Biomarkers of fever: from bench to bedside

M. LIMPER

BIOMARKERS OF FEVER: FROM BENCH TO BEDSIDE

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Cover: "Quarantainehuis" in Curaçao. In 1874, after the revision of the Quarantine Law, this institution arranged for isolation of sailors suffering from yellow fever. Suspected ships were recognizable by their so-called "Yellow Jack", a yellow flag, that was required in case of suspected yellow fever cases. Yellow fever was considered one of the most contagious diseases of that time. In the "Quarantainehuis", suspected patients were incorporated for observation. In 1917, 17 years after the discovery of a vaccine against yellow fever, the building no longer had a quarantine function and was abandoned ever since.

Biomarkers of fever: from bench to bedside

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Promotores:	Prof. dr. E.C.M. van Gorp
	Prof. dr. A.J. Duits
Overige leden:	Prof. dr. P.M. van Hagen
	Prof. dr. P. Patka
	Prof. dr. A. Verbon
	Prof. dr. A.B.J. Groeneveld
	Prof. dr. D.P.M. Brandjes
	Prof. dr. T. van der Poll
Co-promotor:	Dr. M.D. de Kruif

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PART I: INTRODUCTION

CHAPTER 1

General outline of the thesis

This thesis aims to study biomarkers in inflammation and infection, with a special focus on the distinction between infectious and non-infectious fever. The thesis consists of three parts, part I being this introduction, in which the concept of fever in infectious and non-infectious disease is discussed. Furthermore, in this part we provide an overview of the epidemiology of febrile disease, as studied both in a general hospital in the Netherlands and in a general hospital in Curaçao. Also, a review of current literature on biological markers in non-infectious fever is given. Part II describes our clinical studies with focus on biomarkers in different cohorts of patients with infectious and non-infectious fever. In part III, we summarize the findings of this thesis and discuss future research.

PART I: INTRODUCTION

In this part, a general introduction to the concept and pathophysiology of fever is given. To be able to discuss the diagnostic properties of biomarkers in inflammation and infection, it is of great importance to have good insight into the prevalence of febrile disease and diagnosis; the incidence and prevalence of a certain disease influence the diagnostic test properties, such as positive and negative predictive value. In this section, we describe the epidemiology and etiology of febrile disease in the emergency department (ED) both in the Netherlands and in Curaçao. As many cases of fever do not have an infectious etiology, we review current literature on biological markers as diagnostics in patients with non-infectious fever, with special attention to the biological marker procalcitonin (PCT).

PART II: BIOMARKERS

This section focuses on biomarkers, suitable for discrimination between – bacterial - infection and non-infectious inflammation. As a model of non-infectious inflammation, we studied the prognostic value of biomarkers during the acute phase response in rheumatoid arthritis. We present two emergency department studies – one in Amsterdam and one in Curaçao – investigating the diagnostic properties of currently used and new biomarkers, such as PCT and the long pentraxin 3 (PTX3). In addition, we studied the diagnostic behavior of PCT in children with suspected H1N1-influenza during the 2009 pandemic.

PART III: SUMMARY AND APPENDICES

In this part, we summarize the most important findings of our studies and we discuss future research. Data of a prospective pilot study on the ED, using PCT as a guide for antibiotic therapy, are presented.

CHAPTER 2

Introduction and epidemiology of fever

Authors:
M. Limper, A.J. Duits and E.C.M. van Gorp

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Fever as a symptom of disease is an ancient concept, with the oldest known reference dating back to the sixth century BC, when a pictogram of a flaming brazier, symbolizing fever and the local warmth of inflammation was used in Sumerian cuneiform inscriptions[1]. Several centuries later, Hippocratic physicians proposed that body temperature and homeostasis in general was controlled by a delicate balance between four corporal humors - blood, phlegm, black bile and yellow bile -; fever was thought to be the result of an excess of yellow bile[2].

During the Middle Ages, demonic possession was believed to be causing fever. By the 18th century, after the development of improved microscopes by van Leeuwenhoek and the subsequent birth of microbiology, it was hypothesized that body heat was a result of fermentation and putrefaction in the blood[3]. In the late 19th century, metabolic bodily processes were recognized as the source of fever, as well as the fact that tight control of temperature is essential for general well-being[4]. Further research in the 20th century has shown that different endogenously produced molecules of leukocytic origin, such as interleukin 1 (IL-1), -2, 6, tumor necrosis factor (TNF) and interferons (IFN), act on the thermoregulatory center of the hypothalamus, thus eliciting fever[5].

Different definitions of fever for different purposes can be found. In clinical practice, a temperature greater than 38.0 ºC is considered fever, and fever is typically defined as a pyrogen-mediated rise in body temperature above this temperature. Physiologically, it has been defined as "a state of elevated core temperature, which is often, but not necessarily, part of the defensive responses of multicellular organisms (host) to the invasion of live (micro-organisms) or inanimate matter recognized as pathogenic or alien by the host"[6]. The elevated body temperature during fever should be distinguished from that occurring in hyperthermia. In fever, the rise in temperature is a result of well-controlled hypothalamic thermoregulation, whereas in hyperthermia the rise in body temperature is unregulated and pyrogenic cytokines are not directly involved, representing a failure of homeostasis[7].

The function of fever is still under debate, with different studies showing both potentiating and inhibitory effects of the response to infection. Phylogenetic studies have shown that fever is widespread within the animal kingdom; as the rise in temperature is metabolically expensive but still is well-preserved in evolution, it has been argued that fever has to be an adaptive and beneficial response[8]. Furthermore, animal studies have demonstrated enhanced resistance to infection during experimentally increased temperature[7]. In human *in vivo* studies associations between higher temperatures and better disease outcome have been observed[9-11]. On the contrary, it has been suggested that pyrogenic cytokines such as IL-1, IL-2 and TNF are involved in at least part of the local and systemic response to infection, with higher levels of circulating cytokines correlating with less favorable outcome. In experimental studies, the adverse effects of gram-negative sepsis or lipopolysaccharide injections were attenuated by pre-treating animals with IL-1 antagonists and anti-TNF antibodies[12, 13].

Body temperature is controlled deep in the hypothalamus, where thermosensitive receptors are affected by blood temperature, as well as via direct neural connections that measure warmth and cold in skin and muscle. In addition to the peripheral temperature input, the hypothalamus possesses an independent circadian temperature rhythm oscillating around a steady setpoint, unaffected by ambient temperature. The hypothalamus interprets peripheral temperature information and compares this with the independent circadian rhythm, resulting in peripheral heat conservation or loss of heat production. During fever, the hypothalamic setpoint is reset to a higher level while the thermoregulatory mechanisms are still maintained[5].

Substances causing fever, so-called pyrogens, can be divided into exogenous and endogenous pyrogens. Most exogenous pyrogens are microbes, toxins or other microbial products, either working directly on the hypothalamus or inducing the release of endogenous pyrogens, derived form the host's cells. Some endogenously produced molecules are also capable of inducing endogenous pyrogens, such as antigen-antibody complexes and complement factors. Endogenous pyrogens, most importantly IL-1, IL-6, TNF-α and IFN-γ, interact with brain microglia and brain endothelial receptors, thus activating the arachidonic acid pathway. This in turn results in the production of cyclo-oxygenase derived prostaglandins, prostacyclins and thromboxane. Prostaglandin E2, most notably, increases the hypotha-

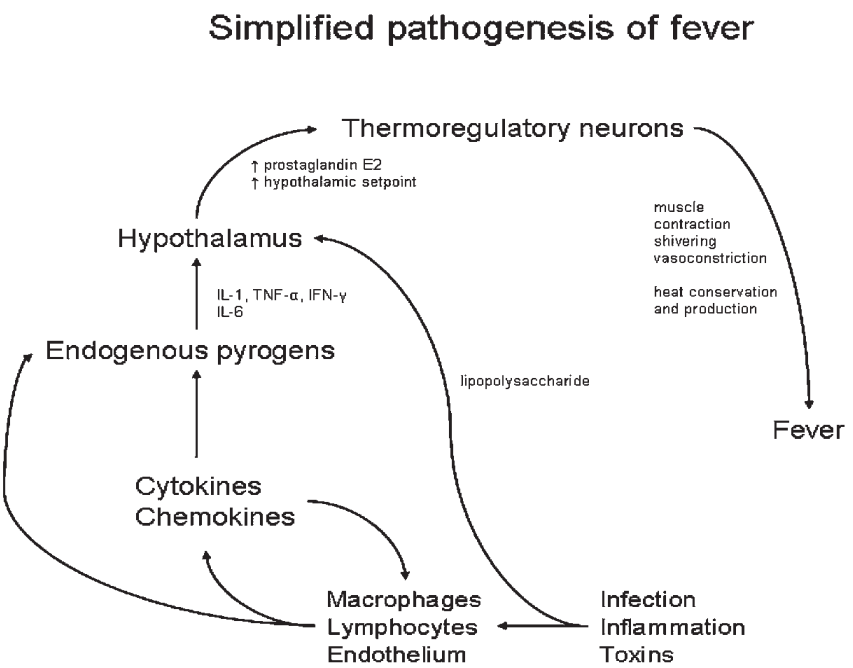

Figure 1: Simplified pathogenesis of fever

lamic thermostatic setpoint. As a result, efferent nerves are activated through sympathetic stimuli and vasoconstriction and heat conservation occurs[5, 14, 15, 16].

Febrile illness is worldwide a very frequent cause of attendance at emergency departments (EDs). Fever was the third reported complaint among most frequently reported specific principal reasons for visiting an ED in the United States in 2005, accounting for 4.4 - 7.5 % of all ED consultations and for up to 30 % in non-surgical patients[17, 18].

Although fever is often caused by bacterial, viral or parasitic infections, an elevated body temperature can be observed in many non-infectious diseases, such as auto-immune diseases, malignancies and thrombo-embolic processes. Distinction between causes of fever is clinically important, because infectious fever will be treated with antibiotics or anti-viral medication, whereas non-infectious fever might be treated with immunosuppressive drugs. Although major breakthroughs have been observed in the treatment and prevention of infections over the past decades, tools for discriminating between infectious and non-infectious causes of fever – either physical examination or supplemental diagnostics - are still very inaccurate and non-specific[19-21].

In order to gain better insight in the epidemiology of fever at the first aid department, we investigated two patient cohorts with non-surgical fever, one at a general hospital in Amsterdam, the Netherlands, and one in a general hospital in Curaçao, a subtropical South-American island close to the coast of Venezuela. In both cohorts, as expected, most patients were diagnosed with a bacterial infection and were treated with antibiotics. Infection could be confirmed in approximately 40% of the patients; approximately 80% of these patients were diagnosed with a confirmed bacterial infection. Leading causes of bacterial infection were pneumonia and urinary tract infections. Approximately 5% of patients with confirmed infection had a viral disease; parasitic or fungal disease was diagnosed in 1.4% and 10.0%, respectively. Non-infectious fever was diagnosed in 4.2% en 5.2% of patients, respectively.

Table 1: patient characteristics Slotervaart Hospital

	n = 213 **n (%) / median (IQR)**
Sex, female	111 (52.1)
Age, yrs.	66 (46 – 79)
Hospitalization	187 (87.8 %)
Admission to Intensive Care Unit	18 (8.5 %)
Mortality	9 (4.2 %)

IQR = interquartile range

Mortality in both cohorts was substantial, 4.2% and 7.9%, respectively. Patient characteristics are given in table 1 (Slotervaart Hospital, Amsterdam, the Netherlands) and table 2 (St. Elisabeth Hospital, Curaçao). Most reported diagnoses are given in figures 1 and 2.

Table 2: patient characteristics St. Elisabeth Hospital

	n = 403 n (%) / median (IQR)
Sex, female	196 (49)
Age, yrs.	52 (32 – 71)
Hospitalization	223 (55.6 %)
Admission to Intensive Care Unit	18 (4.5 %)
Mortality	32 (7.9 %)

IQR = interquartile range

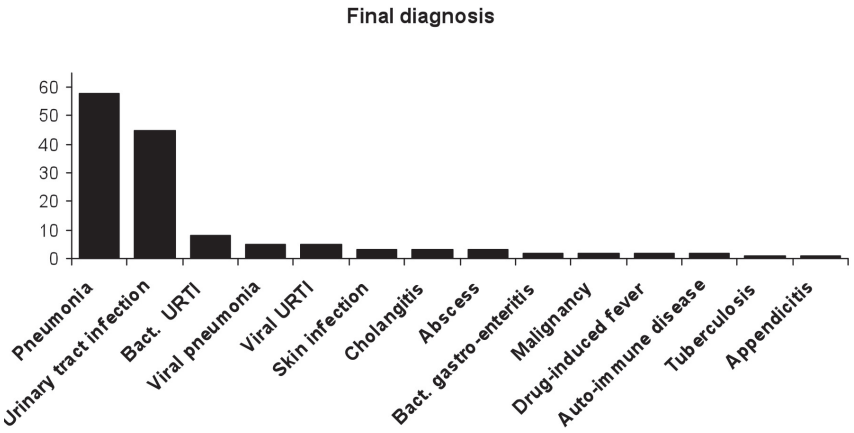

Figure 2: Most reported final diagnosis of febrile patients at the Emergency Department of the Slotervaart Hospital, Amsterdam, the Netherlands, during the year 2008 (URTI: upper respiratory tract infection)

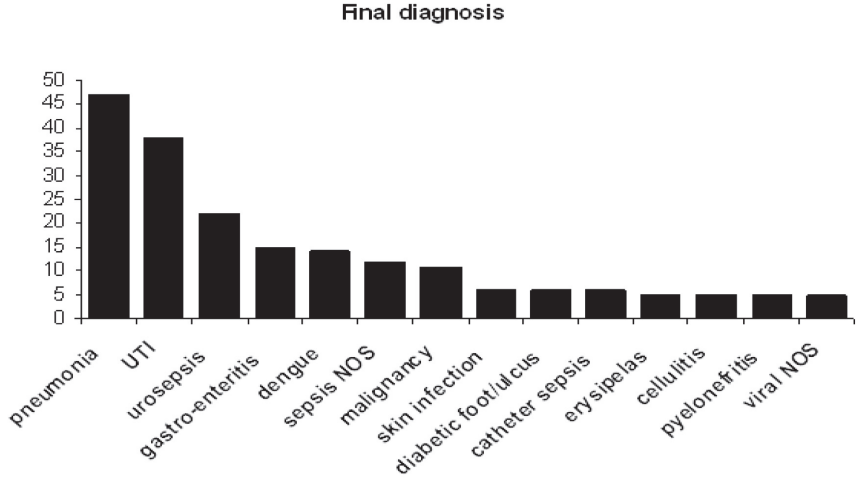

Figure 3: Most reported final diagnosis of febrile patients at the Emergency Department of the St. Elisabeth Hospital, Curaçao, during the year 2008 (UTI: urinary tract infection; NOS: not-otherwise specified)

These two epidemiological studies show that fever in patients at the ED is mainly caused by bacterial infection, but that a substantial part of patients suffer from a non-infectious febrile disease. Moreover, in these patient populations, high mortality numbers are observed. No data on incidence of non-infectious fever at the ED have been published before. The incidence of infectious fever we show, however, is comparable to that found in other studies[17, 18]; we assume that our findings can be extrapolated to other hospitals worldwide, particularly because, despite substantial differences in health care setting, population and climate, results are quite equal between the two investigated hospitals.

