TO TREAT OR NOT TO TREAT

Biomarker-guided therapy for febrile patients in the **emergency department**

YURI VAN DER DOES

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Yuri van der Does

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GENERAL INTRODUCTION

CHAPTER 1

INTRODUCTION AND OUTLINE OF THESIS

Yuri van der Does MD

A revolution in medicine

In the early hours of the morning, on September 3, 1928, Alexander Fleming made a discovery that would change history. Fleming was a biologist and studied Staphylococcus bacteria. By accident, one of his bacterial cultures was contaminated with a fungus, while he was on holiday leave. Upon return, he noticed that the bacteria surrounding the fungus were destroyed. Fleming's famous words: "That's funny" heralded the discovery of the first antibiotic, penicillin1.

The effects of antibiotics were revolutionary, lethal infectious diseases were treatable for the first time[2,3]. Infectious diseases such as pneumonia, the world's leading cause of death in these days, could now be treated[3-5]. The enormous effects of antibiotics became apparent after the second world war, when the average life-expectancy rose as an effect of the decrease in mortality from bacterial infections[6]. The use of antibiotics has increased exponentially since their introduction[6,7]. Today, antibiotics are mostly used in hospitals and other healthcare settings for treatment and prophylaxis of infections, and in the livestock industry, to prevent and treat diseases in farm animals[8,9].

Resistance

In his Nobel prize speech in 1945, Alexander Fleming already warned that bacteria could become resistant to antibiotics[10]. Antibiotic resistance is a prime example of natural selection. Charles Darwin described this theory in his book "On the origin of species by means of natural selection", where he stated that organisms less suited to their environment die, and organisms with favorable traits survive and reproduce[11].

In treatment with antibiotics, most bacteria will die, but some bacteria are neither killed, nor affected in reproduction by the effects of antibiotics. These micro-organisms have acquired mechanisms of resistance to antibiotics, and can multiply at the expense of non-resistant micro-organisms because of selection pressure[7,12].

The use of broad spectrum antibiotics has increased in recent years. This widespread use of broad spectrum antibiotics is considered as one of the main causes of the increase of resistant microorganisms[12-14]. The consequences of antibiotic resistance are disastrous. The world health organization has declared antibiotic resistance as one of the biggest threats to global health today[15]. In Europe, estimated costs associated with antibiotic resistance are in the range of 1.5 billion euros annually[16]. Since the discovery of antibiotics, the problem of antibiotic resistance has grown so extensively, that scientists fear a so-called post-antibiotic era. In this scenario, we would see that antibiotics lose their effectiveness due to resistant bacteria. Consequently, a re-emergence of many infectious diseases that become untreatable would greatly increase global morbidity and mortality[17].

Antimicrobial stewardship

To counter the threat of antibiotic resistance, global initiatives were started to reduce the overuse of antibiotics in healthcare[18,19]. Antimicrobial stewardship is defined as coordinated interventions to optimize antimicrobial use among patients in order to improve patient-centered outcomes, ensure cost-effective therapy and reduce adverse sequelae of antimicrobial use. Antimicrobial stewardship strategies can be implemented throughout healthcare systems. These strategies include education on antibiotic resistance, optimal selection of antibiotics, optimizing regimens, dosage and duration of antibiotics prescriptions. Other strategies consist of clinical guidelines and decision-making aids for physicians, such as treatment algorithms[20-23].

The emergency department

Every day, numerous patients visit emergency departments (EDs) worldwide. Many of these patients have complaints and symptoms that may be caused by infectious diseases. Patients with suspected infections are considered a diagnostic dilemma for physicians in the ED[24,25]. These patients may have clinical signs of an infection, such as fever, coughing, or redness of the skin. However, initially, the etiology (e.g. a bacterial, viral or fungal origin, or a noninfectious cause) of the patients' complaints is often unclear. Physicians in the ED may have a clinical suspicion of the etiology, which is based on findings in patients' history and physical examination, specific laboratory investigations, such as leukocyte count, and focused image techniques, such as chest X-rays. Definitive determination of the pathogen can only be performed using techniques such as cultures and polymerase chain reaction analysis (PCR). The results of these techniques take several days to become available.

However, in the ED, there is a limited time window to start treatment. Patients usually stay in the ED for only a few hours. Therefore, the results of the time-consuming techniques of cultures and PCR are not available to physicians in the ED. Withholding antibiotics to patients with sepsis and septic shock increases mortality in these patient categories[26]. International guidelines of the surviving sepsis campaign recommend administering broad spectrum antibiotics to patients with suspected sepsis to reduce sepsis associated mortality[27]. Consequently, physicians start antibiotics on empiric grounds, without an accurate diagnosis of etiology. This practice results in wide administration of broad spectrum antibiotics in EDs, despite the knowledge of the effects of broad spectrum antibiotics on antibiotic resistance.

The core of the problem - to treat or not to treat - lies in discriminating patients who will benefit from antibiotics, (i.e. patients with bacterial infections) from patients who will not benefit (i.e. patients without bacterial infections).

Biomarkers

Biological markers, or biomarkers in short, are defined as characteristics that are objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention[28]. For example, in current practice, C-reactive protein (CRP) is one of the most commonly used biomarkers[29]. This is an acute phase protein, which is significantly increased in patients with bacterial infections, compared to patients who are not ill[30,31]. One of the most important advantages of CRP, compared to other diagnostic modalities, is that the results are usually available within one hour, so CRP results can be used in medical decision-making in the ED. However, the specificity of CRP is far from perfect, because CRP levels are not only increased in patients with bacterial infections, but in patients with viral infections and non-infectious diseases as well[29].

Procalcitonin

One of the more recent strategies for diagnosing bacterial infections, and consequently reducing the prescription antibiotics in the ED, is procalcitonin (PCT) guided therapy. PCT is a precursor protein of calcitonin. Calcitonin is a hormone which is involved in the calcium homeostasis. In healthy individuals, PCT levels are undetectably low. However, in patients with bacterial infections, blood concentration of PCT is greatly increased. In contrast, the calcitonin blood concentration only varies slightly. Therefore, PCT can be used as a biomarker for bacterial infections[32]. PCT has been studied in several selected populations, and these studies showed PCT to be a more accurate biomarker than CRP in differentiating between bacterial and non-bacterial disease[33.] Still, despite the existing evidence, the real value of PCT in the ED has not yet been determined.

Biomarkers for viral disease

Similar to biomarker strategies that rule-in bacterial infections, biomarkers can be used to rule-in viral diseases. Combinations of these strategies may improve accuracy of determining the etiology. Two candidate biomarkers for ruling-in viral disease are tumor necrosis factor(TNF)-related apoptosis-inducing ligand (TRAIL) and interferon-gamma induced protein-10 (IP-10), also known as C-X-C motif chemokine 10 (CXCL10). TRAIL is a member of the TNF family of cytokines and plays a role in apoptosis of various cell lines during activation of the immune system in response to viral infections[34,35]. IP-10 is a chemokine that is secreted in response to interferon-gamma in case of inflammation. IP-10 has several functions in activating both the innate and adaptive immune system. These roles include activating T1 lymphocytes and natural killer cells, identifying infected cells and regulating cell growth and apoptosis[36]. Blood concentrations of both biomarkers are significantly increased in patients with viral infections, compared to patients with bacterial and non-infectious disease[37.] A recent study by van Houten et al. showed promising results using

TRAIL and IP-10 in combination with CRP in diagnosing bacterial disease in young children with respiratory infections[38]. However, the value of this combined strategy in a general emergency department remains unclear.

Sepsis and severity of disease

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection[39]. Sepsis is no single disease, but a complex condition involving multiple systems, including the immune system, the coagulation system and the endothelium[40,41]. The surviving sepsis campaign guidelines advise to administer broad spectrum antibiotics to patients with sepsis. The rationale of this advice is that early administration of antibiotics can reduce morbidity and mortality, because the cause of sepsis is treated[26,27]. However, not all patients with an infection develop sepsis[41.] Therefore, hypothetically, only patients with sepsis should be treated immediately, and in patients without sepsis, treatment could be delayed until a specific diagnosis is made. If patients who are at risk of becoming critically ill due to sepsis can be identified in the ED, broad spectrum antibiotics may be reserved exclusively for this specific group, and could be withheld in the patients who are not at risk for adverse events of sepsis.

There are several biomarkers that are indicators of specific systems that are involved in sepsis. Mid-regional pro-adrenomedullin (proADM) is a prohormone of adrenomedullin, a peptide with inflammation induced vasodilatory effects[42,43]. Increased levels of proADM in patients with community acquired pneumonia (CAP) are associated with short-term adverse outcomes, such as intensive care unit (ICU) admission and mortality[44]. Pro-endothelin-1 (proET-1) is a precursor of the paracrine hormone endothelin, and has vasoconstrictive properties[45]. Increase in proET-1 is correlated with failure of microvascular homeostasis and organ failure in septic patients[46,47]. The physiologic role of soluble urokinase-type plasminogen activator receptor (suPAR) is unclear at present. However, increases in suPAR blood concentration levels are associated with activation of the immune system due to several stimuli, such as viral, bacterial and parasitic infections, and with malignancies[48]. In observational studies, suPAR predicted adverse outcomes such as readmission to hospital and mortality[49,50]. Although these biomarkers show potential, they are not routinely used in medical practice.

In summary, physicians in the ED need to make the critical decision whether to treat or not to treat patients with suspected infections, under diagnostic uncertainty. And both possibilities have potentially deleterious consequences. In order to make the optimal treatment decision, diagnostic uncertainty needs to be reduced as much as possible, using the principles of evidence based medicine[51]. To determine the likelihood of bacterial infections more accurately, we used a Bayesian approach, by adding the diagnostic values of new biomarkers to the diagnostic values of current standard tests[52].

Aims of this thesis

The overall aims of this thesis were to investigate if biomarkers can improve early identification of bacterial infections and provide early estimation of severity of disease, and if biomarkers can be used to effectively reduce the prescription of antibiotics for febrile patients without bacterial infections in the ED.

Outline of thesis

Part I PCT-guided therapy

Chapter 2 This part begins with an overview of all prospective interventional studies on PCT-guided therapy in the ED in a systematic review. Chapter 3 is a pilot study on PCT-guided therapy, where patients were randomized between standard care and PCT-guided therapy. Chapter 4 clarifies the goals of the HiTEMP study, a randomized clinical trial (RCT) on PCT-guided therapy, featuring the rationale of the study and a thorough description of the study design, methods and statistical analysis. Chapter 5 is the main study of this thesis, the HiTEMP study, a RCT on PCT-guided therapy, including an analysis of efficacy, safety, accuracy and cost-effectiveness.

Part II Additional biomarker strategies

Chapter 6 is a report of a pilot study on the biomarkers TRAIL and IP-10 in a selected patient cohort with patients with confirmed viral, bacterial and non-infectious diagnoses. In Chapter 7 TRAIL and IP-10 are investigated in combination with both CRP and PCT in a cohort of general ED patients. Chapter 8 focuses on severity of disease. We report on the value of single ED measurements of CRP, PCT and the newer biomarkers proADM, proET-1 and suPAR in predicting ICU admission and mortality.

Part III General discussion

Chapter 9 is the concluding part of this thesis. Here, we discuss the findings of this thesis, and describe plans and possibilities for future research.

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PART I
PROCALCITONIN-GUIDED THERAPY

CHAPTER 2

PROCALCITONIN-GUIDED THERAPY FOR THE INITIATION OF ANTIBIOTICS IN THE EMERGENCY DEPARTMENT: A SYSTEMATIC REVIEW

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ABSTRACT

Background

Procalcitonin (PCT) is a new biomarker with a higher accuracy in the diagnosis of bacterial infections. Utilization of PCT may reduce the number of unnecessary antibiotics prescribed to patients, and consequently may decrease the rise in antibiotic resistance.

The aim of this systematic review is to determine if a PCT-guided algorithm can safely reduce the number of antibiotics prescribed to all patients with a suspected of infection in the emergency department(ED).

Methods

MEDLINE, EMBASE, Web-of-science, COCHRANE central, PubMed publisher and Google scholar were searched. Two reviewers performed the screening independently. The QUADAS 2 tool was used to assess quality.

Results

In total, 1621 articles were screened. Nine articles were included in the analysis. In the six studies on adult patients, only patients with respiratory tract infections were investigated. In these studies, a cut-off value of 0.25mcg/L was used, and PCT-guided therapy reduced the number of prescribed antibiotics significantly. Three studies were on pediatric patients, two on fever without source, and one on respiratory complaints. PCT-guided therapy did not reduce antibiotic prescription in children. PCT-guided therapy did not result in an increase in adverse events in any of the studies.

Discussion

PCT-guided therapy in the ED is only studied in subpopulations, where it was effective and safe in adult patients with respiratory tract infections, and not effective but safe nonetheless in specific pediatric populations. Nonadherence is a significant problem in prospective PCT-guided therapy studies. There is not enough evidence to use PCT-guided therapy in a general ED population.

PROSPERO Systematic review registration: CRD42015023534

INTRODUCTION

In the emergency department (ED), immediate treatment of bacterial infections is vital. Delay of administration of antibiotics is associated with morbidity and mortality in patients with severe sepsis and septic shock[1]. On the other side, the use of antibiotics in the ED, without laboratory confirmation of the definitive diagnosis, may result in an overuse of antimicrobial therapy. Consequently, adverse drug events and healthcare costs may rise and antibiotic resistance may increase[2-4]. Antibiotic resistance is a growing global problem[2,3]. Governments worldwide promote the implementation of antimicrobial stewardship programs[5]. Antimicrobial stewardship programs encourage to initiate optimal antimicrobial treatment[6]. Ideally, patients without bacterial infections would not receive antibiotics. However, in the emergency situation, it is difficult to distinguish bacterial infections from viral infections and other febrile conditions.
When a febrile patient presents at the ED, the standard diagnostic approach - besides thorough medical history taking and physical examination - consists of laboratory tests such as C-reactive protein (CRP) and leukocyte count, and different image modalities. Cultures and polymerase chain reaction (PCR) technology can be obtained in the ED, but results are not directly available and therefore not useful for ED decision-making. Procalcitonin (PCT) can be used as a biomarker for bacterial infections. Elevated levels indicate the probable presence of bacterial infections. As levels of PCT rise within approximately six hours after the start of bacterial infection and remain relatively stable, its properties are suitable for ED-application[7,8]. Compared to CRP, PCT has been shown to be more accurate in different age groups, ranging from young children with fever without source(FWS)[9,10], to geriatric patients[11]. Also, for different sites of infection, such as respiratory tract and urogenital tract[12-14], and in multiple clinical settings, including primary care, intensive care units, and in the ED, PCT is more accurate[15-17]. Although the characteristics of PCT are promising, there may be other factors that can influence the initiation of antibiotic therapy. Prospective studies give more insight in these factors, because intention-to-treat analyses can be compared with per-protocol analyses. Also, safety can be addressed in prospective studies, because unwanted undertreatment and consequent adverse events can be quantified. PCT-guided therapy is defined as initiation of antibiotic treatment using PCT measurements, usually using a suggested treatment algorithm based on the height of the PCT measurement[15]. The overall clinical value of PCT-guided therapy in the general ED population of all ages and full spectrum of febrile complaints remains to be investigated.

The aim of this systematic review is to determine if a PCT-guided algorithm can safely reduce the number of antibiotics prescribed to all patients suspected of infection in the ED.

METHODS

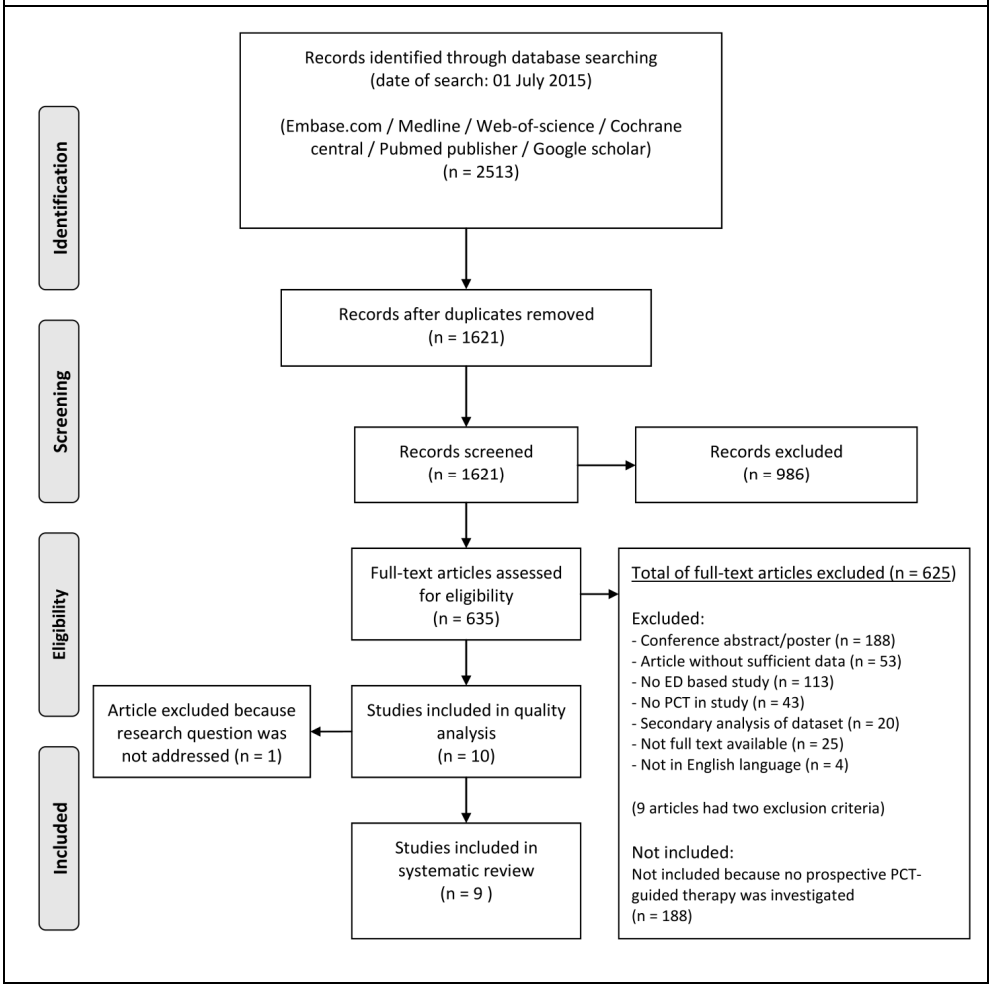
Study design

A systematic review of literature was performed according to the PRISMA guidelines[18]. The design of this systematic review is registered in the PROSPERO database[19], with registration number CRD42015023534 (http://www.crd.york.ac.uk/PROSPERO/). The primary outcome measure was the effectiveness of PCT-guided therapy in the ED, defined as reduction in the initiation of antibiotic therapy.

Search strategy

A comprehensive search, supported by a professional librarian of the Erasmus University Medical Center Rotterdam was performed. The MEDLINE, EMBASE, Web-of-science, COCHRANE central, PubMed publisher and Google scholar, containing all articles up to July 1st, 2015 were searched. The results were limited to the English language. Search terms are listed in Supplement 1. This review was restricted to articles that prospectively reported on an intervention of PCT-guided therapy in an ED setting. Outcome measures were: reduction of antibiotics (defined as number or percentage of antibiotics prescriptions), and safety of PCT-guided therapy (defined as hospital mortality, hospital or intensive care unit (ICU) admission and return visits to the ED). Studies that were not performed in the ED, i.e. in the ICU, medical or surgical wards, or primary care facilities were excluded. Furthermore, studies performed in specific departments such as burns units were excluded, as well as studies where there was no comparison between a PCT-guided therapy group and a control group of standard cares. There was no limit on age distribution or subpopulation of patients. Two authors (Y.D. and M.L.) screened titles and abstracts of the search results, and the full text of the selected articles. In case of disagreement a third reviewer (P.R.) acted as a referee. The QUADAS 2 tool[20] was used for assessing quality and bias in the selected full text studies. The QUADAS 2 tool is the recommended quality assessment tool by the Cochrane library. After positive quality assessment, data were extracted from the remaining articles as reported in supplement 2.

Figure 1. Flow diagram



RESULTS

Literature search

The search results are depicted in Figure 1. The search strategy identified 1621 individual studies. Of these studies, 635 were ED based studies that investigated PCT. A total of 198 studies investigated the accuracy of PCT on various outcomes in the ED; 188 studies did not use a prospective PCT-guided therapy algorithm and were therefore not included for further analysis. After full text screening, 10 articles remained that addressed PCT-guided therapy in a prospective setting. The overall quality of the studies was assessed using the QUADAS 2 guidelines[20].

Quality assessment

The quality assessment is described in Supplement 3, and summarized in Table 1. Although the study of Drozdov et al.[21] was eligible for inclusion in the review based on the selection criteria, it was excluded in the quality assessment. Drozdov et al.[21] did not address the initiation of antibiotics, but instead reported on a PCT-guided stopping algorithm for patients who already received antibiotic treatment for a urinary tract infection. This was not in line with the review question, and therefore the results of this study were not applicable. Stolz et al.[22] excluded patients with another explanation of dyspnea than an acute exacerbation of chronic obstructive pulmonary disease (AECOPD) and patients with psychiatric comorbidity from the study population. The exclusion of a selected part of the total population resulted in a high risk of population selection and possible effect exaggeration, because patients with medical comorbidities were excluded, and patients with possible lower therapy adherence may have been excluded. There was an unclear risk of bias in patient selection in six studies. Lacroix et al.10 used temperature ≥38.0°C as an inclusion criterion. Patients were also included if parents had measured a temperature of ≥38.0°C at home. Lacroix et al.10 reported a low adherence to the combined Lab-score, a prediction model containing a PCT value. No reasons for nonadherence were reported. This raised concerns on the applicability of the results of this study. Furthermore, the authors reported a missed inclusion rate of 75%, but gave no description of individual reasons. This may have resulted in a selection bias. Three studies[9,13,23] used an envelope as randomization method. This method is associated with an increased risk of selection bias, because allocation concealment can be deciphered by holding envelopes against a lightsource[24]. Christ-Crain et al.13 excluded 47 of a total of 597 eligible patients because of “other reasons”. Long et al.[25] excluded 115 patients without specifying the reason of exclusion. The index test description had an unclear risk of bias in two studies. Baer et al.[26] did not report a final diagnosis of the febrile episode. It is not possible to check if the antibiotics were indicated retrospectively. Manzano et al.[9] did not give an antibiotic treatment advice. A concrete cut-off value for PCT with a treatment suggestion could have influenced the results of this study.

Main study results

The selected articles are shown in Table 2. Nine randomized controlled trials met the selection criteria listed in figure 1 and the QUADAS 2 criteria in Table 1. These studies consisted of two multicenter trials [17, 26] and seven single center studies[9,10,13,14,22,23,25]. Six studies[10,13,14,17,22,26] were conducted in Switzerland, five of these[13,14,17,22,26] in the university hospital of Basel. The remaining studies were performed in China[23,25] and Canada[9].

Table 1. QUADAS 2

First author, year, country	Risk of bias				Concerns on applicability		
	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD	FLOW AND TIMING	PATIENT SELECTION	INDEX TEST	REFERENCE STANDARD
Baer 2013, Switzerland	Low	Unclear	Unclear	Low	Low	Unclear	Low
Christ-Crain 2004, Switzerland	Unclear	Low	Low	Low	Low	Low	Low
Christ-Crain 2006, Switzerland	Unclear	Low	Low	Low	Low	Low	Low
Drozdov 2015, Switzerland	Unclear	Low	Low	Low	High	High	Low
Lacroix 2014, Switzerland	Unclear	Unclear	Low	High	Low	Unclear	Low
Long 2011, China	Unclear	Low	Low	Low	Low	Low	Low
Manzano 2010, Canada	Unclear	Unclear	Low	Low	Unclear	Low	Low
Schuetz 2009, Switzerland	Low	Low	Low	Low	Low	Low	Low
Stolz 2007, Switzerland	High	Low	Low	High	Low	Low	Low
Tang 2013, China	Unclear	Low	Low	Low	Low	Low	Low

Study populations

Sample sizes of the studies varied widely, ranging between 15623 and 135917 patients, with six studies[9,10,13,14,22,26] having sample sizes between 200 and 400 patients. Three studies reported on pediatric patient[s9,10,26], of which two[9,10] reported on newborns and infants with FWS, and one on pediatric patients with respiratory tract infections[26].

Six studies reported on adult patients with subcategories of respiratory complaints: community acquired pneumonia[14,25], acute lower respiratory tract infections[13,17], AECOPD22 and exacerbation of asthma23. The age of patients ranged from newborn children between seven days and three months of age[10] to septuagenarians[17]. The majority of the participants were males (>50% men in six[10,13,14,17,25,26] out of the nine studies). One study[9] did not report gender. None of the studies reported ethnicity.

Selection criteria studies

Inclusion criteria of the studies on lower respiratory tract infections[13,14,17,25,26] included body temperature of ≥38°C (100.4° F), combined with at least one symptom of infection, i.e. cough, sputum production or dyspnea, and one clinical sign, i.e. abnormal breath sounds or leukocytosis. The criterion for suspected community acquired pneumonia was an infiltrate on a chest X-ray. Inclusion criteria on asthma and COPD were based on reaction to beta-2-agonist use[22,23]. One study used temperature measured at home as inclusion criterion[26]. Two pediatric studies on FWS[9,10] included a measured body temperature of ≥38°C, without the presence of a suspected cause of fever after history and physical examination[10], and the need for blood and urinary analysis[9]. Eight studies[9,10,13,14,17,22,25,26] reported immunosuppression as an exclusion criterion. This criterion was not uniformly defined. Some studies gave examples of specific conditions, i.e. HIV infection with low CD4+ count[13,26], neutropenic patients[13,26], active tuberculosis[13,14,25] and cystic fibrosis1[3,14,22,25,26]. Four studies[9,10,23,25] excluded patients with current antibiotics use or within 14 days of ED presentation. Schuetz et al.[17] excluded intravenous drug users. Stolz et al.[22] excluded 'vulnerable patients': patients with psychiatric diagnoses, which were not defined.

PCT cut-off values

Seven studies[13,14,17,22,23,25,26] used a cut-off of 0.25 mcg/L to suggest or encourage the initiation of antibiotics. Two pediatric studies did not use a continuous cut-off scale. Lacroix et al.[10] used PCT as part of the Lab-score, a decision rule that combined a semi-quantitative PCT result with a semi-quantitative CRP value and urinary dipstick outcome. The Lab-score is a severity index scale, and the outcome had no directly suggested treatment consequences. Manzano et al.[9] studied PCT prospectively without treatment algorithm, only the PCT result was available, without treatment advice.

Overruling of PCT-guided therapy protocol and nonadherence

Two studies[17,26] described predefined criteria for overruling PCT-guided therapy. These criteria comprised of life threatening illness, defined as respiratory or hemodynamic instability. Schuetz et al.[17] also included a positive screening test for Legionella pneumophilia as criterion. Four[10,13,14,17] studies reported physician nonadherence, ranging from 6% to 20%. In the studies with predefined criteria, Schuetz[17] reported that 9% of the patients were excluded without meeting the nonadherence criteria. Baer et al.[26] only mentioned predefined criteria, and did not report the number of physician nonadherence, nor protocol violations.

Antibiotics reduction

PCT-guided therapy resulted in a significant reduction of antibiotics in all studies in adult patients[13,14,17,22,23,25] (Table 3). In the three pediatric studies, no significant reduction in antibiotics was noted[9,10,26]. No study reported an increase in initiation of antibiotics. All results were from the intention-to-treat analyses.

Patient related outcomes

Five studies[13,14,17,22,23] reported mortality and ICU admission. Death rates varied between 3%13 to 13%14. ICU admittance or mechanical ventilation was required for 5%13 to 14%14 of patients. No studies reported a significant difference between groups for death rates or ICU admission. Seven studies[9,10,13,14,17,22,26] reported hospital admissions. The admission rate ranged from 26%[9] to 97%[14.] No study found a significant difference between hospital admissions. Five studies[13,14,17,22,26] reported length of hospital stay; none found significant differences between groups. One study[25] included patients who were sent home from the ED, and one study[23] did not report a hospital admission number.

Table 2: Study characteristics

First author, year, country	Study population *	Age distribution in years	Gender distribution (male)	Inclusion criteria	Exclusion criteria	PCT cut-off value used	Overruling of algorithm
Baer 2013, Switzerland	337 patients	PCT group: median [IQR] 2.7 [1.1 - 5.2]. Control group: 2.9 [1.2 - 5.7].	PCT group: 98 (58%). Control group: 98 (58%).	Pediatric patients, age 1 month - 18 years, presenting with LRTI, defined as T ≥38°C (measured at home or hospital), with one symptom (cough, sputum production, pleuritic pain, poor feeding) and one clinical sign (tachypnea, dyspnea, wheezing, late inspiratory crackles, bronchial breathing, pleural rub).	Unwillingness or unable to provide written informed consent by patients and care-takers, severe immune suppression (HIV with <15% CD4 count, immunosuppressive treatment, neutropenia (<1000 x 10^9/L)), cystic fibrosis, acute croup, hospital stay within previous 14 days, other severe infection.	Antibiotics definitely (> 0.5 mcg/L), probably (0.26–0.5 mcg/L), probably not (0.1–0.25 mcg/L), and definitely not (0.1 mcg/L).	In patients with life threatening infections, defined as severe co-morbidity, emerging ICU need during initial follow-up, or hemodynamic or respiratory instability.
Christ-Crain 2004, Switzerland	243 patients	PCT group (mean ± SD): 62.8 ± 19.8. Control group: 65.3 ± 17.3 .	PCT group: 67 (54%). Control group 61 (51%).	Adult patients with suspected LRTI, defined as community acquired pneumonia, COPD, asthma, acute bronchitis.	Severely immunocompromised patients i.e. HIV infection with CD4 count <200cells/mL, neutropenic patients, stemcell transplant recipients, cystic fibrosis, active tuberculosis, hospital acquired pneumonia on ED presentation.	Antibiotics discouraged (0.1-0.25mcg/L), suggested (0.25-0.5mcg/L), strongly recommended (>0.5mcg/L).	Not reported.
Christ-Crain 2006, Switzerland	302 patients	PCT group (mean ± SD): 70 ± 17. Control group: 70 ± 17.	PCT group: 94 (62%). Control group 93 (62%).	Adult patients with suspected CAP, defined as infiltrate on chest X-ray, and one of more symptoms or signs: cough, sputum production, dyspnea, temp ≥38.0°C and up, abnormal breath sounds, rales on auscultation, leukocytosis 10 x 10^9/L and up, or less than 4 x 10^9/L.	Cystic fibrosis, active pulmonary tuberculosis, hospital acquired pneumonia on ED presentation., severely immunocompromised patients (not defined).	Antibiotics strongly discouraged (<0.1 mcg/L), discouraged (0.1-0.25mcg/L), encouraged (0.25-0.5mcg/L), strongly encouraged (>0.5mcg/L).	Not reported.
Lacroix 2014, Switzerland	271 patients*	Age in months. PCT group (median [IQR]) 4.8 [1.7 - 10.4]. Control group 3.4 [1.5 - 10.4].	PCT group: 65 (50%). Control group 71 (51%).	Pediatric patients between 7 days and 3 years old with fever without source (FWS): Temperature ≥38°C, with no identified source of infection after thorough history and physical examination. Parental informed consent.	Congenital or acquired immunodeficiency syndromes, antibiotic administration <48h of presentation, fever >7 days.	Lab score: PCT <0.5ng/mL (0), 0.5 - 1.99 ng/mL (2), ≥2 ng/mL (4). In combination with CRP value based score, and Urinary dipstick based score. A Lab score of ≥3 was used as marker for severe bacterial illness.	No algorithm of antibiotic treatment advice reported. Therefore, overruling is also not reported.
Long 2011, China	162 patients*	PCT group (mean ± SD): 44 ± 16. Control group: 47 ± 19.	PCT group: 46 (60%). Control group 49 (62%).	Adult patients with suspected CAP, defined as infiltrate on chest X-ray, and one of more symptoms or signs: fever, cough, purulent sputum, focal chest signs, dyspnea or pleuritic pain. Outpatient treatment.	Pregnancy, commencement of antibiotic therapy >48h before enrolment, systemic immune deficiency, withholding life-support and active tuberculosis.	Antibiotics strongly discouraged (<0.1 mcg/L), discouraged (0.1-0.25mcg/L), encouraged (0.25-0.5mcg/L), strongly encouraged (>0.5mcg/L).	Not reported.
Manzano 2010, Canada	384 patients*	Age in months. PCT group(mean ± SD): 12 ± 8. Control group: 12 ± 8.	Not reported	Pediatric patients, age between 1 and 36 months, a history of rectal temperature ≥38.0°C, no identified source of infection, indication for blood and urine analysis.	Acquired or congenital immunodeficiency, current antibiotics use.	For intention-to-treat analysis no cut-off defined. Semiquantitative test: <0.5ng/mL, 0.5 ng/mL or higher, 2 ng/mL or higher, 10ng/mL or higher. Per protocol analysis with prophylactic antibiotics with PCT level >0.5ng/mL or higher.	No algorithm of antibiotic treatment advice reported. Therefore, overruling is also not reported.
Schuetz 2009, Switzerland	1359 patients*	PCT group: median [IQR] 74 [59-82]. Control group: 72 [59-82].	PCT group: 402 (60%). Control group 380 (55%).	Adult patients with suspected LRTI, defined as at least one respiratory symptom (cough, sputum, dyspnea, tachypnea, pleuritic pain), plus either rales or crepitation on auscultation, or temperature >38.0°C, shivering, leukocytosis.	Inability to give informed consent, active intravenous drug use, severe immunosuppression other than corticosteroid use, life-threatening comorbidity, community acquired pneumonia.	Antibiotics strongly discouraged (<0.1 mcg/L), discouraged (0.1-0.25mcg/L), encouraged (0.25-0.5mcg/L), strongly encouraged (>0.5mcg/L).	Patients in need of ICU admission, respiratory or hemodynamic instability, positive Ag test for Legionella pneumophila, or after consulting with the study center.
Stolz 2007, Switzerland	208 patients*	PCT group: median [IQR] 69.5 [65-77]. Control group: 69.5 [64.8-79].	PCT group: 50 (49%). Control group 44 (42%).	Adult patients with exacerbation of COPD, who met post bronchodilator therapy spirometric criteria according to GOLD guidelines, within 48h of ED admission.	Patients with other explanations for presenting symptoms other than COPD, and "vulnerable patients" ie patients with psychiatric diagnoses (not specified). Immunosupression, asthma, cystic fibrosism presence of infiltrates on chest X-ray on hospital admission.	Antibiotics strongly discouraged (<0.1 mcg/L), discouraged (0.1-0.25mcg/L), encouraged (0.25-0.5mcg/L), strongly encouraged (>0.5mcg/L).	Not reported.
Tang 2013, China	156 patients*	PCT group (mean ± SD): 54 ± 14. Control group: 55 ± 15.	PCT group: 64 (50%). Control group 59 (47%).	Adult patients with suspected exacerbation of asthma, with any or all GINA asthma guidelines criteria: dyspnea, wheeze, acute cough, increased work of breathing, increased beta2 agonist use, O2 saturation <95%, peakflow<80% of known best.	Treatment with antibiotics within two weeks of recruitment, non-respiratory bacterial infection, chest X-ray confirmed pneumonia, other chronic respiratory disease, severe organ dysfunction.	Antibiotics strongly discouraged (<0.1 mcg/L), discouraged (0.1-0.25mcg/L), encouraged (0.25-0.5mcg/L), strongly encouraged (>0.5mcg/L).	Not reported.

