagnostic value of bio-ADM in sepsis. On the other hand, our method of retrospectively identifying patients fulfilling the sepsis criteria has probably identified patients who were not considered clinically septic by the treating physician.

We were confined to admission samples only, and could not investigate dynamic changes in bio-ADM levels and the impact these may have had on reported outcomes.

The mortality rate in our sepsis and septic shock subgroups was somewhat lower than expected, which could make our results difficult to generalise to patient populations outside of Scandinavia.

Conclusion

Elevated admission bio-ADM levels correlate with higher 30-day mortality and an increasing need for organ support in both sepsis and non-sepsis ICU patients. Bio-ADM may be an early morbidity-independent marker of sepsis.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13054-020-03351-1.

Additional file 1. Table S1: Suspected focus of infection and culture findings in the sepsis cohort. **Table S2:** Cutoffs, their corresponding positive and negative predictive values, likelihood ratios and AUCs for the

different biomarkers. **Figure S1:** Sepsis patients according to shock status and 30-day survival with one of the eight most common bacteria found in blood cultures are plotted in relation to the suspected focus of infection on ICU admission.

Abbreviations

ADM: Adrenomedullin; AUC: Area under the curve; bio-ADM: Circulating bioactive adrenomedullin; CI: Confidence interval; CRP: c-reactive protein; CRRT: Continuous renal replacement therapy; $EMR_{30\text{-}day}$: Estimated 30-day mortality risk; ICU: Intensive care unit; IQR: Interquartile range; MR-proADM: Mid regional pro adrenomedullin; N/A: Not applicable; OR: Odds ratio; ROC: Receiver operating characteristic; SAPS3: Simplified acute physiology score III; PASIVA: Patient administrative system for intensive care units; SOFA: Sequential organ failure assessment.

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Authors' contributions

HF and AF designed the study. OL, ML, MA, MS and HL participated in data collection. OL interpreted the data and performed statistical analyses. OL and ML wrote the first draft of the manuscript. All authors read, provided critical revision of, and approved the final manuscript.

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Availability of data materials

The datasets generated and analysed during the current study are not publicly available due to limitations in the ethical approval of the study and data management policies of Region Skåne but are available from the corresponding author on request.

Ethics approval and consent to participate

The study was approved by the Regional Ethical Committee in Lund, Sweden, with reference numbers DNR 2015/267 and DNR 2017/802. An opt-out procedure made a withdrawal of participation possible.

Consent for Publication

Not applicable.

Competing interests

OL, ML, MS, MA, HL, AF and HF declare that they have no competing interests, no financial or any other interests in SphingoTec GmbH and have not been in any way influenced by SphingoTec GmbH in writing this research paper. OM is listed as an inventor on a patent on bio-ADM in dementia prediction. SphingoTec GmbH is the owner of the patent. DB and JS are employed by SphingoTec GmbH and participate in the company's employee stock option program. Bioactive ADM was analysed free of charge by SphingoTec GmbH, Neuendorfstrae 15A, 16761 Hennigsdorf, Germany.

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Publisher's Note

Additional file 1

Circulating bioactive adrenomedullin as a marker of sepsis, septic shock and critical illness

Table S 1: **Suspected focus of infection and culture findings in the sepsis cohort.** Culture negative patients did not have any positive cultures within the time frame 24 hours before/after ICU admission.

	Sepsis cohort
Suspected focus of infection	
Respiratory, n (%)	342 (54%)
Gastrointestinal, n (%)	88 (14%)
Cardiovascular, n (%)	6 (1%)
Genitourinary, n (%)	41 (6%)
Musculo-dermato-haematological, n (%)	27 (4%)
Neurological, n (%)	15 (3%)
Unknown, n (%)	113 (18%)
Sum	632 (100%)
Culture findings	
Positive blood culture, n(%)	139 (22%)
Culture negative	290 (46%)

Table S 2: **Cutoffs, their corresponding positive and negative predictive values, likelihood ratios and AUCs for the different biomarkers.** All cutoffs were Youden's index derived except bio-ADM>70 pg/mL. If data were missing available parameters were specified. *ICU: intensive care unit; AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value; LR+: positive likelihood ratio; LR-: negative likelihood ratio; bio-ADM: circulating bioactive adrenomedullin; CRP: c-reactive protein*

	Sensitivity	**Specificity**	**AUC (95% CI)**	**PPV**	**NPV**	**LR+**	**LR-**
ICU population							
Cutoffs for 30-day mortality							
Bio-ADM>70 pg/mL	42%	73%	0.61 (0.58-0.64)	30%	82%	1.56	0.79
Bio-ADM>45 pg/mL	59%	58%	0.61 (0.58-0.64)	28%	84%	1.40	0.71
Cutoff for identification of sepsis							
Bio-ADM>37 pg/mL	61%	80%	0.76 (0.73-0.78)	51%	86%	2.05	0.33
Sepsis cohort							
Cutoffs for 30 day mortality							
Bio-ADM>70 pg/mL	60%	50%	0.59 (0.53-0.64)	31%	77%	1.20	0.80
Bio-ADM>108 pg/mL	48%	68%	0.59 (0.53-0.64)	36%	77%	1.51	0.77
CRP>117 mg/L (n=600)	59%	54%	0.54 (0.49-0.59)	32%	78%	1.29	0.75
Lactate>3.1 mmol/L (n=626)	55%	59%	0.58 (0.53-0.63)	34%	77%	1.34	0.76

