Sepsis in the intensive care unit

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From definitions to outcomes

Rob G.H. Driessen

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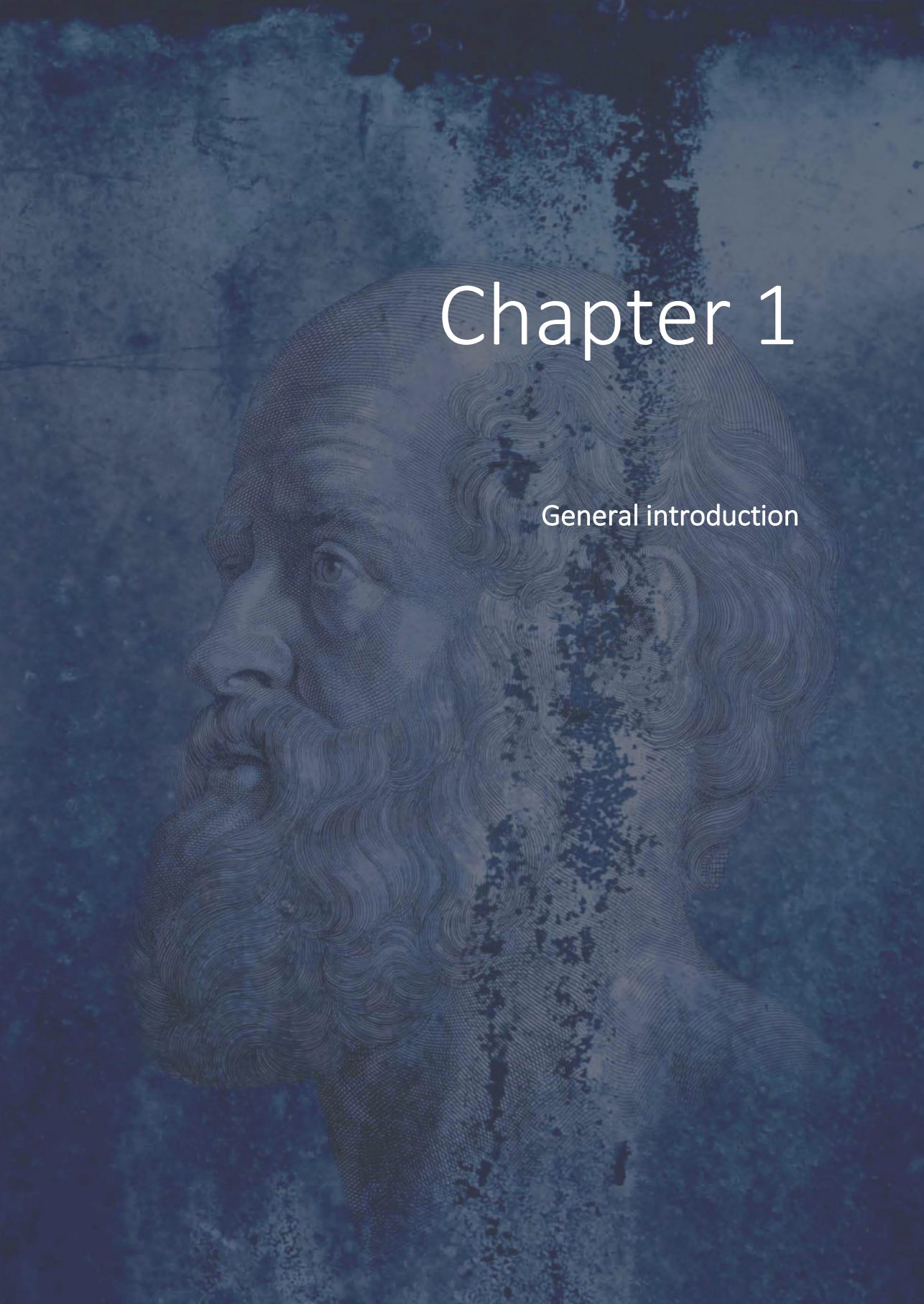

Chapter 1

General introduction

Introduction

The etymology of the word "sepsis" comes from the ancient Greek word "σῆψις" ("sepo"), which means "I rot"[1] and was first documented in medical context in the Poems of Homer (circa 680 BC).[2] Hippocrates (circa 400 BC) used the term "sepsis" in his works and viewed it as a dangerous biological decay occurring in the body. Later, the Roman physician Galen (129-199) described wound healing by second intention and introduced the term "miasma" for emitted putrid fumes created by invisible creatures. Although Van Leeuwenhoek (1632–1723) was able to build a microscope and describe the "animalcules" in 1674, the actual "germ" theory was further developed by Semmelweiss ("father of the antiseptic method", 1818-1865), Pasteur (invented the "germ theory", 1822-1895), Lister (famous for his theories on postoperative infections, 1827-1912) and Koch (one of the "fathers of modern microbiology", 1843-1910).[3] Later, Pfeiffer (1858-1955) discovered that the *Vibrio cholera* bacteria released a substance from its cell wall and named it "endotoxin".[4] Ultimately, our contemporary understanding of sepsis as the presence of pathogenic germs into the bloodstream was developed by Schottmueller (1867-1936) in 1914.[5] After 1914, Fleming (1881-1955) published his work in 1929, concerning a mold that produced a substance causing bacterial inhibition and called it "penicillin". Rich and Lewis described antigen-mediated inhibition of neutrophil and macrophage migration in tuberculin-sensitized tissue, thereby marking the beginning of cytokine research in 1932.[2] In 1944, Menkin (1901-1960) purified factors that could induce a febrile response in animals and called these factors "pyrogens". In fact, the term "cytokine" was first used by Cohen (1922-2020) in 1974.[6]

Shortly after the finding that cytokines play a central role as pro-inflammatory agent in sepsis and septic shock, nitric oxide (NO) was discovered by Furchgott (1916-2009) in 1980[7] as the key factor that dilated blood vessels and caused distributive shock. Since then, the understanding of septic shock progressed with invasive monitoring of hemodynamics. Although Forssmann (1904-1979) described placement of a catheter into the right heart in 1929[8], it was only until the 1970s and 1980s, the understanding of shock progressed when Swan (1922-2005) and Ganz (1919-2009) used a catheter allowing hemodynamic evaluation in patients and referred to it as the Swan-Ganz catheter.[9] After this, a classification of shock based on hemodynamic profiles became generally accepted, and septic shock was regarded as a type of distributive shock, characterized by loss of vasomotor tone and increased cardiac output.[10] In 1991, a consensus conference was organized by the American College of Chest Physicians (ACCP) and the Society of Critical Care Medicine (SCCM), for the first time defining sepsis and septic shock according to the systemic inflammatory response syndrome (SIRS) in association with infection.[11]

Definition(s) of inflammation, sepsis, and septic shock

Inflammation

Inflammation is the basic process whereby tissues of the body respond to harmful stimuli such as pathogens, damaged cells, or irritants.[12] Inflammation is characterized by the presence of five hallmarks described by Celsus, A.D. 25. These are: tumor - swelling of the tissue, calor – elevated tissue temperature, rubor – redness, dolor – pain, and functio laesa – impaired organ function.[13] However, clinical signs alone cannot distinguish a sterile inflammatory response from infection, and non-resolving inflammation can be a major driver of disease.[14] Furthermore, inflammation is primarily a response of innate immunity compared to adaptive immunity, specific for each pathogen. Thus, whereas a microorganism causes infection, inflammation is one of the reactions of the body to the pathogen[15], and the presence of an underlying infectious process is the difference between sepsis and (sterile) inflammation.[3]

Sepsis

Sepsis is currently defined as a life-threatening syndrome caused by a dysregulated host response to infection.[16] The definition of sepsis and septic shock has changed remarkably over the years since the 1990s.[17,18] Up to 2016, sepsis was defined by using the systemic inflammatory response syndrome (SIRS) criteria: tachycardia (heart rate >90 beats/min), tachypnea (respiratory rate >20 breaths/min), fever or hypothermia (temperature >38 or <36°C) and leukocytosis, leukopenia, or bandemia/"left shift" (white blood cells >12,000 cells per mm^3, <4,000 cells per mm^3, or ≥10% immature (band) forms).[18] In this approach, sepsis was seen as a continuum, evolving from infection to sepsis, severe sepsis, and septic shock. Infection was defined as a pathologic process caused by the invasion of normally sterile tissue, fluid, or body cavity by pathogenic microorganisms. Patients were diagnosed with sepsis when there was a suspicion of infection, and two or more SIRS criteria were met. Severe sepsis was defined as sepsis with organ dysfunction and septic shock as severe sepsis with acute circulatory failure.[17]

However, this definition, particularly the SIRS approach, has some limitations, such as that almost all patients admitted in the Intensive Care Unit (ICU) meet these criteria.[19,20] Not all these patients have an underlying infection, as many non-infectious conditions can cause SIRS (trauma, pancreatitis). Moreover, not all patients with an infection have sepsis, and indeed, even mild viral infections cause some degree of host response (tachycardia, tachypnea).[21] Furthermore, when applying the SIRS criteria, one in eight patients with severe sepsis is missed. These SIRS-negative sepsis patients had lower but still substantial mortality as compared to SIRS-positive sepsis.[22]

Therefore, in 2016, the Third International Consensus Definitions Task Force of the Society of Critical Care Medicine (SCCM) and European Society of Intensive Care

Medicine (ESICM) revised the definitions for sepsis and septic shock.[23] Sepsis was now defined as a life-threatening organ dysfunction caused by a dysregulated host response to infection (Sepsis-3 definition).[16] Clinically, sepsis was defined as a rise in Sequential Organ Failure Assessment (SOFA) score of ≥2 points, reflecting an overall mortality risk of 10% in a general population with suspicion of infection. So, the new definition distinguishes between a common uncomplicated infection and the sicker variant, leading to organ dysfunction, and in addition to that, holds a more substantial relationship with outcome. The term "severe sepsis" was abandoned in this definition as the term sepsis itself is already defined as a life-threatening disease with organ dysfunction. Furthermore, by removing the term "severe sepsis" from the definition, the illusion that sepsis is a continuum leading from sepsis to severe sepsis and then to septic shock, is also shattered.

The SOFA score was developed in 1994 and selects six organ systems (respiratory, cardiovascular, renal, hepatic, nervous system, and coagulation) which are scored from 0 (normal function) to 4 (most abnormal function), giving a possible score of 0 to 24 (Table 1.1).[24] In contrast to earlier disease severity scores such as the Acute Physiology And Chronic Health Evaluation (APACHE) II score[25,26] and the Simplified Acute Physiology Score (SAPS)[27], the SOFA score is suitable for serial data and the highest value is often recorded daily.[28] In addition, the SOFA score has been validated in several ICU populations (mixed, medical, and surgical)[29-31], and a higher SOFA score is associated with increased mortality.[32] Therefore, the quick SOFA (qSOFA) was proposed in the consensus definition as a method to recognize patients likely to have sepsis and risk to deteriorate rapidly in the general ward or the emergency department. Altered mentation, systolic blood pressure of 100 mmHg or less, and respiratory rate of 22/min or greater were used as clinical variables (Table 1.2). A score of ≥2 out of 3 was suggestive for sepsis with a predictive validity similar to the full SOFA score.[33] More recent studies show conflicting results regarding the qSOFA compared to the conventional SIRS criteria in identifying patients with sepsis outside of the ICU. One study showed that qSOFA had a lower sensitivity for sepsis screening than the SIRS criteria.[34] In contrast, a prospective cohort study pointed out that the qSOFA score performed better than the SIRS criteria (area under the receiver operating characteristic curve (AUROC) 0.86 vs. 0.67).[35] Overall, qSOFA seems better as a prognostic tool[36], whereas SIRS criteria are superior as a diagnostic instrument.[37]

Septic shock

Whereas the 2001 task force described septic shock as a state of acute circulatory failure[17], the revised definition in 2016 defines it as a subset of sepsis with profound circulatory, cellular, and metabolic abnormalities and substantially increased mortality up to 40%.[16] Patients with septic shock are clinically identified by a vasopressor requirement to maintain a mean arterial blood pressure (MAP) of 65 mmHg or greater and a serum lactate level ≥2 mmol/L in the absence of hypovolemia. This serum lactate

level was not part of the Sepsis-2 definition in 2001. Although elevated lactate levels can reflect cellular dysfunction in sepsis, increased lactate levels can also result from accelerated aerobic glycolysis and reduced hepatic clearance.[38] Furthermore, the cut-off point for serum lactate level in determining a higher risk of ICU mortality is variable in different cohorts. Indeed, a study of more than 28,000 severe sepsis and septic shock patients associated a cut-off value of serum lactate of ≥4 mmol/L with in-hospital mortality.[39]

Table 1.1 The Sequential Organ Failure Assessment (SOFA) score

	SOFA score points				
System	**0**	**1**	**2**	**3**	**4**
Respiration PaO_2/FiO_2, mmHg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7)	<100 (13.3)
Coagulation Platelets ($x10^3/mm^3$)	≥150	<150	<100	<50	<20
Liver Bilirubin, mg/dL (µmol/L)	<1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (204)
Cardiovascular Catecholamine dosage = µg/kg/minute for at least one hour	MAP ≥70	MAP <70	Dopamine <5 or dobutamine (any dose)	Dopamine 5.1 -15 or epinephrine ≤0.1 or norepinephrine <0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Nervous system GCS	15	13-14	10-12	6-9	<6
Renal Creatinine mg/dL (µmol/L, urine output, mL/d	<1.2 (110)	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440) or <500 ml/d	>5.0 (440) or <200 ml/d

Table 1.2 The qSOFA (Quick SOFA) criteria

Criterion	Points
Altered mentation	1
Systolic blood pressure ≤100 mmHg	1
Respiratory rate ≥22 pm	1

Epidemiology of sepsis

Mortality rate from infectious diseases declined in a century from 800/100,000 in 1900 to 70/100,000 in the year 2000 because of the effects of vaccination, chlorination of water, pasteurization of milk, and antibiotics.[40] The pooled incidence of sepsis is estimated at 189 hospital-treated adult sepsis cases per 100,000 person-years with a reported mortality of 27%.[41] However, patients with sepsis treated in the ICU had a mortality of 42%. The incidence of sepsis is increasing, mainly because of increasing age, improved survival of patients with underlying disorders, and related use of immunosuppressive medication and intensive care treatment.[42] Moreover, studies might underestimate the burden of sepsis as less than half of patients are correctly

coded using the International Classification of Disease (ICD) system.[43] The incidence of sepsis peaks in early childhood and elderly adults. Although global sepsis incidence was higher in women than in men (717 vs. 643 cases per 100,000), sepsis-related mortality was higher in men compared to women (164 vs. 134 per 100,000).[44] Finally, the incidence of sepsis seems to be highest among African-American males.[45]

Pathophysiology and characterization of sepsis

Sepsis can be caused by a wide range of bacterial, fungal, or viral infections. After infection, the invading pathogen causes, on the one hand, a disproportionate inflammatory response and, on the other hand, a state of immune suppression with lymphocyte exhaustion and apoptosis.[46] Excessive inflammation in sepsis is initiated by the innate immune system mediated by pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) through activation of pattern recognition receptors (PRRs).[47] Besides the release of pro-inflammatory cytokines, this excessive inflammatory stage is also characterized by complement activation (C3a and C5a)[48], a pro-coagulant state (sometimes with diffuse intravascular coagulation (DIC))[49], and endothelial dysfunction with loss of barrier integrity and capillary leakage.[50] These mechanisms can lead to tissue hypoperfusion, edema, and cardiovascular collapse due to multiple organ failure and shock.[51]

The concept of sepsis as an alternating activation of both pro- and anti-inflammatory mechanisms might partially explain the multitude of negative trials in this field.[52] Indeed, trials aiming to decrease inflammation[53], restore coagulation[54], and reduce mitochondrial dysfunction[55] failed to improve survival rates. Therefore, possible new molecular mechanisms linking inflammation and sepsis to multi-organ failure should be investigated. A possible relevant mechanism that is underinvestigated is the formation and reduced breakdown of the dicarbonyls: methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG).[56] These reactive metabolites are produced by several metabolic processes, such as anaerobic glycolysis, gluconeogenesis, and lipid peroxidation.[57] Inflammation leads to a switch from oxidative phosphorylation to glycolysis, and this Warburg-effect may drive dicarbonyl production.[58] Furthermore, glyoxalase-I (GLO-1), the enzyme clearing dicarbonyl stress by converting MGO into D-lactate, might be downregulated during inflammation.[59] The accumulating dicarbonyls damage intracellular and extracellular proteins due to arginine modifications and the formation of methylglyoxal derived hydro-imidazolone-1 (MG-H1), leading to cell and organ dysfunction[60-62] (Figure 1.1). Gaining insight into the dicarbonyl pathway could be of interest, as therapeutic options by enhancing GLO-1 activity have been described.[63]

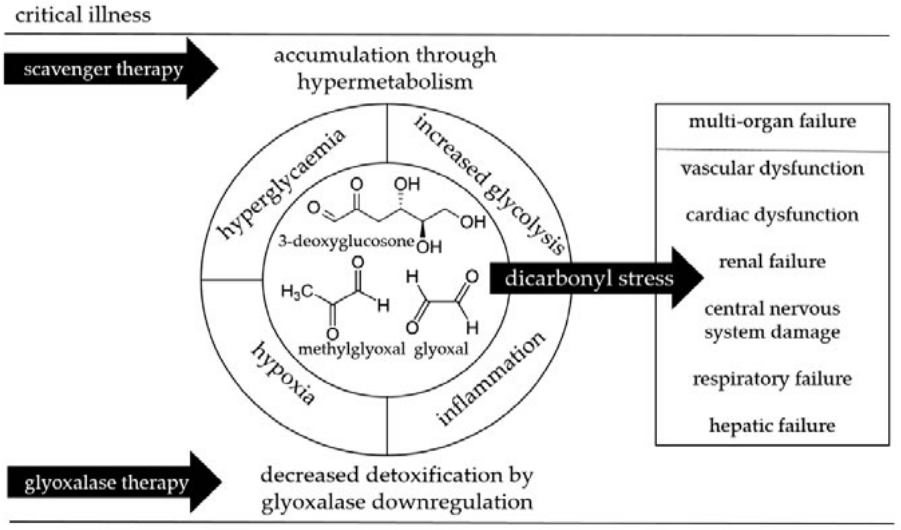

Figure 1.1 Schematic illustration of the hypothesis of whether increased dicarbonyl stress in critically ill patients contributes to the development of multi-organ failure. *Adapted from van Bussel BC, van de Poll MC, Schalkwijk CG, Bergmans DC. Increased Dicarbonyl Stress as a Novel Mechanism of Multi-Organ Failure in Critical Illness. Int J Mol Sci. 2017;18[2], with permission from Multidisciplinary Digital Publishing Institute (MDPI) and the authors.*

Antibiotic treatment of sepsis

After identification of sepsis, swift initiation of therapy is needed. Treatment is based on hemodynamic stabilization and infection control. The Surviving Sepsis Campaign currently guides sepsis treatment according to the International Guidelines for Management of Sepsis and Septic Shock, recently revised in 2021.[64] Briefly, initial resuscitation should start immediately, and hypoperfusion should be treated aggressively with crystalloid fluid (30 ml/kg during the first three hours) and should be guided by dynamic over static variables.[65] A targeted mean arterial blood pressure of 65 mmHg is recommended, and norepinephrine is the vasopressor of choice when necessary.[66] In addition, it is suggested to guide resuscitation based on serum lactate levels, although it is not a direct marker of tissue perfusion.[67] Except for one study in 2001[68], no benefit of early goal-directed treatment was found in more recent trials, although these recent trials included less severely ill patients.[69-71]

The second important treatment goal in sepsis is the timely initiation of appropriate broad-spectrum antibiotic therapy. Guidelines recommend administering broad-spectrum antibiotic therapy as soon as possible, but within one hour and preferably after appropriate microbiologic cultures were obtained.[72] Indeed, failure to start appropriate antibiotic treatment in patients with septic shock substantially increases morbidity and mortality.[73-75] In patients with sepsis without shock, the association of a

delay in treatment and outcome seems less pronounced.[76] Nevertheless, studies report inappropriate antibiotic treatment in ICU patients in 20-30% of patients[77], most often due to extended-spectrum beta-lactamase-producing (ESBL) Gram-negative pathogens.[78] Fungal infections can also play a role, especially in patients with sepsis of abdominal, urogenital, or unknown origin.[79] Although it is suggested to administrate empiric combination therapy (at least two antibiotics from different classes) for initial management of sepsis or septic shock in patients with high risk for multidrug resistant organisms, the level of evidence is low.[74] Furthermore, the effect of combination therapy versus monotherapy has not been investigated in specific subgroups of septic shock with a high risk of infection with resistant microorganisms, especially septic shock with an abdominal focus of infection.[80] A meta-analysis comparing mono- versus combination therapy in ICU patients showed no benefit from empirical combination therapy.[81] However, only 4% of all included patients in this meta-analysis were patients with an abdominal focus of infection, leaving antibiotic treatment regimen of this subgroup of patients underinvestigated.

Outcome, timing, and causes of death in sepsis

Although mortality in sepsis seems to be declining in the last two decades, it still ranges from 20 to 30%.[82] Studies reporting data about mortality in sepsis are abundantly present, but few studies address the exact causes of death in these patients. Refractory shock and withdrawal of care are mentioned in earlier epidemiological studies.[83] In addition, older age, male gender, comorbidities, and higher SOFA score were linked to increased mortality in sepsis.[84]

Timing of death in sepsis is also underinvestigated, and the temporal relationship between death in sepsis patients after ICU admission remains unclear. One-third of all sepsis deaths occur within the first three days of admission in the ICU.[85] Reasons for early death are not well established, and it is unclear if these patients were already in a moribund condition, if there was a delay in admission, or if ICU treatment was futile. Recognizing these reasons for early death might aid in improving clinical care and designing future clinical trials, as some patients possibly do not benefit from an intervention anyway, obscuring the potential effectiveness of the intervention for other patients.[52]

A possible tool that could clarify the causes of death in sepsis is autopsy, sometimes mentioned as the ultimate diagnostic test.[86] Unfortunately, autopsy rates have been declining over the past decades.[87] Even though advances in medical technology might diminish the additional value of an autopsy and an autopsy has limitations[88], discrepancies between clinical and pathological diagnoses persist in critically ill patients.[89-91] Discrepancies between clinical and autopsy diagnoses are classified according to the Goldman criteria. Class I discrepancies represent missed major diagnoses that would have changed therapy and possibly survival, whereas class II

discrepancies are missed major diagnoses that would not have changed survival. Class III discrepancies are missed minor diagnoses related to the terminal disease, and class IV represent missed minor occult diagnoses. Non-discrepant cases are classified as class V, and non-classifiable cases are classified as class VI.[92] Class I discrepancies are reported between 3 and 16% of patients in the ICU.[93] Strikingly, two-thirds of these discrepancies occur in patients with known infection.[94]

Sepsis and COVID-19: inflammation due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection

During this Ph.D. trajectory, the world was struck with the COVID-19 pandemic, caused by infection with SARS-CoV2. Although initially seen as a pulmonary disease, it is recognized as a multi-systemic disease with a broad range of manifestations (e.g., respiratory, cardiovascular, gastrointestinal, hematological, renal, neurological, dermatological, and endocrine symptoms).[95-98] Up to 14 % of infected patients have severe disease with hypoxia requiring hospitalization, of whom 5% develop critical disease requiring mechanical ventilation and admission to the Intensive Care Unit (ICU).[99]

COVID-19 and sepsis share many pathophysiological and clinical features[100], and patients with complications including acute respiratory failure and end-organ damage meet the criteria for sepsis and septic shock.[16] SARS-CoV2 is an infectious agent causing sepsis, as is stated by the Global Sepsis Alliance.[101] Like in sepsis, a pro-inflammatory response sometimes with a cytokine storm is seen in COVID-19.[102] In addition, accumulating evidence suggest that immunosuppression and immunoparalysis play a role. Furthermore, both sepsis and COVID-19 can lead to multiple organ dysfunction[103], abnormal coagulation[104], elevated bilirubin[105], hypoxia, and acute respiratory failure.[106] Apart from the similarities in the acute stages of sepsis and COVID-19, there also seems to be overlap in the late complications during recovery of the diseases.[107] Of course, COVID-19 pathophysiology is not fully understood at this moment, and direct translation of sepsis-related management should be taken with caution.

The COVID-19 pandemic: start of the *MaastrICCht* cohort

At the beginning of the pandemic in 2020, the Maastricht Intensive Care COVID (*MaastrICCht*) cohort was started in our centre.[108,109] Briefly, this prospective cohort study was executed in a patient population admitted to the ICU of the Maastricht University Medical Centre+ (Maastricht UMC+). During the COVID-19 pandemic, the number of ICU beds was rapidly upgraded from 27 to 64 beds. In addition, the study was designed to foster other datasets and registries according to the Findable, Accessible, Interoperable, and Reusable (FAIR) data principle in collaboration.[108] Therefore, the study is registered in the Netherlands Trial Register (registration number NL8613).

This study included all patients with proven COVID-19 infection and respiratory insufficiency requiring mechanical ventilation admitted in the first wave from the 25th of March until the 23rd of June 2020. We defined a positive COVID-19 case as follows: one PCR positive for COVID-19 and/or a chest CT strongly suggestive for COVID-19 infection (CORADS-score of 4-5, scored by a radiologist).[110] Patients were followed until the primary outcome was reached (i.e., either died in the ICU or discharged from the ICU).[108] Earlier studies in this cohort investigated coagulation[111], pulmonary embolism[112], and the relationship of SOFA score with survival in mechanically ventilated COVID 19 patients.[97] Further intended research in this cohort was to investigate the association between coronary artery calcification and organ failure (as reflected by the SOFA score) in inflammation caused by COVID-19 infection. Finally, the development of cardiac biomarkers and electrocardiography (ECG) during the whole course of this inflammatory disease were studied.

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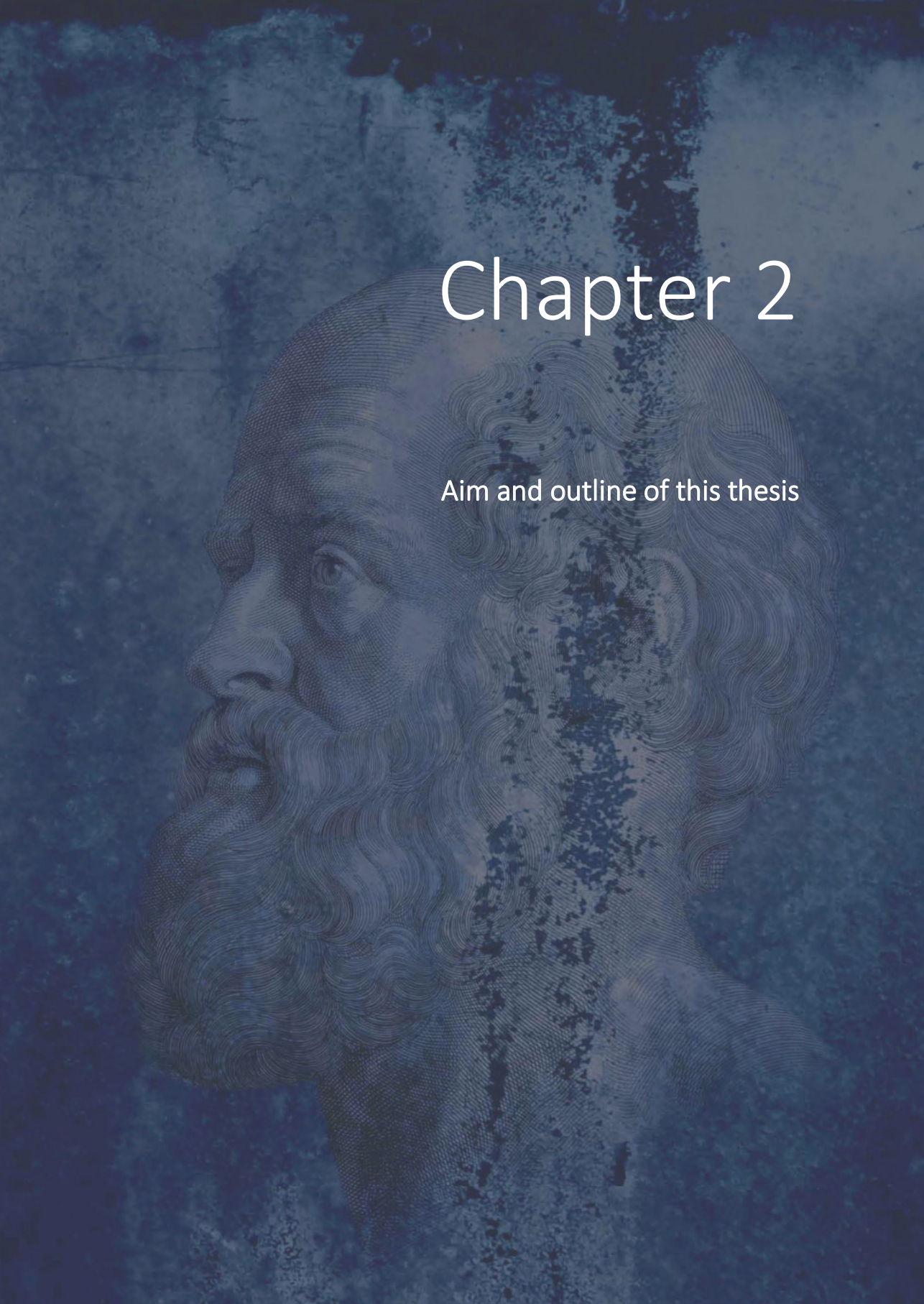


Chapter 2

Aim and outline of this thesis

Aim and outline of this thesis

The overall aim of this thesis was to gain more insight into the epidemiology, causes of death, antibiotic therapy, and outcome of patients with inflammation and sepsis or septic shock. Furthermore, the COVID-19 pandemic striking the world during this Ph.D. created an opportunity to investigate cardiac manifestations during this inflammatory disease.

Chapter 3 of this thesis focuses on the influence of a change in septic shock definition on the epidemiology and outcome of septic shock patients in the ICU by comparing Sepsis-2 and Sepsis 3 definitions. In addition, we assessed the effect of various cut-off values for lactate on the accuracy of the Sepsis-3 criteria for septic shock to identify patients with true high risk of dying in the ICU. Chapter 4 evaluates the incidence and causes of early death in sepsis patients. For this study patients dying within 48 hours after ICU admission were included and contributing factors and variability in clinical judgment between intensivists were also studied. Chapter 5 compares clinical diagnoses and autopsy findings in sepsis patients admitted in the ICU by using the Goldman criteria for discrepancies. Class I discrepancies according to Goldman are diagnostic errors that would have changed clinical management and possibly led to longer survival of patients, whereas class II errors would not have changed therapy. In Chapter 6, we investigate dicarbonyl stress as a possible molecular mechanism with therapeutic options in inflammation. We use an experimental endotoxemia model in healthy men to evaluate the effect of systemic inflammation and hypoxia on glyoxalase-1 expression, which is the enzyme that clears dicarbonyl stress. Chapter 7 analyzes the appropriateness of empirical antibiotic therapy in septic shock patients with an abdominal, urogenital, or unknown focus of infection. We also address the possible added value of adjunctive gentamicin in these patients and compared shock reversal in patients with monotherapy and combination therapy.

Pandemic

At the beginning of 2020, the COVID-19 pandemic struck the world, and in our centre we started the Maastricht Intensive Care COVID (MaastrICCht) cohort. We used this prospective cohort of mechanically ventilated patients with inflammation due to SARS-CoV2 infection to evaluate the association of cardiovascular disease (CVD) reflected by coronary artery calcification (CAC) with organ failure (using the SOFA score) in Chapter 8. In Chapter 9 we evaluated the development of cardiac biomarkers and the electrocardiogram over time during the whole course of this viral inflammatory disease. We compared these serial biomarkers and ECGs in survivors versus non-survivors.

Chapter 10 summarizes our main findings in the general discussion.

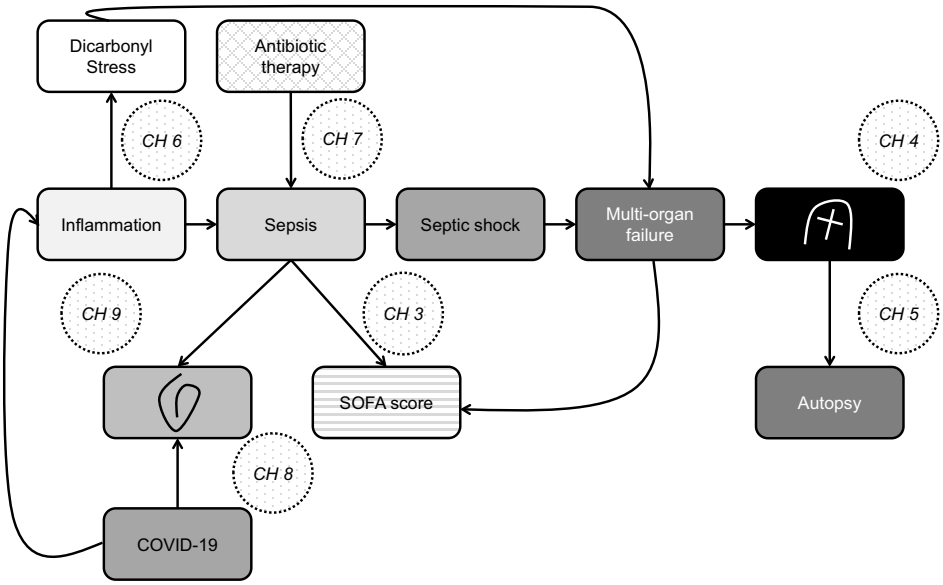
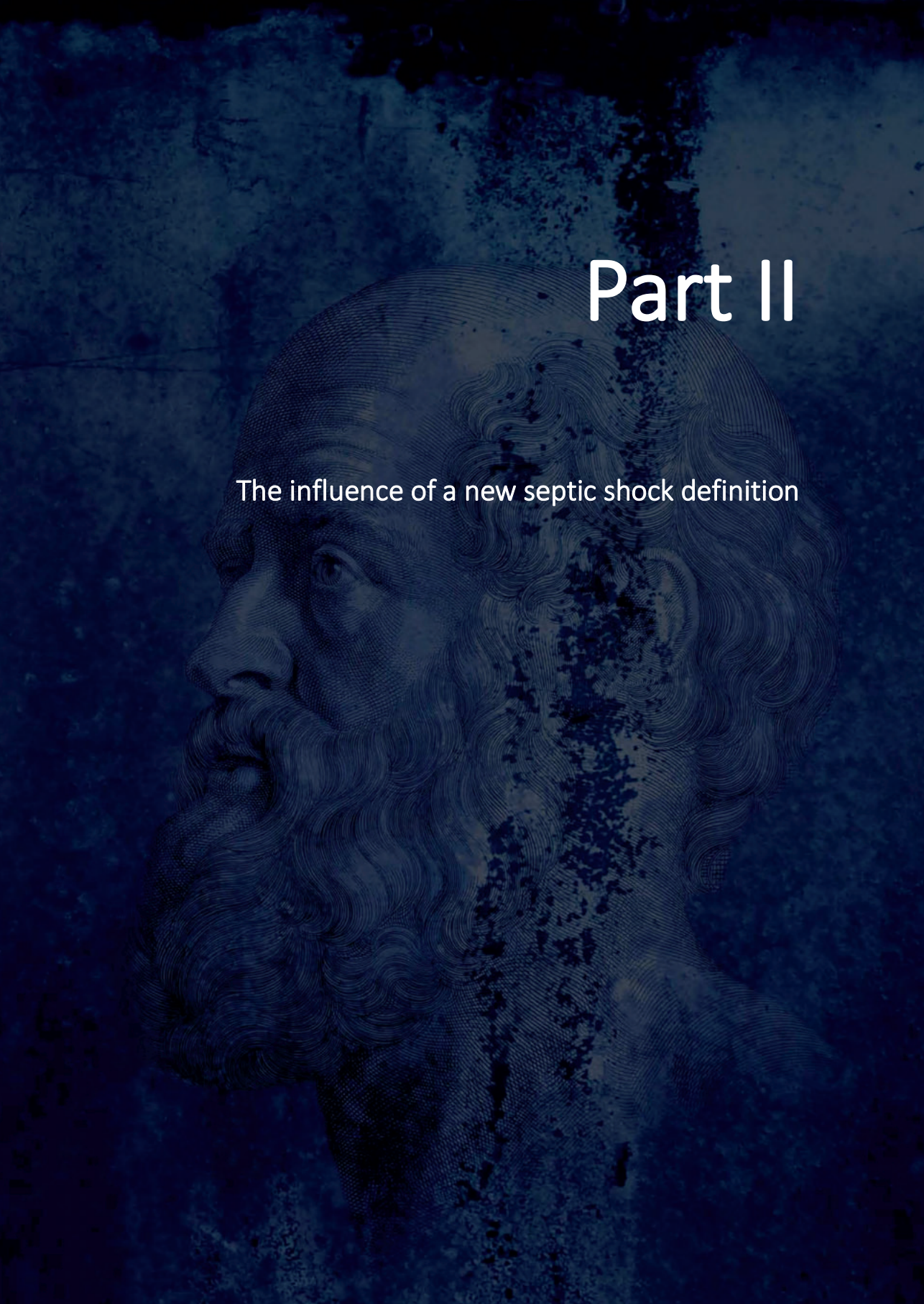


Figure 2.1 Schematic diagram of the thesis outline depicting the coherence between the different aspects of inflammation, sepsis, and septic shock with the individual chapters.



Part II

The influence of a new septic shock definition

Chapter 3

The influence of a change in septic shock definitions on intensive care epidemiology and outcome: comparison of Sepsis-2 and Sepsis-3 definitions

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Infect Dis (Lond) 2018;50(3):207-213

Abstract

Background

Clear definitions for septic shock assist clinicians regarding recognition, treatment, and standardized reporting of outcome of this entity. Sepsis-3 definition of septic shock incorporates a new criterion, a lactate level >2 mmol/L. Differences in epidemiology and outcome of septic shock based upon both definitions were studied in an intensive care (ICU) population of septic patients.

Methods

We analysed a prospectively collected cohort of data in the ICU of the Maastricht University Medical Centre+. 632 septic patients were included. ICU mortality was compared between the patient group fulfilling Sepsis-3 definition for septic shock and those that met Sepsis-2 definition. Furthermore, association between lactate levels and ICU mortality was studied.

Results

Of 632 septic patients, 482 (76.3%) had septic shock according to Sepsis-2 definition and 300 patients (48.4%) according to Sepsis-3 definition. Patients meeting Sepsis-3 definition had a higher mortality than patients meeting Sepsis-2 definition (38.9% vs. 34.0%). Serum lactate levels between 2 and 4 mmol/L (25.0% vs. 26.2%, OR 0.94 (0.5-1.5)) and between 4 and 6 mmol/L (23.8% vs. 26.2%, OR 0.88 (0.4-1.7)) compared to levels ≤2 mmol/L were not associated with significantly higher ICU mortality. Serum lactate values ≥6 mmol/L were significantly associated with increased ICU mortality.

Conclusion

Patients classified according to Sepsis-3 criteria had a higher ICU mortality compared with Sepsis-2 criteria. Lactate levels <6 mmol/L were not able to identify patients with increased ICU mortality. Lactate threshold of 2 mmol/L may be too low to point out patients with actual increased ICU mortality.

Introduction

Sepsis is a life threatening syndrome following a dysregulated host response to infection. It causes major public health concerns[1], has an increasing reported incidence and in-hospital mortality rates greater than 10%.[2] Septic shock is a subset of sepsis in which underlying circulatory and cellular abnormalities are associated with substantially increased ICU mortality rates greater than 40%.

Clear definitions for sepsis and septic shock guide clinicians regarding early recognition and treatment, and facilitate standardised reporting of characteristics and outcome leading to greater consistency of epidemiologic studies and clinical trials. In the past, multiple definitions for sepsis and septic shock were in use, resulting from differences in selected clinical variables.[3] Recently the Third International Consensus Definitions Task Force of the Society of Intensive Care Medicine and European Society of Intensive Care Medicine revised the definitions for sepsis and septic shock (Sepsis-3 definition). Sepsis was herein defined as "an organ dysfunction characterized by a rise in Sequential Organ Failure Assessment (SOFA) score of more than 2, due to an exaggerated host response to infection".[4] Septic shock was defined as a subset of sepsis in which particularly profound circulatory, cellular, and metabolic abnormalities are associated with a greater risk of mortality than with sepsis alone. Sepsis-3 definition stated that patients with septic shock are to be clinically identified by a vasopressor requirement to maintain a mean arterial pressure of 65 mmHg or greater and a serum lactate level >2 mmol/L in the absence of hypovolemia. The optimal cut-off point for serum lactate level to determine ICU mortality in septic shock patients seems variable across different cohorts. The 2001 International Sepsis Definitions Conference (Sepsis-2) clinically identified septic shock as a state of acute circulatory failure characterized by persistent arterial hypotension defined as a systolic arterial pressure below 90 mmHg; a mean arterial pressure below 60 mmHg, or a reduction in systolic blood pressure of more than 40 mmHg from baseline, despite adequate volume resuscitation.[5] Treatment goal in the Surviving Sepsis Campaign international guidelines 2012 is a mean arterial blood pressure of higher than 65 mmHg.[6] In addition, in the Sepsis-2 definition of septic shock, the level of the serum lactate has not been part of the definition. The Third ICD Task Force (Sepsis-3) demonstrated that the combination of hypotension and hyperlactatemia is associated with a significantly higher risk-adjusted ICU mortality compared to hypotension alone.[3] Elevated lactate levels reflect cellular dysfunction in sepsis.[7] To compare differences in epidemiology and outcome of septic shock, the effects of both definitions were studied in a septic intensive care population. In addition, we assessed the effect of various cut-off values for lactate on the accuracy of the Sepsis-3 criteria for septic shock to identify patients with an actual increased risk of ICU mortality compared with septic patients not fulfilling criteria for septic shock.

Materials and methods

Setting

The study was performed at the Maastricht University Medical Centre+, a tertiary care, 715 bed university hospital in the Netherlands with 18 general ICU beds, 9 cardiothoracic ICU beds, 6 high-dependency care unit beds, and approximately 2500 admissions annually.

Patients

All 678 patients that were admitted to our ICUs with a diagnosis of sepsis between 1st January 2013 and 1st January 2016 were entered in a prospectively recorded database. Data on age, gender, reason for admission, co-morbidities, APACHE score, ICU mortality, and in-hospital mortality were recorded. Admission with sepsis was defined as any admission to the intensive care unit clinically coded as infection *and* at least one organ dysfunction.[3] Institutional sepsis guidelines adhere to surviving sepsis campaign guidelines including early fluid resuscitation and antimicrobial therapy. Intravascular volume replacement was guided by either clinical variables and/or pulse contour measurements (PiCCO ©) and/or echocardiography. Norepinephrine is the vasopressor of choice to treat persistent hypotension after adequate fluid resuscitation. Norepinephrine is started when mean arterial blood pressure drops below 65 mmHg. Lactate levels were retrieved from the hospital information system. The highest lactate level within the first 24 hours after admission was used for analysis. Forty-eight patients were excluded from analysis as no lactate measurement was recorded, leaving 632 patient cases available for analysis.

Sepsis definitions

In the Sepsis-2 definition, septic shock was defined as sepsis and circulatory failure (mean arterial blood pressure (MAP) <65 mmHg (according to the treatment goal of Surviving Sepsis Campaign international guidelines 2012), norepinephrine ≥0.1 μg/kg/min). In the Sepsis-3 definition septic shock was defined as sepsis and circulatory failure (MAP<65 mmHg, norepinephrine ≥0.1 μg/kg/min) and lactate level >2 mmol/L.

Statistical analysis

ICU mortality and in-hospital mortality was calculated in patients having septic shock according to the Sepsis-3 definition and in patients having septic shock according to Sepsis-2 definition. As this resulted in two overlapping samples from a single source population no formal statistical analysis could be performed to assess the significance

of the different outcomes following these two approaches. In addition, throughout the entire study population cohorts were created using increasing cut-off values for serum lactate in steps of 2 mmol/L. The association between lactate levels and ICU mortality was assessed using logistic regression analysis adjusting for age, sex, and comorbidity. Outcome data are presented as odds-ratios with 95% confidence intervals. All analyses were performed using SPSS version 22.0 (IBM, Armonk, NY).

Results

Population

A total of 632 patients diagnosed with sepsis between 1st January 2013 and 1st January 2016 were analysed. Details on patient characteristics are presented in Table 3.1. Patients were predominantly male and 54% of patients were older than 65 years of age. Most frequent sources of infection were the lower respiratory tract (38%) and abdominal sepsis (36%). Mean APACHE II score was 25 and 39% of the septic patients were known to have an active malignancy.

Table 3.1 Characteristics of the study population.

