Biomarkers in critically ill patients

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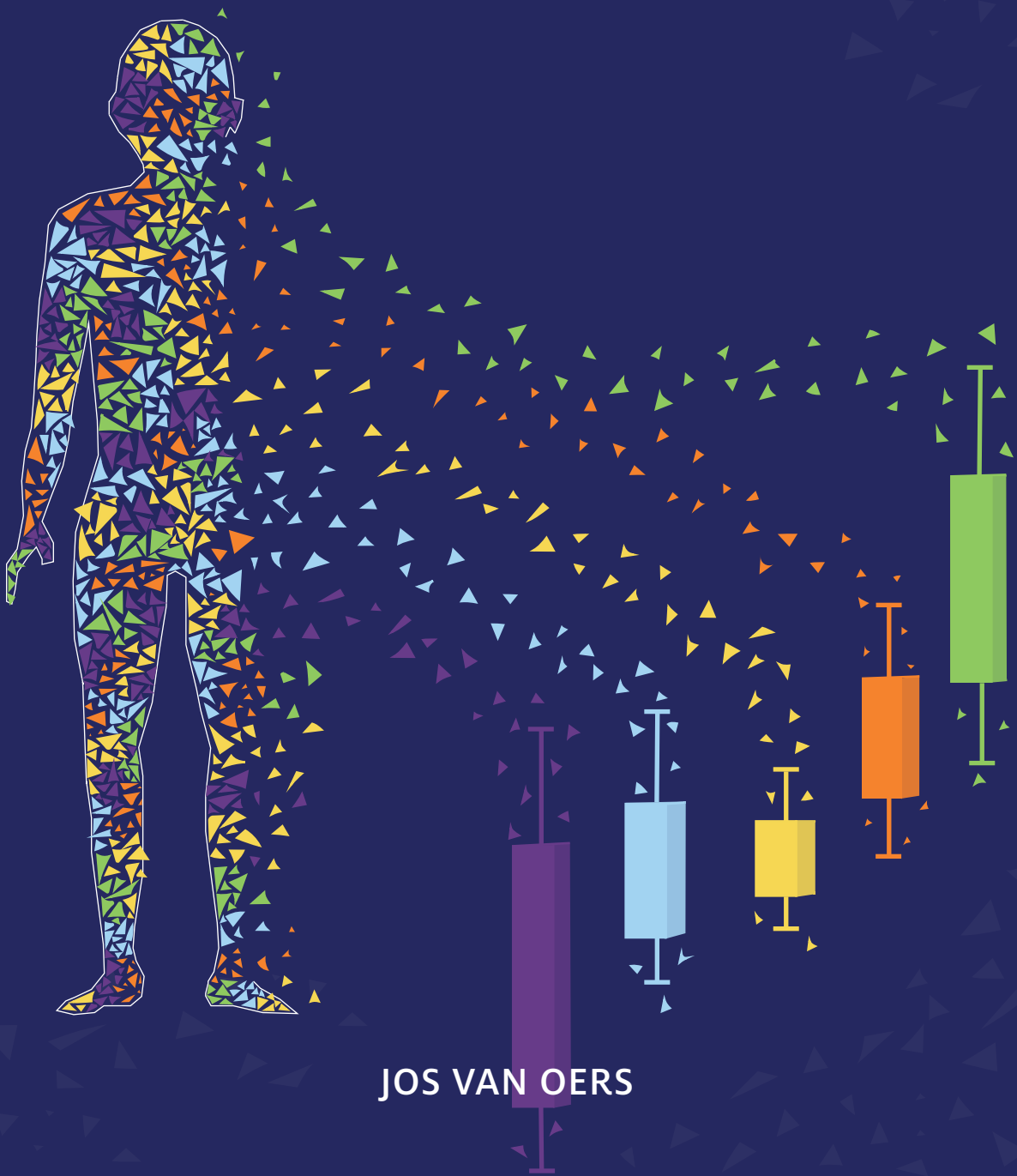
2023

DOI (link to publisher)
10.5463/thesis.328

document version
Publisher's PDF, also known as Version of record

Biomarkers in critically ill patients



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Colofon

Biomarkers in critically ill patients

Coverdesign
Eva van Oers

Design/Lay-out
Proefschriftenbalie, Nijmegen

Print
Ipskamp Printing, Enschede

ISBN: 978-94-6473-208-5
Doi: htpp://doi.org/10.5463/thesis.328

Support for printing of this thesis: Department of Intensive Care Medicine, Amsterdam UMC, VU University Medical Centre, Amsterdam, Elisabeth TweeSteden Ziekenhuis, Zorgsaam Zorggroep Zeeuws-Vlaanderen and Medcaptain Europe B.V.

VRIJE UNIVERSITEIT

BIOMARKERS IN CRITICALLY ILL PATIENTS

ACADEMISCH PROEFSCHRIFT

ter verkrijging van de graad van Doctor of Philosophy aan
de Vrije Universiteit Amsterdam,
op gezag van de rector magnificus
prof.dr. J.J.G. Geurts,
in het openbaar te verdedigen
ten overstaan van de promotiecommissie
van de Faculteit der Geneeskunde
op vrijdag 10 november 2023 om 11.45 uur
in een bijeenkomst van de universiteit,
De Boelelaan 1105

door

Jos Adrianus Henricus van Oers
geboren te Dordrecht

promotoren:	prof.dr. A.R.J. Girbes prof.dr. D.W. De Lange
copromotor:	dr. A. Beishuizen
promotiecommissie:	prof.dr. K.D.P.W.B. Nanayakkara prof.dr. P. Pickkers prof.dr. J. Bakker dr. J.L. Murk prof.dr. J. Chastre prof.dr. P.H.J. Van Der Voort

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Aan Odette,
Eva en Roos

Contents

CHAPTER 1

General introduction and outline of the thesis

Introduction

Biological markers, or biomarkers in short, are characteristics that are objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention [1-3]. Ideally, biomarkers should be independent of renal or hepatic dysfunction and should not be modified by renal replacement therapy. Such an ideal biomarker can help in rapid identification of high-risk patients, early diagnosis of diseases, prediction of prognosis and early assessment of effects of initiated treatment or to guide / personalize therapy [1-3]. Biomarkers have historically been of key importance in a broad range of clinical conditions. Therapeutic advances in field of cardiology (lactate, N-terminal pro-brain natriuretic peptide (NT-proBNP) and cardiac troponins) and oncology (i.e. carcinoembryonic antigen (CEA) in colorectal cancer and human epidermal growth factor receptor 2 (HER2) in breast cancer) have depended on the ability to measure biomarkers that are reliable indicators of the underlying disease.

Currently, diagnosis, follow-up and evaluation of resolution of various diseases in critically ill patients are based on combinations of clinical, laboratory, radiological and/or bacteriological criteria with poor positive predictive values. These parameters are frequently combined into disease severity scores. Clinical severity scores like the Sequential Organ Failure Assessment (SOFA) score [4,5], Acute Physiological and Chronic Health Evaluation (APACHE IV) model [4], World Federation of Neurological Surgeons (WFNS) score [6] were developed to assess disease severity or severity of organ dysfunction and predict outcome in various diseases in critically ill patients. However, incorporation of severity scores in daily routine was hampered due to their complexity and lack of validation. Accurate prediction of outcome still remains difficult and complicates decision making for active treatment aiming at recovery. Biomarkers, as supportive for diagnosis and follow-up or adjunct of clinical severity scores, that are able to identify patients with worst outcome may help early risk assessment and may provide further insights into pathophysiological mechanisms of various diseases [7].

In this thesis we have investigated the following biomarkers: C-reactive protein (CRP), procalcitonin (PCT), mid-regional proadrenomedullin (MR-proADM), mid-regional proartrial natriuretic peptide (MR-proANP), C-terminal pro-arginine Vasopressin (CT-proAVP), and C-terminal proendothelin-1 (CT-proET-1), in critically ill patients with sepsis, pneumonia, aneurysmal subarachnoid hemorrhage (aSAH) and corona virus disease 2019 (COVID-19). The association between obesity and MR-proADM and CT-proET-1 in critically ill patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia was also investigated. In addition, we investigated the value of daily PCT testing in discontinuing antibiotic treatment in critically ill with presumed infection/ sepsis.

Biomarkers

C-reactive protein (CRP) is a rather old biomarker. It was first discovered in a patient with lobar pneumonia in 1930 [8]. CRP is released from the liver in response to stimulation by interleukin-6 [9,10]. It is considered to be an acute phase protein, an early indicator of infectious and inflammatory conditions [9,10]. Normally serum values in healthy individuals are less than 10 mg/L and can reach peak levels of 350-400 mg/L after 30-48 hours in various disease states [10,11]. A sensitivity of 68-92% and specificity of 40-67% was reported for CRP in differentiating bacterial from non-infective causes of inflammation [12,13]. Its low specificity and inability to differentiate bacterial infections from noninfectious causes of inflammation makes CRP of limited diagnostic value. CRP showed promise for evaluating prognosis in patients with ventilator-associated pneumonia (VAP) or sepsis [14-16].

Procalcitonin (PCT), a serum peptide which is a precursor of calcitonin, a calcium regulatory hormone secreted from thyroid tissue in healthy individuals [17]. In infectious conditions, PCT is rapidly released in response to stimulation by inflammatory mediators from all parenchymal cells from nearly all tissues including lung, liver, kidney, pancreas, spleen, colon and adipose tissue [18]. In 1993, PCT was first described as a marker specifically elevated in bacterial infection [17]. PCT started to rise at 2.5 hours, peaked at 13.5 hours, and plasma half-life time was 22.5 hours in bacterial infectious conditions [11]. Normally, serum values are below 0.5 μg/L and patients with values over 1 or 2 μg/L are supposed to carry an increased risk for bacterial infection [17,19]. Some studies showed promising predictive values for PCT [19,20]. PCT was identified as a helpful biomarker to differentiate sepsis from SIRS of non-infectious origin with a mean sensitivity of 77% and specificity of 79% and the area under the receiving operating characteristics curve was 0.85 (95% CI 0.81-0.88) in a recent meta-analysis [21]. Other studies were not able to show that PCT was significantly associated with infection [9]. Luyt was the first to evaluate the usefulness of PCT as a prognostic marker to predict outcome in 63 patients during a proven episode of VAP [22]. Apart from single measurements PCT can also be measured repeatedly and the difference is indicative of amelioration or worsening of the pro-inflammatory condition. A decline of more than 50% in 3 days or 80% in 5 days was described in studies as a predictor of mortality [23,24]. To date, PCT is the most studied biomarker in biomarker guided antimicrobial therapy. PCT was studied in several randomized controlled trials [25-30]. However, while most studies showed an association between decreasing PCT concentrations and the possibility to stop antimicrobial treatment the PCT logarithms between these randomized controlled trials differed a lot. As a result, translation to an universal cut-off value was hampered.

Mid-regional proadrenomedullin (MR-proADM) is the midregion part of the prohormone of ADM [31], a peptide released by endothelial and vascular smooth muscle cells with anti-inflammatory and antiapoptotic effects on vascular endothelial cells, protecting the microcirculation against endothelial permeability in sepsis [32]. ADM release is stimulated by a variety of hormones, cytokines, and physical stress. Moreover, ADM enhances cardiac output, has immune modulating properties, and ADM is also an adipokine, a pro-inflammatory cytokine released by adipose tissue [33, 34]. ADM is rapidly cleared from the circulation, but the midregion part of the prohormone is more stable, and therefore more accessible for clinical purposes [31]. MR-proADM levels were rapidly induced in lower respiratory tract infections [33,35,36]. Baseline MR-proADM measurements proved to be a good predictor of both short and long-term survival in CAP patients admitted to the emergency room or ICU [33,35]. Clearance in serial MR-proADM levels of 30% or more in five days in septic patients admitted to the ICU was associated with better outcome [37].

Mid-regional proatrial peptide (MR-proANP) is the midregion part of the prohormone of ANP [38], a hormone predominantly produced in the atrium of the heart. ANP antagonizes the renin-angiotensin-aldosterone system in response to hypertension and water and salt retention [39]. ANP is secreted as a resultant of atrial stretch mainly, but also by stimulation of pro-inflammatory cytokines. Increased levels of MR-proANP were reported in non-survivors of patients with CAP, VAP and sepsis [39-41].

C-terminal pro-arginine vasopressin (CT-proAVP), also named copeptin, is the C-terminal part of the prohormone of arginine vasopressin (AVP), also known as antidiuretic hormone (ADH), which is produced in the hypothalamus and stored in the posterior pituitary [42,43]. AVP contributes to the regulation of osmotic and cardiovascular homeostasis [42,43]. AVP is stimulated by different stressors. AVP potentiates the action of CRH and leads downstream to release of ACTH and production of cortisol [25], reflecting the individual stress response at hypothalamic level [25]. CT-proAVP concentrations mirror the concentrations of AVP [43]. CT-proAVP is stable for days, and therefore measuring CT-proAVP in blood is more accessible for clinical purposes [43]. High levels of CT-proAVP were reported to be predictive for poor outcome in patients with traumatic brain injury [44], intracerebral hemorrhage [45], and ischemic stroke [46]. CT-proAVP levels at admission were highly predictive for poor functional outcome and mortality in three cohort studies with Asian aneurysmal subarachnoid hemorrhage (aSAH) patients [47-49], and was a good prognostic marker for delayed cerebral ischemia (DCI) [47,48].

C-terminal proendothelin-1 (CT-proET-1) is the C-terminal part of the prohormone of ET-1 [50] which is more stable and therefore a better target for clinical measurements [50].

ET-1 is a very strong vasoconstrictor peptide and pro-inflammatory cytokine that is released from activated endothelial cells [51]. Besides blood vessels, ET-1 receptors are also found in other tissues, with the highest levels in the lungs [51]. Patients with obesity showed increased vascular expression of ET-1 and vasoconstriction activity [52,53]. ET-1 release is stimulated by bacterial toxins and inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha) or interleukin-6 [54]. Elevated concentrations of CT-pro-ET-1 were found in patients with CAP and sepsis [33,54,55].

Characteristics of the diseases in which biomarkers were studied

Sepsis is a heterogeneous syndrome and common cause of death in patients in the intensive care unit (ICU). Recent estimates suggest that, at any given day on the ICU, half of the patients is suspected of having an infection [56]. The mortality of patients with sepsis in the ICU is high and, depending on the severity of illness, mortality rates of 20-60% are reported [56-58]. To counter these high mortality rates most contemporary guidelines advocate swift recognition of infected patients and a rapid, adequate antimicrobial treatment for all septic patients [57]. Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection according to the Sepsis-3 definitions [4]. Organ dysfunction can be assessed by an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 or more points [4]. As SOFA score was never intended as a tool for patient management qSOFA (quick SOFA), i.e. alteration in mental status, systolic blood pressure $\leq$ 100 mm Hg, or respiratory rate $\geq$ 22/min, was advocated for quick identification of septic patients. The Sepsis-3 definitions facilitate early recognition of patients with sepsis. When these Sepsis-3 definitions are used as a fast screening test, there may be a role for biomarkers as a diagnostic aid for proven sepsis?

Pneumonia is characterized by a new or progressive and persistent infiltrate on chest imaging together with fever, leukocytosis, leukopenia or altered mental status and at least one of the following: new onset of purulent sputum, new onset cough, rales or bronchial breath sound or worsening gas exchange [59]. Community-acquired pneumonia (CAP) is defined as a pneumonia acquired outside a hospital or long-term care facility [60-62]. Hospital-acquired pneumonia (HAP) refers to a pneumonia that occurs 48 hours or more after admission [63]. Ventilator-associated pneumonia (VAP) is defined as a pneumonia that arises more than 48 – 72 hours after endotracheal intubation [63]. A pneumonia is an important reason of intensive care unit (ICU) admission, length of stay (LOS) and death [60-62]. Mortality rates for CAP admitted to the ICU of 20 - 30% are reported [62,63]. For HAP mortality rates may be as high as 30 to 70% [64] and an overall attributable mortality of 13% in patients with VAP has been reported in a recent meta-analysis [65]. Knowledge of biomarkers predicting outcome in a pneumonia may help grade its severity and predict treatment response.

Aneurysmal subarachnoid hemorrhage (aSAH) due to a ruptured cerebral aneurysm is an important indication for ICU admission and may lead to significant morbidity and mortality [66-69]. The diagnosis of aSAH is based on clinical symptoms (acute headache, focal neurological deficits, loss of consciousness), presence of blood on CT-cerebrum or presence of xanthochromia in cerebral spinal fluid in combination with an aneurysm, confirmed by computerized tomography angiography (CT-A) or digital substraction angiography (DSA) [66]. Reported incidences of aSAH vary from 6 to 9 aSAHs per 100.000 person-years in the general population [66-69]. About 8% of the patients with aSAH die before arrival at the hospital [70]. Case-fatality rates after one month are around 25% to 35% [70-72]. Although aSAH occurs at a reasonable young age of 55 years [69], estimates of functional independence varied between 36% and 55% at assessments up to 12 months after the bleeding [69]. Also, many patients cannot resume their previous work, have difficulties in relationships and impaired quality of life [73]. The immediate prognosis is determined by the amount of initial intracranial hemorrhage and rebleeding before treatment [66,69]. To prevent rebleeding, the aneurysm is generally obliterated as soon as possible, either by a neurosurgical procedure in which a metal clip is placed over the neck of the aneurysm, or by an endovascular procedure, when platinum coils are inserted inside the aneurysm [66]. Among the secondary complications contributing to morbidity and mortality, delayed cerebral ischemia (DCI) is a major risk factor for bad outcome in aSAH patients [66]. Biomarkers, as a surrogate or adjunct of clinical severity scores, could represent an attractive additional alternative to predict outcome, mortality and DCI in aSAH patients.

Corona virus disease 2019 (COVID-19), the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [74,75], has turned out to be an enormous challenge to ICUs worldwide [76-78]. A substantial part of the patients deteriorated quickly and needed to be admitted to the ICU with signs and symptoms consistent with acute respiratory failure due to viral SARS-CoV 2 pneumonia. SARS-CoV-2 induced disease (COVID-19) can range from mild to severe disease. Both severe and critical type diseases defined by the World Health Organization (WHO) interim guidance were included in our study [79]. Severe disease; severe pneumonia was designated when the patients had clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) plus one of the following symptoms or physiological signs: respiratory rate > 30 breaths/min, severe respiratory distress or SpO_2 < 90% on room air [79]. Critical disease; ARDS was designated when the symptoms of pneumonia lasted less than one week or when there were new or worsening symptoms, chest imaging showed bilateral ground glass lobar opacities, lobar or lung collapse, or nodules and respiratory failure could not be solely explained by cardiac failure or fluid overload. Additionally, signs of oxygenation impairment ($PaO_2/FiO_2 \leq 300$ mmHg with positive end expiratory pressure (PEEP) $\geq$ 5 cmH_2O or continuous positive airway pressure (CPAP) $\geq$ 5 cmH_2O) needed

to be present [79]. As virus-induced endothelial dysfunction and damage, endotheliitis, has been proposed as one of the potential mechanisms of COVID-19 [80,81], there may be a role for endothelium related biomarkers.

COVID-19 and obesity. The prevalence of obesity was high among COVID-19 patients admitted to the ICU [78]. Moreover, the need for invasive mechanical ventilation was higher in COVID-19 patients with obesity admitted to the ICU [82,83]. A state of low-grade chronic inflammation in obesity has been suggested as one of the underlying mechanisms. Obesity may be associated with inflammatory biomarkers MR-proADM and CT-proET-1 in critically ill patients wish SARS-CoV-2 pneumonia.

Antibiotic guidance by PCT. As sepsis is characterized as life-threatening organ dysfunction, the Sepsis-3 definitions highlight the need for early recognition [4]. In addition, most physicians want to start antimicrobial therapy immediately after they have made the presumptive diagnosis of sepsis [4,57,84]. Antibiotic treatment needs to be appropriate (focused on the right pathogen, adequate (at the right dosage), and short [57,85]. However, as no objective measure exists on how long antibiotics should be applied in critically ill patients given the fear of under-treatment, liberal antimicrobial treatment has been an accepted treatment [86]. Using a biomarker as a guide to tailor the duration of antibiotic treatment in critically ill patients with a presumed infection / sepsis would be is an attractive hypothesis [86,87]. To test this hypothesis the Stop Antibiotics on Procalcitonin guidance Study (SAPS) was started in 15 ICUs in the Netherlands. The utility of procalcitonin as a marker to guide antibiotic treatment arises from its unique kinetics: a rapid rise within hours after bacterial infection and an approximate half-life of 24 hours once the infection abates.

Aim of the thesis

The overall aim of this thesis is to investigate whether (1) biomarkers, as replacement of, or adjunct to, severity scores, could improve early diagnosis of sepsis when Sepsis-3 definitions are applied, (2) biomarkers provide early estimation of severity and predict prognosis in critically ill patients with pneumonia and aneurysmal subarachnoid hemorrhage, (3) biomarkers predict prognosis and investigate the association with obesity in critically ill patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia, and (4) biomarkers can be used as a guide to tailor the duration of antibiotic treatment in critically ill patients with presumed bacterial infections / sepsis.

Outline of the thesis

In **chapter 2**, we investigated whether a single PCT or CRP could detect proven sepsis in an ICU population diagnosed with sepsis when Sepsis-3 definitions were applied. We also investigated whether a decline in serial PCT or CRP could predict outcome in 28-day mortality as secondary aim. In **chapter 3**, we aimed to investigate the prognostic value of MR-proADM and MR-proANP at baseline compared with the APACHE IV model and SOFA score to predict 28-day mortality in a cohort of critically ill patients with pneumonia of any cause. Our secondary aim was the prediction of 28-day mortality by clearance of MR-proADM and MR-proANP using serial measurements during 5 days in comparison with APACHE IV model and SOFA score. **Chapter 4** describes the prognostic value of CT-proAVP upon admission to predict poor functional outcome (Glasgow Outcome Scale score 1-3) after one year in critically ill aSAH patients as compared with WFNS and APACHE IV scores. Secondary aims were 30-day and one-year mortality, and DCI. In **chapter 5** we investigated the prognostic value of baseline MR-proADM and CT-proET-1 to predict 28-day mortality in critically ill patients with confirmed SARS-CoV-2 pneumonia. Secondary aim was to study the evolution of these two biomarkers over time during the ICU period. As patients affected by obesity suffering from COVID-19 appear to have a higher risk for ICU admission, we investigated in **chapter 6** whether obesity was associated with differences in endothelium and obesity related inflammatory biomarkers MR-proADM and CT-proET-1 in critically ill patients with SARS-CoV-2 pneumonia. Secondary aim was the association between obesity and clinical outcome (time on a ventilator, ICU length of stay (LOS) and 28-day mortality). **Chapter 7** presents the results of a pragmatic, randomized, controlled, open multicenter intervention trial in the Netherlands focusing on the value of daily PCT testing in discontinuing antibiotic treatment in critically ill patients with presumed bacterial infection / sepsis and provides insights in the optimization of antibiotic therapy in Dutch ICU's. **Chapter 8** contains the English summary, the general discussion and conclusions of this thesis. The main findings of our studies are presented and placed in a broader perspective. **Chapter 9** contains the Dutch summary of the main results and recommendations of this thesis.

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PART I

Biomarkers for diagnosis in sepsis

CHAPTER 2

Diagnostic accuracy of Procalcitonin and C-reactive protein is insufficient to predict proven infection: a retrospective cohort study in unselected patients fulfilling the Sepsis-3 criteria

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J Appl Lab Med. 2020; 5:62-72. doi:10.1373/jalm.2019.029777.

Abstract

Background: New Sepsis-3 definitions facilitate early recognition of patients with sepsis. This study investigated whether a single initial determination of procalcitonin (PCT) or C-reactive protein (CRP) in plasma can predict proven sepsis in Sepsis-3 criteria-positive critically ill patients. And second, whether a decline in serial PCT or CRP can predict outcome in 28-day mortality.

Methods: Patients, 18 or more years old, at the intensive care unit with a suspected infection, a Sequential Organ Failure assessment (SOFA) score of 2 points or more and an index test PCT and CRP at admission were selected from a prospectively collected cohort. PCT and CRP were studied in retrospect with Mann-Whitney U-test and receiver operating characteristics analysis.

Results: In total 157 patients were selected, 63/157 had proven sepsis and in 94/157 sepsis could not be detected. Neither a single PCT nor CRP at admission was able to discriminate proven sepsis from non-proven sepsis (PCT 1.8 and 1.5 µg/L respectively, $p = 0.25$ and CRP 198 and 186 mg/L respectively, $p = 0.53$). Area under the curve for both PCT and CRP for detecting proven sepsis was low (0.55 and 0.53). Furthermore, neither a decline from baseline to day 5 PCT and CRP could predict 28-day mortality (PCT 50% vs 46%, $p = 0.83$ and CRP 30% vs 40%, $p = 0.51$).

Conclusion: PCT and CRP at admission were not able to discern patients with proven sepsis in Sepsis-3 criteria-positive critically ill patients. A decline of PCT and CRP in 5 days was not able to predict 28-day mortality.

Introduction

Sepsis is an important reason of intensive care unit (ICU) admission and common cause of death over the years. Recent estimations suggest that on any given day in the ICU, half of the patients are suspected of having an infection [1]. Mortality of patients with an infection in the ICU is high, with mortality rates of 25-60%, depending on the severity of illness, reported [1–3]. To counteract these high mortality rates most current guidelines advocate rapid identification of infected patients and prompt, adequate antimicrobial treatment for all of these patients [2].

However, how do we recognize septic patients as there are no simple clinical criteria or tests to identify septic patients? Sepsis is defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection according to the latest Sepsis-3 definitions [4]. Organ dysfunction can be assessed by an increase in the Sequential Organ Failure Assessment (SOFA) score of 2 or more points [4]. As SOFA score was never intended as a tool for patient management qSOFA (quick SOFA), i.e. alteration in mental status, systolic blood pressure $\leq$ 100 mm Hg, or respiratory rate $\geq$ 22/min, was advocated for quick identification of septic patients. As a consequence of this sensitive screening tool only a part of these patients had a microbiological proven infection. Literature reveals a percentage of only 30% to 40% microbiological proven sepsis [4]. In an attempt to improve the diagnostic value inflammatory markers like C-reactive protein (CRP) and procalcitonin (PCT) were added to the already existing systemic inflammatory response syndrome (SIRS) criteria during an international sepsis definitions conference in 2001 [5]. PCT is a serum peptide which is elevated in patients with bacterial infection. Normally, serum values are below 0.5 µg/L and patients with values over 1 or 2 µg/L are supposed to carry an increased risk for bacterial infection [6, 7]. Some studies showed promising predictive values for PCT [7, 8]. PCT was identified as a helpful biomarker to differentiate sepsis from SIRS of non-infectious origin with a mean sensitivity of 77% and specificity of 79% and the area under the receiving operating characteristics curve was 0.85 (95% CI 0.81-0.88) in a recent meta-analysis [9]. Other studies were not able to show that PCT was significantly associated with infection [10]. PCT was used as a single value and in serial measurements. A decline of more than 50% in 3 days or 80% in 5 days was described in studies as a predictor of mortality [11, 12]. CRP is considered to be an acute phase protein, an early indicator of infectious and inflammatory conditions [10,13]. Normally serum values in healthy individuals are less than 10 mg/L and can reach peak levels of 350-400 mg/L after 48 hours in disease states [13]. A sensitivity of 79% and specificity of 67% were reported for CRP in differentiating bacterial from non-infective causes of inflammation [14]. Both CRP and PCT are nowadays routinely monitored in ICU patients. When new Sepsis-3 definitions are used as screening test, there may be a value for PCT or CRP as a diagnostic aid for microbiological proven sepsis.

The purpose of this observational cohort study was to evaluate whether a single PCT or CRP can detect microbiological proven sepsis in an ICU population diagnosed with sepsis when new Sepsis-3 definitions were applied. We also investigated whether a decline in serial PCT or CRP can predict outcome in 28-day mortality.

Materials and Methods

Study design and selection criteria

We performed an observational cohort study. Between June 1, 2009 and December 1, 2009 patients with an age of 18 years or more admitted to the intensive care unit (ICU) of the University Medical Center Utrecht (UMCU), an academic hospital in the Netherlands, were prospectively followed. The mixed ICUs received admissions from all specialties (surgery, medical, transplant, cardio-surgical, neurosurgical, trauma etc.) except burns. PCT values are not part of the normal clinical routine, but during this period both PCT and CRP were collected on daily basis. Research nurses then collected all data and constructed the database. In meantime the sepsis definitions were updated in 2016. Sepsis now depends on the suspicion of an infection and the assessment of organ failure with SOFA-score. To answer the primary research question whether PCT or CRP can help in de diagnosis of sepsis we selected patients from the cohort with a suspected infection, SOFA score of 2 points or more and an index test PCT and CRP at admission afterwards, in retrospect. The STARD (Standards for Reporting of Diagnostic Accuracy Studies) 2015 guidelines for reporting diagnostic accuracy studies were followed [15]. Only complete cases were included. Included and excluded patients were described in the patient flow diagram. Secondary outcome measure was the prediction of 28-day mortality by serial PCT and CRP measurements. The study was approved by the Institutional Review Board of the University Medical Center Utrecht and it waived the need for informed consent (UMC Utrecht IRB research protocol 108-188).

Procedures and definitions

Clinical data, microbiological and laboratory results were collected on a daily basis in patients fulfilling the Sepsis-3 criteria during a maximum period of 10 days, or until discharge or death, enabling to identify infections that were not yet clear on the day of admission. The SOFA score was scored retrospectively from clinical and laboratory measures as part of standard clinical practice. Components of SOFA consisted of respiration, coagulation, liver, cardiovascular, central nervous system and renal function. On each item 0 to 4 points could be achieved and points were added to a total score [16]. Blood samples of each included patient were collected for determining

the values of PCT (in heparin plasma on a Kryptor machine, Brahms GmbH, Berlin, Germany) using Time Resolved Amplified Cryptate Emission (TRACE) and CRP (in heparin plasma on a DxC 800 routine chemistry system, Beckman Coulter, Brea, California) and leukocytes (in EDTA-blood on a CD-Saphire routine hematology analyzer, Abbott, Santa Clara, California, USA). As PCT values are not part of the normal clinical routine, they did not influence decision making. Afterwards, when all data were collected, 2 researchers (EdJ and DWL) adjudicated which patients had a "proven infection" or "no proven infection". Both researchers were blinded for the results of PCT and CRP. The Centers of Disease Control (CDC) have published algorithms of health care-associated infection and criteria for specific types of infections in the acute care setting [17]. As infections in immunocompromised patients were not described in these CDC algorithms we used the definitions of immunocompromised patients published in the article of Greenberg, et al. [18]. Clinical evidence for proven infection was based on review of information in the patient chart and cultures of blood, sputum, urine, liquor, abdominal fluid, abscess, wound and tip of central venous catheter. Different types of proven infection are described in Supplemental table 1. Sepsis-3 criteria positive patients were then divided in proven and non-proven sepsis.

Statistical analysis

Normally distributed data were expressed as mean $\pm$ standard deviation (sd), all non-normally distributed data (Kolmogorov-Smirnov test $p < 0.05$) were expressed as median (with interquartile range, IQR) or as number of patients (percentage) where appropriate. Patient characteristics and outcomes were compared using an Independent Samples T test or Mann-Whitney U test for continuous variables and chi square test for categorical variables. The primary analysis compared detection of proven sepsis versus no proven sepsis by CRP, PCT, Leukocytes and temperature at day 1. Sensitivity, specificity, positive and negative predictive values (PPV and NPV), positive and negative likelihood ratios (LR+ and LR-) and diagnostic odds ratio (DOR) were calculated for different cutoff values of both PCT and CRP at day 1. Another way to represent these test characteristics is in a receiver operating characteristics (ROC) curve in which the relationship between sensitivity and specificity is depicted. Secondary analysis compared prediction of 28-day mortality by delta PCT and CRP. Delta PCT and CRP were calculated dividing the decline of PCT or CRP from day 1–5 by day 1 and were presented as percentage (Supplemental table 2). Because in some patients PCT and CRP tend to rise within the first 24 hours after admission, we also investigated the kinetics from the peak value within the first 2 days to day 5 (Supplemental table 2) [12,19]. All tests were two-sided and a p-value ≤ 0.05 was considered statistically significant. All data were analyzed using a statistical software package (SPPS Inc., version 24, Chicago, IL, USA).

Results

During the half-year period 157 out of 361 consecutive admissions were selected. The patient flow diagram shows the flow of patients, along with the primary endpoint of proven sepsis (figure 1). Patients who were excluded (and reasons for this) were noted. Performance of PCT and CRP in these excluded patients is described in Supplemental tables 3 and 4.

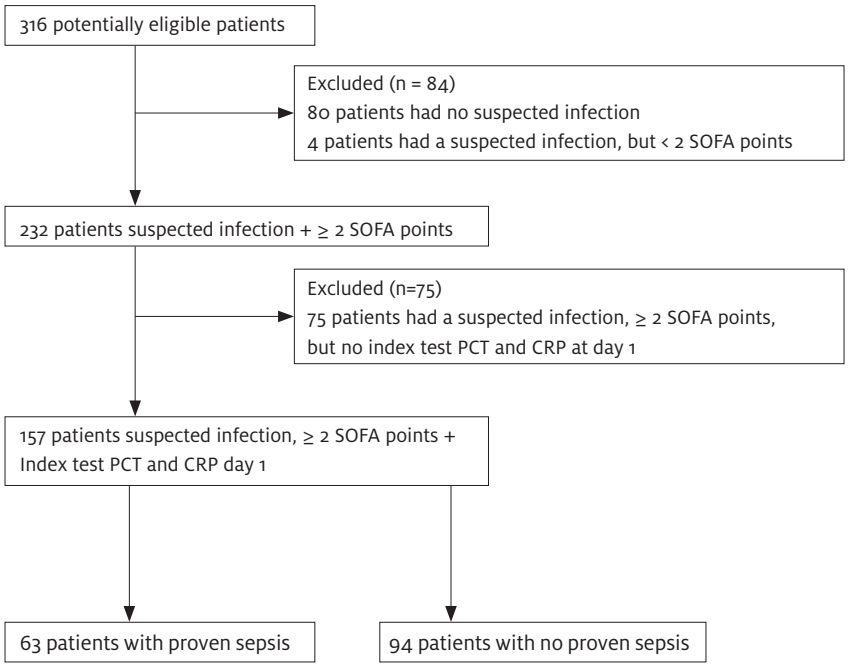

Figure 1 | Patient flow diagram

Demographics and clinical characteristics of the selected patients are shown in Table 1. Patients were divided into those with "proven sepsis" and those with "no proven sepsis". In 63/157 (40%) patients a bacterial infection was considered proven and these patients were assigned to the "proven sepsis" group and in 94/157 (60%) patients no bacterial infection could be confirmed and were assigned to "no proven sepsis" group. The groups were quite comparable except for length of stay (LOS) ICU and hospital in patients with proven sepsis. Referring specialties are described in Supplemental table 5. 58 patients had a community-acquired infection and 99 patients were already hospitalized before admittance to the ICU and 56 patients received already antibiotics before inclusion in the study. But these conditions were well balanced between both

groups. As all patients were suspected of having sepsis according to the Sepsis-3 definitions all patients received antibiotics. Pneumonia and abdominal infection were the most definitive infection sites (Supplemental table 6). In 26 patients Gram positive bacteria were found (*S. pneumoniae* n = 7, *S. pyogenes* n = 2, *Enterococcus spp.* n = 5, *S. aureus* n = 7, *S. epidermidis* n = 5) and in 37 patients Gram negative bacteria (*E. coli* n = 17, *Klebsiella spp.* n = 5, *S. marcescens* n = 3, *Enterobacter spp.* n =5, *H. influenza* n = 2, *P. aeruginosa* n = 4 and *Burkholderia cepacia* n = 1). No viral and fungal infections were found.

In all selected patients PCT and CRP were measured on admission and every subsequent morning. Median PCT value at admission was 1.7 μg/L (IQR 0.5 - 7.3) and median CRP value at admission was 190 mg/L (IQR 109 - 275). Table 2 shows the analysis of clinical and diagnostic data of the patients with proven sepsis versus those with no proven sepsis. No significant differences were seen in median CRP, PCT, temperature and Leukocytes at admission between the 2 groups. As PCT and CRP tend to rise within the first 24 hours after admission peak values of PCT and CRP within the first 2 days were compared between the patients with proven sepsis and those with no proven sepsis. No significant differences were seen (Supplemental Table 7). When the patients were divided in community and hospital-acquired infection no significant differences could be detected between CRP, PCT, leukocytes and temperature at admission. Only in the hospital-acquired group there was a significant difference in CRP between proven and no proven sepsis, with higher CRP levels in no proven sepsis (Supplemental Table 8). No significant differences in median PCT and CRP at admission were seen when surgical patients were excluded (Supplemental table 9). The sensitivity, specificity, positive predicted value (PPV) and negative predictive value (NPV), positive and negative likelihood ratios (LR+ and LR-) and diagnostic odds ratio (DOR) to confirm proven sepsis of various cutoff points of both PCT and CRP are shown in Table 3. A PCT-level ≥ 0.5 μg/L had the best sensitivity (81%) and best DOR (1.71). A PCT-level ≥ 5.0 μg/L had the best specificity (71%). As for CRP a cutoff point of 50 mg/L had the best sensitivity (97%) and best DOR (3.23). A CRP-level ≥ 200 mg/L had the best specificity (54%). The ROC (figure 2) showed an AUC for PCT on day 1 of 0.55 (95% CI 0.46 – 0.64) and an AUC for CRP on day 1 of 0.53 (0.43 – 0.62) for detecting proven sepsis.

Table 1 | Baseline characteristics of the study population ICU-patients with sepsis according to Sepsis-3 definitions

	Total	proven sepsis	no proven sepsis	*p*-value
	no 157	no 63	no 94	
Age	61 (49-73)	61 (42-74)	61 (52-73)	0.42
Gender (male)	100 (64%)	42 (67%)	58 (62%)	0.53
Patient category				
Medical patients	68 (43%)	24 (38%)	44 (47%)	0.28
Surgical patients	89 (57%)	39 (62%)	50 (53%)	0.28
Severity of illness				
SOFA score	6 (5-10)	7 (5-10)	6 (4-9)	0.22
APACHE IV score	79 (29)	77 (29)	80 (30)	0.46
Acquisition of infection				
Community-acquired	58 (37%)	26 (41%)	32 (34%)	0.36
Hospital-acquired	99 (63%)	37 (59%)	62 (66%)	0.36
Presumed infection site				
Pneumonia	54 (34%)	23 (37%)	31 (33%)	0.65
Intra-abdominal infection	34 (22%)	17 (27%)	17 (18%)	0.19
Urinary tract infection	5 (3%)	1 (2%)	4 (4%)	0.35
Meningitis	6 (4%)	2 (3%)	4 (4%)	0.73
Wound infections	9 (6%)	2 (3%)	7 (8%)	0.26
CR-BSI	3 (2%)	2 (3%)	1 (1%)	0.34
Sepsis eci	34 (21%)	12 (19%)	22 (23%)	0.52
Immuuncompromised	3 (2%)	0 (0%)	3 (3%)	0.15
Other sites	9 (6%)	4 (6%)	5 (6%)	0.79
Microbiology				
Gram positive bacteria		26 (41%)	-	-
Gram negative bacteria		37 (59%)	-	-
Treatment				
Antibiotics before inclusion	56 (36%)	23 (37%)	33 (35%)	0.86
MV first 24 hours	85 (54%)	38 (60%)	47 (50%)	0.20
ARF during ICU stay	18 (12%)	7 (11%)	11 (12%)	0.91
Outcome				
ICU LOS in days	11 (7-19)	13 (9-25)	9 (6-16)	< 0.01
Hospital LOS in days	35 (21-56)	41 (21-75)	32 (22-46)	0.05
ICU mortality	28 (18%)	14 (22%)	14 (15%)	0.24
28-day mortality	30 (19%)	10 (16%)	20 (21%)	0.40

Legends: Continuous data are presented as median (interquartile range), except APACHE IV as mean (standard deviation). Categorical data as number (percentage). SOFA: Sequential Organ Failure Assessment; APACHE IV: Acute physiology and chronic health evaluation IV; CR-BSI: Catheter related bloodstream infection; Sepsis eci: Sepsis of unknown cause; Other infection sites: Endocarditis, mediastinitis; MV: Mechanical ventilation; ARF: Acute renal failure; LOS: Length of stay.

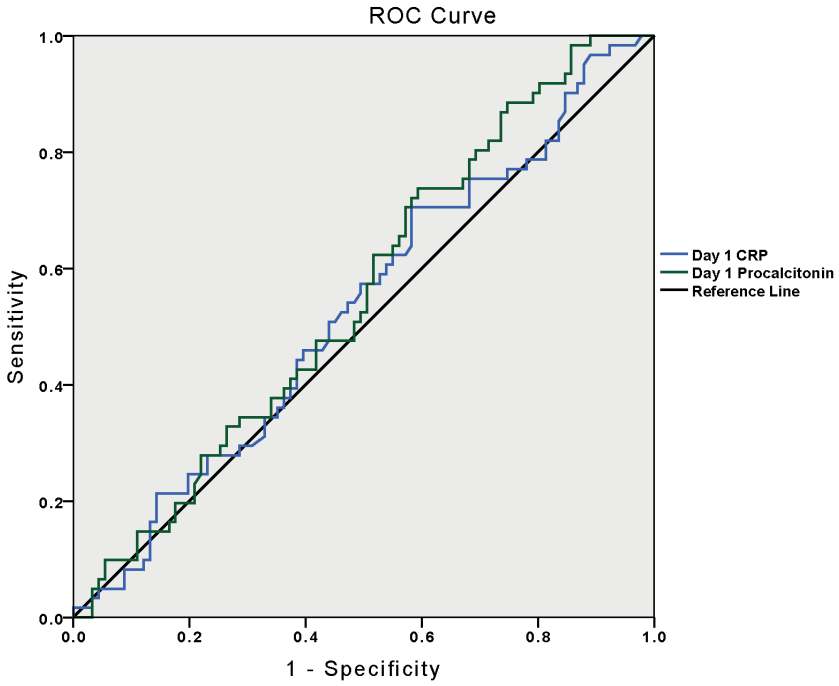

Figure 2 | Receiver operating characteristics curve for day 1 PCT and CRP in predicting proven sepsis in Sepsis-3 criteria positive critically ill patients

References: Green line = day 1 PCT (AUC: 0.55, 95% CI 0.46 -0.64, p 0.25), blue line = day 1 CRP (AUC 0.53, 95% CI 0.43 - 0.62, *p* 0.59), black line = reference line.

Tables 2 | CRP, PCT, leucocytes and temperature at day 1 in Sepsis-3 positive critically ill patients

	Total	**proven sepsis**	**no proven sepsis**	***p*-value**
	no 157	**no 63**	**no 94**	
CRP (mg/L)	190 (109-275)	198 (108-294)	186 (109-266)	0.59
PCT (µg/L)	1.7 (0.5-7.3)	1.8 (0.6-7.5)	1.5 (0.4-7.2)	0.25
Temperature °C	38.3 (37.7-39.0)	38.4 (37.7-39)	38.2 (37.7-38.8)	0.49
Leukocytes (x 10^9 /L)	15.5 (12-20.5)	15.9 (12.3-21.1)	15.2 (11.3 -20.4)	0.77

Legends: All continuous data are presented as median (interquartile range).

Table 3 | Test characteristics of PCT and CRP in patients with proven sepsis

	sensitivity	specificity	PPV	NPV	LR+	LR-	DOR (95% CI)
PCT ≥ 0.5 (µg/L)	81%	29%	43%	69%	1.14	0.67	1.71 (0.79-3.70)
PCT ≥ 1.0 (µg/L)	65%	46%	45%	66%	1.2	0.76	1.57 (0.81-3.03)
PCT ≥ 2.0(µg/L)	48%	52%	40%	60%	1.0	1.0	0.99 (0.52-1.86)
PCT ≥ 5.0 (µg/L)	32%	71%	43%	61%	1.1	0.96	1.15 (0.58-2.31)
CRP ≥ 50 (mg/L)	97%	10%	42%	82%	1.08	0.30	3.23 (0.67-15.48)
CRP ≥ 100 (mg/L)	78%	21%	40%	59%	0.99	1.05	0.95 (0.44-2.05)
CRP ≥ 150 (mg/L)	70%	40%	44%	67%	1.17	0.75	1.57 (0.8-3.09)
CRP ≥ 200 (mg/L)	51%	54%	42%	61%	1.11	0.91	1.15 (0.61-2.18)

Legends: PPV positive predictive value, NPV negative predictive value, LR+ positive likelihood ratio, LR- negative likelihood ratio, DOR diagnostic odds ratio.

The kinetics of CRP, PCT, Leukocytes and temperature from day 1–5 divided by 28-day survivors and non-survivors were presented in Table 4. There were no significant differences in decrease between parameters in 28-day survivors and non-survivors. The kinetics of PCT and CRP from peak value in the first 2 days until day 5 divided by 28-day survivors and non-survivors were calculated. There were no significant differences (Supplemental Table 10). When patients were divided in community and hospital-acquired infection again no significant differences in decrease of parameters could be detected (Supplemental Table 11). In addition to the primary analysis of kinetics of parameters we analyzed the prognostic value for predicting hospital mortality of a PCT decrease of more than 80% between day 1 and day 5 (Supplemental Table 12). Among the 146 patients available for analysis, there was no significant difference in 28-day survival between the patients who had 80% or less PCT decrease and those who had more than 80% PCT decrease ($p = 0.59$). When a cutoff level of 50% PCT decrease was chosen there were also no significant differences in 146 patients available for analysis between 50% or less decrease and more than 50% decrease ($p = 0.61$). A cutoff level of 50% CRP decrease revealed no significant differences in 28-day survival in 125 available patients between 50% or less decrease and more than 50% decrease ($p = 0.77$). When the kinetics of CRP and PCT in 10 days were calculated there were no significant differences between 28-day survivors and non-survivors (Supplemental table 13).

Table 4 | Δ CRP, PCT, Leukocytes and Temperature from day 1- 5 in Sepsis-3 positive critically ill patients.

	Survivors in 28 days	non-survivors in 28 days	*p*-value
	no 127	no 30	
Δ CRP (%)	30% (-8 - 57)	40% (5 - 63)	0.51
Δ PCT (%)	50% (9 - 76)	46% (27 - 78)	0.83
Δ Leukocytes (%)	5% (-21 - 31)	20% (-22 - 40)	0.45
Δ Temperature (%)	0.9% (-0.8 - 3)	2% (0 - 5)	0.14

Legends: All continuous data are presented as median (interquartile range).