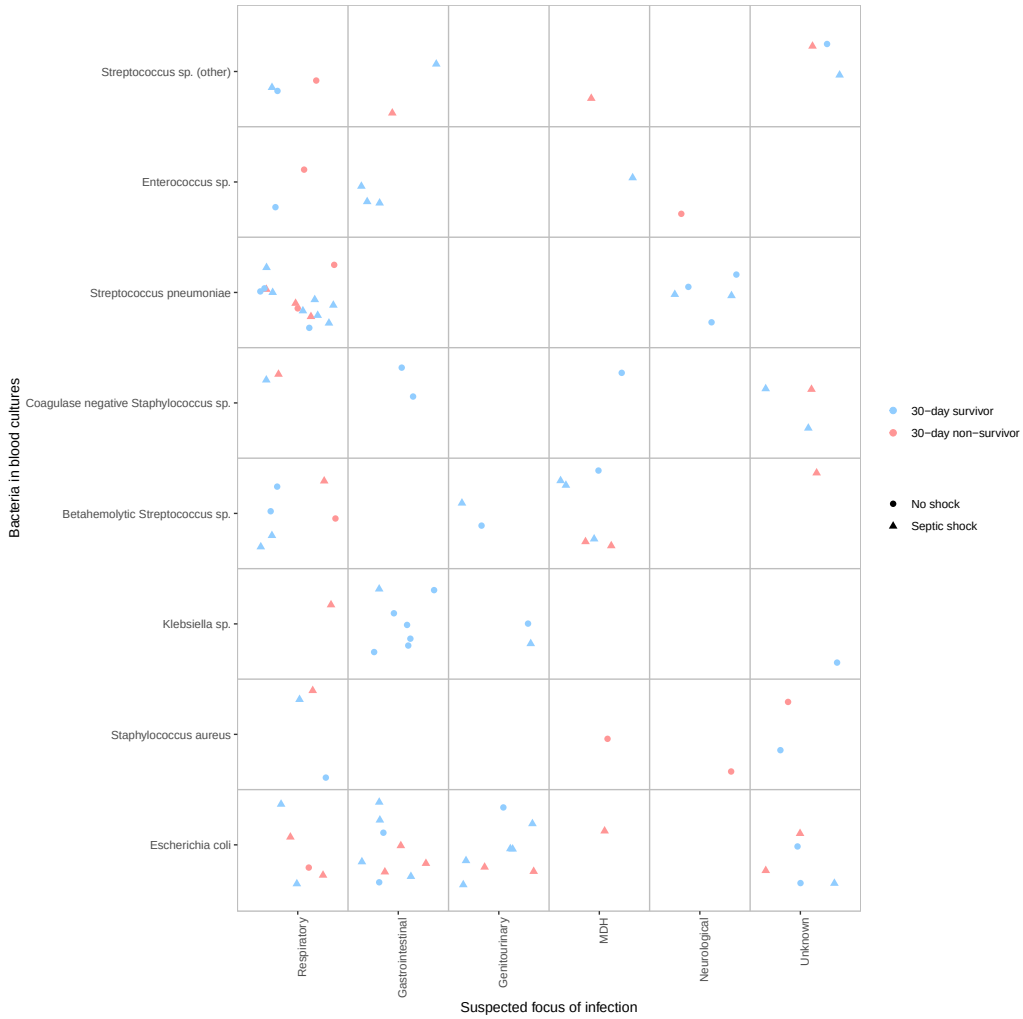

Figure S 1: Sepsis patients according to shock status and 30-day survival with one of the eight most common bacteria found in blood cultures are plotted in relation to the suspected focus of infection on ICU admission. A total of 105 blood culture findings in 98 ICU admissions are included in the figure. Seven admissions had two different bacteria and are thus plotted twice. *MDH, musculo-dermato-haematological*

Paper IV

PLOS ONE

RESEARCH ARTICLE

Bioactive adrenomedullin in sepsis patients in the emergency department is associated with mortality, organ failure and admission to intensive care

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Abstract

Background

Adrenomedullin is a vasoactive hormone with potentially prognostic and therapeutic value, which mainly has been investigated in intensive care unit (ICU) settings. The triaging in the emergency department (ED) of patients to the right level of care is crucial for patient outcome.

Objectives

The primary aim of this study was to investigate the association of bioactive adrenomedullin (bio-ADM) with mortality among sepsis patients in the ED. Secondary aims were to investigate the association of bio-ADM with multiple organ failure (MOF), ICU admission and ED discharge.

Methods

In this prospective observational cohort study, adult sepsis patients in the ED (2013–2015) had blood samples collected for later batch analysis of bio-ADM. Odds ratios (OR) with 95% confidence interval (CI) for bio-ADM were calculated.

Results

Bio-ADM in 594 sepsis patients was analyzed of whom 51 died within 28 days (8.6%), 34 developed severe MOF, 27 were ICU admitted and 67 were discharged from the ED. The median (interquartile range) bio-ADM was 36 (26–56) and 63 (42–132) pg/mL among survivors and non-survivors, respectively, 81 (56–156) pg/mL for patients with severe MOF and 77 (42–133) pg/mL for ICU admitted patients. Each log-2 increment of bio-ADM conferred an OR of 2.30 (95% CI 1.74–3.04) for mortality, the adjusted OR was 2.39 (95% CI 1.69–

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Competing interests: I have read the journal's policy and the authors of this manuscript have the following competing interests: OM is listed as an inventor on a patent on bio-ADM in dementia prediction. SphingoTec GmbH is the owner of the patent. JS is employed by SphingoTec GmbH, the manufacturer of the bio-ADM assay. Bio-ADM was analysed free of charge by SphingoTec GmbH, Neuendorfstrasse 15A, Hennigsdorf, Germany. OL, MR, KB and HF have declared no competing interests. The competing interests have no influence on the restrictions on sharing data we are bound to. However, due to ethical and legal restrictions related to the Swedish Biobanks in Medical Care Act (2002:297) and the Personal Data Act (1998:204) regarding deposition of data, data are not publicly published but available upon request via the authors.