	N (%)
Age	
<44 yrs	65 (10)
45-54 yrs	67 (10)
55-64 yrs	164 (26)
65-74 yrs	199 (32)
>75 yrs	137 (22)
Gender, female n (%)	218 (35)
APACHE II score	25
Surgical n (%)	268 (42)
Medical n (%)	364 (58)
Source of infection n (%)	
Respiratory tract	238 (38)
Abdominal	227 (36)
Urogenital	45 (7)
Skin/soft tissue	22 (3)
Other infections	34 (6)
Unknown	60 (10)
Active malignancy n (%)	244 (39)

Sepsis criteria and ICU mortality

482 patients (76.3%) were classified as having septic shock according to the Sepsis-2 definition with a mean APACHE II score of 26.2 (± 7.8). With addition of the criterion of serum lactate level ≥2 mmol/L, according to the Sepsis-3 definition, only 300 patients

(47.4%) were classified as having septic shock with a mean APACHE II score of 27 (± 7.7) (Table 3.2 and Figure 3.1). ICU mortality was higher in patients with septic shock as classified according to the Sepsis-3 criteria than in patients classified according to the Sepsis-2 criteria (38.9% versus 34.0%). As expected, ICU mortality was lower (20.6%) in the 150 septic patients not classified as having septic shock according to any of the two definitions. In- hospital mortality was 43% for patients classified according to Sepsis-2 criteria versus 47% for patients classified according to Sepsis-3 criteria. ICU mortality in the 182 patients meeting Sepsis-2, but not Sepsis-3 definition of septic shock, was 25.6%. No formal statistical analysis could be performed to assess the significance of the different outcomes following these two approaches because here it concerns overlapping patients from a single source population.

Table 3.2 Number of patients meeting the definitions for septic shock according to the Sepsis-2 and Sepsis-3 definitions and the implications for APACHE II score and ICU mortality.

	No Septic shock (n=150)	Septic shock Sepsis-2 (n=482)	Septic shock Sepsis-3 (n=300)
Age			
<44 yrs	25 (17)	40 (8)	24 (8)
45-54 yrs	11 (7)	56 (12)	34 (11)
55-64 yrs	41 (27)	123 (26)	78 (25)
65-74 yrs	50 (33)	149 (31)	95 (31)
>75 yrs	23 (16)	114 (23)	75 (25)
Gender, female n (%)	51 (34)	167 (35)	100 (33)
APACHE II score (SD)	25 (±8)	26 (±8)	27 (±8)
Surgical n (%)	51 (34)	216 (45)	145 (48)
Medical n (%)	99 (66)	266 (55)	156 (52)
Source of infection n (%)			
Respiratory tract	64 (43)	174 (36)	94 (31)
Abdominal	45 (30)	183 (38)	143 (46)
Urogenital	12 (8)	33 (7)	24 (8)
Skin/soft tissue	1 (1)	21 (4)	10 (4)
Other infections	12 (7)	27 (6)	8 (3)
Unknown	16 (11)	44 (9)	26 (8)
Active malignancy n (%)	63 (42)	181 (38)	118 (39)
ICU mortality (%)	21	34	39
In-hospital mortality (%)	35	43	47

Effects of active malignancy and serum lactate levels on ICU mortality

Patients with sepsis and a known active malignancy had a higher mortality (43%) than patients without an active malignancy (OR 2.4) (Table 3.3). When fulfilling Sepsis-3 criteria for septic shock, ICU mortality in patients with a malignancy was 49.6% and when fulfilling Sepsis-2 criteria ICU mortality was 45%. In conclusion, ICU mortality in septic shock patients with cancer is higher than patients without an active malignancy regardless of the definition used to define septic shock.

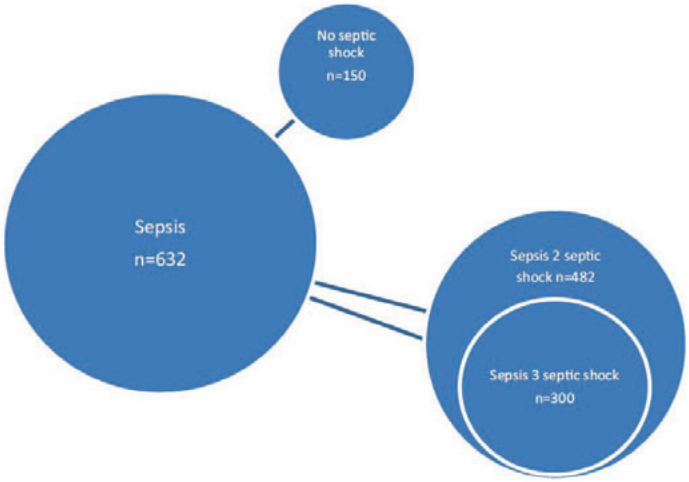

Figure 3.1 Venn diagram showing the overlap of populations.

Table 3.3 Association between sepsis and mortality in patients with active malignancy.

	Sepsis patients surviving ICU	Sepsis patients dying in ICU	OR	95% CI
Active malignancy	140	104	2.4	1.7-3.4
No active malignancy	297	91		

OD, odds ratio, CI, confidence interval

ICU mortality for patients with a serum lactate ≤2 mmol/L was 26.2% and mortality for patients with serum lactate level between 2 and 4 mmol/L was 25.0%. Following Sepsis-2 criteria, septic shock patients with a serum lactate level between 2 and 4 mmol/L did not have a significant higher ICU mortality compared to patients with a lactate level ≤2 mmol/L (OR 0.94 (0.6-1.7)) (Table 3.4). The same was true for patients with lactate level between 4 and 6 mmol/L (OR 0.88 (0.4-1.7)). Lactate levels ≥6 mmol/L were significantly and increasingly associated with ICU mortality, with mortality rate of 51% in patients with lactate levels between 6 and 8 mmol/L. ICU mortality reached 75.8% in the patient group having lactate levels ≥8. The association of in-hospital mortality of patients with septic shock with blood lactate level is shown in Table 3.5. In-hospital mortality for lactate levels below 6 mmol/L did not point out increased risk of dying in the hospital. The in-hospital mortality for septic shock patients with normal blood lactate levels is strikingly high (38.8%), this will be further addressed in the discussion below. We investigated the generalizability of the findings, given the high prevalence of active malignancy, by repeating the lactate analysis, leaving patients with active malignancy out of the analysis. 388 patients with sepsis and

no active malignancy were identified, 301 of these patients (78%) were defined as having septic shock according to Sepsis-2 definition and 188 of the patients (48%) fulfilled Sepsis-3 criteria. Lactate levels between 2 and 4 mmol/L showed no significant increase in ICU mortality (19.8%, OR 0.96 0.5-2.0), and this was also seen for lactate levels between 4 and 6 mmol/L (ICU mortality 23.3%, OR 1.49 0.6-3.7) (Table 3.6).

Table 3.4 Association between blood lactate level and ICU mortality in patients with septic shock defined by Sepsis-2 definition after logistic regression (age, sex, co-morbidity).

Lactate (mmol/L)	N (482)	ICU Mortality	OR	95% CI
<2	172	26.2%		
2 to <4	144	25.0%	0.94	0.5-1.5
4 to <6	63	23.8%	0.88	0.4-1.7
6 to <8	41	51.0%	2.96	1.4-5.9
≥8	62	75.8%	8.84	4.5-17.3

OD, odds ratio, CI, confidence interval

Table 3.5 Association between blood lactate level and in-hospital mortality in patients with septic shock defined by Sepsis-2 definition after logistic regression (age, sex, co-morbidity).

Lactate (mmol/L)	N (475)	In-hospital Mortality	OR	95% CI
<2	170	38.8%		
2 to <4	143	35.7%	0.87	0.5-1.5
4 to <6	61	27.9%	0.63	0.3-1.3
6 to <8	41	53.7%	1.96	0.9-4.3
≥8	60	81.7%	7.33	3.3-16.1

OD, odds ratio, CI, confidence interval

Table 3.6 Association between blood lactate level and ICU mortality in patients with septic shock defined by Sepsis-2 definition and no active malignancy after logistic regression (age, sex, co-morbidity).

Lactate (mmol/L)	N (301)	ICU Mortality	OR	95% CI
<2	111	19.8%		
2 to <4	91	19.8%	0.96	0.5-2.0
4 to <6	43	23.3%	1.49	0.6-3.7
6 to <8	25	52.0%	4.24	1.5-11.3
≥8	31	62.3%	7.22	2.9-18.0

OD, odds ratio, CI, confidence interval

Discussion

This study compares the recent Third International Consensus Definitions of septic shock with the Sepsis-2 definition in a population of 632 septic patients. In the present study population 76% met Sepsis-2 criteria of septic shock, whereas 47% met Sepsis-3

criteria. ICU mortality as well as in-hospital mortality was higher in patients classified according to Sepsis-3 criteria (ICU mortality 38.9%, in-hospital mortality 47.0%) compared to patients classified according to Sepsis-2 criteria (ICU mortality 34.0%, in-hospital mortality 43.0%) of septic shock.

The application of the Sepsis-3 definition in the population of patients with septic shock results in the selection of a smaller but more critically ill subpopulation. These findings are supportive of the aim of the Task Force when designing the consensus definitions to reflect septic shock as a more severe illness with a much higher likelihood of death than sepsis alone.

The septic shock definition according to Sepsis-3 definition differs from Sepsis-2 definition mainly by adding the criterion of serum lactate level >2 mmol/L as a marker for cellular abnormality. It is recommended by the Surviving Sepsis Campaign guidelines to obtain a serum lactate measurement within 6 hours of presentation for all patients with suspected sepsis or septic shock. In the present study population, a lactate level ≥6 mmol/L was associated with increased mortality in septic shock patients. However, no association with increased mortality was found for patients with septic shock and lactate values between 2 and 4 mmol/L and between 4 and 6 mmol/L compared to patients with lactate values ≤2 mmol/L. "Septic shock is a subset of sepsis in which underlying circulatory and cellular/metabolic abnormalities are profound enough to substantially increase mortality", stated by the Task Force in the Sepsis-3 definition on septic shock.[4] However, in this cohort the cellular/metabolic criterion of serum lactate level substantially increases mortality only when it rises above 6 mmol/L. The association between in-hospital mortality and blood lactate level shows a similar pattern, demonstrating that lactate levels below 6 mmol/L are not associated with increased in-hospital mortality.

Striking is the high in-hospital mortality in patients with low lactate levels (<2 mmol/L). This can be explained by the fact that a large proportion of these patients have an underlying malignancy (52%) and 30% of these patients are hematologic patients. Furthermore 54% of these patients left the ICU with treatment restrictions (not to be resuscitate and/or intubate orders). Lack of association between lactate levels below 6 mmol/L and ICU- and in-hospital mortality raises the question if lactate level >2 mmol/L is an accurate cut off value when defining septic shock. Similarly, Casserly et al demonstrated the cut off value of ≥4 mmol/L for lactate in conjunction with hypotension as the only cut off statistically associated with in-hospital mortality in a group of 28150 severe sepsis and septic shock patients from the Surviving Sepsis Campaign database.[8] Intermediate serum lactate values between 2 and 4 mmol/L were associated with increased mortality, but this did not reach statistical significance in this study. Although these intermediate serum lactate values are of clinical importance because of their linear relationship with mortality[9,10], the primary goal of the contemporary Sepsis-3 septic shock definition is to define, recognize, and treat the true high risk patient with significant higher chance of dying than with sepsis alone.

A relatively large subpopulation of the study population had an active malignancy (39%), and this was an independent risk factor for ICU mortality with an odds ratio of 2.4. There are limited epidemiologic data of cancer among septic patients. Cancer is known to be the most common comorbid medical condition in patients with sepsis and septic shock, occurring in 16.8% and 11.6% of patients respectively.[11,12] Danai et al. reported higher mortality rates in a large population of 1.784.445 septic patients with cancer, with a mean case fatality rate of 31.7% vs. 18.8% in non-cancer sepsis patients.[13] Increased mortality in this subpopulation of septic patients may be explained due to the fact that patients are often immune compromised because of the use of chemotherapy or other immune modulating therapy.

In conclusion, the epidemiology and outcome of septic shock patients are influenced by the new Sepsis-3 definition, and its application results in as a smaller but more severely ill subpopulation of septic patients compared to application of the Sepsis-2 definition. This will have influence on inclusion and outcome of clinical trials in septic shock patients.

Strength of the current study is that the study population represents a 'true' septic shock population with corresponding high APACHE scores and mortality rate. Furthermore, mortality rates in association with varying serum lactate levels are comparable with the Surviving Sepsis Campaign database.[4] A large subpopulation of 39% of septic patients had an active malignancy with increased ICU mortality, further defining our population as a more severely ill group of patients.

A limitation of the present study is its observational design and the retrospective nature of the analysis, although data were collected prospectively. Nevertheless, it should be acknowledged that observational studies can provide valuable and accurate information on real life practice. Secondly, in our sepsis database, started in 2013, we applied a cut-off value of mean arterial blood pressure lower than 65 mmHg to define septic shock, following the Surviving Sepsis Campaign Guidelines in 2012. Although this differs from the 2001 Sepsis-2 definition (mean arterial blood pressure lower than 60 mmHg), there will only be a limited yield of patients with a mean arterial pressure between 60 and 65 mmHg without vasopressor. Moreover, our study population is limited in the number of patients included. Furthermore, an elevated serum lactate level is not specific for cellular dysfunction in sepsis and factors such as accelerated aerobic glycolysis and reduced hepatic clearance can also contribute.[14] However, the combination of hyperlactatemia and fluid resistant hypotension is known to identify a patient group with high mortality.[15]

In conclusion, patients classified according to Sepsis-3 criteria had a higher ICU mortality than patients meeting Sepsis-2 criteria. Serum lactate levels <6 mmol/L were not able to identify patients with a decreased chance of ICU survival. The lactate threshold of >2 mmol/L may be too low to point out patient with an actual increased chance of ICU and in-hospital mortality. Future prospective studies should further

validate the proposed clinical criteria of septic shock and may give new insights on cut off values, as well as its generalizability to other comparable university hospitals.

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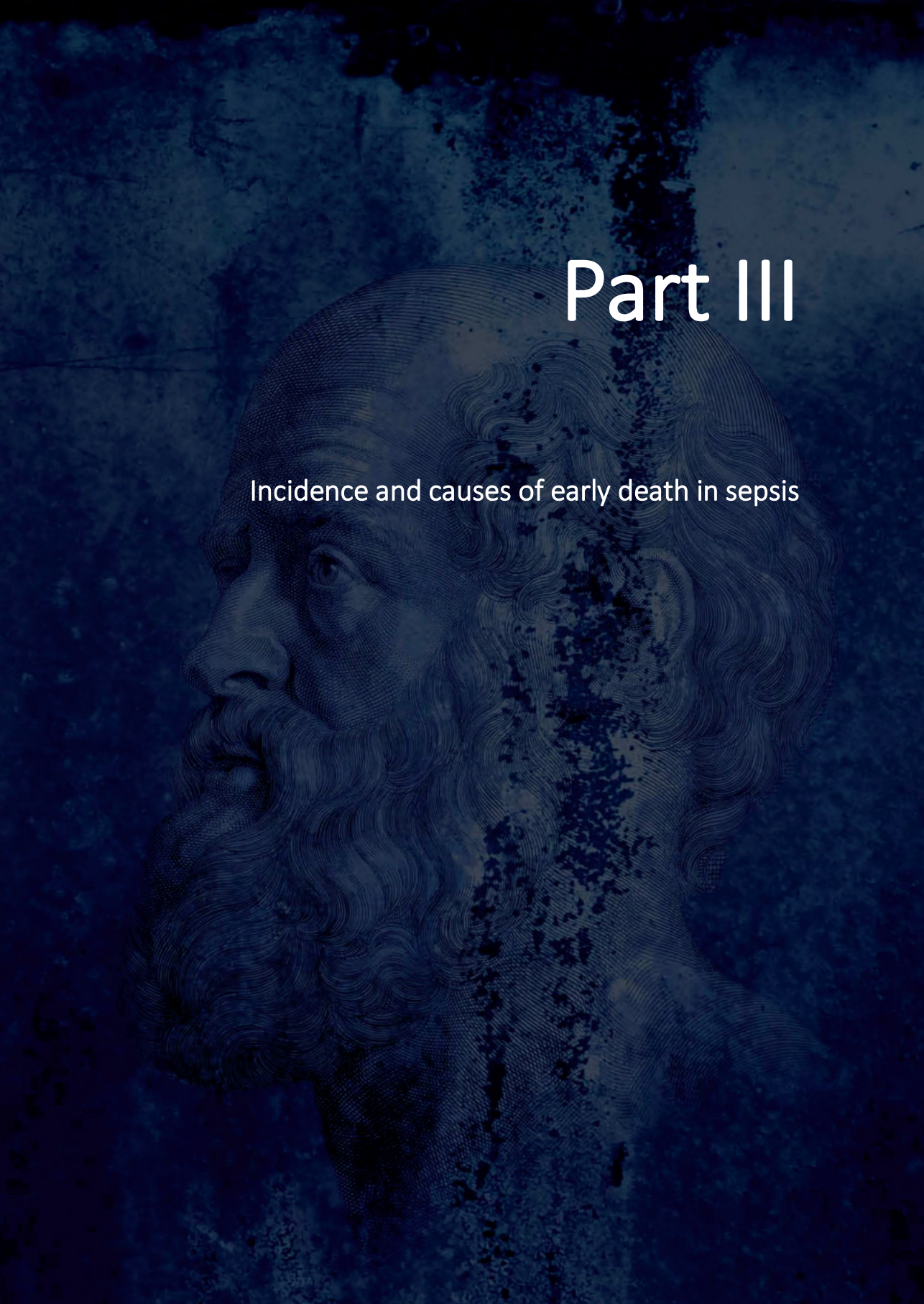
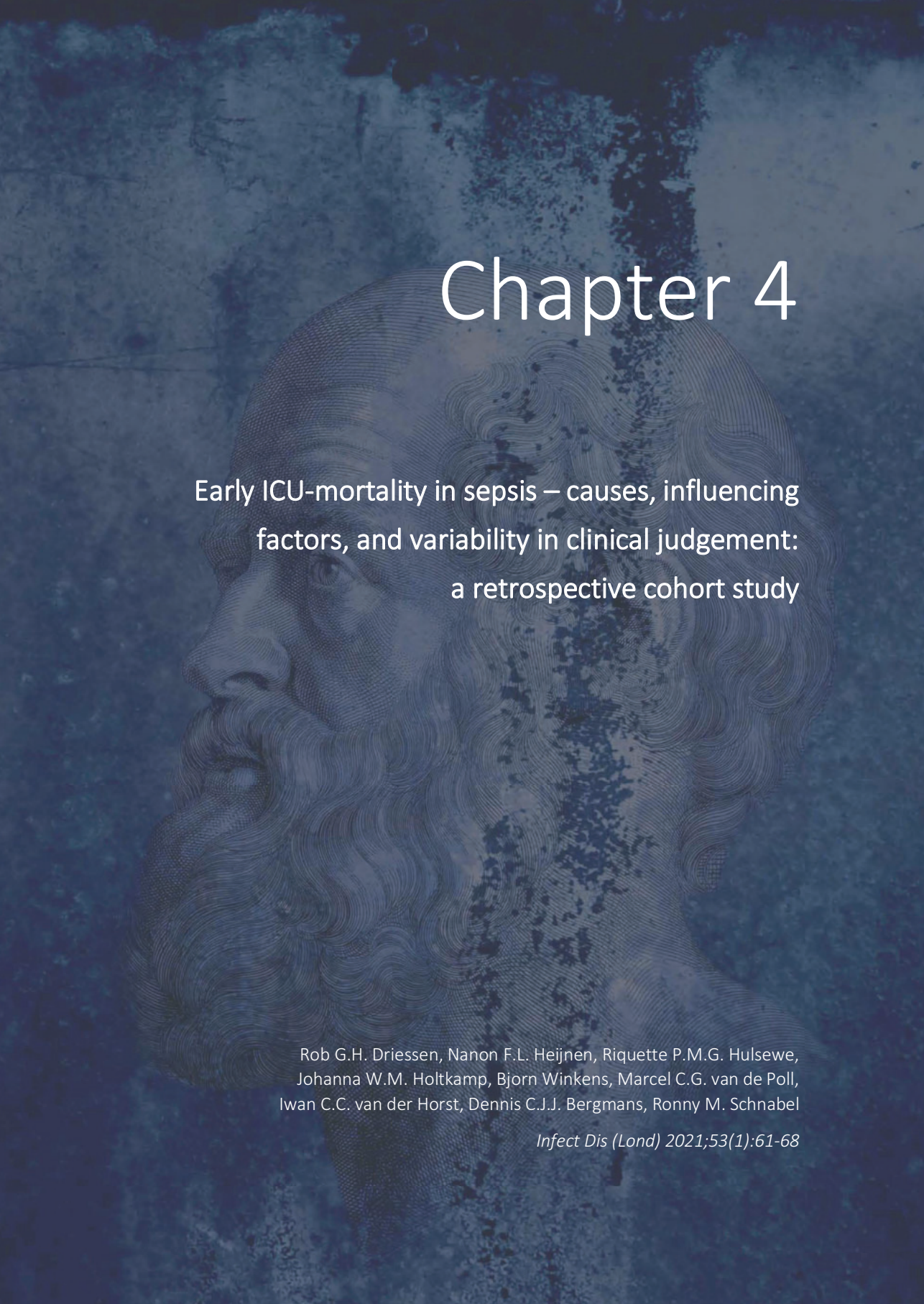


Part III

Incidence and causes of early death in sepsis



Chapter 4

Early ICU-mortality in sepsis – causes, influencing factors, and variability in clinical judgement: a retrospective cohort study

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Abstract

Background

Sepsis is a global health care problem with a high mortality. Early death seems common, however data are sparse. The objective of the present study was to report causes and influencing factors of early death in sepsis and septic shock.

Methods

All septic ICU patients were included from 2012 to 2017. Early death was predefined as occurring within 48 hours. Causes and factors leading up to death were reported by a panel of four intensivists, independently reviewing the medical files. Following factors were assessed: 1. delay in ICU admission; 2. futile ICU treatment; 3. missed diagnosis or inadequate treatment on the ICU. Fleiss' kappa was used to assess inter-observer agreement.

Results

1107 septic patients (APACHE II score 25 ± 8) were included. 344 patients died, of which 97 (28%) within 48 hours. In 33% an autopsy was performed. Primary causes of early death were multiple organ failure, mesenteric ischemia, and death after cardio-pulmonary resuscitation (CPR). Delay in ICU admission was scored in 32% of early deaths with slight agreement (κ=0.180), futile ICU treatment in 29% with moderate agreement (κ=0.415), and missed diagnosis or treatment in 7% of cases with slight agreement (κ=0.122).

Conclusions

Early death after ICU admission in sepsis is common and primarily caused by multiple organ failure, mesenteric ischemia, and death after unsuccessful CPR. Influencing factors were delay in ICU admission and futile ICU admission. Fleiss' kappa indicates substantial variability in clinical judgement between intensivists, strengthening the necessity for shared decision making.

Background

Sepsis is a global healthcare problem with increasing incidence and high mortality attributed mainly to an aging population with increased comorbidity including immunocompromised state and active malignancy.[1,2] Improvement in sepsis recognition and treatment has led to a reduction in 28 day- and in-hospital mortality in the last two decades.[3] Nowadays, mortality rates still range from 20 to 30%.[1,4]
Although data about crude mortality in sepsis are abundantly present in the literature, few studies address the exact causes of death in these patients. To date, some epidemiological and clinical studies have investigated causes of death in these patients (refractory shock and withdrawal of care)[5] and identified possible predictors for death (older age, male gender, comorbidities, and SOFA score) in septic patients.[6] Most studies do not provide or report low autopsy numbers in these critically ill patients, possibly limiting reliable assessment of the causes of death.[7]
Studies investigating the temporal relationship between death in sepsis patients after ICU admission are even more sparse. Apparently, one-third of all sepsis deaths occurs within 3 days of ICU admission.[8] Precise causes of early death in sepsis are also not well established and possible targets to intervene or prevent these early deaths are not well investigated. Recognizing proximal reasons for early death in sepsis after ICU admission is important to improve clinical care and for the design of future interventional clinical trials as some patient categories perhaps do not benefit from an intervention anyway, thereby obscuring potential effectiveness of the intervention for other patients.[9]
The objective of the present retrospective cohort study was to report timing, causes, and influencing factors of early death after ICU admission in patients with sepsis and septic shock.

Methods

Setting

The retrospective cohort study was performed at the Maastricht University Medical Centre+, a tertiary care, 715 bed university hospital in the Netherlands with 33 intensive care unit (ICU) beds with approximately 2500 admissions annually. Our hospital is a reference centre for trauma, neuro-surgical, neurological, and extracorporeal life support (ECLS) patients. The study was approved by the local medical ethics committee.

Study population

Patients admitted to the ICU were systematically screened for sepsis since 2012 and we entered all patients admitted with sepsis in the ICU in our centre in a prospectively

recorded database. We defined admission with sepsis as any admission to the intensive care unit clinically coded as infection and at least one organ dysfunction. All 1107 patients admitted in the ICU with a diagnosis of sepsis were included from January 2012 to December 2017. Septic shock was defined as sepsis with circulatory failure according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) criteria (mean arterial blood pressure <65 mmHg or necessitating norepinephrine ≥0,1 µg/kg/minute to achieve this, despite adequate fluid resuscitation and lactate >2 mmol/L).[10] Patients were treated according to Surviving Sepsis Campaign guidelines, including prompt broad spectrum antibiotics, fluid resuscitation, and vasopressors. Broad-spectrum antibiotic treatment was started in consultation with the medical microbiologist and was de-escalated as soon as possible. Intravascular volume replacement with crystalloids was guided by either clinical variables and/or pulse contour measurement (Pulse Contour Cardiac Output (PiCCO©)) and/or echocardiography. Hypotension, defined as mean arterial blood pressure below 65 mmHg despite adequate volume resuscitation, was treated with norepinephrine as preferred vasopressor. Withholding or withdrawing life-sustaining therapies and end-of-life decisions were taken multidisciplinary by the institutional medical team when continuing treatment was considered futile. Patients who deceased during ICU stay were defined as either early (during the first 48 hours of ICU admission) or late (after 48 hours of ICU admission) deaths.[11]

Collection of data

We collected data on gender, age, source of infection, comorbidities (defined as NYHA class IV cardiac failure, chronic restrictive or obstructive respiratory failure with functional impairment, hepatic cirrhosis, and immunosuppression), active malignancy, and APACHE II score for each patient. Presence of acute kidney injury (defined as creatinine ≥175 mmol/L), coma on admission (defined as Glasgow coma scale ≤8 without sedation) and severe leucocytosis or leukopenia (>40 or <1/mm^3) were also recorded. Furthermore, serum lactate levels were recorded and whether it concerned a medical or surgical patient. Data were extracted from the electronic patient data management system (Intellispace Critical Care and Anaesthesia (ICCA), Philips©).

Review of causes of death

An expert panel of four intensivists (RD, RH, JH, and RS) manually assessed the medical files of all early deaths (n=97) to determine the primary cause of death.

Review of influencing factors in patient management

The expert panel reported the following influencing factors on early mortality: 1. delay in ICU admission; 2. futile ICU treatment (meaning that the patient should not have

been admitted in the ICU because of comorbidity and pre-existing general condition; a missed non-ICU code); 3. missed diagnosis or inadequate treatment on the ICU. These three potential factors were scored by all four panel members, independently and blinded for each other's judgement, in each case as either present, possible (both 1 score), or not present (0 score). The item was considered a contributing cause if at least two or more of the experts agreed on the presence of a flaw. The panel members based their decisions on the analysis of various electronic medical records, documented clinical and diagnostic assessments, imaging, autopsy reports, registration of vital parameters, and Modified Early Warning Scores ahead of ICU admission. Relatives of all early deceased patients were asked for permission by the treating physician to perform an autopsy.

Statistical analysis

Numerical variables were expressed as mean ± standard deviation (SD) and categorical variables as numbers and percentages.
Fleiss' kappa was used for assessing the reliability of agreement between the four physicians scoring patients who died early for three possible categories (delay in admission to the ICU, futile ICU treatment, or inadequate treatment during ICU stay). Interpretation of Fleiss' kappa was performed using Landis and Koch criteria with kappa (κ)<0 indicating poor agreement, 0.01–0.20 slight agreement, 0.21-0.40 fair agreement, 0.41-0.60 moderate agreement, 0.61-0.80 substantial agreement, and 0.81-1.00 indicating almost perfect agreement.[12] All statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NY).

Results

Study population

During the five-year study period, 1107 patients were admitted to ICU with sepsis. Patients were predominantly male (65%), had severe underlying comorbidity (55%) and more than half of the patients (53%) were older than 65 years. Most patients were medical ICU patients (59%), 41% of patients were surgical ICU patients. In 49% of all patients septic shock was present according to Sepsis-3 classification. Mean APACHE II score was 25 ± 8. Active malignancy was present in 426 patients (39%) and almost one third of patients had acute kidney injury. In a majority of patients, we identified the lung as the main source of infection (39%), followed by abdominal infections (33%). In 9% of patients, the definite source of infection could not be determined. Initial lactate levels were almost 10 mmol/L in early deaths versus almost 3 mmol/L in survivors and

4.1 mmol/L in late deaths. An overview of characteristics of included patients is presented in Table 4.1.

Table 4.1 Patient characteristics of survivors, early (≤48 hours), and late (>48 hours) deaths in sepsis patients admitted in the ICU (N=1107).

Variables	All patients N=1107	ICU survivor N=763	ICU non-survivor N=344	p-value*	Early deaths N=97	p-value**	Late deaths N=247
Demographics							
Male gender	719 (65%)	479 (63%)	240 (70%)	0.024	60 (62%)	0.860	180 (73%)
Age				0.001		0.007	
≤44	116 (11%)	99 (13%)	17 (5%)		2 (2%)		15 (6%)
45-54	124 (11%)	88 (12%)	36 (10%)		9 (9%)		27 (11%)
55-64	277 (25%)	181 (24%)	96 (28%)		22 (23%)		74 (30%)
65-74	346 (31%)	235 (31%)	111 (32%)		33 (34%)		78 (32%)
≥75	244 (22%)	160 (21%)	84 (24%)		31 (32%)		53 (21%)
Prognostic scoring							
APACHE II	24.9 ±8	22.8 ±7	29.8 ±8	<0.001	32.7 ±9	<0.001	28.6 ±7
Severe comorbidity[a]	607 (55%)	373 (49%)	234 (68%)	<0.001	56 (58%)	0.101	178 (72%)
Acute renal failure[b]	340 (31%)	200 (26%)	140 (41%)	<0.001	44 (45%)	<0.001	96 (39%)
Comatose at admission[c]	74 (7%)	29 (4%)	45 (13%)	<0.001	22 (23%)	<0.001	23 (9%)
Acidosis at admission pH<7.25	448 (40%)	247 (32%)	201 (58%)	<0.001	80 (82%)	<0.001	121 (49%)
Leucocytes >40 or <1/mm^3	185 (17%)	100 (13%)	85 (25%)	<0.001	28 (29%)	<0.001	57 (23%)
Active Malignancy	426 (38%)	238 (31%)	188 (55%)	<0.001	49 (51%)	<0.001	139 (56%)
Infection source				<0.001		<0.001	
Lung	435 (39%)	283 (37%)	152 (44%)		30 (31%)		122 (49%)
Abdominal	367 (33%)	255 (33%)	112 (33%)		36 (37%)		76 (31%)
Urinary tract	77 (7%)	71 (9%)	6 (2%)		1 (1%)		5 2%)
Cutaneous	43 (4%)	34 (5%)	7 (2%)		5 (5%)		2 (1%)
Mediastinum	11 (1%)	9 (1%)	2 (1%)		1 (1%)		1 (0.4%)
CNS	17 (2%	14 (2%)	3 (1%)		0 (0%)		3 (1%)
Catheter	17 (2%)	15 (2%)	2 (1%)		0 (0%)		2 (1%)
Other	37 (3%)	30 (4%)	7 (2%)		1 (1%)		6 (2%)
Unknown	103 (9%)	50 (7%)	53 (15%)		23 (24%)		30 (12%)
Surgical/Medical				<0.001		0.207	
Surgical	450 (41%)	339 (44%)	111 (32%)		36 (37%)		75 (30%)
Medical	657 (59%)	424 (56%)	233 (68%)		61 (63%)		172 (70%)

[a] NYHA IV cardiac failure; chronic restrictive or obstructive respiratory failure with functional impairment, hepatic cirrhosis, immunosuppression; [b] Creatinine >175 µmol/L; [c] Glasgow coma scale ≤8 without sedation; * p-value comparing ICU survivors with non-survivors; ** p-value comparing ICU survivors with early deaths.

Review of timing and causes of death

During the study period ICU mortality was 31%, which remained stable over time. Of the 344 deceased patients, 97 patients (28%) died within 48 hours after ICU admission (Figure 4.1). The in-hospital mortality of our study population was 40%.

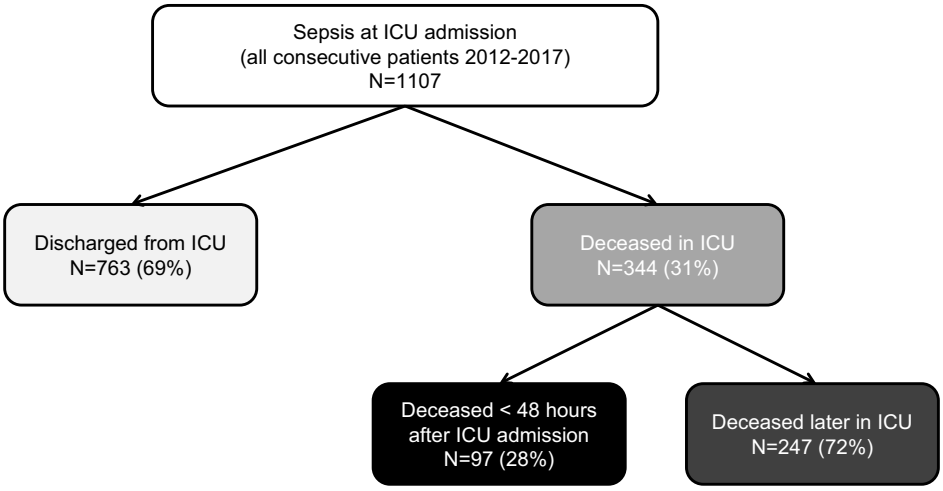

Figure 4.1 Flow chart of the study.

Autopsy was performed in 32 (33%) of early deceased patients. Multiple organ failure related to the primary infection was the main cause of early death in 37% of sepsis patients (n=36). Mesenteric ischemia was diagnosed as main contributor leading to death in 22 (23%) of early deceased patients. The diagnosis of mesenteric ischemia was confirmed by autopsy in nine patients and based on clinical presentation in combination with computed tomography (CT), endoscopy, or surgery findings in 13 patients. Early death in sepsis patients was caused by death after unsuccessful cardiopulmonary resuscitation in 21 (22%) patients. In 13 patients (13%) treatment was withdrawn by the medical team within the first 48 hours after admission because of futility. An overview of the causes of early death investigated by the expert panel is presented in Figure 4.2.

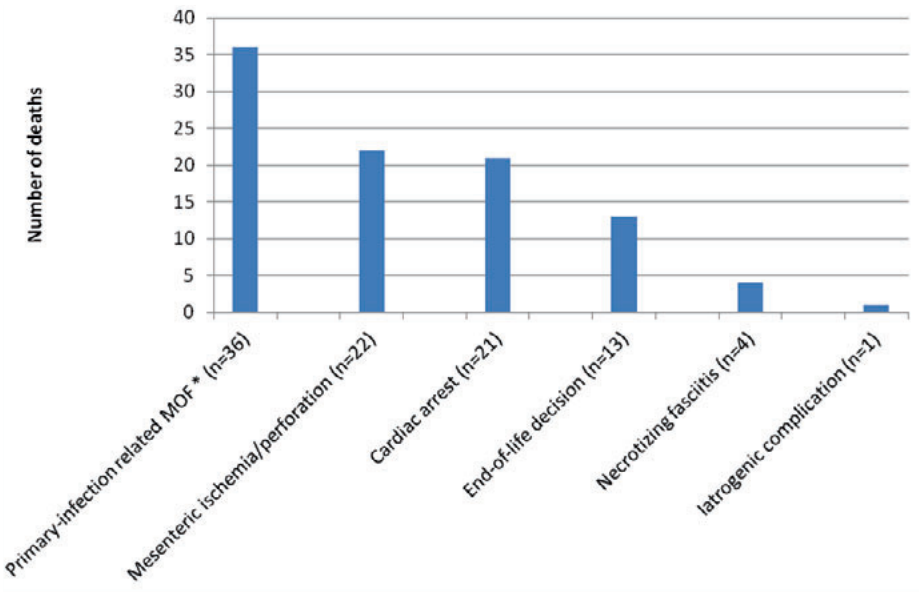

Figure 4.2 Causes of early deaths in the ICU (N=97). * Multiple organ failure.

Assessment of possible influencing factors in patient management in early deaths

In 31 patients (32%) who deceased within 48 hours, two or more physicians from the expert panel scored a possible delay in ICU admission, where Fleiss' kappa is indicating slight agreement (κ=0.180, (95% CI 0.099-0.261)) between the four physicians. Most scored causes of a possible delay in ICU admission were either delayed ICU consultation and/or delayed reaction to a high Modified Early Warning Score (n=13) and delayed (re-) laparotomy (n=6).

In 29% of early deceased patients (n=28), futile commencement of ICU treatment was scored with a moderate agreement between the physicians (κ=0.415, (95% CI 0.334-0.496)). Arguments for a preferable non-ICU code were a moribund general condition (n=10) or metastasized malignancy (n=9).

Inadequate or delayed treatment in the ICU was scored in seven patients with a slight agreement between the physicians (κ=0.121, (95% CI 0.041-0.204)). The results of the expert panel regarding a delay in diagnosis, treatment, and/or decision making are shown in Table 4.2.

Table 4.2 Specification of influencing factors scored by the expert panel in early (≤48 hours) deceased sepsis patients admitted in the ICU.

Early (≤48h) deceased septic ICU patients	N 97	%	K **
Delay in ICU admission	31	32%	0.180 (0.099-0.261)
Delayed ICU consultation or delayed reaction on high MEWS *	13		
Delayed (re-)laparotomy	6		
Patient delay	4		
Delayed CT scan or imaging modality	3		
Delayed reaction on deteriorating blood gas analysis	2		
Delayed drainage of an empyema	1		
Delayed treatment of an ileus	1		
Delayed reaction to severe dehydration	1		
Futile ICU treatment	28	29%	0.415 (0.334-0.496)
Moribund general condition	10		
Metastasized malignancy	9		
Advanced age and/or high level of frailty	3		
Dementia or severe cognitive impairment	2		
Advanced multiple myeloma	2		
Missed documentation on non-icu code	1		
Advanced ALS	1		
Missed / delayed diagnosis and/or treatment in ICU	7	7%	0.122 (0.041-0.204)
Delay in treatment of sepsis	2		
Intra-abdominal bleeding after surgery	1		
Thrombo-embolism mesenteric vessels	1		
Ruptured aneurysm thoraco-abdominal aorta	1		
Fistula between a. iliaca and the bladder	1		
Cardiac arrest after rapid sequence intubation on the ICU	1		

* Modified Early Warning Score; ** Fleiss' kappa (95% confidence intervals).

Discussion

In the present study, we found that early mortality occurs frequently in sepsis and septic shock, mainly caused by multiple organ failure, mesenteric ischemia, and death after unsuccessful CPR. Retrospectively reviewing patient files by an expert panel showed that delay in ICU admission and futile commencement of ICU treatment were frequently reported. Inter-observer agreement, using Fleiss' kappa, between the intensivists on these judgements was, strikingly, rather low, pointing out substantial variability in clinical judgement.

The relevance of differentiating between crude mortality and cause-specific mortality in sepsis syndrome has been pointed out earlier, because the heterogeneous sepsis patients do not always share a uniform pathway leading to death.[13] It is important not only from a research point of view in designing new trials, but also from a clinical point of view in tailoring patient management and when discussing, for instance, end-of-life decisions.

However, research on the causes of mortality in sepsis is sparse. Previous research in 115 sepsis patients showed that refractory shock (44%) and withdrawal of care (44%) are common reasons for death in sepsis patients.[5] Two other studies investigated causes of death in large cohorts of sepsis patients by analysis of death certificates and concluded that in a large proportion of these patients other underlying causes could be identified.[14,15] However, these studies investigated medium- (three months) or long-term mortality. Javed et al. identified initial serum lactate and modified SOFA score as independent predictors of early (within 24 hours) death, occurring in almost 5% of patients with severe sepsis in the emergency department.[16] Daviaud et al. investigated a comparable cohort of 543 septic shock patients and found that 32% of all ICU deaths occurred in an early stage, namely within three days after ICU admission.[8] In line with the present study, most early-onset deaths were related to multiple organ failure causing 82% of early deaths. Strikingly, mesenteric ischemia was identified in only 6.4% as a cause of early death compared to 23% in our study. In 41% of early deceased patients with mesenteric ischemia, the diagnosis was confirmed by autopsy findings in our study, possibly explaining the difference. Although a challenging diagnosis, non-occlusive mesenteric ischemia can occur in septic shock and is associated with multiple organ failure and high mortality reaching up to 75%.[17] In contrast to Daviaud et al, we identified more early deaths following unsuccessful CPR (n=21, 22% versus n=3, 4%), likely representing a patient category admitted in moribund condition.

Interestingly, an autopsy was performed in 33% of early deceased patients, in contrast to most other studies on this subject, reporting low numbers of autopsies.[8] Previous studies have pointed out that autopsies are contributing to a more reliable assessment of the cause of death in critically ill patients.[7,18]

In the present study, futile ICU treatment was scored in 29% (n=28) of early deceased patients. A subgroup of these patients (n=10) was in moribund general condition, possibly limiting the time to clarify the wishes of the patient and the opinion of the medical team regarding treatment or withholding certain treatment, only until after admission on the ICU. We believe ICU admission in some of these cases can be legitimate if the prognosis is unclear and to create time to determine the wishes of the patient and/or relatives and the opinion of the medical team. Another important reason for scoring a deficiency in end-of-life decision making, was the presence of a metastasized malignancy (n=9). Patients with sepsis and cancer have a significantly higher mortality, however outcomes in this subgroup have improved due to advances in care and implementation of adjuvant therapies.[19] Agreement between the four intensivists regarding possible futile ICU treatment was moderate, indicating the importance of careful selection and multi-disciplinary decision making regarding which patients could benefit from ICU treatment.

This study has several strengths. To our knowledge, it is one of few studies reporting causes of early death in sepsis and septic shock patients. The cohort of septic patients used in this study is comparable with several recent intervention studies[20,21] with

regard to a high mean APACHE II score and overall ICU mortality of 31%. Importantly, in one third of all patients dying early, an autopsy was performed, clearly attributing to a reliable assessment on the primary causes of death in these patients. Strikingly, agreement between the intensivists, studying the medical files for possible flaws in management of sepsis patients, was rather low (slight agreement in two categories and moderate agreement in one category). Besides indicating true independence, the retrospective nature of the study may have contributed to the low level of agreement.
Limitations of the present study are the retrospective design of the analysis. The cut-off value of 48 hours defining early death is not a generally agreed time frame and open for debate. It deviates from the earlier work of Daviaud et al., defining 3 days as the cut-off for early death. Predefining early and late deaths within or after 48 hours as the cut-off point, was based on previous work by Law et al., investigating trends in the timing of death among patients with septic shock in a large cohort.[11] Another limitation was the fact that the four intensivists were aware of the outcome of the patients. In the future, upfront prediction if a certain patient will survive the ICU admission, being blinded for the real outcome, would give valuable additional information in these patients.

Conclusions

In the present study, early death (≤48 hours) is common in sepsis and septic shock patients admitted in the ICU, caused mainly by multiple organ failure, mesenteric ischemia, and death after unsuccessful CPR. Delay in ICU admission and futile ICU treatment are frequently encountered by an expert panel retrospectively reviewing the patient files, however, with noticeably low inter-observer agreement indicating variation in clinical judgement. This finding strengthens the necessity for shared decision making in end-of life decision making.