* Studies reported both a number of randomised and number of analyzed patients. Number of analyzed patients is reported. For number of ommited patients in analysis, see table 1. CAP: Community acquired pneumonia. COPD: Chronic obstructive pulmonary disease. ED: Emergency department. GINA: Global initiative for asthma. GOLD: Global initiative for chronic obstructive lung disease. HIV: Human immunodeficiency virus. ICU: Intensive care unit. IQR: Inter quartile range. LRTI: Lower respiratory tract infection. PCT: Procalcitonin. SD: Standard deviation.

Table 3: Study outcomes

First author, year, country	Antibiotics reduction	Hospital admission	Length of hospital stay	ICU admission	ICU length of stay	Return visits to ED	Mortality	Combined safety endpoint	Physician non-adherence with PCT advice
Baer 2013, Switzerland	PCT group 104 (62%), control group 93 (56%). Reduction 6% (95%CI -5% - 16%) in all patients. 28% (95%CI 12% - 43%) in non-CAP patients, -8% (95%CI - 19% - 4%) in CAP patients.	PCT group 104 (62%) Control group 100 (60%) Reduction 2% (95%CI -8% - 12%).	PCT group (days, median,[IQR]) 2.6 (2 [0-4]) Control group 2.7 (2 [0-5]) Reduction: -0.1 (95%CI -0.8 - 0.5).	Not reported separately.	Not reported	Not reported.	None reported.	Defined as hospital readmission, ICU admission, complications or death, complications of LRTI, disease specific failure: PCT group 38 (23%) Control group 33(20%) Reduction 2% (95%CI -5 - 11%)	Not reported.
Christ-Crain 2004, Switzerland	Antibiotics in 154 (63%) patients. PCT group 55 (44%). Control group 99 (83%). P<0.001.	PCT group 101 (81%). Control group 88 (74%). P=0.16.	PCT group (days, mean ± SD) 13.7 ± 7.3. Control group 10.8 ± 7.0. P=0.25.	PCT group 6 (5%). Control group 5 (4%). P=0.71.	Not reported	Not reported.	PCT group 4 (3%). Control group 4 (3%). P=0.95	Not reported.	In 9 patients (7%) antibiotics when PCT was <0.1. In 13 patients (10%) antibiotics when PCT was <0.25. After re-evaluation after 6-24h: of a total of 29 patients in PCT group, 10 received antibiotics, 5 because of elevated PCT levels, 5 because of physician decision.
Christ-Crain 2006, Switzerland	PCT group: 128 (85%). Control group 149 (99%). P<0.001. HR 3.2 (95%CI 2.5 - 4.2).	PCT group 146 (97%). Control group 146 (97%). P=1.0.	PCT group (days, mean ± SD) 12.0 ± 9.1. Control group 13.0 ± 9.0. P=0.35.	PCT group 20 (13%). Control group 21 (14%). P=0.87.	Not reported	Treatment failure (after 6 weeks): 51 patients (17%) PCT group 24 (16%). Control group 27 (18%). P=0.65.	PCT group 18 (12%). Control group 20 (13%). P=0.73.	Not reported.	In 1 patient (0%) antibiotics when PCT was <0.1. (endstage pulmonary fibrosis). In 19 patients (6%) antibiotics when PCT was <0.25. (6 severe COPD, 2 endstage pulmonary fibrosis, 11 other severe comorbidities).
Lacroix 2014, Switzerland	PCT group 54 (41%), control group 42 (42%). P = 1.000.	PCT group 44 (34%). Control group 50 (36%). P = 0.810.	Not reported.	Not reported.	Not reported	Not reported.	Not reported.	Not reported.	In 14 (11%) cases, patients received antibiotics despite the low Lab score. No patients with high Lab scores were witheld antibiotics.
Long 2011, China	PCT group: 69 (85%). Control group 79 (98%). P=0.004. HR 3.2 (95%CI 2.5 - 4.2).	None.	Not applicable.	Not applicable.	Not applicable	Treatment failure (after 4 weeks): 21 patients (13%) PCT group 12 (15%). Control group 9 (11%). No significant difference.	None reported.	Not reported.	Not reported.
Manzano 2010, Canada	PCT group: 48 (25%). Control group: 54 (28%). Risk difference -3 (95%CI -2 to 6).	PCT group: 50 (26%). Control group: 48 (25%). Risk difference 1 (95%CI -8 - 10).	Not reported.	Not reported.	Not reported	Not reported	Not reported.	Not reported.	No antibiotic treatment advice was given.
Schuetz 2009, Switzerland	PCT group 506 (75%), control group 603 (88%). Relative rate difference -12.2 (95%CI -16.3 to -8.1).	PCT group: 628 (93%). Control group: 629 (91%).	PCT group in days: mean (median [IQR]) 9.4 (8 [4-12]) Control group 9.2 (8 [4-12]) Reduction: 1.8 (95%CI -6.9 - 11.0).	PCT group 43 (6%). Control group 60 (9%). Risk difference -2.3 (95%CI -5.2 to 0.4).	Not reported	Recurrence of LRTI/rehospitalisation PCT group 25 (4%). Control group 45 (7%). Risk difference -2.8 (95%CI -5.1 to -0.4).	PCT group 34 (5%). Control group 33 (5%). Risk difference 0.3 (95%CI -2.1 to 2.5).	Death, ICU admission, recurrence of LRTI/rehospitalisation <30 days. PCT group 103 (15%). Control group 130 (19%). Risk difference -3.5 (95%CI -7.6 to 0.4)	In 132 (20%) patients, the PCT algorithm was overruled, of which 62 (9%) were in violation of predefined protocol.
Stolz 2007, Switzerland	PCT group 41 (40%), control group 76 (72%). P <0.0001.	Hospital admission 24h or longer. PCT group: 80 (78%). Control group: 82 (77%). P = 0.852.	PCT group in days: (median [IQR]) 9 [1-15]. Control group 10 [1-15]. P = 0.960.	PCT group 8 (8%). Control group 11 (10%). P = 0.526.	PCT group in days: (mean ± SD) 3.3 ± 2.7. Control group 3.7 ± 2.1. P = 0.351.	Recurrence of ECOPD within 6 months: PCT group 44 (43%). Control group 43 (40%). P = 0.607.	Any cause mortality within 6 months: PCT group 5 (5%). Control group 9 (9%). P = 0.409.	Not reported.	Not reported.
Tang 2013, China	PCT group 59 (46%), control group 95 (75%). P <0.01.	Not reported.	Not reported.	Mechanical ventilation treatment: PCT group 8 (6%). Control group 9 (7%). P = 0.821.	Not reported	Secondary ED visit within 6 weeks. PCT group 8 (6%). Control group 13 (10%) p < 0.05.	PCT group 1. Control group 2. Excluded from further analysis.	Not reported.	Not reported.

CAP: Community acquired pneumonia. CI: Confidence interval. COPD: Chronic obstructive pulmonary disease. ECOPD: Exacerbation of chronic obstructive pulmonary disease. HR: Hazard ratio. ICU: Intensive care unit. IQR: Inter quartile range. LRTI: Lower respiratory tract infection. PCT: Procalcitonin. SD: Standard deviation.

DISCUSSION

Findings

The results of our study show that PCT-guided therapy is only studied prospectively in distinct ED patient populations, adults with respiratory complaints[13,14,17,22,23,25], and in young infants with respiratory complaints[26] and FWS[9,10]. In the studies on adult patients with respiratory complaints, PCT-guided therapy reduced antibiotic prescriptions. In the pediatric subgroups, there was no reduction. In all included studies, there was no undertreatment and there was no increase in adverse events in the intervention group. This suggests that PCT-guided therapy is safe in the patients of these distinct ED populations. PCT-guided therapy has been shown to reduce antibiotic prescriptions in adult patients with respiratory complaints in various clinical settings. In primary care, reduction of antibiotics was 72%[16]. One hospital based study on patients with lower respiratory tract infections did not find a significant reduction in antibiotic prescriptions, due to a reported protocol nonadherence of 41%. The per-protocol did result in a 25% reduction of antibiotics based on a single PCT value27. PCT studies in the ICU mainly focus on stopping antibiotics instead of starting. Several ICU studies show a reduction in duration of antibiotic treatment using PCT-guided therapy[15,28,29.] The most interesting finding was that nonadherence to PCT-guided algorithms was present in several included studies. Lacroix et al.[10] reported that the use of a PCT-guided algorithm, included in the Lab-score, did not result in a reduction in antibiotics in practice. However, per-protocol analysis showed that the algorithm would result in reduction, had it been followed. This illustrates the point that physicians do not always follow the advice of a PCT-guided therapy. This is confirmed by the nonadherence to the PCT-guided therapy algorithms several of the other included studies[10,13,14,17.] Nonadherence is only visible in prospective studies; because, in contrary to observational studies, randomized controlled trials report an intention-to-treat analysis, which includes the physician factor in the results. Prospective PCT-guided therapy studies in other clinical settings also show nonadherence. Briel et al.[16] reported a nonadherence rate of 15% in primary care. Kristoffersen et al.[27] reported a 41% nonadherence in a hospital based setting. In the ICU setting, he PRORATA trial[15] had a protocol nonadherence for stopping antibiotic therapy based on a PCT-guided algorithm of 53%, a recent ICU study reported a 56% nonadherence rate when physicians were asked to stop antibiotics within 24 hours after initiation[29]. PCT-guided therapy is accompanied by protocol nonadherence, and this finding is consistent in multiple clinical settings. We speculate that individual clinical experience is the cause of the lower reduction of antibiotics in intention-to-treat results. This may be caused by the lack of understanding of the factors that influence PCT levels[30]. The results of this systematic review cannot be extrapolated to a general ED population, because the studies included in this systematic review focused on specific subpopu-

lations of patients with respiratory complaints. The study aim was to investigate the value of PCT-guided therapy for all patients in the ED. For this reason, we did not limit the results on specific populations, but included all ED studies from young children to elderly patients. However, our search results only yielded specific subpopulations. We can conclude that PCT-guided therapy is not studied in a wide enough population to use PCT as a standard biomarker for bacterial infections in the ED. The overall quality assessment indicated a low risk on bias in the selected articles. The study by Drozdov et al.[21] did not give information on antibiotic initiation and was therefore excluded. Two studies had a high risk on bias. Stolz et al.[22] excluded patients with possible other explanations for dyspnea than acute exacerbation of COPD (AECOPD). Also, patients with psychiatric comorbidities were excluded. This may have resulted in an exaggeration of the effect of PCT-guided therapy, because merely a part of the total population of patients with AECOPD was analyzed. In the study by Lacroix et al.[10], patient selection issues were noted as well. These studies were included, because the studies both used a PCT-guided algorithm and investigated reduction of antibiotics, and therefore give insight in the effectiveness of PCT-guided therapy in the ED. Because of the high risk of selection bias in these studies, the results cannot be generalized to either the general population of adult ED patients with AECOPD, or to the general population of pediatric ED patients with FWS.

Limitations

It was not possible to pool the data of the nine included studies, because we found insufficient studies with comparable study populations. A pooling of the results of PCT-guided therapy in adults may have resulted in a reduction of antibiotics in adult patients, and to no effect in pediatric patients. However, because of the highly selective populations of the selected studies, these outcomes would not have had added value. This review was performed in 2015. There are several studies being performed at the time of writing, which study PCT-guided therapy, for instance the NeoPInS trial[31]. These results are not available at this moment, but may further clarify the value of PCT-guided therapy. The review is primarily intended for emergency physicians. Therefore, only investigated ED based studies were included. The ED is a unique clinical setting, which has specific problems such as the diagnostic uncertainty at a time when emergency treatment has to be initiated. Hence, the choice for this setting reduces the generalizability of the results to other settings. Five studies[10,13,14,17,23,25] reported on funding. Investigators of three of these studies received funding from the manufacturer of the PCT assay[10,13,17]. The authors of three studies[13,14,17] reported receiving payments for speaking engagements, lecture fees and consultancy work for the manufacturer of the PCT assay. Conflicts of interest might raise concern in the appreciation of the results[32].

CONCLUSION

PCT-guided therapy is a valuable strategy in antimicrobial stewardship, and can theoretically reduce the number of unnecessary antibiotics prescribed to ED patients. However, protocol nonadherence is a significant problem in the prospective PCT-guided therapy studies. In adult patients with suspected respiratory infections, PCT-guided therapy may reduce antibiotic prescriptions, without increasing adverse events. However, physician judgment is still crucial and cannot be replaced by biomarkers in these patient populations based on the available evidence. In pediatric patients, PCT-guided therapy was ineffective, because nonadherence to the PCT-guided algorithm reverses the theoretical reduction in antibiotics. PCT-guided therapy can only become standard therapy in the ED when it is validated in a representative sample. Also, additional evidence on the physiologic properties of PCT may result in more confidence in PCT-guided algorithms.

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Supplement 1. Search terms per database

Embase.com

(procalcitonin/exp OR (procalcitonin* OR (calcitonin* NEAR/3 (precursor* OR prohormone*)) OR (pct NEAR/3 (value* OR level* OR marker* OR biomarker* OR blood* OR sample* OR collect*))):ab,ti) AND ('emergency treatment'/exp OR emergency/exp OR 'emergency nursing'/exp OR 'emergency health service'/exp OR 'emergency ward'/exp OR 'emergency medicine'/exp OR traumatology/exp OR 'multiple trauma'/exp OR (emergenc* OR 'acute care' OR trauma* OR resuscitat* OR ed)) AND ([english]/lim)

Medline (OvidSP)

((Calcitonin/ AND Protein Precursors/) OR (procalcitonin* OR (calcitonin* ADJ3 (precursor* OR prohormone*)) OR (pct ADJ3 (value* OR level* OR marker* OR biomarker* OR blood* OR sample* OR collect*))).ab,ti.) AND (Emergencies/ OR exp Emergency Treatment/ OR emergency nursing/ OR exp Emergency Service, Hospital/ OR exp Emergency Medical Services/ OR exp emergency medicine/ OR traumatology/ OR multiple trauma/ OR (emergenc* OR acute care OR trauma* OR resuscitat* OR ed)) AND (english).la.

Cochrane central

((procalcitonin* OR (calcitonin* NEAR/3 (precursor* OR prohormone*)) OR (pct NEAR/3 (value* OR level* OR marker* OR biomarker* OR blood* OR sample* OR collect*))):ab,ti) AND ((emergenc* OR 'acute care' OR trauma* OR resuscitat* OR ed))

Web-of-science

TS=(((procalcitonin* OR (calcitonin* NEAR/3 (precursor* OR prohormone*)) OR (pct NEAR/3 (value* OR level* OR marker* OR biomarker* OR blood* OR sample* OR collect*)))) AND ((emergenc* OR "acute care" OR trauma* OR resuscitat* OR ed))) AND LA=(english)

PubMed as supplied by publisher

((Calcitonin[mh] AND Protein Precursors[mh]) OR (procalcitonin*[tiab] OR (calcitonin*[tiab] AND (precursor*[tiab] OR prohormone*[tiab])) OR (pct AND (value*[tiab] OR level*[tiab] OR marker*[tiab] OR biomarker*[tiab] OR blood*[tiab] OR sample*[tiab] OR collect*[tiab])))) AND (Emergencies[mh] OR Emergency Treatment[mh] OR emergency nursing[mh] OR Emergency Service, Hospital[mh] OR Emergency Medical Services[mh] OR emergency medicine[mh] OR traumatology[mh] OR multiple trauma[mh] OR (emergenc*[tiab] OR acute care OR trauma*[tiab] OR resuscitat*[tiab] OR ed[tiab])) AND english[la] AND publisher[sb]

Google scholar

Procalcitonin|"pct value|level|sample" emergency|traumatology|trauma|"acute care"

Supplement 2. Data extraction
First author, Year, Country, Journal, Hospital type, Single center/multicenter/ international multicenter, Study design, age distribution, gender distribution, ethnicity, inclusion criteria, exclusion criteria, PCT cut-off value used, overruling of PCT-algorithm, antibiotics reduction, hospital admission, length of hospital stay, ICU admission, ICU length of stay, return visits to ED, mortality, physician non-adherence with PCT advice.

Supplement 3: QUADAS 2 quality assessment **Review question**

Author	Year	Acronym	Title	Journal	Patients, index test, reference standard and target condition	Describe methods of patient selection:	Consecutive or random sample of patients enrolled? (off white = yes, black = no, gray = unclear)
G. Baer, P. Baumann, M. Buettcher, U. Heininger, G. Berthet, J. Schafer, H. C. Bucher, D. Trachsel, J. Schneider, M. Gambon, D. Reppucci, J. M. Bonhoeffer, J. Stahelin-Massik, P. Schuetz, B. Mueller, G. Szinnai, U. B. Schaad and J. Bonhoeffer	2013	ProPAED	Procalcitonin Guidance to Reduce Antibiotic Treatment of Lower Respiratory Tract Infection in Children and Adolescents (ProPAED): A Randomized Controlled Trial	PLoS ONE	Pediatric patients with LRTIs, PCT-guided therapy vs physician assessment and clinical guidelines, antibiotic prescribing rate, duration of antibiotics.	All patients between 1 month and 18 years of age with LRTIs were inlcuded. LRTI was defined with clinical criteria. Missed inclusions were registered.	
M. Christ-Crain, D. Jaccard-Stolz, R. Bingisser, M. M. Gencay, P. R. Huber, M. Tamm and B. Müller	2004		Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial	Lancet	Adult patients with suspected respiratory infections, PCT-guided therapy vs physician assessment, antibiotic prescribing rate.	All adult patients, presenting with dyspnoea, cough or both were eligible. Subpopulations based on clinical signs and symptoms.	
M. Christ-Crain, D. Stolz and R. Bingisser	2006	ProCAP	Procalcitonin guidance of antibiotic therapy in community-acquired pneumonia: a randomized trial	Am J Respir Crit Care Med	Adult ED patients with CAP, PCT-guided therapy vs physician assessment, antibiotic initiation and duration.	Adult patients with CAP admitted in the ED. Criteria were new infiltrate on chest X-ray, and presence of one or several acute respiratory signs and symptoms	
Drozdov, D. Schwarz, S. Kutz, A. Grolimund, E. Rast, A. C. Steiner, D. Regez, K. Schild, U. Guglielmetti, M. Conca, A. Reutlinger, B. Ottiger, C. Buchkremer, F. Haubitz, S. Blum, C. Huber, A. Buergi, U. Schuetz, P. Bock, A. Fux, C. A. Mueller, B. Albrich, W. C.	2015		Procalcitonin and pyuria-based algorithm reduces antibiotic use in urinary tract infections: A randomized controlled trial	BMC Med	Immunocompetent adults with UTI, PCT-pyuria treatment vs standard care, overall antibiotics exposure.	ED patients with non-catheter UTI	
Lacroix, L. Manzano, S. Vandertuin, L. Hugon, F. Galetto-Lacour, A. Gervaix, A.	2014		Impact of the lab-score on antibiotic prescription rate in children with fever without source: A randomized controlled trial	PLoS ONE	ED patients between 7 days and 3 years of age with fever, Lab score vs standard care, antibiotic prescription rate.	Parents of young children visiting tertiary ED were asked to participate.	A total of 1190 patients were assessed for eligibility, however, in 883 patients, participation was not offered. Reasons were not described
W. Long, X. Deng, Y. Zhang, G. Lu, J. Xie and J. Tang	2011		Procalcitonin guidance for reduction of antibiotic use in low-risk outpatients with community-acquired pneumonia	Respirology	ED patients between 18 and 65 years of age, with CAP that were discharged from ED, PCT-guided therapy vs physician assesment, antibiotic use and duration.	ED patients with CAP with PSI of I-III were eligible	
S. Manzano, B. Bailey, J. B. Girodias, A. Galetto-Lacour, J. Cousineau and E. Delvin	2010		Impact of procalcitonin on the management of children aged 1 to 36 months presenting with fever without source: A randomized controlled trial	Am J Emerg Med	Patients between 1 and 36 mnths of age visiting the ED with fever without source, PCT-guided vs standard care, antibiotics initiation.	Parents of patients with FWS were asked for participation when inclusion criteria were met. Randomisation usiong envelopes.	
P. Schuetz, M. Christ-Crain, R. Thomann, C. Falconnier, M. Wolbers, I. Widmer, S. Neidert, T. Fricker, C. Blum, U. Schild, K. Regez, R. Schoenenberger, C. Henzen, T. Bregenzer, C. Hoess, M. Krause, H. C. Bucher, W. Zimmerli and B. Mueller	2009	ProHOSP	Effect of procalcitonin-based guidelines vs standard guidelines on antibiotic use in lower respiratory tract infections: The ProHOSP randomized controlled trial	J Am Med Assoc	Adult ED patients with suspected LRTI, PCT-guided therapy vs standard care, composite safety endpoint, antibiotic exposure and duration, length of hospital stay.	ED patients with 1 respiratory symptom and 1 symptom of infection or auscultatory symptom.	
D. Stolz, M. Christ-Grain, R. Bingisser, J. Leuppi, D. Miedinger, C. Muller, P. Huber, B. Muller and M. Tamm	2007		Antibiotic treatment of exacerbations of COPD: A randomized, controlled trial comparing procalcitonin-guidance with standard therapy	Chest	ECOOPD patients of 40 years and older, PCT-guided therapy vs standard care, total antibiotics use at ED visit and 6 months after	ED patients with COPD who had specific post-bronchodilatator spirometry criteria.	
J. Tang, W. Long, L. Yan, Y. Zhang, J. Xie, G. Lu and C. Yang	2013		Procalcitonin guided antibiotic therapy of acute exacerbations of asthma: A randomized controlled trial	BMC Infect Dis	Adult asthma patients with exacerbation, PCT-guided therapy vs standard care, antibiotics use.	ED patients with criteria for asthma exacerbations.	

DOMAIN 2: Index test(s)

A. Risk of bias		B. Concerns regarding applicability		
If a threshold (cut-off value) was used, was it pre-specified? (off white = yes, black = no, gray = unclear)	**Could the conduct or interpretation of the index test have introduced bias? (RISK: off white = low, black = high, gray = unclear)**	**Is there concern that the index test, its conduct, or interpretation differ from the review question? (RISK: off white = low, black = high, gray = unclear)**	Describe the reference standard and how it was conducted and interpreted:	Is the reference standard likely to correctly classify the target condition? (off white = yes, black = no, gray = unclear)
	It is unclear what the final diagnosis of the febrile episode is. The choice of antibiotics in both the intervention and control group is based on ED assessment, but is not checked with the microbial etiology. Therefore the true indication of antibiotics cannot be verified.	A comparison between PCT per-protocol and intention-to-treat analysis would provide insight in PCT protocol adherence. Only an intention-to-treat analysis is provided, so non-adherence cannot be evaluated.	Reference standard was physician assessment in the ED, based on clinical guidelines.	
			Reference standard was physician assessment in the ED, and microbial laboratory investigations for final diagnosis.	
			Reference standard was physician assessment in the ED, based on clinical guidelines.	
		The review question is PCT guided therapy in the initiation of antibiotics in the ED. Because all patients received antibiotics initially, this study does not answer the primary review question.	Reference standard was physician assessment in the ED, based on clinical guidelines.	
		The study reports low adherence to Lab score recommendations. No reasons for nonadherence were reported.	Reference standard was physician assessment in the ED, based on clinical guidelines.	
			Reference standard was physician assessment in the ED, based on clinical guidelines.	
	The index test had a three-point-scale, either low, moderate or high risk of bacaterial infection. No antibiotic advice was given. Advice on antibiotics may have altered the results of antibiotic prescription or hospital admittance.		Reference standard was physician assessment in the ED, based on clinical guidelines.	
			Reference standard was physician assessment in the ED, based on clinical guidelines.	
			Reference standard was physician assessment in the ED, based on clinical guidelines.	
			Reference standard was physician assessment in the ED, based on clinical guidelines.	

DOMAIN 1: Patient selection

A. Risk of bias				B. Concerns regarding applicability		
Was a case-control design avoided? (off white = yes, black = no, gray = unclear)	Did the study avoid inappropriate exclusions (off white = yes, black = no, gray = unclear)	**Could the selection of patients have introduced bias? (RISK: off white = low, black = high, gray = unclear)**	Describe included patients (prior testing, presentation, intended use of index test and setting):	**Concern that included patients do not match review question? (RISK: off white = low, black = high, gray = unclear)**	Describe the index test and how it was conducted and interpreted:	*Were the index test results interpreted without knowledge of the reference standard? (off white = yes, black = no, gray = unclear)*
			Included pediatric patients all had fever (38.0 and up), with one additional symptom (tachypnea, dyspnoea, wheezing, late inspiratory crackles, bronchial breathing, pleural rub), and were without severe immunesuppression (HIC with low cd4+, immunesuppressive treatment, neutropenia, CF, acute croup, hospital stay within 14 days, or other severe infection. the index test determined if they would receive antibiotics.		PCT testing was performed in the lab using a Kryptor device. A previously described algorithm was used for interpretation, with predefined protocol violations, i.e. severe co-morbidity, ICU need, hemodynamic or respiratory instability	*Not applicable, the reference standard is physician assessment*
		47 patients were excluded because of "other" reasons, not specified, of the total of 597 eligible patients.	Included patients had cough and/or dyspnoea, and were without specific exclusioncriteria, chf, "other pulmonary disease, malignancy, immunocompromised status, or other types of infection. The index test determines if they will receive antibiotics.		PCT testing was performed in the lab using a Kryptor device. A previously described algorithm was used for interpretation, protocol violations were allowed on physician discretion without predefined criteria.	*Not applicable, the reference standard is physician assessment*
		Randomisation by envelopes, a method prone for randomisation bias.	Patients with suspected CAP present in the ED, the index test determines if they will receive antibiotics.		PCT testing was performed in the lab using a Kryptor device. A previously described algorithm was used for interpretation, protocol violations were allowed on physician discretion without predefined criteria.	*Not applicable, the reference standard is physician assessment*
		Randomisation by envelopes, a method prone for randomisation bias.	Immunocompetent patients visiting the ED, were divided in admitted and outpatient groups, and in simple/complicated UTI groups, the PCT level was used for the duration of treatment.	Because all patients received antibiotics initially, this study does not answer the research question.	PCT testing was performed in the lab. A previously described algorithm was used for interpretation, protocol violations were allowed on physician discretion without predefined criteria.	*Not applicable, the reference standard is physician assessment*
		Temperatures taken at home were used as inclusion criteria, these may not be reliable because there is no standard measurement technique.	Patients between 7 days and 3 years of age, who had fever of 38.0, measured either at home or in the ED, with no identified source of infection after history and physical examination, were eligible, parents gave informed consent. The Lab score was used to reduce antibiotics prescription.		The index test comprised of a PCT level (measured using VIDAS Brahms assay), CRP level, and urinary dipstick. Specific results determine a 9-points score.	*Not applicable, the reference standard is physician assessment*
		115 were excluded, but the reason for exclusion is not specified.	CAP patients who were treated as outpatient were included after ED visit. The index test determined if they would receive antibiotics.		PCT testing was performed in the lab using a Kryptor device. A previously described algorithm was used for interpretation, protocol violations were allowed on physician discretion without predefined criteria.	*Not applicable, the reference standard is physician assessment*
		Randomisation by envelopes, a method prone for randomisation bias.	Patients visiting a tertiary pediatric ED, who presented with history of a rectal temperature and where the physician required additional laboratory investigation (defined as fever without source after history and physical examination).	Fever based on history may not as reliable as an objective measurement.	PCT testing was performed using semiquantative PCT-Q test.	*Not applicable, the reference standard is physician assessment*
			Patients with suspected LRTIs of unknown etiology, the index test is to discriminate bacterial from non-bacterial origin		PCT testing was performed in the lab using a Kryptor device. A previously described algorithm was used for interpretation, protocol violations were allowed based on predefined criteria.	*Not applicable, the reference standard is physician assessment*
	Patients in whom there was found an alternative explanation other than AECOPD were excluded, also patients with psychiatric comorbidities were excluded.	By excluding patients with another explanation, the effect of the intervention will be exagerated. Psychiatric comorbidity may be associated with increased nonadherence to therapy. By excluding these patients, the effect also may be exaggerated.	Patients, older than 39 years, with ECOPD with specific spirometric criteria, without any other explanation of complaints, without psychiatric comorbidity or immunosuppression, asthma, CF or infiltrates on chest X-rays on admission. The index test determined if patients would receive antibiotics.		PCT testing was performed in the lab using a Kryptor device. A previously described algorithm was used for interpretation, protocol violations were allowed on physician discretion without predefined criteria.	*Not applicable, the reference standard is physician assessment*
		Randomisation by envelopes, a method prone for randomisation bias.	Adult asthma patients, following international asthma guidelines, without bacterial infection in other parts of body, confirmed pneumonia on chest X ray, other chronic respiratory diseases, or suffering from severe organ disfunction. The index test determined if patients would receive antibiotics.		PCT testing was performed in the lab using a Kryptor device. A previously described algorithm was used for interpretation, protocol violations were allowed on physician discretion without predefined criteria.	*Not applicable, the reference standard is physician assessment*

Domain 3: Reference standard					DOMAIN 4: Flow and timing				
A. Risk of Bias		B. Concerns regarding applicability			A. Risk of Bias				
Were the reference standard results interpreted without knowledge of the results of the index test? (off white = yes, black = no, gray = unclear)	**Could the reference standard, its conduct, or its interpretation have introduced bias? (RISK: off white = low, black = high, gray = unclear)**	**Is there concern that the target condition as defined by the reference standard does not match the review question? (RISK: off white = low, black = high, gray = unclear)**	Describe any patients who did not receive the index test(s) and/or reference standard or who were excluded from the 2x2 table (refer to flow diagram):	Describe the time interval and any interventions between index test(s) and reference standard:	Was there an appropriate interval between index test(s) and reference standard? (off white = yes, black = no, gray = unclear)	Did all patients receive a reference standard? (off white = yes, black = no, gray = unclear)	Did patients receive the same reference standard? (off white = yes, black = no, gray = unclear)	Were all patients included in the analysis? (off white = yes, black = no, gray = unclear)	**Could the patient flow have introduced bias? (RISK: off white = low, black = high, gray = unclear)**
In the control group, the index test results were unknown.	Reference standard was physician assessment in determining initiation of antibiotics. The number of protocol violations in the PCT-guided group (low PCT, still antibiotics, based on physician assessment) are not described.		14 eligible patients who were excluded, were unable to give consent because of language problems with parents, 13 because of "other" reasons that were not specified.	tests were conducted simultaneously in the emergency department					
In the control group, the index test results were unknown.			Patients with chf, other pulmonary disease, hyperventilation, cerebral, URTI, allergy, thromboembolic event, carcinoma, immunocompromised, no-respiratory infection, and " others" were not deemed eligible	tests were conducted simultaneously in the emergency department					
In the control group, the index test results were unknown.			Excluded patients: did not meet CAP criteria, immunocompromised, TB, CF, HAP, not confirmed infiltrate by radiologist, died before randomisation, no informed consent	tests were conducted simultaneously in the emergency department					
In the control group, the index test results were unknown.			All included patients were analysed in the intention-to-treat analysis. They were excluded in the per-protocol analysis, the reasons of exclusion were described.	tests were conducted simultaneously in the emergency department					
In the control group, the index test results were unknown.			This study has not included more than 75% of eligible patients, because of either lack of time for logistics, and physician unwillingness to proceed in diagnostic tests in well appearing patients.	tests were conducted simultaneously in the emergency department					More than 75% of missed inclusions, without reporting of individual reasons.
In the control group, the index test results were unknown.			ED CAP patients who were not eligible for outpatient treatment were excluded, this is not specified	tests were conducted simultaneously in the emergency department					
In the control group, the index test results were unknown.			Patients whose parents did not consent were excluded. Also, in case of insufficient blood sampling, patients were exlcuded.	tests were conducted simultaneously in the emergency department					
In the control group, the indext test results were unknown.			237 patients were not eligible after screening, 12 eligible patients were screened because of "other" reasons, not specified	tests were conducted simultaneously in the emergency department					
In the control group, the index test results were unknown.			62 patients were excluded for analysis, 7 psychiatric patients, 7 with other not specified comorbidities, 3 with chest pain	tests were conducted simultaneously in the emergency department					There is a selection bias due to exclusion criteria.
In the control group, the index test results were unknown.			22 patients were excluded on exclusion criteria, not specified, 2 died before inclusion, 4 did not give informed consent	tests were conducted simultaneously in the emergency department					

PART I
PROCALCITONIN-GUIDED THERAPY

CHAPTER 3

PROCALCITONIN-GUIDED ANTIBIOTIC THERAPY IN PATIENTS PRESENTING WITH FEVER IN THE EMERGENCY DEPARTMENT

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ABSTRACT

Introduction
In the emergency department (ED), patients with fever often receive antibiotics, because physicians have difficulties in determining the etiology of fever. Procalcitonin is a novel biomarker for bacterial infections. We investigated if PCT-guided antibiotic therapy reduced the antibiotic prescription rate in febrile emergency department (ED) patients.