3.39). The area under the receiver operating characteristic curve of a prognostic mortality model based on demographics and biomarkers increased from 0.80 to 0.86 (p = 0.02) when bio-ADM was added. Increasing bio-ADM was associated with severe MOF, ICU admission and ED discharge with adjusted ORs of 3.30 (95% CI 2.13–5.11), 1.75 (95% CI 1.11–2.77) and 0.46 (95% CI 0.32–0.68), respectively.

Conclusion

Bio-ADM in sepsis patients in the ED is associated with mortality, severe MOF, ICU admission and ED discharge, and may be of clinical importance for triage of sepsis patients in the ED.

Introduction

Background

Sepsis is a life-threatening condition which comes in a variety of shapes and severities, affecting millions of people worldwide [1]. In spite of improvements in recent years, the mortality of the most severe form of sepsis, septic shock, is still unacceptably high, up to 38% in North America and Europe [2].

The success rate of treating sepsis is time-sensitive, a short time to recognition and treatment is fundamental for outcomes, exemplified by the recommendation to consider one-hour bundles [3].

Identification of patients with sepsis in the emergency department (ED) is difficult and triaging patients to the correct level of care is a challenge. Biomarkers may be of help in identifying and stratifying sepsis according to severity of disease. An optimal biomarker in the ED setting should thus offer a method to distinguish individuals who can return home from those at high risk of developing multiple organ failure (MOF), thereby guiding clinicians to ensure patients an adequate level of care.

Adrenomedullin

Adrenomedullin (ADM) is a hormone produced by a variety of different cell types and was first derived from pheochromocytoma nearly three decades ago [4]. ADM has homeostatic and regulating effects on renal, immunological, endocrine and cardiovascular systems [4–7]. The effects of ADM on blood vessels include vasodilation [8] and stabilization of the barrier function of endothelial cells maintaining adequate permeability [9, 10]. ADM is typically elevated in patients with the metabolic syndrome [11], heart failure [12–15], chronic kidney failure [16–18] as well as in unselected critically ill patients [19, 20].

There are two predominant methods to measure ADM in peripheral blood. One is based on a part of the pre-cursor pro-hormone of ADM–mid regional pro-adrenomedullin (MR-proADM) [21], while the other measures bioactive adrenomedullin (bio-ADM) directly [22]. Few studies have described the correlation between measured MR-proADM and bio-ADM [22–24]. Although MR-proADM shows prognostic value in disease, it has no known action by itself, which makes the measurement of bio-ADM more attractive and clinically relevant. A median bio-ADM concentration of 20.7 pg/mL with 43 pg/mL as the 99th percentile among 200 healthy subjects has been reported [22].

Adrenomedullin in sepsis

Several studies have reported a strong association between elevated ADM levels and mortality, severity of illness and need for organ support in sepsis patients, using either of the two methods [19, 22, 25–31], proposing ADM to be a predictive biomarker in sepsis. Our group has recently reported that bio-ADM may be a specific marker of sepsis in a general intensive care unit (ICU) population [19].

In addition, the potential of modulating the ADM hormonal system has gained interest since exogenous infusion of ADM in animal models of sepsis has been shown to improve outcomes [10, 32, 33], which has led to the hypothesis that an increment of intravascular bio-ADM may be of therapeutic value in sepsis [9]. This has led to studies of the non-neutralizing anti-ADM antibody Adrecizumab in humans [23, 24, 34]. The formation of Adrecizumab-ADM complexes generates elevated intravascular bio-ADM concentrations where ADM can exert its endothelium-stabilizing effects [9, 35]. The increase of bio-ADM, on the other hand, is not accompanied by an elevation of MR-proADM suggesting a redistribution of ADM rather than an increased synthesis [23, 24]. The clinical implication of the use of Adrecizumab in sepsis is yet unanswered, but clinical trials to investigate this are planned [36].

While most of the studies describing ADM in sepsis are derived from ICU settings, similar findings have been found in populations originating in the ED. Studies performed on infected patients in the ED have reported MR-proADM to have a higher association with mortality and ICU admission compared to other commonly used biomarkers and clinical scores [37–39]. Further, a combination of MR-proADM with clinical scores and other biomarkers in order to improve prognostic accuracy has also been proposed [40–43].

Studies measuring bio-ADM in the ED are sparse. Two recent studies have described bio-ADM in ED populations but patients presented with either acute heart failure or dyspnea [14, 44]. The original paper presenting bio-ADM [22], however, analyzed bio-ADM in patients with suspected sepsis in the ED. In the present study, our aim was to investigate the prognostic capability of bio-ADM in a large sepsis cohort in the ED.

Objectives

We hypothesized that increasing levels of bio-ADM in sepsis patients in the ED were associated with subsequent severity of sepsis and increased mortality.

The primary aim of this study was to investigate the association of bio-ADM with 28-day mortality. Secondary aims were to *I*) assess whether bio-ADM could improve the prognostic precision of a mortality prediction model, *II*) compare the prognostic properties of bio-ADM with other commonly used biomarkers, and III) investigate the association of bio-ADM with *a)* severe MOF, *b*) ICU admission among patients with no limitations of care and *c*) ED discharge.

Material and methods

Study design and setting

This single center prospective observational cohort study was performed in the ED of Skåne University Hospital in Malmö, Sweden. With a catchment population of 400000, the hospital has approximately 85000 emergency visits per year.

Both oral and written consent was obtained by the patients or by their next of kin after they had the opportunity to read and review a written description of the study design and purpose. If a patient at inclusion had a decreased level of consciousness, consent was obtained

retrospectively. This consent procedure and the study as a whole, was approved by the Regional Ethical board in Lund (DNR 2013/635).

The STROBE guidelines were followed [45].