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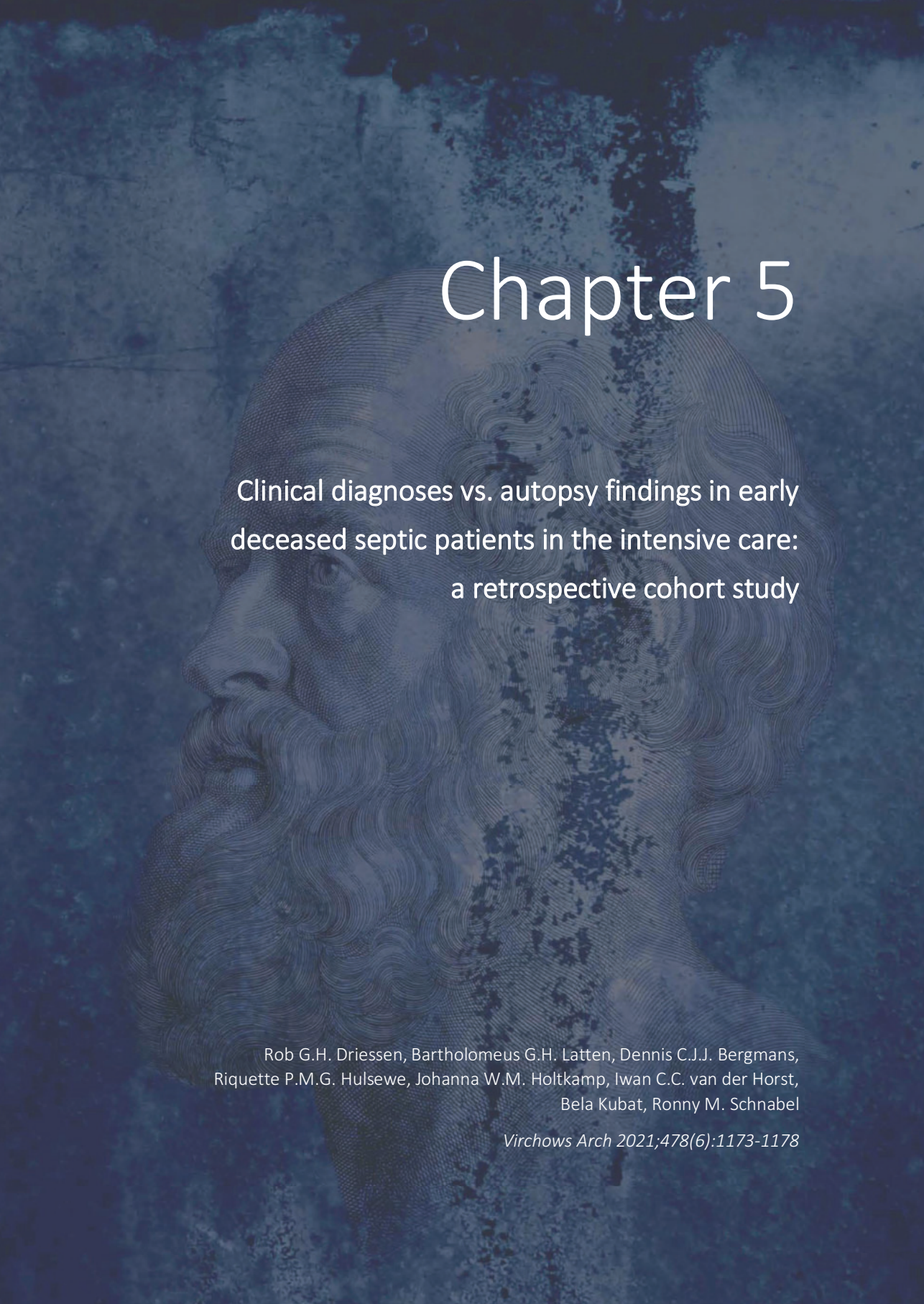

Chapter 5

Clinical diagnoses vs. autopsy findings in early deceased septic patients in the intensive care: a retrospective cohort study

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Abstract

Background

Early death in sepsis occurs frequently; however, specific causes are largely unknown. An autopsy can contribute to ascertain causes of death. The objective of the study was to determine discrepancies in clinical diagnosis and post-mortem findings in septic intensive care unit (ICU) patients deceased within 48 hours after ICU admission.

Methods

All septic ICU patients who deceased within 48 hours after ICU admission were identified and included. Four intensivists determined the clinical cause of death by medical record review. An autopsy was performed within 24 hours of death. Clinical diagnosis and post-mortem findings were compared and classified as autopsy identified-missed clinical diagnoses and autopsy-refuted diagnoses. Class I and II missed major diagnoses using the Goldman criteria were scored.

Results

Between 2012 and 2017, 1107 septic patients were admitted to ICU. Of these, 344 patients (31%) died, of which 97 patients (28%) deceased within 48 hours. In 32 (33%) early deceased patients, an autopsy was agreed. There were 26 autopsy identified-missed clinical diagnoses found, mostly myocardial infarction (n=4) and pneumonia (n=4). In four patients (13%), a class I discrepancy was found. In 14 patients (42%), a class II discrepancy was found.

Conclusions

Autopsy is an important diagnostic tool that can identify definite causes of death. These diagnoses deviate from diagnoses established during admission in early deceased sepsis patients.

Background

Sepsis is a life-threatening syndrome following a dysregulated host response to infection.[1] It still causes major public health concerns and has an increasing incidence.[2] Early death occurs in one-third of these patients.[3] However, studies investigating specific causes of death are scarce.

Autopsy, being the ultimate diagnostic test[4], is a reliable diagnostic tool in determining causes of death in critically ill patients. It also has an educational role, as studies show that attending necropsies foster clinical problem solving[5] and most students describe autopsies as educationally useful.[6] However, autopsy rates have been declining over the past few decades,[7] possibly because of a lack of reimbursement, the fear of disclosing mistakes, and the conception that advances in medical technology diminish the additional value of autopsies.[4]

Nevertheless, several studies have shown persisting discrepancies between clinical and pathological diagnosis in critically ill patients[8-11], reporting class I discrepancies ranging between 3% and 16% of patients.[12] Class I discrepancies, according to the Goldman classification, are diagnostic errors that would have changed clinical management and possibly led to longer survival of patients. Class II discrepancies are errors that probably would not have changed therapy. Almost two-thirds of the class I discrepancies were found in patients with known infection.[11] Furthermore, identifying definite causes of (especially early) death in sepsis patients may improve clinical care and point out patient categories that might not benefit from specific interventions. To our knowledge, studies reporting autopsy findings in septic patients dying within 48 hours of ICU admission are sparse.

This study's main objective is to determine the proportion of discrepancies in clinical diagnoses and post-mortem findings in early deceased septic patients. Therefore, we conducted a retrospective cohort study, comparing clinical and autopsy diagnoses in patients with sepsis and septic shock who died within 48 hours after ICU admission.

Materials and methods

Setting

This study was conducted at the Maastricht University Medical Centre+, a tertiary care, 715-bed university hospital in the Netherlands with 33 intensive care unit (ICU) beds with approximately 2500 annual admissions.

Study population

Patients admitted to ICU are systematically screened for sepsis since 2012, and we entered all patients admitted with sepsis in ICU in a prospectively recorded database. All patients diagnosed with sepsis and deceased within 48 hours after admission in ICU, between 2012 and 2017, were included in the study. Admission with sepsis was defined as any ICU admission clinically coded as infection and at least one organ dysfunction, according to the Surviving Sepsis Campaign guidelines of 2012.[13] Septic shock was defined as sepsis with circulatory failure according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3) criteria.[1] Families of eligible patients who died were routinely approached and requested permission to perform an autopsy on the deceased relative.

Collection of data

For all early deceased septic patients, data on gender, age, source of infection, comorbidities (NYHA class IV cardiac failure, chronic restrictive or obstructive respiratory failure with functional impairment, hepatic cirrhosis, and immunosuppression), active malignancy and APACHE score was recorded. Furthermore, the presence of acute renal failure (defined as creatinine above 175 mmol/L), coma on admission (defined as Glasgow coma scale ≤8 without sedation), and severe leucocytosis or leukopenia (>40 or <1/mm^3) were also recorded.

Clinical diagnosis

Clinical causes of death were investigated by manual analysis of the medical record by an expert panel of four independent physicians. This was based on information at ICU admission and during the length of ICU stay. Data collected included demographics, pre-existing medical conditions and severe comorbidity, presence of active malignancy and source of infection. Clinical causes of death were based on judgement of the four intensivists. In case of non-agreement between the intensivists, consensus could be reached in all cases.

Pathological diagnosis

An autopsy was generally performed within 24 hours of death. Before the autopsy, the pathologist was given relevant clinical information such as underlying diseases and clinical causes of death. This was performed by a standardized written request form for the autopsy, filled in by the treating physician. The pathologist was not blinded for the digital hospital record. A pathology resident, supervised by a pathologist, performed a complete autopsy. The autopsy included macroscopic and microscopic examination, including histological analysis. The pathologists described their findings in a written

report. Clinical and pathological diagnoses were made independently. The pathological diagnoses were determined by manually reviewing all the autopsy reports by the expert panel.

Comparison of clinical and pathologic diagnoses

Clinical diagnoses and post-mortem findings were compared, and discrepancies were classified into two categories: autopsy-identified missed clinical diagnoses and autopsy-refuted clinical diagnoses. The expert panel also classified Class I and Class II discrepancies using the Goldman criteria.[14] A Class I missed major diagnosis being a diagnosis that would have changed patient management and possibly resulted in cure or prolonged survival. Class II missed diagnoses are defined as major discrepancies that probably would not have changed therapy in these patients, because patients were already receiving appropriate therapy, effective therapy was not available, or the patients were too sick to receive appropriate therapy. Discrepancies were classified based on consensus between all four intensivists.

Statistics

Numerical variables were expressed as mean ± standard deviation (SD) and categorical variables as numbers and percentages. Means were compared with the t-test for numerical variables and Chi-square test for categorical variables. All statistical analyses were performed using SPSS version 22.0 (IBM, Armonk, NY).

Results

Study population

During the five-year study period, from 2012 to 2017, 1107 patients were admitted with sepsis in ICU and 344 patients (31%) deceased in ICU. Ninety-seven patients deceased within 48 hours after admission. An autopsy was performed in 32 (33%) of early deceased septic patients. The reason for not performing an autopsy was almost invariably refusal by the relatives.

Patient characteristics are shown in Table 5.1. Of 97 early deceased patients, 62% were men, 56% were older than 65 years of age, and the mean APACHE II score was 32.7 ± 7. Patients often had severe comorbidity (58%), and underlying active malignancy was present in 51%. There were no statistically significant differences in demographic variables or co-morbidity between the patients in the autopsy group vs. non-autopsy group. The primary source of infection was the abdomen (37%), followed by the lung (31%). In 24% of patients, no definite source of infection could be determined. There were significantly more infections of unknown origin in the autopsy group compared to

the non-autopsy group (36% vs. 15%, p=0.021). On the other hand, there were more pulmonary infections in the non-autopsy group compared to the autopsy group (15% vs. 39%, p=0.016).

Table 5.1 Patient characteristics.

Variables	All patients N=97	Autopsy N=33	No Autopsy N=64	p-value
Demographics				
Male gender	60 (62%)	24 (73%)	37 (58%)	0.15
Age				0.30
≤44	2 (2%)	1 (3%)	1 (2%)	
45-54	9 (9%)	3 (9%)	6 (9%)	
55-64	22 (23%)	7 (22%)	15 (22%)	
65-74	33 (34%)	10 (30%)	23 (37%)	
≥75	31 (32%)	12 (36%)	19 (30%)	
Prognostic scoring				
APACHE II	32.7 ± 9	33.7 ± 7	32.2 ± 9	0.40
Severe comorbidity[a]	56 (58%)	16 (45%)	39 (63%)	0.24
Acute renal failure[b]	44 (45%)	18 (55%)	26 (41%)	0.64
Comatose at admission[c]	22 (23%)	7 (21%)	15 (23%)	0.80
Acidosis at admission pH<7.25	80 (82%)	30 (91%)	51 (80%)	0.16
Leucocytes >40 or <1/mm^3	28 (29%)	10 (30%)	18 (28%)	0.82
Active Malignancy	49 (51%)	20 (61%)	29 (45%)	0.15
Infection source				
Lung	30 (31%)	5 (15%)	25 (39%)	0.016
Abdominal	37 (38%)	15 (45%)	22 (34%)	0.29
Urinary tract	1 (1%)	0 (0%)	1 (2%)	
Cutaneous	4 (4%)	0 (0%)	4 (6%)	
Mediastinum	1 (1%)	0 (0%)	1 (2%)	
CNS	0 (0%)	0 (0%)	0 (0%)	
Catheter	0 (0%)	0 (0%)	0 (0%)	
Other	1 (1%)	1 (3%)	0 (0%)	
Unknown	23 (24%)	12 (36%)	10 (15%)	0.021
Clinical diagnosis				
Primary infection related MOF[d]	36 (37%)			
Mesenteric ischemia/perforation	22 (23%)			
Cardiac arrest	21 (22%)			
End of life decision	13 (13%)			
Necrotizing fasciitis	4 (4%)			
Iatrogenic complications	1 (1%)			

[a] NYHA IV cardiac failure; chronic restrictive or obstructive respiratory failure with functional impairment, hepatic cirrhosis, immunosuppression; [b] Creatinine >175mmol/L; [c] Glasgow coma scale ≤8 without sedation; [d] Multiple organ failure.

Clinical diagnosis

The main clinical cause of death in these early deceased septic patients was multiple organ failure related to the primary infection, occurring in 37% of patients. Furthermore, mesenteric ischemia (23%) and death after unsuccessful

cardiopulmonary resuscitation was seen in 22% of deceased patients. In Table 5.1, the clinical causes of death found by the expert panel are mentioned.

Comparison of clinical diagnoses and autopsy findings

Table 5.2 shows the discrepancies between clinical diagnoses and autopsy findings in early deceased sepsis patients. We identified 26 (81%) autopsy-identified missed clinical diagnoses in 32 autopsies, most often myocardial infarction (n=4) and pneumonia (n=4). In three patients, the autopsy revealed a malignancy that was not clinically recognized (one case of lung cancer, one of hepatocellular carcinoma, and one case of gastric cancer). Three other patients had an unidentified haemorrhage, two located intra-abdominal and one intra-cerebral. Clinically undetected mesenteric ischemia was identified in two patients during the autopsy, but also refuted as clinical diagnosis in a patient. The same applied to pancreatitis.

Table 5.2 Major discrepancies between clinical diagnoses and autopsy findings, including Class I errors (˙) (1 myocardial infarction, 1 ruptured aneurysm, 1 bleeding fistula, and 1 abdominal bleeding).

Diagnosis	Autopsy-identified missed clinical diagnosis	Autopsy refuted clinical diagnosis
Myocardial infarction	4˙	
Pneumonia	4	1
Cancer	3	1
Hemorrhage	3˙	
Mesenteric ischemia	2	1
Cirrhosis	2	
Pancreatitis	2	1
Pulmonary embolism	1	1
Ruptured aortic aneurysm	1˙	
Renal infarction	1	
Fistula	1˙	
Intracardiac thrombus	1	
Intracerebral bleeding	1	
Total	26	5

According to the Goldman classification criteria, we identified Class I errors in four patients (13%), meaning this would have changed management and possibly prolonged survival of these patients. This included one myocardial infarction, one ruptured thoracic aortic aneurysm, one bleeding fistula between the iliac artery and the small bowel and one abdominal bleeding after percutaneous drainage of cholecystitis.
In 14 patients (42%), we found Class II errors according to Goldman, meaning these were major discrepancies that would probably not have changed management because treatment was already given or not available, or patients were too sick to receive the appropriate treatment (for instance, surgery for bowel ischemia in a patient with deep

septic shock). Class II errors included bowel ischemia (n=4), myocardial ischemia (n=4) and pneumonia (n=3).

Discussion

The present study aimed to determine the accuracy of clinical diagnoses and identify discrepancies with autopsy findings (especially Class I discrepancies) in patients with sepsis and septic shock dying within 48 hours after ICU admission. We observed an autopsy rate of 33% in this study population, and 26 autopsy-identified missed clinical diagnoses were found. In 13% of patients, a Class I error was identified, meaning this would have changed management and possibly prolonged survival of these patients. In 42% of patients, a Class II error was found.

To our knowledge, this is the first study investigating autopsy findings in this specific group of early deceased septic ICU patients. The autopsy rate of 33% is in range of earlier ICU autopsy studies[12] and much higher than the global autopsy rate in the Netherlands which is 2.7%.[15] The ICU studies report autopsy outcomes in general ICU populations, most often mixed medical and surgical patients. Most of these studies are also retrospective in origin, except for the prospective study by Combes et al.[10] This study reported an autopsy rate of 53% in 315 deceased ICU patients and identified Class I errors in 10% of patients.

Our study identified Class I error in 13% of patients, falling in the upper range of those reported in other ICU studies (3 to 16%). This discrepancy rate is in line with the findings of Silfvast et al., showing that 62% of class I diagnostic errors occur in patients with existing infections.[11] In a systematic review concerning ICU patients, including over 5000 autopsies, a Class I error was reported in 8%.[16] Class I missed diagnoses are clinically the most important, as they would have changed treatment and possibly impacted survival. Despite improvements in technology, including imaging, these major discrepancies between clinical and autopsy diagnoses remain consistent over time at approximately 10%.[14,17]

We identified 26 missed clinical diagnoses, predominantly myocardial infarction, pneumonia, cancer, and haemorrhage. These categories of diagnoses are in line with other ICU autopsy studies[8,10] and also with findings in the general (non-ICU) population.[18] We classified fourteen of these diagnoses as Class II diagnoses, according to Goldman, meaning that they would probably not have changed therapy in these patients. Our study population represents very sick patients, often in deep septic shock, meaning that appropriate therapy for these diagnoses was not always possible because of the severity of the shock. Although pre-mortem diagnosis for some diseases (myocardial infarction and pulmonary embolism) is improved, infections, especially in immunocompromised patients, emerge as a more common cause of death and the anatomic location of infection is sometimes only detected by an autopsy.[4] In the

present study, in 24% of deceased patients the origin of infection was unknown and in the patients undergoing autopsy this percentage (36%) was significantly higher.

Myocardial infarction was described in four septic patients undergoing autopsy in this study. Post-mortem diagnosis of myocardial infarction can be complicated, especially in sepsis patients and the literature describes several diagnostic pitfalls.[19] In three of the four patients a post-mortem angiography was performed, showing three-vessel disease in two patients and one-vessel disease (RCA 75%) in one patient and no discrimination of a thrombus. In all four patients recent ischemia was demonstrated, in two patients by nitro blue tetrazolium (NBT) staining and in all patients this was confirmed with microscopic findings suggestive of ischemia (eosinophilic bands in the cytoplasm of cardiomyocytes). One patient underwent cardiopulmonary resuscitation (CPR) and this could explain the finding of myocardial ischemia at the autopsy. This underlines that unravelling the mechanism leading to myocardial ischemia in these patients can be challenging.[19]

In four patients, pneumonia was found during autopsy. In three of these patients, a purulent effusion was found in the bronchi during the autopsy. Microscopic changes compatible with pneumonia like damaged alveolar septa and intra-alveolar presence of macrophages and neutrophilic granulocytes were found in all four patients. The fact that mesenteric ischemia is identified as both missed as refuted diagnosis with an autopsy, points out that it is a challenging diagnosis, often occurring in patients with septic shock and multiple organ failure.[20] The same seems to be true for pancreatitis in these patients, especially since the pancreas undergoes rapid autolysis after death. It is difficult to distinguish pre-mortem necrosis from autolysis[21], particularly when the inflammatory response is attenuated as can be the case in immunocompromised patients.

Studies have shown that as autopsy rate rises, the number of major misdiagnoses fall[22], pointing out the educational value of autopsy. In our centre, the intensivist who took care of the deceased patient, attends the final part of the autopsy and discusses the results with the pathologist. This facilitates the opportunity to look at the results in a clinical context and learn from each other. Indeed, previous studies demonstrate the importance of attending autopsies for deductive reasoning and awareness of the large proportion of patients with multiple diseases.[23,24]

Our study has several limitations. First, because we are investigating a specific study population (early deceased septic patients), the number of patients is small, limiting generalizability. An autopsy was performed in only 33% of patients, which might introduce bias. However, the autopsy group was largely comparable to the non-autopsy group. Furthermore, the study was performed in a retrospective manner. Nevertheless, given the paucity of data on this matter, our study provides valuable insights concerning the value of autopsy in sepsis patients dying early after admission.

In conclusion, despite technical improvements, in patients with sepsis who died early in the ICU, discrepancies between clinical diagnosis and post-mortem findings are often

identified. In 13% of patients, these discrepancies might have changed therapy or prolonged survival, underlining the importance of autopsies for patient care, understanding pathophysiology and epidemiology of sepsis patients.

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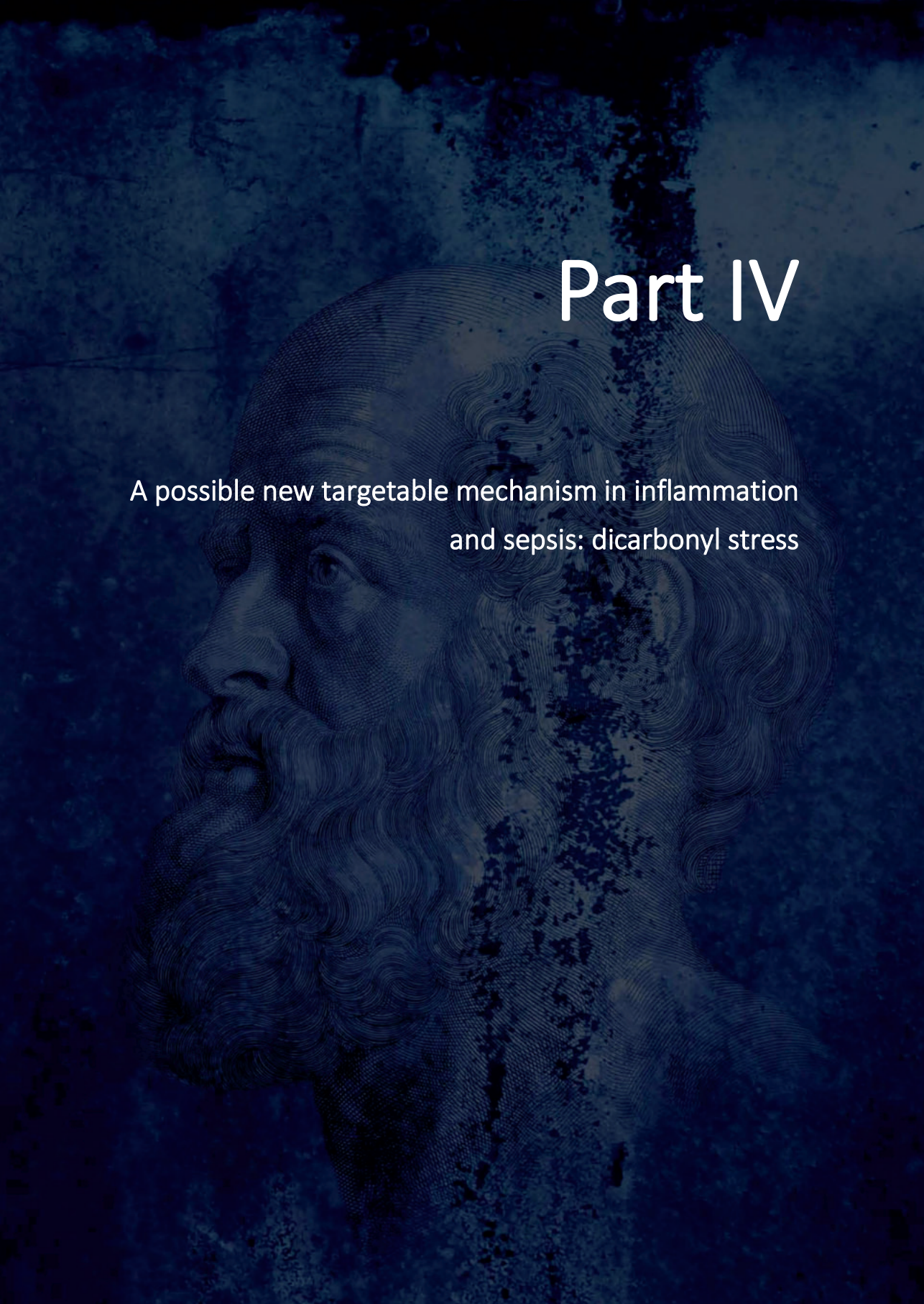
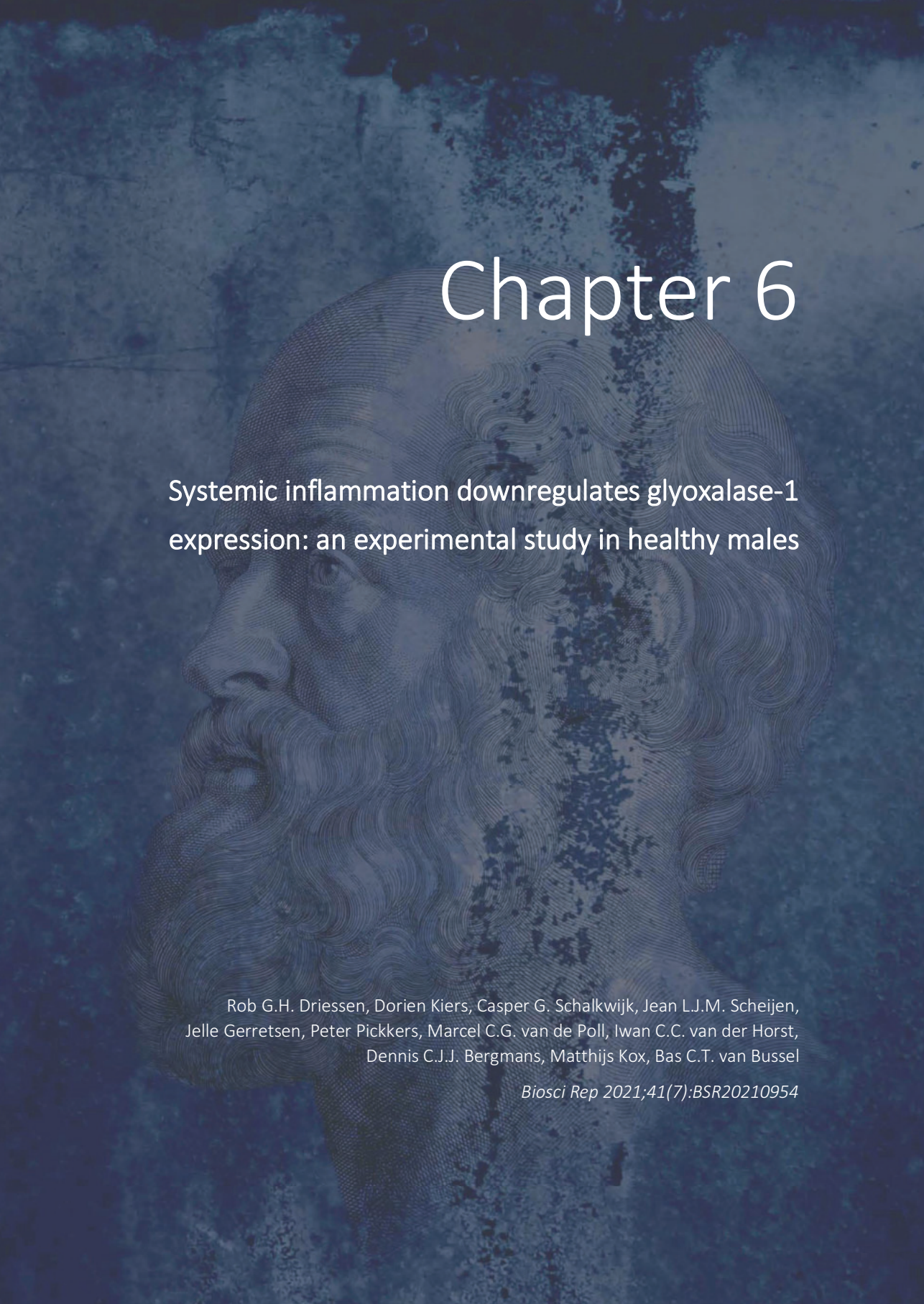


Part IV

A possible new targetable mechanism in inflammation and sepsis: dicarbonyl stress



Chapter 6

Systemic inflammation downregulates glyoxalase-1 expression: an experimental study in healthy males

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Abstract

Background

Hypoxia and inflammation are hallmarks of critical illness, related to multiple organ failure. A possible mechanism leading to multiple organ failure is hypoxia- or inflammation-induced downregulation of the detoxifying glyoxalase system that clears dicarbonyl stress. The dicarbonyl methylglyoxal (MGO) is a highly reactive agent produced by metabolic pathways such as anaerobic glycolysis and gluconeogenesis. MGO leads to protein damage and ultimately multi-organ failure. Whether detoxification of MGO into D-lactate by glyoxalase functions appropriately under conditions of hypoxia and inflammation is largely unknown. We investigated the effect of inflammation and hypoxia on the MGO pathway in humans *in vivo.*

Methods

After prehydration with glucose 2.5% solution, ten healthy males were exposed to hypoxia (arterial saturation 80-85%) for 3.5 hours using an air-tight respiratory helmet, ten males to experimental endotoxemia (LPS 2 ng/kg i.v.), ten males to LPS+hypoxia, and ten males to none of these interventions (control group). Serial blood samples were drawn, and glyoxalase-1 mRNA expression, MGO, methylglyoxal-derived hydroimidazolone-1 (MG-H1), D-lactate and L-lactate levels, were measured serially.

Results

Glyoxalase-1 mRNA expression decreased in the LPS (β (95%CI); -0.87 (-1.24; -0.50) and the LPS+hypoxia groups; -0.78 (-1.07; -0.48) ($p<0.001$). MGO was equal between groups, whereas MG-H1 increased over time in the control group only ($p=0.003$). D-lactate was increased in all four groups. L-lactate was increased in all groups, except in the control group.

Conclusion

Systemic inflammation downregulates glyoxalase-1 mRNA expression in humans. This is a possible mechanism leading to cell damage and multi-organ failure in critical illness with potential for intervention.

Introduction

Severe inflammatory conditions, such as sepsis, leading to multiple organ failure (MOF), are still a major challenge in intensive care units (ICUs).[1,2] Hypoxia is another hallmark of critical illness and sepsis, interacting with severe inflammation at a cellular level causing cytopathic hypoxia.[3] Several pathobiological mechanisms involved in the development of MOF, such as inflammation[4], coagulation[5], endothelial dysfunction,[6] and oxidative stress,[7] have been investigated previously. However, little attention has been paid to another possibly relevant mechanism, the detoxifying glyoxalase system that clears dicarbonyl stress.

It has been postulated that increased formation of the dicarbonyls MGO, glyoxal (GO), and 3-deoxyglucosone (3-DG), induced by inflammation and hypoxia, may contribute to multi-organ failure in critical illness.[8] These reactive dicarbonyls are produced by several metabolic pathways, such as anaerobic glycolysis and gluconeogenesis.[9] Inflammation leads to a switch from oxidative phosphorylation to glycolysis, which may drive production of dicarbonyls.[10] Hypoxia leads to cellular adaptation to low oxygen by activation of hypoxia-inducible factors (HIFs) and activation of anaerobic glycolysis, which also may drive dicarbonyl production.[11] The produced dicarbonyls damage intracellular and extracellular proteins mainly due to arginine modifications and the formation of methylglyoxal derived hydroimidazolone-1 (MG-H1), leading to cell and tissue dysfunction[12], which has been shown to impair organ function.[13-16]

The glyoxalase system clears dicarbonyl stress by detoxifying methylglyoxal, presumably the most reactive and damaging dicarbonyl.[17] It does so by converting methylglyoxal (MGO) into D-lactate, with glyoxalase-1 (GLO-1) as the key enzyme involved.[18] D-lactate concentrations thereby serve as a reflection of cumulative MGO exposure. The glyoxalase system is of particular interest because it has potential for therapeutic intervention by either lowering MGO by arginine or pyridoxamine[18] or by upregulating the glyoxalase-system with isothiocyanate.[19] However, whether detoxification of MGO by GLO-1 functions appropriately during systemic inflammation and/or hypoxia in humans is largely unknown.[20] We hypothesize that inflammation and hypoxia increase MGO, D-lactate, and MG-H1 in humans through reduced GLO-1 expression.

Herein, we investigated the effects of systemic inflammation induced by experimental endotoxemia and hypoxia on GLO-1 expression, MGO, D-lactate, and MG-H1 in healthy males.

Materials and methods

Participants

Data of a total of 40 healthy, non-smoking males, aged 18-29 years are described in this study, who took part in three randomized studies registered at Clinicaltrials.gov (NCT01889823, NCT01978158, and NCT02642237). Data from the hypoxia group (n=10) were obtained from the study registered under NCT01889823[21], data from the LPS (n=10) and LPS+hypoxia (n=10) groups from the study registered under NCT01978158[21], and data from the control group (n=10) from the study registered under NCT02642237.[22] All studies were approved by the local medical ethics committee (CMO Arnhem-Nijmegen), and written informed consent was obtained from all participants. All study procedures were in accordance with the declaration of Helsinki. Participants were screened before the start of the study and had a normal physical examination, electrocardiography, and routine laboratory values. Participants with a pre-existent disease or febrile illness within 4 weeks before the experiment were excluded. Participants were asked to refrain from caffeine and alcohol intake in the preceding 24 hours and food in the preceding 12 hours, before the experiment. Height and weight were measured and recorded.

Study design

The study design with timing and duration of interventions is shown in Figure 6.1. Study procedures were identical in all four groups except the intervention and slightly different time points for blood withdrawal in the control group. Ten participants were exposed to hypoxia for 3.5 hours by titration of FiO_2 to a peripheral saturation (SaO_2) of 80-85% using a nitrogen/medical air mixture and an air-tight respiratory helmet (CaStar, Starmed, Italy) (hypoxia group). A systemic inflammatory response was elicited in ten participants by the administration of 2 ng/kg U.S. Reference Escherichia coli endotoxin (serotype O:113, Clinical Centre Reference endotoxin, National Institute of Health, Bethesda, USA) (LPS group). This human endotoxemia model has been successfully applied as a translational model for sepsis and gives a short-lived, controlled inflammatory response clinically causing fever, tachycardia, and mild hypotension as well as leukocytosis and increased plasma cytokine levels.[23-26] Ten participants were exposed to hypoxia and LPS, with the LPS administered one hour after hypoxia initiation (LPS+hypoxia group). Finally, ten participants underwent the same study protocol as described below; however, these participants were not exposed to LPS or hypoxia (control group). Although the original study by Koch et al. investigated the effect of endotoxin tolerance with a live-attenuated Influenza vaccine, the blood samples used in the present study were taken only from participants receiving placebo (no LPS).[22]

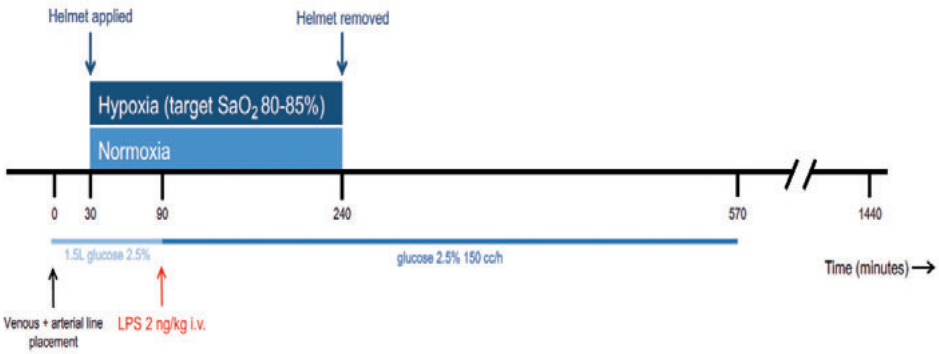

Figure 6.1 Overview of the human endotoxemia and hypoxia model procedures. First, venous and arterial cannula were placed. Subsequently, prehydration with 1.5 L glucose 2.5% infusion (light blue line) was started. After 1 hour, prehydration was ceased, and maintenance fluid infusion of the same solution of 150 mL/hour was commenced. Application of a non-invasive helmet for 210 minutes (blue arrows) to induce hypoxia or normoxia (indicated by the blue square) was initiated after 30 minutes. One hour after the start of prehydration and application of the helmet, 2 ng/kg LPS was administered intravenously (red arrow). Blood samples were drawn at ten time points from 0 until 570 minutes and an additional blood sample was drawn after 24 hours (1440 minutes).

Procedures and recording of vital signs

A venous cannula was placed for fluid infusion. Patients received prehydration with 1.5 L 2.5% glucose/0.45% saline in the hour preceding endotoxin administration, followed by hydration with 150 ml/hour of the same solution for 6 hours. The experimental endotoxemia protocol required an infusion of fluids due to vasodilation and the risk of hypotension. In total 120 mg of glucose was infused during the study protocol. An arterial cannula in the radial artery facilitated blood pressure monitoring and blood withdrawal. Blood was drawn at 10 different points in time; for the hypoxia, LPS, and LPS+hypoxia groups: 0, 90, 150, 180, 210, 240, 270, 330, 450, 570, and 1440 minutes (24 hours); for the control group: 90, 150, 180, 210, 270, 330, 450, and 570 minutes. Unfortunately, no baseline samples of the control group participants were available. Blood samples were collected in Paxgene blood RNA tubes (Qiagen®) and lithium heparin blood tubes. Plasma was separated by centrifugation at 2000 g for 10 minutes at 4˚C. Samples were stored at -80˚C until analysis. Heart rate was monitored using a three-lead electrocardiogram and SaO_2 was monitored using a pulse oximeter. Body temperature was measured using an infrared tympanic thermometer (FirstTemp Genius 2; Covidien, Ireland) every 30 minutes. Leukocyte counts were measured using routine analysis methods also used for patient samples (flow cytometric analysis on a Sysmex XE-5000). Plasma cytokines were measured by

simultaneous Luminex assays (hypoxia, LPS, and LPS+hypoxia groups: Milliplex, Merck Millipore; Billerica, USA; control group: R&D systems; Abingdon Science Park, UK).

Glyoxalase-1 (GLO1) mRNA expression

To determine GLO1 mRNA expression in leukocytes, whole blood was obtained in Paxgene vacutainer tubes (Qiagen, Venlo, the Netherlands). These tubes contain a solution which mixes with blood immediately upon withdrawal, lyses the cells, and stabilizes the RNA, after which tubes were stored at -80°C for subsequent RNA isolation.[27] Samples for this analysis were obtained from the hypoxia, LPS and LPS+hypoxia groups at t=0, 90, 150, 240, 270, 330, 450, and 1440 minutes. RNA was isolated batch wise using the Paxgene blood RNA kit (Qiagen, Venlo, the Netherlands). The iScript cDNA synthesis kit (Bio-Rad laboratories, Lunteren, the Netherlands) was used to convert RNA into cDNA. Quantitative PCR (qPCR) was performed on aCFX96^{tm} Real-Time System (Bio-Rad laboratories, Lunteren, the Netherlands using the following TaqMan primer-probe pairs; human GLO1 Hs00198702_m1 and the reference (housekeeping) gen human RPL27 Hs03044961_g1 (Life technologies, Darmstadt, Germany). We determined GLO1 mRNA expression in the hypoxia, LPS, and the LPS+hypoxia group. There was no mRNA available for the participants in the control group.

Methylglyoxal (MGO)

Plasma concentration of MGO was measured in plasma samples for the hypoxia, LPS, and LPS+hypoxia groups at t=0, 90, 150, 180, 210, 240, 270, 330, 450, 570, and 1440 minutes and for the control group at t=90, 150, 180, 210, 270, 330, 450, 570 minutes. Reversed-phase ultra-performance liquid chromatography-tandem mass spectrometry was used to measure MGO concentrations, as described earlier.[28] The inter-assay variation for MGO was 6.0%.

D-lactate, L-lactate, and Nδ-(5-hydro-5-methyl-4-imidazolone-2-yl)-ornithine (MG-H1)

Plasma D- and L-lactate were measured at t=0 and 450 minutes in the hypoxia, LPS, LPS+hypoxia, and the control group with ultra-performance liquid chromatography mass spectrometry with labelled internal standard.[29] MG-H1 was measured at these same points in time by ultra-performance liquid chromatography-tandem mass spectrometry.[30]

Statistical analysis

Data analyses were performed with SPSS (Statistical Package for Social Sciences, version 24, IBM Corporation, USA), STATA (Data Analyses and Statistical Software, version 13, StataCorp LLC, USA) and GraphPad Prism version 5.00 for Windows (GraphPad Software, USA). Data are presented as mean ± standard error of the mean (SEM). Means were compared using one-way ANOVA and Student's t-tests for paired samples, as appropriate.

We used generalized estimating equations (GEE, using STATA) to investigate longitudinal regression coefficients (β) with their 95% confidence intervals (95%CI) that represent the difference in the development of GLO1 mRNA expression, and MGO plasma concentration over time between the experimental conditions. An exchangeable correlation structure was used. Crude models were adjusted for age and body mass index. GEE has several advantages over, for example, ANOVA for repeated measures to analyze longitudinal data. GEE reports an effect estimate with 95% CI, allows analyses of unequally spaced time intervals, and handles missing data robustly by analyzing all available data. Results were adjusted for age and body-mass index to minimize any effect of random variation between the participants. A two-sided p-value <0.05 was considered statistically significant.

Results

General characteristics of the 40 males, mean age of 22±2 years, were similar across experimental groups (Table 6.1).

Table 6.1 Baseline characteristics of 40 healthy male participants.

	Experimental conditions			Control	
	Hypoxia (n=10)	LPS (n=10)	LPS+hypoxia (n=10)	(n=10)	p-value
Age, years	21 ± 2	21 ± 2	21 ± 2	22 ± 2	0.598
Height, cm	183 ± 5	185 ± 8	184 ± 7	184 ± 7	0.976
Weight, kg	78 ± 11	77 ± 10	77 ± 9	77 ± 12	0.999
Body mass index, kg/m^2	23 ± 3	22 ± 2	23 ± 3	23 ± 3	0.970
Body surface area, m^2	2 ± 0.2	2 ± 0.2	2 ± 0.1	2 ± 0.2	0.999

Data are means ± standard deviation; p-values by one-way ANOVA. LPS, lipopolysaccharide.

Inflammatory parameters

An extensive description of inflammatory parameters in each group is provided elsewhere.[22,31] In Table 6.2, baseline and peak values (post-LPS for the appropriate groups and at the corresponding time points in the non-LPS groups) of leukocyte

counts and body temperature as well as peak plasma levels of the pro-inflammatory cytokines TNF-α and IL-6 are listed.

Table 6.2 Inflammatory parameters in the 40 male participants.

	Experimental conditions						Control	
	Hypoxia baseline (n=10)	Hypoxia peak (n=10)	LPS baseline (n=10)	LPS peak (n=10)	LPS+hypoxia baseline (n=10)	LPS+hypoxia peak (n=10)	Baseline (n=10)	Peak (n=10)
Leukocytes, $*10^9$/L	5.5±1.2	7.8±1.4	4.9±1.1	11.0±1.9	5.2±1.1	14.5±1.6	6.2±1.6	6.2±1.3
Body temperature, ºC	36.4±0.3	36.6±0.4	36.6±0.3	37.9±0.6	36.8±0.3	38.3±0.6	36.7±0.4	37.0±0.4
Plasma TNF-α, pg/mL	5.5±2.9	5.0±2.7	3.8±0.7	284.6±135.1	3.9±0.9	195.5±77.0	4.6±1.9	4.1±1.7
Plasma IL-6, pg/mL	3.2±0.0	3.2± 0.0	3.2±0.0	478.6±241.3	3.2±0.0	292.3±156.4	1.9±0.4	1.8±0.1

Data are means ± standard deviation. LPS, lipopolysaccharide. Peak was measured at 360, 180, 90, and 120 minutes post-LPS administration for leukocytes, body temperature, TNF-α, and IL-6 respectively.

GLO1 mRNA expression

After adjustment for age and BMI, GLO1 expression decreased in the LPS (β (95%CI); -0.87 (-1.24; -0.50)) and LPS+hypoxia (-0.78 (-1.07; -0.48)) groups over time, compared to the hypoxia group (p<0.001, Figure 6.2).

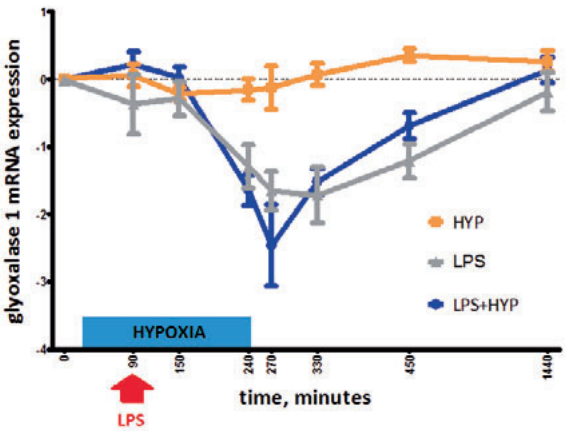

Figure 6.2 Glyoxalase-1 mRNA expression in the hypoxia (HYP, orange line), LPS (grey line), and LPS+hypoxia (LPS+HYP, blue line) groups during the experiment, depicted as means + standard error of the mean. After adjustment for age and BMI, GLO1 expression decreased in the LPS (β (95%CI); -0.87 (-1.24; -0.50)) and LPS+hypoxia (-0.78 (-1.07; -0.48)) groups, compared to the hypoxia group (p < 0.001), calculated using generalized estimating equations.

MGO concentration

After adjustment for age and BMI, MGO concentrations did not differ over time between hypoxia, LPS, and the LPS+hypoxia group (p-values 0.902 for LPS and 0.172 for hypoxia vs. LPS+hypoxia). MGO levels peaked at t=90 minutes (p<0.001) (Figure 6.3) in all three groups, before LPS was administered. MGO also tended to increase over time in the control group, albeit not statistically different (p=0.066). The control group peak was observed later compared with the other groups (t=270 minutes, Figure 6.3).

