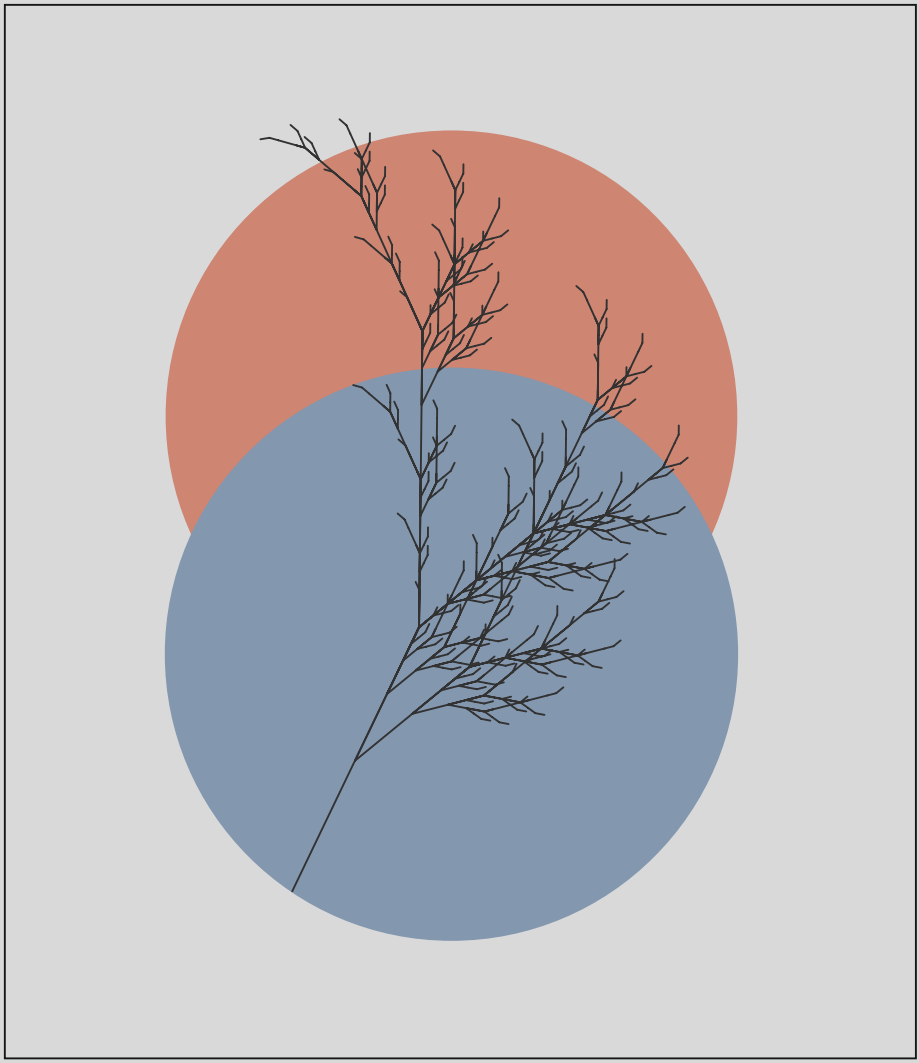
EXPLORING BODY TEMPERATURE ALTERATIONS IN THE CRITICALLY ILL

M.B.A. HARMON



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“Nobody exists on purpose. Nobody belongs anywhere. Everybody’s gonna die. Come watch TV”

- Morty

For mom and dad

Contents

Chapter one

General introduction and outline of this thesis

Body temperature alterations in critically ill patients

Body temperature alterations are common in critically ill patients in the intensive care unit (ICU). In a study examining body temperature alterations on the ICU, 9% of patients presented with or developed hypothermia and 28% fever during their ICU admission.[1] These body temperature alterations can occur spontaneously as a result of underlying pathologic processes, or they may be clinically induced as part of a patient's treatment.

Spontaneous body temperature alterations are associated with a range of different pathologic conditions in the intensive care. Sepsis is the most common cause of both fever and hypothermia. Fever may also occur after brain injury, i.e. after traumatic brain injury or hypoxic-ischemic brain injury post cardiac arrest. Less common causes of fever in the ICU include endocrine- or drug-induced fever.[2] Besides spontaneous hypothermia in sepsis, a number of other factors can cause hypothermia requiring ICU admission, including cold exposure, endocrine abnormalities and drug overdoses.[1,3]

Induced temperature alterations or targeted temperature management (TTM), in which body temperature is physically or pharmacologically altered, is used to treat several pathologic conditions in the ICU. TTM includes normalizing body temperature, for example actively cooling patients to normothermia with traumatic brain injury or rewarming patients with spontaneous hypothermia in sepsis. But it can also imply actively inducing a non-normothermic body temperature. In hypoxic-ischemic brain injury after cardiac arrest, fever is neurologically detrimental[4] and TTM in which patients are cooled to 32°C-36°C, is now a cornerstone in mitigating brain injury.[5] In sepsis, both induced normothermia and induced hypothermia are being explored as experimental clinical therapies.[6,7] The chapters presented in this thesis are introduced in the following paragraphs.

Spontaneous temperature alterations in sepsis

Fever: the pathophysiology of a febrile response

Normal body temperatures in healthy individuals range from 35.6°C to 37.7°C and fluctuate over the course of a day.[8] An elevated body temperature is commonly termed fever or pyrexia. The term hyperthermia is also used but denotes a pathologic increase in body temperature that is not related to an altered hypothalamic set point.[9]

Fever is a highly conserved, phylogenetic response induced by both infectious and noninfectious causes.[10] During an infection, fever is elicited by pathogen associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS),

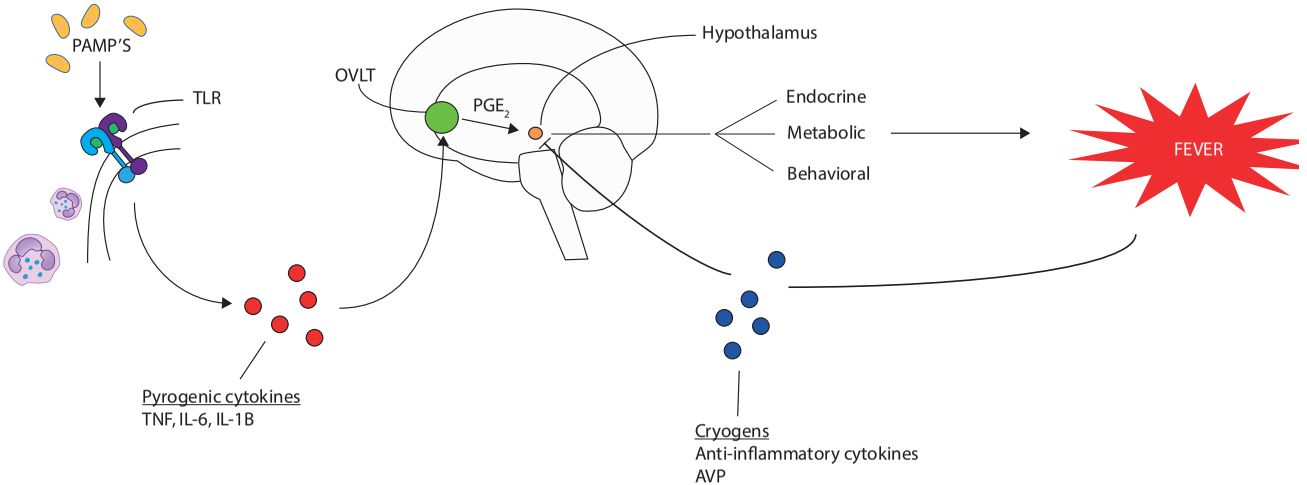
interacting with immune cells through toll-like receptors (TLRs). This interaction induces the synthesis of endogenous pyrogenic cytokines, including interleukin (IL)-1β, tumor necrosis factor (TNF) α, and IL-6. In turn, these pyrogens elicit the release of prostaglandins, mainly type E2, from the brain. Prostaglandin E2 interacts with the pre-optic area of the hypothalamus, activating several post-hypothalamic heat generating and heat conserving mechanisms. These include warmth seeking behavior and shivering as well as autonomic reactions such as decreased sweating and vasoconstriction of superficial vessels in an attempt to conserve heat.[11] The body also initiates several hormonal and metabolic mechanisms including a decrease in vasopressin, which decreases the amount of fluid in the body that needs to be heated, and increased heat thermogenesis from brown adipose tissue. Fever is tightly regulated by glucocorticoids, neuropeptides and anti-inflammatory cytokines.[12] These mechanisms are highlighted in figure 1. There are also other fever pathways, including a pathway that acts independently of pyrogenic cytokines.[13]

The effects of fever

Fever has both beneficial effects and adverse effects. These beneficial effects mostly relate to the body's ability to fight an infection. During an infection, fever enhances components of both the innate and adaptive immune response[10] For the innate immunity these include improved neutrophil taxation and phagocytosis.[14,15] Regarding the adaptive immunity, fever promotes lymphocyte trafficking, increasing the chance that rare antigen specific T-cells come into contact with activating dendritic cells.[10] In addition, fever promotes and stabilizes binding to antigen presenting cells and increase CD-8 T-cell differentiation. Immune cell adhesion to the endothelium via L-selectin is also improved.[16] Fever also promotes the expression of heat shock proteins, which are important for cellular protection and can potentially reduce endothelial and organ damage during proteotoxic stress such as infection.[10] Finally, fever inhibits the growth of pathogens such as *s. pneumoniae* in experimental study designs.[17] However, to inhibit the growth of some pathogens, the bodies temperature has to rise to supraphysiologic temperatures, so this mechanism may not always be viable. Taken together, fever augments several important aspects of the immune response to a pathogen.

Fever can also harm patients. Patients with an injured brain subjected to fever have unfavorable outcome.[18] The mechanism for increased brain injury may include increased circulation of free radicals[19] and also increased metabolism. Increasing body temperature from 38°C to 41°C in anesthetized and mechanically ventilated dogs results in a 20% increase in oxygen consumption.[20] The increased cerebral oxygen consumption augments ischemic injury and can increase cerebral

Figure 1. Schematic overview of the pathogen induced febrile response in humans.



Abbreviations: AVP = arginine vasopressin; iL = interleukin; OVLT = organum vasculosum of the lamina terminalis; PAMPs = pathogen associated molecular patterns; PGE_2 = Prostaglandin E2; TLR = toll like receptors; TNF = tumor necrosis factor;

blood flow, increasing intracranial pressure.[21] In sepsis the increased metabolic demand of fever could exceed the hosts metabolic or cardiopulmonary capacities, exacerbating tissue hypoperfusion and tissue hypoxemia resulting in increased organ failure.[22] This can be exacerbated by sepsis induced cardiopulmonary disfunction. In addition, enhanced host defenses may directly cause collateral tissue injury.[22] In an lipopolysaccharide (LPS) model, exposure to hyperthermia increased neutrophil localization to the lungs potentially increasing the risk for lung injury.[15,23]

Pathophysiology of the hypothermic response in sepsis.

Hypothermia in sepsis is generally defined as a temperature below 36.0°C. Patients with hypothermic sepsis have substantially higher mortality rates compared to nonhypothermic septic patients.[24] Interventions targeting hypothermic septic patients could therefore potentially have a profound impact on clinical outcome. However, our understanding of the hypothermic response in sepsis is limited and mechanisms underlying the hypothermic response remain largely hypothetical.

In light of the perceived immune benefits of fever, the fact that patients develop hypothermia during a severe infection is intriguing and has led to the assumption that hypothermia in sepsis is a result of some physiological or biochemical failure to produce or conserve heat.[25,26] However, body temperature changes during infection have been preserved throughout evolution arguing that both fever and hypothermia provide a survival advantage for vertebrates.[10] In line with this, experimental studies point to hypothermia representing an adaptive response in the face of severe inflammation.[10,26-28] Animals with more severe infections deliberately attempt to lower their body temperature compared to animals with less severe infections which attempt to increase body temperature.[29] Heat conserving or generating mechanisms are intact in hypothermic animals faced with endotoxin shock, indicating that hypothermia may not be a dysfunction of the thermoregulatory system.[30] A recent study found that the conversion from fever to hypothermia may be initiated as a strategy to prevent cellular hypoxia.[31] However, these studies have only been performed in rodents and comparing the thermodynamic response to infection between rodents and humans is difficult, due to differences in their respective thermoregulatory responses.[32]

In contrast, clinical studies to date have mainly focused on impaired thermoregulation as a cause of hypothermia. Of note, interpreting results from observational studies in hypothermic septic patients is inherently difficult due to confounding. Patients with hypothermic sepsis are sicker in comparison to febrile septic patients and any observations in hypothermic patients may also be attributed to increased disease severity.[33,34]

Potential defects along fever induction pathways in figure 1 could hypothetically result in hypothermia. These can be divided in pre-hypothalamic, hypothalamic and post-hypothalamic mechanisms.[35] Pre-hypothalamic mechanisms include the ability to induce fever through proinflammatory cytokines as previously discussed. These not only mediate the febrile response but are also essential in mounting an adequate host response to invading pathogens. Observational clinical studies on these pre-hypothalamic mechanisms have yielded some minimal understanding of the hypothermic response.[26,33-35] Hypothermic patients are not deficient of proinflammatory cytokines, the main driver of the febrile response. TNFα, iL1-b and iL-6 levels have been shown be either similar[33,35] or even increased in hypothermic patients.[26] A retrospective study found that hypothermic septic patients are inclined to develop abnormally low levels of lymphocytes, also called lymphopenia.[34] In line with this finding, afebrile septic patients have reduced human leukocyte antigen (HLA)-DR monocyte expression, a measure of sepsis induced immunosuppression.[36] The increased incidence of lymphopenia in hypothermic septic patients indicates that these patients might be prone to sepsis-induced immunosuppression and subsequent nosocomial infections.[34] However, data is conflicting on whether hypothermic septic patients suffer from an increased incidence of nosocomial infections.[36,37] Hypothalamic and post-hypothalamic mechanisms for spontaneous hypothermia have not been studied in septic patients. Mechanisms could include altered thermal setpoint due to sepsis induced CNS-dysfunction or defective post-hypothalamic heat generating mechanisms such as endothelial dysfunction resulting in inadequate heat preservation.[35]

Clinical aspects of the hypothermic response in sepsis.

Patients presenting with spontaneous hypothermia and sepsis suffer from higher incidences of shock[1,24,38] and organ dysfunction[24,26,35,39], including increased central nervous system dysfunction.[39] Coincidingly, patients with hypothermic sepsis have a higher mortality rate, almost twice that of patients presenting with fever.[24,40] Despite the high mortality rate in hypothermic sepsis, hypothermia does not appear to be a premortal sign in sepsis. Most patients rewarm to normothermia prior to death indicating that thermoregulatory capacity may still be intact in these patients.[26,28] Interestingly, when exploring the temporal relationship between developing hypothermia and the chances of developing respiratory distress or shock, these events appear to occur independently from hypothermia.[28] Other interesting observations in hypothermic septic patients include a lack of shivering.[26] this could indicate a dysfunction of the bodies heat generating mechanisms, but could also be the result of a downward shift in the body temperature threshold

for activating aerobic heat production while the capacity for aerobic metabolism is not yet compromised.[28]

In general, old age is commonly associated with body temperature alterations.[41] Elderly have reduced sweat gland output, altered cutaneous vasomotor control and decreased cardiac output limiting their ability to regulate heat.[42] However, several studies reported no significant difference in age when comparing hypothermic patients to nonhypothermic patients.[24,26,35]

Taken together, the mechanisms underlying the hypothermic response in sepsis are not understood. Further understanding of the hypothermic response is crucial for optimal treatment of these patients and warrants further investigation.

Induced body temperature alterations

Manipulation of body temperature in sepsis

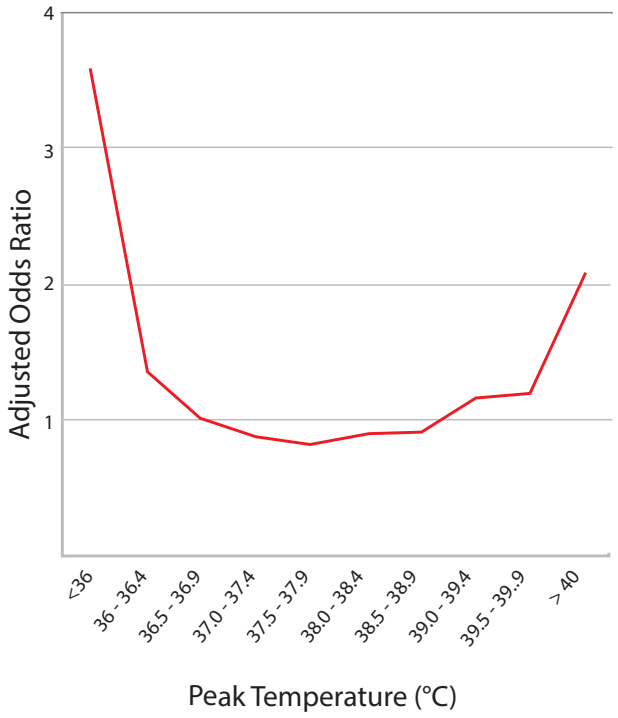
TTM in sepsis encompasses both the practice of actively cooling septic patients, as well as rewarming hypothermic septic patients. These practices are a result of clinical observations on the relationship between body temperature and mortality. Figure 2 shows the odds of mortality for septic patients at different peak body temperatures in the first 24 hours of ICU admission, adjusted for disease severity. These observations suggest that both high grade fever (temperatures > 40°C) as well as hypothermia may be detrimental to patients. In clinical practice, to counteract fever in sepsis, body temperature may pharmacologically or physically lowered, or in the case of hypothermia body temperature is physically increased.[43] However, whether manipulation of body temperature is beneficial in sepsis, is not known.

Induced normothermia and induced hypothermia in sepsis

During sepsis, fever could be driving the cellular metabolic deficit and cytotoxicity due to enhanced immune effectors discussed above.[22] Actively cooling septic patients with fever to normothermic or hypothermic temperatures are experimental therapies aimed at limiting the hyperinflammatory response in sepsis and lowering metabolism. Patients with sepsis and fever can be physically cooled to normothermia, termed induced normothermia or fever control. Patients with sepsis and fever can also be cooled to hypothermic temperatures, termed induced hypothermia (or therapeutic hypothermia).

Applying induced normothermia and induced hypothermia is controversial in sepsis, for the most part due to the perceived benefits of fever in sepsis which could potentially be negated by cooling.[13] However, preclinical studies in animals have shown that induced hypothermia may improve outcome in sepsis. In a rat

Figure 2. The relationship between peak body temperature in the first 24 hours of intensive care admission and adjusted odds ratio for in-hospital mortality patients with an infection.



The figure was taken from Young et al.[44]

model of experimental sepsis, hypothermia reduced proinflammatory cytokine levels.[45] Induced hypothermia also reduced bacterial dissemination in a rat model of pneumococcal pneumosepsis and improved mitochondrial respiration.[46] However, translation of these results to clinical practice has proven difficult. In a subsequent randomized clinical trial, cooling to hypothermia (32°C-34°C) failed to decrease mortality and even delayed recovery of several organ functions indicating an adverse effect of cooling.[7]

A large clinical trial on treatment with induced normothermia to 36.5°C -37.0°C for 48 hours has shown potentially promising results. In patients with fever and septic shock, induced normothermia reduced the need for vasopressors and improved 14-day mortality.[6] These results are contrasted by two other trials that showed adverse hemodynamic effects and increased mortality due to induced

normothermia (target temperatures in these studies were 36.5°C - 38°C and 36.0°C - 37.5°C respectively).[47,48] Comparing the results from these clinical studies is difficult, due to different temperatures management strategies and, in one case, lack of a control group.[6,47,48] The results from these trials warrants further research on the effects and side effects of induced normothermia in sepsis. Induced normothermia is a cheap treatment modality that is easily applicable in ICUs and could potentially benefit septic patients, but needs to be better understood before clinical application in patients.

Rewarming of spontaneous hypothermia in sepsis

TTM in sepsis also encompasses the rewarming of hypothermic septic patients to normothermia. Rewarming spontaneous hypothermic septic patients is likely a common clinical practice, possibly in an attempt to reverse the perceived negative effects of hypothermia.[26,28] However, it is not known whether rewarming improves outcome. Also, there are limited data on the extent and specifics of rewarming practices nor are there clinical studies on the optimal rewarming strategy in hypothermic sepsis. This is important as rewarming can also be harmful; in cardiac arrest rewarming after treatment with therapeutic hypothermia induces iL-6 and complement activation as well other as markers of ischemia reperfusion.[49] Rewarming rates of > 0.5°C can even adversely affect neurologic outcome.[50] If the spontaneous hypothermic response in sepsis is indeed an adaptive response, the practice of rewarming could ultimately turn out to be unnecessary or even harmful.[28]

TTM in cardiac arrest

TTM in which body temperature is actively maintained at 32-36°C for 24 hours after cardiac arrest has become the main treatment modality for mitigating hypoxic-ischemic brain injury after cardiac arrest.[5] TTM may decrease the severity of brain dysfunction after cardiac arrest through several mechanisms; by reducing cerebral metabolism, decreasing ischemic reperfusion injury and preventing subsequent cellular apoptosis.[51] TTM may also decrease the production of free radicals[52] and stabilize disruption of the blood-brain barrier, preventing edema.[51]

Initially, patients post-cardiac arrest were cooled to 33°C following the results of two clinical trials.[53,54] In 2013 the TTM-trial showed that patients who were cooled to 36°C compared to 33°C had similar mortality rates and neurologic outcome.[55] As a result, current guidelines recommend that patients body temperature be maintained anywhere between 32°C and 36°C for 24 hours.[56] The TTM-trial provides a unique opportunity to study physiology during TTM as well as assess the effects of cooling to two different target temperatures.

Respiratory targets such as $PaCO_2$ can influence the risk of secondary brain-injury post cardiac arrest.[57] Both hypercapnia and hypocapnia have been associated with adverse neurologic outcome.[58] Moreover, ventilator settings may affect lung-brain interactions and impact neurologic outcome in cardiac arrest.[59] Therefore, it is important to examine mechanical ventilation practices and their association with outcome in patients with cardiac arrest. This can aid in establishing respiratory targets in order to optimize ventilatory settings post-cardiac arrest aimed at mitigating hypoxic-ischemic brain injury.

Patients with hypoxic-ischemic brain injury are also at risk for nosocomial infections resulting in increased duration of mechanical ventilation and increased ICU stay.[60] There are several interesting aspects relating to the relationship between TTM and these nosocomial infections. For one, cooling to hypothermia can increase the risk for nosocomial infections.[61] Previous TTM research has even suggested that the microbiological make up of these infections could depend on the chosen target temperature, with relatively more gram-negative organisms in patients treated with hypothermia compared to patients not treated with TTM.[60] Also, the difficulties in diagnosing infection during TTM may result in delay in initiation of antibiotic treatment with subsequent increased duration of ICU- and hospital-stay.[62] This may prompt the question whether the use of prophylactic antibiotics may reduce infectious complications in cardiac arrest patients.

Taken together, body temperature can have a profound impact on clinical outcome but our understanding of spontaneous and induced temperature alterations in the ICU is incomplete and warrants further exploration. Induced normothermia is a cheap treatment modality that is easily applicable in ICUs and could potentially benefit septic patients, but needs to be better understood before clinical application in patients.

Part I of this thesis will examine patient characteristics and treatment of patients with hypothermic sepsis, as well as explore mechanisms of the hypothermic response in sepsis.

Part II of this thesis will focus on induced normothermia in sepsis. Using a model of human endotoxemia, which mimics the hyperinflammatory state in sepsis at the physiological and biological level [63], we studied the effects of induced normothermia on physiology, inflammation and coagulation.

Part III of this thesis will explore the effects of different target temperatures in cardiac arrest on mechanical ventilation, gas exchange and nosocomial infections using data collected during and after the TTM-trial.

Outline of this thesis

Part I: Spontaneous hypothermia in sepsis

Chapter 2: We characterized differences in definition and rewarming practices of spontaneous hypothermia during sepsis among physicians in an online survey. We also assessed current opinions on the etiology and treatment of rewarming patients with spontaneous hypothermia during sepsis.
Chapter 3: In a prospective observational study, risk factors for spontaneous hypothermia in sepsis were identified. We also studied the host immune response and markers of endothelial activation. The hypothesis was that patients with spontaneous septic hypothermia could not generate an adequate host immune response and that parameters of endothelial to determine the etiology of the hypothermic response.
Chapter 4: We analyzed whole-genome transcriptome in leukocytes. The hypothesis was that canonical specific signaling pathways in spontaneous hypothermia in sepsis differed from non-hypothermic patients. This study was done in order to generate new hypotheses towards the etiology of the hypothermic response.

Part II: Induced normothermia in human endotoxemia

Chapter 6: We used a human endotoxemia model to study the effects of induced normothermia on physiologic parameters and inflammation. The hypothesis was that induced normothermia would improve hemodynamics and decrease markers of inflammation.
Chapter 7: We used a human endotoxemia model to study the effects of induced normothermia on coagulation and endothelial activation in order to determine if induced normothermia attenuated endotoxemia induced coagulation abnormalities. We hypothesized that induced normothermia would mitigate LPS-induced coagulation abnormalities.

Part III: TTM and cardiac arrest

Chapter 8: We examined the effects of targeted temperature management on mechanical ventilation in patients after cardiac arrest to determine association with outcome. We hypothesized that treatment with TTM at 33°C was associated with a lower minute volume ventilation compared to TTM at 36°C.
Chapter 9: We explored the role that arterial carbon dioxide levels may have in the neurological outcome of cardiac arrest patients, and analyzed the interaction between mild hypercapnia and TTM in relation to neurological outcome.

Chapter 10: We describe the microbiological profile of infectious complications in patients with cardiac arrest and studied the effects of prophylactic antibiotics on these infections. We also examined the impact of TTM at 33°C compared to TTM at 36°C on the microbiological profile of these infections. We hypothesized that use of prophylactic antibiotic use would be associated with less infectious complications in cardiac arrest patients.

The final chapters provide a general discussion of the studies in this thesis in **Chapter 11** and Dutch summary in **chapter 12.**

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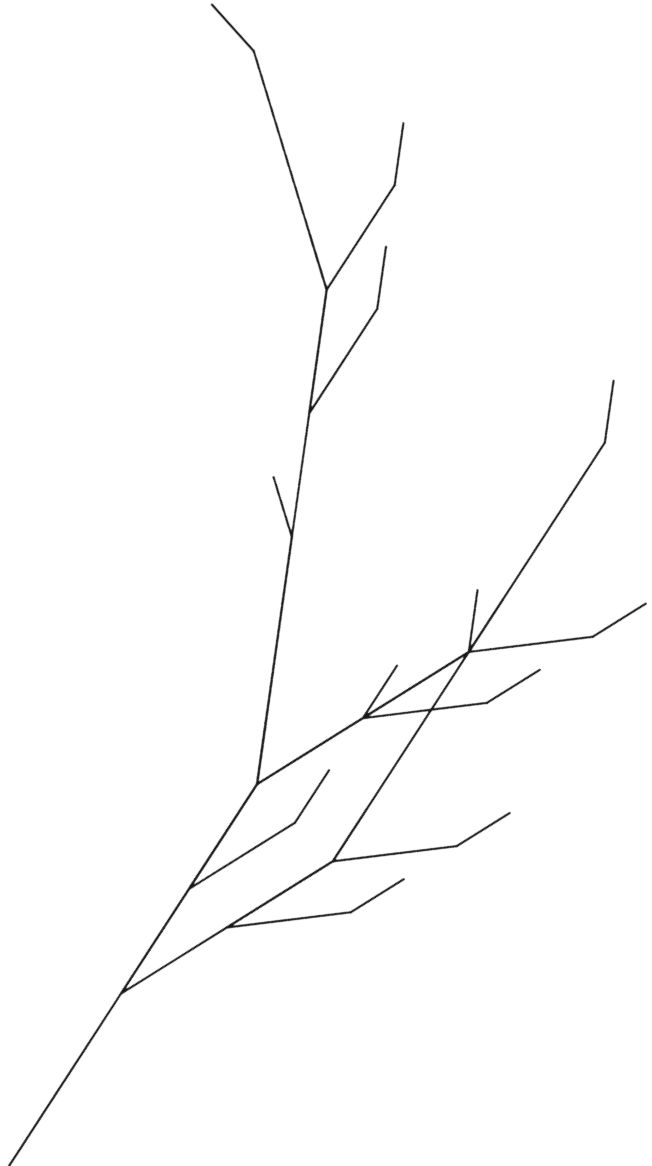

Part one

Chapter two

Opinions and Management of Hypothermic Sepsis: Results from an Online Survey

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Abstract

Introduction Hypothermia is associated with high mortality in sepsis, but it is now recognized that this association may simply reflect its higher prevalence in sicker patients. Furthermore, there is evidence to suggest that hypothermia may not represent a dysfunction in sepsis. In this study, we conducted a survey to assess how this scientific evidence relates to the perceptions of health care professionals regarding septic hypothermia, and how such perceptions drive clinical conduct concerning the use of active rewarming in this population.

Methods A survey with questions on opinions and management of spontaneous hypothermia in sepsis was developed and posted online at the European Society of Intensive Care Medicine (ESICM) website from March 24th, 2017 to the June 26th, 2017 and distributed by electronic email. Respondents were asked to fill in the survey from the perspective of their usual or average practice in their intensive care unit.

Results In total, there were 440 survey respondents. Respondents were predominantly from Europe (66%) The majority of respondents were intensivists (78%) and worked in an academic hospital (66%). One percent of respondents were nurses. Most respondents (96%) reported that there was no protocol for the management of hypothermic sepsis. Of the respondents, 62% actively rewarmed patients with hypothermic sepsis. Hypothermia was defined as a temperature below 36°C (44%) and below 35°C (15%). Rewarming practices showed large variation in terms of the temperature, at which respondents initiate rewarming as well as the target temperature to which patients are rewarmed. The most predominant first-line rewarming method was forced-warm air followed by warm IV fluids. Rewarming decisions were mostly physician driven (58%). Most respondents thought rewarming was beneficial (43%), a small proportion thought rewarming to be harmful (9%).

Conclusions In conclusion, policies, procedures, and beliefs about spontaneous hypothermia and active rewarming in patients with sepsis are variable. This must be taken into consideration in designing future trials. We propose a working group to define hypothermic sepsis to improve comparability of research.

Introduction

An altered core body temperature is a hallmark of sepsis. Although fever is most commonly associated with sepsis, hypothermia occurs spontaneously in 10–35% of septic patients at the time of admission[1] and in an additional 30% of the patients during the intensive care unit (ICU) stay.[2] Development of hypothermia is associated with a twofold increase in mortality compared to patients presenting with fever.[1] This association has led to the hypothesis that hypothermia is a dysregulated, detrimental phenomenon in sepsis.

However, there is an increasing understanding that the association between hypothermia and adverse outcome does not imply that hypothermia directly effects outcome, but that this association may be the result of increased illness severity in patients with hypothermia compared to nonhypothermic patients.[3] The notion that septic hypothermia reflects dysfunction has also been challenged by the finding that hypothermia in septic patients not subjected to active rewarming is a transient, self-limiting response that becomes rare when organ dysfunction is at a maximum in the hours preceding death.[2] The same finding also raises the question as to whether it is necessary to actively rewarm those septic patients who develop hypothermia.

It is currently unknown how these scientific lines of evidence relate to the perceptions of health care professionals regarding septic hypothermia, as is unknown how such perceptions drive clinical conduct concerning the use of active rewarming in these cases. We conducted an online survey on septic hypothermia to address these questions. Using this survey, we aimed to characterize differences in definition and rewarming practices of spontaneous hypothermia during sepsis and determine to what extent these practices are protocolized. Second, we aimed to assess current opinion on the effect of spontaneous hypothermia and rewarming patients with spontaneous hypothermia during sepsis. We hypothesized that rewarming practices and opinions regarding spontaneous hypothermia would be highly variable.

Methods

Survey

A survey containing questions about opinions and management of hypothermic sepsis was developed for this study (Supplementary Data S1). The survey was tested several times for unambiguous interpretation of questions by distributing the survey among intensivists in the ICU of the Academic Medical Center in Amsterdam and was revised according to specific comments of respondents.

It was then posted on the website European Society of Intensive Care Medicine (ESICM) and electronically mailed to members of the following ESICM sections: Systemic Inflammation and Sepsis (3268 members); Trauma and Emergency Medicine (7186 members); and Cardiovascular Dynamics (11,685 members). The survey was online from March 24th, 2017 to June 26th, 2017. Respondents were asked to fill in the survey from the perspective of the usual or average practice on their ICU. IP-addresses were used to provide an indication of the amount of potential duplicate responses.

Statistical analysis

All analyses were performed in R (version 3.1.1) and SPSS version 24. Normally distributed data is presented a mean ± (standard deviation). Non-parametric data is presented as median (25-75th percentile).

Results

In total, there were 440 respondents to the survey. Of these, 288 (66%) of respondents were working in Europe, 67 in Asia (15%), and 39 (9%) in Latin America. 341 (78%) of respondents were intensivists. The main specialty area of respondents was intensive care (288 [59%]) followed by anesthesiology (114 [26%]) and internal medicine (40 [9%]). Respondents mainly worked in an academic hospital (290 [66%]) versus nonacademic hospital (150 [34%]). Reported years of ICU experience were evenly distributed; the largest portion of respondents (28%) reported 0–5 years of ICU experience. Only three (1%) nurses filled in the survey. Table 1 shows the characteristics of respondents in the survey.

Rewarming: definitions and practice.

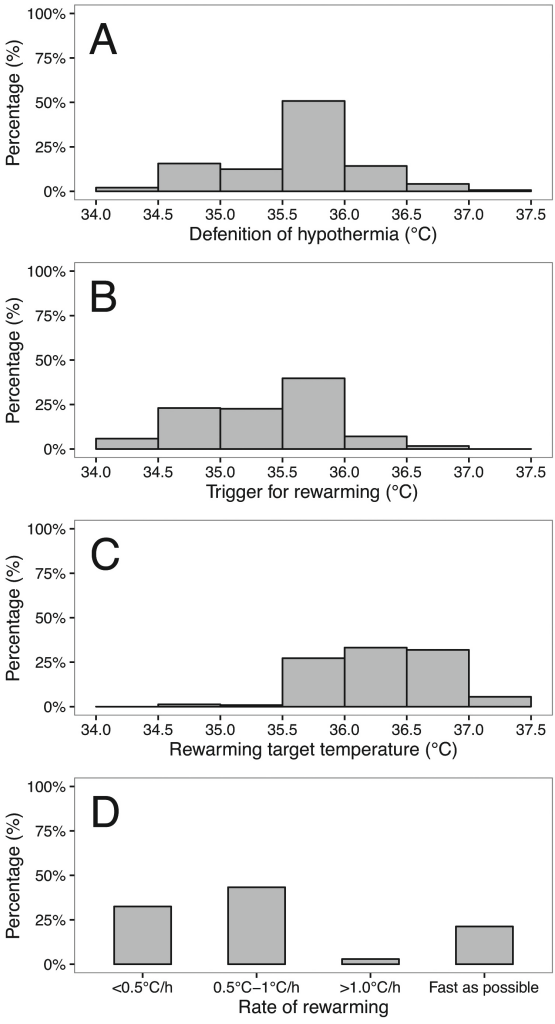
Among the 440 respondents, 96% indicated that there was no written protocol for the management of hypothermic septic patients at their institution. Sixty-two percent of respondents reported that they actively rewarmed patients with spontaneous hypothermia during sepsis, compared to 38% who did not rewarm patients. Respondents were asked to specify per 0.1°C what their definition of hypothermia was. There was a wide range of definitions of hypothermia ranging from 34.0°C to 37.5°C. The most frequent answer was 36.0°C (44%) followed by 35.0°C (15%) (Figure 1A shows answers grouped per 0.5°C).

Respondents who actively rewarmed patients were asked about their rewarming practices. On average, most respondents considered rewarming patients from a temperature of 36.0°C (31%) (Figure 1B). Forty-eight percent of

Table 1. Characteristics of respondents

Characteristic	All respondents
Continent	
Europe	288 (66%)
Asia	67 (15%)
North America	24 (6%)
Latin America	39 (9%)
Oceania	16 (4%)
Africa	6 (1%)
IC Certification level	
Intensivist	341 (78%)
Specialist non-intensivist practicing ICU	45 (10%)
Resident, specialist in training	50 (11%)
Nurse	3 (1%)
Other	1 (0%)
Years of ICU experience	
0-5 years	124 (28%)
6-10 years	106 (24%)
11-15 years	74 (17%)
16-20 years	58 (13%)
>20 years	78 (18%)
Main specialty area	
Intensive Care	258 (59%)
Anesthesiology	114 (26%)
Internal Medicine	40 (9%)
Pulmonology	6 (1%)
Cardiology	5 (1%)
Neurology	2 (0%)
Other	15 (3%)
Hospital type	
Academic	290 (66%)
Non-academic	150 (34%)

Figure 1.



(A) The temperature below which respondents consider a patient with sepsis hypothermic. (B) The trigger temperature at which respondents consider rewarming patients with hypothermic sepsis. (C) The target temperature to which respondents rewarm patients. (D) The reported rewarming rates of respondents. (Answers are grouped per 0.5°C).

respondents delayed rewarming of patients starting at a lower temperature than their hypothermia definition.

The target temperature to which patients are rewarmed also varied; most patients were rewarmed to a target temperature of 37°C (28%) (Figure 1C). There was a large variation in rewarming practice, which ranged from 0.5°C per hour to as fast as possible (Figure 1D). Among respondents, 58% said that the decision to rewarm patients was predominantly physician driven compared with 42% who said that it was nurse driven.

Respondents were asked which factors influenced their decision to rewarm patients on a scale of 0 (not influential at all) to 5 (extremely influential). Shivering was the most influential (3 [2–4]), followed by the presence of shock (3 [2– 3]). The predominant first-line method of rewarming was forced warm air (90%), followed by warmed IV fluids (36%). Respondents were asked which factors influenced their decision to rewarm patients on a scale of 0 (not influential at all) to 5 (extremely influential). Shivering was the most influential (3 (2-4)), followed by the presence of shock (3 (2-3)). The predominant first line method of rewarming was forced warm air (90%), followed by warmed IV fluids (36%).

Respondents opinion

Respondents were also asked their opinion on the effect of hypothermia on the outcome of patients. Most respondents expressed a belief that hypothermia is associated with increased mortality (66%), followed by do not know (24%), does not affect mortality (8%), and decreases mortality (2%). The majority of respondents believe that patients should be rewarmed (55%), compared with 18% who believe that patients should not be rewarmed. Among respondents who believe that patients should be rewarmed, the majority (52%) believe that rewarming negates the negative effects of hypothermia. Among respondents who believe that patients should not be rewarmed, 41% believe that rewarming negated the positive effects of spontaneous hypothermia.

Discussion

In this study, we describe the results of a survey on the management of patients with spontaneous hypothermia. The main findings of this study are as follows: (1) both the definition of spontaneous hypothermia and the practice of active rewarming in these patients are extremely variable; (2) a considerable proportion of respondents do not rewarm patients with spontaneous hypothermia during sepsis; and (3) there is no consensus on the etiology of spontaneous hypothermia.

This study is the first to provide detailed insight into temperature management of patients with spontaneous hypothermia in sepsis. The practice of rewarming remains controversial. Considering that hypothermia is associated with an increased mortality, rewarming is understandable, likely in an attempt to negate any adverse effects of hypothermia. The practice of rewarming may be further motivated by physicians' drive to reach physiological values. A majority of respondents in our survey (62%) indicated that they rewarmed patients with hypothermic sepsis. In a recent survey among UK physicians, a majority of physicians (84%) also rewarmed patients, whereas 16% indicated that they would not rewarm septic patients below a temperature of 34°C[4]. Only 60% of respondents in this study were concerned with a body temperature of 35–36°C in this patient population.[4]

Taken together, the differences in temperature management practice is indicative of a lack of clarity on this issue. The results from this survey make it clear that a robust definition of hypothermia is warranted. This would enable comparisons of incidences and outcome of rewarming practices.

Clinical observational studies overwhelmingly point to hypothermia adversely effecting outcome.[1] In light of these studies, it is noteworthy that none of the respondents reported to have a protocol in place for the management of hypothermic sepsis, most probably due to lack of strong clinical evidence. The aforementioned clinical studies are observational in nature and therefore cannot infer causation between hypothermia and outcome. Moreover, there are no randomized trials studying the impact of rewarming in hypothermic septic patients. If rewarming benefits patients, it is unclear at which temperature they should be rewarmed as well as to which target temperature and how fast patients should be rewarmed. It would be interesting to see the effect that rewarming to target temperatures is in respect to outcome and specifically immune response. In vitro experiments have shown that rewarming can improve immune responses.[5]

On the contrary, experimental evidence points to hypothermia being an adaptive response.[6] In hypothermic rodents with endotoxemia, thermogenic capacity is intact, arguing against hypothermia being a defect in thermogenic capacity.[7] Also, hypothermic rodents actively seek colder environments[8] and spontaneous hypothermia also improved survival in mice infected with E. coli.[9] Moreover, rewarming can potentially have adverse effects such as ischemia reperfusion injury and increased metabolism.[10] A randomized clinical trial would clarify the discrepancy between experimental studies and clinical observations.

There are several limitations that apply to this study. An inherent limitation of this online survey is that we do not know the percentage of respondents or duplicates. To provide an indication of the rate of response to this survey, we have added the number of members of each contacted ESICM section, which

provided most of the responses. We used IP address to address potential duplicate responses to the survey. In total, there were 15 duplicate addresses. The majority of these duplicates had different respondent characteristics. In three cases, there were similar respondent characteristics. We did not remove these. Also, the respondents in this survey were predominantly from European countries, limiting the translatability to other countries. Finally, a large proportion of respondents said that rewarming decisions were nurse driven and only three nurses filled in the survey. We do not know how well the physicians' answers reflect rewarming practices by nurses.

Conclusions

In conclusion, policies, procedures, and beliefs about spontaneous hypothermia and active rewarming in patients with sepsis are variable. The variation in practice must be taken into consideration in designing trials on temperature management in hypothermic sepsis patients. As a starting point, we propose a working group to define hypothermic sepsis to improve comparability of research on this subject.

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Supplemental material

Questionnaire spontaneous hypothermia during sepsis

Thank you very much for taking the time to fill out this survey regarding the definition and treatment of spontaneous hypothermia during sepsis. The questionnaire has a maximum of 25 questions and should take approximately 5-10 minutes to fill out. Please respond from the perspective of the usual or average practice in your ICU.