Participants

Between December 2013 and February 2015, patients 18 years or older, seeking care during office hours (Monday to Friday, 6 AM to 6 PM) in the ED, were screened for inclusion by trained research nurses. The inclusion criteria were based on the sepsis definition at the time [46]: suspected infection in addition to two or more systemic inflammatory response syndrome (SIRS) criteria. Inclusion criteria were: 1) a body temperature lower than 36˚C, or higher than 38˚C, or self-reported fever/chills within 24 hours preceding the ED visit, 2) a respiratory rate higher than 20 breaths/min, 3) a heart rate higher than 90 beats/min. White blood cell count was not part of the inclusion criteria due to unavailability at the time of screening.

The study size was not predefined and consisted of a convenience sample of patients included during the study period.

Variables

The primary outcome was 28-day mortality. Secondary outcomes were number of failing organ systems, ICU admission and ED discharge. Failing organ systems, defined in S1 Table, were registered up to 48 hours after presentation at the ED and trichotomized into 1) no organ failure, 2) intermediate organ failure (one to three failing organ systems) and 3) severe MOF (four or more failing organ systems). ICU admission was registered during the entire follow-up time. Furthermore, a prognostic baseline model including covariates with significantly different distribution in relation to 28-day mortality, and three commonly used biomarkers, lactate, C-reactive protein (CRP) and creatinine was created to investigate whether the addition of bio-ADM improved the model. Premorbid comorbidities were registered and classified as shown in S2 Table.

Data sources

Patient demographics and comorbidities were systematically and prospectively collected from medical records which were reviewed by infectious disease physicians. Site of infection and type of ward, if admitted to the hospital, were recorded.

Biomarkers

Blood was drawn peripherally within one hour of presentation to the ED. All biomarkers except for bio-ADM were analyzed routinely in the certified hospital laboratory. For the analysis of bio-ADM plasma ethylenediaminetetraacetic acid plasma samples were frozen within 2 hours and stored at -80˚C until later batch analysis. Measurements of bio-ADM was undertaken at the laboratory of SphingoTec GmbH in Hennigsdorf, Germany in June 2018 as described elsewhere [47].

Statistics

For all hypotheses tests, we considered p-values <0.05 as significant. Group comparisons of continuous variables were performed using Wilcoxon rank-sum test (Mann-Whitney U test) for two groups. If there were more than two groups to be compared, Kruskal-Wallis rank sum test was used, and if significant, a comparison with pairwise Wilcoxon test, with Holm´s

procedure for adjustment for multiple testing was performed. Differences in proportions were assessed using Pearson's X^2 test. Medians were reported with their corresponding interquartile ranges (IQR). Uni- and multivariable binary logistic regression was used to analyze outcomes. Covariates in the multivariable binary logistic regression analyses were included if they were significantly differently distributed in relation to the primary outcome. The results of the regression analyses were reported as odds ratios (OR) with 95% confidence intervals (CI). The regression models were evaluated with the Hosmer-Lemeshow goodness-of-fit test with ten groups, and only models resulting in non-significant tests were reported [48]. Body mass index (BMI) was stratified according to underweight (<18.5), normal (18.5–25), overweight (25–30) and obese (>35) prior to inclusion in the multivariable binary logistic regressions, with the normal group as reference. If a parameter, due to skewness, needed transformation, the base 2 logarithm was used. The difference in Kaplan-Meier curves was evaluated with the log-rank test [49]. Areas under the receiver operating characteristic curve (AUROC) were calculated [50]. Differences in AUROCs were tested with the method of DeLong et al [51]. Admissions with missing data were excluded from calculations. If a variable had missing values (MV) these were specified. R Studio version 1.2.1335 was used as statistical software.

Results

Participants

Inclusion criteria were met by 647 patients. Due to missing data 50 patients were excluded and bio-ADM was analyzed in 597 patients. Of these, three additional patients had missing mortality follow up data leaving 594 subjects to be included in the study, see Fig 1.

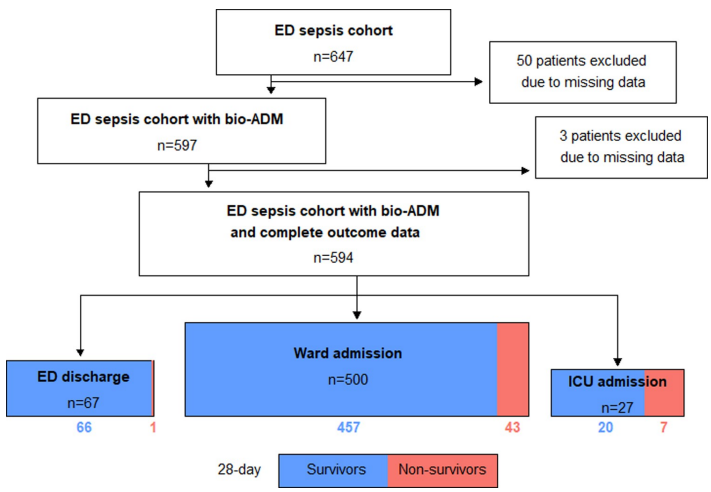

Fig 1. Patient flowchart according to inclusion eligibility, referral after assessment in the emergency department and 28-day mortality. In total 53 patients were excluded due to missing plasma and missing outcome data as 28-day mortality, organ failure and ICU admission. *ED: emergency department; bio-ADM: bioactive adrenomedullin; ICU: intensive care unit.*

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Demographics

Demographics including age, sex, comorbidities and site of infection are shown in Table 1. Non-survivors were generally older, had a lower BMI and a higher burden of cardiovascular disease. Further, non-survivors more often had a decision on limitation of care. The site of infection varied, non-survivors had a higher rate of pulmonary infections, whereas infections

Table 1. Demographics and outcomes of the sepsis cohort and comparisons between 28-day non-survivors and survivors.