Discussion

The primary aim of this study was to analyze whether a single initial PCT or CRP can predict proven sepsis in Sepsis-3 criteria-positive critically ill patients. The secondary aim was to examine the possibility to predict outcome in 28-day mortality with a decline in serial PCT or CRP. It is important to have a diagnostic aid in the search for sepsis, so accurate treatment of bacterial infection and supportive treatment can be tailored to ensure a better survival of the septic patient [2]. The performance of PCT to detect patients with "proven sepsis" was poor (PPV of 40 - 45%, DOR of only 0.99 - 1.71 and an area under the ROC of 0.55). Conversely, the NPV of PCT (61 – 69%) was insufficient to exclude patients who did not have "proven sepsis". CRP's ability to identify or exclude patients with "proven sepsis" was similarly poor. There were no significant differences in decline of both PCT and CRP in 28-day survivors and non-survivors. A chosen cutoff point of 80% or 50% decrease of PCT in 5 days did not identify 28-day survivors from non-survivors. Also, a cutoff point of 50% decrease in CRP in 5 days made no difference in 28-day survival.

Several biomarkers have been proposed which would identify septic patients and would thus benefit from antimicrobial therapy [10]. One of the most studied biomarkers is PCT and its performance to identify infectious from non-infectious states is sometimes called impressive [8]. Our findings are in contrast to previous studies. In a retrospective study PCT testing on day 1 could rule in or out sepsis in critically ill patients retrieved from the US Premier Healthcare Database and was able to reduce antibiotic exposure, total hospital and ICU length of stay and hospital costs [20]. In a prospective observational study in ICU patients both a single CRP and PCT improved the diagnostic accuracy of sepsis (AUC in ROC analysis 0.86 for CRP and 0.82 for PCT) [21]. Our results are in line with previous studies that support the idea that a single PCT measurement is unable to predict sepsis [10]. Research on PCT has shifted toward

serial PCT measurements. Survivors of severe sepsis had a greater PCT decrease in 72 hours compared to non-survivors in a prospective single center observational study in Finland [11]. In a U.S. prospective multicenter study > 80% reduction in 5 days was an independent predictor of mortality in septic patients [12].

A possible explanation for the failure of biomarkers CRP and PCT to identify "proven sepsis" within our cohort Sepsis-3 criteria-positive ICU patients is the fact that both biomarkers will also be elevated in non-infectious inflammatory processes, i.e., trauma, surgery, and acute kidney injury [22–24]. There were slightly more trauma patients in the proven sepsis group, but surgery patients and acute kidney failure were well balanced between the 2 groups. Another reason is the fact that the group "no proven sepsis" will contain a substantial part of patients with a bacterial infection, but cultures stayed false-negative [4]. At last, sepsis is a pathophysiological process too complex to be described by a single measure [4]. Close monitoring of sepsis patients remains the cornerstone of care to reduce mortality. Single measurements of both CRP and PCT lacked sensitivity and specificity for true bacterial infection. So, it was not astonishingly that a decrease in serial measurement was not able to discern 28-day survivors from non-survivors.

Some limitations of our study need to be addressed. First, we did a retrospective observational study of a cohort patients admitted to the ICU during a 6-month period in 2009. It is an old cohort in which clinical, laboratory and microbiological data were prospectively collected. We must rely on older data of clinical practice leading to potential misclassification or observational bias. Some diagnostics for detecting bacterial or viral infection like polymerase chain reaction (PCR) were less or not used in 2009. This explained why we don't have any viral infections collected. Furthermore, the Sepsis-3 definitions were defined in 2016, long after the collection of data in this cohort. Second, by selecting 157 sepsis patients out of 316 eligible patients on basis of Sepsis-3 definitions and an index test CRP and PCT at admission we introduced selection bias. We searched for a proven bacterial infection according to the CDC criteria for infectious diagnosis in acute setting [17]. It was impossible, given the nature of the database, to determine the causes of the negative cultures and we excluded 75 patients, because of no index test CRP and PCT at admission. Antibiotic treatment had already been started in 56 patients, with negative cultures at the ICU as possible result. But there were no significant differences in antibiotics prescribed before inclusion between proven and non-proven sepsis. False negative cultures or cultures that were not properly or not timely performed might result in further misclassification or observational bias. As most physicians want to start antimicrobial therapy immediately after they have made the presumptive diagnosis of sepsis all patients were using antimicrobial therapy on admission and this might have interfered with

cultures becoming positive [2]. Again, this will result in further observational bias We believe that both observational and selection bias in our retrospective study may have led to potential underestimation of accuracy of both PCT and CRP and therefore plan to conduct a prospective observational study in the near future.

However, a strong feature of this analysis is that it is a study in which CRP and PCT were measured on admission and each following day in a mixed surgical and medical ICU in consecutive patients with sepsis according to the new Sepsis-3 definitions. Which may reflect present or future routine clinical practice in a lot of ICUs.

Conclusion

The new Sepsis-3 definitions provide simple bedside criteria to identify septic patients and to start prompt antimicrobial treatment in those with a suspected infection and signs of organ failure [4]. Both CRP and PCT as single measurement at admission were not able to discern patients with a "proven sepsis" from those "with no proven sepsis". A decrease in serial measurements of both CRP and PCT was not different in 28-day survivors and non-survivors. Are both biomarkers completely useless? Several randomized controlled ICU trials have been performed with particularly PCT for the individualization of duration of antibiotic therapy [25-27].

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Supplemental Data

Supplemental Table 1

The definitions of "proven infection" were derived from the Centers of Disease Control (CDC) published algorithms of health care-associated infection and criteria for specific types of infections in the acute care setting [17].

Proven pneumonia
A pneumonia was characterized by a new or progressive and persistent infiltrate on chest imaging. At least one of the following: Fever, leukocytosis or leukopenia or altered mental status and at least of the following:
- new onset of purulent sputum
- new onset cough
- rales or bronchial breath sound
- worsening gas exchange

and positive culture from respiratory secretions, detection of *S. pneumoniae* or *L. pneumophila* antigens in urine or positive blood cultures.

Proven intra-abdominal infection
An intra-abdominal infection was characterized by at least 1 of the following criteria:
1 Patient has at least 2 of the following signs or symptoms: Fever, nausea, vomiting, abdominal pain or jaundice and at least 1 of the following:
   - Organisms cultured from drainage from surgically placed drain.
   - Organisms seen on Gram's stain of drainage or tissue obtained during surgery.
   - Positive blood cultures and radiographic evidence of infection.
2 Positive cultures from purulent material from the intra-abdominal space obtained during surgery or needle aspiration.

Proven urinary tract infection
An urinary tract was characterized by at least 1 of the following signs or symptoms: Fever, urgency, frequency, dysuria or suprapubic tenderness *and* a positive urine culture with $\geq 10^5$ microorganisms per cc urine with no more than 2 species of microorganisms.

Proven meningitis
A meningitis was characterized by at least 1 of the following criteria:
1 Fever, headache, stiff neck, meningeal signs or irritability and at least 1 of the following:
   - Increased white cells, elevated protein, and/or decreased glucose in cerebrospinal fluid.
   - Organisms seen on Gram's stain of cerebrospinal fluid
   - Organisms cultured from blood
   - Positive antigen test of cerebrospinal fluid, blood or urine
2 Organisms cultured from the cerebrospinal fluid.

Proven wound infection
A wound infection was characterized by at least 1 of the following signs or symptoms: Pain or tenderness, localized swelling, redness or heat *and* organisms isolated from an aseptically obtained culture of fluid or tissue from the wound.

Proven catheter related bloodstream infection (CR-BSI)
CR-BSI was characterized by at least 1 of the following criteria:
1 Signs and symptoms of fever, chills or hypotension *and* a central catheter in situ *and* the organism cultured from 1 or more blood cultures is not related to an infection at another site.
2 Signs and symptoms of fever, chills or hypotension, central catheter is removed *and* culture of the tipoff the catheter is positive.

Proven sepsis of unknown cause
Sepsis of unknown cause was characterized by the following signs and symptoms: Fever, chills, tachypnea, tachycardia or hypotension *and* the organism cultured from 1 or more blood cultures is not related to an infection at another site.

Proven immunocompromised infection*
Proven infection in an immunocompromised patient. Immunocompromised patients are patients with HIV/AIDS, hematological malignancy and patients with solid malignancies, organ transplant and rheumatologic/inflammatory conditions who received chemotherapy or systemic steroids.

* Infections in immunocompromised patients are not described in the CDC algorithms published in the article of Horan, et al. [17]. We used the definitions of immunocompromised patients published in the article of Greenberg, et al. [18].

Proven other sites
Proven other sites consisted of patients with proven mediastinitis and proven endocarditis.

Proven endocarditis
Endocarditis was characterized by 2 or more of the following signs and symptoms: Fever, new or changing murmur or skin manifestations (i.e., petechiae, splinter hemorrhages, painful subcutaneous nodules) and at least 1 of the following:
– Organisms cultured from 2 or more blood cultures.
– Evidence of new vegetation seen on echocardiogram.

Proven mediastinitis
Mediastinitis was characterized by at least 1 of the following criteria:
1 Patient has organisms cultured from mediastinal tissue or fluid obtained during surgery or needle aspiration.
2 Patient has at least 1 of the following signs and symptoms: Fever, chest pain, or sternal instability *and* at least 1 of the following:
  – Purulent discharge from mediastinal area.
  – Organisms cultured from blood or discharge from mediastinal area.

Supplemental Table 2 | Definition of PCT and CRP decrease

PCT decrease or ΔPCT was defined: as the difference of PCT concentration between:
– Day 1 [PCT_{day1}] or
– Maximum level of day 1 [PCT_{day1}] and day 2 [PCT_{day2}]
and the PCT concentration level on day 5 [PCT_{day5}]

$$\Delta PCT_{day1\text{-}5}\,(\%) = \frac{[PCT_{day1}] - [PCT_{day5}]}{[PCTd_{ay1}]} \times 100 \quad \text{or}$$

$$\Delta PCT_{Peak1,2\text{-}day5}\,(\%) = \frac{\text{Maximum } [PCT_{day1}]\,[PCT_{day2}] - [PCTday5]}{\text{Maximum } [PCT_{day1}]\,[PCT_{day2}]} \times 100$$

CRP decrease or ΔCRP was defined: as the difference of CRP concentration between:
– Day 1 [CRP_{day1}] or
– Maximum level of day 1 [CRP_{day1}] and day 2 [CRP_{day2}]
and the CRP concentration level on day 5 [CRP_{day5}]

$$\Delta CRP_{day1\text{-}5}\,(\%) = \frac{[CRP_{day1}] - [CRP_{day5}]}{[CRP_{day1}]} \times 100 \quad \text{or}$$

$$\Delta CRP_{Peak1,2\text{-}day5}\,(\%) = \frac{\text{Maximum } [CRP_{day1}]\,[CRP_{day2}] - [CRP_{day5}]}{\text{Maximum } [CRP_{day1}]\,[CRP_{day2}]} \times 100$$

Supplemental Table 3 | First excluded patients: No suspicion of an infection or suspected infection, but less than 2 SOFA points

	Total	**proven infection**	**no proven infection**	***p*-value**
	no 84	**no 13**	**no 71**	
CRP (mg/L)	148 (76 - 231)	148 (81 - 253)	150 (67 - 216)	0.62
PCT (µg/L)	0.7 (0.3 - 2.7)	2.3 (0.4 - 13.9)	0.6 (0.3 - 1.7)	0.08

All median values (interquartile range)

Supplemental Table 4 | Extra excluded patients: Sepsis-3 criteria-positive , but no index test PCT and CRP day 1

	Total	**proven sepsis**	**no proven sepsis**	***p*-value**
	no 75	**no 22**	**no 43**	
CRP (mg/L)	186 (101 - 338)	197 (112 - 364)	186 (95 - 319)	0.42
PCT (µg/L)	No data	_	_	_

All median values (interquartile range)

Supplemental Table 5 | Referring specialties of the study population ICU-patients with sepsis according to Sepsis-3 definitions

	Total	proven sepsis	no proven sepsis	*p*-value
	no 157	no 63	no 94	
Referring specialty				
Cardiology	12 (8%)	3 (5%)	9 (10%)	0.27
Internal medicine	24 (15%)	9 (14%)	15 (16%)	0.76
Pulmonology	10 (6%)	5 (8%)	5 (5%)	0.51
Neurology	11 (7%)	4 (6%)	7 (7%)	0.79
Neurosurgery	12(8%)	2 (3%)	10 (11%)	0.08
Heart lung surgery	17 (11%)	6 (10%)	11 (12%)	0.67
Surgery	46 (29%)	22 (35%)	24 (26%)	0.20
Trauma	14 (9%)	9 (14%)	5 (5%)	0.05
Other	11 (7%)	3 (5%)	8 (8%)	0.37

Legends: All data as number (percentage). Other specialties are urology, gynecology, orthopedics and dermatology.

Supplemental Table 6 | Definitive infection sites

Pneumonia	26 (41%)
Intra-abdominal infection	19 (30%)
Urinary tract infection	1 (2%)
Meningitis	2 (3%)
Wound infection	2 (3%)
CR-BSI	5 (8%)
Laboratory-confirmed bloodstream infection	4 (6%)
Other sites	4 (7%)
Total	63

Legends: definitive infection sites in patients with microbiological proven infection are presented as number (percentage). Other sites are mediastinitis, n = 2 and endocarditis, n = 2.

Supplemental Table 7 | CRP and PCT peak value at day 1_2 in Sepsis-3 criteria-positive patients

	Total	proven sepsis	no proven sepsis	*p*-value
	no 157	no 63	no 94	
CRP (mg/L)	217 (128-305)	234 (141-321)	202 (116-298)	0.25
PCT (µg/L)	1.8 (0.6- 7.4)	2.4 (0.7-7.7)	1.5 (0.5-7.2)	0.18

All median values (interquartile range)

Supplemental Table 8 | CRP, PCT, leucocytes and temperature at day 1 in Sepsis-3 criteria-positive patients

Community-acquired infection				
	Total	**proven sepsis**	**no proven sepsis**	***p*-value**
	no 58	**no 26**	**no 32**	
CRP (mg/L)	177 (106-295)	218 (153-298)	142 (95-270)	0.29
PCT (µg/L)	1.6 (0.5-7.5)	1.9 (0.4-7.5)	1.1 (0.5-7.6)	0.70
Temperature °C	38.3 (37.6-39)	38.3 (37.5-38.9)	38.2 (37.6-39.3)	0.42
Leukocytes (x 10^9 /L)	15 (11.9-19.8)	14.4 (12.2-20.8)	15.4 (10-19)	0.52
Hospital-acquired infection				
	Total	**proven sepsis**	**no proven sepsis**	***p*-value**
	no 99	**no 37**	**no 62**	
CRP (mg/L)	197 (110-268)	185 (88-270)	202 (130-269)	0.03
PCT (µg/L)	1.8 (0.5-5.7)	1.8 (0.7-6.9)	1.9 (0.4-3.6)	0.87
Temperature °C	38.3 (37.8-38.9)	38.5 (37.7-39.1)	38.2 (37.8-38.7)	0.62
Leukocytes (x 10^9 /L)	15.7 (12-20.9)	16.2 (12.2-21.1)	15.1 (11.8-20.7)	0.25

All median values (interquartile range)

Supplemental Table 9 | CRP and PCT at day 1 in Sepsis-3 criteria-positive patients

Medical patients				
	Total	**proven sepsis**	**no proven sepsis**	***p*-value**
	no 68	**no 24**	**no 44**	
CRP (mg/L)	188 (97-257)	191 (86-286)	188 (97-227)	0.86
PCT (µg/L)	1.9 (0.6-8)	2.6 (0.8-8)	1.5 (0.4-8.5)	0.18
Surgical patients				
	Total	**proven sepsis**	**no proven sepsis**	**p-value**
	no 89	**no 39**	**no 50**	
CRP (mg/L)	192 (121-290)	200 (123-294)	175 (116-288)	0.67
PCT (µg/L)	1.6 (0.4-5.2)	1.6 (0.5-5.3)	1.6 (0.4-5.6)	0.72

Medical patients: All patients, without surgery, neurosurgery, thoracic surgery and surgical trauma patients.
All median values (interquartile range).

Supplemental Table 10 | Δ CRP and PCT from peak value day 1_2 until day 5 in Sepsis-3 criteria positive patients

	survivors in 28 days	non-survivors in 28 days	*p*-value
	no 127	no 30	
Δ CRP (%)	38% (12 - 65)	40% (14 - 63)	0.76
Δ PCT (%)	53% (13 - 77)	46% (33 - 78)	0.79

All median values (interquartile range)

Supplemental Table 11 | Δ CRP, PCT, Leukocytes and Temperature from day 1- 5 in Sepsis-3 criteria-positive patients

Community-acquired infection, no 58			
	survivors in 28 days	non-survovors in 28 days	*p*-value
	no 44	no 14	
Δ CRP (%)	37% (-1 - 62)	27% (2 - 62)	0.83
Δ PCT (%)	51% (-6 - 77)	35% (-36 - 72)	0.67
Δ Leukocytes (%)	8% (-21 - 31)	12% (-41 - 42)	0.81
Δ Temperature (%)	0.5% (-1.6 - 3)	2% (-1.4 - 5)	0.45
Hospital-acquired infection, no 99			
	survivors	non-survivors	p-value
	no 83	no 16	
Δ CRP (%)	29% (-11 - 57)	48% (10 - 66)	0.33
Δ PCT (%)	50% (10 - 76)	51% (38 - 79)	0.36
Δ Leukocytes (%)	1% (-22 - 32)	20% (-5 - 39)	0.31
Δ Temperature (%)	1% (-0.5 - 3)	1.7% (0.2- 5)	0.24

All median values (interquartile range).

Supplemental Table 12 | Cross tables and prognostic performance of PCT and CRP decrease (day 1 to 5)

	Alive in 28 days	Dead in 28 days	Total	*p*-value	
Δ PCT day 1-5 decrease > 80%	24	4	28	α^2 0.29	sens 20%
Δ PCT day 1-5 decrease ≤80%	96	22	118	*p*-value 0.59	spec 85%
Total	120	26	146		
Δ PCT day 1-5 decrease > 50%	62	12	74	α^2 0.26	sens 52%
Δ PCT day 1-5 decrease ≤ 50%	58	14	72	*p*-value 0.61	spec 54%
Total	120	26	146		
Δ CRP day 1-5 decrease > 50%	35	6	41	α^2 0.09	sens 33%
Δ CRP day 1-5 decrease ≤ 50%	70	14	84	*p*-value 0.77	spec 70%
Total	105	20	125		

Supplemental Table 13 | Δ CRP and PCT from day 1 until day 10 in Sepsis-3 criteria positive patients

	survivors in 28 days	non-survivors in 28 days	*p*-value
Total 90 patients	**no 78**	**no 12**	
Δ CRP (%)	46% (1 - 70)	7% (-70 - 60)	0.20
Δ PCT (%)	64% (9 - 89)	34% (-6 - 56)	0.14

All median values (interquartile range)

PART II

Biomarkers for prognosis in critically ill patients

Mid-regional proadrenomedullin and mid-regional proatrial natriuretic peptide clearance predicts poor outcome better than single baseline measurements in critically ill patients with pneumonia: a retrospective cohort study

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Cureus. 2021; 13:e15285. doi: 10.7759/cureus.15285.

Abstract

Introduction: We assessed the ability of baseline and serial measurements of mid-regional proadrenomedullin (MR-proADM) and mid-regional proatrial natriuretic peptide (MR-proANP) to predict 28-day mortality in critically ill patients with pneumonia compared with Acute Physiological and Chronic Health Evaluation (APACHE IV) model and Sequential Organ failure Assessment (SOFA) score.

Methods: Biomarkers were collected for the first five days in this retrospective observational cohort study. Biomarker clearance (as percentage) was presented as biomarker decline in five days. We investigated the relationship between biomarkers and mortality in a multivariable Cox regression model. APACHE IV and SOFA were calculated after 24 hours from intensive care unit admission.

Results: In 153 critically ill patients with pneumonia 28-day mortality was 26.8%. Values of baseline MR-proADM, MR-proANP, APACHE IV were significant higher in 28-day non-survivors, but not significant different for SOFA score. Baseline MR-proADM, MR-proANP, APACHE IV and SOFA had a low area under the curve (AUC) in receiver operating characteristics (ROC) curves. No optimal cut-off points could be calculated. Biomarkers and severity scores were divided in tertiles. The highest tertiles baseline MR-proADM and MR-proANP were not significant predictors for 28-day mortality in a multivariable model with age and APACHE IV. SOFA was not a significant predictor in univariable analysis. Clearances of MR-proADM and MR-proANP were significant higher in 28-day survivors. MR-proADM and MR-proANP clearance had similar low accuracy to identify non-survivors in ROC curves and were divided in tertiles, Low clearances of MR-proADM and MR-proANP (first tertiles) were significant predictors for 28-day mortality (hazard ratio (HR) 2.38, 95% CI 1.21 – 4.70, p 0.013 and HR 2.27, 95% CI 1.16 – 4.46, p 0.017) in a model with age and APACHE IV.

Conclusion: MR-proADM and MR-proANP clearance performed better in predicting 28-day mortality in a model with age and APACHE IV compared with single baseline measurements in a mixed population of critically ill with pneumonia.

Introduction

Pneumonia is an important reason of intensive care unit (ICU) admission, length of stay (LOS) and death [1-3]. Mortality rates for community-acquired pneumonia (CAP) admitted to the ICU of 20 - 30% are reported [4]. For hospital-acquired pneumonia (HAP) mortality rates may be as high as 30 to 70% [5] and an attributable mortality of 13% in patients with VAP has been reported in a recent meta-analysis [6]. Knowledge of prognostic factors predicting outcome in a pneumonia may help grade its severity and predict treatment response.

The Acute Physiological and Chronic Health Evaluation (APACHE IV) model and the Sequential Organ failure Assessment (SOFA) score were developed to assess disease severity or severity of organ dysfunction and predict outcome in critically ill patients [7], but due to their complexity incorporation in daily routine is hampered [7]. Additionally, biomarkers have been proposed as surrogate for these clinical scores to predict outcome. Conventional biomarkers white blood count (WBC), C-reactive protein (CRP), procalcitonin (PCT) and lactate have low prognostic value in predicting mortality in patients with CAP or sepsis [8-10]. There may be a role for new cardiovascular biomarkers mid-regional proadrenomedullin (MR-proADM), and mid-regional proartrial natriuretic peptide (MR-proANP) to predict mortality in critically ill patients with a pneumonia. We investigated the role of the precursor fragments of the prohormones of adrenomedullin (ADM) and atrial natriuretic peptide (ANP), because they are more stable than their respective hormones [11,12]. Making these assays more feasible for clinical purposes. MR-proADM is the midregion part of the prohormone of ADM, a peptide released by multiple tissues with anti-inflammatory and antiapoptotic effects on vascular endothelial cells, protecting the microcirculation against endothelial permeability in sepsis [13]. Moreover, ADM enhances cardiac output [10]. MR-proADM levels were rapidly induced in lower respiratory tract infections [7,9,13]. Baseline MR-proADM measurements proved to be a good predictor of both short and long-term survival in CAP patients admitted to the emergency room or ICU [8,10]. Clearance in serial MR-proADM levels of 30% or more in five days in septic patients admitted to the ICU was associated with better outcome [15]. MR-proANP is the midregion part of the prohormone of ANP, a hormone predominantly produced in the atrium of the heart. ANP antagonizes the renin-angiotensin-aldosterone system in response to hypertension and water and salt retention [16]. Increased levels of MR-proANP were reported in non-survivors of patients with CAP, VAP and sepsis [16-18].

In the present study we aimed to investigate the prognostic value of MR-proADM and MR-proANP at baseline compared with the APACHE IV model and SOFA score to predict 28-day mortality in a cohort of critically ill patients with pneumonia of any

cause. Our secondary aim was the prediction of 28-day mortality by clearance of MR-proADM and MR-proANP using serial measurements during five days in comparison with APACHE IV model and SOFA score.

Methods

Study design and selection criteria

This observational cohort study is a post hoc analysis of the Stop Antibiotics on Procalcitonin guidance Study (SAPS) [19], a randomized controlled trial (RCT) in which the efficacy and safety of PCT guidance in reducing the duration of antibiotic treatment in critically ill patients in 15 Dutch ICUs was investigated from 2009 until 2013. Clinical data, microbiological and laboratory results were prospectively collected during this period. The study protocol was approved by the ethics committee of the VU University Medical Centre (Amsterdam, Netherlands). Informed consent was achieved from all participating patients of SAPS and all patients agreed that blood samples were stored for further research. Eligible patients were critically ill patients with pneumonia from one of the participating centers of the SAPS-trial (Elisabeth-Tweesteden hospital). MR-proADM and MR-proANP were measured in samples of these patients. Exclusion criteria were: Age < 18 years, no diagnosis of pneumonia, incomplete data to calculate APACHE IV and SOFA at baseline and unavailability of plasma samples on admission. Pneumonia was characterized by a new or progressive and persistent infiltrate on chest imaging together with fever, leukocytosis, leukopenia or altered mental status and at least one of the following: new onset of purulent sputum, new onset cough, rales or bronchial breath sound or worsening gas exchange [20]. CAP is defined as a pneumonia acquired outside a hospital or long-term care facility [1-3]. HAP refers to a pneumonia that occurs 48 hours or more after admission [5]. VAP is defined as a pneumonia that arises more than 48 – 72 hours after endotracheal intubation [5]. We followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology Statement) guidelines for reporting observational studies [21].

Procedures

Blood samples were collected into EDTA-tubes on a daily basis during the SAPS-trial. Plasma was separated by centrifugation and stored in aliquots at -80 °C. MR-proADM and MR-proANP concentrations were retrospectively measured using an automated immunofluorescent sandwich assay on a Kryptor Compact Plus analyzer (BRAHMS AG, Henningsdorf, Germany) at the clinical laboratory in Enschede, Netherlands. The Kryptor measures the signal that is emitted from an immunocomplex by time-

resolved amplified cryptate emission. MR-proADM and MR-proANP assay have a limit of detection of 0.05 nmol/L and 2.1 pmol/L and functional sensitivity (lowest value with an interassay coefficient of variation (CV) < 20% as described by the manufacturer) of 0.23 nmol/L (MR-proADM) and 4.5 pmol/L (MR-proANP), respectively. Imprecision of both assays were verified according to the Clinical & Laboratory Standards Institute Evaluation Protocol 15 (CLSI EP15), using a low and high sample, measured for 5 days in triplicate. Between- and within-Run CV's were all below 5%. APACHE IV and SOFA were extracted from the SAPS database.

Statistical analysis

All non-normally distributed data (Kolmogorov-Smirnov test $p < 0.05$) were expressed as median (with interquartile range, IQR) or as number of patients (percentage) where appropriate. Patient characteristics and outcomes were compared using a Mann-Whitney U -test for skewed distributed continuous variables and chi-square test for categorical variables. Clearances of MR-proADM and MR-proANP were calculated by dividing the decline of biomarker from day one to day five by the value of the day one and were presented as percentage. The association between mortality and each biomarker and clinical score at admission and clearances of biomarkers was assessed using area under the receiver operating characteristics (ROC) curves. Biomarkers and clinical scores at admission and biomarker clearance were separated in tertiles. Univariable and multivariable Cox proportional hazards regression analyses were done to study the effects on outcome. Potential confounding variables were selected based on univariable regression analysis (variables that yielded p-values < 0.05) and subsequently included in the multivariable regression analysis. The model was checked for intercorrelations among the predictor variables by collinearity statistics. All tests were two-sided and a p-value < 0.05 was considered statistically significant. All data were analyzed using a statistical software package (SPSS Inc., version 24, Chicago, IL, USA).

Results

Descriptive characteristics of the patients

We selected a cohort of 210 patients with a pneumonia from the original SAPS database. In 153 patients MR-proADM and MR-proANP concentrations were measured at baseline in addition to APACHE IV and SOFA scores. The patient flow diagram shows the flow of patients along with the primary endpoint of 28-day survival (Figure 1).

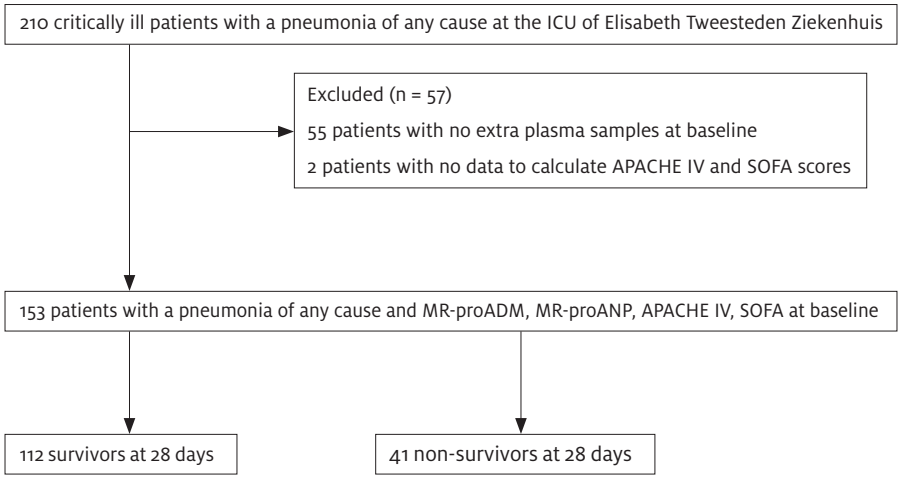

Figure 1 | Patient flow diagram

Demographics and clinical characteristics of these 153 patients, including MR-proADM, MR-proANP, APACHE IV and SOFA score at baseline are shown in Table 1. The 28-day all-cause mortality was 26.8%. Patients were divided in survivors and non-survivors with regards to survival up to 28 days. Both groups were comparable except for older age and higher APACHE IV in non-survivors. There was no significant difference in SOFA score at the first day between both groups. Bacterial pathogens could be detected in 64 patients (41.8%). Gram-positive micro-organisms were found in 18 patients (*S. pneumoniae* n = 14, *Enterococcus spp.* n = 2, other n = 2). Gram-negative micro-organisms were found in 46 patients (*H. influenza* n = 8, *E. coli* n = 8, *Klebsiella spp.* n = 8, *P. aeruginosa* n = 6, *Enterobacter spp.* n = 4, *H. parainfluenza* n = 2, *M. catarrhalis* n = 2 other n = 8). The database revealed no data of viral or fungal infections.

Table 1 | Clinical characteristics of patients at baseline with regards to survival up to 28 days

	Total (N = 153)	Survivors (N = 112)	Non-Survivors (N = 41)	*p* value
Age (years) (median, IQR)	65 (53 - 74)	63 (51 - 71)	69 (63 - 78)	0.003
Male gender (N, %)	94 (61.4%)	66 (58.9%)	28 (68.3%)	0.292
Patient category (N, %)				
Medical	103 (67.3%)	71 (63.4%)	32 (78%)	0.090
Surgical	27 (17.6%)	20 (17.9%)	7(17.1%)	
Trauma	23 (15%)	21 (18.8%)	2 (4.9%)	
Pre-existing comorbidities (N, %)				
Congestive heart failure	15 (9.8%)	8 (7.1%)	7 (17.1%)	0.067
COPD	40 (26.1%)	25 (22.3%)	15 (36.6%)	0.075
Diabetes mellitus	26 (17%)	17 (15.2%)	9 (22%)	0.323
Cerebrovascular disease	37 (24.2%)	23 (20.5%)	14 (34.1%)	0.082
Malignancy	21 (13.7%)	14 (12.5%)	7 (17.1%)	0.467
Chronic renal disease	9 (5.9%)	5 (4.5%)	4 (9.8%)	0.218
Type of pneumonia (N, %)				
Community-acquired pneumonia	60 (39.2%)	43 (38.4%)	17 (41.5%)	0.917
Hospital-acquired pneumonia	76 (49.7%)	56 (50%)	20 (48.8%)	
Ventilator-associated pneumonia	17 (11.1%)	13 (11.6%)	4 (9.8%)	
Microbiology				
Gram-positive (N, %)	18 (11.8%)	14 (12.5%)	4 (9.8%)	0.151
Gram-negative (N, %)	46 (30%)	38 (33.9%)	8 (19.5%)	
No bacterial pathogens (N,%)	89 (58.2%)	60 (53.6%)	29 (70.7%)	
Severity of illness				
Sepsis-3, sepsis (N, %)	148 (96.7%)	109 (97.3%)	39 (95.1%)	0.498
Sepsis-3, septic shock (N, %)	27 (17.8%)	18 (16.1%)	9 (22.5%)	0.361
APACHE IV (points) (median, IQR)	73 (53 - 86)	65 (50 - 84)	81 (61 - 100)	0.003
SOFA score (points) (median, IQR)	5 (3 – 8)	5 (3 - 7)	6 (3 - 10)	0.441
Treatment upon diagnosis (N, %)				
Mechanical ventilation	125 (81.7%)	90 (80.4%)	35 (85.4%)	0.478
Vasopressor use	144 (94.1%)	105 (93,8%)	39 (95.1%)	0.749
Renal replacement therapy	7 (4.6%)	5 (4.5%)	2 (4.9%)	0.914
Length of stay				
ICU LOS (days) (median, IQR)	9 (5 - 18)	9 (4 - 22)	9 (5 - 15)	0.754
Biomarkers				
WBC, 10E9/L, (median, IQR)	12.6 (9.3 - 17.6)	12.3 (9.3 - 16.5)	13.5 (9.5 - 19)	0.407
CRP (mg/L), (median, IQR)	142 (81.8 - 251)	158 (82 - 267)	111 (66 - 230)	0.161
PCT (ng/ml), (median, IQR)	0.7 (0.2 – 4.3)	0.6 (0.2 – 3.1)	1.3 (0.3 – 12.2)	0.066
Lactate (mmol/L), (median, IQR)	1.3 (1 – 1.8)	1.3 (1 - 1.8)	1.5 (1 – 2.1)	0.147
MR-proADM (nmol/L), (median, IQR)	1.3 (0.9 - 2.5)	1.3 (0.9 - 2.1)	1.8 (1 - 3.3)	0.017
MR-proANP (pmol/L), (median, IQR)	159.8 (87.7 - 313)	141.7 (79.1- 208)	201.9 (147.1 - 454.3)	0.001

Legends: All continuous data are presented as median (interquartile range) and categorical data as number (percentage). Abbreviations: COPD: Chronic obstructive pulmonary disease, APACHE IV: Acute physiology and chronic health evaluation IV, SOFA: Sequential organ failure assessment, ICU LOS: Length of stay at the intensive care, WBC: White blood count, CRP: C-reactive protein, PCT: Procalcitonin, MR-proADM: Mid-regional proadrenomedullin, MR-proANP: Mid-regional proatrial natriuretic peptide.

Association between biomarkers at baseline and 28-day mortality

Non-survivors at 28 days had significant higher concentrations MR-proADM and MR-proANP at baseline than survivors (Table 1). WBC, CRP, PCT and lactate concentrations were not significantly different. The area under the curve (AUC) in ROC analysis for the prediction of 28-day mortality of baseline MR-proADM (0.63, 95% confidence interval (CI) 0.53 - 0.73, *p* 0.017)) and MR-proANP (0.68, 95% CI 0.59 - 0.78, *p* 0.001)) was low (Table 2). AUCs for APACHE IV and SOFA were 0.66, (95% CI 0.56 - 0.76), *p* 0.003 and 0.54, (95% CI 0.43 - 0.65), *p* 0.444 (Figure 2) (Table 2). Combinations of biomarkers and scores did not yield a higher AUC (Table 2). Due to a low AUC in the ROC analysis no optimal cut-off points could be calculated. MR-proADM, MR-proANP, APACHE IV and SOFA were divided in tertiles. The highest tertile of MR-proADM, MR-proANP and APACHE IV at day 1 had the strongest association in predicting 28-day mortality compared to patients with the 2 lower tertiles in univariable Cox regression analysis. (Table 3). SOFA score was not a significant predictor of 28-day mortality in univariable analysis and was excluded from further multivariable analysis. The highest tertiles baseline MR-proADM and MR-proANP were not significant predictors for 28-day mortality in a multivariable model with age and APACHE IV. Only APACHE IV had a significant contribution (hazard ratio (HR) 2.07, 95% CI 1.11 – 3.86, *p* 0.021) (Table 3). There were no signs of high correlations between the predictor variables in the model. Tolerance values were between 0.75 and 0.98 (Table 4).

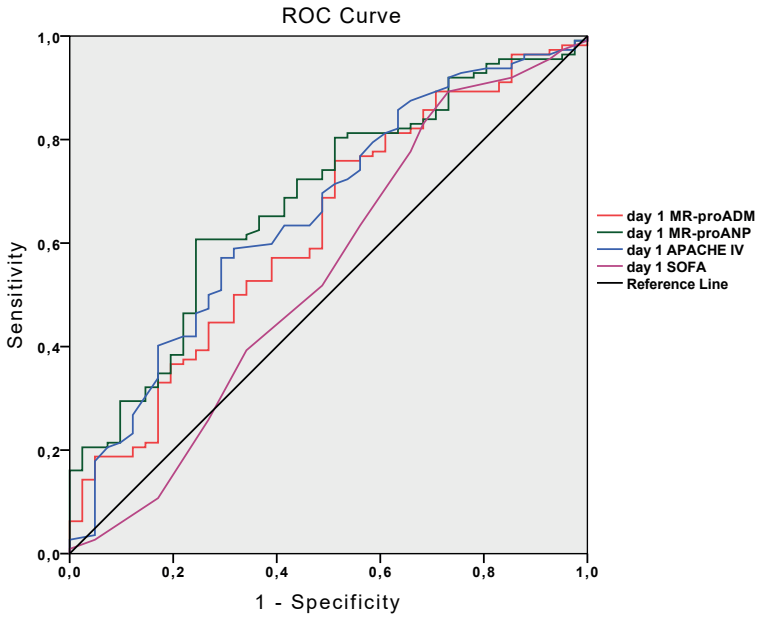

Figure 2 | Prediction of 28-day mortality at baseline

References: Receiver operating characteristics curve for biomarker / clinical score at day 1 in predicting 28-day mortality. Red line = MR-proADM (AUC 0.63, 95% CI 0.53 - 0.73, p 0.017), green line = MR-proANP (AUC 0.68, 95% CI 0.59 - 0.78, *p* 0.001), blue line = APACHE IV (AUC 0.66, 95% CI -.56 - 0.76, *p* 0.003), violet line = SOFA (AUC 0.54, 95% CI 0.43 - 0.65, *p* 0.444), black line = reference line.

Table 2 | Prediction of 28-day mortality by biomarker and clinical score the first day

Biomarker or clinical score	Patients (N)	Mortality (N)	AUC (95% CI)	*p* value
MR-proADM	153	41	0.63 (0.53 – 0.73)	0.017
MR-proANP	153	41	0.68 (0.59 – 0.78)	0.001
APACHE IV	153	41	0.66 (0.56 – 0.76)	0.003
SOFA	153	41	0.54 (0.43 – 0.65)	0.444
APACHE IV + MR-proADM	153	41	0.68 (0.58 – 0.78)	0.001
APACHE IV + MR-proANP	153	41	0.69 ((0.59 – 0.79)	<0.001
SOFA + MR-proADM	153	41	0.61 (0.50 - 0.72)	0.039
SOFA + MR-proANP	153	41	0.68 (0.59 - 0.78)	0.001
MR-proADM + MR-proANP	153	41	0.68 (0.59 – 0.77)	0.001

Legends: Association between biomarkers and clinical scores with 28-day mortality by receiver operating characteristics curves. Abbreviations: AUC: Area under the curve, 95% CI: 95% confidence interval.

Table 3 | Univariable and multivariable Cox regression models for the prediction of 28-day mortality with baseline biomarker values

	Univariable analysis		Multivariable analysis	
	HR (95% CI)	***p* value**	**HR (95% CI)**	***p* value**
Age	1.03 (1.01 – 1.06)	0.014	1.02 (0.99 – 1.05)	0.157
SOFA				
Low (1^{st} + 2^{nd} tertiles)	1.0 (Reference)		-	-
High (3^{rd} tertile)	1.25 (0.68 – 2.32)	0.476	-	-
APACHE IV				
Low (1^{st} + 2^{nd} tertiles)	1.0 (Reference)		1.0 (Reference)	
High (3^{rd} tertile)	2.22 (1.20 – 4.10)	0.011	2.07 (1.11 – 3.86)	0.021
MR-proADM day 1				
Low (1^{st} + 2^{nd} tertiles)	1.0 (Reference)		1.0 (Reference)	
High (3^{rd} tertile)	2.12 (1.15 – 3.91)	0.017	1.09 (0.50 – 2.38)	0.823
MR-proANP day 1				
Low (1^{st} + 2^{nd} tertiles)	1.0 (Reference)		1.0 (Reference)	
High (3^{rd} tertile)	2.42 (1.31 – 4.47)	0.005	1.87 (0.91 – 3.83)	0.089

Legends: Abbreviations: HR = hazard ratio, CI = confidence interval, SOFA sequential organ failure assessment, APACHE IV acute physiology and chronic health evaluation IV, MR-proADM: Mid-regional proadrenomedullin, MR-proANP: Mid-regional proatrial natriuretic peptide.

Table 4 | Collinearity statistics of biomarker/clinical score first day in multivariable Cox regression model

	Tolerance value
Age	0.79
APACHE IV	0.98
MR-proADM	0.69
MR-proANP	0.75

Legends: APACHE IV acute physiology and chronic health evaluation IV, MR-proADM: Mid-regional proadrenomedullin, MR-proANP: Mid-regional proatrial natriuretic peptide

Association between biomarker kinetics and 28-day mortality

Clearance percentages in five days of MR-proADM and MR-proANP were calculated and compared between survivors and non-survivors in 28 days. Clearances of MR-proADM and MR-proANP were significant higher in survivors compared to non-survivors in 28

days (Table 5). MR-proADM and MR-proANP clearance had low accuracy to identify non-survivors in ROC curves, AUC 0.66, (95% CI 0.56 – 0.76), *p* 0.004 and 0.68, (95% CI 0.257 – 0.78), *p* 0.002 (Figure 3) (Table 6). A combination of biomarker clearance did not yield a substantial higher AUC (Table 6). Clearances of MR-proADM and MR-proANP were divided in tertiles. Univariable Cox regression analysis demonstrated that the lowest MR-proADM and MR-proANP clearance (first tertile) in five days had a strong association in predicting 28-day mortality compared to patients with the combined second and third tertiles (Table 7). SOFA score was excluded from further multivariable analysis. Low clearances of MR-proADM and MR-proANP (first tertiles) were significant predictors for 28-day mortality (hazard ratio (HR) 2.38, 95% CI 1.21 – 4.70, *p* 0.013 and HR 2.27, 95% CI 1.16 – 4.46, *p* 0.017) in a model with age and APACHE IV (Table 7). There were no signs of high correlations between the predictor variables, tolerance values were between 0.94 and 0.98 (Table 8).

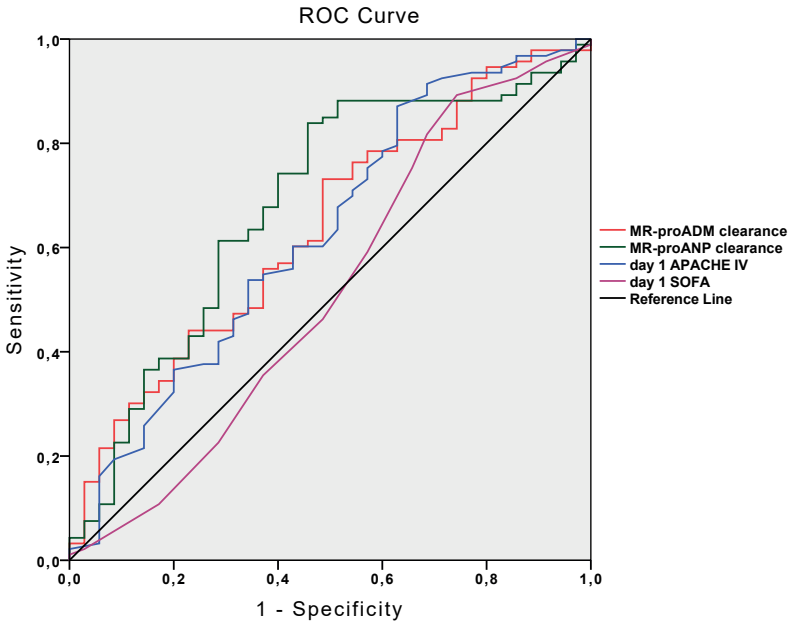

Figure 3 | Prediction of 28-day mortality by biomarker clearance

References: Receiver operating characteristics curve biomarker clearance in five days in predicting 28-day mortality. red line = MR-proADM clearance (AUC 0.66, 95% CI 0.56 - 0.76, *p* = 0.004), green line = MR-proANP clearance (AUC 0.68, 95% CI 0.57 - 0.79, *p* = 0.001), blue line = APACHE IV (AUC 0.66, 95% CI -.56 - 0.76, *p* = 0.003), violet line = SOFA (AUC 0.54, 95% CI 0.43 - 0.65, *p* = 0.444), black line = reference line.

Table 5 | MR-proADM and MR-proANP clearance in five days

28-days survival	survivors	non-survivors	*p* value
	No 112	No 41	
MR-proADM clearance (%)	34% (16 - 50)	16% (-6 - 37)	0.004
MR-proANP clearance (%)	14% (0 - 34)	- 9% (-28 - 18)	0.002

Legends: All continuous data are presented as median % (interquartile range). Abbreviations: MR-proADM: Mid-regional proadrenomedullin, MR-proANP: Mid-regional proatrial natriuretic peptide.

Table 6 | Prediction of 28-day mortality by biomarker clearance in five days

Biomarker	Patients (N)	Mortality (N)	AUC (95% CI)	*p* value
MR-proADM clearance	137	38	0.66 (0.56 – 0.76)	0.004
MR-proANP clearance	130	35	0.68 (0.57 – 0.78)	0.002
APACHE IV + MR-proADM clearance	137	38	0.70 (0.60 – 0.80)	<0.001
APACHE IV + MR-proANP clearance	130	35	0.71 (0.62 – 0.81)	<0.001
SOFA + MR-proADM clearance	137	38	0.68 (0.58 – 0.77)	0.002
SOFA + MR-proANP clearance	130	35	0.69 (0.58 – 0.79)	0.001
MR-proADM clearance + MR-proANP clearance	128	35	0.69 (0.59 – 0.79)	0.001

Legends: Association between biomarker clearance with 28-day mortality by receiver operating characteristics curves. Abbreviations: AUC: Area under the curve, 95% CI: 95% confidence interval

Table 7 | Univariable and multivariable Cox regression models for the prediction of 28-day mortality with biomarker clearance in five days

	Univariable analysis		Multivariable analysis	
	HR (95% CI)	***p* value**	**HR (95% CI)**	***p* value**
Age	1.03 (1.01 – 1.06)	0.014	1.03 (1.01 – 1.06)	0.033
SOFA				
Low (1st + 2nd tertiles)	1.0 (Reference)		-	-
High (3rd tertile)	1.25 (0.68 – 2.32)	0.476	-	-
APACHE IV				
Low (1st + 2nd tertiles)	1.0 (Reference)		1.0 (Reference)	
High (3rd tertile)	2.22 (1.20 – 4.10)	0.011	1.71 (0.87 - 3.37)	0.122
MR-proADM clearance				
2nd + 3rd tertiles (≥ 17% drop)	1.0 (Reference)		1.0 (Reference)	
1st tertile (< 17% drop)	2.52 (1.33 - 4.76)	0.004	2.38 (1.21 - 4.70)	0.013
MR-proANP clearance				
2nd + 3rd tertiles (≥ 0% drop)	1.0 (Reference)		1.0 (Reference)	
1st tertile (< 0% drop)	2.80 (1.44 - 5.45)	0.002	2.27 (1.16 - 4.46)	0.017

Legends: Abbreviations: HR = hazard ratio, CI = confidence interval, SOFA sequential organ failure assessment, APACHE IV acute physiology and chronic health evaluation IV, MR-proADM: Mid-regional proadrenomedullin, MR-proANP: Mid-regional proatrial natriuretic peptide.