Pay attention: this survey concerns spontaneous hypothermia that occurs during sepsis and not medically induced hypothermia or iatrogenic hypothermia (during an operation for example).

Characteristics

1. In which country do you currently work?

2. What is your intensive care certification level?
☐ Intensivist
☐ Specialist non-intensivist practicing ICU
☐ Resident
☐ Nurse
☐ Other

3. Years of ICU experience
☐ 0-5
☐ 6-10
☐ 11-15
☐ 16-20
☐ 20+

4. What is your main specialty area?
☐ Intensive Care
☐ Anesthesiology
☐ Internal Medicine
☐ Pulmonology
☐ Cardiology
☐ Neurology
☐ Other

5. Hospital type
☐ Academic
☐ Non-academic

6. How many ICU beds are there in your ICU?
☐ <10
☐ 10-15
☐ 16-20
☐ >20

Protocol

7. Is there a written protocol for managing spontaneous hypothermia specifically in septic patients on your ICU?
☐ Yes
☐ No

Spontaneous hypothermia definition

8. Below which temperature do you consider a patient with sepsis to be hypothermic?
< [_ | _], _ °C (range 34,0 – 37,5)

Spontaneous hypothermia management

9. Do you actively rewarm patients with hypothermia during sepsis? (all measures excluding the use of normal blankets)
☐ Yes
☐ No

Hypothermia management

10. Below which temperature do you consider actively rewarming patients with spontaneous hypothermia during sepsis?
< [_ | _], _ °C (range 34,0 – 37,5)

11. Is the decision to actively rewarm patients with spontaneous hypothermia during sepsis primarily physician driven or nurse driven?
☐ Physician driven
☐ Nurse driven

12. If the choice is made to actively rewarm a patient with spontaneous hypothermia during sepsis, what is your "first-line" intervention?
    If you combine 2 interventions as a "first-line" intervention, please check both interventions.

☐ Chemically activated heat packs
☐ Electric blankets
☐ Forced warm air blankets (i.e. bairhugger, mistral air)
☐ Warmed IV fluids
☐ Warmed humidified oxygen
☐ Peritoneal dialysis
☐ Hemodialysis
☐ Thoracic lavage
☐ Veno-venous ECMO
☐ Veno-arterial ECMO
☐ Cardiopulmonary bypass

13. Please score how often you use the following interventions to actively rewarm patients with spontaneous hypothermia during sepsis:

	Never used	Rarely (<30% of cases)	Sometimes (30-50% of cases)	Often (50-80% of cases)	Very often (>80% of cases)
Chemically activated heat packs	☐	☐	☐	☐	☐
Electric blankets	☐	☐	☐	☐	☐
Forced warm air blankets (i.e. bear-hugged, mistral air)	☐	☐	☐	☐	☐
Warmed IV fluids	☐	☐	☐	☐	☐
Warmed humidified oxygen	☐	☐	☐	☐	☐
Peritoneal dialysis	☐	☐	☐	☐	☐
Thoracic lavage	☐	☐	☐	☐	☐
Veno-venous ECMO	☐	☐	☐	☐	☐
Veno-arterial ECMO	☐	☐	☐	☐	☐
Cardiopulmonary bypass	☐	☐	☐	☐	☐

14. To what target temperature do you actively rewarm patients with spontaneous hypothermia during sepsis?

< [_ | _], °C (range 34,0 – 37,5)

15. At what rate do you actively rewarm patients with spontaneous hypothermia during sepsis in your ICU?

☐ <0,5°C/hour
☐ 0,5°C -1°C/hour
☐ >1,0°C /hour
☐ As fast as possible

16. Please score how the following factors influence your decision whether or not to actively rewarm patients with spontaneous hypothermia during sepsis:

	1 (Not at all influential)	2 (Slightly influential)	3 (Somewhat influential)	4 (Very influential)	5 (Extremely influential)
Old Age (>80 years)	☐	☐	☐	☐	☐
Shock (use of vasopressors to sustain a MAP > 65mmHg)	☐	☐	☐	☐	☐
Tachypnea (respiratory rate >20/min)	☐	☐	☐	☐	☐
Tachycardia (heart rate >100/min)	☐	☐	☐	☐	☐
Obese patient (BMI>30 kg/m^2)	☐	☐	☐	☐	☐
Immunosuppressed patient	☐	☐	☐	☐	☐
Conscious patient	☐	☐	☐	☐	☐
Shivering patient	☐	☐	☐	☐	☐

Opinion

17. In general, do you believe that spontaneous hypothermia during sepsis _____:

☐ Increases mortality
☐ Does not affect mortality
☐ Decreases mortality
☐ Don't know

18. In general, do you believe that patients with spontaneous hypothermia during sepsis should be rewarmed?

☐ Yes
☐ No
☐ Don't know

19. In general, do you believe that rewarming patients with spontaneous hypothermia during sepsis is_____?

☐ Beneficial
☐ Harmful
☐ Neither
☐ Don't know

20. If beneficial, how do you think active rewarming benefits patients with spontaneous hypothermia during sepsis?

☐ Prevents further damage from hypothermia
☐ Benefits patients through a specific mechanism
☐ Both

21. How likely do you think the following mechanisms are in terms of the benefits of actively rewarming patients with spontaneous hypothermia during sepsis?

	1 (Very unlikely)	2 (Unlikely)	3 (Neutral)	4 (Likely)	5 (Very likely)
Hemodynamic improvement	☐	☐	☐	☐	☐
Respiratory improvement	☐	☐	☐	☐	☐
Immune system defense improvement	☐	☐	☐	☐	☐

22. If harmful, how do you think active rewarming harms patients with spontaneous hypothermia during sepsis?

☐ Negates the positive effects of hypothermia
☐ Harms patients through a specific mechanism
☐ Both

23. How likely do you think the following mechanisms are in terms of the harmful effects of actively rewarming patients with spontaneous hypothermia during sepsis?

	1 (Very unlikely)	2 (Unlikely)	3 (Neutral)	4 (Likely)	5 (Very likely)
Ischemia/ reperfusion injury	☐	☐	☐	☐	☐
Increased shock	☐	☐	☐	☐	☐
Increased metabolism	☐	☐	☐	☐	☐

Randomized controlled trial

The following questions regard the design of a randomized controlled trial (RCT) on rewarming strategies in patients with spontaneous hypothermia during sepsis.

24. Below which temperature would you include patients with spontaneous hypothermia during sepsis in an RCT?

☐ 37,0 °C
☐ 36,5 °C
☐ 36,0 °C
☐ 35,5 °C
☐ 35,0 °C
☐ 34,5 °C
☐ 34,0 °C

25. What is the maximum temperature to which you would actively rewarm patients with spontaneous hypothermia during sepsis in an RCT?

☐ 35,5 °C
☐ 36,0 °C
☐ 36,5 °C
☐ 37,0 °C
☐ 37,5 °C
☐ >38,0 °C

26. Is an RCT warranted to examine the effect of different rewarming strategies in patients with spontaneous hypothermia during sepsis?

☐ Yes
☐ No
☐ Don't know

27. Should a group be included that is not actively rewarmed?

- ☐ Yes
- ☐ No, a group that is not rewarmed is not ethical
- ☐ No, but a control group should be included that is actively rewarmed from significantly lower temperature than the intervention groups

Chapter three

Risk factors, host response and outcome of hypothermic sepsis

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Abstract

Background Hypothermia is associated with adverse outcome in patients with sepsis. The objective of this study was to characterize the host immune response in patients with hypothermic sepsis in order to determine if an excessive anti-inflammatory response could explain immunosuppression and adverse outcome. Markers of endothelial activation and integrity were also measured to explore potential alternative mechanisms of hypothermia. Finally we studied risk factors for hypothermia in an attempt to find new clues to the etiology of hypothermia in sepsis.

Methods Consecutive patients diagnosed with sepsis within 24 hours after admission to ICUs in two tertiary hospitals in the Netherlands were included in the study (n = 525). Hypothermia was defined as body temperature below 36°C in the first 24 hours of ICU admission.

Results Hypothermia was identified in 186 patients and was independently associated with mortality. Levels of proinflammatory and anti-inflammatory cytokines were not different between groups. Hypothermia was also not associated with an altered response to *ex vivo* stimulation with lipopolysaccharide in a subset of 15 patients. Risk factors for hypothermia included low body mass index, hypertension and chronic cardiovascular insufficiency. Levels of the endothelial activation marker fractalkine were increased during the first 4 days of ICU stay.

Conclusions Hypothermia during sepsis is independently associated with mortality, which cannot be attributed to alterations in the host immune responses that were measured in this study. Given that risk factors for hypothermic sepsis are mainly cardiovascular and that the endothelial activation marker fractalkine increased in hypothermia, these findings may suggest that vascular dysfunction plays a role in hypothermic sepsis.

Background

Sepsis is the consequence of a dysregulated immune response to infection, involving both proinflammatory and anti-inflammatory components, as well as a highly activated endothelium, resulting in increased vascular permeability, organ failure and shock.[1] Fever and hypothermia are both hallmark characteristics of sepsis.[2] Hypothermia is observed in 9%-35% of septic patients.[3,4] Whereas fever is generally considered beneficial for patients, hypothermia is independently associated with increased mortality.[4] However the etiology of hypothermia during sepsis is poorly understood.

Studies attempting to elucidate the hypothermic response in sepsis focused on a hypothesized lack of proinflammatory cytokines, in particular interleukin (IL)-6 and tumor necrosis factor (TNF)-α, which are the main mediators of fever. However, these studies could not demonstrate that a depression of the proinflammatory response is associated with hypothermia in sepsis.[5,6] Anti-inflammatory cytokines such as IL-10, which possess antipyretic properties in humans[7] and animals[8], have not been studied before in hypothermic septic patients. An excessive anti-inflammatory response could potentially explain hypothermia[9] and associated lymphopenia which was recently found in hypothermic patients with sepsis.[10]

Other mechanisms have yet to be explored in hypothermic patients with sepsis. Endothelial dysfunction could also play a role in the development of hypothermia, as generalized peripheral vasodilation and loss of endothelial integrity during sepsis may result in heat loss by hampering the body's ability to regulate its core temperature.[11,12]

The aim of this current prospective observational study was to determine if hypothermia is independently associated with 90-day mortality. We subsequently characterized the host immune response in patients with hypothermic sepsis, by determining both proinflammatory and anti-inflammatory cytokines, and whole blood *ex vivo* responsiveness to lipopolysaccharide (LPS). We also measured markers of endothelial activation and integrity. Finally, we studied risk factors for hypothermic sepsis in an attempt to find potential new insights into the etiology of hypothermia in sepsis. Understanding the etiology of hypothermic sepsis may contribute to the identification of potential targets for future interventions.

Methods

Study design, patients and definitions

From January 2011 through July 2013, consecutive patients presenting to the mixed intensive care units (ICU) of two Dutch tertiary teaching hospitals (Academic Medical Center in Amsterdam and University Medical Center in Utrecht) were included. Medical Ethical Committees of both centers approved an opt-out consent method (IRB no.10-056C). Data and plasma samples were prospectively collected as part of the Molecular Diagnosis and Risk Stratification of Sepsis (MARS) project (ClinicalTrials.gov identifier NCT01905033).[13,14] A group of trained investigators collected clinical data. The plausibility of infection was scored *post hoc* classified on a 4-point scale (*none*, *possible*, *probable* or *definite*)[15,16], as described in detail previously.[14] Shock was defined as the use of vasopressors for hypotension in a dose of 0.1 mcg/kg/min during at least 50% of the day. Acute kidney injury (AKI) and acute lung injury (ALI) were scored using pre-set criteria.[17,18]

We selected patients with sepsis, diagnosed within 24 hours of admission, defined as a *definite* or *probable* infection[14] combined with at least one parameter of inflammatory dysfunction, hemodynamic dysfunction, organ dysfunction or deranged tissue perfusion (derived from the 2001 International Sepsis Definitions Conference[2]). Patients with immunodeficiency disorders, use of corticosteroids, immunosuppressive or antineoplastic drugs were excluded. To exclude iatrogenic hypothermia, readmitted patients, patients undergoing active cooling and patients transferred from another ICU or operating room (OR) were also excluded. To control for inadvertent temperatures that may have been entered in the database (i.e. a rectal sensor that has been displaced and is exposed to ambient temperature) patients with unreliable temperatures (below 33°C) were not included. Also patients with a missing minimum temperature were not included. Temperature was measured using a rectal, nasal, inguinal or tympanic temperature probe. Core temperatures were used over inguinal or tympanic measurements. The threshold for hypothermia was set at 36°C, based on previously used cutoff levels.[10,19] Daily (at admission and at 6 a.m. thereafter) left-over EDTA anticoagulated plasma (obtained from blood drawn for patient care) was stored within 4 hours at -80°C. Samples were drawn prior to rewarming patients.

Plasma biomarker measurements

TNF-α, IL-1β, IL-6, IL-8, IL-10, IL-13, soluble intercellular adhesion molecule (ICAM)-1, fractalkine and soluble E-selectin were measured using FlexSet

cytometric bead arrays (BD Bioscience, San Jose, CA) using FACSCalibur (Becton Dickenson, Franklin Lakes, NJ, USA). Angiopoietin-1 and angiopoietin-2 (R&D systems, Abingdon, UK) were measured by Luminex multiplex assay using BioPlex 200 (BioRad, Hercules, CA). Normal biomarker values were acquired from EDTA plasma from 27 age- and gender-matched healthy volunteers, from whom written informed consent was obtained.

The lower limits of detection for the immune assays were: 0.9 pg/mL for TNF-α, 1.3 pg/mL for IL-1β, 0.9 pg/mL for IL-6, 1.3 pg/mL for IL-8, 0.8 pg/mL for IL-10, 0.7 pg/mL for IL-13, 3.1 pg/mL for soluble E-selectin, 6.3 pg/mL for soluble ICAM-1, 4.0 pg/mL for fractalkine, 0.2 pg/mL for angiopoietin-1 and 1.8 pg/mL for angiopoietin-2.

Whole blood stimulations

In a random subset of 15 patients, whole blood was stimulated *ex vivo* with LPS on day 1 of ICU admission, as previously described.[20] Heparin-anticoagulated blood was stimulated for 3h at 37°C in pyrogen-free RPMI 1640 (Life Technologies, Bleiswijk, the Netherlands) with or without 100ng/mL ultrapure LPS (from *Escherichia coli* 0111:B4; InvivoGen, Toulouse, France). TNF-α and IL-1β were measured in supernatants using a cytometric bead array assay (BD Biosciences, San Jose, California). Cytokine release was calculated as the difference in cytokine levels in samples incubated with and without LPS. The medical ethical committee of the Academic Medical Center in Amsterdam gave ethical approval for the conduction of the study (no.NL34294.018.10). Written informed consent was obtained from all patients, or their legal representative, and from healthy volunteers.

Statistical analysis

All analyses were performed in R (version 3.1.1). Student's t-test or Wilcoxon rank-sum test, and chi-square test were used to compare groups. To study factors independently associated with developing hypothermia, we performed multivariable logistic regression. Pre-ICU-admission patient characteristics that were deemed relevant or were associated with hypothermia in univariate analysis ($P < 0.2$) were included in the model. A backward selection procedure using the Akaike information criterion (AIC) including 1000 bootstrap replicates was applied (R-package "rms") to identify risk factors for hypothermia. Age was forced into the model since we considered age an important confounder for all factors incorporated in the model as well as being associated with an altered temperature response.[4,21]

Multivariable logistic regression was used to establish the independent association of hypothermia with 90-day mortality. The Acute Physiology and Chronic Health Evaluation (APACHE) IV score was included in the model to adjust for severity of disease at ICU admission. Age, body mass index (BMI), admission type and source of infection were *a priori* considered potential clinically relevant confounders. Next, risk factors for hypothermia from logistic regression analyses were investigated as confounders for mortality. Significant variables were retained in the model, based on 10% change-in-estimate. In order to determine whether hypothermia was associated with biomarker response irrespective of severity of disease, hypothermic patients were 1:1 matched to nonhypothermic patients by APACHE IV scores, using 'optimal matching' with R-package "MatchIt". $P<0.05$ was considered statistically significant.

Results

Epidemiology of hypothermic sepsis

The selection of study patients is presented in Supplemental Figure 1. From a total of 525 patients, 186 (35.4%) patients were hypothermic during the first 24 hours of admission. Patient characteristics are shown in table 1. Mean body temperature in the first 24 hours was significantly lower in hypothermic versus nonhypothermic patients (median 36.3°C and 37.3°C respectively). Mean age in hypothermic patients was significantly higher and BMI was lower. Hypothermic patients suffered more frequently from cardiovascular disease including chronic cardiovascular insufficiency, hypertension and cerebrovascular disease. Hypothermic patients were most often admitted from the emergency department. Also, a significantly higher proportion of patients with hypothermia had a urinary tract infection. We observed no differences in causative organisms (Supplemental Table 1). Hypothermic patients were more seriously ill, as reflected by higher APACHE IV and Sequential Organ Failure Assessment (SOFA) scores and increased incidence of AKI (and requirement of renal replacement therapy). In line with this, patients with hypothermia had higher maximum white blood cell counts, longer prothrombin times and increased creatinine and lactate levels.

Risk factors for hypothermic sepsis

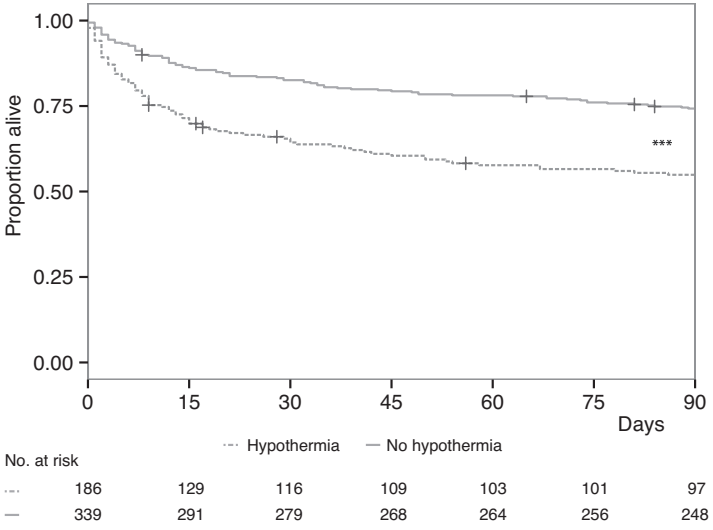
Multivariable analysis was performed to determine whether patient factors were independently associated with hypothermia. The initial model contained age, BMI, cerebrovascular disease, chronic cardiovascular insufficiency, hypertension, chronic renal insufficiency, site of infection and admission origin (Supplemental

Table 2). Interestingly, hypertension (adjusted odds ratio (aOR) 1.98; 95%CI 1.30-3.02) and chronic cardiovascular insufficiency (aOR 3.27; 95%CI 1.25-8.50) were associated with hypothermia. BMI was inversely correlated with hypothermia (aOR 0.96; 95% confidence interval (CI) 0.93-0.99). Age was not independently associated with hypothermia (aOR 1.01; 95%CI 0.999-1.03).

Hypothermia on admission is associated with increased mortality

ICU- and hospital mortality were significantly higher in septic patients with hypothermia (Supplemental Table 3). There were differences in mortality at 30, 60 and 90 days and at 1 year after ICU admission (Figure 1 and Supplemental Table 3). There was an increased incidence of AKI during admission in patients with hypothermia. There was no difference in the incidence of ICU-acquired infections. Multivariable logistic regression including APACHE IV scores was performed to determine if hypothermia was independently associated with mortality. Site of infection was a confounder in our study and thus was retained in the model (Supplemental table 4). Hypothermia was independently associated with an increased risk of death at 90 days (aOR 2.08; 95%CI 1.38-3.16).

Figure 1. Survival curve in patients with and without hypothermia during the first 24 hours of ICU admission.



Kaplan–Meier plot of survival time up to 90 days after intensive care unit admission. *** $P < 0.001$.

Table 1. Baseline characteristics of sepsis patients with and without hypothermia during the first 24 hours of admission

	Hypothermia N = 186	No hypothermia N = 339	p
Demographics			
Age, years, mean [SD]	65.0 [13.8]	61.1 [15.6]	.004
Gender, male (%)	114 (61.3)	206 (60.8)	.94
BMI, kg/m2, mean [SD]	25.6 [5.7]	26.7 [6.7]	.04
Comorbidities			
Charlson score, median [IQR]	5 [3-6]	4 [2-6]	.01
Cerebrovascular disease (%)	28 (15.1)	27 (8)	.01
Chronic cardiovascular insufficiency (%)	13 (7)	7 (2.1)	.009
Chronic renal insufficiency (%)	26 (14)	32 (9.4)	.15
Congestive heart failure (%)	8 (4.3)	17 (5)	.84
COPD (%)	29 (15.6)	61 (18)	.55
Diabetes mellitus (%)	42 (22.6)	67 (19.8)	.51
Hypertension (%)	73 (39.2)	87 (25.7)	.003
Liver cirrhosis	7 (3.8)	6 (1.8)	.23
Peripheral vascular disease (%)	25 (13.4)	44 (13)	.90
Admission			
Admission type, medical (%)	163 (87.6)	298 (87.9)	.55
Admission origin, emergency department (%)	74 (39.8)	99 (29.2)	.04
medium care (%)	26 (14)	47 (13.9)	
ward (%)	86 (46.2)	193 (56.9)	
Site of infection			
Pulmonary (%)	79 (42.5)	161 (47.5)	.28
Abdominal (%)	29 (15.6)	65 (19.2)	.34
Urinary tract (%)	32 (17.2)	33 (9.7)	.02
Other (%)	18 (9.7)	44 (13)	.31
Co-infection (%)	28 (15.1)	36 (10.6)	.15
Severity of disease first 24h			
Mean temperature first 6 h, median [IQR]	36.1 [35.4-37]	37.2 [36.5-38]	<.0001
Mean temperature first 24 h, median [IQR]	36.3 [35.8-36.9]	37.3 [36.8-37.8]	<.0001
APACHE IV score, median [IQR] [a]	82 [67-103]	71 [58-86]	<.0001
SOFA score, median [IQR] [b]	8 [5-10]	7 [4-8]	<.001
Acute kidney injury (%)	92 (49.5)	118 (34.8)	.002
Renal replacement therapy (%)	32 (17.1)	21 (6.2)	<.001
Acute lung injury (%)	49 (26.3)	99 (29.2)	.57
Shock (%)	74 (39.8)	106 (31.3)	.06

Table continues on next page

Table 1. Continued.

	Hypothermia N = 186	No hypothermia N = 339	p
Clinical laboratory parameters first 24h			
WBC count max.(x10^9/l), median [IQR]	16.1 [10.9-25.5]	14.9 [10-19.2]	.02
WBC count min.(x10^9/l), median [IQR]	12.6 [7.1-19.1]	12.2 [7.7-16.2]	.25
Platelets min. (x10^9/l), median [IQR]	189 [120-264]	199 [131-283]	.27
Lactate max. (mmol/l), median [IQR]	3.2 [1.6-6.5]	2.5 [1.6-4.1]	.009
Prothrombin time max. (s), median [IQR]	16.5 [14.1-20.7]	15 [12.6-18.2]	.0001
Creatinine max. (μmol/l), median [IQR]	121 [80-209]	97 [68-162]	<.001
C-reactive protein (mg/l), median [IQR]	146 [82-258]	174 [98-263]	.25

APACHE, acute physiology and chronic health evaluation; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; SD, standard deviation; SOFA, sequential organ failure assessment; WBC, white blood cell.[a] Temperature not included in score

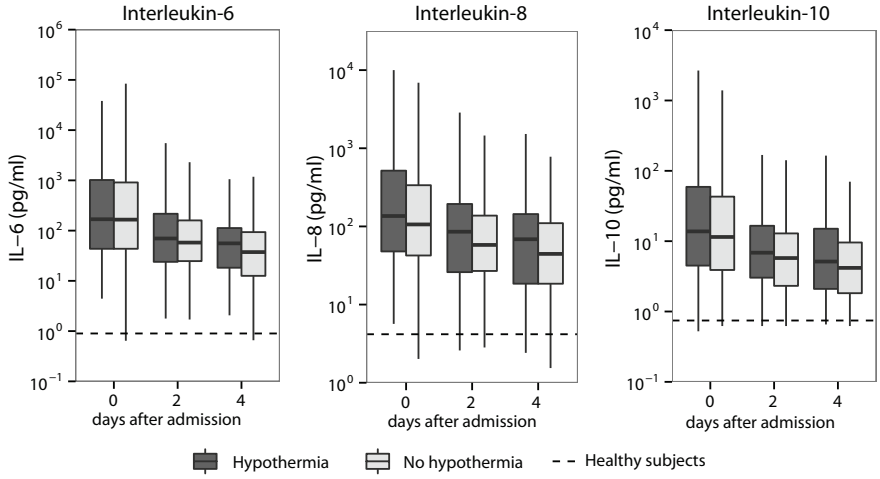
[b] Central nervous system not included in score due to large number of sedated patients

Hypothermic sepsis is not associated with altered anti-inflammatory or proinflammatory cytokine plasma levels

Levels of IL-13 were undetectable or low in the majority of patients and were not different between groups. Levels of IL-10 were increased in sepsis patients compared to healthy subjects, however there was no association with the presence of hypothermia (Figure 2).

Proinflammatory cytokines TNF-α and IL-1β were also undetectable or low in the majority of patients and were not different between groups. IL-6 and IL-8 levels were increased in patients with sepsis however there was also no association with the presence of hypothermia (Figure 2).

Figure 2. Plasma cytokine levels in sepsis patients stratified according to the presence of hypothermia.

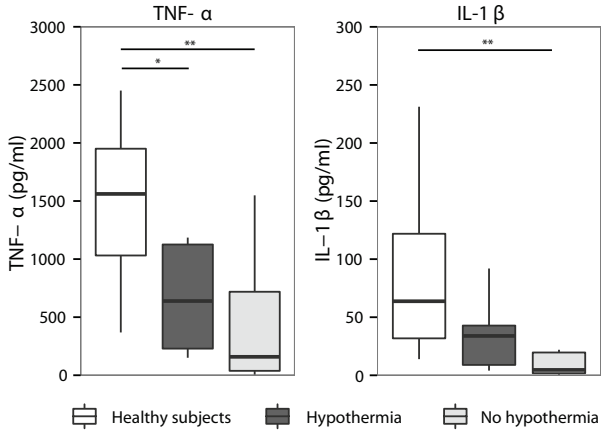


Box-and-whisker diagrams depict the median and lower quartile, upper quartile and respective 1.5 IQR as whiskers. Dashed lines represent median levels in healthy volunteers. Differences between patient groups were not significant.

Hypothermia does not affect leukocyte responsiveness upon ex vivo stimulation

As hypothermia has been postulated to be an early clinical predictor of sepsis-induced immunosuppression[10], we investigated the association between hypothermia and the responsiveness of circulating immune effector cells to LPS, as a marker of sepsis-induced immunosuppression[22]. Whole blood from 15 sepsis patients, of whom 5 hypothermic, was stimulated *ex vivo* and compared with 18 healthy age- and gender-matched volunteers. Clinical characteristics of sepsis patients are displayed in Supplemental Table 5. Patients with sepsis had a reduced capacity to release TNF-α and IL-1β upon LPS stimulation compared to healthy controls (Figure 3). However, no differences in cellular responsiveness were observed between hypothermic and nonhypothermic patients with sepsis.

Figure 3. Whole-blood leukocyte responsiveness to LPS stratified according to the presence of hypothermia.



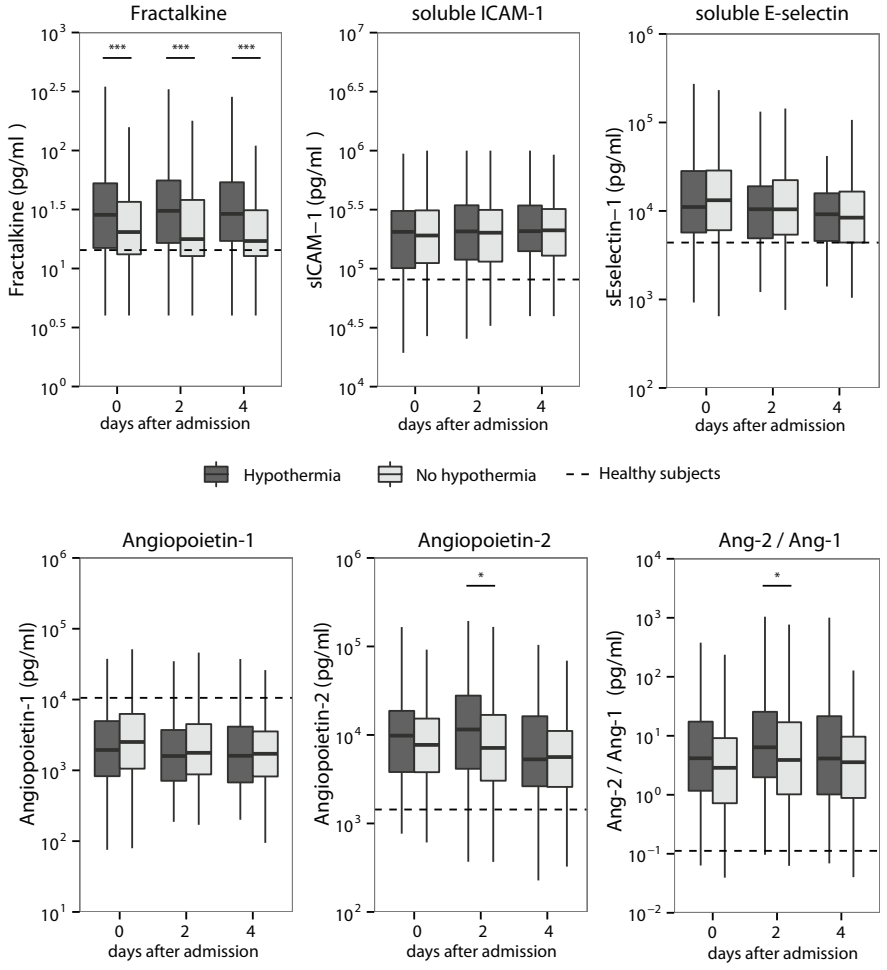
Responsiveness of whole-blood leukocytes to lipopolysaccharide (LPS) was reduced compared to healthy subjects (n = 18), but not different between hypothermic (n = 5) and nonhypothermic (n = 10) patients with sepsis. Box and whisker diagrams depict the median and lower quartile, upper quartile, and respective 1.5 IQR as whiskers. *$P < 0.05$, **$P < 0.01$.

Hypothermia is associated with increased plasma levels of the endothelial cell activation marker fractalkine

All markers of endothelial dysfunction were higher in patients with sepsis compared to healthy controls, except angiopoetin-1, which were lower (Figure 4). Angiopoietin-2 and the ratio of angiopoietin-2/angiopoietin-1 were higher in hypothermic patients on day 2 compared to nonhypothermic patients with sepsis. This difference was not present at the other time points. Strikingly, levels of fractalkine, an endothelial cell derived chemokine, were substantially higher in hypothermic versus nonhypothermic patients on the day of admission (Figure 4). These differences persisted on days 2 and 4 after admission.

To determine whether the higher levels of fractalkine in the hypothermic group were due to differences in disease severity, every hypothermic patient was matched to a nonhypothermic patient with a comparable APACHE IV score. Patient characteristics of the matched cohort are shown in Supplemental Table 6. In the subsequent analysis, fractalkine remained significantly higher in patients with hypothermia (median levels admission 28.5 pg/ml vs 20.8 pg/ml, $P = 0.005$,

Figure 4. Endothelial cell activation in sepsis patients stratified according to the presence of hypothermia.



Box-and-whisker diagrams depict the median and lower quartile, upper quartile and respective 1.5 IQR as whiskers. Dashed lines represent median levels in 27 healthy volunteers. ICAM-1 intercellular adhesion molecule-1. Note: soluble ICAM-1 is also derived from leukocytes. *** $P < 0.001$, * $P < 0.05$.

day 2 18.6 vs 30.8 pg/ml, P =0.001, day 4 17.1 vs 29.0 pg/ml, P =0.001), whereas the other host response biomarkers, including angiopoietin-2 and the ratio of angiopoeietin-2/angiopoietin-1 levels, were not different between groups. There were no differences in soluble ICAM-1, soluble E-selectin and angiopoietin-1 between hypothermic and nonhypothermic patients.

Discussion

Hypothermia at ICU admission is independently associated with adverse outcome in patients with sepsis. In this extensive evaluation of the immune response in hypothermic sepsis, the host immune response was not altered in patients with hypothermia compared to nonhypothermic patients. The endothelial activation marker fractalkine was persistently higher in hypothermic sepsis, irrespective of disease severity. In addition, low BMI, hypertension and cardiovascular insufficiency were identified as risk factors for hypothermic sepsis. Taken together, this data may suggest that vascular dysfunction could play a role in hypothermic sepsis.

An excessive anti-inflammatory response has been proposed as a mechanism for hypothermia.[9] In line with this, a recent study showed increased immunosuppression in hypothermic patients[10], thereby potentially accounting for the association with adverse outcome.[3-5,10] In contrast, we found no difference in either proinflammatory or anti-inflammatory cytokines between hypothermic and nonhypothermic patients, even after correction for disease severity. These data are in line with a study in hypothermic patients showing no difference in circulating levels of proinflammatory cytokines[5] and extend these data by showing that levels of anti-inflammatory are also not affected by hypothermia. Moreover, whole blood stimulations resulted in similar cytokine release in hypothermic *versus* nonhypothermic patients. Therefore a mechanism for hypothermia directly involving anti-inflammatory cytokines seems unlikely. Rather, these data suggest that hypothermic patients do not suffer from increased immunosuppression. In support of this, the incidence of ICU-acquired infections was similar between groups, as found before in sepsis.[22] Of note, according to standard cell stimulation protocols experiments were performed ex vivo at an incubation temperature of 37°C. If whole blood stimulations had been performed at a lower temperature to simulate the temperature of hypothermic patients, one could expect that overall the reactivity of cells in terms of cytokine production might have been slightly higher.[23]

In this systematic study of risk factors for hypothermia during sepsis we identified several interesting associations. BMI was inversely correlated with hypothermia. A physiological explanation is that increased body mass likely slows the dissipation of heat from the body. As low BMI itself has been associated with

poor outcome on the ICU[24], the relationship between hypothermia and BMI and their combined role on outcome is unclear. Leptin, released from adipose tissue, has anti-inflammatory properties and may also mediate the hypothermic response, providing a possible link between the two.[25]

Of interest, we also identified hypertension and chronic cardiovascular insufficiency as risk factors. Patients with cardiovascular disease may be hampered in raising or maintaining core temperature by a dysfunction in autonomic mechanisms such as increased heart rate and blood pressure and by shifting capillary blood flow from cutaneous to deep vascular beds.[26] Alternatively, the association between hypothermia and cardiovascular conditions may reflect the importance of an intact endothelial function in maintaining body temperature during sepsis.

Interestingly, systemic fractalkine levels were significantly higher in hypothermic patients compared to nonhypothermic patients, and this difference was maintained after correcting for disease severity. Fractalkine is a chemokine that has been implicated as a mediator in a diverse spectrum of inflammatory conditions.[27] In critically ill patients with sepsis, increased levels of fractalkine are associated with adverse outcome.[27] Arterial and capillary endothelial cells have been identified as an important source of fractalkine during endotoxemia.[28] Also, levels of angiopoetin-2 and the ratio of angiopoietin-2/angiopoietin-1, which indicate impaired vascular integrity, were increased in the hypothermic patients compared to the nonhypothermic patients, albeit transiently. Taken together with the presence of mainly cardiovascular risk factors in patients with hypothermia, our data may suggest that the endothelium is somehow implicated in hypothermia through an as yet unknown mechanism. Although the current data cannot establish a causal link between fractalkine and hypothermia and the association of hypothermia and fractalkine in a population with significantly increased disease severity warrants further validation, the increased levels of fractalkine in hypothermia are intriguing and a detailed study on the role of the endothelium, in particular fractalkine, is warranted.

There are several shortcomings to this study. First, the timing and method of temperature measurement was not standardized. Although this could have led to an increased variability in this study, we believe this effect will be limited due to the fact that core temperature measurements is standard practice in our ICUs. Second, blood sampling did not exactly coincide with timing of the temperature measurement. Although this might have diluted results, a single hypothermic temperature in 24 hours significantly increases mortality and we feel that the blood sampling does not necessarily need to be on exactly the same time as the hypothermic measurement to characterize this group. Third, results from this study are not applicable to all patients with sepsis as we excluded patients with

a decreased ability to mount an adequate host response (those on steroids, those with immunodeficiency) and patients at risk for iatrogenic hypothermia (patients derived directly from the OR). Multiple testing can cause confounding. However after Bonferroni correction fractalkine remained significantly associated with hypothermia. Also, as fractalkine was significantly elevated at all time-points and remained significant after matching for disease severity, we consider these results to be valid. Lastly, this is an observational study and cause-effect relationships cannot be established due to the nature of this study design.

Conclusions

In conclusion, hypothermia during sepsis is independently associated with 90-day mortality. However, neither the etiology of hypothermia or increased mortality due to hypothermia are explained by a dysfunctional hostimmune response. Low BMI, hypertension and chronic cardiovascular insufficiency are risk factors for hypothermic sepsis. Hypothermia is associated with increased levels of the endothelial derived biomarker fractalkine. The functional role of fractalkine and the endothelium in the context of hypothermic sepsis requires further study.

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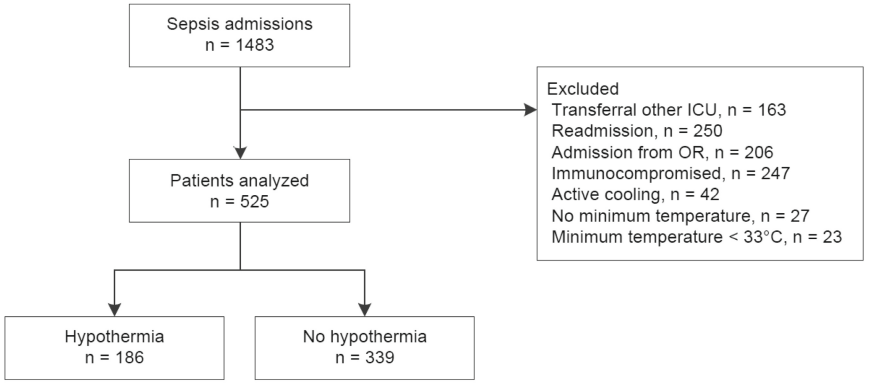
Supplemental material

Supplemental Figure 1. Selection of study patients.



Supplemental Table 1. Causative pathogens

	Hypothermia	No hypothermia	P
	N = 186	N = 339	
Gram-positive bacteria (%)	90 (48.4)	162 (47.8)	.82
Gram-negative bacteria (%)	123 (66.1)	220 (64.9)	.73
Yeast/fungi (%)	13 (7)	37 (10.9)	.18
Other (%)	26 (14)	34 (10)	.19
Unknown (%)	25 (13.4)	60 (17.7)	.28

Percentages represent the pathogens divided by the number of patients. In some cases multiple causative pathogens were isolated.

Supplemental Table 2. Multivariable logistic regression analysis to identify risk factors for hypothermia

	OR	95% C	P
Age	1.01	0.999-1.03	.07
Body mass index	0.96	0.93-0.99	.009
Hypertension	1.98	1.30-3.02	.001
Chronic cardiovascular insufficiency	3.27	1.25-8.50	.02

Supplemental Table 3. Outcomes of sepsis patients with and without hypothermia during the first 24 hours of admission

	Hypothermia N = 186	No hypothermia N = 339	P
Acute kidney injury (%)	105 (56.5)	137 (40.4)	.002
Renal replacement therapy (%)	40 (21.5)	41 (12.1)	.005
Acute lung injury (%)	25 (31.2)	113 (33.3)	.62
ICU-acquired weakness (%)	13 (7)	20 (5.9)	.72
ICU-acquired infections (%)	15 (8.1)	24 (7.1)	.72
ICU-mortality (%)	48 (25.8)	38 (11.2)	<.001
Hospital mortality (%)	69 (37.1)	69 (20.4)	<.001
30 day mortality (%)	66 (35.5)	59 (17.4)	<.001
60 day mortality (%)	78 (41.9)	74 (21.8)	<.001
90 day mortality (%)	84 (45.2)	87 (25.7)	<.001
1 year mortality (%)	104 (55.9)	122 (36)	<.001

Supplemental Table 4. Association between hypothermia and 90-day mortality in patients with sepsis, adjusted for confounders

	OR	95% CI	P
Hypothermia	2.47	1.69 – 3.61	<.0001
Hypothermia + APACHE IV score [a]	1.91	1.28 – 2.87	.0017
Hypothermia + APACHE IV score [a] + Site of infection	2.08	1.38 – 3.16	.0005

APACHE, acute physiology and chronic health evaluation.
[a] Temperature not included in score

Supplemental Table 5. Clinical characteristics of sepsis patients included in ex vivo whole blood stimulation analysis.

	Hypothermia N = 5	No hypothermia N = 10
Demographics		
Age, years, mean [SD]	61.4 [12.9]	67.1 [13.7]
Gender, male (%)	1 (20)	6 (60)
BMI, kg/m2, mean [SD]	29.1 [9.8]	29.3 [8.9]
Charlson score, median [IQR]	3 [0-3]	2 [0-3]

Table continues on next page

Supplemental Table 5. Continued.