Baseline characteristics	Sepsis cohort	Non-survivors	Survivors	p-value
Number, n (% of Sepsis cohort)	594 (100)	51 (8.6)	543 (91.4)	
Age in years, median (IQR)	73 (61–82)	80 (73–88)	72 (59–82)	<0.001
Female sex, n (%)	289 (48.6)	22 (43.1)	267 (49.2)	0.50
Body mass index (MV = 27), median (IQR)	25.7 (22.5–29.6)	24.0 (21.7–27.9)	25.8 (22.6–30)	0.05
Comorbidities				
Cardiovascular disease (MV = 2), n (%)	229 (38.7)	316 (60.8)	198 (36.6)	0.001
Respiratory disease (MV = 2), n (%)	140 (23.6)	18 (35.3)	122 (22.6)	0.06
Neurological disease (MV = 1), n (%)	98 (16.5)	9 (17.6)	89 (16.4)	0.98
Renal disease, n (%)	45 (7.6)	5 (9.8)	40 (7.4)	0.72
Cancer (n = 591), n (%)	165 (27.9)	20 (40)	145 (26.8)	0.13
Immunodeficiency (MV = 9), n (%)	32 (5.5)	6 (12)	26 (4.9)	0.07
Diabetes (MV = 1), n (%)	114 (19.2)	15 (29.4)	99 (18.2)	0.08
Psychiatric disorder (MV = 2), n (%)	63 (10.6)	4 (8)	59 (10.9)	0.69
None of those listed above, n (%)	146 (24.6)	4 (7.8)	142 (26.1)	0.006
Limitation of care (MV = 5), n (%)	90 (15.3)	24 (47.1)	66 (12.3)	<0.001
Site of infection				
Pulmonary, n (%)	199 (33.5)	24 (47.1)	175 (32.2)	0.02
URTI, n (%)	52 (8.8)	0 (0)	52 (9.6)	0.05
Urinary, n (%)	129 (21.7)	4 (7.8)	125 (23)	0.03
Bone and joint, n (%)	7 (1.2)	1 (2.0)	6 (1.1)	1
SSTI, n (%)	58 (9.8)	7 (13.7)	51 (9.9)	0.36
Gastrointestinal, n (%)	23 (3.9)	1 (2.0)	22 (4.1)	0.78
Other, n (%)	76 (12.8)	7 (13.7)	69 (12.7)	0.87
No confirmed infection, n (%)	50 (8.4)	7 (13.7)	43 (7.9)	0.24
Outcomes				
No organ failure, n (%)	278 (46.8)	8 (15.7)	270 (49.7)	<0.001
Intermediate organ failure (1–3), n (%)	282 (47.5)	30 (58.8)	252 (46.4)	0.12
Severe MOF (≥4), n (%)	34 (5,7)	13 (25.4)	21 (3.9)	<0.001
ICU admission, n (%)	27 (4.5)	7 (13.7)	20 (3.7)	0.003
Discharged from ED, n (%)	67 (11.3)	1 (2.0)	66 (12.2)	0.05
Biomarkers				
Bio-ADM pg/mL, median (IQR)	38 (27–60)	63 (42–132)	36 (26–56)	<0.001
Lactate (MV = 25) mmol/L, median (IQR)	1.7 (1.3–2.7)	2.1 (1.3–3.0)	1.7 (1.2–2.6)	0.11
CRP (MV = 7) mg/L, median (IQR)	72 (25–160)	100 (51–178)	69 (23–156)	0.04
Creatinine (MV = 5) μmol/L, median (IQR)	87 (68–120)	105 (79–160)	85 (68–117)	0.006

Data regarding general characteristics, comorbidities, site of infection, outcomes and biomarkers are presented. Non-survivors were compared to survivors, and the p-values refer to that comparison. Proportions (%) are within their subgroups unless otherwise specified. *IQR*: *interquartile range; MV*: *missing values*, *URTI*: *upper respiratory tract infection; SSTI*: *skin and soft tissue infection; ED*: *emergency department; MOF*: *multiple organ failure; ICU*: *intensive care unit; bio-ADM*: *bioactive adrenomedullin; CRP*: *C-reactive protein*

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refrained to the upper respiratory tract and urinary sites were more common among survivors.

Outcomes

Fifty-one patients (8.6%) died within 28 days, of whom 25 patients (4.2%) died within 7 days. Among 316 patients who developed organ failure (53.2%), 34 patients (5.7%) developed severe MOF as shown in Table 1. Twenty-seven patients (4.5%) were admitted to the ICU. Just over every tenth patient (11.3%) was discharged directly from the ED. One of them, the only 28-day non-survivor in the group, was offered admission to the ICU but declined and was discharged to palliative care at home after discussion with the patient and the patient´s family.

Bio-ADM

Levels of bio-ADM ranged 8–813 pg/mL and were logarithmically transformed due to skewness.

Bio-ADM and mortality. Non-survivors had higher levels of bio-ADM than survivors, 63 (42–132) pg/mL versus 36 (26–56) pg/mL, see Table 1. Dividing the patients into quartiles based on levels of bio-ADM a significant separation between the corresponding Kaplan-Meier curves for 28-day mortality, was observed, see Fig 2. The association of bio-ADM with 28-day