The search for new and better biomarkers that predict the presence of bacterial infection in febrile patients has become increasingly relevant, particularly in view of rising antibiotic resistance and medical costs worldwide. Currently used biomarkers, such as C-reactive protein (CRP) have repeatedly been shown to be not sensitive or specific enough to discern between different causes of fever[20].

Recently, use of several new biomarkers for this purpose has been studied. Among many potential markers, procalcitonin (PCT) - a prohormone of calcitonin, under physiological conditions produced by the thyroid gland - appears to be a promising and specific marker of bacterial infection in different patient groups and conditions, varying from neonatal sepsis to outpatients with respiratory complaints. As has been repeatedly shown, procalcitonin has a high specificity (ranging from 90-98%) for predicting bacterial infections and would therefore, theoretically, be suitable for the discrimination between patients with bacterial fever and patients with underlying non-infectious febrile disease[22-25].

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The diagnostic role of procalcitonin and other biomarkers in discriminating infectious from non-infectious fever

Authors:
M. Limper, MD; M.D. de Kruif, MD, PhD; A.J. Duits, PhD; D.P.M. Brandjes, MD, PhD; E.C.M. van Gorp, MD, PhD

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ABSTRACT

Fever is not only observed in the course of a bacterial or viral infection, but can be a symptom of, for instance, auto-immune, malignant or thromboembolic disease. Determining the etiology of fever in a fast and reliable way is of pivotal importance, as different causes of fever may ask for different therapies. Neither clinical signs and symptoms, nor traditional biomarkers, such as CRP, leukocytes and ESR have sufficient sensitivity and specificity to guide treatment decisions. In this review we focus on the value of traditional and newer biomarkers in non-infectious febrile diseases. Procalcitonin (PCT) seems to be the most helpful laboratory marker for the differentiation of causes of fever, particularly in autoimmune, autoinflammatory and malignant diseases.
Keywords**:** fever, biological markers, autoimmune diseases, C-reactive protein, procalcitonin.

INTRODUCTION

Fever is one of the most frequent symptoms seen in patients by both family doctors and in the emergency departments of hospitals. Fever is the third leading cause of visits to an emergency department in the United States; approximately 10% of emergency patients are prescribed antibiotics[1].

Besides infections - bacterial, viral or parasitic – there are several non-infectious medical conditions that can cause an elevated body temperature. Fever is observed in systemic diseases such as systemic lupus erythematosus and rheumatoid arthritis, in inflammatory bowel diseases, in auto-inflammatory syndromes, as a paraneoplastic phenomenon in malignancy or during neutropenia after chemotherapy, as a result of tissue loss in ischemic or thromboembolic processes, in endocrine disorders and as a result of medication.

During the past 10 years, the interest in biomarkers that discriminate between infectious and non-infectious causes of fever has grown. Moreover, the need for new and better biomarkers that predict the presence of bacterial infection in febrile patients has become increasingly relevant. ESR has now been used clinically for almost 90 years, whereas C-reactive protein (CRP) has been used routinely for the past 30. It has repeatedly been shown that the clinically often-used CRP is part of an intrinsically, non-specific acute phase reaction and is therefore not sensitive or specific enough to discern between different causes of fever. The same holds true for other traditional biomarkers. Although major breakthroughs have been observed in the treatment (antibiotics) and prevention (vaccines) of infections during the past decades, tools for discriminating between infectious and non-infectious causes of fever are still very inaccurate and non-specific[2-4]. When treatment is started, currently used laboratory markers are of limited value regarding the guidance of treatment. Finally, a new generation of biomarkers is on the verge of clinical introduction.

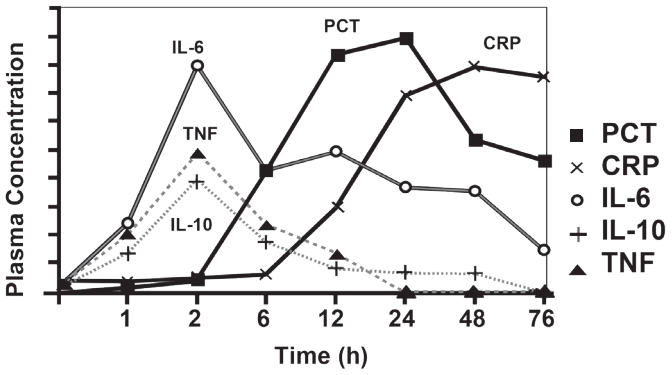

Figure 1: Time course of induction of various parameters of the systemic inflammatory system after stimulus (thoracic surgery). Concentrations are relative and adapted to the Y-axis. Figure courtesy of M. Meisner, 1999, adjusted with permission.

Discriminating between an infectious or non-infectious origin of a fever is of major importance, because the former can be treated with antibiotics, whereas the latter might call for strong, immunosuppressive drugs. An ideal biomarker would have a sensitivity and specificity of 100%, providing the treating physician with certain knowledge about the cause of the fever.

Recently, several studies focusing on new markers, have been undertaken. Procalcitonin (PCT) is such a new marker, about which a number of clinical studies have been published. PCT is a specific marker of bacterial infection in different patient groups and conditions, varying from neonatal sepsis to outpatients with respiratory complaints[5-12]. After a bacterial stimulus in healthy volunteers, PCT levels rise within 4 hours, reaching peak levels after 6 hours and maintaining a plateau through 8 and 24 hours[13] (*figure 1*).

PCT has a half-life time of approximately 24 hours, independent of renal function[14].

As has been repeatedly shown, procalcitonin in general has a high specificity (ranging from 90-98%) for bacterial infections [7, 8, 11, 15] and would therefore, theoretically, be suitable for the discrimination of bacterial fever in patients with underlying non-infectious febrile disease. However, slightly elevated PCT levels can be observed 3-24 hours after aerobic exercise[16] and not all bacterial infections give rise to elevated PCT levels[5]. Moreover, although the negative cut-off value for PCT is generally believed to be < 0.5 ng/mL, optimal cut-off points vary between different studies and patient populations. A mild increase of PCT values could be observed in some patients with an inflammatory, non-infectious condition[17].

The general departure point in the majority of earlier studies, that focus on circulating levels of biomarkers in febrile patients, is infection. In this review we describe, for the first time, the diagnostic applicability of traditional laboratory markers, procalcitonin and other promising biomarkers, taking non-infectious febrile diseases as a departure point. It is particularly within this interesting subgroup of fever patients, who, as a result of the lack of objective parameters to guide the prescribed treatment, are likely to receive antibiotics if the treating physician has reason to doubt the etiology of the fever. Biomarkers with a higher test sensitivity and specificity, regarding the discrimination between fevers with infectious and non-infectious causes, will reduce the amount of antibiotic prescriptions in this patient group and thus lead to a reduction in costs and the development of resistant bacterial strains.

The distinction between viral and bacterial infection, as well as fevers caused by the use of medication, are beyond the scope of this article.

Search terms were "Biological markers [MeSH]", "Fever [MeSH]", "C-reactive protein [MeSH]", "Blood sedimentation [MeSH]", "Procalcitonin [MeSH]", "Serum Amyloid Protein A [MeSH]", "PTX3 Protein [MeSH]", "Autoimmune Diseases [MeSH]", "Inflammatory Bowel Diseases [MeSH]", "Periodic fever syndromes", "Neoplasms [MeSH]", "Neutropenia [MeSH]", "Ischemia", "Thrombo-embolism", "Endocrine System Diseases [MeSH]", "Diagnosis" and "NOT infection". Non-English articles and non-human studies were excluded from this review.

Autoimmune and non-infectious inflammatory diseases

Autoimmune disease is defined as a disease with proof of direct transmissibility of the characteristic lesions of the disease from human to human or from human to animal – i.e., demonstrating the direct pathological effect of autoantibodies -; by indirect re-creation of human disease in an animal model; or by circumstantial evidence such as a positive family history, presence of other autoimmune phenomena in the same patient, infiltrating mononuclear cells in affected tissue, preferential use of certain MHC-II alleles, high serum levels of IgG autoantibodies, deposition of antigen-antibody complexes in affected tissue and/or improvement of symptoms with immunosuppressive therapy[18]. However, in many diseases it remains difficult to prove autoimmunity. Ongoing research has led to the insight that in some of the diseases that were originally categorized as autoimmune diseases – for instance, sarcoidosis, Behcet's disease and temporal arteritis -, the role of auto-antibodies in the pathophysiology was overestimated. These disorders are nowadays coined as non-infectious inflammatory syndromes, with the autoinflammatory disorders as a well-circumscribed subgroup.

Patients suffering from systemic diseases such as rheumatoid arthritis, systemic lupus erythematosus or vasculitides are prone to infection, particularly during immunosuppressive therapy[19]. As most auto-immune and non-infectious inflammatory diseases are characterized by fever, the differentiation of infection from active systemic disease is often difficult, but it has important clinical consequences.

For decades, rheumatologists have been using the erythrocyte sedimentation rate (ESR) as an indicator of activity of autoimmune disease and infection. However, during the past 20 or so years, the value of using ESR has been questioned, as sensitivity and specificity numbers vary strongly between different studies and diseases. ESR is not helpful in discriminating between active autoimmune disease and infection[20]. The only reliable use of ESR seems to be in the diagnostic process of temporal arteritis, where a low ESR gives a high negative predicting value[21].

CRP, although widely used as an early and sensitive marker of infection and inflammation, is not sensitive enough to discriminate between infectious episodes or the exacerbation of an underlying autoimmune disease. Although values of CRP are generally intermediate to low in patients with active SLE, it is not possible to differentiate between SLE activity and infection as a cause of fever, based on CRP alone[22-24]. Patients with SLE and serositis show elevated CRP levels. However, elevated CRP levels in SLE patients without clinical signs of serositis are generally assumed to be indicative of bacterial infection[25].

Persistent elevations of circulating IL-6 levels have been shown to be associated with outcome in different patient groups[26]. However, despite fast and relatively high peak values during acute phase response, interleukin-6 (IL-6) has no additional value over CRP in discriminating systemic infection from absence of infection in patients with SLE, as plasma IL-6 concentrations are elevated in a significant amount of patients with autoimmune diseases in the absence of infection[24].

Serum amyloid A (SAA) has the same initial kinetics as CRP in the acute phase response, with a less rapid decrease after initial elevation. Elevated serum levels of SAA have been described in a wide variety of diseases, including rheumatoid arthritis, ankylosing spondylitis, Behcet's syndrome and Crohn's disease[27].

No studies using SAA as a marker for distinction between infectious and non-infectious fever have been undertaken. Due to the shared acute phase pattern with CRP and its longer half life, it is not to be expected that SAA is a helpful marker for this purpose. SAA may be useful in the evaluation of therapy response and the determination of progression to amyloidosis in these patient groups[28-30].

PCT seems to be a promising marker in differentiating between autoimmune-induced fever and infectious-bacterial fever. Strongly elevated PCT levels have been observed in several systemic and localized bacterial infections[5, 6, 15, 24]. PCT appears to have a higher specificity for bacterial infection than other markers, although it is apparent that not all patients with bacterial infection have elevated PCT levels[5].

In a cohort of patients with Wegener's granulomatosis, elevated PCT levels were described in those suffering from bacterial infection, whereas PCT levels were normal in patients with active autoimmune disease. In the same cohort, CRP could not be used for differentiation purposes[23]. In a study in patients with active Behcet's disease, PCT values were not elevated, whereas CRP and IL-6 levels were higher, when compared to healthy controls[31]. PCT values in patients with diverse systemic autoimmune diseases accompanied by infectious fever were elevated, when compared to values in patients with systemic autoimmune diseases and non-infectious fever[32]. Circulating PCT levels in patients with early RA and reactive arthritis were shown to be normal, whereas PCT levels in patients with bacterial sepsis were elevated [33].

Recent preclinical *in vitro* studies focus on glyco-biomarkers and vascular endothelian growth factor (VEGF). In rheumatoid arthritis (RA), glyco-biomarkers have been proposed as future markers for diagnosis and prognosis. Glyco-biomarkers are sugar molecules that are an integral feature of nearly all biomolecules; they may represent a way in which immunotolerance can be bypassed. IgG-Go (IgG-agalactosyl) may be a diagnostic and prognostic marker in RA, with levels of IgG-Go exhibiting a correlation with the severity and duration of the disease. The relationship between glycosylation and infection still has to be elucidated[34].

Serum levels of VEGF correlate well with RA disease severity and swollen joint counts. VEGF might play an important part in the establishment and promotion of RA and might serve as a marker for disease activity, with a more active disease being associated with higher serum levels[35].

Autoinflammatory diseases

The autoinflammatory diseases are a heterogeneous group of hereditary syndromes characterized by seemingly unprovoked periodic episodes of inflammation without high titers of autoantibodies or antigenspecific T lymphocytes and in the absence of infection[36]. A central role for proteins with a PYRIN domain has been suggested. In normal states, pyrin

downregulates activity of a caspase recruitment domain which promotes elevated levels of IL-Iβ and NF-κB. In autoinflammatory disease, this downregulation is affected, resulting in the production of IL-Iβ and eventual inflammation[36]. Major autoinflammatory syndromes are familial Mediterranean fever (FMF), hyperimmunoglobulinemia D and periodic fever syndrome (HIDS) and tumor necrosis factor receptor superfamily 1A-associated periodic syndrome (TRAPS). Adult onset Still's disease, although in contrast with other autoinflammatory syndromes non-hereditary, associated with longer exacerbations and with possible bone and cartilage destruction, may be classified as an autoinflammatory syndrome as it leads to comparable clinical features, in the absence of evidence for a link to microorganisms or circulating auto-antibodies[37]. Similar to other chronic inflammatory diseases, yet significantly more prevalent, the most severe complication of autoinflammatory syndromes is amyloidosis, leading to end-stage renal failure[38].

Susceptibility genes in most of the autoinflammatory diseases have been identified and insight in molecular pathogenesis has improved. This has led to better diagnosis and more rational therapy, with a growing interest in biological therapy with anti-TNF and anti-IL-I agents[39]. However, only a few studies have focused on the acute phase response during exacerbations of autoinflammatory diseases, with evidence for marked elevations of CRP, the long pentraxin 3 (PTX3), serum amyloid A protein and IgD levels[40-42].

Attacks of FMF can be severe and may mimic acute abdominal syndromes such as appendicitis or pelvic inflammatory disease so closely, that abdominal surgery can be mistakenly performed. A major challenge in the treatment of FMF patients, in particular, is to distinguish FMF attacks from infectious disease. One study showed that levels of CRP and PTX3 were increased during an FMF attack, whereas PCT was elevated above normal cut-off values in only 2 out of 22 patients[43]. Thus, PCT may be a promising marker to distinguish infectious disease from sterile FMF attacks.