Methods
Undifferentiated febrile ED patients were randomized to either PCT-guided therapy or standard-of-care. In the PCT-guided group, a single PCT measurement was added to standard laboratory results. In the PCT-guided therapy group, antibiotic treatment was recommended when PCT was ≥0.5µg/L.
The primary outcome was number of patients who received antibiotics in the ED.

Results
107 Patients were included. Fewer antibiotics were prescribed in the PCT-guided therapy group (80% vs 92% (p= 0.08)). Differences were observed in ICU admission (14(24%) vs 4 (8%) (p=0.03)); mortality (0 (0%) vs 2 (4%) (p=0.12)); temperature (median 38.8 (IQR 38.2 – 39.2) vs median 39.0 (IQR 38.7 – 39.5)) (p=0.03)) and CRP level (mean 138 mg/L (SD 120) vs 179 mg/L (SD 146) (p=0.02)) between PCT-guided therapy and standard-of-care. Mean length of hospital stay was 8 days in both groups. In multivariate regression analysis, PCT-guided therapy resulted in a trend towards reduction of the proportion of patients who received antibiotics (OR 0.47 (95%CI 0.13 – 1.66)).

Discussion
Although no statistically significant reduction in number of patients who received was found, the findings in this study suggest that PCT-guided therapy may reduce antibiotics prescriptions in febrile patients from an undifferentiated adult ED population. PCT-guided therapy may be an important tool in antimicrobial stewardship. Larger trials are needed to validate the value of PCT in the ED.

INTRODUCTION

Antibiotics are the mainstay of treatment of bacterial disease. With the increasing use of antibiotics, resistance of microorganisms is on the rise[1]. The surviving sepsis campaign states that, when patients have a suspected infection with systemic inflammatory response syndrome (SIRS), broad-spectrum antibiotics have to be administered within one hour[2]. This increases the rate of antibiotic prescriptions in the emergency department (ED)[3,4], and may also contribute to further resistance for antibiotics. Antimicrobial stewardship stands for targeted and effective antibacterial therapy, with special attention for the initiation and timely ending of antibiotics use. The goal of antimicrobial stewardship is to contain the increasing resistance of microorganisms[5,6]. Procalcitonin (PCT) is a novel biomarker, which has a higher sensitivity and specificity in the diagnosis of bacterial infection compared to the current standard diagnostic tests available in the ED[7-10]. PCT is a precursor protein of calcitonin. Unlike calcitonin, which is only produced in the C-cells of the thyroid gland, procalcitonin can be produced ubiquitously throughout the human body. The production of PCT is upregulated by pro-inflammatory cytokines like interleukin -1 (IL-1), IL-2, IL-6 and tumor necrosis factor alpha, and directly by bacterial endotoxins and lipopolysaccharide. Interferon gamma, a cytokine associated with viral infections, reduces the upregulation of PCT. Furthermore, an increase in PCT levels can be monitored within 4 to 6 hours after start of infection[11-13]. CRP is an acute phase protein, synthesized exclusively in the liver. CRP levels increase during inflammatory states, but are not specific for bacterial infections and take more time, 6 to 48 hours after start of infection, to be detectable compared to PCT[12,14]. These characteristics give PCT a theoretical advantage over CRP. Previously, we showed that the addition of PCT to the current diagnostic workup of patients presenting with fever helps to discriminate between infectious and non-infectious causes[15].
Moreover, recent primary care and ED based interventional PCT-guided therapy studies reported a reduction in antibiotics use in patients suspected of lower respiratory tract infections[9,16,17]. However, since these studies were limited to specific patient populations, the results are not generalizable to febrile patients without a strong suspicion of a specific infection. Therefore, this study addresses all febrile ED patients, a heterogeneous and more challenging group, in order to determine the value of PCT-guided therapy in this emergency department clinical setting.

The objective of this study was to investigate if PCT-guided therapy reduced unnecessary antibiotics prescription in an undifferentiated febrile ED population.

METHODS

Design
Single center, randomised controlled trial of patients visiting the ED of the Slotervaart Hospital in Amsterdam, the Netherlands. The hospital board of medical ethics approved the study protocol. All patients gave written informed consent.

Setting
The Slotervaart hospital in Amsterdam is a general teaching hospital with 410 beds.

Population
All non-pregnant patients, between 18 and 85 years of age, who presented at the ED with fever and gave written informed consent, were eligible for inclusion.

Design
All eligible patients were asked to participate in the study. After inclusion, patients were randomized to either a PCT-guided therapy arm or a standard-of-care arm, using a computer program. In all patients, blood samples and two sets of blood cultures were obtained. Samples for bacterial and viral cultures and polymerase chain reaction (PCR) were taken from the suspected focus of infection, as judged by the treating physician. In both groups, PCT was determined. PCT results were only available to the physician in the ED in the PCT-guided therapy group. With all available laboratory results, physicians filled out a standard case report form on which was reported whether antibiotics were indicated and whether antibiotics were prescribed. The PCT treatment algorithm recommended withholding antibiotics when PCT was <0.5 µg/L, and recommended prescribing antibiotics with PCT levels ≥0.5 µg/L. This treatment algorithm was described Bouadma et al[18]. Physicians were allowed to prescribe antibiotics in case of low PCT levels if this was according to their clinical judgement. There were no predetermined criteria for disregarding the treatment algorithm.

Outcomes
The primary outcome was the number of patients who received antibiotics. Secondary outcomes were hospital and intensive care unit (ICU) admission and length of stay, and mortality. A definite diagnosis of all patients was reported retrospectively, both by an independent physician and by the primary investigator, blinded for PCT results, and was based on culture results and all diagnostic tests available.

Data analysis
We determined differences in variables between the standard-of-care group and the PCT-guided group using Chi-squared tests for dichotomous variables and Stu-

Table 1: Baseline characteristics and calculated differences of the study population				
	PCT guided	Standard-of-care	Total	p-value*
n	59	48	107	
Female sex, n (%)	20 (34%)	18 (38%)	38 (35%)	p = 0.70
Age, median year (SD)	60 (20)	60 (17)	603 (18)	p = 0.55
Temperature, median ˚C(IQR)	38.8 (38.2 – 39.2)	39.0 (38.7 – 39.5)	38.9 (38.4 – 39.3)	**p = 0.03**
ICU admission, n (%)	14 (24%)	4 (8%)	18 (17%)	**p = 0.03**
Mortality, n (%)	0 (0%)	2 (4%)	2 (2%)	p = 0.12
Hospitalization, n (%)	55 (93%)	42 (88%)	97 (91%)	p = 0.31
Diabetes Mellitus, n (%)	14 (24%)	9 (19%)	23 (22%)	p = 0.53
Immunocompromised, n (%)	6 (10%)	6 (13%)	12 (11%)	p = 0.70
Malignancy, n (%)	10 (17%)	6 (13%)	16 (15%)	p = 0.52
Length of stay, median days (IQR)	6 (3 – 9)	7 (3 – 9)	6 (3 – 9)	p = 0.76
PCT, median µg/L (SD)	0.33 (4.6)	0.39 (5.0)	0.38 (4.7)	p = 0.83
CRP, median mg/mL (SD)	108 (120)	165 (146)	127 (133)	**p = 0.02**
Antibiotics prescribed	47 (80%)	44 (92%)	91(85%)	**p = 0.08**
Antibiotics indicated	50 (85%)	40 (83%)	90 (84%)	p = 0.84
Bacterial infection confirmed	20 (34%)	18 (38%)	38 (36%)	p = 0.70
Abbrevations list: N: number. SD: standard deviation. IQR: Inter quartile range. ICU: intensive care unit. * Students' T-test for continuous variables, Chi-squared test for dichotomous variables.				

dent's T-tests for continuous variables. Variables age and gender, together with variables with significant differences between groups ($p<0.1$) were included in multivariate binominal logistic regression analysis.

RESULTS

Patients were included from May 2010 to May 2012. A total of 342 patients were eligible for inclusion; A total number of 107 patients were included and randomised, 14 patients were excluded because data were incomplete and irretrievable. Of the eligible patients, 221 were not included due to logistical problems.

Primary outcome

In the PCT-guided group, significantly fewer antibiotics were prescribed compared to the standard-of-care group (80% vs. 92%, respectively; $p=0.08$; 13% reduction). The groups did not differ by number of confirmed bacterial infections. A total of 15 patients (25%) in the PCT-guided group received antibiotics despite of their low PCT measurement (PCT <0.5 µg/L).

Secondary outcomes

There was no difference in length of hospital stay or mortality. Yet, significantly more patients in the PCT-guided group were admitted to the intensive care unit, (24% vs. 8%, respectively). Furthermore, the patients in the standard-of-care group had a measured temperature and CRP level that were significantly higher. Consequently, standard therapy was compared to PCT-guided therapy in multivariate logistic regression analysis. The determinants ICU admission, temperature and CRP level had a significant effect in univariate logistic regression and were corrected for in multivariate binomial logistic regression analysis. PCT-guided therapy reduced the rate of antibiotics prescribed, OR 0.47 (95%CI 0.13 – 1.66).

There were 73 (68%) patients with bacterial infections, 22 (21%) patients with viral infections, 1 (1%) patient with a parasitic infection, and 14 (13%) patients with a non-infectious cause of fever.

The types of infections were: respiratory infections in 49 (46%) patients, urinary tract infections in 19 (18%) patients, skin and soft tissue infections in 10 (9%) patients, bloodstream infections in 6 (6%) patients, digestive tract infections in 4 (4%) patients, meningo-encephalic infections in 2 (2%) patients, and other febrile disease, including thyrotoxicosis, malignant neuroleptic syndrome and polymyalgia rheumatic in 17 (15%) patients.

Table 2. Types of infection and most common identified pathogens

Infection	Total of patients (n, %)	Specified infection	(n)	Clinical diagnosis only (n)	Proven by culture (n)	Most common identified pathogens*	(n)
Pneumonia/respiratory infection	49 (46%)	Bacterial	35	22	13	Streptococcus sp. E. Coli M. Mycoplasma H. Influenzae	4 2 2 2
		Viral	14	2	12	Influenza A virus Human rhinovirus Para-influenza virus	5 5 2
Urinary tract infection	19 (18%)	Bacterial	19	13	6	E. Coli P. Aegurinosa K. pneumoniae	8 4 3
Bloodborne infection	6 (6%)	Bacterial	4	-	4	Streptococcus sp. S. Aureus P. Aegurinosa	2 1 1
		Viral	1	-	1	Dengue virus	1
		Parasitic	1	-	1	P. Falciparum	1
Gastro-intestinal infection	4 (4%)	Bacterial	1	-	1	E. Coli (ESBL), K. Pneumoniae (ESBL), P. Aegurinosa	1
		Viral	3	3	-	-	-
Skin and soft tissue infection	10 (9%)	Bacterial	9	6	3	S. Aureus Streptococcus sp. P. Aegurinosa	1 1 1
		Viral	1	-	1	Herpes Simplex	1
Viral respiratory infection with bacterial superinfection	3 (3%)	-	-	-	3	Influenza A virus, M. Pneumoniae Influenza A virus, S. Aureus Para-influenza virus, S. Pneumoniae	1 1 1
Meningo-encephalic infections	2 (2%)	Bacterial	2	1	1	Streptococcus sp. (meningitis) *(CT-confirmed cerebral abscess)*	1 1
No specific suspected infection	5 (5%)	-	-	-	-	-	-
Other	9 (9%)	-	-	-	-	Tumor fever Thyreotoxicosis Malignant neuroleptic syndrome Appendicitis Urea crystal arthritis (gout) Diverticulitis Polymyalgia rheumatica	3 1 1 1 1 1
Total* of infections	107 (100%)	Bacterial	73	42	31		
		Viral	22	5	17		
		Parasitic	1	-	1		
		Other	14				

*In some cultures, multiple microorganisms were present.

Abbrevations: E. Coli : Escherichia Coli, M.Mycoplasma: Mycobacterium Mycoplasma, H. Influenzae: Haemophilus Influenzae, P. Aegurinosa: Pseudomonas Aegurinosa, K.Pneumoniae: Klebshiella Pneumoniae, P. Falciparum: Plasmodium Falciparum, S. Aureus: Staphylococcus Aureus.

N: number. Sp: Species. ESBL: extend spectrum beta-lacatamase. CT: computer tomography.

DISCUSSION

In this randomized clinical trial, we showed that PCT-guided antibiotic therapy for undifferentiated febrile patients in the ED did not result in a significant reduction in prescription of antibiotics, but did show a trend towards reduction of the initiation of unnecessary antibiotic therapy. A recent review of literature[7] shows that the PCT intervention studies in primary care, ED and ICU settings use only subgroups of patients. These studies mainly focus on respiratory tract infections and sepsis. Our study is the first study that included an adult ED population with fever, irrelevant of suspected underlying pathology. Because no selection of patients was made, besides fever, the use of PCT-guided therapy may be expanded beyond patients with specific suspected pathology in the ED.

We demonstrated a trend towards reduction of the initiation of unnecessary antibiotic therapy. Reduction in number of antibiotic prescriptions has been reported for specific patient populations in the ED[17,19-24]. Also, antibiotic reduction in patients with suspected respiratory tract infections and fever has been reported in general practice when PCT-guided antibiotic therapy is used[9]. In the proHOSP study[16] the authors reported a significant reduction in the prescription of antibiotics in patients with lower respiratory tract infections. Furthermore, there were similar rates for adverse events in mortality and ICU admittance.

This is the first PCT-guided therapy study carried out in adult ED patients in the Netherlands, a country known for its restrictive antibiotics prescription policy[25,26]. Other ED based studies were performed in Switzerland[16,20,23], Denmark[24], China[22], and one international multicenter trial in Switzerland, France and the United States[17]. The lower rate of reduction of antibiotic prescription in the current study may be partly explained by the higher threshold of Dutch physicians to prescribe antibiotics.

In the ICU setting, PCT is used as a marker to discontinue antibiotic treatment[18,27,28]. In PCT-guided therapy trials based in the ICU, researchers had access to serial measurements of PCT. However, in this ED based study, a single measurement was used to either start or not to start antimicrobial therapy. Although this resulted in a lower rate of reduction of antibiotic use compared to ICU-based studies, it is nonetheless an interesting result, because PCT can change antibiotic policy in a real-life ED setting in a safe and timely manner.

The study population consisted mainly of patients with respiratory and urinary tract infections. This is in accordance with other ED-based studies, both in the Netherlands and internationally[10,29,30]. As the type of infection in febrile patients is not always clear to the ED physician, the use of fever as sole inclusion criterion reflects the real-life clinical situation.

In the PCT-guided therapy group, there were significantly more ICU admissions, and patients had a higher temperature. Because the patients were randomized, these differences were due to chance. However, this means that the PCT-guided therapy

group may have consisted of generally sicker patients. We performed a statistical correction for this difference; however, the differences between groups may have influenced the results. In a similar population, PCT-guided therapy could therefore reduce the proportion of antibiotic prescriptions even more.

Limitations

There were some limitations in this study. First of all, the sample size was small. A number of 221 patients were not included because of logistical problems in the ED. Patients were not included due to unfamiliarity of the physicians with the study, despite several hospital-wide information sessions. This was because all specialties in the Slotervaart hospital had their own ED consultants. Therefore, eligible patients were not always asked to participate. After inclusion, 14 patients had to be excluded because of incomplete data. In the PCT-guided therapy group, there was a 25% rate of antibiotic prescription with a low PCT level. Patients with a low PCT result were still prescribed antibiotics. This may be attributable to either the unfamiliarity of PCT as an accurate diagnostic marker, or a lack of confidence in the new diagnostic instrument[31].

CONCLUSION

Although no statistically significant reduction in number of patients who received antibiotics was found, the findings in this study suggest that PCT-guided therapy may reduce antibiotics prescriptions in febrile patients from an undifferentiated adult ED population. PCT-guided therapy may be an important tool in antimicrobial stewardship. Larger trials are needed to validate the value of PCT in the ED.

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PART I
PROCALCITONIN-GUIDED THERAPY

CHAPTER 4

HIGHER DIAGNOSTIC ACCURACY AND COST-EFFECTIVENESS USING PROCALCITONIN IN THE TREATMENT OF EMERGENCY MEDICINE PATIENTS WITH FEVER (THE HITEMP STUDY): A MULTICENTER RANDOMIZED STUDY

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ABSTRACT

Background
Fever is a common symptom in the emergency department(ED). Fever can be caused by bacterial infections, which are treated with antibiotics. Often, bacterial infections cannot be ruled out in the ED using standard diagnostics, and empiric antibiotic treatment is started. Procalcitonin(PCT) is a biomarker for bacterial infections, but its role in an undifferentiated ED population remains unclear. We hypothesize that PCT-guided therapy may reduce antibiotics prescription in undifferentiated febrile ED patients. The primary objectives of this study are to determine a) the efficacy, b) the safety of PCT-guided therapy, and c) the accuracy of the biomarker PCT for bacterial infections. The secondary objective is to study the cost-effectiveness of PCT-guided therapy.

Methods and design
This is a multicenter noninferiority randomized controlled trial. All adult ED patients with fever(≥ 38.2°C) are randomized between standard care with and without the addition of a PCT level, after written informed consent.
a) For efficacy, the reduction of patients receiving antibiotics is calculated, using a superiority analysis: differences between the PCT-guided group and control group are assessed using a Fisher's exact test, and a multivariable logistic regression analysis to account for the effects of demographic and medical variables on the percentage of febrile patients receiving antibiotics.
b) Safety consists of a composite endpoint, defined as mortality, intensive care admission and ED return visit within 14 days. Noninferiority of PCT will be tested using a one-sided 95%confidence interval for the difference in the composite safety endpoint between the PCT-guided and control groups using a noninferiority margin of 7.5%.
c)Accuracy of PCT and CRP for the diagnosis of bacterial infections will be reported, using the sensitivity, specificity, and the area under the receiver-operating-characteristic curve in the definitive diagnosis of bacterial infections.
The sample size is 550 patients, which was calculated using a power analysis for all primary objectives. Enrollment of patients started in august 2014 and will last two years.

Discussion
PCT may offer a more tailor-made treatment to the individual ED patient with fever. Prospective costs analyses will reveal the economic consequences of implementing PCT-guided therapy in the ED.

This trial is registered in the Dutch trial register: NTR4949.

BACKGROUND

Fever is one of the most common symptoms of patients visiting the emergency department (ED). The etiology of fever is diverse, ranging from infectious diseases to neoplasms and trauma[1]. Specific etiologies of fever, such as severe bacterial infections, have to be treated within one hour after ED presentation with adequate antibiotic therapy, according to the surviving sepsis guidelines[2].

Because time is of the essence in the initiation of therapy, physicians in the ED have a limited time window for diagnosing the etiology of fever. This results in a "better safe than sorry" approach, in which broad-spectrum antibiotics are administered to febrile patients, only based on history and physical examination, and readily available diagnostic entities.

On the other hand, antibiotic resistance is becoming an increasing problem worldwide. Antimicrobial stewardship advocates thoughtful initiation of antibiotic therapy. Thus, in treatment of bacterial infections, both under-treatment and overtreatment are undesirable. Therefore, it is vital to increase the accuracy of diagnostics of febrile illness.

The mainstay of diagnosing the etiology of fever in the ED consists of history, physical examination and laboratory analysis of serum and other bodily fluids, and chest X-ray examinations. Cultures and viral throat swabs are obtained, but are of no use in the ED, because results take several hours to days and treatment has to be started early after ED presentation[2]. Currently, leukocyte count, with or without leukocyte differentiation, and C-reactive protein (CRP) are the laboratory discriminators of choice in the initial approach in the diagnostic process of febrile diseases.

A higher accuracy of ruling in or ruling out bacterial infections using biomarkers may result in more accurate antimicrobial therapy. On the individual patient level, fewer patients would be treated empirically with antibiotics. Adverse events and drug interactions would be reduced. Also, a more accurate diagnosis could save hospital expenses and result in cost reductions. On a population level, antibiotics resistance could be countered. For patient safety, this should obviously be without an added risk of under-treatment.

Procalcitonin (PCT) is a promising biomarker for bacterial infections. PCT is a precursor protein of calcitonin. Unlike calcitonin, which is only produced in the C-cells of the thyroid gland, PCT can be produced ubiquitously throughout the human body. The production of PCT is upregulated by proinflammatory cytokines like interleukin -1 (IL-1), IL-2, IL-6 and tumor necrosis factor alpha, and directly by bacterial endotoxins and lipopolysaccharide. Interferon gamma, a cytokine associated with viral infections, reduces the upregulation of PCT. It has been shown that PCT levels in non-infectious febrile conditions, such as autoimmune diseases or fever caused by malignant disorders stay low, whereas CRP levels often rise significantly[3]. Furthermore, an increase in PCT levels can be monitored within 4 to 6 hours after start of

infection[4-6]. In comparison, CRP is an acute phase protein synthesized exclusively in the liver. CRP levels increase during inflammatory states, but are not specific for bacterial infections and take more time, 6 to 48 hours after start of infection, to be detectable compared to PCT[5,7]. These characteristics give PCT a theoretical advantage over CRP.
Recently, clinical studies have focused on PCT as a biomarker of bacterial infections, showing better diagnostic properties than commonly used markers such as CRP[3,8-14]. Studies have shown that PCT-guided antibiotic therapy- i.e. starting or withholding antibiotics, based on the PCT-level - is safe and reduces prescription of antibiotics in several distinct patient groups: savings of more than 23% in the intensive care unit (ICU) and up to 78% in the general practice setting[8,11,15]. However, evidence of the effectiveness of PCT-guided antibiotic therapy in an undifferentiated ED population is scarce.

The ED is the gateway of hospital healthcare. Patients enter with symptoms and leave with a diagnosis. Either they are admitted, or are sent home. For almost every diagnosis, there are numerous medical tests and interventions available that can be utilized in the ED. However, budgets are limited and should therefore be used in the most efficient manner possible. ED based cost-effectiveness studies are used to estimate the value for money offered by new technologies. These studies serve as a tool for hospital administrators and decision makers who are responsible for prioritizing interventions under economic constraints[16].

Estimated yearly costs associated with antibiotic resistance in Europe are in the range of 1.5 billion euros, comprising extra healthcare costs and productivity losses caused by infections due to antibiotic-resistant bacteria[17]. PCT-guided therapy may contribute to a reduction of these enormous costs. It may lead to a reduction in costs of antibiotics and additional diagnostics tests, such as blood cultures in non-bacterial disease. Costs of hospital admissions could be reduced as admission for observation may become obsolete. As an added effect, there may be a reduction of productivity losses, related to paid and unpaid work. Patients may be able to return to their daily activities faster.
The current evidence on the cost-effectiveness of PCT-guided therapy consists of retrospective hypothetical models. One study analyzed the cost-effectiveness of PCT testing in patients hospitalized for community-acquired pneumonia with a mathematical model[18]. The authors showed that a PCT-guided protocol would cost less and would be more effective than usual care[18]. Recently, a model-based study from the US assessed the economic impact of PCT testing in patients with acute respiratory tract infections. The authors documented that PCT-guided care was cost saving in inpatient, ICU and outpatient settings, mainly due to a reduction in antibiotic costs. Total yearly savings at the US national level were calculated at 1.6 billion

dollars[19].
These estimates only included costs of PCT testing and savings on costs of hospitalization, excluding savings from other care consumption and productivity losses, which cannot be estimated at this stage. There is a need to broaden the evidence base to promote the efficient use of PCT-guided therapy in patients with fever presenting at EDs.

The primary objectives of this study are to determine a) the efficacy and b) the safety of PCT-guided therapy, and c) the accuracy of the biomarker PCT for bacterial infections. The secondary objective is to study the cost-effectiveness of PCT-guided therapy.

METHODS AND DESIGN

The HiTEMP study is designed as a multicenter noninferiority randomized controlled trial on PCT-guided therapy. The study will be performed in both academic and non-academic teaching hospital settings. Patients will be asked for written informed consent. Patients who consent are randomized using an online computer program, to either the standard-of-care diagnostic workup of febrile patients (control group), or the standard-of-care workup with the addition of the diagnostic biomarker PCT.

Primary outcome measures

a) The efficacy of PCT-guided therapy is defined as the percentage of patients who are prescribed antibiotics in the ED. b) The safety outcome measure consists of a composite endpoint of 30 days mortality, intensive care unit (ICU) admission within 30 days, or a return visit to the ED within 14 days. c) The accuracy of the definitive diagnosis is reported for PCT and CRP using the sensitivity, specificity, and the AUC of the diagnosis of bacterial infections. The definitive diagnosis is defined in two ways. 1. As “confirmed bacterial infection”, in which culture – and PCR results that fit the clinical case presentation are used to report presence of bacterial infections. 2. As “suspected bacterial infection”, in which two independent physicians will give their expert opinion on the presence of a bacterial infection using culture results, image modalities and clinical course, in case of discrepancy, a third physician will decide.

Secondary outcome measures

Secondary outcome measures for cost-effectiveness are hospital treatment costs, related medical consumption during follow-up, costs of absenteeism and reduced productivity while at work.

The adherence to PCT advice is reported, including the percentage of antibiotic prescriptions with low PCT result, and withholding of antibiotics in patients with high PCT result. The percentage of patients who received antibiotics without suspected bacterial infection is reported. Outcome measures are reported for the total patient population, and are stratified by source of infection: respiratory tract infections, urinary tract infections, skin and soft tissue infections, central nervous system infections, abdominal infections, non-infectious fever, fever without source, and other causes of fever.

Inclusion and exclusion criteria

Inclusion criteria: All patients of 18 years and older visiting the ED, with a temperature of ≥ 38.20C (100.80F) are eligible. Temperature is measured using an ear thermometer, in triage. Eligible patients need to provide written informed consent.

Exclusion criteria: Patients with specific immunocompromised status, defined

as neutropenia with absolute neutrophil count less than 0.5x109/L, current chemotherapy, or post-organ transplantation are excluded. Furthermore, pregnant patients, moribund patients and patients with a diagnosis that requires primary surgical intervention, or within 72 hours after surgery are excluded. Patients are randomized using a minimization procedure[20]. The factors for minimization are imbalance in randomization result and study site. The randomization result is allocated at patient enrolment by a computer-generated algorithm.

Sample size calculation and statistical analyses

Sample size and power analysis: For the three primary objectives, the following analyses are used: a) a superiority analysis for the reduction in the percentage of febrile patients receiving antibiotics. b) a noninferiority analysis for safety, and c) an analysis of the test characteristics (sensitivity and specificity) of the biomarker PCT and CRP. The total sample size calculation is based on a power analysis for each of these three primary outcome measures; the total sample size is the maximum of the required sample sizes for the different primary outcome measures.

a) For the superiority analysis of the reduction in the prescription of antibiotics, we assumed that antibiotics are initially prescribed at the ED to 73% of the patients in the control arm, and that the intervention will reduce this percentage to 53%, based on a careful estimate using recent literature[14]. To obtain 80% power to detect a significant difference between the two groups using a two-sided Fisher's exact test with a significance level of 5%, the required sample size is 101 patients per group.

b) The noninferiority analysis for the safety objective uses a composite endpoint consisting of ICU admittance, 30-days mortality or second ED visit within 14 days. For an accurate sample size calculation, we first analyzed ED data from the Erasmus MC from January 2011 until May 2012. In this data set, the prevalence of the composite safety endpoint was 12.7% (102 cases out of 809 patients). We used this percentage as the expected rate in the control group. Using a noninferiority margin of 7.5%, a noninferiority analysis comparing the rates in the control and the intervention groups requires 244 patients in each group to obtain 80% power with a one-sided alpha of 5%. To account for 10% expected dropout, the total sample size must be 550 patients.

c) The third sample size calculation is based on a comparison of sensitivity and specificity of PCT and CRP in patients with infection. We used data from a meta-analysis[21,22] to estimate the accuracy of PCT and CRP for bacterial infection. We conservatively assumed zero correlation between the test results of PCT and CRP for the diagnosis of bacterial infection. For the prevalence of bacterial infections, we used data from our pilot study[3] to obtain a representative estimate of the prevalence of bacterial infections, which led to an estimated prevalence of 68%. Based on the results of the meta-analysis, the sensitivity of PCT was 0.83 and for CRP 0.73; the specificity was 0.88 for PCT and 0.60 for CRP. Using McNemar's test, 340

patients with a bacterial infection (340/0.68=500 patients in total) are required to obtain 80% power for detecting a difference in sensitivity between PCT and CRP. Forty-one patients without a bacterial infection (41/(1-0.68)=129 patients in total) are required to obtain 80% power for detecting a difference in specificity between PCT and CRP. To ensure sufficient power for all three primary outcome measures, we thus use a sample size of 275 patients per group (550 patients in total).

