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ASPECTS OF RISK FACTORS, PATHOPHYSIOLOGY AND OUTCOMES IN TRAUMA

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ASPECTS OF RISK FACTORS, PATHOPHYSIOLOGY AND OUTCOMES IN TRAUMA
THESIS FOR DOCTORAL DEGREE (Ph.D.)

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To my family.

POPULAR SCIENCE SUMMARY

Trauma and injuries are a global health concern. Approximately five million deaths each year, almost 10% of global mortality, are due to injury. In Sweden approximately 5% of deaths each year are due to external causes making this the fifth most common cause of death. Many trauma patients die immediately or in the first hours after trauma. Those who survive the acute phase of trauma are typically treated in our hospital wards and intensive care units. These patients are at risk for severe, potentially lethal, complications such as sepsis and organ failure. This thesis aims to describe patterns of complications after trauma and to identify factors influencing these outcomes.

Study I examined if patients medicating with β-blockers, commonly used in patients with heart disease or hypertension, before trauma had a protective effect of this treatment when exposed to trauma. Patients using β-blockers at the time of trauma were older and had more pre-existing medical conditions than those who did not. We could not show that the use of β-blockers was associated with an increased survival.

Study II examined if thioredoxin (TRX), a bodily molecule that protects the body from stress and damage from oxidation, could predict future development of sepsis after severe trauma. The study results showed that TRX was elevated after trauma, associated with injury severity and blood transfusions. The results also showed that elevated TRX was associated with sepsis development.

Study III was a comparison between different definitions of sepsis performed in severely injured patients. In 2016, a change from the previous definition, sepsis-2 to the current, sepsis-3 was implemented. We showed that using the new sepsis-3 definition resulted in that fewer patients were diagnosed with sepsis as compared to when using the sepsis-2 definition. The sepsis-3, but not the sepsis-2 definition, was associated with death from day 2 after admittance to the intensive care unit. We found that the new sepsis definition was feasible and more accurately predicted mortality than the previous definition in trauma victims.

Study IV aimed to identify risk factors for development of sepsis after trauma. The results showed that patients who received blood transfusions, were older, had injuries to their spine or chest or presented with shock on arrival had a higher risk of developing sepsis. There was also an increased risk of sepsis in patients with positive blood alcohol on admittance. Patients with sepsis after trauma had a complicated course in the intensive care unit and required more circulatory and respiratory support. There was also an increased risk of death in septic patients, but only after excluding patients who died in the early phase due to trauma-related injuries.

Study V identified five different patient groups with different patterns of organ dysfunction after trauma. Each group had different characteristics at admission and showed very diverse outcomes. Further, some groups were possible to identify early after trauma and some patterns might be possible to modify.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Trauma är ett enormt globalt hälsoproblem. Ungefär fem miljoner individer dör varje år på grund av trauma och skador. Det utgör nästan 10% av total global dödlighet. I Sverige är yttre orsaker till död den femte vanligaste dödsorsaken. De flesta traumapatienter som dör, dör direkt efter trauma. De patienter som överlever den akuta fasen behöver ofta vårdas på våra intensivvårdsavdelningar. Dessa patienter löper risk för komplikationer som till exempel blodförgiftningar och organsvikt. Syftet med denna avhandling var att identifiera risk- och skyddsfaktorer för komplikationer såsom blodförgiftning, och dödlighet efter trauma.

Studie I undersökte om patienter som använder β-blockerare, ett läkemedel som vanligtvis används för patienter med hjärtsjukdom eller högt blodtryck, var skyddande efter trauma. Studieresultaten visade att de patienter som använde β-blockerare vid traumatillfället hade fler sjukdomar före traumat än de som inte använde β-blockerare. Vi kunde dock inte visa att användningen av β-blockerare var associerad med en ökad överlevnad.
Studie II undersökte om thioredoxin (TRX), en molekyl som skyddar kroppen från stress och oxiderande skador, kunde förutsäga senare utveckling av sepsis efter trauma. Studieresultaten visade att TRX var förhöjt efter trauma, samt högre hos de patienter som hade mer allvarliga skador och hos de som behövde stora mängder blodtransfusioner. Resultaten visade också att TRX var kopplat till senare utveckling av sepsis.
Studie III var en jämförelse mellan olika definitioner av sepsis utförd hos svårt skadade patienter. Under 2016 genomfördes en övergång från den gamla definitionen, sepsis-2, till den nuvarande, sepsis-3. Vi visade att användning av den nya sepsis-3-definitionen resulterade i att mindre än hälften av patienterna diagnostiserades med sepsis jämfört med att använda den gamla sepsis-2-definitionen. Inget samband mellan någon av definitionerna och dödlighet sågs, förmodligen förklarat av att många patienter dog mycket tidigt på grund av sina svåra skador innan de kunde utveckla sepsis. Däremot sågs ett samband mellan död och den nya sepsis-3 definitionen när de tidiga dödsfallen uteslutits. Det sambandet sågs inte för sepsis-2 definitionen.
Studie IV syftade till att identifiera riskfaktorer för utveckling av sepsis efter trauma. Vi fann att de patienter som fick fler blodtransfusioner, var äldre, hade skador på ryggraden eller bröstkorgen eller inkom med chock till sjukhuset hade en högre risk att utveckla sepsis. Det sågs också en ökad risk för sepsis hos de patienter som hade alkohol i blodet vid ankomst. Patienter med sepsis efter trauma hade ett mer komplicerat förlopp på intensivvårdsavdelningen och krävde mer cirkulations- och andningsstöd. Det fanns också en ökad risk för död hos septiska patienter, men endast efter att de patienter som dog mycket tidigt uteslutits.
Studie V identifierade fem olika grupper med olika mönster av organsvikt efter trauma. Grupperna hade olika ålder och skador vid inkomst till sjukhus och utvecklade olika grader av organsvikt. Vidare är vissa grupper möjliga att identifiera tidigt efter trauma och vissa gruppers förlopp kan vara möjligt att påverka.

ABSTRACT

Trauma is a global health concern. Many trauma patients succumb on the scene or in the immediate phase after trauma. Patients surviving the initial phase may die at a later stage or suffer debilitating consequences in the post-resuscitation phase of trauma care in intensive care units. This thesis is focused on factors associated with outcomes and complications after trauma, as well as early recognition of these complications.

Trauma patients using β-adrenergic receptor antagonists (β-blockers) at the time of injury had more comorbidities and an increased mortality compared to non-users. However, when adjusting for relevant confounders no association between pre-traumatic β-blockade and mortality survival was seen. Previous research suggesting a protective effect of β-blockers in trauma could therefore not be supported.

We investigated thioredoxin (TRX), a potent endogenous antioxidant, and its associations with post-injury sepsis. TRX was elevated after an inflicted femur fracture and subsequent hemorrhage in an animal trauma model. Plasma-levels of thioredoxin was also evaluated in 83 severely injured trauma patients and were significantly higher when compared to healthy controls. This biomarker was associated with injury severity, shock on arrival and massive transfusion. Further, an association between TRX and post-injury sepsis was shown after adjustments for confounders.

The new sepsis definition, sepsis-3, was evaluated and compared with the previous definition, sepsis-2, in 722 severely injured trauma patients. Fewer patients were diagnosed with sepsis when using the new sepsis-3 definition as compared with the old sepsis-2 definition. No association was seen between sepsis, regardless of definition used and overall mortality. However, after censoring patients dying on the first day, before being at risk for sepsis, sepsis-3 was associated with 30-day mortality, whereas sepsis-2 was not. The new definition was feasible and had a stronger association with mortality.

Risk factors for post-injury sepsis as defined by the new sepsis-3 criteria included: age, spine- and chest-injuries, shock on arrival and blood transfusion. Moreover, there was an association between blood alcohol at admission and later development of sepsis previously not described. Patients who developed post-injury sepsis had a complicated clinical course with an increased need for vasopressor treatment, mechanical ventilation and had more days with organ dysfunction. A significant association between post-injury sepsis and mortality was shown, but only after early censoring for trauma-related deaths.

Using a technique for longitudinal clustering, we identified five distinct trajectories of organ dysfunction after trauma. Each one with different baseline characteristics, evolution of organ dysfunction and outcomes. These trajectories had unequal times until stabilization, indicating that some trajectories are easier to identify in an early stage. The study underlines the heterogenous course after trauma and suggests that there exist subsets of traumatically injured patients that might benefit from targeted measures.

LIST OF SCIENTIFIC PAPERS

This thesis is based on the following papers, which will be referred to by their Roman numerals as indicated below:

I. **Effect of preadmission beta blockade on mortality in multiple trauma**
Eriksson M, von Oelreich E, Brattström O, Eriksson J, Larsson E, Oldner A
British journal of surgery Open, 2018, 2, 392-399

II. **Thioredoxin a novel biomarker of post-injury sepsis**
Eriksson J, Gidlöf A, Eriksson M, Larsson E, Brattström O, Oldner A
Free Radical Biology and Medicine, 2017, 104, 138-143

III. **Comparison of the sepsis-2 and sepsis-3 definitions in severely injured trauma patients**
Eriksson J, Eriksson M, Brattström O, Hellgren E, Friman O, Gidlöf A, Larsson E, Oldner A
Journal of Critical Care, 2019, 54, 125-129

IV. **Postinjury sepsis - Associations with risk factors, impact on clinical course, and mortality: A retrospective observational study**
Eriksson J, Lindström A-C, Hellgren E, Friman O, Larsson E, Eriksson M, Oldner A
Critical Care Explorations, 2021, v3(8), e0495

V. **Temporal patterns of organ dysfunction after severe trauma**
Eriksson J, Nelson D, Holst A, Hellgren E, Friman O, Oldner A
Critical Care, 2021, 25, 165

CONTENTS

LIST OF ABBREVIATIONS

AIS	Abbreviated injury scale
AKI	Acute kidney injury
ARDS	Acute respiratory distress syndrome
AUC	Area under the curve
CRP	C-reactive protein
CARS	Compensatory anti-inflammatory response syndrome
CI	Confidence interval
DAMP	Damage-associated molecular patterns
DALY	Disability-adjusted life years
GCS	Glasgow coma scale
GBTM	Group-based trajectory modeling
HDU	High dependency unit
ICU	Intensive care unit
IQR	Inter quartile range
ISF	International sepsis forum classification
ISS	Injury severity score
LISA	The Longitudinal integration database for health insurance and labour market studies
LOS	Length of stay
MAP	Mean arterial pressure
MOF	Multiple organ failure
NBHW	National board of health and welfare
NISS	New injury severity score
OR	Odds ratio
OD	Organ dysfunction
PRBC	Packed red blood cell units
PAMP	Pathogen-associated molecular patterns
PPGM	Posterior probability of group membership
PCT	Procalcitonin
RCT	Randomized controlled trial

ROC	Receiver operating characteristics
SOFA	Sequential organ failure assessment
SIRS	Systemic inflammatory response syndrome
SAP	Systolic arterial pressure
LISA	The longitudinal integration database for health insurance and labor markets
TRX	Thioredoxin
TBI	Traumatic brain injury
WHO	World health organization

1 INTRODUCTION

The introduction of designated trauma centers providing standardized trauma resuscitation has resulted in improved outcomes after trauma.[1] Patients who earlier would succumb to their injuries during the prehospital phase or during initial resuscitation are now surviving to a greater extent. This advance in trauma care and resuscitation results in new challenges as more patients with severe injuries survive long enough to be admitted into the ICU admission. At this stage these patients are at high risk of severe complications and latent death. To further improve survival after trauma, knowledge of both risk- and protective factors for morbidity and mortality is important. Common complications after trauma needs to be identified as early as possible and patients with high risk of complications may benefit from close monitoring and vigilant care.

This thesis evaluates different aspects of the traumatically injured patient and the subsequent care of trauma patients admitted to the ICU. The overall aim of the thesis was to examine factors influencing morbidity and mortality after trauma, with a special focus on post-injury sepsis. In study I, we examined if treatment with β-blockade before the time of trauma was protective. Study II evaluated whether plasma TRX, a potent endogenous antioxidant, was a potential biomarker of post-injury sepsis development. Study III was performed to evaluate the new sequential organ failure assessment (SOFA) based sepsis-3 criteria in trauma patients with high SOFA scores already on admittance. Study IV analyzed risk factors for post-injury sepsis. In study V, we examined organ dysfunction in the first two weeks after trauma and clustered patients according to their organ dysfunction patterns.

2 BACKGROUND

2.1 EPIDEMIOLOGY

Despite many improvements in injury prevention and trauma care, injuries and trauma are still the leading cause of death worldwide. Approximately five million individuals die because of injury each year making up almost 10% of global mortality. In Sweden roughly 5% of all deaths each year are due to external causes making this the fifth most common cause of death in Sweden.[2, 3] Road traffic accidents and intentional injuries account for approximately half of the documented trauma mechanisms.[2] Men are more affected than women, in fact twice as many men succumb to injury each year.[2] The consequences and sequelae of trauma harm and disable many more. Since most trauma victims are young, the burden of trauma on families and society is considerable. When estimating the global burden of diseases using disability-adjusted-life-years (DALYs), the sum of years lost due to premature mortality and years lived with disability, injuries are second only to cardiovascular diseases. The impact of injuries on morbidity are even more pronounced in the younger patients, for individuals between 10-49 years of age, road traffic injuries alone cause most of DALYs. [4]

2.1.1 Abbreviated Injury Scale

The Abbreviated Injury Scale (AIS) is used to describe the anatomical location and severity of injuries. First published in 1971, it uses a seven-digit number to specify body region, specific structure, type and severity of injury.[5] The AIS scale is a measurement for single injuries and is continuously monitored and updated by the Association for the Advancement of Automotive Medicine. It has become the golden standard for injury data collection. Further, it serves as a foundation for several other scoring systems currently in use.

2.1.2 Injury Severity Score, New injury Severity Score

The Injury Severity Score (ISS) was developed in the seventies by Baker and collegues[6]. It was developed to address the problem of grading patients with multiple injuries. It remains one of the most widely used scores for summarizing injury severity and quantifying the total trauma load. ISS is simply calculated by taking the sum of squares of the highest AIS score of the three most severely injured body regions. The body is divided into six regions; head and neck, face, chest, abdomen and pelvic contents, extremities and pelvic skeleton, and external. The maximum score is 75, where ISS above 15 is traditionally defined as severe injury. If any AIS body region score is a 6, the ISS is automatically set to 75. It does not consider multiple injuries to the same body region, hence the development of alternative scoring systems such as the New Injury Severity Score (NISS), calculated as the sum of squares of the three highest AIS scores regardless of body region.[7]

2.1.3 Sequential Organ Failure Assessment score

Initially designed for use in septic patients, this scoring system was originally named the Sepsis Related Organ Failure Assessment.[8] Since the score is not specific to sepsis it was later renamed as Sequential Organ Failure Assessment Score (SOFA). It is one of the most widely used scoring systems for quantifying organ dysfunction. It consists of six organ domains: neurological, respiratory, cardiovascular, renal, coagulation and liver. Each domain is given zero to four points depending on the degree of organ dysfunction, resulting in a maximum score of 24 (Table 1). It has been validated in several studies and settings and is generally accepted to have a good ability to predict outcomes including mortality in general ICU patients as well as in trauma patients in the ICU.[9-11] It is used as a key criterion in the current diagnosis of sepsis.

Table 1. SOFA score

Score	**0**	**1**	**2**	**3**	**4**
Respiration, *PaO_2/FIO_2, kPa*	>53.3	<53.3	<40	<26.7 with respiratory support	<13.3 with respiratory support
Coagulation *Platelets, $x10^3$ μL^{-1}*	≥150	<150	<100	<50	<20
Renal *Creatinine, $\mu mol\ L^{-1}$*	<110	110-170	171-299	300-440	>440
Liver *Bilirubin, $\mu mol\ L^{-1}$*	<20	20-32	33-101	102-204	>204
Cardiovascular	MAP ≥70 mmHg	MAP <70 mmHg	Dopamine <5 or Dobutamine (any dose)	Dopamine 5.1-15 or Epinephrine ≤0.1 or Norepinephrine ≤0.1[a]	Dopamine >15 or Epinephrine >0.1 or Norepinephrine >0.1[a]
Central nervous system *Glasgow Coma Scale, score*	15	13-14	10-12	6-9	< 6

FIO_2, fraction of inspired oxygen; MAP, mean arterial pressure; PaO_2, partial pressure of oxygen;
[a]Catecholamine doses are given as $\mu g\ kg^{-1}\ min^{-1}$ for at least 1 hour

2.2 TRAUMA, PHYSIOLOGICAL CONSEQUENCES AND CHANGES

Trauma patients are known to be prone to develop infections.[12] Not only due to breeches in body barriers, hypothermia and hypoperfusion but also due to functional immunosuppression.[13, 14]

Signature molecules on invading microorganisms are able to activate the innate immune system. These exogenous molecules, or pathogen-associated molecular patterns (PAMPs), are recognized by the innate immune system via different pattern recognition receptors. This recognition initiates several immune responses via different cytokines, interferons, and chemokines. An example is lipopolysaccharide activating toll-like receptor 4, leading to inflammatory cytokine production as well as activation of intracellular signaling pathways.[15] Damage-associated molecular patterns (DAMPs), such as mitochondrial DNA, nuclear DNA or heat shock proteins are released from injured cells after trauma. DAMPs can initiate an immune response similar to the response initiated by PAMPs. DAMPs can be actively secreted from injured cells or released from dead cells as debris. Not surprisingly, DAMPs released after injury can elicit an immune response with systemic inflammation much like sepsis.[16] After trauma, the release of DAMPs is also believed to contribute to the immunosuppressed state seen in post-traumatic patients.