Table 8 | Collinearity statistics of biomarker clearance in five days in multivariable Cox regression model

	Tolerance value
Age	0.94
APACHE IV	0.94
MR-proADM clearance	0.98
MR-proANP clearance	0.98

Legends: APACHE IV acute physiology and chronic health evaluation IV, MR-proADM: Mid-regional proadrenomedullin, MR-proANP: Mid-regional proatrial natriuretic peptide

Discussion

The primary aim of our study was to investigate the prognostic value of baseline MR-proADM and MR-proANP compared with APACHE IV and SOFA scores to predict 28-day mortality in a well-described cohort of critically ill patients with pneumonia. The secondary aim was the prediction of 28-day mortality by clearances of MR-proADM and MR-proANP in five days compared with both severity scores. We reported 2 main findings. First, single baseline MR-proADM and MR-proANP levels were not superior to APACHE IV in both ROC curves and in a multivariable Cox regression model for prediction of 28-day mortality. SOFA score performed poorly in identifying non-survivors. Secondly, MR-proADM and MR-proANP clearance in five days performed better in predicting 28-day mortality in a model with age and APACHE IV. However, APACHE IV and SOFA scores require time and effort to collect data and are only available after 24 hours from admission [7]. A single baseline biomarker, with the ability to provide prognostic information early after admission, would have an advantage. Unfortunately, single baseline MR-proADM and MR-proANP did not perform well in our study. The addition of a single baseline MR-proADM and MR-proANP to the APACHE IV and SOFA models did not increase their predictive value. Clearances of biomarkers MR-proADM and MR-proANP performed better in the Cox regression model, but required five days to calculate in our study.

Our findings of a limited value of baseline MR-proADM and MR-proANP in predicting short-term survival in critically ill patients with pneumonia are in contrast with several studies. These 2 biomarkers have been studied frequently in patients with respiratory infections [8,10,14,17,18]. However, most patients were not admitted to the ICU [8,10,14,17]. Baseline MR-proADM and MR-proANP were studied in an observational cohort study of 728 CAP patients admitted to the emergency department (ED) [10]. Only 18 patients were admitted to the ICU. MR-proADM had the best performance for the prediction of 28-day mortality (HR 3.67 and AUC 0.85). MR-proADM proved to have the highest HR in predicting 28-day mortality in an observational cohort study cohort of 1175 ED patients [22]. Only 32 patients were admitted to the ICU. Baseline MR-proADM performed well in comparison with APACHE II and Simplified Acute Physiology Score II (SAPSII) in another observational study [23]. Pneumonia patients were not selected in this study, as all patients admitted to the ICU were included. Our findings of better performance of MR-proADM clearance in five days as a prognostic factor in predicting mortality was supported by a study in which a clearance of MR-proADM of 30% or more in five days had a higher survival probability in 100 days in septic ICU patients [15].

MR-proADM and MR-proANP are predominantly cardiovascular biomarkers. A possible reason for elevation of cardiovascular biomarkers in acute pulmonary disease may be transient pulmonary hypertension, resulting in right heart strain [8,10]. An explanation for the observed lower prognostic value of the biomarkers in our cohort could be underlying cardiac failure [24]. The database was searched for comorbidities, and only 15 patients had congestive heart failure as a comorbidity, but there were no significant differences between both groups. Septic cardiomyopathy could be another reason of elevated biomarkers and almost all of our patients were septic according to Sepsis-3 definitions, but well balanced between survivors and non-survivors. Renal failure is another reason for elevation of MR-proADM and MR-proANP, most probably due to inappropriate renal clearance or increased strain on the atria due to fluid overload [10,25]. A small number of patients had chronic renal disease as comorbidity and a small portion of the cohort was on renal replacement therapy the first day. As day one biomarkers had low prognostic accuracy regarding survival, biomarkers may need time to differentiate between survival and death. Indeed, MR-proADM and MR-proANP clearance in five days performed better in predicting mortality. Persistently high levels of biomarkers in non-survivors may be due to continued synthesis and release due to active organ dysfunction [15]. This hypothesis is supported by a prospective cohort study with 114 septic critically ill patients with cancer [26]. MR-proADM levels decreased from baseline to follow-up (four - seven days later) in survivors and increased in non-surviving patients in this study. Moreover, MR-proANP levels increased in five days in non-survivors, resulting in a negative calculated clearance percentage of MR-proANP in our cohort. Persistently increased levels of MR-proANP in 10 days following VAP onset were also reported in non-surviving patients in another study [18].

Some limitations of our study need to be addressed. First, we did a retrospective observational study in a cohort of critically ill patients with pneumonia selected from the SAPS database. We must rely on older data of clinical practice leading to potential observational bias. Second, by selecting 153 patients out of 210 potential eligible critically ill patients with a pneumonia and index tests we introduced selection bias. Third, our study population is relatively small and heterogeneous in source, as patients with CAP, HAP and VAP were included. These subgroups were too small to analyze by diagnostic subgroup. Both observational and selection bias may have led to potential underestimation of the prognostic performance of MR-proADM and MR-proANP and we therefore plan to conduct a larger prospective observational study in the near future.

A strong feature of our analysis is that it is a real-life study performed on a mixed surgical and medical ICU with patients with a pneumonia consisting of CAP, HAP and VAP, reflecting routine clinical ICU practice.

Conclusions

Single baseline MR-proADM and MR-proANP were not superior to APACHE IV in predicting 28-day mortality in a mixed population of critically ill with pneumonia. SOFA score was not a significant predictor of 28-day mortality. The predictive value of serial-measured MR-proADM and MR-proANP in predicting 28-day mortality in a model with age and APACHE IV exceeded those of single baseline MR-proADM and MR-proANP measurements in this population. Therefore, clearance of MR-proADM and MR-proANP in time may be better used for prognostication instead of single values.

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CHAPTER 4

C-terminal pro-arginine vasopressin is associated with disease outcome and mortality, but not with delayed cerebral ischemia in critically ill patients with an aneurysmal subarachnoid hemorrhage: a prospective cohort study

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Neurocrit Care. 2022; 37:678-88. doi: 10.1007/s12028-022-01540-0.

Abstract

Background/Objective: An aneurysmal subarachnoid hemorrhage (aSAH) is an important indication for intensive care unit admission and may lead to significant morbidity and mortality. We assessed the ability of C-terminal pro-arginine Vasopressin (CT-proAVP) to predict disease outcome, mortality and delayed cerebral ischemia (DCI) in critically ill patients with aSAH as compared with the World Federation of Neurological Surgeons (WFNS) score and Acute Physiological and Chronic Health Evaluation IV (APACHE IV) model.

Methods: CT-proAVP was collected upon admission in this single-centre prospective observational cohort study. Primary aim was to investigate the relationship between CT-proAVP and one-year poor functional outcome (Glasgow Outcome Scale score 1-3) in a multivariable logistic regression model adjusted for WFNS and APACHE IV scores. Secondary aims were mortality and DCI. The multivariable logistic regression model for DCI was also adjusted for the modified Fisher scale.

Results: In 100 patients, median CT-proAVP level was 24.9 pmol/L (IQR 11.5 – 53.8); Forty-five patients had one-year poor functional outcome, 19 died within 30 days, 25 within one year and DCI occurred in 28 patients. Receiver-operating characteristics (ROC) curves revealed high accuracy for CT-proAVP to identify patients with one-year poor functional outcome (area under the curve (AUC) 0.84, 95% confidence interval (CI) 0.77 - 0.92, $p < 0.001$), 30-day mortality (AUC 0.84, 95% CI 0.76 - 0.93, $p < 0.001$) and one-year mortality (AUC 0.79, 95% CI 0.69 - 0.89, $p < 0.001$). CT-proAVP had a low AUC to identify patients with DCI (AUC 0.67, 95% CI 0.55 - 0.79, p 0.008). CT-proAVP $\geq$ 24.9 pmo/L proved to be a significant predictor for one-year poor functional outcome (odds ratio (OR) 8.04, 95% CI 2.97 - 21.75, $p < 0.001$), and CT-proAVP $\geq$ 29.1 pmol/L and $\geq$ 27.7 pmol/L were significant predictors for 30-day and one-year mortality (OR 9.31, 95% CI 1.55 – 56.07, p 0.015 and OR 5.15, 95% CI 1.48 – 17.93, p 0.010) in multivariable models with WFNS and APACHE IV scores. CT-proAVP $\geq$ 29.5 pmol/L was not a significant predictor for DCI in a multivariable model adjusted for the modified Fisher scale (p 0.061).

Conclusions: CT-proAVP had good ability to predict poor functional outcome and mortality in critically ill aSAH patients. Its prognostic ability to predict DCI was low.

Introduction

A subarachnoid hemorrhage due to a ruptured cerebral aneurysm (aSAH) is an important indication for intensive care unit (ICU) admission and may lead to significant morbidity and mortality [1-4]. Reported incidences vary from 6 to 9 aSAHs per 100.000 person-years in the general population [1-4]. About 8% of the patients with aSAH die before arrival at the hospital [5]. Case-fatality rates after one month are around 25% to 35% [5-7]. Although aSAH occurres at a reasonable young age of 55 years [4], estimates of functional independence varied between 36% and 55% at assessments up to 12 months after the bleeding [4]. Also, many patients cannot resume their previous work, have difficulties in relationships and impaired quality of life [8].

The immediate prognosis is determined by the amount of initial intracranial hemorrhage and rebleeding before treatment [1,3]. To prevent rebleeding, the aneurysm is generally obliterated as soon as possible, either by a neurosurgical procedure in which a metal clip is placed over the neck of the aneurysm, or by an endovascular procedure, when platinum coils are inserted inside the aneurysm [1]. Among the secondary complications contributing to morbidity and mortality, delayed cerebral ischemia (DCI) is a major risk factor for bad outcome in aSAH patients [1,9-12]. The occurrence of DCI is associated with a 1.5–threefold increase in case fatality rate after SAH [9,12]. The World Federation of Neurological Surgeons (WFNS) score, was developed to indicate the severity of neurological injury and provide prognostic information regarding outcome in aSAH patients [13] and the Acute Physiological and Chronic Health Evaluation (APACHE IV) model was developed to assess disease severity or severity of organ dysfunction and predict outcome in critically ill patients [14]. However, accurate prediction of outcome remains difficult and complicates decision making for active treatment aiming at recovery. The modified Fisher scale was designed to predict the risk of DCI in aSAH patients [13,15]. It is entirely based on the amount of blood on neuroimaging at initial presentation. Biomarkers, as a surrogate or adjunct of clinical scores, could represent an attractive alternative to predict outcome. C-terminal pro-arginine vasopressin (CT-proAVP), also termed copeptin, is the C-terminal part of the prohormone of arginine vasopressin (AVP), also termed antidiuretic hormone (ADH), which is produced in the hypothalamus and stored in the posterior pituitary [16,17]. CT-proAVP is stable for days, and therefore measuring CT-proAVP in blood is more feasible for clinical purposes [17]. High levels of CT-proAVP were reported to be predictive for poor outcome in patients with traumatic brain injury [18], intracerebral hemorrhage [19], and ischemic stroke [20]. CT-proAVP levels at admission were highly predictive for poor functional outcome and mortality in three cohort studies with Asian aSAH patients [21-23], and was a good prognostic marker for DCI [21,22]. We studied CT-proAVP in Dutch aSAH patients as there are differences reported between Asian and Caucasian patients with regard to incidence and outcome of aSAH [24,25].

The primary aim of the present study was to investigate the prognostic value of CT-proAVP upon admission to predict poor functional outcome after one year in critically ill aSAH patients as compared with WFNS and APACHE IV scores. Secondary aims were 30-day and one-year mortality, and DCI.

Methods

Study design and selection criteria

In a single-centre prospective observational cohort study, we enrolled patients with aSAH, admitted to the ICU of the Elisabeth Tweesteden (ETZ) hospital (Tilburg, the Netherlands) within 24 hours after bleeding from November 2013 until April 2015. The study protocol was approved by the METC Brabant (Medisch Ethische Toetsingscommissie) (Tilburg, the Netherlands) (NL45096.008.13). Informed consent was achieved from participating patients. Inclusion criteria were adults ≥ 18 years of age, admittance to the ICU with an aSAH within 24 hours after bleeding and a CT-proAVP index test at the day of ICU admission. Exclusion criteria for trial participation were recent (< 30 days): Ischemic or hemorrhagic stroke, intracerebral hemorrhage without subarachnoid blood, head trauma, acute myocardial infarction, acute exacerbation of chronic obstructive pulmonary disease (COPD), sepsis or septic shock, acute pancreatitis, or chronic heart failure and liver cirrhosis. Diagnosis of aSAH was based on clinical symptoms (acute headache, focal neurological deficits, loss of consciousness), presence of blood on CT-cerebrum or presence of xanthochromia in cerebral spinal fluid in combination with an aneurysm, confirmed by computerized tomography angiography (CT-A) or digital substraction angiography (DSA) [1]. Diagnosis of DCI was based on acute clinical deterioration in the patient's neurologic condition between three and 14 days after aSAH, assessed by a decrease of at least two points on the Glasgow Coma Scale (GCS) sum score and/or by the development of new focal neurological deficits, and exclusion of other causes for neurological deterioration [9-12]. In case of suspicion of DCI a CT brain perfusion, CT-angiography or DSA was performed. Other causes for neurological deterioration included: Increasing hydrocephalus, rebleeding of an aneurysm, epileptic seizure, severe infectious disease with associated decrease in level of consciousness, hypoglycaemia (defined as serum glucose < 3 mmol/L), hyponatremia (defined as serum sodium < 125 mmol/L), and metabolic encephalopathy due to renal failure as indicated by rapidly rising serum urea. We followed the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology Statement) guidelines for reporting observational studies [26]. Included and excluded patients were described in the patient flow diagram. A control group consisted of 30 healthy volunteers, all hospital staff, with no vascular risk

factors. Primary aim was the prediction of poor functional outcome after one year, and secondary aims were the prediction of 30-day and one-year mortality and prediction of DCI by baseline CT-proAVP. Patients were contacted after one year by the research nurse (PV) for a questionnaire by telephone. This questionnaire included the Glasgow Outcome Scale (GOS) [27]. The GOS rated from death (one point) to symptom-free full recovery (five points). GOS scores were dichotomized in good and poor functional outcomes (GOS 4-5 vs GOS 1-3). The research nurse was blinded for CT-proAVP levels.

Procedures

Venous blood was drawn to measure CT-proAVP in the control group at the start of the study. Clinical data and laboratory results were collected at the first day of ICU admission in patients enrolled in the study. The initial CT-cerebrum was classified according to the modified Fisher scale [13,15]. Blood samples were collected into clot-tubes at admittance. Serum was separated by centrifugation and stored in aliquots at -80 °C. Serum CT-proAVP levels were measured afterwards using an automated immunofluorescent sandwich assay on a B.R.A.H.M.S. Kryptor Compact Plus analyzer (Thermo Fisher Scientific, Henningsdorf, Germany). The Kryptor measures the signal that is emitted from an immunocomplex by time-resolved amplified cryptate emission. CT-proAVP assays have a lower limit of detection of 0.69 pmol/L. The functional sensitivity (lowest value with an interassay coefficient of variation (CV) < 20% as described by the manufacturer) of 1.08 pmol/L. Imprecision of the assay was verified according to the Clinical & Laboratory Standards Institute Evaluation Protocol 17-A (CLSI EP17-A), using a low and high sample, measured for five days in triplicate. Intra and Inter CV values were all ≤ 10% for CT-proAVP.

Statistical analysis

To study our hypothesis that CT-proAVP was useful as predictor for one-year poor functional outcome, a sample size of 93 subjects will have 80% power to calculate sensitivity and specificity for CT-proAVP in aSAH patients, using a chi-square test with 0.05 two-sided significance level. This power calculation was based on CT-proAVP levels in aSAH patients in a prior study [21]. Normally distributed data were expressed as mean (standard deviation, sd); all non-normally distributed data (Kolmogorov-Smirnov test $p < 0.05$) were expressed as median (with interquartile range, IQR) or as number of patients (percentage) where appropriate. Patient characteristics and outcomes were compared using Mann-Whitney *U*-test for skewed distributed continuous variables and chi-square test for categorical variables. The association between CT-proAVP or severity scores (WFNS and APACHE IV) and one-year poor functional outcome, mortality after 30 days and one year, and DCI was assessed using

area under the receiver operating characteristics (ROC) curves. Youden's index analysis was applied to calculate optimal cut-off points. Youden's index was represented by the next formula J = sensitivity + specificity − 1. Sensitivity, specificity, positive and negative predictive values (PPV, NPV) and positive and negative likelihood ratios (LR+, LR-) were calculated for CT-proAVP and severity scores. CT-proAVP, WFNS, and APACHE IV were transformed to dichotomous variables (below or equal and above the cut-off point) and included in univariable logistic regression models to study the effects on outcome, mortality and DCI. Sex, confirmed predictors of outcome and mortality (age, rebleeding) and DCI (modified Fisher scale) were also tested in univariable regression analysis. Variables that yielded p-values < 0.10 were subsequently included in the multivariable regression analysis. Considering the low number of outcome measures in our study and to avoid overfitting of the model CT-proAVP was tested with only a limited number of other variables. The model was checked for intercorrelations among the predictor variables by collinearity statistics. CT-proAVP levels were also log transformed to calculate the risk of one-year poor functional outcome in a logistic regression analysis formula. All tests were two-sided and a p-value < 0.05 was considered statistically significant. All data were analyzed using a statistical software package (SPSS Inc., version 24, Chicago, IL, USA).

Results

Descriptive characteristics of the patients

During the recruitment period, 155 potentially eligible patients were admitted to the ICU with a presumed diagnosis of aSAH. CT-proAVP levels were measured the first day of admission and data of functional status after one year were obtained in 100 SAH patients with a confirmed aneurysm. The patient flow diagram shows the flow of patients along with the primary endpoint of one-year functional outcome (Figure 1). Table 1 summarized the clinical and laboratory data of these patients.

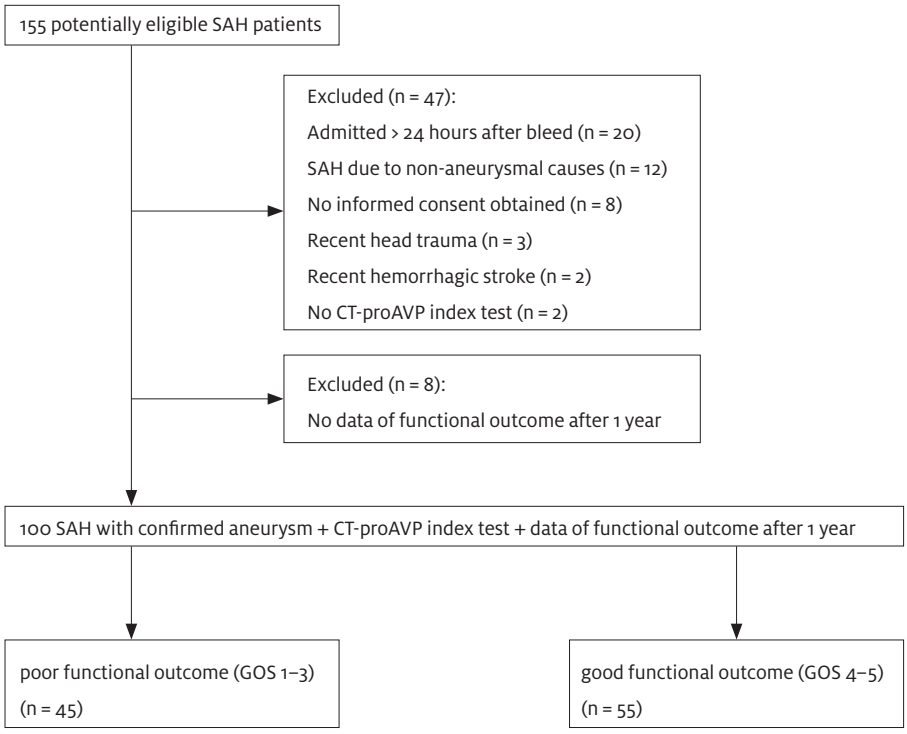

Figure 1 | Patient flow diagram

Legends: SAH: subarachnoid haemorrhage, CT-proAVP: C-terminal pro-arginine vasopressin.

Table 1 | Characteristics of 100 aneurysmal SAH patients

Sex (male/female) (N)	24 / 76
Age, mean (sd)	59.6 (11.8)
GCS at admission, median (IQR)	13 (4 – 15)
WFNS score at admission, median (IQR)	2 (1 - 5)
Modified Fisher scale at admission, median (IQR)	3 (1 – 4)
APACHE IV score ad admission, median (IQR)	49 (31 - 84)
Aneurysmal location (N, %)	
Middle cerebral artery	20 (20%)
Anterior communication artery	40 (40%)
Posterior communication artery	15 (15%)
Posterior inferior cerebelli artery	7 (7%))
Internal carotid artery	3 (3%)
Basilar artery	8 (8%)
Vertebral artery	3 (3%)
Others	4 (4%)
Management (N, %)	
Endovascular coiling	74 (74%)
Neurosurgical clipping	15 (15%)
External ventricular/lumbar drainage	35 (35%)
Adverse events during ICU stay (N, %)	
Rebleeding	16 (16%)
Acute hydrocephalus	39 (39%)
Clinical deterioration caused by DCI	28 (28%)
Intracerebral hemorrhage	13 (13%)
Intraventricular hemorrhage	22 (22%)
Seizures	10 (10%)
Outcome	
ICU LOS (days), median (IQR)	6 (5 - 12)
Hospital LOS (days), median (IQR)	13 (10 - 21)
30-day mortality (N, %)	19 (19%)
One-year mortality (N, %)	25 (25%)
One-year poor functional outcome (GOS 1- 3) (N, %)	45 (45%)
Biomarker	
Time from onset bleeding to serum-sampling in hours, median (IQR)	12 (6 – 17)
Serum CT-proAVP (pmol/L), median (IQR)	24.9 (11.5– 53.8)

Legends: SAH: subarachnoid hemorrhage, GCS: Glasgow Coma Scale, WFNS score: World Federation of Neurological Surgeons score, APACHE IV: Acute physiology and chronic health evaluation IV, DCI: delayed cerebral ischemia, LOS: Length of stay, GOS: Glasgow outcome scale, CT-proAVP: C-terminal pro-arginine vasopressin.

Serum CT-proAVP level on admission in aSAH patients

Serum CT-proAVP levels at the first day of admission in 100 patients were statistically higher compared with 30 healthy controls, 24.9 pmol/L (11.5 – 53.8) vs 3.8 pmol/L (3.1 – 5.3), $p < 0.001$ (Supplemental figure 1).

Association between CT-proAVP and one-year poor functional outcome

After one year 45 patients had poor functional outcome and 55 had good functional outcome. Patients with one-year poor functional outcome had significant higher CT-proAVP levels compared with patients with 1-year good functional outcome (53.1 pmol/L (27.4 – 123.7) vs 14.3 pmol/L (7.3 – 26.8), $p < 0.001$). ROC-curve revealed high accuracy for CT-proAVP to identify patients with one-year poor functional outcome, area under the curve (AUC) 0.84, 95% CI 0.77 - 0.92, $p < 0.001$ (Table 2) (Supplemental figure 2). When CT-proAVP was combined with WFNS or APACHE IV, the combination of APACHE IV and CT-proAVP yielded the highest AUC (Table 2). CT-proAVP and APACHE IV yielded the highest LR+ (Table 2). Eighty-two percent of the patients with both APACHE IV and CT-proAVP ≥ cut-off point had one-year poor outcome and 90% of the patients with both APACHE IV and CT-proAVP < cut-off point had good outcome after one year (Supplemental table 1). Univariable logistic regression analysis demonstrated that CT-proAVP ≥ 24.9 pmol/L and APACHE IV ≥ 44 points had the strongest association with increased risk of one-year poor functional outcome compared to patients with values below the cut-off point (Table 3). CT-proAVP, WFNS, APACHE IV and age were included in a multivariable logistic regression model. CT-proAVP ≥ 24.9 pmol/L proved to be a significant predictor for one-year poor functional outcome (OR 8.04, 95% CI 2.97 - 21.75, $p < 0.001$) (Table 3). There was moderate correlation among predictor variables, variance inflation factors (VIF) of APACHE IV and WNFS were 4.74 and 4.04, respectively (Supplemental table 2). The model was tested for interaction between APACHE IV and WFNS scores in a post-hoc analysis. An APACHE IV*WFNS interaction term made no significant contribution to the multivariable model (p 0.097). The risk of one-year poor functional outcome could be calculated by the following logistic regression analysis formula: $\ln(p/1\text{-}p) = -7.618 + 1.386\alpha1 + 0.047\alpha2$. Where $\alpha1$ is ln (CT-proAVP level) and $\alpha2$ is APACHE IV score, p is the probability of one-year poor functional outcome and $(p/1\text{-}p)$ is the odds of developing poor functional outcome after one year. WFNS score and age were not significant variables in the multivariable regression model.

Table 2 | Prediction of one-year poor outcome by clinical score and CT-proAVP

	AUC (95% CI)	*p*-value	Cutoff	sens	spec	PPV	NPV	LR+	LR-
APACHE IV	0.79 (0.69 – 0.88)	<0.001	44	80%	64%	64%	80%	2.22	0.31
WFNS	0.69 (0.57 – 0.80)	0.001	3	62%	67%	61%	69%	1.88	0.57
CT-proAVP	0.84 (0.77 – 0.92)	<0.001	24.9	78%	73%	70%	80%	2.89	0.30
APACHE IV + CT-proAVP	0.87 (0.80 – 0.94)	<0.001	NA	NA	NA	NA	NA	NA	NA
WFNS + CT-proAVP	0.84 (0.76 – 0.92)	<0.001	NA	NA	NA	NA	NA	NA	NA

Legends: AUC: area under the curve, CI: confidence interval, sens: sensitivity, spec: specificity, PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio, APACHE IV: Acute physiology and chronic health evaluation IV, WFNS: World Federation of Neurological Surgeons score, CT-proAVP: C-terminal pro-arginine vasopressin.

Table 3 | Univariable and multivariable Logistic regression analysis of factors predicting one-year poor functional outcome

	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age	1.04 (0.99 – 1,07)	0.060	1.02 (0.98 – 1.07)	0.359
Sex (female vs male)	1.20 (0.47 – 3.02)	0.707	-	-
Rebleeding	1.71 (0.58 – 5.04)	0.327	-	-
APACHE IV at admission				
< 44	1.0 (Reference)		1.0 (Reference)	
≥ 44	7.00 (2.81 – 17.46)	<0.001	4.26 (1.04 – 17.54)	0.045
WFNS at admission				
< 3	1.0 (Reference)		1.0 (Reference)	
≥ 3	3.39 (1.48 – 7.73)	0.004	1.34 (0.35 – 5.16)	0.675
Serum CT-proAVP (pmol/L) at admission				
< 24.9	1.0 (Reference)		1.0 (Reference)	
≥ 24.9	9.33 (3.72 – 23.42)	<0.001	8.04 (2.97 – 21.75)	<0.001

Legends: OR: odds ratio, CI: confidence interval, APACHE IV: Acute physiology and chronic health evaluation IV, WFNS: World Federation of Neurological Surgeons score, CT-proAVP: C-terminal pro-arginine vasopressin.

Association between CT-proAVP and 30-day and one-year mortality

Nineteen patients died from aSAH in 30 days and 25 patients died within one year. Non-survivors at 30 days and one year had significant higher concentrations of CT-proAVP the first day of admission than survivors (87.8 (30.1 - 228.8) vs 18.4 pmol/L (9.7 - 39.9), $p < 0.001$ for 30-day mortality and 58.4 (29.0 - 163.8) vs 18.4 (8.8 - 38.7), $p < 0.001$ for one-year mortality). ROC-curves revealed high accuracy for CT-proAVP to identify both patients with 30-day (AUC 0.84, 95% CI 0.76 - 0.93, $p < 0.001$) and one-year mortality (AUC 0.79, 95% CI 0.69 - 0.89, $p < 0.001$) (Table 4) (Supplemental figures 3 and 4). The predictive value of CT-proAVP was lower than those of WFNS (30-day mortality) and APACHE IV (30-day and one-year mortality). When CT-proAVP was combined with WFNS or APACHE IV, the combination of APACHE IV and CT-proAVP yielded the highest AUC (Table 4). APACHE IV yielded the highest LR+ for the prediction of 30-day and one-year mortality (Table 4). All (100%) patients with both APACHE IV and CT-proAVP < cut-off point survived 30 days and one year (Supplemental tables 3 and 4). A smaller part (65% and 61%) of the patients with both APACHE IV and CT-proAVP ≥ cut-off point died in 30 days and one year (Supplemental tables 3 and 4). Univariable logistic regression analysis demonstrated that APACHE IV above the cut-off points had the strongest association with increased risk of 30-day and one-year mortality compared to patients with values below the cut-off point (Table 5). CT-proAVP, WFNS, APACHE IV, age and rebleeding were included in a multivariable logistic regression model for 30-day mortality and all, but rebleeding were included in further multivariable analysis for one-year mortality. CT-proAVP ≥ 29.1 pmol/L and 27.7 pmol/L proved to be a significant predictor for 30-day and one-year mortality (OR 9.31, 95% CI 1.55 – 56.07, p 0.015 and OR 5.15, 95% CI 1.48 – 17.93, p 0.010), but not as strong predictor as APACHE IV in predicting 30-day and one-year mortality (OR 18.27, 95% CI 1.19 - 281.53, p 0.037 and OR 10.25, 95% CI 1.45 - 72.48, p 0.020) (Table 5). There was moderate correlation between predictor variables, APACHE IV and WFNS had the highest VIFs (Supplemental tables 5 and 6). The models were tested for interaction between APACHE IV and WFNS scores in a post-hoc analysis. An APACHE IV*WFNS interaction term made no significant contribution to the multivariable model of 30-day and one-year mortality (p 0.276 and p 0.233, respectively).

Table 4 | Prediction of 30-day and one-year mortality by clinical score or CT-proAVP

	AUC (95% CI)	p-value	Cutoff	sens	spec	PPV	NPV	LR+	LR-
30-day mortality									
APACHE IV	0.94 (0.89 – 0.99)	<0.001	70	95%	80%	53%	99%	4.75	0.06
WFNS	0.88 (0.81 – 0.95)	<0.001	3	95%	65%	39%	98%	2.71	0.08
CT-proAVP	0.84 (0.76 – 0.93)	<0.001	29.1	84%	64%	36%	95%	2.33	0.25
APACHE IV + CT-proAVP	0.94 (0.90 – 0.98)	<0.001	NA	NA	NA	NA	NA	NA	NA
WFNS + CT-proAVP	0.92 (0.87 – 0.98)	<0.001	NA	NA	NA	NA	NA	NA	NA
One-year mortality									
APACHE IV	0.87 (0.80 – 0.95)	<0.001	54	88%	71%	50%	95%	3.03	0.17
WFNS	0.77 (0.66 – 0.89)	<0.001	3	80%	65%	44%	91%	2.29	0.33
CT-proAVP	0.79 (0.69 – 0.89)	<0.001	27.7	80%	64%	43%	91%	2.22	0.31
APACHE IV + CT-proAVP	0.88 (0.81 – 0.95)	<0.001	NA	NA	NA	NA	NA	NA	NA
WFNS + CT-proAVP	0.82 (0.73 – 0.92)	<0.001	NA	NA	NA	NA	NA	NA	NA

Legends: AUC: area under the curve, CI: confidence interval, sens: sensitivity, spec: specificity, PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio, APACHE IV: Acute physiology and chronic health evaluation IV, WFNS: World Federation of Neurological Surgeons score, CT-proAVP: C-terminal pro-arginine vasopressin.

Table 5 | Univariable and multivariable Logistic regression analysis of factor predicting 30-day and one-year mortality

30-day mortality				
	Univariable analysis		**Multivariable analysis**	
	OR (95% CI)	***p* value**	**OR (95% CI)**	***p* value**
Age	1.04 (0.99 – 1.09)	0.094	1.04 (0.97 -1.12)	0.246
Sex (female vs male)	1.87 (0.49 – 7.05)	0.357	-	-
Rebleeding	3.28 (1.02 - 10.58)	0.047	1.88 (0.32 - 11.06)	0.488
APACHE IV at admission				
< 70	1.0 (Reference)		1.0 (Reference)	
≥ 70	73.13 (9.08 – 589.23)	<0.001	18.27 (1.19 – 281.53)	0.037
WFNS score at admission				
< 3	1.0 (Reference)		1.0 (Reference)	
≥ 3	34.07 (4.32 – 268.68)	0.001	4.85 (0.22 – 106.80)	0.317
Serum CT-proAVP at admission (pmol/L)				
< 29.1	1.0 (Reference)		1.0 (Reference)	
≥ 29.1	9.56 (2.57 – 35.59)	0.001	9.31 (1.55 – 56.07)	0.015
One-year mortality				
Age	1.05 (1.01 – 1.10)	0.026	1.05 (0.99 – 1.11)	0.062
Sex (female vs male)	1.36 (0.45 – 4.12)	0.590	-	-
Rebleeding	2.05 (0.66 - 6.38)	0.214	-	-
APACHE IV at admission				
< 54	1.0 (Reference)		1.0 (Reference)	
≥ 54	17.67 (4.79 – 65.13)	<0.001	10.25 (1.45 – 72.48)	0.020
WFNS score at admission				
< 3	1.0 (Reference)		1.0 (Reference)	
≥ 3	7.54 (2.54 – 22.41)	0.004	1.54 (0.24– 9.88)	0.649
Serum CT-proAVP (pmol/L) at admission				
< 27.7	1.0 (Reference)		1.0 (Reference)	
≥ 27.7	7.11 (2.40 – 21.10)	<0.001	5.15 (1.48 – 17.93)	0.010

Legends: OR: odds ratio, CI: confidence interval, APACHE IV: Acute physiology and chronic health evaluation IV, WFNS: World Federation of Neurological Surgeons score, CT-proAVP: C-terminal pro-arginine vasopressin.

Association between CT-proAVP and DCI

Twenty-eight aSAH patients suffered from clinical signs of DCI during their ICU stay. Patients who suffered from DCI had significant higher concentrations of CT-proAVP concentrations the first day of admission than patients without DCI (51 pmol/L (15.9 – 116.1) vs 20.8 pmol/L (9.4 – 40.0), *p* 0.008). However, CT-proAVP had a low accuracy

to identify patients with DCI in the ROC analysis (AUC 0.67, 95% CI 0.55 - 0.79, *p* 0.008) (Table 6) (Supplemental figure 5). WFNS, APACHE IV and modified Fisher scale had comparable low accuracy to predict DCI with low AUCs in ROC analysis (Table 6) (Supplemental figure 5). An optimal cut-off point was calculated for CT-proAVP and modified Fisher scale. No optimal cut-off points could be calculated for WFNS and APACHE IV scores and they were not tested in the multivariable model. CT-proAVP yielded a low LR+ for the prediction of DCI. Both CT-proAVP and modified Fisher scale were tested in a multivariable logistic regression model. CT-proAVP ≥ 29.5 pmol/L was not a significant predictor for DCI in a multivariable model adjusted for the modified Fisher scale (OR 2.51, 95% CI 0.96 - 6.56, *p* 0.061) (Table 7). There was no correlation between CT-proAVP and modified Fisher scale (VIF 1.09) (Supplemental table 7).

Table 6 | Prediction of delayed cerebral ischemia during hospitalization by clinical score or CT-proAVP

	AUC (95% CI)	*p*-value	Cutoff	sens	spec	PPV	NPV	LR+	LR-
APACHE IV	0.60 (0.48 – 0.71)	0.136	NA	NA	NA	NA	NA	NA	NA
WFNS	0.51 (0.38 – 0.64)	0.902	NA	NA	NA	NA	NA	NA	NA
Modified Fisher scale	0.65 (0.54 – 0.77)	0.018	3.0	82%	54%	41%	89%	3.72	0.66
CT-proAVP	0.67 (0.55 – 0.79)	0.008	29.5	64%	65%	42%	83%	1.83	0.55
APACHE IV + CT-proAVP	0.61 (0.48 – 0735)	0.105	NA	NA	NA	NA	NA	NA	NA
WFNS + CT-proAVP	0.62 (0.49 – 0.74)	0.071	NA	NA	NA	NA	NA	NA	NA
Mod Fisher scale + CTproAVP	0.70 (0.59 – 0.81)	0.02	NA	NA	NA	NA	NA	NA	NA

Legends: AUC: area under the curve, CI: confidence interval, sens: sensitivity, spec: specificity, PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio, APACHE IV: Acute physiology and chronic health evaluation IV, WFNS: World Federation of Neurological Surgeons score, CT-proAVP: C-terminal pro-arginine vasopressin

Table 7 Univariable and multivariable Logistic regression analysis of factors predicting DCI

	Univariable analysis		Multivariable analysis	
	OR (95% CI)	*p* value	OR (95% CI)	*p* value
Age	1.01 (0.97 – 1.04)	0.786	-	-
Sex (female vs male)	1.65 (0.55 – 4.95)	0.373	-	-
Modified Fisher scale at admission				
< 3	1.0 (Reference)		1.0 (Reference)	
≥ 3	5.44 (1.86 – 15.89)	0.002	4.41 (1.47 – 13.24)	0.008
Serum CT-proAVP (pmol/L) at admission				
< 29.5	1.0 (Reference)		1.0 (Reference)	
≥ 29.5	3.38 (1.36 – 8.43)	0.009	2.51 (0.96 – 6.56)	0.061

Legends: OR: odds ratio, CI: confidence interval, CT-proAVP: C-terminal pro-arginine vasopressin

Discussion

We reported two main findings. First, CT-proAVP had high accuracy to identify critically ill aSAH patients with poor functional outcome and was a significant predictor in a multivariable logistic regression model including WFNS and APACHE IV scores. Combining CT-proAVP with APACHE IV significantly improved the prognostic accuracy for predicting one-year poor functional outcome. Eighty-two percent of the patients with both APACHE IV and CT-proAVP ≥ cut-off point had poor outcome after one year. Secondly, CT-proAVP levels had also high accuracy to identify critically ill aSAH patients who died in 30 days and one year, but CT-proAVP levels were not predictive of DCI during ICU stay. APACHE IV performed better than the WFNS score in predicting outcome and mortality in our study. The WFNS score was based on the GCS and the presence of focal neurological deficit [13], but it can be difficult to assess the neurological status due to sedation or impaired consciousness. A possible explanation for the good performance of APACHE IV in predicting outcome and mortality was capture of the physiologic stress of aSAH by the physiological subscore of APACHE IV, assessing the degree of acute illness. Age and comorbidities are other known predictors of outcome and mortality and are covered by the Age and Chronic Health section of the APACHE IV score. However, incorporation of APACHE IV model in daily routine was hampered due to its complexity. Finding an easily obtainable biomarker, as alternative or adjunct of clinical scores, able to identify patients with worst outcome may help early risk assessment and may provide further insights into pathophysiological mechanisms. It might be argued that especially patients with highest values CT-proAVP would benefit from extended ICU therapy. On the other hand, patients with lower CT-proAVP values have a higher chance of good one-year functional outcome and could be discharged from the ICU to the general ward at an earlier stage.

Our findings of good ability of baseline CT-proAVP levels in serum to predict poor functional outcome and mortality are in line with other studies [21-23]. CT-proAVP, measured during the first day of admission, was frequently studied in Asian patients with aSAH [21-23]. Baseline CT-proAVP levels and WFNS scores in these studies were quite comparable with our study population [21,22]. CT-proAVP levels at baseline were also strongly correlated with WFNS scores, suggesting CT-proAVP as a robust indicator of neurological outcome following aSAH [22,28]. In contrast to our findings, combining CT-proAVP with WFNS scores further improved the predictive performance of WFNS scores for poor outcome and mortality in several studies [21,22]. Elevated baseline CT-proAVP levels correlated with clinical deterioration caused by DCI in several studies [21,22] and CT-proAVP was an independent predictor of clinical deterioration caused by DCI in logistic regression models [21]. This was considered an important finding, as DCI is the main treatable determinant of poor outcome after aSAH [29]. Unexpectedly,

CT-proAVP levels had a low ability to predict DCI in our study. We used the term DCI to address clinical deterioration caused by DCI [11]. There are some disadvantages of this clinical diagnosis. The clinical spectrum of DCI is wide. Typical features are neurological deficits or decrease in levels of consciousness. However, neck stiffness, fever or mutism have also been reported as clinical signs of DCI in some studies [11]. A proportion of aSAH patients are comatose or sedated. Last, clinical deterioration is a diagnosis per exclusionem. Zhu et al. [21] and Zheng et al. [22] used the term cerebral vasospasm for describing clinical deterioration from DCI, but the term vasospasm should be reserved for the results of radiological tests (either CT angiography, DSA or MRA) [11]. We studied CT-proAVP levels measured once at baseline and the occurrence of DCI during ICU stay. However, significant differences in plasma CT-proAVP levels between DCI and non-DCI patients and at different time points were only found from day seven, when consecutive CT-proAVP levels were collected for DCI prediction the first two weeks in aSAH patients [30], suggesting a dynamic secretion of CT-proAVP which necessitates serial CT-proAVP measurements to more accurately predict DCI [30]. In addition, it was found that increased CT-proAVP levels in cerebrospinal fluid were also associated with DCI in aSAH patients [31].

CT-proAVP, the C-terminal part of the prohormone of AVP, is produced in the hypothalamus [16,17]. AVP contributes to the regulation of osmotic and cardiovascular homeostasis [16,17]. AVP is stimulated by different stressors. AVP potentiates the action of CRH and leads downstream to release of ACTH and production of cortisol [16], reflecting the individual stress response at hypothalamic level [16]. CT-proAVP concentrations mirror the concentrations of AVP [17]. CT-proAVP is stable for days, and therefore measuring CT-proAVP in blood is more feasible for clinical purposes [17]. CT-proAVP is known to have prognostic value in various diseases, as it reflects disease severity and the chance of recovery [18-20]. Therefore, it has been hypothesized that the close relationship of CT-proAVP levels to the degree of activation of the stress axis is the basis of its usefulness as prognostic biomarker in aSAH patients [16]. Baseline CT-proAVP levels were predictive for outcome and mortality in our study, but not for DCI. The exact underlying pathophysiological mechanisms of DCI are multifactorial and not fully understood [9-12]. Animal studies suggest that AVP could be involved in the development of DCI [32] and ischemic brain edema [33]. Intracisternal injection of AVP induced acute vasospasm in model of SAH in rats [32]. Treatment with vasopressin receptor antagonists reduced the infarction volume in an embolic focal ischemia model in rats [33].

Some limitations of our study need to be addressed. First, we did a single-centre prospective observational study in a cohort aSAH patients admitted to the ICU within 24 hours after bleeding from November 2013 until April 2015. Results of single-

centre studies are determined by the case-mix (which varies with the age profile and comorbidities of the patients) and resources (number of physicians, nurse/patient ratio) of the particular ICU) [34]. We must be careful about extrapolating these results to the general population. Second, we collected baseline CT-proAVP levels and did not collect serial CT-proAVP levels during ICU stay. Third, by selecting 100 patients out of 155 potential eligible critically ill SAH patients on basis of inclusion criteria, an index test CT-proAVP at admission and data of functional outcome after one year we introduced selection bias. We believe that both observational and selection bias in our study may have led to potential underestimation of the prognostic performance of CT-proAVP and therefore plan to conduct a larger multi-centre prospective observational study with serial CT-proAVP measurements in the near future.

CT-proAVP, as single baseline value, will always oversimplify prognostic assessment and therefore, CT-proAVP is meant, rather than to supersede, to complement clinician's judgement. Prognosis cannot be based on a biomarker alone, even though it is highly sensitive and specific.

Conclusions

We conclude that single baseline CT-proAVP had good ability to predict one-year poor functional outcome in critically ill aSAH patients. Baseline CT-proAVP as adjunct to the APACHE IV model may help clinicians to identify patients at higher risk of poor outcome. CT-proAVP levels had also high accuracy to predict mortality, but the prognostic ability of single baseline CT-proAVP to predict DCI during ICU stay was low.

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Appendix

Supplemental Table 1 | Predicted probability of one-year poor functional outcome for combinations CT-proAVP and APACHE IV

	APACHE IV < 44	APACHE IV ≥ 44
CT-proAVP < 24.9 pmol/	10%	36%
CT-proAVP ≥ 24.9 pmol/L	40%	82%

Legends: APACHE IV: Acute physiology and chronic health evaluation IV, CT-proAVP: C-terminal pro-arginine vasopressin. Values indicate the probability (in percentage) of one-year poor functional outcome given the ranges of CT-proAVP and APACHE IV.

Supplemental Table 2 | Collinearity statistics of predictor variables in multivariable logistic regression model for one-year poor functional outcome

	Variance Inflation Factor (VIF)
Age	1.19
APACHE IV	4.74
WFNS	4.04
CT-proAVP	1.30

Supplemental Table 3 | Predicted probability of 30-day mortality for combinations of CT-proAVP and APACHE IV

	APACHE IV < 70	APACHE IV ≥ 70
CT-proAVP < 29.1 pmol/L	0%	21%
CT-proAVP ≥ 29.1 pmol/L	11%	65%

Legends: APACHE IV: Acute physiology and chronic health evaluation IV, CT-proAVP: C-terminal pro-arginine vasopressin. Values indicate the probability (in percentage) of 30-day mortality given the ranges of CT-proAVP and APACHE IV.

Supplemental Table 4 | Predicted probability of one-year mortality for combinations of CT-proAVP and APACHE IV (Values indicate the probability (in percentage) of one-year mortality given the ranges of CT-proAVP and APACHE IV)

	APACHE IV < 54	APACHE IV ≥ 54
CT-proAVP < 27.7 pmol/L	0%	27%
CT-proAVP ≥ 27.7 pmol/L	44%	61%

Legends: APACHE IV: Acute physiology and chronic health evaluation IV, CT-proAVP: C-terminal pro-arginine vasopressin. Values indicate the probability (in percentage) of one-year mortality given the ranges of CT-proAVP and APACHE IV.