	Hypothermia N = 5	No hypothermia N = 10
Site of infection		
Pulmonary (%)	2 (40)	6 (60)
Abdominal (%)	0 (0)	1 (10)
Urinary tract (%)	2 (40)	2 (20)
Other (%)	5 (100)	10 (100)
Co-infection (%)	1 (20)	1 (10)
Severity of disease first 24h		
Mean temperature first 6 h, median [IQR]	36.1 [35-36.9]	37.1 [36.9-38]
Mean temperature first 24 h, median [IQR]	36 [35.6-36.3]	37.4 [37.2-37.8]
APACHE IV score, median [IQR] [a]	75 [65-113]	73 [63-80]
SOFA score, median [IQR] [b]	9 [8-9]	7.5 [6-9]
Acute kidney injury (%)	3 (60)	4 (40)
Acute lung injury (%)	2 (40)	4 (40)
Shock (%)	1 (20)	4 (40)
Clinical laboratory parameters first 24h		
WBC count max.(x10^9/l), median [IQR]	13.1 [8.3-13.5]	12.2 [10.5-18.8]
WBC count min.(x10^9/l), median [IQR]	9.4 [7-12.7]	9.2 [7.8-15.2]
Platelets min. (x10^9/l), median [IQR]	109 [72-276]	158 [138-199]
Lactate max. (mmol/l), median [IQR]	1.1 [1.1-1.9]	2.4 [2.124]
Prothrombin time max. (s), median [IQR]	16 [13.5-16.7]	16.8 [12.1-19.9]
Creatinine max. (µmol/l), median [IQR]	257 [200-421]	113 [88-142]
C-reactive protein (mg/l), median [IQR]	160 [128-219]	128 [71-177]
Outcome		
ICU-mortality (%)	1 (20)	1 (10)
Hospital mortality (%)	2 (40)	4 (40)
30 day mortality (%)	2 (40)	2 (20)
60 day mortality (%)	3 (60)	3 (30)
90 day mortality (%)	3 (60)	4 (40)
1 year mortality (%)	3 (60)	4 (40)

APACHE, acute physiology and chronic health evaluation; IQR, interquartile range; SD, standard deviation; SOFA, sequential organ failure
assessment; WBC, white blood cell.
[a] Temperature not included in score
[b] Central nervous system not included in score due to large number of sedated patients

Supplemental Table 6. Clinical characteristics of sepsis patients with and without hypothermia of admission matched for APACHE IV score.

	Hypothermia N = 186	No hypothermia N = 186	p
Demographics			
Age, years, mean [SD]	65.0 [13.8]	62.1 [15.9]	.06
Gender, male (%)	114 (61.3)	114 (61.3)	>.99
BMI, kg/m2, mean [SD]	25.6 [5.7]	27.1 [6.5]	.02
Charlson score, median [IQR]	5 [3-6]	4 [2-6]	.20
Site of infection			
Pulmonary (%)	79 (42.5)	81 (43.5)	.91
Abdominal (%)	29 (15.6)	36 (19.4)	.40
Urinary tract (%)	32 (17.2)	21 (11.3)	.13
Other (%)	18 (9.7)	26 (14)	.27
Co-infection (%)	28 (15.1)	22 (11.8)	.43
Severity of disease first 24h			
Mean temperature first 6 h, median [IQR]	36.1 [35.4-37]	37.1 [36.5-37.9]	<.0001
Mean temperature first 24 h, median [IQR]	36.3 [35.8-36.9]	37.3 [36.7-37.8]	<.0001
APACHE IV score, median [IQR] [a]	82 [67-103]	82 [67-100]	.54
SOFA score, median [IQR] [b]	8 [5-10]	7 [4-9]	.008
Acute kidney injury (%)	92 (49.5)	72 (38.7)	.06
Acute lung injury (%)	49 (26.3)	51 (27.4)	.90
Shock (%)	74 (39.8)	62 (33.3)	.24
Clinical laboratory parameters first 24h			
WBC count max.(x10^9/l), median [IQR]	16.1 [10.9-25.5]	14.9 [10-19.4]	.07
WBC count min.(x10^9/l), median [IQR]	12.6 [7.1-19.1]	12.7 [8-16.5]	.47
Platelets min. (x10^9/l), median [IQR]	189 [120-264]	200 [130-275]	.54
Lactate max. (mmol/l), median [IQR]	3.2 [1.6-6.5]	2.7 [1.6-5.1]	.16
Prothrombin time max. (s), median [IQR]	16.5 [14.1-20.7]	15.6 [13.2-19.2]	.03
Creatinine max. (µmol/l), median [IQR]	121 [80-209]	103 [73-174]	.03
C-reactive protein (mg/l), median [IQR]	146 [82-258]	154 [93-248]	.81
Outcome			
ICU-mortality (%)	48 (25.8)	26 (14)	.008
Hospital mortality (%)	69 (37.1)	47 (25.3)	.01
30 day mortality (%)	66 (35.5)	41 (22)	.003

Table continues on next page

Supplemental Table 6. Continued

	Hypothermia N = 186	No hypothermia N = 186	p
60 day mortality (%)	78 (41.9)	49 (26.3)	.0005
90 day mortality (%)	84 (45.2)	61 (32.8)	.01
1 year mortality (%)	104 (55.9)	79 (42.5)	.007

APACHE, acute physiology and chronic health evaluation; IQR, interquartile range; SD, standard deviation; SOFA, sequential organ failure assessment; WBC, white blood cell.

[a] Temperature not included in score

[b] Central nervous system not included in score due to large number of sedated patients

Chapter 3a

To the editor: Should we assume that hypothermia is a dysfunction in sepsis?

Alexandre A. Steiner, Monique T. Fonseca and Francisco G. Soriano

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Wiewel et al.[1] clearly showed that development of hypothermia instead of fever in sepsis is not tied to a switch from a pro-inflammatory to an anti-inflammatory state. The authors then suggest that vascular dysfunction could play a role in hypothermia. While this hypothesis deserves attention, we urge researchers to consider that there is no hard evidence indicating that hypothermia is a dysfunction in sepsis.

Not all systems fail simultaneously in sepsis, and those with preserved function are likely to launch evolutionarily conserved compensatory responses. Could thermoregulation be preserved during septic hypothermia? Could hypothermia be adaptive when the costs of fever exceed its benefits? According to evidence from rat models of systemic inflammation, the answers to these questions may be yes. First, hypothermia in endotoxemic rats is an early, transient phenomenon that is not consequential to circulatory shock.[2] Second, hypothermia in endotoxic shock is brought about by downregulation of thermogenesis when thermogenic capacity is unimpaired.[2,3] Third, rats with endotoxic shock do not attempt to restore normothermia when given the chance to select a warmer environment; on the contrary, they seek a cooler environment.[3] Last, spontaneous hypothermia has been shown to be more advantageous than fever in rats with severe forms of endotoxemia and *Escherichia coli* sepsis.[2,4]

There has been a complete disconnect between these experimental data and clinical studies on this subject. Recently, though, Fonseca et al.[5] published the first effort to reconcile experimental and clinical evidence on septic hypothermia.

That study revealed that, similarly to animal models of endotoxemia, hypothermia in human sepsis is usually self-limiting and transient. Perhaps most importantly, hypothermia was rarely observed in the moments that preceded death, when multiple organ failure is presumably at its peak. Hence, it is possible that an early, regulated form of hypothermia exists in human sepsis. By the same token, the reported association between hypothermia and higher mortality should not be taken as evidence that hypothermia is a dysfunction that worsens sepsis. This association could merely reflect the fact that hypothermia replaces fever in the most severe cases of sepsis, both in rats and humans. In our opinion, the impact of septic hypothermia on clinical outcomes can only be adequately addressed by an interventional study in which spontaneous hypothermia is allowed or prevented within the hypothermic subset of septic patients. We are planning such a study and invite those interested to join us.

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Chapter 3b

Reply: Should we assume that hypothermia is a dysfunction in sepsis?

Matthew B. Harmon, Maryse A. Wiewel, W. Joost Wiersinga and Nicole P. Juffermans

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DOI: 10.1186/s13054-016-1584-y

We are thankful for the letter of Steiner and colleagues in response to our paper on risk factors, host response and outcome of hypothermic sepsis.[1] We fully acknowledge the authors' contributions to the field and their efforts to reconcile experimental and clinical evidence on septic hypothermia.[2]

We agree with the authors that hypothermia could be an adaptive response during sepsis. It may be hypothesized that once the metabolic cost of fever outweighs its immune stimulatory benefits, the host may become hypothermic, thereby decreasing metabolism and also potentially decreasing inflammation. We also agree that our study was not designed to provide definitive evidence that hypothermia is a dysfunction in sepsis. As mentioned in the limitation section, our study was observational and cause–effect relationships cannot be established due to the nature of the study design. Indeed, findings which have been associated with hypothermia in previous studies, such as increased lymphopenia[3] and increased levels of fractalkine[1], can also be linked to increased disease severity and not to hypothermia per se.

Some of the experimental work may relate to clinical findings. Spontaneous hypothermia in rat endotoxemia may be a pre-emptive strategy to prevent hypoxia.[4] In comparison, patients who are more prone to hypoxia or a metabolic deficit may also develop hypothermia more often, such as those with preexisting circulatory dysfunction (i.e., chronic cardiovascular dysfunction) or those with few metabolic reserves (i.e., low body mass index). That said, however, it is difficult

to reconcile an adaptive response in rodents to an evidently increased mortality noted in observational studies in patients.[1]

A remark on the interpretation of findings in experimental models is that regulation of body temperature in rodents is profoundly different than in humans due to differences in the ratio of body content to body surface.[5] Therefore, experimental results need to be validated in clinical studies. We look forward to the results of an interventional study in which spontaneous hypothermia is allowed or prevented within the hypothermic subset of patients with sepsis. We would like to participate in this effort and we suggest that this trial includes analyses on the host response, including markers of immune suppression and endothelial dysfunction, to provide further insight into the etiology of hypothermia in sepsis pathogenesis.

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Chapter four

Patients with hypothermic sepsis have a unique gene expression profile compared to patients with fever and sepsis

Matthew B.A. Harmon*, Brendon Scicluna*, Maryse Wiewel, Marcus J. Schultz, Janneke Horn, Olaf L. Cremer, Tom van der Poll, W. Joost Wiersinga and Nicole P. Juffermans on behalf of the MARS consortium

* Contributed equally to this article

Submitted

Abstract

Objective The pathophysiology of hypothermia during sepsis is unclear. Using genomic profiling of blood leukocytes, we aimed to determine if hypothermia is associated with a different gene expression profile compared to fever during sepsis.

Design Prospective observational study.

Setting Two ICUs in tertiary hospitals in the Netherlands.

Patients Patients with sepsis and either hypothermia or fever within 24 hours after ICU admission were included in the study (n = 168). Hypothermia was defined as body temperature below 36 °C. Fever was defined as body temperature equal to or above 38.3°C.

Interventions Not applicable.

Measurements and Main results We compared blood gene expression (whole-genome transcriptome in leukocytes) in hypothermic septic compared to febrile septic patients in an unmatched analysis and matched for APACHE IV score and the presence of shock. In total 67 septic patients were hypothermic and 101 patients were febrile. Hypothermia was associated with a distinct gene expression profile in both unmatched and matched analyses. There were significant differences related to the up- and downregulation of canonical signaling pathways. In the matched analysis the top upregulated gene was Cold-inducible mRNA binding protein (CIRBP) which plays a role in cold-induced suppression of cell proliferation. In addition, we found three signaling pathways significantly upregulated in hypothermic patients compared to febrile patients; tryptophan degradation X, phenylalanine degradation IV and putrescine degradation III.

Conclusions There are distinct signaling pathways and genes associated with hypothermia, including tryptophan degradation and CIRBP expression, providing a possible link to the modulation of body temperature and immunosuppression. Future studies may focus on the canonical signaling pathways presented in this paper to further investigate spontaneous hypothermia in sepsis.

Introduction

Body temperature changes are common in sepsis.[1] Patients who present with spontaneous hypothermia suffer from substantially increased morbidity and mortality compared to their normothermic or febrile counterparts.[2,3] It is unclear whether hypothermia simply represents a symptom of severe inflammation or that hypothermia itself drives mortality through a yet unknown mechanism.[4,5] Animal studies even indicate that hypothermia may be an adaptive response to severe inflammation in order to limit metabolism and prevent hypoxia.[6]

To determine the etiology of the hypothermic response during sepsis, studies have mainly focused on the ability to generate an adequate host immune response, often with levels of pro-inflammatory cytokines as a read-out. However, studies have not confirmed a defective immune host response, as septic patients with hypothermia had similar[7,8], or even increased proinflammatory cytokine levels[9] in comparison to normothermic or febrile patients. Patients with hypothermic sepsis do develop persistent lymphopenia, a marker of immunosuppression.[10] Alternatively, the cardiovascular system may play a role. We previously showed that markers of endothelial injury are increased in hypothermic sepsis compared to nonhypothermic controls.[7]

Taken together, the pathophysiology of hypothermic response in sepsis remains ill defined. Whole blood transcriptome analysis has provided valuable insights in the complex pathophysiology of the sepsis syndrome.[11] In this study we aimed to determine if hypothermia in sepsis patients is associated with a different blood leukocyte gene expression profile compared to fever. We hypothesized that the blood transcriptomes of hypothermic sepsis patients differed from those obtained in febrile sepsis patients, which in turn reflect on variations in the host response.

Materials and methods

Study Design, Setting, and Patient Identification

This study was performed within the 'Molecular Diagnosis and Risk Stratification of Sepsis (MARS) project, a prospective observational cohort study in mixed ICUs of two tertiary teaching hospitals (Academic Medical Center in Amsterdam and University Medical Center in Utrecht) in the Netherlands (ClinicalTrials.gov identifier NCT01905033).[12,13] Between January 2011 and July 2012, patients older than 18 years of age with an expected length of stay longer than 24 hours were included via an opt-out consent method approved by ethical committees of both hospitals (IRB no. 10-056). During this study demographic, clinical, microbiology,

and interventional data were collected daily by trained research physicians. The plausibility of an infection was assessed using a four-point scale (none, possible, probable or definite) using Centers for Disease Control and Prevention and International Sepsis Forum consensus definitions[14,15] as described previously.[12]

We included patients diagnosed with sepsis and having blood microarray data obtained within the first 24 hours of ICU admission. Sepsis was defined on ICU admission as having definite or probable infection[12], combined with at least one parameter of inflammatory dysfunction, hemodynamic dysfunction, organ dysfunction or deranged tissue perfusion.[16] To limit the occurrence of iatrogenic hypothermia, patients admitted from the operating room (OR), ICU readmissions, patients undergoing active cooling, patients transferred from another ICU and patients with immunosuppression were excluded from this study.[7] Shock was defined as hypotension requiring treatment with vasopressors at a dose of 0.1 mcg/kg/min during at least 50% of the day. Clinical severity was assessed by Acute Physiology and Chronic Health Evaluation (APACHE) IV and Sequential Organ Failure Assessment (SOFA) scores.

To control for body temperatures that may have been inadvertently entered in the database (i.e. a rectal sensor that has been displaced and is exposed to ambient temperature), patients with unreliably low measurements of temperature (below 33°C) were excluded. Also, patients with only one registered temperature measurement during the first 24 hours were not included. Temperature was measured using a rectal, nasal, inguinal or tympanic temperature probes. Core temperatures were used in preference to inguinal or tympanic measurements.

Hypothermia was defined as a minimum body temperature <36.0°C and a maximum body temperature <38.3°C in the first 24 hours of ICU admission. Conversely, fever was defined as a maximum body temperature ³38.3°C and a minimum body temperature ³36.0°C as defined in previous studies.[10,17] Normothermia was defined a minimum and maximum body temperature between ³36.0°C and <38.3°C. Patients were defined as 'both' if they had both hypothermia and fever in the first 24 hours of ICU admission.

Blood Gene Expression Microarrays and bioinformatics

Whole blood was collected in PAXgene tubes (Becton-Dickinson, Breda, the Netherlands) within 24 hours after ICU admission and total RNA was isolated using the PAXgene blood mRNA kit (Qiagen, Venlo, the Netherlands) in combination with QIAcube automated system (Qiagen), as previously described.[13,18,19] Microarray data (Affymetrix Human Genome U219 96-array plates) are accessible to the public via the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) accession GSE65682.

Briefly, raw scans were pre-processed by means of the robust multi-average (RMA) method, normalized (quantile), summarized by median polish and log2 transformed using the affy method.[20] Non-experimental chip effects were assessed and corrected by means of the combat method in the surrogate variable analysis R package.[21] Comparisons between groups was done using multi-variate linear models, including age and gender as covariates, implemented in the limma method.[22] Benjamini-Hochberg (BH) adjusted p-values < 0.05 defined genome-wide significance. To assess the association with canonical signaling pathways we used Ingenuity Pathway Analysis software (Qiagen Bioinformatics). Fisher exact test BH-adjusted p-values < 0.05 demarcated significance. Human species and Ingenuity gene knowledgebase were specified. All other parameters were default.

Statistical analysis

All data were analyzed using R studio (version 3.2.2, R Core Team 2013, Vienna, Austria). In this study we performed two analyses. Firstly, we compared genomic profiles between septic hypothermic and febrile patients without correcting for disease severity. Subsequently, in order to determine whether hypothermia was associated with a specific genomic profile irrespective of severity of disease, hypothermic patients were 1:1 matched to fever patients using their APACHE IV scores and presence of shock. Data is presented as numbers (percentages), parametric data as mean ± SD and non-parametric data as median and 25^{th} – 75^{th} percentages; Q1-Q3). Data distribution was assessed by the Kolmogorov-Smirnov test. Mann-Whitney U or a Kruskal-Wallis test was used to analyze continuous nonparametric data, whereas continuous parametric data were analyzed using Student's t-test or analysis of variance (two-sided analysis of variance). All categorical data were analyzed using a chi-square or Fisher exact test. A p-value less than 0.05 was considered to be of statistical significance for clinical data. Matching was done using "optimal matching" with R-package "MatchIt" (caliper 0.35 standard deviations of the logit).

Results

Patients

The selection of study patients can be seen in figure 1. Out of a total of 579 sepsis admissions, 168 patients were included in the microarray analysis. Table 1 shows the baseline characteristics of these patients arranged by temperature group (baseline characteristics of patients that were normothermic (n=75) or both

Table 1. Baseline characteristics and outcome of sepsis patients according to different temperatures

	Hypothermia N=67	Normothermia N= 75	Fever N=101	Both N=18	P-value
Demographics					
Age, years, mean [SD]	68.1 [10.9]	64.7 [14.6]	60.0 [16.6]	58.1 [17.3]	0.002
Gender, male (%)	37 (55)	34 (45)	66 (65)	12 (67)	0.051
BMI, kg/m2, mean [SD]	25.5 [5.5]	25.9 [6.4]	27.0 [7.3]	27.1 [7.2]	0.498
Comorbidities					
Charlson score, median [IQR]	5 [3-6]	4 [3-7]	4 [2-6]	3 [2-4]	0.003
Chronic cardiovascular insufficiency (%)	4 (6)	2 (3)	2 (2)	4 (22)	0.003
Chronic renal insufficiency (%)	11 (16)	12 (16)	6 (6)	2 (11)	0.109
Congestive heart failure (%)	3 (4)	3 (4)	4 (4)	1 (6)	1
COPD (%)	9 (13)	16 (21)	17 (17)	2 (11)	0.57
Diabetes mellitus (%)	19 (28)	16 (21)	16 (16)	4 (22)	0.276
Site of infection					
Pulmonary (%)	28 (42)	31 (41)	50 (50)	6 (33)	0.524
Abdominal (%)	13 (19)	19 (25)	21 (21)	3 (17)	-
Urinary tract (%)	10 (15)	10 (13)	9 (9)	3 (17)	-
Other (%)	3 (4)	9 (12)	8 (8)	3 (17)	-
Co-infection (%)	13 (19)	6 (8)	13 (13)	3 (17)	-
Severity of disease first 24h					
Min temp first 24 h, mean [SD]	35.0 [0.9]	36.6 [0.5]	37.2 [0.7]	34.9 [0.7]	<0.0001
Max temp first 24 h, mean [SD]	37.1 [0.9]	37.6 [0.9]	39.3 [0.9]	38.8 [0.9]	<0.0001
APACHE IV score, median [IQR] [a]	82 [71.5-104.5]	80 [66.5-93.5]	68 [55-84]	92 [65.8-111.3]	<0.0001
SOFA score, median [IQR] [b]	9 [6-11]	8 [4-10]	7 [4-8]	6[4-11]	0.001
Acute kidney injury (%)	38 (57)	28 (37)	31 (31)	9 (50)	0.009
Renal replacement therapy (%)	14 (21)	4 (5)	8 (8)	4 (22)	0.008

Table continues on next page

Table 1. Continued.

	Hypothermia N=67	Normothermia N= 75	Fever N=101	Both N=18	P-value
Acute lung injury (%)	21 (31)	26 (35)	30 (30)	49 (22)	0.751
Shock (%)	32 (48)	26 (35)	25 (25)	7 (39)	0.023
Clinical laboratory parameters first 24h					
WBC count max. (x10^9/l), median [IQR]	17.4 [10.6-27.8]	15.5 [9.5-21.4]	14.0 [9.9-17.9]	15.0 [10.8-23.5]	0.33
Platelets min. (x10^9/l), median [IQR]	186 [114-254]	207 [129-297]	208 [131-305]	217 [146-248]	0.458
Lactate max. (mmol/l), median [IQR]	3.2 [2.1-9.1]	2.9 [1.7-5.2]	2.5 [1.6-3.7]	4.7 [1.95-6.38]	0.036
Prothrombin time max. (s), median [IQR]	16.3 [14.0-22-2]	14.8 [12.5-19.6]	14.1 [12.1-16.8]	15.8 [13.5-19.3]	0.004
Creatinine max. (µmol/l), median [IQR]	114 [76-200]	90 [65-162.5]	99 [71.5-161.5]	133 [82.3-234.5]	0.114
Outcome					
ICU-mortality (%)	21 (31)	13 (17)	9 (9)	4 (22)	0.003
30-day mortality (%)	29 (43)	20 (27)	16 (16)	4 (22)	0.001
90-day mortality (%)	35 (52)	28 (37)	22 (22)	7 (39)	0.002

APACHE, acute physiology and chronic health evaluation; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; SD, standard deviation; SOFA, sequential organ failure assessment; WBC, white blood cell. a Temperature not included in score
b Central nervous system not included in score due to large number of sedated patients

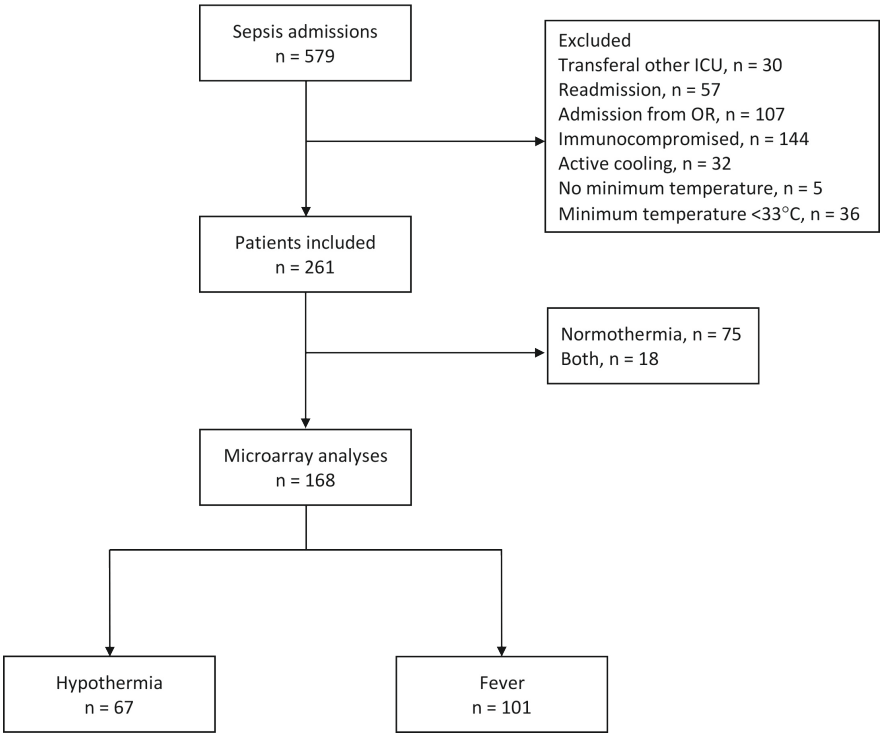
hypothermic and febrile (n=18) are shown in supplemental table 1 for the purpose of interpretation, but these were not included in the microarray analysis).
Of the 168 included patients, 67 patients were hypothermic and 101 patients were febrile. Minimum temperature in the hypothermic group was lower compared to the febrile group (35.0°C±0.9 *vs.* 37.2°C±0.7) as was the maximum temperature (37.1°C±0.9 *vs.* 39.3°C±0.9). Hypothermic patients were also older compared to febrile patients but BMI and gender distribution were similar between groups. There was also a similar distribution of site of infection between hypothermic and febrile patients.

Patients in the hypothermic group compared to febrile patients had higher APACHE IV scores and SOFA scores, increased incidence of shock and higher rates of mortality at 30-days post-ICU admission (29 (43%) *vs.* 22 (22%)).

Alterations in microarray gene expression in hypothermia compared to fever

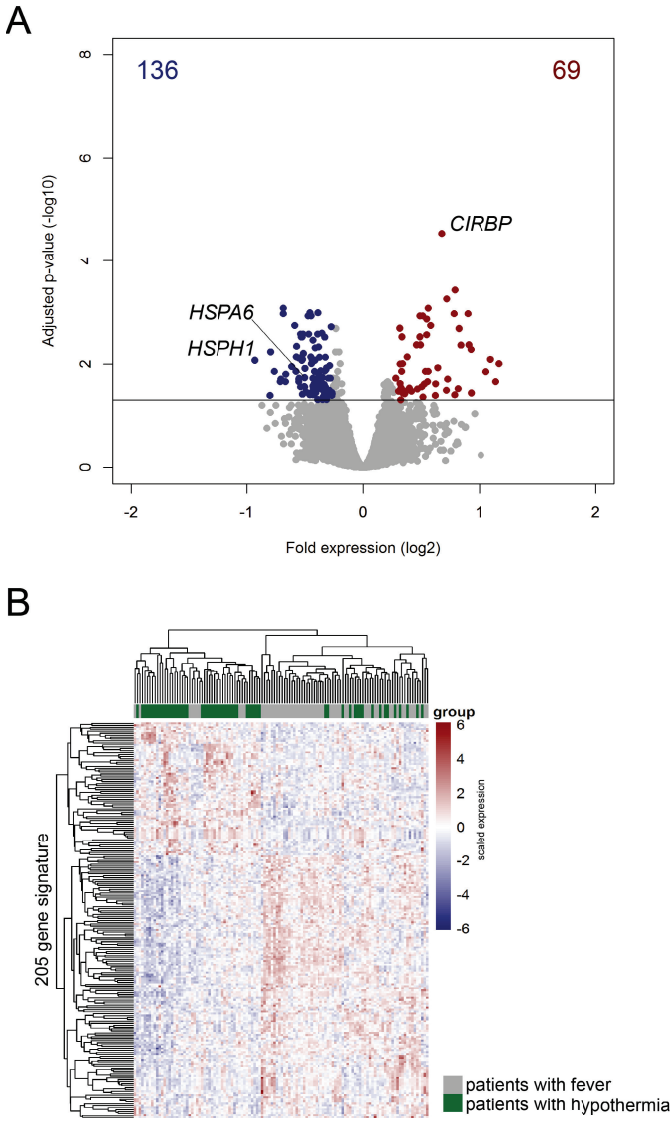
A total of 1930 transcripts were significantly altered in hypothermic patients compared to febrile patients, of which 1425 were reduced and 505 transcripts were elevated (supplemental figure 1A). Supplemental figure 1B shows the significant canonical pathways associated with these genes. Subsequently, we matched patients for APACHE IV scores and presence of shock. In total, 55 patients in each group remained for further analysis (characteristics of whom are shown in supplemental table 1). After matching there was no difference between groups in terms of APACHE IV scores and SOFA scores as well as incidence of shock. The 30-day mortality remained significantly increased in hypothermic patients (21 (38%) *vs.* 10 (18%), p = 0.041). Despite matching for APACHE IV scores, which includes age, patients in the hypothermic group were significantly older (67.3 years±11.6 vs. 61.8 years±16.3, p = 0.044). In total, 205 transcripts were significantly altered in hypothermic patients compared to febrile patients, of which 136 were reduced and 69 were elevated (figure 2A and B). The top-most significant gene was *CIRBP*, encoding cold-induced RNA binding protein, which plays a role in cold-induced suppression of cell proliferation. Among the genes with decreased expression in hypothermic patients we found members of the heat shock protein 70 complex (HSP70), namely *HSPH1* and *HSPA6*, encoding chaperone proteins that play essential roles in stress-induced misfolded protein responses (figure 2A). Pathway analysis of significantly altered, high expression genes resulted in significant associations with tryptophan degradation X, phenylalanine degradation IV and putrescine degradation III canonical signaling pathways (figure 2C). These signaling pathways relate to immunometabolic reactions that function in the degradation of amino acids and (poly)amines.

Figure 1. Flowchart showing the selection of study patients.

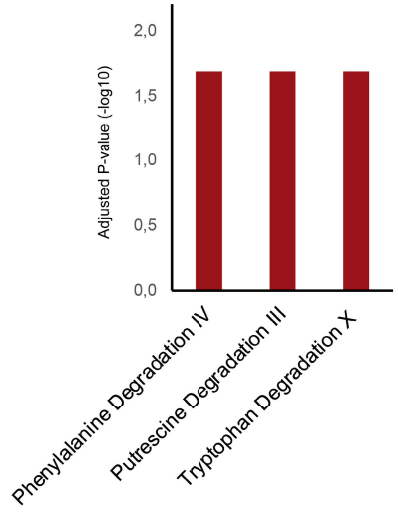


Some patients were excluded for multiple reasons and were counted multiple times for reason of exclusion.

Figure 2. Gene expression profiles from whole blood leukocyte microarray analysis in hypothermic septic patients versus febrile septic patients.



C



(A). Volcano plot (integrating adjusted p-values and fold expression indices) of gene expression differences in hypothermic septic patients compared to febrile patients in a cohort matched for APACHE IV scores and shock. Red dots, high expression genes; blue dots, low expression genes. (B) Unsupervised heatmap representation of the 205 significantly altered genes. (C) Bar plot showing high expression genes significantly associated with Ingenuity's canonical signaling pathways in hypothermia compared to fever.

Discussion

In this study of canonical pathways in blood leukocytes in septic patients we found that hypothermic septic patients have a unique gene expression profile compared to sepsis patient presenting with fever. After correcting for disease severity hypothermic septic patients showed a surprisingly similar gene expression profile compared patients with febrile sepsis. However, there were distinct upregulated signaling pathways related to degradation of amino acids and (poly)amines were strongly associated with hypothermia, both in uncorrected analyses and analyses corrected for disease severity.

Patients with hypothermic sepsis show significant alterations in genomic pathways compared to febrile patients. These included downregulated pathways related to protein catabolism and translation, cell growth proliferation and mobility, cardiovascular signaling, pattern recognition receptor and cytokine signaling and lymphocyte pathways. In addition, pathways relating to amino acid and (poly)amine degradation were significantly upregulated. In a different sepsis cohort similar defects in metabolic and immunologic signaling pathways have been associated with underlying immunoparalysis.[23] In line with this finding, a recent retrospective analysis of hypothermic septic patients found increased incidence of lymphopenia associated with hypothermia compared to nonhypothermic septic patients.[10] However, other studies focusing on the host response have revealed remarkably few differences between hypothermic and nonhypothermic or febrile patients regarding typical pro- and anti-inflammatory cytokine responses and thereby signal that hypothermic patients are initially able to mount an adequate host response.[7,8]

Subsequently, to address confounding due to increased disease severity in hypothermic patients, we adjusted our model for illness severity by matching of patients on APACHE IV score and presence of shock. In this analysis, many of the identified genetic pathways found in the unmatched cohort were no longer present in the matched cohort, suggesting that these pathways may be related to disease severity.

CIRBP expression, which encodes cold-induced RNA binding protein was significantly elevated in patients discordant for hypothermia sepsis, importantly, after matching patients for disease severity. Altered CIRBP expression in several different species in response to lower temperatures, suggesting that CIRBP is a conserved response to cold stress.[24] CIRPB expression is upregulated during hypoxia, hypothermia, and oxidative stress.[25] It plays a role in cold induced cell suppression. In addition, it can trigger an inflammatory response in sepsis[26] and may be a critical mediator in organ failure during sepsis.[25] Interestingly,

antagonizing CIRBP mitigates inflammation and improves survival in a mouse model of sepsis.[25]

HSPH1 and HSPA6, members of the HSP70 complex, were downregulated in hypothermic septic patients. HSP70 expression is temperature dependent and important for sustaining immune function during sepsis as well as cell protection.[27] Among other things it can upregulate the expression of proinflammatory and pyrogenic cytokines, such as tumor necrosis factor alpha and interleukin-1 beta.[28] HSP70 deficiency can aggravate peritonitis in mice.[29]

In the matched analysis, three canonical pathways associated with amino acid and polyamine degradation were significantly upregulated in hypothermic septic patients compared to febrile septic patients. First, the tryptophan degradation X pathway was upregulated. Tryptophan is an essential amino acid and degradation occurs along two different pathways.[30] Metabolites of one, the kynurenine pathway, mainly regulate anti-inflammatory effects of the immune response.[31] Tryptophan degradation to kynurenine is dependent on indoleamine-2,3-dioxygenase (IDO) enzyme activity.[30] In sepsis, increased degradation from tryptophan to kynurenine is associated with decreased lymphocyte counts.[32] In vitro and in vivo evidence shows that tryptophan metabolites are fatal for T-cell survival.[33] Hypothetically this could provide an explanation the association of prolonged lymphopenia and the occurrence of hypothermia in sepsis in the first 24 hours of ICU admission.[10] Tryptophan is also degraded to serotonin. Interestingly, serotonin deficient mice are extremely susceptible to temperature variations and show a profound hypothermic response when placed in a cold environment.[34] Serotonin also induces permeability of endothelial cells in sepsis.[35] Depending on the mechanisms involved in serotonin and sepsis, future studies could evaluate the effect of interventions on the tryptophan-serotonin axis, such as the use of serotonin receptor antagonists, which has improved survival in experimental settings, but has not been evaluated in humans.[36]

Secondly, the putrescine degradation III pathway was upregulated. Putrescine is a polyamine found in almost all living organisms. In humans it has shown the potential to modulate the innate immune response.[37] In a retrospective study in patients with community acquired pneumonia, putrescine levels were associated with disease severity and mortality.[38] In addition, putrescine is essential to the survival of important pathogens such as *Streptococcus pneumoniae*[39,40] and *Escherischia coli*.[41]

Phenylalanine degradation IV pathway was the third pathway which was significantly upregulated; phenylalanine is a precursor to L-dopa and catecholamines. Increased serum levels of phenylalanine have been shown to be increased in patients after trauma, burns and sepsis[42,43] and predict mortality in patients with a severe infection[44]. Increased serum levels of phenylalanine are related to insufficient tissue perfusion and impaired cellular energy production.

Impaired phenylalanine metabolism can interfere with the production of catecholamine and augment shock. Interestingly, tetrahydrobiopterin (BH4) is a co-factor for the degradation of both phenylalanine and tryptophan[45], providing a link between the two pathways found in this study.

Taken together, the presence of pathways that are upregulated irrespective of disease severity, suggests that hypothermic sepsis is associated with a distinct genomic profile. Our results suggest an important relation to the modulation of body temperature and immunosuppression. The genes and pathways found in this study could serve as potential therapeutic targets and warrant more investigation. The importance of these findings is not only limited to understanding the pathophysiology hypothermic response. Although not the primary aim of our study, the pathways identified in this study could also be used to identify at risk septic patients and monitor illness severity and efficacy of treatment.[44]

In general, the etiology and pathophysiology of the hypothermic response remains unclear. Our study suggests that patients with hypothermia distinct differences in gene expression profiles. Although several pathways were only altered in the unmatched analysis, these pathways may also hold valuable insights in the pathophysiology of hypothermic sepsis. By matching for disease severity, important pathways relating hypothermia and sepsis may be overlooked as hypothermia may represent a symptom of disease severity.[46] How the genes and pathways found in this study relate to the hypothermic response and increased mortality remains to be seen. While our study was not designed to address the relationship between transcriptomes of hypothermic adult and pediatric sepsis patients, generalizability of our findings to pediatric sepsis patients would represent an important extension. Based on previous reports on endotype classification of adult and pediatric sepsis patients[47], we envisage that transcriptional profiles in hypothermic/normothermic/febrile pediatric sepsis patients will reflect, at least in part, those observed in adult sepsis patients.

This study has several strengths. In this prospective cohort we looked at the extremes of temperatures in sepsis (hypothermia *vs.* fever). By doing this we reason we had the highest likelihood of finding pathways that relate to the hypothermic septic response. Of note, our study is based on a prospective observational cohort that was not designed to address the scope of our study. Future studies in larger cohorts of sepsis with or without hypothermia are certainly warranted. Secondly, instead of using conventional biomarkers, we used microarray in blood leukocytes to identify potential clues to the etiology of the hypothermic response in sepsis. Blood leukocytes represent a clinically relevant and easily accessible body compartment that has been extensively employed in clinical studies to identify fundamental features of the immune response during sepsis.[11] Results

from this study can guide future studies on the pathophysiology of the hypothermic response.

There are also several limitations to this study. First of all, pathway analysis based on transcriptional profiles does not clarify whether the upregulation or downregulation of pathways indicate a lack, or overabundance of a specific protein substrate. Future studies on the metabolite and protein products of the herein inferred canonical signaling pathways are warranted. Second, we did not standardize the timing and method of temperature measurements. However, core temperature measurements are common practice in the ICU setting and we also controlled for potentially incorrect temperature measurements. Furthermore, in this analysis we chose only to compare hypothermic patients to those with fever. As a result, there are large temperature differences between these two groups potentially minimizing the effect of any measurement inaccuracies. Also, blood sampling was performed in the first 24 hours of ICU admission. The sampling did not necessarily coincide with the hypothermic or febrile temperature measurement. However, we do not think that sampling needs to be simultaneous with temperature measurements in order to characterize this group of patients. A single hypothermic measurement in the first 24 hours of ICU admission is significantly associated with adverse outcome[1,7], and changes associated with hypothermia likely persist beyond the hypothermic measurement. Finally, in the matched analysis, age was significantly higher in the hypothermic group compared to the febrile group. Though age (and gender) was added as a covariate in our transcriptome analysis, the former may confound results, at least in part, since prior studies have shown that age is an independent risk factor for hypothermia.[48] Finally, due to the inherent nature of observational studies, cause-effect relationships cannot be established.

Conclusions

In conclusion, hypothermic patients were characterized by largely similar, but also significant changes in leukocyte transcriptomes. Genes were associated with distinct cellular biological pathways, including tryptophan metabolism and serotonin-signaling. Cold-inducible mRNA binding protein (CIRBP) expression was particularly elevated in sepsis patients with hypothermia. These signaling pathways provide a possible link to the modulation of body temperature and immunosuppression. Future functional studies on the canonical signaling pathways and specific genes presented in this paper are warranted.

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Supplemental material

Supplemental Table 1. Baseline characteristics in matched analysis for APACHE IV and SOFA score

	Hypothermic N=55	Fever N=55	P-value
Demographics			
Age, years, mean [SD]	67.3 [11.6]	61.8 [16.3]	0.044
Gender, male (%)	30 (55)	35 (64)	0.455
BMI, kg/m2, mean [SD]	25.1 [5.6]	27.3 [8.1]	0.11
Comorbidities			
Charlson score, median [IQR]	4 [3-6]	4 [2-6]	0.208
Chronic cardiovascular insufficiency (%)	4 (7)	0 (0)	0.139
Chronic renal insufficiency (%)	7 (13)	5 (9)	0.783
Congestive heart failure (%)	2 (4)	1 (2)	1
COPD (%)	6 (11)	10 (18)	0.42
Diabetes mellitus (%)	13 (24)	9 (16)	0.44
Site of infection			
Pulmonary (%)	23 (42)	27 (49)	0.431
Abdominal (%)	10 (18)	11 (20)	-
Urinary tract (%)	9 (16)	3 (5)	-
Other (%)	2 (4)	4 (7)	-
Co-infection (%)	11 (29)	10 (18)	-
Severity of disease first 24h			
Min temp first 24 h, mean [SD]	35.0 [0.9]	37.2 [0.6]	<0.0001
Max temp first 24 h, mean [SD]	37.1 [0.9]	39.2 [0.8]	<0.0001
APACHE IV score, median [IQR] a	81 [67.5-92.5]	77 [64.5-90.5]	0.76
SOFA score, median [IQR] b	8 [6-10]	7 [5-9]	0.258
Acute kidney injury (%)	28 (51)	21 (38)	0.259
Renal replacement therapy (%)	9 (16)	7 (13)	0.798
Acute lung injury (%)	15 (27)	18 (32)	0.682
Shock (%)	22 (40)	21 (38)	1
Clinical laboratory parameters first 24h			
WBC count max. (x10^9/l), median [IQR]	16.2 [10.4-27.8]	14.2 [9.7-17.7]	0.187
Platelets min. (x10^9/l), median [IQR]	188 [108-254]	200 [121-293]	0.365
Lactate max. (mmol/l), median [IQR]	2.9 [1.8-8.2]	2.8 [2-5.2]	0.4
Prothrombin time max. (s), median [IQR]	16.5 [15.0-22.5]	14.6 [12.1-17.8]	0.004
Creatinine max. (µmol/l), median [IQR]	112 [76-176]	129 [77-174]	0.805

Table continues on next page

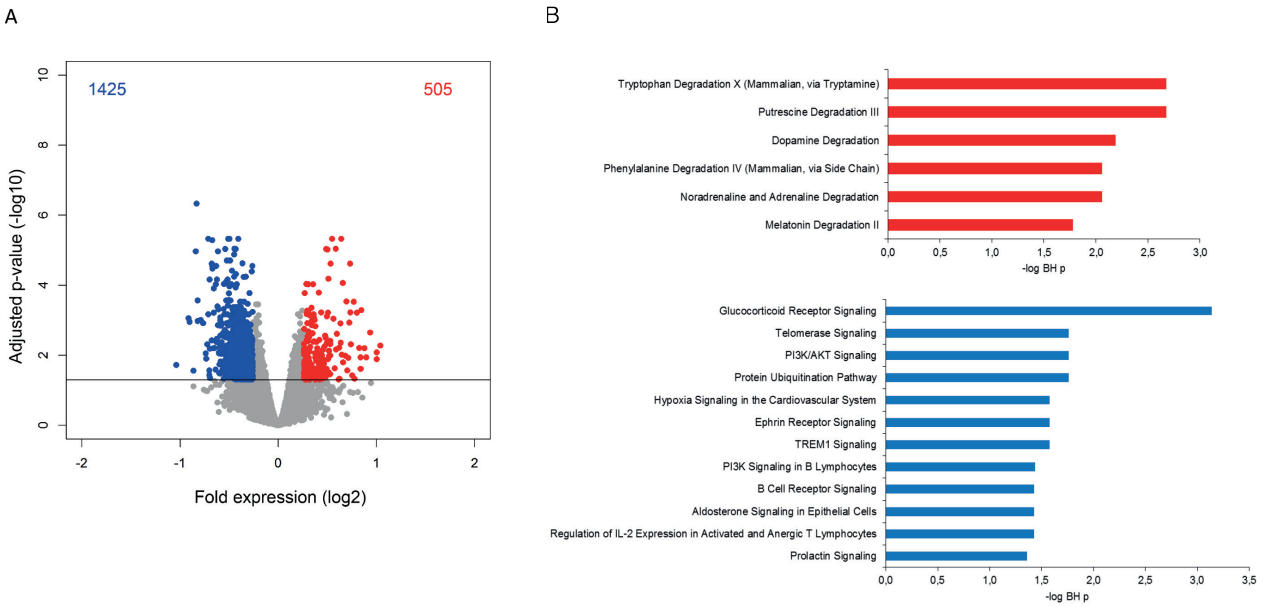
Supplemental table 1. Continued

	Hypothermic N=55	Fever N=55	P-value
Outcome			
ICU-mortality (%)	14(25)	6 (11)	0.077
30-day mortality (%)	21 (38)	10 (18)	0.041
90-day mortality (%)	26 (47)	12 (22)	0.008

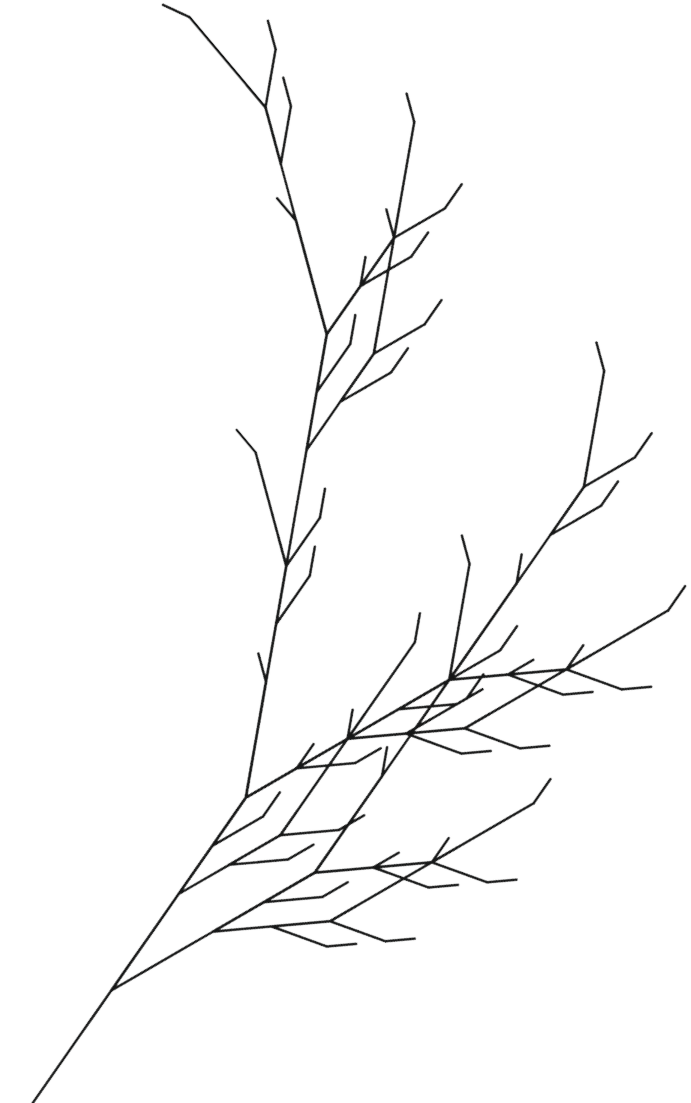
APACHE, acute physiology and chronic health evaluation; COPD, chronic obstructive pulmonary disease; IQR, interquartile range; SD, standard deviation; SOFA, sequential organ failure assessment; WBC, white blood cell. a Temperature not included in score

b Central nervous system not included in score due to large number of sedated patients

Supplemental figure 1.