The initial systemic hyperinflammation is associated with a long-lasting compensatory anti-inflammatory response syndrome (CARS), resulting in the immunosuppression often seen after trauma. CARS is viewed as a homeostatic phenomenon, aiming to mitigate the effects and potential organ injury caused by hyperinflammation. When persisting, or too excessive it makes the patient vulnerable to secondary infections such as post-injury sepsis. Lately it has been suggested that the hyper- and the anti-inflammatory processes occur simultaneously.[17] A study from the Netherlands could show that trauma patients exhibit this state of immunosuppression, characterized by an anti-inflammatory cytokine pattern as well as low expression of genes linked to a competent immune system. DAMPs were heavily increased directly after trauma and significantly associated with the subsequent extent of immunosuppression.[18] It is further shown that increases in DAMPs are associated with multiple organ failure and mortality.[19]

Microcirculatory changes after trauma are also seen in the period following injury. The catecholamine surge seen after trauma is proposed to cause damage to endothelial structures.[20] Hemorrhage and hypovolemia results in swelling of the endothelial wall.[21] Inflammatory cytokines cause endothelial activation, including upregulation of inducible nitric oxide synthase, which is partly responsible for the vascular hypo-responsiveness seen after trauma.[22] This activated endothelium entails adhesion of leukocytes, that diapedese through the capillary wall. Leukocytes are stimulated via inflammatory cytokines from ischemic or injured cells and these activated leukocytes in turn further promote the swelling and cellular dysfunction of the endothelium. This phenomenon is believed to affect substrate supply to tissues, decreasing oxygen delivery and increasing arterio-venous shunting through affected areas.[23-25]

2.3 COMPLICATIONS IN THE POST-RESUSCITATION PHASE

Mortality after trauma was classically described by Baker and Trunkey as trimodal.[26] They described immediate deaths at the scene and early deaths in the initial hours after trauma, both most commonly due to severe central nervous system injuries or exsanguination. Further, late deaths within days to weeks were usually from sepsis or sepsis-induced multiple organ failure. This trimodal distribution of trauma deaths has, however, not been reproduced in more recent studies. Resuscitation strategies, damage control surgery and improved trauma care has resulted in that more severely injured patients survive through the early phase of trauma (Figure 1). Contemporary studies show more of a heterogenous or bimodal pattern of death after trauma.[27-29] Traumatic brain injury (TBI) and hemorrhage accounts for most of the mortality after trauma. These victims generally die early, patients succumbing from hemorrhage generally within the first hours and patients succumbing from TBI within the first days. In the post-resuscitation phase, sepsis and multiple organ failure (MOF) are accountable for the majority of deaths.[30, 31]

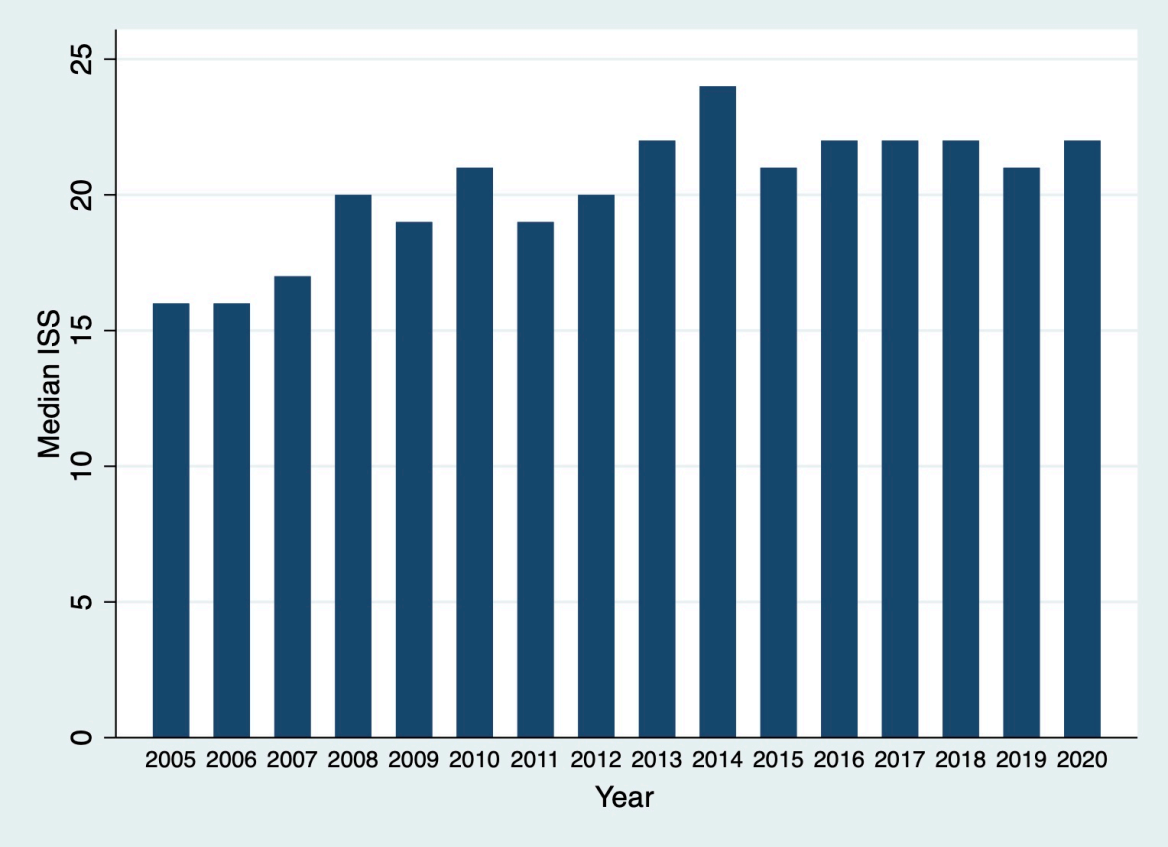

Figure 1. Median Injury Severity Score per year for patients admitted to the ICU at Karolinska University Hospital.

Mortality after multiple trauma has decreased in the latest decades, but there has not been a similar decrease in mortality for the subgroup of patients developing sepsis after trauma.[12] Post-injury complications entail both economic and human costs. Ingraham *et al.* estimated the attributable mortality for different complications in a matched case control study. Cardiovascular events, acute kidney injury (AKI), acute respiratory distress syndrome (ARDS) and sepsis were responsible for between 13-33% of the attributable mortality with cardiovascular events as the main reason of excess mortality. In contrast, infectious complications and AKI were associated with the greatest excess length of stay (LOS).[32] Shafi *et al*. found that the single most potent determinant of LOS was development of complications after trauma, with infections including sepsis as the main contributor.[33]

Long-term morbidity has been shown to be greatly increased in patients who developed post-injury MOF compared to trauma patients with single organ failure.[34] Not surprisingly, excess costs due to complications increase in the same manner. A US study showed that trauma patients experiencing a major complication such as sepsis or AKI increased their hospital costs more than four times.[35] One of the main roles of critical care is to reduce the impact of post-traumatic complications. Although all complications may not be avoidable several studies conclude that early identification together with appropriate care decreases the risk and severity of post-traumatic complications.[36-38]

2.3.1 Post-injury sepsis

2.3.1.1 The changing definitions of sepsis

The term sepsis syndrome was first used by Bone and colleagues, based on the combination of suspected or confirmed infection in conjunction with signs of systemic inflammation. In 1992 the first sepsis consensus definition ("Sepsis-1") was published.[39] The consensus statement differed between the infection and the immune response from the host, the latter was defined as sepsis. Further the term severe sepsis was coined as sepsis in conjunction with organ dysfunction. Lastly, septic shock was used to describe patients with hypotension and impaired tissue oxygenation. These terms were acknowledged to exist also in situations without infection such as burns or pancreatitis. Thus, the term systemic inflammatory response syndrome (SIRS) was proposed, an activated systemic immune response, regardless of cause (Table 2). Sepsis-1 was later revised in 2001 ("Sepsis-2") with the addition of clinical criteria. Although this widened definition may have reflected a more realistic clinical scenario, it was criticized for lack of a strict standardization of the definition. Further, concerns about inadequate sensitivity and specificity of SIRS were raised. For example, 90% of patients admitted to the ICU met these criteria regardless of infection or not, and 1 of 8 patients with infection and organ dysfunction did not fulfill the SIRS criteria [40, 41]

Table 2. Systemic Inflammatory Response Syndrome (SIRS)

	Value
Temperature	<36 degrees Celsius or >38 degrees Celsius
Heart rate	>90 / minute
Respiratory rate	>20 / minute or PaCO2 <4.3 kPa
White blood cell count	<4 x 10^9/litre or >12 x 10^9/litre

PaCO2, partial arterial pressure of carbon dioxide; kPa, kilopascal

The current definition of sepsis (sepsis-3) was the result of a collaboration between the European Society of Intensive Care Medicine and the North American Society of Critical Care Medicine. The definitions and the clinical criteria for sepsis and septic shock were published in 2016.[42-44] The Third International Consensus Definitions for Sepsis and Septic Shock (sepsis- 3) defined sepsis as "life-threatening organ dysfunction caused by a dysregulated host response to infection".

The concept of dysregulated host response was evaluated by comparing different scoring systems against mortality and morbidity outcomes. This process led to the recommendation that a change in baseline SOFA score of 2 points or more was to represent organ dysfunction. Septic shock was defined as a "subset of sepsis in which underlying circulatory and cellular metabolism abnormalities are profound enough to substantially increase mortality". Clinical criteria for septic shock were defined as hypotension requiring vasopressor to maintain mean arterial pressure above 65 mm Hg and a serum lactate level above 2 mmol/l after adequate fluid resuscitation. It should be noted that neither infection nor adequate fluid resuscitation were defined in the process of the new sepsis-3 definitions. The operational, clinical criteria for sepsis and septic shock according to the sepsis-3 consensus statement, as well as the previous sepsis definitions are summarized in table 3.

Table 3. Sepsis criteria according to sepsis definitions

	Sepsis-3	Sepsis-2	Sepsis-1
Sepsis	Increase in SOFA score ≥2 + suspected infection	≥2 SIRS criteria or other clinical signs of systemic inflammation+ suspected infection	≥2 SIRS criteria + suspected infection
Severe sepsis	Not applicable	Sepsis associated with organ dysfunction, hypoperfusion or hypotension	Sepsis associated with organ dysfunction, hypoperfusion or hypotension
Septic shock	Vasopressor needed to maintain MAP ≥65 + serum lactate ≥2, despite fluid resuscitation	Sepsis-induced hypotension (SAP <90 or reduction by ≥40 mm Hg from baseline or MAP <60) persisting despite adequate fluid resuscitation	Sepsis-induced hypotension (SAP <90 or reduction by ≥40 mm Hg from baseline or MAP <60) persisting despite adequate fluid resuscitation

SOFA, sequential organ failure assessment; MAP, mean arterial pressure; SIRS, systemic inflammatory response syndrome; SAP, systolic arterial pressure

There are some non-trauma studies comparing the two definitions. These generally show a higher mortality for patients with sepsis according to the sepsis-3 criteria, compared to patients with sepsis defined according to sepsis-2. The same relationship between the two definitions is generally seen with patients in septic shock.[45-47] Further, sepsis-3 seems to better predict mortality than sepsis-2.[43] However, studies comparing different sepsis definitions in a trauma setting are few, if any.

2.3.1.2 Post-injury sepsis, epidemiology, and risk factors

Post-injury sepsis is a common complication. However, incidences vary considerably depending on degrees of injury, definition of infection and sepsis. Incidences ranging from 2% to over 45% are reported.[12, 48-51] The mortality rate for post-injury sepsis, with the sepsis-2 definition, varies depending on case mix and setting but has been reported to be approximately 10-20%.[13, 32, 49] Interestingly, where the mortality after trauma seems to have decreased the latest decades, the mortality in post-injury sepsis has not. A large German retrospective study analyzed 30000 trauma patients from 1993-2008. The mortality after trauma decreased from 17% to 12%, but the mortality for patients with post-injury sepsis did not. Instead, the researchers could show a slight increase from 16% to 18%.[12]

The transition to the sepsis-3 criteria complicates comparisons with studies performed with the previous sepsis definitions. The inherent SOFA elevation at admission in traumatically injured patients further complicates, the SOFA-based, sepsis-3 diagnosis. Few studies on post-injury sepsis with the sepsis-3 definition exist at the time of writing. Comorbidities and severity of injury are still shown to be risk factors using the new sepsis-3 definition. Other risk factors that were previously associated with post-injury sepsis-2, such as blood

transfusions[12, 52, 53], low Glasgow coma scale (GCS) score at admission[12, 48, 49], age[13] and male gender[12, 13, 49, 54] had before this thesis not been reproduced under the sepsis-3 definitions.

2.3.2 Post-injury multiple organ dysfunction

2.3.2.1 Definition

The first description of multiple organ failure was the Sequential Systems Failure in 1973 when Tilney described a syndrome of organ problems occurring after rupture of aortic abdominal aneurysms.[55] In 1977 the term multiple organ failure (MOF) was first mentioned by Eiseman and colleagues.[56] Since then numerous scoring systems for MOF have been developed. A review from 2006 found 20 different scoring systems or definitions of MOF.[57] The Sequential Organ Failure Assessment score is today one of the most widely used, at least in a European context. A comparison between three commonly used scoring systems, Denver score, SOFA score and Marshall score concluded that the SOFA score showed the most balanced relation between sensitivity and specificity to predict outcome in trauma patients.[9] Interestingly, neither the original SOFA definition nor the later validation[8, 11] by Vincent et al defined *multiple* organ failure. A commonly used SOFA-based definition for MOF is a score of 3 or more in at least two different organ systems.

2.3.2.2 Multiple organ failure, epidemiology and risk factors

A decrease in the incidence of post-injury MOF has been reported, but the mortality is not decreasing in the same clear manner. A US study showed a decrease in post-injury MOF from 17% to 10% between 2003 to 2010, however, deaths related to MOF did not change during this time. Most MOF-related deaths occurred during the first days after onset. [58] Other studies show a decrease in MOF-related deaths.[1] Causes of death in traumatically injured patients are difficult to establish and together with different definitions of MOF and case-mixes this might explain the varying numbers. However, post-injury MOF remains the main cause of late mortality after trauma. Between 15-40% of trauma patients develop post-injury MOF and between 25-40% of these patients succumb to MOF-related death.[59-61]
Male sex, age, degree of injury severity, low blood pressure at admission, blood transfusions, neurological impairment are all shown to be risk factors for the development of post-injury MOF.[48, 54, 59-62]

2.3.2.3 Clinical evolution of organ dysfunction

Traditionally, ICU and trauma research have been focused more on temporally static approaches than on temporal evolution and trends. This might seem odd since monitoring trends and evolution of disease and treating patients accordingly are routine to the ICU physician. One reason for the lack of temporal research might be that analyzing temporal patterns, in contrast to static measures, is often more cumbersome, require more data and more elaborate statistical methods. Lately, more research has been performed to identify patterns and composite phenotypes of disease in the ICU. Studies on ARDS and sepsis have resulted in new insights on pathophysiology and phenotypes.[63-67]. However, most rely on

admission presentation and characteristics. Few studies have investigated the temporal patterns and evolution of post-traumatic organ dysfunction. Further, no studies have evaluated the individual organ's contribution to post-traumatic organ dysfunction.

2.4 BETABLOCKADE AND TRAUMA

The impact of traumatic injury results in several reactions. One relatively recent theory is that the large amount of catecholamines released after trauma exerts damaging effects on the endothelium. Plasma catecholamine increase is associated with syndecan-1, a marker of endothelial damage, and with the coagulopathy often seen in trauma victims.[20, 68] Accordingly, efforts have been made to modulate this excessive release of catecholamines and dampen its effects, possibly improving survival and organ function. It is believed that trauma-induced coagulopathy is present already at the scene of the trauma accident.[69, 70] Given this, it is reasonable to assume that damage to endothelium occurs in the initial phase as well. Thus, pre-injury β-blockade might be protective. However, studies on pre-injury β-blockade have not been consistent. Some studies show a protective effect, mainly in traumatic brain injury, where others have showed no difference, or in some instances, even a decreased survival.[71-73] Reasons for the absence of a protective effect in many studies could have many reasons. β-blockade might decrease the natural response to trauma and mask the shock-state of the patient, leading to a period of under-resuscitation. Chronic medication with β-blockers is also a marker of comorbidity, although adjusted for in study design, imperfection or imbalances in adjustments may lead to bias in results. Further, data on pre-traumatic medications may be inaccurate or incomplete.

2.5 BIOMARKERS IN POST-INJURY SEPSIS

The search for the "holy grail" of sepsis, a biomarker that can differentiate between inflammation and sepsis has been the subject of research for many years but still no golden standard sepsis biomarker exists. Bacterial cultures are the standard test in diagnosing the pathogen, but it takes time, prophylactic antibiotics may result in negative cultures, and cultures might be contaminated by common skin bacteria. Since the time to treatment in septic patients is of the utmost importance, treatment of suspected sepsis is commonly decided and commenced before definitive diagnosis. Thus, a biomarker capable of distinguishing between inflammation and sepsis, readily available and capable of indicating sepsis in an early stage would be much desirable. Trauma invokes a particular challenge in the diagnosis of sepsis since the trauma *per se* induces an inflammatory response that obscures and masks the signs and symptoms of sepsis. Several potential biomarkers have been evaluated, to date, none are able to distinguish between inflammation, such as after injury, and infection.

C-Reactive Protein (CRP) is one of the most common biomarkers. Transcription is enhanced by cytokines in response to inflammation, infection and tissue damage. This biomarker peaks within the first 3-4 days after trauma but has a protracted trajectory and is not able to discriminate between non-septic and septic conditions. Several studies have investigated CRP in a trauma setting, none were able to show any predictive power for post-injury sepsis.[74-76]

Procalcitonin (PCT) is the most reliable of the biomarkers commonly used. Normally produced in the thyroid gland but under stimulation from endotoxin or pro-inflammatory cytokines several tissues not normally producing PCT are able to release PCT. Levels increase 2-4 hours after trauma peaking in around 24 hours. PCT is generally considered a valuable sepsis biomarker.[77] But, in the trauma patient, particularly in the early post-traumatic phase, studies indicate that the predictive value of PCT for subsequent sepsis development is more ambiguous. PCT is correlated to injury severity and is usually elevated during the immediate phase after trauma, peaking in around 24-48 hours, but generally returns to baseline values in uncomplicated cases after a few days.[78, 79] Some studies support early PCT measurement to aid early recognition of subsequent post-injury sepsis in traumatically injured patients.[75, 80] Other studies indicate that PCT measurement has a more limited role in the early post-traumatic phase and recommend trend following and repeated sampling.[74, 79, 81] To be noted, PCT is removed during continuous renal replacement therapy further complicating diagnosis in trauma patients often suffering from acute kidney injury and on dialysis.[82]

Several other biomarkers have been evaluated in studies of post-injury sepsis. Some examples are heparin-binding protein, interleukin-10, interleukin-1, tumor necrosis factor alfa and pancreatic stone protein.[81, 83] None are used in contemporary clinical practice.

2.5.1 Thioredoxin

The thioredoxin (TRX) system consists of TRX, TRX reductase and NADPH as well as an inhibitor molecule, TRX interacting protein. This system is of the outmost importance for balancing and keeping the homeostasis of the cellular redox status. This system is also involved in several other functions such as anti-apoptosis, inflammatory regulation, growth promotion and much more. It has previously been showed that TRX is elevated in septic non-trauma patients[84-86], and that TRX outperformed conventional markers such as CRP and PCT in prediction of 28-days mortality in this setting.[87] However, TRX has never been evaluated in a trauma setting.

2.6 ANIMAL MODELS, FOCUS ON TRAUMA MODELS

Animal models are inherently limited. They are performed to study processes that are not possible to study in human subjects. Human and animals share physiological properties, but they are not the same. While we can treat patients in our ICUs for weeks or more, this is seldom possible in animal models. Hence, trauma models generally focus on the pathophysiology during the first hours after trauma, and therefore lack information on later

complications such as post-injury sepsis and MOF. Nevertheless, animal models provide the researcher with highly relevant means for studying pathophysiology, especially in the initial phase of disease. The possibility to perform standardized, reproducible research under highly monitored conditions and interventions is appealing.