Statistical analyses for the primary study parameters

a) Antibiotics use. The percentage of patients that are prescribed antibiotics is compared between the PCT-guided group and control group using a Fisher's exact test. Demographic parameters (age, sex, mortality), medical parameters (medication use, comorbidity, temperature, CRP and PCT measurements), hospital admission related parameters (hospital admittance, hospital length of stay, ICU admittance, ICU length of stay) are compared between the PCT-guided group and control group using Fisher's exact tests for dichotomous variables, chi-square tests for categorical variables with more than two categories, t-tests for continuous variables that are normally distributed, and Kruskal-Wallis tests for continuous variables that are not normally distributed. A logistic regression analysis will be used to determine the influence of these parameters on the proportion of antibiotics prescriptions in the control group and the PCT-guided therapy group. To select the independent variables in this logistic regression model, we will use a stepwise backward approach in which only the independent variables are retained that have a significant effect using a significance level of 5%; however, the variable group (PCT-guided group versus control group) will be included in the model irrespective of the results. In the event of missing values in possible confounding data, like intoxications and prescription drugs use, we will use multiple imputation. Both an intention to treat analysis and a per protocol analysis will be performed. Physician adherence to the PCT guidance will be determined.

b) Safety. Significant differences between the PCT-guided group and control group in the composite safety endpoint are determined using a one-sided upper 95% confidence limit for the difference in proportion between the PCT-guided group and the control group. This confidence interval will be calculated using the method of Agresti and Caffo[23]. Noninferiority of the intervention will be established if the 95% confidence interval excludes 7.5%, i.e. if PCT-guided prescription of antibiotics does not increase the rate of the composite endpoint by more than 7.5 percentage points. Differences in hospital length of stay will be assessed using the Kruskal-Wallis test.

c) Accuracy of PCT and CRP. In all patients, the final diagnosis of the etiology of fever will be determined. Also, it will be determined if there is a bacterial infection. The variables "confirmed bacterial infection" and "suspected bacterial infection" will serve as gold standards for the evaluation of the accuracy of the biomarkers CRP and PCT. These will be determined retrospectively, using culture results and all

diagnostic tests available. In all patients, both PCT and CRP levels will be available for statistical analysis. The ability to predict bacterial infection of both PCT and CRP will be evaluated using receiver operating characteristic curves, and the area under the curve will be calculated. In addition, logistic regression will be performed to analyze the effects of PCT and CRP levels on the probability of a bacterial infection. The independent variables in this analysis are age, sex, PCT, CRP, temperature, co-morbidity, and other variables that have a $p<0.1$ for the difference between groups. We will account for possible non-linear effects of age, PCT, CRP and temperature by using appropriate transformations of these variables.

Statistical analyses for the secondary study parameters

The study will involve an economic evaluation from the societal perspective comparing PCT-guided therapy with usual care. The economic evaluation will use the technique of cost-minimization analysis, which compares two interventions of identical effectiveness to find out which is less costly. Total treatment costs will be compared between the PCT-guided therapy arm and the control arm, including costs for PCT testing (intervention group only), other diagnostic tests, ED visits, antibiotics and other medications, adverse effects of antibiotics, hospital admissions, return visits to the general practitioner (GP) and other related medical consumption. These costs will be taken into account during the one-month follow-up period. Unit prices will be calculated using real economic cost prices or using standard cost-prices for health economic evaluations[24]. Unit prices will be multiplied by the quantities for each resource used, and then summed over the separate types of resource to give a total cost per patient. In addition, differences in labor productivity losses will be evaluated by comparing costs of absence from work (absenteeism) and reduced productivity while at work (presenteeism). Moreover, productivity losses related to unpaid work (e.g., household work, shopping, odd jobs, and voluntary work) will be included. Productivity losses will be evaluated using the Productivity Costs Questionnaire[25]. Mean total costs will be calculated for patients in each treatment group.

Laboratory examinations

Blood samples will be obtained at inclusion. Samples will be centrifuged (3000 N Relative centrifugal force (Rcf) at room temperature for 5 minutes). The serum will be measured on the routine analyzer of the clinical chemistry laboratory (Roche Cobas 8000 system, Roche Diagnostics Netherlands). PCT-measurements will be performed by using an electro-chemiluminiscent immunoassay (ECLIA)(Roche diagnostics, Brahms, Henningsdorf, Germany). All samples will be measured and reported without knowledge of the clinical status of the subjects.

PCT-guided therapy

PCT-guided therapy is defined as the initiation of antibiotics, based on all available diagnostics with the addition of PCT-levels. The PCT results are appraised using a two-point scale, in which bacterial infections are respectively deemed unlikely (PCT < 0.5 μg/L) and likely (PCT ≥ 0.5 μg/L). These cut-off values are used in other trials[26-28].

Follow-up

One month after inclusion, patients will be contacted by telephone by one of the investigators. Course of the disease, including medicine use, related GP hospital visits (and diagnostics/prescriptions), and labor productivity losses, will be evaluated. Three months after inclusion, one of the investigators will contact patient's GP in order to evaluate the final outcome of the febrile episode. Patients are allowed to participate only once.

Adverse events

The composite safety endpoint is continually monitored during the trial. Also, adverse events, not in the composite safety endpoint - death after 30 days, life threatening events or any other important medical event that may jeopardize patients in any way - are monitored by the data safety monitoring board of the study.

Ethics approval and trial registration

The ethics committee of the Erasmus University Medical Center, in Rotterdam, the Netherlands, approved this trial. It is conducted according to the principles of the Declaration of Helsinki, version March 1, 2013 and in accordance with the Dutch Medical Research Involving Human Subjects Act (Wet Medisch wetenschappelijk Onderzoek met mensen, WMO). The CONSORT statements and its revised extension for reporting noninferiority trials were consulted and taken into account when designing and writing this protocol[29,30]. The trial has been registered in the Dutch trial registry, NTR4949.

DISCUSSION

Although observational studies have shown that PCT is more sensitive and specific for bacterial infections in comparison to CRP[14,31], and prospective trials have investigated the reduction of antibiotics in patients with respiratory complaints[32,33], the role of PCT in the ED remains unclear. This study is designed to answer the question whether PCT can aid in improving the accuracy of diagnosing bacterial infections in an ED setting. The real-life clinical ED setting differs from a research setting in which patients have very specific symptoms. Consequently, the value of PCT for ED patients without specific complaints has not been determined.
Because of this clinical problem, we used an objective measurement of temperature of 38.2°C or higher as sole inclusion criterion. As a result, we have a more heterogeneous population compared to other trials[32,33], allowing us to extrapolate the results to the majority of the adult ED patient population. Furthermore, we will be able to identify cases where physicians disregard the PCT level, and investigate the causes. These are urgent questions in emergency medicine.
The costs of PCT-guided therapy may be of influence in implementation in clinical practice. In the Netherlands alone, 94.2 billion euros was spent on healthcare in 2013, and the costs are rising[34]. Therefore, there is much interest in healthcare initiatives that benefit patients' health and are not associated with additional costs. Currently, there are no prospective cost-effectiveness studies on PCT-guided therapy. Two economic evaluation studies[18,19] report that a PCT-guided antibiotic algorithm may reduce costs. However, physician adherence to the PCT-guided therapy protocol could not be accurately estimated, and productivity losses are not analyzed. Therefore, these studies do not provide concrete evidence. In the HiTEMP study, costs are analyzed prospectively to provide a more accurate recommendation.

To reduce the risk of harming patients who receive PCT-guided therapy, we designed a noninferiority study protocol for the primary safety objective. Our study is in its design largely similar to the ProHOSP trial[33]. This ED based noninferiority trial, which investigates the start of antibiotic therapy using PCT in patients with suspected respiratory tract infections, uses a composite endpoint with a noninferiority margin of 7.5%. Moreover, in the Cochrane review on PCT-guided therapy, no differences in mortality or treatment failure were found[35]. We therefore consider the noninferiority margin of 7.5% of the composite endpoint ethically acceptable. We use a composite endpoint, consisting of patient centered outcomes. These outcomes, i.e. mortality, ICU admittance <30 days or return to ED within two weeks, are a potential sign of treatment failure.
There are logistical issues that may arise during the course of the study. One of the most important issues may be physician protocol adherence. Physicians need to

learn how they should interpret the value of the PCT level, and develop a certain clinical 'feel' towards it. The investigators will facilitate adoption of PCT, by informing every physician prior and during the study. Possibly, a 'learning curve' can be seen in the treatment according to PCT guidance. However, the physician motivates every choice of treatment that is not in accordance with the treatment advice based on the PCT result. Moreover, the accuracy of PCT will be calculated using the definitive diagnosis. Physician adherence to PCT guidance will also be investigated.

CONCLUSION

The HiTEMP trial addresses critical clinical questions in emergency medicine. PCT may offer a more tailor-made treatment to the individual ED patient with fever. The study will also shed light on the cost consequences of implementing PCT-guided therapy in the ED.

Prospective
The HiTEMP study is open for inclusion. Results are expected at the end of 2016.

This study protocol is approved by the medical ethics committee (Medisch ethische toetsingscommissie, METC) of the Erasmus University Medical Center, in Rotterdam, the Netherlands. Reference number MEC 2013-149.

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PART I
PROCALCITONIN-GUIDED THERAPY

CHAPTER 5

PROCALCITONIN-GUIDED ANTIBIOTIC THERAPY IN PATIENTS WITH FEVER IN A GENERAL EMERGENCY DEPARTMENT POPULATION: A MULTICENTER NONINFERIORITY RANDOMIZED CLINICAL TRIAL (HITEMP STUDY)

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ABSTRACT

Introduction

Importance: Procalcitonin(PCT)-guided therapy may reduce the number of antibiotics prescribed, but the effect in a general emergency department(ED) population is unclear.

Objective: To determine efficacy, safety, accuracy and economic consequences of PCT-guided therapy in ED patients with fever.

Methods

Design: Noninferiority randomized multicenter clinical trial of PCT-guided therapy versus standard care.

Setting: Two Dutch hospitals.

Participants: Adult patients with fever(≥38.2°C/100.8°F) in triage. Exclusion criteria: no written informed consent, specific immunocompromised conditions, pregnancy, moribund patients, patients who were within 72 hours after surgery or who required primary surgical intervention.

Intervention: Randomization between PCT-guided therapy and standard care. In the PCT-guided group, a single PCT value was available to the treating physician in the ED. The treatment protocol advised antibiotics when PCT level was ≥0.5µg/L.

Main Outcomes and Measures: Primary outcomes. Efficacy: number of patients who were prescribed antibiotics. Safety: composite safety outcome. Accuracy: AUC of PCT and CRP in diagnosing suspected and confirmed bacterial infections. Secondary outcome. Economic consequences: medical and non-medical costs.

Results

A total of 551 patients were included. Efficacy(n=551): PCT-guided therapy did not reduce the number of patients who received antibiotics compared to the control group, 200(73%) versus 212(77%) of patients received antibiotics(p=0.28). Multivariable logistic regression analysis: OR 0.83(95% CI0.53–1.30) for prescription of antibiotics using PCT-guided therapy. Safety(n=526): No statistically significant difference in composite safety outcome between groups (p=0.12). PCT-guided therapy was noninferior to standard care, upper limit of the one-sided 95% CI was 0.46.

Accuracy(n=529): PCT ≥0.5µg/L for confirmed bacterial infections: sensitivity 0.52(95% CI0.45–0.60), specificity 0.74(95% CI0.68–0.78). AUC of confirmed bacterial infections: PCT 0.681(95% CI0.633–0.730), CRP 0.619(95% CI0.569–0.669). AUC of suspected and confirmed bacterial infections: PCT 0.683(95% CI0.635–0.731), CRP 0.695(95% CI0.646–0.744).

Economic consequences: The total average costs per person: € 5386 for patients in the control group, € 4853 for patients in the PCT-guided group, mean difference was -€533(95% CI -€1570-€505).

Conclusion
PCT-guided therapy did not reduce antibiotics in a general ED population with fever. PCT-guided therapy was noninferior to standard care by means of safety. Although the accuracy of PCT for bacterial infections was higher than CRP, it was still poor. Future studies should focus on more accurate diagnostic modalities of bacterial infections.

Trial Registration Dutch trial register: NTR4949.
http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=4949

INTRODUCTION

In patients with bacterial infections, the treatment goal is to provide optimal antimicrobial therapy in accordance with antibiotic stewardship guidelines[1]. However, in the emergency department(ED), antimicrobial treatment is often initiated before a definitive diagnosis is made, since bacterial infections cannot always be ruled out using routine biomarkers such as C-reactive protein(CRP)[2].
When considering antibiotic treatment in patients with a suspected bacterial infection, ED physicians face a dilemma: On the one hand, antibiotics have to be prescribed sparingly, because overuse of antibiotics increases antibiotic resistance and raises healthcare costs[3,4]. On the other hand, withholding antibiotics from patients with bacterial infections and sepsis increases mortality[5,6]. Consequently, patients with suspected infections are often given antibiotics in the ED – a decision based on empirical grounds, rather than on a definitive diagnosis[2,7-9].
For the correct diagnosis of bacterial infection, accurate diagnostic modalities are crucial. Procalcitonin(PCT) is a biomarker with a higher diagnostic accuracy than CRP for bacterial infections in specific adult patient populations[10-12]. PCT can be applied in an algorithm that determines whether antibiotic treatment is necessary, a strategy known as PCT-guided therapy[13]. PCT-guided therapy reduces antibiotics in various clinical settings, such as in primary care and intensive care units(ICU)[13,14]. In the ED, PCT-guided therapy reduces antibiotics in subpopulations of patients with respiratory complaints and patients with suspected community acquired pneumonia(CAP), without increase in adverse events[12,15,16]. However, the effect of PCT-guided therapy in a general ED population remains unclear, because previous ED studies either used specific patient groups, or lacked statistical power[12,15-17]. Furthermore, current evidence on the economic consequences of PCT-guided therapy is incomplete, as studies so far have mainly been retrospective model-based studies, limited to hospital costs[18-21].

The aim of this study was to determine if PCT-guided therapy can be used for a general ED population of patients with fever, by determining the efficacy, safety, diagnostic accuracy, and economic consequences of this strategy.

METHODS

Study design

The HiTEMP study(Higher diagnostic accuracy and cost-effectiveness using procalcitonin in the treatment of emergency medicine patients with fever) was performed in a tertiary referral hospital, Erasmus University Medical Center(EMC) in Rotterdam, and in the Jeroen Bosch hospital(JBZ) in 's-Hertogenbosch, both in the Netherlands. The study was a multicenter, randomized clinical trial with a noninferiority design. The study design has been published previously[22]. In brief, patients were randomized in ED triage between standard care(control group) and PCT-guided therapy(PCT-guided group) and were followed up for 90 days, by ways of medical database review, telephone interviews and general practitioner(GP) inquiry. This study was approved by the Erasmus University Medical Center ethics review board(NL44227.078.13).

Study population

ED patients with a temperature of ≥38.2°C(100.8°F) were eligible. The temperature of 38.2°C was based on the epidemiology of fever in ED patients[23]. Patients were excluded if they had specific immunocompromised conditions, defined as neutropenia with absolute neutrophil count of <0.5x109cells/L, current chemotherapy, or post-solid organ transplantation. Furthermore, pregnant patients, moribund patients and patients with a diagnosis that required primary surgical intervention, or who were within 72 hours after surgery were excluded.

Intervention

PCT levels were determined in all patients. However, only PCT levels of patients in the PCT-guided group were available to the ED physician. A bacterial infection was deemed unlikely when PCT was <0.5µg/L, and antibiotics were discouraged. When PCT was ≥0.5µg/L, a bacterial infection was likely and antibiotics were encouraged. All treating physicians were trained in interpreting PCT values. Following institutional guidelines, patients with suspected infections and systemic inflammatory response syndrome(SIRS) received a single dose of broad-spectrum antibiotics within one hour after ED arrival. This was no main outcome measure, because this treatment took place before laboratory results were available.

Primary outcomes

The efficacy of PCT-guided therapy was defined as the reduction in percentage of patients who were prescribed antibiotic therapy in the ED.

Safety was assessed with a composite safety endpoint(CSE) consisting of 30-days mortality, ICU admission within 30 days after ED visit, and return visits to the ED within 14 days as indication of treatment failure.

Accuracy was defined as the accuracy of PCT and CRP in diagnosing confirmed bacterial infections only, and confirmed and suspected bacterial infections combined. Confirmed bacterial infections were defined as clinically significant positive blood cultures and focus cultures. Suspected bacterial infections were determined by chart review with predefined clinical criteria, by two independent physicians(supplement 1). In case of disagreement, a third physician was referee.

Secondary outcomes

Efficacy of PCT-guided therapy for patients with respiratory sources of infections was assessed in a subgroup analysis, in order to compare results with studies on PCT-guided therapy in this specific subgroup. Secondary safety outcome measures were hospital admission, length-of-hospital-stay and length-of-ICU-stay. We calculated the percentage of protocol nonadherence. Secondary accuracy outcome was accuracy of PCT and CRP in diagnosing bacteremia.

The economic analysis with cost-minimization design included hospital and societal costs. Medical costs included ED visits, PCT testing, antibiotics administered in ED and first three days of antibiotic treatment, hospital and ICU admissions, and general practitioner(GP)consultations. Non-medical costs were defined as productivity losses, which comprised costs of absence from work(absenteeism), reduced productivity while at work(presenteeism), and productivity losses related to unpaid work. Costs of productivity losses were assessed using the iMTA Productivity Cost Questionnaire(iPCQ)[24]. The time horizon for this analysis was 30 days, except for the out-of-hospital medical costs, which was 90 days.

Statistical analysis

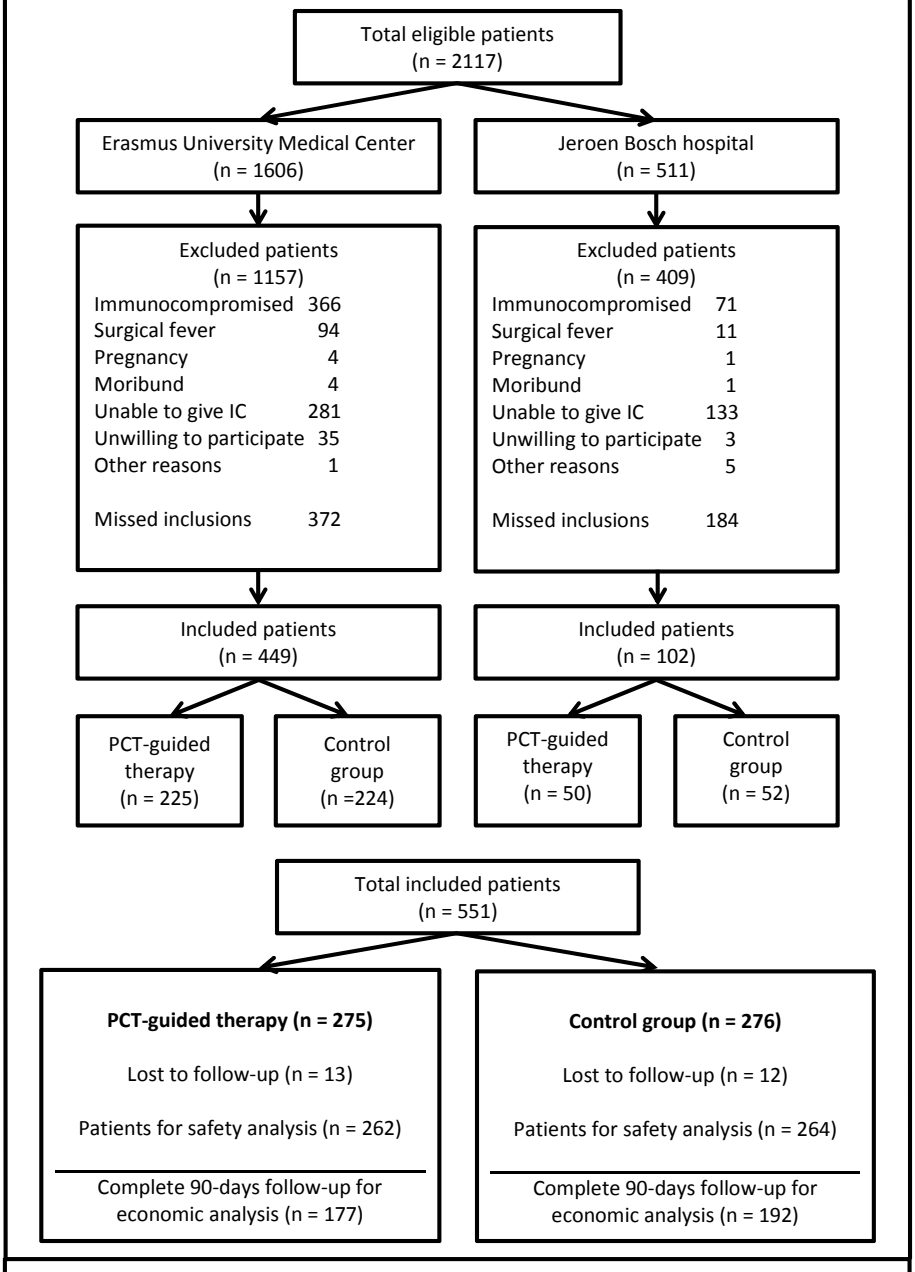
Efficacy was determined using multivariable logistic regression analysis with stepwise backward selection of variables, with a p-value cut-off of 0.05. Age, sex, temperature, medication use, comorbidity, PCT and CRP levels and other variables were considered as independent variables in this analysis. This analysis was performed according the intention-to-treat principle. PCT-guided therapy was deemed noninferior if the upper limit of the one-sided 95 confidence interval(95% CI) for the difference in the CSE between the PCT-guided group and the control group was not higher than 7.5 percentage points. The 95% CI was calculated according to the method described by Agresti and Caffo[25]. The study was powered for the CSE, based on the noninferiority margin of 7.5%. The calculated sample size was 550 patients. Accuracy was reported in three ways. First, as sensitivity and specificity, with binomial proportion CIs calculated using the Clopper-Pearson method. Second, as a receiver operator characteristic curve(ROC) curve. Third, using multivariable logistic regression, corrected for the independent variables age, sex, PCT and CRP levels, temperature, comorbidity, and other variables with $p < 0.1$ for the difference between groups. We accounted for non-linear effects of PCT with logarithmic transfor-

mation. Differences in secondary outcomes were analyzed with Fisher's exact test for dichotomous variables, the independent samples T-test for normally distributed variables, and the Mann-Whitney U test for continuous variables that were not normally distributed. We calculated the optimal cut-off of PCT for confirmed bacterial infections with the Youden's index[26]. Cost-minimization: Costs were derived from the Dutch guidelines for economic evaluations in healthcare[27]. For medical costs, the quantities for each resource used were multiplied by standard unit prices.
Non-medical costs were calculated by multiplying the number of hours of productivity losses by an average hourly wage, for patients who were employed, and by multiplying the number of hours of inability to do unpaid work by standard housekeeper replacement costs for all patients. We calculated mean values for all cost categories and for total costs for both the PCT-guided and control groups. Mean differences between the groups and, using nonparametric bootstrapping, 95% CIs were calculated. All costs were reported in Euros(€). Unless otherwise specified, all statistical tests were two-sided with a significance level of 0.05. Statistical analyses were performed with the statistical package for the social sciences(SPSS v.23), IBM corporation, and Excel, Microsoft corporation.

RESULTS

Between August 2014 and January 2017, 2117 patients were screened for inclusion. Of these 2117 patients, 551 patients were included, of whom 449 in EMC and 102 in JBZ(figure 1). There were 372 missed inclusions in EMC, and 184 in JBZ. Missed inclusions were eligible patients who were not randomized because physicians were unable to perform the randomization procedure due to time constraints. All 551 cases were included in the intention-to-treat analysis for efficacy. There were 25(5%) patients lost-to-follow-up after 30 days, making the number of patients for the safety analysis 526. Patients who were lost-to-follow-up could not be contacted by telephone, and there was no response from their GP. PCT results were unavailable for 19 patients, of whom 8 in the PCT-guided group. This was due to failure to obtain blood samples in the ED. For three additional patients, no CRP result was available, making the number of cases for the accuracy analyses 529. Baseline characteristics are reported in table 1.

Figure 1. Flowchart



IC: informed consent, N: number of patients, PCT: procalcitonin

Table 1. Baseline characteristics

		All (n = 551)	Control group (n= 276)	PCT-guided group (n = 275)
Demographic characteristics				
Age	median [IQR]	63 [43 - 71]	62 [44 - 73]	61 [43 - 70]
Female sex	n (%)	253 (46)	122 (44)	131 (48)
Vital signs at presentation				
Temperature	median [IQR]	38.8 [38.4 - 39.2]	38.8 [38.4 - 39.2]	38.8 [38.4 - 39.3]
Heart rate	median [IQR] n = 550	105 [92 - 119]	105 [92 - 119]	105 [90 - 118]
Systolic bloodpressure	median [IQR] n = 549	130 [118 - 145]	130 [118 - 145]	130 [117 - 145]
Diastolic bloodpressure	median [IQR] n = 549	75 [67 - 85]	75 [67 - 85]	75 [67 - 85]
Respiratory rate	median [IQR] n = 410	20 [17 - 25]	22 [18 - 25]	20 [16 - 25]
Comorbidity				
Diabetes	n (%)	97 (18)	51 (19)	46 (17)
Malignancy	n (%)	106 (19)	44 (16)	62 (23)
HIV	n (%)	17 (3)	10 (4)	7 (3)
Current medication use				
Current antibiotics use (before ED v	n (%)	88 (16)	40 (15)	48 (18)
Steroids	n (%)	78 (14)	37 (13)	41 (15)
Oral anticoagulants	n (%)	64 (12)	31 (11)	33 (12)
Acetylsalicylic acid	n (%)	67 (12)	33 (12)	34 (12)
Biomarkers				
PCT in mcg/L	median [IQR] n = 532	0.26 [0.10 - 0.96]	0.26 [0.10 - 1.00]	0.28 [0.10 - 0.93]
CRP in mg/L	median [IQR] n = 548	62 [19 - 150]	67 [21 - 159]	57 [17 - 132]
Leukocytes in count*10^9 cells/L	median [IQR]	12.0 [8.5 - 15.6]	11.7 [8.6 - 14.9]	11.6 [8.3 - 16.0]
Diagnosis				
Confirmed bacterial infections	n (%)	198 (36)	103 (37)	95 (35)
Confirmed viral infections	n (%)	41 (7)	20 (7)	21 (41)
Confirmed fungal infections	n (%)	1 (0)	0 (0)	1 (0)
Confirmed parasite infections	n (%)	1 (0)	0 (0)	1 (0)
Confirmed non-infectious fever	n (%)	37 (7)	14 (3)	23 (4)
Bacteremia	n (%)	99 (18)	57 (21)	42 (15)
Categories of fever by focus				
- Skin and soft tissue	n (%)	47 (9)	27 (10)	20 (7)
- Respiratory tract	n (%)	212 (39)	102 (37)	110 (40)
- Urogenital tract	n (%)	108 (20)	54 (20)	54 (20)
- Abdominal	n (%)	69 (13)	36 (13)	33 (12)
- Central nervous system	n (%)	4 (1)	1 (0)	3 (1)
- Other source	n (%)	13 (2)	9 (3)	4 (2)
- Fever without source	n (%)	60 (11)	33 (12)	27 (10)
- Non infectious fever	n (%)	37 (7)	15 (5)	22 (8)
Initial ED treatment				
Antibiotics in ED because of SIRS	n (%)	283 (51)	148 (54)	135 (49)

CRP: C-reactive protein, ED: emergency department, HIV: human immunodeficiency virus, IQR: interquartile range, PCT: procalcitonin

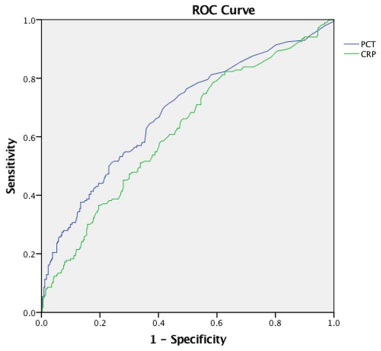
Primary outcomes

Efficacy: 200(73%) of patients in the PCT-guided group received antibiotics, compared to 212(77%) of patients in the control group (p = 0.28)(table 2). PCT-guided therapy did not reduce the probability of antibiotics prescription, odds ratio(OR) 0.83(95% CI 0.53–1.30) when corrected for age, sex, temperature, medication use, comorbidity and PCT and CRP levels. No other independent variables had significant effect(table 3).

Safety: There was no significant difference in the CSE between groups(table 2). The upper limit of the one-sided 95% CI for the difference in the CSE between the PCT-guided group and the control group was 0.46, which was below the defined noninferiority margin of 7.5, making PCT-guided therapy noninferior to standard care by means of safety.

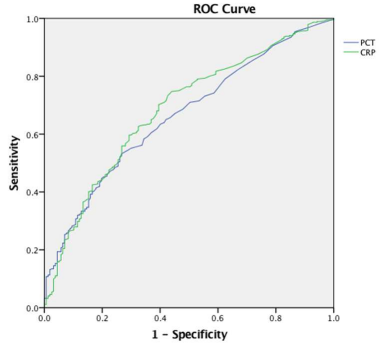
Figure 2. ROC curves

ROC curve of PCT and CRP for confirmed bacterial infection (n = 529)



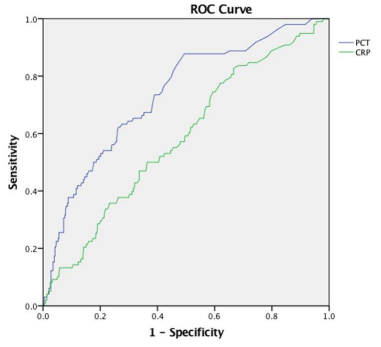
PCT AUC 0.681 (95%CI 0.633 – 0.730)
CRP AUC 0.619 (95%CI 0.569 – 0.669)

ROC curve of PCT and CRP for confirmed and suspected bacterial infection (n = 529)



PCT AUC 0.683 (95%CI 0.635 – 0.731)
CRP AUC 0.695 (95%CI 0.646 – 0.744)

ROC curve of PCT and CRP for bacteremia (n = 529)



PCT AUC 0.736 (95%CI 0.681 – 0.790)
CRP AUC 0.585 (95%CI 0.523 – 0.646)

AUC: Area under curve, CRP: C- reactive protein, PCT: Procalcitonin, ROC curve: Receiver operator characteristic curve

Accuracy: For confirmed bacterial infections only, sensitivity of PCT ≥0.5µg/L was 0.52(95% CI 0.45–0.60), and specificity 0.73(95% CI 0.68–0.78). The ROC curve of PCT yielded an AUC of 0.681(95% CI 0.633–0.730), which was higher than the AUC of CRP, 0.619(95% CI 0.569–0.669) (figure 2).

Multivariable logistic regression analysis showed an OR of PCT of 1.37(95% CI 1.20–1.56), corrected for age, sex, temperature, comorbidity, CRP level and leukocyte count (table 3). This OR showed that a 1% relative increase in PCT level increases the odds for a patient having a bacterial infection 0.37.
For confirmed and suspected infections combined, sensitivity of PCT ≥0.5µg/L was 0.43(95% CI 0.38–0.48), and specificity 0.85(95% CI 0.77–0.89). The AUC of PCT was 0.683(95% CI 0.635–0.731), and was lower than the AUC of CRP, 0.695(95% CI 0.646–0.744). The OR of PCT of 1.29(95% CI 1.09–1.53), corrected for age, sex, temperature, comorbidity, CRP level and leukocyte count.