Supplemental Table 5 | Collinearity statistics of predictor variables in multivariable logistic regression model for 30-day mortality

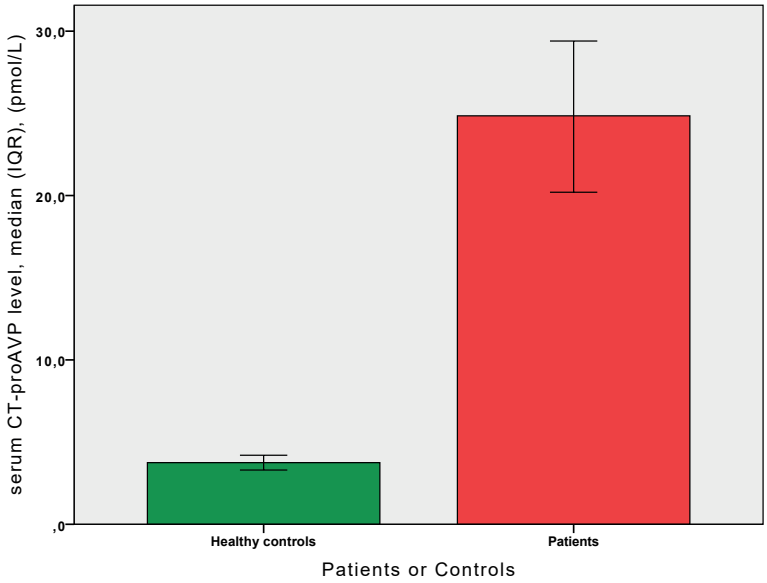
	Variance Inflation Factor (VIF)
Age	1.20
Rebleeding	1.09
APACHE IV	4.75
WFNS	4.06
CT-proAVP	1.31

Supplemental Table 6 | Collinearity statistics of predictor variables in multivariable logistic regression model for one-year mortality

	Variance Inflation Factor (VIF)
Age	1.19
APACHE IV	4.74
WFNS	4.04
CT-proAVP	1.30

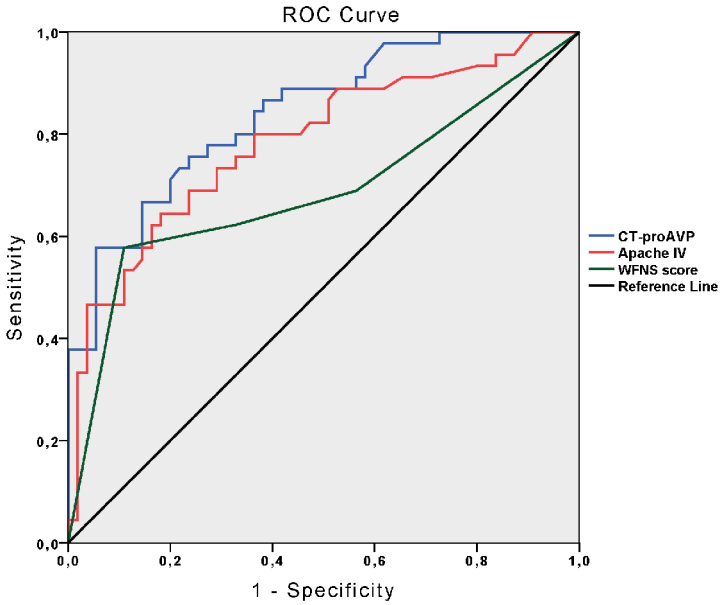
Supplemental Table 7 | Collinearity statistics of predictor variables in multivariable logistic regression model for DCI

	Variance Inflation Factor (VIF)
Modified Fisher scale	1.09
CT-proAVP	1.09



Supplemental Figure 1 | Serum CT-proAVP concentrations in aneurysmal SAH patients and healthy controls

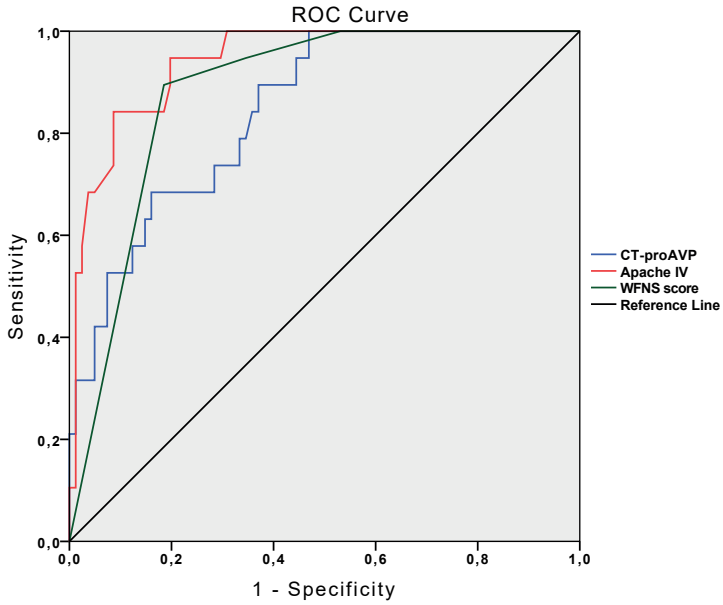
Legends: CT-proAVP: C-terminal pro-arginine vasopressin.



Supplemental Figure 2 | Receiver operating characteristics curve for clinical score and CT-proAVP in predicting one-year poor functional outcome

References: Red line = APACHE IV (AUC: 0.79, 95% CI: 0.69 – 0.88, *p* <0.001), green line = WFNS (AUC: 0.69, 95% CI: 0.57 – 0.80, *p* 0.001), blue line = CT-proAVP (AUC: 0.84, 95% CI: 0.77 - 0.92, *p* < 0.001) and black line = reference line.

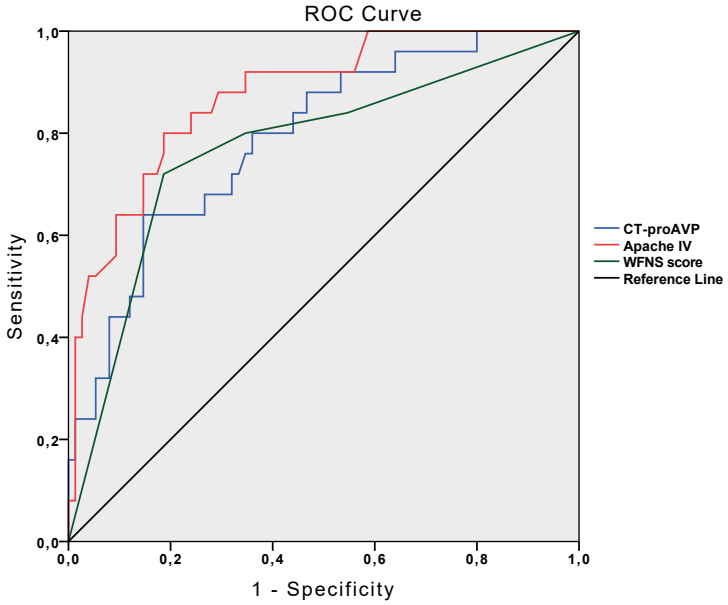
Legends: APACHE IV: Acute physiology and chronic health evaluation IV, WFNS: World Federation of Neurological Surgeons score, CT-proAVP: C-terminal pro-arginine vasopressin.



Supplemental Figure 3 | Receiver operating characteristics curve for clinical score and CT-proAVP in predicting 30-day mortality

References: Red line = APACHE IV (AUC: 0.94, 95% CI: 0.89 – 0.99, p <0.001), green line = WFNS (AUC: 0.88, 95% CI: 0.81 – 0.95, p < 0.001), blue line = CT-proAVP (AUC: 0.84, 95% CI: 0.76 - 0.93, p < 0.001) and black line = reference line. .

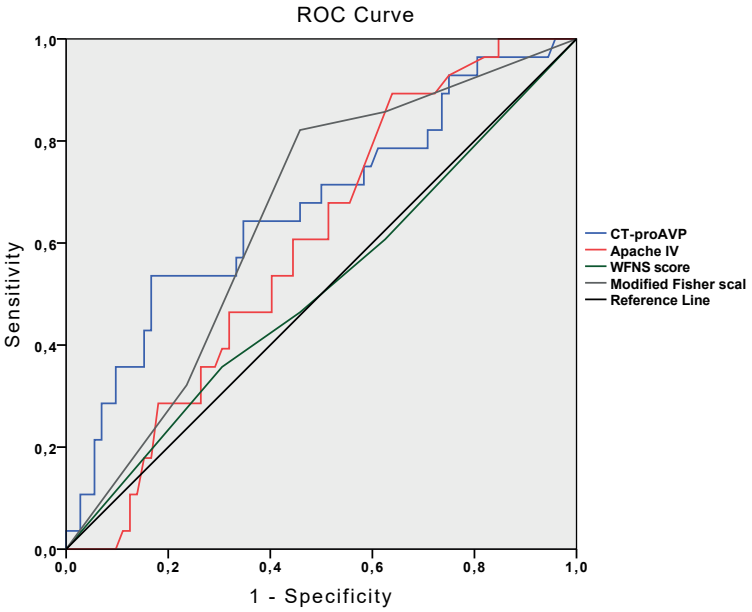
Legends: APACHE IV: Acute physiology and chronic health evaluation IV, WFNS: World Federation of Neurological Surgeons score, CT-proAVP: C-terminal pro-arginine vasopressin.



Supplemental Figure 4 | Receiver operating characteristics curve for clinical score and CT-proAVP in predicting one-year mortality

References: Red line = APACHE IV (AUC: 0.87, 95% CI: 0.80 – 0.98, p <0.001), green line = WFNS (AUC: 0.77, 95% CI: 0.66 – 0.89, p < 0.001), blue line = CT-proAVP (AUC: 0.79, 95% CI: 0.69 - 0.89, p < 0.001) and black line = reference line.

Legends: APACHE IV: Acute physiology and chronic health evaluation IV, WFNS: World Federation of Neurological Surgeons score, CT-proAVP: C-terminal pro-arginine vasopressin.



Supplemental Figure 5 | Receiver operating characteristics curve for clinical score and CT-proAVP in predicting delayed cerebral ischemia during hospitalization

References: Red line = APACHE IV (AUC: 0.60, 95% CI: 0.48 – 0.71, *p* 0.136), green line = WFNS (AUC: 0.51, 95% CI: 0.38 – 0.64, *p* 0.902), grey line = Modified Fisher scale (AUC 0.65, 95% CI: 0.54 - 0.77, *p* 0.018), blue line = CT-proAVP (AUC: 0.67, 95% CI: 0.55 - 0.79, *p* 0.008) and black line = reference line.

Legends: APACHE IV: Acute physiology and chronic health evaluation IV, WFNS: World Federation of Neurological Surgeons score, CT-proAVP: C-terminal pro-arginine vasopressin.

PART III

Biomarkers in critically ill patients with SARS-CoV-2 pneumonia

Endothelium-associated biomarkers mid-regional proadrenomedullin and C-terminal proendothelin-1 have good ability to predict 28-day mortality in critically ill patients with SARS-CoV-2 pneumonia: a prospective cohort study

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J Crit Care. 2021; 66:173-180. doi: 10.1016/j.jcrc.2021.07.017.

Abstract

Purpose: We assessed the ability of mid-regional proadrenomedullin (MR-proADM) and C-terminal proendothelin-1 (CT-proET-1) to predict 28-day mortality in critically ill patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia.

Methods: Biomarkers were collected during the first seven days in this prospective observational cohort study. We investigated the relationship between biomarkers and mortality in a multivariable Cox regression model adjusted for age and SOFA score.

Results: In 105 critically ill patients with confirmed SARS-CoV-2 pneumonia 28-day mortality was 28.6%. MR-proADM and CT-proET-1 were significantly higher in 28-day non-survivors at baseline and over time. ROC curves revealed high accuracy to identify non-survivors for baseline MR-proADM and CT-proET-1, AUC 0.84, (95% CI 0.76 – 0.92), $p < 0.001$ and 0.79, (95% CI 0.69 – 0.89), $p < 0.001$, respectively. The AUC for prediction of 28-day mortality for MR-proADM and CT-proET-1 remained high over time. MR-proADM ≥ 1.57 nmol/L and CT-proET-1 ≥ 111 pmol/L at baseline were significant predictors for 28-day mortality (HR 6.80, 95% CI 3.12 – 14.84, $p < 0.001$ and HR 3.72, 95% CI 1.71 – 8.08, p 0.01).

Conclusion: Baseline and serial MR-proADM and CT-proET-1 had good ability to predict 28-day mortality in critically ill patients with SARS-CoV-2 pneumonia.

Introduction

Corona virus disease 2019 (COVID-19), the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1,2], has turned out to be an enormous challenge to intensive care units (ICU) worldwide [3-5]. A substantial part of the patients deteriorated quickly and needed to be admitted to the ICU with signs and symptoms consistent with acute respiratory failure and/or acute respiratory distress syndrome (ARDS). Many of those patients had prolonged treatment periods at the ICU and large variations in mortality (26 – 61.5%) were reported [3-5]. Up to 25% of the patients with COVID-19 developed prothrombotic complications, like deep venous thrombosis or pulmonary embolisms [6].

As virus-induced endothelial dysfunction and damage, endotheliitis, has been proposed as one of the potential mechanisms of COVID-19 [7,8], there may be a role for endothelium related adrenomedullin (ADM) and Endothelin-1 (ET-1). The midregion and C-terminal part of these prohormones are more stable [9,10], and therefore measuring the midregion or C-terminal prohormone is more feasible for clinical purposes. Mid-regional proadrenomedullin (MR-proADM) is the midregion of the prohormone of ADM [9]. ADM is a peptide generated by endothelial and vascular smooth muscle cells, with anti-inflammatory effects on vascular endothelial cells, protecting the microcirculation against endothelial permeability in sepsis [11,12]. In lower respiratory tract infections MR-proADM levels were rapidly induced and baseline MR-proADM measurements proved to be a good predictor of short and long-term survival in community-acquired pneumonia (CAP) patients admitted to the emergency room or ICU [13,14]. C-terminal proendothelin-1 (CT-proET-1) is the C-terminal part of the prohormone of ET-1 [10]. ET-1 is a strong vasoconstrictor peptide and pro-inflammatory cytokine that is released from activated endothelial cells [15]. Elevated concentrations of CT-pro-ET-1 were found in patients with CAP and sepsis [14,16,17].

In the present study we aimed to investigate the prognostic value of MR-proADM and CT-proET-1 at baseline to predict 28-day mortality in critically ill patients with confirmed SARS-CoV-2 pneumonia. Secondary aim was testing of these two biomarkers over time in the ICU.

Material and methods

Study design and selection criteria

In a single centre prospective observational cohort study, we enrolled patients with confirmed SARS-CoV-2 pneumonia, admitted to the ICU of the Elisabeth-Tweesteden (ETZ) Hospital (Tilburg, the Netherlands) from March 11 until May 27, 2020. The study protocol was approved by the METC Brabant (Medisch Ethische Toetsingscommissie Brabant) (Tilburg, the Netherlands) (NW 2020-86). Informed consent was achieved from participating patients. Inclusion criteria were adults ≥ 18 years of age, admitted to the ICU with pneumonia and SARS-CoV-2 infection confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay of nasopharyngeal or bronchial swabs. Patients who did not meet the inclusion criteria or without informed consent were excluded. SARS-CoV-2 pneumonia was defined according to the interim guidance of World Health Organization (WHO) for clinical management of COVID-19 [18]. Both severe and critical type diseases defined by the WHO interim guidance were included. Severe disease; severe pneumonia was designated when the patients had clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) plus one of the following symptoms or physiological signs: respiratory rate > 30 breaths/min, severe respiratory distress or SpO_2 < 90% on room air. Chest imaging (radiograph, CT scan or lung ultrasound) may assist in diagnosis and identify or exclude pulmonary complications [18]. Critical disease; ARDS was designated when the symptoms of pneumonia lasted less than one week or when there were new or worsening symptoms, chest imaging showed bilateral ground glass lobar opacities, lobar or lung collapse, or nodules and respiratory failure could not be solely explained by cardiac failure or fluid overload. Additionally, signs of oxygenation impairment (PaO_2/FiO_2 ≤ 300 mmHg with positive end expiratory pressure (PEEP) ≥ 5 cmH_2O or continuous positive airway pressure (CPAP) ≥ 5 cmH_2O) needed to be present [18]. All patients received selective decontamination of the digestive tract. Prophylactic antibiotics were given during the first four days to all patients as part of this decontamination strategy. STROBE (Strengthening the Reporting of Observational Studies in Epidemiology Statement) guidelines for reporting observational studies were followed [19]. Only cases with an index test comprising MR-proADM and CT-pro-ET-1 at baseline were included. Primary outcome measure was the prediction of 28-day mortality by baseline biomarker. Testing of the changes of biomarkers in COVID-19 patients over time in the ICU was the secondary aim of the study.

Procedures

Clinical data, microbiological and laboratory results were collected on a daily basis in patients enrolled in the study. These data were obtained from the accepting hospitals if patients had an early transfer to another ICU. Additional blood samples were collected into EDTA-tubes on a daily basis for seven days, or until discharge or death. Plasma was separated by centrifugation and stored in aliquots at -80 °C. MR-proADM and CT-pro-ET-1 levels were measured using an automated immunofluorescent sandwich assay on a Kryptor Compact Plus analyzer (BRAHMS AG, Henningsdorf, Germany) at the central diagnostic laboratory in Maastricht, the Netherlands. The Kryptor measures the signal that is emitted from an immunocomplex by time-resolved amplified cryptate emission. MR-proADM and CT-proET-1 assays have a limit of detection of 0.05 nmol/L and 2.94 pmol/L. The functional sensitivity (lowest value with an interassay coefficient of variation (CV) < 20% as described by the manufacturer) of 0.25 nmol/L (MR-proADM) and 9.78 pmol/L (CT-proET-1), respectively. Imprecision of the assays were verified according to the Clinical & Laboratory Standards Institute Evaluation Protocol 17-A (CLSI EP17-A), using a low and high sample, measured for five days in triplicate. Intra and Inter CV values were all ≤ 10% for MR-proADM and CT-proET-1.

Statistical analysis

All non-normally distributed data (Kolmogorov-Smirnov test $p < 0.05$) were expressed as median (with interquartile range, IQR) or as number of patients (percentage) where appropriate. Patient characteristics and outcomes were compared using a Mann-Whitney *U*-test for skewed distributed continuous variables and a chi-square test was used to analyse categorical variables. To analyse the time course of MR-proADM and CT-proET-1 profiles in the different patient groups a linear mixed-models analysis for repeated measures was used, including time and 28-day survival as independent factors. Testing for interaction was performed. The association between mortality and each biomarker or severity score at admission was assessed using area under the receiver operating characteristics (ROC) curves. Optimal cut-off points were calculated for each biomarker and severity score. Continuous variables were transformed to dichotomous variables (below or equal and above the cut-off point) and then included in the Cox regression model to study the effects on outcome. Considering the total number of deaths within 28 days in our study (n=30) and to avoid overfitting in the model, MR-proADM and CT-proET-1 were tested in a separate multivariable model with two other variables: age and Sequential Organ failure Assessment (SOFA) score. The cumulative survival was analysed by applying the Kaplan-Meier curves and differences in mortality were compared with the Log Rank test. All tests were two-sided

and a p-value < 0.05 was considered statistically significant. All data were analyzed using a statistical software package (SPSS Inc., version 24, Chicago, IL, USA).

Results

Descriptive characteristics of the patients

A cohort of 133 critically ill patients with a suspected SARS-CoV-2 pneumonia was identified during the study period. In 105 patients, SARS-CoV-2 was confirmed by RT-PCR (in 88 nasopharyngeal swabs and 17 tracheal aspirations), informed consent was achieved and MR-proADM and CT-pro-ET-1 levels were measured at admittance and subsequent days. The patient flow diagram shows the flow of patients along with the primary endpoint of 28-day survival (figure 1). Fifty-five (52.4%) patients were transferred to another ICU due to national government policy in order to distribute COVID-19 patients over the country. The median ICU-time before transfer to another ICU was three days (IQR 2 - 5).

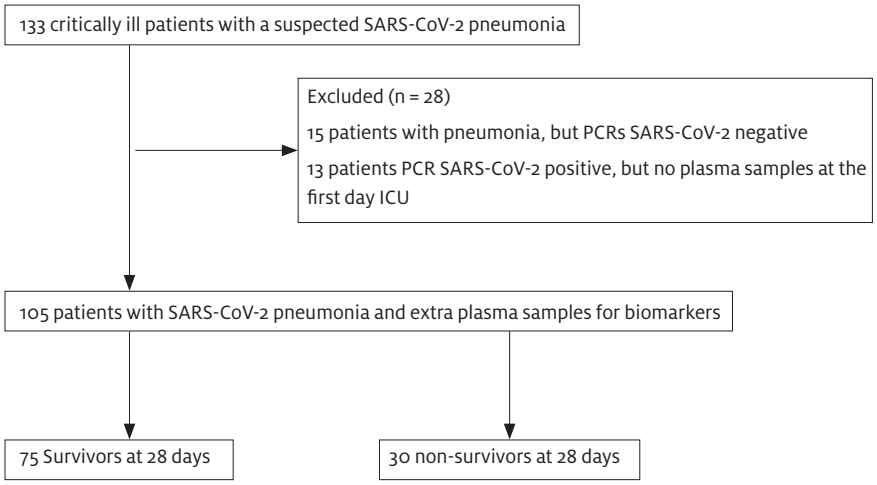

Figure 1 | Patient flow diagram

Legends: Patient flow diagram.

Demographics and clinical characteristics of the 105 included patients are shown in table 1. Twenty-three (22%) of the 105 included patients had severe pneumonia and 82 (78%) fulfilled the Berlin criteria for ARDS [20], with severe ARDS in 19 (18%) patients. There was a low number of coinfections with bacteria, fungi or other viruses (table S1, appendix p 2). *Enterococcus spp* (n = 5) and *S. pneumonia* (n = 3) were most frequently found as bacterial coinfections and *A fumigatus* was found in deep respiratory tract secretions in seven patients.

The 28-day all-cause mortality was 28.6%. Patients were divided in survivors and non-survivors with regards to survival up to 28 days. Both groups were comparable except for older age, higher SOFA score, Acute Physiological and Chronic Health Evaluation IV (APACHE IV) and Confusion blood Urea nitrogen Respiratory rate Blood pressure age 65 or older (CURB-65) score in non-survivors. Duration of invasive mechanical ventilation, Length of stay (LOS) at the ICU and hospital were, due to early death, significant lower in 28-day non-survivors.

Table 1 | Characteristics of patients with SARS-CoV-2 pneumonia with regards to survival up to 28 days

	Total (N = 105)	**Survivors (N = 75)**	**Non-Survivors (N = 30)**	***p* value**
Age (years) (median, IQR)	68 (59 - 74)	65 (58 - 73)	72 (67 - 76)	0.01
Male gender (N, %)	80 (76.2%)	56 (74.7%)	24 (80%)	0.56
BMI (kg/m²) (median, IQR)	28.4 (25.8 - 32.7)	28.3 (25.8 - 32.3)	29.2 (25.5 - 33.3)	0.65
Pre-existing comorbidities (N, %)				
Obesity (BMI ≥ 30 kg/m²)	42 (40%)	29 (38.7%)	13 (43.3%)	0.66
Hypertension	29 (27.9%)	20 (27%)	9 (30%)	0.76
Congestive heart failure	17 (16.2%)	11 (14.7.7%)	6 (20%)	0.50
COPD	16 (15.2%)	12 (16%)	4 (13.3%)	0.73
Diabetes mellitus	24 (22.9%)	17 (22.7%)	7 (23.3%)	0.94
Cerebrovascular disease	7 (6.7%)	3 (4%)	4 (13.3%)	0.08
Malignancy	15 (14.3%)	10 (13.3%)	5 (16.7%)	0.66
Chronic renal disease	5 (4.8%)	2 (2.7%)	3 (10%)	0.11
Auto-immune disorder	8 (7.6%)	7 (9.3%)	1 (3.3%)	0.30
Initial symptoms (N, %)				
Fever (temp > 38.0°C)	79 (75.2%)	54 (72%)	25 (83.3%)	0.22
Cough	98 (93.3%)	71 (94.7%)	27 (90%)	0.39
sputum	24 (22.9%)	14 (18.7%)	10 (33.3%)	0.11
Dyspnea	86 (81.9%)	61 (81.3%)	25 (83.3%)	0.81
Nausea or vomiting	15 (14.3%)	12 (16%)	3 (10%)	0.43
Diarrhoea	19 (18.1%)	16 (21.3%)	3 (10%)	0.17

Myalgia	15 (14.3%)	12 (16%)	4 (10%)	0.43
Time course of illness – days				
Time from illness onset to ICU admission (days) (median, IQR)	8 (7 - 11)	8 (7 - 11)	7 (5 - 12)	0.06
Time from RT-PCR diagnosis to ICU admission (days) (median, IQR)	0 (-2.5 - 1)	-1 (-3 - 0)	0 (0 - 1)	0.01
Severity of illness at baseline				
Sepsis-3, sepsis (N, %)	102 (97.1%)	72 (96%)	30 (100 %)	0.27
Sepsis-3, septic shock (N, %)	11 (10.5%)	6 (8%)	5 (16.7%)	0.19
SOFA (points) (median, IQR)	6 (3 – 7)	5 (3 - 6)	7 (4 - 7)	0.01
APACHE IV (points) (median, IQR)	47 (40 - 59)	43 (35 - 53)	53 (45 - 71)	<0.001
CURB-65 (points) (median, IQR)	2 (1 -2)	1 (0 – 2)	2 (2 – 3)	<0.001
PaO2 /FiO2 ratio, mmHg	163 (119 – 194)	167 (116 – 199)	162 (132 – 177)	0.51
Therapy during ICU (N, %)				
HFNO (only)	7 (6.7%)	5 (6.7%)	2 (6.7%)	1.00
IMV	98 (93.3%)	70 (93.3%)	28 (93.3%)	1.00
Prone position ventilation	58 (55.2%)	41 (54.7%)	17 (56.7%)	0.85
Vasopressor	87 (82.9%)	60 (80%)	27 (90%)	0.22
CRRT	9 (8.6%)	4 (5.3%)	5 (16.7%)	0.06
Anti-COVID-19 treatment				
Chloroquine only	79 (75.2%)	59 (78.7%)	20 (66.7%)	0.20
Chloroquine + Lopinavir/ Ritonavir	26 (24.8%)	16 (21.3%)	10 (33.3%)	0.20
Methylprednisolone	6 (5.7%)	3 (4%)	3 (10%)	0.23
IL-1RA	2 (1.9%)	1 (1.3%)	1 (3.3%)	0.50
Outcome (median, IQR)				
Duration IMV (days)	14 (9 - 26)	19 (10 -30)	10 (3 - 17)	<0.001
ICU LOS (days)	17 (10 -32)	24 (12 - 35)	11 (4 - 18)	<0.001
Hospital LOS (days)	23 (12 - 37)	30 (18 - 44)	12 (6 - 18)	<0.001
Biomarkers at baseline (median, IQR)				
WBC, 10E9/L	8.2 (6.1 - 11.2)	7.9 (6.0 - 11.3)	8.8 (6.5 - 11.2)	0.51
Neutrophil, 10E9/L	5.9 (4.0 – 8.9)	5.9 (3.8 – 8.9)	6.8 (4.2 - 9.2)	0.45
Lymphocyte, 10E9/L	0..7(0.5 - 0.9)	0.8 (0.5 - 1.0)	0.7 (0.4 - 0.9)	0.22
Platelets, 10E9/L	227 (180 - 287)	228 (1749- 278)	223 (178 - 305)	0.94
D-dimer (ng/mL)	1381 (797 – 4080)	1279 (751 – 3405)	2223 (1173 – 11838)	0.06
cTnT (ng/mL)	0.02 (0.01 – 0.03)	0.01 (0.01 – 0.02)	0.03 (0.02 – 0.07)	<0.001
CRP (mg/L)	141 (90 - 207)	141 (91 - 196)	146 (89 - 240)	0.60
PCT (ng/mL)	0.5 (0.2 – 1.1)	0.4 (0.2 – 0.9)	0.9 (0.3 – 2.4)	0.04

Ferritin (mcg/L)	1320 (720 - 2317)	1115 (583 - 1917)	1449 (1047 - 3604)	0.06
Lactate (mmol/L)	1.2 (0.9 – 1.6)	1.1 (0.8 - 1.5)	1.6 (1.1 – 1.9)	0.01
MR-proADM (nmol/L)	1.16 (0.85 – 1.71)	1.01 (0.80 – 1.28)	1.88 (1.35 - 2.64)	<0.001
CT-proET-1 (pmol/L)	93.5 (72.1 – 122.9)	84.2 (66.7 - 101.1)	132.1 (100.7 – 156.2)	<0.001

Legends: All continuous data are presented as median (interquartile range) and categorical data as number (percentage). BMI: body mass index, COPD: Chronic obstructive pulmonary disease, SOFA: Sequential organ failure assessment, APACHE IV: Acute physiology and chronic health evaluation IV, CURB-65: Confusion blood Urea nitrogen Respiratory rate Blood pressure age 65 or older, HFNO: high flow nasal oxygen, IMV: Invasive mechanical ventilation, CRRT: continuous renal replacement therapy, IL-1RA: Recombinant interleukin-1 receptor antagonist, LOS: Length of stay, WBC: White blood cells, cTnT: cardiac Troponin T, CRP: C-reactive protein, PCT: Procalcitonin, MR-proADM: Mid-regional proadrenomedullin, CT-proET-1: C-terminal pro-endothelin-1.

Association between baseline MR-proADM, CT-proET-1 and 28-day mortality

Non-survivors at 28 days had significant higher concentrations MR-proADM and CT-proET-1 at baseline than survivors (table 1). Baseline cardiac Troponin T (cTnT), procalcitonin (PCT) and lactate concentrations were also significant higher in non-survivors. There were no significant differences in other biomarkers at baseline between 28-day survivors and non-survivors (table 1). ROC curves revealed that baseline MR-proADM and CT-proET-1 had high accuracy to identify non-survivors, area under the curve (AUC) 0.84, (95% confidence interval (CI) 0.76 – 0.92), $p < 0.001$ and 0.79, (95% CI 0.69 – 0.89), $p < 0.001$, respectively (table 2). CURB-65 and cTnT had comparable high AUCs in ROC analysis and SOFA, APACHE IV and other biomarkers had lower AUCs (table 2). When MR-proADM or CT-proET-1 at baseline were combined with SOFA, APACHE IV or CURB-65, the combination of MR-proADM and CURB-65 yielded the highest AUC (0.86. 95% CI 0.78 - 0.94, $p < 0.001$) (table S2, appendix p 3) (figure S1, appendix p 6). The combination of baseline MR-proADM and CT-proET-1 did not yield a higher AUC (table S2, appendix p 3). Optimal cut-off points were calculated for each biomarker and sensitivity, specificity, positive and negative predictive values (PPV, NPV) and positive and negative likelihood ratios (LR+, LR-) for each severity score and biomarker were calculated. MR-proADM, CT-proET-1 and cTnT yielded the highest LR+ (table 2). Continuous variables were transformed to dichotomous variables and included in the univariable Cox regression analysis. Univariable Cox regression analysis demonstrated that, MR-proADM ≥ 1.57 nmol/L, CT-proET-1 ≥ 111 pmol/L and cTnT ≥ 0.025 ng/mL had the strongest association with increased risk of 28-day mortality compared to patients with values below the cut-off point (table S3, appendix p 4). MR-proADM and CT-proET-1 were separately included in a multivariable Cox regression model with age and SOFA as co-predictors (table 3). MR-proADM ≥ 1.57 nmol/L and CT-proET-1 ≥ 111 pmol/L were significant predictors for 28-day mortality

with high hazard ratios (HR 6.80, 95% CI 3.12 – 14.84, $p < 0.001$ and HR 3.72, 95% CI 1.71 – 8.08, p 0.01). Patients with baseline MR-proADM and CT-proET-1 equal or above their cut-off point had increased risk of 28-day mortality in Kaplan-Meier analysis compared with the values below the cut-off points (figure S2 a-b, appendix p 7-8).

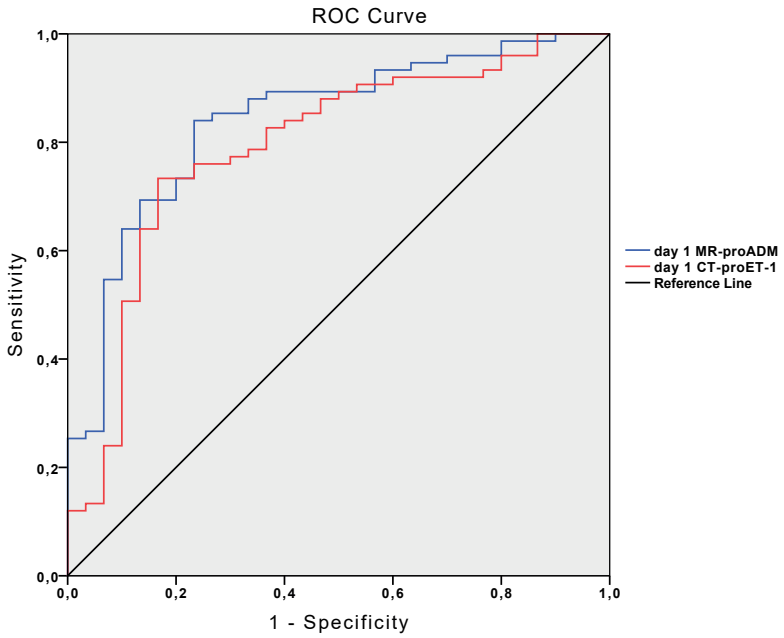

Figure 2 | ROC curve for biomarker at baseline in predicting 28-day mortality

Legends: Blue line = MR-proADM (AUC 0.84, 95% CI 0.76 - 0.92, p < 0.001), red line = CT-proET-1 (AUC 0.79, 95% CI 0.69 - 0.89, p < 0.001), black line = reference line.

Table 2 | Prediction of 28-day mortality by clinical score and biomarker at baseline

	AUC (95% CI)	*p* value	cut-off	sens	spec	PPV	NPV	LR+	LR-
SOFA	0.66 (0.54 - 0.78)	0.01	7	76%	57%	81%	49%	1.77	0.42
APACHE IV	0.73 (0.63 - 0.83)	<0.001	52	71%	53%	79%	43%	1.51	0.55
CURB-65	0.78 (0.68 - 0.87)	<0.001	3	88%	47%	80%	61%	1.57	0.26
D-dimer	0.63 (0.50 - 0.75)	0.06	NA	NA	NA	NA	NA	NA	NA
cTnT	0.76 (0.66 - 0.86)	<0.001	0.025	77%	70%	87%	55%	2.57	0.33
CRP	0.53 (0.41 - 0.66)	0.60	NA	NA	NA	NA	NA	NA	NA
PCT	0.63 (0.51 - 0.75)	0.04	1.0	77%	50%	80%	45%	1.54	0.46
Ferritin	0.64 (0.50 - 0.78)	0.06	NA	NA	NA	NA	NA	NA	NA
Lactate	0.68 (0.57 - 0.79)	0.01	1.5	73%	57%	81%	46%	1.70	0.47
MR-proADM	0.84 (0.76 - 0.92)	<0.001	1.57	88%	67%	87%	69%	2.67	0.18
CT-proET-1	0.79 (0.69 - 0.89)	<0.001	111	83%	63%	85%	59%	2.24	0.27

Legends: AUC: area under the curve, sens: sensitivity, spec: specificity, PPV: positive predictive value, NPV: negative predictive value, LR+: positive likelihood ratio, LR-: negative likelihood ratio, SOFA: Sequential organ failure assessment, APACHE IV: Acute physiology and chronic health evaluation IV, CURB-65: Confusion blood Urea nitrogen Respiratory rate Blood pressure age 65 or older, cTnT: cardiac Troponin T, PCT: Procalcitonin, MR-proADM: Mid-regional proadrenomedullin, CT-proET-1: C-terminal pro-endothelin-1.

Table 3 | Multivariable Cox regression models for the prediction of 28-day mortality with baseline biomarker values

			Multivariable analysis	
	Patients (N)	**Events (N)**	**HR (95% CI)**	***P* value**
Model 1				
Age	105	30	1.07 (1.01 – 1.13)	0.03
SOFA day 1	105	30		
< 7			1.0 (Reference)	
≥ 7			2.36 (1.13 – 4.92)	0.02
MR-proADM day 1	105	30		
< 1.57 nmol/L			1.0 (Reference)	
≥ 1.57 nmol/L			6.80 (3.12 - 14.84)	<0.001
Model 2				
Age	105	30	1.05 (0.99 - 1.11)	0.07
SOFA day 1	105	30		
< 7			1.0 (Reference)	
≥ 7			2.41 (1.16 - 5.01)	0.02
CT-proET-1 day 1	105	30		
< 111 pmol/L			1.0 (Reference)	
≥ 111 pmol/L			3.72 (1.71 - 8.08)	0.01

Legends: HR = hazard ratio, CI = confidence interval, SOFA: Sequential organ failure assessment, MR-proADM: Mid-regional proadrenomedullin, CT-proET-1: C-terminal pro-endothelin-1.

Dynamic changes in MR-proADM and CT-proET-1 during ICU stay

Time dependent analysis of MR-proADM, CT-proET-1, PCT, and C-reactive protein (CRP) were performed. MR-proADM levels were significantly different between non-survivors and survivors, with higher levels MR-proADM in non-survivors (p 0.01). MR-proADM increased faster over time in non-survivors, as demonstrated by a significant time*28-day survival interaction term, (p 0.01) (figure 3a). CT-proET-1 levels were significantly different between non-survivors and survivors, with higher levels in non-survivors ($p < 0.001$). These differences in CT-proET-1 levels between non-survivors and survivors were constant over time, with a non-significant time*28-day survival interaction term (p 0.43) (figure 3b). Moreover, the AUC for prediction of 28-day non-survivors remained high over days for MR-proADM and CT-proET-1 (table S4, appendix p 5). There were no significant differences in PCT and CRP levels between both groups at any of the timepoints (p 0.18 and p 0.64, respectively). Only CRP differed over time (time*28-day survival interaction term p 0.02). (figure 3c and 3d).

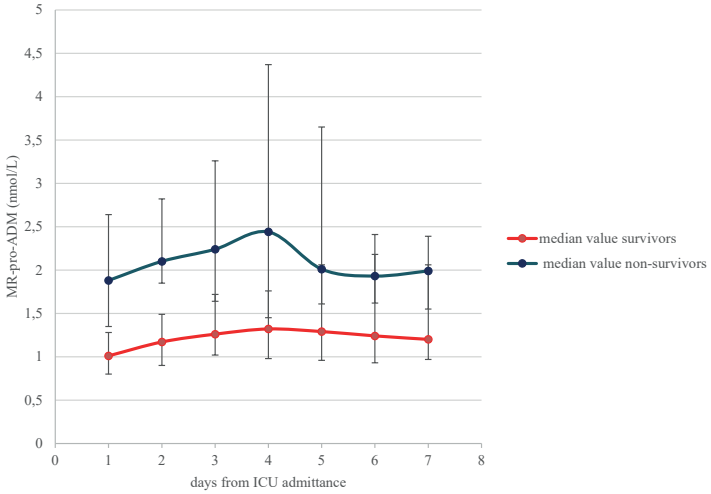

Figure 3 | A
Legends: Temporal changes in MR-proADM. Median values with IQR. Time dependent analysis of MR-proADM was significant different between non-survivors and survivors (*p* 0.01) and significant over time (time*group interaction term, *p* 0.01).

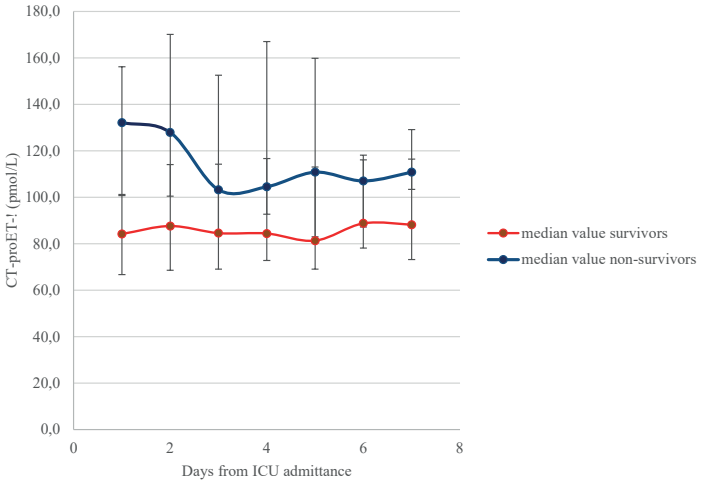

Figure 3 | B
Legends: Temporal changes in CT-proET-1. Median values with IQR. Time dependent analysis of CT-proET-1 was significant different between survivors and non-survivors (*p* < 0.001), but constant over time (time*group interaction term *p* 0.43).

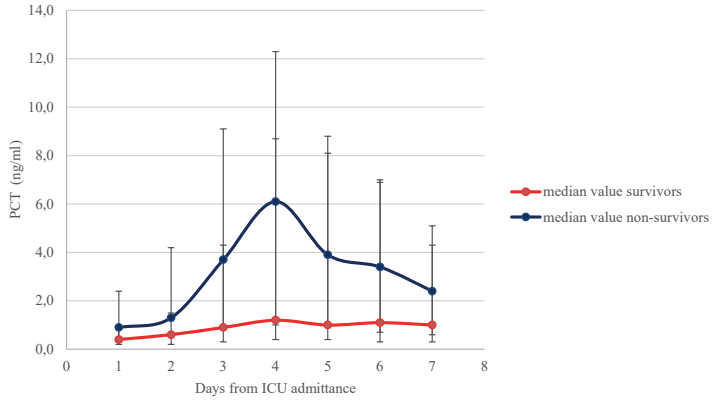

Figure 3 | C
Legends: Temporal changes in PCT. Median values with IQR. Time dependent analysis of PCT was non-significant different between survivors and non-survivors (*p* 0.18) and non-significant over time (*p* 0.46).

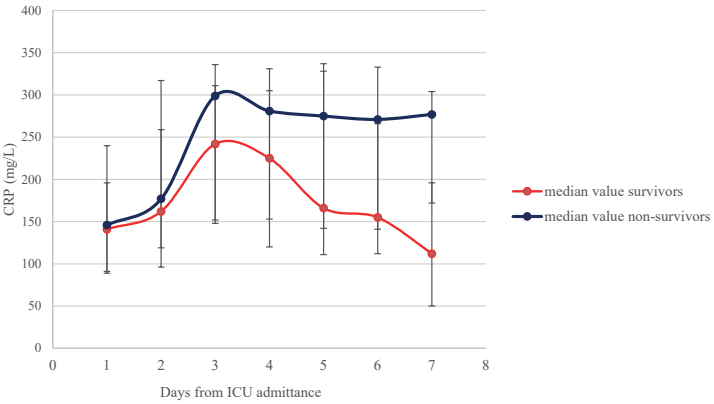

Figure 3 | D
Legends: Temporal changes in CRP.Median values with IQR. Time dependent analysis of CRP was non-significant different between survivors and non-survivors (*p* 0.64), but significant over time (time*group interaction term, *p* 0.02).

Discussion

The primary aim of the study was to investigate the prognostic value of baseline MR-proADM and CT-proET-1 to predict 28-day mortality in a well-described cohort critically ill patients with SARS-CoV-2 pneumonia. Testing of the changes of the two biomarkers over time was the secondary aim. We reported two main findings. First, baseline MR-proADM and CT-proET-1 had high accuracy to identify 28-day non-

survivors in ROC curves and were significant predictors for 28-day mortality with high hazard ratios in a multivariable Cox regression model with age and SOFA score. A higher mortality characterized patients presenting with MR-proADM and CT-proET-1 equal or exceeding their cut-off points (1.57 nmol/L and 111 pmol/L, respectively). The prognostic accuracy of baseline MR-proADM and CT-proET-1 to identify 28-day non-survivors was higher compared with most commonly measured laboratory parameters and APACHE IV and SOFA severity scores. Secondly, these significant higher levels of MR-proADM and CT-proET-1 in non-survivors persisted over time. Whereby MR-proADM increased faster in non-survivors. The differences in CT-proET-1 levels between non-survivors and survivors were constant over time. Moreover, the AUC for the prediction of 28-day mortality remained high for both MR-proADM and CT-proET-1 over time. Finding a biomarker able to identify patients with worst outcome in the ICU emerged as a priority during the COVID-19 pandemic. Both MR-proADM and CT-proET-1 appeared to be biomarkers with strong prognostic values. It might be argued that patients with highest values would benefit most from anti-inflammatory therapies such as steroids and interleukin-6 receptor antagonists.

Laboratory abnormalities are frequently reported in COVID-19 patients. Lymphocytopenia, increased values of CRP and D-dimer were most frequent predictive of adverse outcome [21]. We could not report similar results for these biomarkers in our study. Our findings of good ability of MR-proADM to predict 28-day mortality are in line with several studies. MR-proADM was frequently studied in patients with non-COVID-19 respiratory infections [13,14,22]. However, most of these patients were not admitted to the ICU [13,14,22]. Baseline MR-proADM was studied in an observational cohort study of 728 CAP patients admitted to the emergency department (ED) [14]. MR-proADM had the best performance for prediction of 28-day mortality (HR 3.67 and AUC 0.85). In another observational cohort study of 1175 ED patients MR-proADM was able to identify patients requiring rapid administration of antibiotics or triage to the ICU [22]. Baseline MR-proADM performed well in predicting 28-day mortality in comparison with APACHE II and Simplified Acute Physiology Score II (SAPSII) when all patients admitted to the ICU were included [23]. The value of MR-proADM as prognostic marker in adult patients hospitalized with SARS-CoV-2 infection was studied in several observational studies [24-29]. Increased levels of MR-proADM were independently associated with mortality [24-29]. However, not all patients were admitted to the ICU [24,25,28,29]. CT-proET-1 was studied in non-COVID-19 patients admitted to the ED with CAP [14,16]. CT-proET-1 correlated with disease severity of CAP and was an independent predictor of mortality in both studies with CAP patients [14,16]. CT-proET-1 was studied in in critically ill patients with sepsis admitted to the ICU [17]. CT-proET-1 levels > 74 pmol/L at ICU admission independently predicted ICU death [17]. Our search did not reveal studies with CT-proET-1 as marker in patients with SARS-CoV-2 infection.