Juvenile pigs are often used in animal trauma models. They are large enough for instrumentation and medical equipment normally used in humans. Pigs share many physiological properties with humans. Their blood volume is large enough for frequent blood sampling and this animal model is suitable for trauma and hemorrhage models. Most circulatory functions are fully developed at birth, making the use of 2-3 months old pigs suitable as models for trauma and hemorrhage. Further, swine are shown to have similar cardiovascular, hematologic and electrolyte profiles to humans.[88, 89] Ventilation parameters are similar to those in humans. However, pigs have lung vascular smooth muscle cells that are sensitive and prone to increased pulmonary vascular resistance. Moreover, pigs are able to contract their spleens in response to hemorrhage resulting in a form of autotransfusion, contributing to around 20-25% of the red cell volume. This has caused many researchers to ligate or remove the spleen initially in the trauma model.[89] In comparison to humans, pigs are hypercoagulable, and states of coagulopathy are difficult to simulate in these models.[90, 91]

Animal models are only mimicking the real world, and findings in animal experiments are merely the basis for further investigations in humans.

3 AIMS OF THE THESIS

To investigate whether medication with β-blockers at the time of injury could be protective in trauma.

To evaluate plasma-thioredoxin in trauma patients as a potential biomarker of post-injury sepsis.

To compare the discriminatory properties for mortality for the previous sepsis-2 definition with the new sepsis-3 definition, in ICU-treated trauma patients.

To estimate incidence and risk factors for post-injury sepsis, and associations with mortality.

To analyze patterns of organ dysfunction in ICU-admitted trauma patients.

4 MATERIAL AND METHODS

4.1 NATIONAL REGISTRIES

Sweden has a long tradition of record keeping. All Swedish residents are at birth or after permanent immigration given a unique ten-digit identity number. This number is used for interactions with authorities, healthcare, and several administrative purposes. The identification number allows linkage of national registries, giving researchers almost complete coverage of the population.

4.1.1 The national patient register

This register is administered by national board of health and welfare (NBHW). It was initiated in the 1960's and gradually expanded. Since 1987 it contains all inpatient care in Sweden and since 2001 also outpatient visits, excluding primary care. Information on each care episode, admission and discharge dates, hospital, or clinic, main- and secondary diagnosis and procedures are registered. Diagnoses is coded according to the World health organization (WHO) International Classification of Disease (ICD 10) since 1996.

4.1.2 The cause of death register

The cause of death register is a high quality, in essence complete, register containing details on time and cause of death for all Swedish citizens and residents with a national identification number since 1952. Since 1961 it is updated annually. NBHW is responsible for the registry since 1994. Since 2012 it contains all deaths in Sweden regardless of nationality of the deceased. Swedish nationals dying abroad are also included. Information on the immediate cause of death and underlying causes is provided in line with WHO standards and 96% of all individuals in the cause of death register have a specific cause of death registered. Misclassification of the cause of death is around 20% but varies depending on age and diagnosis of the deceased.[92]

4.1.3 The prescribed drug register

Administered by NBHW, this register provides statistics about prescribed drugs in Sweden. Established in 2005, it contains all prescribed drugs dispensed at pharmacies. Drugs administered in hospitals and nursing homes are not included and neither are vaccines. It is considered to have 100% coverage regarding prescribed drugs.

4.1.4 The longitudinal integration database for health insurance and labour market studies (LISA)

This register contains data on all individuals aged 16 years or older since 1990. It provides information on employment, education, income, and other socioeconomic variables.

4.2 LOCAL REGISTRIES

4.2.1 The trauma register Karolinska

The trauma register at Karolinska University hospital was established in 2005. It includes all admissions that result in activation of the trauma team. This activation is based on specific anatomic injuries, mechanisms of injury or physiological derangements. Patients who later are found to have an ISS ≥9 are retrospectively added to the register. The register contains data on pre-hospital and in-hospital care. Information such as time to scene, trauma mechanism, initial physiological data as well as outcome variables such as survival status 30 days after injury are collected. Patients pronounced dead after brief resuscitation on arrival are also included. Patients suffering from isolated fractures of upper or lower extremities, chronic subdural hematoma, drowning and hypothermia without simultaneous trauma are not included.

4.2.2 ICU register Karolinska (TRAUMAREG)

This registry, that was active between 2007-2016, included trauma patients 15 years or older that were expected to stay in the ICU for more than 24 hours. Data on physiological variables, lab variables, organ dysfunctions and treatments were collected daily by research nurses and entered into the registry. Data was collected until ICU discharge or death. The data was validated twice to assure quality.

4.2.3 Biobank of trauma patients (TRAUMABIO)

This biobank was active 2007-2016 with the purpose to collect plasma samples from trauma patients admitted to the ICU. If informed consent was given by the patient or the patient's next of kin, samples were collected, centrifugated and stored once daily until death, discharge or until ICU day 10, whichever came first.

4.3 STUDY DESIGN AND OUTCOME MEASURES

Study designs are summarized in table 4.

Table 4. Study design and outcome measures.

Study	I	II	**III**	**IV**	**V**
Design	Cohort study	Cohort study and animal study	Cohort study	Cohort study	Cohort study
Data source	Trauma register Karolinska, Patient register, LISA, Register of total population, Cause of death register, Prescribed drug register	TRAUMAREG, Trauma register Karolinska, TRAUMABIO, Porcine trauma model, Healthy volunteers	TRAUMAREG, Trauma register Karolinska	TRAUMAREG, Trauma register Karolinska	TRAUMAREG, Trauma register Karolinska
Sample size	1376 patients	83 patients 15 healthy volunteers 4 landrace pigs	722 patients	722 patients	660 patients
Follow-up	30 days	ICU stay	30 days	1 year	1 year
Outcome measures	Associations between β-blocker use pre-trauma and mortality	Thioredoxin levels in trauma patients, associations between thioredoxin and post-injury sepsis	30-day mortality in patients with sepsis according to the sepsis-2 and sepsis-3 criteria respectively	30-day mortality, 1-year mortality, incidence of post-injury sepsis, risk factors for post-injury sepsis,	Different trajectories of organ dysfunction, time to stabilization of these trajectories

LISA, The longitudinal integration database for health insurance and labor market studies; ICU, intensive care unit

4.4 STATISTICS

Data is generally presented with counts and proportions (%) or median with interquartile range. Comparisons of continuous data were made by the Mann-Whitney U test or Kruskal-Wallis test. Differences between proportions were made with the chi-square test, or Fisher's exact test where appropriate. In study I, correlation between variables were analyzed with Spearmans correlation coefficient. Differences in survival in paper II and III were made with the log rank test. Predictive properties in paper II were analyzed with receiver operating

characteristics curves (ROC) and presented as area under the curve with corresponding confidence interval (CI), equality of ROC areas were made with the non-parametric approach as suggested by de Long.[93] In study I-IV , associations between outcomes and predictors were made with univariate and multivariable logistic regression and presented as odds ratios with corresponding 95% CI. In paper V we used group-based multi trajectory modeling to find trajectories of organ dysfunction. Data was analyzed as complete cases (paper II and IV) and with simple (paper III) or multiple imputations (paper I and V).

Stata/SE v14.2 - v16.1 (StataCorp, College Station, TX, USA), GraphPad Prism version 6.0 (GraphPad Software, La Jolla, CA, USA), R Core Team (2021) (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna Austria) and RStudio Team (2021) (Rstudio, PBC, Boston, MA, USA) were used for statistical analyses.

Statistical tests were two-sided and *p*-values below 0.05 were considered significant.

4.4.1 Study I

Data from the Trauma register Karolinska between 2006-2015 were linked with LISA, the Patient register and the Prescribed drug register to gather socio-economic, comorbidity data as well as to be able to define β-blocker use at the time of trauma. Users were defined as having filled at least one prescription of β-blockers six months before trauma. We excluded patients under 50 years of age and patients who had an ISS <15 or ISS of 75. Associations between β-blocker use and 30-day mortality were explored using multivariable logistic regression.

4.4.2 Study II

This study consisted of two parts, one small animal model and one on trauma patients admitted to the ICU.

Landrace pigs were anesthetized, ventilated and monitored. A traumatic femur fracture was inflicted followed by controlled hemorrhage. Blood samples for TRX analyzing were taken at three time points.

Patient data from the TRAUMAREG 2007-2014 were extracted if patients had plasma samples saved in TRAUMABIO taken on day one and three during their ICU stay. Admittance data were linked from the Trauma register Karolinska. Plasma from volunteers was analyzed for comparative measures. A commercially available Enzyme-Linked Immunosorbent Assay were used for the analysis of TRX in plasma. Samples were analyzed in duplicates and mean of two values were used. The association between TRX and severe sepsis was analyzed in a multivariable logistic regression model. ROC curves were used to analyze TRX as a predictor for post-injury sepsis.

4.4.3 Study III

Data from patients included in the TRAUMAREG 2007-2016 was extracted until day 10, discharge or death whichever occurred first. Admittance data was linked from the Trauma register Karolinska. Primary outcome was 30-day mortality. Infection was defined according to the International sepsis forum classification (ISF).[94] Sepsis-2 was defined according to the criteria from Bone *et al*.[39] Sepsis-3 was defined according to the criteria defined by Singer *et al*[42], specifically as infection in conjunction with an increase in SOFA score of ≥2 from the previous day. Predictive properties of the two sepsis definitions were analyzed with ROC curves. Difference in survival was analyzed with the log-rank test. To account for the competing risk of early trauma-related deaths before being at risk for sepsis a temporal analysis was made by consecutive censoring of patients dying on day 1 and forward. Analyses of risk of death and discriminatory properties were then made for each censoring step.

4.4.4 Study IV

Data from patients included in the TRAUMAREG 2007-2016 were extracted. The primary outcome measure was 30-day mortality, secondary outcomes were 1-year mortality and impact on clinical course. Sepsis was defined according to the sepsis-3 definition.[42] Analysis of risk factors for post-injury sepsis were made by uni- and multivariable logistic regression. A logistic regression analysis of risk for post-injury sepsis and association to the number of packed red blood cells administered were also performed. To account for the competing risk of early trauma-related deaths before being at risk for sepsis a temporal analysis was made by consecutive censoring of patients dying on day one and forward. Analyses of risk of death were then made for each censoring step.

4.4.5 Study V

Data from patients included in the TRAUMAREG 2007-2016 were extracted. Data was retrieved during the ICU and, where applicable, high dependency unit (HDU) stay until discharge, death or up to 28 days after trauma, whichever occurred first. Patients transferred to another hospital during the ICU or high dependency unit (HDU) stay were excluded. Group-based trajectory modeling (GBTM) was performed to identify different trajectory groups of organ dysfunction. GBTM yields a probability of assignment to a particular group (posterior probability of group membership, PPGM). Time to stabilized trajectory group assignment was analyzed as well. We defined trajectory group assignment as stabilized when the highest PPGM did not change as compared to their final assignment.

4.5 ETHICAL CONSIDERATIONS

All studies in this thesis are approved by the regional ethics committee of Stockholm, Sweden. The studies are conducted in accordance with the Helsinki declaration and good clinical practice. Studies I and III-V are registry-based, observational, carried no deviation

from clinical routine care and no direct contact between researchers and study participants existed. No procedures involving pain, discomfort or risk for complication existed. Informed consent was waived by the ethical committee. Ethical aspects are related to integrity violations when collecting data from patients' charts, this potential integrity violation must be weighed against the benefit of increasing knowledge about risk factors and complications of the disease they are treated for.

Study II involved an animal model and blood sampling from patients as well as from healthy volunteers after informed consent. All animals were handled according to the Animal ethics board guidelines and food and water was ad libitum until 1h before the experiment. The animal model was approved by the animal ethics board, Stockholm, Sweden. The blood samples taken from patients and healthy volunteers were approved by the regional ethics committee of Stockholm. Blood sampling of patients are part of routine care during the ICU stay and one extra vial of blood does not pose any significant discomfort and the risk of complications are deemed small.

5 RESULTS

5.1 STUDY I

A total number of 1376 patients were included in the final cohort. Of these, 338 patients were defined as β-blocker users. Baseline characteristics differed in that β-blocker users had more co-morbidities and were older than non-users (Table 5).

Table 5. General characteristics and outcomes of the study cohort divided by β-blocker usage.

	β-blocker (-)	**β-blocker (+)**	***p*-value**
n (%)	1038 (75.4)	338 (24.6)	
Age, median (IQR)	63.5 (56-73)	71.5 (63-82)	< 0.001
Male, n (%)	733 (70.6)	223 (66.0)	0.108
Education level, n (%)			0.175
Low	240 (25.1)	88 (30.6)	
Medium	444 (46.3)	125 (43.4)	
High	274 (28.6)	75 (26.0)	
CCI, median (IQR)	0 (0-1)	1 (0-2)	< 0.001
CCI category, n (%)			
0	693 (66.8)	113 (34.9)	< 0.001
1	168 (16.2)	88 (26.0)	
>1	177 (17.1)	132 (39.1)	
Ischemic heart disease, n (%)	27 (2.6)	96 (28.4)	< 0.001
Congestive heart failure, n (%)	28 (2.7)	60 (17.8)	< 0.001
Hypertension, n (%)	118 (11.4)	141 (41.7)	< 0.001
Diabetes mellitus, n (%)	69 (6.6)	62 (18.3)	< 0.001
Anticoagulation therapy, n (%)	31 (3.0)	65 (19.2)	< 0.001
Psychiatric co-morbidity, n (%)	142 (13.7)	39 (11.5)	0.312
Substance abuse, n (%)	172 (16.6)	48 (14.2)	0.302
ISS, median (IQR)	24 (17-27)	25 (17-26)	0.911
Blunt trauma, n (%)	1020 (98.3)	331 (97.9)	0.689
Severe head injury, n (%)	651 (62.7)	216 (63.9)	0.694
Severe thoracic injury, n (%)	400 (38.5)	132 (39.1)	0.865
Severe abdominal injury, n (%)	89 (8.6)	28 (8.3)	0.868
SAP*, median (IQR)	144 (120-164)	150 (120-170)	0.073
SAP* < 90 mm Hg, n (%)	83 (8.0)	32 (9.5)	0.396
ICU admittance, n (%)	602 (58.0)	190 (56.2)	0.565
30-day post-injury mortality, n (%)	205 (19.7)	111 (32.8)	< 0.001

*Continuous parameters presented as median with interquartile range (IQR), categorical parameters as n (%). CCI, Charlson Comorbidity Index; ISS, Injury Severity Score; SAP, Systolic Arterial Pressure; ICU, Intensive Care Unit. *On arrival to the trauma unit.*

β-blocker users had a higher unadjusted mortality than non-users, 32.8% vs 19.7% (p <0.001, log-rank test). In the univariate analysis, β-blocker users had an increased odds of 30-day mortality (odds ratio (OR) 1.99, 95% CI 1.51-2.61, p <0.001). However, in the fully adjusted model (Figure 2) no such association was seen (OR 1.09, 95% CI 0.7-1.7, p <0.703) between β-blocker users and non-users. Further, no interaction was seen between β-blocker use and severe head injury or β-blocker user and shock on arrival. In a separate analysis, no association was seen between β-blocker users and mortality or individuals with or without head injury.

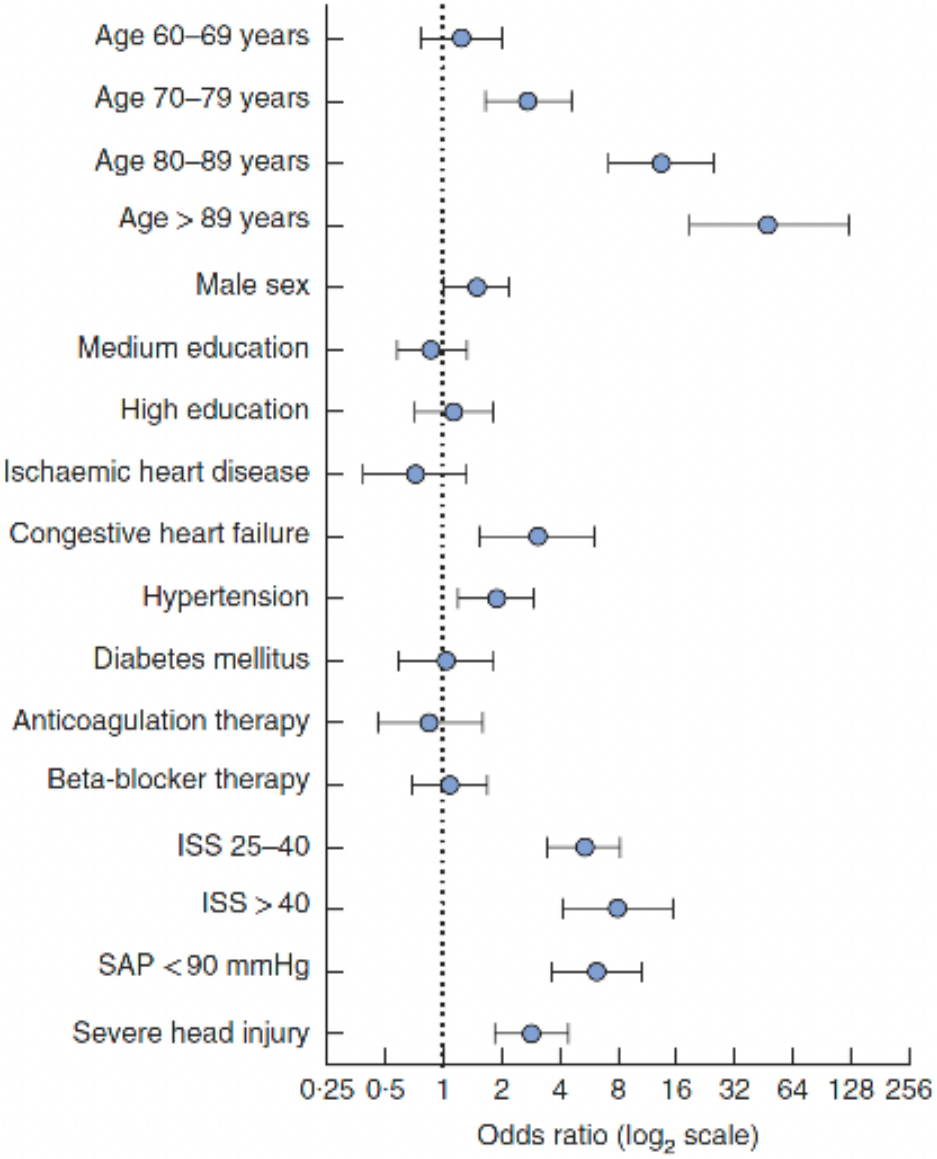

Figure 2. Multivariable model for 30-day mortality with odds ratios and 95% confidence interval. ISS, Injury Severity Score; SAP, Systolic Arterial Pressure.

5.2 STUDY II

In the porcine trauma model, all four pigs survived throughout the experiment. There was an increase in plasma TRX after femur fracture, hemorrhage, and resuscitation however not significant ($p = 0.069$). Cardiac index and lactate levels were markedly affected after trauma and returned to near normal levels at the end of the experiment.

Eighty-three patients were included in the study. Median time from trauma to first blood sampling was in median 16 h (inter quartile range (IQR) 10-21h). General characteristics of the study cohort are depicted in table 6.

Table 6. General characteristics and outcomes of the study cohort.