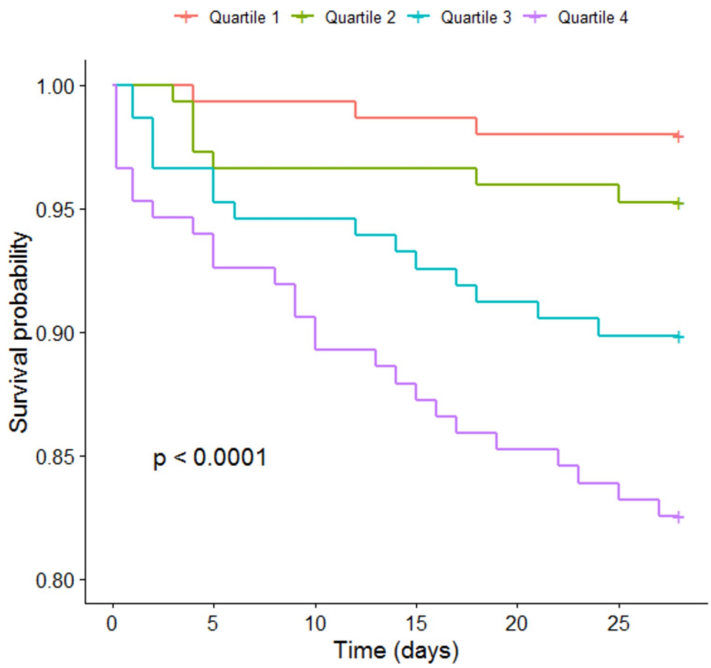

Fig 2. Kaplan-Meier curve according to quartiles of bio-ADM and 28-day mortality. The range of bio-ADM (pg/mL) was for Quartile 1: <27; Quartile 2: 27–38; Quartile 3: 38–60; Quartile 4: >60. The p-value was derived from the log-rank test. *bio-ADM*: *bioactive adrenomedullin*.

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Table 2. Odds ratios for bio-ADM from uni- and multivariate binary logistic regression analyses for primary and secondary outcomes.

Univariate				Multivariate			
Primary outcome	**OR**	**95% CI**	**p-value**	**Primary outcome**	**OR**	**95% CI**	**p-value**
28-day mortality	2.30	1.74–3.04	<0.001	28-day mortality (MV = 29)	2.39	1.69–3.39	<0.001
Secondary outcome	**OR**	**95% CI**	**p-value**	**Secondary outcome**	**OR**	**95% CI**	**p-value**
Severe MOF	3.22	2.26–4.59	<0.001	Severe MOF (MV = 29)	3.30	2.13–5.11	<0.001
ICU admission (MV = 5)	2.21	1.50–3.24	<0.001	ICU admission (MV = 27)	1.75	1.11–2.77	0.02
ED discharge	0.41	0.29–0.56	<0.001	ED discharge (MV = 29)	0.46	0.32–0.68	<0.001

The odds ratio for bio-ADM was calculated on a base 2 logarithmic scale. Multivariate included covariates bio-ADM, age, known cardiovascular disease, BMI, urinary, URTI and pulmonary site of infection. The outcome ICU admission was only calculated among patients with no limitations of care (n = 499). *bio-ADM*: *bioactive adrenomedullin; BMI*: *body mass index; ICU*: *intensive care unit; URTI*: *upper respiratory tract infection; OR*: *odds ratio; CI*: *confidence interval; MV*: *missing values; ED*: *emergency department*.

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mortality showed a univariate OR of 2.30 (95% CI 1.74–3.04), which remained significant after adjustments, 2.39 (95% CI 1.69–3.39), see Table 2.

A baseline mortality prediction model including age, previous cardiovascular disease, BMI, URTI, urinary or pulmonary infection site and routine biomarkers (CRP, lactate, creatinine) resulted in an AUROC of 0.80, which significantly improved with the addition of bio-ADM to an AUROC of 0.86 (p = 0.02), see Fig 3.

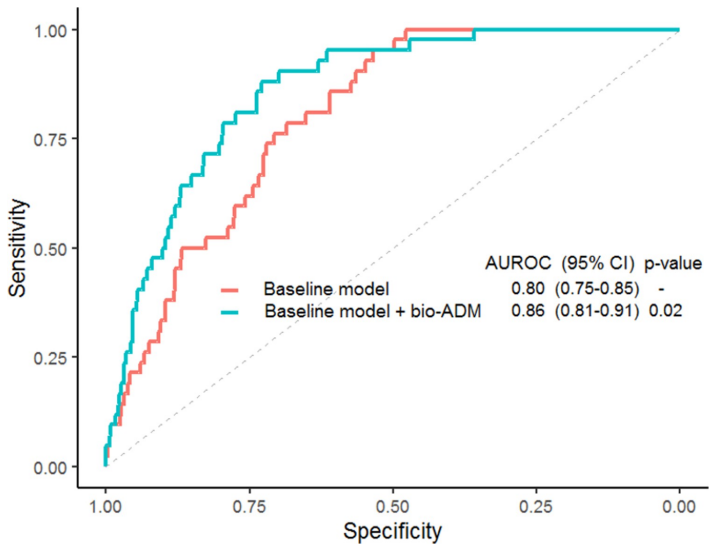

Fig 3. Receiver operating characteristics curves for mortality predictive models. Baseline model with covariates age, known cardiovascular, BMI, URTI, urinary and pulmonary site of infection, C-reactive protein, lactate and creatinine. The additive value of bio-ADM is shown in Baseline + bio-ADM. The p-value is derived from the DeLong's test for comparison between the two AUROCs. *BMI*: *body mass index; URTI*: *upper respiratory tract infections; bio-ADM*: *bioactive adrenomedullin; AUROC*: *area under the receiver operating characteristic; CI*: *confidence interval*.

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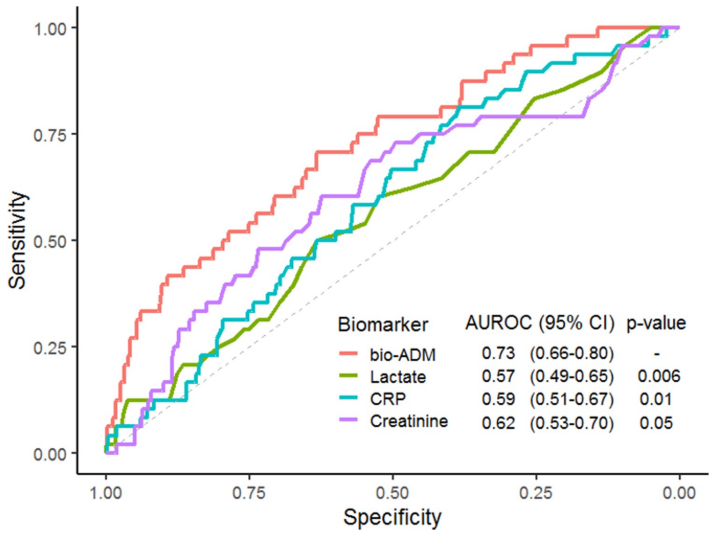