Table 3. Multivariable analysis of prescription of antibiotics and confirmed and suspected bacterial infections

Efficacy outcome: multivariable logistic regression analysis

Dependent variable:	All patients (n = 551)* Antibiotics prescribed Odds ratio (95% CI)	Subgroup analysis of patients suspected and Antibiotics prescribed Odds ratio (95% CI)
Randomisation (PCT-guided group)	**0.83 (0.53 - 1.30)**	**1.29 (0.59 - 2.82)**
Age (years)	1.04 (1.02 - 1.05)	1.04 (1.02 - 1.06)
Female	0.99 (0.63 - 1.56)	1.14 (0.51 - 2.55)
Temperature (degrees Celcius)	2.37 (1.50 - 3.75)	2.11 (0.94 - 4.74)
Comorbidity: malignancy	1.74 (0.88 - 3.45)	3.81 (0.75 - 19.35)
Comorbidity: diabetes	0.96 (0.48 - 1.91)	0.98 (0.29 - 3.38)
Comorbidity: HIV	1.76 (0.46 - 6.85)	2.31 (0.35 - 15.24)
Medication use: previous antibiotics	3.12 (1.45 - 6.71)	2.31 (0.74 - 7.19)
Medication use: steroids	1.07 (0.55 - 2.10)	3.48 (1.11 - 10.88)
Medication use: oral anticoagulants	1.09 (0.50 - 2.37)	2.69 (0.60 - 11.94)
Medication use: acetylsalicyl acid	2.05 (0.86 - 4.88)	2.86 (0.60 - 13.65)
PCT level (µg/L)	1.18 (1.00 - 1.38)	1.19 (0.74 - 1.90)
CRP level (mg/L)	1.01 (1.00 - 1.01)	1.01 (1.00 - 1.02)

* Multivariable logistic regression analysis with predefined variables. No additional baseline variables met criteria for

Accuracy outcomes: multivariable logistic regression analysis

Dependent variable:	All patients (n = 529)** Confirmed bacterial infections Odds ratio (95% CI)	All patients (n = 529)** Confirmed and suspected bacterial infections Odds ratio (95% CI)
Logarithmic PCT level	**1.37 (1.20 - 1.56)**	**1.29 (1.08 - 1.52)**
CRP level (mg/L)	1.00 (1.00 - 1.01)	1.01 (1.00 - 1.01)
Age (years)	1.01 (1.00 - 1.03)	1.03 (1.02 - 1.04)
Female	0.77 (0.52 - 1.14)	0.59 (0.38 - 0.92)
Temperature (degrees Celcius)	1.67 (1.17 - 2.37)	2.18 (1.41 - 3.36)
Comorbidity: malignancy	1.62 (1.00 - 2.64)	2.06 (1.06 - 4.00)
Comorbidity: diabetes	1.00 (0.59 - 1.70)	1.65 (0.85 - 3.22)
Comorbidity: HIV	0.70 (0.21 - 2.36)	0.70 (0.21 - 2.25)
Leukocyte count (10^9 cells/L)	1.00 (0.99 - 1.02)	1.02 (0.99 - 1.05)

**Multivariable logistic regression analysis with predefined variables, with the addition of leukocyte count.

CI: confidence interval, CRP: C-reactive protein, n: number, PCT: procalcitonin.

Secondary outcomes

In the subgroup analysis of patients with respiratory sources of infections(n = 212), 83(75%) of patients in the PCT-group received antibiotics, compared to 73(72%) patients in the control group (p=0.76). The OR for prescription of antibiotics was 1.29(95% CI 0.59–2.82) (table 3).

In the total population, there were no statistically significant differences in individual endpoints of the CSE (n=526), second ED visit within 14 days (p=0.20), ICU admission within 30 days after ED visit(p=1.00) and 30-days mortality (p=0.11), nor in endpoints hospital admission(p=0.10), length-of-hospital-stay(p=0.25) and length-of-ICU-stay(p=0.32)(table 2).

Protocol nonadherence was 49%(n=529), which consisted of 232 patients who received antibiotics with PCT<0.5µg/L, and 26 patients who did not receive antibiotics with PCT≥0.5µg/L (supplement 2).

A total of 89(48%) of patients with a confirmed bacterial infection(n=186) had PCT<0.5µg/L (supplement 2). Of these 89 patients, 12 patients did not receive antibiotics. Of these 12 patients, two patients suffered adverse events, one return visit to the ED, and one ICU admission. The AUC of PCT for bacteremia was 0.736(95% CI 0.681–0.790), the AUC of CRP for bacteremia was 0.585(95% CI 0.523–0.646). The OR of PCT for bacteremia was 1.37(95% CI 1.20–1.56), corrected for age, sex, temperature, comorbidity, CRP level. No additional variables met criteria for inclusion (supplement 3).

The calculated optimal cut-off of PCT for confirmed bacterial infection was 0.25µg/L. Sensitivity of PCT ≥0.25µg/L was 0.70(95% CI 0.63–0.77), and specificity 0.58(95% CI 0.52–0.63).

A total of 369 patients had a complete follow-up for the cost-minimization analysis, 177(64%) in the control group and 192(70%) in the PCT-guided group. The total average costs per person were €4853 for patients in the PCT-guided group and €5386 for patients in the control group, with a mean difference of -€533 (95% CI -€1570 to €505).

Table 4: Economic analysis

	PCT-guided group (n = 192)		Control group (n = 177)		Difference	
	Mean	Standard deviation	Mean	Standard deviation	Mean	95% CI
Hospital costs	**€ 3899**	**€ 4704**	**€ 4162**	**€ 4680**	**€ 263-**	**-€ 1225 to € 698**
PCT testing	€ 56	€ -	€ -	€ -	€ 56	€ 56 to € 56
Antibiotics administered in ED	€ 7	€ 12	€ 8	€ 15	€ 1-	-€ 3 to € 2
Antibiotics first three days	€ 82	€ 136	€ 73	€ 135	€ 9	-€ 18 to € 37
ED admission	€ 275	€ 63	€ 278	€ 68	€ 3-	-€ 16 to € 11
Admission university hospital	€ 2966	€ 3714	€ 3330	€ 4087	€ 364-	-€ 1162 to € 435
Admission non-university hospital	€ 261	€ 885	€ 235	€ 911	€ 25	-€ 158 to € 209
ICU admission	€ 252	€ 1838	€ 239	€ 1762	€ 13	-€ 356 to € 382
Costs of extramural care	**€ 89**	**€ 117**	**€ 109**	**€ 144**	**€ 20-**	**-€ 47 to € 6**
General practitioner visit	€ 54	€ 75	€ 74	€ 102	€ 20-	-€ 38 to -€ 1
General practitioner house call	€ 34	€ 91	€ 35	€ 98	€ 1-	-€ 20 to € 18
Productivity costs	**€ 866**	**€ 1742**	**€ 1115**	**€ 2118**	**€ 249-**	**-€ 644 to € 147**
Costs of absenteeism from work	€ 632	€ 1628	€ 830	€ 1875	€ 198-	-€ 557 to € 160
Costs of productivity loss while at w	€ 28	€ 142	€ 42	€ 223	€ 14-	-€ 52 to € 24
Replacement for unpaid work	€ 206	€ 521	€ 243	€ 694	€ 37-	-€ 162 to € 88
Total costs	**€ 4853**	**€ 4969**	**€ 5386**	**€ 5168**	**€ 533-**	**-€ 1570 to € 505**

ED: Emergency department, ICU: intensive care unit, PCT: procalcitonin

DISCUSSION

Our study showed that PCT-guided therapy did not reduce the number of febrile patients who were prescribed antibiotics in the ED. PCT-guided therapy was noninferior to standard therapy regarding the CSE. PCT was more accurate in the diagnosis of confirmed bacterial infections than CRP, but the accuracy was poor. For confirmed and suspected infections, the accuracy of PCT was lower than the accuracy of CRP, which suggests that, in patients with suspected infections, PCT results can be incongruent with clinical judgement. PCT-guided therapy did not result in a statistically significant costs reduction.

Our findings are in contrast with other studies on PCT-guided therapy in the ED[10,12,15,16,28,29]. These studies reported antibiotic reductions of 13 to 39 with PCT-guided therapy, in adult patient populations of patients with lower respiratory tract infections, CAP and COPD. However, in our study, we found no difference in the number of antibiotics prescriptions; not in the total cohort, nor in the subgroup analysis of patients with respiratory infections. We speculate that physicians based treatment decisions on clinical judgement and other diagnostic modalities instead of PCT advice in conflicting situations.

The essence of PCT-guided therapy is to reduce antibiotics in patients without bacterial infections. For this strategy to work, PCT levels have to identify patients without bacterial infections accurately, and on top of that, physicians have to trust the results. In the ProHOSP study, Schuetz et al.[12] reported that PCT-guided therapy significantly reduced antibiotics prescriptions in ED patients with suspected CAP. The authors suggested that Swiss physicians' experience with PCT resulted in an increased adherence to PCT advice in the ProHOSP study, because physicians in Switzerland were used to working with PCT. Physicians in Dutch EDs did not have extensive previous clinical experience with the PCT biomarker before this study started. However, we hypothesize that this lack of previous clinical experience, and possible concomitant lack of trust in the accuracy of PCT results, cannot be the only explanation for our findings. Following the 49% PCT-guided therapy protocol nonadherence, we presume that, in a general ED population, PCT is insufficient as a single biomarker in the diagnosis of bacterial infections, and that clinical judgement and other diagnostic modalities are crucical for physicians in order to prevent undertreatment. This was also suggested by Kristoffersen et al., who found a PCT-guided therapy protocol nonadherence of 41% [30].

Although PCT was more accurate for confirmed bacterial infections than CRP and although an increase in PCT level made the presence of a patient having a confirmed infection more likely, a single cut-off of PCT ≥0.5µg/L resulted in an erroneous treatment advice to withhold antibiotics in 89(49%) patients with a confirmed bacterial infection. This cut-off value was higher than in other ED and primary care studies[12,14,15]. The calculated optimal cut-off of PCT for confirmed bacterial infec-

tions, ≥0.25µg/L, had a sensitivity of 0.70, and coincidentally corresponded with the cut-off used in these studies. Out of 253 patients with PCT levels of ≥0.25µg/L, 55 patients(28%) had a confirmed bacterial infection. PCT levels, both with the cut-off of ≥0.5µg/L and with the calculated optimal cut-off of ≥0.25µg/L for confirmed bacterial infections resulted in false negative outcomes in respectively 89(17%) and 55(10%) patients(supplement 2). From these findings we conclude that a single PCT result gave inaccurate treatment advice and should not be used as only criterion to start or withhold antibiotics in a general ED population.

The accuracy of PCT for bacterial infections in our study is lower than in two recent meta-analyses on the accuracy of PCT in the ED (e.g. an AUC of PCT for bacteremia of 0.736 in our study versus 0.84 reported by Jones et al.)[31,32]. This may be due to the fact that our patient population was designed to resemble a general ED population, thereby making it a heterogeneous group with only 36 of patients with a confirmed bacterial infection. Studies with highly selected populations such as the ProHOSP study, consisted of higher percentages of bacterial infections, with 68 of patients having a CAP12.

In the PCT-guided group, patients were admitted to the hospital less often, and had a lower absolute 30-days mortality. Although both findings did not meet the criteria for statistical significance, we speculate that physicians may have used PCT as a biomarker for disease severity. On the one hand, low PCT results may have influenced physicians not to admit patients to the hospital. On the other hand, high PCT results may have prompted physicians to treat patients more aggressively, resulting in a reduction in 30-days mortality. Shehabi et al.[33] described that a single PCT level at ICU admission was predictive of sepsis severity. The value of PCT in disease severity in a general ED patient population has not yet been determined.

The economic analysis demonstrated that there was no statistically significant difference in costs between groups. This was contrary to our hypothesis that PCT-guided therapy reduced overall costs. Hospital admissions and consequent productivity losses account for the highest expenses. Future biomarker strategies that can reduce hospital admissions may reduce healthcare costs.

Limitations

We used body temperature as sole inclusion criterion. This was both a strength and a weakness of the study. A strength, because it was an objective measurement and makes the results generalizable to all ED patients with fever. A weakness, because fever is not a perfect marker of infectious disease. The use of this inclusion criterion resulted in a selection bias, where patients with infectious diseases and normothermia or hypothermia were not eligible for inclusion. Patients with altered mental status and severely ill patients were de facto excluded, because these patients were unable to give written informed consent. Therefore, the results are not generalizable for the entire adult ED patient population.

CONCLUSION

PCT-guided therapy was noninferior to standard care by means of safety, but did not reduce the prescription of antibiotics in patients with fever in a general ED population. In this heterogeneous patient population, PCT was more accurate in the diagnosis of confirmed bacterial infections than CRP, but the accuracy of PCT for bacterial infections was poor.
Future studies should focus on more accurate diagnostic modalities of bacterial infections to reduce antibiotics prescriptions in the ED. Furthermore, PCT-guided therapy may be used for assessing disease severity.

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Supplement 1. Criteria for confirmed and suspected infections

Confirmed infections

Confirmed bacterial infections:
Positive culture result for bacteria (from an otherwise sterile location), positive Legionella or pneumococcus urine test. ³
(Coagulase negative Staphylococcus bacteremia is considered as contamination and not as infection.)

Confirmed viral infections:
Positive viral PCR or serology. *

Confirmed fungal infections:
Positive blood culture or PCR.

Confirmed parasitical infections:
Positive blood culture or PCR, positive blood smear for malaria.

Suspected infections

Suspected bacterial infections:
Negative findings in cultures and PCR.
Positive infiltrate on chest x-ray with as at least 1 respiratory symptom (cough, sputum, dyspnea, tachypnea, pleuritic pain), plus either rales or crepitation on auscultation, shivering, leukocytosis. Positive image modality: infiltrate, abscess. Positive urine nitrite, with positive leukocytes in urine and leukocytosis, or with dysuria complaints in history. Skin: local red and painfull swelling. Abscess found on endoscopy. CSF analysis one of the following criteria: high opening pressure (>18cm H_2O), pleiocytosis (> 5.109/L), elevated protein level (>0.4 g/L), low glucose (<2.5 mmol/L), low glucose CSF/blood ratio (< 0.4), high lactate (>4.2 mmol/L). ERCP findings suggestive of cholangitis. Clinical improvement with antibiotics treatment, when no highly suspected other cause is present.

Suspected viral infections:
Negative findings in cultures and PCR.
History mentions flu-like symptoms. Dry cough. Pathognomic findings of viral infections, such as, but not limited to Koplik spots. Improvement without antibiotics.

Noninfectious fever:
Negative findings in cultures and PCR.
Negative image modality findings suspected for infection. Confirmed or highly likely other diagnosis. Improvement without antibiotics.

Unknown:
Negative findings in cultures and PCR.
Insufficient clinical data in medical chart to determine suspected infection category.

*when a patient has both a confirmed bacterial and viral infection, it is considered a bacterial infection because this has treatment consequences.

CSF: cerebrospinal fluid. ERCP: endoscopic retrograde cholangiopancreatoscopy. PCR: polymerase chain reaction diagnostics.

Supplement 2: Accuracy of PCT for confirmed and suspected infection:

Total number of patients in whom PCT levels were available:		**n = 529**				
Cut-off value: PCT <0.5µg/L	**n =**	**341**	**PCT ≥0.5µg/L**	**n =**	**188**	**Total**
Confirmed bacterial infection:	**n =**	**89**	**Confirmed bacterial infection:**	**n =**	**97**	**186**
Received antibiotics:	n =	77	Received antibiotics:	n =	92	169
Not received antibiotics:	n =	12	Not received antibiotics:	n =	5	17
No confirmed bacterial infection:	**n =**	**252**	**No confirmed bacterial infection:**	**n =**	**91**	**343**
Received antibiotics:	n =	155	Received antibiotics:	n =	70	225
Not received antibiotics:	n =	97	Not received antibiotics:	n =	21	118
Sensitivity of PCT ≥0.5µg/L for confirmed bacterial infections:			0.52 (95% CI 0.45 - 0.60)			
Specificity of PCT ≥0.5µg/L for confirmed bacterial infections:			0.73 (95% CI 0.68 - 0.78)			
Cut-off value: PCT <0.5µg/L	**n =**	**341**	**PCT ≥0.5µg/L**	**n =**	**188**	**Total**
Confirmed and suspected bacterial infection:	**n =**	**216**	**Confirmed and suspected bacterial infection:**	**n =**	**164**	**380**
Received antibiotics:	n =	190	Received antibiotics:	n =	152	342
Not received antibiotics:	n =	26	Not received antibiotics:	n =	12	38
No confirmed and suspected bacterial infection:	**n =**	**125**	**No confirmed and suspected bacterial infection:**	**n =**	**24**	**149**
Received antibiotics:	n =	42	Received antibiotics:	n =	10	52
Not received antibiotics:	n =	83	Not received antibiotics:	n =	14	97
Sensitivity of PCT ≥0.5µg/L for confirmed and suspected bacterial infections:			0.43 (95% CI 0.38 - 0.48)			
Specificity of PCT <0.5µg/L for confirmed and suspected bacterial infections:			0.84 (95% CI 0.77 - 0.89)			
Optimal cut-off value: PCT <0.25µg/L	**n =**	**253**	**PCT ≥0.25µg/L**	**n =**	**276**	**Total**
Confirmed bacterial infection:	**n =**	**55**	**Confirmed bacterial infection:**	**n =**	**131**	**186**
Received antibiotics:	n =	48	Received antibiotics:	n =	121	169
Not received antibiotics:	n =	7	Not received antibiotics:	n =	10	17
No confirmed bacterial infection:	**n =**	**198**	**No confirmed bacterial infection:**	**n =**	**145**	**343**
Received antibiotics:	n =	117	Received antibiotics:	n =	108	225
Not received antibiotics:	n =	81	Not received antibiotics:	n =	37	118
Sensitivity of PCT ≥0.25µg/L for confirmed and suspected bacterial infections:			0.70 (95% CI 0.63 - 0.77)			
Specificity of PCT <0.25µg/L for confirmed and suspected bacterial infections:			0.58 (95% CI 0.52 - 0.63)			

N: number of patients, PCT: procalcitonir

Supplement 3: Multivariable analysis of bacteremia

Multivariable logistic regression analysis

	All patients (n = 529)*
Dependent variable:	Bacteremia Odds ratio (95% CI)
Logaritmic PCT level	**1.37 (1.20 - 1.56)**
CRP level (mg/L)	1.00 (1.00 - 1.01)
Age (years)	1.01 (1.00 - 1.03)
Female	0.77 (0.52 - 1.14)
Temperature (degrees Celcius)	1.67 (1.17 - 2.37)
Comorbidity: malignancy	1.62 (1.00 - 2.64)
Comorbidity: diabetes	1.00 (0.59 - 1.70)
Comorbidity: HIV	0.70 (0.21 - 2.36)

Multivariable logistic regression analysis with predefined variables. No additional baseline variables met criteria for inclusion in analysis.

CI: confidence interval, CRP: C-reactive protein, n: number, OR : Odds ratio, PCT: procalcitonin.

PART II
ADDITIONAL BIOMARKER STRATEGIES

CHAPTER 6

TRAIL AND IP-10 AS BIOMARKERS OF VIRAL INFECTION IN THE EMERGENCY DEPARTMENT

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ABSTRACT

Introduction

Fever is a common symptom in the Emergency department (ED). If viral infections can be identified more accurately, the overuse of antibiotics, attributed adverse events and antibiotics resistance may be reduced. TNF-related apoptosis-inducing ligand (TRAIL) and interferon-gamma-inducible protein 10 (IP-10) are described as novel biomarkers for viral disease. We investigated if TRAIL and IP-10 can differentiate etiologies of fever in the ED, both as a single biomarker, and in combination with procalcitonin (PCT).

Methods

Adult patients with fever in the ED were included. TRAIL and IP-10 levels were determined in patient cases of confirmed viral, bacterial and non-infectious fever. Confirmed viral and confirmed bacterial infection were defined as laboratory evidence of presence of disease and clinical signs, confirmed non-infectious as absence of laboratory evidence and strong alternative diagnosis. A Kruskal-Wallis test was utilized to determine difference between etiologies of fever for TRAIL and IP-10 levels, and confirmed viral infections were compared with other etiologies of fever using Mann Whitney U tests. The area-under-the-curve (AUC) was calculated for TRAIL and IP-10 and optimal cut-off values were derived. In binary logistic regression analysis, a combined biomarker model including PCT, TRAIL and IP-10 was created and the AUC was calculated.

Results

A total of 54 patients were included, of whom 13 with a confirmed viral disease, 33 with a confirmed bacterial disease and 8 patients with confirmed non-infectious disease. TRAIL levels were significantly higher in patients with confirmed viral infections, compared to patients with a confirmed bacterial infection and non-infectious disease, ($p < 0.001$). IP-10 levels were not significantly different ($p = 0.052$). For the discrimination of confirmed viral infections from other febrile etiologies, levels of both TRAIL ($p = 0.016$) and IP-10 ($p = 0.017$) were significantly higher in confirmed viral disease. AUCs were 0.72 (95%CI 0.56 – 0.88) for TRAIL and 0.72 (95%CI 0.59 – 0.86) for IP-10. The combined biomarker model showed an AUC of 0.84 (95%CI 0.72 – 0.97) for discrimination between confirmed viral and non-viral disease.

Discussion

Levels of TRAIL and IP-10 were significantly elevated in viral infections, compared to bacterial and non-infectious febrile disease in an undifferentiated ED patient cohort. A combined biomarker model with PCT resulted in an even higher diagnostic accuracy of viral disease than single biomarkers individually. The cut-off values of the novel biomarkers require validation, but the results of this study are a proof of principle.

INTRODUCTION

Diagnosing the cause of fever in a patient in the emergency department (ED) is difficult. Fever is a common presenting symptom in both viral and bacterial disease. Also, many non-infectious diseases may cause fever[1-3].
There is a dilemma in the treatment of febrile illness in the ED. On the one hand, patients with a severe bacterial infection have to be treated with antibiotics as soon as possible. Delay is associated with a rise in morbidity and mortality[4]. On the other hand, particularly in this time of antimicrobial stewardship, it is good clinical practice to use antibiotics for treatment of bacterial disease as efficient as possible[5,6]. Antibiotic overtreatment of patients may result in an increase of antibiotic resistance[5,6].
Bacterial cultures and specific viral assays are obtained in the ED, but results often take several days to become available. Due to time restraints, it is not possible to wait for these results in daily ED practice. Treatment has to be started before the definitive diagnosis is available. New strategies for appropriate use of antimicrobial therapy are needed[7,8].
In the standard diagnostic workup of febrile patients, biomarkers are used to focus on predicting the presence of bacteria[2,9,10]. In current practice, C-reactive protein (CRP) and to a lesser extent procalcitonin (PCT) are used as clinical biomarkers for identification of bacterial infection. Although the use of these markers reduces antibiotics in selected populations, diagnostic uncertainty remains[9,10].
Recently, TNF-related apoptosis-inducing ligand (TRAIL) and interferon-gamma-inducible protein 10 (IP-10) were described as novel biomarkers for viral disease[11]. TRAIL is expressed in various cells of the adaptive immune system, and plays a role in the response to viral infections[12]. IP-10 has a role in the inflammation cascade in viral and bacterial disease[13].
Biomarkers focusing on both bacterial and viral disease can be combined. Low levels of PCT have been associated with the absence of bacteria. Therefore, a combination of PCT, which is TRAIL and IP-10 may be valuable for the additional diagnostic accuracy diagnosis of viral infection.

The novel biomarkers for the differentiation of viral disease may be of clinical significance. Therefore, we investigated if TRAIL and IP-10 can differentiate viral and bacterial or non-infectious causes of fever in the ED. Furthermore, we combined the results of TRAIL and IP-10 with PCT in a combined biomarker model.

METHODS

This was was a substudy of a previous study on PCT-guided therapy for febrile patients in the ED at the Slotervaart Hospital, Amsterdam, the Netherlands[14]. The local ethics committee approved the study. From May 2010 to May 2012, a total of 107 adult febrile patients (T > 38.0 °C) were included after written informed consent was obtained. From the cohort of this study, patients with a confirmed diagnosis of bacterial infection, viral infection, or non-infectious disease were selected. The patients were confined to one of the following groups. 1)Confirmed bacterial infection, defined as a positive culture result in concordance with clinical findings. 2) Confirmed viral infection, defined as positive viral PCR in concordance with clinical findings. 3) Non-infectious disease, no evidence of infectious fever despite extensive supplementary diagnostics, and a strong alternative diagnosis. Patients with both a confirmed bacterial and viral infection were excluded. In all patients, levels of TRAIL and IP-10 were determined, using Human TRAIL/TNFSF10 Quantikine ELISA Kit and Human CXCL10/IP-10 Quantikine ELISA Kit of R&D Systems according to the manufacturer's manual.

Data-analysis

A Kruskal-Wallis test was used to determine differences of levels of TRAIL and IP-10 between the three defined groups of patients. Additionally, patients with confirmed viral disease were compared with patients with confirmed non-viral disease (groups of confirmed bacterial infection and non-infectious combined). Furthermore, patients with non-infectious disease and infectious disease (groups confirmed bacterial infection and confirmed viral disease combined), using Mann-Whitney U tests. P-values of <0,05 were considered significant. The area under the ROC for TRAIL and IP-10 was calculated for confirmed viral disease versus non-viral disease. Using the ROC curves, optimal cut-offs of both TRAIL and IP-10 were determined. The value of the square of distance between point (0,1) and the ROC curve (d2) was calculated using d2 = (1-sensitivity)2 + ((1-specificity)2. The lowest value indicated the optimal cut-off. PCT, TRAIL and IP-10 results were combined in a binary logistic regression model. This combined biomarker model for discriminating viral disease used a cut-off of <0.5 μg/L for PCT, and the optimal cut-off values for TRAIL and IP-10. The area under the curve was calculated for the model of the combined biomarkers for confirmed viral disease versus non-viral disease. Data-analysis was performed using statistical package for the social sciences (SPSS) version 21, IBM corporation.

Table 1: Baseline characteristics of study population

		Confirmed viral	Confirmed bacterial	Confirmed non-infectious	p-value (one-way ANOVA)
N		13	33	8	
Female	n (%)	6 (46)	14 (42)	2 (25)	p = 0.616
Age in years	n (%)	53 (36)	65 (30)	56 (37)	p = 0.349
Temperature in ˚C	median [IQR]	38.5 (1.1)	39.0 (0.8)	38.8 (1.7)	p = 0.320
Hospitalization	n (%)	12 (93)	31 (94)	6 (75)	p = 0.257
Hospital length of stay	median [IQR]	6 (5)	7 (9)	7 (15)	p = 0.338
ICU admission	n (%)	2 (15)	6 (18)	1 (11)	p = 0.923
Mortality	n (%)	0 (0)	2 (6)	0 (0)	p = 0.532
Diabetes Mellitus	n (%)	4 (31)	7 (21)	0 (0)	p = 0.241
Immunocompromised	n (%)	1 (8)	5 (12)	1 (13)	p = 0.908
Malignancy	n (%)	1 (8)	5 (15)	4 (50)	p = 0.038
HIV	n (%)	0 (0)	2 (6)	1 (13)	p = 0.484
Steroids	n (%)	2 (15)	7 (21)	2 (25)	p = 0.860
CRP, median mg/mL	median [IQR]	107 (98)	202 (249)	96 (105)	p = 0.060
PCT, median µg/mL	median [IQR]	0.21 (0.38)	1.26 (2.55)	0.30 (0.40)	p = 0.102
TRAIL, median pg/mL	median [IQR]	51 (21)	34 (35)	8 (14)	p = 0.078
IP-10, median pg/mL	median [IQR]	1295 (611)	585 (963)	466 (1234)	p = 0.036
Leukocyte count 10^9 cells/L	median [IQR]	9.7 (5.7)	14.2 (7.0)	6.6 (7.1)	p = 0.002
Respiratory infection	n (%)	11 (85)	12 (36)		
Urinary tract infection	n (%)	0 (0)	13 (39)		
Skin infection	n (%)	1 (8)	3 (9)		
Viremia / bacteriemia only	n (%)	1 (8)	3 (9)		
Cholangitis	n (%)	none	1 (3)		
Meningitis	n (%)	none	1 (3)		

Abbrevations list: N: number. IQR: Inter quartile range. ICU: intensive care unit. HIV: human immunodeficiency virus. CRP: C-reactive protein. PCT: Procalcitonin. TRAIL: TNF-related apoptosis-inducing ligand. IP-10: interferon-γ-inducible protein 10

RESULTS

A total of 54 patients were selected; 13 patients had a confirmed viral infection, 33 a confirmed bacterial infection and 8 patients had confirmed non-infectious disease. Respiratory infection was present in 11 patients with viral disease, and in 12 patients with bacterial disease. Baseline characteristics are reported in table 1, specific pathogens of the defined groups are reported in table 2.

Table 2: Pathogens in the confirmed viral, confirmed bacterial and confirmed non-infectious disease group

Confirmed viral disease		Confirmed bacterial disease		Confirmed non-infectious disease	
Pathogen	N*	Pathogen	N**	Cause	N
Adenovirus	1	E. Coli	11	Fever without known cause, confirmed non-infectious	2
Dengue	1	H. influenzae	2	Gout	1
Herpes simplex virus	1	K. Pneumoniae	4	Hyperthyroidism	1
Influenza A virus	4	M. pneumoniae	2	Malignant neuroleptic syndrome	1
parainfluenza virus	2	P.aegurinosa	8	Tumor fever	3
Rhinovirus	5	S. agalactiae	1		
		S. aureus	2		
		S. pneumoniae	2		
		Enterococcus sp	1		
		Legionella sp	1		
		Micrococcus sp	1		
		Streptococcus sp	3		
Total	14	Total	38	Total	8

N: number. * One patient had both a confirmed adenovirus and rhinovirus infection ** Three patients had confirmed infections with two different bacterial pathogens, one patient had confirmed infection with three different bacterial pathogens.

Figure 1.

Concentration of TRAIL in pg/ml in confirmed viral, bacterial and non-infectious disease

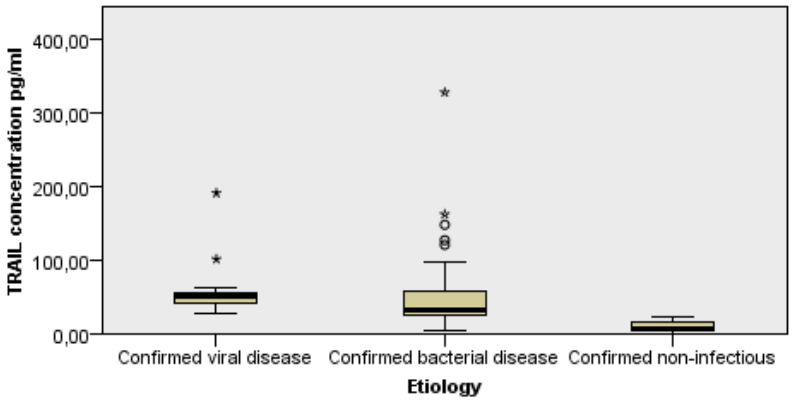
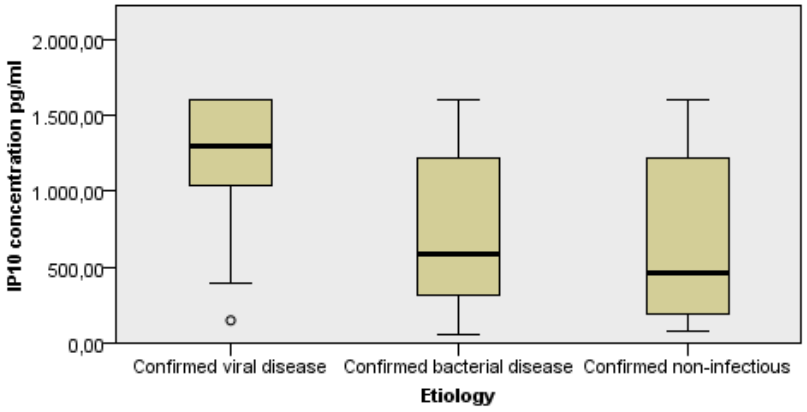


Figure 2.

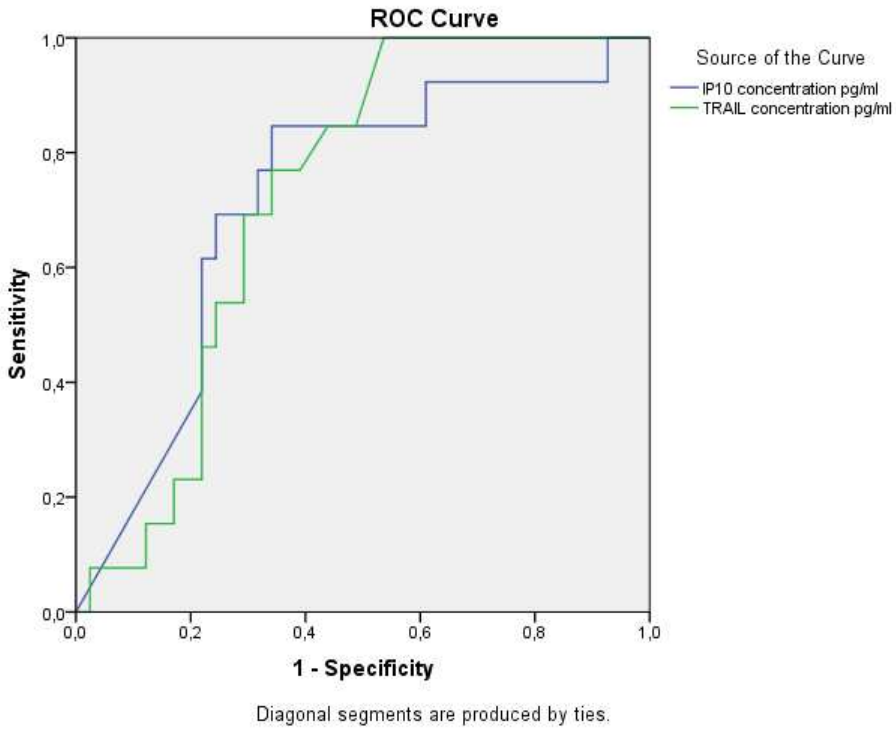
Concentration of IP-10 in pg/ml in confirmed viral, bacterial and non-infectious disease



The levels of TRAIL were significantly higher in patients with a confirmed viral infection, compared to patients with a confirmed bacterial infection and confirmed non-infectious fever ($p > 0.001$). IP-10 did not show a significant difference ($p = 0.052$). Results are shown in figure 1 and 2. TRAIL levels were not significantly different between patients with confirmed viral and confirmed bacterial infections ($p = 0.100$). IP-10 levels were significantly elevated ($p = 0.022$) in confirmed viral versus confirmed bacterial infections.