Age, years, median (IQR)	49 (28-62)
Male, n (%)	63 (75.9)
APACHE II, median (IQR)	17 (12-22)
Mechanism of injury, n (%)	
Traffic related	45 (54.2)
Fall	10 (12.0)
Assault	7 (8.4)
Self-inflicted	15 (18.1)
Others	6 (7.2)
Admission SAP <90, n (%)	22 (27)
Admission GCS, median (IQR)	14 (8-15)
Massive transfusion (≥10 units /24h), n (%)	28 (34)
Sepsis, n (%)	48 (58)
SOFA admission score, median (IQR)	8 (6-11)
ISS, median (IQR)	29 (21-42)
Invasive mechanical ventilation, n (%)	76 (92)
ICU length of stay, days, median (IQR)	8 (5-14)
ICU mortality, n (%)	4 (4.8)
30-day post-injury mortality, n (%)	7 (8.3)

Continuous parameters presented as median (inter quartile range, IQR), categorical parameters as count (percent). APACHE II, acute physiology and chronic health evaluation; SAP, systolic arterial pressure; GCS, Glasgow coma scale; SOFA, sequential organ failure assessment score; ISS, injury severity score. Admission refers to the admission to the trauma unit.

Trauma patients had significantly higher plasma TRX on day 1 compared to healthy controls. Plasma TRX increased with severity of injury. Higher levels of TRX were seen in patients who received massive transfusion and those who were in shock at the time of admission. No

gender or age differences were seen. A weak, significant correlation between ISS and TRX sampled on day one was seen (Spearman correlation coefficient, 0,3448). Area under the curve (AUC) of TRX sampled day 1 as a predictor of sepsis was 0.66. In the multivariable analysis, TRX was the only variable associated with post-injury sepsis (Table 7).

Table 7. Uni- and multivariable logistic regression analysis for risk factors for later development of sepsis.

	Univariate OR (95% CI)	***p*-value**	**Multivariable OR (95% CI)**	***p*-value**
CRP (mg/l)	1.005 (0.997-1.012)	0.234		
ISS (points)	1.022 (0.993-1.053	0.139	1.012 (0.978-1.047)	0.488
Massive transfusion (Y/N)	1.452 (0.570-3.699)	0.435		
Admission SAP* (mm Hg)	0.992 (0.981-1.004)	0.182	1.000 (0.987-1.013)	0.964
TRX day 1 (ng/ml)	1.011 (1.002-1.020)	0.017	1.010 (1.001-1.019)	0.038

*OR, odds ratio; CI, confidence interval; CRP, C-reactive protein; ISS, injury severity score; SAP, systolic arterial pressure; TRX, thioredoxin. *On arrival to the trauma unit*

5.3 STUDY III

The study cohort included 722 severely injured patients with median ISS of 26 (IQR 18-38), median age 41 (IQR 28-58), 78% were male. Overall length of stay was 3.7 days and overall mortality was 9.3% at 30 days. Admission and outcomes for the total cohort and for the two sepsis definitions are shown in table 8.

Table 8. Demographic, admission data and outcomes for all patients, sepsis-2 and sepsis-3 patients respectively

	All (n=722)	**Sepsis-2 (n=315)**	**Non sepsis-2 (n=407)**	***p*-value**	**Sepsis-3 (n=148)**	**Non sepsis-3 (n=574)**	***p*-value**
Male gender, n (%)	561 (77.7)	251 (79.7)	310 (76.2)	0.260	117 (79.1)	444 (77.4)	0.657
Age, years, median (IQR)	41 (28-58)	43 (29-59)	39 (26-56)	0.023	46 (29-63)	40 (27-56)	0.007
History of comorbidity, n (%)	369 (51)	169 (53.7)	200 (49.1)	0.229	86 (58.1)	283 (49.3)	0.056
Mechanism of injury n (%)							
Traffic related	302 (41.8)	140 (44.4)	162 (39.8)		68 (46.0)	234 (40.8)	
Fall	123 (17.0)	44 (14.0)	79 (19.4)		27 (18.2)	96 (16.7)	
Assault	86 (11.9)	30 (9.5)	56 (13.8)	0.031	10 (6.8)	76 (13.2)	0.161
Self-inflicted	120 (16.6)	63 (20.0)	57 14.0)		28 (18.9)	92 (16.0)	
Others	91 (12.6)	38 (12.0)	53 (13.0)		15 (10.1)	76 (13.2)	
Penetrating injury, n (%)	88 (12.2)	35 (11.1)	53 (13.0)	0.436	13 (8.8)	75 (13.1)	0.156
Admission SAP < 90, n (%)	115 (15.9)	76 (24.1)	39 (9.6)	0.000	42 (28.4)	73 (12.7)	0.000
Massive transfusion, n (%)	125 (17.3)	78 (24.8)	47 (11.6)	0.000	40 (27.0)	85 (14.8)	0.000
ISS, median (IQR)	26 (18-38)	33 (22-43)	24 (17-33)	0.000	34 (23-43)	25 (17-35)	0.000
ISS >15, n (%)	605 (83.8)	279 (88.6)	326 (80.1)	0.002	136 (91.9)	469 (81.7)	0.003
AIS head ≥3, n (%)	298 (41.3)	150 (47.6)	148 (36.4)	0.002	66 (44.6)	232 (40.4)	0.357
SOFA admission score (Without GCS), median (IQR)	5 (3-7)	7 (5-8)	4 (2-6)	0.000	7 (5-9)	5 (3-7)	0.000
SOFA admission score (Including GCS), median (IQR)	7 (4-10)	9 (6-11)	5 (3-8)	0.000	9 (6-11)	6 (4-9)	0.000
Mechanical ventilation, n (%)	573 (79.4)	304 (96.5)	269 (66.1)	0.000	146 (98.7)	427 (74.4)	0.000
SIRS, n (%)	704 (97.5)	315 (100)	389 (95.6)	0.001	148 (100)	556 (96.9)	0.029
SOFA total max, median (IQR)	8 (5-10)	10 (8-12)	5 (4-8)	0.000	11 (9-13)	6 (4-9)	0.000
ICU LOS, day, median (IQR)s	3.7 (2.0-8.4)	9.7 (5.5-16.5)	2.3 (1.5-3.3)	0.000	11.9 (7.1-19.2)	2.9 (1.8-5.1)	0.000
30-day mortality, n (%)	67 (9.3)	24 (7.6)	43 (10.6)	0.176	18 (12.2)	49 (8.5)	0.175

SAP, systolic arterial blood pressure; ISS, injury severity score; SOFA, sequential organ failure assessment; AIS, abbreviated injury score; GCS, Glasgow coma score; SIRS, systemic inflammatory response syndrome; ICU LOS, intensive care unit length of stay. SOFA total max is the sum of each SOFA-domains maximum score during the study period. Data on SOFA, SIRS, and ventilation during the study period. Continuous parameters presented as median (inter quartile range, IQR), categorical parameters as count and percent.

Forty percent of the patients fulfilled the criteria for sepsis-2 vs 20% for the sepsis-3 definition during the study period of the first ten days in the ICU (Figure 3). Further, all patients fulfilling the sepsis-2 criteria also fulfilled the sepsis-3 criteria.

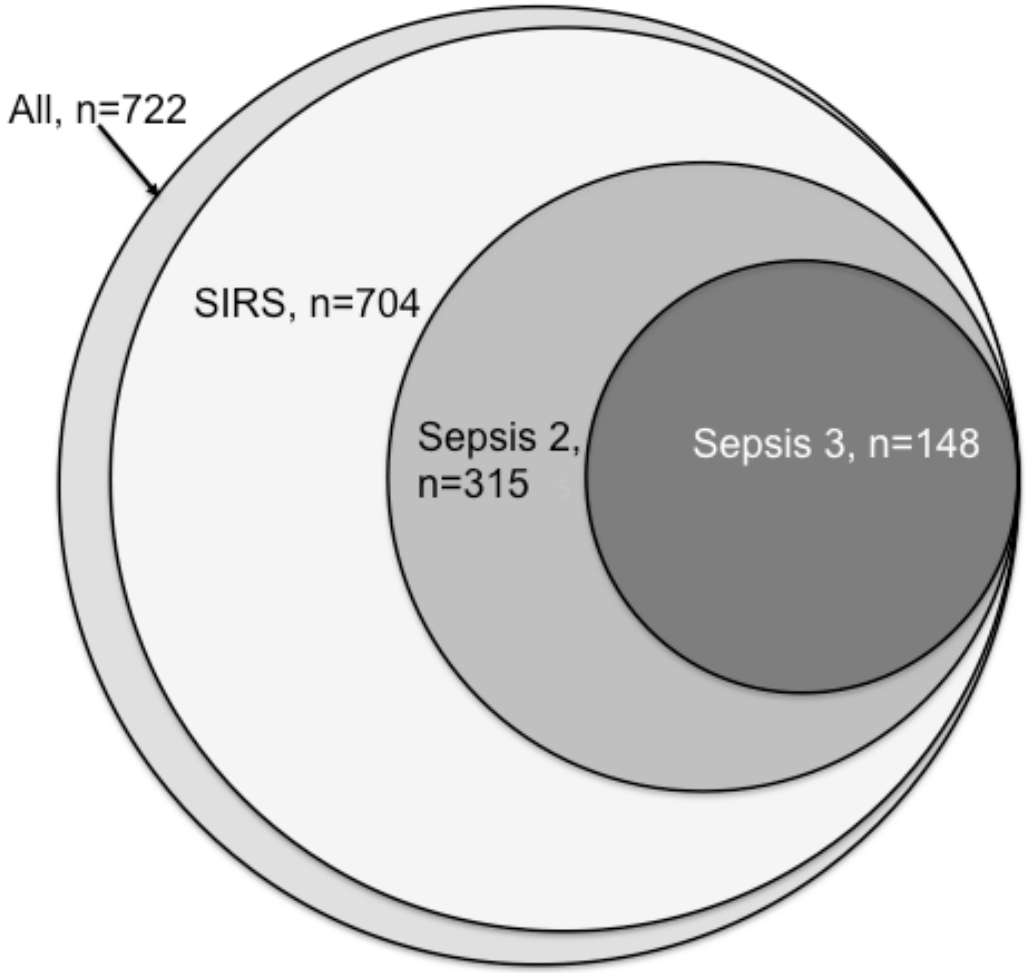

Figure 3 Venn diagram of systemic inflammatory response syndrome (SIRS), sepsis-2 and sepsis-3.

ICU LOS was markedly longer in septic patients, 10 days in sepsis-2 and 12 days in sepsis-3 patients, respectively compared with 2–3 days in the non-septic patients (Table 7). No significant differences in 30-day mortality were seen between neither sepsis-2 patients (OR 0.7 (CI 0.4–1.2)) nor sepsis-3 patients (OR 1.5 (CI 0.8-2.6) and their respective non-septic controls. However, when censoring patients dying early after admission to the ICU, the risk of 30-day mortality increased and became significant for sepsis-3 already after censoring patients dying at day 1. For sepsis-2 this association never reached significance (Figure 4).

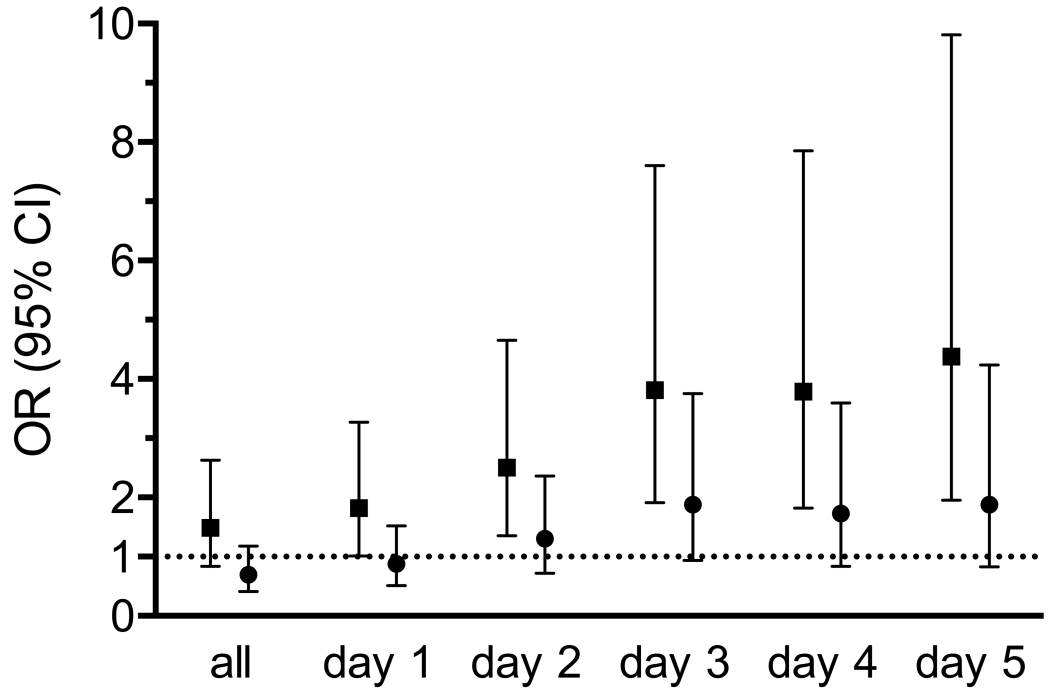

Figure. 4. Temporal analyses of odds ratio for 30-day mortality. Logistic regression analyses exploring 30-day mortality consecutively censoring patients dying at the early stages. Odds ratio (OR) and 95% confidence intervals (CI) for 30-day mortality for sepsis-2 (circles) and sepsis-3 (squares). The x-axis depicts all patients and subsequently censoring patients dying on day 1 and on, up until day 5.

Sensitivity analyses in form of imputing median as well as highest score instead of zero points for missing SOFA or SIRS scores did not change the major findings, neither did inclusion of the neurological component of the SOFA score. Using only confirmed infections, according to ISF guidelines, as a prerequisite for sepsis, decreased the number of patients with sepsis. However, the pattern remained with increasing and significant odds ratios and AUC for 30-day mortality with gradual censoring of early deaths for sepsis-3, but not for sepsis-2 (data not shown).

5.4 STUDY IV

The study population consisted of 722 trauma patients admitted to the ICU. They were predominantly male, median age of 41 and a quarter of the patients had pre-existing comorbidities. They were severely injured and 80% had an ISS over 15. One sixth of the patients were in shock on arrival and about half of the patients required surgery during the first 24 hours. Admission characteristics for the total cohort and for non-septic and septic patients are shown in table 9.

Table 9. Admission data.

	All	Missing (n)	Nonsepsis	Sepsis
Number of patients, n (%)	722 (100)	0	564 (78)	158 (22)
Age, median (IQR)	41(28-58)	0	39 (27-56)	47 (31-63)
Female sex, n (%)	161 (22)	0	129 (23)	32 (20)
Charlson Comorbidity Index >0 points, n (%)	166 (23)	0	122 (22)	44 (28)
Charlson Comorbidity Index, points, median (IQR)	0 (0-0)	0	0 (0-0)	0 (0-1)
Injury mechanism, n (%)				
Traffic	302 (42)	0	229 (41)	73 (46)
Fall	123 (17)	0	96 (17)	27 (17)
Self-inflicted	120 (17)	0	89 (16)	31 (20)
Assault	86 (12)	0	75 (13)	11 (7)
Others	91 (13)	0	75 (13)	16 (10)
Intubated at scene, n (%)	140 (19)	0	103 (18)	37 (23)
Injury Severity Score, median (IQR)	26 (18-38)	2	24 (17-35)	34 (24-43)
Injury Severity Score > 15, n (%)	605 (84)	2	460 (82)	145 (92)
AIS head >2, n (%)	294 (41)	0	223 (40)	71 (45)
AIS face > 2, n (%)	20 (3)	0	14 (3)	6 (4)
AIS neck > 2, n (%)	42 (6)	0	32 (6)	10 (6)
AIS spine > 2, n (%)	175 (24)	0	115 (20)	60 (38)
AIS upper extremity > 2, n (%)	36 (5)	0	25 (4)	11 (7)
AIS thorax > 2, n (%)	421 (58)	0	311 (55)	110 (70)
AIS abdomen > 2, n (%)	178 (25)	0	125 (22)	53 (34)
AIS lower extremity > 2, n (%)	233 (32)	0	166 (29)	67 (42)
Penetrating trauma, n (%)	88 (12)	0	75 (13)	13 (8)
Shock on arrival, n (%)	115 (16)	9	67 (12)	48 (30)
Admission systolic arterial pressure, median (IQR)	122 (103-148)	9	126 (109-150)	110 (84-135)
Admission Glasgow Coma Scale, median (IQR)	13 (8-15)	59	14 (8-15)	11 (8-15)
Blood alcohol concentration> 0 mM, n (%)	184 (27)	33	138 (26)	46 (31)
Admission creatinine, median (IQR)	92 (77-112)	34	91 (75-110)	99 (84-119)
Admission trauma-induced coagulopathy, n (%)	105 (16)	76	72 (14)	33 (23)
Massive transfusion, n (%)	125 (17)	0	76 (14)	49 (31)
Nr of packed red blood cells units 24 hrs, median (IQR)	2 (0-7)	0	1 (0-5)	5 (0- 11)
Total fluid load 24 hrs, litres, median (IQR)	5.6 (3.4-8.8)	0	5.1 (3.1-8.2)	7.8 (4.8-12.1)
Surgery first 24 hrs, n (%)	378 (52)	0	286 (51)	92 (58)
Acute and Chronic Health Evaluation II, median (IQR)	15 (11-21)	0	14 (10-20)	18 (14-23)
Admission Sequential Organ Failure Assessment, median (IQR)	5 (3-7)	0	5 (3-7)	7 (5-9)

Admission refers to the admission to the trauma unit. AIS, abbreviated injury scale. IQR, inter quartile range.

The daily prevalence of sepsis increased during the first 5 days, and 22% of the patients developed sepsis during the study period.

Risk factors for post-injury sepsis were analyzed, first with univariate logistic regression and variables with a *p*-value below 0.2, as well as sex, were forwarded to the multivariable regression. In the adjusted analysis, age, spine and chest injury, shock on arrival, positive blood alcohol, and packed red blood cell units transfusion were associated with later sepsis development (Table 10).