Fig 4. Receiver operating characteristics curves for the biomarkers bio-ADM, lactate, CRP and creatinine corresponding to 28 day mortality. Only patients with all four biomarkers analyzed were included (n = 562). P-values are derived from the DeLong's test for comparison with the AUROC of bio-ADM. *bio-ADM: bioactive adrenomedullin; CRP: C-reactive protein; AUROC: area under the receiver operating characteristic curve.*

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Bio-ADM and other biomarkers. The receiver operating characteristics curves with corresponding AUROCs for lactate, CRP, creatinine and bio-ADM in relation to 28-day mortality are shown in Fig 4. Bio-ADM had a significantly higher AUROC than lactate, CRP and creatinine.

Bio-ADM and organ failure. Bio-ADM concentrations among patients without organ failure, 31 (21–44) pg/mL, intermediate organ failure, 45 (31–72) pg/mL, and severe MOF, 81 (56–156) pg/mL, are shown in Fig 5. A significant separation between the groups was seen ($p<0.001$).

ORs from uni- and multivariate regressions for bio-ADM for the development of severe MOF were 3.22 (95% CI 2.26–4.59) and 3.30 (95% CI 2.13–5.11), respectively, see Table 2.

Bio-ADM and ICU admission. Patients admitted to the ICU had significantly higher levels of bio-ADM, 77 (42–133) pg/mL, than patients not admitted to the ICU, 41 (28–61) pg/mL, and patients discharged from the ED, 26 (19–32) pg/mL ($p<0.001$). Fig 6 shows the distribution of bio-ADM according to patient referral after assessment in the ED. The distribution was significantly separated between the groups ($p<0.001$). There was a significant association between ICU admission and increasing levels of bio-ADM, both before and after adjustment, see Table 2.

Bio-ADM and ED discharge. The median bio-ADM among patients discharged from ED was 26 (19–32) pg/mL, significantly lower than the corresponding median of 41 (28–63) pg/mL among patients admitted to a hospital ward or admitted to the ICU, 73 (41–130) pg/mL ($p<0.001$).

Uni- and multivariate logistic regression analyses showed an inverse association of increasing levels of bio-ADM and ED discharge, see Table 2.

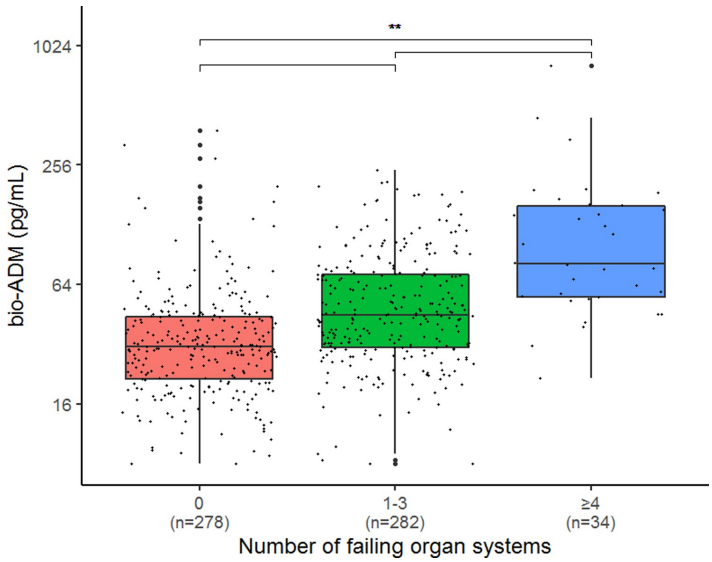

Fig 5. Boxplots showing levels of bio-ADM according to number of failing organ systems. P-values are derived from the pairwise Wilcoxon test. **: p<0.001 *bio-ADM*: *bioactive adrenomedullin*.

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Discussion

To our knowledge, this is the largest study to date investigating bio-ADM as a prognostic biomarker in patients with sepsis in the ED. Our data show that high levels of bio-ADM in the ED are associated with mortality, development of severe MOF and referral to intensive care. Moreover, we found that bio-ADM adds important prognostic information to the commonly used prognostic factors age, comorbidities, site of infection and routine biomarkers, and that low levels of bio-ADM are related to less severe disease and discharge from the ED.

Our study suggests that bio-ADM is of potential clinical use for early stratification of unselected sepsis patients in the ED. Alongside with the first study describing bio-ADM [22] and recent reports on possible applications of bio-ADM in patients with dyspnea [44] as well as heart failure [14], our data show that bio-ADM is a potentially important clinical biomarker in the ED. Whether these results are generalizable to a broader unselected ED population remains unknown and needs to be addressed in future studies. However, reports where MR-proADM was measured in broader ED populations show promising results [42, 52].