Evidence of direct viral infection of the endothelial cell and diffuse endothelial inflammation was found in post-mortem analysis of COVID-19 patients [8]. These findings suggested that SARS-CoV-2 infection enabled the induction of endothelial dysfunction and damage, endotheliitis [8]. ADM is a peptide hormone, produced by endothelial and vascular smooth muscle cells due to pro-inflammatory cytokines, bacterial toxins, hypoxia or volume overload. ADM binds to receptors in especially cardiovascular and pulmonary tissues and has anti-inflammatory effects on vascular endothelial cells, stabilizing the endothelial barrier function and protects the microcirculation against permeability in sepsis [11,12,30]. Besides its action on the endothelium ADM has important effects on the vascular system, ADM reduces vasoconstriction through inhibition of the renin-angiotensin-aldosterone system [11,30]. ET-1 is released from activated endothelial cells. It is a strong vasoconstrictor peptide and pro-inflammatory cytokine [15]. Besides blood vessels, ET-1 receptors are also found in other tissues, with the highest levels in the lungs [15]. ET-1 release is stimulated by bacterial toxins and inflammatory cytokines such as tumor necrosis factor alpha (TNF-alpha) or interleukin-6 [17]. Both MR-proADM and CT-proET-1 are the more stable midregion and C-terminal part of the prohormones that correlate with the release of the active peptides [9,10]. Renal failure could be another reason for elevated levels MR-proADM and CT-proET-1, most probably due to inappropriate renal clearance [31]. A small portion of the patients had chronic renal disease as comorbidity, and only two patients were on renal replacement therapy when biomarkers were collected. MR-proADM and CT-proET-1 levels could decrease faster during renal replacement therapy [31].

Some limitations of our study need to be addressed. First, we did a prospective observational study in a cohort critically ill patients with SARS-CoV-2 pneumonia from March 11 until May 27, 2020, which was the first period of the COVID-19 pandemic in the Netherlands. The treatment of COVID-19 has changed during the last year. Treatment with Chloroquine and Lopinavir/Ritonavir is obsolete and none of these patients were treated with dexamethasone during the first 10 days of hospitalization or interleukin-6 receptor antagonists. There may be differences in 28-day mortality due to changes in COVID-19 treatment. We must rely on older data of clinical practice leading to potential observational bias. Second, plasma samples could not be collected for seven days in all patients due to early ICU discharge, transfer to another ICU or early death. Incomplete longitudinal biomarker data might result in withdrawal bias. Clinical and microbiological data were obtained from the accepting hospitals if patients had an early transfer to another ICU. We may have lost some data by this method of collecting. Third, asymmetry in numbers survivors and non-survivors could influence the statistical significance between both groups. Fourth, although we have lost only a small number (28/133) of the total eligible patients during the study period

by selecting only patients with SARS-CoV-2 infection confirmed by RT-PCR, with index tests at the first day and informed consent we may have introduced selection bias. Both observational, withdrawal and selection bias may have led to potential underestimation of the prognostic performance of MR-proADM and CT-proET-1.

A biomarker, whether measured as single baseline value or measured serially, will always oversimplify the interpretation of important clinical and other laboratory variables and therefore, MR-proADM and CT-proET-1 are meant, rather than to replace, to complement clinician's judgement.

A strong feature of our analysis is that it is a real-life study performed on a mixed ICU in the Netherlands with patients with SARS-CoV-2 pneumonia, reflecting routine clinical ICU practice during the first period of the COVID-19 pandemic.

Conclusions

Baseline MR-proADM and CT-proET-1 had good ability to predict 28-day mortality in critically ill patients with SARS-CoV-2 pneumonia. Moreover, MR-proADM and CT-proET-1 appeared to be biomarkers with persistent strong prognostic value in the following days. MR-proADM and CT-proET-1 may help clinicians to identify patients at higher risk of adverse outcome and improve the decisions about ICU treatment.

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Appendix

Supplemental Table 1 | Secondary infections

	Total
Positive blood cultures (N, %)	10 (9.5%)
Positive sputum cultures (N, %)	3 (2.9%)
Positive pleural fluid cultures (N, %)	1 (1%)
Positive urine antigen testing (N, %)	2 (1.9%)
Gram-positive bacteria	
S. pneumoniae	3 (2.9%)
S. aureus	1 (1%)
Enterococcus spp.	5 (4.8%)
Gram-negative bacteria	
Legionella spp.	1 (1%)
Enterobacter spp.	1 (1%)
B. fragilis	1 (1%)
P. aeroginosa	2 (1.9%)
S. maltophilia	1 (1%)
Fungi	
A. fumigatus	7 (6.7%)
Viruses	
Rhinovirus	2 (1.9%)

Supplemental Table 2 | Combination severity score and biomarker at baseline

	Number of patients	**Number of events**	**AUC (95% CI)**	***p* value**
SOFA + MR-proADM	105	30	0.85 (0.78 - 0.93)	<0.001
APACHE IV + MR-proADM	102	30	0.84 (0.76 - 0.93)	<0.001
CURB-65 + MR-proADM	104	30	0.86 (0.78 - 0.94)	<0.001
SOFA + CT-proET-1	105	30	0.80 (0.71 - 0.90)	<0.001
APACHE IV + CT-proET-1	102	30	0.79 (0.70 - 0.89)	<0.001
CURB-65 + CT-proET-1	104	30	0.84 (0.76 - 0.87)	<0.001
MR-proADM + CT-proET-1	105	30	0.84 (0.75 - 0.93)	<0.001

Legends: AUC = Area under the curve, CI = Confidence interval, SOFA: Sequential organ failure assessment, APACHE IV: Acute physiology and chronic health evaluation IV, CURB-65: Confusion blood Urea nitrogen Respiratory rate Blood pressure age 65 or older, MR-proADM: Mid-regional proadrenomedullin, CT-proET-1: C-terminal pro-endothelin-1.

Supplemental Table 3 | Univariable Cox regression analysis for the prediction of 28-day mortality with baseline biomarker values and severity scores

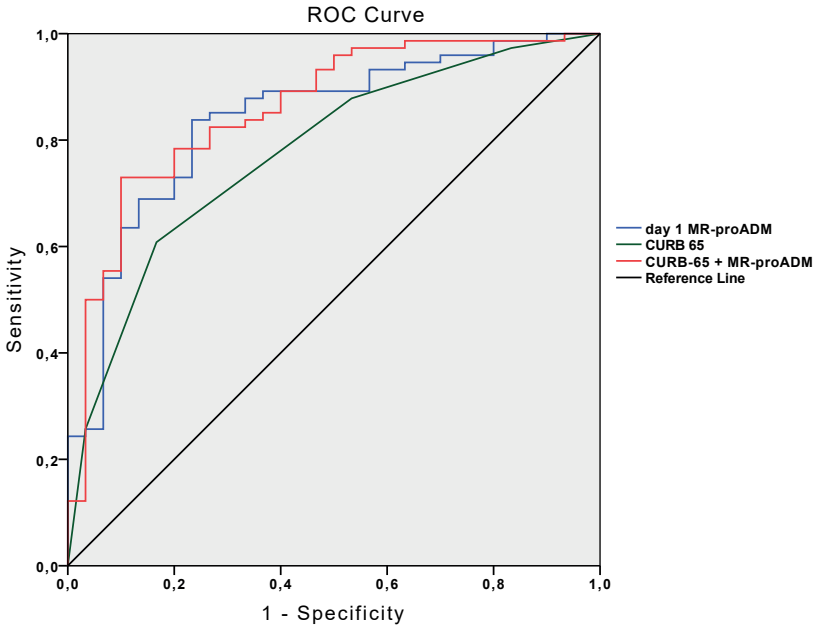
			Univariable analysis	
	Patients (N)	**Events (N)**	**HR (95% CI)**	***P* value**
Age	105	30	1.07 (1.02 – 1.12)	0.01
SOFA day 1	105	30		
< 7			1.0 (Reference)	
≥ 7			3.18 (1.54 – 6.57)	0.01
APACHE IV	102	30		
< 52			1.0 (Reference)	
≥ 52			2.41 (1.17 – 4.94)	0.02
CURB-65	104	30		
< 3			1.0 (Reference)	
≥ 3			3.78 (1.84 - 7.76)	<0.001
cTnT day 1	105	30		
< 0.025 ng/mL			1.0 (Reference)	
≥ 0.025 ng/mL			5.52 (2.52 – 12.08)	<0.001
PCT day 1	105	30		
< 1.0 ng/ml			1.0 (Reference)	
≥ 1.0 ng.ml			2.70 (1.32 - 5.53)	0.01
Lactate day 1	104	30		
< 1.5 mmol/L			1.0 (Reference)	
≥ 1.5 mmol/L			2.71 (1.31 - 5.59)	0.01
MR-proADM day 1	105	30		
< 1.57 nmol/L			1.0 (Reference)	
≥ 1.57 nmol/L			8.72 (4.04 - 18.80)	<0.001
CT-proET-1 day 1	105	30		
< 111 pmol/L			1.0 (Reference)	
≥ 111 pmol/L			3.32 (1.62 - 6.83)	<0.001

Legends: HR = hazard ratio, CI = confidence interval, SOFA: Sequential organ failure assessment, APACHE IV: Acute physiology and chronic health evaluation IV, CURB-65: Confusion blood Urea nitrogen Respiratory rate Blood pressure age 65 or older, cTnT: cardiac Troponin T, PCT: Procalcitonin, MR-proADM: Mid-regional proadrenomedullin, CT-proET-1: C-terminal pro-endothelin-1.

Supplemental Table 4 | Prediction of 28-day mortality by biomarker over time

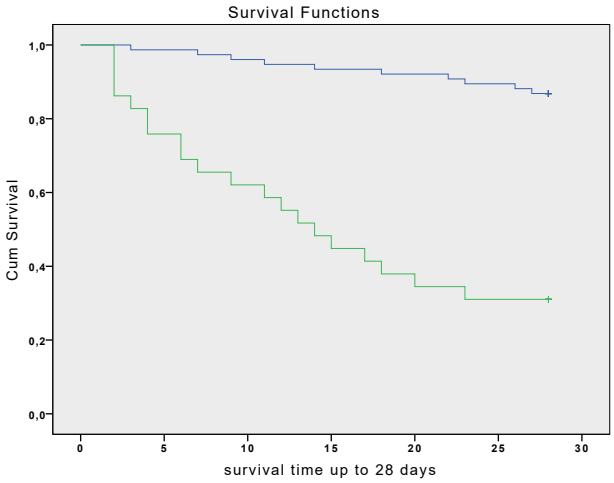
MR-proADM				
days	**Patients (N)**	**Events (N)**	**AUC (95% CI)**	***P* value**
1	105	30	0.84 (0.76 - 0.93)	<0.001
2	82	22	0.86 (0.77 - 0.95)	<0.001
3	65	15	0.84 (0.73 - 0.94)	<0.001
4	61	15	0.81 (0.69 - 0.93)	<0.001
5	51	11	0.78 (0.65 - 0.91)	0.01
6	43	8	0.72 (0.57 - 0.88)	0.05
7	44	9	0.75 (0.64 - 0.90)	0.02
CT-proET-1				
days	**Patients (N)**	**Events (N)**	**AUC (95% CI)**	***P* value**
1	105	30	0.79 (0.69 - 0.89)	<0.001
2	82	22	0.75 (0.63 - 0.87)	0.01
3	65	15	0.70 (0.57 - 0.84)	0.02
4	61	15	0.72 (0.58 - 0.86)	0.01
5	51	11	0.72 (0.56 - 0.88)	0.03
6	43	8	0.63 (0.44 - 0.82)	0.26
7	44	9	0.74 (0.58 - 0.89)	0.03

Legends: AUC: Area under the curve, MR-proADM: Mid-regional proadrenomedullin, CT-proET-1: C-terminal pro-endothelin-1.



Supplemental Figure 1 | Receiver operating characteristics curve for day 1 MR-proADM in predicting 28-day mortality.

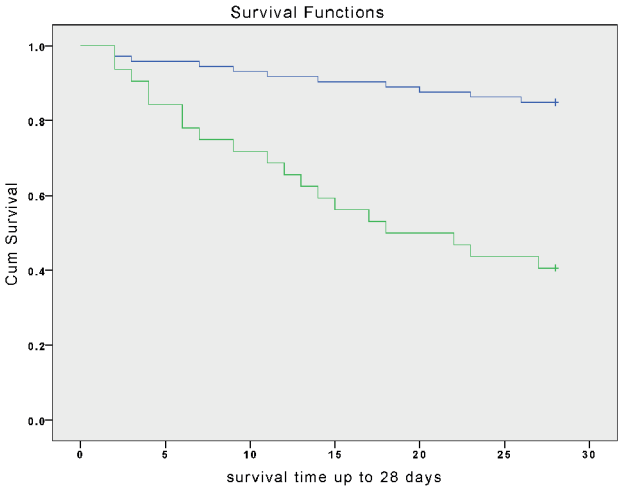
References: Blue line = MR-proADM day 1 (AUC 0.84, 95% CI 0.76 - 0.92, *p*<0.001), green line = CURB-65 (AUC 0.78, 95% CI 0.68 - 0.87, *p*<0.001), red line = CURB-65 + MR-proADM day 1 (AUC 0.86, 95% CI 0.78 - 0.94, *p*<0.001), black line = reference line.



Supplemental Figure 2a

Number at risk				
MR-proADM < 1.57 nmol/L	76	73	70	66
MR-proADM ≥ 1.57 nmol/L	29	18	10	9

Legends: Kaplan Meier 28-day survival curve for optimal MR-proADM cut-off of 1.57 nmol/L at baseline (Blue line = MR-proADM < 1.57 nmol/L, green line ≥ 1.57 nmol/L, Log Rank $p < 0.001$).



Supplemental Figure 2b

Number at risk				
CT-proET-1 < 111 pmol/L	73	68	64	62
CT-proET-1 ≥ 111 pmol/L	32	23	16	13

Legends: Kaplan Meier 28-day survival curve for optimal CT-proET-1 cut-off of 111 pmol/L at baseline
(Blue line = CT-proET-1 cut-off < 111 pmol/L, green line ≥ 111 pmol/L, Log Rank $p < 0.001$).

Response to: MR-proADM has a good ability to predict mortality in critically ill patients with SARS-CoV-2 pneumonia: Beware of some potential confounders!

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J Crit Care. 2022; 67:214-215. doi: 10.1016/j.jcrc.2021.10.004.

To the editor

We appreciate the comments of professor Honore and colleagues concerning the potential confounding effect of continuous renal replacement therapy (CRRT) on the validity of mid-regional proadrenomedullin (MR-proADM) measurements in severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2) pneumonia patients. As MR-proADM can be removed by CRRT and non-survivors were treated with CRRT three times more compared with survivors, the letter authors propose that CRRT could have artificially lowered MR-proADM levels and introduced confounding.

We note that professor Honore and colleagues have reversed the key results in their letter: Our study showed MR-proADM to be lower rather than higher in survivors (1.01 vs. 1.88 in survivors vs. non-survivors at baseline). The true MR-proADM difference between survivors and non-survivors would therefore be larger rather than smaller.

Nevertheless, the authors make an interesting point and we ourselves have mentioned that MR-proADM levels could decrease faster during CRRT [1]. Nine out of 105 patients (8.6%) received CRRT during ICU stay and it was used three times more (16.7% vs 5.3%) in the non-survivor group. However, biomarkers were collected during the first seven days and only two patients were on CRRT when biomarkers were collected [1]. When we excluded these two patients the difference in baseline MR-proADM between survivors and non survivors did not change (median value 1.01 nmol/L (IQR 0.80 - 1.28) vs. median value 1.88 nmol/L (IQR 1.29 - 2.61), $p < 0.001$).

In all, we are confident that this specific biasing mechanism did not artificially inflate the difference between survivors and non-survivors. Even so, we need to account for other potential biases. By performing a single-centre prospective observational study with a limited number of patients we may have introduced selection, collider, and observational bias, which may have led to underestimation of the prognostic performance of MR-proADM. We therefore plan to perform a larger multi-centre study in the near future.

References

1 van Oers JAH, Kluiters Y, Bons JAP, de Jongh M, Pouwels S, Ramnarain D, et al. Endothelium-associated biomarkers mid-regional proadrenomedullin and C-terminal proendothelin-1 have good ability to predict 28-day mortality in critically ill patients with SARS-CoV-2 pneumonia: A prospective cohort study. J Crit Care. 2021; 66:173-89.

CHAPTER 6

Mid-regional proadrenomedullin, C-terminal proendothelin-1 values, and disease course are not different in critically ill SARS-CoV-2 pneumonia patients with obesity

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Int J Obes. 2022; 46:1801-7. doi: 10.1038/s41366-022-01184-2.

Abstract

Background/objectives: Patients affected by obesity and Corona virus disease 2019 (COVID-19), the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), appear to have a higher risk for intensive care (ICU) admission. A state of low-grade chronic inflammation in obesity has been suggested as one of the underlying mechanisms. We investigated whether obesity is associated with differences in new inflammatory biomarkers mid-regional proadrenomedullin (MR-proADM), C-terminal proendothelin-1 (CT-proET-1), and clinical outcome in critically ill patients with SARS-CoV-2 pneumonia.

Subjects/methods: Hundred-five critically ill patients with SARS-CoV-2 pneumonia were divided in patients with obesity (body mass index (BMI) $\geq$ 30 kg/m^2, n = 42) and patients without obesity (BMI < 30 kg/m^2, n = 63) and studied in a retrospective observational cohort study. MR-proADM, CT-proET-1 concentrations and conventional markers white blood count (WBC), C-reactive protein (CRP), procalcitonin (PCT) were collected during the first seven days.

Results: BMI was 33.5 (32 - 36.1) and 26.2 (24.7 - 27.8) kg/m^2 in the group with and without obesity. There were no significant differences in concentrations MR-proADM, CT-proET-1, WBC, CRP, and PCT at baseline and the next six days between patients with and without obesity. Only MR-proADM changed significantly over time (p 0.039). Also, BMI did not correlate with inflammatory biomarkers (MR-proADM rho = 0.150, p 0.125, CT-proET-1 rho = 0.179, p 0.067, WBC rho = -0.044, p 0.654, CRP rho = 0.057, p 0.564, PCT rho = 0.022, p 0.842). Finally, no significant differences in time on a ventilator, ICU length of stay and 28-day mortality between patients with or without obesity were observed.

Conclusions: In critically ill patients with confirmed SARS-CoV-2 pneumonia, obesity was not associated with differences in MR-proADM, and CT-proET-1, or impaired outcome.

Introduction

Corona virus disease 2019 (COVID-19), the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1,2], has turned out to be an enormous challenge to intensive care units (ICU) worldwide [3-5]. A substantial part of the patients deteriorated quickly and needed to be admitted to the ICU with signs and symptoms consistent with acute respiratory failure and/or acute respiratory distress syndrome (ARDS). Age, male sex, hypertension, type 2 diabetes and coronary artery disease were reported as risk factors for ICU admission in COVID-19 patients [1-5]. Also, the prevalence of obesity was high among COVID-19 patients admitted to the ICU [5,6]. Moreover, the need for invasive mechanical ventilation was higher in COVID-19 patients with obesity admitted to the ICU [7,8].

The potential pathophysiological mechanisms between SARS-CoV-2 infection and obesity are not yet fully understood. SARS-CoV-2 virus infection of the host, using the angiotensin-converting enzyme 2 (ACE2) receptor, is one of the proposed mechanisms in COVID-19 [9]. The ACE2 receptors are highly expressed by adipocytes of patients affected by obesity and type 2 diabetes [10]. The adipose tissue may serve as a reservoir for the virus and excrete the virus over a long period, resulting in a continuous augmented inflammatory response [10].

Cytokines were measured to observe the inflammatory response in a prospective observational cohort study with 67 COVID-19 ICU patients [11]. There were no differences in cytokine response between ICU patients with and without obesity. But, as cytokines have a short half-life and can therefore only be measured during a short period, there may be a role for more stable biomarkers as measure of inflammation. There were no differences in conventional inflammatory biomarkers white blood count (WBC), C-reactive protein (CRP) and procalcitonin (PCT) in this observational study [11]. As virus-induced endothelial dysfunction and damage, endotheliitis, has been proposed as one of the potential mechanisms of COVID-19 [12,13], there may be a role for endothelium related inflammatory biomarkers Mid-regional proadrenomedullin (MR-proADM) and C-terminal proendothelin-1 (CT-proET-1). MR-proADM and CT-proET-1 are the precursor fragments of the prohormones of adrenomedullin (ADM) and endothelin-1. (ET-1) [14,15]. The precursor fragments are more stable and therefore measuring the fragments are more feasible for clinical purposes. ADM is a peptide generated by endothelial and vascular smooth muscle cells, with anti-inflammatory effects on vascular endothelial cells, protecting the microcirculation against endothelial permeability in sepsis [16,17]. ADM is also an adipokine, a pro-inflammatory cytokine released by adipose tissue [18]. ET-1 is a strong vasoconstrictor peptide and pro-inflammatory cytokine that is released from activated endothelial cells [19]. Patients

with obesity showed increased vascular expression of ET-1 and vasoconstriction activity [20,21].

The primary aim of the present study was to investigate whether obesity was associated with differences in endothelium and obesity related inflammatory biomarkers MR-proADM and CT-proET-1 in critically ill patients with SARS-CoV-2 pneumonia. Secondary aim was the association between obesity and clinical outcome (time on a ventilator, ICU length of stay (LOS) and 28-day mortality).

Materials/Subjects and Methods

Study design and selection criteria

This single centre observational cohort study is a secondary analysis of a prospective observational biomarker study [22], in which patients were enrolled with confirmed SARS-CoV-2 pneumonia, admitted to the ICU of the Elisabeh-Tweesteden Hospital (Tilburg Netherlands) from March 11 until May 27, 2020. All clinical data, microbiological and laboratory results, including MR-pro-ADM and CT-proET-1, were prospectively collected during this period. The study protocol was approved by the METC Brabant (Medisch Ethische Toetsingscommissie Brabant) (Tilburg, Netherlands) (NW 2020-86). Informed consent was received from all participating patients. Inclusion criteria were adults ≥ 18 years of age, admitted to the ICU with pneumonia and SARS-CoV-2 infection confirmed by real-time reverse transcriptase-polymerase chain reaction (RT-PCR) assay of nasopharyngeal or bronchial swabs. Patients who did not meet the inclusion criteria or without informed consent were excluded. Both severe and critical type diseases defined by the World health Organization (WHO) interim guidance were included. Severe disease; severe pneumonia was designated when the patients had clinical signs of pneumonia (fever, cough, dyspnea, fast breathing) plus one of the following symptoms or physiological signs: respiratory rate > 30 breaths/min, severe respiratory distress or SpO_2 < 90% on room air. Chest imaging (radiograph, CT scan or lung ultrasound) may assist in diagnosis and identify or exclude pulmonary complications [23]. Critical disease; ARDS was designated when the symptoms of pneumonia lasted less than one week or when there were new or worsening symptoms, chest imaging showed bilateral ground glass lobar opacities, lobar or lung collapse, or nodules and respiratory failure could not be solely explained by cardiac failure or fluid overload. Additionally, signs of oxygenation impairment ($PaO_2/FiO_2 \leq 300$ mmHg with positive end expiratory pressure (PEEP) ≥ 5 cmH2O or continuous positive airway pressure (CPAP) ≥ 5 cmH_2O) needed to be present [23]. The included patients were divided in two categories; 1) patients with obesity (body mass index (BMI) ≥ 30 kg/m²)

and 2) without obesity (BMI < 30 kg/m²) according to the classification by the WHO [24]. All patients received selective decontamination of the digestive tract, as described in previous publications [25]. Prophylactic antibiotics were given during the first four days to all patients as part of this decontamination strategy. STROBE (Strengthening the Reporting of Observational Studies in Epidemiology Statement) guidelines for reporting observational studies were followed [26].

Procedures

For clinical purposes serial values of conventional biomarkers WBC, CRP and PCT were collected on a daily basis in patients enrolled in the study. Additional blood samples were collected into EDTA-tubes on a daily basis for seven days, or until discharge or death. Plasma was separated by centrifugation and stored in aliquots at -80 °C. MR-proADM and CT-pro-ET-1 concentrations were measured using an automated immunofluorescent sandwich assay on a B.R.A.H.M.S. Kryptor Compact Plus analyzer (Thermo Fisher Scientific, Henningsdorf, Germany) at the central diagnostic laboratory in Maastricht, the Netherlands. The Kryptor measures the signal that is emitted from an immunocomplex by time-resolved amplified cryptate emission. MR-proADM and CT-proET-1 assays have a limit of detection of 0.05 nmol/L and 2.94 pmol/L. The functional sensitivity (lowest value with an interassay coefficient of variation (CV) < 20% as described by the manufacturer) of 0.25 nmol/L (MR-proADM) and 9.78 pmol/L (CT-proET-1), respectively. Imprecision of the assays were verified according to the Clinical & Laboratory Standards Institute Evaluation Protocol 17-A (CLSI EP17-A), using a low and high sample, measured for five days in triplicate. Intra and Inter CV values were all ≤ 10% for MR-proADM and CT-proET-1.

Statistical analysis

All non-normally distributed data (Kolmogorov-Smirnov test $p < 0.05$) will be expressed as median (with interquartile range, IQR) or as number of patients (percentage) where appropriate. Patient characteristics and outcomes will be compared using a Mann-Whitney *U*-test for continuous variables and chi-square test for categorical variables. To analyze the time course of biomarker profiles a linear mixed model for repeated measures with time and obesity as independent factors was used. Testing for interaction was performed. Individual BMI values were correlated with biomarker concentrations. The time on a ventilator, length of stay intensive care and cumulative survival will be analyzed by applying the Kaplan-Meier curves and differences will be compared with the log-rank test. Patients who died in hospital or those who were still in the ICU and/or receiving mechanical ventilation on day 28 were censored at day 29 for the analysis of time on mechanical ventilation and ICU LOS. Patients who

were discharged alive from the hospital or were still in the ICU or hospital on day 28 were censored at day 29 for the mortality analysis. We assessed the proportional hazards assumption in time on ventilator, LOS ICU and 28-day survival analysis using Schoenfeld residuals. The proportional hazards assumption was not violated (all *p* values > 0.30). All tests are two-sided and a *p*-value < 0.05 was considered statistically significant. All data are analyzed using a statistical software package (SPSS Inc., version 24, Chicago, IL, USA).

Results

Characteristics of the patients

We selected a cohort of 133 critically ill patients with a suspected SARS-CoV-2 pneumonia during the study period. In 105 patients, SARS-CoV-2 was confirmed by RT-PCR, informed consent was achieved and MR-proADM, CT-proET-1, WBC, CRP, and PCT concentrations were measured at day 1 and subsequent days. The patient flow diagram shows the flow of patients with and without obesity along with the endpoint of 28-day survival (Supplemental Fig. 1). Fifty-five (52.4%) patients were transferred to another ICU to distribute COVID-19 patients equally over the country. Median ICU-time before transfer to another ICU was 3 days (IQR 2 - 5).

Demographics and clinical characteristics of the 105 selected patients are shown in table 1. Twenty-three (22%) patients had severe disease and 82 (78%) had critical disease, i.e. ARDS, 12 (11%) had mild ARDS, 51 (49%) moderate ARDS and 19 (18%) severe ARDS according to the Berlin definition [27]. There was a low number of bacterial and fungal coinfections. Gram-positive bacterial microorganisms were found in nine patients (*Enterococcus spp* n = 5, *S. pneumonia* n = 3, *S. aureus* n = 1). Gram-negative bacterial microorganisms were found in six patients (*P. aeruginosa* n = 2, *Legionella spp*. n =. 1, *Enterobacter spp*. n = 1, *B. fragilis* n = 1, *S. maltophilia* n = 1). *A. fumigatus* was found in deep respiratory tract secretions in seven patients.

Patients were divided in two categories: patients with obesity, n = 42, of which 26.6% with class I obesity (BMI 30 - 34.99), 8.6% with class II (BMI 35 - 39.99), and 4.8% with class III obesity (BMI ≥ 40) [24] and patients without obesity, n = 63, 19% with normal weight (BMI 18.50 - 24.99), and 41% overweight (BMI 25 - 29.99) [24] (Supplemental Fig. 2). Both groups were comparable except for age, BMI.

Table 1 | Characteristics of patients with SARS-CoV-2 pneumonia at ICU admissiona

	Total	Non-obese BMI < 30 kg/m²	Obese BMI ≥ 30 kg/m²	*p* value
	(N = 105)	(N = 63)	(N = 42)	
Age (years) (median, IQR)	68 (59 - 74)	71 (62 - 75)	64 (55 - 70)	0.002
Male (N, %)	80 (76.2%)	50 (79.4%)	30 (71.4%)	0.562
BMI (kg/m2) (median, IQR)	28.4 (25.8 - 32.7)	26.2 (24.7 – 27.8)	33.5 (32 - 36.1)	<0.001
Pre-existing comorbidities (N, %)				
Hypertension	29 (27.9%)	17 (27.4%)	12 (28.6%)	0.898
Congestive heart failure	17 (16.2%)	8 (12.7%)	9 (21.4%)	0.234
COPD	16 (15.2%)	9 (14.3%)	7 (16.7%)	0.739
Diabetes mellitus	24 (22.9%)	13 (20.6%)	11 (26.2%)	0.507
Cerebrovascular disease	7 (6.7%)	5 (7.9%)	2 (4.8%)	0.523
Malignancy	15 (14.3%)	11 (17.5%)	4 (9.5%)	0.255
Chronic renal disease	5 (4.8%)	1 (1.6%)	4 (9.5%)	0.061
Initial symptoms (N, %)				
Fever (temp > 38.0°C)	79 (75.2%)	49 (77.8%)	30 (71.4%)	0.460
Cough	98 (93.3%)	59 (93.7%)	39 (92.9%)	0.873
Sputum production	24 (22.9%)	15 (23.8%)	9 (21.4%)	0.776
Dyspnea	86 (81.9%)	53 (84.1%)	33 (78.6%)	0.469
Nausea or vomiting	15 (14.3%)	8 (12.7%)	7 (16.7%)	0.569
Diarrhoea	19 (18.1%)	11 (17.5 %)	8 (19%)	0.836
Myalgia	15 (14.3%)	6 (9.5%)	9 (21.4%)	0.088
Severity of illness at baseline				
Sepsis-3, sepsis (N, %)	102 (97.1%)	62 (98.4%)	40 (95.2 %)	0.399
Sepsis-3, septic shock (N, %)	11 (10.5%)	6 (9.5%)	5 (11.9%)	0.696
SOFA (points) (median, IQR)	6 (3 – 7)	6 (4 - 7)	5 (3 - 7)	0.411
APACHE IV (points) (median, IQR)	47 (40 - 59)	50 (40 - 61)	44 (40 - 52)	0.301
Therapy during ICU (N, %)				
HFNO (only)	7 (6.7%)	4 (6.3%)	3 (7.1%)	0.873
IMV	98 (93.3%)	59 (93.7%)	39 (92.9%)	0.873
Vasopressor	87 (82.9%)	60 (80%)	27 (90%)	0.219
CRRT	9 (8.6%)	3 (4.8%)	6 (14.3%)	0.088
Anti-COVID-19 treatment				
Chloroquine only	79 (75.2%)	49 (77.8%)	30 (71.4%)	0.460
Chloroquine + Lopinavir / Ritonavir	26 (24.8%)	14 (22.2%)	12 (28.6%)	0.460
Methylprednisolone	6 (5.7%)	1 (1.6%)	5 (11.9%)	0.026
IL-1RA	2 (1.9%)	0	2 (4.8%)	0.080

Outcome (median, IQR)				
Duration IMV [b]	15 (9 - 26)	18 (9 -26)	14 (9 - 27)	0.992
ICU LOS (days) [b]	17 (10 -32)	20 (10 - 32)	14 (10 - 33)	0.671
28-day mortality (N, %) [b]	30 (28.6%)	17 (27%)	13 (31%)	0.570
Biomarkers at baseline (median, IQR)				
WBC, 10E9/L	8.2 (6.1 - 11.2)	8.4 (6.6 - 11.5)	7.4 (5.4 – 10.8)	0.204
CRP (mg/L)	141 (90 - 207)	138 (89 - 225)	149 (96 – 190)	0.685
PCT (ng/mL)	0.47 (0.22 – 1.14)	0.55 (0.21 – 1.07)	0.46 (0.22 – 1.19)	0.974
MR-proADM (nmol/L)	1.16 (0.85 – 1.71)	1.12 (0.85 – 1.45)	1.24 (0.85 - 2.17)	0.250
CT-proET-1 (pmol/L)	93.5 (72.1 - 122.9)	88.3 (69.3 - 116.2)	101.5 (77.0 - 136.4)	0.127

[a] All continuous data are presented as median (interquartile range) and categorical data as number (percentage). Differences in continuous variables were compared by Mann-Whitney U test and differences in categorical variables by chi-square test.
[b] Differences in outcome measures were assessed by log-rank test.

Legends: BMI: body mass index, COPD: Chronic obstructive pulmonary disease, SOFA: Sequential organ failure assessment, APACHE IV: Acute physiology and chronic health evaluation IV, HFNO: high flow nasal oxygen therapy, IMV: Invasive mechanical ventilation, CRRT: continuous renal replacement therapy, IL-1RA: Recombinant interleukin-1 receptor antagonist LOS: Length of stay, WBC: White blood cells, CRP: C-reactive protein, PCT: Procalcitonin, MR-proADM: Mid-regional proadrenomedullin, CT-proET-1: C-terminal pro-endothelin-1

Association biomarkers and obesity

There were no significant differences in plasma concentrations inflammatory biomarkers at ICU admission between patients with and without obesity ($p < 0.05$ for all biomarkers) (Table 1). Despite lower values of WBC and CRP in patients with obesity at all the timepoints, there were no significant differences in concentrations MR-proADM, CT-proET-1, WBC, CRP, and PCT at baseline and the next six days between patients with and without obesity (Fig. 1 a-e). Only MR-proADM changed significantly over time ($p = 0.039$). Furthermore, BMI did not correlate with concentrations MR-proADM, CT-proET-1, WBC, CRP, and PCT, at ICU admission (rho = 0.150 ($p = 0.125$), rho = 0.179 ($p = 0.067$), rho = -0.044 ($p = 0.654$), rho = 0.057 ($p = 0.564$), rho = 0.022 ($p = 0.842$), respectively) (Supplemental Fig. 3 a-e).

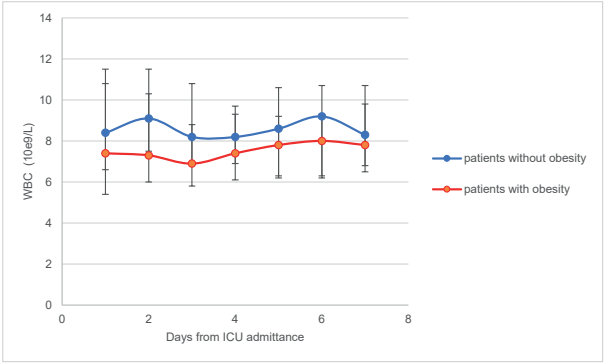

Figure 1a: Temporal changes in WBC

Legends: Median values with IQR. WBC was non-significant different between patients with obesity and without obesity (*p* 0.375) and non-significant over time (*p* 0.302).

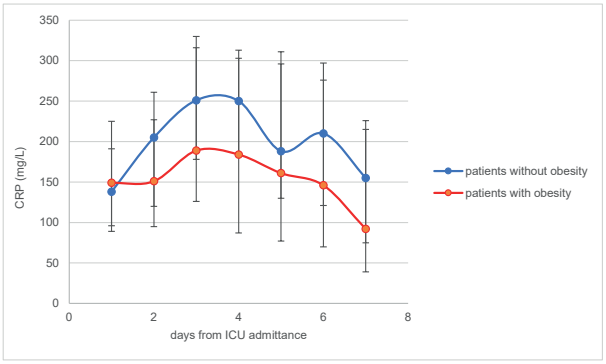

Figure 1b: Temporal changes in CRP

Legends: Median values with IQR. CRP was non-significant different between patients with obesity and without obesity (*p* 0.401) and non-significant over time (*p* 0.461).

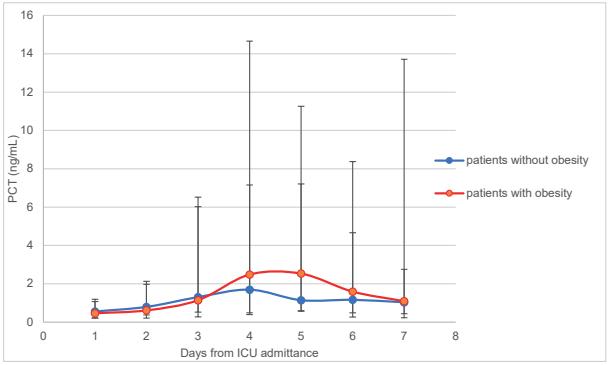

Figure 1c: Temporal changes in PCT

Legends: Median values with IQR. PCT was non-significant different between patients with obesity and without obesity (*p*= 0.945) and non-significant over time (*p*= 0.504).

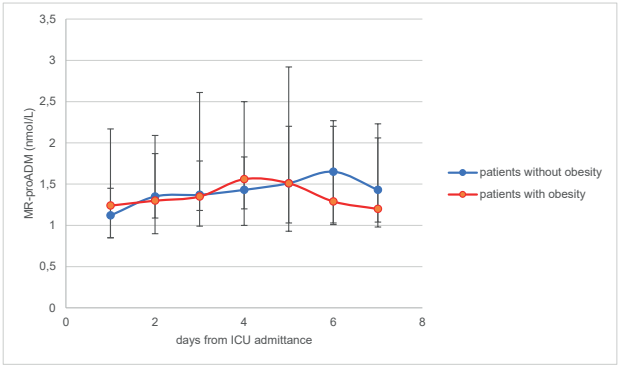

Figure 1d: Temporal changes in MR-proADM

Legends: Median values with IQR. MR-proADM was non-significant different between patients with obesity and without obesity (p= 0.121) and significant over time (p= 0.039).

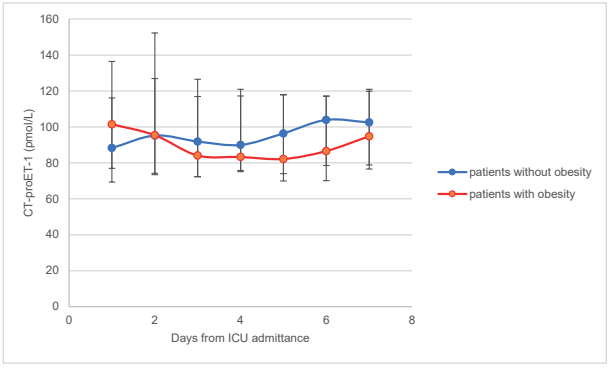

Figure 1e: Temporal changes in CT-proET-1

Legends: Median values with IQR. CT-proET-1 was non-significant different between patients with obesity and without obesity (p 0.074) and non-significant over time (p 0.273).

Clinical outcomes

On day 28 of ICU admission, 9 (23%) of the patients with obesity were still mechanically ventilated and 13 (31%) were still in the ICU, while 11 (19%) of the patients without obesity were still mechanically ventilated and 20 (32%) were still in the ICU (p = 0.594, p = 0.932, respectively). Time on the ventilator was 14 days (IQR 9 - 27) in patients with obesity and 18 days (IQR 9 - 26) in patients without obesity (log-rank p = 0.992). ICU LOS was 14 days (IQR 10 - 33) in patients with obesity and 20 days (10 - 32) in patients without obesity (log-rank p = 0.671). There was no significant difference in 28-day mortality between both groups, 31% versus 27%, log-rank p = 0.570. The Kaplan-Meier curves for time on mechanical ventilation, ICU LOS and 28-day mortality are presented in Supplemental Fig. 4 a-c.

Discussion

The primary aim of the study was to investigate whether obesity was associated with differences in endothelium and obesity related inflammatory biomarkers MR-proADM and CT-proET-1 in a well-described cohort critically ill patients with SARS-CoV-2 pneumonia. Secondary outcomes were the association between obesity and time on a ventilator, ICU LOS and 28-day mortality. We reported two main findings. First, obesity was not related to different responses in MR-proADM and CT-proET-1. Secondly, we found no relationship between obesity and clinical outcome. What our study adds pertaining to the role of obesity in critically ill patients with SARS-CoV-2 pneumonia are data of inflammatory biomarkers MR-proADM and CT-proET-1 during the first seven days of ICU admission.

Worldwide changes in lifestyle, consumer markets and urbanization are important causes of a high prevalence of obesity, especially in Western countries [28]. Patients affected by obesity have an increased risk of developing type 2 diabetes mellitus, hypertension and dyslipidemia [28,29]. It is no surprise that obesity has a high impact on health care providers [28]. Obesity was found to be an independent risk factor for ICU admission and mortality during the H1N1 pandemic 10 years ago [30]. Two single-centre studies showed a high prevalence of patients affected by obesity and severe obesity in patients with SARS-CoV-2 in need of invasive mechanical ventilation [7,8]. Mortality doubled among different classes of obesity in patients hospitalized with SARS-CoV-2 infection in a multicentre prospective cohort study with 5795 hospitalized patients [31]. The present study is a secondary analysis of a prospective observational biomarker study, in which 105 critically ill patients with confirmed SARS-CoV-2 pneumonia were enrolled [22]. Baseline MR-proADM ≥ 1.57 nmol/L and CT-proET-1 ≥ 111 pmol/L were significant predictors of 28-day mortality for all 105 patients in multivariable Cox regression models adjusted for age and SOFA score (HR 6.80, 95% CI 3.12 – 14.84, $p <$ 0.001 and HR 3.72, 95% CI 1.71 – 8.08, p 0.01). The value of MR-proADM as prognostic marker in adult patients hospitalized with SARS-CoV-2 infection was studied in several other observational studies [32-37]. Increased levels of MR-proADM were independently associated with mortality [32-37]. However, none of these studies investigated whether obesity was associated with differences in MR-proADM. Obesity was associated with lower 6-year mortality and higher MR-proADM levels in non-COVID-19 community-acquired pneumonia patients in a secondary analysis of the ProHosp Trial [38]. Baseline median MR-proADM level was 1.21 nmol/L (IQR 0.81 - 1.88), but no details of the number of patients admitted to the ICU were reported. MR-proADM levels were measured in a cohort of 153 critically ill patients with presumed bacterial pneumonia [39]. Median baseline MR-proADM levels were significant higher compared with the SARS-CoV-2 cohort, 1.32 nmol/L (IQR 0.91 - 2.45) vs 1.16 nmol/L (0.86 - 1.71), p 0.015.

However, median APACHE IV scores of the bacterial pneumonia cohort were also significant higher compared with the SARS-CoV-2 cohort, 73 (IQR 53 - 86) vs 47 (40 - 59), $p < 0.001$. Increased levels of CT-pro-ET-1 were independently associated with mortality in critically ill patients with SARS-CoV-2 pneumonia in one observational cohort study [22], but without differences in BMI classes. Epidemiologic studies in non-COVID-19 critically ill patients have shown beneficial effects of higher BMI on mortality in hospitalized patients with specific disease conditions. It has been observed in chronic diseases such as heart failure, coronary artery disease, sepsis, ARDS, or critical illness in general [40-42]. A phenomenon called the "obesity paradox" [40-43]. The existence of the obesity paradox has been challenged [40,43]. The J-shaped relationship between BMI and outcome may result from increased mortality at the extremes on both sides (underweight and severe obesity). Moderate obesity may reflect relatively good health. The paradoxical association may be mediated by confounding variables. Patients with obesity might be admitted to the ICU at a lower level of severity or because of different admission criteria, due to difficulties in providing adequate care in the ward setting or to avoid possible complications [40]. High-risk patients with obesity and comorbidities may have already died before hospital admission, a phenomenon called "selective survivor" effect [44]. On the other hand, the obesity paradox is increasingly adopted [40,41]. Several mechanisms have been proposed. Patients with obesity have higher metabolic reserves [40,41]. An activated renin-angiotensin system in obesity may have protective hemodynamic effects in critically ill patients due to decreased need for fluid or vasopressor support [41]. Obesity may constitute to a status of low-grade chronic inflammation, which protects against the detrimental effects of an aggressive second hit during critical illness [40-42]. Conflicting data on the obesity paradox in critically ill patients are reported in different studies. The relationship between obesity and in-hospital mortality was analyzed in 222 critically ill COVID-19 patients with respiratory failure in a French observational cohort study [45]. Patients with moderate obesity (defined as BMI 30 - 39.9 kg/m^2) had a lower risk of death than patients with normal weight, overweight or severe obesity, suggesting a possible obesity paradox. We could not detect significant differences in 28-day mortality between patients with and without obesity in our smaller study, as previously reported [46]. Our findings are in line with a much larger Dutch multicenter observational cohort study with 2635 COVID-19 patients admitted to the ICU [47].

Regarding the pathophysiology, the relation between SARS-CoV-2 and obesity might be explained by a state of low-grade chronic inflammation, associated with obesity [40,48,49]. Adipose tissue is considered to be more than a long-term energy storage organ [49]. Proinflammatory adipokines, released by adipocytes, are thought to play a crucial role in the pathogenesis of obesity and related adverse outcome [40,48,49]. Studies have shown increased plasma levels of leptin, tumor necrosis factor-alpha (TNF-α), interleukin-1

and 6 (IL-1, IL-6), reactive oxygen species (ROS) and ADM, leading to a dysregulated chronic immune response, which disrupt vascular homeostasis and contribute to endothelial dysfunction and damage in patients affected by obesity [18,48-51]. Evidence of diffuse endothelial inflammation was also found in post-mortem analysis of COVID-19 patients [13]. ADM is a peptide hormone, produced by endothelial, vascular smooth muscle cells and adipose tissue due to pro-inflammatory cytokines, bacterial toxins, hypoxia or volume overload. ADM binds to receptors in especially cardiovascular and pulmonary tissues and has anti-inflammatory effects on vascular endothelial cells, stabilizing the endothelial barrier function and protects the microcirculation against permeability in sepsis [16,17]. Besides its action on the endothelium ADM has important effects on the vascular system, ADM reduces vasoconstriction through inhibition of the renin-angiotensin-aldosterone system [16,52]. ET-1 is released from activated endothelial cells. It is a strong vasoconstrictor peptide and pro-inflammatory cytokine [19]. Patients with obesity showed increased vascular expression of ET-1 and vasoconstriction activity [20,21]. ET-1 release is stimulated by bacterial toxins and inflammatory cytokines such as (TNF-α or IL-6 [53]. MR-proADM and CT-proET-1 are the more stable precursor fragments of the prohormones that correlate with the release of the active peptides [14,15]. In addition, there are studies showing a positive association between obesity and MR-proADM [50,51,54]. BMI correlated significantly to plasma MR-proADM ($r = 0.714$, $p < 0.001$) in a cohort of 357 subjects in a study reported by Vila et al. [50]. We could not detect any correlation between BMI and MR-proADM and there were no significant differences in MR-proADM between patients with and without obesity at baseline and the next days. Lower MR-proADM values, median 0.39 nmol/L (IQR 0.33 - 0.45) and median 0.41 nmol/L (IQR 0.23 - 0.64), were reported in reference populations of healthy individuals [55,56]. We measured higher baseline median values in our cohort critically ill patients with SARS-CoV-2 pneumonia. A state of hyper-inflammation in all SARS-CoV-2 patients, as illustrated by high baseline CRP values could be a possible explanation for higher baseline MR-proADM values. Moreover, we found no differences in MR-proADM levels between SARS-CoV-2 patients with and without obesity, but 68% of the patients in the non-obese group had overweight and only 32% had normal weight. A negative association with CT-proET-1 and obesity was observed in human participants in an observational cohort study of 8592 participants [54] and lower levels of endothelin-1 were found in mice with obesity in an animal model [57]. We could not detect any significant differences in CT-proET-1, at baseline and the next six days, in patients with and without obesity. Again, due to hyper-inflammation in critically ill patients with SARS-CoV-2 pneumonia. It appears that the pathophysiological influences of obesity and COVID-19 comprise multifactorial mechanisms, which are still not fully understood.