Table 10. Univariate and multivariable analysis of risk factors for post injury sepsis

	Univariate OR (95% CI)	***p*-value**	**Multivariable OR (95% CI)**	***p*-value**
Age (continuous)	1.01 (1.01-1.02)	0.002	1.02 (1.01-1.03)	0.002
Male sex	1.2 (0.8- 1.8)	0.485	1.3 (0.7-2.1)	0.390
Charlson Comorbidity Index, points, >0	1.4 (0.9-2.1)	0.102	1.1 (0.7- 1.8)	0.676
AIS head >2	1.2 (0.9-1.8)	0.223		
AIS face > 2	1.6 (0.6-4.1)	0.377		
AIS neck > 2	1.1 (0.5-2.3)	0.756		
AIS spine > 2	2.4 (1.6-3.5)	<0.001	2.0 (1.3-3.2)	0.002
AIS upper extremity > 2	1.6 (0.8-3.4)	0.200		
AIS chest > 2	1.9 (1.3-2.7)	0.001	1.6 (1.0-2.4)	0.047
AIS abdomen > 2	1.8 (1.2-2.6)	0.004	1.4 (0.9-2.3)	0.139
AIS lower extremity > 2	1.8 (1.2-2.5)	0.002	1.5 (0.9-2.3)	0.088
Penetrating trauma	0.6 (0.3-1.1)	0.088	0.5 (0.2-1.1)	0.087
Shock on arrival	3.2 (2.1-4.9)	<0.001	2.0 (1.2-3.3)	0.011
Admission creatinine > 100 mM	1.8 (1.2-2.5)	0.003	1.4 (0.9-2.2)	0.109
Blood alcohol concentration > 0 mM	1.3 (0.9-2.0)	0.175	1.8 (1.2-2.9)	0.010
Nr of packed red blood cells, units 24 hrs (continuous)	1.06 (1.04-1.08)	<0.001	1.04 (1.01-1.06)	0.005
Surgery first 24 hrs	1.4 (0.95-1.9)	0.095	1.0 (0.6-1.5)	0.924

Odds ratio (OR) and 95% confidence intervals (95% CI). AIS, abbreviated injury scale. Admission refers to the admission to the trauma unit. Variables with a p < 0.2 in the univariate analysis and sex forwarded to the multivariable analysis.

The association between blood transfusion and later sepsis development was further analyzed in a separate analysis where the risk of sepsis increased in a dose related manner with the number of units of blood transfused (Figure 5).

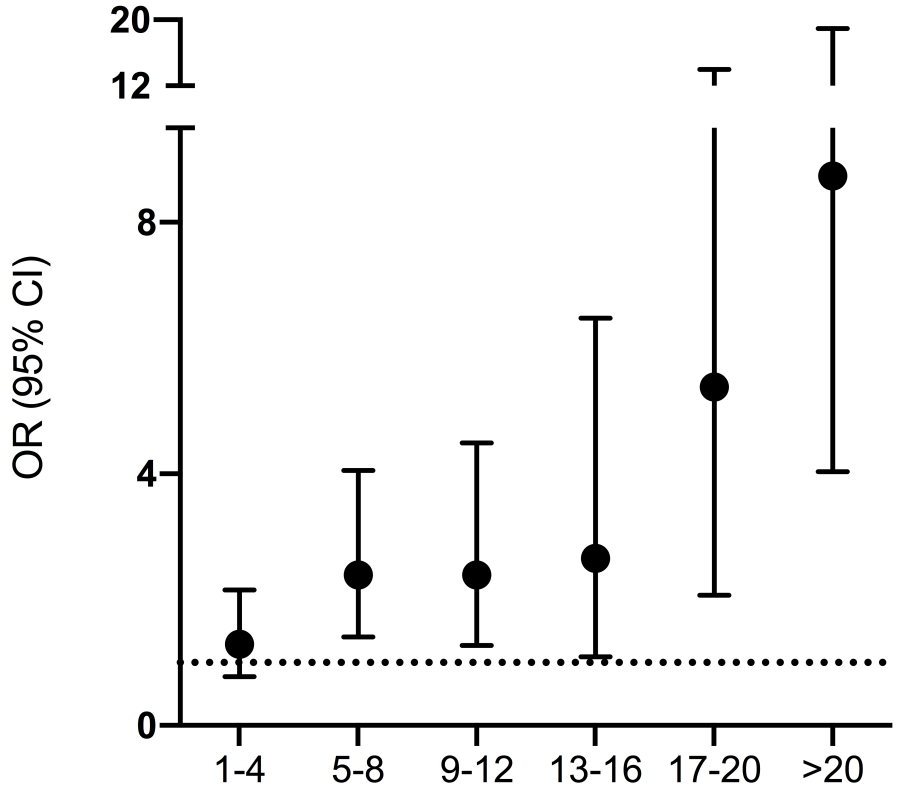

Figure 5. Logistic regression analyses exploring odds ratio (OR) and 95% confidence intervals (CI) for postinjury sepsis in relation to the transfused number of packed red blood cell units during the first 24 hours (x-axis).

Sepsis development was associated with a complicated clinical course, septic patients had more organ failure, need for dialysis and longer length of stay in the ICU than their non-septic counterparts. No significant differences in 30-day mortality between septic and non-septic patients were seen (Table 11). However, when censoring patients dying in the early phase after trauma, sepsis was associated with death beyond day 2 (data not shown).

Table 11. Clinical course and outcomes.

	Non-sepsis	**Sepsis**	***p*-value**
Number of patients	564	158	
SOFA score, total max, median (IQR)	6 (4-9)	11 (9-14)	<0.001
MODS days, median (IQR)	1 (0-3)	8 (4-13)	<0.001
ICU days on vasopressor, median (IQR)	1 (0-3)	7 (4-12)	<0.001
ICU days on mechanical ventilation, median (IQR)	2 (0-4)	11 (7-19)	<0.001
ICU days on CRRT, median (IQR)	0 (0-0)	0 (0-1)	<0.001
ICU LOS, median (IQR)	2.8 (1.8-4.9)	13 (8.0-20)	<0.001
Hospital LOS, median (IQR)	14 (8-25)	28 (17-57)	<0.001
ICU mortality, n (%)	38 (6.7)	12 (7.6)	0.71
Hospital mortality, n (%)	47 (8.3)	21 (13.3)	0.059
30-day mortality, n (%)	50 (8.9)	17 (10.8)	0.47
1-year mortality, n (%)	62 (11.0)	28 (17.7)	0.025

LOS, length of stay; SOFA, sequential organ assessment score; MODS, multiple organ dysfunction syndrome (≥ 6 SOFA points); IQR, inter quartile range; ICU, intensive care unit; LOS, length of stay. One-year follow-up was missing for one non-sepsis patient.

5.5 STUDY V

After exclusion of patients transferred to other hospitals, 660 patients were included in the final cohort. Median age was 40 years, 22% had pre-existing comorbidity. There was a male dominance and a high median ISS of 26. One-fifth developed sepsis during the ICU stay.

We identified five trajectories of organ dysfunction (OD). These five trajectories of organ dysfunction after trauma displayed differences in admission characteristics, organ dysfunction trajectories and outcomes. Data on admission characteristics for the total cohort and for the five trajectory groups are shown in table 12.

Table 12. Admission data

	All patients	Group 1	Group 2	Group 3	Group 4	Group 5
Age	40 (27-56)	38 (26-51)	41 (27-56)	45 (28-63)	44 (31-60)	44 (27-64)
Sex (male)	517 (78%)	237 (79%)	102 (76%)	70 (80%)	35 (88%)	73 (74%)
Charlson comorbidity index ≥ 1	145 (22%)	59 (20%)	35 (26%)	24 (28%)	11 (28%)	16 (16%)
Injury mechanisms						
Traffic	273 (41%)	121 (40%)	58 (43%)	31 (36%)	19 (48%)	44 (45%)
Fall	113 (17%)	49 (16%)	19 (14%)	17 (19%)	6 (15%)	22 (22%)
Self-inflicted	109 (16%)	41 (14%)	22 (16%)	23 (26%)	8 (20%)	15 (15%)
Assault	83 (12%)	49 (16%)	14 (10%)	7 (8.0%)	3 (7.5%)	10 (10%)
Others	82 (12%)	40 (13%)	22 (16%)	9 (10%)	4 (10%)	7 (7.1%)
Intubated at scene	128 (19%)	31 (10%)	27 (20%)	12 (14%)	9 (22%)	49 (50%)
Blunt trauma	524 (79%)	234 (78%)	104 (77%)	73 (84%)	29 (72%)	84 (86%)
ISS, points	26 (17-38)	20 (14-27)	25 (18-38)	34 (22-43)	41 (29-50)	41 (29-54)
ISS > 15	545 (83%)	218 (73%)	115 (85%)	78 (90%)	39 (98%)	95 (97%)
AIS head ≥ 3	275 (42%)	70 (23%)	53 (39%)	47 (54%)	19 (48%)	86 (88%)
AIS chest ≥3	370 (56%)	139 (46%)	77 (57%)	55 (63%)	31 (78%)	68 (69%)
AIS abdomen ≥3	161 (24%)	70 (23%)	27 (20%)	27 (31%)	19 (48%)	18 (18%)
AIS spine ≥3	152 (23%)	52 (17%)	27 (20%)	34 (39%)	17 (42%)	22 (22%)
AIS lower extremity ≥3	207 (31%)	65 (22%)	54 (40%)	39 (45%)	24 (60%)	25 (26%)
Admission SAP, mmHg	123 (104-149)	130 (114-150)	120 (95-150)	126 (105-149)	90 (64-112)	120 (90-150)
Shock on arrival	99 (15%)	19 (6.3%)	26 (19%)	14 (16%)	18 (45%)	22 (22%)
Admission GCS	13 (8.0-15)	15 (12-15)	13 (8.0-15)	13 (8.0-15)	8.0 (3.0-14)	5.0 (3.0-8.0)
Admission creatinine, µM/L	92 (76-112)	90 (71-107)	87 (75-102)	101 (83-116)	119 (102-148)	92 (80-111)
Admission blood glucose, mM/L	8.8 (7.1-10)	8.2 (6.8-10)	8.9 (7.1-11)	9.1 (7.8-11)	9.8 (7.2-11)	10 (8.5-13)
Blood alcohol level > 0	171 (27%)	81 (28%)	31 (24%)	23 (28%)	11 (30%)	25 (26%)
Admission TIC	90 (15%)	32 (12%)	16 (14%)	18 (22%)	8 (24%)	16 (17%)
Admission INR	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1.1 (1.0-1.2)	1.2 (1.1-1.2)	1.1 (1.0-1.2)
Admission platelet count, 10^9/L	234 (188-282)	242 (199-288)	234 (190-283)	230 (190-276)	193 (157-271)	224 (179-268)
Admission fibrinogen level, g/L	2.2 (1.8-2.6)	2.2 (1.9-2.7)	2.2 (1.8-2.6)	2.2 (1.7-2.6)	1.5 (0.9-2.4)	1.9 (1.4-2.6)
Massive transfusion	109 (16%)	27 (9.0%)	23 (17%)	15 (17%)	27 (68%)	17 (17%)
Number of PRBC 24 hrs	2 (0-7)	0 (0-4)	2 (0-7)	4 (0-8)	12 (6-27)	2 (0-8)
Total fluid load 24 hrs, L	5.5 (3.5-8.6)	4.7 (2.7-7.2)	5.6 (33.7-8.7)	6.5 (4.2-9.5)	14 (8.1-21)	5.9 (4.0-9.0)
Surgery during the first 24 hrs	350 (53%)	140 (47%)	77 (57%)	56 (64%)	27 (68%)	50 (51%)

Admission data. Continuous parameters presented as median (IQR), and categorical parameters presented as n (%). Admission refers to the admission to the trauma unit. ISS, injury severity score; AIS, abbreviated injury scale; SAP, systolic arterial blood pressure; shock on arrival defined as admission systolic blood pressure < 90 mmHg; GCS, Glasgow coma scale; TIC, trauma-induced coagulopathy; INR, international normalized ratio; PRBC, packed red blood cell units

We summarized and arbitrarily named the five identified groups as follows: Group 1, Mild OD; Group 2, Moderate OD; Group 3, Severe OD; Group 4 Extreme OD; Group 5, Traumatic brain injury (TBI) and OD. The trajectories of the five groups are depicted in figure 6.

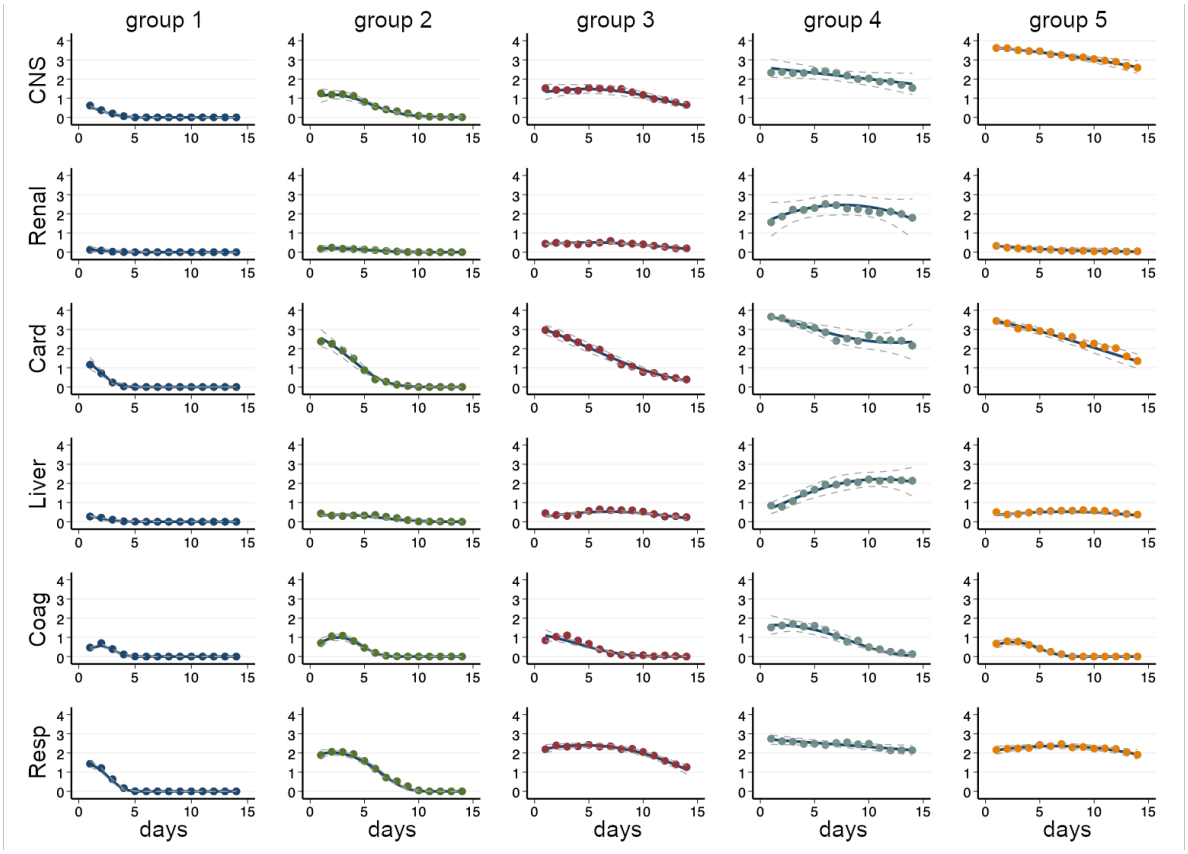

Figure 6. Trajectory group classification. The five identified trajectory groups of organ dysfunction represented by the columns. Sequential organ failure assessment (SOFA) points for each domain (y-axis) are shown for the first 14 days after trauma (x-axis). Final trajectory model (blue line) with corresponding 95% confidence intervals (dashed lines). Mean true observed SOFA score for each time point (dots). Central nervous system domain (CNS), renal domain (Renal), cardiovascular domain (Card), liver domain (Liver), coagulation domain (Coag), and respiratory domain (Resp). Reading example: Group 4 experienced relative stationary CNS SOFA scores during the first week. They experienced an increase in both renal and liver scores during the first week, after that renal scores gradually decreased, but liver scores continued to increase during the full study period of 14 days.

When analyzing the time to stabilized group assignment, we saw major differences between the groups. The patients belonging to the groups with the lowest and highest mortalities, group 1, and group 5, stabilized early. This contrasted with the groups with moderate mortality, groups 2 and 3 where stabilization occurred at a much later stage (Figure 7).

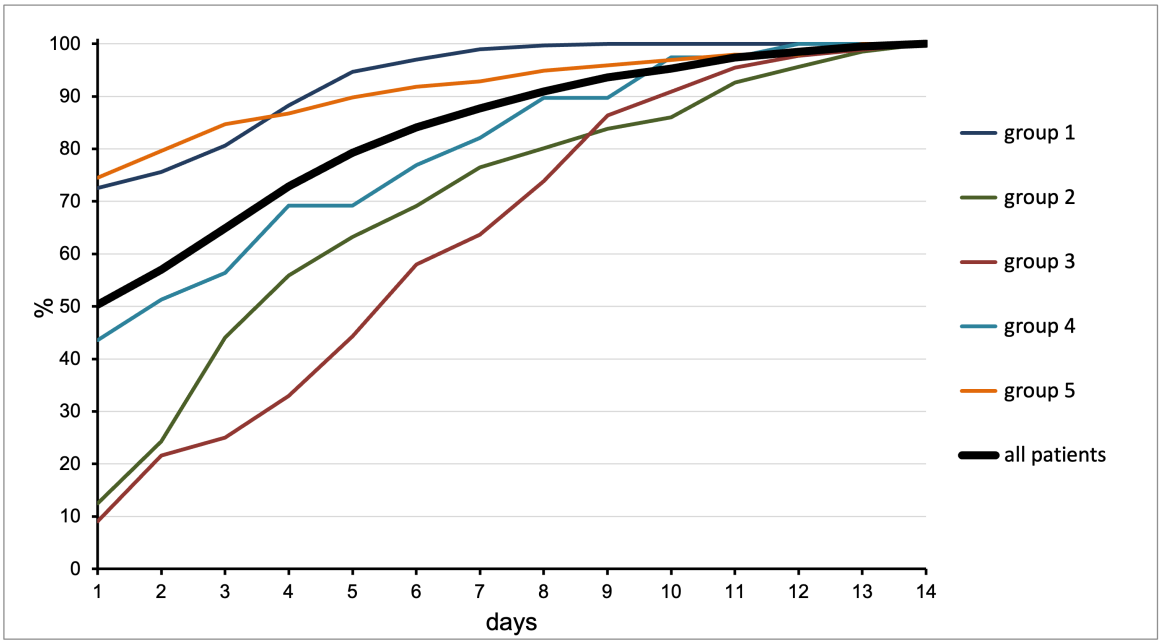

Figure 7. Trajectory stabilization over time. Cumulative percentage of patients (y-axis) for whom the posterior probability of group membership stabilizes at a given time point (x-axis). The legend depicts the colors for the respective trajectory groups as well as for the total cohort.

6 DISCUSSION

6.1 METHODOLOGICAL CONSIDERATIONS

6.1.1 Study design considerations

All studies in this thesis, except for part of study II, are observational, retrospective studies. Hence, they are considered to have lower evidence grade than randomized controlled trials (RCT), generally accepted to be the golden standard of study design. The main advantage of RCTs are their high internal validity, meaning superior control over possible bias second to randomization and blinding. However, well-performed observational studies are shown not to overestimate results and provides results similar to RCTs.[95] Two drawbacks with RCTs are their often rigid design control, making generalization of results problematic and the inability to perform RCT due to unethical or unfeasible reasons. For example, to evaluate an association between pre traumatic B-blockers and mortality, as we did in study I, would be problematic with an RCT design.

In study II, we used an animal model to explore levels of TRX after trauma in comparison to their baseline values, which would have been difficult with human participants.