In a study comparing PCT values in patients with adult onset Still's disease with and without infectious fever, high levels of PCT were also observed in patients without bacterial infection, indicating that PCT is not useful for the differentiation between exacerbations of Still's disease and infection[32]. High levels of serum ferritin are a common finding in Still's disease. Glycosylated ferritin, in non-pathological conditions representing more than half of the total serum ferritin, is often very low (< 20%) in adult onset Still's disease, while the level is higher (20-50%) in other inflammatory conditions. However, also during severe systemic infections, glycosylated ferritin levels < 20% can be found[44].

Inflammatory bowel diseases

The two major inflammatory bowel diseases (IBD) are ulcerative colitis and Crohn's disease. Both diseases are treated with corticosteroids and immune modifying agents, resulting in a relatively immune compromised state. Patients with IBD are particularly susceptible to *Clostridium difficile* infection and have a four times higher chance of dying as a result of infection than patients without IBD[45].

The pathogenesis of IBD has still to be elucidated. A cascade of immunologic events occurs, resulting in increased local release of pro-inflammatory cytokines in the gut. Il-

6, IL-10 and TNF-α, in particular, have been shown to play a pivotal role in the chronic inflammatory process[46, 47]. As ulcerative colitis and Crohn's disease are different disease entities both clinically and pathophysiologically, different acute phase responses during exacerbations can be expected. However, the scarce research addressing the differentiation between exacerbations of IBD and infectious colitis does not focus on ulcerative colitis and Crohn's disease separately. Hence, in this review we are forced to consider the two diseases as one group.

As diverse cytokines are produced in large amounts during exacerbations of IBD, levels of regularly used laboratory markers such as CRP or ESR will be elevated, and they therefore possess little discriminatory value[48]. However, an accumulation of evidence points towards an association between oxidative stress and exacerbations. Markers for lipid peroxidation and antioxidant status have been shown to be higher in patients with active IBD, indicating enhanced oxidative stress and decreased anti-oxidant status[49]. There is a proven correlation between oxidative stress and the severity of sepsis; in non-septic infections the levels of oxidative stress should be low[50]. Emergency patients with bacterial infection were shown to have normal markers for oxidative stress[51]. A combination of elevated acute phase proteins and normal markers for oxidative stress suggests the presence of infection in patients with IBD, whereas an exacerbation of IBD might be indicated by elevated acute phase proteins and markers for oxidative stress. As a complicating factor, patients suffering from Chronic Granulomatous Disease (CGD) - a rare disorder caused by defects in the phagocyte NADPH oxidase resulting in the inability to generate reactive oxygen intermediates - may present with a clinical picture mimicking Crohn's disease[52], which makes a pathogenic role for oxidative stress less likely.

One study assessed the value of PCT in a cohort of 51 IBD patients and 25 patients with self-limiting colitis. All IBD patients exhibited low levels of PCT in serum, independent of disease activity, whereas patients with infectious colitis were shown to have elevated PCT values. In this study the positive, predictive value for diagnosing infectious colitis was 96%, with a negative predictive value of 93%[53].

Malignant disorders

Neoplasms contribute to between 7-20% of the cases of fever of unknown origin[54, 55]. Fever is particularly associated with non-Hodgkin lymphoma, leukemia, renal cell carcinoma and hepatocellular carcinoma. Although the pathophysiology of neoplastic fever is not well understood, it is suspected to be cytokine mediated, with emphasis on IL-1, IL-6, TNF-α and interferon. It has been suggested that tumor cells produce pyrogens; another proposed explanation for neoplastic fever is the release of TNF by necrotic bone marrow. In neutropenic patients receiving chemotherapy, the discrimination between neoplastic fever and infectious fever is essential[55].

The value of CRP in differentiating infectious from non-infectious fever in neutropenic patients has been studied extensively. It has repeatedly been shown that CRP is an unreliable marker of bacterial infection in this patient group[56-58].

A recent systematic review identifies PCT as a valuable tool in determining the etiology of fever in neutropenic patients, with elevated levels of circulating PCT in patients with bacterial fever[10]. One study comparing the value of PCT, neopterin, CRP, IL-6 and IL-8 as diagnostic markers for this purpose showed a high, negative predictive value of PCT for Gram-negative bacteremia and non-significant results for the other markers[9]. It has been argued that a risk-assessment model, including clinical parameters together with laboratory markers such as PCT or IL-8, should be constructed, thus enabling physicians to accurately define a low-risk group of febrile neutropenic patients[59].

Ischemic diseases

During the first days after myocardial fever, low grade fever is a common observation. A relationship between the extent of necrosis and the rise in temperature has been shown[60, 61]. Animal studies indicate that elevated corporal temperature might be harmful prior to and directly after an acute coronary syndrome or myocardial infarction[62]. Fever after myocardial tissue loss occurs due to ischemic cell death, resulting in the production of cytokines. TNF, a very potent inflammatory mediator, has been shown to be present to an excessive degree in plasma during this process[63].

In patients who have suffered an acute stroke, fever is also a common observation. Although approximately 25% of patients who have suffered an acute stroke and fever suffer from concomitant infection, about 15% of patients have a fever without a recorded infection. Fever in this patient group is associated with poor outcome and severe cerebrovascular damage. A correlation between cerebral tissue loss and fever has been noted. The only discriminating characteristic between the infectious and non-infectious causes of fever is the early onset of fever in non-infectious patients[64, 65].

No clinical studies, investigating the role of biomarkers in differentiating between infectious and non-infectious etiology of fever in patients suffering from myocardial infarction or acute stroke, have been undertaken.

Fever is observed in a substantial number of patients with pulmonary embolism (PE). The exact etiology of this fever is unclear. However, similar cytokine cascades as in true ischemic conditions may be expected[66]. Based on ESR and leukocyte counts, no differentiation between PE and community acquired pneumonia can be made[67]. One study assessed the value of PCT in patients with possible PE. Out of 40 patients with fever and clinical and radiological findings consistent with pleuritis or pneumonia, 10 patients were diagnosed with PE and 30 with pneumonia. But while high CRP levels were measured in these 10 patients, PCT levels were normal[68]. These findings need to be confirmed within the context of a larger trial.

Endocrine disorders

Phaeochromocytoma is classically associated with spiking fever. In some phaeochromocytomas, a central role for IL-6 can be observed. Based on laboratory markers, phaeochromocytoma may be difficult to distinguish from an abscess, for instance, as the elevation of IL-6

is followed by a general inflammatory reaction. High levels of leukocytes, fibrinogen and CRP will be observed[69].

In acute adrenal insufficiency, fever is one of many aspecific findings. It is unclear whether this fever is indicative of general inflammation or whether it is due to the precipitating infection that is most often the direct cause of adrenal insufficiency[70]. As most cases occur after infection, and patients are likely to develop severe shock, initial empirical antibiotic treatment is warranted. No studies that try to discriminate between infectious and non-infectious causes of acute adrenal insufficiency have been undertaken. This is because treatment should not be delayed while diagnostic tests are performed.

To our knowledge, procalcitonin has not been evaluated in endocrine disorders.

Conclusion

In this review we have described the value of commonly used biomarkers, procalcitonin, and other experimental markers in patients with non-infectious fever. Fever is a very common symptom of many diseases, reflecting general inflammatory processes in the body that are not necessarily caused by infection. The proper treatment of febrile patients requires adequate and quick diagnoses. Laboratory markers with high sensitivity and specificity regarding the differentiation between infectious and non-infectious causes of fever may

Table 1: Differential diagnosis of non-infectious febrile diseases and relative values of CRP and PCT during steady state, exacerbation of underlying disease and bacterial infection (no change from baseline indicated by "=", relative elevation from baseline indicated by "↑" or "↑↑", insufficient data indicated by "??")

	Steady state		Exacerbation		Bact. infection	
Auto-immune/systemic	**CRP**	**PCT**	**CRP**	**PCT**	**CRP**	**PCT**
· RA	=	=	↑	=	↑↑	↑↑
· SLE	=	=	=/↑	=	↑↑	↑↑
· Arteriitis temporalis	↑/↑↑	=	n/a	n/a	n/a	n/a
· Vasculitis other	=/↑	=	=/↑↑	=	↑/↑↑	↑/↑↑
· Sarcoidosis	=/↑	??	=/↑	??	↑/↑↑	??
· Behcet's	=	=	↑	=	↑/↑↑	↑/↑↑
Auto-inflammatory						
· FMF	=	=	↑↑	=/↑	↑/↑↑	↑/↑↑
· TRAPS/HIDS	=	??	↑↑	??	??	??
· Still's disease	=	=	↑↑	↑↑	↑/↑↑	↑/↑↑
IBD						
· Crohn's disease	=	=	↑/↑↑	=	↑/↑↑	↑/↑↑
· Colitis ulcerosa	=	=	↑/↑↑	=	↑/↑↑	↑/↑↑
Malignancy	=/↑↑	=	n/a	n/a	=/↑↑	↑/↑↑
Tissue loss/ ischemia	??	??	??	??	??	??
Endocrine	??	??	??	??	??	??

support the treating physician in deciding to withhold the prescription of antibiotics, thus leading to cost reductions and the decrease of bacterial resistance.

Traditional biomarkers, such as CRP, leukocytes and ESR do not have sufficient sensitivity and specificity to guide treatment decisions. To date, PCT seems to be the most helpful laboratory marker for this purpose, particularly in autoimmune, autoinflammatory and malignant diseases. PCT is not useful for the differentiation between exacerbations of Still's disease and infection *(Table 1)*.

PCT has recently been introduced as a clinical marker in various hospitals all over the world, and is more and more accepted as a useful marker in the diagnostic process in febrile patients. However, the optimal cut-off values for PCT in different patient groups with different pathology still have to be constructed. At this moment, the PCT assay is still costly with a range of $ 20,- to $ 40,- per measurement, which makes it less suitable for resource-poor countries.

Other novel biomarkers, such as SAA and PTX3, are still experimental; further research on these markers is required to determine their clinical value.

It should be clear that no single biomarker is sensitive or specific enough to be relied on completely. Therapy decisions should still be based on a combination of medical history, physical examination and adjunctive tests. In specific cases, specific biomarkers can be used to discriminate infectious from non-infectious fever. Further research in specific patient groups should focus on integrating biological markers and clinical parameters into decision rules. As the usefulness of laboratory markers varies within different diseases, decision rules applicable to specific settings and diseases have to be developed.

Despite the added value of procalcitonin and other "new kids on the block", medicine still remains the science of uncertainty and the art of probability.

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PART II: BIOMARKERS

The acute phase response is not predictive for the development of arthritis in seropositive arthralgia – a prospective cohort study

Authors:
M. Limper, MD*, L.A. van de Stadt, MD*, W.H. Bos, MD, PhD, M.D. de Kruif, MD, PhD, C.A. Spek, PhD, G. Wolbink, MD, PhD, D. van Schaardenburg, MD, PhD, E.C.M. van Gorp, MD, PhD

** The first two authors contributed equally to this work*
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ABSTRACT

Introduction

In clinically active rheumatoid arthritis (RA) acute phase reactants such as C-reactive protein (CRP) can be elevated. Patients presenting with arthralgia and a positive test for anti-cyclic citrullinated peptide antibodies (aCCP) and/or IgM rheumatoid factor (IgM-RF) are at risk for developing RA. Elevated acute phase reactants in this phase could be predictive for the development of arthritis.

Methods

137 aCCP and/or IgM-RF positive patients were included. Patients were followed annually for the development of arthritis, which was defined as presence of one or more swollen joints at clinical examination. High sensitive CRP (hsCRP), Procalcitonin (PCT), SPLA2, Tumor necrosis factor (TNF)-α, IL-6, IL-12p70, IL-10 and interferon (IFN)-γ were measured in baseline serum samples. Gene expression focusing on a predefined panel of genes coding for inflammatory molecules was measured by means of multiplex ligation-dependent probe amplification (MLPA).

Results

35 patients (26 %) developed arthritis within a median time of 11 months (IQR 3.7 – 18 months). Circulating levels of cytokines, sPLA2, hsCRP and PCT were not different between patients with progression to clinical arthritis and patients without progression. However, a trend for IL12p70, TNF-α, IL-10, IL-6 and sPLA2 could be observed. No significant correlation between mRNA expression levels of inflammatory genes was found. Subgroup analysis of patients with early progression to arthritis showed significantly higher levels of mRNA expression of PARN and BMI1 as compared to patients without progression to arthritis.

Conclusions

Although low grade inflammation is present before the onset of clinical arthritis in large cohorts and can be detected using consecutive measurements, a single measurement of acute phase reactants seems to be of limited value for prediction of development of arthritis in individual patients.

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic, systemic inflammatory disease, of which the exact etiology remains to be elucidated. In the pathophysiology of RA, inflamed synovia play a central role, exhibiting varying changes with disease progression. In early, pre-clinical disease, hyperplasia and edema of synovial lining can already be observed. The synovial sublining is infiltrated by mononuclear cells, mainly macrophages and monocytes, T cells and B cells, producing cytokines and chemokines including IL-1, IL-6 and TNF-α. In clinically active RA, inflammation is represented in the blood by elevated levels of acute phase reactants such as high sensitivity C-reactive protein (hsCRP), which can thereby be used as a diagnostic marker for disease activity.

Approximately half of patients with RA have specific serologic abnormalities several years before the onset of symptoms. The presence of an elevated serum level of IgM-Rheumatoid factor (IgM-RF) or anti citrullinated peptide antibodies (ACPA) in a healthy individual implies an increased risk for the development of RA[1]. Furthermore, a rise in CRP and secretory phospholipase A2 (SPLA2) levels has been associated with the development of RA[2]. However, it remains unclear whether these serological markers can be used to predict the development of arthritis prior to disease onset.

This study was conducted to test the hypothesis that acute phase proteins can be used as markers to predict the development of RA in patients at increased risk.

PATIENTS AND METHODS

Study population

The study was conducted at the Jan van Breemen Research Institute, Reade, Amsterdam. A detailed description of the study population can be found in previous publications[3, 4]. In short, patients with arthralgia and a positive anti-CCP2 and/or IgM-Rheumatoid Factor (IgM-RF) status were recruited. At the first visit, a trained investigator completed a questionnaire regarding the presenting symptoms. The absence of arthritis was confirmed by two independent investigators (WB or LAS and DS) of which one was a senior rheumatologist blinded for antibody status and medical history. Patients were excluded if one or both investigators observed any swollen joint and/or if chart review revealed past arthritis observed by a rheumatologist. Furthermore, patients previously treated with a disease modifying anti-rheumatic drug (DMARD) and patients with systemic lupus erythematosus or Sjögren's syndrome were excluded due to the possibility of false-positive RF in these patients. During yearly follow-up visits, development of arthritis was independently confirmed by two investigators (WB or LAS and DS). Extra visits were planned if arthritis developed. Median follow-up was 21 months (range 6–48 months). Analysis for the current study started after inclusion of the first 137 consecutive patients from this cohort, recruited between September 2004 and November 2007.