Some limitations of our study need to be addressed. First, this was a small retrospective observational single-centre study in which results are hypothesis generating, but no direct cause-and-effect relationship can be deduced. Second, all clinical, microbiological and laboratory data were prospectively collected between March 11 until May 27, 2020, which was the first period of the COVID-19 pandemic in the Netherlands. The treatment of COVID-19 has changed during the last year. Treatment with Chloroquine and Lopinavir/Ritonavir is obsolete and none of these patients were treated with dexamethasone during the first 10 days of hospitalization or interleukin-6 receptor antagonists. We must rely on older data of clinical practice leading to potential observational bias. Third, by only investigating ICU patients we may have introduced selection and collider bias between SARS-CoV-2 infection and adiposity [58]. Fourth, plasma samples could not be collected for seven days in all patients due to early ICU discharge, transfer to another ICU or early death. Incomplete longitudinal biomarker data might result in withdrawal bias. Fifth, the group of patients with obesity consisted largely of patients with class I obesity and only a low number of patients had a BMI above 40 kg/m^2. Again, selection bias needs to be taken in account. Both observation, selection, collider and withdrawal bias may have led to potential underestimation of the association between obesity and the biomarkers MR-proADM and CT-proET-1.

Conclusions

Obesity was not associated with differences in new endothelium and obesity related MR-proADM, CT-proET-1 and conventional inflammatory biomarkers in critically ill patients with confirmed SARS-CoV-2 pneumonia. We found no relation between obesity, time on the ventilator, ICU LOS and 28-day mortality.

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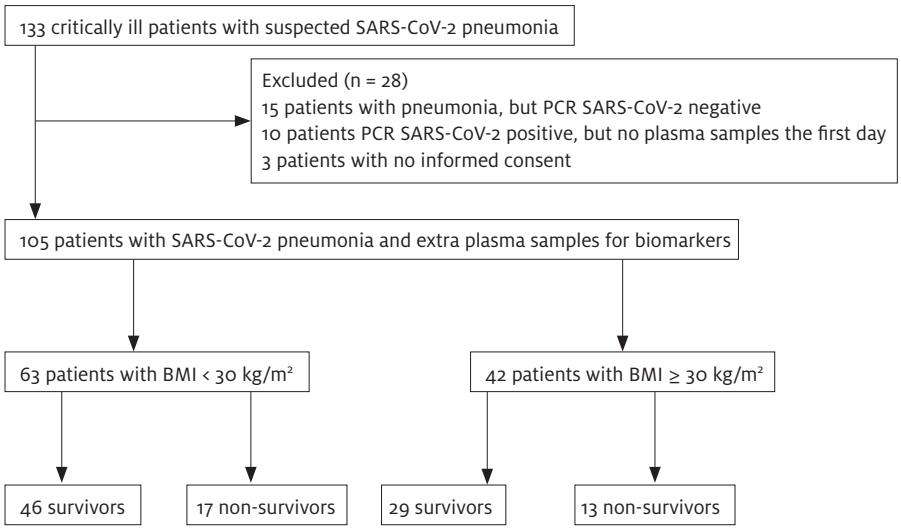
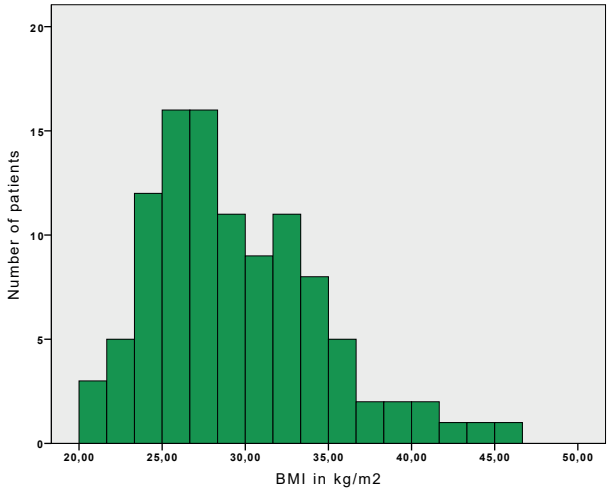
Supplemental data



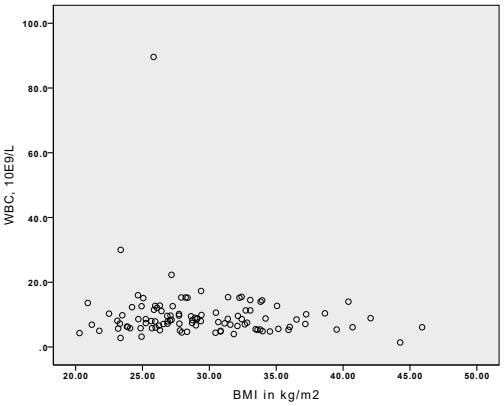
Supplemental Figure 1



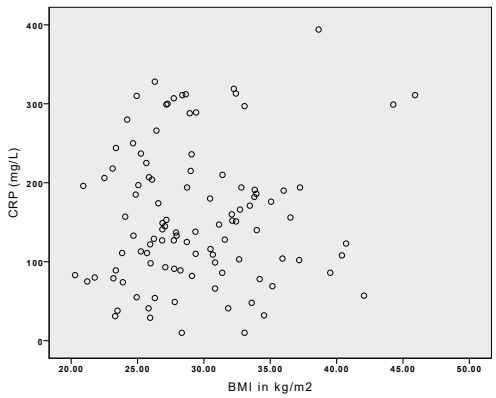
Supplemental Figure 2 | Distribution of BMI

Legends: Histogram depicting BMI frequencies. Patients with obesity (n = 42) and patients without obesity (n = 63).

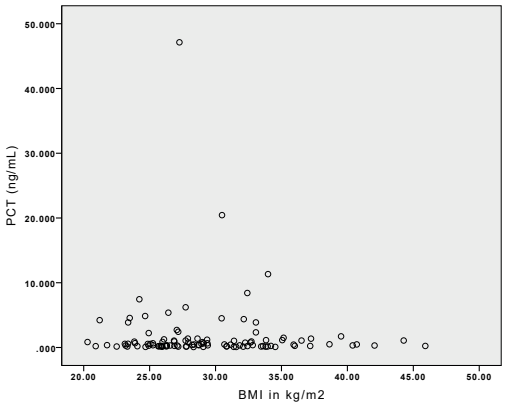
A


WBC, 10E9/L
100.0
80.0
60.0
40.0
20.0
.0
20.00
25.00
30.00
35.00
40.00
45.00
50.00
BMI in kg/m2

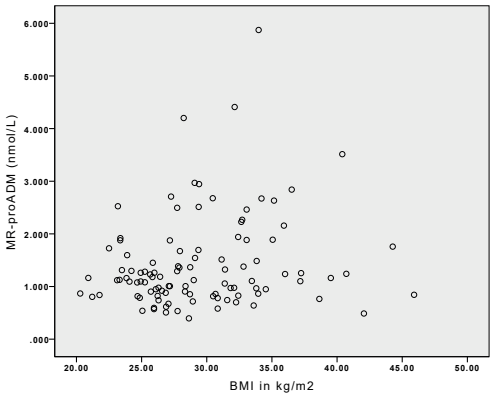
B


CRP (mg/L)
400
300
200
100
0
20.00
25.00
30.00
35.00
40.00
45.00
50.00
BMI in kg/m2

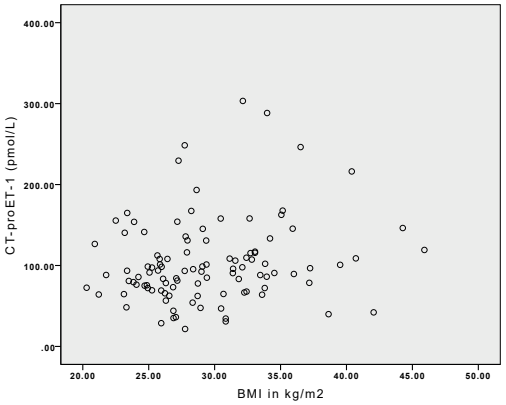
C


PCT (ng/mL)
50.000
40.000
30.000
20.000
10.000
.000
20.00
25.00
30.00
35.00
40.00
45.00
50.00
BMI in kg/m2

D

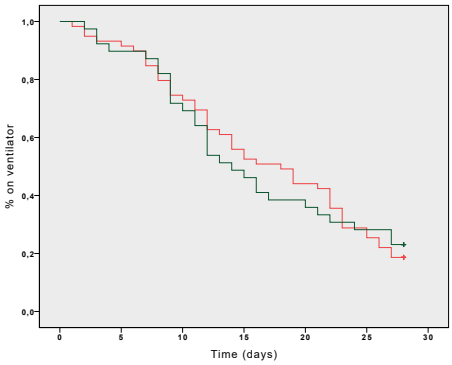


E

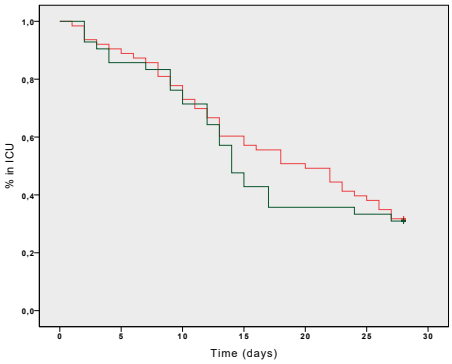


Supplemental Figure 3 | Relationship between BMI and levels of biomarkers on day of ICU admission

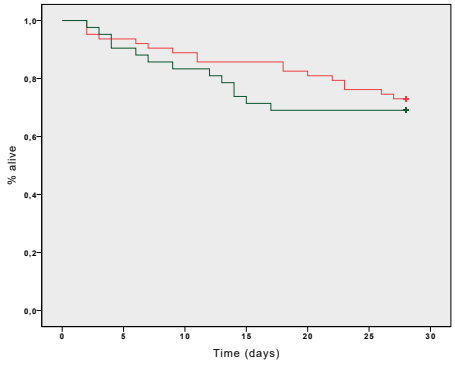
Legends: **A:** WBC (rho = -0.044, *p* 0.654) **B:** CRP (rho = 0.057, *p* 0.564) , **C:** PCT (rho = 0.022, *p* 0.824), **D:** MR-proADM (rho = 0.150, *p* 0.125), **E:** CT-proET-1 (rho = 0.179, *p* 0.067) rho and p values were calculated using Spearman's correlation.



Supplemental Figure 4a | Clinical outcomes
Legends: Time on ventilator. Kaplan-Meier graph of time on a ventilator. Green line: Patients with obesity, red line: Patients without obesity, log-rank *p* 0.992.



Supplemental Figure 4b
Legends: Length of stay ICU. Kaplan-Meier graph of length of stay ICU. Green line: Patients with obesity, red line: Patients without obesity, log-rank *p* 0.671



Supplemental Figure 4c
Legends: 28-day mortality. Kaplan-Meier graph of 28-day mortality. Green line: Patients with obesity, red line: Patients without obesity, log-rank *p* 0.570.

PART IV

Clinical therapeutic applicability of biomarkers

Efficacy and safety of procalcitonin guidance in reducing the duration of antibiotic treatment in critically ill patients: a randomized, controlled open-label trial

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Lancet Infect Dis. 2016; 16:819-27. doi: 10.1016/S1473-3099(16)00053-0.

Abstract

Introduction: In critically ill patients, antibiotic therapy is of great importance but prolonged duration of treatment is associated with the development of antimicrobial resistance. Procalcitonin is a marker used to guide antibacterial therapy and reduce its duration but data on safety of this reduction are lacking. We assessed the efficacy and safety of procalcitonin-guided antibiotic treatment in patients in intensive care units (ICUs) in a health-care system with a comparatively low use of antibiotics.

Methods: We did a prospective, multicentre, randomized, controlled open-label, intervention trial in 15 hospitals in the Netherlands. Critically ill patients aged at least 18 years, admitted to the ICU, and who received their first dose of antibiotics no longer than 24 h before inclusion in the study for an assumed or proven infection were eligible to participate. Patients who received antibiotics for presumed infection were randomly assigned (1:1), using a computer-generated list, and stratified (according to treatment centre, whether infection was acquired before or during ICU stay, and dependent on severity of infection [ie, sepsis, severe sepsis, or septic shock]) to receive either procalcitonin-guided or standard-of-care antibiotic discontinuation. Both patients and investigators were aware of group assignment. In the procalcitonin-guided group, a non-binding advice to discontinue antibiotics was provided if procalcitonin concentration had decreased by 80% or more of its peak value or to 0.5 ug/L or lower. In the standard-of-care group, patients were treated according to local antibiotic protocols. Primary endpoints were antibiotic daily defined doses and duration of antibiotic treatment. All analyses were done by intention to treat. Mortality analyses were completed for all patients (intention to treat) and for patients in whom antibiotics were stopped while being on the ICU (per-protocol analysis). Safety endpoints were reinstitution of antibiotics and recurrent inflammation measured by C-reactive protein concentrations and they were measured in the population adhering to the stopping rules (per protocol analysis). The study is registered with ClinicalTrials.gov, number NCT01139489, and was completed in August, 2014.

Results: Between Sept 18, 2009 and July 1, 2013, 1575 of the 4507 patients assessed for eligibility were randomly assigned to the procalcitonin-guided group (761) or to the standard-of-care (785). In 538 patients (71%) in the procalcitonin-guided group antibiotics were discontinued in the ICU. Median consumption of antibiotics was 7.5 daily defined doses (IQR 4.0-12.7) in the procalcitonin-guide group versus 9.3 daily defined doses (5.0-16.6) in the standard-of-care group (between group absolute difference 2.69, 95% CI 1.26-4.12, $p < 0.0001$). Median duration of treatment was 5 days (3-9) in the procalcitonin-guide group and 7 days (4-11) in the standard-of-care group (between group absolute difference 1.22, 0.65-1.78, $p < 0.0001$). Mortality at 28 days

was 149 (20%) of 761 patients in the procalcitonin-guided group and 196 (25.0%) of 785 patients in the standard-of-care group (between group absolute difference 5.4%, 95% CI from 1.2-9.5, $p = 0.0122$) according to the intention-to-treat analysis, and 107 (20%) of 538 patients in the procalcitonin-guided group versus 121 (27%) of 457 patients in the standard-of-care group (between group absolute difference 6.6%, 95% CI 1.3 -11.9, $p = 0.0154$) in the per-protocol analysis. One-year mortality in the per protocol analyses was 191 (36%) of 538 patients in the procalcitonin-guided and 196 (43%) of 457 patients in the standard-of-care group (between group absolute difference 7.4%, 95% CI 1.3-13.8; $p = 0.0188$).

Conclusion: Procalcitonin guidance stimulates reduction of duration of treatment and daily defined doses in critically ill patients with a presumed bacterial infection. This reduction was associated with a significant decrease in mortality. We hypothesize that procalcitonin concentrations help the physician in deciding whether or not the presumed infection is truly bacterial which leads to more adequate diagnosis and treatment, the cornerstones of antibiotic stewardship.

Introduction

Sepsis remains a major cause of death in critically ill patients. Rapid and adequate antibiotic therapy is of great importance in critically ill patients, but overly prolonged antimicrobial treatment is undesirable because of increasing resistance [1]. However, with critically ill patients, physicians might be reluctant to shorten the duration of antimicrobial treatment [2]. Therefore, specific markers for resolution of infection might assist physicians in making individualized antibiotic therapy decisions. Regularly used markers for this purpose are the leukocyte count and C-reactive protein (CRP). However, procalcitonin (PCT) has been advocated as a marker with a better specificity and sensitivity than CRP for follow-up of severe bacterial infections [3-10].

Findings from several studies [11-20] have shown that procalcitonin guidance can reduce the duration of antibiotic treatment for patients with bacterial infection, but the safety of such protocols has not been firmly established [21-22]. Additionally, most of these intensive care units (ICU) trials were done in countries with a high baseline consumption of antibiotics. In the Netherlands the antibiotic consumption per capita is relatively low. By contrast, in terms of defined daily dosages (DDDs) per 1000 patient days, the consumption in the United Kingdom, the United States, France and Greece is 1.5 to 3.3 times higher [23].

The objective of this trial was to evaluate the efficacy and safety of procalcitonin-guided antibiotic treatment in a large heterogeneous set of ICU-patients in a health-care system with a comparatively low use of antibiotics. Our hypothesis was that addition of procalcitonin to the standard-of-care could reduce the duration of antibiotic treatment and thus the amount of antibiotics administered, without increasing mortality or recurrent infections.

Methods

Study design

The Stop Antibiotics on PCT guidance Study (SAPS) was a prospective, multicentre, randomized, open-label, intervention trial in patients admitted to the ICU of three university medical centres and 12 teaching hospitals in the Netherlands. This study was approved for all centres by ethics committee of the VU University Medical Center (Amsterdam, Netherlands) and is in full compliance with the Helsinki declaration. The study protocol is available online [24].

Participants

Eligible patients were at least 18 years of age, be admitted to the ICU, and have received their first dose of antibiotics no longer than 24 hours before inclusion to the trial for an assumed or proven infection. Patients were excluded in cases of systemic antibiotics as prophylaxis only, antibiotics solely as part of selective decontamination of the digestive tract, prolonged therapy (e.g. endocarditis), expected ICU stay of less than 24 h, severe immunosuppression severe infections (due to viruses, parasites, or Mycobacterium tuberculosis), and moribund patients. Patients who received corticosteroids were not excluded. Patients could only participate once in this trial. All patients or their legal representatives provided written informed consent.

Randomisation and masking

Patients were randomly assigned (in a 1:1 ratio) to receive either treatment according to procalcitonin guidance (procalcitonin-guided group) or standard-of-care. Randomization was done centrally by use of a computer-generated list produced by an independent research organization (the Julius Centre for Human Research, Utrecht, Netherlands). Randomization was stratified according to treatment centre, whether the infection was acquired before or during ICU stay and depending on severity of infection (i.e. sepsis, severe sepsis or septic shock) [25]. Patients and investigators were aware of treatment assignment.

Procedures

For patients randomly assigned to the procalcitonin-guided group, once a day measurements of procalcitonin were taken and made available to the attending physicians, including a baseline measurement as close to initiation of antibiotics as possible, at least within 24 h. Procalcitonin concentration was not measured in the standard-of-care group. Except for the procalcitonin measurements, all monitoring was similar between the procalcitonin-guided and the standard-of-care groups. Procalcitonin was measured on analyzers available at the site (Kryptor machine [Thermo Fisher Scientific, Waltham, MA, USA] or a suitable Vidas [Marchy-l'Etoile, France] or Roche [Basel, Switzerland] immunoanalyzer) that were maintained according to national quality standards. In the procalcitonin-guided group, procalcitonin was measured until ICU-discharge or until the third day after systemic antibiotics were stopped. The study protocol advised to stop the prescribed antibiotics if procalcitonin concentration had decreased by 80% or more of its peak value (relative stopping threshold) or when it reached a value of 0.5 ug/L or lower (absolute stopping threshold). The attending physician was free to decide to continue antibiotic

treatment in patients who had reached these thresholds. Reasons for non-adherence were recorded. Antibiotics in the standard-of-care group were stopped according to local or national guidelines and according to the discretion of attending physicians. The number of antibiotic-free days in the first 28 days after study inclusion were recorded (including antibiotic days on subsequent nursing wards). In both groups CRP concentrations were analyzed once a day until 28 days after inclusion as an additional safety measure. Patients were followed up to 1 year after entering the study, allowing assessment of 28-day and 1-year mortality.

Outcomes

The primary outcome was the consumption of antibiotics (expressed as DDDs) and duration of antibiotic treatment (defined as the number of 24 h periods between start and end of antibiotic treatment) in the two groups for all randomized patients who were not excluded (the modified intention-to-treat population). For every participant, the total amount of antibiotics given during the study period was assessed on the basis of individual drug administration records. Our definition of DDDs accords with the recommendations of the World Health Organization (supplementary material) [26]. The route of administration was incorporated in the daily dose calculations. The primary safety outcome was mortality at 28 days and 1 year, assessed in the modified intention-to-treat population and per-protocol population. Secondary outcomes were the percentage of patients who had recurrent infections, length of stay in hospital and ICU, costs of antibiotics, and costs of procalcitonin tests. The total direct costs of antibiotic treatment per patient were calculated by multiplying the total amounts of all antibiotics used with the lowest Dutch list price according to the Dutch National Health Care Institute, which reports the lowest and highest pharmacy purchase prices including 6% tax for all registered drugs. The SAPS trial was supervised by an independent Data Safety Monitoring Board (consisting of an intensivist, statistician, and a pulmonologist), which was not involved in the design, completion of the trial, or recruitment of patients. The Data Safety Monitoring Board concluded after the interim analysis (after the first 750 patients had been; about 2 years after start of the study) that the trial could be continued.

Statistical analysis

The goal of this trial was to establish whether the procalcitonin-guided strategy was superior in terms of antibiotic use and duration, length of stay in the ICU, and cost-effectiveness and to show non-inferiority of the procalcitonin-guided antibiotic management regarding 28-day mortality and recurrent infections. For the superiority primary outcome, the power calculation was based on an estimated 15% reduction in

duration of antibiotic treatment. We assumed a mean duration of antibiotic treatment of 8 days and a standard deviation of 6 days [17]. With an α of 0.05 and a β of 0.1 we would need 526 patients in each arm. However, some patients would be discharged from the ICU before reaching the stopping rules. These patients would not be stopped according to the procalcitonin guidelines. We assumed that 20% of the patients were going to be discharged before the stopping rule was effectuated. Hence, the need for 631 patients per study arm.

We did not want the intervention to lead to excess mortality in the procalcitonin-guided group. In view of a 28% mortality in a previously published study [17], for the procalcitonin-guided group to be non-inferior to standard of care in terms of safety, the non-inferiority margin for procalcitonin-guided treatment regarding 28-day mortality was set to 8%. This margin would lead to a mortality of 28% in the standard-of-care group versus 30% in the procalcitonin-guided group. On basis of these assumptions and with an α of 0.025, a β of 0.1 we would need 663 patients in each group for 90% power that the one-sided 97.5% confidence interval excludes a difference in the standard-of-care group of more than 8%. On basis of these two calculations the study needed at least 1326 patients.

We compared baseline characteristics and outcomes with a t test or Mann-Whitney U test for continuous outcomes, α^2 for nominal outcomes and a log-rank test to compare Kaplan-Meier survival curves. We calculated a cumulative event estimate was calculated by a hazard ratio (HR, 95% CI). All tests were two-sided. All analyses were completed using SPSS, version 20 (IBM software). The study is registered with ClinicalTrials.gov, NCT01139489.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

From September 18, 2009, to July 1, 2013, 4507 patients were screened in the 15 participating ICUs. Of these patients, 1575 (35%) were enrolled, including 29 patients who subsequently withdrew from the study or had major protocol violations, resulting in the modified intention-to-treat population of 1546 patients (761 in the

procalcitonin-guided group and 785 in the standard-of-care group (Fig. 1). 223 (29%) of the 761 patients in the procalcitonin-guide group had died or were discharged from the ICU before antibiotics were stopped. Although these patients did not discontinue their antibiotic treatment, they were included in the analyses as part of the procalcitonin-guided group (intention-to-treat principle). 761 patients in the procalcitonin-guided group and 785 patients in the standard-of-care group were included in the modified intention-to-treat analyses. Baseline characteristics of the 1546 patients were similar between the two groups (Table 1).

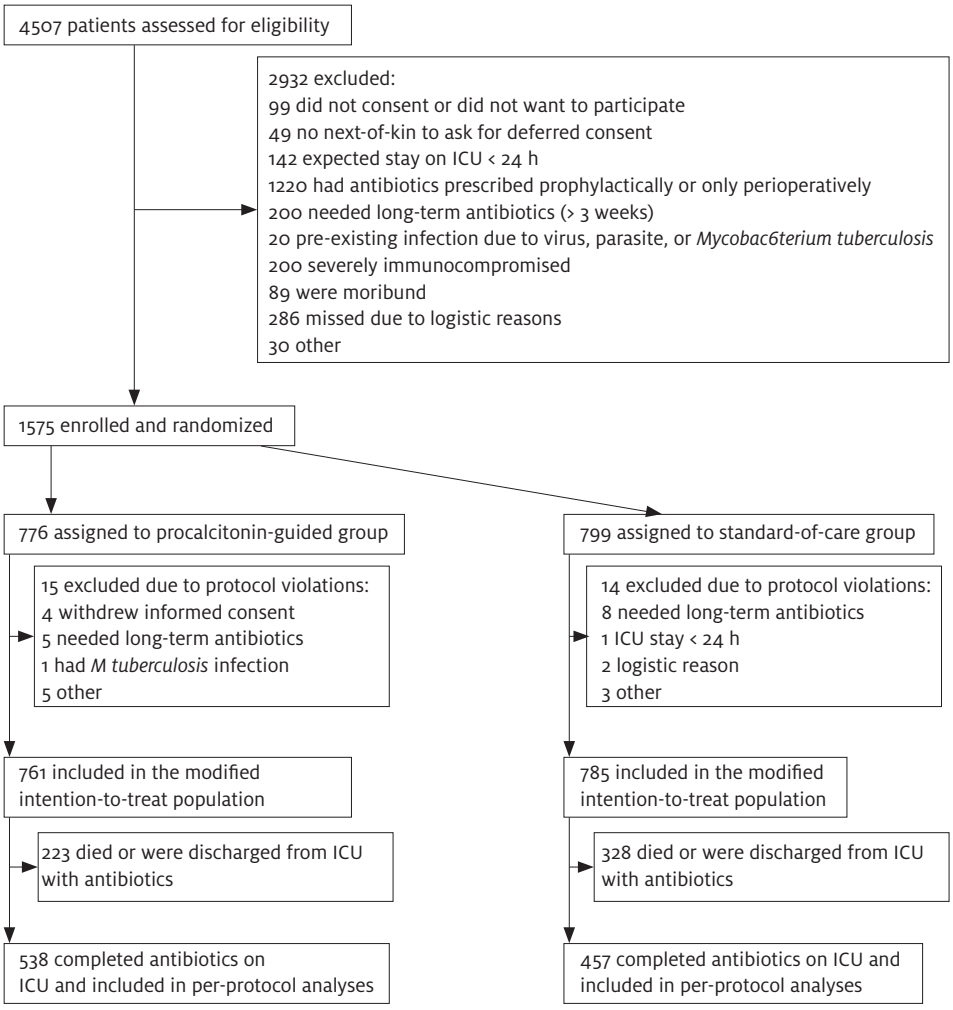

Figure 1 | Trial profile

Table 1 | Baseline characteristics of the patients at baseline

Characteristic	Procalcitonin group (N = 761)	Standard-of-care-group (N = 785)
Age - years	65 (54 – 75)	65 (57 – 75)
Male sex – no. (%)	466 (61%)	470 (60%)
Severity of illness		
APACHE IV score	72.0 (52.0 – 92.0)	71.0 (55.0 – 95.0)
Sepsis or severe sepsis – no. (%)	625 (82%)	634 (81%)
Septic shock – no. (%)	136 (18%)	151 (19%)
SOFA score¶	6.0 (3.0 - 9.0)	6.0 (4.0 - 9.0)
Respiratory	3 (2-3)	3 (2-3)
Cardiovascular	3 (0-4)	3 (0-4)
Renal	0 (0-1)	0 (0-1)
Hepatic	0 (0-0)	0 (0-0)
Neurological	0 (0-2)	0(0-1)
Coagulation	0 (0-0)	0 (0-1)
Acquisition of infection		
Community-acquired – no. (%)	392 (52%)	400 (51%)
Hospital-acquired – no. (%)	189 (25%)	186 (24%)
ICU-acquired – no. (%)	180 (24%)	199 (25%)
Presumed infection site		
Pulmonary – no. (%)	491 (65%)	503 (64%)
CNS – no. (%)	29 (4%)	30 (4%)
Skin and soft tissue – no. (%)	13 (2%)	23 (3%)
Catheter related infection – no. (%)	8 (1%)	11 (1%)
Intra-abdominal infection – no. (%)	109 (14%)	129 (16%)
Urinary tract infection – no. (%)	27 (4%)	24 (3%)
ENT– no. (%)	7 (1%)	7 (1%)
Bloodstream infection – no. (%)	4 (1%)	4 (1%)
Unknown focus – no. (%)	74 (10%)	54 (7%)
Infection and inflammation		
Procalcitonin (mg/L)	1.9 (0.40 – 14.1)	NA
C-reactive protein (mg/L)	202.0 (99.0 – 306.3)	204.0 (105.5 – 307.5)
Leucocytes (10^9 cells per L)	14.7 (10.6 - 21.3)	14.9 (10.4 - 21.0)
Temperature (°C)	38.0 (37.4 - 38.8)	38.0 (37.4 -3 8.7)

Treatment in first 24 h		
Mechanical ventilation	617 (81%)	628 (80%)
Renal replacement in first 24 h	72 (9%)	86 (11%)
Inotropic or vasopressor support	729 (965)	751 (96%)
Selective decontamination of the digestive tract	399 (52%)	421 (54%)
Corticosteroids	412 (54%)	420 (54%)

Legends: All values between brackets are interquartile ranges because of skewed distributions, unless otherwise stated. There were no statistical significant differences between the two groups. APACHE IV denotes Acute and Chronic Health Evaluation IV score. ICU means Intensive Care Unit, and ENT means an infectious focus on ear-nose-throat area.

¶ SOFA denotes Sequential Organ Failure Assessment score, which contains six subscores (respiratory, coagulation, liver, cardiovascular, renal and neurological). Each subscore can be attributed 0 - 4 points depending on the amount of organ dysfunction. The original SOFA-score was used, including the mean arterial pressure of < 70 mmHg to obtain 1 point for cardiovascular failure.

In the study population of 1546 patients, median consumption of antibiotics was 7.5 defined daily doses (IQR 4-12.8 days) in the procalcitonin-guided group versus 9.3 defined daily doses (5.0-16.5) in the standard-of-care group between-group absolute difference 2.69, 95% CI 1.26-4.12, $p < 0.0001$). Median duration of treatment in the first 28 days was 5.0 days (IQR 3.0-9.0) in the procalcitonin-guided group versus 7.0 days (IQR 4.0-11.0 in the standard-of-care group (between group absolute difference 1.22, 95% CI 0.65 to 1.78, $p < 0.0001$). The median antibiotic-free days within the first 28 days after randomization was 7.0 (IQR 0.0-14.5 days) in the PCT-guided group versus 5.0 days (IQR 0.0-13.0 days) in the standard-of-care group (absolute difference 1.31, 95% CI 0.52-2.09, $p = 0.0016$).

At 28 days after randomization 149 (20%) of 761 patients had died in the procalcitonin-guided group versus 196 (25%) of 785 patients in the standard-of-care group. The between group absolute difference was 5.4% (95% CI 1.2- 9.5, $p = 0.0122$). One year after randomization this difference remained with 265 (35%) deaths of 761 patients in the procalcitonin-guided group versus 321 (41%) deaths of 785 patients in the standard-of-care group (log-rank test $p = 0.0070$). The between group absolute difference was 6.1% (95% CI 1.2-10.9, $p < 0.0158$; HR 1.26, 95% CI 1.07-1.49, $p = 0.0060$) in the intention-to-treat analysis. The Kaplan-Meier survival curves of both groups are shown in Fig. 2.

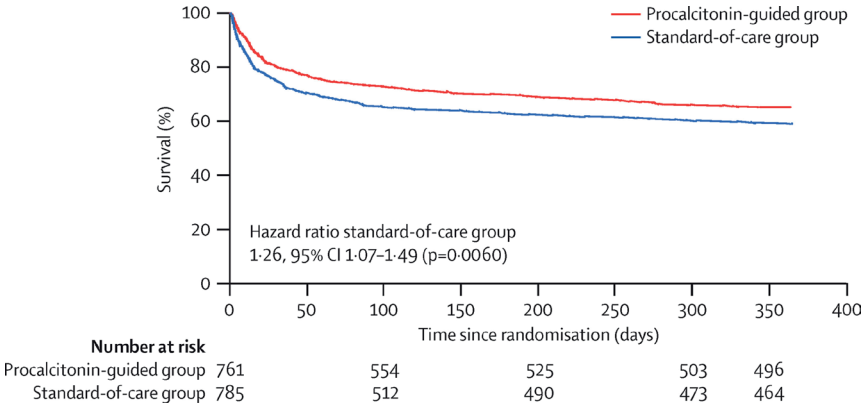

Figure 2 | Probability of survival from randomization through day 365

Legends: The graph shows the Kaplan-Meier estimates for the probability of survival among patients whose antibiotic treatment was guided by procalcitonin and among those whose antibiotic treatment was according to the standard-of-care.

The remaining 538 (71%) of 761 patients in the procalcitonin-guided and 457 (58%) of 785 patients in the standard-of-care group completed their antibiotic treatment in the ICU; these two groups were compared as per-protocol analysis. The 28-day mortality in this analysis was 107 (20%) of 538 patients in the procalcitonin-guided group versus 121 (27%) of 457 patients in the standard-of-care group (between-group absolute difference was 6.6% (95% CI 1.3-11.9, $p = 0.0154$). The one-year mortality in the per protocol analyses was 191 (36%) of 538 patients in the procalcitonin-guided group and 196 (43%) of 457 patients (42.9%) in the standard-of-care group (between-group absolute difference 7.4%, 95% CI 1.3-13.8, $p = 0.0188$). The differences in various other subgroups are shown in the appendix.

In the first 28 days after being assigned to a group, 175 (23%) of 761 patients in the procalcitonin-guided group received an additional course of systemic antibiotics within the first 28 days of randomization versus 173 (22%) of 785 patients in the standard-of-care group (intention-to-treat $p = 0.67$). These additional antibiotics were given after a median interval of 4.0 days (IQR 2.0-8.0) in both the procalcitonin-guided group and the standard-of-care group ($p = 0.96$). In 38 (5%) of 761 patients in the procalcitonin-guided group versus 23 (3%) of 785 patients in the standard-of-care group ($p = 0.0492$), a second course of antibiotic treatment was given for a reinfection that was proven by culture to be the same pathogen and the same organ as the original infection. When asked if the “reinfection" was caused by an overly short initial course of antibiotics, physicians answered this affirmatively for 16 (26%) of 61 patients with a

recurrent infection. The non-inferiority analysis for the re-institution of antibiotics in the per protocol population was 151 (28%) of 538 patients in the procalcitonin-guided group versus 117 (26%) of 457 in the standard-of-care group (between-group absolute difference was -2.5%, 95% CI -7.9 to 3.1, $p = 0.39$).

A stopping criterion was reached in 557 patients in the procalcitonin-guided group during their ICU-stay. Adherence to this stopping advice was for 243 patients who had their antibiotics stopped within 24 h and 297 patients (53.3%) treatments were stopped within 48 h after reaching the stopping threshold. 17 patients (3%) did not have their antibiotics stopped. Of the reasons why intensivists decided to continue antibiotics in patients who reached the stopping rule, various non-specific concerns about stopping antibiotics were mentioned (appendix).

In 38 (7%) of 557 patients, antibiotics were already discontinued before reaching either stopping rule. Of the patients in whom physicians adhered to one of the stopping rules, 126 (42%) of 297 patients were stopped because of a decrease in procalcitonin concentrations to 20% or lower of the peak value, 154 (52%) of 297 patients were stopped as the procalcitonin concentration was 0.5 ug/L or lower, and 17 (6%) of 297 patients reached both stopping rules simultaneously. Thus, both components of the stopping rule seem to be of relevance. For both study groups the CRP concentrations showed no difference for day 1 through 28 (Fig. 3), even without Bonferroni correction for multiple testing [28]. Median length of stay on the ICU was 8.5 days (IQR 5.0-17.0) in the procalcitonin-guided group versus 9.0 (IQR 4.0-17.0) in the standard-of-care group ($p = 0.56$; Table 2). Median length of stay in the hospital was the same for both groups at 22 days (IQR 13.0-39.3 procalcitonin-guided vs 12.0-40.0 standard-of-care; $p = 0.77$; Table 2).

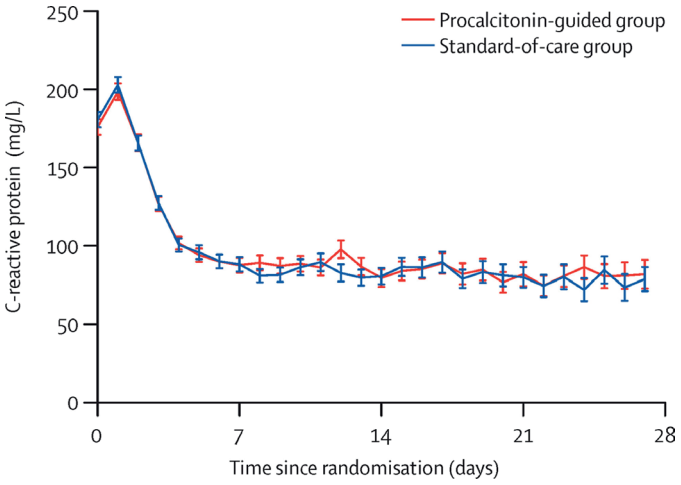

Figure 3 | Serial measurements of CRP in both study groups

Legends: The levels of C-reactive protein (CRP) were measured in both study groups. Depicted are the mean values and standard errors during the first 30 days after randomization. Patients who were discharged from the hospital before day 30 were included for as long as they were admitted to the hospital

Table 2 | Primary and secondary outcome measures

	Procalcitonin-guided group (n = 761)	Standard-of-care group (n = 785)	Between-group absolute difference in means (95% CI)	*p* value
Antibiotic consumption				
Daily defined doses in first 28 h	7.5 (4.0 - 12.8)	9.3 (5.0 - 16.5)	2.69 (1.26 - 4.12)	< 0.0001
Duration of treatment	5.0 (3.0 - 9.0)	7.0 (4.0 - 11.0)	1.22 (0.65 - 1.78)	< 0.0001
Antibiotic-free days in first 28 days	7.0 (0.0 - 14.5)	5.0 (0.0 - 13.0)	1.31 (0.52 - 2.09)	0.0016
Mortality (%)				
28-day mortality	149 (19.6%)	196 (25.0%)	5.4% (1.2 - 9.5)	0.0122
1-year mortality	265 (34.8%)	321 (40.9%)	6.1% (1.2 - 10.9)	0.0158
Adverse events				
Reinfection	38 (5%)	23 (2.9%)	-2.1% (-4.1 - -0.1)	0.0492
Repeated coarse of antibiotics	175 (23.0%)	173 (22.0%)	-1.0% (-5.1 - 3.2)	0.67
Time (days) between stop and reinstitution of antibiotics	4.0 (2.0-8.0)	4.0 (2.0-8.0)	-0.22 (-1.31 - 0.88)	0.96

Costs				
Total cumulative costs antibiotics (€)	€150.082	€181.263	NA	NA
Median cumulative costs antibiotics per patient (€)	€107 (51 – 229)	€129 (66 – 273)	€33.6 (2.5 – 64.8)	0.0006
Length of stay (days)				
LOS on the ICU	8.5 (5.0 - 17.0)	9.0 (4.0 - 17.0)	-0.21 (-0.92 - 1.60)	0.56
LOS in hospital	22.0 (13.0-39.3)	22.0 (12.0-40.0)	0.39 (-2.69 - 3.46)	0.77

Legends: The between group absolute differences are calculated on the mean values, percentage differences and 95% confidence intervals.

The median costs for the first course of antibiotics were €107 (IQR 51-229) in the procalcitonin-guided group versus €129 (IQR 66-273) in the standard-of-care group (p = 0.0006; Table 2). The cumulative estimated cost for the first course of antibiotics in the procalcitonin-guided group was €150,082 versus €181,263 in the standard-of-care group. These cost savings should be balanced against the costs of 5425 PCT measurements that were performed in the intervention arm.

Discussion

In the SAPS trial we noted a clear reduction of antibiotic treatment duration from 7 days in the standard-of-care group to 5 days in the procalcitonin-guided group. Early discontinuation of antibiotics was not associated with more subsequent antibiotic prescriptions or higher CRP concentrations levels in the procalcitonin-guided patients. Furthermore, this reduction was non-inferior in terms of 28-day mortality and was even accompanied by a decrease in mortality in the procalcitonin-guided group (19.6%) than in the standard-of-care group (25.0%).

Additionally, the reduction in antibiotic treatment duration achieved with procalcitonin guidance constitutes a relevant decrease in the volume of prescribed antibiotics on ICUs from 9.3 daily defined doses in the standard-of-care group to 7.5 defined daily doses in the procalcitonin-guide group. This decrease corresponded with a relative reduction in antibiotic consumption of 19%. The close similarity of the two CRP curves also suggests that the earlier discontinuation in the procalcitonin-guided group did not result in a higher rate of re-infection.

The total reduction in antibiotic costs using procalcitonin guidance was a mean of €34 per patient. In our study about a mean of seven procalcitonin measurements were taken per patient. Therefore, the reduction in antibiotic costs will only outweigh the

costs of additional procalcitonin measurements if procalcitonin tests costs less than about €4 per measurement. In other settings this value might differ, but procalcitonin monitoring could offer many more important benefits than only reduction of antibiotic costs.

Reduction in 28-day mortality and 1-year mortality associated with the procalcitonin strategy was unexpected as this study was aiming for non-inferiority. If physicians suspect that a patient has a bacterial infection they will (pre-emptively) start antibiotics. If procalcitonin concentration is high, as expected, then these physicians are reassured about their initial diagnosis. However, if procalcitonin concentrations are low, it makes severe bacterial infection improbable, and the initial diagnosis is questioned. Physicians then need to reconsider their diagnosis at an earlier stage. Therefore, knowledge of procalcitonin concentrations might lead to earlier and more adequate diagnoses and treatments, reducing mortality. Furthermore, antibiotics that are unnecessary might lead to adverse effects without benefits (eg. antibiotic resistance, selection of more resilient pathogens such as Clostridium, and drug reactions). Such adverse effects of antibiotic treatment have been previously noted [29,30]. In a de-escalation study in ICU patients with severe sepsis and septic shock, the odds for mortality were reduced in patients in whom antibiotics were stopped or better aimed on the pathogens [29]. The authors proposed that the reduction of toxic effects of antibiotics might have contributed to the survival benefit, eg. low nephrotoxicity of some classes of antibiotics. The percentages of patients who received a repeated course of antibiotics were similar between the groups (23% in the procalcitonin guided vs 22% in the standard of care; Table 2). However, the cases considered to be re-infectious by physicians were much lower in the standard-of-care group (3%) than in the procalcitonin-guide group (5%; Table 2). Although the difference in re-infections was significant (Table 2), the numbers suggest under-reporting, given the much higher reinstitution rate of antibiotics. Additionally, physicians might have been biased to considering re-infection earlier in patients in whom procalcitonin guidance contributed to the decision to discontinue antibiotics. The adequacy of the antibiotics, a more timely recognition of alternative diagnoses, and lower toxicity of antibiotics might all account for the lower mortality in our procalcitonin-guided study group [30]. However, this remains speculative and bias or a type I error might still play a part.

Previous studies have addressed the possibility to stop antibiotic treatment based upon a PCT-guided strategy in the critically ill patient [14-20]. A small proof of principle study found that PCT was able to decrease antibiotic treatment in severe sepsis and septic shock [14]. This strategy was confirmed in two small ICU-studies, but none was powered for mortality [16,18]. The French PRORATA-trial, however, was larger and aimed to demonstrate efficacy as well as safety [17]. In that study, procalcitonin guidance

led to a reduction of 23% in antibiotic exposure and 2.7 more antibiotic-free days. Unfortunately, the 60-day mortality was 3.8% higher in the procalcitonin-guided group. Therefore, some debate remained whether PCT can safely reduce antibiotic duration in critically ill patients. This debate was fueled by the recent ProGuard study, which showed no significant reduction in duration of treatment, antibiotic-free days and overall antibiotic exposure between a standard-of-care group versus a procalcitonin-guided group [20]. However, this trial [20] used only an absolute stopping rule and a strict procalcitonin threshold of 0.1 ug/L. Our results show that both the 'absolute' (ie, procalcitonin ≤0.5 ug/L) and the relative (ie, procalcitonin ≤ 20% of its peak value) stopping rules assisted in antibiotic discontinuation. Furthermore, the study was designed with a size to detect - a rather ambitious - reduction of duration of treatment of at least 3.75 days. Although a reduction of two days was noted, it was not statistically significant. Our study suggests that reduction in antibiotic exposure can be achieved without an increase in mortality, even in a context of low background usage of antibiotics in critically ill patients. Lowering the antibiotic exposure might have a beneficial impact on emergence of resistance. However, prophylactic use of antibiotics was not evaluated in this study and such patients were not considered eligible. This is of importance because nine of the participating ICUs routinely used selective decontamination of the digestive tract. Antibiotics given as part of this decontamination strategy were only counted if the patient was considered having an infection. Patients on selective decontamination of the digestive tract who had, or were suspected of having an infection were not eligible (appendix).

Several studies show that demonstrate that a well considered reduction of antibiotics, although not necessarily equal to early discontinuation, is associated with a decreased mortality [29]. In patients with pulmonary infections a reduction in antibiotic use is associated with a reduction in mortality. In an individual patient meta-analysis [30], studying 4211 patients, the mortality in the procalcitonin-guided group was 5.6% versus 6.3% in the control group. Although this difference was not statistically significant, it corroborates our reduced mortality. Our study was conceived to include a heterogeneous ICU patient population in a real-life setting, focusing only on the additional value of procalcitonin in responsible discontinuation of antibiotic treatment. To our knowledge, this is the largest procalcitonin study in the intensive care setting so far, with more than 1500 patients included. To emphasize the importance of safety, our study set the non-inferiority margin at 8% and estimated the sample size with a power of 90% instead of 80%. Ideally, if a lower non-inferiority margin such as 4% would be desirable, this would have required over 5500 patients. An unexpected finding was the observed lower mortality in the procalcitonin-guided group. We hypothesized that the reduced mortality in the procalcitonin-guided group was the result of an earlier focus on an alternative diagnosis if procalcitonin concentrations

were low. Alternatively, persistently high levels of procalcitonin concentrations might suggest the need to critically review antimicrobial treatment [31].