(A) Volcano plot of gene expression profiles of hypothermic septic patients compared to febrile patients in the unmatched cohort. The volcano plot shows the differences in gene expression in hypothermia compared to fever (x-axis) and multiple-comparison adjusted p values for hypothermia compared to fever (y-axis). (B) shows the up- and downregulated canonical pathways associated with the gene expression profiles in hypothermia compared to fever.



Part two

Chapter five

Physiologic and host immune effects of induced normothermia in human endotoxemia

Matthew B.A. Harmon, Nanon F.L. Heijnen, S. de Bruin, Niek H. Sperna Weiland, Anita M. de Boer, Marcus J. Schultz, Janneke Horn and Nicole P. Juffermans

In preparation

Abstract

Background Induced normothermia as a treatment for sepsis is controversial. In this study, we aimed to investigate the effect of induced normothermia on the host physiological parameters and the immune response during lipopolysaccharide (LPS)-induced endotoxemia in healthy human volunteers.

Methods A total of 12 volunteers received an LPS-infusion of 2ng/kg. Subjects were assigned to either the induced normothermia group or the fever group. In the induced normothermia group, normothermia consisted of external surface cooling, cooled intravenous fluids (4°C) and medication to reduce shivering (buspirone, clonidine and magnesium sulphate). Blood samples were taken prior to- and at 1, 3, 6, and 8 hours after LPS-infusion. We measured plasma cytokine levels of tumor necrosis factor alpha (TNFa), interleukin (iL)-6 and iL-10. A mixed effects model was used to compare variables between groups over time. An area under the curve (AUC) was calculated for cytokine levels and compared using a Wilcoxon signed rank test.

Results Induced normothermia significantly reduced peak core body temperature compared to the control group (37.2°C (±0.3) vs. 38.7°C (±0.3); $p < 0.0001$). LPS resulted in an increased heart rate (59 ± 6 beats per minute (BPM) at baseline to 93 ± 11 BPM at t=3 hours, $p = 0.002$). Induced normothermia resulted in a significantly lower heart rate compared to controls ($p < 0.0001$) while mean arterial pressure ($p = 0.7$) and lactate levels were unchanged ($p=0.06$). Normothermia also lowered iL-10 levels ($p = 0.04$) but did not lower C-reactive protein (CRP) ($p = 0.08$) or plasma cytokine levels of iL-6 or TNFa ($p = 0.4$ and $p = 0.5$ respectively). Induced normothermia with external surface cooling was well tolerated in the awake subjects.

Conclusions In conclusion, induced normothermia with external cooling and pharmacological adjuncts in awake subjects after LPS infusion was feasible. Induced normothermia lowered heart rate while maintaining perfusion compared to febrile controls. Induced normothermia decreased iL-10 levels, but did not lower pro-inflammatory cytokine levels or CRP.

Background

Sepsis is characterized by an excessive hyper-inflammatory host response.[1] This response is partially mediated by pro- and anti-inflammatory cytokines, ultimately leading to organ failure and death.[2]

Fever, often defined as a temperature ³ 38.3°C, is common in sepsis and augments the inflammatory response during infections and sepsis. This response may be considered beneficial by promoting an adequate host immune response to invading organisms.[3] However, fever also has adverse effects, such as vasodilation, increased metabolic rate and tissue injury.[3] It is unclear to what extent critically ill septic patients can increase their metabolism without exacerbating tissue hypoperfusion.

Induced normothermia using external cooling could potentially benefit patients with sepsis. In a randomized controlled trial, cooling to a target temperature of 37°C lowered heart rate, decreased vasopressor use and possibly decreased 14-day mortality.[4,5] The mechanism is not known but may include inhibition of an excessive hyper-inflammatory response.[6,7] Also, induced normothermia could potentially reduce fever induced adrenergic stress by reducing heart rate, similar to beta-adrenergic blockade treatment.[5,8] However, intervening in the febrile response is controversial due to the perceived immune benefits of fever in addition to the potential side effects of cooling to normothermia. Induced normothermia may be associated with increased rates of nosocomial infections.[4] Also, although lowering body temperature decreases oxygen consumption, induced normothermia could potentially exacerbate tissue perfusion by decreasing cardiac output and causing vasoconstriction.[9] Lastly, induced normothermia in awake patients is perceived to cause discomfort. Data are limited on the effects of induced normothermia on physiology and the host immune response in hyperinflammatory states such as sepsis. Human endotoxemia models provide a controlled setting to study the physiologic and host immune effects of induced normothermia.[10]

In this study we examined the physiologic response to induced normothermia and studied the effects of induced normothermia on the host immune response during human endotoxemia. We hypothesized that induced normothermia improves hemodynamic parameters and mitigates the lipopolysaccharide (LPS) induced inflammatory cytokine release.

Methods

Study Design

The ESCIMO-study (External Surface Cooling In huMan endOtoxemia) was a human volunteer open-label non-randomized controlled trial. This study was reviewed and approved by the Amsterdam university medical center Medical Ethical Committee (NL53460.018.15) and performed according to the Declaration of Helsinki, including Good Clinical Practice. We included 12 healthy volunteers, aged 18-35 years with a body mass index (BMI) between 20-25 kg/m^2. The volunteers were screened before the start of the study and had no abnormalities on physical examinations, routine laboratory tests and electrocardiography. Subjects were excluded if they had a history of drug abuse, medication use on prescription, or had participated in any medical drug study 3 months prior to inclusion or in a previous volunteer studies using LPS. Supplemental table 1 shows the complete in- and exclusion criteria for this study. Volunteers were assigned either to the intervention group receiving LPS and induced normothermia or to a fever group only receiving LPS (n=6 per group).

Study model

The experiments took place at the Daycare center of an academic teaching hospital in Amsterdam, the Netherlands. An arterial catheter was placed for the monitoring of the blood pressure and taking blood samples, and a rectal temperature probe was inserted to measure core temperature. Other vital parameters were monitored with electrocardiography (ECG) and pulse oximetry. Ambient temperature in the room was set at 21°C.

At T=0 hours, all subjects received 2ng/kg Escherichia coli LPS (National Institutes of Health Clinical Center, Bethesda, United States of America). In the intervention group, induced normothermia was initiated at T=1 hour and continued to T=8 hours after LPS infusion using an external surface cooling device (Artic Sun© temperature management system). Cooling pads were applied to the torso and upper legs after which subjects were cooled to a core target temperature between 36.0 - 37.0°C via a closed loop system with feedback provided via the rectal temperature probe. Subjects also received cooled intravenous (IV) fluids for 150 minutes (4°C normal saline).

Cooling awake subjects without pharmacological adjuncts can result in increasing shivering with increases in the metabolic rate as well as inducing thermal discomfort. Moreover, cooling the skin can result in a compensatory increase in core temperature due to autonomic temperature defense mechanisms

such as vasoconstriction and shivering.[11] To counteract shivering and thermal discomfort during cooling, 30mg of buspirone was given orally at the initiation of induced normothermia. In addition, the subjects received clonidine (75mcg bolus followed by a continuous infusion of 1-2mcg/kg/h) and magnesium sulphate (4g bolus followed by a maximum continuous infusion of 2g/hour for 150 min). These doses were adapted from previous studies on cooling awake subjects.[12,13] To prevent nausea, ondansetron 4mg was given intravenously.

Prior to LPS and at 1, 3, 6, and 8 hours after LPS-infusion, subjects scored their own thermal discomfort on a scale ranging from 0 - 10, with 0 meaning extreme cold discomfort, 5 neutral, and 10 extreme warmth discomfort. Simultaneously, shivering was measured hourly using the bedside shivering assessment scale.[14]

Sample collection and analysis and outcomes

Blood samples were taken from the arterial catheter just before and at 1, 3, 6, and 8 hours after LPS administration for measurement of full blood count, chemistry and blood gas analysis. EDTA whole blood was centrifugated at 1500g and the supernatant was stored at – 80°C for later analysis of cytokines.

ELISA

Standard sandwich enzyme-linked immunosorbent assays (ELISA) were used to measure plasma levels of interleukin (iL)-6, iL-10, and tumor necrosis factor alpha (TNFα) according manufacturer's protocols (eBioscience, San Diego, CA, USA). ELISAs were performed on the supernatant of the EDTA anticoagulated samples. To account for interplate variability, two samples were included on each plate.

Statistical analysis

Statistical analyses were performed using Rstudio version 1.2. Depending on normality of the data, differences in baseline characteristics between groups were calculated with either the students T-test or the Wilcoxon ranked sums test. Linear mixed models were used to analyze differences in continuous variables between groups over time, using timepoint and group as fixed effects and subject ID as random effect. If data was non-parametric, it was log transformed prior to statistical testing.

For the cytokine measurements an area under the curve (AUC) was calculated based on the absolute change. Subsequently, the AUC between groups was compared using the Wilcoxon ranked sums test. Normally distributed data was presented a mean ± standard deviation (SD). Non-parametric data was presented

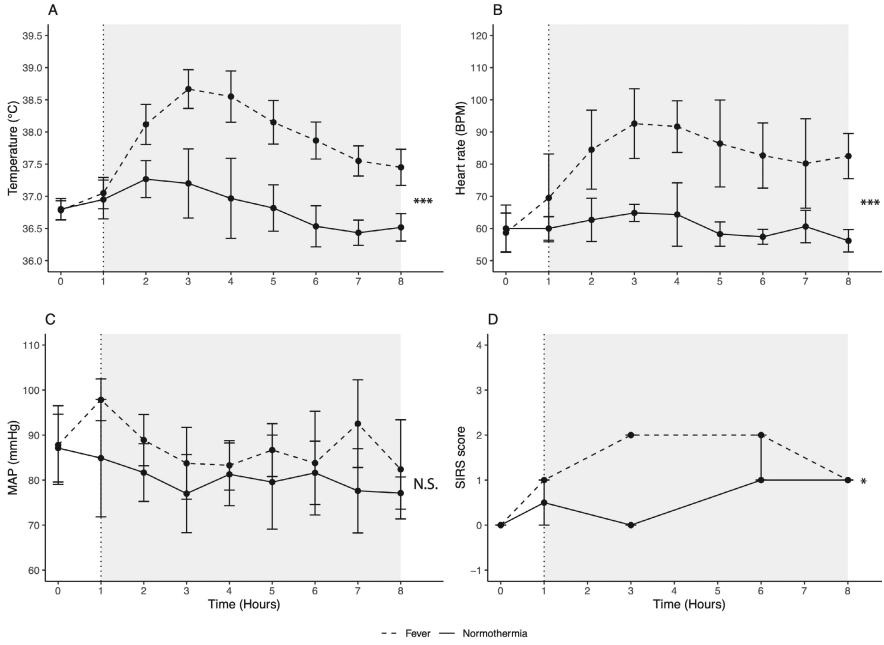
as median (25-75th percentile). Results from the linear mixed models were presented as Beta-coefficient (b) and 95% confidence interval (95% CI). A p-value below 0.05 was considered statistically significant.

Results

The impact of induced normothermia on the physiologic response to LPS-infusion

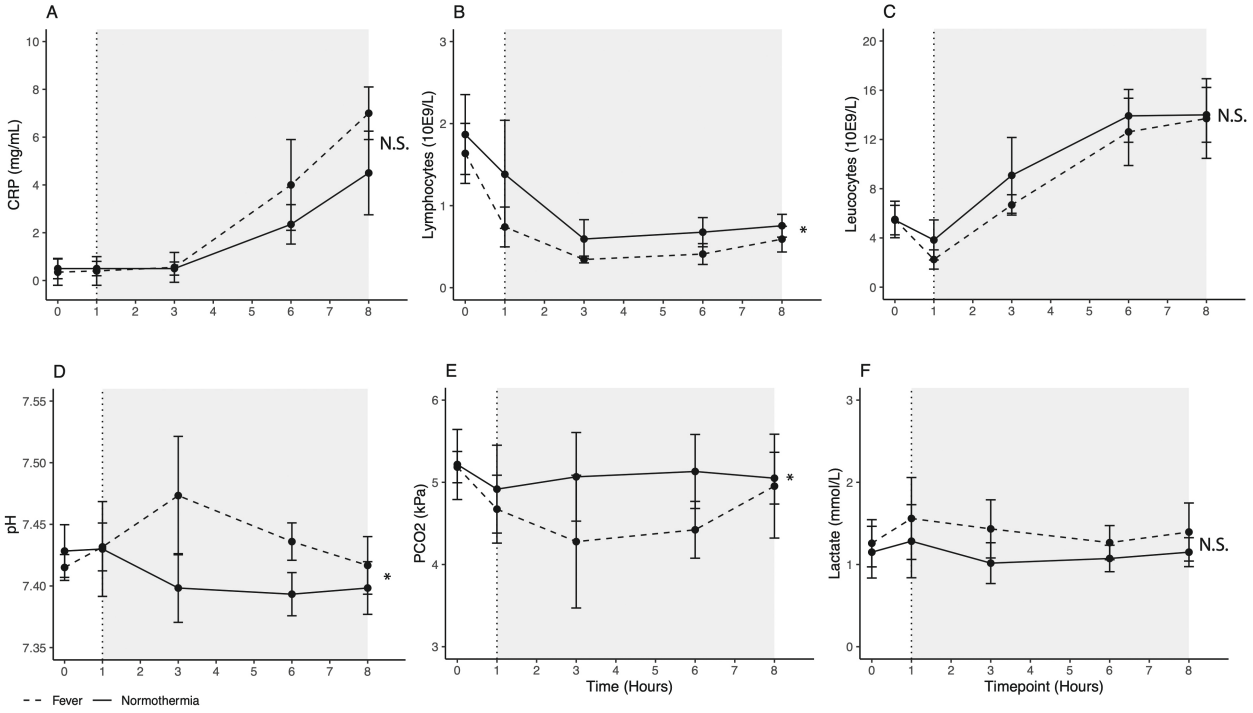
There were no baseline differences in heart rate, mean arterial pressure (MAP) and lab values between groups (data not shown). Subjects in both groups received similar amounts of fluids during the study period. The normothermia group received a total of 7.8g (±1.1g) magnesium and 321mg (±63mg) clonidine.
In the fever group, LPS induced shivering, resulting in fever with a peak temperature of 38.7°C (±0.3) at T=3 hours after LPS infusion (figure 1A). In the normothermia group, body temperature was significantly lower compared to the fever group (figure 1A, $p<0.0001$). The mean peak body temperature was 37.2°C (±0.3) in the normothermia group, at T=3 hours. Supplemental figure 1 shows the individual temperature plots for each volunteer. LPS resulted in an increased heart rate (figure 1B; 59 ± 6 beats per minute (BPM) at baseline to 93 ± 11 BPM at t=3 hours, $p=0.002$). Induced normothermia resulted in a significantly lower heart rate compared to volunteers with fever ($p < 0.0001$). MAP values did not differ between groups over time (figure 1C; $p = 0.08$). At T=3 hours after admission of LPS, Systemic inflammatory response syndrome (SIRS) scores peaked at 2 [2-2] (figure 1D). In the normothermia group, SIRS score was lower compared to the fever group ($p = 0.001$). LPS increased pH (from 7.42 ± 0.01 at baseline to 7.47 ± 0.05 at t=3 hours, $p=0.02$) and decreased PCO2 levels (from 5.19 ± 0.19 kPa at baseline to 4.28 ± 0.81 kPa at t=3 hours, $p= 0.04$), likely due to hyperventilation (figure 2D & E respectively). Induced normothermia lowered pH ($p = 0.01$) and PCO2 $p = (0.05)$ during the study period compared to the fever group. Lactate levels increased after LPS infusion, but not significantly (figure 2F; from 1.26 ± 0.29 mmol/L at baseline to 1.56 ± 0.50 mmol/L at T=1-hour, $p=0.26$). Lactate levels were consistently lower in the normothermia group compared to the fever but were not significantly different ($p = 0.06$). Shivering was largely prevented in the normothermia group (figure 3A). One hour after LPS infusion, volunteers in both groups reported cold discomfort. In the fever group, this gradually changed to heat discomfort at T=4 hours. The normothermia group reported cold discomfort at T=1 hour gradually returned to neutral at T=4 hours (figure 3B).

Figure 1. Physiology parameters over time in volunteers with endotoxemia and fever and volunteers with endotoxemia treated with induced normothermia.



Lipopolysaccharide was given to all volunteers at T=0. Cooling to normothermia (37°C) was initiated at T=1 in the normothermia group. Dashed line indicates volunteers in the fever group. Solid line indicates volunteers in the normothermia group. Grey box represents the period of induced normothermia.
Figure 1A-C: Dots represent means with bars representing standard deviation. Figure 1D: Dots represent medians with bars representing the interquartile ranges *P < 0.05, ***P <0.0001, N.S. = Not significant.
Abbreviations: BPM = beats per minute, LPS = lipopolysaccharide, MAP = mean arterial pressure, SIRS = systemic inflammatory response syndrome

Figure 2. Laboratory parameters over time in volunteers with endotoxemia and fever and volunteers with endotoxemia treated with induced normothermi



Lipopolysaccharide was given to all volunteers at T=0. Cooling to normothermia (37°C) was initiated at T=1 in the normothermia group. Dashed line indicates volunteers in the fever group. Solid line indicates volunteers in the normothermia group. Grey box represents the period of induced normothermia. Figure 2A: Data are represented as medians with bars representing the interquartile ranges. Figure 2B-F: Dots represent means with bars representing standard deviation. *P < 0.05, N.S. = Not significant.

Abbreviations: CRP= C-reactive protein, LPS = lipopolysaccharide

Figure 3. Shivering and thermal discomfort scores of volunteers during the study period.

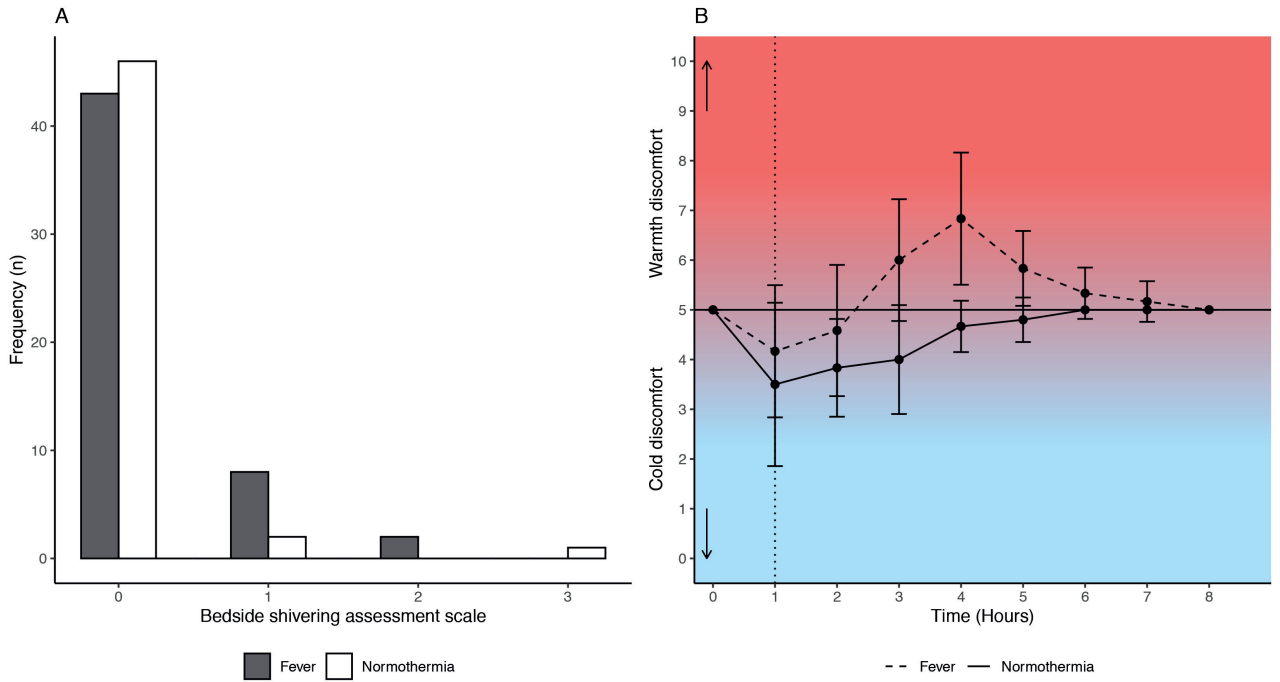


Figure 3A: shows the cumulative frequency of observed shivering scores during the study period. Grey bars show the fever group. White bars show the normothermia group. Figure 3B: shows the warmth and cold discomfort scored over time. Dots represent means with bars representing standard deviation. Lipopolysaccharide was given to all volunteers at T=0. Cooling to normothermia (37°C) was initiated at T=1 in the normothermia group. Dashed line indicates volunteers in the fever group. Solid line indicates volunteers in the normothermia group. Abbreviations: LPS = lipopolysaccharide

The effect of induced normothermia on the inflammatory host response.

LPS resulted in an increase in C-reactive protein (CRP) (figure 2A; from 0.4 mg/mL [0.3-0.9] at baseline to 7.7 mg/mL [6.85-10.0] at t=8 hours, p=0.03) and in leukocyte count (figure 2B; from 5.5 ± 1.5 x 10E9/L cells at baseline to 13.7 ± 3.2 x 10E9/L cells at t=8 hours, p <0.0001). Lymphocytes decreased after LPS infusion (figure 2C; from 1.6 ± 0.4 x 10E9/L cells at baseline to 0.3 ± 0.0 x 10E9/L cells at t=8 hours, p<0.0001). Induced normothermia was associated with lower CRP levels albeit not statistically significantly altered (p = 0.08). Induced normothermia did not alter leukocyte levels (p = 0.13). Lymphocyte counts were significantly lower in the normothermia compared to the fever group (p = 0.007), however the difference between groups was the largest at T=1 and likely not an effect of cooling.

In the fever group, infusion of LPS resulted in an increase in IL-6 and IL-10 plasma levels with a peak at T=3 hours. TNFa levels peaked at T=1 hour after LPS infusion. Figure 4A-C shows the change in cytokine levels from baseline. Induced normothermia lowered plasma cytokine levels of iL-10 compared to febrile controls (figure 4F, p=0.04). but did not alter plasma cytokine levels of iL-6 and TNFa (figure 4D, E, p = 0.4 & p = 0.5 respectively). Supplemental table 2 and 3 show all results from the mixed model and AUC analyses.

Discussion

In this model of human endotoxemia, we found that induced normothermia (using external cooling and medication to prevent shivering) was well tolerated by awake volunteers. Cooling to normothermia successfully prevented the LPS induced febrile response. Induced normothermia also lowered heart rate but did not affect perfusion as measured by MAP and lactate levels. Induced normothermia lowered iL-10 levels compared to the fever group but did not lower CRP or plasma cytokine levels of iL-6 or TNFa.

Induced normothermia did not have a profound effect on makers of inflammation in this study as only iL-10 levels were affected. Of note, the TNFa response is known to be very early. As cooling was initiated after 1 hour, the window to prevent a TNFa response possibly had already passed. However, it is surprising that iL-6 levels were not lower following induced normothermia. Experimental studies in animals have shown that cooling has a profound effect on proinflammatory cytokine levels.[15] However, these studies were mostly performed in animals cooled to hypothermia. Possibly, the temperature difference in our study was not large enough to exert a significant effect on iL-6. Additionally, the sample size of our study may have been too small to find an effect of cooling to

Figure 4. Changes in cytokine profile in volunteers with endotoxemia and fever and volunteers with endotoxemia treated with induced normothermia.

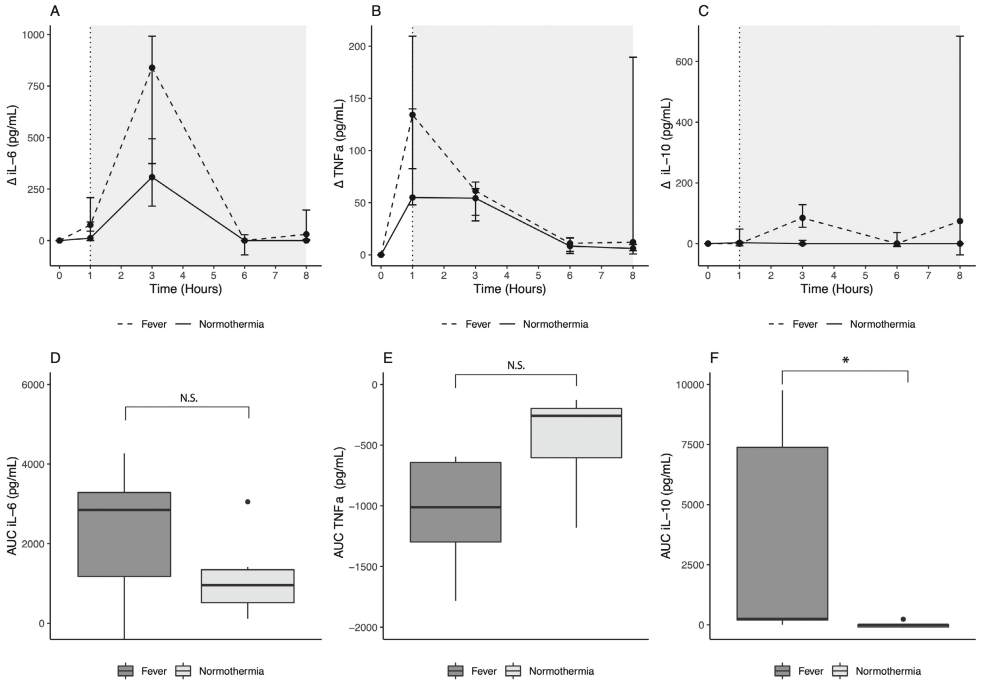


Figure 4A-C: Shows changes in cytokine profile in plasma from baseline over time. Lipopolysaccharide was given to all volunteers at T=0. Cooling to normothermia (37°C) was initiated at T=1 in the normothermia group. Dashed line indicates volunteers in the fever group. Solid line indicates volunteers in the normothermia group. Grey box represents the period of induced normothermia. Dots represent means with bars representing the standard deviation. Figure 4D-F: Boxplot showing the area under the curve of cytokine levels between the fever group (dark grey boxes) and normothermia group (light grey boxes). *P < 0.05, N.S. = Not significant.
Abbreviations: AUC = area under the curve, iL = interleukin, LPS = lipopolysaccharide, TNFa = tumor necrosis factor alpha

normothermia, as we found larger than expected individual variation in cytokine levels. Also, sedation due to pharmacological adjuncts may have partially negated the effects of cooling by reducing hyperventilation and resultant hypocapnia. Hyperventilation has previously been shown to lower cytokine levels in an endotoxemia model.[16]

Induced normothermia lowered heart rate while maintaining perfusion as measured by MAP and lactate, suggesting cooling did not adversely affect tissue perfusion. Previously, cooling of febrile sepsis patients reduced the amount of vasopressor dose needed to maintain an adequate MAP.[4] Reducing heart rate during sepsis could directly benefit patients, by reducing myocardial oxygen consumption and improving diastolic relaxation time which improves coronary perfusion.[17] Interestingly, in patients with septic shock, treatment with esmolol decreased mortality. Esmolol reduced heart rate and endogenous norepinephrine levels while increasing stroke volume index,[8] and microcirculatory flow.[17] Thereby, cooling may be of benefit in patients with supposedly high adrenergic activity. However, not all studies show hemodynamic benefits from cooling to normothermia. In previous studies in patients with septic shock and fever, cooling to normothermia decreased MAP, cardiac output and increased lactate.[6,7] These apparent differences with our results may in part relate to timing and intensity of cooling.[7] Although these studies are difficult to compare due to the use of different target temperatures and lack of a control group, the results indicate that during cooling, some form of tissue perfusion monitoring is warranted.

In general, clinical or biological markers to assess the risks and benefits of fever in individual septic patients is lacking. Subsequently, treating all septic patients with induced normothermia will likely result in a heterogeneity of the treatment effect. Future clinical studies should focus on finding markers to determine a patient's appropriate body temperature in sepsis, in order to determine which patients may benefit from cooling.

Importantly, cooling was well tolerated in the awake volunteers. Cooling in awake volunteers without pharmacological adjuncts to reduce shivering results in an increased metabolic rate and actually aggravates fever due to compensatory autonomic mechanisms.[11] Several different pharmacological interventions have been previously used to overcome shivering during cooling of awake volunteers.[13,18] Clonidine, an a2-agonist used in this study, is effective in preventing shivering in healthy individuals undergoing external cooling, possibly by impairing central thermoregulatory control.[12] $MgSO_4$ has been shown to increase the rate of cooling, likely in part by preventing peripheral vasoconstriction.[19] In addition, $MgSO_4$ can mitigate thermal discomfort due to surface cooling.[19] Buspirone is a serotonin 5-HT1A partial agonist and directly lowers body temperature. In a previous

study, buspirone also acted synergistically with meperidine to lower the shivering threshold.[20]

However, as a result of the use of pharmacological adjuncts during cooling, the noted changes in this study could have been due to external cooling or to the effects of magnesium and clonidine. In an attempt to dissect the effects of external cooling and the effects of magnesium and clonidine, we aimed to add a third group to this study, which received LPS with the same dose of magnesium and clonidine but without external cooling. However, following administration of these drugs, the two included volunteers became hypotensive towards the end of the study and could not continue the intervention. We terminated this study group for safety reasons. However, as external cooling often requires some drugs to counteract shivering, the combination of these interventions may reflect pragmatic clinical practice.

Conclusions

In conclusion, induced normothermia with external cooling and pharmacological adjuncts was well tolerated in awake subjects. Induced normothermia lowered heart rate but did not affect perfusion compared to febrile controls. Induced normothermia decreased iL-10 levels compared to febrile controls but did not lower proinflammatory cytokine levels or CRP.

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Supplemental material

Supplemental Table 1. In- and exclusion criteria.

Inclusion criteria
1. Male
2. Age 18 – 35 years
Exclusion criteria
1. No informed consent
2. Any abnormal test result during the screening prior to inclusion (medical history, physical examination, ECG, blood and urine examination).
3. History of drug abuse
4. Use of any medication on prescription
5. Smoking < 6 months
6. Participation in any other medical study < 3 months
7. Participation in previous volunteer studies using LPS

Supplemental Table 2. Overview of mixed model results of individual physiological laboratory and calculated parameters comparing volunteers with endotoxemia and fever and volunteers with endotoxemia treated with induced normothermia (37°C).

Variable	Beta-coefficient	95% Confidence interval	P-value
Temperature °C	-1.1	-1.4 - -0.8	<.0001
Heart rate (BPM)	-22.8	-30.7 - -14.8	<.0001
Mean arterial pressure (mmHg)	-6.7	-14.6 – 1.2	0.08
SIRS score *	-0.5	-0.7 - -0.2	0.001
CRP (mg/mL) *	-0.2	-0.5 - 0.0	0.08
Leukocytes (10E9/L)	1.4	-0.6 - 3.4	0.13
Lymphocytes (10E9/L)	0.3	0.1 - 0.6	0.007
pH	-0.03	-0.06 - - 0.01	0.01
PCO2 (kPa)	0.4	0.0 - 0.9	0.05
Lactate (mmol/L)	-0.3	-0.6 - 0.0	0.06

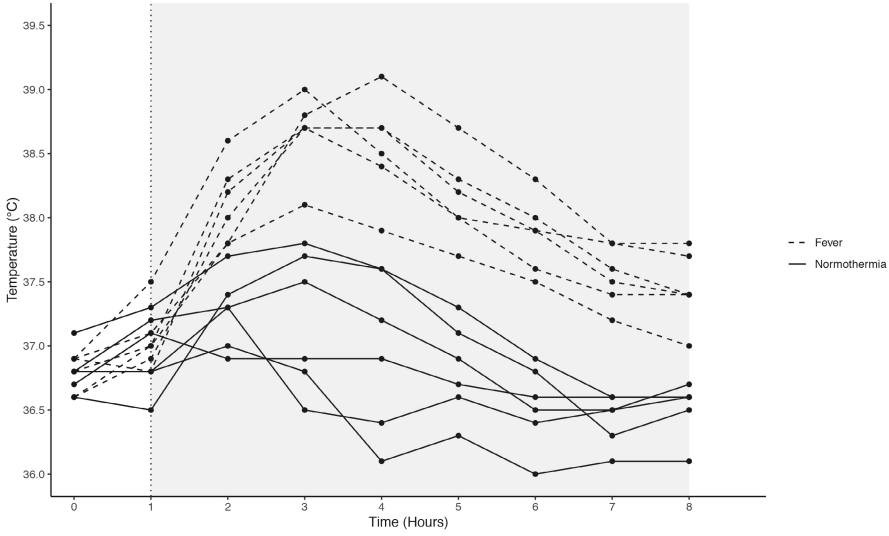
* Log transformed data. Abbreviations: BPM = beats per minute, CRP = C-reactive protein, SIRS = systemic inflammatory response syndrome

Supplemental Table 3. Overview of different cytokine AUC levels between volunteers with endotoxemia and fever and volunteers with endotoxemia treated with induced normothermia (37°C).

	Fever (n=6)	Normothermia (n=6)	
Variable	**Median [IQR]**	**Median [IQR]**	**P-value**
iL-6 AUC (pg/mL)	1841 [414 - 2252]	812 [62 - 1401]	0.4
TNFa AUC (pg/mL)	-1012 [-1298 - -643]	- 259 [-604 - -198]	0.5
iL-10 AUC (pg/mL)	242 [202 - 7383.6]	0 [-89 - 5.9]	0.04

Abbreviations: AUC = area under the curve, iL = interleukin, IQR = interquartile range, LPS = lipopolysaccharide, TNFa = tumor necrosis factor alpha

Supplemental Figure 1. Individual temperature plots of each healthy volunteer.



Lipopolysaccharide was given to all volunteers at T=0. Cooling to normothermia (37°C) was initiated at T=1 in the normothermia group. Dashed line indicates volunteers in the fever group. Solid line indicates volunteers in the normothermia group. Grey box represents the period of induced normothermia.

Chapter six

Induced normothermia ameliorates the procoagulant host response in human endotoxemia.

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Abstract

Background Dysregulation of coagulation is an important characteristic of sepsis, ranging from mild consumption coagulopathy to disseminated intravascular coagulation (DIC). In this study, we aimed to investigate the effect of induced normothermia on coagulation during lipopolysaccharide (LPS)-induced endotoxemia in healthy volunteers.

Methods A total of 12 volunteers received an LPS-infusion of 2ng/kg and were assigned to either the fever group or the induced normothermia group. Induced normothermia (37°C) consisted of external surface cooling using the Arctic Sun© device, cooled intravenous fluids (4°C) and medication to reduce shivering (buspirone, clonidine and magnesium sulphate). Conventional coagulation tests, plasma levels of von Willebrand factor (vWf) and rotational thromboelastometry (ROTEM) were measured prior to- and at 1, 3, 6, and 8 hours after LPS-infusion. Differences between groups were tested with a mixed effects model.

Results LPS caused a transient decrease in platelet levels and in activated partial thromboplastin time (aPTT), while prolonging prothrombin time (PT). LPS also increased D-Dimer and vWf levels compared to baseline. Induced normothermia inhibited the decrease in platelet levels ($p= 0.002$), aPTT ($p=0.005$) and vWf levels ($p=0.03$) compared to the fever group. Induced normothermia also improved calculated DIC scores compared to volunteers with fever ($p=0.04$). ROTEM measurements were largely unaffected by LPS.

Conclusion In human endotoxemia, induced normothermia decreases makers of endothelial activation and DIC and should be further studied as a potential treatment in hyperinflammatory states with consumption coagulopathy, such as sepsis.

Background

The dysregulated host-immune response in sepsis involves activation of a procoagulant response and impairment of anticoagulant mechanisms, resulting in consumption coagulopathy with low platelet count, prolonged prothrombin time (PT) and increased D-dimer levels. The most severe septic patients develop overt disseminated intravascular coagulation (DIC). These coagulation changes are associated with multiple organ failure.[1] Sepsis often presents with fever, defined as a core body temperature of > 38.3°C.[2] Fever is commonly thought to be a functional response to infection as it improves pathogen clearance and immune cell mobility.[3] However, fever may also have harmful effects. The increased metabolic cost of fever may exacerbate oxygen deficits at a cellular level, resulting in increased tissue injury, such as cardiac distress and encephalopathy.[3] Also, fever may contribute to an exaggerated pro-inflammatory response, with increased cytokine production and vasodilatory shock. In line with this, induced normothermia in patients with septic shock presenting with fever was found to decrease vasopressor need compared to the febrile control arm[4], although not all studies have reported beneficial effects.[5]

Data are scant on how cooling affects the procoagulant host response. Whereas profound hypothermia decreases platelet function and impairs the synthesis and kinetics of clotting factors[6], cooling to normothermia is not likely to illicit these processes. Conversely, as induced normothermia has been shown to limit inflammation[5], this could actually reduce the activation of coagulation.

Experimental human endotoxemia provides for a controlled setting to study the effects of fever control on the host immune response. The model of endotoxemia induced by lipopolysaccharide (LPS) is characterized by a procoagulant response, with increased levels of von Willebrand factor (vWf) and tissue factor, together with a (transient) profound decrease in platelet count and prolonged prothrombin time (PT), thereby closely resembling sepsis-associated coagulopathy.[7-9]

In this study, we aimed to investigate the effect of induced normothermia on the coagulation processes during LPS-induced endotoxemia in healthy volunteers. We hypothesized that cooling to normothermia limits the activation of coagulation.

Methods

Study Design, Setting, and Patient Identification

This study is part of a larger study on the mechanisms and safety of fever control in healthy volunteers. In short, the ESCIMO-study (External Surface Cooling In huMan endOtoxemia) was a human volunteer open-label non-randomized controlled trial in which 12 healthy male volunteers aged 18 to 35 were included at the Amsterdam University Medical Centre. Inclusion criteria were an unremarkable medical history and physical examination. This study was reviewed and approved by the Amsterdam university medical centre Medical Ethical Committee (NL53460.018.15) and performed according to the Declaration of Helsinki, including Good Clinical Practice.

Study model

Every subject received an indwelling arterial catheter for measurement of blood pressure and blood sampling. A rectal temperature probe was inserted to measure core temperature. Other vital parameters were monitored with electrocardiogram and pulse oximetry. Ambient temperature in the room was set at 21°C.
In this study, the first 6 participants were designated to the fever group which received LPS. The following 6 participants were included in the normothermia group, which received LPS and were subsequently cooled to normothermia. At T=0 hours, all subjects received 2ng/kg Escherichia coli LPS (National Institutes of Health Clinical Centre, Bethesda, United States of America). In the normothermia group, cooling was initiated at T=1 hour and continued to T=8 hours after LPS-infusion using an external surface cooling device (Arctic Sun© temperature management system), set at a target temperature of 36.0-37.0°C. To minimize shivering, subjects in the normothermia group received buspirone, magnesium sulphate and clonidine. To prevent nausea, ondansetron 4mg was given intravenously.