Figure 3.

Receiver-Operator-Curve of TRAIL and IP-10 in differentiation between confirmed viral disease versus non-viral disease

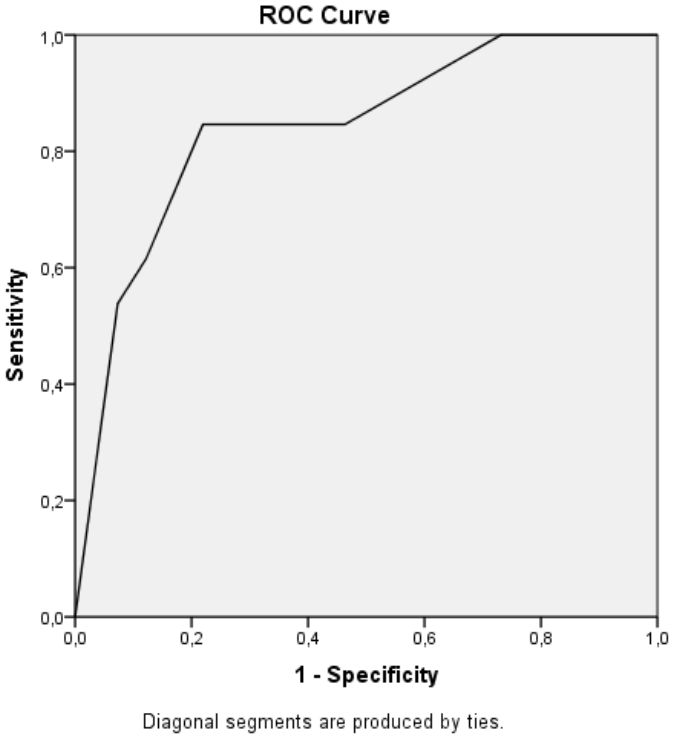


When compared between confirmed viral and confirmed non-viral disease, levels of both TRAIL and IP-10 were significantly higher in confirmed viral disease, ($p = 0.016$, and $p = 0.017$, respectively). TRAIL levels were significantly elevated in patients with confirmed infections, compared to patients with confirmed non-infectious fever, ($p < 0.000$), no significant difference between IP-10 levels was observed ($p = 0.319$). ROC for TRAIL and for IP-10 (figure 3) showed an area under the curve of 0.72 (95%CI 0.56 – 0.88) for TRAIL and 0.72 (95%CI 0.59 – 0.86) for IP-10 respectively, in the discrimination of confirmed viral disease from confirmed non-viral disease (figure 3). The optimal cut-off in differentiating confirmed viral disease from confirmed non-viral disease of TRAIL was 93.83 pg/ml, and 911.43pg/ml for IP-10.

Binary logistic regression analysis for PCT <0.5 µg/L for confirmed viral disease resulted in an odds ratio (OR) of 3.18 (95%CI 0.76 – 13.24). The AUC of PCT <0.5 µg/L for confirmed viral disease was 0.629 (95%CI 0.46 – 0.80). The combined biomarker model for confirmed viral disease versus confirmed non-viral disease consisted of a PCT level of <0.5 µg/L, a TRAIL level of >93.83 pg/ml and an IP-10 level of > 911.43pg/ml. The binary logistic regression analysis of the combined biomarker model resulted in an OR of 5.10 (95%CI 0.96 – 27.04). The AUC of the combined biomarker model was 0.84 (95%CI 0.72 – 0.97). AUC is shown in figure 4.

Figure 4.

Receiver-Operator-Curve of the combined biomarker model, consisting of optimal cut-off values of TRAIL (≥93.83 pg/ml), IP-10 (≥911.49 pg/ml) and procalcitonin (<0.5 µg/L) in differentiation between Confirmed viral disease versus non-viral disease



1

DISCUSSION

In this study of febrile ED patients, there was a strong association between elevated levels of TRAIL and IP-10 and the presence of viral infection. The combination of TRAIL and IP-10 with PCT resulted in an even higher accuracy in discriminating confirmed viral disease from confirmed non-viral disease.

Oved et al.[11] reported TRAIL levels to be lower in patients with bacterial infections. In our study, we demonstrated that this finding is reproducible in an undifferentiated cohort of ED patients. This result further strengthens the evidence that TRAIL may be utilized as a biomarker for viral disease in clinical practice. Although a significant difference in IP-10 levels between groups in our population could not be observed, a trend towards significance was shown. Larger validation studies may show the discriminative value of IP-10 in more detail.

This is the first study to report on biological markers for differentiating between confirmed viral and confirmed non-viral infection in an ED setting. These biomarkers may be helpful in ED treatment decision-making. Currently, the initiation of antibiotics in the ED is based on the rule-out of bacterial infections[2,3]. Additional viral rule-in or rule-out may further reduce the over-prescription of antibiotics in the ED. However, the clinical significance of TRAIL and IP-10 in the treatment of febrile patients is still unclear. There is a need for larger validation studies of these novel biomarkers; a larger follow-up study is currently being set-up at our institution. Most importantly, the cut-off values have to be clinically validated in order to use TRAIL and IP-10 to guide antibiotic therapy.

The findings of this study are in line with theoretically favorable characteristics of TRAIL and IP-10. The interferon-gamma (IFN-γ) pathway is activated in reaction to viral infections[15]. TRAIL is in turn upregulated by IFN alpha (IFN-α) and beta (IFN-β), and by IFN-γ, produced autocrinely by T-helper cells. TRAIL binds to the TRAIL receptor and induces apoptosis of the infected cells[12,16-18].

IP-10 is also upregulated by IFN-γ, and less elevated in bacterial infections compared to viral infections[11,19]. This in line with our findings. IP-10 is a CXC chemokine secreted by several cell types including macrophages and is induced in response to diverse stimuli, such as IFN α, β and γ ,but also directly by viruses[20]. IP10 has been shown to play an important role in the recruiting of virus-specific T-cells and viral clearance in simian varicella virus infection[21] and during acute hepatitis C infection[22].

Limitations

A selection of patients of a cohort with undifferentiated febrile patients was used. Of a total of 107 patients, there was a definitive diagnosis in 54 patients. The cut-off values found in our analyses were derived from a small sample size. The cut-off values used in this study show additional accuracy in the diagnosis of confirmed viral disease in a combined biomarker model, and thereby suggest that both TRAIL and

IP-10 are of discriminatory value. However, these cut-off values are not validated in a sufficiently large cohort and can therefore not be utilized in clinical practice yet. Notwithstanding, these findings are a proof of principle for the use of TRAIL and IP-10 in the ED. In this study, patients with either a confirmed bacterial infection, a confirmed viral infection, or confirmed non-infectious disease were included. We excluded patients with both confirmed bacterial and viral infections. In clinical practice, it may be difficult to distinguish a community-acquired pneumonia from a viral upper respiratory infection with bacterial superinfection. Our cohort was a sample of an ED population, consisting of a variety of viral and bacterial pathogens. The patients in the confirmed viral infections group mainly had respiratory tract infections, whereas the patients in the confirmed bacterial infections group had more diverse sites of infection. Besides respiratory tract infections, they also had urinary tract and skin infections. It could well be possible that different inflammatory cascades are activated in different sites of infection. This may account for the differences in TRAIL and IP-10 levels between confirmed viral and confirmed bacterial infections. However, in this clinical study, a difference between markers is shown nonetheless, and these findings advocate further research. At the moment, TRAIL and IP10 are only available as Enzyme Linked Immunosorbent Assay (ELISA), making them less suitable for clinical use in the ED. Further studies in larger ED cohorts are necessary to establish the added value of these viral infection markers in order to motivate diagnostic companies to develop immunoassays on automated immunochemistry platforms with more favorable turn-around times (i.e. < 60 minutes) making these markers more suitable for ED use.

CONCLUSION

Measurement of TRAIL and IP-10 in febrile patients in the ED may be of added value in the diagnostic process, with elevated levels indicating the presence of confirmed viral infection. The addition of TRAIL and IP-10 to PCT in differentiating between confirmed viral and confirmed non-viral disease in a combined biomarker model results in a higher discriminating value than the single biomarkers on their own. These results are a proof of principle. Validation in a larger cohort may determine the clinical value of TRAIL and IP-10 in the ED.

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PART II
ADDITIONAL BIOMARKER STRATEGIES

CHAPTER 7

IDENTIFYING PATIENTS WITH BACTERIAL INFECTIONS USING A COMBINATION OF BIOMARKERS IN THE EMERGENCY DEPARTMENT

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Submitted for publication

ABSTRACT

Background

To effectively reduce the unnecessary use of broad spectrum antibiotics in the emergency department(ED), patients with bacterial infections need to be identified accurately. We investigate the diagnostic value of a combination of biomarkers for bacterial infections CRP and PCT, together with biomarkers for viral infections, TRAIL and IP-10, in identifying bacterial infections in a general ED population with fever.

Methods

This is a sub-study in the HiTEMP cohort. Patients with fever were included during ED triage, and blood samples were obtained. Using both diagnostics and expert panel analysis, all patients were classified as having either (suspected or confirmed) bacterial, or non-bacterial disease. Using multivariable logistic regression analysis, three biomarker models were calculated, model 1:(CRP,TRAIL,IP-10), model 2:(PCT,TRAIL,IP-10) and model 3:(CRP, PCT, TRAIL,IP-10).

Results

A total of 315 patients were included, of whom 228 patients had a bacterial infection. The areas under the curve for the combined models were, for model 1: 0.730(95%CI 0.665–0.795), for model 2: 0.748 (95%CI 0.685–0.811), and for model 3: 0.767(95%CI 0.704–0.829).

Discussion

These findings show that a combination of CRP, PCT, TRAIL and IP-10 can identify bacterial infections with higher accuracy than single biomarkers and combinations of a single bacterial biomarkers combined with TRAIL and IP-10.

INTRODUCTION

Antibiotic resistance is a threat to global health[1,2]. The widespread use of broad spectrum antibiotics contributes to the selection pressure of antibiotic resistant bacteria[3,4].

Patients with suspected infections in the emergency department (ED) are often treated with broad spectrum antibiotics, because bacterial infections cannot be ruled out[5]. Currently, the diagnostic workup in EDs consists of clinical assessment and laboratory investigations such as C-reactive protein (CRP) and procalcitonin (PCT). PCT-guided therapy has successfully reduced antibiotics in selected populations of patients with respiratory complaints in the ED[6-8]. However, in a general ED population, PCT-guided therapy proved to be ineffective, due to inaccuracy of PCT in differentiating between bacterial and non-bacterial disease[9]. In order to reduce antibiotics prescriptions in a general ED population, the discrimination of bacterial from non-bacterial disease has to be as accurate as possible. Recently, studies have shown that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), and interferon-gamma induced protein-10 (IP-10), two immune response derived biomarkers, can accurately differentiate between viral and bacterial infections in the ED, both as single markers and in combination with CRP or PCT[10-12]. These study populations consisted either of young children, or had highly selected patient populations. Moreover, the combination of both CRP and PCT, together with TRAIL and IP-10, has not been investigated in an adult ED population. Furthermore, the clinical value of the combination of these biomarkers has not been fully elucidated.

The aim of this study is to investigate the predictive value of a combination of CRP, PCT, TRAIL and IP-10 in diagnosing bacterial infections in a general ED population.

METHODS

This was a sub-study of the HiTEMP study cohort, which is described previously[9,13]. In brief, the cohort of this study consisted of adult patients who visited the ED of the Erasmus University Medical Center between August 2014 and June 2016 with a temperature of ≥38.2 ˚C/ ≥100.7 ˚F in ED triage.

Study population

All adult febrile patients were eligible for inclusion. All patients gave written informed consent. Pregnant patients, patients with a solid organ transplant, severe neutropenia, or active chemotherapy, post-operative patients (up to 72 hours), and patients with a confirmed surgical diagnosis before ED triage and patients with a life expectancy of less than 24 hours were excluded. Patients who opted out of participating in additional studies after the HiTEMP study, were excluded.

Study design

In the ED, blood samples were obtained for clinical use and for additional research purposes. In all patients, CRP, PCT, TRAIL and IP-10 were determined. In this study, predictive values of three combined models of multiple biomarkers were investigated, for differentiating between bacterial and non-bacterial disease. All models contained optimal cut-off values of TRAIL and IP-10. Model 1 further included CRP, model 2 included PCT, and model 3 included both CRP and PCT.

Primary outcome

The primary outcome was the presence of either a confirmed or suspected bacterial infection, and defined as “bacterial infection”. Patients were classified in either the confirmed and suspected bacterial infections group, or the non-bacterial infections group, consisting of patients with confirmed and suspected viral infections, patients with non-infectious fever, and patients with undetermined disease, but not suspected of bacterial infection.

Confirmed infections were defined as clinically significant cultures. The presence of a coagulase negative staphylococcus (CNS) in a blood culture was deemed as contamination. Suspected bacterial infections were determined by an expert panel analysis, a structured medical chart review by two independent physicians, using predefined criteria (suppl 1). In case of disagreement, a third expert physician acted as referee. In case of the presence of both a confirmed viral and bacterial infection, the patient was classified in the bacterial infections group, because a bacterial infection was considered clinically relevant.

Data analysis

Differences in baseline characteristics were compared between patients with bac-

terial infections and patients with non-bacterial disease using Fisher's exact test for dichotomous variables and independent samples T-test for continuous variables, and Mann Whitney U test for not normally distributed continuous variables.
Accuracy of CRP, PCT, TRAIL and IP-10 for bacterial infections was reported as sensitivity and specificity and area under receiver operating characteristic curve (AUC) for optimal cut-offs of individual biomarkers. We calculated the optimal cut-off of CRP, PCT, TRAIL and IP-10 using Youden's index. For CRP and PCT, the optimal cut-off was defined as the lowest value that predicted the presence of a bacterial infection. TRAIL and IP-10 were used as a rule-out of bacterial infections. The optimal cut-off was defined as the highest value that still predicted the presence of a bacterial infection. Higher values of TRAIL and IP-10 predicted an absence of a bacterial infection. Sensitivity and specificity for CRP, PCT, TRAIL and IP-10 in diagnosing bacterial infections were reported with binominal proportion confidence intervals (CIs), using the Clopper-Pearson method. We created three multivariable binary logistic regression models to predict combined accuracy of bacterial infections. The models included the optimal cut-offs of the following biomarkers, model 1: CRP, TRAIL, IP-10, model 2: PCT, TRAIL, IP-10, and model 3: CRP, PCT, TRAIL, IP-10. An AUC for each of the models was reported. All statistical tests were two-sided with a significance level of 0.05. Data-analysis was performed with the statistical package for the social sciences (SPSS), version 23, IBM cooperation.

RESULTS

In the HiTEMP study, a total of 449 patients were included in the Erasmus University Medical Center. In this analysis, the total number of patients 315. Nine patients did not consent for additional studies other than the HiTEMP study, and in 125 patients, insufficient additional material for analysis of TRAIL and IP-10 was available. Of these 315 patients, there was no respiratory rate available in 95 patients, and in two patients no blood pressure was available because these variables were not measured in ED triage. Of all patients included in the study, 228 had either a suspected or confirmed bacterial infection. Of these 228 patients, 7 (3%) had a concomitant confirmed viral infection. Another 87 patients were not suspected of having a bacterial infection. Of these 87 patients, 10 (12%) had a confirmed viral infection, 48 (55%) had a suspected viral infection, 23 (26%) had confirmed non-infectious fever, and in 6 (7%) patients the cause of fever was unknown (Table 1).

There were statistically significant differences baseline characteristics between patients with bacterial and non-bacterial disease in age ($p = 0.00$), temperature ($p = 0.02$), malignancy as comorbidity ($p = 0.01$) and diabetes mellitus as comorbidity ($p = 0.00$). The AUC for bacterial infections for CRP was 0.679(95% CI 0.613 – 0.746), for PCT 0.680 (95% CI 0.619 – 0.742), and the ROC for ruling out bacterial infections

Table 1. Baseline characteristics

		All (n = 315)	Non-bacterial infections (n = 87)	Bacterial infections (n = 228)	P-value
Demographic characteristics					
Age	median [IQR]	58 [39 - 69]	47 [27 - 63]	61 [45 - 70]	p < 0.001
Female sex	n (%)	149 (47)	40 (46)	109 (48)	p = 0.80
Vital signs at presentation					
Temperature	median [IQR]	38.7 [38.5 - 39.2]	38.6 [38.4 - 39.1]	38.8 [38.5 - 39.3]	p = 0.15
Heart rate	median [IQR]	105 [95 - 120]	107 [90 - 120]	105 [95 - 120] n = 229	p = 0.96
Systolic bloodpressure	median [IQR] n = 313	130 [118 - 145]	128 [117 - 140] n = 85	130 [119 - 146]	p = 0.18
Diastolic bloodpressure	median [IQR] n = 313	75 [67 - 85]	75 [69 - 85] n = 85	75 [66 - 85]	p = 0.42
Respiratory rate	median [IQR] n = 220	20 [16 - 25]	24 [16 - 24] n = 63	20 [16 - 25] n = 159	p = 0.11
Comorbidity					
Diabetes	n (%)	50 (16)	5 (6)	45 (20)	p = 0.00
Malignancy	n (%)	69 (22)	10 (12)	59 (26)	p = 0.01
HIV	n (%)	16 (5)	6 (7)	10 (4)	p = 0.39
Current medication use					
Current antibiotics use (before ED vis	n (%)	42 (13)	7 (8)	35 (15)	p = 0.10
Corticosteroids	n (%)	45 (14)	16 (18)	29 (13)	p = 0.21
Oral anticoagulants	n (%)	37 (12)	6 (7)	31 (14)	p = 0.12
Acetylsalicylic acid	n (%)	32 (10)	6 (7)	26 (11)	p = 0.30
Biomarkers					
CRP in mg/L	median [IQR]	62 [19 - 142]	24 [13 - 82]	71 [28 - 161]	p < 0.001*
PCT in mcg/L	median [IQR]	0.22 [0.10 - 0.65]	0.13 [0.07 - 0.27]	0.31 [0.11 - 1.12]	p < 0.001*
TRAIL in pg/ml	median [IQR]	28.0 [0.0 - 74.5]	37.6 [0.0 - 145.0]	24.8 [0.0 - 62.4]	p = 0.00*
IP-10 in pg/mL	median [IQR]	470 [197 - 825]	774 [340 - 825]	351 [183 - 723]	p < 0.001
Clinical syndrome at presentation					
Skin	n (%)	31 (10)	1 (1)	30 (13)	
Respiratory	n (%)	120 (38)	45 (52)	75 (33)	
Urogenital	n (%)	65 (21)	0 (0)	65 (28)	
Abdominal	n (%)	35 (11)	4 (5)	31 (14)	
Central nervous system	n (%)	3(1)	0 (0)	3 (1)	
Other	n (%)	4 (1)	0 (0)	4(2)	
Noninfectious	n (%)	25 (8)	24(28)	1(0)	
Unknown	n (%)	34 (11)	13 (15)	21 (9)	
Final diagnosis after expert review					
Suspected bacterial infections	n (%)	113 (36)	0 (0)	113 (50)	
Confirmed bacterial infections	n (%)	115 (37)	0 (0)	115 (50)	
Suspected viral infections	n (%)	48 (15)	48 (55)	0 (0)	
Confirmed viral infections	n (%)	17 (5)	10 (12)	7 (3)	
Confirmed non-infectious fever	n (%)	23 (7)	23 (26)	0 (0)	
Fever of unknown etiology	n (%)	6 (2)	6 (7)	0 (0)	
Additional diagnostics					
Bacteremia	n (%)	58 (18)	1 (1)	57 (18)	

* P-values were calculated with Fisher's exact test for dichotomous variables, and independent samples T-test for continuous variables. Continuous variables that were not normally distributed, were calculated using the Mann-Whitney U test with an *. ** This posititive blood culture was a coagulase negative staphylococcus, and was considered contamination. CRP: C-reactive protein, ED: emergency department, HIV: human immunodeficiency virus, IQR: interquartile range, IP-10: interferon-gamma induced protein-10, PCT: procalcitonin

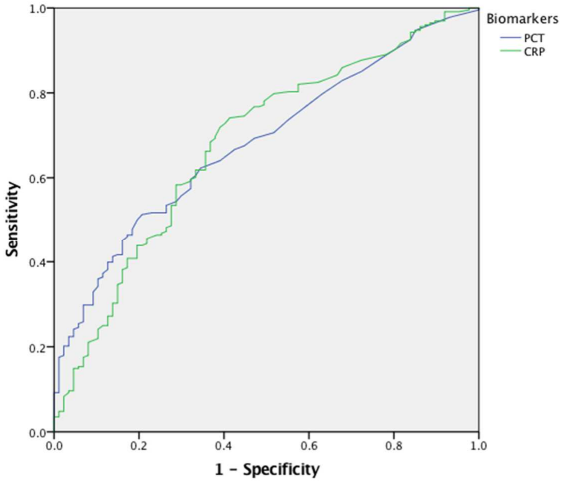
was 0.607 (95% CI 0.532 – 0.683) for TRAIL and 0.665 (0.597 – 0.734) for IP-10. The ROCs are reported in figure 1.

The optimal cut-offs, Youden's index, sensitivity and specificity were reported in table 2. In multivariable logistic regression analysis, the odds ratios (OR) of the optimal cut-offs for biomarkers for bacterial infections in model 1 were: for CRP, OR 3.07 (95% CI 1.78 – 5.31), for TRAIL OR 1.94 (95% CI 1.05 – 3.58) and IP-10 OR 2.58

Figure 1. ROC curves of CRP, PCT, TRAIL and IP10 for suspected and confirmed bacterial infections

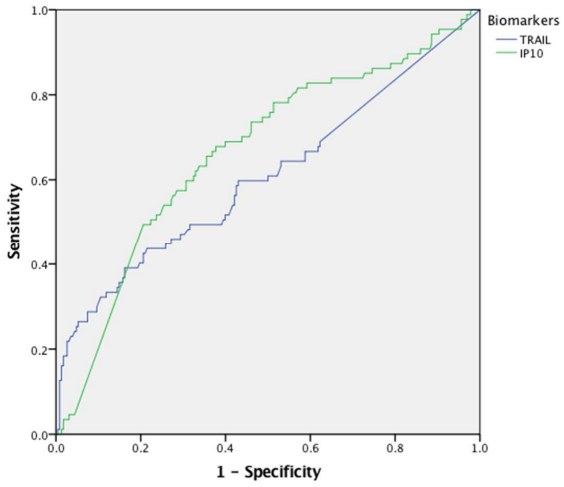
AUC of CRP and PCT for suspected and confirmed bacterial infections

CRP: AUC 0.679 (95% CI 0.613 – 0.746)
PCT: AUC 0.680 (95% CI 0.619 – 0.742)



AUC of TRAIL and IP-10 for ruling out suspected and confirmed bacterial infections

TRAIL: AUC 0.607 (95% CI 0.532 – 0.683)
IP-10: AUC 0.665 (95% CI 0.597 – 0.734)



AUC: Area under curve, CRP: C- reactive protein, IP-10: interferon-gamma induced protein-10 PCT: Procalcitonin, ROC curve: Receiver operator characteristic curve, TRAIL: tumor necrosis factor-related apoptosis-inducing ligand

Table 2. Analyses of predictive values of biomarkers for primary outcome

n = 315

Primary outcome: suspected and confirmed bacterial infections

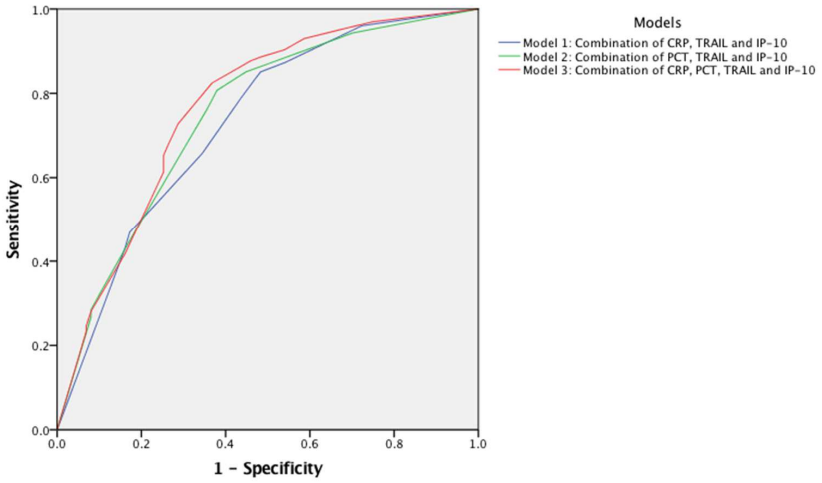
Predictors	AUC	(95% CI)	Youden's index	Optimal cut-off		Sensitivity	(95% CI)	Specificity	(95% CI)
Biomarkers									
CRP	0.679	0.613 – 0.746	0.33	32	mg/ml	0.72	0.66 - 0.78	0.61	0.50 - 0.71
PCT	0.680	0.619 – 0.742	0.31	0.30	mg/ml	0.51	0.45 - 0.58	0.79	0.69 - 0.87
TRAIL*	0.607	0.532 – 0.683	0.23	547	pg/ml	0.83	0.78 - 0.88	0.39	0.29 - 0.50
IP-10*	0.665	0.597 – 0.734	0.30	79.3	pg/ml	0.63	0.56 - 0.69	0.68	0.57 - 0.77

* Results for TRAIL and IP-10 for the abscence of primary outcome. AUC: area under curve, CI: confidence interval, CRP: C-reactive protein, ICU: Intensive care unit, mg/ml: milligrams per milliliter, PCT: procalcitonin, pg/ml: picograms per milliliter, proADM: proadrenomedullin, proET-1: proendothelin-1, qSOFA: quick SOFA score, SIRS: systemic inflammatory response syndrome, suPAR: soluble urokinase-type plasminogen

(95% CI 1.48 – 4.51).
The ORs for model 2 were: for PCT, OR 4.10 (95% CI 2.22 – 7.63), for TRAIL, OR 1.79 (95% CI 0.97 – 3.33) and IP-10, OR 3.45 (95% CI 1.94 – 6.12). The ORs for model 3 were: for CRP, OR 2.33 (95% CI 1.31 – 4.13), for PCT OR 3.30 (95% CI 1.74 -6.28), for TRAIL OR 1.56 (95% CI 0.82 – 2.95) and IP-10 OR 3.09 (95% CI 1.72 – 5.55).

The AUCs of the combined optimal cut-offs of biomarkers models for bacterial infections were, for model 1: AUC of 0.730 (95% CI 0.665 – 0.795), for model 2: 0.748 (95% CI 0.685 – 0.811), and for model 3: 0.767 (95% CI 0.704 – 0.829). The ROCs were reported in figure 2.

Figure 2. ROC curves of combined biomarker models for suspected and confirmed bacterial infections



Model 1: AUC 0.730 (95% CI 0.665 – 0.795)
Model 2: AUC 0.748 (95% CI 0.685 – 0.811)
Model 3: AUC 0.767 (95% CI 0.704 – 0.829)

AUC: Area under curve, CRP: C- reactive protein, IP-10: interferon-gamma induced protein-10 PCT: Procalcitonin, ROC curve: Receiver operator characteristic curve, TRAIL: tumor necrosis factor-related apoptosis-inducing ligand

DISCUSSION

The results of this study showed that a combined model containing optimal cut-offs of CRP, PCT, TRAIL and IP-10 predicted the presence of bacterial infections with higher probability than individual measurements of the currently used biomarkers CRP and PCT. Moreover, the model combining both CRP and PCT, together with TRAIL and IP-10, was more accurate than models with either CRP or PCT as a single marker.

A previous study by van Houten et al. showed that a combination of CRP, TRAIL and IP-10 was superior in diagnosing bacterial infections compared to PCT in young children[11]. Another study, in adult ED patients, showed that PCT in combination both TRAIL and IP-10 was more accurate in ruling in viral infections in patients with confirmed infections than individual measurements of these biomarkers. Our results are in line with these findings. Furthermore, by comparing three combined models, we showed that a combination of both CRP and PCT with TRAIL and IP-10 is superior than either individual biomarker.

In our results, we found a lower AUC than other studies that used a combination of biomarkers in differentiating between bacterial and non-bacterial disease[11,14]. These studies both used a previously described combination, called the "signature test" or "index test"[10]. This test is a logistic regression formula with predefined cut-off levels of CRP (40mg/l), TRAIL (70pg/ml) and IP-10 (500 pg/ml). Furthermore, in the index test, patients were divided into three groups, classified as either having a viral, or equivocal, or bacterial infection. The results presented in these studies showed the accuracy in differentiating bacterial from viral infections, with exclusion of the equivocal group, such as in the study by van Houten et al., who reported a AUC of 0.90 (95% CI 0.86 – 0.95). To effectively reduce antibiotics in patients with infectious diseases in the ED, bacterial infections have to be ruled-out unequivocally. When diagnostic uncertainty remains, biomarker-guided therapy is not effective9. Therefore, future prospective interventional studies should investigate if this approach, with a classification with three categories, or a category which consists of patients with a very low probability of having a bacterial infection, may reduce prescription of antibiotics in patients in this category.

An additional explanation of the differences in diagnostic accuracy between previous studies and our results, is the selection of study populations. Van Houten et al. only included children between 2 and 60 months of age, with either a suspected respiratory tract infection, or fever without source[11]. In pediatric patients, fever is most commonly the result of respiratory infections[15]. In this study, only 38% of patients had respiratory focus of fever. Differences in etiology of fever may account for a lower accuracy in our population.

Limitations

In this study, we used a cohort of the HiTEMP study[9]. The main inclusion criterion was fever. Although this is an objectively measurable variable, it created a selection bias, because patients with suspected infections without fever were excluded from participation. As in similar studies on differentiating between bacterial and non-bacterial disease, the reference standard of suspected and confirmed bacterial infections we used in our study is no gold standard[11,16]. In the structured medical chart review, one of the criteria was “clinical improvement under antibiotics”. Some of the patients who were classified using this criterion, may also have improved without antibiotics. Therefore, there may have been overestimation of the number of patients in the group of suspected bacterial infections, resulting in a lower accuracy of the combination of biomarkers. The multivariable logistic regression model with a combination of biomarkers was calculated using optimal cut-offs. The use of these binary cut-offs made the model user-friendly, at the cost of accuracy. Furthermore, this model was not validated. Therefore, we suggest a validation study of a multivariable model including biomarkers CRP, PCT, TRAIL and IP-10, with the incorporation of a group with intermediate probability of bacterial infections.

CONCLUSION

Using a combination of biomarkers CRP, PCT, TRAIL and IP-10, bacterial infections could be diagnosed with higher accuracy compared to single biomarkers or a combination of either CRP or PCT with TRAIL and IP-10, in adult patients with fever in a general ED. Interventional studies may determine the clinical value of the combination of these biomarkers.

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PART II
ADDITIONAL BIOMARKER STRATEGIES

CHAPTER 8

EARLY IDENTIFICATION OF DISEASE SEVERITY USING BIOMARKERS IN THE EMERGENCY DEPARTMENT

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Submitted for publication

ABSTRACT

Objective

In the emergency department(ED), it is important to identify patients with sepsis early, in order to start optimal treatment. Risk stratification in the ED is performed with clinical scores SIRS and qSOFA. The objective of this study is to compare the predictive values of SIRS and qSOFA with biomarkers C-reactive protein(CRP), procalcitonin(PCT), proadrenomedullin(proADM), pro-endothelin-1(proET-1) and soluble urokinase-type plasminogen activator receptor(suPAR) for prediction of intensive care unit(ICU) admission and 30-days and 90-days mortality.

Methods

Post-hoc analysis of the HiTEMP study, a multicenter study in a general ED population with fever. Patients were followed-up for 90 days. In all patients, blood samples for biomarker analysis were obtained in the ED. Single-center study, Erasmus University Medical Center, Rotterdam, the Netherlands. All adult patients who visited the ED with fever were eligible. Exclusion criteria were: no written informed consent, pregnancy, solid organ transplant, severe neutropenia, or current chemotherapy, post-operative patients, and moribund patients. Only patients with complete 90-days follow-up were included in analysis.