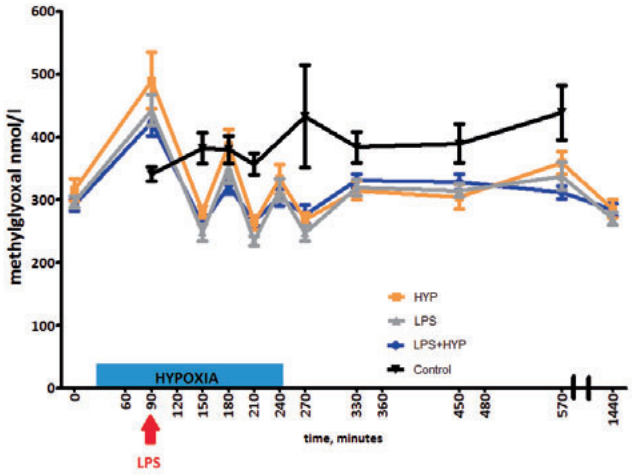

Figure 6.3 Methylglyoxal (MGO) concentrations for the hypoxia (orange line), LPS (grey line), combined LPS and hypoxia (blue line), and control (black line) groups during the experiment depicted as means + standard error of the mean. No between-group differences were found using generalized estimating equations.

D-lactate, L-lactate, and MG-H1 levels

D-lactate, the product of MGO broken down by glyoxalase, increased significantly between 0 and 450 minutes in all the intervention groups (hypoxia: p=0.002, LPS: p<0.001 and LPS+hypoxia: p<0.001), but also in the control group (p=0.013) (Figure 6.4). L-lactate levels, an end product of glucose metabolism and also a marker for tissue hypoxia, increased over time in the hypoxia (p=0.022), LPS (p<0.001), and LPS+hypoxia group (p=0.013), but not in the control group (p=0.437). MG-H1, the major advanced glycation end product (AGE) of MGO, did not significantly change over time within the intervention groups (hypoxia: p=0.062, LPS: p=0.17 and LPS+hypoxia p=0.26). A trend might be observed suggesting an increase in MG-H1 over time in the three experimental groups, however, MG-H1 levels also increased over time in the control group (p=0.003) (Figure 6.4).

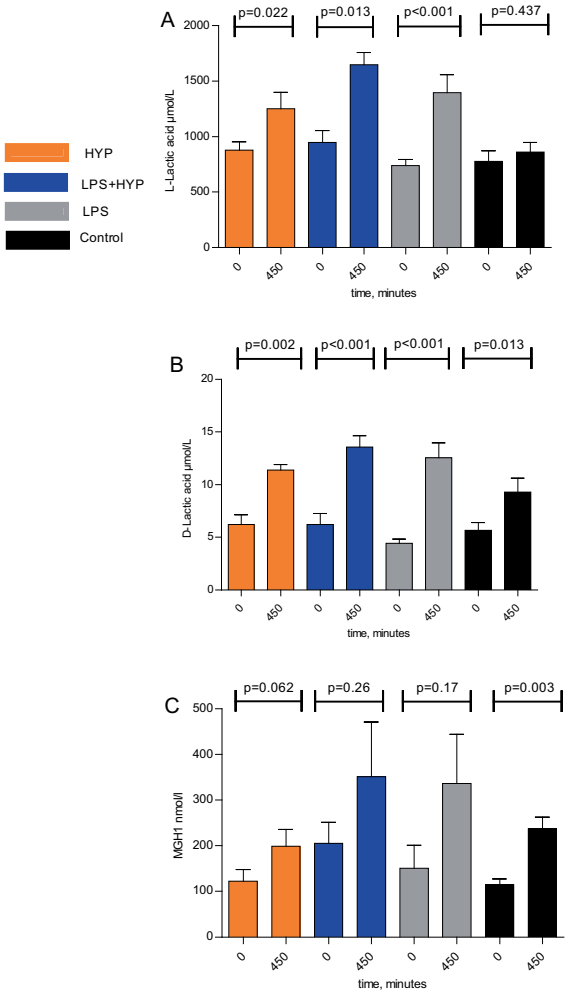

Figure 6.4 Concentrations of L- and D-lactate and MG-H1 for the different conditions and the control group. A: Panel A: L-lactate, B: Panel B: D-lactate, C: Panel C: MG-H1. L- (panel A) and D-lactate (panel B), and MG-H1 (panel C) concentrations in the hypoxia (orange boxes), LPS (grey boxes), combined LPS and hypoxia (blue boxes), and the control group (black boxes) at 0 and 450 minutes. Results are reported as means + standard error of the mean (SEM). Student-t-test was used for determining p-values.

Discussion

This experimental study in healthy males yields two main findings. First, a significant downregulation of GLO-1-expression was identified in response to inflammation, but not hypoxia. Second, experimental hypoxia and inflammation did not lead to a relevant and unequivocal difference in MGO and MG-H1 concentrations over time between the conditions.

We comprehensively investigated the effect of both inflammation and hypoxia on the dicarbonyl stress pathway in humans. Previously, *in vitro* research described a cascade linking inflammation to reduced GLO-1 expression and accumulation of both MGO and AGEs in ruptured human carotid plaques.[32] Furthermore, in mice, it was shown that increased MGO levels augmented vascular inflammation partially independent of hyperglycemia.[33] In a case-control study in sepsis patients, MGO was higher at sepsis onset and after 24 hours compared to controls, and MGO was an early predictor for survival in these patients.[20] GLO-1 was reduced in patients with septic shock after 24 hours. However, no measurements of D-lactate or MG-H1 were conducted, and the effect of hypoxia on the dicarbonyl pathway was not investigated.

In the present study, GLO-1 expression was significantly downregulated in healthy males receiving LPS but was not influenced by hypoxia. The glyoxalase detoxifying system with GLO-1 as its key enzyme depends on the presence of glutathione.[18] Inflammatory conditions are associated with an increased state of oxidative stress, affecting glutathione and inhibiting the cytosolic glutathione-dependent glyoxalase system.[34] This could lead to an accumulation of dicarbonyls. However, in these healthy men, the GLO-1 expression normalized during the eight-hour experiment, reflecting a transient effect of endotoxemia. The swift recovery of the glyoxalase system in healthy volunteers could explain why MGO blood concentrations were not higher in the LPS groups compared to the control group and suggest intact compensation mechanisms. Moreover, the endotoxemia model does not fully resemble a full-blown septic shock state in which, as alluded to before, increased MGO concentrations were found.[20] Because we used Paxgene tubes for the determination of GLO-1 mRNA expression, which result in immediate lysis of leukocytes and stabilization of RNA following blood withdrawal, leukocyte viability was not an issue, and stability of RNA stored in these tubes was previously shown to be excellent.[27] Furthermore, the LPS dosage given to the participants in this experiment is not expected to cause cell death or apoptosis.

Although MGO concentrations peaked between 0 and 90 minutes in all conditions in our experiment, there were no differences between the three experimental conditions. This peak in MGO concentration occurred before LPS administration and was also present in the LPS group (with normoxia) and thus can be explained by neither hypoxia

nor inflammation. The effect of the prehydration with 1,5 L 2,5% glucose/0,45% saline (i.e., glucose infusion) could play a role in this peak and the observed increase in D-lactate as a breakdown product of MGO, as this increase over time was also observed in the control experiment. Indeed, previous research has demonstrated that dicarbonyl concentrations increase during an oral glucose tolerance test, even in individuals with normal glucose metabolism.[35]
Post-translational modification of proteins, forming advanced glycation end products, is an important consequence of increased dicarbonyl stress. Previous studies have pointed out that MG-H1 is a major MGO protein modification product in humans[36] and is considered a key pathway leading to hyperglycemia-induced complications of diabetes mellitus.[12,37] To our knowledge, no previous studies are investigating MG-H1 concentrations in inflammatory states *in vivo*. Although in the present study a trend in increase in MG-H1 concentrations was present, this result was not statistically significant for the experimental conditions possibly due to the small sample size.

The study has several strengths and limitations. First, we used a homogenous study population consisting of healthy males. Furthermore, all the study participants underwent the same standardized study protocol and this human endotoxemia model is described in detail earlier[21] and used in several other studies.[22,31,38] This has the advantage of studying hypoxia and inflammation in humans in a highly standardized way. Notably, glyoxalase expression measurements, dicarbonyls, and their modifications end products were performed using the gold standard techniques intended to investigate this pathway comprehensively.[28] The limitation of this study, including healthy males only, is that this limits generalizability to women and patients with comorbidities. Data and blood samples from volunteers who took part in three separate studies[21,22] were used for the present investigation and we cannot rule out that this influenced the results. We tried to minimize this effect by adjusting for age and BMI in the four groups, also because ageing and obesity are both associated with dicarbonyl stress.[9] Because of limited availability of sample that we could assay, we had to prioritize which components of the dicarbonyl pathway we could measure. For instance, we did not measure glutathione, an important catalyzing factor in the glyoxalase pathway. However, as glyoxalase-I is the key-limiting enzyme in this pathway, we believe it is justifiable to emphasize on this enzyme. Furthermore, although applied in several earlier studies[22,31,38] the human endotoxemia model does not entirely resemble a full-blown sepsis state observed in critically ill patients. This might have caused an underestimation of the effects of hypoxia and inflammation on dicarbonyl stress and may explain that we did not observe the hypothesized increase in MGO concentration after GLO-1 decrease. Nevertheless, the significant peak in MGO concentration occurring early in the experimental groups suggests that our study was sensitive to reveal any effect of LPS and hypoxia on MGO, if present, in healthy males. Therefore, with regards to our initial hypothesis, the lack of effect on MGO and MG-H1

of hypoxia and inflammation can be regarded as a negative result of this study. Because Paxgene samples of the control experiment were not available, we cannot entirely exclude that we missed a downregulating effect of hypoxia on GLO-1 expression. In fact, prehydration with 1.5 L 2.5% glucose/0.45% saline (i.e., glucose infusion) could have biased the effect of hypoxia on GLO-1 expression towards zero as it has been shown that hyperglycemia upregulates GLO-1-expression.[39] Furthermore, we did not collect blood cells for functional assays, which limits further investigation of GLO-1 activity. In addition, the experiment cannot exclude that D- and L-lactate levels were determined by intercurrent changes in gut permeability caused by LPS or hypoxia.[8,40] Nevertheless, given the paucity of human *in vivo* data on this pathway, our study provides valuable insights into the interactions between inflammation, hypoxia, and dicarbonyl stress. This is important as therapeutic options by enhancing glyoxalase activity by a combination of trans-resveratrol and hesperetin showed to reduce methylglyoxal and protein modifications by MGO in overweight and obese individuals.[41] Other therapeutic options are GLO-1 induction by isothiocyanates[19] or scavenging MGO by pyridoxamine or arginine (18) to lower toxic dicarbonyl stress.

In conclusion, our study shows that systemic inflammation downregulates GLO-1 in humans. Downregulation of GLO-1 is a possible mechanism leading to cell damage and multi-organ failure in sepsis with intervention potential. We did not observe significant differences in MGO concentrations in healthy males. The results urge further investigation of the glyoxalase pathway in sepsis.

Clinical perspectives

- Hypoxia and inflammation may lead to multiple organ failure in critical illness due to downregulation of the detoxifying enzyme glyoxalase, which clears the highly reactive and protein damaging dicarbonyl methylglyoxal.
- In the present study, glyoxalase-1 mRNA expression was significantly downregulated by induced inflammation, but not by hypoxia, in humans.
- Downregulation of GLO-1 is a possible mechanism leading to cell damage and multi-organ failure in sepsis with intervention potential, urging further investigation of the glyoxalase pathway in sepsis.

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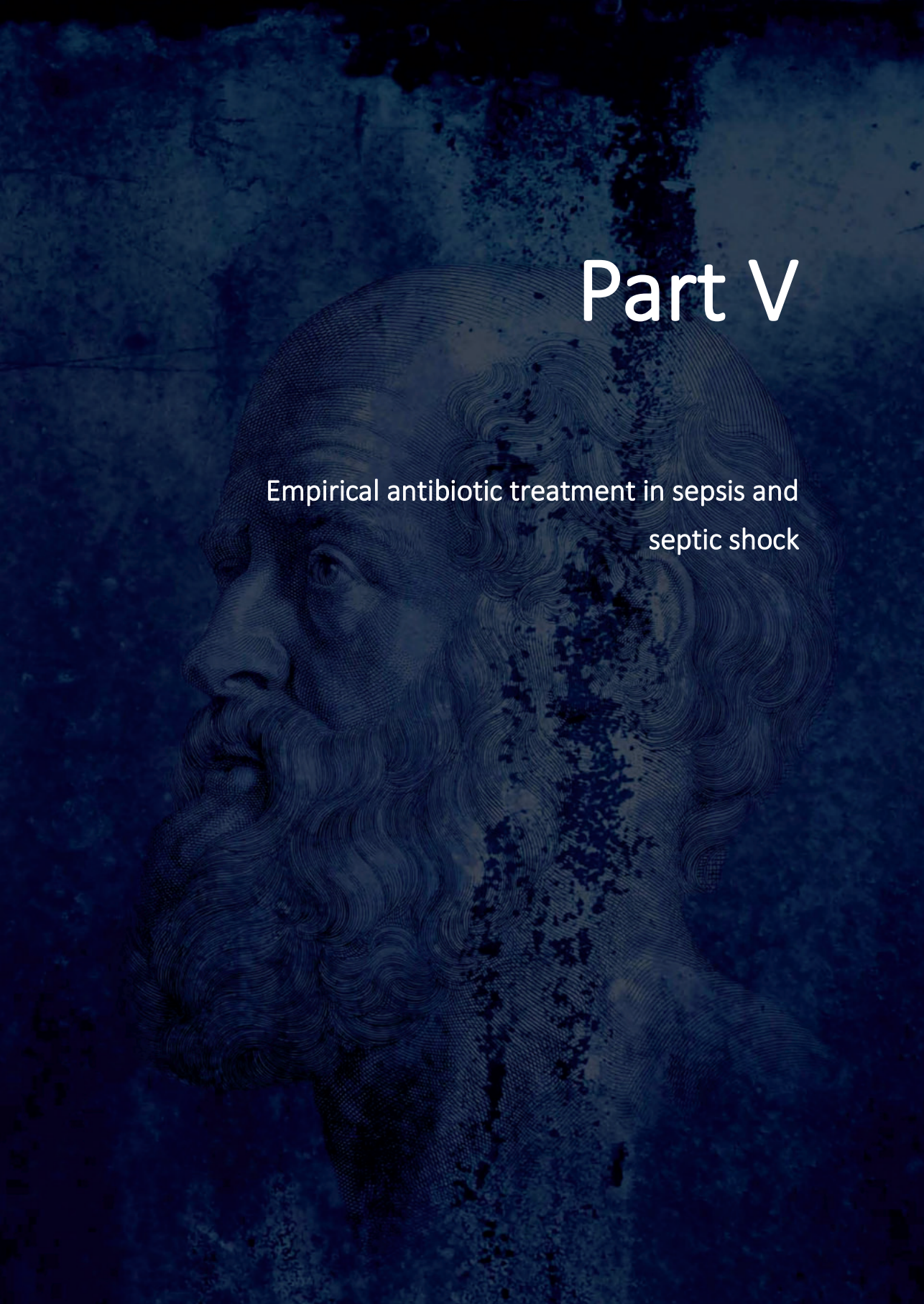

Part V

Empirical antibiotic treatment in sepsis and septic shock

Chapter 7

Appropriateness of empirical antibiotic therapy and added value of adjunctive gentamicin in patients with septic shock: a prospective cohort study in the ICU

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Abstract

Objectives

To determine the appropriateness of empiric antibiotic therapy and the possible benefit of adding short-course gentamicin in septic shock patients with abdominal, urogenital, or an unknown focus. Secondary objectives were the effect of gentamicin addition on shock reversal and the incidence of a fungal infection.

Methods

Microbiological cultures, antibiotic treatment, and antibiotic resistance patterns of the cultured microorganisms were recorded during the first 5 days of admission. Inappropriate antibiotic therapy was defined as a prescription within the first 24 hours that did not cover cultured bacteria during the first 5 days of admission and was determined in the overall group and in patients receiving adjunctive gentamicin (combination therapy) versus patients receiving monotherapy. Binomial logistic regression analysis was used to investigate the association of gentamicin addition with shock reversal.

Results

Of 203 septic shock patients, with abdominal (n=143), urogenital (n=27), or unknown (n=33) focus, 115 patients received monotherapy, and 88 patients received combination therapy. Inappropriate therapy occurred in 29 patients (14%), more frequently in monotherapy (17%) versus combination therapy (10%). Combination therapy would have been effective in 55% of patients with inappropriate monotherapy. We found no association between gentamicin addition and shock reversal (p=0.223). A fungal infection was present in 22 patients (11%).

Conclusion

Inappropriate empirical antibiotic therapy occurs in 17% of septic shock patients receiving monotherapy. In 55% of these patients, additional gentamicin would have resulted in appropriate therapy. When clinical course is unfavorable, lowering the threshold for administering adjunctive aminoglycoside and antifungal therapy should be considered.

Introduction

Septic shock is a subcategory of sepsis with profound circulatory, cellular, and metabolic abnormalities[1], associated with high mortality.[2] One of the most important treatments of these critically ill patients is the swift initiation of appropriate broad-spectrum antibiotic therapy.[3] Failure to start appropriate antibiotic treatment in patients with septic shock substantially increases morbidity and mortality.[4-6]

Inappropriate antibiotic treatment can result from an infection with extended spectrum beta-lactamase-producing (ESBL) Gram-negative pathogens or fungal infection. ESBL fecal carriage is increasing worldwide, especially in the ICU.[7] The risk of infection with these pathogens is higher in patients with sepsis of suspected abdominal, urogenital, or unknown origin.[8] Several studies report inappropriate antibiotic treatment for intensive care unit (ICU) patients in up to 20-30% of cases.[5,6,9]

The addition of an aminoglycoside broadens the empirical antibiotic spectrum, thereby reducing the risk of inappropriate treatment compared to monotherapy.[10] It also has the property of killing bacteria fast and concentration-dependent, working in synergy with beta-lactam antibiotics.[11] Although short courses (one or two doses) are advocated in antibiotic guidelines[12], controversy remains on the added value of aminoglycoside therapy. A meta-analysis did not show faster shock reversal or improved survival in sepsis patients treated with an adjunctive aminoglycoside. In this meta-analysis, an increased risk of nephrotoxicity and renal failure was observed[13], in contrast to other studies showing that the addition of an aminoglycoside seems to be safe regarding nephrotoxicity in sepsis patients.[14-16]

Current Surviving Sepsis Guidelines recommend adding an agent targeting Gram-negative bacteria to the empiric regimen in critically ill patients at high risk of infection with multi-drug resistant pathogens to ensure at least one of the agents is effective.[3] However, the appropriateness of empirical antibiotic therapy and the potential of gentamicin, as the agent targeting Gram-negative bacteria, in such a highly selective group of septic shock patients with a high risk of suffering from either potentially resistant Gram-negative microorganisms, or fungal infection is largely unknown in the ICU.

The aim of this study was to investigate both the appropriateness of empiric antibiotic therapy and the possible benefit of adding short-course gentamicin in septic shock patients with abdominal, urogenital, or unknown focus. Secondary objectives were investigating the effect of gentamicin on shock reversal and assessing the incidence of fungal infection in this population.

Methods

Settings

This study was conducted at the ICU of the Maastricht University Medical Centre+ (MUMC+), a tertiary care, 715-bed university hospital in the Netherlands with 33 intensive care unit beds and approximately 2200 admissions annually. Our hospital is a tertiary referral centre for trauma, neuro-surgical, neurological, and extracorporeal life support (ECLS) patients.

Patient population

We systematically screened all patients admitted to the ICU for sepsis since 2012 and enrolled all patients admitted with sepsis in a prospectively recorded database. Admission with sepsis was defined as any ICU admission clinically coded as infection and at least one organ dysfunction, according to the Surviving Sepsis Campaign guidelines of 2012.[17] For this study, we included the subset of patients with septic shock due to (suspected) abdominal, urogenital, and unknown focus of infection between 2012 and 2017. Septic shock was defined as sepsis with circulatory failure and lactate levels >2 mmol/L despite adequate fluid resuscitation and requiring vasopressor treatment to maintain adequate mean arterial pressure (MAP) of ≥65 mmHg, according to the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-)3 criteria.[1]

Patients were treated according to Surviving Sepsis Campaign guidelines, with the prompt administration of broad-spectrum antibiotics, fluid resuscitation, and vasopressors. Intravascular volume replacement was guided by either clinical variables and/or pulse contour measurements (PiCCO®, Pulse index Contour Continuous Cardiac Output, Pulsion Medical Systems, Germany) and/or echocardiography. Norepinephrine was the vasopressor of choice to treat persistent hypotension after adequate fluid resuscitation. Empirical antibiotic treatment was started in consultation with the clinical microbiologist and was de-escalated when considered appropriate. In our centre, the first-choice antibiotic therapy for sepsis with abdominal, urogenital, or unknown focus is an extended-spectrum penicillin/beta-lactamase inhibitor combination (amoxicillin/clavulanic acid or piperacillin/tazobactam) with (combination therapy group) or without (monotherapy group) gentamicin (5 or 7 mg/kg dosage). According to local guidelines, patients with community-acquired sepsis without neutropenia were treated with amoxicillin/clavulanic acid and gentamicin whereas patients with nosocomial sepsis without neutropenia were treated with piperacillin/tazobactam.[12] In selective patients with suspicion of or cultured earlier with microorganisms that were not susceptible to these antibiotics, an even broader antibiotic such as meropenem was administered. It was at the discretion of the treating

physician to add a short course (no more than 3 dosages) of adjunctive aminoglycoside treatment (gentamicin 5 or 7 mg/kg). Patient management and antibiotic strategy were discussed daily in the multidisciplinary meeting in the presence of a clinical microbiologist. All mechanically ventilated patients received selective digestive decontamination (SDD) from the day of tracheal intubation until ICU discharge. The SDD suspension consisted of Tobramycin, Colistin, and Amphotericin-B and was administered in the oral cavity and into the gut through a nasogastric tube. In patients not receiving broad-spectrum antibiotics, Cefotaxime intravenously was administered during the first four days to bridge the first period in which SDD is not yet fully functional.

Collection of data and study-design

Data were recorded regarding sex, age, source of infection, comorbidities, active malignancy, and severity of disease (APACHE-II score) for all included patients. Furthermore, the need for invasive mechanical ventilation, vasopressor need, serum lactate levels, renal function, and need for continuous veno-venous hemofiltration (CVVH) were also recorded. The study was approved by the local Medical Ethical Committee of the MUMC+ (reference number 2018-0689).

Microbiological data and antibiotic resistance

The administered antibiotics and relevant microbiological cultures were recorded by manually analyzing the medical records of the included patients from the Patient Data Management Systems (PDMS): Systems, Applications, and Products in Data Processing (SAP©, Walldorf, Germany) and Intellispace Critical Care and Anaesthesia (ICCA© Philips Healthcare, Amsterdam, the Netherlands). Microbiological cultures, antibiotic treatment, and antibiotic resistance patterns of the cultured microorganisms were recorded during the first 5 days (120 hours) of ICU admission. Blood cultures were registered, and results from other cultures were expressed either quantitatively by colony forming units (CFU) of the cultured micro-organism(s) or, in case of a liquid culture, semi-quantitatively by a four-point scale; sporadic, little, intermediate, or abundant growth. We included all positive blood cultures and other cultures with growth of bacteria when present at levels greater than 10^4 CFU or labeled as abundantly present on the four-point scale. Bacterial resistance was defined as both intermediately susceptible as well as truly resistant.

Definitions

Appropriateness of antibiotic therapy

Inappropriate antibiotic therapy was defined as a prescription of antibiotics within the first 24 hours that did not cover bacteria present in cultures obtained between day 0 and day 5 after admission to the ICU.

Shock reversal

Shock reversal definition was based on vasopressor requirement and was defined as a decrease of 25% per day in norepinephrine requirement or an absolute requirement of <0.15 mcg/kg/minute.

Outcome measures

The primary outcome measure was the occurrence of inappropriate antibiotic therapy. The primary outcome was determined both in the overall patient group as well as in patients treated with gentamicin (combination therapy) versus patients not treated with gentamicin (monotherapy).
Secondary outcomes were shock reversal during the first five days of ICU admission and the presence of (invasive) fungal infection.

Statistical analysis

Statistical analyses were performed using IBM SPSS version 25 (SPSS Inc., Chicago, USA). Descriptive statistics, independent samples t-tests, Chi-square tests, and one-way ANOVA were used to describe patient demographics and compare the treatment groups. Binomial logistic regression was performed to investigate parameters associated with shock reversal. Sex, age, comorbidity, severity of disease, and variables with $p<0.10$ (norepinephrine dosage on day 1, highest serum lactate level in the first 24 hours) and treatment with gentamicin were applied in the model. Goodness of fit for the logistic regression was assessed by the Hosmer-Lemeshow statistic. All tests were two-tailed and an $\alpha < 0.05$ was considered statistically significant. Data are presented as mean (±standard deviation), median (± interquartile range), mode, or number (%) as appropriate.

Results

Study population

The flowchart of the study is depicted in Figure 7.1. We excluded 78 patients, 77 because of negative cultures or because no cultures were taken (22%, 3%, and 2% of patients with abdominal, urogenital, and unknown focus of septic shock, respectively), and one patient due to lack of consent. In all, 203 septic shock patients were included in the analysis, 115 (57%) patients with monotherapy, and 88 (43%) patients with combination therapy.

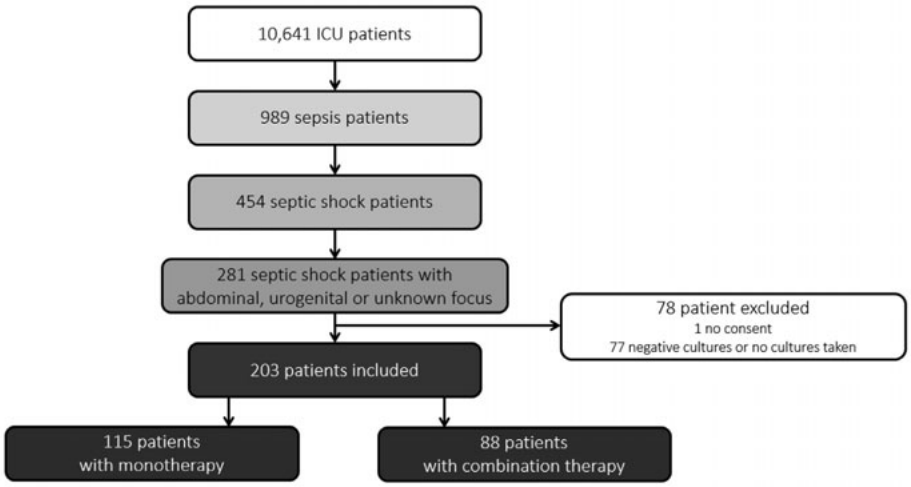

Figure 7.1 Flowchart of the study.

Baseline characteristics (Table 7.1), show a septic shock population with predominantly male (62%), older than 65 years of age (56%), invasively ventilated (79%), surgical (64%) patients, who were already admitted to hospital (47% of patients in the ward, 21% in the operating theatre versus 29% of patients admitted directly from the emergency department). Mean APACHE II score was 27 ± 8 with a mean norepinephrine dosage of 0.37 ± 0.35 mcg/kg/minute, and mean lactate levels were 6.1 ± 4.2 mmol/L. Both comorbidity and active malignancy were present in almost half of the included patients, and ICU mortality was 40%.

Table 7.1 Baseline characteristics of the study population.

	Overall group (N=203)	Abdominal (N=143)	Urogenital (N=27)	Unknown (N=33)	p-value*
Sex					*0.069*
Male	126 (62%)	86 (60%)	14 (52%)	26 (79%)	
Age					*0.245*
<44 yr	15 (7%)	10 (7%)	1 (4%)	4 (12%)	
45 - 54 yr	25 (12%)	19 (13%)	1 (4%)	5 (16%)	
55 - 64 yr	50 (25%)	36 (25%)	4 (15%)	10 (30%)	
65 - 74 yr	63 (31%)	43 (30%)	10 (37%)	10 (30%)	
>75 yr	50 (25%)	35 (25%)	11 (40%)	4 (12%)	
Invasive ventilation	160 (79%)	122 (85%)	13 (48%)	25 (76%)	*<0.001*
Severe comorbidity[a]	94 (46%)	59 (41%)	12 (44%)	23 (70%)	*0.012*
Acute renal failure[b]	84 (41%)	51 (36%)	11 (40%)	22 (67%)	*0.005*
Comatose at admission[c]	13 (6%)	8 (6%)	0 (0%)	5 (15%)	*0.045*
Acidosis at admission[d]	102 (50%)	69 (48%)	11 (40%)	22 (67%)	*0.092*
Leucocytes >40 or <1 mm^3	48 (24%)	24 (17%)	5 (19%)	19 (58%)	*<0.001*
Patient category					*<0.001*
Surgical	130 (64%)	112 (78%)	16 (59%)	2 (6%)	
Medical	73 (36%)	31 (22%)	11 (41%)	31 (94%)	
Patient admitted from					*<0.001*
Emergency department	59 (29%)	31 (22%)	16 (59%)	12 (36%)	
Ward	95 (47%)	70 (49%)	6 (22%)	19 (58%)	
Other ICU	7 (3%)	4 (3%)	1 (4%)	2 (6%)	
Operating room	42 (21%)	38 (26%)	4 (15%)	0 (0%)	
Norepinephrine mcg/kg/min ± *SD*	0.37 ± 0.35	0.35 ± 0.32	0.24 ± 0.21	0.56 ± 0.49	*0.001*
Lactate[f] mmol/L ± *SD*	6.1 ± 4.2	5.8 ± 4.0	4.4 ± 1.6	8.7 ± 5.5	*<0.001*
Thrombocytes 10^9/L ± *SD*	214 ± 149	244 ± 146	153 ± 84	131 ± 156	*<0.001*
Active malignancy n *(%)*	98 (48%)	69 (48%)	9 (33%)	20 (61%)	*0.110*
APACHE-II score	27 ± 8	25 ± 8	26 ± 4	34 ± 6	*<0.001*
ICU mortality	82 (40%)	57 (40%)	3 (11%)	22 (67%)	*<0.001*
Empirical therapy					*0.338*
Piperacillin/tazobactam	100 (49%)	75 (53%)	7 (26%)	18 (55%)	
Amoxicillin/clavulanic acid	66 (33%)	43 (30%)	15 (54%)	8 (24%)	
Meropenem	15 (7%)	12 (8%)	1 (5%)	2 (6%)	
Others	22 (11%)	13 (9%)	4 (15%)	5 (15%)	

Data are n (%) unless otherwise specified. [a] NYHA IV cardiac failure; chronic restrictive or obstructive respiratory failure with functional impairment, hepatic cirrhosis, immunosuppression; [b] Creatinine >175 µmol/L; [c] Glasgow Coma Scale ≤ 8 without sedation; [d] pH <7.25; [e] Highest dosage during the first 24 hours after admission; [f] Highest value during the first 24 hours after admission; * p-value derived from one-way ANOVA or Chi-square test as appropriate.

Septic shock was predominantly caused by an abdominal focus (143 patients, 70%). Baseline characteristics differed significantly between the three distinct infectious foci, showing that patients with a urogenital focus had a lower incidence of invasive ventilation, lower need for vasopressors, lower lactate (4.4 ± 1.6), and lower mortality (11%) than the two other groups. The APACHE II score (34 ± 6), vasopressor need (0.56 ± 0.49 mcg/kg/minute), lactate (8.7 ± 5.5), comorbidity (70%), presence of an

active malignancy (61%), and mortality (67%) were highest in the patients with an unknown focus of infection.

Comparison of patients receiving combination therapy and monotherapy

Patients receiving gentamicin had a higher need for vasopressor treatment than those treated with monotherapy (0.45 ± 0.39 vs. 0.31 ± 0.31 µg/kg/min, p=0.006). Furthermore, patients receiving gentamicin were more often admitted from the emergency department (35 patients (40%) versus 24 patients (21%), p=0.018). There was also a difference in antibiotic therapy as the patients in the combination group were treated more often with amoxicillin/clavulanic acid (44% vs. 24%, p=0.002). Treatment with piperacillin/tazobactam and meropenem did not significantly differ between both groups. Only two patients (both in the monotherapy group) received vancomycin and four patients (all in the monotherapy group) received ciprofloxacin. We found no differences between the monotherapy and the combination group in the other characteristics, including renal function and number of patients requiring renal replacement therapy (Table 7.2).

Occurrence of (in)appropriate antibiotic therapy

In the overall group, inappropriate antibiotic therapy was found in 29 (14%) patients. In 20 out of 115 (17%) patients treated with monotherapy, therapy was inadequate. If an aminoglycoside would have been added, appropriate antibacterial therapy would increase from 83% (95/115 patients) to 92% (106/115 patients. Inappropriate therapy was most frequently encountered in patients receiving amoxicillin/clavulanic acid (30%) or piperacillin/tazobactam (16%) monotherapy.

Inappropriate treatment occurred in 9 out of 88 (10%) patients receiving combination therapy. In 17 out of 88 (19%) patients in the combination group, gentamicin was the only effective component of the antibiotic therapy because the pathogen showed resistance against the primary agent (amoxicillin/clavulanic acid or piperacillin/ tazobactam) (Table 7.3). We found inadequate therapy in 14% of piperacillin/ tazobactam/gentamicin prescriptions and 10% of amoxicillin/clavulanic acid/ gentamicin prescriptions. *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Enterobacter cloacae* were the most frequent cultured Gram-negative microorganisms resistant to the prescribed antibiotic (Table S1).

Table 7.2 Patient characteristics per antibiotic treatment group.

	No gentamicin addition N=115	Gentamicin addition N=88	p-value[•]
Sex			*0.291*
Male	75 (65%)	51 (58%)	
Age			*0.242*
≤44 yr	7 (6%)	8 (9%)	
45 - 54 yr	18 (15%)	7 (8%)	
55 - 64 yr	25 (22%)	25 (28%)	
65 - 74 yr	33 (29%)	30 (34%)	
≥75 yr	32 (28%)	18 (21%)	
Invasive ventilation	92 (80%)	68 (77%)	*0.637*
Severe comorbidity[a]	51 (44%)	43 (49%)	*0.523*
Acute renal failure[b]	47 (41%)	37 (42%)	*0.866*
Comatose at admission[c]	8 (7%)	5 (6%)	*0.713*
Acidosis at admission[d]	54 (47%)	48 (55%)	*0.284*
Leucocytes >40 or <1 mm^3	26 (23%)	22 (25%)	*0.691*
Patient category			*0.199*
Surgical	78 (68%)	52 (59%)	
Medical	37 (32%)	36 (41%)	
Patient admitted from			*0.018*
Emergency department	24 (21%)	35 (40%)	
Ward	60 (52%)	35 (40%)	
Other ICU	3 (3%)	4 (4%)	
Operating room	28 (24%)	14 (16%)	
Norepinephrine ± *SD*, * µg/kg/min	0.31 ± 0.31	0.45 ± 0.39	*0.006*
Lactate ± *SD*, mmol/L	5.6 ± 3.9	6.7 ± 4.6	*0.069*
Thrombocytes 10^9/L ± *SD*	220 ± 153	205 ± 142	*0.475*
Active malignancy *(%)*	54 (47%)	44 (50%)	*0.667*
APACHE-II score	26 ± 8	28 ± 8	*0.207*
ICU mortality	42 (37%)	40 (46%)	*0.199*
Shock reversibility	90 (79%)	56 (63%)	0.016
Empirical therapy			
Piperacillin/tazobactam	63 (55%)	37 (42%)	0.037
Amoxicillin/clavulanic acid	27 (24%)	39 (44%)	0.072
Meropenem	11 (10%)	4 (5%)	0.002
Ciprofloxacin	4 (4%)	0 (0%)	0.175
Cefotaxim	2 (2%)	0 (0%)	0.135
Ceftriaxon	2 (2%)	3 (3%)	0.51
Vancomycin	2 (2%)	0 (0%)	0.65
Cefuroxim	1 (1%)	1 (1%)	0.51
Amoxicillin	1 (1%)	0 (0%)	1.0
Tobramycin	1 (1%)	0 (0%)	1.0
Others/missing	1 (1%)	4 (5%)	1.0
Sepsis focus			*0.449*
Abdominal	85 (74%)	58 (66%)	
Urogenital	13 (11%)	14 (16%)	
Unknown	17 (15%)	16 (18%)	

Table 7.2 (continued)

	No gentamicin addition N=115	Gentamicin addition N=88	p-value*
Creatinine µmol/L			
Day 1	174 ± 123	166 ± 102	0.636
Day 2	172 ± 114	179 ± 119	0.661
Day 3	164 ± 103	173 ± 107	0.582
Day 4	155 ± 106	164 ± 107	0.610
Day 5	138 ± 92	156 ± 107	0.330
CVVH[e]	25 (22%)	13 (15%)	*0.722*

Data are n (%) unless otherwise specified. [a] NYHA IV cardiac failure; chronic restrictive or obstructive respiratory failure with functional impairment, hepatic cirrhosis, immunosuppression; [b] Creatinine >175 µmol/L; [c] Glasgow Coma Scale ≤8 without sedation; [d] pH <7.25; [e] Continuous Venovenous Hemofiltration needed on day 5 after ICU admission; * p values derived from either independent samples T-test, Chi-square or Fishers' exact test as appropriate.

Table 7.3 Antibiotic prescriptions, number of inappropriate antibiotic treatments and incidence of fungal infections.

No Gentamicin added	**#prescriptions**	**Inappropriate therapy**	**Gentamicin would have been effective**	**Fungal infection**
Meropenem	11	1	-	2
Piperacillin/tazobactam	63	10	6	6
Amoxicillin/clavulanic acid	27	8	3	7
Other	14	1	2	2
Total	115	20	11	17
Gentamicin added			**Gentamicin is the only effective component of combination therapy**	
Meropenem & Gentamicin	4	-	-	-
Piperacillin/tazobactam & Gentamicin	37	5	7	3
Amoxicillin/clavulanic acid & Gentamicin	39	4	10	2
Other	8	-	-	-
Total	88	9	17	5

Incidence of fungal infection

A fungal infection was present in 11% (22/203 patients, 17 in the group without gentamicin and 5 in the group treated with gentamicin). Fungi were cultured from abscesses in 21 patients, in five patients fungi were sampled from the blood cultures. In total, six different *Candida species* were found in the cultures, most often *Candida albicans* (18 cases), occasionally patients had positive cultures with more than one *Candida* species (Table S7.2).

Effect of antibiotic therapy on shock reversal

Mortality was 46% in the combination group vs. 37% in the monotherapy group (p=0.199). Shock reversibility was significantly higher in the monotherapy group (90 out of 115 patients, 79%) when compared to the combination group (56 out of 88 patients, 63%) (p=0.016) (Table 7.2). Logistic regression analysis was performed to identify possible predictors for shock reversal. Hosmer-Lemeshow goodness of fit test indicated no evidence of poor fit (Chi-square 5.9, p=0.658). Binomial logistic regression analysis pointed out that the addition of gentamicin was not associated with shock reversal (B 1.578 (0.758 – 3.286), p=0.223). Norepinephrine dosage at day 1, APACHE II score, and highest lactate in the first 24 hours were associated with shock reversal. (Figure 7.2).

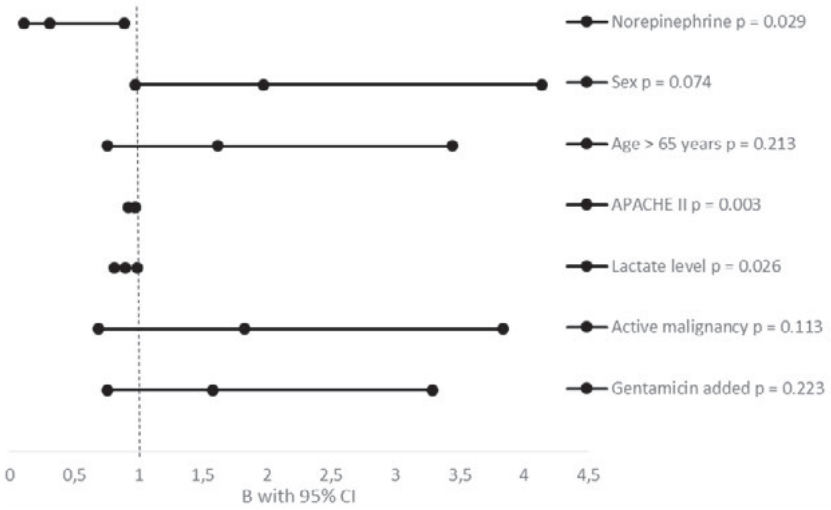

Figure 7.2 Forest plot of binomial logistic regression analysis for possible predictors of shock reversal.

Discussion

Inappropriate empirical antibiotic therapy was prescribed in 14% (29/203) of patients admitted to the ICU with septic shock with an abdominal, urogenital, or unknown focus. Inappropriate antibiotic therapy occurred more often in the monotherapy group when compared to the combination therapy group with adjunctive gentamicin (17% vs. 10%). In 55% of patients treated with inadequate monotherapy, gentamicin addition would have resulted in appropriate antibiotic therapy. In 11% of septic shock patients, a fungal infection was present. We found no difference in ICU mortality between the monotherapy and the combination group. Although shock reversal occurred

significantly more in the monotherapy group, we found no association between shock reversal and administration of gentamicin.

Several studies have assessed the added value of aminoglycoside therapy in the non-ICU[18] and ICU population[10,14,19] with sepsis. A non-ICU study, including 626 sepsis patients with Gram-negative bloodstream infection, did not show clinical benefit of short-course aminoglycoside addition regarding mortality, although the risk of inappropriate therapy was reduced eight-fold with combination therapy. In this study, the influence of clinical severity of disease on whether or not to add aminoglycoside was addressed. The association between severity of disease and adding an aminoglycoside could confound by indication and seems to be the case in our study. Gentamicin was prescribed to more severely ill patients who needed significantly higher vasopressor dosage and tended to have a higher APACHE II score and lactate levels.
In a prospective ICU trial, including 648 severe sepsis or septic shock patients (245 of them receiving gentamicin), short-course gentamicin therapy was not associated with faster reversal of shock or improved survival. Still, it did increase the incidence of renal failure. Two retrospective ICU studies did not associate short-course aminoglycoside addition with nephrotoxicity, nor did they show a clinical benefit for gentamicin. The study results in our centre extend these findings to a septic shock population at high risk (abdominal, urogenital, or unknown focus) for extended- spectrum-beta-lactamase Gram-negative microorganisms and fungal infection, despite the higher number of appropriate therapy courses in the gentamicin group (90% vs. 83%).
The number of inappropriate therapies in our centre (17 vs. 10% in the monotherapy and combination therapy, respectively) falls in the upper range of other studies reporting appropriateness of mono- or combination- antibiotic therapy, ranging from 6 – 30%.[6,9,20] This can be explained by the fact that included patients in our study were severely ill and at relatively high risk for resistant Gram-negative microorganisms and fungal infections, due to the etiology of the infection. Thus, the clinician should still take in account the possibility of inadequate therapy, even in this severely ill patient group with broad antibiotic coverage.
Patients receiving gentamicin were more often treated with amoxicillin/clavulanic acid than patients receiving monotherapy in this study (44% vs. 24%, p=0.002). We believe this can be explained by the fact that in the gentamicin group, more patients are admitted directly from the emergency department (40% vs. 21%). Amoxicillin/clavulanic acid is the first choice antibiotic therapy for patients admitted from out-of-hospital according to the antibiotic treatment protocol in our centre. Although more patients in the monotherapy group received meropenem, this difference was not statistically different between both groups (10% vs. 5%, p=0.175). The fact that less patients in the combination group received broad-spectrum

antibiotics could also be explained as an advantage of gentamicin combination therapies.

Fungi were cultured in 11% of included patients. Two-thirds of the study population were surgical patients, and 78% of abdominal infections were surgical ICU patients. Recent major surgery is a known risk factor for fungal infection next to total parental nutrition, immunocompromised state, and comorbidities like chronic liver and renal failure.[21,22] Strikingly, in a recent prospective matched case-control study including 192 patients with *Candida* bloodstream infection and 411 control patients, exposure to aminoglycoside treatment was associated with candidemia.[23] In our study, *Candida* infection occurred more in the monotherapy group (15%) than in the gentamicin treated group (6%).

Our study has several strengths. First of all, the study population comprises a representative septic shock population with very ill patients and high mortality. Appropriateness of antibiotic therapy is investigated in a relevant group with a high risk of putatively resistant microorganisms, not earlier explored in detail, to the best of our knowledge. Furthermore, randomized controlled trials are not to be expected in this complex setting. All included patients were treated according to the same local protocol in a setting with a low prevalence of antibiotic resistance.

There are several limitations to this study. First, this was a single-centre study in a hospital with secondary and tertiary care, so the generalizability and extrapolation of the results can be debated. Although the results apply strongly to our centre and environment and can be different in other settings, we believe that they give valuable insights into the pathogens involved and the appropriateness of therapy in this severely ill patient group. The limited sample size of this highly selected population might influence power in this study. Moreover, there were 76 patients excluded from the analysis because there were no cultures taken (for blood cultures ranging from 3% in urogenital infections to 22% in abdominal infections) or cultures were all negative. We further analyzed this finding, and the absence of blood cultures in patients with abdominal sepsis could partially be explained by the fact that more than half of these patients died within 24 hours. Apparently, these patients were in end-stage septic shock, and other more urgent treatments had priority. Furthermore, confounding by indication might be reflected by the fact that the treating physician decided to administrate gentamicin, and patients receiving gentamicin tended to be more severely ill. Nevertheless, the only significant difference between the treatment groups was the vasopressor dosage, which was higher in the gentamicin group. Data on dosage and drug monitoring are not presented in the present study; however, the intended administered dosage was 5 or 7 mg/kg gentamicin in all treated patients during the study period.