Sample collection and analysis and outcomes

Citrated blood samples (BD™ Vacutainer™ Citrate Tubes) were taken from the arterial catheter just before and at 1, 3, 6, and 8 hours after LPS administration. Blood was centrifugated at 1500g and the supernatant was stored at –80°C for later analysis of vWf antigen analysis by Enzyme-linked immunosorbent assay (ELISA) using a homemade assay with antibodies from DAKO (Glostrup, Denmark).

Citrated whole blood was analysed with the rotational thromboelastometry (ROTEM) delta device at 37°C. The variables measured were: coagulation time (CT), clot formation time (CFT), clot amplitude after 5, 10, 15, 20, 25, and 30 minutes (CA-5, -10, -15, -20, -25, -30), α-angle (alpha), maximum clot firmness (MCF), clot lysis at 30, 45, and 60 min (LI30, 45, and 60), and the maximum lysis in percentage (ML). If an error appeared during the test or it seemed that a subtest (like extrinsically activated test (EXTEM), intrinsically activated test (INTEM) or fibrin-based extrinsically activated test (FIBTEM)) did not run properly, that particular test was repeated immediately with blood retrieved from the same sample in order to provide a reliable result. The G-value was assessed using the formula (5,000 × MCF) / (100 – MCF) and expressed as dynes/cm2.[10] The systemic inflammatory response syndrome (SIRS) score was calculated according to the Bone criteria.[11] The DIC-score was calculated according to the International Society on Thrombosis and Haemostasis (ISTH) guideline.[12]

Statistical analysis

Statistical analyses were performed using R studio version 1.3. Depending on normality of the data, baseline differences between groups were calculated with either the students T-test or the Wilcoxon ranked sums test. To compare changes over time within groups, a paired students T-test or the Wilcoxon ranked sums test was performed between T=0 and the maximum or minimum value during the study period. Linear mixed models were used to analyse differences in continuous variables between groups over time, using timepoint and group as fixed effects and subject ID as random effect. Nested models with and without group as a variable were compared to determine differences between groups. If data were non-parametric, data were transformed prior to statistical testing. Normally distributed data were presented as mean ± standard deviation. Non-parametric data were presented as median (25-75th percentile). Results from the linear mixed models were presented as Beta-coefficient (b) and 95% confidence interval (95% CI). A p-value below 0.05 was considered statistically significant.

Results

The impact of fever control on the physiologic response to LPS-infusion

LPS resulted in fever in the fever group with a mean peak temperature of 38.7°C (±0.3) at t=3 hours after LPS-infusion. In the normothermia group, body temperature was significantly lower ($p<0.0001$), with mean peak temperature at t=3 hours of 37.2°C (±0.3). LPS resulted in an increased heart rate (58.7 ± 6.1

beats per minute (BPM) at baseline to 92.6 ± 10.9 BPM at t=4 hours). LPS caused a leucocytosis (5.5 ± 1.5 × 10^9/L cells at baseline to 13.7 ± 3.2 × 10^9/L cells at t=8 hours) and an increase in C-reactive protein (CRP) (0.4 [0.3 -0.9] mg/mL at baseline to 7.7 [6.9 – 10.0] at t=8 hours).

The impact of fever control on LPS-induced consumption coagulopathy

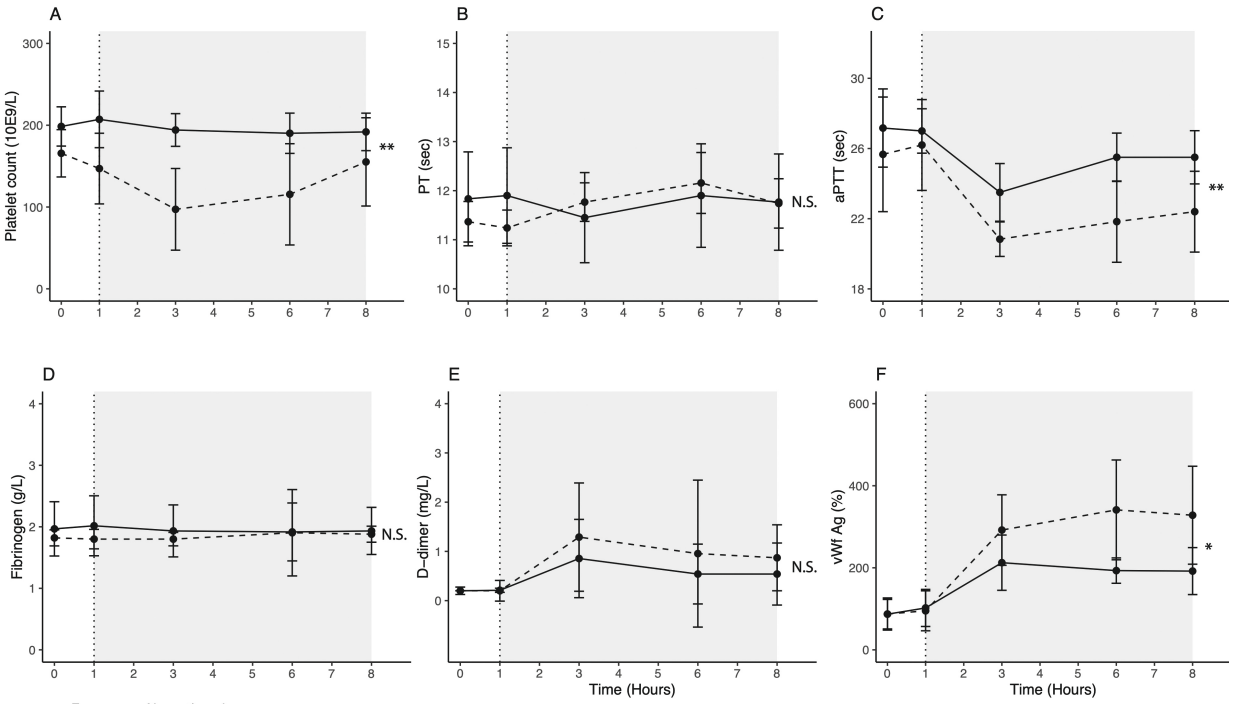
LPS resulted in a thrombocytopenia in the fever group (from 166 ± 29 × 10^9/L cells at baseline to 97 ± 50 × 10^9/L cells at t=3 hours, $p < 0.0001$). Platelet counts were significantly higher in the normothermia group compared to the fever group (194 ± 20 × 10^9/L cells at t=3 hours, figure 1A; $p = 0.003$). LPS resulted in PT prolongation in the fever group (from 11.4 sec ± 0.4 at baseline to 12.2 sec ± 0.6 at t=6 hours, $p = 0.03$). PT was not altered in the normothermia group (figure 1B; $p = 0.99$). LPS decreased activated partial prothrombin time (aPTT) (from 25.7 ± 3.3 sec at baseline to 20.8 ± 1.0 sec at t=3 hours, 0.006), which was prevented in the normothermia group compared to the fever group (figure 1C; $p = 0.005$). LPS did not alter fibrinogen levels, nor were there differences between the fever group and the normothermia group (figure 1D; $p = 0.78$). LPS increased vWf antigen levels (from 87% ± 39 at baseline to 341% ± 122 at t=6 hours, $p < 0.0001$), which was prevented in the normothermia group (figure 1E; $p = 0.03$). LPS-infusion resulted in an increase in D-dimer at T=3 hours (from 0.2 mg/L [0.2-0.3] at baseline to 1.3 mg/L [0.8-1.9] at t=3 hours, $p < 0.0001$). This increase persisted until the end of the study period. D-dimer values did not differ between the groups (figure 1F; $p = 0.06$).

Calculated ISTH DIC-scores were significantly lower in the normothermia group compared to the fever group (figure 2A, $p = 0.04$). Figure 2B shows the distribution of DIC scores between groups. In total, half of the subjects in the fever group reached a DIC-score above 4 at some time point during the study period whereas none of the subjects in the normothermia group reached a DIC-score above 4. Supplemental table 1 shows all the results of the mixed model used to assess correlations between the intervention and the measurements of interest.

The impact of LPS and fever control on ROTEM values

There was a large variation in ROTEM values while largely remaining within reference ranges. Figure 3 shows selected parameters from the ROTEM analyses. Cooling to normothermia did not alter most ROTEM parameters between groups (figure 3, supplemental table 2)., INTEM CT was higher in the normothermia group compared to the fever group (Figure 3D, $p= 0.007$). Of note, differences in INTEM CT levels between groups were already observable at T=1 hour, before

Figure 1. Conventional coagulation tests over time in volunteers with endotoxemia and fever and volunteers with endotoxemia treated with induced normothermia.



Lipopolysaccharide was given to all volunteers at T=0. Cooling to normothermia (37°C) was initiated at T=1 in the normothermia group. Grey box represents the period of induced normothermia. The dashed line represents the fever group and the solid line represents the normothermia group. Figure 3A-E: Data are presented as means with standard deviation. Figure 3F: Data are presented as medians with interquartile range. *P<0.05, **P<0.01, N.S. = Not significant. Abbreviations: aPTT = activated Partial Thromboplastin Time. PT = prothrombin time. vWf Ag = von Willebrand factor Antigen

Figure 2. DIC scores in volunteers with endotoxemia and fever and volunteers with endotoxemia treated with induced normothermia.

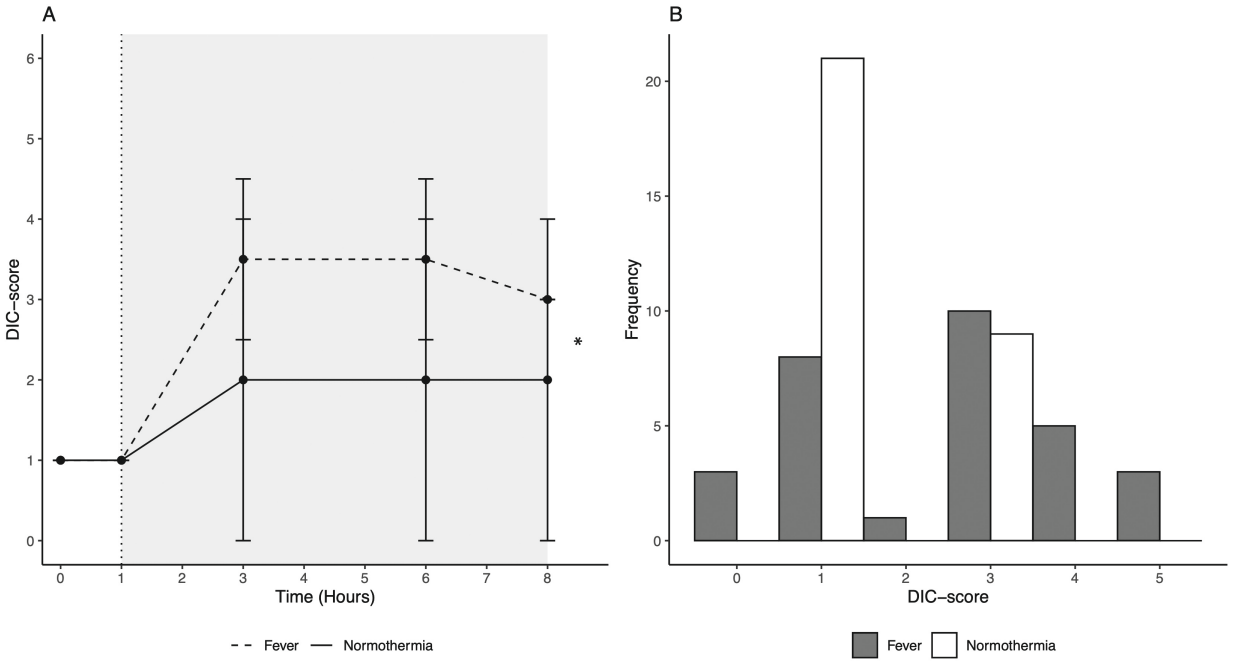
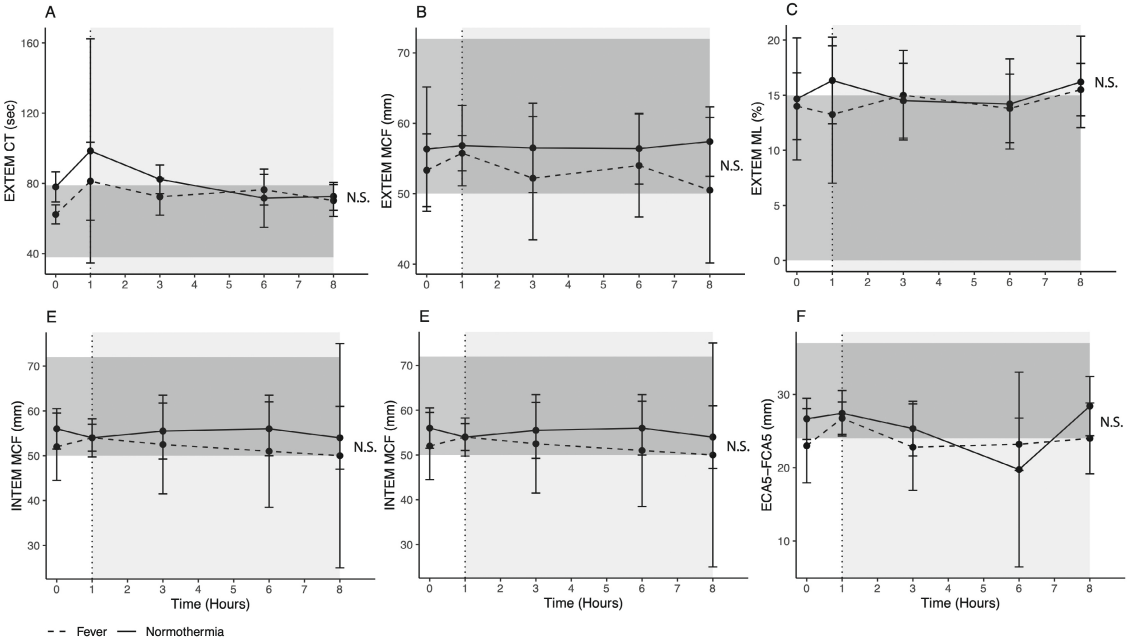


Figure 2A shows DIC scores over time. Lipopolysaccharide was given to all volunteers at T=0. Cooling to normothermia (37°C) was initiated at T=1 in the normothermia group. Grey box in figure 2A represents the period of induced normothermia. The dashed line represents the fever group and the solid line represents the normothermia group. Data are presented medians with bars representing the interquartile ranges. Figure 2B shows the frequency of DIC scores in both groups over all timepoints.

*$P < 0.05$, N.S. = Not significant.

Abbreviations: DIC = Disseminated intravascular coagulation.

Figure 3. Clot formation and lysis parameters of rotational thromboelastometry (ROTEM) in volunteers with endotoxemia and fever and volunteers with endotoxemia treated with induced normothermia.



Lipopolysaccharide was given to all volunteers at T=0. Cooling to normothermia (37°C) was initiated at T=1 in the normothermia group. Light grey box represents the period of normothermia. Dark grey box represents the reference range for specific ROTEM tests. The dashed line represents the fever group and the solid line represents the normothermia group. Data are presented as means with standard deviation. **P <0.01, N.S. = Not significant. Abbreviations: CT = clotting time. DIC = Disseminated intravascular coagulation. EXTEM = extrinsically activated test. ECA5-FCA5 = EXTEM clot amplitude after 5 minutes – fibrin-based extrinsically activated test (FIBTEM) clot amplitude after 5 minutes. INTEM = intrinsically activated test. MCF = Maximum clot firmness. ML = Maximum lysis.

cooling was initiated. There was no difference in EXTEM CA5 – FIBTEM CA5 (a measure of platelet function) between the fever group and the normothermia group (Figure 3F, p = 0.38) despite differences in platelet levels between groups. G-values, which may indicate a hypercoagulable state, were not different between groups (p = 0.15).

Discussion

In this study, we investigated the effect of induced normothermia on coagulation status during LPS-induced endotoxemia in healthy volunteers. The main finding of this study is that induced normothermia reversed LPS induced thrombocytopenia and DIC scores compared patients with fever. Also, elevated vWf antigen levels were decreased. Thereby, normothermia reverses coagulation derangements as seen during sepsis-induced coagulopathy.

LPS-induced endotoxemia in human volunteers resulted in derangements of the coagulation system resembling those noted in sepsis-induced consumption coagulopathy and DIC. The observed coagulation abnormalities induced by LPS are concurrent with previous studies on sepsis induced coagulopathy, including activation of the endothelium and increased secretion of vWf, resulting in activation of platelets.[13] These platelets have a high capacity to aggregate, causing clumping of platelets with an ensuing decrease in circulating platelets.[14, 15] LPS also prolonged PT, presumably due to activation and consumption of coagulation factors in the formation of microthrombi. Together, LPS induces an increase in DIC scores.[16] The decrease in aPTT following LPS is most likely caused by coagulation factor VIII as LPS is known to increase factor VIII.[17]

Cooling to normothermia prevented several LPS-induced derangements. Induced normothermia maintained platelet levels compared to the fever group. Overall, prolongation of PT and increase in D-dimer levels were less outspoken in the normothermia group compared to the fever group. This resulted in decreased DIC scores in the normothermia group, as well as in a reduction of the number of volunteers with severe DIC scores. Of note, fibrinogen levels were unaffected by cooling. Possibly, an absence of effect is due to the chosen timeframe. In LPS-induced endotoxemia, fibrinogen levels increase after 24 hours, which was beyond the time frame of this study.[17]

Induced normothermia may reduce DIC through an effect on the endothelium, as we found that cooling to normothermia reduced levels of vWf which is a marker of endothelial activation.[17-19] Our study cannot dissect cause from effect. Induced normothermia may inhibit consumption coagulopathy and subsequent microthrombi formation, which may have prevented endothelial damage.[17] However, this may not be in line with the finding that LPS-induced

thrombocytopenia is transient. More likely, induced normothermia reduced endothelial cell activation with less activation of the ensuing coagulation cascade. The effect on the APTT may also be explained by less endothelial cell activation with less vWF release. As vWf is the carrier of factor VIII in plasma, decrease in vWf in the normothermia group compared to controls may in part explain normalization of aPTT levels in the normothermia group.

In line with the decrease in APTT, INTEM CT in the fever group was prolonged compared to the normothermia group. Of note though, the differences in INTEM CT between groups were already apparent at T=1, so the differences between groups may not have been a result of normothermia. Other LPS-induced coagulation derangements were not detected by ROTEM results. As ROTEM values are influenced by platelet function and counts[20] and we previously showed that ROTEM has diagnostic value in detecting DIC, this finding was unexpected.[20-22] Also, ROTEM values were previously shown to be altered in LPS induced endotoxemia.[20] However, the impact of LPS on ROTEM values seems subtle and there was a large spread compared to conventional coagulation tests. Therefore, the number of subjects in this trial may have been too small to detect any subtle coagulation changes.

There are limitations to this study. To reduce shivering, medication including magnesium sulphate was used. Thereby, we cannot dissect whether the external cooling or the medications have contributed to observed effects. High magnesium levels inhibit blood coagulation and thrombus formation[23] and potentially limit inflammation in experimental studies.[24-26] An α2-adrenoreceptor agonist such as clonidine does not induce platelet aggregation[27,28] or affect coagulation[29] but may have anti-inflammatory effects.[27,28,30]. We attempted to include a third control group receiving only medication but without induced normothermia. Due to hypotension, we prematurely stopped inclusion in this group. However, as external cooling requires some form of sedation or analgesia to combat shivering, this study reflects pragmatic clinical practice.

Conclusion

In conclusion, induced normothermia reverses LPS-induced derangements of the coagulation system resembling those noted in sepsis-induced consumption coagulopathy and DIC, suggesting a decrease in endothelial driven coagulation. Induced normothermia should be further studied as a potential treatment in hyperinflammatory states such as sepsis.

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Supplemental material

Supplemental Table 1: Overview of results from the mixed effects models of conventional coagulation tests and derived coagulation abnormality scores comparing volunteers with endotoxemia and fever and volunteers with endotoxemia treated with induced normothermia (37°C).

Variable	Beta-coefficient	95% Confidence interval	P-value
Platelet count (109/L cells)	67.1	27.1 - 107.2	0.002
Fibrinogen (g/L)	0.1	-0.4 - 0.5	0.78
D-dimer (mg/L)	-0.3	-0.7 - 0.0	0.06*
vWf (%)	-89.2	-171.7 - -6.6	0.03
aPTT (sec)	2.7	0.9 - 4.5	0.005
PT (sec)	0.0	-0.8 - 0.8	0.99
G-value (dynes/cm2)	1.1	-0.6 - 2.7	0.15
DIC-score	-0.3	-0.6 - 0.0	0.04*

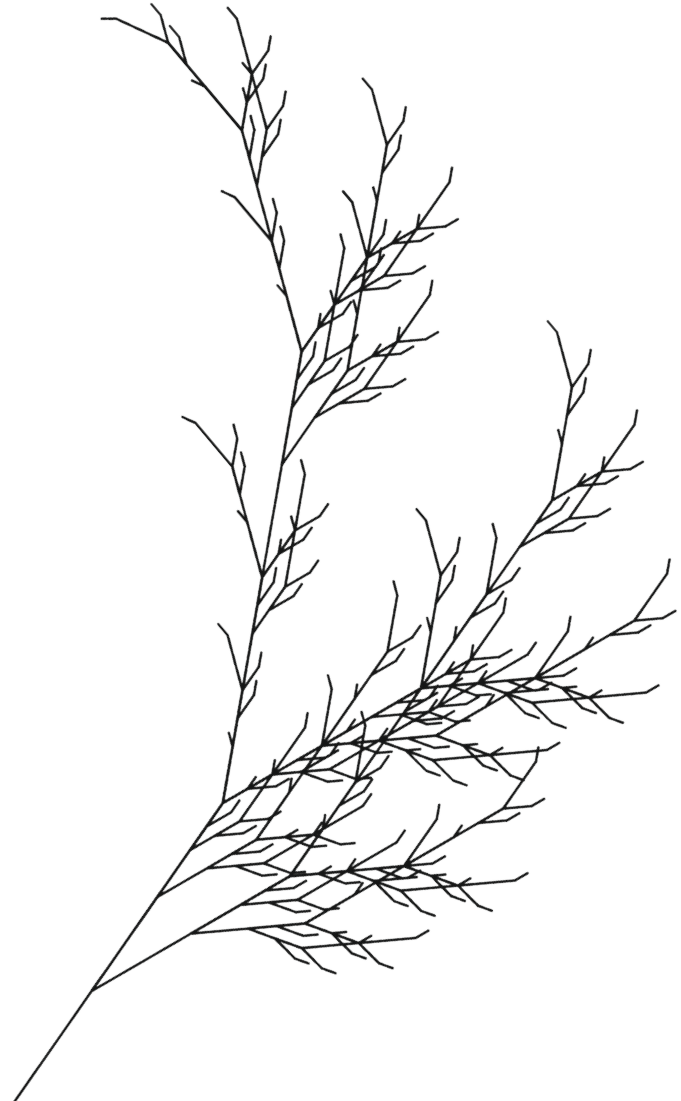
* Log transformed data.
Abbreviations: aPTT= activated partial thromboplastin time, DIC= disseminated intravascular coagulation, vWf= von Willebrand factor

Supplemental Table 2: Overview of results from the mixed effects model analyses of the rotational thromboelastometry (ROTEM) tests comparing volunteers with endotoxemia and fever and volunteers with endotoxemia treated with induced normothermia (37°C).

	EXTEM			INTEM			FIBTEM		
Subtest	**b**	**95% CI**	**P-value**	**b**	**95% CI**	**P-value**	**b**	**95% CI**	**P-value**
Coagulation time (sec)	6.4	-13.0 - 25.6	0.47	43.9	13.0 – 74.8	0.007	22.5	-8.3 – 53.0	0.15
Maximum clot firmness (mm)	4.8	-2.0 - 11.6	0.13	9.7	-1.1 - 20.4	0.06	4.74	-2.0 - 11.4	0.13
Amplitude 5 minutes (mm)	5.1	-1.5 - 11.7	0.10	7.14	-2.2 - 16.5	0.11	3.07	-1.9 - 8.0	0.18
Amplitude 10 minutes (mm)	4.1	-3.2 - 11.3	0.22	8.1	-2.9 - 19.1	0.12	4.29	-1.7 - 10.2	0.13
Maximum lysis (%)	0.7	-3.8 - 5.1	0.73	0.1	-5.7 - 5.9	0.96	0.16	-0.5 - 0.8	0.35*
EXTEM amplitude 5 minutes – FIBTEM amplitude 5 minutes (mm)	-	-	-	-	-	-	0.1	-0.2 – 0.4	0.38

* Log transformed data.

Abbreviations: CI = confidence interval. EXTEM = extrinsically activated test. FIBTEM = fibrin-based extrinsically activated test. INTEM = intrinsically activated test, IQR = interquartile range



Part three

Chapter seven

Practice of mechanical ventilation in cardiac arrest patients and effects of targeted temperature management: A substudy of the targeted temperature management trial

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Abstract

Aims Mechanical ventilation practices in patients with cardiac arrest are not well described. Also, the effect of temperature on mechanical ventilation settings is not known. The aims of this study were 1) to describe practice of mechanical ventilation and its relation with outcome 2) to determine effects of different target temperatures strategies (33°C versus 36°C) on mechanical ventilation settings.

Methods This is a substudy of the TTM-trial in which unconscious survivors of a cardiac arrest due to a cardiac cause were randomized to two TTM strategies, 33°C (TTM33) and 36°C (TTM36). Mechanical ventilation data were obtained at three time points: 1) before TTM; 2) at the end of TTM (before rewarming) and 3) after rewarming. Logistic regression was used to determine an association between mechanical ventilation variables and outcome. Repeated-measures mixed modelling was performed to determine the effect of TTM on ventilation settings.

Results Mechanical ventilation data was available for 567 of the 950 TTM patients. Of these, 81% was male with a mean (SD) age of 64 (12) years. At the end of TTM median tidal volume was 7.7 ml/kg predicted body weight (PBW) (6.4-8.7) and 60% of patients were ventilated with a tidal volume ≤ 8ml/kg PBW. Median PEEP was 7.7cmH_2O (6.4-8.7) and mean driving pressure was 14.6 cmH_2O (± 4.3). The median FiO_2 fraction was 0.35 (0.30-0.45). Multivariate analysis showed an independent relationship between increased respiratory rate and 28-day mortality. TTM33 resulted in lower end-tidal CO_2 (Pgroup=0.0003) and higher alveolar dead space fraction (Pgroup=0.003) compared to TTM36, while PCO_2 levels and respiratory minute volume were similar between groups.

Conclusions In the majority of the cardiac arrest patients, protective ventilation settings are applied, including low tidal volumes and driving pressures. High respiratory rate was associated with mortality. TTM33 results in lower end-tidal CO_2 levels and a higher alveolar dead space fraction compared to TTTM36.

Introduction

Targeted temperature management (TTM) is the main treatment modality for survivors of a cardiac arrest.[1] Large scale studies on mechanical ventilation practice after cardiac arrest and during TTM are scant. Ventilation strategies such as low tidal volume ventilation can improve outcome in patients with acute lung injury[2] and possibly also in patients without lung injury.[3,4] However implementation of lung protective ventilation strategies is difficult.[5,6] Recently, observational studies examined ventilation practices in ARDS[7], and non-ARDS[8], but these studies did not focus on cardiac arrest. Following the multi-center randomized target temperature management after cardiac arrest trial(TTM-trial)[9], the recommendation to control temperature between 32°C and 36°C[10] yielded variation in practice of TTM management.[11] The effect TTM on mechanical ventilation settings is largely unknown. In a retrospective study in cardiac arrest patients, application of 33°C was associated with improved gas exchange.[12]

Studying mechanical ventilation practices in patients following cardiac arrest may lead to optimization of ventilation during TTM and may ultimately improve outcome of these patients. The TTM trial provides a unique opportunity to study mechanical ventilation practices in cardiac arrest and to study the effect of temperature on parameters of mechanical ventilation. Therefore, the aims of this study were the following:1) to describe practice of mechanical ventilation and its independent relation with outcome.2) to determine effects of different target temperatures on mechanical ventilation parameters. We hypothesized that TTM at 33°C would lower ventilation settings needed for a minute volume ventilation.

Methods

Patients

This study is a retrospective substudy of the multi-centre, randomized, parallel-group, assessor-blinded TTM-trial. The TTM-trial included adult (≥18 years) unconscious patients (Glasgow Coma Scale <8) resuscitated from cardiac arrest of a presumed cardiac cause with return of spontaneous circulation during at least 20 minutes. Following informed consent according to the regulations as approved by local IRB, patients were included at 36 centres in Europe and Australia. Further details on the exclusion criteria, trial protocol and main results were published previously.[9]

TTM protocol

The entire duration of the TTM-protocol was 36 hours, which started at randomization to either 33°C (TTM33) or 36°C (TTM36). The assigned target temperature was achieved as soon as possible after which patients were kept at their target temperature until 28 hours after randomization. After 28 hours, patients were gradually rewarmed to 37°C at a maximum speed of 0.5°C per hour. Sedation was mandatory for both groups during the entire TTM intervention. Patients were ventilated in either a pressure or a volume controlled mode.

Post-hoc survey

Participating centers of the TTM-trial were asked to complete an additional online case report form with data on mechanical ventilation at three time points: prior to the TTM intervention, after 24 hours of TTM (before rewarming) and after rewarming was completed (at the start of the normothermic phase). Collected data are tidal volume (V_T), positive end expiratory pressure (PEEP), plateau pressure of the respiratory system (Pplat), respiratory rate (RR) and end tidal CO_2 ($etCO_2$). Data on lactate, arterial PO_2 (PaO_2), arterial PCO_2 ($PaCO_2$), base excess, pHa and mean arterial pressure were derived from the original TTM-database. Blood gasses were measured alpha-stat. Respiratory minute volume (RMV) was calculated as the V_T x RR. Static compliance of the respiratory system (Cstat) was calculated as V_T/Pplat – PEEP. Driving pressure (ΔP) was calculated as V_T/Cstat. Alveolar dead space ventilation was calculated as ($PaCO_2$-$etCO_2$)/$PaCO_2$.[13] Supranormal arterial oxygen content was defined as a PaO_2 level >13.3 kPa.[14]

Statistical Analysis

All analyses were performed in R (version 3.1.1). Baseline differences and differences in mechanical ventilation parameters were assessed using either the students t-test or the Wilcoxon rank sum test depending on normality of the data. To study the relationship between mechanical ventilation on 28-day mortality, a logistic regression model was performed. A priori, age, SOFA score, asthma/COPD, time from cardiac arrest to ROSC, first measured body temperature, lactate and cardiovascular diseases were put into the model. Mechanical ventilation variables with a P-value below 0.2 were also included. Collinearity diagnostics were performed using the variance inflation factor to check for variable independence. Missing data was imputed using the "MICE" package.[15] A restricted cubic spline function was used for non-linear variables. Variables were sequentially removed from the model based on likelihood ratio tests.

LOESS regression with a polynomial regression of 1 and a span of 0.75 was used to visualize the relationship between mechanical ventilation variables and 28-day mortality. To study differences between target temperatures over time, continuous variables of interest were analyzed by repeated-measures mixed model. Overall differences between groups were measured using TTM group and time point as a fixed effect, expressed as β-coefficient, confidence interval and P value. Post-hoc analysis of estimates between time points were assessed with the interaction term TTM group and time point using the "LSmeans" package.[16] Normally distributed data was presented a mean ± (standard deviation). Non-parametric data was presented as median (25-75th percentile). P values < 0.05 were considered significant.

Results

Of the 950 patients randomized in the TTM-trial in 36 centers, mechanical ventilation data was available for a total 567 patients from 24 centers. The centers that were not able to submit data comprise of both academic and non-academic hospitals. Age, sex and mortality did not differ between the included and non-included patients.

Baseline characteristics

Baseline characteristics of included patients are shown in table 1. Patients were mostly male and frequently had cardiovascular risk factors. Of note, patients were hypothermic upon start of study. The number of patients with asthma or COPD Gold I-IV did not differ between survivors and non-survivors. Non-survivors had longer time from cardiac arrest to basic life support and higher incidence of circulatory shock compared to the survivor group.[17]

Mechanical ventilation settings during TTM

Mechanical ventilation settings for patients are shown in table 2 and cumulative frequency distributions in figure 1. The median V_T was 7.7 ml/kg (6.4-8.7) predicted body weight (PBW) and in total, 60% of patients were ventilated with a V_T equal to or below 8 ml/kg (figure 1). Median PEEP was 6 cmH_2O (5-8) and mean driving pressure 14.7 cmH_2O (± 4.2). More than half of patients were ventilated with > 5 mmHg of PEEP. Plateau pressure was above 30 cm H_20 in 8% of patients and above 20 cm H_2O in 60% of patients. Compliance of the respiratory system, was below 50 mL/cmH_2O in the majority of patients (83%).

Table 1. Baseline patient characteristics in all patients and survivors vs. non-survivors at 28-days

	All N=567	Survivors N=328	Non-survivors N=239	P-value
Demographic characteristics				
Age (yr) - mean ±SD	64.07 ±12.31	60,94 ±12.4	68,37 ±10.81	<0.0001
Height (cm) - mean ±SD	175,13 ±8.55	176,19 ±7.9	173,66 ±9.19	0.001
Weight (kg) - mean ±SD	81,82 ±16.05	81,76 ±15.24	81,89 ±17.15	0,929
Male sex – no. (%)	460 (81.1)	273 (83.23)	187 (78.24)	0.175
Medical History - no.(%)				
Chronic heart failure	35 (6.2)	14 (4.27)	21 (8.79)	0.038
Previous AMI	125 (22.0)	58 (17.68)	67 (28.03)	0.004
Ischemic heart disease	163 (28.8)	85 (25.91)	78 (32.64)	0.077
Previous cardiac arrhythmia	93 (16.6)	43 (13.11)	51 (21.34)	0.01
Previous cardiac arrest	11 (1.9)	4 (1.22)	7 (2.93)	0.221
Arterial hypertension	220 (38.9)	117 (35.67)	103 (43.1)	0.081
TIA or stroke	41 (7.2)	13 (3.96)	28 (11.72)	<0.0001
Diabetes	86 (15.3)	37 (11.28)	49 (20.5)	0.003
Asthma or COPD	65 (11.5)	30 (9.15)	35 (14.64)	0.051
Characteristics of the cardiac arrest				
Bystander performed CPR - no. (%)	400 (70.7)	252 (76.83)	148 (61.92)	<0.0001
Time from cardiac arrest to BLS - median (IQR)	1 (0-2)	1 (0-2)	1 (0-3)	0.071
Time from cardiac arrest to ROSC - median (IQR)	25 (16.5- 40)	20 (14-31)	35 (23-51.5)	<0.0001
First monitored rhythm - no. (%)				<0.0001
Asystole - no. (%)	68 (12.0)	12 (3.66)	56 (23.43)	-
Non-perfusing VT - no. (%)	12 (2.1)	7 (2.13)	5 (2.09)	-
PEA - no. (%)	35 (6.2)	10 (3.05)	25 (10.46)	-
ROSC after bystander defibrillation - no. (%)	12 (2.1)	10 (3.05)	2 (0.84)	-
VF - no. (%)	428 (75.5)	284 (86.59)	144 (60.25)	-
Unknown - no. (%)	12 (2.1)	5 (1.52)	7 (2.93)	-
Clinical characteristics on admission				
First Body temperature (°C) - mean ±SD	35.32 ±1.14	35.4 ±1.07	35.19 ±1.23	0.039
Circulatory shock - no. (%)	57 (10.1)	22 (6.71)	36 (15.06)	0.001
SOFA score - mean ±SD	10.55 ±2.62	10.02 ±2.43	11.23 ±2.7	<0.0001

Abbreviations: AMI, acute myocardial infarction, BLS, basic life support, CPR, cardiopulmonary resuscitation, COPD, chronic obstructive pulmonary disease, IQR, interquartile range, PEA, pulseless electrical activity, ROSC, return of spontaneous circulation , SOFA, sequential organ failure assessment, TIA, transient ischemic attack, VT, ventricular tachycardia, VF, ventricular fibrillation.

Table 2. Mechanical ventilation parameters and blood gas values at the end of targeted temperature management (T=28 hours) in survivors at 28-days vs. non-survivors at 28-days

Variable (no. of patients)	All N=567	Survivors N= 328	Non-survivors N= 239	P-value
Tidal volume (mL/kg PBW) *(n=492)*	7.7 (6.4-8.7)	7,8 (6.5-8.7)	7,52 (6.4-8.7)	0.247
PEEP (cmH_2O) *(n=489)*	6 (5-8)	6 (5-8)	7 (5-8)	0.372
Pplat (cmH_2O) *(n=325)*	21.0 (18.0-24.0)	20.8 (18.0-24.0)	21.0 (18.0-25.0)	0.654
Respiratory rate (breaths/min) *(n=535)*	16 (14-20)	16 (13-18)	16.5 (15-20)	<0.0001
Driving pressure (cmH_2O) *(n=300)*	14.7 ±4.2	14.4 ±3.9	14.8 ±4.5	0.573
Power (J/min) *(n=302)*	6.1 (4.4-8.1)	6,1 (4.5-7.8)	6.1 (4.2-8.1)	0.916
Static compliance (mL/H_2O) *(n=302)*	37.1 (29.2-47.1)	38.2 (31.5-48.8)	34.7 (27.4-44.7)	0.013
Respiratory minute volume (L/min) *(n=496)*	9.3 ± 3.3	9.2 ±3.3	9.4 ±3.3	0.588
End tidal CO_2 (kPa) *(n=307)*	4.2 ±1.2	4.2 ±1.1	4.2 ±1.3	0.725
Alveolar dead space fraction *(n=235)*	0.20 ±0.25	0.21 ±0.18	0.19 ±0.31	0.567
$PaCO_2$ (kPa) *(n=445)*	5.3 ±0.9	5.3 ±0.9	5.3 ±1.0	0.351
PaO_2 (kPa) *(n=442)*	12.8 ±3.1	13.0 ±3.4	12.3 ±2.9	0.019
FIO_2 fraction *(n=502)*	0.35 (0.30-0.45)	0.35 (0.3-0.42)	0.35 (0.3-0.45)	0.342
P/F ratio (mmHg) *(n=441)*	267 ±93	274 ±89	256 ±98	0.052
pHa *(n=444)*	7.36 ±0.07	7.36 ±0.07	7.35 ±0.09	0.051
Lactate (mmol/L) *(n=401)*	1.4 (1.0-2.1)	1.2 (0.9-1.8)	1.7 (1.1-2.7)	<0.0001
Base excess (mEq/L) *(n=442)*	-3.0 ±3.8	-2.5 ±3.4	-3.6 ±4.4	0.005

Abbreviations: PEEP, positive end-expiratory pressure, Pplat = plateau pressure of the respiratory system, P/F ratio, PaO_2/FIO_2 ratio Data is presented as mean ± standard deviation or median (interquartile range)

The median FIO_2 fraction was 0.35 (0.30-0.45). In patients with a supranormal arterial oxygen content (> 13.3 kPa), 48% were ventilated with an $FiO_2 \geq 0.4$.

Mechanical ventilation settings in hypoxemic vs. normoxemic patients

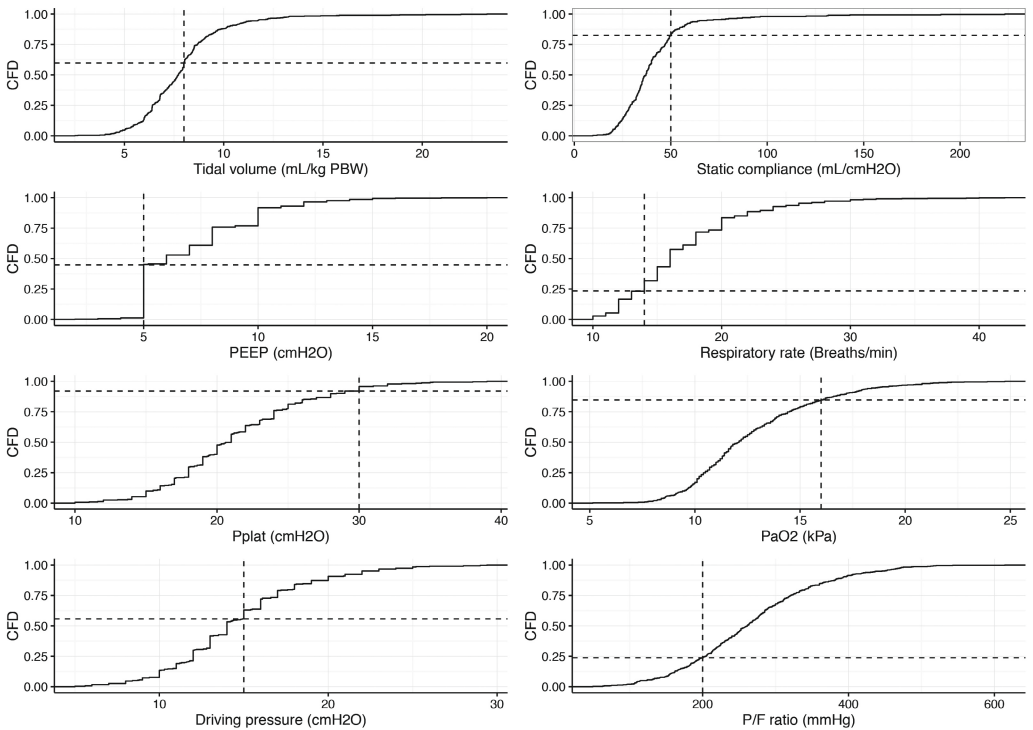
As a surrogate marker of pulmonary complications, hypoxemic patients (P/F ratio < 200 mmHg) were compared to normoxemic patients (P/F ratio ≥ 200 mmHg) (supplemental table 2). PEEP was significantly higher in hypoxemic patients compared to normoxemic patients as was Pplat driving pressure V_T and respiratory rate. Static compliance was lower in hypoxemic patients compared to normoxemic patients (34.3 (27.2-41.4) vs. 38.7 (32.8-48.4), p = 0.002).

Mechanical ventilation settings during TTM in survivors vs. non-survivors at 28-days

Baseline differences are given in table 1. V_T, PEEP, Pplat and driving pressure did not differ between groups (table 2). Static compliance of the respiratory system was significantly lower while respiratory rate was significantly higher in non-survivors compared to survivors. Figure 2 shows the distribution of ventilation parameters plotted against V_T and PaO_2 levels. The distribution for survivors and non-survivors at 28-days was largely the same.