Results

A total of 353 patients were included in the study. Nine patients were admitted to the ICU, 9 patients died within 30 days, and a total of 13 within 90 days. For ICU admission, clinical scores had similar predictive values as biomarkers, (AUC(95%CI) of SIRS 0.727 (0.542-0.912), qSOFA 0.688 (0.499-0.876), CRP 0.528 (0.322-0.734),PCT 0.570 (0.352 - 0.788), proADM 0.730 (0.614-0.846), proET-1 0.773 (0.660-0.886), suPAR 0.672(0.488-0.857), respectively). For both 30-days and 90-days mortality, biomarkers outperformed clinical scores, (AUC(95%CI) for 30-days mortality: SIRS 0.522 (0.317-0.726), qSOFA 0.518 (0.312-0.723), CRP 0.742 (0.610-0.873), PCT 0.668 (0.481-0.855), proADM 0.836 (0.743-0.930), proET-1 0.945 (0.896-0.993), suPAR 0.842(0.685-1.000), respectively).

Conclusion

Biomarkers proADM, proET-1 and suPAR, and to a lesser extent CRP and PCT were more accurate in predicting mortality than clinical scores SIRS and qSOFA. ProADM, proET-1 and suPAR and clinical scores predicted ICU admission with comparable accuracy. Biomarkers can be used for timely diagnosis of a severe course of disease in sepsis.

INTRODUCTION

Sepsis is a global health problem causing high rates of intensive care unit (ICU) admissions and mortality[1,2].
In the emergency department (ED), patients who are at risk for developing a severe course of disease need to be identified early, so timely treatment can be initiated, and patients will receive optimal sepsis care[3,4].
Sepsis is a multi-system disease, with involvement of different inflammatory pathways, the coagulation system and the endothelial system[5,6]. Currently, physicians in the ED classify disease severity with clinical scores, such as the systemic inflammatory response syndrome (SIRS) criteria and with the quickSOFA (qSOFA) score[3,7]. These clinical scores have fair accuracy for adverse outcome prediction8. However, these clinical scores mainly include vital signs, and have limited predictive value in specific patient groups, such as the elderly[9].
Biomarkers can predict disease severity by indicating the state of activation of pathways in different systems, even before patients have abnormal vital parameters. Procalcitonin (PCT) can be used as a prognostic marker for severity of sepsis in the ED and ICU[10,11]. Indicators of activation of the microvascular system and endothelial dysfunction, mid-regional proadrenomedullin (proADM), pro-endothelin-1 (proET-1) and soluble urokinase-type plasminogen activator receptor (suPAR), a biomarker for activation of inflammatory systems, can predict mortality in patients with community acquired pneumonia and in patients with sepsis[12-16].
Despite the multi-system involvement in sepsis, a comparison of the predictive value of clinical scores and biomarkers of both inflammatory activity and endothelial dysfunction has not been studied extensively in a general ED population.

The aim of this study is to determine and compare the predictive value of clinical scores with single measurements of biomarkers CRP, PCT, proADM, proET-1 and suPAR on ICU admission and all-cause 30-days and 90-days mortality in a general ED population.

METHODS

This study was a post-hoc study of the HiTEMP (Higher diagnostic accuracy and cost-effectiveness using procalcitonin in the treatment of emergency medicine patients with fever) study cohort, which has been described previously[11,17]. In brief, the cohort of this study consists of adult patients who visited the ED of the Erasmus University Medical Center between August 2014 and June 2016 with a temperature of ≥38.2 ˚C/ ≥100.7 ˚F in ED triage.

Study population

All adult febrile patients were eligible for inclusion. All patients gave written informed consent. Pregnant patients, patients with a solid organ transplant, severe neutropenia, or current chemotherapy, post-operative patients (up to 72 hours), and patients with a confirmed surgical diagnosis before ED triage and patients with a life expectancy of less than 24 hours were excluded. In this post-hoc analysis only patients with a complete follow-up were included, to ensure validity of data on mortality.

Study design

In the ED, blood samples were obtained for clinical use and for additional research purposes. Patients were followed up after 30 days by a telephone interview, after 90 days by contacting their general practitioner and with medical chart review.

Outcomes

The primary outcomes of the study were ICU admission within 30 days after ED visit, and mortality within 30 and 90 days after ED visit.

Measurements

In all patients, we calculated a SIRS and qSOFA score. In case of missing values in vital parameters, we set the missing values to a non-divergent value, so the patients would not score an extra point on the clinical score. The assumption was that the triage nurse would have reported all vital parameters if an abnormal value was expected. Furthermore, in all patients, CRP, PCT, proADM, proET-1 and suPAR levels were measured from blood samples obtained in the ED. CRP and PCT measurements were performed on the routine analyzer of the clinical chemistry laboratory (using an electro-chemiluminiscent immunoassay (ECLIA) (Roche diagnostics, Brahms, Henningsdorf, Germany). ProADM and proET-1 measurements were performed on a Kryptor Compact Plus in Thermofisher laboratories, Henningsdorf, Germany. ELISA SuPAR measurements (Virogates, Denmark) were performed in NUTOPI laboratories, Poznan, Poland.

Data analysis

Using vital parameters measured at ED admission, we calculated the SIRS and qSOFA clinical scores. For the primary outcomes, baseline differences and differences in qSOFA, SIRS and biomarkers were analyzed using the Fisher's exact test for dichotomous variables, with the independent T-test for normally distributed continuous variables, and with the Mann-Whitney U test for non-normally distributed variables. For the outcomes ICU admission and 30-days and 90-days mortality, receiver operator characteristic (ROC) curves were used to calculate the area under the curve (AUC) for each biomarker and the SIRS and qSOFA severity scores. We calculated the optimal cut-off for each biomarker with Youden's index[18]. The optimal cut-offs were used to calculate sensitivity and specificity for the primary outcomes, binomial proportion confidence intervals (CIs) were calculated using the using the Clopper-Pearson method. All statistical tests were two-sided with a significance level of 0.05, unless otherwise specified. Data-analysis was performed with the statistical package for the social sciences (SPSS), version 23, IBM cooperation.

RESULTS

Of a total of 449 patients who were included, 9 patients did not give consent for additional studies, there were insufficient additional blood samples to determine all biomarkers from 68 patients, and 19 patients did not have complete 90-days follow-up. This resulted in a total number of 353 patients who were included in the analysis. Of the total cohort, 9 (3%) patients were admitted to the ICU within 30 days, of whom 8 within 72 hours after ED visit. Nine patients (3%) died within 30 days of their ED visit. Four patients died within the period of 31 and 90 days after ED visit, making the total of patients who died within 90 days 13(4%).

Baseline characteristics

Baseline characteristics are reported in table 1. In 108 patients, no respiratory rate was available, and in 2 patients no blood pressure was available because these data were not recorded in ED triage. Between patients who were admitted to the ICU, there were statistically significant differences in baseline characteristics for age ($p = 0.03$), diabetes as comorbidity ($p = 0.05$) and respiratory rate ($n = 237$) ($p < 0.001$). Between 30-days survivors and non-survivors, there were statistically significant differences in age, ($p = 0.01$), sex ($p = 0.04$), malignancy as comorbidity ($p = 0.02$) and respiratory rate ($n = 237$) ($p = 0.00$). Between 90-days survivors and non-survivors, there were statistically significant differences in age ($p = 0.01$), sex ($p = 0.03$) and malignancy as comorbidity ($p = 0.01$) (supplement 1).

Table 1. Baseline characteristics

		All (n = 353)	ICU admissions (n = 9)	30-days non-survivors (n = 9)	90-days non-survivors (n = 13)
Demographic characteristics					
Age	median [IQR]	59 [41 - 69]	68 [60 - 76]	75 [61 - 77]	72 [54 - 77]
Female sex	n (%)	163 (46)	6 (67)	1 (11)	2 (15)
Vital signs at presentation					
Temperature	median [IQR]	38.8 [38.4 - 39.2]	38.8 [38.4 - 39.5]	38.6 [38.5 - 39.0]	38.6 [38.4 - 38.9]
Heart rate	median [IQR]	105 [92 - 120]	113 [98 - 122]	110 [89 - 126]	110 [95 - 126]
Systolic bloodpressure	median [IQR] n = 351	130 [119 - 145]	127 [107 - 146]	121 [114 - 126]	121 [113 - 139]
Diastolic bloodpressure	median [IQR] n = 351	76 [67 - 85]	85 [60 - 94]	80 [72 - 86]	78 [64 - 84]
Respiratory rate	median [IQR] n = 245	20 [16 - 25]	28 [23 - 40] (n = 8)	32 [21 - 40] (n = 5)	25 [19 - 38](n = 8)
Comorbidity					
Malignancy	n (%)	73 (21)	3 (33)	5 (56)	7 (54)
Diabetes mellitus	n (%)	59 (17)	4 (44)	2 (22)	2 (15)
HIV	n (%)	11 (3)	0 (0)	0 (0)	0 (0)
Clinical scores					
SIRS	median [IQR]	3 [2 -3]	3 [3 - 4]	3 [2 - 3]	3 [2 - 3]
qSOFA	median [IQR]	0 [0-1]	1 [0 - 1]	0 [0 - 1]	0 [0 - 1]
Biomarkers					
CRP in mg/L	median [IQR]	61 [18 - 143]	101 [17 - 155]	129 [96 - 229]	122 [79 - 198]
PCT in mcg/L	median [IQR]	0.23 [0.09 - 0.82]	0.31 [0.08 - 10.09]	1.21 [0.25 - 1.76]	1.21 [0.47 - 2.33]
ProADM in nmol/L	median [IQR]	0.99 [0.70 - 1.49]	1.41 [1.20 - 2.00]	2.04 [1.42 - 3.26]	2.21 [1.65 - 3.26]
ProET-1 in pmol/L	median [IQR]	90.6 [63.6 - 127.4]	139.2 [100.5 - 239.3]	301.9 [179.1 - 573.0]	255.1 [177.0 - 485.9]
suPAR in ng/mL	median [IQR]	3.73 [2.91 - 5.46]	5.02 [3.21 - 10.39]	9.77 [5.96 - 13.69]	9.77 [5.04 - 13.69]
Etiology of fever					
Confirmed bacterial infection	n (%)	122 (35)	4 (44)	3 (33)	6 (46)
Confirmed viral infection	n (%)	28 (8)	1 (11)	0 (0)	0 (0)
Confirmed bacteremia	n (%)	64 (18)	3 (33)	1 (11)	3 (23)
Initial ED treatment					
Antibiotics in ED because of SIRS	n (%)	167 (47)	7 (78)	6 (67)	8 (62)
Antibiotic therapy started	n (%)	252 (72)	7 (78)	8 (89)	12 (92)
Disposition					
Hospital admission	n (%)	259 (73)	9 (100)	8 (89)	12 (92)
ICU admission	n (%)	9 (3)	9 (100)	3 (33)	3 (23)
30-days mortality	n (%)	9 (3)	3 (33)	9 (100)	9 (69)
90-days mortality	n (%)	13 (4)	3 (33)	9 (100)	13 (100)

CRP: C-reactive protein, ED: emergency department, HIV: human immunodeficiency virus, ICU: intensive care unit, IQR: interquartile range, PCT: procalcitonin, proADM: proadrenomedullin, proET-1: pro-endothelin-1, qSOFA: quick SOFA, SIRS: systemic inflammatory response syndrome, suPAR: soluble urokinase-type plasminogen activator receptor.

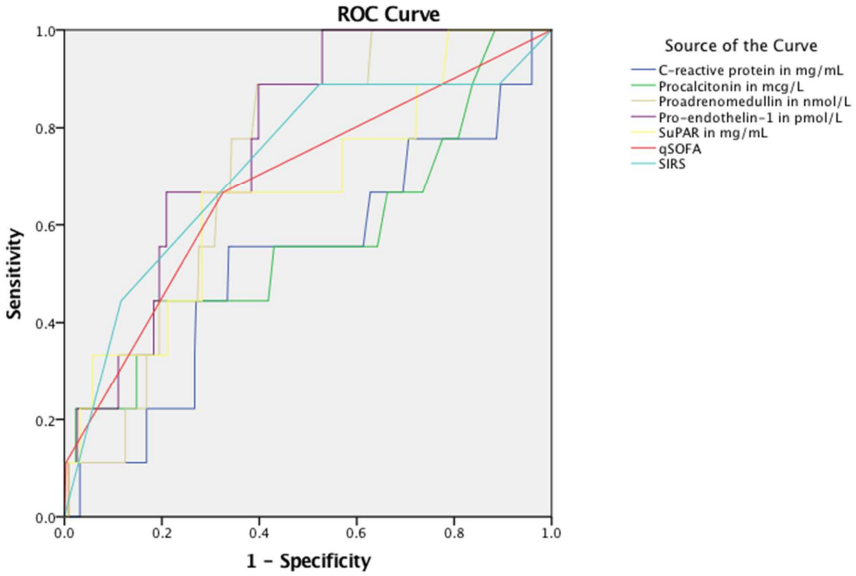
Biomarkers

Patients who were admitted to the ICU had significantly higher levels of proADM, proET-1, SIRS score, and a qSOFA score in the ED than patients who were not admitted to the ICU (proADM ($p = 0.02$), proET-1 ($p = 0.02$), SIRS (0.01), qSOFA ($p = 0.01$)). Patients who died within 30 days after ED visit, had significantly higher levels of CRP, proADM, proET-1 and suPAR than survivors (CRP ($p = 0.02$), proADM ($p = 0.01$), proET-1 ($p < 0.001$), suPAR ($p < 0.001$)).

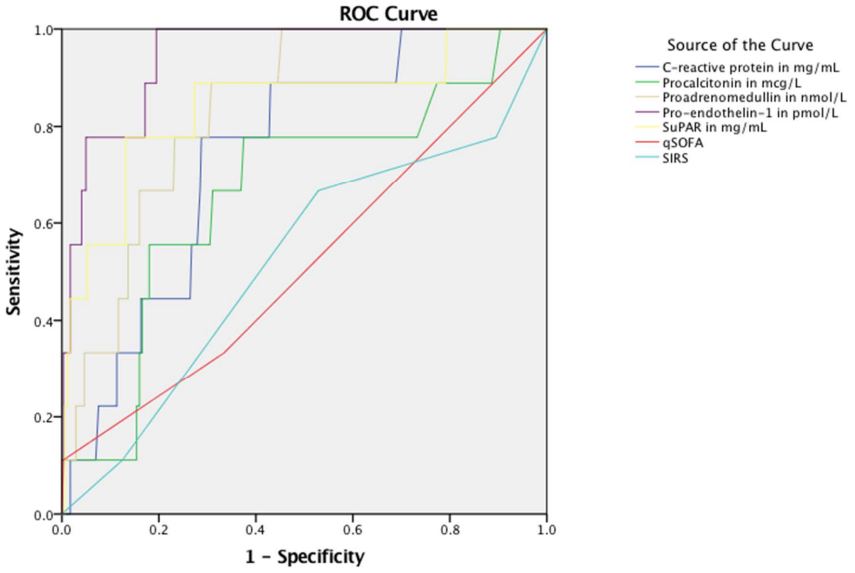
Patients who died within 90 days after ED visit, had significantly higher levels of PCT, proADM, proET-1 and suPAR than survivors (CRP ($p = 0.05$), PCT ($p = 0.01$), proADM ($p < 0.001$), proET-1 ($p < 0.001$), suPAR ($p < 0.001$) (supplement 1)). The ROC curves of the primary endpoints are reported in figure 1. For ICU admission, proADM, proET-1, and SIRS and qSOFA scores had statistically significant predictive values. For both 30-days and 90-days mortality, CRP, PCT, proADM, proET-1 and suPAR had statistically significant predictive values. The AUCs of biomarkers and clinical scores are reported in table 2.

Figure 1. ROC curves of clinical scores and biomarkers for primary outcomes

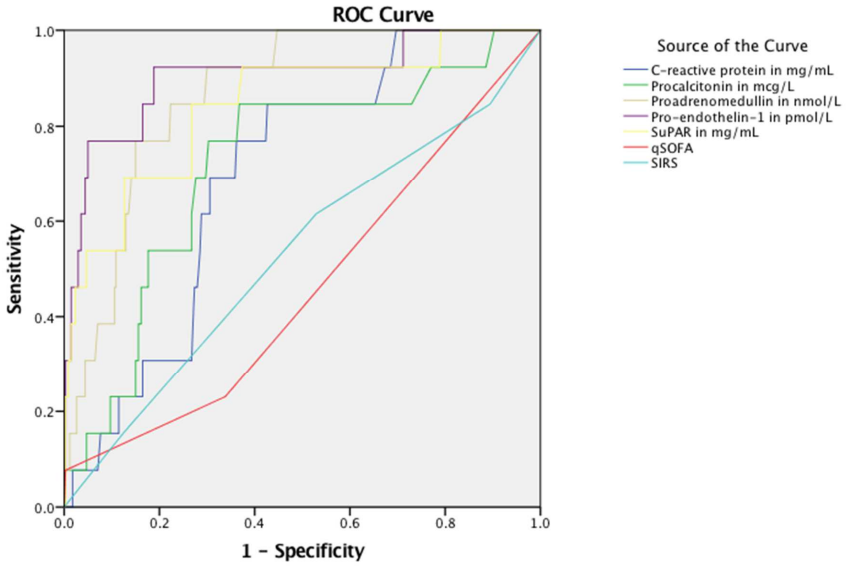
a. ROC curve of clinical scores and biomarkers for intensive care unit admission



b. ROC curve of clinical scores and biomarkers for 30-days mortality



c. ROC curve of clinical scores and biomarkers for 90-days mortality



Areas under curve are reported in table 2.

qSOFA: quick SOFA, ROC: Receiver operator curve, SIRS: systemic inflammatory response syndrome, ROC curve: Receiver operator characteristic curve, suPAR: soluble urokinase-type plasminogen activator receptor.

Optimal cut-offs and Youden's index for ICU admission, 30-days and 90-days mortality of CRP, PCT, proADM, proET-1, suPAR and SIRS and qSOFA criteria are reported in table 2.

Sensitivity and specificity of clinical scores and biomarkers for primary outcomes are reported in table 2. For ICU admission within 30 days after ED visit, clinical scores had predictive values that were comparable to the predictive values of biomarkers proADM, proET-1 and suPAR, and to a lesser extent CRP and PCT. For both 30-days and 90-days mortality, all biomarkers had higher predictive values than clinical scores.

Table 2. Analyses of predictive values of biomarkers and clinical scores for primary outcomes

ICU admission within 30 days (n = 353)									
Predictors	AUC	(95% CI)	Youden's index	Optimal cut-off		Sensitivity	(95% CI)	Specificity	(95% CI)
Biomarkers									
CRP	0.528	(0.322 - 0.734)	0.22	101	mg/ml	0.56	(0.21 - 0.86)	0.66	(0.61 - 0.71)
PCT	0.570	(0.352 - 0.788)	0.26	1.21	mg/ml	0.44	(0.14 - 0.79)	0.82	(0.77 - 0.86)
proADM	0.730	(0.614 - 0.846)	0.49	1.12	mg/ml	0.89	(0.52 - 1.00)	0.60	(0.55 - 0.66)
proET-1	0.773	(0.660 - 0.886)	0.49	99.6	mg/ml	0.89	(0.52 - 1.00)	0.60	(0.55 - 0.65)
suPAR	0.672	(0.488 - 0.857)	0.39	4.96	mg/ml	0.67	(0.30 - 0.93)	0.72	(0.67 - 0.77)
Clinical scores									
SIRS	0.727	(0.542 - 0.912)	0.37	3	criteria	0.89	(0.52 - 1.00)	0.48	(0.43 - 0.53)
qSOFA	0.688	(0.499 - 0.876)	0.34	1	criterion	0.67	(0.30 - 0.93)	0.67	(0.62 - 0.72)
30-days mortality (n = 353)									
Predictors	AUC	(95% CI)	Youden's index	Optimal cut-off		Sensitivity	(95% CI)	Specificity	(95% CI)
Biomarkers									
CRP	0.742	(0.610 - 0.873)	0.49	120	mg/ml	0.78	(0.40 - 0.97)	0.71	(0.66 - 0.76)
PCT	0.668	(0.481 - 0.855)	0.40	0.41	mg/ml	0.78	(0.40 - 0.97)	0.63	(0.57 - 0.68)
proADM	0.836	(0.743 - 0.930)	0.58	1.33	mg/ml	0.89	(0.52 - 1.00)	0.69	(0.64 - 0.74)
proET-1	0.945	(0.896 - 0.993)	0.81	136.3	mg/ml	1.00	(0.66)*	0.81	(0.76 - 0.85)
suPAR	0.842	(0.685 - 1.000)	0.65	6.90	mg/ml	0.78	(0.40 - 0.97)	0.87	(0.83 - 0.90)
Clinical scores									
SIRS	0.522	(0.317 - 0.726)	0.11	3	criteria	0.67	(0.30 - 0.93)	0.47	(0.42 - 0.52)
qSOFA	0.518	(0.312 - 0.723)	0.14	2	criteria	0.11	(0.00 - 0.48)	0.99	(0.98 - 1.00)
90-days mortality (n = 353)									
Predictors	AUC	(95% CI)	Youden's index	Optimal cut-off		Sensitivity	(95% CI)	Specificity	(95% CI)
Biomarkers									
CRP	0.698	(0.587 - 0.809)	0.42	71	mg/ml	0.85	(0.55 - 0.98)	0.57	(0.52 - 0.63)
PCT	0.720	(0.580 - 0.861)	0.48	0.41	mg/ml	0.85	(0.55 - 0.98)	0.63	(0.58 - 0.68)
proADM	0.866	(0.796 - 0.936)	0.62	1.33	mg/ml	0.92	(0.64 - 1.00)	0.70	(0.65 - 0.75)
proET-1	0.903	(0.800 - 1.000)	0.74	136.3	mg/ml	0.92	(0.64 - 1.00)	0.81	(0.77 - 0.85)
suPAR	0.842	(0.722 - 0.962)	0.58	5.00	mg/ml	0.85	(0.55 - 0.98)	0.73	(0.68 - 0.78)
Clinical scores									
SIRS	0.530	(0.361 - 0.698)	0.09	3	criteria	0.62	(0.32 - 0.86)	0.47	(0.42 - 0.53)
qSOFA	0.459	(0.297 - 0.621)	0.07	2	criteria	0.08	(0.00 - 0.36)	1.00	(0.98 - 1.00)

* one-sided 97.5% confidence interval. AUC: area under curve, CI: confidence interval, CRP: C-reactive protein, ICU: Intensive care unit, PCT: procalcitonin, proADM: proadrenomedullin, proET-1: proendothelin-1, qSOFA: quick SOFA score, SIRS: systemic inflammatory response syndrome, suPAR: soluble urokinase-type plasminogen activator receptor

DISCUSSION

The results of our study show that biomarkers proADM, proET-1 and suPAR predicted ICU admission, 30-days and 90-days mortality with good to excellent accuracy in a general ED population of patients with fever, and outperformed clinical scores in predicting mortality.

Our findings are in line with several studies on the biomarkers PCT, proADM, proET-1 and suPAR, that showed fair prognostic accuracy for ICU admission and in-hospital or 30-days mortality[10,12,16]. A meta-analysis of the predictive value of proADM in ED patients with community acquired pneumonia yielded a combined AUC of 0.76 (95 % CI, 0.72-0.80)19.

For both clinical scores SIRS and qSOFA, we found a lower predictive value for both 30-days and 90-days mortality than a recent study by Seymour et al. In the study by Seymour et al., the qSOFA score and SIRS score identified in-hospital mortality in patients with a suspected infection with an AUC of 0.76 (0.75 – 0.77) for SIRS and 0.81 (95% CI 0.80 – 0.82) for qSOFA, respectively8. Singer et al reported an AUC of qSOFA for in-hospital mortality of 0.76 (95% CI 0.73 – 0.78), in a retrospective study of a general ED population20. Contrary to these studies, our results showed that SIRS and qSOFA did not predict mortality, with a AUC for 30-days mortality for SIRS of 0.522 (95% CI 0.317 – 0.726) and for qSOFA of 0.518 (95% CI 0.312 – 0.723). These differences may be attributable to a selection bias in our study; severely ill patients who could not give written informed consent were not included. Yet, lower accuracy of qSOFA was also found in geriatric patients with suspected fever[9]. In our study, the accuracy of both SIRS and qSOFA for ICU admission was higher than the accuracy for 30-days and 90-days mortality. However, abnormal clinical scores may have prompted physicians to admit specific patients to the ICU. Because this potential influence on clinical decision making, clinical scores may have had a confounding role in the prediction of ICU admission in this study.

The difference between clinical scores and biomarkers is that biomarkers are indicators of specific activated systems and inflammatory pathways in sepsis, contrary to clinical scores, which only represent the combined end-organ effects of all activated systems in sepsis. End-organ effects, such as increased respiratory rate, tachycardia and hypotension are compensatory mechanisms, which are activated to counter organ dysfunction. Patients with sepsis who still have normal vital parameters cannot be identified using a clinical score. However, because activation of specific activated systems and inflammatory pathways can be measured using biomarkers, patients with severe course of disease can be detected earlier. In short, clinical scores are lagging behind in identifying critically ill patients. This is a crucial difference. When patients with severe disease are identified earlier, interventions can be started earlier, and end-organ failure and subsequent adverse events may be prevented.

Sepsis is a complex syndrome, and has no uniform manifestation[7]. Therefore, bio-

marker guided treatment in sepsis can be effective in selected groups of patients, but inaccurate and ineffective in a general, real-life population. In cardiology, myocardial infarction is ruled out based on measurements of a single biomarker, high sensitive troponin T, with an accuracy of >99%[21]. Consequently, troponin guided risk assessment and treatment is incorporated in international treatment guidelines[22,23]. Likewise, using the biomarkers in this study, the activity of specific pathophysiologic pathways can be identified with high accuracy. To effectively treat patients based on risk stratification tools, the accuracy of these tools needs to be unequivocal. The findings in this study are based on a small number of patients with adverse events. Hence, these findings have to be validated in a larger cohort in order to start biomarker-guided interventions.
We hypothesize that combining both biomarkers for severity of disease with clinical scores in a combined prediction model will yield a higher accuracy for predicting disease severity than individual biomarkers or clinical scores. Therefore, we suggest a combined prediction model of both the qSOFA clinical score and biomarkers CRP, PCT, proADM, proET-1 and suPAR for identifying patients who are at risk for both ICU admission and 30-days and 90-days mortality. This model needs to be validated in larger populations to determine if interventional studies are feasible. If feasible, implementation of this strategy can result in timely and accurate identification of patients who are at risk for adverse events. Consequently, end-organ failure and subsequent adverse events can be prevented, unnecessary hospital admissions and ICU admissions can be reduced, and critically ill patients receive optimal care.

Limitations
Fever as inclusion criterion was used to select patients with a suspected infection objectively, and to make the results generalizable for a real-life ED population. However, not all patients with suspected infectious diseases who visit the ED have fever. Additionally, patients in this study were required to give written informed consent in the ED. Severely ill patients with reduced consciousness were therefore not included. Hence, these factors created a selection bias in our cohort, and consequently, results of this study cannot be generalized to all ED patients with suspected infections. The respiratory rate was not reported in several patients. Because this variable is part of both the qSOFA and SIRS scores, we made the assumption that these parameters were non-divergent in the patients with missing values. If there were abnormal variables in these patients, the accuracy of the clinical scores could deviate from our results. Our population had a low number of patients who were admitted to the ICU and who had 30-days and 90-days mortality. Therefore, our results may be an overestimation of the predictive value due to the small number of patients. This study is therefore intended as a proof of concept that biomarkers can be used as early predictive indicators for mortality in a general ED population. Validation of the accuracy of the proposed model is required.

CONCLUSION

In our study, we showed that in a general ED population, biomarkers proADM, pro-ET-1 and suPAR, and to a lesser extent CRP and PCT were more accurate in predicting 30-days and 90-days mortality than clinical scores SIRS and qSOFA. ProADM, proET-1 and suPAR and clinical scores predicted ICU admission with comparable accuracy. Biomarkers can be used for timely diagnosis of a severe course of disease in sepsis.

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Supplement 1. Analysis of differences between baseline and predictor variables between groups per primary outcome

		ICU admission			30-days mortality			90-days mortality		
		Non-ICU admissions (n = 344)	ICU admissions (n = 9)	p-value	30-days survivors (n = 344)	30-days non-survivors (n = 9)	p-value	90-days survivors (n = 340)	90-days non-survivors (n = 13)	p-value
Demographic characteristics										
Age	median [IQR]	58 [40 - 69]	68 [60 - 76]	p = 0.03*	58 [40 - 69]	75 [61 - 77]	p = 0.01*	58 [39 - 69]	72 [54 - 77]	p = 0.01*
Female sex	n (%)	157 (46)	6 (67)	p = 0.31	162 (47)	1 (11)	p = 0.04	161 (47)	2 (15)	p = 0.03
Vital signs at presentation										
Temperature	median [IQR]	38.8 [38.4 - 39.2]	38.8 [38.4 - 39.5]	p = 0.81	38.8 [38.4 - 39.2]	38.6 [38.5 - 39.0]	p = 0.51*	38.8 [38.4 - 39.2]	38.6 [38.4 - 38.9]	p = 0.12*
Heart rate	median [IQR]	105 [92 - 120]	113 [98 - 122]	p = 0.61	105 [92 - 119]	110 [89 - 126]	p = 0.57	105 [92 - 119]	110 [95 - 126]	p = 0.47
Systolic bloodpressu	median [IQR]	130 [119 - 145] (n = 342)	127 [107 - 146]	p = 0.43	130 [119 - 145] (n = 342)	121 [114 - 126]	p = 0.44*	130 [120 - 146] (n = 338)	121 [113 - 139]	p = 0.64
Diastolic bloodpressu	median [IQR]	76 [67 - 85] (n = 342)	85 [60 - 94]	p = 0.57	76 [67 - 85] (n = 342)	80 [72 - 86]	p = 0.13	76 [67 - 85] (n = 338)	78 [64 - 84]	p = 0.83
Respiratory rate	median [IQR]	20 [16 - 24] (n = 237)	28 [23 - 40] (n = 8)	p < 0.001	20 [16 - 25] (n = 240)	32 [21 - 40] (n = 5)	p = 0.00	20 [16 - 25] (n = 237)	25 [19 - 38](n = 8)	p = 0.07*
Comorbidity										
Malignancy	n (%)	70 (20)	3 (33)	p = 0.40	68 (20)	5 (56)	p = 0.02	66 (19)	7 (54)	p = 0.01
Diabetes mellitus	n (%)	55 (16)	4 (44)	p = 0.05	57 (17)	2 (22)	p = 0.65	57 (17)	2 (15)	p = 1.00
HIV	n (%)	11 (3)	0 (0)	p = 1.00	11 (3)	0 (0)	p = 1.00	11 (3)	0 (0)	p = 1.00
Clinical scores										
SIRS	median [IQR]	3 [2 - 3]	3 [3 - 4]	p = 0.02	3 [2 - 3]	3 [2 - 3]	p = 0.98	3 [2 - 3]	3 [2 - 3]	p = 0.78
qSOFA	median [IQR]	1 [0 - 1]	1 [0 - 1]	p = 0.01	0 [0 -1]	0 [0 - 1]	p = 0.83*	0 [0 - 1]	0 [0 - 1]	p = 0.81
Biomarkers										
CRP in mg/L	median [IQR]	61 [18 - 143]	101 [17 - 155]	p = 0.68	59 [17 - 141]	129 [96 - 229]	p = 0.02	59 [17 - 142]	122 [79 - 198]	p = 0.05
PCT in mcg/L	median [IQR]	0.23 [0.09 - 0.77]	0.31 [0.08 - 10.09]	p = 0.47*	0.22 [0.09 - 0.75]	1.21 [0.25 - 1.76]	p = 0.09*	0.22 [0.09 - 0.74]	1.21 [0.47 - 2.33]	p = 0.01*
ProADM in nmol/L	median [IQR]	0.97 [0.68 - 1.48]	1.41 [1.20 - 2.00]	p = 0.02*	0.97 [0.68 - 1.44]	2.04 [1.42 - 3.26]	p = 0.01*	0.96 [0.68 - 1.42]	2.21 [1.65 - 3.26]	p < 0.001*
ProET-1 in pmol/L	median [IQR]	89.3 [63.0 - 124.4]	139.2 [100.5 - 239.3]	p = 0.02	89.1 [63.0 - 122.2]	301.9 [179.1 - 573.0]	p <0.001*	89.0 [62.7 - 121.1]	255.1 [177.0 - 485.9]	p < 0.001*
suPAR in ng/mL	median [IQR]	3.73 [2.89 - 5.43]	5.02 [3.21 - 10.39]	p = 0.08*	3.71 [2.89 - 5.29]	9.77 [5.96 - 13.69]	p <0.001*	3.69 [2.89 - 5.24]	9.77 [5.04 - 13.69]	p < 0.001*

P-values were calculated using the Fisher's exact test for dichotomous variables and using independent sample T-test for continouos variables, when a Mann-WIthney U test was used because variables were not normally distributed, this was indicated with an *.