6.1.2 External validity

We perform studies to draw wider conclusions, inferences, on the population from where the sample is drawn. These studies in this thesis are all conducted in a single center, possibly limiting external validity.

In study I only patients aged 50 years or more were included, hence we cannot state that β-blockade could be beneficial or harmful in younger patients.

Study II-V include patients with severe trauma admitted to the ICU, meaning that inferences to less injured and physiologically stable patients are limited. Due to lack of resources (research nurse availability) and since informed consent was a prerequisite for inclusion of patients to the biobank (TRAUMABIO), potentially eligible patients were not always included. However, patients included in these studies have baseline characteristics, comorbidities and trauma mechanism that were very similar to other studies in the field, although patients included tend to be more injured.[68, 96-99]

6.1.2.1 Random error

Random error describes the influence of chance on our estimates. The risk of random errors decreases with increasing sample size, in contrast to systematic errors which do not. The precision of estimates is influenced by random error and is typically described via confidence intervals and p-values. P-values describe the probability that we would have our sample data given that there was no difference between groups compared (i.e., that the null hypothesis

was true). A p-value of 0.05 was used in our studies, this is an arbitrary level but nevertheless the conventional limit.

Confidence intervals reflects the range of values likely to contain the measure of interest. Or more specifically, if we would have re-sampled our study cohort from the source population 95% of our point estimates would be within that range.

In study I, large sample sizes reduce the impact of random errors and thus results in accordingly smaller CI. However, the CI for the association of β-blocker use and mortality crosses one, meaning that there is a possibility that there is indeed an undetected effect due to lack of power (i.e., type II error). In study II, the sample size was small resulting in for some estimates wide confidence intervals reflecting the uncertainty of the estimates. In studies III-V, the sample size was intermediate. These cohorts illustrate the typical tradeoff between number of study participants and high-resolution, validated data.

6.1.3 Internal validity

6.1.3.1 Misclassification bias

This term is also known as classification bias, information bias, observation bias or measurement bias. It involves the risk of incorrect determination, classification or measurement of exposure, outcome, or important confounders. Has the information on outcomes been collected in the same way for exposed and non-exposed? To minimize the risk of misclassification bias, ideally a researcher unknown to the exposure should gather data regarding the outcome, and vice versa a researcher unknown to the outcome should gather data regarding the exposure. Further, the effect of misclassification bias depends largely on its type. If the information is collected differently for one group of the patients than for the other, the estimates of risk are subsequently affected, falsely raised, or lowered depending on the direction of the bias. If the bias is non-differential, meaning "random noise" and equally affecting both groups, then the bias instead usually tends to mask real differences, often called "bias towards the null".

In study I, we used several national registries. LISA, used for income and education data is considered robust. However, missing data existed, 9% of patients lacked data on education and 5% on income. The national patient register does not carry information on primary care and hence follows the possibility of misclassification of comorbidities, however, possible bias is likely to be non-differential. The analysis with other comorbidity definitions indicates that this may be of minor importance. In study II-V, the data used was gathered prospectively by research nurses unbeknown to the exposure and outcome of interest and had no prior information on planned studies which lower the risk of misclassification bias.

Since the definition of sepsis is of central importance in Study II-IV, this deserves some elaboration. The definition of sepsis is based on suspected or proven infection in conjunction with, for sepsis-2 the SIRS criteria, and for sepsis-3 the SOFA criteria. The problem with those definitions in retrospective trauma studies are twofold.

Firstly, the criteria for both sepsis-2 and sepsis-3 are based on that SIRS or the increase in SOFA should be caused by infection. More specifically, for sepsis-2 "When SIRS is the result of a confirmed infectious process, it is termed sepsis"[39] and for sepsis-3 " Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection".[42] Defining cause and effect is problematic in many circumstances, even more so in retrospective observational studies. We cannot with certainty say that our septic patients, regardless of definition, fulfilled their non-infectious criteria due to infection and not to other non-infectious causes. This problem is inherent to most sepsis studies.

Secondly, for the sepsis-3 definition we used an increase of SOFA score from the previous day as our criteria. This was decided since trauma patients have a highly elevated SOFA score at admission due to the trauma *per se*. Hence, using the admission SOFA score as baseline would result in very few patients developing sepsis. Nevertheless, regardless of SOFA-baseline chosen, a patient in the ICU need not only to increase their SOFA score by two points or more but also override their natural decline in SOFA when recovering from their trauma-related injuries. This aspect is easy to extrapolate to all critically ill patients admitted to the ICU.

The first issue, cause of SIRS or SOFA increase, most likely affects both groups and as such is considered non-differential, however, it may affect incidences.

The second issue, the difficulty of injured patients to increase SOFA by two or more, is more problematic. This would explain our findings in study III where sepsis-3 was much less common than sepsis-2, which is based on SIRS. However, the issue of highly elevated SOFA scores at admission was not closely discussed in the consensus definition of sepsis-3 and we believe that our definition of the sepsis-3 baseline provides a balanced approach to this problem.

6.1.3.2 Selection bias

To address the possibility of selection bias, we may ask ourselves: are the groups similar in all important aspects except for the exposure studied? For study I, all patients included in the trauma registry were also included in the study cohort which limits selection bias. For studies II-V the sample sizes were smaller, in some cases based on informed consent and the availability of research nurses. This may certainly impact generalizability. However, since the data was collected prospectively and without knowledge of future study questions, i.e., the data was collected in the same manner for both exposed and unexposed, this will lessen the probability of selection bias.

6.1.3.3 Confounding

Defined as factors associated with the exposure and affecting the outcome, confounding may result in mixing or blurring of effects. It is different from intermediate factors which are on a causal pathway between exposure and outcome. Confounding can, as opposed to selection and misclassification bias, be controlled for with restriction (excluding subjects with

suspected confounding factors), matching (classically done in case-control studies) and stratification (a form of *post hoc* restriction where the researcher analyses data separately for subjects with and without the confounding factor).

In studies I-IV, we used multivariable logistic regression to control for confounding, however, we were limited in particularly study II by the small sample size. The effect of unmeasured or unadjusted confounders is impossible to rule out. Further, there exists little consensus as to which variables to include or not.

6.1.3.4 Competing risks

Competing risks are traditionally defined as when subjects can experience one or more events or outcomes which "compete" with the outcome of interest. In study III and IV, we anticipated a variant of this potential bias, an event (death) competed with the exposure of interest. In these studies, post-injury sepsis was the exposure of interest and death due to trauma-related injuries was the competing risk of that exposure.

We chose to do a sensitivity analysis where we gradually censored patients dying during the early days after trauma. We hence treated the early deaths as non-informative. In other words, we assumed that the early deaths were not related to the exposure of interest, namely post-injury sepsis. This assumption was based on the fact that sepsis rarely develops on the first day after trauma and as such that patients were not at risk for the exposure. As shown in figure 4, the OR for post-injury sepsis and its association with mortality increases when censoring patients dying at the early stages, this as an effect of censoring non-exposed patients. Our approach could be debated; therefore, we chose to show all days of consecutive censoring.

6.1.3.5 Missing data

The potential bias due to missing data depends on the mechanism causing the data to be missing. In study I, 9% of the patients had missing data on education and 5% on income. The patient register does not include information on comorbidities from primary care and the outpatient part had incomplete coverage during the initial years. The unchanged results regardless of comorbidity definition in Study I suggest that this may be of minor importance. In study II-V the amount of missing data was low, however, these studies suffer from a variant of missing data namely the inability to include potentially eligible patients. Hence, inference must be based on the characteristics of those patients finally included.

6.2 INTERPRETATION OF FINDINGS

6.2.1 Study I

The catecholamine surge seen after severe injury is complex. It is proposed that the catecholamine-induced damage to the endothelium seen after trauma is responsible for post-

traumatic coagulopathy and organ failure.[20, 68] Further, catecholamines, levels of which increase significantly during severe injury, causes an increase and modulation of immune cells.[100, 101] β-blockers are shown to modify this catecholamine induced immune response.[102, 103] In the last decades, the role of β-blockers role in several diseases and conditions has been evaluated. In the trauma setting, most studies focus on TBI. β-blockers are shown in animal TBI models to improve cerebral blood flow and reduce cerebral hypoxia[104] and some authors recommend giving β-blockers to patients with severe TBI after stabilization and resuscitation as a part of a standardized neurocritical protocol.[105] β-blockers have also gained interest as potential treatment for other groups of critically ill patients such as COVID-19 patients, burn victims and septic patients.,

There are several mechanisms suggested for the protective role of β-blockers in a trauma setting. Trauma patients are still generally young; however, we are seeing an aging population with more comorbidities in our trauma-ICUs, a general cardiovascular protective effect in these patients is not unlikely.[106] This is supported by evidence that post-traumatic administration of atenolol is associated with a reduction in the incidence of myocardial injury.[107]

In study I, no major differences in injury severity, mechanisms of injury, proportion of severe head injuries or shock on arrival were seen between patients using β-blockers at the time of trauma and non-users. However, users were older and had more comorbidities. The unadjusted OR for death at 30-days was two times higher for β-blocker users. After adjustments for relevant confounders, no such association was seen. Pre-trauma medication with β-blockers at admission should probably be viewed, and could possibly be used, as a proxy for frailty by the clinician. Head injuries are a major cause of mortality after trauma, and previous studies have indicated a protective effect of β-blockade in TBI patients. In our study, no association was seen in the subgroup of patients with severe TBI.

Our hypothesis was that pre-traumatic β-blockade would be beneficial in trauma patients, but no such effect could be seen. We cannot rule out that such an effect exists, lack of power to detect such a difference could be an explanation. A post hoc power analysis, with all its weaknesses, indicates otherwise with a post hoc power of >90% (data not shown). Another explanation for the lack of association could be residual confounding. Another aspect is that we did not have data on medication, including β-blockade, in the ICU. It is the authors assumption that many if not most of the β-blocker users did not receive β-blockade during the initial days after trauma. Studies have shown that discontinuation of β-blockade during hospitalization is associated with higher risk of mortality.[108, 109] Further, to continue the use of β-blockade during non-cardiac surgery is routine. It is possible that discontinuation of β-blockade for these patients had negative effects.

Baseline characteristics regarding SAP on admission were the same for users and non-users, and no interaction between β-blockade and shock on arrival was seen. Considering the negative effects showed with discontinuation of β-blockade in similar cohorts of patients this might support early re-institution of β-blockade in circulatory stable patients.

In conclusion, β-blockers seem to be a valid choice for the traumatically injured patient with hypertension, but more studies are needed before a general recommendation could be issued.

6.2.2 Study II

A reliable biomarker for sepsis has been termed the holy grail of sepsis. This is not less true for post-injury sepsis, a diagnosis notoriously difficult to identify in the trauma patient with multiple injuries. Clinical signs and common biomarkers of infection are typically masked by the hyper-inflammatory state induced by multiple injuries. Several sepsis biomarkers have been evaluated in the post-traumatic setting.

Oxidative stress occurs continuously during normal physiological conditions. In the generation of ATP via the mitochondrial oxidative phosphorylation, reactive oxygen and nitrogen species are generated as by-products. These species are short-lived and have an essential role in cell-signaling but are highly reactive and cause indiscriminate damage to surrounding molecules if not controlled.[110] Their role is highly regulated by antioxidant systems and in conditions with severe oxidative stress, the thioredoxin system is believed to be one of the most important.[111] In sepsis where antioxidant defenses are overwhelmed, oxidative stress results, which cause significant damage to lipids, proteins, and nucleic acids, both within mitochondria and cells.[112] Further, a link between oxidative stress in sepsis, subsequent mitochondrial dysfunction, failure of energy production and organ dysfunction has been proposed.[113]

In patients with sepsis, serum levels of TRX are significantly elevated.[85, 86] Interestingly treatment with TRX in septic mice increase survival, and neutralizing TRX antibodies showed deleterious effects.[85] These results suggests that TRX plays a critical role in the protection against damage from infection and inflammation, and it could be a potential therapeutic target for modifying a septic course.

We found that TRX was elevated in trauma patients compared to healthy controls and that TRX measured early after trauma was associated with later development of sepsis. The relationship between increasing injury severity, massive transfusion and shock on arrival suggests a link between the degree of injury and oxidative stress. This is supported by other non-trauma studies showing that higher levels of TRX are associated with a more severe disease state.[114-116]

The only variable independently associated with development of post-injury sepsis was plasma TRX at day one. A more thorough analysis with more potential confounders would perhaps reveal other findings. We were, however, limited by the number of patients included in the study.

The association with TRX, sampled in median 16 hours from trauma, and the later development of sepsis (in median 74 hours after trauma) leads us to the question if it is plausible to assume that a biomarker sampled so early after trauma and before sepsis diagnosis could be considered a biomarker instead of a proxy for injury or a mediator

between injury and sepsis. We know that TRX is elevated in several conditions in the critically ill including sepsis. TRX levels in trauma patients could be considered a marker of oxidative stress, a more sensitive and objective measure of the total degree of physiological stress after injury than ISS, massive transfusion, or shock on arrival. Levels of oxidative stress, and possibly exhaustion of anti-oxidative defenses, is associated with complications such as sepsis, and this would support our findings.[112, 117]

The elevation of TRX in trauma patients and the animal model is well in line with previous studies on patients with severe medical conditions such as aortic aneurysms and burns.[114, 118] These findings are consistent with the notion that injury severity, presence of shock and exposure to massive transfusion were associated with high plasma TRX levels in our study.

Being a single-center study with a limited number of patents and samples, our findings should be considered merely as hypothesis-generating that need to be reproduced and validated.

6.2.3 Study III

The lack of a golden standard for the diagnosis of sepsis has resulted in numerous efforts to define this condition with major impact on morbidity and mortality. The ongoing work of defining sepsis reflects upon the complexity of the disease, symptoms and biological processes shared with other disease states, the heterogenous response in patients and its possibility to be evoked by several different pathogens. The definition of sepsis establishes a set of criteria for what sepsis is, as well as what it is not, and subsequently what interventions and treatments may be appropriate. It provides the basis for inclusion in studies, allows benchmarking and performance monitoring as well as providing clinicians a common language in communication.

Understanding the impact of changes to the definition is important for comparing previous studies, designing future studies, healthcare planning and allocation of resources. Accordingly, several studies have compared the sepsis-2 definition with the sepsis-3 definition. The two large validation studies published after the sepsis-3 consensus definition show a similar incidence of both definitions; however, the sepsis-3 definition seems to better discriminate for in-hospital mortality than the sepsis-2 criteria.[43, 119] At the time of publication no comparisons between the two definitions in a trauma context existed.

Our hypothesis in study III was that the SOFA-based sepsis-3 definition would result in fewer patients developing sepsis as compared with the previous sepsis-2 definition. The results from this study confirm our hypothesis, the incidence of sepsis was less than half using the new as compared with the previous definition.

The reason for the large difference in incidence between the two definitions might have several explanations. We had a high incidence of infections compared to other studies. This could partly be explained by the severity of injuries in our cohort with a median ISS of 26. The high injury severity, a known risk factor for post-injury infections and sepsis has likely

contributed to the incidence of infections.[13, 120] Further, the incidence of SIRS, being an unspecific entity, was very high in our cohort. This, together with the noted infection rate may explain the high sepsis-2 incidence. In the assessment of the sepsis-3 criteria, Seymour and colleagues could show that of the scoring systems evaluated, SOFA was the best discriminator of patients with an infection and a high risk of mortality.[43] We could not reproduce their high discriminatory performances for neither sepsis-3 nor sepsis-2 in our trauma cohort. The high SOFA and SIRS frequency at admittance, which both persisted for several days most likely influenced this.

The original work by Singer, validated by Seymour, assumed zero SOFA points as baseline SOFA in patients without pre-existing organ dysfunction. This approach is problematic in trauma patients, our median admission SOFA was five. We instead chose to use the SOFA score from the previous day for calculating the ≥2 increase in points necessary for the sepsis-3 diagnosis. An elevated baseline SOFA score is a general finding in all critically ill patients admitted to an ICU for non-infectious causes. Our approach is debatable, but in the context of trauma patients with a high trauma-related admission SOFA score we found no other approach feasible.

Many patients succumb to their injuries during the first days after trauma when the prevalence of infections is low. Because of this anticipated competing risk of exposure, we performed a continuous censoring of early deaths, which revealed an association for sepsis-3, but not for sepsis-2, with 30-day mortality.

The presented results, with the much lower incidence of post-injury sepsis-3 compared to sepsis-2 highlight the problem of using an organ dysfunction-based score as a prerequisite for diagnosis in patients with organ dysfunction already at admittance. This needs to be addressed in studies of both trauma patients and other cohorts of critically ill patients admitted for non-infectious causes.

6.2.4 **Study IV**

Post-injury sepsis is thought to be an entity different from non-traumatic sepsis. Patients suffering from trauma are generally younger and have fewer comorbidities. The traumatic injury, breaching of body barriers, exogenous bacterial contamination and the release of DAMPs is thought to predispose for post-traumatic infections. The prevention and recognition of infections and sepsis in the traumatically injured patients differs from primary sepsis as it typically presents days after admission.[120, 121]

The introduction of the organ dysfunction-based sepsis-3 criteria poses potentially new challenges. Many patients with trauma present with trauma-related organ dysfunction at admittance. Discriminating between trauma-related and infectious organ dysfunction is challenging and adds complexity to an already heterogenous clinical picture. At the time of writing study IV, few trauma-studies were performed under the organ dysfunction-based sepsis-3 criteria.

Age, spine- and chest-injuries, shock on arrival and blood transfusion were all significantly associated with later sepsis development during the ICU stay in our study. These findings are in line with previous findings performed under the sepsis-2 era and define a high-risk patient in need of vigilance and close monitoring.[12, 13, 53, 122, 123]

The association between sepsis and shock and number of transfusions indicate the need for bleeding control and restoration of homeostasis. Improved survival of severely injured and exsanguinated patients results in survivors that are prone to develop infectious complications.[124] Our finding that blood transfusions were, in a dose-dependent manner, associated with sepsis are in line with other studies in severely injured patients.[122] There are reports that blood transfusions interact with the immune system increasing proneness to develop infections and systemic inflammation.[125-127] Further, following transfusion it is reported that up to 25% of red blood cells are hemolyzed within 24 hours. Red blood cells contain DAMPs and the subsequent release is shown to stimulate the innate immune system.[128] The dose-dependent appearance for the risk of infectious complications gives support to the concept of limited instead of overly aggressive resuscitation, damage-control strategies and goal-directed therapy.

A third of the patients had detectable blood alcohol levels at admission which, in our study was a risk factor for post-injury sepsis. Associations between presence of blood alcohol and pneumonia have been reported[129] and chronic alcohol consumption has been shown to associate with pneumonia and organ dysfunction in trauma patients.[130, 131] The implications of post-injury sepsis on the clinical course, where septic patients experienced a several-fold increase in days with multiple organ dysfunction, mechanical ventilation and vasopressor treatment. The increase in length of stay could be explained by the fact that the septic patients were older and had more severe injuries. Nevertheless, sepsis is known to have a significant effect on LOS in the ICU.[132]

We could not show an association between post-injury sepsis and mortality. This was not unexpected since our severely injured cohort experienced a high initial mortality, largely occurring before these patients had the risk to develop sepsis. The subsequent analysis, where we censored patients dying at the early stages, showed a significant association between post-injury sepsis and mortality.