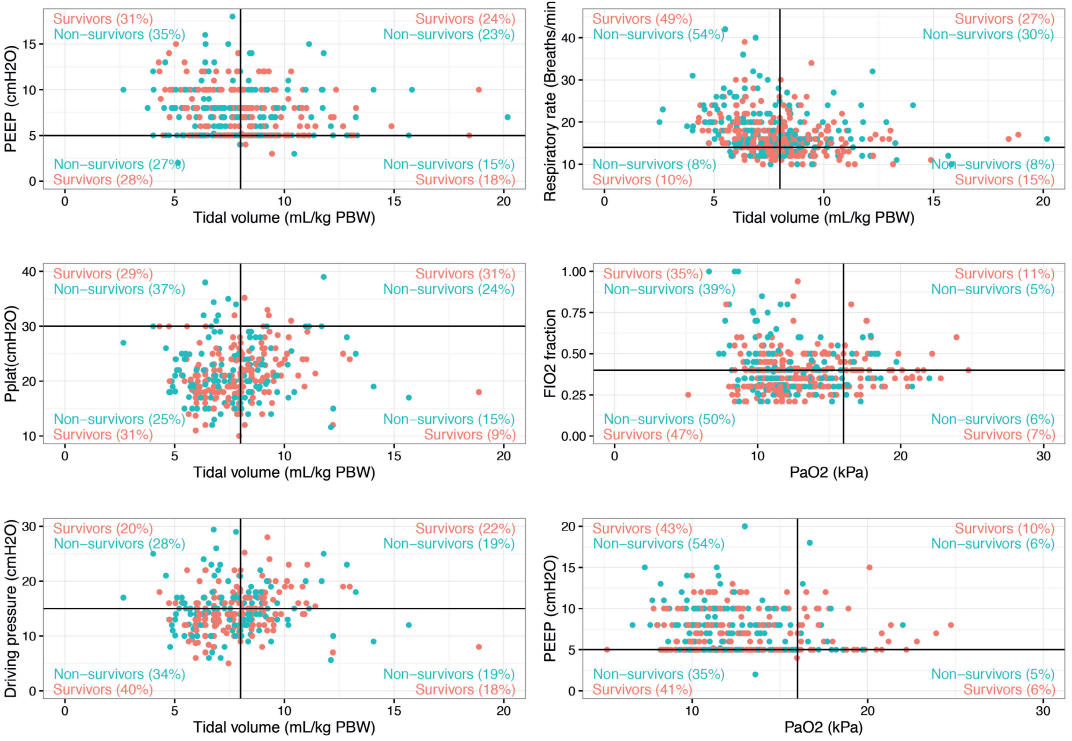
Figure 3 shows the relationship between mortality and ventilation settings using Loess regression curves. Mortality appears to be higher when driving pressures are above 20 cmH_2O and when plateau pressures are above 25 cmH_2O. Also, mortality is higher in patients with P/F ratios below 200 and in patients with low compliance. Of interest, high tidal volumes were not apparently related to mortality. Mechanical ventilation factors were included in a multivariable logistic regression model with a priori selected variables for disease severity and medical history to assess which factors were independently associated with mortality. In this analysis, with the exception of high respiratory rate, ventilator parameters were not associated with 28-day mortality (supplemental table 1).

Figure 1. Cumulative frequency distributions of ventilation parameters during targeted temperature management.



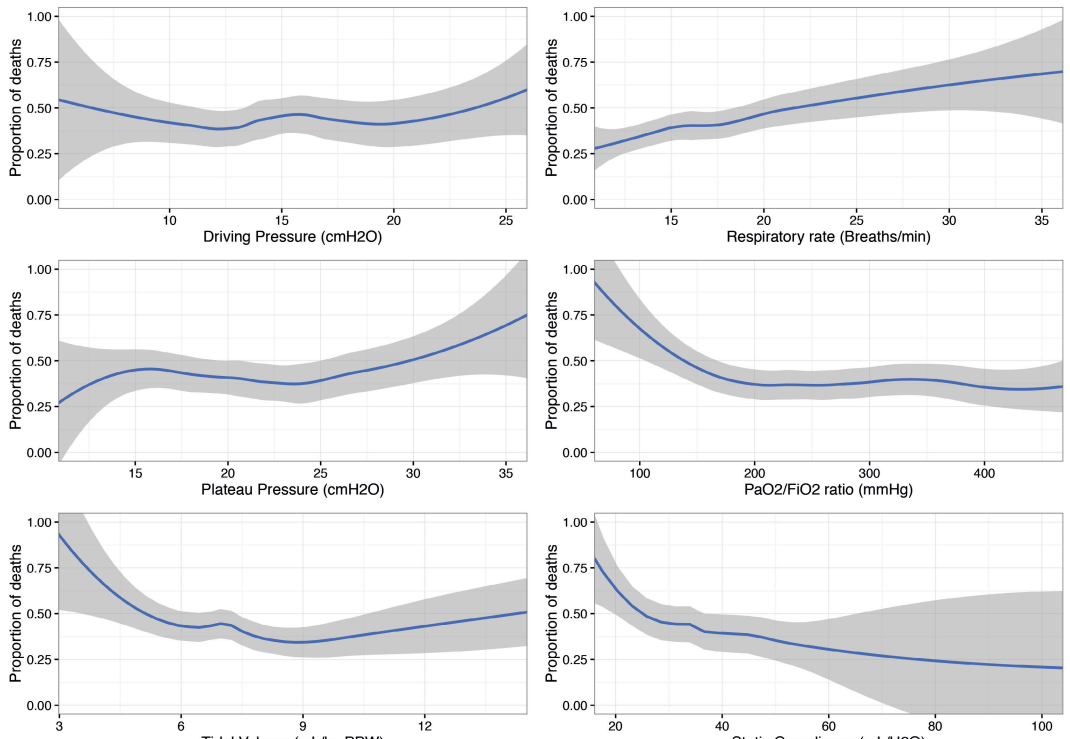
Cumulative frequency distributions of tidal volume, PEEP, Pplat, Driving pressure, Static compliance, respiratory rate, PaO_2 and P/F ratio. The dotted line represents the proportion of patients reaching the cutoff value. Abbreviations: CFD, cumulative frequency distribution. PEEP, positive end-expiratory pressure. Pplat, plateau pressure.

Figure 2. The distribution of ventilation parameters at the end of targeted temperature management in survivors and non-survivors at 28-days.



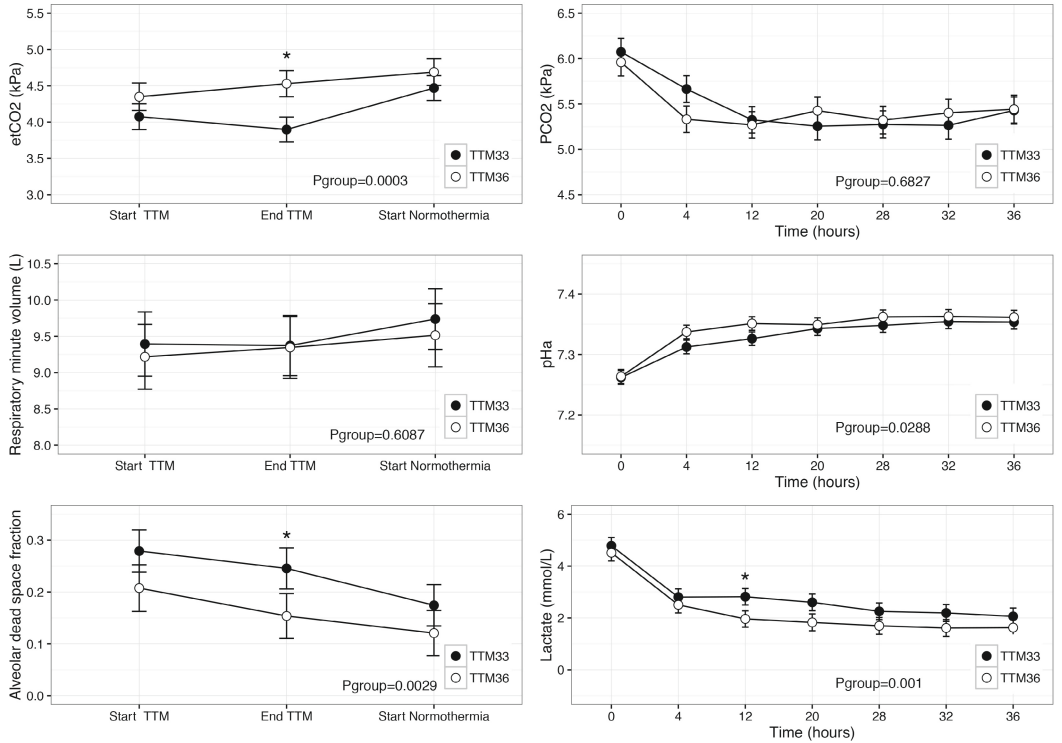
Distribution of tidal volume against PEEP, Pplat, Driving pressure, Respiratory rate. In addition, the distribution of PaO_2 levels against FIO_2 fraction and PEEP. Abbreviations: PEEP, positive end-expiratory pressure. Pplat, plateau pressure.

Figure 3. Relationship between ventilation parameters at the end of targeted temperature management and 28-day mortality.



LOESS regression plots showing the relationship between 28-day mortality and driving pressure, Pplat, tidal volume, respiratory rate, P/F ratio and static compliance respectively. Abbreviations: PEEP, positive end-expiratory pressure. Pplat, plateau pressure.

Figure 4. The effect of different target temperatures on mechanical ventilation.



Repeated-measures mixed model on the effect of target temperatures on mechanical ventilation. Circles represent estimates including error bars for 95% confidence limits from mixed effect modeling. Asterisks indicate a significant interaction between groups at each time point. Pgroup represents the overall difference between target temperature management (TTM) groups. Abbreviations: $etCO_2$, end-tidal CO_2. TTM33, TTM at 33°C. TTM36, TTM at 36°C

The effect of different target temperature levels on mechanical ventilation parameters

At the end of the TTM period, patients kept at TTM33 had significantly lower $etCO_2$ when compared to patients kept at TTM36 (3.9 kPa ± 0.98 vs. 4.55 kPa ± 1.31, p<0.0001), whereas $PaCO_2$ levels and respiratory minute volume were similar between groups (supplemental table 3). In addition, alveolar dead space fraction was significantly higher in TTM33 vs. TTM36 (0.24 ± 0.19 vs. 0.15 ± 0.29, p=0.004). PEEP, V_T and Pplat did not differ between groups (supplemental table 3).

To study the effect of TTM on $etCO_2$ and $PaCO_2$ over time, we performed a mixed effects model (supplemental table 4, figure 4). $PaCO_2$ levels were significantly lower after 4 hours compared to baseline in both the TTM33 group (β = 0.41 kPa [95% confidence limit, 0.09 - 0.73 kPa]; p = 0.0014) and the TTM36 group (β = 0.63 kPa [95% confidence limit, 0.31 - 0.95 kPa]; p = <0.0001). However, there was no difference between groups in $PaCO_2$ levels. In contrast, $etCO_2$ levels were lower in the TTM33 group compared to the TTM36group, whereas alveolar dead space fraction was higher. These results were found at a similar respiratory minute volume between both groups. Metabolic acidosis was more pronounced in the TTM33 group, with lower pHa, lower base excess and increased lactate levels.

Discussion

In this substudy of the TTM-trial, we describe ventilation practice in patients after a cardiac arrest and the effects of different target temperatures on mechanical ventilation settings and parameters of gas-exchange. At the end of TTM (before rewarming), we found that patients are predominantly ventilated with low tidal volumes, equal or below 8 ml/kg. Oxygen was applied liberally, with 48% of patients with supranormal (>13.3 kPa) oxygenation receiving high FiO_2 levels. Non-survivors had lower oxygenation, higher respiratory rates, lower compliance, higher driving pressures and were ventilated with lower tidal volumes compared to survivors at 28-days.

In multivariate analysis, respiratory rate, but none of the other ventilation parameters, was independently associated with 28-day mortality. In studying the effect of different target temperatures on mechanical ventilation, we found that TTM33 resulted in lower $etCO_2$ levels and higher alveolar dead space fraction compared to TTTM36 at similar minute ventilation.

This study suggests that a majority of cardiac arrest patients are ventilated according to lung protective standards. These results are similar to the PRoVENT

study, an observational study focusing on mechanical ventilation practices in patients without ARDS.[8] In addition, a previous study in patients with cardiac arrest found that low V_T ventilation was increasingly applied in ICUs in 40 countries over a period of 12 years.[6] Of note, compliance of the respiratory system was low in the majority of patients and PEEP level was set at > 5 cm H_20 in more than half of patients. This may suggest that most of the patients may have had a pulmonary complication following cardiac arrest, such as lung contusion following chest compressions, pulmonary edema, atelectasis or aspiration. As a surrogate marker of pulmonary complications, we compared hypoxemic patients to normoxemic patients. In total, 105 patients out of 336 had a P/F ratio below 200, indicating that a relatively large portion of patients may have suffered from pulmonary complications. These patients were ventilated with markedly higher pressure levels. Unfortunately, we did not collect X-rays, so we cannot conclude with certainty about pulmonary conditions of patients. Of note, impaired circulation may also result in hypoxemia.

In the loess regression plots, both driving pressure > 20 cmH_2O as well as plateau pressure > 30 cmH_20 show a linear relation with mortality. Thereby, the relationship between ventilation settings and mortality following cardiac arrest appear follow similar trends as in ARDS, although in patients with ARDS the linear relation to proportion of deaths occurs at driving pressures > 10 cmH_2O and plateau pressures >20 cmH_20.[18] Of note, V_T was not associated with mortality in this cohort of patients, likely due to the fact that the majority of them was already ventilated at relatively low V_T. In multivariate analysis, other ventilation factors were also not independently associated with outcome, as mortality was predominantly associated with non-respiratory factors. In ARDS patients, driving pressure is associated with adverse outcome.[19] The lack of a relationship between driving pressure and 28-day mortality in our cohort may be explained by a low effect size or a limited number of patients with high driving pressure. We are unsure how to explain the association between respiratory rate and 28-day mortality. An explanation may be that neurological damage may have driven the relation between hyperventilation and outcome. Of note, paralysis was not part of standard care. Alternatively, given that respiratory rate was increased in the hypoxemic patients compared to normoxemic patients, increased respiratory rate may have been a consequence of lung injury or altered gas exchange, which may have had a negative interaction with outcome. The clinical relevance of this finding remains to be determined.

$PaCO_2$ levels decreased in both TTM groups, most likely due to sedation. In contrast, $etCO_2$ levels were lower in the TTM33 group compared to the TTM36 group. This coincided with a significantly higher alveolar dead space fraction in TTM33 compared to the TTM36 group. A possible explanation for this finding

may be that TTM33 resulted in lower pulmonary perfusion due to increased vasoconstriction. This may be in line with the finding of higher lactate levels in TTM33 compared to TTM36.In ARDS, increased dead space in associated with increased mortality[20], and this finding could have implications for cardiac arrest patients at risk for lung injury. Taken together, although TTM33 does not result in reduced survival compared to TTM36[9], we feel that decreased pulmonary perfusion with increased dead space fraction are unwanted effects, arguing against maintaining cardiac arrest patients at 33°C. Of note, blood gas was not corrected for temperature in this study. This means that $PaCO_2$ may have been lower in the TTM33 group, as the solubility of CO_2 increases at lower temperatures, Perhaps, correcting $PaCO_2$ levels for body temperature may have ultimately allowed for lower ventilation settings in the TTM33 group.

There are several limitations to this study. Although the study was predefined, several parameters were retrospectively collected through a post-hoc survey, and results should be considered within the limitations of this study design, including lacking data on cause of death. Also, data could not be obtained from all patients included in the original TTM trial. However, centers in whom patients were missing are both large and small centers, from all countries which contributed to the TTM trial. In comparing patients in this study to those that were left out, there was no difference between most baseline variables, nor in mortality. Also, several variables suffered from missing data, resulting in the necessity to impute data for the logistic regression model. However, running the model with and without imputed data did not alter the outcome, suggesting a stable model. We also lacked information about pulmonary complications. In addition to missing data, another limitation is that paralysis was not applied as per study protocol, which may have allowed for spontaneous breathing efforts, which in turn may have affected respiratory rate and other ventilation data.

Conclusions

Cardiac arrest patients predominantly receive protective ventilation with low V_T and low driving pressures. Higher respiratory rate is associated with increased mortality. TTM33 resulted in decreased $etCO_2$ with increased alveolar dead space fraction compared to TTM36. Optimization of ventilator parameters and gas-exchange should be considered to improve outcome after cardiac arrest.

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Supplemental material

Supplemental Table 1. Multivariable regression for factors associated with 28-day mortality

Variable	OR	95%CI	P-value
Age (yr)	1.06	1.04 - 1.08	< 0.0001
SOFA	1.10	1.02 - 1.19	0.01
CA to ROSC (min)	1.02	1.01 - 1.03	< 0.0001
Respiratory rate (breaths/min)	1.09	1.04 - 1.13	< 0.0001

Abbreviations: CA, cardiac arrest. CI, confidence interval. OR, odds ratio ROSC, return of SOFA, sequential organ failure assessment.

Supplemental Table 2. Mechanical ventilation parameters and blood gas values at the end of targeted temperature management (T=28 hours) in normoxemic patients (P/F ratio ≥ 200 mmHg) vs. hypoxemic patients (P/F ratio < 200 mmHg).

	Normoxemic N= 336	Hypoxemic N= 105	P-value
Tidal volume (mL/kg PBW)	7.6(6.6-8.5)	8.0 (7-9)	0.022
PEEP (cmH_2O)	5.2 (5-8)	8.0 (5-10)	<0.0001
Pplat (cmH_2O)	20.0 (17.9-23.0)	24.0 (20.8-26.4)	<0.0001
Respiratory rate (breaths/min)	15 (13-18)	16 (15-20)	0.001
Driving pressure (cmH_2O)	13.9±3.7	16.3 ±4.2	<0.0001
Static compliance (mL/H_2O)	38.7 (32.8-48.4)	34.3 (27.2-41.4)	0.002
Respiratory minute volume (L/min)	8.8 ±2.8	9.8 ±3.1	0.005
$etCO_2$ (kPa)	4.3 ±1.2	4.0 ±0.8	0.035
Alveolar dead space fraction	0.18 ±0.26	0.26 ±0.18	0.01
$PaCO_2$ (kPa)	5.2 ±0.8	5.6 ±1.1	0.001
PaO_2 (kPa)	13.5 ±3.1	10.4 ±2.0	<0.0001
FIO_2	0.35 (0.3-0.4)	0.5 (0.45-0.6)	<0.0001
P/F ratio (mmHg)	302 ±76	153 ±36	<0.0001
pHa	7.36 ±0.07	7.33 ±0.08	0.001
Lactate (mmol/L)	1.3 (0.9-1.9)	1.6 (1.1-2.7)	0.002
Base excess (mEq/L)	-2.8 ±3.5	-3.6 ±4.3	0.126

Abbreviations: $EtCO_2$, end tidal CO_2. PEEP, positive end-expiratory pressure, P/F ratio, PaO2/FIO2 ratio. Data is presented as mean ± standard deviation or median (interquartile range)
Abbreviations: PEEP. positive end-expiratory pressure

Supplemental Table 3. Mechanical ventilation parameters and blood gas values at the end of targeted temperature management (T=28 hours) in TTM33 vs. TTM36

	TTM33 N= 281	TTM36 N= 286	P-value
Tidal volume (mL/kg PBW)	7.7(6.5-8.7)	7.7 (6.4-8.7)	0.827
PEEP (cmH_2O)	6 (5-8)	6 (5-8)	0.356
Pplat (cmH_2O)	21 (18-25.0)	20 (18-24)	0.021
Respiratory rate (breaths/min)	16 (14-20)	16 (14-20)	0.588
Driving pressure (cmH_2O)	15.0 ±4.3	14.1 ±4.3	0.102
Static compliance (mL/H_2O)	35.9 (29.0-45.8)	37.5 (29.2-47.9)	0.368
Respiratory minute volume (L/min)	9.3 ±3.1	9.3 ±3.5	0.842
$etCO_2$ (kPa)	3.9 ±1.0	4.6 ±1.3	<0.0001
Alveolar dead space fraction	0.24 ±0.19	0.15 ±0.29	0.004
$PaCO_2$ (kPa)	5.3 ±0.9	5.3 ±0.9	0.513
PaO_2 (kPa)	12.6 ±3.1	12.9 ±3.2	0.238
FIO_2	0.35 (0.30-0.45)	0.35 (0.30-0.45)	0.572
P/F ratio (mmHg)	263 ±92	271 ±95	0.349
pHa	7.35 ±0.08	7.36 ±0.07	0.035
Lactate (mmol/L)	1.5 (1.1-2.7)	1.2 (0.9-1.9)	<0.0001
Base excess (mEq/L)	-3.6 ±4.1	-2.4 ±3.5	0.001

Abbreviations: $EtCO_2$, end tidal CO_2. PEEP, positive end-expiratory pressure, P/F ratio, PaO2/FIO2 ratio. Data is presented as mean ± standard deviation or median (interquartile range)
Abbreviations: PEEP. positive end-expiratory pressure

Supplemental Table 4. Outcome Mixed model analysis between different target temperatures

	B-coefficient	95%CI	P-value
$PaCO_2$ (kPa)	-0.02	-0.14 - 0.09	0.68
$EtCO_2$ (kPa)	0.38	0.18 - 0.59	0.0003
Alveolar dead space fraction	-0.07	-0.12 - -0.03	0.003
Respiratory minute volume (L/min)	-0.13	-0.63 - 0.37	0.61
pHa	0.012	0.001 - 0.023	0.029
Lactate (mmol/L)	-0.53	-0.84 - -0.21	0.001
Base excess (mEq/L)	0.75	0.20 - 1.31	0.008

Abbreviations: CI, confidence interval, etCO2, end tidal CO2

Chapter eight

Carbon dioxide dynamics in relation to neurological outcome in resuscitated out-of-hospital cardiac arrest patients: an exploratory Target Temperature Management Trial substudy

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Abstract

Background Dyscarbia is common in out-of-hospital cardiac arrest (OHCA) patients and its association to neurological outcome is undetermined.

Methods Exploratory post-hoc sub-study of the Target Temperature Management (TTM) trial, including resuscitated OHCA patients, investigating the association between serial measurements of arterial partial carbon dioxide pressure ($PaCO_2$) and neurological outcome at 6 months, defined by the cerebral performance category (CPC) scale, dichotomized to good outcome (CPC 1,2) and poor outcome (CPC 3 - 5). The effects of hypercapnia and hypocapnia, time weighted mean $PaCO_2$ and absolute $PaCO_2$ difference were analyzed. Additionally, the association between mild hypercapnia (6.0 - 7.30 kPa) and neurological outcome, its interaction with target temperature (33°C and 36°C), and the association between $PaCO_2$ and peak serum-Tau were evaluated.

Results Of the 939 patients in the TTM-trial 869 were eligible for analysis. 96% of patients were exposed to hypo- or hypercapnia. None of the analyses indicated a statistically significant association between $PaCO_2$ and neurological outcome (P = 0.13-0.96). Mild hypercapnia was not associated with neurological outcome (P = 0.78) and there was no statistically significant interaction with target temperature (Pinteraction = 0.95). There was no association between $PaCO_2$ and peak serum-Tau levels 48 or 72 hours after ROSC.

Conclusions Dyscarbia is common after ROSC. No statistically significant association between $PaCO_2$ in the post cardiac arrest phase and neurological outcome at six months after cardiac arrest was detected. There was no significant interaction between mild hypercapnia and temperature in relation to neurological outcome.

Background

Out-of-hospital cardiac arrest (OHCA) is a common reason for critical care admission.[1-3] Despite increasing survival after OHCA with initial shockable rhythm in the last decades[4,5], overall survival is still low[6,7], and brain injury continues to be the principal cause of death and disability.[5,8] Guidelines recommend ventilation to normal carbon dioxide levels after return of spontaneous circulation (ROSC).[9,10] However, unintended hyperventilation during and after resuscitation frequently occurs and variability in ventilation leading to dyscarbia is a prevalent finding in the post cardiac arrest phase.[11,12] Elevated arterial carbon dioxide partial pressure ($PaCO_2$), hypercapnia, may lead to cerebral vasodilatation and increased cerebral blood flow (CBF), while a decrease in $PaCO_2$, hypocapnia, can exert the opposite effect.[13] $PaCO_2$ is also a central variable in acid-base homeostasis encompassing hypercapnic acidosis or hypocapnic alkalosis.[14] In recent studies, $PaCO_2$ was associated with neurological outcome after OHCA; hypocapnia was related to poor neurological outcome[15-17], whilst hypercapnia was associated with good as well as poor neurological outcome.[16-21]

As hypercapnia has been associated to good neurological outcome[16], the novel concept of targeted therapeutic mild hypercapnia (TTMH) has been tested in the pilot randomized Carbon Control and Cardiac Arrest (CCC)-trial and shown to be safe, feasible to perform, lowering biomarkers of brain damage and a tendency of improved global functional outcome.[21] A phase-III randomized clinical trial has recently started recruiting patients to further investigate the impact of TTMH on neurological outcome (NCT03114033) and there are active plans to co-enrol patients with the ongoing TTM2-trial (NCT02908308) comparing hypothermia to normothermia after cardiac arrest.[22]

We conducted this exploratory sub-study of the TTM-trial to describe the evolution of $PaCO_2$ in serial measurements at predefined time points during the first hours after ROSC, to explore the role that $PaCO_2$ may have in the neurological outcome of OHCA patients and to specifically analyze the interaction between mild hypercapnia and targeted temperature management in relation to neurological outcome. To further strengthen the analyzes, we investigated the association of $PaCO_2$ to a surrogate marker of neurological outcome: peak levels of serum-protein tau, a marker of neuronal damage, that has shown to be more accurate than neuron specific enolase (NSE) in predicting outcome.[23]

Methods

This study is based on the TTM-trial conducted between 2010 and 2013 and was approved by the TTM-trial steering group before the completion of the trial.[24] Ethical committees in each participating country approved the TTM-trial protocol and informed consent was waived or obtained according to national legislations, in line with the Helsinki declaration. The patients included were unconscious (GCS < 8) adults (≥ 18 years of age) with stable ROSC after OHCA of presumed cardiac cause. Main exclusion criteria were unwitnessed cardiac arrest with asystole as primary rhythm, known or suspected intracranial hemorrhage or stroke, and time from ROSC to screening > 240 minutes.[24] All patients were admitted to an intensive care unit (ICU), intubated, sedated and mechanically ventilated. After inclusion, the patients were randomized to the 33°C or the 36°C group and temperature controlled during the intervention period of 36 hours, which commenced at inclusion into the trial. Mandatory sedation was discontinued 36 hours after inclusion. The TTM study analyzed 939 eligible patients with no difference in survival or neurological outcome at 6 months between the two allocation groups.[24] For this sub-study we included patients surviving the intervention period in order to have a defined exposure period for carbon dioxide.

Patient data and blood sampling

Pre-hospital data were reported according to the Utstein criteria.[25] Baseline, intervention related and physiological variables, comorbidities, demographic, pre-hospital and admission data, as well as characteristics of the cardiac arrest and baseline laboratory analyses were collected. A complete arterial blood gas analysis was performed in all patients at admission to hospital, start of intervention (T0, which also was the time of randomization) and after 4, 12, 20, 28, 32 and 36 hours. All arterial blood gases were managed according to the alpha-stat method. The median time from ROSC to randomization was 133 (interquartile range 83 - 188) minutes. To include the admission blood gas (after ROSC, but before randomization) we timed this $PaCO_2$ value to one hour before T0; (T-1). $PaCO_2$ data were surveyed for physiological plausibility and in 4 of measurements we corrected a misplaced decimal point. Corrections were conducted by FE and NN in accordance with other data registered on the same patient.

Outcome

The primary outcome was overall neurological function at 6-month follow-up, using the Cerebral Performance Category (CPC)-Scale (CPC 1 good cerebral

performance, CPC 2 moderate cerebral disability, independent in activities of daily life, CPC 3 severe cerebral disability, dependent on others for daily support, 4 vegetative state and CPC 5 dead).[26-28] The CPC-scale was dichotomized to good (CPC 1 – 2) and poor (CPC 3 – 5) outcome.[29] In a secondary analysis we used the biomarker protein Tau as outcome to strengthen the analyses using neurologic functional outcome.

1. Main analysis

Levels of carbon dioxide

As our main analysis, we studied the association of dyscarbia with neurological outcome by dividing the cohort into three groups according to the single highest or lowest $PaCO_2$ value during the observation period. The groups were defined as hypocapnia (< 4.5 kPa), normocapnia (4.5 – 6.0 kPa) and hypercapnia (> 6.0 kPa) in keeping with a previous investigation.[16] The outcome of the hypercapnia and hypocapnia group were each compared with the normocapnia group. Then we compared the outcome of the hypercapnia and hypocapnia group with the outcome of a compound group of the remaining patients.

2. Secondary analyses

2.1 Carbon dioxide amplitude

The amplitude in $PaCO_2$ ($\Delta PaCO_2$) was calculated as a continuous variable, investigating an association of maximum amplitude in $PaCO_2$ during the observation period and neurological outcome.

2.2 Carbon dioxide over time

We obtained an approximation of the time weighted mean carbon dioxide exposure as an area under curve (AUC) by integrating $PaCO_2$ over time. The AUC including eight $PaCO_2$ values was analyzed, investigating an association with neurological outcome over the whole observation period, as well as the AUC of the first four $PaCO_2$ values, in order to study the influence of early dyscarbia.

2.3 Maximum $PaCO_2$ and lowest pH analysis

The association between maximum $PaCO_2$ and lowest pH as continuous variables and neurological outcome was evaluated in univariable analyses. Thereafter both variables were introduced into a combined logistic regression model.

2.4 Therapeutic targeted mild hypercapnia (TTMH)
From the AUC analysis, we extracted two $PaCO_2$ groups approximating $PaCO_2$ ranges employed by Eastwood et al, 4.5 - 6.0 kPa and 6.0 - 7.30 kPa and subdivided them according to target temperature (33°C and 36°C) in order to detect possible interactions between $PaCO_2$ and temperature in relation to outcome.[21]

3. Analyses using a biomarker as outcome

Association of $PaCO_2$ and s-Tau
A nested cohort analysis was performed in 689 patients in a previous sub-study of the TTM-trial, evaluating s-Tau levels at 24, 48 and 72 hours after ROSC, showing the highest accuracy of predicting poor outcome after 6 months for peak s-Tau at 48 and 72 hours.[23] Therefore, we analyzed the association of $PaCO_2$ and peak s-Tau at these time points, employing the multivariable models used for our primary analyses.

4. Sensitivity analyses

For sensitivity analysis we used the complete case cohort consisting of 485 patients (56%) with blood gas samples registered from all measuring points and the total case (n=939) cohort, including 100% of the patients, also those not surviving the full exposure period.

Statistical considerations

Proportions are expressed as percentages and continuous data as mean with standard deviations (SD). The association between $PaCO_2$ and neurological outcome was analyzed using logistic regression. Except for the maximum $PaCO_2$ and lowest pH analyses, all analyses were corrected for pre-specified, and relevant co-variates: age (years), sex (male/female), chronic heart failure (yes/no), asthma/chronic obstructive pulmonary disease (yes/no), cardiac arrest witnessed (yes/no), bystander CPR (yes/no), first rhythm shockable (yes/no), time to ROSC (minutes), GCS-Motor Score (1 versus 2 - 5), shock on admission (yes/no), pH at admission (units). Whether pooling of the two temperature groups was feasible was established by a term of interaction model between the $PaCO_2$ analysis groups and the two temperature groups. A significant term of interaction entailed subgroup analyses for the 33°C and the 36°C groups. A non-significant term of interaction entailed a combined group analysis.

Logistic regressions are presented as odds ratios (OR) with 95% confidence intervals (CI) with OR below 1 indicating better, and above 1 indicating worse neurological outcome. OR relating to continuous data describe the variation per one unit (1 kPa for $PaCO_2$ and 1 unit for pH).

For s-Tau analysis, multivariable linear regression was used including the same co-variables and interaction analyses as above. S-Tau values where transformed to a logarithmic scale and used as outcome in the linear regression analyses. The regression coefficients achieved for each independent variable were transformed back and reflect the multiplicative change in s-Tau. This means that coefficients below 1 correspond to a decrease in s-Tau and above 1 to an increase. Linear regressions are presented as coefficient estimates with 95% CI.

Multiple imputation was used to compensate for missing values; 20 imputations were generated using chained equations and evaluated by graphical methods. The estimates from the logistic and linear regression for each imputed sample were combined into one estimate with 95% CI including the uncertainty from the multiple imputations. The primary analyses were performed on a multiple imputation cohort. A complete case cohort was used for sensitivity analysis. We regarded a two-sided P-value < 0.05 as significant. Analyses were conducted using IBM SPSS statistics for Windows version 22.0, Armok NY and R:A language and environment for statistical Computing version 3.3.3R Foundation for Statistical Computing, Vienna, Austria and the package mice was used for multiple imputations.[30]

Results

From the 939 patients included in the TTM-trial we excluded patients who did not survive the analysis period (n = 62), patients with no $PaCO_2$ data (n = 2) and patients with no data on neurological outcome (n = 6) (Figure 1), leaving 869 (93%) patients for analysis. Additional data on the number of excluded patients at each measuring point are displayed in Supplementary Table 1, Additional File 1. Baseline characteristics of the included patients are presented in Table 1. 878 of 6952 analyzed measuring points were missing (12.6%), detailed information regarding the number of missing values at each time point is shown in Table 2 and multiple imputation was used to overcome the missingness. 485 patients had no missing values. Overall, 440 (50.6%) of 869 patients had a good outcome while the outcome of 429 (49.4%) was considered poor.

Table 1. Patient baseline characteristics

Demographic characteristics	**Total no. N= 869**
Age – years (mean, s.d.)	63.9 ± 12.2
Male sex no. (%)	707 (81.4)
Background no. (%)	
Chronic heart failure	55 (6.3)
TIA or stroke	69 (8.0)
Arterial hypertension	347 (40.1)
Asthma/COPD	86 (9.9)
Diabetes mellitus	128 (14.8)
Previous PCI	101 (11.6)
Previous CABG	82 (9.5)
Cardiac arrest characteristics	
Bystander witnessed arrest no. (%)	783 (90.1)
Bystander CPR no. (%)	638 (73.4)
Shock on admission no. (%)	111 (12.8)
Prehospital intubation no. (%)	576 (67.2)
Time to ROSC (min) (mean s.d.)	30.4 ± 21.7
Characteristics on admission	
pH	7.21 ± 0.15
$PaCO_2$ (kPa) (mean, s.d.)	6.4 ± 2
PaO_2 (kPa) (mean, s.d.)	25.1 ± 17
Lactate (mmol/L) (mean, s.d.)	6.5 ± 4.3
BE ≥ -5 (mmol/l) no. (%)	579 (7.3)
GCS – Motor 1 no. (%)	443 (51.3)
Sedated on arrival no. (%)	254 (29.4)

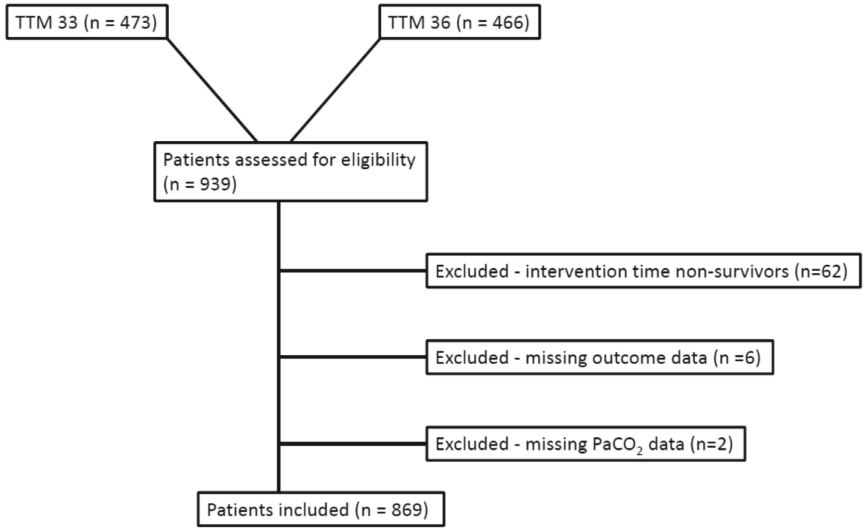
SD = Standard deviation. TIA = transient ischemic attack. COPD = Chronic obstructive pulmonary disease. PCI = Percutaneous coronary intervention. CABG = Coronary artery bypass graft. GCS = Glasgow coma scale. ROSC = return of spontaneous circulation. $PaCO_2$ = arterial carbon dioxide pressure. PaO_2 = arterial oxygen pressure. kPa = kilopascal. CPR = cardiopulmonary resuscitation. BE = Base excess.

Table 2. Number of missing measurements at each time point. Total no. N = 869

Time (h)	T -1	0	4	12	20	28	32	36
Missing n	45	154	94	91	118	117	135	124
% of total	5.18	17.7	10.8	10.4	13.6	13.5	15.5	14.2

n = numbers. h = hours. T -1 = time at admission, after ROSC but before randomization.

Figure 1. Patient selection pathway for the $PaCO_2$ outcome analyses.

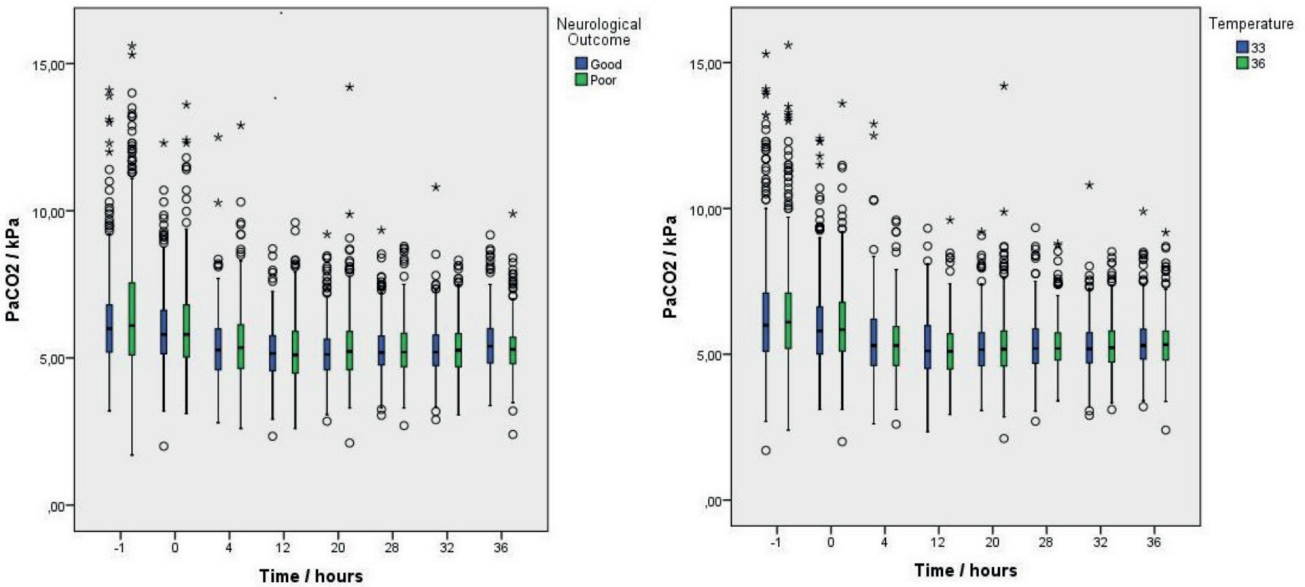


TTM = targeted temperature management. TTM 33 and TTM 36 = TTM group, 33 or 36°C core body temperature derived from TTM-trial study [24]. n = number of patients. The diagram does not display the selection pathway for the s-Tau analysis or the sensitivity analyses.

On arrival, the mean $PaCO_2$ was 6.40 (SD 1.99) kPa and decreased over time in both temperature (Figure 2a) and outcome (Figure 2b) groups. 516 of 869 (59%) patients were hypocapnic and 685 of 869 (79%) were hypercapnic at some time point during the analysis period. 371 (43%) were both hypo- and hypercapnic and only 39 patients (4%) were normocapnic throughout. 6-month outcome data of the exposure groups are displayed in Supplementary Table 2, Additional File 1. The $\Delta PaCO_2$ group had a mean range of 2.88 (SD 1.60) kPa. $PaCO_2$-AUC for the first four measuring points was mean 5.51 (SD 0.92) kPa while $PaCO_2$-AUC for all measurements was mean 5.37 (SD 0.62) kPa.

The analysis of interaction between temperature groups (33°C or 36°C) and $PaCO_2$ group showed no significant difference for main outcome (Pmain-outcome = 0.072 – 0.98) in all comparisons. The terms of interaction were, except for $\Delta PaCO_2$ (p=0.046) also non-significant (Pinteraction = 0.255 – 0.947). For the $\Delta PaCO_2$ group, the 33°C and the 36°C subgroups were analyzed separately, showing no significant association with outcome in any of the groups which made pooling of temperature groups feasible.

Figure 2. Distributional characteristics of $PaCO_2$ over time.



Boxplots depicting the distributional characteristics of $PaCO_2$ at 8 measurement points from admission to hospital to the end of intervention at 36 hours for the TTM 33 and TTM 36 groups and the investigated combined cohort dichotomized into good and poor outcome. Boxplot values are displayed as median, 25% quartiles from median and range. TTM = Targeted temperature management. $PaCO_2$ = arterial carbon dioxide pressure. kPa = kilopascal. Core body temperature, 33° or 36°, in degrees Celsius.

Multivariable models with all co-variables included in the analyses for hyper- and hypocapnia versus normocapnia are presented in Table 3a and b.

Our main analysis revealed no statistically significant difference between the hypercapnia and the normocapnia group (OR 0.70, 95% CI 0.44 - 1.11; P = 0.13) or the hypercapnia and the non-hypercapnia group (OR 0.80, 95% CI 0.51 - 1.22; P = 0.31) in relation to poor neurological outcome. In a similar analysis the hypocapnia group was compared to the normocapnia and subsequently to the non-hypocapnia group with no significant differences (OR 0.96, 95% CI 0.64 -1.45; P = 0.85); (OR 1.04, 95% CI 0.72 - 1.49; P = 0.82). The $\Delta PaCO_2$ analysis did not reveal a statistically significant association with poor neurological outcome, neither for the 33°C nor the 36°C subgroup (OR 1.08, 95% CI 0.9 - 1.29; P = 0.37); (OR 1.00, 95% CI 0.82 - 1.20; P = 0.96), or for the combined group (OR 1.04, 95% CI 0.91 - 1.18; P = 0.56). The $PaCO_2$-AUC from admission to end of intervention time or the first four measured $PaCO_2$ values (early dyscarbia) were not associated with poor neurological outcome (OR 1.09, 95% CI 0.83 - 1.42; P = 0.53) and (OR 0.99, 95% CI 0.81 - 1.22; P = 0.96) respectively.