Several limitations of our study should be mentioned. First, approximately 30% of the patients randomized to the procalcitonin-guided strategy were discharged from the ICU before the algorithm recommended to stop the antibiotics. This figure was higher than the 20% we had anticipated when designing this study. Further reduction of antibiotics might have been achieved if procalcitonin guidance would have been continued on the wards. However, this study was designed for patients during ICU stay and continuation of the protocol on the ward was not deemed possible for logistic reasons.

Second, physicians did not adhere to the stopping advice in more half of the patients. The patients in whom antibiotic treatment was continued did differ in certain baseline characteristics from those that actually stopped antibiotics (appendix). Apparently, physicians use procalcitonin concentrations to show that antibiotics can be safely stopped in stable patients. They are, however, hesitant to stop when patients are not yet stable. Clearly, use of procalcitonin concentrations cannot convince them in such cases [32]. Whether discontinuation of antibiotics in these subjectively unstable patients would have led to higher mortality cannot be determined by this study. Procalcitonin measurements can be used to support decision making in stable patients, but does not abolish proper clinical reasoning. Despite this limitation overall antibiotic consumption was reduced, indicating that especially inappropriate antibiotics were the first to be discontinued. This is might turn out to be a major contributor to antibiotic stewardship.

Third, specific certain patients who were immunocompromised or treated for illnesses needing long durations of antibiotic treatment were excluded. These exclusions were chosen for safety and pragmatic reasons. Advice to stop antibiotic use in these patients would often be ignored and therefore considered not useful. However, we are not aware of any principal reasons why measuring procalcitonin would not be useful in reducing duration of treatment in these infections too, albeit over longer timescales or with other thresholds. Particularly in these patient groups earlier termination of antibiotic treatment might affect the overall consumption of antibiotics.

Fourth, clinicians were aware of the study group assignments and not all co-interventions that might have been affected by this knowledge could be assessed.

Fifth, we did not collect data for antibiotic resistance and, therefore, we are unaware of the appropriateness of the empirical antibiotic strategy. Additionally, in many patients

cultures were negative or did reveal bacteria that were not considered as true pathogens (eg. candida colonization in sputum cultures). The patients who did not reach a stopping rule might be the patients for whom the initial therapy was inappropriate or inadequate. Such patients might be detected earlier in the procalcitonin-guided arm than in the standard-of-care group, leading to an earlier antibiotic switch.

Conclusion

This large and pragmatic study shows that a reduction in antibiotic treatment duration and consumption can be achieved by the addition of a procalcitonin-guided algorithm to aid clinical judgment. This reduction of antibiotic duration was achieved in a setting with an already low background consumption of antibiotics without an increase in mortality.

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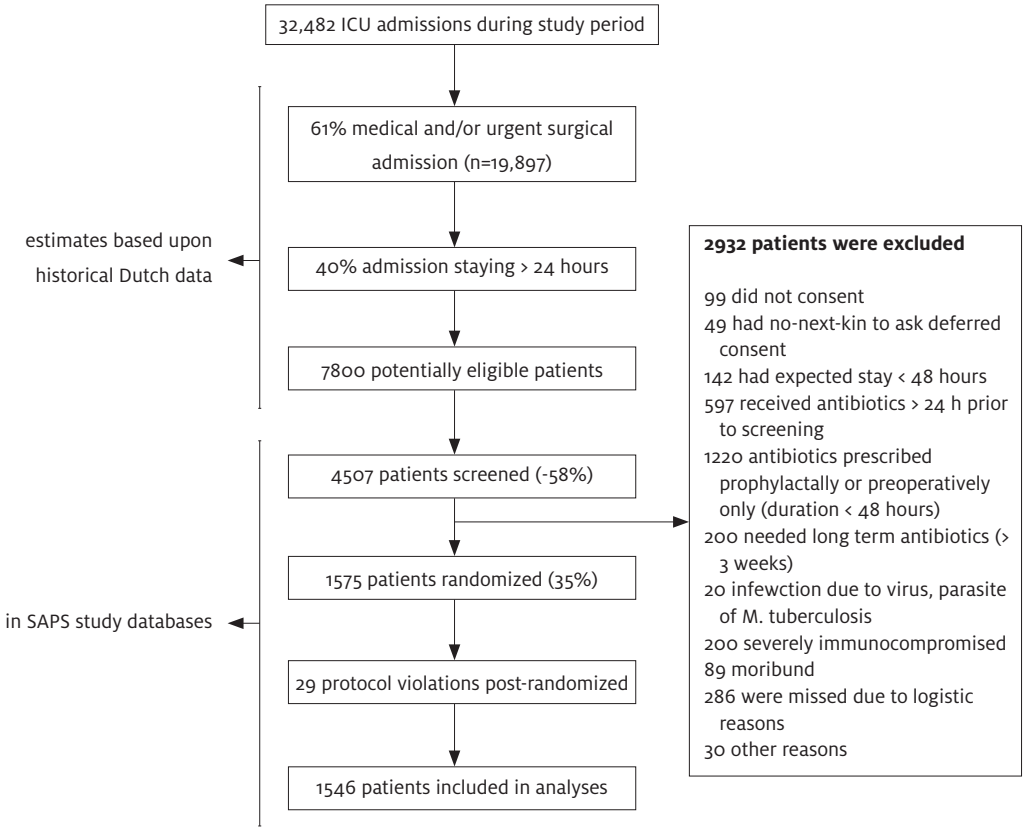
Appendix

Flow of patients in the participating centers



During the varying times the 15 ICUs participated in the SAPS study 32,482 patients were admitted. We estimated the loss of patients before screening leading to roughly 7800 patients that could have been eligible. Of those "potentially eligible patients" 4507 were screened according to the study logs and 1575 were randomized.

Daily defined dosage calculation and relationship with prices*

Name	1DDD (mg) i.v.	1DDD (mg) po	1DDD(€) i.v.	1DDD(€) p.o.
Amikacin	1000		19.74	
Amoxicillin	1000	1000	1.38	0.29
Amoxicillin and clavulanic acid	3000	1000	9.90	0.34
Azitromycin	500	300	0.22	0.22
Benzylpenicillin (units)	3000000		2.64	
Cefazolin	3000		8.70	
Cefotaxime	4000		22.72	
Ceftazidime	4000		36.55	
Ceftriaxone	2000		16.59	
Cefuroxime	3000		10.07	
Ciprofloxacin	500	1000	25.93	0.24
Clindamycin	1800	1200	14.67	1.56
Colistin	4000		30.06	
Trimethoprim-sulfamethoxazole	960	960	2.59	0.15
Doxycycline	100	100	3.72	0.15
Ertapenem	1000		46.79	
Erytromycin	1000	1000	13.37	0.35
Flucloxacillin	2000	2000	8.84	0.41
Gentamicin	240		5.60	
Glycylcycline (Tygecycline)	100		113.15	
Imipenem	2000		43.06	
Levofloxacin	500	500	105.15	0.15
Linezolid		1200		118.32
Meropenem	2000		44.45	
Metronidazol	1500	1500	10.81	0.63
Moxifloxacin	400	400	59.36	2.41
Norfloxacin		800		0.49
Ofloxacin		400		0.40
Piperacillin plus tazobactam	14000		34.10	
Rifampicin	600		6.67	
Teicoplanin	400		40.33	
Tetracycline		1000		0.49
Tobramycin	240		20.71	
Trimethoprim		400		0.27
Vancomycine	2000		29.63	

*This is not a fully complete list of antibiotics. although the most prescribed antibiotics are mentioned.

Daily Defined Dose (DDD) calculation

In 1996 the WHO developed the Anatomical Therapeutic Chemical (ATC)/DDD system as an international standard for drug utilization studies. In the Anatomical Therapeutic Chemical (ATC) classification system. the drugs are divided into different groups according to the organ or system on which they act and their chemical, pharmacological and therapeutic properties. Access to standardized and validated information on drug use is essential to allow audit of patterns of drug utilization, identification of problems, educational or other interventions and monitoring of the outcomes of the interventions.

In daily practice the ATC/DDD system of recommended prescribing can vary substantially from daily practice. In the instance of antibiotics the ATC/DDD system acts on the assumption of average infections that need a certain amount of antibiotics. In practice infections may be worse or less severe. warranting a different dosing. For more information. See http://www.whocc.no/atc_ddd_index (last visited 30th March 2015).

The basic definition of the defined daily dose (DDD) is:

The DDD is the assumed average maintenance dose per day for a drug used for its main indication in adults.

A DDD will only be assigned for drugs that already have an ATC code.

Example 1: Imipenem iv has a DDD of 2000 mg, which means that an average patient who receives imipenem uses 2 gram per day. If a patient consumes 10 grams over six days he has consumed 5 DDDs of this drug.

Example 2: Cefotaxime iv has a DDD of 4g, which means that an average patient who receives cefotaxim uses 4 gram a day. Gentamicin iv has a DDD of 240 mg. Realize that this represents an index figure rather than a clinically advised dosage. If a patient receives 480 mg gentamicin iv once daily during the first two days of admission and 4 gram of cefotaxim daily during five days. he can have said to have received 4 DDD of gentamicin and 5 DDD of cefotaxim, which makes 9 DDD in total for this patient.

Example 3: Ceftriaxone iv has a DDD of 2g, which means that an average patient who receives ceftriaxone uses 2 gram a day. If a patients admitted with meningitis receives 4g a day for seven days, he can have said to have received 14 DDD of this drug.

Selective decontamination of the digestive tract (SDD)

Introduction

Selective decontamination of the digestive tract has long been in used in several ICUs in the Netherlands. but it gained momentum after publication of a large randomized controlled trial in 2009 (De Smet et al. NEJM 2009;360:20-31). In this study patients were block-randomized between a standard-of-care period. a period in which selective decontamination of the digestive tract (SDD) was applied or selective oropharyngeal decontamination (SOD) was applied. This study resulted in an absolute mortality reduction of 3.5 and 2.9 percentage points for SDD and SOD respectively when compared to standard-of-care. After publication in 2009 more and more Dutch ICUs implemented either the SDD or the SOD strategy.

SDD/SOD

Most ICUs adhere to the protocol described in the study by De Smet et al. (NEJM 2009;360:20-31). SDD consists of a third generation cephalosporin (usually cefotaxime 4 times per day 1000 mg given

intravenously) during the first days (maximum duration of treatment was 4 days) and the application of three non-absorbable antimicrobials in the mouth (mouth paste. 4 times per day) and in the stomach (suspension through the nasogastric tube 4 times per day).The mouth paste contains polymyxin E, tobramycin and amphotericin B each in a 2% concentration and administration and was applied every 6 h. The gastric suspension (10 ml) contains 100 mg polymyxin E. 80 mg tobramycin and 500 mg amphotericin B was applied every 6h.

SDD and eligibility for the SAPS- study

If the patient was not suspected of having an infection, and the SDD was given according to protocol. then this patient was not eligible for inclusion in the SAPS-study.

If the patient was considered to have an infection and cefotaxime was used as part of the SDD-regimen as well as part of empirical antimicrobial treatment. then this patient was considered eligible for inclusion in our study. The entire course of intravenously given antimicrobials is counted for our defined daily dosage (DDD) and duration of treatment (DOT) calculations. The non-absorbable mouth paste and gastric suspension are not counted in our DDD and DOT calculations.

Example 1

A patient is seen on the emergency department (ED) for severe community-acquired pneumonia at 11 AM. This patient is immediately treated with ceftriaxone 2000 mg intravenously on the ED and subsequently intubated. Within 1h the patient is transferred to the ICU where he is started on SDD at 12 AM (cefotaxime 1000 mg every 6 h and mouth paste and gastric suspension every 6h). Additionally this patient is treated with ciprofloxacin 400 mg intravenously every 12h to cover atypical pathogens.

Such a patient could be included in the SAPS-study.

On day 1 (inclusion day):	
ceftriaxone 2000 mg once daily	= 1DDD
cefotaxime 1000 mg every 6h for a half day	= 0.5 DDD
ciprofloxacin 400 mg twice daily for a half day	= 0.5 DDD
total inclusion day	= 2 DDD

On day 2:	
cefotaxime 1000 mg every 6 h for a full day	= 1 DDD
ciprofloxacin 400 mg every 12 h for a full day	= 1 DDD
total day 2	= 2 DDD

Example 2

Another patient is seen on the emergency room with cardiogenic shock and subsequently admitted to the ICU. SDD is started, including a course of cefotaxime 4 times per day 1000 mg. After 4 days the cefotaxime is discontinued but the mouth paste and stomach suspension are continued until discharge from the ICU. However, on day 10 the patient develops a spiking fever and a catheter-related blood stream infection is suspected. The central venous catheter is removed and vancomycin 1000 mg twice daily is given. The patient is included in the SAPS-study on ICU-day 10.

On day 10:

Vancomycin 1000 mg every 12 h for a half day = 0.5 DDD

On day 11:

Vancomycin 1000 mg every 12 h for a full day = 1 DDD

On day 12:

Vancomycin dosage was adjusted according to therapeutic drug monitoring and dosage was decreased to 750 mg every 12 h. = 0.75 DDD

On day 13:

Based upon preliminary culture results ciprofloxacin 400 mg every 12 h was added.

Vancomycin 750 mg every 12 h for a full day = 0.75 DDD

Ciprofloxacin 400 mg every 12 h for a full day = 1 DDD

Total day 13 = 1.75 DDD

Table Reasons for non-adherence

	No.patients	Percentage (%)	Reason why physician did not adhere to stopping advice
	120	51.7%	Other reasons or not specified
	37	15.9%	Physician considers risk of discontinuation of antibiotics too high
	23	9.9%	Inflammation parameters are still too high
	21	9.1%	Stopping rule is given much earlier than the normal protocol
	18	7.8%	Patient still has fever
	13	5.6%	Patient is not stable enough to stop antibiotics
total	232	100%	

In 538/761 patients a stopping advice was issued while the patients were still on the ICU. In 306 patients this advice was followed within 24 hours and in 380 patients within 48 hours. In the 232 patients in which the antibiotics were not stopped within 24 hours the physicians provided the following reasons for "non-adherence" to the stopping advices.

PART V

Summary and future perspectives

CHAPTER 8

Summary and general discussion

In this thesis, we investigated whether biomarkers, as replacement of or adjunct to severity scores, could (1) improve early diagnosis of sepsis when Sepsis-3 definitions are applied, (2) provide early estimation of severity and predict prognosis in critically ill patients with pneumonia and aneurysmal subarachnoid hemorrhage, (3) predict prognosis and investigate the association with obesity in critically ill patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia, and (4) whether biomarkers can be used as a guide to tailor the duration of antibiotic treatment in critically ill patients with presumed bacterial infections / sepsis.

Biomarkers for diagnosis in sepsis

After a general introduction (**chapter 1**) we focused in **chapter 2** on the diagnostic accuracy of procalcitonin (PCT) [1,2] and C-reactive protein (CRP) [3] to predict proven infection, according to the Centers of Disease Control (CDC) criteria for specific types of infections in the acute care setting [4], in critically ill fulfilling the Sepsis-3 criteria. New Sepsis-3 definitions facilitate early recognition of patients with sepsis [5] and they are more specific for a detrimental outcome in critically ill patients with infection [6]. When these Sepsis-3 definitions are used as a fast screening test, there may be a role for biomarkers as a diagnostic aid for proven sepsis? We showed that PCT and CRP were not able to distinguish proven sepsis from non-proven sepsis in Sepsis-3 criteria-positive ICU patients. Furthermore, a decline in PCT and CRP in 5 days was not able to predict 28-day mortality. PCT and CRP measurements will not further help us as a diagnostic aid in predicting sepsis.

Biomarkers for prognosis in critically ill patients

In **chapter 3** we aimed to investigate the prognostic value of mid-regional pro-adrenomedullin (MR-proADM) [7] and mid-regional proatrial peptide (MR-proANP) [8] at baseline compared with the Acute Physiological and Chronic Health Evaluation (APACHE) IV model [9] and Sequential Organ failure Assessment (SOFA) score [9] to predict 28-day mortality in a mixed cohort of critically ill patients with proven pneumonia. The secondary aim was the prediction of 28-day mortality by clearance of MR-proADM and MR-proANP using serial measurements during 5 days when compared to APACHE IV model and SOFA score. No optimal cut-off points could be calculated in receiver operating characteristics (ROC) curves. Biomarkers and severity scores were divided in tertiles for the prediction of 28-day mortality in this study. The highest tertiles baseline MR-proADM and MR-proANP were not significant predictors for 28-day mortality in a multivariable model with age and APACHE IV. SOFA was not a significant predictor in

univariable analysis. Single baseline MR-proADM and MR-proANP were not superior to APACHE IV in predicting 28-day mortality in a mixed population of critically ill with pneumonia. Patients with low MR-proADM and MR-proANP clearance (first tertile) were significant predictors for 28-day mortality (hazard ratio (HR) 2.38, 95% CI 1.21 – 4.70, *p* 0.013 and HR 2.27, 95% CI 1.16 – 4.46, *p* 0.017) in a model with age and APACHE IV. We concluded that the predictive value of serial-measured MR-proADM and MR-proANP in predicting 28-day mortality in a model with age and APACHE IV exceeded those of single baseline measurements. Therefore, clearance of MR-proADM and MR-proANP in time may be more suitable for prognostication instead of single biomarker values. In **chapter 4** we assessed the ability of baseline C-terminal pro-arginine Vasopressin (CT-proAVP) [10] to predict disease outcome, mortality and delayed cerebral ischemia (DCI)[11,12] in critically ill patients with an aneurysmal subarachnoid hemorrhage (aSAH) [13] as compared with the World Federation of Neurological Surgeons (WFNS) [14] score and APACHE IV model [9]. Primary aim was to investigate the relationship between CT-proAVP and one-year poor functional outcome (Glasgow Outcome Scale score 1-3) [15] in a multivariable logistic regression model adjusted for WFNS and APACHE IV scores. Secondary aims were mortality and DCI. Diagnosis of DCI was based on acute clinical deterioration in the patient's neurologic condition and/or by the development of new focal neurological deficits. A CT brain perfusion, CT-angiography or DSA was performed in case of suspicion of DCI. The multivariable logistic regression model for DCI was also adjusted for the modified Fisher scale [16]. We concluded that single baseline CT-proAVP had good ability to predict one-year poor functional outcome in critically ill aSAH patients. ROC curves revealed high accuracy for CT-proAVP to identify patients with one-year poor functional outcome (area under the curve (AUC) 0.84, 95% confidence interval (CI) 0.77 - 0.92, $p < 0.001$). CT-proAVP $\geq$ 24.9 pmo/L proved to be a significant predictor for one-year poor functional outcome (odds ratio (OR) 8.04, 95% CI 2.97 - 21.75, $p < 0.001$) in multivariable models with WFNS and APACHE IV score. When CT-proAVP was combined with APACHE IV, the combination of APACHE IV and CT-proAVP yielded the highest AUC (0.87 (0.80 – 0.94)). CT-proAVP levels had also high accuracy to predict mortality (30-day mortality (AUC 0.84, 95% CI 0.76 - 0.93, $p < 0.001$) and one-year mortality (AUC 0.79, 95% CI 0.69 - 0.89, $p < 0.001$). CT-proAVP $\geq$ 29.1 pmol/L and $\geq$ 27.7 pmol/L, respectively, were significant predictors for 30-day and one-year mortality (OR 9.31, 95% CI 1.55 – 56.07, *p* 0.015 and OR 5.15, 95% CI 1.48 – 17.93, *p* 0.010) in multivariable models with WFNS and APACHE IV scores. Unexpectedly, CT-proAVP had a low AUC to identify patients with DCI (AUC 0.67, 95% CI 0.55 - 0.79, *p* 0.008), and CT-proAVP $\geq$ 29.5 pmol/L was not a significant predictor for DCI in a multivariable model adjusted for the modified Fisher scale (*p* 0.061). This was considered an important finding, as DCI was the most important treatable determinant of poor outcome after aSAH [11,12].

Biomarkers in critically ill patients with SARS-CoV-2 pneumonia

Finding a biomarker able to identify patients with worst outcome in the ICU emerged as a priority during the COVID-19 pandemic. In **chapter 5** we assessed the ability of MR-proADM and C-terminal proendothelin-1 (CT-proET-1) [17] to predict 28-day mortality in critically ill patients with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonia [18,19]. Biomarkers were collected during the first seven days in this prospective observational cohort study. We investigated the relationship between biomarkers and mortality in a multivariable Cox regression model adjusted for age and SOFA score [9]. MR-proADM and CT-proET-1 were significantly higher in 28-day non-survivors at baseline and over time. ROC curves revealed high accuracy to identify non-survivors for baseline MR-proADM and CT-proET-1, AUC 0.84, (95% CI 0.76 – 0.92), $p < 0.001$ and 0.79, (95% CI 0.69 – 0.89), $p < 0.001$, respectively. These AUCs were higher compared with existing severity scores. A combination of MR-proADM or CT-proET-1 with severity scores did not improve the already high AUC of the biomarkers to identify non-survivors. The AUC for prediction of 28-day mortality for MR-proADM and CT-proET-1 remained high over time. MR-proADM ≥ 1.57 nmol/L and CT-proET-1 ≥ 111 pmol/L at baseline were significant predictors for 28-day mortality (HR 6.80, 95% CI 3.12 – 14.84, $p < 0.001$ and HR 3.72, 95% CI 1.71 – 8.08, p 0.01). We concluded that baseline MR-proADM and CT-proET-1 had good ability to predict 28-day mortality in critically ill patients with SARS-CoV-2 pneumonia. Moreover, MR-proADM and CT-proET-1 appeared to be biomarkers with persistent strong prognostic value in the following days. MR-proADM and CT-proET-1 may help clinicians to identify patients at higher risk of adverse outcome and improve the decisions about ICU treatment. Patients affected by obesity and Corona virus disease 2019 (COVID-19), the disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), appear to have a higher risk for intensive care (ICU) admission [20,21]. In **chapter 6** we investigated whether obesity is associated with differences in new inflammatory biomarkers MR-proADM, CT-proET-1, and clinical outcome in critically ill patients with SARS-CoV-2 pneumonia. Hundred-five critically ill patients with SARS-CoV-2 pneumonia were divided in patients with obesity (defined as a body mass index (BMI) ≥ 30 kg/m², n = 42) and patients without obesity (BMI < 30 kg/m², n = 63) and studied in a retrospective observational cohort study. MR-proADM, CT-proET-1 concentrations and conventional markers white blood count (WBC), C-reactive protein (CRP), procalcitonin (PCT) were collected during the first seven days. BMI was 33.5 (IQR 32 - 36.1) kg/m² and 26.2 (IQR 24.7 - 27.8) kg/m² in the group with and without obesity. There were no significant differences in concentrations MR-proADM, CT-proET-1, WBC, CRP, and PCT at baseline and the next six days between patients with and without obesity. Only MR-

proADM changed significantly over time (p 0.039). Also, BMI did not correlate with inflammatory biomarkers (MR-proADM rho = 0.150, *p* 0.125, CT-proET-1 rho = 0.179, *p* 0.067, WBC rho = -0.044, *p* 0.654, CRP rho = 0.057, *p* 0.564, PCT rho = 0.022, *p* 0.842). Finally, no significant differences in time on a ventilator, ICU length of stay and 28-day mortality between patients with or without obesity were observed. We concluded that in critically ill patients with confirmed SARS-CoV-2 pneumonia, obesity was neither associated with baseline and time-course of MR-proADM, and CT-proET-1, nor with impaired outcome.

Clinical therapeutic applicability of biomarkers

Chapter 7 described the Stop Antibiotics on guidance of Procalcitonin Study (SAPS) in which the biomarker PCT was tested as a guide to tailor the duration of antibiotic treatment in critically ill patients with a presumed infection / sepsis in 15 ICUs in the Netherlands. The SAPS-trial revealed a significant reduction in median duration of prescribed antibiotics in the first 28 days for the PCT-guided intervention group (5 days [IQR 3 - 8 days] vs 7 days [IQR 4 - 10 days], $p < 0.001$) in 1546 critically ill patients. Guidance of antibiotic treatment by daily PCT measurements had to be safe. Based on earlier studies [22,23], we anticipated a mortality rate similar in both intervention and control group. However, SAPS revealed a survival benefit of PCT-guidance at 28 days (19.6% vs 25%, *p* 0.0012) and 1 year after randomization this survival benefit persisted (34.8% vs 40.9%, *p* 0.006). We supposed that PCT concentrations helped physicians in deciding whether or not the presumed infection was truly bacterial, leading to a more adequate diagnosis and treatment as possible explanation. Several limitations of the SAPS trial were addressed in the published article. First, the mechanisms underlying the survival benefit of PCT-guidance in the SAPS trial were not further clarified. Second, 30% of the patients in the PCT-guided intervention group were discharged from the ICU with antibiotics or died before reaching the stopping advice. Further reduction of antibiotic therapy could have been possible in the patients with early discharge if PCT-guidance would have been continued on the general wards. Third, a stopping criterion was reached in 557 patients in the PCT-guided group during their ICU stay. Antibiotic treatment was stopped in 53% of the patients, but non-adherence to this stopping criterion was 47%. This non-adherence percentage was comparable with previous PCT studies [23]. It was a stopping advice. Sensitivity and specificity were not high enough to withhold antibiotics on PCT alone [24]. Fourth, the SAPS trial revealed only limited data of costs reductions of PCT-guided therapy, a reduction of € 34,- in median cumulative costs of antibiotics per patient was reported. Fifth, no effects on the antimicrobial resistance due to shorter antibiotic treatment by PCT-guidance were reported in the SAPS trial.

Future perspectives

Sepsis is the most common cause of death in patients in the intensive care unit (ICU). Mortality of patients with sepsis in the ICU is persistently high and, depending on the severity of illness, mortality rates of 25-60% are reported [25-27]. Therefore, with the birth of the Sepsis-3 definitions more emphasis was placed on the early recognition and early goal-directed therapy of sepsis [5]. Hence, most physicians nowadays want to start antimicrobial therapy immediately after they have made the presumptive diagnosis of sepsis [5,26]. A clinical suspicion of infection is mandatory for diagnosing sepsis according to Sepsis-3 definitions [5]. However, the clinical diagnosis of sepsis upon ICU admittance corresponded poorly with the presence of infection in a retrospective cohort study with 2579 patients treated for sepsis in the ICU [28]. The plausibility of infection was determined post hoc in this study, based on CDC criteria for specific types of infections in the acute care setting [4]. Up to 43% of the patients treated for sepsis were unlikely to have an infection. These results corresponded with a French study found that 49% of the patients were unnecessarily treated for a new infection on the ICU [29]. This finding was based on the level of microbiological evidence. As the discrimination between infectious and non-infectious causes of critical illness in the ICU using clinical parameters only has proved challenging, multiple attempts have been performed using biomarkers, in particular CRP and PCT for diagnosis of infection in sepsis [30,31]. It is clear that CRP and PCT are neither "magic markers" nor "holy grail" for the positive diagnosis of proven infection in sepsis. Vice versa, low values of CRP and PCT have only a moderate negative predictive value and cannot be used to exclude sepsis in critical ill patients [32,33] As a matter of pragmatism, no biomarker alone should ever be used for diagnosis of sepsis. It is one tool in the clinician's armamentarium and is meant, rather than to replace, to complement clinician's judgement based on clinical examination, other laboratory tests, and microbiological results [32]. As a consequence, a physician cannot depend on a single CRP or PCT level to start or withhold antibiotics in the case of a presumed infection or sepsis in critically ill patients [34,35].

Research on biomarkers has shifted from single measurements to serial measurements for the discontinuation of antimicrobial therapy. To date, PCT is the most studied biomarker investigated in randomized controlled trials. The landmark randomized controlled trials of PCT-guided antimicrobial therapy in critically ill patients are listed in table 1. The first proof-of-concept study looking at ICU patients with sepsis found a reduction of antibiotics exposure, 3.5 days lower median duration of antibiotic therapy in the PCT guided group compared with the control group, without negative impact on outcome [22]. Antibiotics were stopped when PCT levels had decreased $\geq$ 90% from the peak value. The later multicenter PRORATA trial used daily PCT measurements to start and stop antibiotics in ICU patients with a presumed infection [23]. A PCT level of

0.5 μg/L was used as cut-off to start antibiotics and antibiotics were stopped when PCT levels dropped ≥ 80% from the peak concentration or when PCT were < 0.5 μg/L. The intervention group had significant more days without antibiotics, without significant differences in mortality. However, there was a trend toward a higher mortality. In the Stop Antibiotics on guidance of Procalcitonin (SAPS) study antibiotics were started in all 1546 critically ill patients with clinical suspicion of infection or sepsis, but were recommended to be discontinued when PCT levels declined ≥ 80% from the peak level and/or when PCT levels were ≤ 0.5 μg/L [36]. PCT-guided therapy resulted in lower duration antibiotic treatment (from 7 to 5 days) and in improved survival (5.4% and 6.1% better survival at 28 days and 1 year). The recently published PROGRESS trial investigated the value of serial PCT measurements to guide antibiotic treatment in 266 septic patients [37]. Antibiotics were prescribed for 5 days and were advised to discontinue when PCT had decreased ≥ 80% from baseline value or PCT values ≤ 0.5 μg/L. The rate of infection-associated adverse events was significantly lower (7.2% vs 15.3%) (primary endpoint). A significant reduction in median length of antibiotic therapy and 28-day mortality were reported as secondary endpoints. There have also been some studies with negative results. The PASS trial was an interventional study with the rational to improve survival by early antibiotic intervention and escalation any time when PCT was rising in critically ill patients [38]. Although diagnostic and therapeutic measures were escalated in the PCT-guided intervention group, there was no outcome benefit. The SisPCT trial was a randomized, clinical, 2x2 factorial trial in which patients were randomly assigned to receive sodium selenite and/or PCT-guided antimicrobial therapy [39]. There were no changes in outcome and duration of antibiotic therapy, but the PCT algorithm differed from previously used PCT algorithms. The ProACT trial, which studied the effects of single PCT measurements in lower respiratory tract infection in the emergency department (ED) of US hospitals, failed to demonstrate any effect of PCT guidance [40]. They used a PCT algorithm similar to prior studies with a cut-off of 0.5 μg/L, but included patients with low severity of disease and low likelihood of bacterial infection. The HiTEMP study investigated the value of PCT to guide antibiotic therapy in ED patients with fever in regard to antibiotic prescription, clinical outcomes and costs [41]. Single PCT measurements upon ED admission with a cut-off of 0.5 μg/L were used to initiate antibiotic treatment. They did not find any added benefit of PCT measurements in unselected patients with fever. Most probably due to the use of a high PCT cut-off point. In an open RCT in 2 Brazilian ICUs a CRP-based algorithm was compared with a PCT-based algorithm in critically ill patients with severe sepsis and septic shock [42]. The PCT algorithm used was similar to a previous algorithm [22]. There were no differences in duration of antibiotic therapy and 28-day mortality reported. Few RCTs have been reported on the use of CRP versus standard therapy for antibiotic management in adult critically ill patients [43,44]. Median antibiotic duration could be significantly reduced by CRP guidance in a smaller RCT

with 130 septic critically ill patients in Brazil [43] and CRP-guided therapy resulted in a median antibiotic duration of 7 days (IQR 6-10), non-inferior in terms of clinical failure to a fixed treatment of 14 days, in 170 Swiss patients with gram-negative bacteremia [44].

Table 1 | Randomized controlled trials of PCT-guided antimicrobial therapy in critically ill patients

Study	Patients	AB-stewardship design	Stopping criteria	AB duration in day PCT vs control	28-day mortality
Nobre (2008) [22]	Severe sepsis-septic shock	AB stop design	3-5 days AB + PCT ≥ 90% decrease to stop AB	6 (3-34) vs 9.5 (2-33) median (IQR) *p* 0.15	ns
Bouadma (2010) PRORATA trial [23]	Suspected infection / sepsis	AB start and stop design	PCT > 0.5 µg/L start AB PCT ≥ 80% decrease or < 0.5 µg/L stop AB	6.1 (6.0) vs 9.9 (7.1) mean (sd) *p* < 0.001	ns
Jensen (2011) PASS trial [38]	Critically ill patients	AB start, escalation and stop design	PCT > 1.0 µg/L escalation of diagnostics and AB PCT < 0.1 µg/L de-escalation	6 (3-11) vs 4 (3-10) median (IQR) ns	ns
Oliveira (2013) [42]*	Severe sepsis - septic shock	AB stop design	3 days AB + PCT ≥ 90% decrease to stop AB	7 (6 -8.5) vs 6 (5-7) median (IQR) ns	ns
de Jong (2016) SAPS trial [36]	Suspected infection / sepsis	AB stop design	PCT ≥ 80% decrease or ≤ 0.5 µg/L stop AB	5 (3 - 9) vs 7 (4 - 11) median (IQR) *p* < 0.001	19.6% vs 25% *p* 0.0012
Bloos (2016) SISPCT trial [39]	Severe sepsis – septic shock	AB stop + escalation design	PCT ≤ 1.0 µg/L or ≥50% decrease at day 7, 10, or 14 to stop AB	7 (3-12) vs 7 (3-12) median (IQR) ns	ns
Kyriazopoulou (2021) PROGRESS trial [37]	Suspected infection / sepsis	AB stop design	5 days AB + PCT ≥ 80% decrease or ≤ 0.5 µg/L at day 5 or later to stop AB	5 (5-7) vs 10 (7-15) median (IQR) *p* < 0.001	15.2% vs 28.2% *p* 0.02

*PCT compared with CRP as control group

Although the SAPS trial convincingly demonstrated that PCT-guidance was effective and safe in reducing the duration of antibiotic therapy and antibiotic usage in adult critically ill patients the 2016 Dutch Working Party on Antibiotics Policy (SWAB) guidelines for antimicrobial stewardship gave a weak recommendation to consider PCT-guidance in the ICU setting [45]. Absence of cost-effectiveness analysis and limited availability of PCT in Dutch hospitals were reported as reasons for the weak

recommendation [45]. Moreover, the Guideline committee was of the opinion that PCT-guidance of treatment duration of respiratory tract infections will have little effect on patient outcomes, as the recommended antibiotic therapy duration in Dutch hospitals (5 days) is already short [45]. However, the median duration of antibiotic treatment in the control group of the SAPS-trial, with predominantly patients with respiratory tract infections, was 7 days (IQR 4-11 days). The wide IQR suggests that physicians were reluctant to trust guidelines and preferred to prolong antibiotic treatment if they believed it was necessary. A cost-effectiveness analysis based on the patient data from the SAPS trial was published in 2018 [46]. The impact of PCT-guidance on in-hospital costs was negligible (minus € 65,-), and the lower in-hospital mortality resulted in a non-significant increase in costs over 1 year time frame (+ 3.8%). A weak recommendation was given by the 2021 international Surviving Sepsis Campaign guidelines using PCT to decide when to discontinue antibiotic therapy in adult patients with sepsis or septic shock and adequate source control if the optimal duration of therapy is unclear [26]. The overall quality of evidence of trials that assessed use of PCT to guide antibiotic treatment was judged to be low. The total duration of antibiotic therapy in many trials was already 7 days or longer in the intervention group and the algorithms for antibiotic therapy, frequency of PCT monitoring and thresholds for discontinuation differed a lot across the trials. As a result, nowadays serial PCT measurements are used on a limited scale to discontinue antibiotic duration in Dutch ICUs.

In addition to costs, inexperience with PCT and already short duration of antibiotic therapy in the Netherlands, another reason for limited use of serial PCT measurements might be absence of a clear explanation of the survival benefit achieved in the SAPS trial. The SAPS trial showed an unexpected, but significant reduction in both 28-day and 1-year mortality for PCT-guided discontinuation of antibiotic treatment (table 1) [36]. We hypothesized that PCT concentrations might have helped physicians in deciding whether or not the presumed infection was truly bacterial, leading to a more adequate diagnosis and treatment. This hypothesis was supported by a retrospective cohort study with 2579 patients treated for presumed sepsis at admittance of 2 Dutch ICUs [28]. Patients who were initially treated for sepsis, but subsequently diagnosed with noninfectious disease had a significant higher mortality rate compared with patients with an infection. The authors suggested that this observation may likely be due to variations in underlying pathology, but may also be due in part, to a delay in diagnosis and therapy resulting from misdiagnosis [28]. In addition, the survival benefit of PCT-guided antibiotic discontinuation in the SAPS trial was later confirmed by the smaller PROGRESS trial, in which the median length of antibiotic therapy and 28-day mortality were significantly reduced (table 1) [37]. The survival benefit was explained by a reduction in antibiotic related adverse events, in particular diarrhoea and acute kidney injury. The PROGRESS trial showed a significant higher incidence of diarrhea,

acute kidney injury, electrolyte disturbances, dehydration, elevated liver function tests and cardiac arrhythmias in the standard of care arm of the trial. We searched the SAPS database for diarrhoea, acute kidney injury, electrolyte disturbances, dehydration, elevated liver function tests and cardiac arrhythmias as adverse effects of antibiotics. Unfortunately, the database did only reveal the number of patients with acute kidney injury during the trial. There were no significant differences in serum creatinine and number of patients on renal replacement therapy between PCT-guided intervention group and controls during the first 14 days. In future research we should focus on diarrhoea, acute kidney injury, electrolyte disturbances, dehydration, elevated liver function tests and cardiac arrhythmias on a patient-level as possible explanations of the survival benefit from PCT-guided antibiotic therapy.

How to proceed with biomarkers in ICUs? Are biomarkers completely useless in critically ill patients? Concluding from this thesis, we can state that a single biomarker has limited diagnostic or prognostic value in heterogeneous populations. We demonstrated that PCT and CRP were not able to distinguish proven sepsis from non-proven sepsis in Sepsis-3 criteria-positive ICU patients. Both PCT and CRP failed to narrow down the potential patient group of patients with sepsis according to the Sepsis-3 definitions. Baseline and serial MR-proADM and MR-proANP measurements performed poorly as predictor of outcome in a mixed population of critically ill with pneumonia. On the other hand, biomarkers were very capable to provide early estimation of severity and predict prognosis in more homogeneous study populations. Especially, when these biomarkers were associated with dysregulated physiological pathways [47]. Baseline CT-proAVP had good ability to predict poor functional outcome and mortality in critically ill patients with aneurysmal subarachnoid hemorrhage. It might be argued that especially patients with highest values CT-proAVP at baseline would benefit from extended ICU therapy and patients with lower CT-proAVP values have a higher chance of good one-year functional outcome and could be discharged from the ICU to the general ward at an earlier stage. It has been hypothesized that the close relationship of CT-proAVP levels to the degree of activation of the pituitary-adrenal axis is the basis of its usefulness as prognostic biomarker in aSAH patients [10]. We must be careful about extrapolating these results to the general population. Results of single-centre studies are determined by the case-mix and resources of the particular ICU [48]. Therefore, the prognostic value of CT-proAVP in aSAH should first be tested in a larger multicenter prospective observational study compared to APACHE IV model and WFNS score. The combination of baseline CT-proAVP and APACHE IV [9] or WFNS [14] failed to improve outcome and mortality prediction, which may be a limiting factor to the implementation of CT-proAVP in clinical care in aSAH patients. Baseline and serial MR-proADM and CT-proET-1 had good ability to predict 28-day mortality in critically ill patients with confirmed SARS-CoV-2 pneumonia, but a combination with already

existing severity scores failed to improve prediction of 28-day mortality. Evidence of direct viral infection of the endothelial cell and diffuse endothelial inflammation was found in post-mortem analysis of COVID-19 patients [49]. These findings suggested that SARS-CoV-2 infection facilitated the induction of endothelial cell dysfunction and damage, endotheliitis [49,50]. Both adrenomedullin (ADM) and Endothelin-1 (ET-1) are released by endothelial and vascular smooth muscle cells due to pro-inflammatory cytokines. MR-proADM and CT-proET-1 are the more stable midregion and C-terminal part of the prohormones that correlate with the release of the active peptides [7,17]. It might be argued that patients with highest values MR-proADM and CT-proET-1 would benefit most from anti-inflammatory therapies such as steroids and interleukin-6 receptor antagonists.

Conclusions

Single baseline nor serial measurements of biomarkers alone should not be used to start or withhold antibiotic therapy in critically ill patients. The biomarkers PCT and CRP failed to narrow down the potential patient group of patients with sepsis according to the Sepsis-3 definitions. Biomarkers have limited value in prognosis in non-specified heterogeneous populations of critically ill patients. Biomarkers did have strong prognostic value in more homogeneous populations, especially if they fit dysregulated physiological pathways. Combinations of biomarkers and common severity scores failed to improve outcome prediction. Finally, serial PCT measurements have been proven safe, cost-effective and helpful to shorten or individualize antibiotic therapy in critically ill patients.

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CHAPTER 9

Algemene discussie en toekomstperspectief

In dit proefschrift hebben we onderzocht of biologische laboratoriumbepalingen (biomarkers) (1) de vroege diagnose van sepsis (bloedvergiftiging) door de nieuwe Sepsis-3 definitie konden verbeteren. Door enerzijds de bestaande voorspellende modellen te verbeteren of door deze volledig te vervangen. (2) We hebben ook onderzocht of deze biomarkers de ernst van ziekte en de uitkomt van intensive care patiënten met een longontsteking of een aneurysmatische subarachnoïdale hersenbloeding konden voorspellen. (3) Bij intensive care patiënten met een longontsteking door het coronavirus hebben we onderzocht of biomarkers de overleving in 28 dagen konden voorspellen en of de hoogte en het verloop van de biomarkers door obesitas bij deze patiënten beïnvloed zou worden. (4) Als laatst hebben we onderzocht of de duur van de antibiotica behandeling bij intensive care patiënten met behulp van dagelijkse bepalingen van biomarkers verkort kon worden.

Biomarkers voor sepsis diagnosis

Na de introductie in **hoofdstuk 1** onderzochten we in **hoofdstuk 2** de diagnostische kwaliteit van procalcitonine (PCT) [1,2] en C-reactive protein (CRP) [3] om bewezen infectie, zoals die beschreven zijn in de definities van de Centers of Disease Control (CDC) criteria voor specifieke type infecties in de acute zorg [4], aan te tonen bij intensive care patiënten die volgens de Sepsis-3 definities septisch waren. De nieuwe Sepsis-3 definities faciliteren de vroege herkenning van patiënten met sepsis [5] en zijn meer specifiek voor het aantonen van een slechte uitkomst in patiënten met een infectie [6]. Als deze Sepsis-3 definities gebruikt worden als test om patiënten snel te kunnen onderzoeken op sepsis, dan zou er een rol kunnen zijn voor biomarkers bij de diagnose van sepsis. Wij toonden aan dat zowel bepaling van PCT als CRP een onvoldoende discriminerend vermogen heeft om infectie te onderscheiden van niet-infectie. Een daling in PCT en CRP over 5 dagen was ook niet in staat om de overleden van de levenden in de eerste 28 dagen van elkaar te onderscheiden. PCT- en CRP-metingen zullen artsen niet helpen bij het verder aanscherpen van de diagnose sepsis.

Biomarkers voor prognose bij intensive care patiënten

In **hoofdstuk 3** hebben we de voorspellende waarde voor sterfte in de eerste 28 dagen van de biomarkers mid-regional proadrenomedullin (MR-proADM) [7] en mid-regional proatrial peptide (MR-proANP) [8] bij opname vergeleken met het Acute Physiological and Chronic Health Evaluation APACHE) IV model [9] en de Sequential Organ failure

Assessment (SOFA) score [9] in een groep van intensive care patiënten met een longontsteking. Het secundaire doel was de voorspelling van sterfte in 28 dagen door het verloop van de biomarkers in de eerste 5 dagen (biomarkerklaring) vergeleken met APACHE IV model en SOFA-score. In de receiver operating characteristics (ROC) curves konden we geen optimale afkappunt voor de biomarkers en voorspellende scores bepalen. Deze werden verdeeld in tertielen. De hoogste tertielen MR-proADM en MR-proANP bij opname waren geen significante voorspellers van sterfte in 28 dagen in een multivariabel model met leeftijd en APACHE IV. In deze groep intensive care patiënten met een pneumonie waren MR-proADM en MR-proANP bij opname niet beter dan APACHE IV in het voorspellen van sterfte in 28 dagen. De SOFA score was geen significante voorspeller van sterfte in 28 dagen in een eerdere univariabele analyse. Patiënten met de laagste klaring van MR-proADM en MR-proANP (1e tertiel) waren significante voorspellers van sterfte in 28 dagen (hazard ratio (HR) 2.38, 95% CI 1.21 – 4.70, *p* 0.013 en HR 2.27, 95% CI 1.16 – 4.46, *p* 0.017) in een model met leeftijd en APACHE IV. Wij concludeerden dat opeenvolgende metingen MR-proADM en MR-proANP beter sterfte in 28 dagen voorspelden dan enkele metingen bij opname. In **hoofdstuk 4** hebben we het vermogen van C-terminal pro-arginine Vasopressin (CT-proAVP) [10], vergeleken met de World Federation of Neurological Surgeons (WFNS) [14] score en APACHE IV model [9], om uitkomst van ziekte, mortaliteit en secundaire cerebrale ischemie [11,12] bij intensive care patiënten met een aneurysmatische subarachnoïdale hersenbloeding [13] te voorspellen onderzocht. Het primaire doel was het voorspellen van het slecht functioneren 1 jaar na ziekte (Glasgow Outcome Scale score 1-3) [15] door CT-proAVP in een multivariabel logistisch regressie model aangepast voor WFNS en APACHE IV scores. Secundaire doelen waren mortaliteit en secundaire cerebrale ischemie. Het model voor secundaire cerebrale ischemie was aangepast voor de modified Fisher scale [16]. Wij concludeerden dat CT-proAVP goed in staat was slecht functioneren 1 jaar na ziekte te voorspellen. Oppervlakte onder de curve 0.84, 95% betrouwbaarheidsinterval 0.77 - 0.92, $p < 0.001$ in ROC curves. CT-proAVP ≥ 24.9 pmo/L was een significante voorspeller voor slecht functioneren 1 jaar na ziekte (odds ratio (OR) 8.04, 95% betrouwbaarheidsinterval 2.97 - 21.75, $p < 0.001$) in een multivariabel model met WFNS en APACHE IV score. Het combineren van APACHE IV en CT-proAVP toonde het grootste oppervlakte onder de curve. CT-proAVP was goed in staat mortaliteit (na 30 dagen (oppervlakte onder de curve 0.84, 95% betrouwbaarheidsinterval 0.76 - 0.93, $p < 0.001$) en na 1 jaar (AUC 0.79, 95% betrouwbaarheidsinterval 0.69 - 0.89, $p < 0.001$). CT-proAVP ≥ 29.1 pmol/L en ≥ 27.7 pmol/L waren significante voorspellers voor mortaliteit na 30 dagen en 1 jaar (OR 9.31, 95% betrouwbaarheidsinterval 1.55 – 56.07, *p* 0.015 and OR 5.15, 95% betrouwbaarheidsinterval CI 1.48 – 17.93, *p* 0.010) in multivariabele modellen met WFNS en APACHE IV scores. CT-proAVP had een laag oppervlakte onder de curve voor het aantonen van patiënten met secundaire cerebrale ischemie 0.67, 95% betrouwbaarheidsinterval 0.55 - 0.79, *p* 0.008) en CT-proAVP ≥ 29.5

pmol/L was geen significante voorspeller voor secundaire cerebrale ischemie in een multivariabel model gecorrigeerd voor de modified Fisher scale (p 0.061). Dit was een belangrijke bevinding daar secundaire ischemie een behandelbare oorzaak is van slecht functioneren 1 jaar na ziekte[11,12].