Furthermore, 20 patients with RA fulfilling the 1987 ACR criteria were selected as a positive control for hsCRP and SPLA2 measurements and 40 healthy blood bank donors were used as negative control.

Measurements

Blood samples were obtained by venapuncture. RNA was isolated from PAXgene^{tm} tubes (PreAnalytiX GmbH, Hombrechtikon, Switzerland) containing RNA stabilizing buffer according to protocol and gene expression was measured by means of multiplex ligation-dependent probe amplification (MLPA), focusing on a predefined panel of genes coding for inflammatory molecules, as described previously[5]. EDTA anticoagulated plasma was aliquoted and stored at -80°C. The blood was routinely screened for haematological and biochemical variables. hsCRP was measured using a highly sensitive latex-enhanced assay on a Hitachi 911 analyzer (Roche Diagnostics), according to the manufacturer's instructions. Procalcitonin (PCT) levels were measured by a chemiluminescence sandwich immunoassay (BRAHMS AG, Hennigsdorf, Germany) as previously described[6]. SPLA2 levels were measured by ELISA as previously described[7]. Tumor necrosis factor (TNF)-α, IL-6, IL-12p70, IL-10 and interferon (IFN)-γ were measured by cytometric bead array (CBA) multiplex assay (BD Biosciences, San Jose, CA, USA). All samples were measured in a blinded fashion without knowledge of the clinical status of the patients.

Statistical Analysis

Baseline demographic and clinical variables are tabulated and descriptive statistics are presented as numbers with percentages, medians with inter quartile ranges (IQR) or means with standard deviations when a normal distribution could be assumed.

Differences in marker levels between groups were determined with an unpaired T-test. Correlations were determined by means of logistic regression analysis. CBA, MLPA and biomarker levels were transformed with their natural logarithm prior to statistical analysis to normalize the data.

Subgroup analysis comparing early progressors, defined as patients with development of arthritis within the first IQR after inclusion in the study, with non-progressors was performed for all markers. A two-tailed p-value <0.05 was considered to indicate statistical significance.

RESULTS

Patient characteristics

137 Anti-CCP and/or IgM-RF positive arthralgia patients were included. Patient characteristics are shown in table 1. Among the patients, 82 (60%) were IgM-RF positive, 94 (69%) were anti-CCP positive and 39 (29%) were both IgM-RF and anti-CCP positive. Overall, 35

patients (26 %) developed arthritis within a median time of 11 months (IQR 3.7 – 18 months); 18 patients developed arthritis within the first IQR after inclusion and were considered early progressors.

Table 1: Baseline characteristics of patients with arthralgia and positive anti-CCP or IgM-Rheumatoid Factor.

	Total n = 137
Female sex, n (%)	104 (76 %)
Age, median (IQR)	47 yrs (39 – 55 yrs)
Follow-up, median (IQR)	21 months (15 – 31 months)
Progression to arthritis, n (%)	35 (26 %)
Time to arthritis, median (IQR)	11 months (3.7 – 18 months)
TJC 53, median (IQR)	0 (0 – 2)
RF positive, n (%)	82 (60 %)
Anti-CCP2 positive, n (%)	94 (69 %)
RF + anti-CCP positive, n (%)	39 (29 %)

Circulating levels of cytokines

Circulating levels of cytokines were not different between patients with progression to clinical arthritis and patients without progression. However, a trend for IL12p70, TNF-α, IL-10 and IL-6 could be observed, with higher levels in patients who progressed to arthritis ($p = 0.07$, $p = 0.07$, $p = 0.10$ and $p = 0.05$, respectively). Subgroup analysis of circulating levels of cytokines in early progressors as compared to patients without progression to arthritis showed no significant difference (see Table 2).

mRNA biomarkers

No significant correlation between mRNA expression levels of inflammatory genes - TNF–α, IL-1β, IL-1RA, IL-8, IL-15, nuclear factor (NF)-κB1A, NF-κB1, serpin B9, MYC, cyclin-dependent kinase inhibitor 1A (CDKN1A), small inducible cytokine A4 (SCYa4), platelet-derived growth factor subunit B (PDGFb), poly(A)-specific ribonuclease (PARN), thrombospondin 1 (THBS1), tumor necrosis factor receptor superfamily member 1A (TNFRS1A), polycomb complex protein BMI-1, macrophage migration inhibitory factor (MIF), phosphodiesterase 4B (PDE4B), tyrosine-protein phosphatase non-receptor type 1 (PTPN1), protein tyrosine phosphatase type IVA 2 (PTP4A2) and glutathione S-transferase P (GSTP1) – and progression to arthritis could be observed (see Table 2).

Subgroup analysis of patients with early progression to arthritis (that is, within 10 weeks after inclusion) showed significantly higher levels of mRNA expression of PARN and BMI1 as compared to patients without progression to arthritis. The same results could be observed comparing levels of mRNA expression of PARN and BMI1 in early progressors to levels in all other included patients.

Table 2: levels of mRNA expression, circulating cytokines and circulating protein biomarkers in arthralgia patients with and without progression to arthritis

	Arthritis **n= 35**	**No arthritis** **n= 102**	**P-value**
CBA	*Geometric mean (95% CI)*	*Geometric mean (95% CI)*	
IL-12p70	7.08 (2.04 – 24.55)	1.91 (1.35 – 2.69)	0.07
TNF-α	2.95 (1.54 – 5.62)	1.45 (1.15 – 1.82)	0.07
IL-10	2.29 (1.26 – 4.17)	1.32 (1.15 – 1.51)	0.10
IL-6	2.69 (1.66 – 4.37)	1.58 (1.38 – 1.82)	0.05
IL-1β	10.0 (3.16 – 31.6)	5.50 (2.88 – 10.47)	0.39
IL-8	2.95 (1.70 – 5.13)	2.69 (2.04 – 3.55)	0.78
MLPA			
IL-15	0.05 (0.04 – 0.05)	0.05 (0.04 – 0.05)	0.54
NF-κB1A	0.39 (0.35 – 0.44)	0.40 (0.38 – 0.42)	0.65
TNF	0.02 (0.02 – 0.02)	0.02 (0.02 – 0.02)	0.41
IL-1β	0.11 (0.10 – 0.13)	0.11 (0.10 – 0.12)	0.95
IL-1RN	0.35 (0.30 – 0.42)	0.35 (0.33 – 0.38)	0.99
IL-8	0.03 (0.03 – 0.04)	0.03 (0.03 – 0.04)	0.97
MYC	0.43 (0.38 – 0.49)	0.39 (0.36 – 0.42)	0.19
SCYa4	0.09 (0.08 – 0.10)	0.09 (0.08 – 0.10)	0.92
Serpin-B9	0.45 (0.39 – 0.51)	0.43 (0.41 – 0.45)	0.63
PDGF-β	0.03 (0.03 – 0.04	0.03 (0.03 – 0.03)	0.23
PARN	0.19 (0.18 – 0.20)	0.18 (0.17 – 0.19)	0.09
THBS1	0.05 (0.04 – 0.05)	0.04 (0.04 – 0.05)	0.16
LTA	0.05 (0.04 – 0.05)	0.05 (0.04 – 0.05)	0.09
CDKN1A	0.13 (0.11 – 0.16)	0.12 (0.11 – 0.13)	0.27
Tnfrsf1a	0.35 (0.32 – 0.39)	0.35 (0.34 – 0.36)	0.80
BMI1	0.11 (0.10 – 0.13)	0.10 (0.09 – 0.11)	0.08
MIF	0.26 (0.22 – 0.30)	0.25 (0.23 – 0.27)	0.73
PDE4b	0.34 (0.32 – 0.37)	0.34 (0.32 – 0.35)	0.84
PTPN1	0.07 (0.06 – 0.08)	0.07 (0.06 – 0.07)	0.53
PTP4A2	0.81 (0.76 – 0.87)	0.78 (0.74 – 0.81)	0.20
GSTP1	0.05 (0.04 – 0.05)	0.04 (0.03 – 0.05)	0.42
Biomarkers			
Procalcitonin	0.04 (0.03 – 0.05)	0.04 (0.03 – 0.05)	0.69
hs-CRP (mg/L)	2.51 (1.72 – 3.66)	2.30 (1.82 – 2.90)	0.71
SPLA2 (ng/ml)	5.79 (4.75 – 7.07)	4.20 (3.20 – 5.53)	0.07
Subgroup analysis	Early progressors n = 18	No arthritis n = 102	
PARN	0.20 (0.18 – 0.22)	0.18 (0.17 – 0.19)	0.008
BMI1	0.13 (0.11 – 0.15)	0.10 (0.09 – 0.10)	0.02

Biomarker levels

Mean sPLA2, hsCRP and PCT levels were not different between patients who developed arthritis and patients who did not; a trend towards higher sPLA2 levels in patients with arthritis could be observed (p = 0.07, see table 2). SPLA2 levels of arthralgia patients did not differ from SPLA2 levels of healthy controls (geometric mean (GM), 95%CI): 4.56, 3.69-5.64 and 4.65, 3.84-5.64 respectively; p = 0.93), in contrast to the levels of RA patients (GM, 95%CI: 10.99, 6.93-17.42), that were significantly higher than both the levels of healthy controls and arthralgia patients (p <0.001). hsCRP levels of arthralgia patients on the other hand were higher than hsCRP levels of healthy controls (GM, 95%CI: 2.35, 1.93-2.86 and 1.06, 0.72-1.55 respectively, p<0.001). hsCRP levels of RA patients were even higher (GM, 95%CI: 7.58, 4.06-14.13; p <0.001).

DISCUSSION

This study of patients with an increased risk of developing RA was conducted to investigate whether differences in inflammatory patterns in those patients who developed arthritis could be observed before clinical signs of arthritis could be confirmed. We found no evidence of a more pronounced systemic acute phase response, higher cytokine levels or a higher expression of pro-inflammatory gene markers in patients who later progressed to arthritis than in those who did not, although a number of the biomarkers showed a trend towards higher levels in those that later developed arthritis. These data indicate that any possible local inflammatory processes in the joints of seropositive arthralgia patients do not result in a discernible systemic acute phase response activation.

These results are in contrast with our previous studies in which we reported elevation of CRP and SPLA2 levels in pre-clinical RA[8, 9]. A possible explanation for this discrepancy could be the difference in study design. In these previous studies multiple, longitudinally collected serum samples were analyzed and a slight but significant rise in CRP and SPLA2 levels could be observed before RA onset, in contrast to stable levels in healthy controls. However, CRP and SPLA2 levels in preclinical RA were still within the normal range and therefore large numbers of samples are needed to detect such small changes. In this study a trend was observed toward higher levels of IL-12p70,TNF-α, IL-10, IL-6, PARN, LTA, BMI1,and SPLA2 in arthritis progressors; in early progressors higher levels of PARN and BMI-1 were observed. More significant differences might be observed when larger numbers of samples are analyzed. Also, in this study, hsCRP levels of arthralgia patients were higher than hsCRP levels of healthy controls, indicating that arthralgia patients have a slightly activated acute phase response on the group level.

Thus, although evidence exists for an induction of inflammation prior to the onset of RA, our data indicate that acute phase response markers such as CRP and SPLA2 are not useful in clinical practice as predictors of development of arthritis in individual high risk patients. This is in line with results from other studies in which hsCRP and SPLA2 in a single pre-RA serum sample were not increased in comparison to healthy controls[10].

In conclusion, although low grade inflammation is present before the onset of clinical arthritis in large cohorts and can be detected using consecutive measurements, a single measurement of acute phase reactants seems to be of limited value for prediction of development of arthritis in individual patients.

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Additional value of procalcitonin for diagnosis of infection in patients with fever at the emergency department

Authors:
M.D. de Kruif, MD; M. Limper, MD; H. Gerritsen, MD; C.A. Spek, PhD;
D.P.M. Brandjes, MD, PhD; H. ten Cate, MD, PhD; P.M. Bossuyt, PhD;
P.H. Reitsma, PhD; E.C.M. van Gorp, MD, PhD

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ABSTRACT

Objective

First, to determine whether procalcitonin (PCT) significantly adds diagnostic value in terms of sensitivity and specificity to a common set of markers of infection, including C-reactive protein (CRP), at the Emergency Department. Second, to create a simple scoring rule implementing PCT values. Third, to determine and compare associations of CRP and PCT with clinical outcomes.

Design

The additional diagnostic value of PCT was determined using multiple logistic regression analysis. A score was developed to help distinguish patients with a culture-proven bacterial infection from patients not needing antibiotic treatment using 16 potential clinical and laboratory variables. The prognostic value of CRP and PCT was determined using Spearman's correlation and logistic regression.

Setting

Emergency Department of a 310-bed teaching hospital.

Patients

Patients between 18 and 85 years old presenting with fever to the Emergency Department.

Interventions

None.

Measurements and Main Results

A total of 211 patients were studied (infection confirmed, n = 73; infection likely, n = 58; infection not excluded, n = 46; no infection, n = 34). CRP and chills were the strongest predictors for the diagnosis of bacterial infection. After addition of PCT to these parameters, model fit significantly improved (p = .003). The resulting scoring rule (score = 0.01 * CRP + 2 * chills + 1 * PCT) was characterized by an AUC value of 0.83 (sensitivity 79%; specificity of 71%), which was more accurate than physician judgment or SIRS (systemic inflammatory response syndrome). PCT levels were significantly associated with admission to a special care unit, duration of intravenous antibiotic use, total duration of antibiotic treatment, and length of hospital stay, whereas CRP was related only to the latter two variables.

Conclusions

These data suggest that PCT may be a valuable addition to currently used markers of infection for diagnosis of infection and prognosis in patients with fever at the Emergency Department.

INTRODUCTION

Fever is a common symptom at the Emergency Department and highly suggestive for microbial infection. Other clinical symptoms associated with infection are tachycardia, tachypnea, and abnormal leukocyte counts. Together these symptoms have been used to define the systemic inflammatory response syndrome (SIRS)[1]. Although the concept of SIRS is useful for detection of any inflammatory condition in patients, this syndrome is not specific enough to distinguish infectious from noninfectious causes of inflammation[2, 3]. In clinical practice, fever is still the most critical sign initiating a search for infection.

Currently, the differentiation between various causes of fever in clinical practice is based upon a combination of clinical parameters as well as laboratory values, including C-reactive protein (CRP) and leukocyte counts. In addition to these "conventional" markers, procalcitonin (PCT) has been suggested as a novel infection biomarker[4, 5]. Several meta-analyses concluded that in critically ill patients, PCT is probably superior to CRP for diagnosing bacterial infection[6-9]. Nevertheless, the use of PCT in clinical practice is-at least in Emergency Department settings-still not established. An important reason is that despite this evidence, it remains unclear whether PCT adds significantly to the discriminative properties of the already used set of diagnostic markers, although this is an essential matter of concern in diagnostic research[10].