We found a strong association between bio-ADM in the ED and mortality, which remained after adjustments for known prognostic factors. Similar findings have been described in previous studies for both septic [19, 29–31] and non-septic [19, 20] patients treated in the ICU, but not as clearly among septic patients in the ED [22]. The prognostic ability of bio-ADM to predict mortality by itself was modest in the present study, but superior to three commonly used biomarkers, lactate, CRP and creatinine. Importantly, a baseline prediction model was improved when bio-ADM was added, indicating strong additional prognostic properties for bio-ADM. Our findings resemble results from a study in a similar setting where ADM was analyzed using the MR-proADM method. In that study, Scheutz et al. reported an improvement of

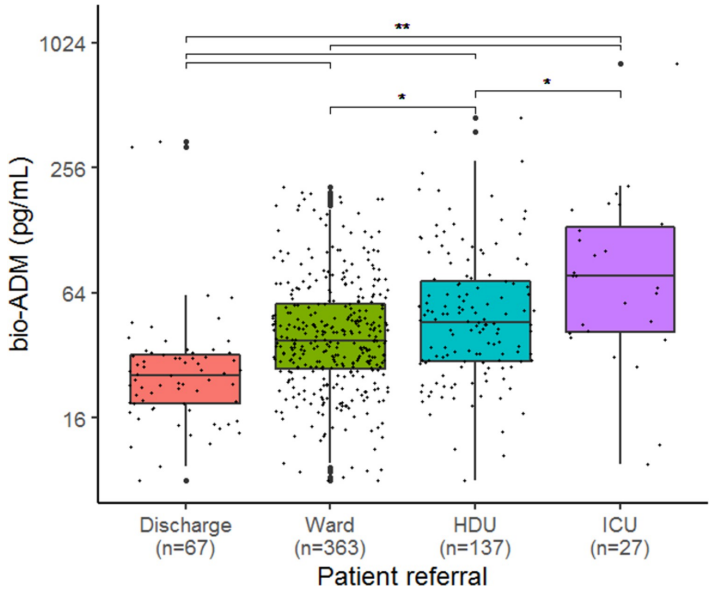

Fig 6. Boxplots showing levels of bio-ADM according to patient referral after assessment in the emergency department. P-values are derived from the pairwise Wilcoxon test. *: p<0.05, **: p<0.001. *bio-ADM*: *bioactive adrenomedullin; HDU*: *high dependency unit; ICU*: *intensive care unit.*

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a predictive model with an increased AUROC from 0.79 to 0.84, when MR-proADM was added [52].

The highest levels of bio-ADM in our study were found among patients admitted who developed severe MOF. Rising levels of bio-ADM were associated with increasing number of failing organ systems in sepsis patients. These results are in line with previous findings that septic patients with high levels of bio-ADM in the ICU had an increased need of organ support [19, 20, 29–31].

Interestingly, in the present ED cohort the median bio-ADM of 73 (41–130) pg/mL in the group of patients admitted to the ICU was similar to the distribution of bio-ADM in an ICU sepsis population where the median bio-ADM was 74 (42–145) pg/mL [19]. This is the first report to describe that bio-ADM is predictive of ICU admission in a sepsis cohort in the ED, which is a novel finding. Due to known variations in the availability of ICU beds across countries, this may however not be generalizable to other hospital environments [53].

The patients discharged from the ED in our cohort had low levels of bio-ADM with levels close to those in healthy subjects [22]. There were some extreme outliers within the group, making a clear threshold of bio-ADM difficult to identify. To our knowledge, no previous study has reported levels of bio-ADM in patients with sepsis discharged from ED.

Strengths and limitations

This large prospective observational cohort study affirms previous findings from ICU settings and demonstrates the potential applicability of bio-ADM in the ED setting. Furthermore, all

patient records in this study were thoroughly revised by infectious disease physicians to assure correct diagnoses. Also, this study included patients with limitations of care.

This study has several limitations. First, we only enrolled participants during office hours which may have led to a selection bias. Second, we were confined to admission samples, making it impossible to analyze dynamic changes and how these could correlate with outcomes. Third, this was a single-center study why generalizability of our results to other hospital settings may be limited. Finally, the study was initiated when sepsis was defined by the Sepsis-2 criteria and thus SOFA score was not recorded.

Conclusions

Bio-ADM in sepsis patients in the ED is associated with mortality, MOF, ICU admission and ED discharge. Bio-ADM exceeds the prognostic properties of routine biomarkers as lactate, CRP and creatinine and may be of clinical importance for triage of sepsis patients in the ED.

Supporting information

S1 Table. Dysfunction criteria for organ failure up to 48 hours after ED presentation.
(DOCX)

S2 Table Comorbidities and examples of corresponding diagnoses.
(DOCX)

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S1 Table. Dysfunction criteria for organ failure up to 48 hours after ED presentation.

Failing organ system	Dysfunction criteria
Central nervous system	Confusion, drowsiness or loss of consciousness
Circulatory failure	Systolic blood pressure < 90 mmHg, mean arterial pressure < 70 mmHg, decrease of systolic blood pressure greater than 40 mmHg or need for vasopressor to maintain blood pressure
Respiratory failure	SaO_2 < 90% or need for mechanical ventilation
Kidney failure	Serum creatinine increase of > 44 μmol/L between any two measurements, need for acute renal replacement therapy or an increase in creatinine corresponding to 1.5-fold of baseline with an initial value of > 160 μmol/L within 48 h
Liver failure	Total serum bilirubin > 40 μmol/L
Hematologic dysfunction	Platelet count < 100×10^9/L, INR > 1.5 or an aPTT > 60 s
Metabolic dysfunction	Serum lactate > 3.5 mmol/L.

S2 Table. Comorbidities and examples of corresponding diagnoses.

Comorbidities	Diseases
Cardivascular disease	Ischemic heart disease, heart failure, atrial fibrillation/flutter
Respiratory disease	Chronic obstructive pulmonary disease, asthma, restrictive pulmonary disease (fibrosis, interstitial lung disease, asbestosis), other pulmonary disease (including pulmonary hypertension).
Neurological disease	Neuromuscular disease (including post-polio syndrome), cerebral stroke, transient ischemic attack,
Renal disease	Parenchymatic renal disease, glomerular filtration rate <30 ml/min
Psychiatric disorder	Dementia, anxiety, depression,

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OSCAR HM LUNDBERG is a specialist in anesthesiology and intensive care medicine at Skåne University Hospital in Malmö, Sweden. In this thesis dr. Lundberg explores the potential role of the hormone adrenomedullin, alone or in combination with other biomarkers, among sepsis patients in the intensive care unit and in the emergency department.

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