Biomarkers in intensive care patiënten met SARS-CoV-2 pneumonie

Tijdens de COVID-19 pandemie was er grote behoefte aan biomarkers die patiënten met een slechte prognose op de intensive care snel konden identificeren. In **hoofdstuk 5** hebben we het vermogen van MR-proADM en C-terminal proendothelin-1 (CT-proET-1) [17] om sterfte binnen 28 dagen bij intensive care patiënten met een severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pneumonie [18,19] te voorspellen onderzocht. In deze prospectieve observationale cohortstudie werden de biomarkers gedurende de eerste 7 dagen intensive care verzameld. Wij onderzochten de relatie tussen biomarkers en mortaliteit in en multivariabel Cox regressiemodel gecorrigeerd voor leeftijd en SOFA score [9]. MR-proADM en CT-proET-1 waarden bij opname en de volgende dagen waren significant hoger in patiënten die stierven in 28 dagen. ROC-curves toonde dat MR-proADM en CT-proET-1 bij opname patiënten die binnen 28 dagen stierven met grote accuraatheid konden identificeren. Oppervlaktes onder de curve 0.84, (95% betrouwbaarheidsinterval 0.76 – 0.92), $p < 0.001$ en 0.79, (95% betrouwbaarheidsinterval 0.69 – 0.89), $p < 0.001$. De voorspelende waarde van de biomarkers op niet overleven in 28 dagen was hoger dan van de bestaande prognostische modellen. MR-proADM of CT-proET-1 gecombineerd met de bestaande prognostische modellen verhoogden de voorspellende waarde op niet overleven niet verder. MR-proADM $\geq$ 1.57 nmol/L en CT-proET-1 $\geq$ 111 pmol/L bij opname waren significante voorspellers voor sterfte binnen 28 dagen (HR 6.80, 95% CI 3.12 – 14.84, $p < 0.001$ en HR 3.72, 95% CI 1.71 – 8.08, p 0.01). Wij concluderen dat MR-proADM en CT-proET-1 goed in staat zijn om sterfte binnen 28 dagen bij intensive care patiënten met een SARS-CoV-2 pneumonie te voorspellen. Deze sterke prognostische waarde behielden de biomarkers in de opeenvolgende dagen. MR-proADM en CT-proET-1 zouden artsen kunnen helpen om patiënten met een hoger risico op overlijden sneller te identificeren en zo de intensive care behandeling te verbeteren. Patiënten met obesitas en COVID-19, de ziekte veroorzaakt door het severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) lijken een groter risico op intensive care opname te hebben [20,21]. In **hoofdstuk 6** onderzochten we de relatie tussen obesitas en nieuwe inflammatoire biomarkers MR-proADM, CT-proET-1 en het klinisch verloop in intensive care patiënten met een SARS-CoV-2 pneumonie. Honderdvijf intensive care

patiënten met een SARS-CoV-2 pneumonie warden verdeeld in patiënten met obesitas (body mass index (BMI) ≥ 30 kg/m², n = 42) en patiënten zonder obesitas (BMI < 30 kg/m², n = 63) en bestudeerd in een retrospectieve observationele cohortstudie. MR-proADM, CT-proET-1 en conventionele markers leukocytengetal, C-reactive protein (CRP), procalcitonine (PCT) werden gedurende de eerste 7 dagen verzameld. BMI was 33.5 (32 - 36.1) en 26.2 (24.7 - 27.8) kg/m² in de groepen met en zonder obesitas. Er waren geen verschillen in de concentraties MR-proADM, CT-proET-1, leukocytengetal, CRP en PCT tussen de 2 groepen gedurende de eerste 7 dagen. Alleen het MR-proADM veranderde over de tijd (*p* 0.039). BMI waardes correleerden niet met inflammatoire biomarkers (MR-proADM rho = 0.150, *p* 0.125, CT-proET-1 rho = 0.179, *p* 0.067, WBC rho = -0.044, *p* 0.654, CRP rho = 0.057, *p* 0.564, PCT rho = 0.022, *p* 0.842). Er waren tenslotte geen significante verschillen in beademingsduur, verblijfsduur op de intensive care en sterfte binnen 28 dagen tussen de patiënten met en zonder obesitas. Wij concludeerden dat obesitas geen relatie had met verschillen in MR-proADM, CT-proET-1 of een slechtere uitkomst van ziekte.

Therapeutische toepassingen van biomarkers in de kliniek

Hoofdstuk 7 beschrijft de Stop Antibiotica op geleide van de Procalcitonine Studie (SAPS), waarin PCT de duur van de behandeling met antibiotica bij intensive care patiënten met de verdenking op een infectie / sepsis in 15 intensive care units in Nederland begeleidde. De SAPS-studie toonde in de PCT-geleide interventie groep een significante vermindering in de mediane antibiotica duur gedurende de eerste 28 dagen (5 dagen [IQR 3 - 8 dagen] versus 7 dagen [IQR 4 - 10 dagen], *p* < 0.001) in 1546 intensive care patiënten. Sturing van antibiotica door dagelijkse PCT-metingen moest veilig zijn. Op basis van eerdere studies [22,23] verwachtten wij geen verschil in sterfte tussen interventie - en controlegroep. Tot onze verbazing was er een overlevingsvoordeel op 28 dagen en 1 jaar voor de interventiegroep (19.6% versus 25%, *p* 0.0012 en 34.8% versus 40.9%, *p* 0.006). Een mogelijke verklaring voor het overlevingsvoordeel kan zijn dat PCT-bepalingen artsen kunnen helpen een ware bacteriële infectie te identificeren, tot een adequate diagnose te komen en zo de behandeling te verbeteren. Een aantal beperkingen van deze studie werden in het gepubliceerde artikel besproken. Ten eerste, de onderliggende mechanismen aan het overlevingsvoordeel van PCT zijn in de SAPS-studie niet verder uitgewerkt. Ten tweede, 30% van de patiënten in de PCT-interventiegroep waren al van de intensive care ontslagen of overleden voordat het antibiotica stop advies bereikt was. PCT-begeleiding had een nog groter effect kunnen hebben indien PCT- begeleiding doorgegaan was op de verpleegafdeling.

Ten derde, 557 patiënten kregen een antibiotica stop advies. Antibiotica werden in 53% van de patiënten gestaakt en in 47% van de patiënten werd dit advies genegeerd. Het percentage van het niet-naleven was vergelijkbaar met eerdere PCT-studies [23]. Het was een stop-advies. De sensitiviteit en specificiteit van PCT waren niet hoog genoeg om antibiotica te staken op PCT-metingen alleen [24]. Ten vierde, de SAPS-studie toonde beperkt informatie over reductie in kosten door PCT-begeleiding, een € 34,- kostenbesparing aan antibiotica door PCT-begeleiding werd vermeld. Tot slot, de gevolgen van een korte antibioticaduur door PCT-metingen op de antimicrobiële resistentie werd in de SAPS-studie niet gerapporteerd.

Toekomstperspectieven

Sepsis is vandaag de dag de meest voorkomende oorzaak van sterfte en intensive care (IC) verblijf. Het sterftegetal van sepsis patiënten is hoog en afhankelijk van de ernst van ziekte worden sterftegetallen van 25-60% vermeld [25-27]. Er wordt daarom met de komst van de Sepsis-3 definities meer de nadruk op vroege herkenning en vroeg starten van antibiotica therapie [5]. Vandaar dat de meeste artsen vandaag de dag de antibiotica behandeling direct willen starten het moment dat zij sepsis vermoeden [5,26]. Verdenking op een infectie is een verplicht onderdeel voor de diagnose sepsis volgens de Sepsis-3 definitie [5]. De klinische diagnose sepsis bij opname intensive care correleerde matig met de aanwezigheid van een infectie in een retrospectieve cohortstudie met 2579 patiënten die op de intensive care voor sepsis behandeld werden [28]. De aannemelijkheid van een infectie, gebaseerd op de CDC-criteria voor specifieke types van infectie [4], werd achteraf bepaald. Bij in totaal 43% van de patiënten behandeld voor sepsis was een infectie zeer onwaarschijnlijk. Deze resultaten komen overeen met een Franse studie waarin 49% van de patiënten onnodig werden behandeld voor een nieuwe infectie op de intensive care [29]. De diagnose infectie was gebaseerd op microbiologische kweekuitslagen. Het onderscheid tussen een infectieuze en niet-infectieuze oorzaak van ziekte binnen de intensive care op basis van alleen klinische parameters is moeilijk. Diverse pogingen zijn gedaan om met biomarkers (CRP en PCT) infectie binnen sepsis vast te stellen [30,31]. Het is duidelijk geworden dat CRP en PCT geen magische markers zijn voor het aantonen van een bewezen infectie binnen de sepsis definitie [32,33]. Anderzijds, lage waarden van CRP en PCT hebben een beperkte negatief voorspellende waarde en kunnen daarom niet gebruikt worden om sepsis uit te sluiten in intensive care patiënten [32,33]. Pragmatisch gezien moet de diagnose sepsis niet gesteld worden op alleen een biomarker. De biomarker is onderdeel van de gereedschapskist van de arts en kan als aanvulling dienen op de klinische beoordeling door de arts. Deze klinische beoordeling is gebaseerd op lichamelijk onderzoek, overige laboratoriumbepalingen en microbiologische kweekuitslagen [32]. Daardoor

kan een arts niet alleen CRP- of PCT -bepalingen vertrouwen om antibiotica te starten of te onthouden bij intensive care patiënten met de verdenking op een infectie of sepsis [34,35].

Tabel 1 | Randomized controlled trials met PCT-geleide antimicrobiële therapie bij intensive care patiënten

Studie	Patiënten	AB-begeleiding design	Stop criteria	AB-duur in dagen PCT versus controles	28-dagen sterfte
Nobre (2008) [22]	Ernstige sepsis-septische shock	AB stop design	3-5 dagen AB + PCT ≥ 90% daling to stop AB	6 (3-34) vs 9.5 (2-33)	ns
Bouadma (2010) PRORATA trial [23]	Verdenking infectie / sepsis	AB start and stop design	PCT > 0.5 µg/L start AB	6.1 (6.0) vs 9.9 (7.1) mean (sd) $p < 0.001$	ns
Jensen (2011) PASS trial [38]	Intensive Care patiënten	AB start, escalatie en stop design	PCT > 1.0 µg/L escalatie van diagnostiek en AB PCT < 0.1 µg/L de-escalatie	6 (3-11) vs 4 (3-10) median (IQR) ns	ns
Oliveira (2013) [42]*	Ernstige sepsis - septische shock	AB stop design	3 days AB + PCT ≥ 90% daling stop AB	7 (6 -8.5) vs 6 (5-7) median (IQR) ns	ns
de Jong (2016) SAPS trial [36]	Verdenking infectie / sepsis	AB stop design	PCT ≥ 80% daling of ≤ 0.5 µg/L stop AB	5 (3 - 9) vs 7 (4 - 11) median (IQR) $p < 0.001$	19.6% vs 25% p 0.0012
Bloos (2016) SISPCT trial [39]	Ernstige sepsis - septische shock	AB stop + escalatie design	PCT ≤ 1.0 µg/L or ≥50% daling op dag 7, 10, or 14 stop AB	7 (3-12) vs 7 (3-12) median (IQR) ns	ns
Kyriazopoulou (2021) PROGRESS trial [37]	Verdenking infectie / sepsis	AB stop design	5 dagen AB + PCT ≥ 80% daling of ≤ 0.5 µg/L dag 5 of later stop AB	5 (5-7) vs 10 (7-15) median (IQR) $p < 0.001$	15.2% vs 28.2% p 0.02

*PCT vergeleken met CRP als controlegroep

Onderzoek naar biomarkers is verschoven van enkele bepalingen naar opeenvolgende metingen voor het verkorten van de antimicrobiële therapie. Vandaag de dag is PCT de meest onderzochte biomarker in gerandomiseerde studies. De belangrijkste gerandomiseerde gecontroleerde studie met PCT-geleide antimicrobiële therapie in intensive care patiënten worden in tabel 1 vermeld. De eerste proof-of-concept studie die zich richtte op intensive care patiënten met sepsis vond een reductie van 3.5 dag antibiotica therapie in de PCT-geleide interventiegroep, zonder negatieve gevolgen [22].

Antibiotica werden gestaakt indien PCT ≥ 90% van de piekwaarde gedaald was. De latere multicenter PRORATA-studie maakte gebruik van dagelijkse PCT-metingen om antibiotica te starten en staken bij intensive care patiënten met een vermeende infectie [23]. Bij een PCT-waarde van 0.5 ≥ 90% werden antibiotica gestart en antibiotica werden gestaakt indien PCT > 80% van de piekwaarde gedaald was of indien PCT < 0.5 μg/L was. De interventiegroep had significant meer dagen zonder antibiotica, zonder significante verschillen in mortaliteit. Er was wel een trend naar een hogere mortaliteit in de interventiegroep. In de Stop Antibiotica op geleide van de Procalcitonine Studie (SAPS) werden antibiotica gestart in alle 1546 intensive care patiënten met een klinische verdenking op een infectie of sepsis, maar een stopadvies werd gegeven indien PCT-waarden ≥ 80% van de piekwaarde gedaald waren of indien PCT-waarden ≤ 0.5 μg/L waren [36]. PCT-geleide therapie resulteerde in een kortere duur antibiotica (van 7 naar 5 dagen) en in een verbeterde overleving (5.4% en 6.1% betere overleving na 28 dagen en 1 jaar). De recent gepubliceerde PROGRESS-studie onderzocht de waarde van dagelijkse PCT-metingen bij het begeleiden van de antibioticaduur in 266 septische patiënten [37]. Antibiotica werden voor 5 dagen voorgeschreven en een stopadvies werd daarna gegeven indien PCT ≥ 80% ten opzichte van de baseline waarde gedaald was of indien PCT ≤ 0.5 μg/L was. Ook zij rapporteerden een significante reductie in de mediane antibioticaduur, sterfte binnen 28 dagen en een significante daling in infectie-gerelateerde bijwerkingen (7.2% versus 15.3%). Er zijn ook een aantal negatieve studies gepubliceerd. De PASS-studie was een interventiestudie met als doel de overleving te verbeteren bij vroegtijdig antibiotica en escalatie van therapie bij stijgend PCT-waarden [38]. In de interventiegroep was er een toename van therapeutische interventies, maar geen verbetering in uitkomst van ziekte. De SisPCT-studie was een gerandomiseerde, 2x2 factorial studie waarin patiënten gerandomiseerd werden in sodium-selenium therapie en/of PCT-geleide sturing van de antibioticaduur [39]. Er waren geen verschillen in uitkomst en duur van de antibiotica therapie. Het PCT-algoritme verschilde wel van de eerdere studies. De ProACT-studie bestudeerde het effect van enkelvoudige PCT-bepalingen op patiënten met lage luchtweginfecties op de spoedeisende hulp in de VS [40]. PCT-geleide therapie gaf geen voordeel. Het gebruikte PCT-algoritme was vergelijkbaar met eerdere studies met een PCT-afkappunt van 0.5 μg/L, maar veel van de geïncludeerde patiënten waren niet ernstig ziek en hadden een lage a priori kans op een infectie. De HiTEMP-studie onderzocht de waarde van PCT-begeleiding van antibiotica bij patiënten met koorts op de spoedeisende hulp met betrekking op het voorschrijven van antibiotica, uitkomst van zieke en kosten [41]. Antibiotica werden gestart indien PCT-waarden > 0.5 μg/L waren. PCT-bepalingen hadden een geen effect bij niet-geselecteerde patiënten met koorts op de spoedeisende hulp. Meest waarschijnlijk was een te hoog PCT-afkappunt gebruikt. In 2 een open gerandomiseerde studie op 2 Braziliaanse intensive care units werd een CRP-geleid algoritme met een PCT-geleid algoritme vergeleken bij patiënten

met ernstige sepsis en septische shock [42]. Het PCT-algoritme was vergelijkbaar met eerdere PCT-algoritmes [22]. Er waren geen verschillen in antibioticaduur en sterfte binnen 28 dagen gerapporteerd. In slechts weinig gerandomiseerde studies werd een CRP-geleid algoritme voor antibiotica behandelduur met standaardtherapie in volwassen intensive care patiënten vergeleken [43,44]. De mediane antibiotica duur kon significant gereduceerd worden met CRP-begeleiding in een kleinere gerandomiseerde studie met 130 septische patiënten in Brazilië [43] en CRP-begeleiding resulteerde in een mediane antibiotica behandelduur van 7 dagen (IQR 6-10), niet inferieur vergeleken met een standaard 14 dagen antibioticabeleid in 170 patiënten met gramnegatieve bacteriemie in Zwitserland [44].

Hoewel de SAPS-studie duidelijk demonstreerde dat PCT-begeleiding effectief en veilig was in het verkorten van de antibioticaduur bij intensive care patiënten gaf richtlijn "Antimicrobial Stewardship" van de Stichting Werkgroep Antibioticabeleid (SWAB) een zwakke aanbeveling voor het gebruik van PCT [45]. Afwezigheid van een analyse naar kosten en effectiviteit en een beperkt gebruik van PCT in Nederlandse ziekenhuizen waren de belangrijkste reden voor een zwakke aanbeveling. SWAB was bovendien van mening dat begeleiding van de antibioticaduur met PCT bij respiratoire infecties weinig effect op de uitkomst van ziekte zou hebben, daar de aanbevolen duur van antibiotica (5 dagen) in Nederlandse ziekenhuizen al kort is. De mediane duur van antibiotica was echter 7 dagen (IQR 4-11 dagen) in de controlegroep van de SAPS-studie, met overwegend patiënten met luchtweginfecties. De wijde IQR suggereerde dat artsen terughoudend waren in het volgen van de richtlijnen en er de voorkeur aan gaven de antibioticaduur te verlengen als zij geloofden dat dit nodig was. Een kosten-effectiviteitanalyse gebaseerd op de patiëntengegevens van de SAPS-studie werd in 2018 gepubliceerd [46]. De impact van PCT-sturing op ziekenhuiskosten was verwaarloosbaar (min €65,-) en de lagere ziekenhuissterfte resulteerde in een niet significante toename in kosten in 1 jaar (+ 3.8%). Een zwakke aanbeveling werd ook gegeven door de 2021 internationale Surviving Sepsis Campaign richtlijn om PCT te gebruiken antibiotica te staken in patiënten met sepsis of septische shock en adequate controle van de bron van infectie bij wie de optimale duur van infectie onbekend is [26]. De Surviving Sepsis Campaign oordeelde dat het geleverde bewijs voor begeleiding van de antibioticaduur door PCT laag was. De totale antibioticaduur was in de interventiegroep vaak al 7 of meer dagen en de PCT-algoritmes verschilden veel van elkaar. Met als gevolgd dat vandaag de dag maar op een beperkte schaal gebruik wordt gemaakt van PCT in Nederlandse intensive care units.

In aanvulling op kosten, onervarenheid met PCT en een al korte antibioticaduur in Nederland, is een andere reden voor het beperkt gebruiken van PCT het afwezig zijn van een duidelijke verklaring voor het overlevingsvoordeel in de SAPS-studie. De SAPS-

studie toonde een significante verlaging van zowel de sterfte in 28 dagen als 1 jaar in de PCT-geleide interventiegroep (tabel 1) [36]. Wij vermoedden dat PCT-waarden artsen kan helpen in het onderscheiden of een vermeende infectie daadwerkelijk een bacteriële origine heeft, dat dan weer kan leiden tot een adequate diagnose en een meer gerichte therapie. Deze verklaring wordt ondersteund door een retrospectieve cohortstudie met 2579 patiënten met verdenking sepsis bij opname op 2 Nederlandse intensive care units [28]. De patiënten die initieel behandeld werden voor sepsis, maar in retrospectie een niet-infectieuze diagnose hadden, hadden een significant hogere sterfte vergeleken met de patiënten die wel septisch bleken. De auteurs veronderstelden dat deze observatie ten deel het gevolg was van de onderliggende pathologie, maar ook voor een deel gevolg kan zijn van een vertraging in diagnostiek en therapie ten gevolge van de verkeerde diagnose [28]. Een voordeel in overleving van PCT-geleide antibiotica therapie werd later bevestigd in de kleinere PROGRESS-studie, waarin de mediane antibioticaduur en de sterfte binnen 28 dagen significant lager uitvielen in de PCT-interventiegroep (tabel 1) [37]. Het voordeel in overleving werd verklaard door een vermindering in het aantal antibiotica gerelateerde bijwerkingen, in het bijzonder diarree en acute nierinsufficiëntie. De PROGRESS-studie toonde een significant hogere incidentie diarree, acute nierinsufficiëntie, elektrolyten stoornissen, dehydratie, acute leverproefstoornissen, hartritmestoornissen als nadelige effecten van antibiotica. Helaas had de SAPS-database alleen gegevens over het aantal patiënten met acute nierinsufficiëntie. Er waren geen significante verschillen in serum creatinine en het aantal patiënten aan niervervangende therapie tussen de interventie- en controlegroep gedurende de eerste 14 dagen. Verder onderzoek zou zich meer moeten focussen op diarree, acute nierinsufficiëntie, elektrolytstoornissen, dehydratie, gestoorde leverfunctieproeven en hartritmestoornissen op patiënten niveau als mogelijke verklaring voor het voordeel in overleving voor PCT-geleid antibioticabeleid.

Hoe nu verder met biomarkers op intensive care units? Zijn biomarkers volkomen nutteloos bij intensive care patiënten? Concluderend uit dit promotieonderzoek kunnen we stellen dat een enkele biomarker bepaling beperkte diagnostische en prognostische waarde heeft in heterogene populaties. Wij toonden aan dat PCT en CRP niet in staat waren bewezen sepsis van niet-bewezen sepsis in Sepsis-3 positieve patiënten te onderscheiden. PCT en CRP waren niet in staat de potentiële groep patiënten met sepsis te verkleinen. MR-proADM en MR-proANP bepalingen deden het matig als voorspeller van uitkomst van ziekte in een gemende populatie intensive care patiënten met een pneumonie. Aan de andere kant waren biomarkers goed in staat een vroege indicatie van ernst van ziekte te geven en prognose te voorspellen in een meer homogene studiepopulatie. In het bijzonder wanneer deze biomarkers geassocieerd waren met dysregulatie van fysiologische orgaansystemen. [47]. CT-proAVP bij opname was goed in staat om een slechte ziekte uitkomst en sterfte te voorspellen in intensive

care patiënten met een aneurysmatische subarachnoïdale hersenbloeding. We zouden kunnen stellen dat patiënten met hoge CT-proAVP waarden bij opname voordeel kunnen hebben van extra intensive care behandeling en anderzijds dat patiënten met lage CT-proAVP waarden een betere kans op goed herstel na 1 jaar hebben en eerder naar de verpleegafdeling gestuurd kunnen worden. De nauwe relatie van CT-proAVP waarden met de activatie van de hypofyse-bijnierschors as zou de bruikbaarheid van deze biomarker voor prognose in aneurysmatische subarachnoïdale hersenbloeding kunnen verklaren [10]. Met het extrapoleren van de studieresultaten naar de algemene populatie moeten we voorzichtig zijn. Resultaten van één centrum worden bepaald door de case-mix en middelen van die intensive care unit [48]. De prognostische waarde van CT-proAVP vergeleken met APACHE IV en WFNS score moet daarom eerst in een observationele studie met meerdere centra getest worden. De combinatie van CT-proAVP bij opname met APACHE IV [9] en WFNS [14] verbeterde niet de voorspelling van uitkomst van ziekte en sterfte. Dit kan een beperkende factor voor implementatie van CT-proAVP in de klinische zorg van patiënten met een aneurysmatische subarachnoïdale hersenbloeding zijn. MR-proADM en CT-proET-1 bij opname en de opéénvolgende dagen waren goed in staat om 28 dagen sterfte te voorspellen in intensive care patiënten met een bewezen SARS-CoV-2 longontsteking, maar de combinatie met de al langer bestaande voorspellende modellen was niet in staat de sterfte binnen 28 dagen beter te voorspellen. Bewijs voor een virale infectie van de endotheelcel en diffuse ontsteking van het endotheel werd gevonden in post-mortem onderzoek van COVID-19 patiënten [49]. Deze bevindingen zijn suggestief voor de inductie van endotheelcel dysfunctie en schade, endotheliitis, door de SARS-CoV-2 infectie [49,50]. Zowel adrenomedullin (ADM) en Endothelin-1 (ET-1) worden vrijgegeven door endotheelcellen en de gladde spiercellen van de bloedvaten als respons op pro-inflammatoire cytokines. MR-proADM en CT-proET-1 zijn de meer stabiele midregion en C-terminal gedeelte van de prohormonen die correleren met de uitscheiding van de actieve peptiden [6,17]. Men zou kunnen stellen dat patiënten met de hoogste waarden MR-proADM en CT-proET-1 het meeste profijt kunnen hebben van corticosteroïden en interleukine-6 receptor antagonisten

Conclusies

Enkelvoudige biomarker bepalingen bij opname of tijdens opéénvolgende dagen moeten niet gebruikt worden om antibiotica enerzijds te starten of anderzijds te onthouden bij intensive care patiënten. De biomarkers PCT en CRP waren niet in staat de groep intensive care patiënten met verdenking sepsis volgens de Sepsis-3 definities te versmallen. Biomarkers hebben slechts beperkte prognostische waarde in heterogene populaties intensive care patiënten met diverse ziektebeelden.

Biomarkers kunnen een sterke prognostische waarde hebben in meer homogene patiëntengroepen, vooral als ze passen bij ontregelde fysiologische orgaansystemen. Combinaties biomarker met langer bestaande voorspellende modellen gaven geen verbetering van voorspelling van uitkomst van ziekte. Tot slot, opéénvolgende metingen PCT zijn veilig, kosten-efficiënt en doeltreffend gebleken in het verkorten of individualiseren van de antibioticakuur in intensive care patiënten.

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PART VI

Appendices

List of abbreviations

ACE2	angiotensin converting enzyme 2
ACTH	adrenocorticotrophic hormone
ADH	antidiuretic hormone
ADM	adrenomedullin
ANP	atrial-natriuretic peptide
APACHE IV	acute physiology and chronic health evaluation IV
ARDS	acute respiratory distress syndrome
aSAH	aneurysmal subarachnoid hemorrhage
AUC	area under the curve
AVP	arginine vasopressin
BMI	body mass index
CAP	community-acquired pneumonia
CDC	Centers of Disease Control
CI	confidence interval
CLSI EP15	Clinical & Laboratory Standards Institute Evaluation Protocol 15
COPD	chronic obstructive pulmonary disease
COVID-19	corona virus disease 2019
CPAP	continuous positive airway pressure
CRH	corticotrophin releasing hormone
CRP	C-reactive protein
CRRT	continuous renal replacement therapy
CT-A	computerized tomography angiography
cTnT	cardiac troponin T
CT-proAVP	C-terminal pro-arginine vasopressin
CT-proET-1	C-terminal proendothelin-1
CURB-65	Confusion blood Urea nitrogen Respiratory rate Blood pressure age 65 or older,
CV	coefficient of variation,
DCI	delayed cerebral ischemia
DDDs	defined daily dosages
DOR	diagnostic odds ratio
DSA	digital substraction angiography
ED	emergency department
EDTA	Ethylene Diamine Tetra Acetic acid
ET-1	Endothelin-1
ETZ	Elisabeth Tweesteden Ziekenhuis
GCS	Glasgow Coma Scale
GOS	Glasgow Outcome Scale

HAP	hospital-acquired pneumonia
HiTEMP	Higher diagnostic accuracy and cost-effectiveness using procalcitonin in treatment of emergency medicine patients with fever
HR	hazard ratio
ICU	Intensive Care Unit
IL-1	interleukin-1
IL-6	interleukin 6
IQR	interquartile range
LOS	length of stay
LR+	positive likelihood ratios
LR-	negative likelihood ratio
METC	Medisch Ethische Toetsingscommissie
MR-proADM	mid-regional proadrenomedullin
MR-proANP	mid-regional proatrial natriuretic peptide
NIH Stroke Scale	National Institutes of Health Stroke scale
NPV	negative predictive value
PASS	Procalcitonin AND Survival Study
PCT	Procalcitonin
PEEP	positive end expiratory pressure,
PPV	positive predictive value
ProACT	Procalcitonin Antibiotic Consensus Trial
PRORATA	Procalcitonin to Reduce Antibiotic Treatments in Acute ill patients
PROGRESS	Procalcitonin-guided antimicrobial therapy to reduce long-term sequelae of infections
RCT	randomized controlled trial
ROC	curve receiver operating characteristics curve
ROS	reactive oxygen species
RT-PCR	real-time reverse transcriptase-polymerase chain reaction
SAPS	Stop Antibiotics on Procalcitonin Study
SAPS II	Simplified Acute Physiology Score II,
SARS-CoV-2	severe acute respiratory syndrome coronavirus
SCCM	Society of Critical Care Medicine
SD	standard deviation
SisPCT	Sodium Selenite and Procalcitonin guided antimicrobial; therapy in severe sepsis
SOFA	sequential organ failure assessment
STARD	Standards for Reporting of Diagnostic Accuracy Studies
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology Statement
TNF-alpha	tumor necrosis factor alpha.

TRACE	time-resolved amplified cryptate emission
UMCU	University Medical Center Utrecht
VAP	ventilator-acquired pneumonia
VIF	variance inflation factors
WBC	white blood count
WFNS score	World Federation of Neurological Surgeons score
WHO	World Health Organization

Curriculum Vitae

Jos van Oers was born in Dordrecht on May 4th 1968. He graduated from Titus Brandsma College in Dordrecht in 1987 after which he attended medical school at the Erasmus University in Rotterdam. After receiving his Medical Doctor degree in 1995 he was drafted into military services and served as a military physician in Seedorf, Germany. Later, he worked as a resident in Allergy and Immunology at Medical Centre de Klokkenberg in Breda and as a resident Internal Medicine at Albert Schweitzer Hospital in Dordrecht. During this period he became interested in medical research and was stimulated to participate in writing research articles. He started his training Internal Medicine at Deventer Hospital and completed his training at University Medical Center Groningen in 2005. During his training in Internal Medicine he started a fellowship in Intensive Care Medicine to become an intensivist. After becoming registered as an intensivist in 2006 he started working at Elisabeth Tweesteden Hospital in Tilburg. He became involved in the Stop Antibiotics on Procalcitonin Guidance Study group in 2011 and participated in several publications and presentations on biomarkers and antibiotic stewardship. In 2017, he started as a PhD candidate under supervision of prof.dr. A.R.J. Girbes and prof.dr. D.W. De Lange at the department of Intensive Care Medicine at Amsterdam UMC, Medical Centre, VU University Medical Centre. His PhD project concerned the use of biomarkers for diagnosis, prognosis and clinical applicability of biomarkers in critically ill patients. The results of this PhD project are described in this dissertation. Since June 2021, he is working in both Elisabeth-Tweesteden Hospital Tilburg and Zorgsaam Hospital Zeeuws-Vlaanderen. He is living in Tilburg with Odette and their daughters Eva and Roos.

PhD portfolio

Summary of PhD training and teaching

Name student	Jos van Oers
PhD period	01-1-2017 – 10-11-2023
Research school	VU University Amsterdam
promotoren	prof.dr. A.R.J. Girbes
	prof.dr. D.W. De Lange
copromotor	dr. A. Beishuizen

1 PhD training

Courses	Year	Workload (hours)	ECTS
GCP-WMO course (Elisabeth Tweesteden Ziekenhuis)	2012	28	1.0
Statistics course TIAS (Tilburg University)	2014	56	2.0
GCP-WMO refresher course (Elisabeth Tweesteden Ziekenhuis)	2016	14	0.5
Scientific writing in English (Elisabeth Tweesteden Ziekenhuis)	2016	28	1.0
Scientific Integrity (Amsterdam UMC, VUMC)	2022	56	2.0

2 Presentations on PhD research topic at (inter)national conferences

Multidrug resistant organisms in a Dutch ICU *ISICEM, Brussels*	2017	14	0.5
Antibiotic resistance in the ICU *VentiCare, Utrecht*	2017	14	0.5
Optimizing antibiotic therapy *ISICEM, Brussels*	2017	14	0.5
Biomarkers for antibiotic stewardship and prognosis in pneumonia patients in the ICU *SCCM congress, San Antonio, Tx*	2018	14	0.5
Procalcitonin guided antibiotic stewardship In 15 Dutch ICUs *Symposium to treat or not to treat, Rotterdam*	2018	14	0.5
Impact of sepsis on the ICU *Beckman Coulter Symposium, Alphen aan de Rijn*	2018	14	0.5
CT-proAVP, but not D-dimer is associated with poor outcome in critically ill patients with aSAH *ISICEM, Brussels*	2023	14	0.5

3 Conferences

Intensivistendagen	2017	11	0.4
International Symposium of Intensive Care and Emergency Medicine	2017	24	0.9
Intensivistendagen	2018	11	0.4
Annual Meeting Society of Critical Care Medicine Congress	2018	30	1.1
Intensivistendagen	2019	11	0.4
International Symposium of Intensive Care and Emergency Medicine	2019	24	0.9
Intensivistendagen	2021	11	0.4
Annual Congress European Society of Intensive Care Medicine	2021	24	0.9
Intensivistendagen	2022	12	0.4
8th Netherlands International Sepsis Symposium	2022	5	0.2
Annual Congress European Society of Intensive Care Medicine	2022	22	0.8
International Symposium of Intensive Care and Emergency Medicine	2023	24	0.9

4 Teaching

Therapy of community-acquired pneumonia *(physicians & trainees) (ETZ, Tilburg)*	2018	14	0.5
Antibiotic stewardship *(physicians & trainees) (ETZ, Tilburg)*	2018	14	0.5
Prehospital trauma care *(physicians & trainees) (ETZ, Tilburg)*	2019	14	0.5
Neurotrauma *(physicians & trainees) (ETZ, Tilburg)*	2019	14	0.5
Biomarkers in the ICU *(physicians & trainees) (ETZ, Tilburg)*	2019	14	0.5
Vitamin C in sepsis *(physicians & trainees) (ETZ, Tilburg)*	2020	14	0.5
Sepsis in the ICU *(physicians & trainees) (ETZ, Tilburg)*	2021	14	0.5
Prognosis of elderly COVID-19 patients in the ICU *(physicians & trainees) (ETZ, Tilburg)*	2022	14	0.5

5 Other

Simulatiestudie competenties physician assistants vs arts-assistenten op een intensive care *HAN-University of Applied Sciences, Nijmegen*	2017	28	1.0
Fundamental Critical Care Support (FCCS) course *NSTZ, Ede*	2017	48	1.8
Balance in Pain, Sedation and Delirium *Oral presentation Orion satellite symposium, Brussels*	2017	14	0.5
Hemodynamic monitoring in the ICU *Oral presentation symposium IC-regio Zuid-West Nederland*	2017	14	0.5
Fundamental Critical Care Support (FCCS) course *NSTZ, Ede*	2018	48	1.8
Skills of physician assistants and residents tested with simulated intensive care patients *Oral presentation NVIC-dagen 2018*	2018	14	0.5
Dexdor Q&A workshop *Oral presentation IC Bravis Ziekenhuis, Roosendaal*	2018	14	0.5
Research project Biomarkers in Critically Ill Patients *Oral presentation (physicians & trainees) (ETZ, Tilburg)*	2018	14	0.5
Pain, Agitation and Delirium workshop *Oral presentation PADIS workshop AZ Maria Middelares, Gant*	2018	14	0.5
Fundamental Critical Care Support (FCCS) course *NSTZ, Ede*	2019	48	1.8
Fundamental Critical Care Support (FCCS) course *NSTZ, Ede*	2020	32	1.2
Workshop “Het Donatiegesprek”	2020	14	0.5
Supervision trainee ICU *(ETZ, Tilburg)*	2021	28	1.0
Fundamental Critical Care Support (FCCS) course *NSTZ, Ede*	2021	16	0.6
ATLS refresher course	2021	10	0.4
Fundamental Critical Care Support (FCCS) course *NVIC, Houten*	2021	16	0.6
Supervision trainee ICU *(ETZ, Tilburg)*	2022	28	1.0
Advantages of powernaps for ICU nurses *Oral presentation NVIC-dagen 2022*	2022	28	0.5
Fundamental Critical Care Support (FCCS) course *NSTZ, Ede*	2022	16	0.6
Update Research project Biomarkers in Critically Ill Patients *Oral presentation (physicians & trainees) (ETZ, Tilburg)*	2022	14	0.5
Fundamental Critical Care Support (FCCS) course *NVIC, Houten*	2022	16	0.6

List of publications

1 Pelikan Z, van Oers JA, Levens WJL, Fouchier SM. De rol van allergie bij interstitiële cystitis. Ned Tijdschr Geneeskd. 1999; 143:1289-92.

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Dankwoord (Word of Gratitude)

Het schrijven van dit dankwoord viel mij zwaarder dan ik aanvankelijk dacht. Enige tijd geleden ben ik begonnen met de eerste steekwoorden op papier te zetten en heb het af en toe weer opgepakt en het gevormd tot het document dat nu voor u ligt. Dit hoofdstuk is anders dan de eerdere hoofdstukken, die veelal gevuld zijn met met cijfers en feiten. In dit hoofdstuk wil ik eenieder bedanken, die aan dit proefschrift heeft bijgedragen. Dit proefschrift was niet tot stand gekomen zonder de hulp en ondersteuning die ik heb mogen ontvangen van collega's, gezin en familie.

Ik wil een aantal mensen in het bijzonder bedanken:

Allereerst alle patiënten, die ten tijde van hun ziek zijn, belangeloos hebben deelgenomen aan de studies in dit proefschrift.

Mijn promotoren, prof.dr. A.R.J. Girbes en prof.dr. D.W. De Lange
Armand, wil ik bedanken voor de mogelijkheid om dit promotieonderzoek aan de Vrije Universiteit Amsterdam te verrichten naast mijn werk als intensivist in een andere kliniek. Als buiten-promovendus, niet bepaald "dicht bij het vuur", wanneer je elkaar minder frequent ziet, is het niet altijd makkelijk geweest. Bij vragen was er echter altijd snel contact via telefoon of mail. Ik wil je danken voor je geduld en vertrouwen op een goede afloop. Jouw scherpe inzichten en commentaren hebben dit proefschrift naar een hoger niveau getild.
Dylan, ik wil je bedanken voor de energie en inzet waarmee jij mij hebt begeleid in mijn promotietraject. Emails en manuscripten werden voorzien van op,- en aanmerkingen altijd binnen twee dagen weer teruggestuurd. Vaak op tijdstippen, waarbij de meeste mensen onder de wol liggen.

Mijn co-promotor, dr. A. Beishuizen
Bert, ik wil je bedanken voor je begeleiding en steun, maar ook voor je humor en relativeringsvermogen over de afgelopen jaren heen. De data zijn verzameld, analyses gedaan en manuscripten zijn geschreven, de eindstreep is gehaald. Bepaald geen laaghangend fruit.

Geachte leden van de promotiecommissie bestaande uit, prof.dr. K.D.P.W.B. Nanayakkara, prof.dr. P. Pickkers, prof.dr. J. Bakker, dr. J.L. Murk, prof.dr. J. Chastre en prof.dr. P.H.J. Van Der Voort dank voor jullie bereidheid om mijn proefschrift te beoordelen en plaats te nemen in mijn commissie.

Maarten Nijsten, we kenden elkaar al uit de tijd dat ik fellow intensive care geneeskunde in Groningen was en later als collega-onderzoeker binnen de SAPS-studie. Ik wil je bedanken om mij bij professor Girbes in 2016 te introduceren als nieuwe promovendus op een PhD traject over biomarkers volgend op de SAPS-studie.

Evelien de Jong, samen met Dylan, Bert en Maarten waren wij de SAPS-stuurgroep en hadden wij vele vergaderingen, teleconferenties en etentjes met elkaar, uiteindelijk resulterend in The lancet Infectious Diseases publicatie. Jij was mijn voorganger en voorbeeld. Jouw opdracht om kort volgend op jouw eigen promotie mijn proefschrift de verdedigen was toch moeilijker te volbrengen.

Jürgen Reimer en Henk Nijzingh wil ik bedanken voor het bij elkaar brengen van de leden van de SAPS-studiegroep.

Harm-Jan de Grooth, dank voor je uitleg en ondersteuning bij vragen over methodologie en statistiek bij de biomarker studies. Altijd was je weer bereid om een extra analyse te draaien en de uitkomst nauwkeurig te rapporteren en te bespreken.

Yvette Kluiters, als klinische chemicus in het Elisabeth Tweesteden Ziekenhuis hebben wij intensief samengewerkt tijdens de SAPS-studie. Dankzij jou werden niet alleen de vele PCT-metingen gedaan, maar jij hebt ook serum samples ingevroren, die weer de basis voor een nieuwe studie vormden.

Hans Krabbe, dank voor de extra MR-proADM en MR-proANP metingen in Enschede en voor je niet aflatende steun en motivatie om hoofdstuk 4 van dit proefschrift te voltooien.

Judith Bons, dank voor de extra MR-proADM en CT-proET-1 metingen in Maastricht tijdens de COVID-19 pandemie.

Mijn collega's van de afdeling intensive care van het Elisabeth-Tweesteden Ziekenhuis: Intensivisten, arts-assistenten, physician assistants, verpleegkundig specialisten, verpleegkundigen, fysiotherapeuten, voedingsassistenten, medewerkers algemene dienst en secretariaat. Samen, als één team, leveren wij de beste zorg aan onze kritiek zieke patiënten. Speciaal dank aan Dharmanand Ramnarain en Angela Kotsopoulos. Dharmanand, dank voor onze gesprekken over methodologie en statistiek. Een onderwerp waar jij je als epidemioloog verder in hebt gekwalificeerd. Angela, mijn "roommate", als klankbord, vanachter jouw computerscherm, heb jij mijn frustraties moeten aanhoren over weer een net niet significante uitkomst of wanneer een manuscript geweigerd werd voor publicatie. Dank voor je opbeurende opmerkingen en adviezen.

Piet Vos, aanvankelijk werkzaam als research-verpleegkundige op de intensive care van het Elisabeth-Tweesteden Ziekenhuis en later verbonden aan Erasmus MC. Dank voor jouw hulp bij het aanmelden en starten van wetenschappelijk onderzoek en het verwerken van data uit de eerste studies van dit proefschrift.

De dames van het secretariaat Intensive Care Elisabeth-Tweesteden Ziekenhuis (Liesbeth Savelsberg, Christel Goertz, Rita Maas, Erica Bekers, Monique Broers) wil ik bedanken voor hun steun bij het nabellen van patiënten en families voor het verkrijgen van informed consent tijdens de COVID-19 pandemie, het verzamelen van de vragenlijsten over functioneren na IC opname en het verspreiden van dit proefschrift.

Ik wil de medewerkers van de medische bibliotheek van het Elisabeth-Tweesteden Ziekenhuis bedanken voor het opzoeken van de vele artikelen die ik aangevraagd heb.

Twee van de hoofdstukken van dit proefschrift beschrijven het beloop van biomarkers bij COVID-19 patiënten. Ik wil alle collega-specialisten, (oud)arts-assistenten en (oud)verpleegkundigen uit het ETZ bedanken die extra op de intensive care zijn komen werken tijdens de COVID-19 pandemie. In het bijzonder wil ik Sjaak Pouwels bedanken die naast de patiëntenzorg tijdens deze periode ook tijd en energie vond voor wetenschappelijk onderzoek bij deze nieuwe patiëntenpopulatie. Hetgeen resulteerde in diverse artikelen en hoofdstuk 6 van dit proefschrift.

Ik wil mijn collega's van de afdeling intensive care van Zorgsaam Ziekenhuis Terneuzen bedanken voor hun collegiale samenwerking. Voor een luisterend oor en relativerende opmerking wanneer nodig.

Ik wil mijn dankbaarheid, liefde en respect uitspreken voor mijn ouders, Jan en Ria, die mij geleerd hebben altijd nieuwsgierig te blijven en de moeilijke vragen te stellen. Door jullie ben ik geworden tot de persoon die ik nu ben. Jullie hebben altijd in mij geloofd en zijn mij blijven steunen. Ook wanneer de weg naar het doel lang en moeilijk was. Papa, met jouw eigen promotieonderzoek heb je laten zien wat met inzet, discipline en volharding te bereiken is. Hoe trots was jij toen ik je vertelde dat het proefschrift in manuscript vorm door de (co)promotoren goedkeurd was. Hoe graag was jij bij de verdediging aanwezig geweest. Helaas was die tijd jou niet meer gegeven.

Dit proefschrift is tot stand gekomen zonder structurele onderzoeksdagen, naast het reguliere intensive care werk, "in de verloren uurtjes" en vooral in veel privétijd. Ik wil daarom de allerbelangrijkste mensen in mijn leven bedanken, mijn vrouw Odette en onze dochters Eva en Roos, voor jullie steun en toewijding gedurende de tijd dat ik met het onderzoek bezig was. Lieve Odette, samen hebben we al veel meegemaakt,

goede, maar ook minder goede tijden. Het leven heeft jou hard geraakt het moment dat bij jou de diagnose borstkanker werd gesteld en het chemotherapie traject begon. Ik bewonder je voor je wilskracht, doorzettingsvermogen en vechtlust om de ziekte te overwinnen en om je toewijding een goede partner en moeder te zijn voor onze dochters. Jij laat mij zien dat er meer is dan alleen werken. Lieve Eva en Roos, we hebben als gezin zoveel dingen al gezien en ondernomen en ik hoop dat daar nog veel meer avonturen gaan volgen. Maar het allerbelangrijkste vinden wij als ouders dat jullie gelukkig en veilig opgroeien en plezier beleven in jullie leven. Jullie staan voor ons onvoorwaardelijk op nummer één.

Biomarkers in critically ill patients