PART III
CONCLUSIONS

CHAPTER 9

GENERAL DISCUSSION

Yuri van der Does MD

Aims of this thesis

The overall aims of this thesis were to investigate if biomarkers can improve early identification of bacterial infections and provide early estimation of severity of disease, and if biomarkers can be used to effectively reduce the prescription of antibiotics for febrile patients without bacterial infections in the ED.

Main results

In the emergency department (ED), biomarker-guided treatment with procalcitonin (PCT) did not result in a reduction of prescription of antibiotics to patients with fever. However, combinations of biomarkers for both viral and bacterial infections (CRP, PCT, TRAIL and IP-10) could discriminate between bacterial and non-bacterial disease with higher accuracy than individual markers and previously described combinations of biomarkers. Biomarkers (CRP, PCT, proADM, proET-1 and suPAR) could identify patients who were at risk for a severe course of illness and mortality.

PCT-guided therapy

In part I of this thesis we focused on PCT-guided therapy, a biomarker strategy to reduce antibiotic prescriptions in the ED. Because PCT-levels are generally elevated in case of bacterial infections, PCT can be theoretically used as a biomarker to discriminate between bacterial and non-bacterial disease[1]. The diagnostic value of PCT has been studied extensively, and PCT has been found to be more accurate than the conventional biomarker C-reactive protein (CRP)[2]. With antimicrobial stewardship in mind, several distinct PCT-guided therapies were designed, with different goals. In one strategy, PCT-guided therapy is used to reduce the length of antibiotic treatment. This strategy has been studied mostly in the intensive care unit (ICU), and was shown to be safe, and effective in reducing the overall exposure to antibiotics in ICU patients[3]. Another strategy of PCT-guided therapy is the guidance of initiation of antibiotics.

Initiation of antibiotics

In this thesis, we focused on initiation of antibiotics using PCT-guided therapy. Accuracy of a biomarker is only one part of a biomarker-guided therapy. Physicians make the decision to start antibiotics. Therefore, the adherence to the treatment algorithm is another important factor in the efficacy of biomarker-guided therapy. To assess the previously reported clinical value and efficacy of PCT-guided therapy, we performed a systematic review on studies that prospectively investigated efficacy. We limited the setting of the studies to the ED. Patients only spend a limited amount of time in the ED. Physicians have to make critical decisions on initiation of treatment under time constraints, with limited information of the patients' medical history and with limited availability of diagnostic modalities[4]. Because these conditions may influence treatment decisions, and because these conditions are sig-

nificantly different from primary care, medical wards and ICUs, we only compared studies from ED settings.
In our systematic review, a common finding of all studies was that PCT-guided therapy did not result in an increase in adverse events[5]. Furthermore, we found that PCT-guided therapy did reduce antibiotic prescriptions in adult patients with respiratory infections. However, in young children PCT-guided therapy did not reduce antibiotics. Additionally, several of the studies included in the systematic review reported nonadherence to study protocols. Nonadherence was not limited to ED settings, and is also reported in PCT-guided therapy studies in primary care, medical wards and ICUs[5]. Nonadherence was defined as the discrepancy between the treatment advice based on the PCT-guided advice, and the actual antibiotic treatment physicians initiated. Nonadherence could be either withholding antibiotics despite the advice to start treatment, or by starting treatment in spite of the advice to withhold antibiotics.

The practice of emergency medicine
None of the studies on PCT-guided therapy addressed a general ED population. Instead, populations were classified by age (pediatric patients) or specific complaints (respiratory complaints). Therefore, findings of these studies are not applicable to daily practice in the ED, because 1. Most patients who visit the ED are adults. 2. Most patients who have suspected infections do not have specific classified findings, such as unilateral rales on auscultation of the lungs, or a lobar infiltrate on a chest x-ray. Moreover, the incidence of bacterial infections was higher in selected populations than in a general ED population. For example, the incidence of community acquired pneumonia in the proHosp study was 68%, whereas in the study by Limper et al. the incidence of CAP in a general ED population was only 27%[6,7]. In the later study, 39% of patients did not have a confirmed diagnosis. Physicians in the ED need adequate decision-making support tools to treat all patients with suspected infections, not only patients with clearly identifiable symptoms, but also, or maybe even more so, patients with vague or indeterminate complaints. With this rationale, we started a pilot study on PCT-guided therapy in the ED.

PCT-guided therapy in a general ED population
To select patients with a suspected infection in an objective manner, we used fever as single inclusion criterion. Fever is an objectively and easily measurable vital parameter, and a fair indicator of infections[6,8]. With this criterion, we could include a heterogeneous population of patients with infectious diseases without the risk of selection of specific classes of patients, making the results generalizable to the general ED population. In the pilot study, we did not find a statistically significant reduction of antibiotic treatment with the use of PCT-guided therapy, although a trend towards reduction was observered[9]. Based on the findings in the pilot study,

we designed a larger study with a similar research question, with the acronym Hi-TEMP, for fever, or hyperthermia, which was an abbreviation of "Higher diagnostic accuracy and cost-effectiveness using procalcitonin in the treatment of emergency medicine patients with fever"[10]. In addition to the outcomes of the pilot study, we added a healthcare costs analysis, including hospital costs and societal costs. Furthermore, this was a multicenter study, in both a tertiary care academic hospital, and a non-academic hospital[11].

Based on the results of the largest ED study on PCT-guided therapy, the proHosp study, and our pilot study, our hypothesis was to find a significant reduction of prescription of antibiotics. However, to our surprise, we did not. Results of the study showed no significant difference in antibiotic prescriptions between patients who received standard care and patients who received PCT-guided therapy[12]. In our view, the explanation of this finding was attributable to another unexpected finding, namely the low accuracy of PCT for both confirmed and suspected bacterial infections compared to previous studies on PCT2.

In the HITEMP study, our goal was to design a study in which all patients with suspected infections in the ED were included, using objective inclusion criteria. Although selection bias occurred in our population on several areas, (patients with severe disease were not included because they could not give written informed consent in the ED, patients with mild disease without fever were not included because they did not meet the inclusion criterion of fever), the population of the Hi-TEMP study approximated the heterogeneity of a general ED population to a higher extend than the previous studies on PCT-guided therapy[5]. We found that the AUC of the predictive value of PCT for bacterial infections in a general ED population was 0.681 (95%CI 0.633–0.730), which was lower than AUCs of PCT for bacterial infections in previously reported in studies on specific patient groups. There was nonadherence to PCT-guided treatment advice in 49% of cases in our study12. We expect that physicians who were in a conflicting situation, in other words, when their clinical judgement was in contrast with the PCT advice, would trust on their own clinical judgement and disregard the PCT-guided treatment advice. This means that physicians implicitly compared the diagnostic value of PCT with the diagnostic value of clinical judgement. Additionally, in subsequent encounters with PCT results after a conflicting situation, physicians may have had a biased attitude, an anchor, towards PCT results. With a previous negative experience with PCT values in mind, physicians could have been even more inclined to disregard treatment advice[4,13].

Additional biomarker strategies

Our main conclusion of the HITEMP study was that PCT-guided therapy was not effective in reducing antibiotics in a general ED population. The antibiotic treatment advice, based on a single PCT measurement with a cut-off of ≥0.5µg/l, was disregarded by physicians due to conflicts of PCT-guided treatment advice with clinical

judgement. To reduce antibiotics in the ED, bacterial infections needed to be determined with higher accuracy. With this idea, we performed two studies combining PCT with TRAIL and IP-10. We hypothesized that if a patient was diagnosed with a viral infection, it was less likely that a bacterial infection was present. In the pilot study on viral biomarkers, we showed that TRAIL and IP-10 could differentiate patients with confirmed viral infections from patients with confirmed bacterial infections and non-infectious fever. However, this was a selected sample of patients with a single confirmed infection. In real-life, patients can have both a viral and bacterial infection simultaneously. Moreover, by selecting only patients with confirmed infections, there was a possibility of an overestimation of accuracy. Therefore, we determined the accuracy of a combination of biomarkers for viral and bacterial infections in the HiTEMP cohort, to approximate a general ED population. In this subsequent study, we found that the combination of CRP, PCT, TRAIL and IP-10 could differentiate between bacterial and non-bacterial disease with higher accuracy than individual biomarkers. The classification "suspected infections" was performed by two independent physicians, with a systematic approach. However, expert panel review is no gold standard for diagnosing bacterial infections. To determine the true value of this combination of biomarkers, an interventional study is needed. Before an interventional study can be initiated, the accuracy of the combination of biomarkers needs to be validated in prospective studies.

Severity of disease

In the last study in this thesis, we focused on a different outcome, severity of disease. We defined severe disease as either need for intensive care unit (ICU) admission, or mortality. In this study, we showed that an increase of biomarkers for activation of different systems in sepsis predicted ICU admission with comparable accuracy as validated clinical scores, and predicted mortality with even higher accuracy than clinical scores[14]. Because of the low number of patients who had severe disease, we could not calculate a combined prediction model with both biomarkers and clinical scores. Theoretically, a combination of multiple biomarkers for different systems that are activated in sepsis, together with clinical scores that indicate the effects of sepsis, could yield a detailed indication of disease severity of sepsis. Although an indication of disease severity in patients with suspected infections does not determine if patients have bacterial infections or not, an indication of disease severity may help physicians in treatment decisions. Clinical guidelines advise to treat patients with suspected sepsis with broad spectrum antibiotics, in order to reduce mortality[15,16]. If patients who are at risk for a severe course of illness could be identified in the ED, these patients could receive optimal treatment in an early stage. On the other hand, if patients are not at risk for a severe course of illness, antibiotic treatment could be avoided, and a watchful waiting approach could be initiated in specific patient groups. For example, such an approach has been previously proposed for pediatric

patients with uncomplicated otitis media[17]. Additionally, even hospital admissions could be reduced, if patients are not severely ill, and hence do not require clinical observation. This will eventually reduce overall healthcare costs. Although the predictive value of biomarkers for disease severity should first be validated in prospective studies before an interventional study can be performed, withholding antibiotics for not severely ill patients may be an option to reduce antibiotic prescriptions in the ED. Of course, careful consideration of inclusion criteria and acceptable consequences should be part of the study design.

Next steps

Based on the results of the studies in this thesis, we expect that combinations or sets of biomarkers are a key step to increase diagnostic accuracy in differentiation between bacterial and non-bacterial disease. In our study on the combination of biomarkers for viral and bacterial infections, we have used a single cut-off for all biomarkers in the model. However, there may be other approaches that may be more effective in ruling out bacterial infections and consequently reducing the use of antibiotics. Recent studies in pediatric patients have used the combination of CRP, TRAIL and IP-10 using the so-called "index test"[18,19]. In this test, patients have a high, low or equivocal probability for bacterial infections. This approach can be used to effectively rule-out bacterial infections in patients with low probability, and antibiotic treatment can be avoided. One of our next projects will be the design and validation of a clinical decision support tool to differentiate between bacterial and non-bacterial disease in adult ED patients, with a combination of CRP, PCT, TRAIL and IP-10. Although PCT-guided therapy was shown to be ineffective in the HiTEMP study, a combination of several biomarkers can be more accurate in differentiating between bacterial and non-bacterial disease[12,20]. In this project, we will explore different approaches, a "classical" dichotomous outcome, an approach with an equivocal group, and a numerical scoring system which indicates probability of bacterial disease. This project is the logical next step after the HiTEMP study, by a refinement of diagnostic means. The goal of this project is to reduce unnecessary prescription of antibiotics.

The second project is a further study into severity of disease. In sepsis, multiple systems eventually cause organ failure. Our hypothesis is that if we can measure activity in all these systems, together with measurement of clinical parameters, we can identify those patients who are at risk for a severe course of illness in an earlier stage, and intervene earlier. Furthermore, patients with a predicted mild course of disease may be exempt from unnecessary treatment and observation. One of the first steps of the project will be an observational study of the biomarkers proADM, proET-1 and suPAR for hospital admission, ICU admission and mortality in the ED. To specifically assess disease severity in patients with sepsis, we plan to investigate

multiple systems, such as activation of the immune system and the microvascular system, with the addition of the activation of the coagulation system. A study on ICU patients will be initiated where we will measure biomarkers before, during and after ICU admission. With this approach, we expect to gain more insight in the pathophysiology of sepsis, and to be able to quantify disease severity in an objective way. In the future, these insights may lead to early interventions that may optimize critical care, reduce healthcare costs by reducing hospital admissions, and possibly, reduce antibiotics.

Future diagnostic modalities

In this thesis, and in our newly proposed projects, the goal was, and remains, to determine the diagnostic value of indicators that can differentiate between pathogens, and can assess activity of different physiological systems. The indicators we studied were protein-based biomarkers and clinical scores. Clinical scores contain vital parameters, such as respiratory rate and blood pressure. In sepsis, abnormal vital parameters can be seen as a compensation mechanism for failing underlying systems, such as the vascular system and endothelium. Likewise, abnormal protein-based biomarkers represent a reaction to pathogen-initiated activation of specific genes that encode for these biomarkers. The so-called “omics” diagnostics (genomics, transcriptomics, proteomics and metabolomics) are diagnostic tests that utilize the human genome, and provide information on activation and inhibition of a multitude of (patho)physiological systems, on a deeper level than ever before. The omics diagnostics are part of the upcoming field of systems biology[21,22]. The translation of systems biology from laboratories to clinical practice is one of the challenges for the future.

Future treatments

Antibiotics are most common treatment for bacterial infections. However, due to the threat of antibiotic resistance and the looming post-antibiotic era, alternative treatment options should be investigated.

In this thesis, we investigated several biomarkers as indicators for bacterial and viral disease. However, these biomarkers themselves also have physiological functions. In a study in septic hamsters who were administered exogenous PCT, mortality rose significantly compared to the control group who did not receive exogenous PCT. In another experiment, septic hamsters were treated with an anti-serum to hamster PCT. The group of hamsters who received the anti-serum to PCT, and had a subsequent neutralization of PCT, had a lower mortality rate than the control group, who did not receive PCT neutralization[23]. In another study, mice with a S. pneumoniae lung infection were treated with exogenous TRAIL. The intervention group of mice had a higher survival rate than the control group[24]. These animal experiments show that there may be possibilities to target therapies on the host organism it-

self, instead of on the pathogen. However, these findings have to be validated and developed into treatments for humans first, and then tested rigorously for efficacy and safety before they can be utilized in clinical practice. A recently rediscovered, and possible future treatment is bacteriophage therapy. Bacteriophages are viruses that target specific bacteria. Bacteriophage treatment was investigated in the early twentieth century. Because of the success of antibiotics, research on bacteriophage therapy was discontinued in the Western world. Today, in a search for alternatives for antibiotics, bacteriophage therapy could be a solution to the problem of antibiotic resistance. Further well-conducted studies are required to determine the role of bacteriophage therapy[25].

Putting it in perspective

The rationale of this thesis was to find and investigate ways to address the problem of antibiotic resistance. We focused our question on how we could reduce antibiotic prescriptions to patients who did not need antibiotics, and limited our studies to a specific clinical setting, the ED. We concluded that PCT-guided therapy did not effectively reduce antibiotic prescriptions in our study setting. Hence, we investigated two strategies, one to make the differentiation of bacterial and non-bacterial disease more accurate, another to determine disease severity. For both strategies, we presented our plans for further study. This is where we are now. We have added our tiny bits of evidence and insights to the vast quantity of medical evidence in the field of infectious diseases, that started with Alexander Fleming, when he discovered penicillin. Antibiotic resistance remains a threat to global health. However, current strategies will be refined. Newer and more accurate diagnostics will become available. New treatments will be discovered. And, following the principle of natural selection, with continuous effort of physicians and scientists, with hits and misses, failures and successes, we will find a way.

Closing comments

Although the ultimate goal of is this thesis is investigating ways to combat antibiotic resistance, the practice of medicine revolves around people. The primary interest of physicians should always be their patients. Therefore, physicians who treat patients with infectious diseases have the self-proclaimed duty to critically appraise all evidence on antibiotic resistance, in order to decide what will keep their patients from harm. Moreover, physicians have to think critically about their own clinical judgement. Because eventually, it is the physician who will make the decision, whether – to treat or not to treat -.

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PART III
CONCLUSIONS

CHAPTER 10

SUMMARY OF FINDINGS

Yuri van der Does MD

SUMMARY OF FINDINGS

Part I Procalcitonin-guided therapy

Chapter 2: In this systematic review, we report on prospective studies on PCT-guided antibiotic therapy. In the six studies on adult patients with respiratory complaints, PCT-guided therapy reduced antibiotic prescriptions. In three studies on pediatric populations, there was no reduction in antibiotics, due to nonadherence to the PCT-guided therapy protocol. PCT-guided therapy did not increase adverse events. Chapter 3: In this pilot study, we randomized 106 patients with fever in the ED between PCT-guided antibiotic therapy and standard care. There was no significant reduction in antibiotics in the PCT-guided group, although a trend was noted (92% vs 80% of patients received antibiotics). The PCT-guided group had significantly less ICU admissions and mortality. We concluded that the sample size was insufficient and hypothesized that effects could be shown in a sufficiently powered study. Chapter 4: The protocol of the HiTEMP study: Higher diagnostic accuracy and cost-effectiveness using procalcitonin in the treatment of emergency medicine patients with fever. Chapter 5: In the HiTEMP study, 551 patients were included. We found no significant reduction in antibiotics in the PCT-guided group (73% vs 77%, p = 0.28). PCT-guided therapy was noninferior by means of adverse events. The accuracy of PCT ≥0.5µg/L for confirmed bacterial infections was low, with a sensitivity 0.52(95% CI 0.45–0.60) and a specificity 0.74(95% CI 0.68–0.78). However, the accuracy for confirmed bacterial infections was higher for PCT than for CRP. The costs of treatment were equal between groups, € 5386 for patients in the control group, and € 4853 for patients in the PCT-guided group, with a mean difference of -€533(95% CI -€1570 to €505). Our hypothesis was that low accuracy of PCT for bacterial infections explained the lack of effect of PCT-guided therapy.

Part II Additional biomarker strategies

Chapter 6: In this pilot study of 54 patients with confirmed infections, we showed that the blood concentrations of biomarkers TRAIL and IP-10 were significantly higher in patients with viral disease compared to patients with non-viral disease (TRAIL: p <0.001) (IP-10: p = 0.05). A combined model of PCT, TRAIL and IP-10 resulted in an area under curve (AUC) of 0.84 (95% CI 0.72 – 0.97) for identifying confirmed viral disease. Chapter 7: In this study of 315 patients from the HiTEMP cohort, we used a combined biomarker model of CRP, PCT, TRAIL and IP-10 for the prediction of suspected and confirmed bacterial infections resulted in an AUC of 0.77 (95% CI 0.70 – 0.83). Chapter 8: In a study on disease severity on 353 patients of the HiTEMP cohort, we found that the biomarkers proADM, proET-1 and suPAR predicted ICU admission with similar accuracy as the clinical score SIRS and qSOFA, and predicted mortality with a higher accuracy than the clinical scores. CRP and PCT had limited predictive value for ICU admission, and predicted mortality to a lesser extent than

proADM, proET-1 and suPAR, but more accurate than SIRS and qSOFA.

Part III Conclusions

Chapter 9: In this last part, we summarize the findings of the studies in this thesis. PCT-guided therapy did not reduce antibiotics in a general population with fever in the ED. Combinations of biomarkers (CRP, PCT, TRAIL and IP-10) could discriminate between bacterial and non-bacterial disease with higher accuracy than individual markers. Biomarkers (CRP, PCT, proADM, proET-1 and suPAR) could identify patients who were at risk for a severe course of illness and mortality.

Furthermore, two new research projects are described. The first project will continue differentiating bacterial from non-bacterial disease using the combined biomarker model of CRP, PCT, TRAIL and IP-10 in the ED. The second project will further deepen the knowledge on severity of disease in patients with suspected infections in the acute and critical care setting. The future diagnostic modalities of systems biology are addressed, and future treatments, including bacteriophage therapy, are described.

PART III
CONCLUSIONS

CHAPTER 10

NEDERLANDSE SAMENVATTING

Yuri van der Does MD

SAMENVATTING

Deel I Procalcitonine-geleide therapie

Hoofdstuk 2: In deze systematische review beschrijven we de prospectieve studies over PCT-geleide antibiotische therapie. In de zes onderzoeken in volwassen patiënten met luchtwegklachten, werd het percentage patiënten dat antibiotica kreeg kleiner door PCT-geleide therapie. In drie onderzoeken bij pediatrische patiënten was er geen afname van antibiotica, als gevolg van het niet volgen van het PCT-advies door de artsen. PCT-geleide therapie leidde niet tot meer morbiditeit of mortaliteit. Hoofdstuk 3: In deze pilotstudie hebben wij 106 patiënten met koorts op de SEH gerandomiseerd tussen PCT-geleide antibiotische therapie en standaardzorg. Er was geen significante afname van antibiotica in de PCT-geleide groep, hoewel er wel een trend werd opgemerkt (92% versus 80% van de patiënten kreeg antibiotica). De PCT-groep had statistisch significant minder IC-opnames en mortaliteit. We concludeerden dat de steekproefomvang onvoldoende was en veronderstelden dat effecten konden worden aangetoond in een onderzoek met voldoende power. Hoofdstuk 4: Dit hoofdstuk is het protocol van de HiTEMP-studie: Higher diagnostic accuracy and cost-effectiveness using procalcitonin in the treatment of emergency medicine patients with fever. Hoofdstuk 5: In de HiTEMP-studie werden 551 patiënten geïncludeerd. We vonden geen significante vermindering van antibiotica in de PCT-geleide groep (73% versus 77%, p = 0.28). PCT-geleide therapie was niet-inferieur in termen van veiligheid. De nauwkeurigheid van PCT ≥ 0,5 µg/l voor bewezen bacteriële infecties was laag, met een sensitiviteit van 0,52 (95% BI 0,45-0,60) en een specificiteit van 0,74 (95% CI 0,68-0,78). Echter, de nauwkeurigheid voor bewezen bacteriële infecties was hoger voor PCT dan voor CRP. De behandelingskosten waren gelijk voor beide groepen, € 5386 voor patiënten in de controlegroep en € 4853 voor patiënten in de PCT-begeleide groep, met een gemiddeld verschil van - € 533 (95% BI -€ 1570 tot € 505). Onze hypothese was dat een lage nauwkeurigheid van PCT voor bacteriële infecties het gebrek aan effect van PCT-geleide therapie verklaarde.

Deel II Additionele biomarker strategieën

Hoofdstuk 6: In deze pilotstudie onder 54 patiënten met bewezen infecties toonden wij aan dat de bloedconcentraties van biomarkers TRAIL en IP-10 significant hoger waren bij patiënten met een virale ziekte, in vergelijking met patiënten met niet-virale aandoeningen (TRAIL: p <0,001) (IP-10: p = 0.05). Een gecombineerd model van PCT, TRAIL en IP-10 resulteerde in een area-under-curve (AUC) van 0,84 (95% CI 0,72 - 0,97) voor het identificeren van bewezen virale ziekte. Hoofdstuk 7: In deze studie onder 315 patiënten uit het HiTEMP-cohort gebruikten we een gecombineerd biomarkermodel van CRP, PCT, TRAIL en IP-10 voor de predictie van vermoedelijke en bewezen bacteriële infecties. Dit model resulteerde in een AUC van

0,77 (95% CI 0,70 - 0,83). Hoofdstuk 8: In een onderzoek naar ernst van ziekte bij 353 patiënten van het HiTEMP-cohort, ontdekten wij dat de biomarkers proADM, proET-1 en suPAR ICU-opnames voorspelden met dezelfde nauwkeurigheid als de klinische score SIRS en qSOFA. Daarnaast voorspelden deze biomarkers mortaliteit met een hogere nauwkeurigheid dan de klinische scores. CRP en PCT hadden beperkte voorspellende waarde voor ICU-opname. CRP en PCT voorspelden mortaliteit minder nauwkeurig dan proADM, proET-1 en suPAR, maar alle biomarkers voorspelden mortaliteit nauwkeuriger dan SIRS en qSOFA.

Deel III Conclusies

Hoofdstuk 9: In dit laatste deel vatten we de bevindingen van de studies in dit proefschrift samen. PCT-geleide therapie verminderde antibiotica niet bij een algemene patiëntenpopulatie met koorts op de SEH. Combinaties van biomarkers (CRP, PCT, TRAIL en IP-10) waren nauwkeuriger in het maken van een onderscheid tussen bacteriële en niet-bacteriële ziekte dan individuele markers. Biomarkers (CRP, PCT, proADM, proET-1 en suPAR) konden patiënten identificeren die het risico liepen op een ernstige ziekte en sterfte.

Verder worden in dit laatste deel twee nieuwe toekomstige onderzoeksprojecten beschreven. Het eerste project zal verder gaan op de vraag hoe bacteriële en niet-bacteriële ziekten van elkaar onderscheden kunnen worden, met behulp van het gecombineerde biomarkermodel van CRP, PCT, TRAIL en IP-10. Deze studie zal op de spoedeisende hulp worden uitgevoerd. Het tweede project heeft als doel om de kennis over ernst van ziekte bij patiënten met infecties in de acute zorg verder verdiepen. Aan het einde van het hoofdstuk worden toekomstige diagnostische modaliteiten van systeembiologie en toekomstige behandelingen, waaronder bacteriofaagtherapie, beschreven.

APPENDICES

LIST OF PUBLICATIONS

Yuri van der Does MD

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CURRICULUM VITAE

Yuri van der Does MD

Yuri van der Does was born on August 8th, 1985 in Haaren, the Netherlands. After graduating high school (VWO, gymnasium) at Maurick college Vught in 2003, he studied medicine at Utrecht University. During his medical education, Yuri worked on a number of research projects, ranging from medical ethics (prof. dr. J.J.M. van Delden) to psychiatric epidemiology (prof. dr. N. J. de Wit). After graduation in October 2009, he worked in the emergency department of the Jeroen Bosch hospital in Den Bosch, and in the intensive care unit of Tergooi hospital in Hilversum. In 2011, he started as a junior physician in the emergency department of the Erasmus University Medical Center in Rotterdam. Here, he investigated non-invasive techniques for assessing hemodynamic status in emergency department triage (dr. P.P.M. Rood, dr. S.C.E. Klein Nagelvoort-Schuit). In January 2012, he started emergency medicine residency, at the Erasmus University Medical Center, under supervision of residency director prof. dr. P. Patka. During his residency, he started a PhD track under supervision of prof. dr. P. Patka and prof. dr. E.C.M. van Gorp. In December 2014, he finished his residency, and currently works as an emergency physician in the emergency department of the Erasmus University Medical Center.

PHD PORTFOLIO

Summary of PhD training and teaching

Name PhD student: Yuri van der Does	PhD period: 2013 - 2018
Erasmus MC Department: Emergency Medicine	Promotors: Prof. Dr. P. Patka/Prof. Dr. E. van Gorp
Research School: Erasmus University Rotterdam	Supervisors: Dr. P.P.M. Rood / Dr. M. Limper

1. PhD training	**Year**	**Workload (Hours)**
General courses		
- BROK ('Basiscursus Regelgeving Klinisch Onderzoek')	2013	22
- Didactic skills – Teach the Teacher I	2014	12
- Research Integrity	2015	14
- Statistics	2015	20
- Methodology (Principals of clinical epidemiology)	2015	20
- Biomedical English Writing and Communication	2016	35
- Didactic skills – Teach the Teacher III	2017	12
Specific courses (e.g. Research school, Medical Training)		
- Emergency medicine residency, Erasmus MC	2012 – 2014	3 years
- Generic Instructor course, SBOH	2012	20
- BKO Basis Kwalificatie Onderwijs traject (deel BKO), Erasmus MC	2013 – 2016	56
- Fellowship Management and administration, NVSHA	2015 – 2016	220
- Starting supervision in science, Erasmus MC	2017	20
Seminars and workshops		
- Regional education courses in emergency medicine residency, NVSHA	2012 – 2014	120
- National education courses in emergency medicine residency, NVSHA	2012 – 2014	28
Presentations (at conferences, see below)		
- Poster presentation, Dutch North Sea EM Conference 2013, Egmond aan zee	2013	12
- Oral presentation, International Congress of EM 2014, Hong Kong, China	2014	20
- Poster presentation, European Society of EM Conference 2014, Amsterdam	2014	12
- Workshop on research (EHBO) Dutch North Sea EM conference 2015, Egmond aan zee	2015	20
- Invited speaker, Symposium on Sepsis, Leiden University Medical Center	2016	12
- Oral presentation (2x), Dutch North Sea EM Conference 2016, Egmond aan zee	2016	12
- Invited speaker, Dutch North Sea EM Conference 2016, Egmond aan zee	2016	12
- Keynote speaker, European Society of EM Conference 2016, Vienna, Austria	2016	20
- Keynote speaker, Intensive Care and EM Conference 2017, Belo Horizonte, Brazil	2017	40
(Inter)national conferences		
- Dutch North Sea EM conference, Egmond aan zee, the Netherlands	2013	10
- International Congress of EM, ICEM2014, Hong Kong, China	2014	40
- European Society of EM conference EuSEM 2014, Amsterdam, the Netherlands	2014	10
- Dutch North Sea EM conference, Egmond aan zee, the Netherlands	2015	10
- Dutch North Sea EM conference, Egmond aan zee, the Netherlands	2016	10
- European Society of EM Conference 2016 Vienna, Austria	2016	10
- Intensive Care and EM Conference 2017 Belo Horizonte, Brazil	2017	40
- Dutch North Sea EM conference, Egmond aan zee, the Netherlands	2017	10
Other		
- Awarded Erasmus MC Efficacy grant 2013 with HiTEMP researchers, 150k for PhD track funding	2013	40

2. Teaching

	Year	Workload (Hours)
Lecturing		
- Emergency medicine for physicians treating mentally disabled patients	2013	10
- Desiderius School, advanced nursing training	2013 – 2018	30
- Students of team "acute medicine"	2013 – 2018	40
- General practice residency, lecture on altered mental status	2015 – 2018	40
- Erasmus Medical School, minor "steun- en bewegingsapparaat", lecture on dislocations	2015 – 2018	12
- Erasmus Medical School, minor "spoedeisende hulp", lecture on sepsis	2017	10
- Erasmus Medical School, minor "spoedeisende hulp", lecture on critical thinking	2017	10
- Erasmus Medical School, minor "acute medicine", lecture on coma	2017	10
- Emergency medicine residency, lectures and workshops on scientific research	2015 – 2018	40
- Emergency medicine residency, lectures on critical thinking	2018	10
Supervising practicals and excursions, Tutoring		
- Practical training in traumatology for medical students	2012 – 2016	18
- Tutoring first-year medical students	2013	28
- Systematic review minor: urgentiegeneeskunde	2015 – 2016	12
- Systematic review minor: steun- en bewegingsapparaat	2016 – 2018	18
- Systematic review minor: spoedeisende hulp	2017	12
Supervising Master's theses		
- A. Tjikhoeri – TRAIL and IP-10 in febrile emergency medicine patients	2015	56
- S. Dorst – PCT and coagulation in febrile emergency department patients	2016	56
- Drs. D. de Snoo – Emergency medicine resident – PTSS in ED personnel	2017 – 2018	30
Other		
- Instructor START class for general practitioners, SBOH	2012 – 2014	80
- Instructor Advanced Pediatric Life Support (APLS), SSHK	2013 – 2018	240
- Peer reviewer: Am. J. EM, Am. Heart J., BMJ open, the Lancet infectious diseases	2014 – 2018	100
- Member of scientific committee NVSHA	2015 – 2018	20
- Coach Viruskenner, project on virology for high school students, January - March	2016	20
- Chair of scientific committee NVSHA	2016 – 2018	60
- Chair scientific jury committee, Dutch North Sea EM conference, Egmond aan zee	2017	10
- Peer support for emergency department personnel	2018	20
	Total	1931 hours = 69 ECTS

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TO TREAT OR NOT TO TREAT

Biomarker-guided therapy for febrile patients in the **emergency department**