In conclusion, inappropriate empirical antibiotic therapy is frequently encountered in severely ill septic shock patients with an abdominal, urogenital, or unknown etiology.

Empirical monotherapy was inappropriate in almost one in five patients, and in more than half of these cases, adding gentamicin would have resulted in appropriate coverage. In 11% of all patients, a fungal infection was present. In case of unfavorable clinical course under antibiotic monotherapy, lowering the threshold for administering adjunctive aminoglycoside and antifungal therapy should be considered.

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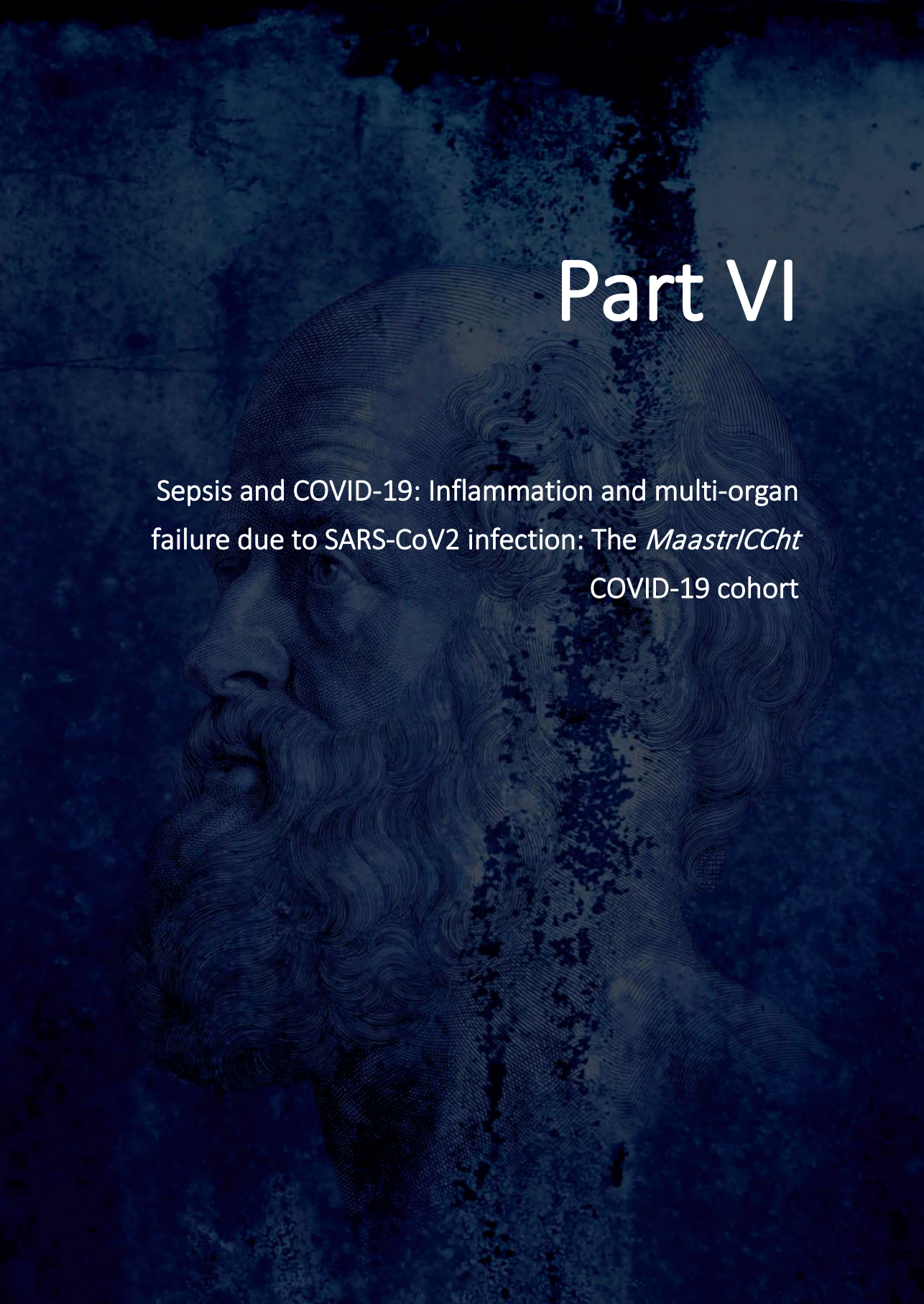
Supplemental material

Table S7.1 Most frequently cultured Gram-negative microorganisms with resistance to antibiotic therapy.

Microorganism	Positive cultures	Meropenem resistant	Piperacillin/tazobactam resistant	Amoxicillin/clavulanic acid resistant	Gentamicin resistant
Escherichia coli	130	-	20 (15%)	50 (38%)	11 (8%)
Klebsiella pneumonia	24	-	8 (33%)	8 (33%)	1 (4%)
Pseudomonas aeruginosa	22	-	5 (23%)	22 (100%)	1 (5%)
Enterobacter cloacae	20	-	5 (25%)	20 (100%)	-

Table S7.2 Most frequently cultured fungi (in some patients more than one *Candida species* was cultured).

Type fungi	Frequency
Candida albicans	18
Candida glabrata	13
Candida parapsilosis	3
Candida dubliniensis	2
Candida tropicalis	1
Candida inconspicua	1



Part VI

Sepsis and COVID-19: Inflammation and multi-organ failure due to SARS-CoV2 infection: The *MaastrICCht* COVID-19 cohort

Chapter 8

Coronary artery calcifications are associated with more severe multi-organ failure in patients with a severe COVID-19 infection; longitudinal results of the Maastricht Intensive Care COVID cohort

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Abstract

Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is regarded as a multi-systemic disease. Patients with pre-existing cardiovascular disease (CVD) have an increased risk for a more severe disease course. This study aimed to investigate if a higher degree of coronary artery calcifications (CAC) on a standard chest computed tomography (CT) scan in mechanically ventilated patients was associated with more severe multi-organ failure over time.

Methods

All mechanically ventilated ICU patients with SARS-CoV-2 infection who underwent a chest CT were prospectively included. CT was used to establish the extent of CAC using a semi-quantitative grading system. We categorized patients into three sex-specific tertiles of CAC: lowest, intermediate, and highest CAC-score. Daily, the Sequential Organ Failure Assessment (SOFA) scores were collected to evaluate organ failure over time. Linear mixed-effects regression was used to investigate differences in SOFA scores between tertiles. The models were adjusted for age, sex, APACHE II score, cardiovascular risk factors, and chronic liver, lung, and renal disease.

Results

Seventy-one patients were included. Patients in the highest CAC tertile had, on average over time, a 1.8 [0.5-3.1] points higher SOFA score, compared to the lowest CAC tertile (p=0.005). This association remained significant after adjustment for age, sex and APACHE-II score (1.4 [0.1-2.7], p=0.042) and clinically relevant after adjustment for cardiovascular risk factors (1.3 [0.0-2.7], p=0.06) and chronic diseases (1.3 [-0.2-2.7], p=0.085).

Conclusion

A greater extent of CAC is associated with a more severe multi-organ failure in mechanically ventilated COVID-19 patients.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection was initially thought to mainly affect the pulmonary system.[1,2] Nowadays, it is recognized as a multi-systemic disease whereas patients with comorbidities are at increased risk of developing severe disease.[3-6] Up to 14% of infected patients had severe disease with hypoxia requiring hospitalization, of whom 5% required admission to the intensive care unit (ICU) and mechanical ventilation.[7]

In the ICU, the Sequential Organ Failure Assessment (SOFA) score is widely used to evaluate a patients' organ function during admission, as SOFA is designed to capture changes in clinical status over time. The SOFA score includes components reflecting pulmonary, cardiovascular, hepatic, coagulation, renal, and neurological function.[8,9] Previous studies showed that a decrease in score is associated with improved survival in mechanically ventilated COVID-19 patients, irrespective of existing comorbidities.[5] In contrast, an increasing SOFA score indicates worsening of organ function and is associated with increased morbidity and mortality.

Patients with COVID-19 and pre-existing cardiovascular disease (CVD) tend to have a more severe disease course[10] and serial SOFA scores are particularly suitable for such evaluation over time in a pandemic. Early identification of patients with a high risk of developing multi-organ failure and death is needed to aid clinical decision making, to tailor patient management, and to recognize patient categories that might not benefit from ICU treatment at all.

In COVID-19 patients, coronary artery calcification (CAC), as detected on computed tomography (CT) is associated with a worse outcome.[11-13] However, most studies have a cross-sectional design and do not include mechanically ventilated patients. Thus, whether a higher degree of CAC is associated with a worse SOFA score over time in this population, irrespective of preexisting cardiovascular risk factors, is unknown.

We hypothesize that a higher degree of CAC is associated with a worse disease course reflected by a higher SOFA score over time. In addition, this association is independent of patient characteristics, disease severity, cardiovascular risk factors, and comorbidity. Thus, the aim of the present study was to investigate whether a higher degree of CAC, as an integrated quantification tool of cardiovascular risk, is associated with more severe multi-organ failure over time in mechanically ventilated patients with COVID-19. Quantifying the extent of CAC could identify patients at risk for multi-organ failure, which is associated with worse outcome.

Materials and methods

Patient population

The Maastricht Intensive Care COVID (*MaastrICCht*) cohort study design has been described more extensively elsewhere.[14,15] Briefly, this prospective cohort study was executed in a patient population admitted to the ICU of the Maastricht University Medical Centre+ (Maastricht UMC+). During the COVID-19 pandemic, the number of ICU beds was rapidly upgraded from 27 to 64 beds. The study was designed to foster other datasets and registries according to the FAIR data principle in collaboration.[14] The local institutional review board (Medisch Ethische Toetsings Commissie (METC) 2020-1565/ 300523) of the Maastricht UMC+ approved the study, which was performed based on the regulations of Helsinki. During the pandemic, the board of directors of Maastricht UMC+ adopted a policy to inform patients and ask for their consent to use the collected data for research purposes. The study is registered in the Netherlands Trial Register (registration number NL8613) and was written following the STrengtening and Reporting of Observational studies in Epidemiology (STROBE) guideline.[16]
The *MaastrICCht* cohort included all patients with COVID-19 infection and respiratory insufficiency requiring mechanical ventilation that were admitted in the first wave from the 25th of March until the 23rd of June 2020. A positive COVID-19 case was defined as follows: one polymerase chain reaction (PCR) test positive for COVID-19 and/or a chest CT strongly suggestive for COVID-19 infection, indicated by a COVID-19 Reporting and Data System (CO-RADS)-score of 4-5, scored by a radiologist and no alternative diagnosis.[17-20] Patients were followed until the primary outcome was reached (i.e., either death in the ICU or discharge from the ICU).[14] For the present study, only patients who underwent a chest CT scan were included.

Imaging protocol

All eligible patients underwent a chest CT scan, either at the Maastricht UMC+ or the referring centre for patients transported for logistical reasons due to the pandemic. Chest CT scans from patients transferred from elsewhere were requested and reassessed. As a result, vendors as well as scan parameters and reconstruction techniques differed between patients. Scans in our centre were performed on four different scanners. In case of a new, clinically stable triage patient, scans were performed on a mobile CT scan unit (Alliance Medical equipped with Lightspeed 16, GE Healthcare, Milwaukee, WI, USA), which was placed temporarily outside the hospital. When the outpatient was unstable, a more advanced system at the emergency ward was chosen (SOMATOM Definition Flash; (Siemens Healthineers, Forchheim, Germany). Clinical in-patients were scanned within the department of radiology and nuclear medicine following the regular clinical pathway (SOMATOM Force; SOMATOM

Definition AS (Siemens Healthineers). Tube voltage on these scanners varied between 90-140 kV. Additional to these scans, CTs from different hospitals throughout the Netherlands were included as well. Therefore, scan- and reconstruction parameters differed. CT scans were performed in caudo-cranial direction with and without the use of intravenous contrast material.

Coronary calcium score

CAC was graded with a semi-quantitative grading system and graded on the data available on the PACS workstation (IMPAX version 6.6.1.5003; AGFA HealthCare N.V., Mortsel, Belgium). All data were rated in consensus by two readers (BM and CM), experienced in cardiac imaging. The readers were blinded for patient outcome and were allowed to adjust the window level. A semi-quantitative grading system was used to assess the calcifications according to their location in the left main (LM), left anterior descending (LAD), left circumflex (Cx) and right coronary artery (RCA) as 0 = absent; 1 = mild; 2 = moderate and 3 = severe.[21-23] The four separate scores can be summed up to get an overall grade reaching from 0-12[24,25], where 0 is the absence of CAC and 12 is severe calcified plaques in all coronary arteries (LM, LAD, Cx, and RCA).

Serial outcome variable of multi-organ failure: the SOFA score

In mechanically ventilated patients with a COVID-19 infection within the *MaastrICCht* cohort, every component of the SOFA score was collected daily as previously described in more detail.[5,14] The SOFA score includes components reflecting the pulmonary, cardiovascular, hepatic, coagulation, renal, and neurological status. Each organ system component is scored as one of five categories, ranging from 0 (normal organ function) to 4 (most abnormal organ function).[8] The SOFA score is the sum of the six organ system component scores ranging from 0 to 24 (Supplemental Table S8.2). A higher score indicates worse multiple organ function and is associated with a higher morbidity and mortality.

Confounders

Comorbidities were proposed as confounders as these can be associated with organ function at baseline and the course of multi-organ failure over time.[26]

For the present study, in addition to age, time since intubation (days, continuous) and sex (male/female), APACHE II score (continuous), hypertension (yes/no), dyslipidemia (yes/no), obesity (BMI≥30 kg/m^2; yes/no), current smoker (yes/no), physician-diagnosed diabetes mellitus type 2 (yes/no), chronic lung disease (yes/no), liver disease (yes/no), and renal disease (yes/no) were considered as potential confounders. The APACHE II score is a physiologically based classification system for measuring the

severity of illness in groups of critically ill patients.[27] APACHE II and SOFA scores differ, although both score severity of critical illness.

Statistical analyses

The sample characteristics were described using median and interquartile range (IQR), mean and standard deviation (SD), or percentages, as appropriate.
First, the cohort was categorized into sex-specific tertiles of CAC. The first tertile was the patient group with the lowest CAC-score, the middle with intermediate and the third tertile with the highest CAC-score. Then, baseline characteristics were compared across tertiles using Kruskal-Wallis, one-way ANOVA, Chi-square, or Fisher's Exact test as appropriate.
Linear mixed-effects regression was used with a random intercept for participant and time since intubation to investigate the association between CAC and SOFA score by computing differences in average SOFA scores between tertiles (with the lowest tertile as reference). In addition to estimating longitudinal SOFA score differences between CAC tertiles, a full longitudinal assessment requires addressing an increase/decrease in SOFA scores over time. Therefore, linear mixed-effects regression was used with a random intercept and random slope to compute average differences in the slope over time (i.e., increased/decreased) between groups. When the difference in the slope over time was not statistically significant, models for average differences were presented. Specifically, we used unstructured variance-covariance matrix and an autoregressive correlation structure of the first order for longitudinal measures.
SOFA score differences were assessed using crude sex-specific CAC tertiles (Model 1). Next, the hypothesis was subsequently challenged that a higher degree of CAC is associated with a higher SOFA score over time by adjusting for patient characteristics, admission disease severity, other cardiovascular risk factors and comorbidity. Hence, the model was adjusted for age, sex, and APACHE II score (Model 2). Additionally, the latter was adjusted for cardiovascular risk (hypertension, dyslipidemia, obesity, smoking, and diabetes mellitus type 2), as these are associated with CAC[28-30] (Model 3), and finally, adjustments for chronic liver, lung, and renal disease were made (Model 4). Potential interaction of the association between tertiles of CAC and SOFA scores by time and sex was also tested, by adding an interaction term to Model 2. A two-side P-value < 0.05 and P-interaction < 0.05 were considered statistically significant. All the analyses were conducted using R version 3.6.1 (R studio, Boston, Massachusetts).

Results

Patient population

In total, 94 COVID-19 patients were admitted to the ICU of our hospital during the study period. A standard chest-CT was available in 71 patients (Figure 8.1). Characteristics of the 71 patients who underwent a chest CT were compared to the 23 patients without chest CT (Supplemental Table 8.1). Patients with an available chest CT scan had a longer ICU stay (18 vs. 11 days (p=0.005) and a lower arterial blood gas PCO_2 of 5.3 kPa vs. 6.7 kPa (p=0.030).
Patients were divided in three tertiles based on the sum of the CAC: tertile 1: score 0-1; tertile 2: score 2-6 and tertile 3: score 7-12. Patients within the highest CAC tertile were older (p<0.001), had more presence of diabetes mellitus type 2 (p=0.015), dyslipidaemia (p=0.010), more vasopressor use (p=0.022), a lower urine production (p=0.017) and lower thrombocytes (p=0.031) as compared to low and intermediate CAC tertiles (Table 8.1).

Longitudinal associations between coronary calcium and multi-organ failure

In the crude analyses, patients in the highest CAC tertile had, on average over time, a 1.8 [0.6-3.1] points higher SOFA score when compared to those in the lowest CAC tertile (p=0.005) (Table 8.2, Model 1). This association remained statistically significant after adjustment for age, sex, and APACHE-II-score (1.4 [0.1-2.7] points, p=0.042) (Table 8.2, Model 2). Regression coefficients showed a higher SOFA score in the highest CAC tertile, after adjustment for cardiovascular risk factors (hypertension, dyslipidemia, obesity, smoking, diabetes mellitus type 2) (1.3 [0.0-2.7], p=0.059) (Table 8.2, Model 3) and adjustment for chronic liver, lung, and renal disease (1.3 [-0.2-2.7], p=0.085), although not statistically significant (Table 8.2, Model 4).
Longitudinal regression coefficients in SOFA scores over time between CAC tertiles are reported for average differences only, as no changes (i.e., increase/decrease) in SOFA scores between CAC tertiles were observed (i.e., no statistically significant interaction in time since intubation and CAC tertiles was present) (Figure 8.2).
Furthermore, no significant interaction between sex, association of CAC tertiles and SOFA score over time was observed (p for interaction = 0.712 and 0.566 for the middle and highest tertiles, respectively).
When analysing the development of the individual SOFA score components, a specific component contributing significantly more to the association between CAC and SOFA score when compared to the other components, was not identified (Table 8.3).
Figure 8.3 shows examples of three patients with no, mild, and severe CAC.

Table 8.1 Demographic characteristics, medical history, cardiorespiratory indices, and risk indicators of study patients across tertiles of coronary artery calcification.

	Degree of coronary artery calcification in study population			
	Tertile 1 n=25	Tertile 2 n=23	Tertile 3 n=23	p-value for difference
Age (years), median (IQR)	57 (14)	67 (11)	73 (5)	<0.001
Sex, men	72.0%	78.2%	78.2%	0.840
Length of ICU* stay (days), median (IQR)	18.0 (17.0)	22.0 (25.0)	15.0 (15.5)	0.177
ICU mortality	24.0%	30.4%	52.2%	0.481
Height (cm), median (IQR)	180 (15.0)	175 (10.0)	173 (8.5)	0.082
Weight (kg), median (IQR)	90 (20.0)	83 (12.5)	80 (13.3)	0.330
Body mass index (kg/m^2), median (IQR)	27.8 (4.0)	27.1 (3.5)	27.1 (5.4)	0.852
Chronic cardiac disease	0.0%	0.0%	4.3%	0.648 [b]
Chronic pulmonary disease	4.0%	8.7%	13.0%	0.508 [b]
Chronic kidney disease	4.0%	0.00%	0.00%	1.000 [b]
Liver disease	0.0%	0.0%	4.3%	0.648 [b]
Diabetes mellitus type 2	8.0%	0.0%	26.1%	0.015 [b]
Presence of cardiovascular risk factors				
Hypertension	20.0%	31.8%	47.8%	0.121
Dyslipidemia	4.0%	18.2%	39.1%	0.010 [b]
Smoking	8.0%	9.1%	13.0%	0.888 [b]
Obesity	8.0%	4.5%	21.7%	0.197
APACHE II score (points), median (IQR)	15.0 (4.0)	15.0 (4.5)	18.0 (6.5)	0.052
SOFA score on admission, median (IQR)	5.0 (4.0)	6.0 (3.0)	7.0 (4.0)	0.211
Mechanical ventilation, yes %	88.0%	95.5%	95.7%	0.610 [b]
Pressure control, %	44.0%	56.5%	60.7%	
Pressure support, %	8.0%	4.3%	4.3%	
CPAP, %	36.0%	30.4%	30.4%	
FiO_2 high admission, median (IQR)	80.0 (35.0)	70.0 (23.8)	70.0 (25.0)	0.163
Respiration rate high admission (per minute), median (IQR)	30.0 (12.0)	26.0 (3.5)	25.0 (4.5)	0.149
Inspiratory pressure (cm H_2O), median (IQR)	20.0 (8.0)	24.0 (10.0)	24.0 (9.3)	0.258
Positive end-expiratory pressure (cm H_2O), mean (± SD)	29.3 (± 5.0)	29.2 (± 1.8)	27.0 (± 4.5)	0.513[a]
Tidal volume (ml), median (IQR)	497.0 (155.0)	464.0 (114.0)	437.0 (93.0)	0.609
Arterial blood gas pO_2 (kPa), median (IQR)	10.5 (4.5)	9.2 (3.2)	9.6 (2.0)	0.440
Arterial blood gas pCO_2 (kPa), median (IQR)	5.0 (2.3)	6.2 (1.6)	5.2 (1.5)	0.295
Arterial blood gas (pH), median (IQR)	7.34 (0.15)	7.29 (0.22)	7.29 (0.14)	0.487
Mean arterial pressure (mmHg), mean (± SD)	100.4 (± 14.0)	99.7 (± 13.3)	96.8 (± 13.5)	0.629[a]
Vasopressor use, yes %	40.0%	65.2%	78.3%	0.022
Bilirubin (µg/L), median (IQR)	9.4 (5.8)	10.5 (5.5)	9.6 (10.8)	0.795
Dialysis, yes %	12.0%	8.7%	21.7%	0.481 [b]
Creatinine (µmol/L, median (IQR)	77.0 (30.0)	72.0 (37.0)	80.0 (95.3)	0.758
Urine production (ml/24hours), median (IQR)	1350 (1230)	1590 (1615)	630 (1118)	0.017
Glasgow coma score, median (IQR)	15 (0)	15 (0)	15 (0)	0.062
Thrombocytes (10E^9/L), median (IQR)	330.5 (108.5)	374.0 (140.0)	254.0 (131.0)	0.031

* ICU stands for Intensive Care Unit; IQR, interquartile range; SD, standard deviation; APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment. Data are presented as mean (standard deviation) or count (percentage), unless indicated otherwise. Differences were tested using the independent-samples t-test or Pearson's Chi-square, unless indicated otherwise. [a] One-way ANOVA instead of Kruskal-Wallis test [b] Fishers' Exact test instead of Chi-square test due to low expected values.

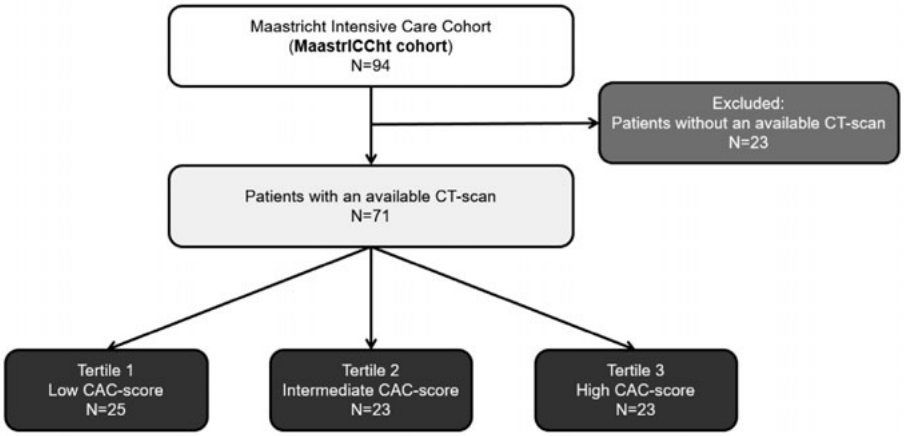

Figure 8.1 Flow diagram. CT indicates computed tomography; CAC, coronary artery calcifications.

Table 8.2 The longitudinal association between coronary calcium score and development of SOFA scores over time.

Model	Regression coefficient (95% CI)	p-value
Model 1: Crude		
Tertile 1, degree of coronary artery calcification in study population	Reference category	
Tertile 2, degree of coronary artery calcification in study population	0.9 (-0.4-2.1)	0.189
Tertile 3, degree of coronary artery calcification in study population	1.8 (0.6- 3.1)	0.005
Model 2: Model 1 adjusted for age and sex, APACHE-II-score		
Tertile 1, degree of coronary artery calcification in study population	Reference category	
Tertile 2, degree of coronary artery calcification in study population	0.7 (-0.5-1.9)	0.241
Tertile 3, degree of coronary artery calcification in study population	1.4 (0.1-2.7)	0.042
Model 3: Model 2 adjusted for cardiovascular risk (hypertension, dyslipidemia, obesity, smoking, diabetes mellitus type 2)		
Tertile 1, degree of coronary artery calcification in study population	Reference category	
Tertile 2, degree of coronary artery calcification in study population	0.5 (-0.8-1.7)	0.463
Tertile 3, degree of coronary artery calcification in study population	1.3 (-0.0-2.7)	0.059
Model 4: Model 3 adjusted for liver conditions, chronic lung disease, and chronic kidney conditions		
Tertile 1, degree of coronary artery calcification in study population	Reference	
Tertile 2, degree of coronary artery calcification in study population	0.4 (-0.8-1.7)	0.482
Tertile 3, degree of coronary artery calcification in study population	1.3 (-0.2-2.7)	0.085

CI, confidence interval; ICU, Intensive Care Unit;, APACHE, Acute Physiology and Chronic Health Evaluation; SOFA, Sequential Organ Failure Assessment.

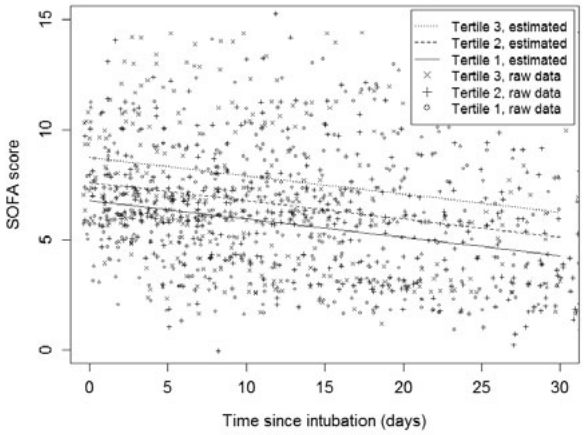

Figure 8.2 Observed and predicted Sequential Organ Failure Assessment (SOFA) scores over time for the tertiles of coronary artery calcification (CAC). The figure shows that patients in the highest CAC tertile have the highest SOFA scores. In addition, SOFA scores improve gradually over time, similar for the three tertiles.

Table 8.3 Results of linear mixed-effect models: development of individual SOFA component scores.

	Model 2 adjusted regression coefficient (95% CI)	p-value	Model 4 adjusted regression coefficient (95% CI)	p-value
Tertile 1, degree of coronary artery calcification in the study population	Reference		Reference	
PaO_2/FiO_2 ratio				
Tertile 2	-0.02 (-0.10 - 0.06)	0.707	-0.01 (-0.10 - 0.08)	0.829
Tertile 3	-0.03 (-0.12 - 0.06)	0.500	-0.03 (-0.10 - 0.08)	0.564
PaO_2, kPa				
Tertile 2	-0.41(-0.97 - 0.16)	0.154	-0.40 (-0.95 - 0.14)	0.145
Tertile 3	-0.28 (-0.92- 0.36)	0.393	-0.36 (-1.03 - 0.31)	0.285
FiO_2, %				
Tertile 2	2.67 (-3.38 – 8.72)	0.382	2.20 (-3.59- 7.99)	0.451
Tertile 3	1.64 (-4.97 – 8.26)	0.622	1.68 (-5.05- 8.41)	0.620
SOFA cardiovascular component score				
Tertile 2	0.38 (-0.12 - 0.88)	0.135	0.38 (-0.15 - 0.90)	0.155
Tertile 3	0.33 (-0.21 - 0.87)	0.226	0.49 (-0.10 - 1.09)	0.106
Bilirubin, µmol/L				
Tertile 2	-0.75 (-5.55 - 4.05)	0.756	-2.17 (-6.95 - 2.61)	0.370
Tertile 3	2.01 (-3.25 - 7.27)	0.449	1.23 (-4.31 - 6.77)	0.660
SOFA renal component score				
Tertile 2	-0.04 (-0.73 - 0.65)	0.913	-0.21 (-0.89 - 0.47)	0.537
Tertile 3	0.62 (-0.13 - 1.38)	0.104	0.40 (-0.39 - 1.19)	0.315
Glasgow coma score				
Tertile 2	-0.07 (-0.20 - 0.07)	0.343	-0.54 (-1.60 - 0.52)	0.317
Tertile 3	-0.03 (-0.18 - 0.12)	0.660	-0.54 (-1.79 - 0.71)	0.390
Thrombocytes, 10^9/L				
Tertile 2	39.5 (-28.2 – 107.2)	0.250	26.6 (-42.2- 95.4)	0.444
Tertile 3	-53.9 (-130 - 22.2)	0.163	-40.1 (-121.8- 41.7)	0.333

Data are longitudinal regression coefficients that show the average difference per Sequential Organ Failure Assessment (SOFA) score component over time between coronary artery calcifications (CAC) tertiles, with the lowest CAC tertile as the reference category. Data are adjusted for age, sex, and APACHE II score (Model 2) and additionally for chronic liver, lung, and renal disease (Model 4). PaO_2 indicates partial pressure of oxygen; FiO_2, fraction of inspired oxygen; CI, confidence interval.

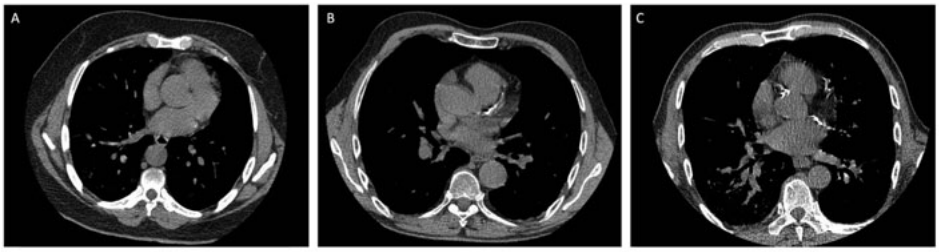

Figure 8.3 Three examples of chest CTs on coronary artery calcification (CAC) **A**) is of a patient with no CAC; **B**) a patient with CAC localized only in the left main and left anterior descending coronary artery and **C**) example of a patient with extensive CAC in all the coronary arteries.

Discussion

The present study showed that a greater extent of CAC in mechanically ventilated ICU patients with COVID-19, was associated with more severe organ failure (indicated by a higher average SOFA score), independent of age, sex, and APACHE II-score. The same order of magnitude of association is observed after adjustment for cardiovascular risk factor and chronic liver, lung, and renal disease, however not statistically significant. No changes in SOFA scores over time (i.e., increase/decrease) between tertiles of CAC were observed. In addition, no significant interaction between sex and the association between SOFA score over time and the extent of CAC was found.

Previous studies investigated the association between CAC and the outcome of COVID-19 patients. However, most studies included non-ICU patients only, used a cross-sectional design, and did not study serial data as in the present study.[11,13,31] Luo et al. showed, in a retrospective cohort study, that a higher level of CAC was associated with more in-hospital deaths and other adverse events in COVID-19 patients. We add evidence that CAC is associated with multi-organ failure in a serial design.[31] A study by Dillinger et al. included 209 hospitalized patients with a proven COVID-19 infection, in which the presence of CAC was analyzed. CAC was associated with a worse disease course in terms of respiratory failure requiring mechanical ventilation, extracorporeal membrane oxygenation (ECMO), or death.[12] This study did not include mechanically ventilated patients and described CAC only as absent or present. In addition, Gupta et al. showed in a non-ICU population of 180 COVID-19 patients that CAC was associated with a higher mortality and more patients required intubation.[32] Another single-centre, retrospective, observational study applied the Agatston score to non-gated chest CTs and observed more adverse events (e.g., transfer to the ICU, death, or both) in hospitalized, non-ICU patients with CAC.[13]

The standard scoring system for CAC is the Agatston score. However, this technique is fully standardized in terms of imaging protocol (3 mm slice thickness and a tube voltage of 120 kV) and interpretation.[33] Since the chest CT scans in the present study were performed for diagnosing COVID-19 and no dedicated cardiac scans were performed at the height of the pandemic, we used a semi-quantitative grading system, validated in earlier studies to be used on routine chest CTs to assess CAC.[21,22,32]

This study tested the hypothesis that CVD, reflected by CAC, is a potential risk factor for more severe organ failure in patients with severe COVID-19 requiring mechanical ventilation. During a pandemic, identifying patients at higher risk of a worse outcome, is essential for predicting which patients will benefit from admission to the ICU. Grading CAC on a standard chest-CT comes at no additional costs and may help clinicians aid decision-making. In addition, the results show that CAC may hold important prognostic information, regarding multi-organ failure as assessed by the SOFA score. Therefore, reporting CAC in all radiological chest CT reports in COVID-19 patients might benefit clinical decision support.

This study has several strengths. First, the study is prospective by design, including many serial measurements in patients with COVID-19 infection, including a systematic data collection following a predefined protocol.[14] Furthermore, CAC was assessed using a semi-quantitative grading system, as validated in the literature for determining CAC in non-gated chest-CT scans.[21,22] CAC was assessed in consensus by two readers experienced in cardiac imaging. In addition, we used the SOFA score in a longitudinal design. The SOFA score, in contrast to other disease severity scores such as the APACHE II score[27,34] and the Simplified Acute Physiology Score (SAPS)[35], was developed for serial data and thus longitudinal evaluation. Moreover, earlier research in COVID-19 patients has shown that a worse course of the SOFA score over time is associated with a worse outcome, independent of the APACHE II score.[5] Lastly, assessing a clinical SOFA score is less complex than APACHE and SAPS scores. Therefore, it is applicable when both time and resources are scarce, such as in a pandemic situation.

A limitation of this study is that it includes mechanically ventilated ICU patients only, thereby limiting the generalizability of the results to patients not in need of mechanical ventilation. Furthermore, our study was limited to patients who had an available chest CT scan. Characteristics of these patients were similar as those without available chest CT scan, except the latter had a shorter ICU stay and a higher arterial blood gas PCO_2. Therefore, it is unlikely that selection bias had a major influence on the associations. If less severely affected patients might have had a lower degree of multi-organ failure and CVD (i.e., lower CAC), the reported associations might have even been underestimated. The association between CAC and SOFA score loses statistical significance after adjustment for cardiovascular risk factors and chronic liver, lung, and

renal disease, likely because of low power. However, the same order of magnitude of clinically relevant association (1.3 point higher SOFA score in both models) suggests that these cardiovascular risk factors and chronic diseases do, at least, not fully, explain the observed observations between CAC and SOFA scores.[36] The SOFA score uses a limited number of organ systems and weighting is applied to each organ score. Using a limited set of variables could lead to underestimation of the degree of organ failure in patients. However, the score is widely used, can be easily calculated at the bedside, and appears effective in detecting associations with CAC compared to its component scores. CO-RADS scores were not reported, as they are not related to the extent of the pulmonary involvement.[37] In addition, all included patients had severe respiratory failure requiring mechanical ventilation. Therefore, the authors believe that severity of pulmonary findings on CTs most likely do not influence the results of the study.

Conclusion

A higher degree of CAC is associated with more severe organ failure (indicated by a higher SOFA score) over time, that drives worse outcome. Reporting CAC on standard chest CTs in COVID-19 patients might guide clinical decision-making.

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Supplemental material

Table S8.1 Differences between included and excluded patients, based on the availability of a chest CT.

	Included n=71	Excluded n=23	p-value for difference
Age (years), median (IQR)	67.0 (14.0)	59.0 (± 15.8)	0.295
Sex, % men	76.1%	85.0%	0.545
Length of ICU[a] stay (days), median (IQR)	18 (20.5)	11 (12.5)	0.005
Height (cm), median (IQR)	175 (11.5)	178 (10.0)	0.197
Weight (kg), median (IQR)	85 (15.0)	89 (14.5)	0.397
Body mass index (kg/m^2), median (IQR)	27 (5.2)	28 (±3.9)	0.977
Chronic cardiac disease	1.4%	0.0%	1.000
Chronic pulmonary disease	8.5%	10.0%	1.000
Chronic renal disease	1.4%	5.0%	0.393
Liver disease	1.4%	0.0%	1.000
Diabetes mellitus type 2	11.4%	20.0%	
Hypertension	32.9%	60.0%	0.744[b]
Dyslipidemia	20.0%	20.0%	1.000
Smoking	10.0%	0.0%	0.341
Obesity	11.4%	15.0%	0.703
APACHE II score (points), median (IQR)	16 (5.5)	15 (7.0)	0.122
Mechanical ventilation, yes %	92.9%	90.0%	0.649
Pressure control, %	54%	60%	
Pressure support, %	6%	15%	
CPAP, %	32%	15%	
FiO_2 high admission, median (IQR)	70 (26.3)	75 (20.0)	0.905
Respiration rate high admission (per minute), median (IQR)	26.0 (6.5)	23.5 (10.5)	0.249
Inspiratory pressure (cm H_2O), median (IQR)	23.5 (11.8)	26.0 (± 5.8)	0.193
Positive end-expiratory pressure (cm H_2O), median (IQR)	28.5 (3.5)	22.0 (7.0)	0.066
Tidal volume (ml), median (IQR)	447 (132)	438 (82)	0.617
Arterial blood gas pO_2 (kPa), median (IQR)	9.7 (3.2)	10.0 (2.5)	0.770
Arterial blood gas pCO_2 (kPa), median (IQR)	5.3 (2.0)	6.7 (1.5)	0.030
Arterial blood gas (pH), median (IQR)	7.3 (0.2)	7.5 (0.2)	0.070
Mean arterial pressure (mmHg), mean (SD)	99.0 (13.5)	97.7 (15.4)	0.737[c]
Vasopressor use, yes %	60.6%	52.6%	0.718[b]
Bilirubin (µg/L**), median (IQR)	10.3 (8.5)	9.9 (10.4)	0.585
Dialysis, yes %	14.1%	10.0%	1.000
Creatinine (µmol/L), median (IQR)	75.5 (50.3)	81.0 (58.3)	0.275
Urine production, (ml/24hours**), median (IQR)	1110 (1583)	1200 (1423)	0.996
Glasgow coma score, median (IQR)	15 (0)	15 (0)	0.800
Thrombocytes (10E^9/L), median (IQR)	323 (152)	340 (158)	0.441
Mortality, % deceased	35.2%	40.0%	0.896[b]

[a] ICU indicates Intensive Care Unit, [b] Chi-square test instead of Fisher's Exact test. [c] Independent sample T-test instead of Mann-Whitney U test. Differences were tested using Mann-Whitney U test or Fisher's Exact test, unless indicated otherwise.

Table S8.2 The Sequential Organ Failure Assessment (SOFA) score.

	SOFA score points				
System	**0**	**1**	**2**	**3**	**4**
Respiration PaO_2/FiO_2, mmHg (kPa)	≥400 (53.3)	<400 (53.3)	<300 (40)	<200 (26.7)	<100 (13.3)
Coagulation Platelets ($x10^3/mm^3$)	≥150	<150	<100	<50	<20
Liver Bilirubin, mg/dL (µmol/L)	<1.2 (20)	1.2-1.9 (20-32)	2.0-5.9 (33-101)	6.0-11.9 (102-204)	>12.0 (204)
Cardiovascular Catecholamine dosage =µg/kg/minute for at least one hour	MAP ≥70	MAP <70	Dopamine <5 or dobutamine (any dose)	Dopamine 5.1-15 or epinephrine ≤0.1 or norepinephrine <0.1	Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1
Nervous system GCS	15	13-14	10-12	6-9	<6
Renal Creatinine mg/dL (µmol/L, urine output, mL/d	<1.2 (110)	1.2-1.9 (110-170)	2.0-3.4 (171-299)	3.5-4.9 (300-440) or <500 ml/d	>5.0 (440) or <200 ml/d

Chapter 9

Serial assessment of myocardial injury markers in mechanically ventilated SARS-CoV-2 patients: the prospective *MaastrICCht* cohort

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Abstract

Background

Myocardial injury in coronavirus disease 2019 (COVID-19) is associated with in-hospital mortality. However, the development of myocardial injury over time and whether myocardial injury in COVID-19 patients at the Intensive Care Unit (ICU) is associated with outcome is unclear.

Objectives

To prospectively investigate myocardial injury with serial measurements over the full course of ICU admission in mechanically ventilated COVID-19 patients.

Methods

As part of the prospective *MaastriCCht cohort,* predefined myocardial injury markers, including high-sensitivity cardiac troponin T (hs-cTnT), N-terminal pro-B-type Natriuretic Peptide (NT-proBNP), and electrocardiography (ECG) characteristics were serially collected in mechanically ventilated COVID-19 patients. Linear mixed-effects regression was used to compare survivors with non-survivors, adjusting for sex, age, APACHE-II score, daily creatinine concentration, hypertension, diabetes mellitus, and obesity.

Results

In 90 patients, 57 (63%) were survivors and 33 (37%) non-survivors, a total of 628 serial ECGs, 1565 hs-cTnT, and 1559 NT-proBNP concentrations were assessed. Log-Hs-cTnT was lower in survivors compared to non-survivors at day 1 (ß -0.93 (-1.37; -0.49, p<0.001) and did not change over time. Log-NT-proBNP did not differ at day 1 between both groups but decreased over time in the survivor group (ß -0.08 (-0.11; -0.04, p<0.001) compared to non-survivors. Many ECG abnormalities were present in the whole population, without significant differences between both groups.

Conclusion

Baseline hs-cTnT and change in NT-proBNP were strongly associated with mortality. Two-thirds of COVID-19 patients showed ECG abnormalities. Our serial assessment suggests that myocardial injury is common in mechanically ventilated COVID-19 patients and is associated with outcome.

Introduction

Severe Acute Respiratory Syndrome-Coronavirus-2 (SARS-CoV-2) resulting in coronavirus disease 2019 (COVID-19) can lead to respiratory failure requiring mechanical ventilation in severe cases. The disease course in mechanically ventilated patients is often complicated by multi-organ failure, including myocardial injury[1] and thrombosis.[2-4] Myocardial injury is reported in 4-37% of COVID-19 patients and may be the consequence of viral myocarditis, type I myocardial infarction caused by atherosclerotic plaque disruption, or type II myocardial infarction caused by an imbalance between oxygen demand and supply.[5-7]

Most studies on COVID-19 define myocardial injury based on elevated cardiac troponin (cTn) as described in the Fourth Universal Definition of Myocardial Infarction because of their unique cardiac origin.[8] They should be preferably be measured using high-sensitivity (hs) cTn assays. Other biomarkers of interest might be serum creatine-kinase (CK), creatine-kinase MB type (CK-MB), and N-terminal pro-B-type Natriuretic Peptide (NT-proBNP).[9] NT-proBNP, is a marker of hemodynamic myocardial stress and heart failure but can also be elevated in patients with severe inflammatory and respiratory disease.[10,11] Despite all this, cTn and NT-proBNP concentrations are also affected by impaired renal function.[12]

Moreover, abnormal ECG findings upon or during admission, including arrhythmias, ST-segment deviation, prolonged PR and QTc intervals are associated with major adverse events, infection severity, and transfer to the ICU.[13,14]

Myocardial injury in COVID-19 patients is strongly associated with in-hospital mortality[15-17] and may relate to pre-existing cardiovascular disease (CVD), or is primarily due to COVID-19 infection. The latter may, in turn, worsen underlying CVD or even precipitate de novo cardiac complications.[18-21] Actually, it was shown that the prognosis of patients with underlying CVD *without* myocardial injury was relatively favourable.[15] This suggests an independent role for COVID-induced myocardial injury over sole pre-existent CVD.

Current evidence as used in COVID-19 guidelines is based on retrospective and cross-sectional studies on myocardial injury in COVID-19 patients without serial measurements.[22]

We hypothesized that elevated cardiac biomarkers and altered ECGs are prevalent in mechanically ventilated COVID-19 patients and, when assessed over time, are associated with outcome, independent of clinical characteristics, disease severity, renal function[12], and COVID-19 related risk factors, such as hypertension, diabetes mellitus, and obesity.[23,24]

Therefore, in the present study, we investigated myocardial injury development by serial cardiac biomarkers and serial ECGs from intubation onwards over the disease course, comparing ICU survivors and non-survivors.