In summary, study IV confirms many of the risk factors previously shown under the sepsis-2 era. In addition, blood alcohol at admission was shown to be associated with post-injury sepsis. The results from study IV serve to better identify patients at risk for post-injury sepsis and target preventive measures and close monitoring.

6.2.5 **Study V**

Organ dysfunction after trauma has classically been studied as an arbitrarily defined entity, using in a European context for example a total SOFA >5 or >2 SOFA points in at least two organ systems. Criticism against this static approach has been raised. Patients with 3 SOFA points in the central nervous and respiratory domains have most likely not experienced the

same injuries or share the same prognosis as patients with 3 points in other domains. Further, the temporal aspect of organ dysfunction has often been neglected or in best case arbitrarily summarized. Steps have been taken to identifying phenotypes of disease in the critically ill. ARDS and sepsis are examples.[63, 65] These rely predominately on early presentation data and patterns. The temporal aspect of phenotypes and evolution of different trajectories are less explored. This is no less true for organ dysfunction.

Study V analyses the temporal trajectories of SOFA-based organ dysfunction using information on all individual SOFA domains over 14 days after trauma.

Several techniques are available to model temporal trajectories. We used group-based trajectory modeling (GBTM) for this purpose. The advantage of this technique, compared to another used variant of finite mixture modeling: growth mixture modelling, is the simpler estimation and less probability to experience convergence issues. Further, GBTM has been shown to perform well against other forms of longitudinal modeling.[133]

To the best of our knowledge no previous studies have incorporated both the temporal aspect and different patterns of organ dysfunction. A previous study analyzed the patterns of total SOFA score over time in trauma patients. This study identified three groups, one mainly consisting of TBI patients, one group severe injury and one with lesser injuries.[97] Unfortunately, the use of the total SOFA score, as compared to the individual SOFA domains, limits comparison.

In study V, five groups were identified, each with their unique combination of the six SOFA organ domains.

Group 1, mild OD, in general had a favorable outcome. These patients were younger, less injured and had a low burden of comorbidity. The use of organ supportive therapy and incidence of sepsis was negligible. The mild OD group found its trajectory quickly and exemplify a trauma patient with low risk of complications that possibly can be identified at an early stage.

Group 2 and 3, moderate and severe organ dysfunction, are perhaps the most interesting. They had similar admission characteristics and organ dysfunction patterns during the first days after trauma. However, group 3 were somewhat more injured and older, whereas group 2 had more patients in shock on admission. The time to trajectory stabilization for these groups were by far the longest. At day 5 after trauma only 50% of the patients in these two groups had found their trajectory. The incidence of post-injury sepsis differed in these groups; for group 2, moderate organ dysfunction, 18% experienced sepsis during the ICU and HDU stay. This contrasts with group 3 were 56% developed sepsis. One explanation for the long time to stabilization could be that these patients with initially similar organ dysfunction patterns changed their trajectory due to complications such as sepsis. It is shown in several studies that post-injury sepsis complicates the clinical course, increases LOS and mortality.

This possibly contributed to the more complicated clinical course in group 3 as compared to group 2.[12, 134]

Group 4, extreme organ dysfunction, were severely injured and massively transfused. They experienced a complicated clinical course with sepsis seen in 72% of these patients. The organ dysfunction was considerable and sustained. Renal and liver dysfunction was marked and increased during the first week and they had the most days on renal support of all groups. The high need of renal replacement therapy in this group is not surprising, hemorrhagic shock and hypoperfusion are known risk factors for acute kidney injury after trauma.[135, 136] The need of blood transfusions and its association with post-injury sepsis was noted in study IV as well as in previous studies.[12] This group had a very high mortality, but the time to death was in median over a week indicating a complicated clinical course with vast organ supportive therapy. This group of highly injured patients are readily recognizable clinically and pose a significant challenge in the ICU.

Group 5, TBI with organ dysfunction, the group with most severe head injuries and lowest GCS had the highest mortality. This is not surprising. TBI is reported to be the main cause of death after trauma thus a high mortality is expected.[30, 137] The short time to death most likely reflects the high prevalence of refractory head injuries. This group also had a significant amount of cardiovascular and respiratory support, at least partly explained by vasopressors use and respiratory support to control cerebral perfusion pressure and ventilation. This specific entity of trauma patients found their trajectory rapidly, with over 80% of the patients assigned to their group within 3 days after trauma. Accordingly, TBI patients are highly discernible and exhibit an early well-defined trajectory of organ dysfunction.

Study V underlines the heterogenous clinical course after trauma. It indicates that there are subsets of patients with an initially undefined clinical course that might benefit from targeted support, increased monitoring and vigilance. Further, study V provides a methodology to identify and separate different phenotypes of organ dysfunction after trauma. It exemplifies a way to use the full range of available clinical data instead of expert-based, arbitrarily chosen cut-offs or only baseline data for defining organ dysfunction and clustering of patients.

7 FUTURE PERSPECTIVES AND CLINICAL CONSIDERATIONS

Post-traumatic complications, and in particular post-injury sepsis and organ dysfunction, is still a major concern in our patients. Protective and preventive measures are much needed to improve morbidity and mortality.

It is possible that β-blockers do possess a protective effect despite our negative findings in study I. A possible explanation for our lack of association could be that patients need a longer, continuous β-blockade after trauma to achieve a protective effect. Studies incorporating medications during the ICU and hospital stay might reveal other findings. Patients admitted for general surgery are recommended to continue their medication of β-blockade and it might be that a protective effect was blurred due to the negative consequences of potential withdrawal of β-blockade in many severely injured trauma patients.

Early identification of complications is essential for prompt treatment and improving outcomes. TRX, evaluated in study II, is a promising candidate for a post-injury sepsis biomarker. However, given the small number of included patients and samples in study II and the resulting limitation of confounder adjustments, our results must be seen as hypothesis generating. We are currently collecting plasma samples from trauma patients, and we hope that new studies on TRX, especially on the temporal kinetics might prove useful in the evaluation of TRX in post-injury sepsis.

Criticism against the sepsis-2 criteria, including the lack of specificity of SIRS, exemplified by reports that 50% of hospitalized patients had SIRS and 84% of all patient-days in a surgical ICU were SIRS-positive, motivated the move to sepsis-3.[138, 139] However, the introduction of the sepsis-3 criteria raises new, but similar issues which were examined in study III. In the trauma setting there is a problem with sepsis-3 given its SOFA-based criteria and the issue of baseline SOFA. Using admission SOFA as baseline results in few trauma patients ever fulfilling the sepsis-3 criteria as the baseline score is elevated by trauma *per se*. On the other hand, assuming zero points (or 1-2 points for known comorbidities) as a baseline result in virtually all infected trauma patients fulfilling the sepsis-3 criteria. This is also true for other critically ill patients being admitted for non-infectious causes. This might not be a major problem in a clinical context as a patient with suspected infection and sepsis should be treated regardless of formal criteria. However, it does pose a problem in a research context. When including septic patients in a trial, evaluating risk factors for sepsis, or estimating incidences of sepsis after some condition, this problem must be considered. In the last decades, many interventional trials in patients with sepsis have resulted in neutral findings. This was followed by a discussion on the sepsis-2 definition being too unspecific, allowing non-specific inclusion of patients, resulting in heterogenous study populations with diverse clinical courses. This could be an explanation for why many of these large trials have failed to show an effect of potentially important interventions. The sepsis-3 definition generally

results in fewer patients diagnosed than when using the old definition. The patients diagnosed according to sepsis-3 seem to have an increased risk of adverse outcomes and possibly also have more to gain from therapeutic interventions. This knowledge might prove useful in future trials in traumatically injured patients with sepsis.

Most likely there is a window of opportunity when early treatment can attenuate or negate the dire consequences of post-injury sepsis. Biomarkers and risk factors are helpful in this context. However, many risk factors for post-injury sepsis are non-modifiable from a hospital or ICU point of view. Those identified in study IV; injury localisation, age, shock on arrival and blood alcohol levels on admission are examples if this. Blood transfusions showed a dose-dependent pattern for the risk of post-injury sepsis. This finding may very well be a proxy for severity of injury, despite our efforts to adjust for this. The recognition of a patient at high risk for post-injury sepsis should alert the treating physician and call for close monitoring and vigilance.

Study V showed that some groups of severely injured patients consume many resources in the intensive care and a large difference in mortality was seen between the groups identified. Furthermore, it seems that some of these severely injured patients are predestined at an early stage to follow a certain trajectory, for example those with mild OD and those with TBI, group 1 and 5 respectively. These early defined trajectories may be difficult to influence in the ICU setting. However, the trajectories of patients that do not stabilize early, group 2 and 3, are perhaps modifiable. Close monitoring, repetitive sampling of infectious markers and blood cultures, early antibiotics and radiology might be motivated in these patients.

8 CONCLUSIONS

Treatment with β-blockers in the peri-traumatic period did not have an association with short-term mortality after adjustment for relevant confounders. However, the use of β-blockers could be considered a sign of a high-risk patient with an increased mortality risk because of the high prevalence of comorbidities

Thioredoxin was elevated in response to severe trauma and associated with post-injury sepsis after adjustment for injury related factors.

The change from the old sepsis-2 definition to the current sepsis-3 definition cuts the incidence of sepsis in half. Both definitions show poor discriminatory properties for overall 30-day mortality.

Sepsis is common among ICU treated patients after severe injury. Age, spine- and chest-injuries, shock on arrival, blood alcohol levels at admission and blood transfusions were found to be risk factors for post-injury sepsis. Post-injury sepsis was associated with 30-day mortality but only after censoring of early deaths.

We identified five distinct trajectories of organ dysfunction in intensive care treated trauma patients, each with substantial differences in admission characteristics, clinical course, and outcomes. The findings indicate subsets of patients with an initial undefined course that might benefit from targeted support.

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10 REFERENCES

1. van Breugel JMM, Niemeyer MJS, Houwert RM, Groenwold RHH, Leenen LPH, van Wessem KJP. Global changes in mortality rates in polytrauma patients admitted to the ICU-a systematic review. *World J Emerg Surg*. 2020;15(1):55.
2. *Injuries and violence, the facts 2014.* 2014. www.who.int Accessed October 28, 2021.
3. Swedish Board of Health and Welfare, Causes of death 2019. www.socialstyrelsen.se Accessed October 28, 2021
4. Diseases GBD, Injuries C. Global burden of 369 diseases and injuries in 204 countries and territories, 1990-2019: a systematic analysis for the Global Burden of Disease Study 2019. *Lancet*. 2020;396(10258):1204-1222.
5. Rating the severity of tissue damage. I. The abbreviated scale. *Jama*. 1971;215(2):277-80.
6. Baker SP, O'Neill B, Haddon W, Jr., Long WB. The injury severity score: a method for describing patients with multiple injuries and evaluating emergency care. *The Journal of trauma*. 1974;14(3):187-96.
7. Osler T, Baker SP, Long W. A modification of the injury severity score that both improves accuracy and simplifies scoring. *The Journal of trauma*. 1997;43(6):922-5; discussion 925-6.
8. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med*. 1996;22(7):707-10.
9. Frohlich M, Wafaisade A, Mansuri A, et al. Which score should be used for posttraumatic multiple organ failure? - Comparison of the MODS, Denver- and SOFA- Scores. *Scand J Trauma Resusc Emerg Med*. 2016;24(1):130.
10. Moreno R, Vincent JL, Matos R, et al. The use of maximum SOFA score to quantify organ dysfunction/failure in intensive care. Results of a prospective, multicentre study. Working Group on Sepsis related Problems of the ESICM. *Intensive Care Med*. 1999;25(7):686-96.
11. Vincent JL, de Mendonca A, Cantraine F, et al. Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. *Critical care medicine*. 1998;26(11):1793-800.
12. Wafaisade A, Lefering R, Bouillon B, et al. Epidemiology and risk factors of sepsis after multiple trauma: an analysis of 29,829 patients from the Trauma Registry of the German Society for Trauma Surgery. *Critical care medicine*. 2011;39(4):621-8.
13. Kisat M, Villegas CV, Onguti S, et al. Predictors of sepsis in moderately severely injured patients: an analysis of the National Trauma Data Bank. *Surgical infections*. 2013;14(1):62-8.
14. Weuster M, Bruck A, Lippross S, et al. Epidemiology of accidental hypothermia in polytrauma patients: An analysis of 15,230 patients of the TraumaRegister DGU. *The journal of trauma and acute care surgery*. 2016;81(5):905-912.

15. Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. *Int Rev Immunol*. 2011;30(1):16-34.

16. Zhang Q, Raoof M, Chen Y, et al. Circulating mitochondrial DAMPs cause inflammatory responses to injury. *Nature*. 2010;464(7285):104-7.

17. Relja B, Land WG. Damage-associated molecular patterns in trauma. *Eur J Trauma Emerg Surg*. 2020;46(4):751-775.

18. Timmermans K, Kox M, Vaneker M, et al. Plasma levels of danger-associated molecular patterns are associated with immune suppression in trauma patients. *Intensive Care Med*. 2016;42(4):551-561.

19. Simmons JD, Lee YL, Mulekar S, et al. Elevated levels of plasma mitochondrial DNA DAMPs are linked to clinical outcome in severely injured human subjects. *Annals of surgery*. 2013;258(4):591-6; discussion 596-8.

20. Johansson PI, Stensballe J, Ostrowski SR. Shock induced endotheliopathy (SHINE) in acute critical illness - a unifying pathophysiologic mechanism. *Crit Care*. 2017;21(1):25.

21. Mazzoni MC, Intaglietta M, Cragoe EJ, Jr., Arfors KE. Amiloride-sensitive Na+ pathways in capillary endothelial cell swelling during hemorrhagic shock. *J Appl Physiol (1985)*. 1992;73(4):1467-73.

22. van Meurs M, Wulfert FM, Knol AJ, et al. Early organ-specific endothelial activation during hemorrhagic shock and resuscitation. *Shock*. 2008;29(2):291-9.

23. Czabanka M, Peter C, Martin E, Walther A. Microcirculatory endothelial dysfunction during endotoxemia--insights into pathophysiology, pathologic mechanisms and clinical relevance. *Curr Vasc Pharmacol*. 2007;5(4):266-75.

24. Ivanov KP, Mel'nikova NN. Leukocytes as a cause of microcirculatory dysfunction. *Bull Exp Biol Med*. 2006;141(6):666-8.

25. Szopinski J, Kusza K, Semionow M. Microcirculatory responses to hypovolemic shock. *The Journal of trauma*. 2011;71(6):1779-88.

26. Baker CC, Oppenheimer L, Stephens B, Lewis FR, Trunkey DD. Epidemiology of trauma deaths. *Am J Surg*. 1980;140(1):144-50.

27. Demetriades D, Kimbrell B, Salim A, et al. Trauma deaths in a mature urban trauma system: is "trimodal" distribution a valid concept? *Journal of the American College of Surgeons*. 2005;201(3):343-8.

28. de Knegt C, Meylaerts SA, Leenen LP. Applicability of the trimodal distribution of trauma deaths in a Level I trauma centre in the Netherlands with a population of mainly blunt trauma. *Injury*. 2008;39(9):993-1000.

29. Gunst M, Ghaemmaghami V, Gruszecki A, Urban J, Frankel H, Shafi S. Changing epidemiology of trauma deaths leads to a bimodal distribution. *Proc (Bayl Univ Med Cent)*. 2010;23(4):349-54.

30. Dutton RP, Stansbury LG, Leone S, Kramer E, Hess JR, Scalea TM. Trauma mortality in mature trauma systems: are we doing better? An analysis of trauma mortality patterns, 1997-2008. *The Journal of trauma*. 2010;69(3):620-6.

31. Pfeifer R, Tarkin IS, Rocos B, Pape HC. Patterns of mortality and causes of death in polytrauma patients--has anything changed? *Injury*. 2009;40(9):907-11.

32. Ingraham AM, Xiong W, Hemmila MR, et al. The attributable mortality and length of stay of trauma-related complications: a matched cohort study. *Annals of surgery*. 2010;252(2):358-62.

33. Shafi S, Barnes S, Nicewander D, et al. Health care reform at trauma centers--mortality, complications, and length of stay. *The Journal of trauma*. 2010;69(6):1367-71.

34. Ulvik A, Kvale R, Wentzel-Larsen T, Flaatten H. Multiple organ failure after trauma affects even long-term survival and functional status. *Crit Care*. 2007;11(5):R95.

35. Hemmila MR, Jakubus JL, Maggio PM, et al. Real money: complications and hospital costs in trauma patients. *Surgery*. 2008;144(2):307-16.

36. Bastani A, Galens S, Rocchini A, et al. ED identification of patients with severe sepsis/septic shock decreases mortality in a community hospital. *Am J Emerg Med*. 2012;30(8):1561-6.

37. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. *Critical care medicine*. 2006;34(6):1589-96.

38. Rivers EP, Katranji M, Jaehne KA, et al. Early interventions in severe sepsis and septic shock: a review of the evidence one decade later. *Minerva Anestesiol*. 2012;78(6):712-24.

39. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest*. 1992;101(6):1644-55.

40. Sprung CL, Sakr Y, Vincent JL, et al. An evaluation of systemic inflammatory response syndrome signs in the Sepsis Occurrence In Acutely Ill Patients (SOAP) study. *Intensive Care Med*. 2006;32(3):421-7.

41. Kaukonen KM, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. *The New England journal of medicine*. 2015;372(17):1629-38.

42. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama*. 2016;315(8):801-10.

43. Seymour CW, Liu VX, Iwashyna TJ, et al. Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama*. 2016;315(8):762-74.

44. Shankar-Hari M, Phillips GS, Levy ML, et al. Developing a New Definition and Assessing New Clinical Criteria for Septic Shock: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *Jama*. 2016;315(8):775-87.

45. Shankar-Hari M, Harrison DA, Rubenfeld GD, Rowan K. Epidemiology of sepsis and septic shock in critical care units: comparison between sepsis-2 and sepsis-3 populations using a national critical care database. *British journal of anaesthesia*. 2017;119(4):626-636.

46. Fang X, Wang Z, Yang J, et al. Clinical Evaluation of Sepsis-1 and Sepsis-3 in the ICU. *Chest*. 2017;

47. Donnelly JP, Safford MM, Shapiro NI, Baddley JW, Wang HE. Application of the Third International Consensus Definitions for Sepsis (Sepsis-3) Classification: a retrospective population-based cohort study. *The Lancet Infectious diseases*. 2017;17(6):661-670.

48. Brattstrom O, Granath F, Rossi P, Oldner A. Early predictors of morbidity and mortality in trauma patients treated in the intensive care unit. *Acta anaesthesiologica Scandinavica*. 2010;54(8):1007-17.

49. Osborn TM, Tracy JK, Dunne JR, Pasquale M, Napolitano LM. Epidemiology of sepsis in patients with traumatic injury. *Critical care medicine*. 2004;32(11):2234-40.