This study aimed to determine the additional value of PCT as a biomarker in patients presenting with fever at the Emergency Department. We investigated the additional value of PCT to current markers in a multivariable logistic regression model and subsequently created a clinical scoring rule for diagnosis of bacterial infection. In addition, the role of PCT and CRP was further explored by studying associations with clinical outcomes.

METHODS

Patients

Between April 2004 and September 2006, patients presenting with fever admitted to the Department of Internal Medicine of the Slotervaart Hospital in Amsterdam, The Netherlands, were included in the study. The study was approved by the Institutional Scientific and Ethics Committees. Eligible were nonpregnant adult patients, ages 18 to 85 yrs, presenting with fever. They were included in the study within 36 hrs after presentation. In close agreement with common clinical practice, fever was defined by a tympanic temperature >=38.0 ^{o}C (Genius First Temp M3000A, Tyco Healthcare, Princeton, NJ) recorded either at admission or, when subfebrile at admission, between admission and inclusion. Written informed consent was obtained from all participating subjects.

Definitions

After completion of the study, the medical record of each included patient was reviewed by two blinded medical investigators, and in case of disagreement, reviewed by a third independent expert in infectious disease. Confirmed bacterial infection was defined by a positive culture at a likely focus (group 1). In the second group, with "possible infection," infection was either likely (defined by positive imaging findings on a radiograph, computed tomography, or magnetic resonance imaging scan, such as an infiltrate or abscess confirmed by a radiologist and visual findings such as a localized warm, red and painful swelling or an abscess found by endoscopy) or could not be excluded. In the third, "noninfectious" group, bacterial infection was excluded because culture and imaging findings were negative and/or an alternative diagnosis was confirmed. Chills were defined as a (sub) acute feeling of coldness together with shivering and feeling the need for a thick blanket. The judgment of the treating physician (who was not aware of PCT levels) about the likelihood of bacterial infection was recorded at admission by personal inquiry by the investigator, or when this was not possible, by careful examination of the original patient's medical records and medication use records.

Measurements

Blood samples were obtained by venapuncture, collected on ice, and centrifuged within 15 min. (2 x 3000 revolutions per minute at 5 °C for 10 minutes). EDTA anticoagulated plasma was aliquoted and stored at -80 °C. The blood was routinely screened for hematologic and biochemical variables. CRP was measured using an automated analyzer, Synchron LX20 (Beckman Coulter, Fullerton, CA). PCT levels were measured by a chemiluminescence sandwich immunoassay (BRAHMS Aktiengesellschaft, Hennigsdorf, Germany) as previously described[11].

The functional assay sensitivity is <0.007 ng/mL. The intra-assay coefficient of variation is <8% in samples containing 0.008-4 ng/mL. All samples were measured in a blinded fashion without knowledge of the clinical status of the patients.

Data Analysis

Group differences were calculated using Mann-Whitney U test for continuous variables and chi-square test for binominal variables. The association between the presence and absence of bacterial infection (group 1 and group 3, respectively) and each potential diagnostic determinant (chosen based upon literature[3, 12-21] was first quantified using univariate logistic regression analyses. Determinants with significant associations (p value <.20) were used to build a multivariable logistic regression model. Determinants from patient history, physical examination, and traditional laboratory markers of infection, including CRP, were included first, and the optimal model was determined. Then, PCT was added to this model. Differences in the discriminative value between the models with and without PCT were determined by comparing -2log likelihood ratios according to the chi-square goodness-of-fit test.

For all variables included in the analysis, 95% or more of the data points were complete. Since multivariable analysis requires all data to be present in all subjects, we used regression imputation techniques to complete the missing values.

To adjust for the optimism in the regression coefficients, random bootstrapping techniques were applied using StatsDirect version 2.6.5 (StatsDirect Ltd, Altrincham, United Kingdom). Bootstrapping involves taking numerous samples with replacement from the study population sample. It is an internal validation technique (that is, it estimates the future performance of the model without using new data)[22]. The data were bootstrapped 1000x and a final model was created. This model was transformed into a clinical scoring rule and the receiver operating curve area and other descriptive variables of this score were estimated. The derived scoring rule was then applied, for validation, to the likely infection group. Associations between PCT and CRP and relevant clinical parameters were determined by logistic regression analysis for binominal variables and Spearman's ranked correlation test for continuous variables. Data are presented as medians with quartiles or as numbers with percentages; odds ratios (OR) with 95% confidence intervals (CI) and Spearman's rho coefficients are shown when appropriate. Data were analyzed using SPSS version 15.0.

RESULTS

Patients

A flow diagram of the 211 patients included is presented in Figure 1.

Presence of bacterial infection was confirmed for 73 patients (group 1), considered likely (n = 58) or could not be excluded (n = 46) for 104 other patients (possible infection, group 2), and was excluded for 34 patients (group 3). Patient characteristics are presented in Table 1.

Diagnosis of Infection

Univariate regression analysis was performed to identify markers of infection among 16 clinical and laboratory candidate markers (Table 2). Groups 1 and 3 were compared. Significant markers ($p < .20$) identified for the discrimination between confirmed infection and no infection were CRP, ESR, leukocyte count, (low) thrombocyte count, (low) albumin, tachypnea, and chills.

These variables were then included in a multivariable model (Table 2). Only levels of CRP and the presence of chills were significantly associated with confirmed infection. A new model was created using these variables. The area under the curve (AUC) of this model was 0.79 (95% CI, 0.70 to 0.88). When PCT was added to this model, model fit was significantly improved ($p = .003$). The model with PCT had an AUC of 0.82 (95 CI, 0.74 to 0.90).

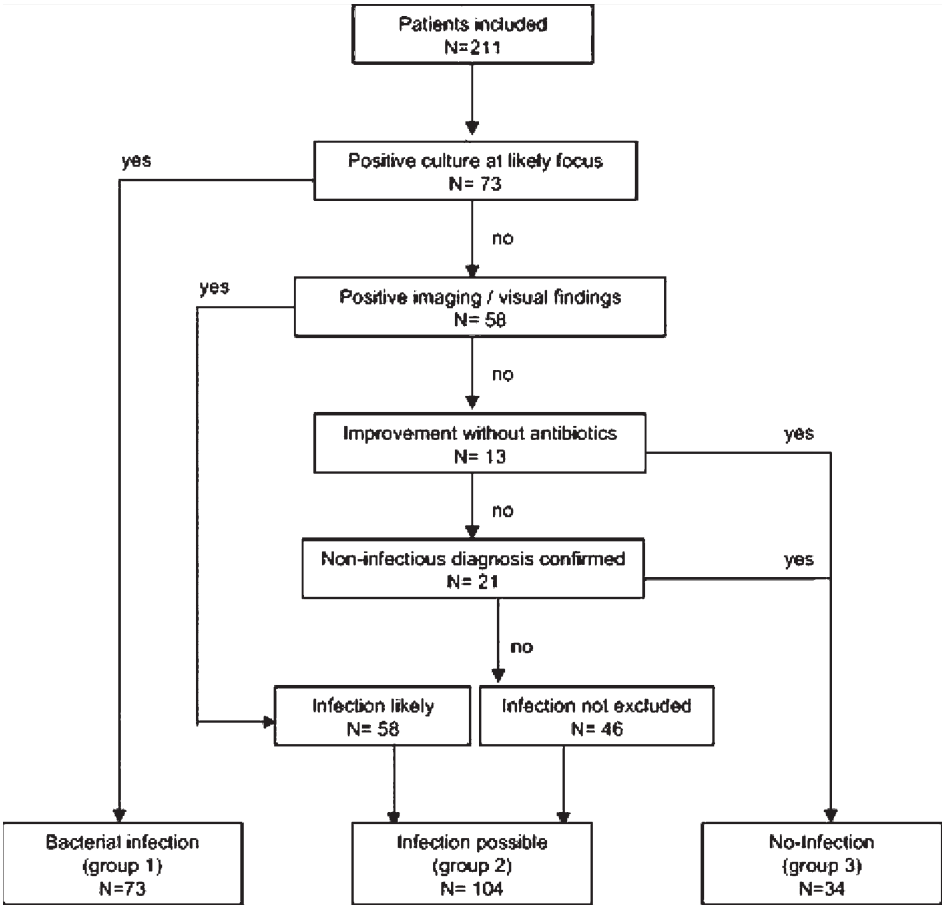

Figure 1. Flow chart: Patient cohort of adults (n = 211) presenting with fever to the Emergency Department.

We then adjusted the coefficients for overfitting by bootstrapping the data. Subsequently, the regression coefficients of the bootstrapped model were rounded to assign a score to each predictive variable. Thus, the following scoring rule was defined:

Score = 0.01 * Crp + 2 * Chills + 1 * Pct

Here, CRP is given in mg/l, PCT in ng/mL and chills is scored either 1 (present) or 0 (absent). Figure 2 shows the ROC curves of the several models. The scoring rule demonstrated an AUC of 0.83 (95% CI, 0.74 to 0.90). A model combining PCT and chills without CRP showed an AUC value of 0.76 (95% CI, 0.67 to 0.86). The optimal cut-off point (best diagnostic accuracy) of the scoring rule was 2.4, which showed a sensitivity of 79% and a specificity of 71%. This score corresponded with an 85% chance of having an infection.

Table 1. Patient characteristics of adults presenting with fever to the Emergency Department (n = 211)

	All N=211 n (%) / median (IQR)	Bacterial Infection N=73 n (%) / median (IQR)	No bacterial infection N=34 n (%) / median (IQR)	P-value
Age, yrs	64 (46-74)	68 (52-74)	52 (43-67)	0.001
Sex, male n (%)	115 (55)	42 (58)	18 (53)	NS
Underlying Morbidity, n (%)				
diabetes mellitus	37 (18)	13 (18)	5 (15)	NS
Malignancy	17 (8)	4 (6)	7 (21)	0.017
HIV	12 (6)	4 (6)	3 (9)	NS
COPD	53 (25)	21 (29)	5 (15)	NS
congestive heart failure	20 (10)	10 (14)	1 (3)	NS
Medication, n (%)				
Immunosuppressives	23 (11)	10 (14)	3 (9)	NS
Antibiotics	48 (23)	17 (23)	6 (18)	NS
Location, n (%)				
Cardiovascular	1 (1)	0 (0)	1 (3)	NS
Respiratory	101 (48)	29 (40)	8 (24)	NS
Gastro-intestinal	34 (16)	13 (18)	11 (32)	NS
Musculo-skeletal	3 (1)	1 (1)	2 (6)	NS
Urogenital	32 (15)	22 (30)	1 (3)	0.002
Skin	12 (6)	6 (8)	1 (3)	NS
lymph nodes	8 (4)	2 (3)	2 (6)	NS
Other	4 (2)	0 (0)	3 (9)	NS
Unknown	13 (6)	0 (0)	0 (0)	NS
Duration of fever before inclusion, days	3 (2-6)	3 (2-7)	4 (2-9)	NS
Duration of inclusion delay, days	1 (1-2)	1 (1-2)	1 (1-2)	NS
Clinical symptoms, (%)				
Red painful swelling	32 (15)	10 (14)	8 (24)	NS
Cough	101 (48)	35 (48)	9 (27)	0.036
Chills	94 (44)	36 (49)	10 (30)	NS
Night sweating	48 (23)	17 (23)	9 (27)	NS
Peaking temperatures	36 (17)	13 (18)	9 (27)	NS
Nausea	64 (30)	25 (34)	12 (35)	NS
Diarrhea	32 (15)	14 (19)	8 (24)	NS
Dysuria	21 (10)	13 (18)	0 (0)	0.034
Clinical signs				
temperature, °C	38.9 (38.5-39.0)	39.0 (38.5-39.5)	38.9 (38.5-39.4)	NS
tachypnea, n (%)	91 (43)	37 (51)	9 (27)	0.018
tachycardia, n (%)	83 (39)	27 (38)	12 (37)	NS

	All N=211 n (%) / median (IQR)	Bacterial Infection N=73 n (%) / median (IQR)	No bacterial infection N=34 n (%) / median (IQR)	P-value
mean arterial blood pressure, mmHg	105 (95-120)	106 (91-121)	108 (97-119)	NS
SIRS	166 (79)	63 (86)	22 (65)	0.010
Laboratory values				
CRP, mg/l	139 (67-247)	179 (86-338)	73 (22-132)	0.000
BSE, mm/h	51 (31-84)	57 (37-87)	35 (20-75)	0.018
Leucocyte count, 109/l	11.0 (7.5-15.9)	11.1 (8.0-16.3)	8.8 (6.8-13.3)	NS
Thrombocyte count, 109/l	230 (175-306)	220 (171-265)	246 (176-310)	NS
AF, U/l	81 (63-108)	78 (68-109)	84 (60-113)	NS
GGT, U/l	38 (23-64)	38 (23-78)	38 (20-64)	NS
ASAT, U/l	22 (14-41)	26 (14-53)	21 (14-42)	NS
ALAT, U,l	25 (16-45)	30 (18-56)	21 (14-41)	NS
Albumin, g/l	29 (25-34)	27 (23-33)	33 (28-36)	0.001
PCT, ng/ml	0.27 (0.06-1.38)	0.67 (0.15-3.3)	0.15 (0.04-0.35)	0.000
Physician judgment infection present, n (%)	182 (86)	70 (96)	17 (50)	0.000
Mortality	5 (2)	1 (1)	0 (0)	NS

Various other cut-off levels are presented in Table 3, together with a comparison to alternative diagnostic approaches. The AUC of the scoring rule was superior to SIRS criteria and physician judgment of presence of bacterial infection. The rule was tested by applying it to the patient subgroup with likely infection determined by positive imaging findings, together with the no infection group. In these groups, the rule had an AUC of 0.80 (95% CI, 0.71 to 0.89).

Prognosis

The prognostic value of the scoring rule determinants CRP and PCT is shown in Table 4. CRP levels were significantly associated with length of hospital stay and total duration of antibiotic treatment. PCT levels were also associated with these prognostic factors, but, in addition, were associated with duration of intravenously administered antibiotics and admission to a special care unit.