Methods

The present study is part of the Maastricht Intensive Care COVID (*MaastrICCht*) cohort, which is a prospective observational study that included all patients on mechanical ventilation for COVID-19 admitted to our ICU (Trial Register number [NL8613]). The study protocol has been described before in more detail[25] and has been approved by the institutional review board of Maastricht University Medical Centre+ (MUMC+) (METc, 2020-1565/ 300523). The manuscript was written following the "STrengthening the Reporting of OBservational studies in Epidemiology" (STROBE) guideline.[26]

Participants

Maastricht UMC+ is a tertiary care university teaching hospital in the Netherlands. Usually, the Maastricht UMC+ ICU had a capacity of 27 ICU beds. However, during the COVID-19 pandemic, our ICU was rapidly upgraded to a maximum of 64 beds.
All patients with respiratory insufficiency requiring mechanical ventilation and at least one PCR positive for SARS-CoV-2 and/or a chest CT scan strongly suggestive for SARS-CoV-2 infection, based on a CORADS-score of 4-5 scored by a radiologist[27] were included. After training by qualified research staff and with daily supervision by a senior investigator, medical research interns and Ph.D. candidates included participants and collected clinical, physiological, and laboratory variables using a predefined study protocol as previously described.[25] Participants were included from March 25th until June 23rd of 2020.
Participants were followed until death in the ICU or ICU discharge, and categorized based on the primary outcome. Patients who were transferred to other hospitals were followed up and reclassified to the non-survivor group if they had died in-hospital. Population characteristics and potential confounding variables have been described extensively elsewhere.[1,3,28]

Cardiac biomarkers

Cardiomyocyte injury was assessed daily in serum samples using hs-cTnT (ng/L), CK (U/L) and CK-MB (µg/L) using the Cobas 8000 analyzer (Roche Diagnostics, Mannheim, Germany). In addition, as hemodynamic myocardial stress and heart failure biomarker, we assessed NT-proBNP (pmol/L) in serum, as measured on the Cobas 8000 analyzer. Also, creatinine (µmol/L) was measured on the Cobas 8000 analyzer. Assay characteristics were all according to the package inserts.
The cardiac biomarkers were scored as follows:

1. Hs-cTnT: concentration at inclusion (day of intubation), lowest, highest, and delta (between lowest and highest) concentration. Normal concentration: <14 ng/L

2. CK: concentration at inclusion, highest, lowest, and delta concentration. Normal concentration: 0 – 225 U/L
3. CK-MB: concentration at inclusion, highest, lowest, and delta concentration. Normal concentration: <4.9 µg/L
4. NT-proBNP: concentration at inclusion, highest, lowest, and delta concentration. Normal concentration: <35 pmol/L

ECG analysis

Within the *MaastrICCht* cohort, ECGs were performed at least every other day. All ECGs were recorded at 25 mm/s speed and 10 mm/mV amplitude. ECG assessment, with an appropriate scoring system, was predesigned (supplemental 1) by a team of four physicians, including a cardiologist specialized in electrophysiology (KV), a cardiologist-intensivist (RD), and two cardiologists in training (MG and CG). The first 10 ECGs were assessed and discussed by all four to score the ECG as objectively as possible. After the scoring was objective, one investigator assessed all ECGs using a pre-set protocol and solved uncertainties within the team.

The ECG characteristics were scored systematically based on 1) Rhythm, 2) Conduction, 3) RV strain, 4) Repolarisation abnormalities, 5) T-wave abnormalities, and 6) Other.

1. Rhythm was classified as sinus rhythm, supraventricular tachycardia (i.e., atrial fibrillation, atrial flutter, and atrial tachycardia), escape rhythm, and pacemaker rhythm.
2. Conduction was scored based on: presence of a left bundle branch block (LBBB) based on the conventional criteria with QRS duration >0.12 seconds, QS or rS complex in V1, and an R wave peak time >60 ms in lead I, V5, or V6 along with the absence of a Q wave in these leads.[29] Presence of a first-degree atrio-ventricular (AV) block defined as PR interval was >200ms.
3. RV strain was defined as having either right bundle branch block (RBBB), right or extreme axis, or two or more of the following: RsR' pattern, qR pattern, Broad S in I, aVL, V5, or V6, Slurred S in I, aVL, V5, or V6, or p-pulmonale. RBBB was defined as QRS duration >0.12 seconds, rsr', rsR', rSR' in leads V1 or V2, S wave of greater duration than R wave or greater than 40 ms in leads I and V6, normal R peak time in leads V5 and V6 but greater than 50 ms in lead V1[29]; right axis was defined as from 90° - 180°; extreme axis was defined as from -90° - 180°; p-pulmonale was defined as p-wave in lead II >2.5 mm in height.[30]. Slurred S was defined as slowing in the rate of fall of the S wave, and broad S was defined as width of S >40 ms.[31]
4. Repolarization abnormalities were scored based on whether ST-segment elevation or depression was present in at least two contiguous leads. Elevation and depression were defined as a deviation of 0.1 mv in the limb leads and 0.2 mv in the precordial leads, measured 40 ms after the J-point.[9]

5. T-wave abnormalities were defined as T-wave inversion, flat T-waves, or biphasic T-waves in at least two contiguous leads.
6. Other ECG characteristics scored were QTc time (ms), heart rate, height of the R wave in V1-V2, depth S-wave in I, p-wave split, fragmentation of the QRS complex, and microvoltages defined as QRS amplitude <5 mm in the limb leads.[32] QTc interval was calculated based on the Bazett formula.[33] Prolonged QTc was defined as QTc longer than 500 ms.

Statistical analyses

All participants eligible for the study were included in the cohort until June 23rd, 2020. Data were analysed with R version 3.6.1 (R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria).

First, the cohort was categorized into ICU-survivors and ICU-non-survivors. Then, the sample characteristics were described using mean and standard deviation (SD), median and interquartile range (IQR), or percentage, as appropriate. Finally, differences were tested using the independent-samples T-test, Chi-square test, Fisher's Exact test, or Mann-Whitney U test, as appropriate.

We used linear mixed-effects regression models with a random intercept and random slope with time to compute differences in cardiac biomarkers over time between both groups. We used an unstructured variance-covariance matrix for random effects and an autoregressive correlation structure of the first order for longitudinal measures. Using the Akaike Information Criterion, the best fitting model for change over time was selected. Biomarkers were log-transformed due to skewed residuals.

We computed the crude group differences (Model 1). Subsequently, we investigated potential confounding by clinical characteristics, disease severity, daily renal function, and COVID-19 related risk factors for CVD to challenge our hypothesis. Therefore, the model was first adjusted for sex and age (Model 2), and additionally for APACHE-II score at baseline (Model 3), daily creatinine concentrations (Model 4), and hypertension, diabetes mellitus, and obesity (Model 5). Results of the regression models were expressed as regression coefficient ß, including 95% confidence interval (CI). P-value <0.05 was considered statistically significant.

Results

Baseline characteristics

The *MaastrICCht* cohort included a total of 94 participants at the time of data extraction. Four patients were excluded because there were no ECGs or biomarkers

available, because these patients were transported or died within 48 hours after ICU admission. Of all 90 included patients, 33 patients (37%) had died (ICU non-survivor group), and 57 (63%) were discharged alive from the ICU (ICU survivor group) (Figure 9.1). The non-survivor group was on average older (69 versus 62 years, p=0.006), had a shorter ICU stay (13 versus 22 days, p=0.001), had more often a history of myocardial infarction (15% versus 2%, p=0.020), and had a higher APACHE II score (18 versus 15, p=0.040) (Table 9.1). In total, we studied 628 serial ECGs and 1565 hs-cTnT, 1615 CK, 1535 CK-MB, and 1559 NT-proBNP concentrations.

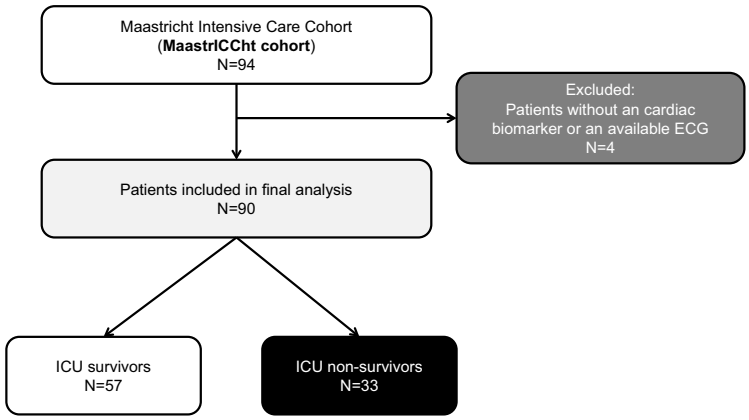


Figure 9.1 Flowchart of the study. The Maastricht Intensive Care COVID (*MaastrICCht*) cohort; ECG, electrocardiogram; ICU, Intensive Care Unit.

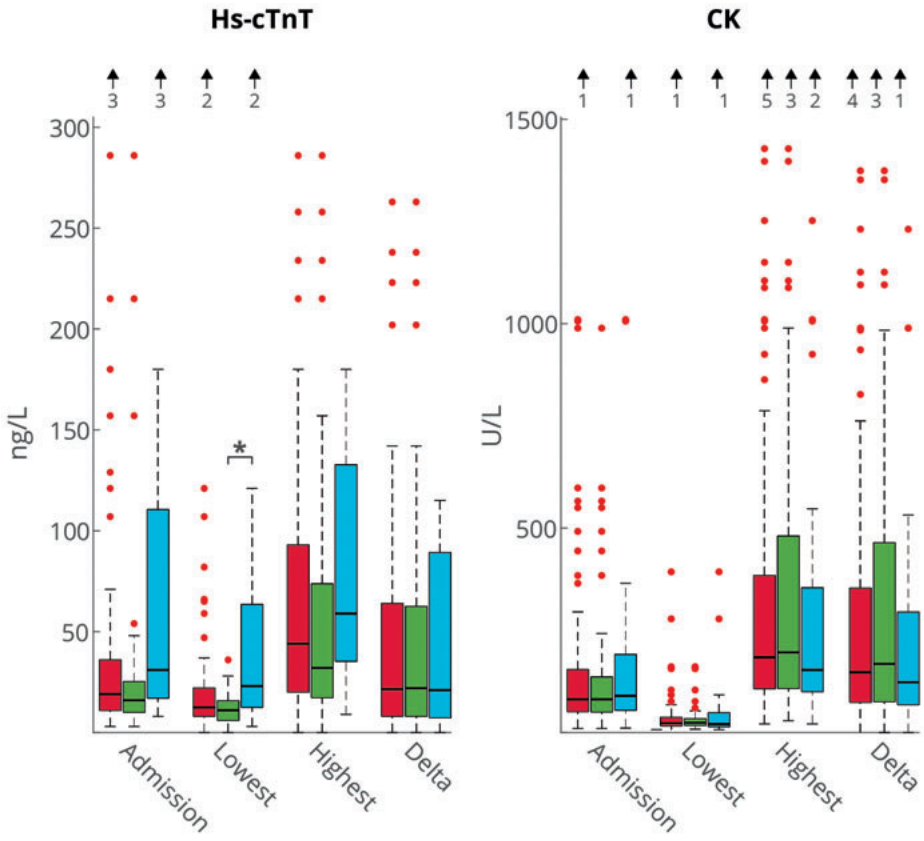
Table 9.1 Characteristics of the study population in ICU-survivors and ICU-non-survivors.

	ICU-survivors (N=57)	ICU-non-survivors (N=33)	p-value for difference
Age, year	62 (±12)	69 (±10)	0.006
Sex, men	42 (74%)	28 (85%)	0.220
Time of ICU stay, days*	22 (17)	13 (9)	0.001
Body mass index, kg/m^2	27.9 (±4.2)	27.2 (±4.0)	0.430
Admission location:			
emergency room	12 (21%)	8 (24%)	0.600‡
ward	28 (49%)	15 (45%)	0.740
transfer from other hospital	17 (30%)	10 (30%)	0.960
Previous myocardial infarction	1 (2%)	5 (15%)	0.020‡
Cardiomyopathy/LV dysfunction	2 (4%)	0 (0%)	0.530‡
Coronary artery disease	3 (5%)	5 (15%)	0.140‡
Chronic renal disease	1 (2%)	1 (3%)	1.00‡
Presence of any cardiovascular risk factor#	25 (44%)	21 (64%)	0.070
APACHE II score, points	15 (5.5)	18 (5.8)	0.040
Mean arterial pressure, mmHg (lowest in first 24 hours)	61.8 (23.9)	63.2 (13.0)	0.760
Hydroxychloroquine administered	38 (67%)	23 (69%)	0.880

Data are presented as mean (standard deviation) or count (percentage), unless indicated otherwise. Differences were tested using the independent-samples t-test or Pearson's Chi-square test unless indicated otherwise. *Median and 1st and 3rd quartiles; ‡Fisher's Exact test; † Mann Whitney U test; # Diabetes mellitus, hypertension, dyslipidaemia, smoking, obesity). ICU, Intensive Care Unit

Cardiac biomarkers

Figure 9.2 shows the median with interquartile range (IQR) cardiac biomarker concentrations at admission and lowest, highest, and delta (change between lowest and highest) during ICU stay for ICU survivors and non-survivors. Hs-cTnT at admission was 23 (15-144) ng/L for non-survivors and 14 (9-22) ng/L for survivors (p=0.177). The lowest hs-cTnT was significantly higher in non-survivors versus survivors (23 (12-65) ng/L vs. 11 (6-16) ng/L, p=0.001). For CK and CK-MB, the concentrations did not differ between the two groups. For NT-proBNP the highest (469 (280-2261) vs. 230 (129-435) pmol/L, p=0.003), lowest (109 (56-176) vs. 37 (19-108) pmol/L, p=0.003) and delta (362 (215-1866) vs. 155 (88-354), p=0.046) concentrations were all significantly higher in non-survivors compared to survivors.



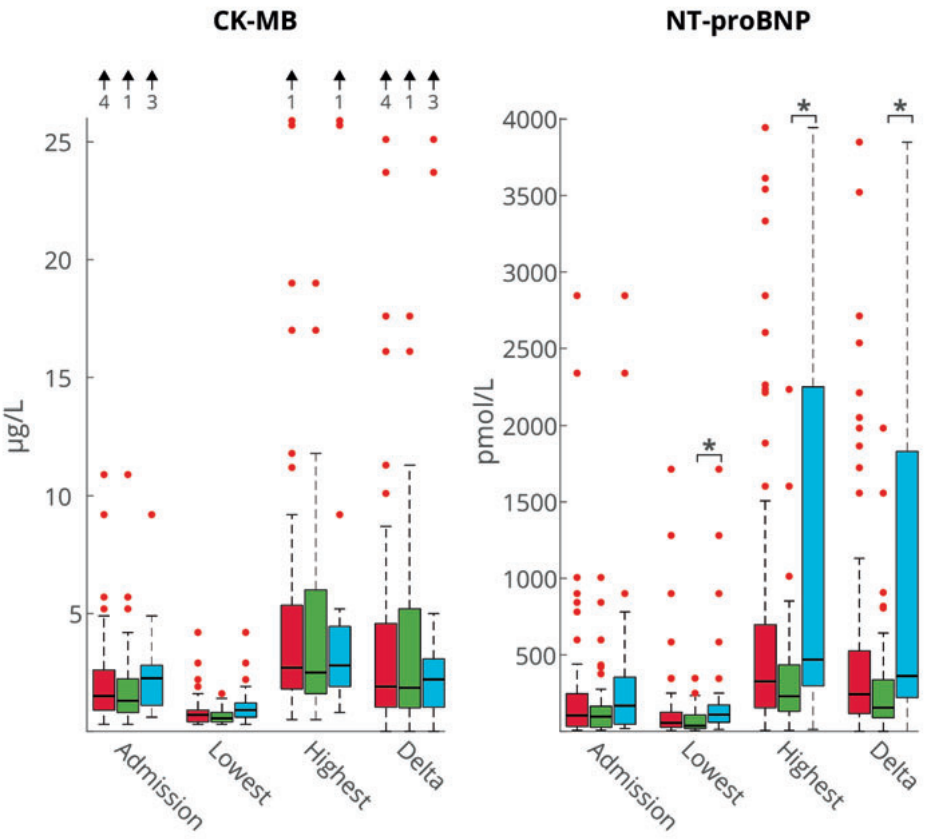


Figure 9.2 Cardiac biomarkers (hs-cTnT, CK, CK-MB, and NT-proBNP) on day 1. Concentrations are median (interquartile range (IQR)). Red bars depict all patients, green bars survivors, and blue bars non-survivors. Arrows above the figures indicate number of outliers exceeding the range of this figure. * p<0.05.

Table 9.2 depicts the results of the linear mixed-effect models showing the difference in biomarker concentrations at the start of mechanical ventilation and the difference in development over time between the two groups.

Log-Hs-cTnT was lower for survivors compared to non-survivors at day 1 (ß: -0.93, 95% CI: [-1.37;-0.49], p<0.001) and this association remained statistically significant after adjustment for sex and age (ß -0.74 [-1.17; -0.30], p=0.001) (model 2), APACHE II score (ß -0.66 [-1.08; -0.24], p=0.003) (model 3), daily creatinine concentrations (ß -0.47 [-0.95; 0.00], p=0.049) (model 4) and cardiovascular risk factors (ß -0.55 [-1.04; -0.07], p=0.028) (model 5). The change over time in log-hs-cTnT did not differ statistically significantly between the groups (Table 9.2 and Figure 9.3 Panel A).

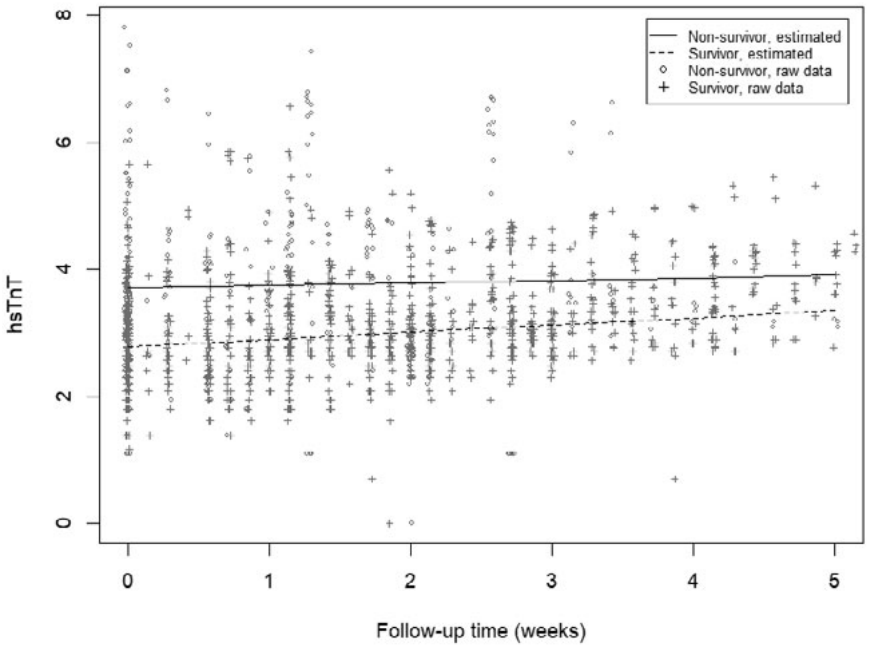
Table 9.2 Associations between serial biomarkers over time and survival in mechanically ventilated COVID-19 patients. Biomarker data were log-transformed for reasons of normality and skewed residuals.

Model	log(hs-cTnT) Regression coefficient (95% CI)	p-value	log(CK) Regression coefficient (95% CI)	p-value	log(CKmb) Regression coefficient (95% CI)	p-value	log(NT-proBNP) Regression coefficient (95% CI)	p-value
Model 1								
ICU-non-survivor (reference)	ref	ref	ref	ref	ref	ref	ref	Ref
ICU-survivor*	-0.93 (-1.37; -0.49)	<0.001	-0.20 (-0.57; 0.17)	0.289	-0.41 (-0.70; -0.13)	0.005	-0.39 (-0.96; 0.19)	0.182
Interaction between group and time‡	0.01 (-0.01; 0.03)	0.348	0.03 (0.01; 0.06)	0.059	0.02 (0.00; 0.03)	0.049	-0.08 (-0.11; -0.04)	<0.001
Model 2								
ICU-non-survivor (reference)	ref	ref	ref	ref	ref	ref	ref	ref
ICU-survivor*	-0.74 (-1.17; -0.30)	0.001	-0.18 (-0.55; 0.20)	0.360	-0.29 (-0.57; 0.00)	0.048	-0.32 (-0.90; 0.26)	0.279
Interaction between group and time‡	0.01 (-0.01; 0.03)	0.449	0.04 (0.01; 0.06)	0.003	0.02 (0.00; 0.03)	0.059	-0.08 (-0.11; -0.05)	<0.001
Model 3								
ICU-non-survivor (reference)	ref	ref	ref	ref	ref	ref	ref	ref
ICU-survivor*	-0.66 (-1.08; -0.24)	0.003	-0.14 (-0.52; 0.24)	0.460	-0.25 (-0.52; 0.03)	0.084	-0.28 (-0.85; 0.29)	0.331
Interaction between group and time‡	0.01 (-0.02; 0.03)	0.560	0.03 (0.01; 0.06)	0.005	0.01 (0.00; 0.03)	0.088	-0.08 (-0.11; -0.05)	<0.001
Model 4								
ICU-non-survivor (reference)	ref	ref	ref	ref	ref	ref	ref	ref
ICU-survivor*	-0.47 (-0.95; 0.00)	0.049	-0.03 (-0.41; 0.35)	0.871	-0.13 (-0.40; 0.14)	0.342	-0.10 (-0.62; 0.43)	0.717
Interaction between group and time‡	0.01 (-0.02; 0.03)	0.588	0.01 (0.03; 0.05)	0.014	0.02 (0.00; 0.03)	0.179	-0.08 (-0.12; -0.05)	<0.001
Model 5								
ICU-non-survivor (reference)	ref	ref	ref	ref	ref	ref	ref	ref
ICU-survivor*	-0.55 (-1.04; -0.07)	0.028	-0.09 (-0.47; 0.29)	0.634	-0.15 (-0.42; 0.12)	0.284	-0.13 (-0.67; 0.40)	0.631
Interaction between group and time‡	0.01 (-0.02; 0.03)	0.511	0.03 (0.01; 0.06)	0.008	0.02 (0.00; 0.03)	0.161	-0.08 (-0.12; -0.05)	<0.001

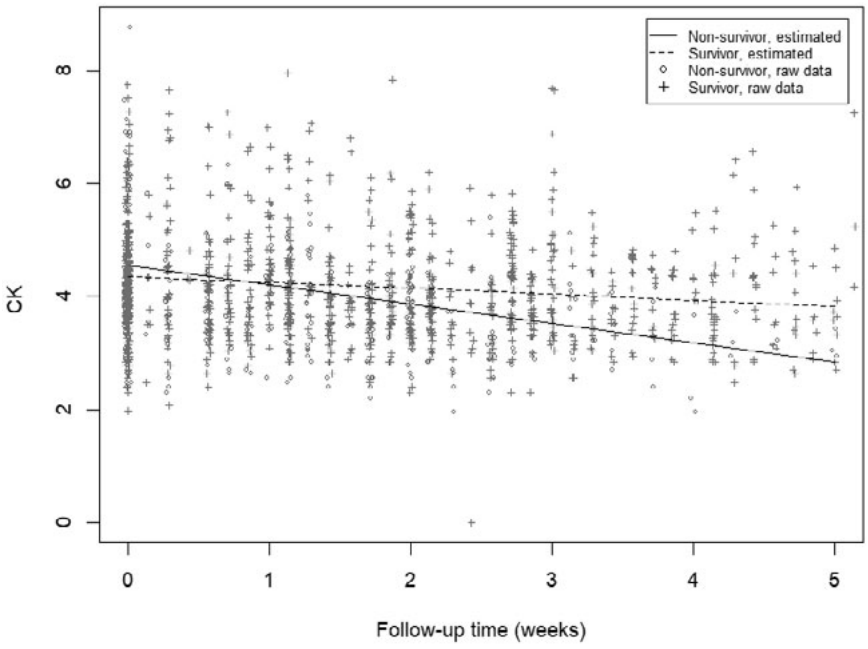
Results of the linear mixed-effects models show the difference in log-biomarker development over time between ICU-survivors and ICU-non-survivors, with ICU-non-survivors as the reference category. Data are regression coefficients (β) with 95% confidence intervals (CI). The regression coefficient ß indicates the mean difference in log biomarker over time. The regression ß for the between-group and time interaction indicates the changes in log biomarker over time between ICU-survivors and ICU-non-survivors (with ICU-non-survivors as reference (ref)). Model 1 is the crude model; model 2 adjusts for sex and age; model 3 additionally adjusts for APACHE-II score at baseline; model 4 also adjusts for daily creatinine concentration; model 5 also adjusts for smoking status, the presence of hypertension, diabetes, and obesity. CI, confidence interval; ICU, Intensive Care Unit; APACHE, Acute Physiology and Chronic Health Evaluation. *A negative regression coefficient indicates that the average log-biomarker concentration of survivors at day 1 is lower compared to the non-survivors. ‡A negative regression coefficient for the interaction term indicates that the log-biomarker concentration of survivors decreases more over time compared to the non-survivors. (i.e. the interaction between group and time models the change over time for both groups separately).

Log-CK concentration did not differ between the groups at day 1 in any model, but over time decreased less in survivors compared to non-survivors (Table 9.2 and Figure 9.3 Panel B). For log-CK-MB the crude model (ß -0.41 [-0.70; -0.13], p=0.005) and the model after adjustment for sex and age (ß -0.29 [-0.57; 0.00], p=0.048) show statistically significantly lower concentrations for survivors at day 1. This association did not remain after further adjustments. Over time, log-CK-MB concentration decreased statistically significantly more in non-survivors versus survivors in the crude model (ß 0.02 (0.00; 0.03, p=0.049), however this association did not remain after adjustments (Table 9.2, Figure 9.3 Panel C).
Log-NT-proBNP at day 1 did not differ statistically significantly between the two groups, but over time it decreased significantly more in the survivor group (ß -0.08 [-0.11; -0.04], p<0.001). This association remained statistically significant after adjustment for sex and age (ß -0.08 [-0.11; -0.05], p<0.001), APACHE II score (ß -0.08 [-0.11; -0.05], p<0.001), daily creatinine concentrations (ß -0.08 [-0.12; -0.05], p<0.001) and COVID-19 related cardiovascular risk factors (ß -0.08 [-0.12; -0.05], p<0.001), (Table 9.2 and Figure 9.3 Panel D).

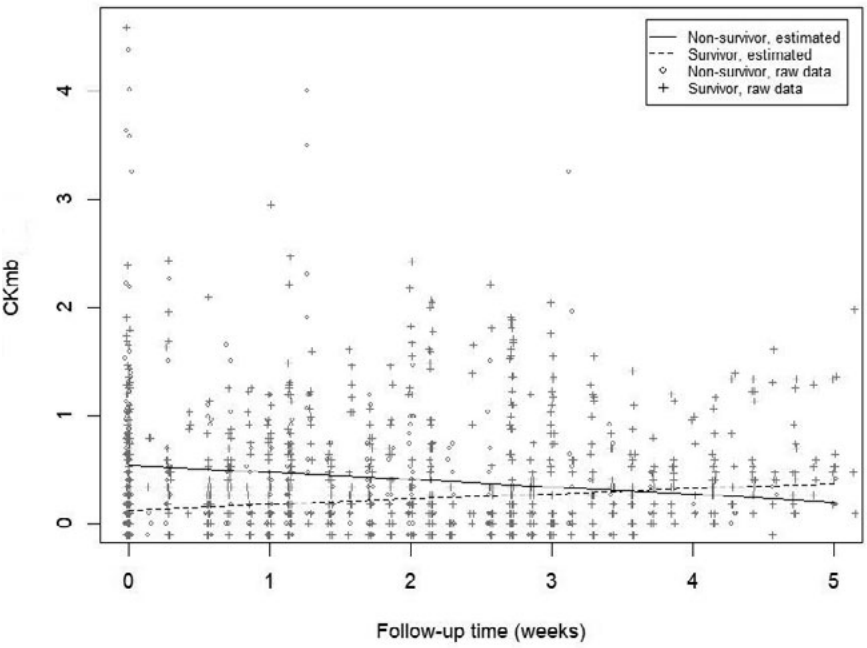
Panel A



Panel B



Panel C



Panel D

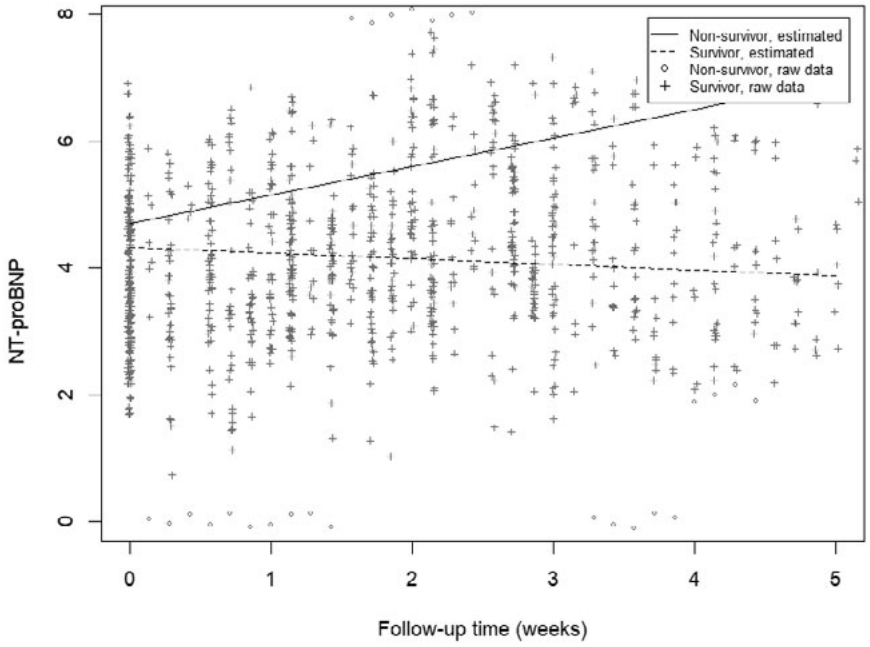


Figure 9.3 Observed and predicted log-biomarker values over time for ICU-survivors and ICU-non-survivors. Biomarkers were log-transferred due to non-normal distribution.

Electrocardiographic characteristics

In general, ECG abnormalities occurred in a high number of COVID-19 patients with the highest presence at baseline of flat T waves (n=83, 92%), QRS fragmentations (n=71, 79%), p-wave split (n=39, 43%), RV strain (n=38, 42%), and occurrence of repolarisation abnormalities (n=20, 22%). When assessing not only baseline, but all ECGs, the highest presence of abnormalities was QRS fragmentations (n=86, 96%), p-wave split (n=69, 77%), flat T waves (n=67, 74%), RV strain (n=65, 72%), and occurrence of repolarisation abnormalities (n=57, 61%). These data are summarized in Table 9.3 and Supplemental Table S9.1.

Rhythm

At baseline, 80 out of 90 patients (89%) were in sinus rhythm and seven patients had atrial fibrillation (8%). Rhythm at baseline ECG did not differ between survivors and non-survivors (Supplemental Table S9.1). However, over time, nine patients (10%) developed atrial fibrillation, five (9%) in survivors and four (12%) in non-survivors (p=0.72) (Table 9.3).

Table 9.3 ECG characteristics in serial ECGs (n=628 ECGs) for the whole population and stratified for ICU-survivors and ICU-non-survivors.

	All (N=90)	ICU-survivors (N=57)	ICU-non-survivors (N=33)	p-value for difference
Rhythm				
Sinus rhythm, n (%)	83 (92%)	53 (90%)	30 (91%)	0.70‡
Supraventricular tachycardia, n (%)	12 (13%)	7 (12%)	5 (15%)	0.70
Atrial fibrillation, n (%)	9 (10%)	5 (9%)	4 (12%)	0.72‡
Atrial flutter, n (%)	1 (1%)	1 (2%)	0 (0%)	1.0‡
Atrial tachycardia, n (%)	3 (3%)	2 (4%)	1 (3%)	1.0‡
Escape rhythm, n (%)	4 (4%)	2 (4%)	2 (6%)	0.62‡
Paced rhythm, n (%)	1 (1%)	1 (2%)	0 (0%)	1.0‡
Conduction				
LBBB, n (%)	5 (6%)	2 (4%)	3 (9%)	0.27
QRS duration (ms)	98±16	98±15	99±17	0.49
QRS > 120 ms	10 (11%)	5 (9%)	5 (15%)	0.35
1st degree AV block n, (%)	14 (16%)	7 (12%)	7 (21%)	0.26
PR time (ms)	150±25	149±24	153±26	0.05
RV strain				
RBBB, n (%)	4 (4%)	2 (4%)	2 (6%)	0.62‡
Right or extreme axis, n (%)	6 (7%)	4 (7%)	2 (6%)	1.0‡
At least two of the following n, (%)	65 (72%)	40 (70%)	25 (76%)	0.57
RsR' pattern, n (%)	33 (37%)	19 (33%)	14 (42%)	0.39
qR pattern, n (%)	12 (13%)	4 (7%)	8 (24%)	0.027‡
Broad S in I, aVL, V5 or V6, n (%)	67 (74%)	39 (68%)	28 (85%)	0.09
Slurred S in I, aVL, V5 or V6, n (%)	66 (73%)	44 (77%)	22 (67%)	0.28
P-pulmonale, n (%)	12 (13%)	8 (11%)	4 (12%)	1.0‡
Repolarization abnormalities, n (%)				
ST-segment deviation in at least two consecutive leads n (%)	57 (61%)	36 (61%)	21 (60%)	0.92
ST-segment elevation in at least two consecutive leads, n (%)	22 (24%)	14 (25%)	8 (24%)	0.97
ST-segment depression in at least two consecutive leads, n (%)	44 (49%)	28 (49%)	16 (49%)	0.95
T-wave abnormalities, n (%)				
Any T-wave abnormality n (%)	73 (81%)	47 (83%)	26 (79%)	0.67
T-waves inversion in at least consecutive two leads, n (%)	20 (22%)	13 (23%)	7 (21%)	0.86
Flat T-waves in at least two consecutive leads, n (%)	67 (74%)	44 (77%)	23 (70%)	0.43
Biphasic T-waves in at least two consecutive leads, n (%)	15 (17%)	9 (14%)	6 (18%)	0.60
Other				
QTc time (ms)	436 (55)	430 (51)	450 (61)	< 0.001
Prolonged QTc time (ms) > 500 ms	34 (38%)	18 (32%)	16 (49%)	0.11
Heartrate (bpm)	86 (21)	86 (21)	85 (21)	0.46
Height R-wave				
V1 (mV)	0.15 (0.14)	0.15 (0.13)	0.16 (0.17)	0.162
V2 (mV)	0.50 (0.36)	0.48 (0.32)	0.55 (0.44)	0.04

Table 9.3 (continued)

	All (N=90)	ICU-survivors (N=57)	ICU-non-survivors (N=33)	p-value for difference
P-wave split, n (%)	69 (77%)	43 (75%)	26 (79%)	0.71
QRS fragmentations, n (%)	86 (96%)	55 (96%)	31 (94%)	0.62‡
Microvoltages, n (%)	15 (17%)	11 (19%)	4 (12%)	0.56‡
Left heart axis n (%)	25 (28%)	12 (21%)	13 (39%)	0.06

Data are presented as mean (standard deviation) or count (percentage), unless indicated otherwise. Differences were tested using the independent-samples t-test or Pearson's Chi-square test unless indicated otherwise. ‡Fisher's Exact test. ICU, Intensive Care Unit.

Conduction

At baseline (Supplemental Table S9.1), LBBB was present in five patients (6%), 2 (4%) in the survivor group, and three (9%) in the non-survivors (p=0.35) and did not change over time (Table 9.3). QRS duration did not differ between survivors and non-survivors (98±15 ms versus 101±19 ms, p=0.50). The PR time at baseline did not differ statistically significantly between groups (150±20 ms versus 153±25 ms). When assessing baseline and all subsequent ECGs, the prevalence of first-degree AV block was comparable in both groups.

RV-strain

At baseline, the prevalence of RBBB did not differ between both groups and was seen in two (4%) survivors compared to two (6%) non-survivors (p=0.62). Over time, this number did not change in both groups. Right axis deviation was also comparable between groups and present in two (4%) survivors and two (6%) non-survivors (p=0.62). RV-strain was found in 21 (37%) survivors and 17 (52%) of non-survivors and was comparable between both groups as well (p=0.17). Of the individual criteria, a broad S wave in I, aVL, V5, or V6 was statistically significantly more present in non-survivors (23 patients, 70%) compared to survivors (27 patients, 47%) (p=0.04). When comparing all ECGs, RV-strain did not differ between both groups (Table 9.3).

Repolarisation abnormalities

At baseline, ST-segment elevation in at least two contiguous leads did not differ between groups and was present in three (5%) survivors and one (3%) non-survivor (p=1.0). Over time, the presence of ST-segment elevation increased but remained comparable between both groups. ST-segment depression was present in ten (18%) and seven (21%) survivors and non-survivors, respectively (p=0.67).

T-wave abnormalities

T-wave abnormalities neither differed between both groups at baseline nor when comparing serial ECGs, with a markedly higher presence for flat T-waves in both groups (52 (91%) and 31 (94%) for survivors and non-survivors, respectively) (Supplemental Table S9.1). When comparing serial ECGs, no difference in ST-segment abnormalities and T-wave abnormalities were seen between both groups (Table 9.3).

Other

At baseline, QTc was 429±52 ms in the survivor group and 448±58 ms in the non-survivors (p=0.118). Over time, QTc was statistically significantly of longer duration in non-survivors when compared to survivors (450±61 ms vs. 430±51 ms, p<0.001) (Table 9.3). We did not observe a statistically significant difference in the occurrence of a prolonged QTc (>500 ms) between patients who received hydroxychloroquine and those who did not (34% vs. 45% p=0.362). The average heart rate was 92 beats per minute on average at baseline and comparable between both groups. R-wave height in lead V2 over time was statistically significantly higher in non-survivors compared to survivors (0.55±0.44 mV vs. 0.48±0.32 mV, p=0.040). At baseline, p-wave splitting, QRS-fragmentations and microvoltages occurred in 43%, 79%, and 4% and over time in 77%, 96%, and 17%, respectively. These characteristics did not differ between both groups (Table 9.3).

Discussion

We assessed serial cardiac biomarkers and ECG characteristics in mechanically ventilated patients of the Intensive Care COVID (*MaastriCCht*) cohort and compared them in survivors to non-survivors. Our main findings are: 1) higher hs-cTnT at day 1 was associated with mortality (p<0.001), and this association remained significant after adjustment for sex, age, APACHE II score, daily creatinine concentrations, and COVID-19 related cardiovascular risk factors. 2) The lowest, highest, and delta NT-proBNP concentrations were higher in non-survivors versus survivors, with serial NT-proBNP concentrations that decreased more in survivors compared to non-survivors in adjusted models (p-values <0.001). 3) The presence of ECG abnormalities in all patients, irrespective of survival was high. 4) In non-survivors, QTc time was longer, R wave in lead V2 was higher, and a broad S wave in left lateral leads was more often present. Our findings underscore the importance of serial assessment of the cardiac biomarkers, especially NT-proBNP, as opposed to single measurements. Hs-cTnT was associated with mortality on admission in the ICU.

Cardiac biomarkers

Previous studies of myocardial injury in COVID-19 were retrospective by design[17], did not include mechanically ventilated patients[16], and solely investigated either biomarker[34] or ECG parameters.[35] These studies consistently showed that patients with signs of myocardial injury had an up to ten times higher mortality rate compared to patients without myocardial injury.[17,21,36] Several studies showed that 6-10% of COVID-19 patients had elevated cTn concentrations and 13-15% had elevated NT-proBNP, and both were associated with increased mortality. Furthermore, although myocardial injury is associated with fatal outcomes, the prognosis of COVID-19 patients with underlying cardiovascular disease without myocardial injury was shown to be relatively favourable compared to those with underlying CVD with myocardial injury.[15] As the risk factors for both CVD and COVID-19 disease severity are similar, adjustment for confounders such as hypertension, diabetes, and obesity is important. Renal function is modified over the course of critical illness and affects hs-cTnT, NT-proBNP concentrations, and survival, thus acting as a potential confounder.

Although the mechanism of cardiac injury in COVID-19 is not fully elucidated, the downregulation of ACE-2 by SARS-CoV-2 may lead to increased microvascular damage, myocardial hypertrophy, atrial dilatation, and diastolic dysfunction during COVID-19 infection.[37,38] In addition, SARS-CoV-2 elicits a host response that triggers wide-ranging, immune-inflammatory thrombotic and parenchymal derangements[3,39], also leading to myocardial damage reflecting myocarditis, thromboembolism[3], endothelitis, cardiac arrhythmias, or myocardial ischemia (either type I or II).

ECG characteristics

Previous studies investigating ECG alterations in COVID-19 patients included primarily hospitalized, non-ICU patients.[13,35] Therefore, we should be careful translating these findings to the situation where patients are admitted to the ICU with mechanical ventilation and hemodynamic support. These factors may very well affect ECG alterations. Furthermore, previous studies did not perform serial ECGs every other day as was done in the present study. Thus, on the one hand, we found a high incidence of ECG abnormalities in COVID patients, which is in line with previous observations.[13,35] On the other hand, we found that most ECG characteristics did not differ between survivors and non-survivors. This can be explained by the fact that our study population consisted of mechanically ventilated ICU patients and thus more severely affected COVID-19 patients. In contrast, other studies investigated ECGs at the emergency department or non-ICU departments, including less severe cases.[40]

Atrial fibrillation occurred in eight percent of our patients, which is in line with the ten percent reported by others.[13] The present study found an increase in PR time, in line with other studies.[14,35] LBBB and ST-T segment alterations occurred in our study almost

twice as much as previously reported (6% vs. 3% and 19% vs. 8.2%, respectively).[13] Bergamaschi et al. found no differences in QTc interval between survivors and non-survivors while in our study QTc was longer in non-survivors (450 vs. 430, p<0.001).[13] Hydroxychloroquine use was not associated with the occurrence of a prolonged QTc (>500 ms) in our study (p=0.362). Strikingly, we found fragmentation of the QRS in 79% of patients whereas previously, fragmented QRS was identified in 36.8% and was a predictor of cardiac mortality in COVID-19 patients.[41]

Strength and limitations

This study has several strengths. First, this is a comprehensive prospective cohort study. We determined biomarkers daily in all patients, thereby including many serial measurements over time. Furthermore, we performed an ECG every other day in all patients, thereby investigating ECG features and changes over time during ICU admission due to COVID-19. Second, all ECGs (n=632) were interpreted manually, which has several advantages, including improved accuracy over automated interpretation.[42] Except for the first ten ECGs, one physician manually evaluated the ECGs. However, uncertainty was solved within the team of four physicians. Third, we addressed potential confounding extensively by adjusting the models for sex, age, APACHE II score, daily creatinine concentrations, and COVID-19 related cardiovascular risk factors.

A limitation of this study is that it is a single-centre study, solely including ICU patients on mechanical ventilation with COVID-19. This limits generalizability to other mechanically ventilated COVID-19 patients only. Further, we studied variables over time, and during admission, many interventions might influence these variables. For example, except for hydroxychloroquine, we did not report/adjust for daily medication, which can influence some ECG characteristics like QTc time.

Conclusion

This prospective study is the first to serially investigate cardiac biomarker and ECG characteristics over time in mechanically ventilated ICU patients with COVID-19. We show that higher hs-cTnT at admission and increasing NT-proBNP over time are associated with mortality, irrespective of sex, age, APACHE II score, daily creatinine concentrations, and COVID-19 related cardiovascular risk factors. Significant ECG abnormalities are prevalent in more than two-thirds of mechanically ventilated COVID-19 patients. These findings shed light on the cardiac pathophysiology over time in critically ill COVID-19 patients. This suggests that serial measurement can provide direction to physicians for clinical decision-making and prognostication in future COVID-19 patients and studies.

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Supplemental material

Table S9.1 ECG characteristics at baseline in ICU-survivors and ICU-non-survivors.