Table 3a. Multivariate model of hypercapnia versus normocapnia in relation to neurological outcome.

	OR	CI	P-value
Hypercapnia (normocapnia reference)	0.70	0.44 – 1.11	0.13
TTM group (33°C reference)	1.00	0.71 – 1.42	0.99
Age (per year)	1.07	1.05 – 1.08	<0.001
Sex (male reference)	1.34	0.84 – 2.15	0.22
Chronic heart failure (yes/no)	2.09	0.98 – 4.46	0.06
Asthma/COPD (yes/no)	1.32	0.72 – 2.43	0.37
Bystander witnessed arrest (yes/no)	0.61	0.35 – 1.07	0.09
Bystander CPR (yes/no)	0.87	0.57 – 1.33	0.53
Time to ROSC (per min)	1.03	1.02 – 1.04	<0.001
GCS - M (1 vs 2 – 5)	2.5	1.72 – 3.57	<0.001
Shock on admission (yes/no)	1.56	0.88 – 2.75	0.13
First rhythm shockable (yes/no)	0.19	0.11 – 0.33	<0.001
pH (per 1.0 unit increase)	0.28	0.07 – 1.17	0.08

Table 3b. Multivariate model of hypocapnia versus normocapnia in relation to neurological outcome.

	OR	CI	P-value
Hypocapnia (normocapnia reference)	0.96	0.64 – 1.45	0.85
TTM group (33°C reference)	1.00	0.69 – 1.46	0.99
Age (per year)	1.06	1.04 – 1.08	<0.001
Sex (male reference)	1.58	0.95 – 2.63	0.08
Chronic heart failure (yes/no)	1.95	0.87 – 4.37	0.10
Asthma/COPD (yes/no)	1.41	0.74 – 2.66	0.29
Bystander witnessed arrest (yes/no)	0.55	0.29 – 1.05	0.07
Bystander CPR (yes/no)	0.97	0.62 – 1.53	0.91
Time to ROSC (per min)	1.03	1.02 – 1.05	<0.001
GCS – M (1 vs 2-5)	1.92	1.32 – 2.86	0.001
Shock on admission (yes/no)	2.4	1.33 – 4.34	0.004
First rhythm shockable (yes/no)	0.16	0.09 – 0.29	<0.001
pH (per 1.0 unit increase)	0.22	0.05 – 0.89	0.03

Hypercapnia = $PaCO_2$ > 6.0 kPa, normocapnia = $PaCO_2$ 4.5 - 6.0 kPa, hypocapnia = $PaCO_2$ < 4.5 kPa. CI = 95% Confidence interval. OR = Odds Ratio. TTM = Targeted Temperature Management. COPD = Chronic obstructive pulmonary disease. CPR = Cardiopulmonary resuscitation. GCS - M = Glasgow coma scale – Motor. ROSC = return of spontaneous circulation. $PaCO_2$ = arterial carbon dioxide pressure. OR < 1 indicates better outcome.

When analyzed separately in univariable logistic regression models, maximum $PaCO_2$ as well as lowest pH showed highly significant associations with poor neurological outcome (OR 1.17, 95% CI 1.06 - 1.28; P < 0.001); (OR 0.03, 95% CI 0.01 - 0.09; P < 0.001). When analyzed in a combined logistic regression model only the significant association between lowest pH and poor neurological outcome prevailed, (OR 0.02, 95% CI 0.05 - 0.11; P < 0.001) per unit decrease in pH. $PaCO_2$ and pH were not highly correlated with a collinearity between the regression coefficients of -0.64.

The TTMH-analysis, comparing a normocapnia to a mild hypercapnia group, showed a non-significant term of interaction (P = 0.79) between temperature and $PaCO_2$ in relation to outcome; thus, we continued with a multivariable model without interaction. This analysis showed neither a significant difference between the mildly elevated (n = 121) and normocapnic (n = 675) $PaCO_2$ groups, (OR 1.01, 95% CI 0.60 - 1.67; P = 0.98) in relation to neurological outcome nor in the temperature groups (OR 0.96, 95% CI 0.68 - 1.35; P = 0.83).

Of the 689 patients in the s-Tau nested cohort analysis, 100 were excluded, either due to our exclusion criteria (n = 64) or missing peak s-tau values (n = 36). The multivariable analysis of the remaining 589 patients, showed no association between $PaCO_2$ and s-Tau in our models (P = 0.12–1.00). The terms of interaction

Table 4. Results of the peak s-Tau nested cohort analysis for the employed multivariable models.

Multivariable model	Estimate	CI	P-value
Hypocapnia vs non-hypocapnia*	1.07	0.73 – 1.57	0.71
Hypocapnia vs normocapnia*	1.37	0.45 – 4.15	0.57
Hypercapnia vs non-hypercapnia*	0.68	0.42 – 1.10	0.12
Hypercapnia vs normocapnia*	1.00	0.38 – 2.64	1.00
Amplitude**	1.04	0.91 – 1.20	0.53
AUC, all values**	1.08	0.83 – 1.42	0.56
AUC, first four values**	0.94	0.76 – 1.17	0.59
TTMH Mild hypercapnia vs normocapnia*	0.75	0.43 – 1.28	0.29

CI = 95% Confidence Interval. S-Tau = Serum Tau. AUC = Area under curve. TTMH = Therapeutic Targeted Mild Hypercapnia. For analyses of categorical data* the estimate indicates how many times higher the s-Tau is compared to reference group. For analyses of continuous data** the estimate indicates how much higher s-Tau is per 1kPa $PaCO_2$ increase.

analysis were non-significant (Pinteraction = 0.11–0.83). Complete results are displayed in Table 4.

Both sensitivity analyses revealed similar results as the analyses on the imputed dataset, for the complete sample cohort with 485 patients (P = 0.32-0.96) and the all-patient cohort with 939 patients (P = 0.15- 0.98). For details concerning the sensitivity analyses, see Supplementary Table 3 and 4, Additional File 1.

Discussion

In this exploratory sub-study of the TTM-trial, dyscarbia after ROSC was frequent. We were not able to detect a statistically significant association between hypercapnia, hypocapnia, $PaCO_2$-AUC or $\Delta PaCO_2$ and neurological outcome. There was no significant interaction between temperature group and carbon dioxide level in relation to outcome. $PaCO_2$ was not associated with peak s-Tau levels after 48 or 72 hours after randomization.

Our results differ from a prospective single-center study by Roberts et al including 193 post cardiac arrest patients, suggesting an independent association between hypocapnia and hypercapnia and poor neurological function at hospital discharge.[17] In contrast to our study Roberts et al included mainly patients after in-hospital cardiac arrest and used TTM in 6 patients only.[17] Dyscarbia was less common compared to the present study (69% versus 96%). Our results also differ from database study by Schneider et al, analyzing $PaCO_2$ values of 16542 patients admitted after cardiac arrest showing a higher likelihood of discharge home for the group of patients exposed to hypercapnia after ROSC compared to normocapnia

or hypocapnia 16. As in our study, dyscarbia after ROSC was common. However, important confounders on background information on the nature of cardiac arrest (initial rhythm, time to ROSC etc.) were not available and, apart from a nested cohort analysis, only single $PaCO_2$ values were analyzed. With exception of the CCC-trial21, randomizing to different targets of $PaCO_2$, we are only aware of one study analyzing multiple $PaCO_2$ values over time during the post cardiac arrest phase.[20] This prospective observational study, including 409 OHCA patients, analyzed serial blood gases during the first 24 hours after ROSC and found that exposure to a moderately increased $PaCO_2$ level was an independent predictor for good outcome at 12 months. We chose a comparable approach in our analysis of $PaCO_2$-AUC, but could not confirm this finding. In their study, blood gases were analyzed by either alpha- or pH-stat20, while the blood gas management method employed in our study was exclusively alpha-stat. The solubility of carbon dioxide in blood is temperature dependent and might influence the ventilation strategy. Ventilation has, via the coupling of CO_2 and cerebral vascular tone, influence on CBF in OHCA patients treated with TTM.31 Voicu et al showed a significant difference in $PaCO_2$, arterial pH and CBF when alpha-stat was compared to pH-stat.[31] These findings might identify a source of error in studies using mixed blood gas management32, and when comparing studies using different methods, which might explain the deviance of our results from other studies. pH was in our study independently associated with neurological outcome whereas maximum $PaCO_2$ was not. This confirms previous findings that pH is an independent outcome predictor after ROSC.[33,34]

Our study does not indicate benefit of TTMH as investigated by Eastwood et al21, but also no harm. It is imperative to appreciate that we, in contrast to the pilot CCC-trial21, compared time weighted mean $PaCO_2$ values (observed, non-targeted $PaCO_2$), while the CCC-trial randomized patients to specific $PaCO_2$ ranges (prescribed, targeted $PaCO_2$). Additionally, we widened the mild hypercapnia group for our analysis to 6.0 – 7.30 kPa for increased robustness of our results. Whether TTMH is indeed beneficial remains to be proven in a definitive clinical trial.[21] Importantly, there was no significant interaction between temperature level and $PaCO_2$ in terms of outcome, supporting the possibility to co-enroll in trials investigating carbon dioxide and temperature targets.[22]

The effects of $PaCO_2$ on biomarkers have to date only been evaluated in the above mentioned CCC-trial where mild hypercapnia reduced NSE and S100B (S100 calcium-binding protein B) levels.[21] In our cohort, $PaCO_2$ showed no association with peak s-Tau levels, which is in line with the lack of association between $PaCO_2$ and neurological outcome in our study. We have previously reported that s-Tau is superior to NSE in predicting outcome and that S-100 does not add to a prediction model including NSE and clinical information.[23,35]

Study strengths and limitations

There is no consensus how to report carbon dioxide levels in relation to outcome in cardiac arrest patients and previous studies have employed methods suited to the nature of their data (single lowest/highest values versus serial measurements, within a defined time period versus not, using a pre-specified sampling plan or not). In this study we have employed many different analytic approaches and used different outcomes (functional outcome and biomarkers) in order to provide an as robust as possible investigation. It is important to emphasize that the study was conceived post-hoc and with a definite exploratory approach. All results must be regarded as hypothesis generating, and due to the observational design, we cannot make causality statements. Blood gases represent the $PaCO_2$ at a certain point in time and we assumed that the $PaCO_2$ in between blood samples was linear. It is also important to point out that patients not surviving the analysis period were excluded from the analysis to allow a defined exposure period of carbon dioxide. There are however considerable strengths in our analysis as data were derived from a large, well-controlled cohort of OHCA patients with availability of important confounders. Physiological and biochemical data were collected prospectively at specified time points according to a pre-defined protocol and blood gasses were analyzed in a uniform way. Measurements were serial and therefore likely to demonstrate the association of $PaCO_2$ with outcome in the post cardiac arrest phase more accurately than single measurements. Follow-up data were acquired with face-to-face interviews using a structured protocol and the loss of patients in the follow-up period was minimal.[24] We performed sensitivity analyses of patients with all data registered at all sampling points and including all patients, also those not surviving the full analysis period, and obtained similar results.

Conclusion

Dyscarbia after ROSC was common in OHCA patients, but measured as extreme values and over time not associated with neurological outcome at 6-month follow-up. Mild hypercapnia was not associated with adverse outcome and there was no interaction with temperature group affiliation.

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Supplemental material

Supplemental Table 1. Number of patients excluded and $PaCO_2$ at each measuring point.

Time	T -1	T 0	T 4	T 12	T 20	T 28	T 32	T 36
N Valid	59	50	38	26	18	7	3	2
N Missing	11	20	32	44	52	63	67	68
$PaCO_2$ mean (kPa)	8.07	6.38	6.12	5.37	5.96	6.21	5.44	5.65
$PaCO_2$ median (kPa)	7.10	6.13	5.62	5.04	5.35	5.80	5.70	5.65
Standard deviation	3.69	1.79	1.87	1.51	2.19	2.61	1.10	0.49
Minimum (kPa)	3.30	3.50	3.40	3.06	3.00	4.18	4.23	5.30
Maximum (kPa)	20.00	12.90	11.20	10.00	11.40	11.90	6.38	6.00
Percentile 25 (kPa)	5.80	5.37	4.60	4.20	4.40	4.60	4.23	5.30
Percentile 50 (kPa)	7.10	6.14	5.62	5.04	5.35	5.80	5.70	5.65
Percentile 75 (kPa)	9.40	7.23	7.40	6.21	6.95	6.10		

N = Number. T = measuring point in hours before/after randomization. $PaCO_2$ = partial arterial carbon dioxide pressure. kPa = kilopascal

Supplemental Table 2. 6-month neurological outcome in $PaCO_2$ extreme value exposure groups dichotomized to good and poor. n=869

$PaCO_2$ Group	Total n (%)	Good outcome n (%)	Poor outcome n (%)
Hypercapnia (> 6.0 kPa)	685 (79)	349 (51)	336 (49)
Hypocapnia (< 4.5 kPa)	516 (59)	260 (50)	256 (50)
Normocapnia (4.5 – 6.0 kPa)	39 (4)	23 (59)	16 (41)
Hyper- and hypocapnia	371 (43)	192 (52)	179 (48)

N = number. $PaCO_2$ = partial arterial carbon dioxide pressure. kPa = kilopascal. CPC = Cerebral performance category. Good outcome = CPC 1 and 2, poor outcome = CPC 3-5. CPC 1 good cerebral performance, CPC 2 moderate cerebral disability, independent in activities of daily life, CPC 3 severe cerebral disability, dependent on others for daily support, 4 vegetative state and CPC 5 dead.

Supplemental Table 3. Sensitivity analysis of $PaCO_2$ groups including all patients (n=939), adjusted for confounders.

Analysis	OR	CI	P-value
Hypercapnia vs normocapnia	0.70	0.30-1.64	0.41
Hypercapnia vs non-hypercapnia	0.73	0.48-1.12	0.15
Hypocapnia vs normocapnia	1.04	0.41-2.60	0.94
Hypocapnia vs non-hypocapnia	0.99	0.70-1.41	0.96
$PaCO_2$-AUC first four measurements	1.03	0.86-1.24	0.71
$PaCO_2$-AUC all measurements	1.09	0.89-1.34	0.40
Amplitude	1.00	0.88-1.13	0.98

OR = Odds Ratio. CI = Confidence Interval. $PaCO_2$ = partial arterial carbon dioxide pressure. AUC = Area under curve. OR < 1 indicates better outcome. Patients with no outcome data (n=6), no $PaCO_2$ data (n=2) and lack of confounders (n=5) were excluded, 926 patients we included in the final analysis. Confounders corrected for: age (years), sex (male/female), chronic heart failure (yes/no), asthma/chronic obstructive pulmonary disease (yes/no), cardiac arrest witnessed (yes/no), bystander CPR (yes/no), first rhythm shockable (yes/no), time to ROSC (minutes), GCS-Motor Score (1 versus 2 - 5), shock on admission (yes/no), pH at admission (units).

Supplemental Table 4. Sensitivity analysis of $PaCO_2$ groups including complete cases (n=485), adjusted for confounders.

Analysis	OR	CI	P-value
Hypercapnia vs normocapnia	0.60	0.21 – 1.68	0.33
Hypercapnia vs non-hypercapnia	0.76	0.44 – 1.30	0.32
Hypocapnia vs normocapnia	1.21	0.41 – 3.56	0.73
Hypocapnia vs non-hypocapnia	1.19	0.76 – 1.87	0.45
$PaCO_2$-AUC first four measurements	0.97	0.74 – 1.27	0.84
$PaCO_2$-AUC all measurements	1.00	0.71 – 1.43	0.96
Amplitude	1.06	0.89 – 1.24	0.52

OR = Odds Ratio. CI = Confidence Interval. $PaCO_2$ = partial arterial carbon dioxide pressure. AUC = Area under curve. OR < 1 indicates better outcome. Confounders corrected for: age (years), sex (male/female), chronic heart failure (yes/no), asthma/chronic obstructive pulmonary disease (yes/no), cardiac arrest witnessed (yes/no), bystander CPR (yes/no), first rhythm shockable (yes/no), time to ROSC (minutes), GCS-Motor Score (1 versus 2 - 5), shock on admission (yes/no), pH at admission (units).

Chapter nine

Microbiological profile of nosocomial infections following cardiac arrest: Insights from the targeted temperature management (TTM) trial

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Abstract

Aims Infectious complications frequently occur in intensive care unit patients admitted after out-of-hospital cardiac arrest. There is debate on the effects of temperature management on the incidence of infections, as well as on the efficacy and choice of antibiotic prophylaxis. In this substudy of the targeted temperature management (TTM) trial, we describe the microbiological profile of infectious complications in patients with cardiac arrest and examined the impact of TTM at 33°C compared to TTM at 36°C. Furthermore, we aimed to determine the association between antibiotic prophylaxis and the incidence of infections.

Methods This is a posthoc analysis of the TTM cohort. Microbiological data was retrospectively collected for the first 14-days of ICU-admission. Logistic regression was used to determine the relationship between antibiotic prophylaxis and pneumonia adjusted for mortality.

Results Of 696 patients included in this analysis, 158 (23%) developed pneumonia and 28 (4%) had bacteremia with a clinically relevant pathogen. *Staphylococcus aureus* was the most common pathogen isolated in patients with pneumonia (23%) and in patients with bacteremia (24%). Gram-negative pathogens were most common overall. TTM did not have an impact on the microbiological profile. The use of antibiotic prophylaxis was significantly associated with a reduced risk of infection (OR 0.59, 95%CI 0.43-0.79, p = 0.0005). This association remained significant after correcting for confounders (OR 0.64, 95%CI 0.46-0.90; p = 0.01). The association is not present in a model after correction for clustering within centers (aOR 0.55, 95%CI 0.20-1.47, p = 0.22). Adjustment for mortality did not influence the outcome.

Conclusion Gram-negative pathogens are the most common causes of nosocomial infections following cardiac arrest. TTM does not impact the microbiological profile. It remains unclear whether patients in ICUs using antibiotic prophylaxis have a reduced risk of pneumonia and bacteremia that is unrelated to center effects.

Background

Infectious complications are common in cardiac arrest patients and may contribute to mortality.[1-5] The high rate of nosocomial infections may be due to an impaired immune response following cardiac arrest.[6] There is a debate whether induced hypothermia may further hamper the body's ability to adequately respond to infections.[7] One study suggests that hypothermia is associated with an increased incidence of pneumonia within the first 3 days following cardiac arrest.[1] In particular, a higher incidence of infections due to gram-negative organisms was found.[1] In contrast, we recently showed that there were no differences between risk of infection in patients randomized to targeted temperature management at 33°C versus 36°C.[5]

Discrepancies between studies may be explained by difficulties in diagnosis of infections during temperature management strategies, not only due to temperature modulation but also due to systemic inflammatory reaction following a cardiac arrest. Difficulties in diagnosing infection may result in delay in initiation of antibiotic treatment[8,9] with subsequent increased duration of ICU- and hospital-stay.[10] This may prompt the question whether the use of prophylactic antibiotics may reduce infectious complications in cardiac arrest patients. In retrospective analyses, antibiotic use in the first 7 days following cardiac arrest was associated with improved survival[8] and a four-fold reduction of pneumonia.[11]

The Targeted Temperature Management (TTM) was a randomized clinical trial in which the impact of temperature on neurological outcome was assessed. In this substudy, we describe the microbiological profile of nosocomial infections in patients with cardiac arrest and examined the impact of TTM33 compared to TTM36 on this profile. Also, the association between prophylactic antibiotics and the incidence of infectious complications was investigated. We hypothesized that use of prophylactic antibiotic use would be associated with less infectious complications in cardiac arrest patients.

Methods

Patients

This is posthoc analysis of the TTM cohort, that following informed consent from next of kin included adult (≥18 years) unconscious patients (Glasgow Coma Scale <8) resuscitated from cardiac arrest of a presumed cardiac cause with return of spontaneous circulation with a duration of at least 20 minutes. Inclusion occurred at 36 centers in Europe and Australia. Further details on the exclusion criteria, trial protocol and main results were published previously.[12]

TTM protocol

Within the TTM-trial patients were randomized to a temperature of 33°C (TTM33) or 36°C (TTM36). 28 hours after randomization patients were gradually rewarmed to 37°C at a maximum speed of 0.5°C per hour. The total intervention period was 36 hours. Antibiotics were administered according to local protocols at each site. Final treatment decisions regarding antibiotics were taken by the treating physician.

Infectious complications

Data on infectious complications and clinical parameters were gathered by the treating physician, using an electronic case report form (eCRF). Daily scoring of suspected pneumonia was based on a new pulmonary or progressive infiltrate on chest X-ray and the presence of one or more of the following clinical features: fever (>38,0°C), leukocytosis (>12.000 cells/L) and purulent tracheobronchial secretions.[13,14] Due to temperature management, no minimum number of criteria was specified for the diagnosis of pneumonia. Pneumonia was considered confirmed if the local laboratory identified a causative organism in the pulmonary secretion samples in combination with above mentioned clinical symptoms. Bacteremia was defined as a positive blood culture with a pathogenic organism. Blood cultures that were positive with commensal skin flora were considered contaminated unless two separate, consecutive blood cultures were positive with the same microorganism.

In this post-hoc study, participating centers of the TTM-trial were asked to complete an eCRF with data on microbiological culture results and antibiotic use in the first 14 days of admission. This included queries on microorganisms in pulmonary secretion samples, blood cultures and urine cultures and use of prophylactic antibiotics, including selective digestive tract decontamination (SDD), which comprises of oral and gastric delivery of non-absorbable antibiotics for the duration of IC stay as well as a cephalosporin for the duration of four days.

Statistical analysis

Statistical significance in baseline characteristics between groups was evaluated by the Student's T-test for normally distributed continuous variables and the Mann-Whitney test for non-normally distributed continuous variables. The Chi-square test was used for comparing categorical variables. Categorical variables were expressed in absolute numbers and percentages.

To study the relationship between prophylactic antibiotic use on the risk of infection while accounting for mortality in the course following cardiac arrest, a linear mixed logistic regression model was used, with the participating hospitals as a random intercept. 'A priori, age, SOFA score day 1, asthma/COPD, time from cardiac arrest to ROSC, and immunosuppression (defined as either an immunosuppressive condition (i.e. HIV, hematologic or other malignancy, alcoholism or IV drug abuse), or the use immunosuppressive medication were selected as confounders. The variables were sequentially added to the model to analyze the relationship between prophylactic antibiotics and pneumonia in the first 14 days of ICU admission. Covariates remained in the model when the Likelihood Ratio test was significant, $p \leq 0.05$. The Likelihood-Ratio test compares nested models for which the parameters of one model are a subset of the second model. Collinearity diagnostics were performed using the variance inflation factor to check for variable independence.

In this model, mortality is not accounted for, as the exact time of death was not known. We made the assumption that mortality in the first 3 days of ICU admission is unlikely to be affected by prophylactic antibiotic use, and we consider the mortality in the first 3 days of ICU admission as a competing risk. Patients who died within 3 days of ICU admission could have been part of the outcome with or without an infection. To estimate the robustness of the model we repeated the initial model with inclusion of patients who died in the first 3 days as having an infection and subsequently also repeated the model including these patients as not having an infection and compared the standardized beta values for the three models. A p-value less than 0.05 was considered significant. All analyses were performed in R (version 3.1.1).

Results

Patients

Of the 950 patients randomized in the TTM-trial in 36 centers, data on microbiology results was available for 696 patients in 23 centers. In this cohort, the mean age was 64 (12) and 568 (82%) were male (table 1 shows all baseline characteristics of patients). Of the 696 patients, 186 patients (27%) developed a confirmed infection (either confirmed pneumonia or bacteremia). Within the confirmed infection group, 158 (89%) patients had pneumonia with confirmed microbiological culture and 28 (11%) patients had bacteremia. There were no differences in baseline characteristics between patients with a confirmed infection compared to patients without a confirmed infection (patients with suspected pneumonia and no infection).

Table 1. Baseline characteristics in patients with and without an infection

	All N=696	No confirmed infection N=518	Confirmed infection N=178	P-value
Demographic characteristics				
Age (yr) - mean ±SD	64.0 ±12.0	64.2 ±12.0	63.4 ±12.1	0.43
Weight (kg) - mean ±SD	81.9 ±15.4	82.1 ±16.0	81.6 ±13.9	0.671
Male sex – no. (%)	568 (82)	425 (82)	143 (80)	0.659
Medical History - no.(%)				
Chronic heart failure	43 (6)	30 (6)	13 (5)	0.711
Previous AMI	144 (21)	102 (20)	42 (22)	0.531
Ischemic heart disease	191 (28)	140 (28)	51 (27)	1
Previous cardiac arrhythmia	110 (16)	81 (16)	29 (16)	0.906
Previous cardiac arrest	15 (2)	10 (2)	5 (3)	0.772
Arterial hypertension	272 (39)	193 (38)	79 (42)	0.331
TIA or stroke	50 (7)	41 (8)	9 (5)	0.183
Diabetes	108 (16)	78 (15)	30 (16)	0.811
Asthma or COPD	73 (11)	47 (9)	26 (14)	0.088
Immunodeficiency	24 (3)	9 (5)	15 (3)	0.168
Characteristics of the cardiac arrest				
Bystander performed CPR - no. (%)	503 (73)	369 (73)	134 (72)	0.856
Time from cardiac arrest to ROSC - median (IQR)	32 (22-50)	25 (16-39)	25 (18.5-40)	0.15
First monitored rhythm - no. (%)				0.176
Asystole - no. (%)	78 (11)	60 (12)	18 (10)	-
Non-perfusing VT - no. (%)	14 (2)	11 (2)	3 (2)	-
PEA - no. (%)	49 (7)	40 (8)	9 (5)	-
ROSC after bystander defibrillation - no. (%)	12 (2)	8 (2)	4 (2)	-
VF - no. (%)	530 (76)	254 (72)	242 (92)	-
Unknown - no. (%)	13 (2)	6 (1)	7 (4)	-
Clinical characteristics on admission				
First Body temperature (°C) - mean ±SD	35.3 ±1.2	35.3 ±1.2	35.4 ±1.1	0.313
Circulatory shock - no. (%)	75 (11)	58 (11)	17 (9)	0.404
SOFA score - mean ±SD	10.4 ±2.6	10.4 ±2.6	10.6 ±2.5	0.479

Abbreviations: AMI, acute myocardial infarction, BLS, basic life support, CPR, cardiopulmonary resuscitation, COPD, chronic obstructive pulmonary disease, IQR, interquartile range, PEA, pulseless electrical activity, ROSC, return of spontaneous circulation , SD, standard deviation, SOFA, sequential organ failure assessment , TIA, transient ischemic attack, VT, ventricular tachycardia, VF, ventricular fibrillation.

Pneumonia

In total, 331 (48%) of the total 696 patients had a suspected or confirmed pneumonia. Of these patients, 158 (48%) had confirmed microbiologic cultures. Pathogens comprised of both gram-negative (61.1%) and gram-positive (38.2%) micro-organisms. The most frequently isolated organisms in the sputum cultures were *Staphylococcus aureus* (22.9%), *Escherichia coli* (9.1%), *Haemophilus influenzae* (9.1%) and *Streptococcus spp* (7.6%) *Pseudomonas aeruginosa* infections were infrequent (2.5%) (figure 1). Supplemental table 1 shows an overview of all isolated pathogens from sputum.

Bacteremia

In total 28 (4% of all patients) patients had a positive blood culture associated with clinically relevant infection. Gram-negative pathogens and gram-positive pathogens were both prevalent. The most frequently isolated pathogens were *Staphylococcus aureus* (24.2%), *Klebsiella pneumoniae* (12.1%) and *Enterococcus faecalis* (9.1%) (figure 2). Supplemental table 2 shows an overview of all isolated micro-organisms from blood cultures.

Figure 1. Pathogens in sputum cultures in confirmed pneumonia cases in the first 14 days of ICU-admission.

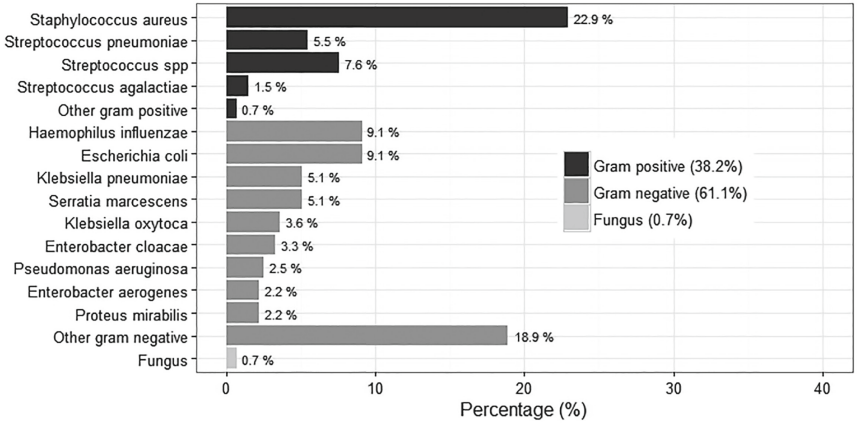


Figure 2. Pathogens in blood cultures in bacteremia cases in the first 14 days of ICU-admission.

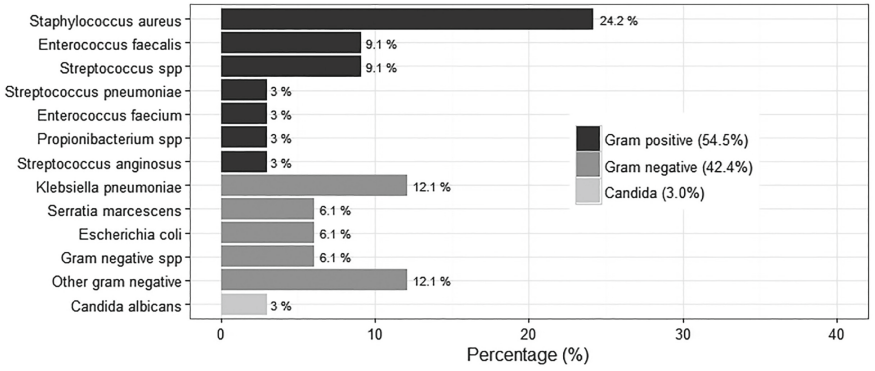


Table 2. Infections in patients admitted to centers with and without the use of antibiotic prophylaxis

Infections, n (%)	No antibiotic prophylaxis N=351	Antibiotic Prophylaxis N=345	P-value
Pneumonia	190 (54)	141 (41)	0.001
Suspected pneumonia	94 (27)	79 (23)	0.245
Confirmed pneumonia	96 (27)	62 (18)	0.002
Gram-positive in culture	55 (16)	34 (10)	0.018
Gram-negative in culture	68 (19)	48 (14)	0.066
Bacteremia	24 (7)	4 (1)	0.0001
Gram-positive in culture	15 (4)	3 (1)	0.011
Gram-negative in culture	12 (3)	2 (1)	0.013

Bacteriological distribution between different target temperatures

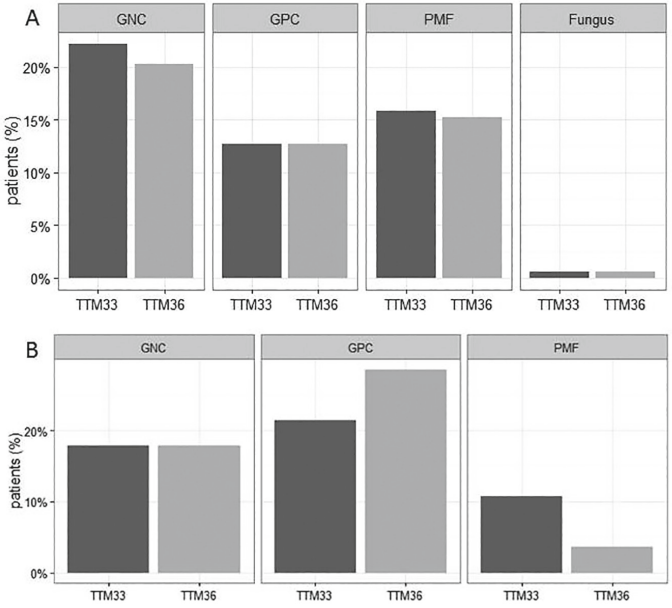
Baseline characteristics between the TTM groups can be seen in supplemental table 3. Figure 3 shows the bacteriological profile of sputum and blood cultures according to different target temperatures. The frequency of pneumonia patients with gram-positive, gram-negative or polymicrobial flora culture was not significantly different between TTM33 and TTM36 (figure 3A, supplemental table 4). In addition, we did not find a significant difference between TTM33 and TTM36 in patients with a gram-positive or gram-negative bacteremia (p=0.53) (figure 3B, supplemental table 4).

Table 3. Association between antibiotic prophylaxis and pneumonia (both confirmed and suspected) in the first 14 days of ICU admission, adjusted for confounders.

	aOR	95% CI	P-value
Prophylaxis	0.59	0.43 - 0.79	0.0005
Prophylaxis + age	0.57	0.42 - 0.78	0.0003
Prophylaxis + age + SOFA	0.64	0.46 - 0.89	0.009
Prophylaxis + age + SOFA + asthma/COPD	0.65	0.46 - 0.90	0.01
Prophylaxis + age + SOFA + asthma/COPD + time to ROSC	0.64	0.46 - 0.90	0.009
Prophylaxis + age + SOFA + asthma/COPD + time to ROSC + immunosuppression	0.64	0.46 - 0.90	0.01
Prophylaxis + age + SOFA + clustering within centers	0.55	0.20 - 1.47	0.22

Abbreviations: CI, confidence interval. COPD, chronic obstructive pulmonary disease. aOR, adjusted odds ratio. SOFA, sequential organ failure assessment. ROSC, return of spontaneous circulation.

Figure 3. Pathogen distribution in sputum cultures in confirmed pneumonia and bacteremia cases in the first 14 days of ICU-admission, according to different target temperatures.



GNC, Gram negative culture, GPC, Gram positive culture, PMF, polymicrobial flora, TTM, target temperature management.

The effect of prophylactic antibiotics on the incidence of pneumonia and bacteremia

Within the scope of this sub-study, five centers used SDD, seven centers used prophylactic cephalosporin treatment, one center used amoxicillin/clavulanic acid and two used a β-lactam that was not further specified (supplemental table 5 and 6 respectively show the site-specific prophylaxis and total antibiotic use in patients, supplemental table 7 shows the distribution of pathogens per center). We analyzed the incidence of infections in centers using antibiotic prophylaxis compared to centers that do not. Baseline characteristics between groups can be seen in supplemental table 8. In univariate analysis, patients in centers using prophylactic antibiotics had a lower incidence of both pneumonia (141 (41%) vs. 190 (54%), p=0.001) and bacteremia (4 (1%) vs. 24 (7%), p=0.0001) compared to patients in centers not receiving antibiotic prophylaxis (table 2). In the initial logistic regression model, prophylactic antibiotics was associated to a lower risk of pneumonia in the first 14 days of ICU-admission (adjusted odds ratio (aOR) 0.59, 95% confidence interval (CI) 0.43-0.79, p = 0.0005). After correcting for confounders, the relationship between prophylaxis and pneumonia remained significant (aOR 0.64, 95%CI (0.46-0.90), p = 0.01) (table 3). To adjust for potential clustering within centers, an additional model was run correcting for a potential cluster effect. In this model, the risk of acquiring pneumonia in the first 14 days of ICU admission further declines with prophylactic use of antibiotics, but statistical significance is lost. (aOR 0.55, 95%CI (0.20 – 1.47), p = 0.22) As the exact time of death was not noted and we wanted to account for the effect of mortality on outcome, we made the assumption that death within 3 days of ICU admission after cardiac arrest is likely not due to infection. The model was repeated with classification of all patients with early deaths as having an infection and as not having an infection. There was no difference between these models (Supplemental table 9 and figure 1).

Discussion

Incidence of nosocomial infections following cardiac arrest was high in this study, primarily driven by pneumonia. Nosocomial infection was most often due to gram-negative bacteria, although *Staphylococcus aureus* was the most commonly cultured micro-organism. Target temperature did not impact the distribution of pathogens in terms of gram-negative or gram-positive organisms. Antibiotic prophylaxis was significantly associated with a lower incidence of pneumonia and bacteremia in cardiac arrest patients in a model uncorrected for center effect.

Detailed description of the distribution of pathogenic organisms is important as it can help guide empiric antibiotic treatment. Inappropriate antibiotic therapy occurs in a significant portion of cardiac arrest patients and is associated with increased ICU-stay.[1] Our results are in line with previous descriptions of pathogen distribution, showing that *Staphylococcus aureus* is the most commonly cultured pathogen.[1-4] In our study, gram-negative pathogens in pneumonia were the most commonly cultured. Although we did not prospectively document aspiration, it can be hypothesized that aspiration may have contributed to the high incidence of pneumonia in this patient population. Only a limited number of anaerobic micro-organisms were found in this study, likely due to the fact that anaerobic cultures are not routinely performed on sputum. Also, *Pseudomonas aeruginosa* was rarely cultured. Taken together, depending on local resistance patterns, a cephalosporin of the 2nd or 3rd generation may be a reasonable empiric approach. In line with this, the incidence of pneumonia was lower in centers that used a cephalosporin of the 2nd or 3rd generation as prophylaxis.

Staphylococcus aureus is the most commonly cultured pathogen in sputum.[1-4] This finding has similarities to the observation that *Staphylococcus aureus* is also frequently cultured in trauma patients.[15-17] Also, as relatively large proportion of patients receive antibiotic prophylaxis with limited activity against *Staphylococcus aureus*, this micro-organism may be selected due to antibiotic use. In general, a high incidence of nosocomial infections is thought to be associated with immune paralysis[18], which occurs within an hour after trauma.[19] In line with this, we previously showed that the ability of immune cells to react to gram-positive and gram-negative bacterial components is reduced following cardiac arrest, irrespective of the temperature management regime.[6]

Bacteremia was less common, comprising 4% of patients in this study. This is lower than previously reported.[2-4] *Staphylococcus aureus* was the most commonly cultured pathogen in our study compared to *Escherichia coli* in a previous study.[2] Only 2 of the 8 patients with *Staphylococcus aureus* bacteremia had positive sputum cultures with *Staphylococcus aureus*. It remains unclear whether the concept of the use of prophylactic antibiotics reduces infection risk. In a systematic review, prophylaxis with an SDD regime has been shown to reduce nosocomial infections.[20] Specifically, in the group of cardiac arrest patients, prophylaxis was found to reduce the incidence of early pneumonia.[11] This finding was partly confirmed in a prospective randomized pilot trial of 83 patients with cardiac arrest, in which prophylactic versus clinically driven antibiotics decreased the rate of positive cultures from the lower respiratory tract, although outcomes did not improve.[5] However, in the same cohort, we previously reported that infectious complications are associated with increased mortality following cardiac arrest.[5] The potential benefits of antibiotic prophylaxis need to be weighed against potentially increased

antibiotic resistance of organisms due to prophylaxis.[20] At present, resistance during SDD treatment is low, at least in countries with low resistance rates.[21,22] However, we did not collect data on adverse effects of prophylactic antibiotics, which besides resistance also include drug reactions, drug interactions, etc. Our initial analysis showed a statistically significant association between prophylactic antibiotics and reduction of infections. After accounting for potential clustering in participating TTM centers, the association between prophylactic antibiotics and reduction of infections was lost. In order to have generalizable inferences of the effect of prophylactic antibiotics on outcome, centers must be taken into account, however accounting for a center effect reduces the statistical power. Thereby, it remains unclear from our study whether antibiotics reduce the infection risk unrelated to a center effect.

Given that the incidence of nosocomial infection is high in the cardiac arrest population, and that infection contributes to adverse outcome in these patients, investigations of the risk-benefit of prophylaxis in future randomized trials is warranted, including the optimal type of antibiotics as well as the duration of prophylaxis.

There are several limitations to this study. Recognizing fever as a sign of infection is hampered during TTM, which may lead to under-diagnosis. Also, frequency of taking cultures was not pre-specified and may have differed between centers. In particular, centers using SDD may have cultured more often than centers not using SD, thereby skewing results. However, this would have probably resulted in more infections in the SDD centers instead of less. Data on microbiological cultures were collected retrospectively and results from this study should be considered within the limitations of this design. As an example, tracheal aspirates were not further specified as protected vs. unprotected. Second, we did not perform a quantitative analysis with broncho-alveolar lavage, and positive cultures of tracheal aspirates could also reflect colonization. However, the effects of prophylaxis were also seen on the incidence of bacteremia. Finally, not all TTM centers participated in this substudy and this may have induced systematic bias. However, patients in the TTM were stratified per center and patients were missing from both large and small centers. We believe this study's cohort is a fair representation of cardiac arrest patients treated with TTM.

Conclusions

Gram-negative pathogens are the most common causes of nosocomial infections following cardiac arrest, irrespective of different TTM strategies. It remains unclear whether patients in centers using antibiotic have a reduced incidence of pneumonia and bacteremia that is unrelated to center effects.

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Supplemental material

Supplemental Table 1. Overview of pathogens detected in sputum cultures in patients with confirmed pneumonia

Micro-organisms, n (%)	Patients (N= 158)
Gram-positive bacteria	
Staphylococcus aureus	63 (22.9)
Streptococcus spp	21 (7.6)
Streptococcus pneumoniae	15 (5.5)
Streptococcus agalactiae	4 (1.5)
viridans streptococci	1 (0.4)
Gram-positive *spp*	1 (0.4)
Gram-negative bacteria	
Haemophilus influenzae	25 (9.1)
Escherichia coli	25 (9.1)
Klebsiella pneumoniae	14 (5.1)
Serratia marcescens	14 (5.1)
Klebsiella oxytoca	10 (3.6)
Enterobacter cloacae	9 (3.3)
Pseudomonas aeruginosa	7 (2.5)
Enterobacter aerogenes	6 (2.2)
Proteus mirabilis	6 (2.2)
Moraxella catarrhalis	4 (1.5)
Klebsiella spp	4 (1.5)
Gram-negative *spp*	4 (1.5)
Haemophilus parainfluenzae	4 (1.5)
Enterobacter spp	3 (1.1)
Morganella morganii	3 (1.1)
Haemophilus spp	3 (1.1)
Stenotrophomonas maltophilia	3 (1.1)
Serratia spp	3 (1.1)
Neisseria spp	3 (1.1)
Hafnia alvei	3 (1.1)
Citrobacter freundii	3 (1.1)
Pseudomonas spp	2 (0.7)
Acinetobacter spp	2 (0.7)
Morganella spp	2 (0.7)
Burkholderia spp	1 (0.4)
Citrobacter koseri	1 (0.4)
Escherichia spp	1 (0.4)

Table continues on next page

Supplemental Table 1. Continued.