Table 2. Univariate and multivariable analysis

	Missing values, n (%)	OR (CI)	P-value
CRP	1 (0.5)	1.010 (1.005-1.015)	**0.000**
ESR	3 (1.4)	1.013 (1.000-1.026)	**0.056**
Leucocyte count	1 (0.5)	1.080 (0.996-1.172)	**0.064**
Thrombocyte count	8 (3.8)	0.997 (0.993-1.001)	**0.132**
AF	5 (2.4)	0.935 (0.995-1.004)	0.935
GGT	5 (2.4)	1.001 (0.997-1.005)	0.595
ASAT	1 (0.5)	1.000 (0.995-1.005)	0.943
ALAT	0 (0)	0.999 (0.996-1.001)	0.310
Albumin	7 (3.3)	0.881 (0.815-0.952)	**0.001**
Temperature	0 (0)	1.265 (0.692-2.314)	0.445
Tachypnea	0 (0)	2.855 (1.173-6.948)	**0.021**
Tachycardia	0 (0)	1.302 (0.575-2.949)	0.527
Pulse	0 (0)	1.014 (0.991-1.038)	0.229
Chills	0 (0)	2.335 (0.980-5.567)	**0.056**
Peaking temperature	0 (0)	0.602 (0.228-1.587)	0.305
Night sweating	0 (0)	0.843 (0.331-2.149)	0.721
PCT	2 (0.9)	2.576 (1.170-5.671)	**0.019**

Abbreviations: CRP= C-reactive protein, ESR= erythrocyte sedimentation rate, AF= alkaline phosphatase, GGT= gamma-glutamyl transferase, ASAT= aspartyl aminotransferase, ALAT= alanine transaminase, PCT= procalcitonin

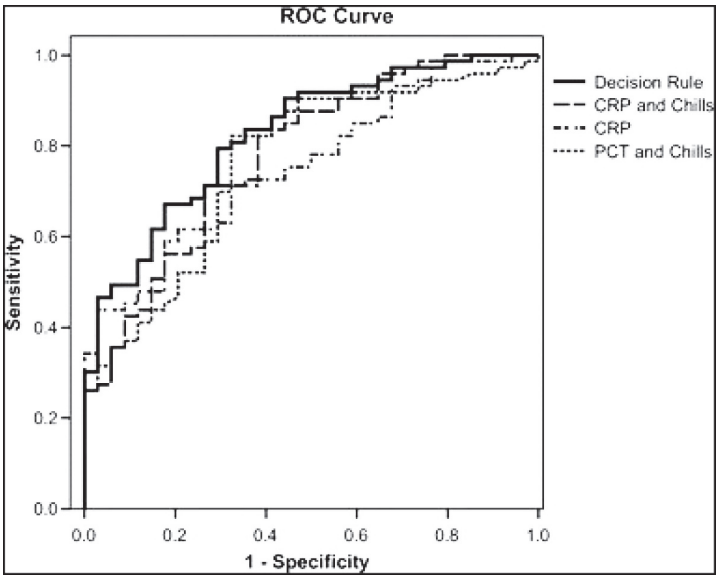

Figure 2. Receiver operating curve (ROC) curve. Graph plotting sensitivity against specificity of a novel scoring rule in adults presenting with fever to the Emergency Department. The scoring rule score was determined by 0.01 * [CRP] + 1 * [PCT] + 2 * [chills]. CRP, C-reactive protein; PCT, procalcitonin.

Table 3. Diagnostic accuracy-comparison to other diagnostic parameters of a novel scoring rule for the discrimination of bacterial infection in patients with fever at various cut-off levels (n = 211)

Diagnostic parameter	AUC[3]	CI[3]	cut-off[3]	Sensitivity, %[3]	Specificity, %[3]
Decision rule[1]	0.83	0.74-0.90	2.4	0.79	0.71
			0.7	0.95	0.32
			4.3	0.49	0.95
CRP (mg/L)	0.76	0.67-0.85	133	0.62	0.79
			9	0.99	0.18
PCT (ng/mL)	0.73	0.63-0.82	0.7	0.52	0.91
			0.5	0.53	0.85
			2.0	0.34	0.97
			10	0.16	1.00
Chills	0.60	0.49-0.71	Yes/no	0.46	0.71
SIRS[2]	0.61	0.49-0.73	Yes/no	0.86	0.35
Physician judgment[4]	0.79	0.66-0.92	Yes/no	0.97	0.62

1Decision rule Score was determined by 0.01*[CRP] +1*[PCT] + 2*[chills]. CRP= C-reactive protein. PCT= procalcitonin.
2SIRS= Systemic Inflammatory Response Syndrome
3Area under the curves (AUC) with 95%-confidence intervals (CI) are presented from ROC curves summarizing performance of the decision rule score. Optimal cut-off values were defined as maximum sensitivity and specificity. Binominal variables were scored either present (yes) or not present (no).
4Judgment of treating physician of presence of bacterial infection as determined in the subgroups of proven bacterial infection versus infection excluded.

Table 4. Prognostic properties: Associations between plasma levels of C-reactive protein and procalcitonin and markers of prognosis in a patient cohort of adults presenting with fever to the Emergency Department (n = 211)

	Median (IQR) /n (%)[1]	CRP			PCT		
		rho/ OR[1]	CI[1]	p-value[1]	rho / OR[1]	CI[1]	p-value[1]
duration hospital stay[2], days	8 (5-13)	0.177	NA	0.011*	0.244	NA	0.000*
Duration antibiotics[2], days	10 (8-14)	0.318	NA	0.000*	0.308	NA	0.000*
duration iv antibiotics[2], days	6.5 (5-10)	0.113	NA	0.182	0.227	NA	0.007*
ICU/MC admission	13 (6%)	1.002	0.997-1.006	0.434	1.078	1.036-1.122	0.000*
30-day mortality	5 (2%)	0.996	0.987-1.005	0.389	1.030	0.968-1.096	0.346

1For continuous variables: median values are presented with interquartile ranges (IQR) and Spearman correlation coefficients (rho). For dichotomous variables: numbers of patients (n) are presented with odds ratio's (OR) including the 95%-confidence interval (CI). A $P<0.05$ was considered statistically significant (*).NA= Not applicable. iv= intravenous.
2non-survivors excluded.

DISCUSSION

This study first aimed to determine the additional value of PCT for use as a biomarker in patients presenting to the hospital with fever. Patients proven to have or not have a bacterial infection were compared with one another. The addition of PCT appeared to significantly improve a regression model using the level of CRP and the presence of chills, which were identified as the best discriminative markers among a set of commonly used markers for infection.

Second, by using these variables, we created a simple and easy to calculate scoring rule for clinical decision making. The rule showed an AUC of 0.83, which was superior to the use of SIRS or the clinical physician judgment. Third, PCT was associated more closely with clinical outcomes than CRP. Together the data demonstrate that PCT is a valuable addition to the currently applied set of biomarkers in patients presenting with fever to the Emergency Department.

In our study, at clinical cut-off levels, PCT tended to be more specific than CRP, whereas CRP was more sensitive. These different diagnostic properties may account for the additional diagnostic value of PCT in the scoring rule. One study compared sensitivity and specificity of CRP and PCT in patients presenting with fever at the Emergency Department before and showed more or less similar figures, but did not investigate the additional value of PCT to current markers[23]. The additional value of PCT was explored in two other studies with a different setup. One study presented a scoring rule for children with meningitis to distinguish between aseptic and bacterial meningitis[24]. PCT levels appeared to add diagnostic value to common clinical variables. Another study, using a different statistical approach via a classification and regression tree model, showed that PCT significantly added diagnostic value to currently determined variables for the identification of bloodstream infection in patients with fever[25].

The scoring rule finally created showed comparable diagnostic properties in a validation cohort of patients with likely infection, which indicates that the rule may be applicable to other, similar cohorts of patients. The sensitivity and specificity of the scoring rule were 79% and 71%, respectively. These figures were better than the diagnostic properties of PCT, CRP, or chills alone. Thus, the rule demonstrates a proof of principle that it is possible to combine various markers of infection in a way that is superior to the use of single markers only. It needs to be noted, however, that the negative predictive value of the rule (62%) was less than the negative predictive value of the physician judgment (85%); hence, the rule will not justify clinical decision making without consideration of the clinical condition of the patient and the (subjective) judgment of the treating physician. Furthermore, it must be emphasized that the rule must first be validated in a new cohort of patients ("external validation"), before it may be applicable in a clinical practice setting.

In addition to the diagnostic value of the markers, we investigated and compared prognostic values of PCT and CRP. The prognosis of a patient with fever is highly relevant for treatment decisions such as the choice of ward for admission or the choice of antibiotics, their

route of administration, and the need for supportive measures. PCT levels in our study were predictive for multiple prognostic variables, including duration of stay in the hospital, duration of intravenously administered antibiotics, total duration of antibiotic treatment, and admission to a special care unit. In contrast, levels of CRP were associated only with duration of stay in the hospital and total duration of antibiotic treatment. This difference in prognostic properties has also been found by others and suggests that PCT is superior to CRP in terms of prognostic properties[23, 26-29].

An ideal biomarker is not only useful for detection of the presence of illness, but also predicts absence of illness after recovery[30]. It has been demonstrated that the timing of discontinuation of antibiotic therapy in patients with pneumonia or sepsis could be safely guided by levels of PCT. In comparison to conventional, empirically-based antibiotic decision making, PCT-guided therapy greatly reduced numbers of antibiotic prescriptions and duration of antibiotic therapy[31-35]. Notably, the current scoring rule has not been designed for this purpose, but based upon the superior diagnostic properties of the rule as shown in this study in comparison with its single determinants, one could speculate that an approach that aims to combine both clinical and biological markers may also hold promise for refinement of PCT-guided therapy algorithms.

The current study is an observational study. Observational studies are limited by their inability to distinguish between primary and secondary effects and by difficulties to control for confounding factors[36, 37]. Because of definitions used in our study, the primary aim of the scoring rule was to identify the subset of patients with positive cultures as judged by the treating physician. Despite the confirmed presence of a bacterial infection, however, not all of these patients may ultimately require antibiotics, since a portion of patients may also recover without antibiotics. The reliability of the clinical-scoring rule may be further limited by the accumulation of error intervals of the single determinants, caused by technical limitations of the assays and a potentially subjective interpretation of the definition of chills. By reducing the amount of variables included in the rule to a minimum, we tried to minimize this error as much as possible. Furthermore, in the derivation cohort, only patients with proven infection were included to prevent mixing of results, but this selection may have caused a certain optimism in the AUC of the rule because of exclusion of patients with likely infection. Nevertheless, no differences were observed in patient characteristics in these groups. Taken together, the limitations of the rule emphasize the need for further validation of the rule in a new patient cohort.

CONCLUSIONS

In conclusion, we showed that it was possible to create a simple, easy-to-calculate clinical scoring rule for the detection of infection in patients presenting with fever at the Emergency Department which was diagnostically superior to physician judgment and SIRS. The biomarker PCT appeared to add significant diagnostic value in terms of sensitivity and

specificity to currently used markers of infection. In addition, PCT was a better prognostic marker than CRP. These data suggest that PCT may be a valuable addition to currently used markers of infection at the Emergency Department.

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Procalcitonin as a potent marker of bacterial infection in febrile Afro-Caribbean patients at the emergency department

Authors:
M. Limper, MD; M.D. de Kruif, MD, PhD; N.E. Ajubi, PhD; A.P. van Zanten, PhD; D.P.M. Brandjes, MD, PhD; A.J. Duits, PhD; E.C.M. van Gorp, MD, PhD

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ABSTRACT

Purpose

Procalcitonin (PCT) has been shown to be of additional value in the work-up of a febrile patient. This study is the first to investigate the additional value of PCT in an Afro-Caribbean febrile population at the emergency department (ED) of a general hospital.

Methods

Febrile patients were included at the ED. Prospective, blinded PCT-measurements were performed in patients with a microbiologically or serologically confirmed diagnosis or a strongly suspected diagnosis on clinical grounds.

Results

PCT analysis was performed in 93 patients. PCT levels differentiated well between confirmed bacterial and confirmed viral infection (AUC of 0.82, sensitivity 85 %, specificity 69 %, cut-off 0.24 ng/mL), between confirmed bacterial infection and non-infectious fever (AUC of 0.84, sensitivity 90 %, specificity 71 %, cut-off 0.21 ng/mL) and between all bacterial infections (confirmed and suspected) and non-infectious fever (AUC of 0.80, sensitivity 85 %, specificity 71 %, cut-off 0.21 ng/mL). CRP levels were shown to be less accurate when comparing the same groups.

Conclusion

This is the first study, showing that in a non-Caucasian febrile population at the ED, PCT is a more valuable marker of bacterial infection than CRP. These results may improve diagnostics and eventually decrease antibiotic prescriptions in resource-limited settings.

Keywords

Procalcitonin; infection; diagnostics; Afro-Caribbean; emergency department

INTRODUCTION

Infectious diseases are a major cause of morbidity and mortality worldwide, particularly in tropical regions[1-3]. Traditionally, fever has been associated with bacterial infection. However, in up to 50 % of febrile people, fever is caused by non-bacterial infection or other inflammatory conditions, such as malignancy or auto-immune disease[4, 5]. Because untreated bacterial infections may result in serious complications, the majority of patients with fever receive antibiotic treatment, leading to overprescription of antibiotics and contributing to the development of antimicrobial resistance, increasing costs and possible adverse effects[6].

Neither clinical signs and symptoms, nor current biomarkers such as C-reactive protein (CRP) have, as a single marker, sufficient power to discriminate the etiology of fever[7-9]. Lately, procalcitonin (PCT) has been added to the diagnostic work-up of febrile patients. Levels of PCT, a prohormone of calcitonin normally produced by the thyroid in physiological conditions, rise dramatically during bacterial infection, whereas low levels are detected during viral infections or non-infectious febrile conditions[10-13]. Both the diagnostic and prognostic properties of PCT have been shown to be superior to CRP in numerous studies[11, 13-16].

PCT is (still) relatively expensive, which limits its application in resource-poor settings in tropical regions where infectious diseases are widely prevalent. Since antibiotic overuse also tends to be a large problem in these regions, improvement of the diagnostic work-up of fever is required here in particular. Unfortunately, research on PCT has almost exclusively focused on Caucasian populations in Northern countries. As such, it is difficult to apply these results directly to patients in tropical regions with different disease spectra and different genetic backgrounds.

Therefore, this study aims to investigate the diagnostic and prognostic value of PCT and CRP in an Afro-Caribbean febrile population at the emergency department (ED) of a general hospital.

MATERIALS AND METHODS

Study design and patients

The study was conducted at the ED of the St. Elisabeth Hospital, Curaçao, Netherlands Antilles and was designed as a single center, observational cohort study. The study was approved by the local ethics committee for human studies. Written informed consent was obtained from all patients.

Between March 2008 and September 2009, all patients with fever (rectal T > 38.5 °C) were included within 24 hrs. after admission. Clinical data were registered, routine laboratory measurements were performed and cultures were taken. Patient follow-up was 60 days.

Supplementary diagnostics were ordered by the attending physician. At the St. Elisabeth Hospital, bacterial diagnostics (i.e., cultures of body fluids and faeces) can be ordered routinely; viral diagnostics are limited to serology only. Regular imaging by means of X-ray, CT and MRI can be performed. No nuclear imaging can be performed.

Definitions

Final diagnoses were made at discharge by an independent physician and again in retrospect by the main investigator, using all available data, including culture results, serology, radiology and pathology data. In case of a discrepancy between discharge diagnosis and the diagnosis in retrospect, an independent senior internist was asked to give a final clinical diagnosis.