	All (N=90)	ICU-survivors (N=57)	ICU-non-survivors (N=33)	p-value for difference
Rhythm				
Sinus rhythm, n (%)	80 (89%	51 (85%)	29 (88%)	0.14‡
Supraventricular tachycardia, n (%)	8 (9%)	4 (7%)	4 (12%)	0.41
Atrial fibrillation, n(%)	7 8%)	4 (7%)	3 (9%)	0.70‡
Atrial flutter, n(%)	0 (0%)	0 (0%)	0 (0%)	
Atrial tachycardia, n (%)	1 (1%)	0 (0%)	1 (3%)	0.37‡
Escape rhythm, n (%)	1 (1%)	1 (2%)	0 (0%)	1.0‡
Paced rhythm, n (%)	1 (1%)	1 (2%)	0 (0%)	1.0‡
Conduction				
LBBB, n (%)	5 (6%)	2 (4%)	3 (9%)	0.35‡
QRS duration (ms)	100±17	98±15	101±19	0.50
QRS >120 ms	8 (9%)	3 (5%)	5 (15%)	0.14‡
1st degree AV block n, (%)	2 (2%)	0 (0%)	2 (6%)	0.13‡
PR time (ms)	151±22	150±20	153±25	0.68
RV strain, n (%)				
RBBB, n (%)	4 (4%)	2 (4%)	2 (6%)	0.62‡
Right or extreme axis, n (%)	4 (4%)	2 (4%)	2 (6%)	0.62‡
At least two of the following n, (%)	38 (42%)	21 (37%)	17 (52%)	0.17
RsR' pattern, n (%)	14 (16%)	7 (12%)	7 (21%)	0.26
qR pattern, n (%)	4 (4%)	1 (2%)	3 (9%)	0.14‡
Broad S in I, aVL, V5 or V6, n (%)	50 (56%)	27 (47%)	23 (70%)	**0.04**
Slurred S in I, aVL, V5 or V6, n (%)	46 (51%)	27 (47%)	19 (57%)	0.35
P-pulmonale, n (%)	6 (7%)	4 (7%)	2 (6%)	1.0‡
Repolarization deviation in two consecutive leads, n (%)	20 (22%)	12 (21%)	8 (24%)	0.73
ST-elevation in at least two consecutive leads, n (%)	4 (4%)	3 (5%)	1 (3%)	1.0‡
ST-depression in at least two consecutive leads, n (%)	17 (19%)	10 (18%)	7 (21%)	0.67
Any T-wave abnormalities, n (%)	84 (89%)	53 (90%)	31 (89%)	0.85
T-waves inversion in at least consecutive two leads, n (%)	3 (3%)	2 (5%)	1 (3%)	1.0‡
Flat T-waves in at least two consecutive leads, n (%)	83 (92%)	52 (91%)	31 (94%)	1.0‡
Biphasic T-waves in at least two consecutive leads, n (%)	1 (1%)	0 (0%)	1 (3%)	0.37‡
General/other				
QTc time (ms)	437±55	429±52	448±58	0.118
Prolonged QTc time (ms) > 500 ms	3 (3%)	2 (4%)	1 (3%)	1.0‡
Heartrate (bpm)	92±22	92±22	92±23	0.95
Heigth R-wave				
V1 (mV)	0.13±0.12	0.13±0.11	0.12±0.13	0.65
V2 (mV)	0.44±0.34	0.44±0.31	0.45±0.40	0.82
Depth S-wave in I (mV)	0.28±0.17	0.27 0.17	0.30±0.19	0.68
P-wave split, n (%)	39 (43%)	27 (47%)	12 (36%)	0.58
QRS fragmentations, n (%)	71 (79%)	43 (75%)	28 (85%)	0.29
Microvoltages, n (%)	4 (4%)	3 (5%)	1 (3%)	1.0‡
Left heart axis	20 (22%)	10 (30%)	10 (18%)	0.16

Data are presented as mean (standard deviation) or count (percentage), unless indicated otherwise. Differences were tested using the independent-samples t-test or Pearson's Chi-square test, unless indicated otherwise. ‡Fisher's Exact test. ICU, Intensive Care Unit.

Table S9.2 Association between baseline and cumulative ECG abnormalities and survival, corrected for age and sex.

Baseline	OR (p value)	In any	OR (p value)
SVT	0.862 0.168 – 4.427 p 0.86		1.483 0.365 – 6.025 p 0.58
LBBB	0.386 0.033 – 4.545 p 0.45		0.464 0.052 – 4.137 p 0.49
RBBB	1.085 0.029 – 40.269 p 0.97		0.622 0.072 – 5.378 p 0.67
RV strain	0.668 0.037 – 12.102 p 0.79		1.138 0.358 – 3.614 p 0.83
ST-segment abnormalities	1.189 0.358 – 3.951 p 0.78		1.226 0.457 – 3.287 p 0.685
T-wave abnormalities	2.158 0.087 – 53.697 p 0.64		1.652 0.485 – 5.620 p 0.422
QTc prolongation	0.533 0.036 – 7.885 p 0.65		0.565 0.208 – 1.534 p 0.263

Table S9.3 Cardiac biomarkers in ICU survivors and non-survivors.

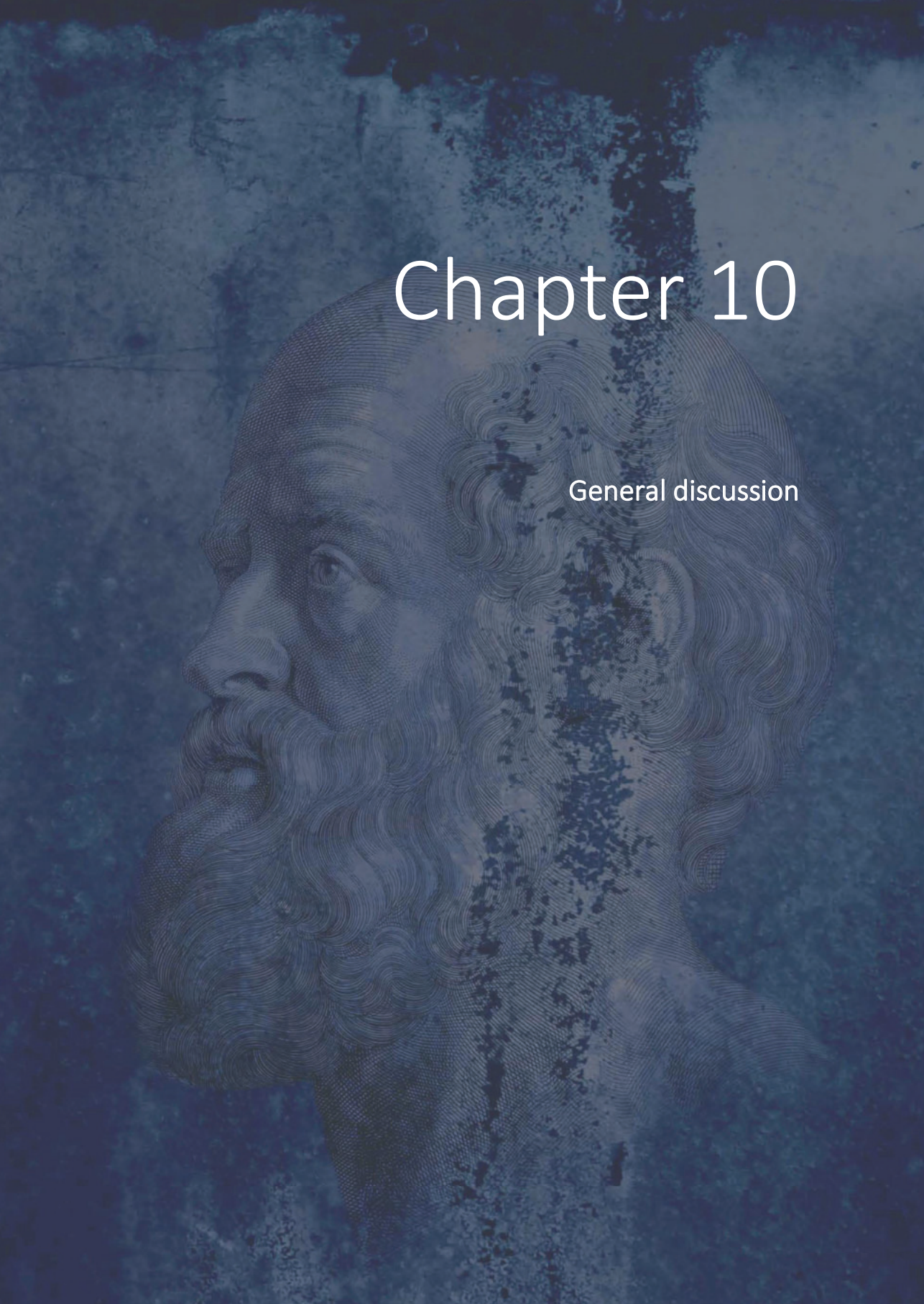
	All N=90	ICU survivors N=57	ICU non survivors N=33	p value
Hs-cTnT (ng/L)				
Admission	19 (10-33)	14 (9-22)	23 (15-144)	0.177
Highest	44 (20-94)	32 (17-74)	59 (35-134)	0.062
Lowest	13 (8-22)	11 (6-16)	23 (12-65)	**0.001**
Delta	22 (8-64)	22 (8-63)	21 (7-91)	1.0
CK (U/L)				
Admission	101 (61-156)	93 (55-156)	111 (64-168)	0.759
Highest	184 (107-401)	196 (107-491)	153 (97-365)	0.789
Lowest	23 (16-37)	24 (17-34)	21 (14-51)	0.592
Delta	148 (74-356)	168 (74-472)	123 (66-304)	0.200
CK-MB (µg/L)				
Admission	2.1 (1.1-2.9)	1.6 (1.0-2.6)	2.8 (2.0-9.2)	0.152
Highest	2.7 (1.8-5.4)	2.5 (1.6-6.2)	2.8 (1.9-4.6)	0.937
Lowest	0.7 (0.4-0.9)	0.6 (0.4-0.8)	0.9 (0.6-1.2)	0.100
Delta	1.9 (1.0-4.8)	1.9 (1.0-5.4)	2.2 (1.0-3.2)	0.937
NT-proBNP (pmol/L)				
Admission	116 (28-261)	98 (28-181)	131 (28-410)	0.428
Highest	327 (154-707)	230 (129-435)	469 (280-2261)	**0.003**
Lowest	56 (26-128)	37 (19-108)	109 (56-176)	**0.003**
Delta	243 (116-547)	155 (88-354)	362 (215-1866)	**0.046**

Table S9.4 Associations of cardiac biomarkers with survival, corrected for age, sex, and renal function.

	Baseline	Highest	Lowest	Delta
Hs-cTnT	1.002 [0.992 – 1.012] p 0.643	1.002 [0.999 – 1.0004] p 0.270	**1.081 [1.026 – 1.140] p 0.004**	1.001 [0.999 – 1.004] p 0.364
CK	0.999 [0.993 – 1.005] p 0.754	0.999 [0.998 – 1.001] p 0.257	1.002 [0.989 – 1.014] p 0.808	0.999 [0.998 – 1.001] p 0.241
CK-MB	0.986 [0.939 – 1.035] p 0.573	0.994 [0.958 – 1.030] p 0.731	3.723[0.880 – 15.755] p 0.074	0.992 [0.957 – 1.029] p 0.676
NT-proBNP	1.000 [0.998 – 1.002] p 0.809	**1.002 [1.001 – 1.002] p 0.002**	**1.008 [1.000 – 1.016] p 0.047**	**1.002 [1.001 – 1.003] p 0.003**

Part VII

General discussion



Chapter 10

General discussion

General discussion

Sepsis is defined as a life-threatening syndrome caused by a dysregulated host response to infection.[1] Its overall incidence is increasing due to increasing age and improved survival of patients with comorbidity and immunosuppressive medication use.[2] Mortality seems to be decreasing but is still high, with a reported mortality of 27% overall and up to 42% in the intensive care unit (ICU).[3] Sepsis is heterogeneous and this affects diagnostic criteria, pathophysiology, and hampers treatment evaluation. However, sepsis seems to be the final common pathway to death for a variety of severe infectious diseases (bacterial, viral, and fungal infections). Multiple mechanisms are involved, although the whole pathophysiology of sepsis toward multi-organ failure and death is not fully understood. Although our understanding of the pathophysiology has improved considerably[4,5], multiple trials modulating the response to infection have not led to new treatments[6], and mortality remains unacceptably high. Consequently, research on this topic is important. Therefore, in this thesis, we studied various aspects of sepsis. Like, the effect of a change in definition, the causes of early death, a potential new molecular mechanism, the appropriateness of antibiotic treatment, and (cardiac) complications in patients with inflammation and sepsis. **Chapter 10** summarizes the main findings of this thesis, methodological issues, potential clinical implications, and future perspectives.

10.1 Main Findings

Part II The influence of a new septic shock definition

In 2016, the Third International Consensus Definitions Task Force of the Society of Intensive Care Medicine and the European Society of Intensive Care Medicine revised the definitions for sepsis and septic shock (Sepsis-3 definition). Septic shock is now defined clinically by a vasopressor requirement to maintain a mean arterial pressure of 65 mm Hg or greater and a serum lactate level ≥2 mmol/L in the absence of hypovolemia.[1] The serum lactate level, as a reflection of cellular and metabolic dysfunction in septic shock[7], is not incorporated in the Sepsis-2 definition. The goal was that the new definition would guide clinicians in early recognition and lead to greater consistency of epidemiologic studies and trials.

Therefore, in **Chapter 3**, we compared the Sepsis-3 definition of septic shock with the Sepsis-2 definition in an ICU population of 632 septic patients. We found that the Sepsis-3 definition identified a smaller but more severely ill subpopulation than the Sepsis-2 definition. Indeed, 76% (482 out of 632 patients) of sepsis patients met Sepsis-2 criteria of septic shock, whereas 47% (300 out of 632 patients) met Sepsis-3

criteria. In addition, both ICU mortality (39% vs. 34%) and in-hospital mortality (47% vs. 43%) were higher in patients classified according to Sepsis-3 criteria than patients classified according to Sepsis-2 criteria of septic shock. These findings support the aim of the Task Force, reflecting septic shock as a more severe illness with a much higher likelihood of death than sepsis alone.

The study's second finding was that no association with increased mortality was found for patients with septic shock and lactate values between 2 and 4 mmol/L as well as between 4 and 6 mmol/L compared to patients with lactate values ≤2 mmol/L. Thus, in this cohort, the criterion of serum lactate level substantially increases mortality only when it rises above 6 mmol/L. Although intermediate serum lactate values are of clinical importance because of their linear relationship with mortality[8,9], the primary goal of the contemporary Sepsis-3 septic shock definition is to define, recognize, and treat the factual high-risk patient with a significantly higher chance of dying than with sepsis alone. Therefore, the appropriate cut-off value for serum lactate for the prognosis might have to change for intensive care patients with septic shock. These patients are already admitted in the ICU and are severely ill and this may have led to confounding by indication. Furthermore, these results are only generalizable for intensive care patients.

Finally, we found that a large subpopulation of the study population had an active malignancy (39%), which proved to be an independent risk factor for ICU mortality with an odds ratio of 2.4. Cancer is a common comorbid medical condition in patients with sepsis and septic shock[10,11], with reported higher mortality rates when compared to non-cancer sepsis patients.[12] Increased mortality rates in this subpopulation of septic patients may be explained because patients are often immunocompromised because of chemotherapy or other immune-modulating therapy. Still, over the recent years, outcomes in patients with cancer admitted to the ICU have improved.[13] When considering admission to the ICU in immunocompromised patients, the delay between admission and possible intubation should be as short as possible, as this is a strong predictor of mortality.[14] Communication with the patients and their families is also important to prevent fragmented care, reduce anxiety and post-traumatic stress[15] and to counsel patient and family with regard to chances towards outcome.

Part III Early death in sepsis

Few studies address the exact causes and the timing of death in sepsis and septic shock, although one-third of sepsis patients die within three days of admission to the ICU.[16] Recognizing patients with sepsis at risk for early death can influence clinical care and guide the design of future clinical trials to identify patients that do not benefit from an intervention anyway.[6]

This was the rationale to study the timing, causes, and influencing factors of early death in septic ICU patients in **Chapter 4**. Our main finding was that 97 out of 344

(28%) deceased patients died within 48 hours after admission with sepsis in the ICU. Early death was mainly caused by multiple organ failure (37%), mesenteric ischemia (23%), death after unsuccessful CPR (22%), and after withdrawal of treatment (13%), in line with previous research.[17]

Additionally, an expert panel of four intensivists retrospectively reviewed patient files of early deceased patients and found that delay in ICU admission (32%) and futile commencement of ICU treatment (29%) were frequently reported, however with low to moderate interobserver agreement (Fleiss' kappa of 0.180 and 0.415 respectively) between the intensivists. The latter indicates the subjectivity of reasons to admit patients in the ICU, although carefully considered by each physician. Careful selection and multi-disciplinary decision-making regarding which patients could benefit from ICU treatment might reduce this subjectivity. A subgroup of patients (n=10) reported as futile ICU treatment was in moribund general condition, limiting the time to clarify the wishes of the patient and the opinion of the medical team regarding treatment or withholding specific treatment. In some cases, ICU admission can be legitimate to create time to determine the wishes of the patient and/or relatives and the opinion of the medical team. The other important reason for reporting futile ICU treatment was the presence of a metastasized malignancy (n=9). As described earlier in **Chapter 3**, patients with sepsis and cancer have significantly higher mortality, although outcomes in this subgroup have improved due to advances in care and implementation of adjuvant therapies.[13]

Interestingly, in our study autopsy was performed in 33% of early deceased patients, which is in contrast to most other studies on this subject, reporting low numbers of autopsies.[16] Autopsies contribute to a more reliable assessment of the cause of death in these individual critically ill patients.[18,19] Therefore, we reported discrepancies between clinical diagnoses versus autopsy findings in these early (<48 hours after ICU admission) deceased septic patients and reported the results in **Chapter 5**. We used the Goldman criteria to classify the discrepancies between clinical and autopsy diagnoses.[20] In this retrospective cohort study, we observed an autopsy rate of 33%, much higher than the global autopsy rate in the Netherlands of 2,7%.[21] We found 26 autopsy-identified missed clinical diagnoses, predominantly myocardial infarction, pneumonia, cancer, and hemorrhage, in line with other ICU autopsy studies.[18,19] In 13% of patients, a Class I error according to the Goldman criteria was identified, meaning this would have changed management and possibly prolonged survival. In 42% of patients, a Class II error was found, meaning that these missed diagnoses would not have influenced survival. Major discrepancies between clinical and autopsy diagnoses remain consistent over time at approximately 10%[20,22] despite improvements in technology. This study underlines the ongoing importance of autopsies for patient care, understanding the pathophysiology of sepsis patients and also for educational purpose because if autopsy rate rises, the number of major misdiagnoses decrease[23]. A

limitation is the fact that not all relatives consent to an autopsy performed on their deceased family member.

Part IV A possible new molecular mechanism in inflammation and sepsis

In addition to the fact that sepsis is a heterogeneous diagnosis as shown above, it is a complicated syndrome with alternating pro- and anti-inflammatory mechanisms[5] affecting the endothelium[24], lung and other organ function[25], coagulation[26], and microcirculation.[27] This may be why no single treatment[6] or biomarker[28] has successfully improved survival in sepsis trials. Therefore, novel molecular mechanisms linking increased inflammation, impaired coagulation, endothelial dysfunction, and increased oxidative stress to multi-organ failure should be investigated. Chapter 6 investigated such a possible mechanism, the detoxifying glyoxalase system that clears dicarbonyl stress.[29] The dicarbonyls (i.e., methylglyoxal (MGO), glyoxal (GO), and 3-deoxyglucosone (3-DG)) are highly reactive metabolites produced by several metabolic processes such as anaerobic glycolysis, gluconeogenesis, and lipid peroxidation.[30] Inflammation and hypoxia may increase glycolytic flux and consequently increase the production of dicarbonyls. Furthermore, inflammation and hypoxia can downregulate glyoxalase-1 (GLO-1) activity, which is the enzyme that breaks down dicarbonyls into D-lactate.[29] The produced dicarbonyls can damage intracellular and extracellular proteins due to arginine modifications and the formation of methyl-glyoxal derived hydroimidazolone-1 (MG-H1), leading to cell and tissue dysfunction.[31] Indeed, in diabetes mellitus these advanced glycation endproducts (AGEs) have been extensively studied and were involved in complications affecting multiple organs (i.e., renal, neurological, micro- and macrovascular disease).[31]

We comprehensively investigated the effect of inflammation and hypoxia on the dicarbonyl stress pathway in humans using a homogenous study population of healthy males. The human endotoxemia model, described in detail earlier[32], was used in several studies[33,34] and facilitated studying hypoxia and inflammation in humans in a highly standardized way. Briefly, 40 healthy males were included, of which ten were exposed to hypoxia (hypoxia group), ten to endotoxin (lipopolysaccharide (LPS) group), ten to both conditions (LPS+hypoxia group), and ten participants were not exposed to hypoxia or endotoxin (control group). Blood samples were drawn at ten time points from 0 to 570 minutes and after 24 hours and GLO-1 expression, levels of MGO, D-lactate (the end product of methylglyoxal breakdown by GLO-1), and MG-H1 were determined.

The study yielded two main findings. First, GLO-1 expression was significantly downregulated in healthy males receiving LPS but was not influenced by hypoxia. Second, experimental hypoxia and inflammation did not lead to a relevant and unequivocal difference in MGO and MG-H1 concentrations over time between the conditions.

GLO-1 expression normalized during the eight-hour experiment, reflecting a transient effect of endotoxemia. The swift recovery of the glyoxalase system in healthy volunteers could explain why MGO blood concentrations were not higher in the LPS groups compared to the control group. Moreover, the endotoxemia model does not entirely resemble a full-blown septic shock state in which, as alluded to before, increased MGO concentrations were found.[35]

MGO concentrations peaked between 0 and 90 minutes (before LPS administration) in all conditions in our experiment, and this may be an effect of the prehydration with 1,5 L 2,5% glucose/0,45% saline (i.e., glucose infusion). Glucose infusion may also explain increased D-lactate as a breakdown product of MGO, as this increase was also observed in the control experiment. Indeed, previous research has demonstrated that dicarbonyl concentrations increase during an oral glucose tolerance test, even in individuals with normal glucose metabolism.[36] Infusion of fluids was required to counter the clinical symptoms (hypotension, tachycardia) of LPS infusion.

To summarize, the study showed that systemic inflammation downregulates GLO-1 in humans. Downregulation of GLO-1 is a possible mechanism leading to cell damage and multi-organ failure in sepsis. We did not observe significant differences in MGO or MG-H1 concentrations in healthy males. The results still urge further investigation of the whole dicarbonyl pathway in sepsis, because it is a plausible mechanism with potential for intervention.[37] It would be interesting to see if these healthy men's results can be extrapolated to sepsis patients.

Part V Appropriateness of antibiotic therapy in septic shock patients

Timely initiation of the appropriate antibiotic treatment is, next to resuscitation and hemodynamic stabilization, the most essential treatment goal in sepsis and septic shock. It is recommended by the guidelines to administer a broad-spectrum antibiotic as soon as possible, but at least within one hour.[38] Even though failure to start appropriate antibiotic treatment is associated with increased mortality in septic ICU patients[39,40], studies still report inappropriate treatment in 20-30% of patients.[41] Although, Surviving Sepsis Campaign (SSC) guidelines recommend starting empiric combination therapy (at least two antibiotics from different classes) in patients with high risk for multidrug resistant organisms, the level of evidence is low[38] and the effect has not been investigated in specific subgroups of septic shock at high risk of resistant microorganisms.[42] The Dutch guidelines, in contrast to SSC guidlines, recommend against double active therapy for sepsis or septic shock, however, it is stated that they do not recommend against broadening the inital empirical antibiotic spectrum using combination therapy. Even in the largest meta-analysis comparing mono- versus combination therapy in ICU patients, only 4% of included patients had an abdominal focus of infection.[43] Therefore, **Chapter 7** investigated the appropriateness of empiric antibiotic therapy and the possible benefit of combination therapy (adding a short

course of gentamicin, an aminoglycoside) in septic shock patients with an abdominal, urogenital, or unknown focus of infection.

Inappropriate empirical antibiotic therapy was prescribed in 14% (29/203) patients admitted to the ICU with septic shock with an abdominal, urogenital, or unknown focus and occurred more often in the monotherapy group when compared to the combination therapy group with adjunctive gentamicin (17% vs. 10%). In 55% of patients treated with inadequate monotherapy, gentamicin addition would have resulted in appropriate antibiotic therapy. We found no difference in ICU mortality between the monotherapy and the combination group. Although shock reversal occurred significantly more in the monotherapy group, we found no association between shock reversal and administration of gentamicin.

A previous prospective ICU trial included 648 severe sepsis or septic shock patients (245 receiving gentamicin). It did not associate short-course gentamicin therapy with faster reversal of shock or improved survival.[44] Our study extends these findings to a septic shock population at high risk for extended-spectrum beta-lactamase Gram-negative microorganisms and fungal infection, despite the higher number of appropriate therapy courses in the gentamicin group (90% vs. 83%).

Fungi were cultured in 11% of included patients. Two-thirds of the study population were surgical patients and 78% of abdominal infections were surgical ICU patients. Recent major surgery is a known risk factor for fungal infection next to total parental nutrition, immunocompromised state, and comorbidities like chronic liver and renal failure.[45,46] Strikingly, in a recent prospective matched case-control study including 192 patients with *Candida* bloodstream infection and 411 control patients, exposure to aminoglycoside treatment was associated with candidemia.[47] In our study, *Candida* infection occurred more in the monotherapy group (15%) than in the gentamicin-treated group (6%). The largest randomized controlled trial comparing empirical antifungal therapy versus no antifungals showed no benefit in ICU patients. However, the study included a low number of patients at (very) high risk of invasive candidiasis.

In conclusion, empirical monotherapy was inappropriate in almost one in five patients, and in more than half of these cases, adding gentamicin would have resulted in appropriate coverage. Thus, the clinician should still consider the possibility of inadequate therapy, even in this severely ill patient group with broad antibiotic coverage. Moreover, in 11% of all patients, a fungal infection was present. Therefore, in case of unfavorable clinical course under antibiotic monotherapy lowering the threshold for administering adjunctive aminoglycoside and antifungal therapy should be considered in patients at (very) high risk for extended-spectrum beta-lactamase Gram-negative microorganisms and fungal infection.

Part VI Sepsis and COVID-19: Inflammation and multi-organ failure due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) infection: The *MaastrICCht* COVID-19 cohort

At the start of 2020, during this Ph.D., SARS-CoV-2 caused a worldwide pandemic, stretching medical resources, especially in the ICU. Initially, COVID-19 appeared as a pulmonary disease, but now it is recognized as a multi-systemic disease[48], sharing similarities with sepsis. Both can lead to multiple organ dysfunction[49], abnormal coagulation[50], elevated bilirubin[51], hypoxia, and acute respiratory failure.[52] Although the pathophysiological mechanism of COVID-19 is not fully elucidated, it is clear that COVID-19 can indeed cause sepsis.[53]

The Maastricht Intensive Care COVID (*MaastrICCht*) cohort was started in our centre at the beginning of the pandemic in March 2020, including all mechanically ventilated COVID-19 patients. Patients were followed until the primary outcome was reached (i.e., either died in the ICU or discharged from the ICU).[54] We used the *MaastrICCht* cohort to investigate both the association between coronary artery calcification (CAC) and organ failure (as reflected by the SOFA score) **(Chapter 8)** and the development of cardiac biomarkers and the electrocardiogram **(Chapter 9)** during the disease course of mechanically ventilated COVID-19 patients. In addition, determining the association between inflammation, sepsis, and cardiovascular risk is relevant in identifying patients at high risk during the disease course and after survival, as surviving sepsis is associated with a high risk of late cardiovascular events.[55]

Chapter 8 showed in a longitudinal study, that patients in the highest tertile of CAC, had on average over time, 1.8 [0.5-3.1] points higher SOFA score when compared to those in the lowest CAC tertile (p=0.005). Thus, a higher degree of CAC was associated with worse longitudinal development of organ failure, independent of age, sex, Acute Physiology And Chronic Health Evaluation (APACHE II)-score. The association remained clinically relevant after adjustment for cardiovascular risk factors (1.3 [0.0-2.7], p=0.06) and chronic liver, lung, and kidney diseases (1.3 [0.0-2.7], p=0.085). No significant interaction between sex and the association between SOFA score over time and degree of CAC was found. The SOFA score was used in a longitudinal design. It was developed for serial data, in contrast to other disease severity scores such as the APACHE II[56] and the Simplified Acute Physiology Score (SAPS).[57]

Identifying patients at higher risk of worse outcomes during a pandemic is essential in predicting which patients will benefit from admission to the ICU. In addition, the results show that CAC may hold crucial prognostic information regarding multi-organ failure as assessed by the SOFA score. Thus, reporting CAC in all radiological chest CT reports in COVID-19 patients might benefit clinical decision support, at least for ICU patients, mainly because it comes at no additional costs.

Concerning COVID-19 patients, the prognosis of patients with underlying cardiovascular disease (CVD) but *without* myocardial injury was relatively favourable.[58]

This suggests an independent role for myocardial injury over sole pre-existent CVD. Previous studies of myocardial injury in COVID-19 were retrospective by design[59] and did not include mechanically ventilated patients.[60] These studies consistently showed that patients with signs of myocardial injury had an up to ten times higher mortality rate than patients without myocardial injury.[59,61,62] Therefore, Chapter 9 investigated serial cardiac biomarkers and ECGs over the course of the ICU admission in mechanically ventilated patients of the Intensive Care COVID Cohort (*MaastriCCht*) and compared survivors to non-survivors. The main findings were:
1) Higher high-sensitivity cardiac troponin T (hs-cTnT) at day one was associated with mortality ($p<0.001$). This association remained significant after adjusting for sex, age, APACHE II score, daily creatinine concentrations, and COVID-19 related cardiovascular risk factors.
2) The highest, lowest, and delta N-terminal pro-B-type Natriuretic Peptide (NT-proBNP were higher in non-survivors versus survivors, with serial NT-proBNP values that decreased more in survivors than non-survivors in adjusted models (p-values <0.001).
3) The incidence of ECG abnormalities in all patients, irrespective of survival, was high, especially for p-wave split (96%), QRS fragmentations (96%), flat T waves (74%), RV strain (72%), and repolarization abnormalities (61%). Most ECG abnormalities did not differ between survivors and non-survivors, except for QTc time (longer in non-survivors), R wave in lead V2 (higher in non-survivors), and presence of a broad S wave in left lateral leads (more often in non-survivors).
The findings underscore the importance of serial assessment of the cardiac biomarkers, especially hs-cTnT and NT-proBNP. Taken together, the results suggest that structural and functional cardiac alterations (i.e., elevated cardiac biomarkers and altered ECGs) are prevalent in mechanically ventilated COVID-19 patients and, when assessed over time, drive the outcome, independent of clinical characteristics, disease severity, renal function[63] and COVID-19 related risk factors such as hypertension, diabetes mellitus, and obesity.[64,65]
Although the mechanism of cardiac injury in COVID-19 is not fully elucidated, SARS-CoV-2 elicits a host response that triggers wide-ranging, immune-inflammatory, thrombotic, and parenchymal derangements.[66,67] These mechanisms may cause myocardial damage reflecting myocarditis, thromboembolism[67], endothelitis, cardiac arrhythmias, or myocardial ischemia (either type I or II). This phenomenon has previously been described in patients with acute respiratory disease admitted to the ICU due to the influenza virus.[68-70]

Our serial assessment of cardiac biomarkers and ECGs suggests that myocardial injury is highly prevalent in these patients. Therefore assessing biomarkers and ECG serially throughout the disease course is useful.

10.2 Methodological aspects

Internal validity

Observational studies might be influenced by selection bias, information bias, and confounding.[71] These factors and their potential impact need to be addressed to evaluate the results of this thesis properly.

Selection bias

Selection bias might lead to over- or underestimation of the results and we tried to minimize this across the different studies. First, the sepsis database used for the studies in **Chapters 3, 4, 5, and 7** included *all* sepsis patients admitted to the ICU. However, the study investigating the influence of a new septic shock definition (**Chapter 3**), excluded 48 patients because no serum lactate measurement was available. We do not report on the outcome of these patients and possibly these patients either died before lactate measurements could be performed or patients were not sick enough to warrant lactate measurement. Therefore, we cannot exclude over- or underestimation of the results. Furthermore, in our study comparing clinical diagnoses with autopsy findings (**Chapter 5**), only 33% of patients had an autopsy performed, which might introduce bias. However, except for the focus of infection, this group was comparable with the non-autopsy group. Furthermore, by including all performed autopsies in this specific population, bias is minimized as much as possible.
The *MaastrICCht* cohort included *all* mechanically ventilated patients with COVID-19, admitted in the ICU during the first wave of the disease and these data were studied in **Chapters 8 and 9.** However, in **Chapter 8**, only patients with an available chest CT scan were included in the study to investigate the association of coronary artery calcification and multi-organ failure. This could have led to selection bias because the patients with the most severe infection, which can be associated to cardiovascular disease, had an indication for a chest CT scan. As patients not undergoing a chest CT might therefore have less coronary calcium and lower SOFA scores, this may have underestimated the presented association between CAC and SOFA score.
For the longitudinal data analysed in **Chapter 6,** generalized estimating equations (GEE) was used. GEE has the advantage of handling missing data robustly by analysing all available data.[72] Therefore, missing data is minimized and the number of study participants is maximised in the analyses. Hence, it is less likely that selection bias influenced the results.
As all these studies were performed in a tertiary referral centre, we cannot exclude some form of referral bias, as more complex patients might have been referred to our centre. For example, **Chapter 3 and 4** pointed out that a large proportion of patients with sepsis and septic shock had an underlying (hematologic) malignancy and were possibly referred to our centre for treatment. It is clear that these patients have worse

prognosis when admitted with sepsis in the ICU and this might have influenced the results.

Information bias

Information bias may influence the results and associations in this thesis due to inaccurate measurements of determinants or confounders and misclassification. This can be minimized by repeated measures and more precise measurements.[71] The present thesis applied repeated measurements and longitudinal assessment of determinants and/or outcome variables in several studies. In **Chapter 6**, repeated measurement of MGO, GLO-1 expression, MG-H1, and D- and L-lactate was performed. Inter-assay variation for MGO was 6.0%. Furthermore, a highly standardized study protocol was used in a homogenous study population of healthy males. **Chapter 8** also has a longitudinal design with daily measurement of SOFA scores, following a predefined protocol. The SOFA score was developed for serial data and is suitable for longitudinal evaluation.[73] This repeated measurement design reduces the influence of random error in this study. CAC was assessed by two readers during consensus meetings. Both readers were experienced in cardiac imaging and blinded for the outcome of patients. Cardiac biomarkers and ECGs were assessed serially in **Chapter 9**, and the ECGs were interpreted manually which has improved accuracy over automated interpretation.[74] Only one observer manually evaluated the ECGs which might have led to systematic over- or underestimation of the results. However, this was minimized by discussing the first ten ECGs within a team of four experts and by solving uncertainties within this same expert panel. Moreover, we used a predefined protocol to systematically and objectively determine ECG abnormalities.
For the stratification of serum lactate values, only the highest serum lactate value during the first 24 hours was used in **Chapter 3**. Thus, we cannot entirely exclude measurement error and misclassification. In particular, multiple measurements increase precision. In our study investigating early death in sepsis patients **(Chapter 4)**, the expert panel of four intensivists were aware of the outcome of the patients (death). This may have led to information bias, as panel members may have searched more extensively for recognition and/or treatment flaws, knowing that all the included patients died. This may have overestimated scoring results of the different categories of possible flaws in recognition and treatment of these patients.

Confounding

Residual confounding cannot be entirely excluded in studies with an observational design and can lead to over- or underestimating the effect and even an opposite effect.[71]
In **Chapter 3**, we used a single-source population with overlapping patients, when comparing two septic shock definitions. This means that an individual patient could

meet both Sepsis-2 and Sepsis-3 criteria. Due to this fact, no formal statistical analyses could be performed to assess the significance of the different outcomes. The goal was to establish the effect of a new septic shock definition on our sepsis population. However, it would also be interesting to use septic shock as an additional variable taking all ICU patients as a starting point. The latter was beyond the scope of the paper and, as a result, no correction of confounding by a form of regression analysis could be performed.

For measuring dicarbonylstress in **Chapter 6,** a highly standardized population of healthy males was selected. Furthermore, results were adjusted for age and body-mass index to minimize any effects of these potential confounders, as these parameters may also influence the dicarbonyl pathway. Moreover, mass spectrometry produces very precise measurements with minimal error.

Confounding by indication plays a role in the study investigating the appropriateness of antibiotic therapy and added value of gentamicin in septic shock patients **(Chapter 7)**. Patients in the monotherapy group (without gentamicin) more often had shock reversal than the combination therapy group. The treating physician decided whether gentamicin was associated, and patients receiving gentamicin tended to be sicker and needed more vasopressor therapy. A binomial regression analysis was performed to correct these possible confounders, and no association of gentamicin administration and shock reversal was demonstrated.

The studies from the Maastricht Intensive Care COVID (*MaastrICCht*) cohort investigating CAC and SOFA score **(Chapter 8)** and cardiac biomarkers and ECGs **(Chapter 9)** in mechanically ventilated COVID-19 patients, addressed confounding extensively. Linear mixed-effects regression was used and models were corrected for potential confounders such as age, sex, APACHE-II score, cardiovascular risk (hypertension, dyslipidemia, obesity, smoking, and diabetes mellitus type 2), chronic lung, liver, and renal disease in **Chapter 8**. In addition, in **Chapter 9**, a model correcting for daily creatinine concentrations was added because of the influence of renal function on cardiac biomarkers.[63] By correcting for these factors, the potential influence of confounding was minimized in these studies.

External validity

The studies investigating the influence of septic shock definitions **(Chapter 3)**, early ICU mortality and autopsy findings in these patients **(Chapter 4 and 5)** and appropriateness of empirical antibiotic therapy **(Chapter 7)** are all performed in an ICU population with sepsis and/or septic shock. Therefore, these findings may not be generalizable to sepsis patients admitted in the general ward or the emergency department. Furthermore, these studies were performed in a tertiary care, 715-bed, university hospital. Our hospital is a tertiary referral centre for trauma, neuro-surgical, neurological, and

extracorporeal life support (ECLS) patients. Although our centre also functions as a peripheral centre, our patient population may not represent non-university centres.
In **Chapter 6**, we investigate the effect of hypoxia and inflammation on the dicarbonyl pathway in healthy young males to create a homogenous study population. This limits generalizability to women and older patients with comorbidities.
For the studies described in **Chapter 8** and **Chapter 9**, the prospective Maastricht Intensive Care COVID cohort (*MaastrICCht*) included intubated and mechanically ventilated patients with confirmed COVID-19 infection. Therefore, generalizability is limited regarding COVID-19 patients not in need of mechanical ventilation.

Longitudinal designs and causality

Although data regarding sepsis patients were collected prospectively in our sepsis database, the studies investigating the influence of septic shock definition and occurrence and causes of early death in **Chapters 3, 4, and 5** predominantly have a cross-sectional character.
In comparison, the study regarding inflammation and its influence on dicarbonyl stress **(Chapter 6)** has a longitudinal design with repeated measures within subjects and between groups. Longitudinal studies may add more insight regarding causality when compared to cross-sectional data (71). This is also the case for our study investigating the association between coronary artery calcification and the development of multi-organ failure in mechanically ventilated COVID-19 patients **(Chapter 8)**. In this study, daily SOFA scores were collected and assessed within subjects. In **Chapter 9**, cardiac biomarkers and ECGs are investigated over time within subjects and compared between survivors and non-survivors, allowing for many serial measurements over time.
In conclusion, the longitudinal and serial designs applied in the studies in the second half of this thesis allow for more insight into the associations over time. Although the studies in the present thesis are observational and cannot prove causality by itself, longitudinal and serial designs and extensive dealing with confounding make the associations as reliable as possible.

10.3 Clinical implications

Sepsis incidence is increasing, causes 20% of all global deaths, and mortality of patients admitted in the ICU with sepsis is still unacceptably high at 42%.[3] Therefore, research on this topic remains of the utmost importance. This thesis contributes to gaining insight into the definitions of septic shock, causes of early death, new molecular mechanisms, antibiotic treatment, and cardiac manifestations in inflammation, sepsis, and septic shock. It also confirms that sepsis is a very heterogeneous disorder

regarding diagnostic criteria, multi-organ (including cardiac) involvement, different pathophysiologic pathways and treatments. Below, some clinical implications of our studies give direction for future research.

First, although Sepsis-3 criteria for discriminating *sepsis* in the ICU are still a matter of debate[75], for *septic shock*, the Sepsis-3 definition identified a smaller and sicker population with higher APACHE II score and mortality compared to the Sepsis-2 definition in our sepsis population. Therefore, applying the Sepsis-3 criteria for septic shock not only supports the aim of the task force at identifying a population at higher risk of dying, but it can also lead to a greater consistency of epidemiological studies and clinical trials in the future.

Second, the guidelines suggest using serum lactate to guide resuscitation in sepsis and septic shock[38], and there is a more than reasonable body of evidence suggesting serum lactate use as a tool during resuscitation. However, considering intermediate serum lactate levels (between 2 and 6 mmol/L), a more delicate approach with advanced hemodynamic monitoring (dynamic variables using pulse contour analysis or echocardiography) might be indicated to guide fluid resuscitation, especially since there is growing evidence that serum lactate is a relatively poor surrogate for tissue perfusion.[76] Nevertheless, stratifying lactate levels seems to increase accuracy in diagnosing septic shock.[77]

Third, as early death (<48 hours) in sepsis patients occurs in almost one in three patients and is caused by a delay in ICU admission in 32%, this might suggest a role for further and more strict implementation of early warning systems, although evidence is still conflicting.[78,79] Furthermore, it should be acknowledged that most sepsis deaths occur in older patients with substantial frailty and comorbidity and thus might not be preventable.[80]

Fourth, careful selection and multi-disciplinary decision-making are important regarding which patients could benefit from ICU treatment. This is highlighted by both the fact that futile ICU treatment is often scored (in 29% of early deaths) and that consensus between the four intensivists scoring the items was moderate to low.

Fifth, this thesis further establishes the ever-remaining value of the autopsy as an ultimate diagnostic test in sepsis patients, as discrepancies between clinical diagnoses and postmortem findings are often identified. In addition, the number of these misdiagnoses decreases when the autopsy rate rises.[23] Therefore, these results encourage the clinician to obtain an autopsy more frequently as it facilitates the opportunity to look at the results in a clinical context and learn from each other.

Sixth, the clinician should still be aware of the possibility of inadequate antibiotic therapy in a selected population of septic shock patients with an abdominal, urogenital, or unknown focus of infection, despite broad coverage in these patients. The addition of a short course of aminoglycoside therapy in patients with inadequate monotherapy results in appropriate therapy in more than half of these cases. Moreover, fungal infections occur in 11% of patients, and therefore in case of unfavorable clinical course

under monotherapy, administering adjunctive aminoglycoside, and antifungal therapy should be considered in this patient population.
Seventh, reporting coronary calcification (CAC) in standard chest-CT scans comes at little cost. Moreover, it gives valuable prognostic information in mechanically ventilated COVID-19 patients, as this thesis shows that a higher degree of CAC is related to more severe organ failure.
Finally, serial measurements of the cardiac biomarkers hs-cTnT and NT-proBNP can guide physicians for clinical decision-making and prognostication in future COVID-19 patients. This thesis describes the strong association of hs-cTnT at admission and NT-proBNP over time with mortality. Therefore, in case of increased hs-cTnT or rising NT-proBNP, more advanced diagnostic modalities might be indicated, for instance, echocardiography, to tailor patient management further.

10.4 Future perspectives

Our sepsis database (until 2020) and the *MaastrICCht* COVID-19 cohort (up to this day) have included more patients over the years. For example, the *MaastrICCht* COVID-19 cohort now includes more than 500 mechanically ventilated COVID-19 patients with serial measurements during the whole disease course. In combination with the findings in this thesis, it creates opportunity to build and perform further observational investigations on sepsis. Below we summarize some additional research questions and hypotheses generated from this thesis:

Diagnostic

- Determining major discrepancies (Goldman class I and II) between clinical diagnoses and autopsies in a sufficiently large cohort of sepsis patients.
- Determining the value of electrocardiography, cardiac biomarkers, and echocardiography in critically ill septic patients and identifying which septic shock patients require further cardiac follow-up.

Prognostic

- Validating and comparing the results describing the influence of a change in septic shock definition in a cohort from another centre, including the effect of various cut-off values for serum lactate levels.
- Establishing the optimal cut-off point of serum lactate level or serum lactate clearance in sepsis and septic shock in terms of prognostic value.
- Upfront predicting outcome in sepsis patients by physicians in a prospective design, instead of retrospectively analyzing causes of death in deceased patients (and thus awareness of the outcome for the physician).
- Establishing whether downregulation of glyoxalase-1 in sepsis and septic shock patients leads to the accumulation of methylglyoxal and MG-H1.
- Verifying whether the degree of coronary calcium on a standard chest CT predicts adverse outcomes and cardiac events in mechanically ventilated patients with COVID-19.
- Studying whether cardiac biomarkers predict adverse outcomes (i.e., mortality) in mechanically ventilated patients with COVID-19.

Other high potential fields for future studies

Below, other potential fields of interest related to this thesis are summarized. These research questions have a more general character and relate to the heterogeneity of sepsis, biomarker research, use of artificial intelligence, and early recognition and treatment of sepsis.[81] Overall, both studies reducing heterogeneity (like the study in healthy males in Chapter 6) when investigating a certain mechanism in sepsis, as well as observational studies are needed, because in the first type of study generalizability is compromised.