Micro-organisms, *n* (%)	Patients (N= 158)
Haemophilus parahaemolyticus	1 (0.4)
Pantoea agglomerans	1 (0.4)
Proteus spp	1 (0.4)
Other	
Aspergillus fumigatus	1 (0.4)
fungus *spp*	1 (0.4)

Supplemental Table 2. Overview of pathogens detected in blood culture in patients with bacteremia

Micro-organisms , n (%)	Patients (n=28)
Gram-positive	
Staphylococcus aureus	8 (24.2)
Enterococcus faecalis	3 (9.1)
Streptococcus spp	3 (9.1)
Streptococcus anginosus	1 (3)
Streptococcus pneumoniae	1 (3)
Enterococcus faecium	1 (3)
Propionibacterium spp	1 (3)
Gram-Negative	
Klebsiella pneumoniae	4 (12.1)
Escherichia coli	2 (6.1)
Serratia marcescens	2 (6.1)
Gram-negative spp	2 (6.1)
Proteus vulgaris	1 (3)
Enterobacter spp	1 (3)
Enterobacter cloacae	1 (3)
Enterobacter aerogenes	1 (3)
Other	
Candida albicans	1 (3)

Supplemental Table 3. Baseline characteristics in patients treated with TTM33 vs. TTM36

	TTM33 N=350	TTM36 N=346	P-value
Demographic characteristics			
Age (yr) - mean ±SD	65.5 (11.7)	63.5 (12.3)	0.258
Weight (kg) - mean ±SD	82.6 (15.9)	81.3 (14.9)	0.703
Male sex – no. (%)	293(84)	275 (79)	0.182
Medical History - no.(%)			
Prior cardiovascular disease	216 (62)	204 (59)	0.494
Asthma or COPD	32 (9)	41 (12)	0.266
Characteristics of the cardiac arrest			
Bystander performed CPR - no. (%)	309 (88)	310 (90)	0.928
Time from cardiac arrest to ROSC – median (IQR)	25 (17-40)	25 (15-39.8)	0.469
Clinical characteristics on admission			
First Body temperature (°C) - mean ±SD	35.2 (1.2)	35.3 (1.1)	0.148
Circulatory shock - no. (%)	39 (11)	36 (10)	0.806

Abbreviations: CPR, cardiopulmonary resuscitation, COPD, chronic obstructive pulmonary disease, IQR, interquartile range, ROSC, return of spontaneous circulation, SD, standard deviation, TTM, targeted temperature management

Supplemental Table 4. Microbiological characteristics of confirmed pneumonia and bacteremia in TTM33 vs TTM36

	TTM33 (N=350)	TTM36 (N=346)	P-value
Pneumonia, n (% of TTM patients)	81 (23)	77 (22)	1
GPC	20 (25)	20 (26)	-
GNC	35 (43)	32 (42)	-
PMF	31 (25)	31 (24)	-
Fungus	1 (1)	1 (1)	-
Bacteremia, n (% of TTM patients)	14 (4)	14 (4)	0.53
GPC	6 (43)	8 (57)	-
GNC	5 (36)	5 (36)	-
PMF	3 (21)	1 (7)	-
Fungus	0 (0)	0 (0)	-

Abbreviations: GNC, gram negative culture, GPC, gram positive culture, PMF, poly microbial flora. Percentages of cultures are relative to total amount of patients with pneumonia or bacteremia, unless stated otherwise.

Supplemental Table 5. Site-specific prophylaxis use of antibiotics in prophylaxis group

Center	Antibiotic prophylaxis
DKRIG	Continuous cephalosporin
ITTRI	Continuous cephalosporin + SDD
NLAMC	Continuous cephalosporin + SDD
NLARN	Continuous cephalosporin + SDD
NLLEE	Single dose cephalosporin + SDD
NLOLV	Continuous cephalosporin + SDD
SEDAN	β-lactam not further specified
SELUN	β-lactam not further specified
SEOST	Continuous cephalosporin
UKGST	Amoxicillin/clavulanic acid

Abbreviations: SDD, selective digestive decontamination

Supplemental Table 6. Reported antibiotic use in patients with cardiac arrest.

Antibiotic, n (%)	Patients N=696
Betalactam	340 (48.9)
Aminoglycosides	21 (3)
Quinolones	78 (11.2)
Macrolides	31 (4.5)
Glycopeptides	18 (2.6)
Other antibiotics	269 (38.6)

Supplemental Table 7. Distribution of pathogens per TTM center

Site	Fungus n=2	GNC N=67	GPC N=40	PMF N=49
CHSTG (n=27)	0 (0.0)	4 (6.0)	1 (2.5)	4 (8.2)
CZPRA (n=30)	0 (0.0)	9 (13.4)	1 (2.5)	5 (10.2)
DKRIG (n=171)	1 (50.0)	5 (7.5)	4 (10.0)	1 (2.0)
ITPOR (n=26)	0 (0.0)	4 (6.0)	3 (7.5)	2 (4.1)
ITTRI (n=10)	0 (0.0)	0 (0.0)	1 (2.5)	0 (0.0)
LUCHL(n=38)	0 (0.0)	2 (3.0)	0 (0.0)	4 (8.2)
NLAMC(n=28)	0 (0.0)	2 (3.0)	1 (2.5)	2 (4.1)
NLARN (n=26)	1 (50.0)	4 (6.0)	2 (5.0)	1 (2.0)
NLLEE (n=38)	0 (0.0)	4 (6.0)	0 (0.0)	0 (0.0)
NOBER (n=36)	0 (0.0)	4 (6.0)	6 (15.0)	3 (6.1)
SEHBG (n=22)	0 (0.0)	1 (1.5)	1 (2.5)	3 (6.1)
SEKAR (n=7)	0 (0.0)	1 (1.5)	1 (2.5)	0 (0.0)
SELUN (n=41)	0 (0.0)	6 (9.0)	1 (2.5)	18 (36.7)
SEMMO (n=21)	0 (0.0)	6 (9.0)	4 (10.0)	1 (2.0)
UKBER (n=29)	0 (0.0)	1 (1.5)	2 (5.0)	0 (0.0)
UKBOU (n=20)	0 (0.0)	1 (1.5)	3 (7.5)	0 (0.0)
UKCAR (n=28)	0 (0.0)	3 (4.5)	3 (7.5)	3 (6.1)
UKGST (n=18)	0 (0.0)	5 (7.5)	3 (7.5)	0 (0.0)
UKSTG (n=34)	0 (0.0)	5 (7.5)	3 (7.5)	2 (4.1)

Supplemental Table 8. Baseline characteristics in patients receiving antibiotic prophylaxis vs no antibiotic prophylaxis

	No antibiotic prophylaxis N=351	Antibiotic Prophylaxis N=345	P-value
Demographic characteristics			
Age (yr) - mean ±SD	65.2 (12.2)	62.79 (11.8)	0.01
Weight (kg) - mean ±SD	80.1 (16)	83.8 (14.7)	0.002
Male sex – no. (%)	274 (78)	294 (85)	0.014
Medical History - no.(%)			
Chronic heart failure	22 (6)	21 (6)	1
Previous AMI	81 (23)	63 (18)	0.138
Ischemic heart disease	115 (33)	76 (22)	0.004
Previous cardiac arrhythmia	61 (17)	49 (14)	0.261
Previous cardiac arrest	11 (3)	4 (1)	0.121
Arterial hypertension	162 (46)	110 (32)	0.001
TIA or stroke	26 (8)	24 (7)	0.87
Diabetes	59 (17)	49 (14)	0.361
Asthma or COPD	46 (13)	27 (8)	0.031
Immunosuppression	9 (3)	17 (5)	0.118
Characteristics of the cardiac arrest			
Bystander performed CPR - no. (%)	249 (71)	254 (74)	0.399
Time from cardiac arrest to BLS - median (IQR)	1 (0-3)	0 (0-2)	<0.0001
Time from cardiac arrest to ROSC - median (IQR)	26 (18-42)	24 (15-36)	0.004
First monitored rhythm - no. (%)			0.013
Asystole - no. (%)	54 (15)	24 (7)	-
Non-perfusing VT - no. (%)	6 (2)	8 (2)	-
PEA - no. (%)	23 (7)	26 (8)	-
ROSC after bystander defibrillation - no. (%)	6 (2)	6 (2)	-
VF - no. (%)	254 (72)	276 (80)	-
Unknown - no. (%)	8 (2)	5 (1)	-
Clinical characteristics on admission			
First Body temperature (°C) - mean ±SD	35.3 (1.1)	35.3 (1.6)	0.758
Circulatory shock - no. (%)	45 (13)	30 (9)	0.08
SOFA score - mean ±SD	10.6 (2.7)	10.3 (2.4)	0.183

Abbreviations: AMI, acute myocardial infarction, BLS, basic life support, CPR, cardiopulmonary resuscitation, COPD, chronic obstructive pulmonary disease, IQR, interquartile range, PEA, pulseless electrical activity, ROSC, return of spontaneous circulation , SD, standard deviation, SOFA, sequential organ failure assessment, TIA, transient ischemic attack, VT, ventricular tachycardia, VF, ventricular fibrillation.

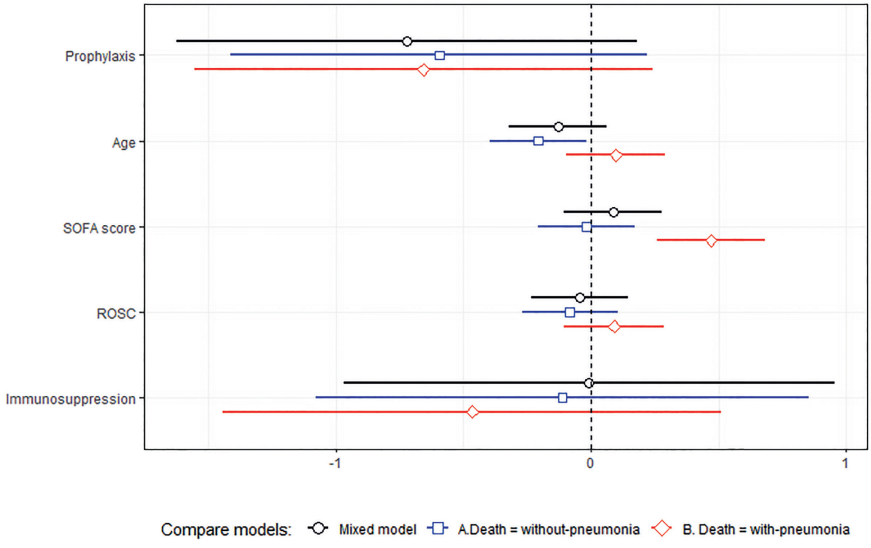
Supplemental Table 9. Multivariate model with death within 3 days of ICU admission as a competing risk for the outcome 'infection'

Multiple mixed logistic regression model (Hospitals as random intercept)	aOR (95% CI), p value
Prophylaxis + age + SOFA + asthma/COPD + time to ROSC + immunosuppression	0.55 (0.20 to 1.47), 0.22
A*. Model adjusted for death in the first three days (without pneumonia)	0.55 (0.24 to 1.25), 0.15
B*. Model adjusted for death in the first three days (with pneumonia)	0.52 (0.21 to 1.27), 0.15

* Model A outcome adjusted; all patients died in the first three days are relabelled as not having pneumonia.
* Model B outcome adjusted; all patients died in the first three days are relabelled as having pneumonia.

Abbreviations: CI, confidence interval. COPD, chronic obstructive pulmonary disease. aOR, adjusted odds ratio. SOFA, sequential organ failure assessment. ROSC, return of spontaneous circulation.

Supplemental Figure 1. Beta coefficients of 3 multivariate models accounting for the effect of mortality within 3 days of ICU admission.



The comparison of models by standardized beta coefficients shows that only the sofa score will be influenced if all patients that died in the first three days of admission would have had a pneumonia.

Chapter ten

Summary and general discussion

The aim of this thesis was to explore aspects of spontaneous and induced body temperature alterations in critically ill patients. The results from the studies presented in this thesis are discussed in the following paragraphs.

Body temperature and sepsis

Body temperature alterations are common in sepsis, but whether these temperature changes are beneficial or harmful to the host is controversial. The studies in this thesis on spontaneous hypothermia and induced normothermia in sepsis are seemingly at odds. On the one hand, spontaneous body temperature alterations in sepsis (i.e. fever and hypothermia), could both reflect beneficial adaptive responses at different stages of an infection.[1] Alternatively, actively cooling patients with fever and sepsis implies that fever is detrimental and should be treated. We do not fundamentally understand pathophysiologic mechanisms during sepsis and their relationship with body temperature. Therefore, the studies on sepsis and endotoxemia in this thesis were primarily aimed at understanding the pathophysiology underlying spontaneous and induced temperature alterations in sepsis in order to guide future temperature management strategies and ultimately optimize treatment for septic patients.

Hypothermic sepsis.

The survey presented in **chapter two** highlights how little we know about the optimal management of hypothermic sepsis. We found that definitions of spontaneous hypothermia and the practice of active rewarming in these patients are extremely variable. As a uniform definition of hypothermia will improve comparability of studies on hypothermia and probably would increase awareness, we call for a consensus definition of hypothermia. Among respondents there was also no consensus on the etiology of spontaneous hypothermia. The diverging opinions among physicians likely reflect a paucity on data on the hypothermic septic response and the difficulty in interpreting etiologic studies on hypothermic sepsis.

Several studies have observed increased mortality in hypothermic septic patients. This mortality may be attributed to hypothermia, but the prognostic value of hypothermia could also simply reflect more severe sepsis.[2] Animal studies even suggest that hypothermia may be an adaptive response.[3] Determining the etiology of body temperature response is imperative, as this understanding could ultimately have treatment implications for patients. If hypothermic sepsis is indeed an adaptive response, this could imply that hypothermic patients should not be rewarmed to normothermia, which is often done in clinical practice. On the

other hand, if hypothermia during sepsis is a deleterious response, rewarming to normothermia may benefit patients. Some authors have even hypothesized that patients should be rewarmed to febrile temperatures to invoke the beneficial immune response attributed to fever.[4]

In order to gain insight in the etiology of the hypothermic response in sepsis, we studied risk factors, host immune markers and endothelial markers in the hypothermic septic population in **chapters three and four**. In **chapter three** we found that patients with cardiovascular risk factors and low BMI were at risk for developing hypothermia. We also found signs that hypothermic patients may suffer from increased endothelial dysfunction. We subsequently proposed a mechanism to reconcile results from experimental studies and our clinical findings.[5] Animal studies show that spontaneous hypothermia in endotoxemia may be a pre-emptive strategy to prevent hypoxia.[6] Patients with sepsis and preexisting circulatory dysfunction (i.e. chronic cardiovascular dysfunction) or a metabolic deficit (i.e. lower body mass index) may reach a cellular hypoxic threshold sooner than other patients. Cellular hypoxia may be further exacerbated in these patients as we found signals of increased endothelial dysfunction in hypothermic septic patients.[5] Moreover, in **chapter four** we found that the tryptophan degradation X pathway was upregulated in patients with hypothermia compared to fever. Tryptophan has the potential, through serotonin mediated pathways, to lower body temperature.[7,8] But tryptophan metabolites are also toxic for some immune cells, potentially explaining the observation of prolonged lymphopenia in hypothermic septic patients.[9] Hypothetically, the hypothermic response could be a short-term strategy by the body to (temporarily) ward off hypoxia or extend metabolic reserves by lowering body temperature and decreasing oxygen consumption. This strategy may leave patients immunocompromised, ultimately resulting in increased mortality later on. Perhaps patients with hypothermic sepsis should be allowed to stay hypothermic. However, they may need some form of immune stimulation to potentially compensate for the immunosuppressive effects of hypothermia.

Induced hypothermia and induced normothermia in sepsis patients

Induced hypothermia and induced normothermia (also called fever control), in which patients with fever are cooled to hypothermia or normothermia, are experimental treatment strategies aimed at limiting metabolism and decreasing inflammation in sepsis. Animals studies have overwhelmingly shown that therapeutic hypothermia, in which animals with sepsis or endotoxemia are actively cooled to below 36°C, is associated with decreased inflammation and improved outcome.[10,11] However, the difficulty in translating results from these controlled

preclinical studies to clinical patients was underlined by the cooling and surviving septic shock (CASS) trial, an randomized controlled trial (RCT) in which patients with septic shock were treated with therapeutic hypothermia (32°C - 34°C). This study was stopped early due to futility, but cooling actually adversely effected several markers of organ failure.[12]

Induced normothermia however, may be an alternative intervention in sepsis. Treatment with induced normothermia in patients with fever and septic shock showed promising results with improved hemodynamic status and 14-day mortality compared to controls. More recently however, results of two clinical trials showing potentially adverse effects of induced normothermia, specifically regarding tissue perfusion.[13,14] The reason for these conflicting results is unclear. Therefore, we investigated the effects of induced normothermia in a highly reproducible healthy volunteer model of endotoxemia. In **chapter eight,** induced normothermia lowered heart rate while maintaining perfusion compared to healthy volunteers who only received LPS. Induced normothermia decreased interleukin-10 levels, but did not lower pro-inflammatory cytokine levels or CRP. In **chapter nine,** induced normothermia decreased disseminated intravascular coagulation (DIC) scores and plasma von Willebrand factor (vWf) levels and maintained platelet levels compared to volunteers that only received LPS, possibly indicating decreased endothelial activation. The results indicate that treatment with induced normothermia could be targeted to specific patient subpopulations, for example, patients with deranged coagulation or excessive endothelial activation.

Targeted temperature management in patients with cardiac arrest

In non-infectious etiologies such as hypoxic-ischemic brain injury after cardiac arrest, the effects of fever are well-defined; fever harms the injured brain.[15] Targeted temperature management (TTM), in which patients with cardiac arrest are actively cooled to 32°C-36°C, has improved survival and neurologic outcome since its implementation.[16] But TTM treatment can still be improved, to tailor the treatment to the specific needs of cardiac arrest patients. Towards this goal, we studied mechanical ventilation practices in patients following cardiac arrest in **chapter five**. We found that in the majority of the cardiac arrest patients, protective ventilation settings are applied, including low tidal volumes and driving pressures. TTM at 33°C resulted in lower end-tidal CO2 levels and a higher alveolar dead space fraction compared to TTM at 36°C, but did not result in a lower minute volume ventilation. The higher dead space in patients treated with TTM at 33°C could be a result of lower pulmonary perfusion due to increased vasoconstriction and this may be an unwanted side effect of cooling to 33°C. In

ARDS for example, increased dead space in associated with increased mortality.[17] TTM at 36°C may therefore be preferable in patients with pulmonary aspiration following cardiac arrest, who are at high risk for lung injury. In **chapter six** we explored the effects of hypo- and hypercarbia on neurologic outcome, but found no relationship between PaCO2 and neurologic outcome. There are two RCT's and one observational study that support these findings[18,19], although other observational studies have been inconsistent showing both negative and beneficial effects of hypo- and hypercarbia.[20-23] These findings do not necessarily support specific carbon dioxide targets post cardiac arrest. Therefore, carbon dioxide levels should be titrated to the specific needs of patients.

In general, prospective clinical trials on mechanical ventilation in ICU patients have largely excluded patients with cardiac arrest[24], despite this population being at risk for lung injury. The studies presented in this thesis these results should lead to recommendations on optimal mechanical ventilation strategies specifically for patients with cardiac arrest.[24]

In **chapter seven** we described the effect of TTM on the microbiological profile of infectious complications in patients with cardiac arrest. We found an 11% incidence of bacteremia in these patients.[25] In this study, nosocomial infection was most often due to gram-negative bacteria, although *Staphylococcus aureus* was the most commonly cultured micro-organism. The results from this study can help guide empiric antibiotic treatment as inappropriate antibiotic therapy occurs in a significant proportion of cardiac arrest patient and is associated with increased ICU-stay.[25] Subsequently, we investigated the association between antibiotic prophylaxis and nosocomial infections. We found that antibiotic prophylaxis was associated with a decreased incidence of pneumonia and bacteremia. In line with this finding, a large RCT has shown that prophylactic antibiotic treatment with amoxicillin–clavulanate reduces early ventilator-associated pneumonia in patients admitted to the ICU after cardiac arrest.[26] We suggest antibiotic prophylaxis as a standard practice in patients with cardiac arrest. Based on our results, a cephalosporin of the 2nd or 3rd generation may be a reasonable empiric approach. However, antibiotic prophylaxis selection should also be based on local resistance patterns. Sites in this study were predominantly from northern European countries, which have notably different antibiotic resistance patterns compared to southern European countries.[27] Also, there is a paucity of data on the relationship between nosocomial pneumonia and associated bacteremia and our results can aid in the epidemiologic understanding of bacteremia in mechanically ventilated patients.[28]

Taken together, studies examining the characteristics of TTM treatment in cardiac arrest are imperative as they serve to improve our understanding of TTM and adjacent therapies. TTM is complex and substitutes a combination of multiple

interventions that require optimization and standardization in order to maximize TTMs neuroprotective effects.[29] The studies presented in this thesis aid in the optimization and standardization of TTM and ultimately could lead to TTM treatment that is adapted towards specific patient characteristics and needs.

A future outlook on the appropriate body temperature in cardiac arrest and sepsis; moving away from a one size fits all approach.

There likely is an optimum body temperature, or body temperature range, for individual patients in specific disease states. Studies to date have given us a general idea of what acceptable temperature ranges are for patients, depending on their underlying illness. However, any further progress in identifying the optimal body temperature and TTM strategy for patients in cardiac arrest and sepsis risks a multitude of large, expensive and likley negative clinical trials. To advance TTM research in cardiac arrest and sepsis we need new biomarkers, in order to better understand underlying pathophysiologic processes in critically ill patients and their relationship to body temperature. Using these biomarkers, we could potentially individualize temperature management and improve clinical outcomes.

In cardiac arrest, there is evidence that the optimum body temperature is likely patient dependent. Results from the HYPERION trial showed that in cardiac arrest patients with non-shockable rhythms, TTM to 33°C led to a higher percentage of patients who survived with a favorable neurologic outcome as compared to 37°C.[30] The results from the HYPERION trial indicate that the necessary depth of TTM in patients may be dependent on the extent of neurologic damage as patients with non-shockable rhythms have significantly worse cardiac arrest characteristics including longer low-flow states. Unfortunately, TTM is currently a one-size fits all treatment and the chosen target temperature is usually based on a physician's preference or local protocol.[31] This approach diminishes the underlying complexities of brain injury after cardiac arrest.

In cardiac arrest, monitoring tools are needed to assess and monitor the appropriate depth and duration of TTM treatment in individual patients. Continuous electroencephalography (EEG) monitoring is being validated as a prognostication tool to predict clinical outcome after cardiac arrest.[32] In a recent study, cardiac arrest patients with cerebral edema or a malignant EEG fared better at lower target temperatures.[33] These results indicate that EEG monitoring could be used to determine the necessary depth and duration of TTM treatment. Studies should also focus on identifying brain injury specific biomarkers such as serum Tau protein levels. The use of these tools should not be limited to pre-TTM brain injury stratification and treatment allocation. Using these tools

TTM-treatment could be monitored in real-time based on the brains response and titrated according to specific patient needs.[31]

In sepsis, body temperature and the road towards TTM as a treatment is more complex, mostly due to the fact that we have yet to determine whether body temperature alterations in sepsis are beneficial or detrimental to the host. Towards this goal RCTs have been proposed to determine the etiology of the hypothermic response, in which patients are either rewarmed to a certain target temperature or left hypothermic.[2] If patients that are left hypothermic have better outcomes than those that are rewarmed, this would point to hypothermia being an adaptive response. Although the results of such a study would be intriguing, this type of study may suffer from the same pitfall as in previous clinical hypothermia and induced normothermia studies in sepsis. Treating sepsis with active cooling aims to attenuate the excessive immune response in sepsis. However, to what extent this "excessive" immune response still modulates vital physiologic processes in patients is not clear. Treating every septic patient with an identical temperature strategy will therefore likely result in benefit for some but detrimental effects for others. Moreover, previous studies have used clinical parameters such as the occurrence of shock, to categorize patients prior to TTM treatment. But these clinical parameters do not necessarily reflect underlying pathologic processes [34].This could explain the fact that promising preclinical results on the application of therapeutic hypothermia in sepsis resulted in mixed clinical results.[12]

Before initiating more TTM trials in sepsis we need a better understanding of the underlying pathophysiology of body temperature during sepsis. We should focus also on identifying biomarkers that can be used to monitor the adverse or beneficial effects of body temperature. Using these biomarkers, we may be able to determine whether a patient's body temperature is appropriate or warrants intervening. A starting point for future studies could be cellular hypoxia, which may drive body temperature alterations in sepsis. In both animals and humans, hypoxia regulates body temperature[35] and animal studies have found that spontaneous hypothermic response may a be preemptive attempt by the body to prevent hypoxia.[6] Interestingly, in the unmatched microarray analysis in chapter four, we found that hypothermic patients have a downregulation of cellular hypoxia signaling pathways despite these patients having a higher incidence of shock compared to patients with fever. Hypothetically, hypoxia signaling could be functional until a certain hypoxic threshold is reached. Once tolerable cellular hypoxic levels are exceeded, cellular hypoxia signaling pathways are downregulated and the bodies temperature setpoint is lowered to mitigate further hypoxic damage. Continuous observation of cellular hypoxia markers during spontaneous temperature alterations in septic patients would aid in better understanding the interplay between body temperature and hypoxia. Cutaneous

mitochondrial respirometry has emerged as a technique to continuously measure mitochondrial pO2 and mitochondrial oxygen consumption (mitoVO2).[36] It would be interesting to monitor parameters of cellular hypoxia during temperature alterations in sepsis.

Similarly, studying the genomic profile of septic patients using microarray technology can help us comprehend underlying pathophysiologic mechanisms during temperature alterations in sepsis. Intriguingly, there even seems to be an overlap between spontaneous temperature alterations in sepsis, which results in an upregulation of the trypotophan degradation pathway and TTM applied in cardiac arrest, which induces tryptophan catabolism.[37] Although the relationship between these two findings is unclear, these findings indicate that pathways like tryptophan are modifiable and could be used to monitor spontaneous and induced temperature alterations.

Taken together, we need a better understanding of the relationship between body temperature and underlying pathophysiologic mechanisms in critically ill patients before new clinical TTM trials are initiated. Although mechanistic studies in cardiac arrest and sepsis may not directly be as rewarding, they will provide a more focused direction to future TTM studies and help us move away from the one-size fits all approach in clinical trials.

Conclusion

In summary, body temperature and the application of TTM in critically ill patients is decidedly complex. The future will likely warrant a tailored temperature management approach in which we monitor the risk-benefits of a critically ill patient's body temperature in real-time. This thesis describes several aspects of the spontaneous hypothermic response to sepsis. It also examines the effects of TTM in hyperinflammatory states such as sepsis and cardiac arrest. Although this thesis does not definitively answer fundamental questions on the etiology of the body temperature alterations in the critically ill, it does provide important pieces of our understanding of spontaneous and induced body temperature alterations in critically ill and a foundation to further study these phenomena.

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Chapter eleven

Nederlandse samenvatting

Op de intensive care (IC) vinden bij ernstig zieke patiënten vaak veranderingen in lichaamstemperatuur plaats. Onder deze temperatuurveranderingen vallen zowel ondertemperatuur, ofwel hypothermie, en koorts. Deze veranderingen kunnen allerlei oorzaken hebben. Eén van de belangrijkste oorzaken van deze temperatuurveranderingen op de IC is sepsis, of bloedvergiftiging. Bij sepsis kan zowel koorts als hypothermie optreden. Interessant aan hypothermie tijdens sepsis is dat deze patiënten een zeer sombere prognose hebben, zonder dat we goed weten waarom. Verder wordt lichaamstemperatuur op de IC soms opzettelijk aangepast als onderdeel van de behandeling. Zo wordt momenteel onderzocht of koelen mogelijk een gunstige werking heeft bij sepsis. Bij sepsis is het immuunsysteem op hol geslagen en het experimentele koelen van patiënten heeft als doel deze extreme immuunreactie in te perken. Maar deze therapie bevindt zich nog in de experimentele fase; er is nog veel onduidelijk over de effecten van koelen tijdens sepsis.

Koelen is al wel onderdeel van de behandeling bij patiënten die opgenomen worden na een hartstilstand (in het Engels: cardiac arrest). Deze patiënten worden 24 uur lang gekoeld om verdere hersenschade te voorkomen die op kan treden als gevolg van zuurstoftekort in de hersenen tijdens cardiac arrest. Deze therapie wordt targeted temperature management (TTM) genoemd. Hoewel duidelijk is dat TTM verdere hersenschade voorkomt, kan nog veel verbeterd worden aan de behandeling met TTM en de ondersteunende therapieën rondom TTM. Door verder onderzoek naar TTM te doen, kan deze therapie in de toekomst doelgerichter en effectiever worden toegepast.

In dit proefschrift worden verschillende aspecten van spontane en geïnduceerde temperatuurverandering tijdens sepsis en cardiac arrest onderzocht. In het eerste deel van dit proefschrift onderzoeken wij mechanismen van de hypotherme respons in sepsis. In het tweede deel onderzoeken wij het effect van koelen op ontsteking en stolling in gezonde vrijwilligers met koorts en een ontstekingsreactie. In het derde deel van dit proefschrift onderzoeken wij verschillende aspecten van de behandeling met TTM in patiënten met een cardiac arrest.

DEEL I

In **hoofdstuk 2** hebben we internationaal artsen en verpleegkundigen gevraagd naar hun mening over definities, mechanismen en behandeling van spontane hypothermie tijdens sepsis. In deze studie vonden we dat de meningen over definities en behandeling van spontante hypothermie bij sepsis erg uiteenliepen. Wij hebben in deze studie gepleit voor een eenduidige definitie van hypothermie om de vergelijkbaarheid onder studies te verbeteren. Verder waren er verschillende meningen over de etiologie van de hypothermierespons tijdens sepsis. Wij denken dat hieraan ten grondslag ligt dat er weinig studies naar dit onderwerp zijn.

In **hoofdstuk 3** hebben we mogelijke risicofactoren voor het ontwikkelen van spontane hypothermie tijdens sepsis onderzocht. Verder hebben we mogelijke mechanismen van spontane hypothermie tijdens sepsis onderzocht met als doel te achterhalen waarom deze patiënten hypothermie ontwikkelen en een hogere mortaliteit hebben. In deze studie zijn patiënten met sepsis en hypothermie vergeleken met patiënten die geen hypothermie ontwikkelden tijdens sepsis. Het blijkt dat patiënten met een lage BMI en cardiovasculaire risicofactoren verhoogd kans hebben om hypothermie te ontwikkelen. Verder vonden we dat patiënten met hypothermie tijdens sepsis verhoogde markers van endotheel dysfunctie hadden.

Om een beter beeld te krijgen van mogelijke oorzaken van de hypothermie tijdens sepsis is in **hoofdstuk 4** de genexpressie van leukocyten bestudeerd bij patiënten met sepsis. Hiermee kunnen we gen-specifieke netwerken (in het Engels: pathways) in kaart brengen die mogelijk relevant zijn voor de hypotherme respons tijdens sepsis. Zo hoopten we nieuwe hypothesen te vormen over de mechanismen voor hypothermie en mortaliteit in deze patiëntenpopulatie. In deze studie waren een aantal pathways versterkt in hypotherme sepsis patiënten, o.a. één pathway die de afbraak van de aminozuur tryptofaan reguleert. Dit is relevant omdat stoffen die betrokken zijn bij de afbraak van tryptofaan zowel temperatuur kunnen beïnvloeden als het immuunsysteem. Met behulp van de resultaten uit deze studie kan verder onderzoek gedaan worden naar de hypothermie respons tijdens sepsis.

DEEL II

In **hoofdstuk 5** is in gezonde vrijwilligers met koorts en een gecontroleerde ontstekingsreactie gekeken naar de effecten van koelen op fysiologie en ontsteking. Om deze ontstekingsreactie te initiëren, gaven we vrijwilligers intraveneus lipopolysacharide (LPS). Dit is een onderdeel van de celwand van een gramnegatieve bacterie waar vrijwilligers kortdurend ziek van worden, koorts krijgen en in het bloed een stijging in ontstekingswaardenwaarden laten zien, een situatie die lijkt op hyperinflammatiore ziektebeelden als sepsis. In deze studie hebben we vrijwilligers in twee groepen opgedeeld. Zes vrijwilligers kregen alleen LPS. De andere zes vrijwilligers kregen LPS, en toen zij koorts begonnen te ontwikkelen gekoeld werden naar een normale lichaamstemperatuur (normothermie). Om het koelen van deze wakkere vrijwilligers te faciliteren was ook medicatie nodig om rillen tegen te gaan en de vrijwilligers comfortabel te houden.

In deze studie vonden we dat koelen de hartslag van vrijwilligers verlaagt, maar dat dit niet ten koste lijkt te gaan van de doorbloeding van weefsel. Koelen had echter weinig effect of de gemeten ontstekingswaarden. Dit was verrassend aangezien bij dierstudies koelen een duidelijke verlaging van ontstekingswaarden

geeft. Omdat er een grote spreiding was in de gemeten ontstekingswaarden en we kleine groepen vrijwilligers hadden, verklaart dit mogelijk het uitblijven van het effect van koelen op de ontstekingsparameters.

In **hoofdstuk 6** onderzochten we de effecten van koelen op de stolling en de activatie van endotheel (een belangrijk component van ontsteking en stolling in sepsis) in hetzelfde studiemodel als hoofdstuk 5. Koelen leek in deze studie endotheel activatie tegen te gaan. Twee markers van endotheel activatie normaliseerden als gevolg van koelen. Verder verminderde koelen de mate van diffuse intravasale stolling (DIS) een maat voor ernstige ontregelde stolling tijdens sepsis. Met de resultaten van deze studie kan in de toekomst koelen gerichter worden toegepast, bijvoorbeeld bij patiënten die ernstige stollingsstoornissen hebben of aanwijzingen voor endotheel activatie tijdens sepsis.

DEEL III

Het laatste deel van dit proefschrift richt zich op koelen (TTM) na een reanimatie. Naar aanleiding van een grote klinische studie (de TTM-trial) worden patiënten na cardiac arrest gekoeld tot temperaturen tussen 32°C en 36°C. Uit de TTM-trial bleek namelijk dat koelen naar 36°C even effectief was als koelen naar 33°C voor het verbeteren van neurologische uitkomst. Behandeling met TTM is echter complex en vereist standaardisering van verschillende onderdelen van behandeling rondom TTM, zodat de effecten van TTM optimaal benut kunnen worden. Wij hebben verschillende aspecten van TTM-behandeling onderzocht, met als doel de behandeling met TTM te optimaliseren.

In **hoofdstuk 7** gekeken hoe patiënten beademd worden tijdens behandeling met TTM. Het gros van deze patiënten werd longprotectief beademd, met lage teugvolumes en driving pressures. Dit is een belangrijke observatie omdat grote teugvolumes en grote driving pressures longschade kunnen geven. In deze studie vonden we verder aanwijzingen voor een nadelig effect op de gasuitwisseling in de longen bij koelen naar lagere temperaturen. Bij een lagere temperatuur is er mogelijk meer vasoconstrictie in het longvaatbed waardoor er minder gasuitwisseling is tussen het longweefsel en de bloedvaten die door kou samengeknepen zijn. Dit kan een ongewenst effect zijn van koelen naar lagere temperaturen, zeker in patiënten met risico op longschade.

In het verlengde van hoofdstuk 7 hebben we in **hoofdstuk 8** gekeken naar de effecten van het effect van koolstofdioxide (CO2) op neurologische uitkomst na cardiac arrest. Sommige studies suggereren dat te lage of te hoge CO2 waarden na een cardiac arrest neurologische uitkomst kunnen verslechteren. Fysiologisch is hier ook een goede verklaring voor; CO2 kan de bloeddoorstroming in de hersenen sterk beïnvloeden en te veel of te weinig bloedtoevoer in de hersenen

kan beschadigd hersenweefsel verder beschadigen. Wij vonden in onze studie echter geen aanwijzingen voor een relatie tussen neurologische uitkomst en CO2 waarden in het bloed.

Patiënten hebben na een cardiac arrest een hoog risico op infecties. In **hoofdstuk 9** hebben we in kaart gebracht welke micro-organismen infecties veroorzaken bij patiënten na een cardiac arrest. Door in kaart te brengen welke bacteriën vaak infecties veroorzaken bij deze patiënten, kunnen we onze empirische antibioticatherapie (behandeling met antibiotica voordat bekend is welke bacterie de infectie veroorzaakt) beter afstemmen. Hiernaast hebben we ook onderzocht of profylactische behandeling met antibiotica infecties voorkomt. Antibioticaprofylaxe was in onze analyse geassocieerd met minder infecties. Wij stellen daarom voor dat profylactische behandeling met antibiotica standaard moet worden bij patiënten na cardiac arrest.

In **hoofdstuk 10** volgt een samenvatting en met name verdere interpretatie van de resultaten en wordt gefilosofeerd over toekomstig onderzoek en de implicaties van dit proefschrift.

Appendices

List of publications

Curriculum Vitae

PhD portfolio

Acknowledgements

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British Journal of Anaesthesia. 2021.

Curriculum Vitae

Matthew Bruce André Harmon was born on the 11th of August 1986 in Washington D.C., United States of America (USA). He lived in Alexandria, Virginia for the first eight years of his life. When he was eight years old he moved with his family to The Hague in the Netherlands. In The Hague he attended high school at the "Vrijzinnig Christelijk Lyceum". He graduated in 2005. Matthew then attended Claremont Mckenna College in Los Angeles, California for one year. After that, he began his medical studies at the Vrije Universiteit Medical Center (VUmc) in Amsterdam in 2006.

He started his scientific career in his third year of medical studies, at the Department of Anatomy and Neurosciences at the VU looking at the Leucine-rich repeat kinase 2 (LRRK2) in pathology specimens from patients with Parkinson's Disease under supervision of dr. W.D.J. van de Berg. For his final research thesis of medical school, he examined anastomotic stricture formation after treatment of esophageal atresia at the Department of Pediatric Surgery at the VUmc together with dr. A.F.W. van der Steeg. After a clinical internship at the Department of Intensive Care at the VUmc, he developed a special interest in the field of anesthesiology and intensive care medicine.

To follow this interest in intensive care medicine, Matthew started his PhD studies at the Department of Intensive Care of the Academic Medical Center (AMC) in Amsterdam, with a thesis titled "Exploring body temperature alterations in the critically ill", under supervision of prof. dr. M.J. Schultz and prof dr. N.P. Juffermans. During this period, he participated in the Cooling and Surviving Septic Shock study, a collaboration between the Rigshospitalet in Copenhagen and the AMC in Amsterdam. In 2019, Matthew won the best abstract award at the "Nederlandse intensivisten dagen" in Rotterdam, the Netherlands, for his presentation on the effects of induced normothermia on coagulation in endotoxemia. In January of 2018 Matthew started his anesthesiology residency at the Leiden University Medical Center.

PhD portfolio

PhD student: M.B.A. Harmon
PhD period: January 2014 – December 2017
PhD supervisors: Prof. dr. N.P. Juffermans and Prof. dr. M.J. Schultz

	Year	ECTS
1. PhD training		
AMC Graduate School courses		
- Basic Couse Legislation and Organization for Clinical Researchers (BROK)	2014	0.9
- Practical biostatistics	2014	1.1
- Clinical Data Management	2014	0.3
- Computing in R	2015	0.4
- Advanced biostatistics	2015	2.1
- Clinical epidemiology: Randomized Controlled Trials	2015	1.0
- Clinical epidemiology: Observational Epidemiology	2015	1.0
- Laboratory Animal Science	2017	3.9
External courses, workshops & master classes		
- NVIC Cursus Echografie, Houten	2016	0.4
- TTM teaching course, Targeted Temperature Management, Berlin	2014	0.4
Presentations		
- Nederlandse Vereniging voor Intensive Care, Intensivistendagen, Rotterdam. "*Fever control reduces the severity of diffuse intravascular dissemination in human endotoxemia*." (Oral presentation)	2019	0.5
- European Society of Intensive Care Medicine, Paris. "*Blood genomic profile in hypothermic sepsis differs from febrile sepsis patients*." (Oral presentation)	2018	0.5
- European Society of Intensive Care Medicine, Paris. "*Fever control reduces the severity of diffuse intravascular dissemination in human endotoxemia*." (Poster discussion)	2018	0.5
- International symposium on Acute Pulmonary Injury Translational Research, Madrid. "*The effects of body temperature on ventilation parameters*." (Oral presentation)	2017	0.5
- International Symposium on Intensive Care and Emergency Medicine, Brussels. "*Host response and outcome of hypothermic sepsis*." (Poster discussion)	2016	0.5
Conferences		
- Nederlandse Vereniging voor Intensive Care, Intensivistendagen, Rotterdam	2019	0.25
- European Society of Intensive Care Medicine Congress, Paris	2018	1
- International Symposium on Acute Pulmonary Injury Translational Research, Madrid	2017	0.5
- International Symposium on Intensive Care and Emergency Medicine, Brussels	2016	1

Other activities			
-	Intensive Care Research Meeting (weekly)	2014-2017	12
-	Intensive Care Journal Club (monthly)	2014-2017	4
-	Laboratory of Experimental Intensive Care and Anesthesiology (LEICA) research meeting (weekly)	2014-2017	12
2. Teaching			
-	Eline Kho, student technische geneeskunde *"Temperature curve complexity and associated host immune response during sepsis"*	2016	1
-	Raymond van Wijk, student technische geneeskunde	2017	1
-	*"Characterization and application of temperature complexity on the Intensive Care"*		
-	Claudia van Zwienen, master student geneeksunde *"The effect of external surface cooling in human endotoxemia on the immune system"*	2017	1
-	Nanon Heijnen, master student geneeskunde *"The effect of external surface cooling on the coagulation in human volunteers with LPS-induced endotoxemia"*	2017-2018	1
3. Parameters of esteem			
-	NVIC Best Abstract Award: Fever control tijdens humane endotoxemie is geassocieerd met minder DIS en inhibitie van endotheelactivatie.	2019	

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