When a causative bacterium or virus could be identified, either by culture or viral serology, in concordance with patient history and clinical signs and symptoms, patients were assigned to the 'confirmed bacterial' or 'confirmed viral' groups. Confirmed parasites and fungi were classified as 'confirmed infectious – other'.

When a specific micro-organism could not be identified, but clinical signs and symptoms were consistent with radiological or pathology findings – for instance, a clear history of fever, productive cough and dyspnea, abnormal pulmonary auscultation and an infiltrate on the X-thorax -, a clinical diagnosis was assigned and patients were assigned to the 'suspected bacterial' or 'suspected viral' group. Patients who were discharged without a clear – infectious or non-infectious - diagnosis were included in the 'rest' group. When a non-infectious cause of the fever, without the presence of pathogenic micro-organisms, could be identified, patients were confined to the 'non-infectious' group.

Measurements

Blood samples were obtained by venapuncture at inclusion. Samples were centrifuged within 15 minutes (2x 4000 rpm at 4 °C for ten minutes), aliquoted in 1 mL portions and stored at – 70 °C. The blood was routinely analyzed for hematological and biochemical variables, including CRP. Leukocyte count and differential was performed on the Siemens ADVIA 120 hematology analyzer, using light scatter, differential WBC lysis, and myeloperoxidase staining to determine WBC parameters. CRP measurements were performed on the Abbott c8000 chemistry analyzer using a latex-enhanced immunoturbidimetric method. Prospective, blinded PCT-measurements were performed by using a time-resolved amplified cryptate emission (TRACE) technology assay (Kryptor PCT, Brahms, Henningsdorf, Germany) in those patients assigned to the 'confirmed bacterial', 'confirmed viral', 'confirmed infectious – other' , 'suspected bacterial', 'suspected viral' and 'non-infectious' group.

All samples were measured in a blinded fashion without knowledge of the clinical status of the subjects.

Data analysis

Data were analyzed using SPSS version 17.0. Data are presented as numbers with percentages or as medians with corresponding interquartile ranges. Associations between the presence and absence of bacterial infection and levels of PCT, CRP and leukocytes were quantified using logistic regression analysis and are expressed as odds ratios (OR) with 95 % confidence intervals (CI) and *p* values. Areas under the curve (AUC) of the Receiver Operating Characteristics (ROC) curve were calculated. A *p* value < 0.05 was considered statistically significant.

RESULTS

Patients

During the study period, 462 patients with fever were identified. In 120 patients (94 % Afro-Caribbean), a confirmed or strongly suspected diagnosis could be established. Due to logistical problems – i.e. blood not processed or stored properly -, PCT could not be measured

Table 1: Patient characteristics. Patient cohort of adults (n = 93) presenting with fever to the emergency department

	All n = 93 n (%) / median (IQR)	**Bacterial infection n = 68 n (%) / median (IQR)**	**No bacterial infection n = 25 n (%) / median (IQR)**	**p-value**
Age (yrs)	56 (36 – 72)	62 (43.5 – 73.5)	36 (28 – 53.8)	< 0.001
Sex, male	55 (59.1)	40 (58.8)	16 (64.0)	NS
Duration of fever before inclusion (days)	0 (0 – 1)	0 (0 – 1)	0 (0 – 1)	NS
Clinical signs				
Temperature (°C)	39.0 (38.8 – 39.8)	39.3 (38.9 – 39.8)	39.0 (38.7 – 39.5)	NS
Tachypnea	20 (21.5)	16 (25.4)	2 (8.0)	< 0.01
Tachycardia	57 (61.3)	43 (68.2)	14 (56.0)	NS
Mean arterial blood pressure (mmHg)	110 (97 – 127)	111 (98 – 127)	107 (97 – 125)	NS
Laboratory values				
CRP (mg %)	12.1 (2.6 – 21.9)	15.3 (6.2 – 25.3)	2.8 (1.0 – 6.8)	< 0.001
Leukocytes (giga/L)	11.4 (6.9 – 18.3)	13.1 (8.1 – 19.0)	9.0 (5.0 – 12.3)	NS
PCT (ng/mL)	0.96 (0.21 – 5.16)	1.91 (0.32 – 8.73)	0.19 (0.12 – 0.66)	< 0.001
Outcome				
Mortality	12 (12.9)	11 (17.5)	1 (4.0)	< 0.001
Hospitalization	66 (71.0)	49 (77.8)	18 (72.0)	NS
Duration of hospital stay (days)	9 (4 - 15)	7 (1 – 13)	4 (0 – 7)	0.01

in 27 of these patients. Analysis was performed in 93 patients. Patient characteristics are shown in table 1. A number of 67 patients (72.0 %) were hospitalized; from these patients, 13 patients (14.0 %) died within the follow-up period of 60 days (median 9 days after inclusions, IQR 2 -31 days) and 7 patients (7.5 %) were admitted to the Intensive Care Unit.

Final diagnoses are given in table 2. In 42 patients (45.2 %), bacterial infection was confirmed; in 26 patients (28.0 %), bacterial infection was suspected. In 4 patients (4.3 %), viral infection was confirmed; in 14 patients (15.1 %), viral infection was suspected. In 8 patients (8.6 %), a non-infectious cause of fever was identified (3 malignancy, 2 drug-induced fever, extra-uterine gravidity, sickle cell crisis, anaphylaxis).

Table 2: Final diagnosis of patients (n = 93) presenting with fever to the emergency department

Diagnosis	**n**
Urinary tract infection	21
Bacterial pneumonia	20
Dengue fever	9
Skin infection	7
Bacterial sepsis, focus unknown	6
Diabetic foot	3
HIV	3
Bacterial meningitis	2
Central line sepsis	2
Viral gastro-enteritis	2
Pelvic inflammatory disease	2
Abscess	1
Viral pneumonia	1
Viral meningitis	1
Viral respiratory tract infection	1
Diverticulitis	1
Cholangitis	1
Bacterial gastro-enteritis	1
Influenza	1
Non-infectious fever	8

Markers of infection

Levels of PCT, CRP and leukocytes are shown in *figure 1*. Median PCT levels were 0.96 ng/mL (IQR 0.19 – 5.22 ng/mL) in the overall cohort, 1.91 ng/mL (IQR 0.31 – 9.01 ng/mL) in patients with confirmed and suspected bacterial infection and 0.22 ng/mL (IQR 0.11 – 1.52

ng/mL) in patients with confirmed and suspected viral infection; median CRP levels were 12.1 mg % (IQR 2.4 – 22.0 mg %), 15.3 mg % (IQR 6.0 – 25.6 mg %) and 2.7 mg % (IQR 1.3 – 5.8 mg %); median leukocyte levels were 11.4 giga/L (IQR 6.9 – 18.6 giga/L), 13.1 giga/L (IQR 8.0 – 19.7 giga/L) and 6.6 giga/L (IQR 4.8 – 10.0 giga/L), in the same groups respectively.

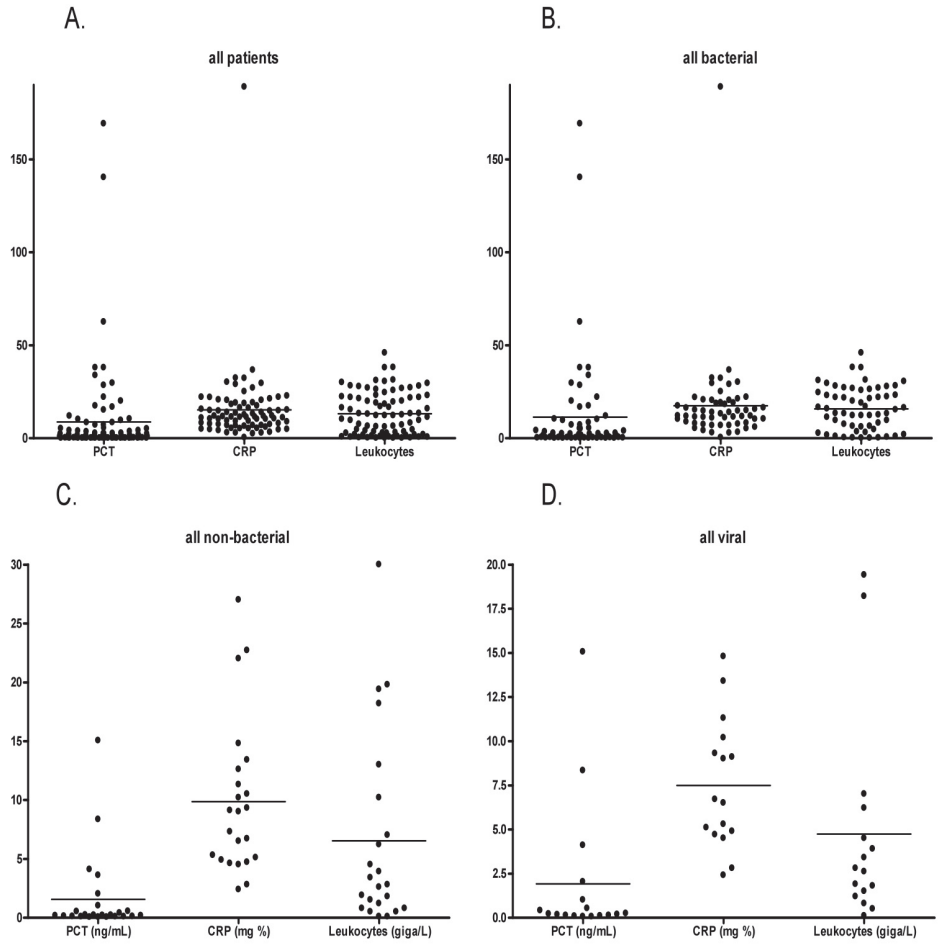

Figure 1: Levels of CRP, PCT and leukocytes in patients presenting with fever to the emergency department. Panel A shows levels of all patients together, panel B shows levels in patients with (confirmed or suspected) bacterial infection, panel C shows levels in patients with non-bacterial fever and panel D shows levels in patients with (confirmed or suspected) viral infection

Diagnostic properties

PCT levels differentiated well between infectious and non-infectious fever (AUC of 0.76, sensitivity 78 %, specificity 71 %, cut-off 0.21 ng/mL; *figure 2*), between confirmed bacterial

and confirmed viral infection (AUC of 0.82, sensitivity 85 %, specificity 69 %, cut-off 0.24 ng/mL), between confirmed bacterial infection and non-infectious fever (AUC of 0.84, sensitivity 90 %, specificity 71 %, cut-off 0.21 ng/mL) and between all bacterial infections (confirmed and suspected) and non-infectious fever (AUC of 0.80, sensitivity 85 %, specificity 71 %, cut-off 0.21 ng/mL). CRP levels were shown to be less accurate when comparing the same groups (AUC of 0.69, sensitivity 71 %, specificity 75 %, cut-off 8.3 mg %; AUC of 0.65, sensitivity 89 %, specificity 43 %, cut-off 8.5 mg %; AUC of 0.64, sensitivity 90 %, specificity 43 %, cut-off 8.5 mg %, respectively).

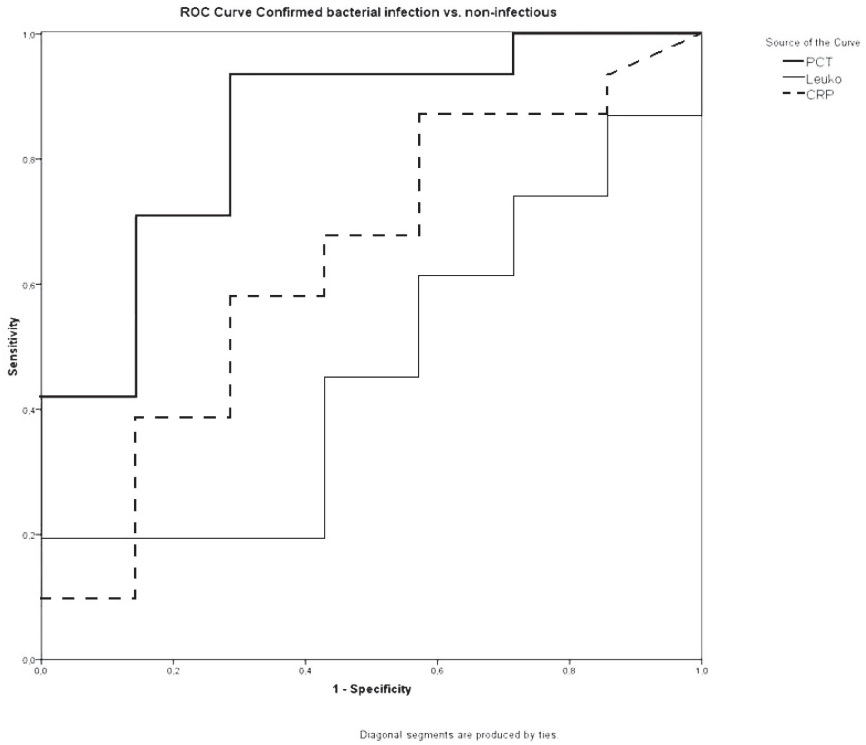

Figure 2: Receiver operating characteristic (ROC) curve, showing the diagnostic value of CRP, leukocytes and PCT for the differentiation between infectious fever and non-infectious fever. AUC PCT 0.76 (sensitivity 78% / specificity 71% at cut-off 0.21 ng/mL); CRP 0.59 (88% / 43% at 0.85 mg%); Leukocytes 0.41 (10% / 100 % at 28.0 giga/L), respectively

Prognostic properties

A significant correlation between PCT levels and confirmed bacterial infection was observed (OR 1.088; 95 % CI 1.018 – 1.162; p = 0.013). A less strong, but still significant correlation between CRP levels and confirmed bacterial infection could be found (OR 1.046; 95 % CI 1.004 – 1.090; p = 0.03). Leukocyte levels did not correlate with bacterial infection (OR 1.043; 95 % CI 0.986 – 1.104; p = 0.14).

DISCUSSION

In this study, we investigated the diagnostic and prognostic value of PCT and CRP in the diagnostic work-up of febrile patients at the ED of a general Caribbean hospital. Levels of PCT showed better diagnostic and prognostic properties than CRP. This is the first study to show that in a non-Caucasian febrile population at the emergency department, PCT is a more valuable marker of bacterial infection than CRP.

It has recently been shown in many studies that inter- and intra-racial genetic polymorphisms of inflammatory genes, including polymorphisms in the CRP-complex, are widespread and strongly influence the inflammatory response and the susceptibility to infectious diseases[17, 18]. Our study suggest that, regardless of multiple differences between genetic backgrounds and the different disease spectra, the positive results of PCT studies in Northern, Caucasian populations can likely be applied to an Afro-Caribbean population in a tropical region.