50. Shalhub S, Junker CE, Imahara SD, Mindrinos MN, Dissanaike S, O'Keefe GE. Variation in the TLR4 gene influences the risk of organ failure and shock posttrauma: a cohort study. *The Journal of trauma*. 2009;66(1):115-22; discussion 122-3.

51. Balci C, Sivaci R, Akbulut G, Karabekir HS. Procalcitonin levels as an early marker in patients with multiple trauma under intensive care. *The Journal of international medical research*. 2009;37(6):1709-17.

52. Croce MA, Fabian TC, Waddle-Smith L, Maxwell RA. Identification of early predictors for post-traumatic pneumonia. *Am Surg*. 2001;67(2):105-10.

53. Papia G, McLellan BA, El-Helou P, et al. Infection in hospitalized trauma patients: incidence, risk factors, and complications. *The Journal of trauma*. 1999;47(5):923-7.

54. Trentzsch H, Lefering R, Nienaber U, Kraft R, Faist E, Piltz S. The role of biological sex in severely traumatized patients on outcomes: a matched-pair analysis. *Annals of surgery*. 2015;261(4):774-80.

55. Tilney NL, Bailey GL, Morgan AP. Sequential system failure after rupture of abdominal aortic aneurysms: an unsolved problem in postoperative care. *Annals of surgery*. 1973;178(2):117-22.

56. Eiseman B, Beart R, Norton L. Multiple organ failure. *Surg Gynecol Obstet*. 1977;144(3):323-6.

57. Baue AE. MOF, MODS, and SIRS: what is in a name or an acronym? *Shock*. 2006;26(5):438-49.

58. Sauaia A, Moore EE, Johnson JL, et al. Temporal trends of postinjury multiple-organ failure: still resource intensive, morbid, and lethal. *The journal of trauma and acute care surgery*. 2014;76(3):582-92, discussion 592-3.

59. Frohlich M, Lefering R, Probst C, et al. Epidemiology and risk factors of multiple-organ failure after multiple trauma: an analysis of 31,154 patients from the TraumaRegister DGU. *The journal of trauma and acute care surgery*. 2014;76(4):921-7.

60. Ciesla DJ, Moore EE, Johnson JL, Burch JM, Cothren CC, Sauaia A. A 12-year prospective study of postinjury multiple organ failure: has anything changed? *Arch Surg*. 2005;140(5):432-8; discussion 438-40.

61. Minei JP, Cuschieri J, Sperry J, et al. The changing pattern and implications of multiple organ failure after blunt injury with hemorrhagic shock. *Critical care medicine*. 2012;40(4):1129-35.

62. Dewar DC, Tarrant SM, King KL, Balogh ZJ. Changes in the epidemiology and prediction of multiple-organ failure after injury. *The journal of trauma and acute care surgery*. 2013;74(3):774-9.

63. Calfee CS, Delucchi K, Parsons PE, et al. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. *Lancet Respir Med*. 2014;2(8):611-20.

64. Liu X, Jiang Y, Jia X, et al. Identification of distinct clinical phenotypes of acute respiratory distress syndrome with differential responses to treatment. *Crit Care*. 2021;25(1):320.

65. Seymour CW, Kennedy JN, Wang S, et al. Derivation, Validation, and Potential Treatment Implications of Novel Clinical Phenotypes for Sepsis. *Jama*. 2019;

66. Gardlund B, Dmitrieva NO, Pieper CF, Finfer S, Marshall JC, Taylor Thompson B. Six subphenotypes in septic shock: Latent class analysis of the PROWESS Shock study. *Journal of critical care*. 2018;47:70-79.

67. Knox DB, Lanspa MJ, Kuttler KG, Brewer SC, Brown SM. Phenotypic clusters within sepsis-associated multiple organ dysfunction syndrome. *Intensive Care Med*. 2015;41(5):814-22.

68. Johansson PI, Henriksen HH, Stensballe J, et al. Traumatic Endotheliopathy: A Prospective Observational Study of 424 Severely Injured Patients. *Annals of surgery*. 2017;265(3):597-603.

69. Spasiano A, Barbarino C, Marangone A, et al. Early thromboelastography in acute traumatic coagulopathy: an observational study focusing on pre-hospital trauma care. *Eur J Trauma Emerg Surg*. 2020;

70. Carroll RC, Craft RM, Langdon RJ, et al. Early evaluation of acute traumatic coagulopathy by thrombelastography. *Transl Res*. 2009;154(1):34-9.

71. Arbabi S, Campion EM, Hemmila MR, et al. Beta-blocker use is associated with improved outcomes in adult trauma patients. *The Journal of trauma*. 2007;62(1):56-61; discussion 61-2.

72. Neideen T, Lam M, Brasel KJ. Preinjury beta blockers are associated with increased mortality in geriatric trauma patients. *The Journal of trauma*. 2008;65(5):1016-20.

73. Ferraris VA, Ferraris SP, Saha SP. The relationship between mortality and preexisting cardiac disease in 5,971 trauma patients. *The Journal of trauma*. 2010;69(3):645-52.

74. Castelli GP, Pognani C, Cita M, Paladini R. Procalcitonin as a prognostic and diagnostic tool for septic complications after major trauma. *Critical care medicine*. 2009;37(6):1845-9.

75. Meisner M, Adina H, Schmidt J. Correlation of procalcitonin and C-reactive protein to inflammation, complications, and outcome during the intensive care unit course of multiple-trauma patients. *Crit Care*. 2006;10(1):R1.

76. Egger G, Aigner R, Glasner A, Hofer HP, Mitterhammer H, Zelzer S. Blood polymorphonuclear leukocyte migration as a predictive marker for infections in severe trauma: comparison with various inflammation parameters. *Intensive Care Med*. 2004;30(2):331-334.

77. Wacker C, Prkno A, Brunkhorst FM, Schlattmann P. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. *The Lancet Infectious diseases*. 2013;13(5):426-35.

78. Wanner GA, Keel M, Steckholzer U, Beier W, Stocker R, Ertel W. Relationship between procalcitonin plasma levels and severity of injury, sepsis, organ failure, and mortality in injured patients. *Critical care medicine*. 2000;28(4):950-7.

79. Sakran JV, Michetti CP, Sheridan MJ, et al. The utility of procalcitonin in critically ill trauma patients. *The journal of trauma and acute care surgery*. 2012;73(2):413-8; discussion 418.

80. Billeter A, Turina M, Seifert B, Mica L, Stocker R, Keel M. Early serum procalcitonin, interleukin-6, and 24-hour lactate clearance: useful indicators of septic infections in severely traumatized patients. *World journal of surgery*. 2009;33(3):558-66.

81. Ciriello V, Gudipati S, Stavrou PZ, Kanakaris NK, Bellamy MC, Giannoudis PV. Biomarkers predicting sepsis in polytrauma patients: Current evidence. *Injury*. 2013;44(12):1680-92.

82. Level C, Chauveau P, Guisset O, et al. Mass transfer, clearance and plasma concentration of procalcitonin during continuous venovenous hemofiltration in patients with septic shock and acute oliguric renal failure. *Crit Care*. 2003;7(6):R160-6.

83. Halldorsdottir HD, Eriksson J, Persson BP, et al. Heparin-binding protein as a biomarker of post-injury sepsis in trauma patients. *Acta anaesthesiologica Scandinavica*. 2018;62(7):962-973.

84. Leaver SK, MacCallum NS, Pingle V, et al. Increased plasma thioredoxin levels in patients with sepsis: positive association with macrophage migration inhibitory factor. *Intensive Care Med*. 2010;36(2):336-41.

85. Hofer S, Rosenhagen C, Nakamura H, et al. Thioredoxin in human and experimental sepsis. *Critical care medicine*. 2009;37(7):2155-9.

86. Brenner T, Rosenhagen C, Steppan J, et al. Redox responses in patients with sepsis: high correlation of thioredoxin-1 and macrophage migration inhibitory factor plasma levels. *Mediators of inflammation*. 2010;2010:985614.

87. Li X, Shen H, Zhou T, et al. Early Elevation of Thioredoxin-1 Serum Levels Predicts 28-Day Mortality in Patients with Sepsis. *J Inflamm Res*. 2021;14:3837-3848.

88. Chiara O, Pelosi P, Brazzi L, et al. Resuscitation from hemorrhagic shock: experimental model comparing normal saline, dextran, and hypertonic saline solutions. *Critical care medicine*. 2003;31(7):1915-22.

89. Hildebrand F, Andruszkow H, Huber-Lang M, Pape HC, van Griensven M. Combined hemorrhage/trauma models in pigs-current state and future perspectives. *Shock*. 2013;40(4):247-73.

90. Velik-Salchner C, Schnurer C, Fries D, et al. Normal values for thrombelastography (ROTEM) and selected coagulation parameters in porcine blood. *Thromb Res*. 2006;117(5):597-602.

91. Frith D, Cohen MJ, Brohi K. Animal models of trauma-induced coagulopathy. *Thromb Res*. 2012;129(5):551-6.

92. Swedish Board of Health and Welfare, Dödsorsaksstatistik. Historik, produktionsmetoder och tillförlitlighet. 2010.

93. DeLong ER, DeLong DM, Clarke-Pearson DL. Comparing the areas under two or more correlated receiver operating characteristic curves: a nonparametric approach. *Biometrics*. 1988;44(3):837-45.

94. Calandra T, Cohen J, International Sepsis Forum Definition of Infection in the ICUCC. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. *Critical care medicine*. 2005;33(7):1538-48.

95. Concato J, Shah N, Horwitz RI. Randomized, controlled trials, observational studies, and the hierarchy of research designs. *The New England journal of medicine*. 2000;342(25):1887-92.

96. Shepherd JM, Cole E, Brohi K. Contemporary Patterns of Multiple Organ Dysfunction in Trauma. *Shock*. 2017;47(4):429-435.

97. Cole E, Gillespie S, Vulliamy P, Brohi K. Multiple organ dysfunction after trauma. *The British journal of surgery*. 2020;107(4):402-412.

98. Bardes JM, Inaba K, Schellenberg M, et al. The contemporary timing of trauma deaths. *The journal of trauma and acute care surgery*. 2018;84(6):893-899.

99. Hutchings L, Watkinson P, Young JD, Willett K. Defining multiple organ failure after major trauma: A comparison of the Denver, Sequential Organ Failure Assessment, and Marshall scoring systems. *The journal of trauma and acute care surgery*. 2017;82(3):534-541.

100. Schedlowski M, Hosch W, Oberbeck R, et al. Catecholamines modulate human NK cell circulation and function via spleen-independent beta 2-adrenergic mechanisms. *J Immunol*. 1996;156(1):93-9.

101. Jetschmann JU, Benschop RJ, Jacobs R, et al. Expression and in-vivo modulation of alpha- and beta-adrenoceptors on human natural killer (CD16+) cells. *J Neuroimmunol*. 1997;74(1-2):159-64.

102. Friese RS, Barber R, McBride D, Bender J, Gentilello LM. Could Beta blockade improve outcome after injury by modulating inflammatory profiles? *The Journal of trauma*. 2008;64(4):1061-8.

103. Bible LE, Pasupuleti LV, Alzate WD, et al. Early propranolol administration to severely injured patients can improve bone marrow dysfunction. *The journal of trauma and acute care surgery*. 2014;77(1):54-60; discussion 59-60.

104. Ley EJ, Scehnet J, Park R, et al. The in vivo effect of propranolol on cerebral perfusion and hypoxia after traumatic brain injury. *The Journal of trauma*. 2009;66(1):154-9; discussion 159-61.

105. Khalili H, Ahl R, Paydar S, et al. Beta-Blocker Therapy in Severe Traumatic Brain Injury: A Prospective Randomized Controlled Trial. *World journal of surgery*. 2020;44(6):1844-1853.

106. Burstow M, Civil I, Hsee L. Trauma in the Elderly: Demographic Trends (1995-2014) in a Major New Zealand Trauma Centre. *World journal of surgery*. 2019;43(2):466-475.

107. Cruickshank JM, Neil-Dwyer G, Degaute JP, et al. Reduction of stress/catecholamine-induced cardiac necrosis by beta 1-selective blockade. *Lancet*. 1987;2(8559):585-9.

108. Prins KW, Neill JM, Tyler JO, Eckman PM, Duval S. Effects of Beta-Blocker Withdrawal in Acute Decompensated Heart Failure: A Systematic Review and Meta-Analysis. *JACC Heart Fail*. 2015;3(8):647-53.

109. Noveanu M, Breidthardt T, Reichlin T, et al. Effect of oral beta-blocker on short and long-term mortality in patients with acute respiratory failure: results from the BASEL-II-ICU study. *Crit Care*. 2010;14(6):R198.

110. Galley HF. Oxidative stress and mitochondrial dysfunction in sepsis. *British journal of anaesthesia*. 2011;107(1):57-64.

111. Lowes DA, Galley HF. Mitochondrial protection by the thioredoxin-2 and glutathione systems in an in vitro endothelial model of sepsis. *Biochem J*. 2011;436(1):123-32.

112. Goode HF, Cowley HC, Walker BE, Howdle PD, Webster NR. Decreased antioxidant status and increased lipid peroxidation in patients with septic shock and secondary organ dysfunction. *Critical care medicine*. 1995;23(4):646-51.

113. Crouser ED. Mitochondrial dysfunction in septic shock and multiple organ dysfunction syndrome. *Mitochondrion*. 2004;4(5-6):729-41.

114. Martinez-Pinna R, Lindholt JS, Blanco-Colio LM, et al. Increased levels of thioredoxin in patients with abdominal aortic aneurysms (AAAs). A potential link of oxidative stress with AAA evolution. *Atherosclerosis*. 2010;212(1):333-8.

115. Shim YK, J-T. Serum Thioredoxin 1 Level Has Close Relation with Myocardial Damage Amount in Acute Myocardial Infarction Patients. 2012;

116. Takahashi K, Chin K, Nakamura H, et al. Plasma thioredoxin, a novel oxidative stress marker, in patients with obstructive sleep apnea before and after nasal continuous positive airway pressure. *Antioxidants & redox signaling*. 2008;10(4):715-26.

117. Cowley HC, Bacon PJ, Goode HF, Webster NR, Jones JG, Menon DK. Plasma antioxidant potential in severe sepsis: a comparison of survivors and nonsurvivors. *Critical care medicine*. 1996;24(7):1179-83.

118. Abdiu A, Nakamura H, Sahaf B, Yodoi J, Holmgren A, Rosen A. Thioredoxin blood level increases after severe burn injury. *Antioxidants & redox signaling*. 2000;2(4):707-16.

119. Raith EP, Udy AA, Bailey M, et al. Prognostic Accuracy of the SOFA Score, SIRS Criteria, and qSOFA Score for In-Hospital Mortality Among Adults With Suspected Infection Admitted to the Intensive Care Unit. *Jama*. 2017;317(3):290-300.

120. Eguia E, Cobb AN, Baker MS, et al. Risk factors for infection and evaluation of Sepsis-3 in patients with trauma. *Am J Surg*. 2019;

121. Mas-Celis F, Olea-Lopez J, Parroquin-Maldonado JA. Sepsis in Trauma: A Deadly Complication. *Arch Med Res*. 2021;

122. Nederpelt CJ, El Hechi M, Parks J, et al. The dose-dependent relationship between blood transfusions and infections after trauma: A population-based study. *The journal of trauma and acute care surgery*. 2020;89(1):51-57.

123. Jaja BNR, Jiang F, Badhiwala JH, et al. Association of Pneumonia, Wound Infection, and Sepsis with Clinical Outcomes after Acute Traumatic Spinal Cord Injury. *Journal of neurotrauma*. 2019;36(21):3044-3050.

124. Lord JM, Midwinter MJ, Chen YF, et al. The systemic immune response to trauma: an overview of pathophysiology and treatment. *Lancet*. 2014;384(9952):1455-65.

125. Hensler T, Heinemann B, Sauerland S, et al. Immunologic alterations associated with high blood transfusion volume after multiple injury: effects on plasmatic cytokine and cytokine receptor concentrations. *Shock*. 2003;20(6):497-502.

126. Dunne JR, Malone DL, Tracy JK, Napolitano LM. Allogenic blood transfusion in the first 24 hours after trauma is associated with increased systemic inflammatory response syndrome (SIRS) and death. *Surgical infections*. 2004;5(4):395-404.

127. Beale E, Zhu J, Chan L, Shulman I, Harwood R, Demetriades D. Blood transfusion in critically injured patients: a prospective study. *Injury*. 2006;37(5):455-65.

128. Vourc'h M, Roquilly A, Asehnoune K. Trauma-Induced Damage-Associated Molecular Patterns-Mediated Remote Organ Injury and Immunosuppression in the Acutely Ill Patient. *Front Immunol*. 2018;9:1330.

129. Ahmed N, Greenberg P. Examining the influence of blood alcohol level on the incidence of pneumonia & sepsis complications following traumatic injury. *Alcohol*. 2019;76:111-115.

130. Moss M, Burnham EL. Chronic alcohol abuse, acute respiratory distress syndrome, and multiple organ dysfunction. *Critical care medicine*. 2003;31(4 Suppl):S207-12.

131. von Heymann C, Langenkamp J, Dubisz N, et al. Posttraumatic immune modulation in chronic alcoholics is associated with multiple organ dysfunction syndrome. *The Journal of trauma*. 2002;52(1):95-103.

132. Bohmer AB, Just KS, Lefering R, et al. Factors influencing lengths of stay in the intensive care unit for surviving trauma patients: a retrospective analysis of 30,157 cases. *Crit Care*. 2014;18(4):R143.

133. Elmer J, Jones BL, Nagin DS. Comparison of parametric and nonparametric methods for outcome prediction using longitudinal data after cardiac arrest. *Resuscitation*. 2020;148:152-160.

134. Eguia E, Bunn C, Kulshrestha S, et al. Trends, Cost, and Mortality From Sepsis After Trauma in the United States: An Evaluation of the National Inpatient Sample of Hospitalizations, 2012-2016. *Critical care medicine*. 2020;

135. Harrois A, Soyer B, Gauss T, et al. Prevalence and risk factors for acute kidney injury among trauma patients: a multicenter cohort study. *Crit Care*. 2018;22(1):344.

136. Nasu T, Ueda K, Kawashima S, et al. Prehospital Blood Pressure and Lactate are Early Predictors of Acute Kidney Injury After Trauma. *The Journal of surgical research*. 2021;265:180-186.

137. Lansink KW, Gunning AC, Leenen LP. Cause of death and time of death distribution of trauma patients in a Level I trauma centre in the Netherlands. *Eur J Trauma Emerg Surg*. 2013;39(4):375-83.

138. Churpek MM, Zadravecz FJ, Winslow C, Howell MD, Edelson DP. Incidence and Prognostic Value of the Systemic Inflammatory Response Syndrome and Organ Dysfunctions in Ward Patients. *Am J Respir Crit Care Med*. 2015;192(8):958-64.

139. Pittet D, Rangel-Frausto S, Li N, et al. Systemic inflammatory response syndrome, sepsis, severe sepsis and septic shock: incidence, morbidities and outcomes in surgical ICU patients. *Intensive Care Med*. 1995;21(4):302-9.