Diagnostic and early intervention

- Identifying subgroups of patients, early in the disease course, based on clinical or molecular phenotypes to reduce heterogeneity.[82]
- Investigating whether biomarkers in septic shock help identify it.[81]
- Optimizing data collection and data analyzing techniques to show if data-driven models and machine learning are valuable in clinical decision-making and prediction in sepsis patients.[83]
- Studying whether screening for sepsis can lead to improved outcomes.[81]

Treatment optimization

- Identifying the best endpoints for volume resuscitation, and how to titrate volume resuscitation.[84]
- Establishing whether vasopressor use and target mean arterial blood pressure should and can be personalized, based on patient characteristics, and what agent should be used as a second vasopressor.[84,85]
- Unravelling the impact of timeliness of antibiotic treatment and determining the time threshold for administering antibiotics.[86,87]

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Clinical and societal impact

Sepsis is a life-threatening condition marked by severe organ failure. It is regarded as a worldwide health threat affecting individuals of any age and sex. In fact, it is the costliest healthcare condition, as the average hospital-wide cost of sepsis is estimated at more than US$ 32 000 per patient.[1] Sepsis is a leading cause of death in Intensive Care Units (ICU's).[2] In 2017, 49 million individuals were affected, and sepsis causes 11 million deaths worldwide.[3] Overall mortality is almost 27%. However, for patients needing ICU treatment, the in-hospital mortality reaches 42%.[3] Furthermore, the epidemiology of sepsis is not well understood and results from existing epidemiologic studies can often not be compared due to heterogeneity of methods and patients.[4] This thesis contributes to gaining more insight into sepsis epidemiology and heterogeneity regarding definition, diagnosis, causes of early death, and development of multi-organ failure and treatment.

Chapter 3 shows that the Sepsis-3 definition for septic shock defines a more severely ill group of patients with higher mortality than the Sepsis-2 definition. Furthermore, mortality rises significantly when serum lactate levels rise >6 mmol/L. These findings are in line with the purpose of the Sepsis-3 criteria: identifying patients at higher risk of dying than patients with sepsis alone.[5] Consequently, regarding clinical impact, these critically ill patients may be monitored more meticulously and receive more aggressive treatment. As for societal impact, a better discrimination of patients at high risk of dying from septic shock versus patients with an uncomplicated infection may be more cost-effective.[6] For sepsis, however, the added value of Sepsis-3 definition is limited because almost all ICU patients seem to meet these criteria.[7] For the diagnosis of sepsis, application of a SOFA score change of more than 2 points did not result in a more specific criterion, as this resulted in a higher sepsis frequency than the Systemic Inflammatory Response Syndrome criteria.[8] However, the (q)SOFA was never designed as a screening tool for sepsis, as is acknowledged by the authors in the Sepsis-3 definition paper.[9] Indeed, in the latest Surviving Sepsis Guidelines, revised in 2021, usage of the qSOFA as a single screening tool for sepsis and septic shock is not recommended.[10] Furthermore, as a clinician, we should treat the patient and their symptoms (i.e. oliguria, hypoxemia, etc.) and not wait for a score to indicate that the patient is deteriorating.

In **Chapter 4**, we show that early death (<48 hours) in sepsis is common (one in three patients), and flaws in recognition and possible futile treatment are scored in one-third of cases each. Thus, there might be a role for further implementation and optimization of early warning systems. This might have its impact on recognizing patients requiring intensive care monitoring and treatment in an earlier stage and prevent early death. Indeed, early warning systems seem to perform well for prediction of cardiac arrest

and death within 48 hours, but impact on outcome and resource utilization remain to be determined.[11] The careful selection and multi-disciplinary decision-making regarding which patients benefit from an expensive and invasive ICU treatment are relevant. Indeed, this thesis showed a large inter-observer variability between intensivists scoring possible flaws in the recognition and management of early deceased patients. Thus, there seems to be a large variability in clinical judgement regarding these patients, underlining the importance of shared decision-making.

Chapter 5 underlines the value of an autopsy in early deceased sepsis patients, as discrepancies between clinical diagnoses and postmortem findings are often identified. In addition, these findings encourage clinicians to obtain an autopsy more often, not only from a diagnostic point of view, but also from an educational perspective. Furthermore, the autopsy rate has an impact on society, regarding public health, statistics of diseases, and forensic issues.[12] Moreover, autopsy plays an important role in counseling the families of deceased patients, especially in the intensive care unit. Indeed, it can establish the exact cause of death and help to understand the reason why their relative died. Finally, autopsy can help understand the pathology and the pathogenesis of new diseases, like COVID-19, and this may influence patient management.[13]

In Chapter 6, we propose a new possible mechanism contributing to the development of multi-organ failure in inflammation and hypoxia: the detoxifying glyoxalase system that clears dicarbonyl stress. We investigated this mechanism during a highly standardized experiment in a homogeneous population of healthy young males. Although our hypothesis regarding downregulation of glyoxalase-1 (GLO-1) by inflammation was confirmed, the hypothesized increase in methylglyoxal (MGO) was not established. Although the mechanism has potential for intervention by upregulating GLO-1[14] or scavenging MGO[15], the presumed impact on septic patients remains to be determined. In general, however, investigating possible new mechanisms with a plausible pathophysiological background relating sepsis, may contribute to better patient care and improved outcomes.

Chapter 7 handles with inappropriateness of antibiotic therapy in septic shock patients with an abdominal, urogenital, or unknown focus. Adequacy of initial empirical antimicrobial treatment is crucial in terms of outcome for ICU patients with sepsis.[16] We found that 14% of patients received inappropriate therapy and 11% of patients had a fungal infection. This might impact patient management as the clinician should still take in account the possibility of inadequate therapy, even in this severely ill patient category with broad antibiotic coverage. Furthermore, the study results indicate a better antibiotic coverage but no earlier shock reversal by adding gentamicin, even in this septic shock population at high risk for extended-spectrum beta-lactamase Gram-negative bacteria and fungal infection. However, in case of unfavourable disease course under antibiotic monotherapy, lowering the threshold for administering

adjunctive aminoglycoside and antifungal therapy should be considered in this high risk patient group.

Chapter 8 and **Chapter 9** investigate the association of coronary artery calcification (CAC) and serial cardiac biomarkers and electrocardiography with more severe organ failure and survival. Patient selection, clinical decision making, and prognostication is important in mechanically ventilated patients with COVID-19 infection, as a pandemic can stretch intensive care resources. Cardiovascular disease (CVD) has an important impact on fatal outcomes in COVID-19 patients across all ages.[17] Identifying COVID-19 patients at high risk of worse outcome can tailor patient management in these patients and also increase patient awareness regarding risk factors for COVID-19.[18] In patients with known CAC or increased hs-cTnT or NT-pro-BNP, a more thorough diagnostic work-up might be justified. Indeed, transthoracic echocardiography is now performed in a minority of COVID-19 patients and patients with elevated cardiac biomarkers more often had reduced left ventricular function.[19] Patients with CAC or elevated biomarkers might represent a subgroup benefiting from echocardiography early in the disease course to tailor clinical management.

Scientific impact

Our sepsis database (until 2020) and the *MaastrICCht* COVID-19 cohort (up to this day) have included more patients over the years, and the latter database has now included over 500 mechanically ventilated COVID-19 patients with serial measurements during the whole disease course. This provides the opportunity to investigate further additional hypotheses in a more significant number of patients in the future. Thus, the present thesis has multiple implications regarding scientific impact. The sepsis database can be enriched by adding additional measures like ECG, echocardiography, and chest CT scans analyzed in a uniform matter and can be updated and function as an ongoing prospective cohort.

Chapter 3 points out that the Sepsis-3 definition for septic shock indeed identifies a more severely ill patient population with higher mortality than the Sepsis-2 definition, according to the goal of the guidelines.[9] For future research on septic shock, a clear and uniform definition of septic shock facilitates more standardized reporting on characteristics and outcomes and greater consistency of epidemiologic studies and clinical trials.

Few studies have addressed the temporal relationship between death in sepsis patients. Unfortunately, this is also true for these patients' exact causes of death because autopsy numbers are declining in the Netherlands.[20]

Chapter 4 confirms that early death is common in sepsis patients and flaws in early recognition and possible futility of treatment are often encountered. This should be considered in future research; for example, research on the effect of early warning systems report inconsistent results regarding health outcomes and resource utilization,

owing to methodological limitations.[11] Furthermore, patients admitted in moribund condition or with pending cardiac arrest might not benefit from an intervention anyway, thereby obscuring potential effectiveness of an intervention for other patients. The latter is important in sepsis research concerning highly heterogenic patients in different stages of the disease process.

The value of reporting autopsy results is confirmed in Chapter 5 and the fact that clinical causes of death in these patients are confirmed by autopsy findings improves the reliability of these scientific findings. In addition, autopsies can provide material for advancing medical research, in order to study the pathogenesis of different disease processes and their influence on different organ systems.[21] Finally, implementing contemporary approaches to autopsy (post-mortem CT scans and molecular pathology) can lead to improved quality of health care and research.

The study results in Chapter 6 investigating the effect of hypoxia and inflammation on glyoxalase-1 (GLO-1) as a possible mechanism leading to multi-organ failure urge for further investigation. On the one hand, this study confirmed the hypothesis that inflammation downregulates the protective enzyme glyoxalase-1. However, on the other hand, no significant difference between groups in methylglyoxal (MGO) and methyl-glyoxal derived hydroimidazolone-1 (MG-H1) was observed. The effects of hypoxia and inflammation on the dicarbonyl pathway are performed in a highly homogeneous population of healthy males and in a highly standardized way. The question if the results in healthy males can be extrapolated to sepsis patients remains to be answered. This would be of interest because the dicarbonyl pathway is a mechanism with potential for intervention, either by reducing protein modifications by a combination of trans-resveratrol and hesperitin or by GLO-1 induction by isothiocynates.[14]

Chapter 7 handled the appropriateness of antibiotic therapy in septic shock patients, reporting inappropriate therapy in 14% of septic shock patients. It also investigates the effect of adjunctive aminoglycoside treatment and compares monotherapy and combination therapy groups. Although inappropriate treatment is more common in the monotherapy group versus the combination group (17% vs. 10%), there was no association between aminoglycoside administration and the occurrence of shock reversal in these patients. This study extends the findings of two other studies[22,23] to an underinvestigated septic shock population at high risk for extended-spectrum beta-lactamase Gram-negative bacteria and fungal infection.

Chapter 8 and Chapter 9 investigate the association between CAC and organ failure and the value of serial assessment of cardiac biomarker and electrocardiography in mechanically ventilated COVID-19 patients. A higher degree of CAC on chest CT scans is linked to more severe organ failure development over time. Furthermore, higher hs-cTnT at admission and an increase in NT-proBNP over time were associated with lower survival. These findings are scientifically relevant and might be used for future prognostication in patients. The latter seems feasible to investigate in our *MaastrICCht*

COVID-19 cohort, as this cohort now included over 500 mechanically ventilated COVID-19 patients. Moreover, because cardiac events like myocardial infarction, also occur more often during or after non-COVID related infections[24], the value of CAC detection and serial cardiac biomarkers and ECGs might also apply to these patient populations. This is a possible future research question to investigate in a larger prospective cohort of sepsis patients.

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Summary

Summary

Sepsis, a life-threatening syndrome caused by a dysregulated response to infection, has an increasing incidence and unacceptably high mortality, despite the improved understanding of its pathophysiology. Therefore, research on this topic is essential, and this thesis studies various aspects of inflammation, sepsis, and septic shock. The overall aim was to gain more insight into the epidemiology, causes of death and outcomes of sepsis and septic shock. In addition, the COVID-19 pandemic created an opportunity to investigate cardiac manifestations in inflammatory disease.

Part I of the thesis starts with a general introduction on sepsis and septic shock in **Chapter 1**, introducing the different aspects of sepsis studied. Subsequently, **Chapter 2** discusses the aims and outline of this thesis.

In **Part II** of this thesis we discuss the influence of a change in septic shock definition on epidemiology and outcome in intensive care. **Chapter 3** describes a study that compares Sepsis-2 and Sepsis-3 definitions of septic shock in 632 included patients admitted with sepsis in the intensive care unit (ICU). The new Sepsis-3 definition of septic shock identified a smaller but more severely ill subgroup of patients with higher mortality when compared to Sepsis-2 definition. More than one-third of included sepsis patients had cancer, and this was associated with increased mortality. Stratification of serum lactate levels revealed that lactate levels below 6 mmol/L were not associated with increased ICU-mortality in our study population. Thus, the threshold of serum lactate level >2 mmol/L, as an added criterion in the Sepsis-3 definition of septic shock, might be too low to identify patients at higher risk of dying in the hospital or the ICU.

The incidence and causes of early death in sepsis patients are studied in **Part III** of the thesis. **Chapter 4** describes a study in 1107 sepsis patients, indicating that early death (within 48 hours after ICU admission) is common in septic ICU patients and occurs in almost one-third of all deaths. Primary causes of early death were multiple organ failure, mesenteric ischemia, and death after cardio-pulmonary resuscitation in the ICU. An expert panel consisting of four intensivists scored possible influencing factors by assessing the medical files of all these early deaths. A delay in ICU admission was scored in almost one-third of early deceased patients, with a slight agreement between the panel members. Furthermore, futile ICU treatment was scored in one-third of patients, with a moderate agreement between the intensivists. It indicates substantial clinical variability between physicians, strengthening the need for multi-disciplinary decision-making and careful selection regarding which patients could benefit from ICU treatment. In one-third of the early deceased patients, an autopsy was performed, and the study described in **Chapter 5** compared clinical diagnoses versus autopsy findings in these patients. The autopsy revealed 26 missed clinical diagnoses, for instance, four myocardial infarctions and four pneumonias. In 13% of patients, a diagnostic error was found that would have changed clinical management and possibly led to more

prolonged survival. The study underlines the importance of an autopsy as an ultimate diagnostic test, revealing significant discrepancies despite technical improvements nowadays.

Part IV of this thesis describes a possible new targetable mechanism in inflammation and sepsis. Chapter 6 investigated the possible effect of hypoxia and inflammation, both hallmarks of critical illness, on glyoxalase-1 (GLO-1). GLO-1 is a detoxifying enzyme that converts the highly reactive dicarbonyl methylglyoxal (MGO) into D-lactate. MGO can react with proteins due to arginine modifications forming advanced glycation endproducts (AGE's), known to be involved in the complications in multiple organs in diabetes mellitus. An example of such an AGE is methyl-glyoxal derived hydroimidazolone-1 (MG-H1) and by damaging proteins, it can lead to cell and organ dysfunction. The study investigated inflammation (due to lipopolysaccharide (LPS) administration), hypoxia, and both conditions combined on the dicarbonyl pathway in healthy young males and compared them to a control group. Expression of the protective enzyme GLO-1 was decreased by inflammation, but not hypoxia, in these males. However, experimental hypoxia and inflammation did not significantly differ in MGO concentrations over time. The results urge further investigation of this pathway, for instance, in actual sepsis patients, because it is a plausible mechanism with potential for intervention.

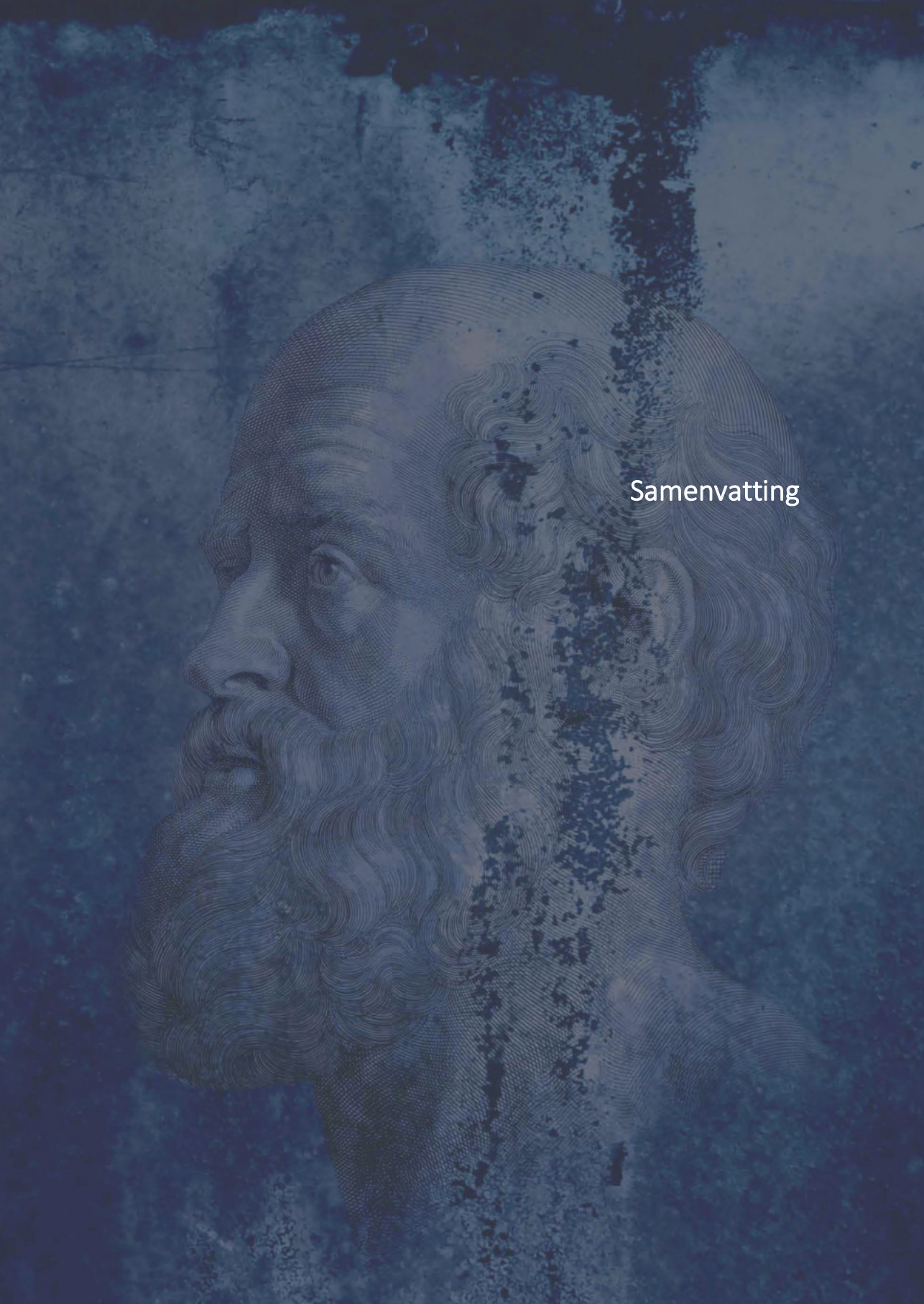
Part V of the thesis handles the appropriateness of antibiotic treatment in septic shock patients admitted to the ICU. The study described in Chapter 7 investigated the appropriateness of antibiotic treatment in 203 septic shock patients with an abdominal, urogenital, or unknown focus of infection. Inappropriate antibiotic therapy was defined as a prescription within the first 24 hours that did not cover cultured bacteria during the first 5 days of admission. This outcome was studied in the overall group, patients receiving adjunctive gentamicin (combination therapy), and patients receiving monotherapy (no adjunctive gentamicin). Mortality and shock reversal were also studied in these groups. In 14% of septic shock patients, antibiotic coverage was inappropriate, which occurred more often in patients with monotherapy when compared to combination therapy (17% vs. 10%). In 11% of patients, fungal infection was found. Shock reversal occurred significantly more often in the monotherapy group. However, binomial logistic regression analysis showed no association of gentamicin administration with shock reversal. The clinician should still consider the possibility of inadequate therapy due to extended-spectrum beta-lactamase-producing (ESBL) Gram-negative bacteria or fungi, even in this severely ill patient group with broad-spectrum antibiotic coverage. Additional aminoglycoside and/or antifungal therapy should be considered in these patients if the clinical course of these patients is unfavourable.

Part VI of this thesis uses the Maastricht Intensive Care COVID (*MaastrICCht*) cohort to investigate both the association between coronary artery calcification (CAC) and organ failure (as assessed by the Sequential Organ Failure Assessment (SOFA) score) (Chapter 8) and the development of the cardiac biomarkers and the electrocardiogram (ECG)

(Chapter 9) during the disease course in mechanically ventilated COVID-19 patients. COVID-19, first appearing as mainly a pulmonary disease, proved to be a multi-systemic disease, sharing similarities with sepsis, such as multiple organ failure, abnormal coagulation, hypoxia, and acute respiratory failure. Chapter 8 showed that patients in the highest tertile of CAC had, on average, over time, 1.8 points higher SOFA score when compared to those in the lowest tertile. The association was independent of age, sex, Acute Physiology And Chronic Health Evaluation (APACHE) II score. Therefore, reporting CAC in all radiological chest CT reports in COVID-19 patients might benefit clinical decision-making because it comes at no additional costs. Chapter 9 investigated serial cardiac biomarkers and electrocardiograms during the disease course of mechanically ventilated COVID-19 patients, comparing survivors to non-survivors. Higher high-sensitive cardiac troponin T (hs-cTnT) at admission was associated with mortality, whereas serial NT-proBNP values decreased more in survivors than in non-survivors. ECG abnormalities were common in these patients. However, no essential differences between survivors and non-survivors were found. The findings underscore the importance of serial assessment of cardiac biomarkers in these patients, as they drive outcomes independent of confounders like sex, age, and COVID-19 related cardiovascular risk factors.

Finally, Part VII contains the general discussion of this thesis in Chapter 10. Herein, I place the results of this thesis in the context of the available literature and discuss the methodological issues that need to be addressed to evaluate the results of this thesis properly. Furthermore, the clinical implications of the results of this thesis are discussed. Finally, I propose several additional research hypotheses in the future perspectives section of the general discussion.

Sepsis is a final common pathway to death for several infectious diseases and although our understanding of the pathophysiology has remarkably improved, multiple trials targeting the response to infection have not led to new treatments. This thesis contributes to gaining insight into the definitions of septic shock, the causes of early death, new possible mechanisms, appropriateness of antibiotic treatment, and cardiac manifestations in patients with inflammation, sepsis, and septic shock in the ICU. In addition, it confirms that sepsis is a heterogeneous disorder regarding diagnostic criteria, different pathophysiologic pathways, multi-organ (including cardiac) involvement, and treatments.



Samenvatting

Samenvatting

Sepsis, een levensbedreigend syndroom veroorzaakt door een ontregelde reactie op een infectie, heeft een toenemende incidentie en onaanvaardbaar hoge mortaliteit, ondanks het verbeterde inzicht in de pathofysiologie. Onderzoek naar dit onderwerp is derhalve essentieel en dit proefschrift bestudeert verschillende aspecten van inflammatie, sepsis, en septische shock. Het algemene doel van de thesis was om meer inzicht te krijgen in de epidemiologie, doodsoorzaken, nieuwe mechanismen, antibiotische behandeling, en uitkomsten van sepsis en septische shock. Bovendien ontstond door de COVID-19-pandemie een mogelijkheid om cardiale manifestaties bij inflammatie en sepsis te onderzoeken.

Deel I van het proefschrift begint met een algemene inleiding over sepsis en septische shock in **Hoofdstuk 1**, waarin de verschillende aspecten van sepsis besproken in dit proefschrift worden geïntroduceerd. Vervolgens wordt in **Hoofdstuk 2** ingegaan op de doelstellingen en hoofdlijnen van dit proefschrift.

In **Deel II** van dit proefschrift bespreken we de invloed van een nieuwe definitie van septische shock op de epidemiologie en uitkomstmaten van patiënten op de intensive care. **Hoofdstuk 3** beschrijft een studie die de Sepsis-2 en Sepsis-3 definities van septische shock vergelijkt bij 632 geïncludeerde patiënten opgenomen met sepsis op de intensive care (ICU). De nieuwe Sepsis-3 definitie van septische shock identificeerde een kleinere, maar ziekere subgroep van patiënten met een hogere sterfte in vergelijking met Sepsis-2 definitie. Meer dan een derde van de opgenomen sepsispatiënten had een maligniteit (kanker) en dit was geassocieerd met een verhoogde mortaliteit. Stratificatie van serum lactaatspiegels toonde aan dat lactaatspiegels lager dan 6 mmol/L niet geassocieerd waren met verhoogde IC-mortaliteit in onze onderzoekspopulatie. De drempel van serumlactaatniveau >2 mmol/L, als een toegevoegd criterium in de Sepsis-3-definitie van septische shock, kan dus te laag zijn om patiënten met een hoger risico op overlijden in het ziekenhuis of de ICU te identificeren.

De incidentie en oorzaken van vroege dood bij sepsispatiënten worden bestudeerd in **Deel III** van het proefschrift. **Hoofdstuk 4** beschrijft een studie onder 1107 sepsispatiënten, waaruit blijkt dat vroege dood (binnen 48 uur na IC-opname) vaak voorkomt bij septische IC-patiënten, namelijk in bijna een derde van alle overlijdens. Belangrijkste oorzaken van vroegtijdig overlijden waren multi-orgaanfalen, darmischemie en overlijden na reanimatie op de ICU. Een expertpanel bestaande uit vier intensivisten scoorde mogelijke beïnvloedende factoren bij vroeg overlijden, door de medische dossiers van deze sterfgevallen te beoordelen. Er werd bij bijna een derde deel van de vroeg overleden patiënten een vertraging in intensive care opname

gescoord, met een geringe overeenstemming tussen de panelleden. Verder werd bij een derde van de patiënten gescoord dat een IC-behandeling eigenlijk niet (meer) zinvol was, met een middelmatige overeenstemming tussen de intensivisten. De matige overeenstemming tussen de verschillende intensivisten bij het scoren van deze factoren duidt op een aanzienlijke variabiliteit in klinische beoordeling tussen artsen. Dit versterkt de behoefte aan multidisciplinaire besluitvorming en zorgvuldige selectie met betrekking tot welke patiënten baat kunnen hebben bij IC-behandeling. Bij een derde van de vroeg overleden patiënten werd een obductie (lijkschouw) uitgevoerd en de studie beschreven in Hoofdstuk 5 vergelijkt klinische diagnoses versus obductie bevindingen bij deze patiënten. Bij obductie werden er 26 gemiste klinische diagnoses gevonden, waaronder vier hartinfarcten en vier longontstekingen. Bij 13% van de patiënten werd een gemiste diagnose gevonden die de behandeling zou hebben veranderd en mogelijk zou hebben geleid tot een langere overleving van de betreffende patiënt. Deze studie onderstreept het belang van een obductie als een ultieme diagnostische test en laat zien dat er aanzienlijke discrepanties blijven bestaan tussen kliniek en obductie ondanks alle technische ontwikkelingen in de geneeskunde van tegenwoordig.

Deel IV van dit proefschrift beschrijft een potentieel nieuw mechanisme bij inflammatie en sepsis met ook aangrijpingspunten voor interventie. Hoofdstuk 6 onderzocht het mogelijke effect van hypoxie en inflammatie, beide kenmerken van kritische ziekte, op glyoxalase-1 (GLO-1). GLO-1 is een enzym dat het zeer reactieve dicarbonyl methylglyoxal (MGO) omzet in D-lactaat. MGO kan reageren met eiwitten in en buiten de cellen met als gevolg van arginine-modificaties die advanced glycation-endproducts (AGE's) vormen, waarvan bekend is dat ze betrokken zijn bij de complicaties in meerdere organen bij diabetes mellitus. Een voorbeeld van zo'n AGE is methylglyoxal afgeleid hydroimidazolone-1 (MG-H1). Deze stof kan, door eiwitten te beschadigen, leiden tot cel- en orgaandisfunctie. De studie onderzocht de invloed van inflammatie (als gevolg van toediening van lipopolysaccharide (LPS)), hypoxie, en beide condities gecombineerd op de dicarbonyl pathway bij gezonde jonge mannen en vergeleek ze met een controlegroep zonder blootstelling aan LPS of hypoxie. Genexpressie van het beschermende enzym GLO-1 werd duidelijk verminderd door inflammatie, maar niet door hypoxie, bij deze mannen. MGO-concentraties verschilden echter niet significant tussen de verschillende condities (inflammatie, hypoxie en beiden gecombineerd) en de controlegroep over de tijd. Verder onderzoek van deze potentiele pathway zou interessant zijn, bijvoorbeeld bij daadwerkelijke sepsispatiënten, omdat het een biologisch plausibel mechanisme is met potentieel voor interventie.

Deel V van het proefschrift behandelt de vraag hoe adequaat de antibioticabehandeling is bij patiënten met septische shock die op de IC worden opgenomen. De studie beschreven in Hoofdstuk 7 onderzocht de adequaatheid van

antibiotica bij 203 septische shockpatiënten met een abdominaal, urogenitaal, of onbekend focus van infectie. Inadequate antibiotische therapie werd gedefinieerd als een middel toegediend binnen de eerste 24 uur dat geen adequate dekking gaf voor gekweekte bacteriën tijdens de eerste 5 dagen van opname. Deze uitkomst werd onderzocht in de totale groep, maar ook bij de patiënten die aanvullend gentamicine (combinatietherapie) kregen en patiënten die monotherapie kregen (geen gentamicine). Verder werden sterfte en shock reversibiliteit bestudeerd in deze groepen. Bij 14% van de septische shock patiënten was de initiële antibiotische dekking niet adequaat. Dit kwam vaker voor bij patiënten met monotherapie in vergelijking met combinatietherapie (17% versus 10%). Bij 11% van de patiënten werd een schimmelinfectie gevonden. Shock reversibiliteit kwam significant vaker voor in de groep die monotherapie kreeg. Binomiale logistische regressieanalyse toonde echter geen associatie van gentamicine toediening met shock reversibiliteit. De clinicus dient dus nog steeds de mogelijkheid van ontoereikende therapie te overwegen als gevolg van extended-spectrum bèta-lactamase-producerende (ESBL) Gram-negatieve bacteriën of schimmels, zelfs in deze ernstig zieke patiëntengroep met breedspectrum antibiotische dekking. Aanvullende aminoglycoside en/of antifungale therapie zouden bij deze patiënten overwogen kunnen worden bij een ongunstig klinische beloop.

Deel VI van dit proefschrift gebruikt het Maastricht Intensive Care COVID (*MaastrICCht*) cohort om de associatie tussen kransslagaderverkalking (coronary artery calcification, CAC) op een computed tomografie (CT) scan en orgaanfalen (bepaald door middel van de Sequential Organ Failure Assessment (SOFA) score) te onderzoeken (Hoofdstuk 8). Tevens wordt het beloop van de cardiale biomarkers en het elektrocardiogram (ECG) (Hoofdstuk 9) tijdens het gehele ziekteverloop bij beademde COVID-19 patiënten onderzocht. COVID-19 presenteerde zich initieel als een longaandoening maar bleek later veel meer een multi-systeemziekte te zijn, die veel overeenkomsten heeft met sepsis, zoals multi-orgaanfalen, abnormale stolling, hypoxie, en acute respiratoire insufficiëntie. Hoofdstuk 8 toonde aan dat patiënten in het hoogste tertiel van kransslagader verkalking gemiddeld, na verloop van tijd, een 1.8 punten hogere SOFA-score hadden in vergelijking met die in het laagste tertiel. Deze associatie was onafhankelijk van leeftijd, geslacht, en Acute Physiology and Chronic Health Evaluation (APACHE) II-score. Het standaard beschrijven van de mate van kransslagader verkalking in alle radiologische CT-verslagen bij COVID-19-patiënten kan de klinische besluitvorming ten goede komen, temeer ook omdat het geen extra kosten met zich meebrengt. Hoofdstuk 9 onderzocht het seriële beloop van de cardiale biomarkers en elektrocardiogrammen tijdens het ziekteverloop van beademde COVID-19-patiënten, waarbij overlevenden met niet-overlevenden werd vergeleken. Verhoogde high sensitive cardiac troponin T (hs-cTnT) bij opname was geassocieerd met een hogere kans op sterfte, terwijl NT-proBNP-waarden meer afnamen over de tijd bij overlevenden dan bij niet-overlevenden. ECG-afwijkingen kwamen vaak voor bij deze

patiënten. Er werden echter geen essentiële verschillen tussen overlevenden en niet-overlevenden gevonden. De bevindingen tonen het belang aan van seriële bepaling van de cardiale biomarkers bij deze patiënten, omdat deze gerelateerd zijn aan uitkomsten van patiënten, onafhankelijk van geslacht, leeftijd, en COVID-19-gerelateerde cardiovasculaire risicofactoren.

Ten slotte bevat Deel VII de algemene bespreking van dit proefschrift en de belangrijkste bevindingen worden besproken in Hoofdstuk 10. Hierin plaats ik de resultaten van dit proefschrift in de context van de beschikbare literatuur en bespreek ik de belangrijkste methodologische kwesties om de resultaten van dit proefschrift goed te evalueren. Verder worden de klinische implicaties van de resultaten van dit proefschrift besproken. Tot slot stel ik een aantal aanvullende onderzoekshypothesen voor de toekomst voor.

Concluderend is sepsis een levensbedreigend syndroom met hoge sterfte, veroorzaakt door een reactie van het lichaam op verschillende infectieziekten. Hoewel ons begrip van de pathofysiologie van sepsis de laatste jaren aanmerkelijk is verbeterd, hebben meerdere onderzoeken gericht op de respons op infectie niet geleid tot werkzame nieuwe behandelingen. Dit proefschrift draagt bij aan het verkrijgen van inzicht in de definities van septische shock, de oorzaken van vroege dood, nieuwe mogelijke onderliggende mechanismen, adequaatheid van antibiotische behandeling, en cardiale manifestaties bij patiënten met ontsteking, sepsis, en septische shock op de IC. Bovendien bevestigt dit proefschrift het beeld dat sepsis een heterogene aandoening is met betrekking tot diagnostische criteria, verschillende pathofysiologische routes, multi-orgaan (inclusief cardiale) betrokkenheid en behandelingen.

Dankwoord

Dankwoord

Een proefschrift schrijven, zeker na je specialisatie, doe je niet alleen. Zonder samenwerking en begeleiding was het niet mogelijk geweest dit traject te voltooien. Ik wil dan ook graag een aantal mensen in het bijzonder bedanken.

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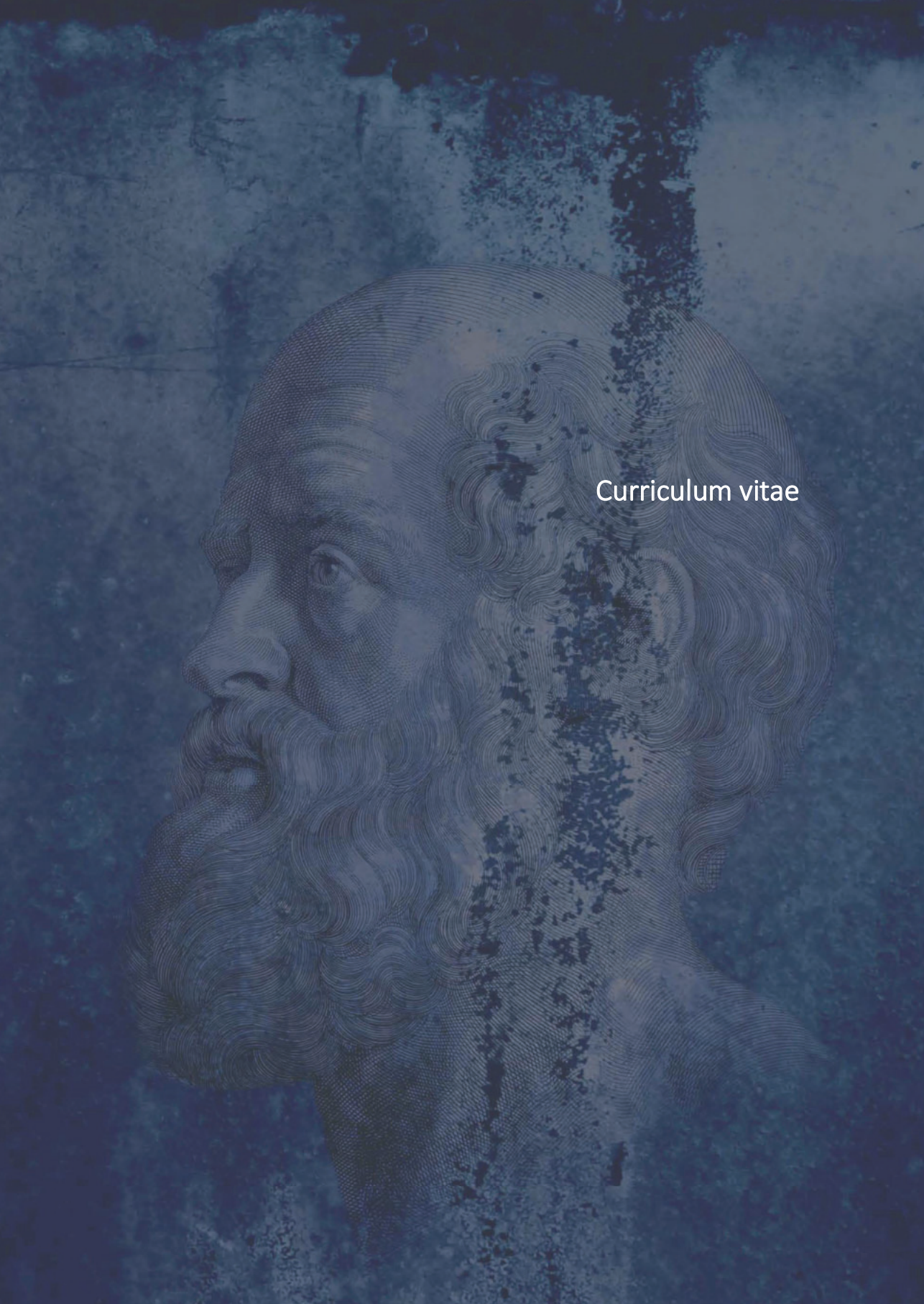
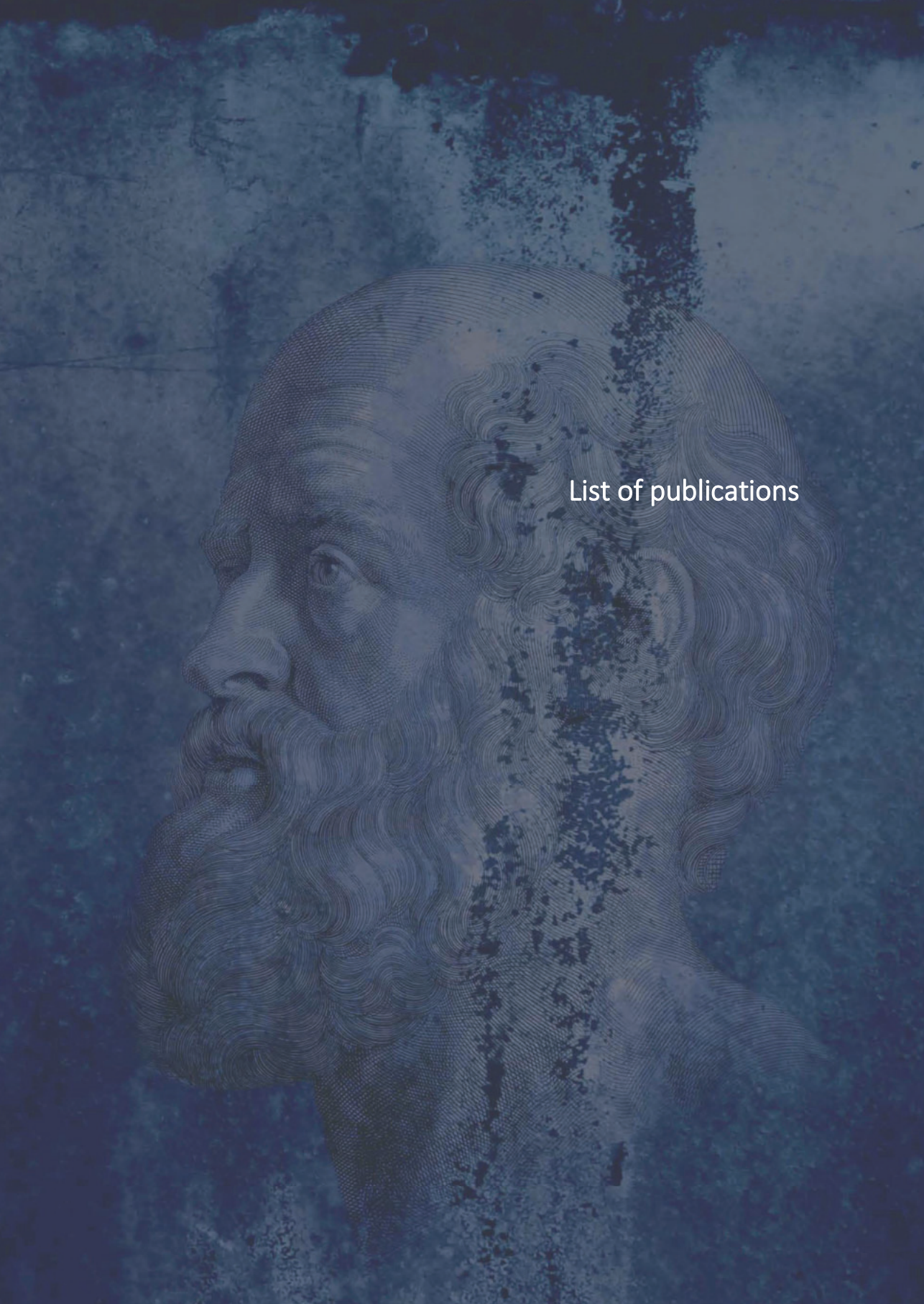


Curriculum vitae

Curriculum vitae

Rob Driessen, full name Rob Godefridus Hubertus Driessen, was born on December 22th, 1980, in Elsloo (a village 16 km of Maastricht), the Netherlands. After primary school, he attended secondary school at the S.G. Groenewald, Stein, the Netherlands. He graduated in 1999 after which he studied Biomedical Sciences from 1999 to 2000 at Maastricht University, Maastricht, the Netherlands. In September 2000, he started to study Medicine at the Faculty of Health, Medicine, and Life Sciences of the Maastricht University. In 2006, he received his medical degree after which he started as a cardiology resident not in training in the Maastricht University Medical Centre+ (MUMC+). In September 2007, he was allowed to start his training in cardiology under supervision of prof. dr. H.J.G.M. Crijns and dr. E.C. Cheriex., which led to his registration as a cardiologist in September 2013. He started a fellowship in intensive care medicine in the same year, under supervision of Dr. D.C.J.J. Bergmans and prof. dr. W.N.K.A. van Mook. In March 2016, he was registered as a cardiologist-intensivist and he started working in the MUMC+ at the department of Intensive Care (led by prof. dr. P.M.H.J. Roekaerts and since 2019 by prof. dr. J.C.C. van der Horst) and the department of cardiology (led by prof. dr. H.J.G.M. Crijns and since 2021 by prof. dr. K. Vernooy). The research trajectory that led to this thesis was started in 2017 and completed in 2022 under supervision of Prof. dr. J.C.C. van der Horst, dr. D.C.J.J. Bergmans and dr. R.M. Schnabel.



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Oral presentations and abstracts

8th Annual International Euregio intensive care symposium, Lanaken Belgium, 2016, oral poster presentation
The influence of a change in septic shock definitions on intensive care epidemiology and outcome: comparison of sepsis-2 and sepsis-3 definitions
Rob G.H. Driessen, van de Poll MCG, Mol MF, van Mook WNKA, Schnabel RM

37th International Symposium on Intensive Care and Emergency Medicine
Brussels, Belgium, 2017, oral poster presentation
P93 Intra operator variability in visualization of microcirculation in ICU patients using sidestream dark field imaging
M. Bol, M. Suverein, T. Delnoij, **Rob G.H. Driessen**, S. Heines, T. Delhaas, M. Vd Poll, J. Sels
MUMC, Netherlands

31th (European Society of Intensive Care Medicine (ESICM) Paris, 2018, oral poster presentation
Human endotoxemia and hypoxia increase dicarbonyl stress in healthy men
Rob G.H. Driessen, Dennis CJJ Bergmans, Dorien Kiers, Paul Roekaerts, Marcel CG van de Poll, Casper G Schalkwijk, Peter Pickkers, Matthijs Kox, Bas CT van Bussel

NVIC dagen, Eindhoven, the Netherlands, 2020, oral poster presentation
Endotoxemie en hypoxie verhogen methylglyoxal stress in gezonde mannen.
Rob G.H. Driessen, Dennis CJJ Bergmans, Casper CG Schalkwijk, Iwan CC van der Horst, Dorien Kiers, Marcel CG van de Poll, Peter Pickkers, Matthijs Kox, Bas CT van Bussel[1]

NVVC dagen, Arnhem, the Netherlands, 2022, oral poster presentation
Serial measurements of cardiac biomarkers and electrocardiography in mechanically ventilated COVID-19 patients: the prospective Maastricht Intensive Care cohort.
Rob G.H. Driessen, Mohammed A. Ghossein, Frank van Rosmalen, Jan-Willem E.M. Sels, Thijs Delnoij, Zafer Geyik, Alma M.A. Mingels, Antonius M.W. van Stipdonk, Frits W. Prinzen, Chahinda Ghossein-Doha, Sander M.J. van Kuijk, Iwan C.C. van der Horst, Kevin Vernooy, Bas C.T. van Bussel.

Chairs

Co-Chairman 7th Annual international Euregio Intensive care symposium: "Challenges in Clinical Nutrition and Intestinal Failure". Maastricht, Netherlands, September 2016.