The relatively high costs of PCT measurements discourage application of PCT in resource-poor settings. Furthermore, the PCT test is not readily available on most routine clinical chemistry analyzers. However, an improved diagnostic work-up of fever is likely to result in a decreased use of antibiotics, which will not only limit the costs of the drugs themselves, but also side-effect related costs. In the long term, increased costs due to antibiotic resistance are avoided. Indeed, studies have shown that the use of a PCT based algorithm reduced use of antibiotics in a general practice setting[11]. Moreover, recent studies have demonstrated that single PCT measurements may also be used to safely guide the duration of antibiotic therapy. In the landmark ProHosp trial, a large trial investigating 1359 patients with lower respiratory tract infections, a PCT based algorithm significantly reduced the duration of antibiotic therapy as well as frequencies of antibiotic side-effects and duration of stay in the hospital[13]. Although no cost-effectiveness analysis was carried out, costs were obviously reduced this way.

Some limitations concerning this study have to be noted. First, the study was an observational study, thus providing weaker empirical evidence than a randomized controlled study would provide. Also, in many febrile patients, no definite diagnosis could be established and PCT was not measured. This may have caused some selection bias; it could be suggested that patients with a clear diagnosis were more severely ill, resulting in more aggressive diagnostic procedures. This may explain the very high AUCs in this study, assuming that in more severely ill subjects, the acute phase reaction is activated more significantly in case of a bacterial infection. Nevertheless, the primary aim of our study was to compare PCT and CRP within the same study subjects. In addition, diagnostic possibilities in Curaçao are relatively limited. Viral diagnostics generally only consist of serology with emphasis on dengue fever; PCR is not used routinely. Therefore, our study may underestimate the presence of viral disease, which may have influenced overall outcomes.

In conclusion, PCT is a valuable marker of bacterial infection in Afro-Caribbean febrile patients at the ED, with greater predictive value than CRP and other markers of infection. By implementing PCT as a routine marker in the work-up of febrile patients, diagnostic power will be improved. This may eventually lead to a reduction in antibiotic prescriptions, costs and adverse events.

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Procalcitonin in children with suspected novel influenza A (H1N1) infection

Authors:
M. Limper, MD; P.M. Smit, MD; K.M. Bongers, MD; A.P. van Zanten, PhD; P.H.M. Smits, PhD; D.P.M. Brandjes, MD, PhD; J.W. Mulder, MD, PhD; I.A. von Rosenstiel, MD; E.C.M. van Gorp, MD, PhD

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ABSTRACT

Aims and Methods

To gain insight in the acute phase response and to investigate the value of procalcitonin (PCT) in children with suspected novel influenza A (H1N1) infection, 30 patients with suspected H1N1 infection were included.

Results

Twenty-five patients (83.3 %) were diagnosed with a viral illness, including 9 patients with H1N1 infection. Median PCT levels, CRP levels and leukocyte counts in the subgroup with proven viral infections were 0.153 ng/mL with IQR 0.094–0.261 ng/mL; 19.0 mg/L with IQR 5.6–28.3 mg/L; and 13.1 giga/L with IQR 9.5–16.0 giga/L, respectively. PCT levels were below the lower cut-off value in 22 of 25 children (88%) with H1N1 infection and other viral diseases, whereas CRP levels and leukocyte counts were shown to exceed the lower cut-off value in 6 and 5 patients, respectively.

Conclusion

Low PCT values seem to be of added value to support a diagnosis of viral infection during the diagnostic procedure in children with flu-like symptoms.

INTRODUCTION

In June 2009, the first patients with Novel Influenza A (H1N1) were diagnosed in The Netherlands. Children under 5 years of age were particularly at risk for hospitalization, with a total of 574 hospitalized children under 5 years of age until December 2009, according to The National Institute for Public Health and the Environment.

Rapid diagnosis of children with flu-like symptoms is of great clinical importance, in particular for the guidance of clinical management. In case of a suspected influenza infection, there might be an indication to prescribe neuraminidase inhibitors, whereas in case of a suspected bacterial infection, the patient should be treated with antibiotics. Both therapies are associated with possible side-effects, which urges the development of clinical and laboratory markers to distinguish between viral and/or bacterial infection[1, 2]. It has been shown that the discrimination of influenza from other viral and bacterial infections in children during an influenza epidemic, based on clinical signs and symptoms, is unreliable[3]. As testing for influenza by means of nucleic acid amplification techniques in practice takes one day or more, children with flu-like symptoms will often initially get treated with double-therapy until the final diagnosis is clear.

Earlier studies showed that the differentiated white blood cell count (WBC) and the C-reactive protein (CRP) may exceed the upper limits of normal in children with adenovirus infection or influenza, sometimes mimicking findings as can be observed during bacterial infection[4, 5]. Discriminating between bacterial and viral infections, based on these parameters, might not be sufficiently adequate. In a number of pediatric studies, circulating levels of procalcitonin (PCT), the 116 amino acid pro-form of calcitonin, were mainly raised in the early stage of severe bacterial infection and only mildly raised in the absence of bacteria[6-8]. As a lower cut-off point for the exclusion of bacterial infection in children, values between 0.53-0.9 ng/mL have been suggested[9-11]. We hypothesize that PCT might be a useful tool for the discrimination of children with bacterial infection from children with viral infection during an influenza pandemic.

To our knowledge, the acute phase response in children with H1N1 infection has not been described before. To be able to evaluate the value of PCT in children with suspected viral infection during the H1N1 pandemic, more information on the acute phase response is needed. This study was performed to gain insight into the acute phase response in children with suspected H1N1 infection. Furthermore, the value of PCT in children with suspected H1N1 infection was investigated.

MATERIALS AND METHODS

Patients

From October until December 2009, 30 consecutive children with suspected novel influenza A (H1N1) infection (i.e.: patients with fever (rectal temperature >38.2 °C or tympanic temperature >38 °C) and at least two of the following complaints: cough, rhinorrhea, myalgia, sore throat, headache, chills, malaise) who underwent venapuncture for routine diagnostic purposes, were included at the freely accessible (i.e. no referral needed) influenza outpatient clinic of the Slotervaart Hospital, Amsterdam, The Netherlands. As no extra blood for specific research purposes was needed and no additional patient discomfort was caused, informed consent was not deemed necessary.

Diagnostic procedures

Standard blood tests included C-reactive protein (CRP) and differentiated white blood cell count (WBC). Quantitative PCT levels were measured using a specific immunometric assay (BRAHMS Ag, Hennigsdorf/Berlin, Germany) according to the protocol. From all patients a standard pharyngeal swab was obtained and, using primers as advised by the World Health Organization (WHO), real-time one-step polymerase-chain-reaction (RT-PCR) assays were used to detect the following pathogens: influenza A and subtype pH1N1, influenza B, rhinovirus, adenovirus, parainfluenza virus 1-4, enterovirus, human coronavirus, human metapneumovirus, respiratory syncytial virus, *Legionella* species, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. Blood and sputum cultures, lumbar punctures and radiologic examinations were routinely ordered at the discretion of the treating physician.

Statistical analysis

Values are presented as numbers with percentages or as medians with interquartile ranges (IQR). Correlations between CRP, leukocytes and PCT were assessed using Spearman's correlation test. Student's t-test was used for inter-group comparison. A p-value of less than 0.05 was considered significant. Statistical calculations were performed with SPSS software (version 17.0, SPSS Inc., Chicago, Illinois).

RESULTS

Thirty consecutive patients were included. Patient characteristics are shown in Table 1.

Table 1: Patient characteristics.

Characteristics	Total *n*=30
Female sex, n (%)	19 (63.3)
Age (months), mean ± SD	24 ± 29.8
Body temperature (°C), mean ± SD	38.1 ± 1.01
Heart rate (bpm), mean ± SD	140 ± 24.5
Oxygen saturation (peripheral), mean ± SD	97.2 ± 3.2
Hospitalized, n (%)	18 (60)

Twenty-five patients (83.3%) were diagnosed with RT-PCR confirmed viral illness, three patients were diagnosed with a - clinically suspected - viral upper airway infection without positive RT-PCR result, one patient was diagnosed with a *Mycoplasma pneumoniae* infection and one patient was diagnosed with a non-confirmed bacterial pneumonia (double-sided infiltrates on the thoracic X-ray and a good response to antibiotics; cultures negative). Overall, 9 cases of H1N1 (30%), 11 cases of rhinovirus (36.7%), 5 cases of respiratory syncytial virus (16.7%), 4 cases of adenovirus (13.3%) and one case of human metapneumovirus (3.3%) were diagnosed (Table 2). A total of 18 children (60%) were hospitalized. Patients with respiratory syncytial virus, rhinovirus and H1N1 were most frequently hospitalized (hospitalization rate 100%, 54.5% and 44.4%, respectively).

Table 2: Detected viruses by means of RT-PCR in children with suspected Novel Influenza A (H1N1) (n=30) during the H1N1 pandemic.

Virus	***n***	**%**
Influenza A (H1N1)	9	30.0
Rhinovirus	11	36.7
Adenovirus	4	13.3
Respiratory syncytial virus	5	16.7
Human metapneumovirus	1	3.3

Sum of percentages for each virus exceeds the percentage of children diagnosed with a confirmed viral illness (83.3%) due to double infections.

Median PCT value in the subgroup with confirmed viral infections was 0.153 ng/mL with IQR 0.094–0.261 ng/mL, median CRP value was 19.0 mg/L with IQR 5.6–28.3 mg/L and median leukocyte count was 13.1 giga/L with IQR 9.5–16.0 giga/L (Figure 1).

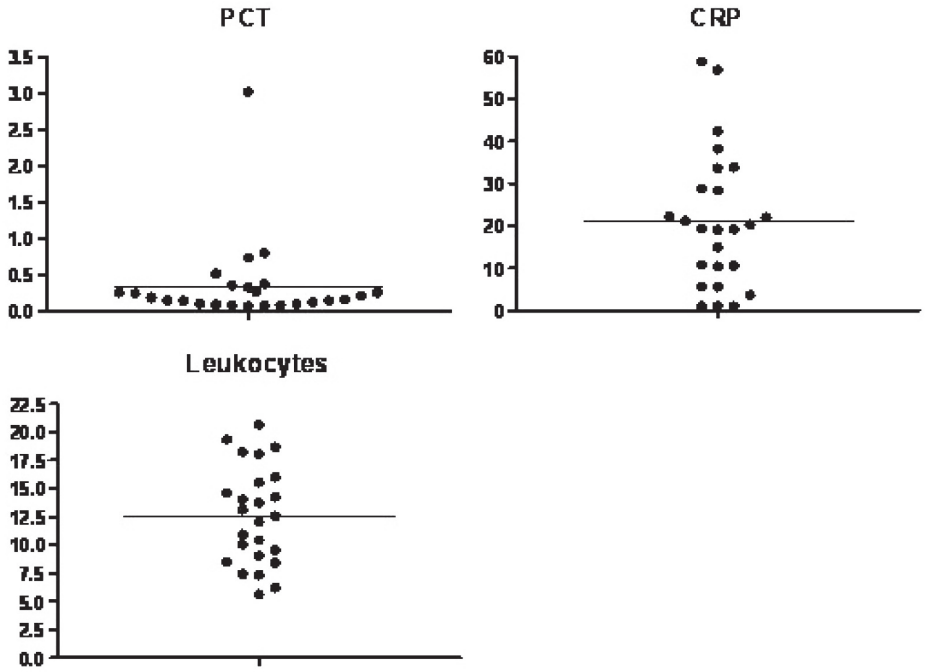

Figure 1: PCT levels (ng/mL), CRP levels (mg/mL) and leukocyte counts (giga/L) in patients (n=25) with a laboratory confirmed (RT-PCR) viral infection at presentation.

Combining this subgroup with the three cases with suspected, but non-confirmed viral infection, did not significantly influence the results (median 0.155 ng/mL, IQR 0.09–0.262 ng/mL; median 19.05 mg/L, IQR 5.53–28.53 mg/L; median 12.55 giga/L, IQR 9.13–15.88 giga/L for PCT, CRP and leukocyte count, respectively). The one case with non-confirmed bacterial pneumonia showed a marked acute phase response, with a PCT value of 32.17 ng/mL and a CRP value of 444.1 mg/L. The one case with metapneumovirus infection also exhibited elevated PCT and CRP (3.02 ng/mL and 42.3 mg/L), with normal leukocyte counts. The sensitivity of PCT for viral infection was 88%, at a cut-off <0.71 ng/mL - the earlier described lower cut-off point in healthy neonates -[10], whereas the sensitivity of CRP was 28% at a cut-off <10 mg/L and 76% at a cut-off <30 mg/L.

Subgroup analysis per virus of PCT, CRP and leukocyte counts did not show any significant difference (data not shown), although a trend towards significance could be observed in leukocyte counts, with lower counts in patients with H1N1 compared to the other viral infections (p=0.07).

In the overall cohort, a significant correlation between PCT and CRP (ρ 0.531, p=0.003), between CRP and leukocyte count (ρ 0.445, p=0.016), but not between PCT and leukocyte count (ρ 0.041, p=0.83) could be observed. Exclusion of the one case with suspected bacterial infection still resulted in a significant correlation between PCT and CRP (ρ 0.479,

p=0.010), between CRP and leukocyte count (ρ 0.383, p=0.044), and not between PCT and leukocyte count (ρ -0.065, p=0.74).

CONCLUSION

Our study was aimed to characterize the acute phase response and to assess the value of PCT in children with suspected novel influenza A (H1N1) infection during the pandemic outbreak. Based on the results of this small cohort study, we can conclude that the acute phase response in children with H1N1 infection and other viral infections is mild, with low levels of CRP, PCT and leukocyte count. For CRP and leukocyte count, this is in conjuncture with earlier studies in children and adults[4, 5, 12, 13].

We show that values of PCT are mostly below 0.71 ng/mL - the earlier described lower cut-off point in healthy neonates -[10] in children with viral infection of any cause. One patient with a human metapneumovirus infection had a PCT value of 3.02 ng/mL. The reason for this finding is unclear; one explanation might be that this patient was suffering from an undetected bacterial co-infection.

Levels of CRP were considerably low in the subgroup with viral infections and showed a significant correlation with PCT levels. However, in addition to the one patient with human metapneumovirus infection (CRP 42.3 mg/L), 5 other patients exhibited CRP values ≥30 mg/L, whereas the generally accepted lower cut-off point is <10 mg/L[14]. In this cohort, in 5 patients with a viral infection the leukocyte count was elevated (i.e. >17.5 giga/L). A correlation with CRP, but not with PCT could be found.

In this cohort, PCT measurement in children with suspected H1N1 infection seems to be of added value during the diagnostic process. Low circulating levels of PCT appear to correlate with the presence of viral infection and/or the absence of bacterial infection. Although circulating levels of CRP show a correlation with PCT and are generally low in patients with viral infection, a considerable amount of patients had moderately elevated levels of CRP. The same holds true for leukocyte counts.

Given this findings, low PCT levels seem to be of added value to support a diagnosis of viral infection during the diagnostic procedure in children with flu-like symptoms.

This study has some limitations. Apart from the small cohort size, a major drawback lies in the fact that we almost uniformly included patients with viral infections. To be able to analyze the prognostic properties of PCT, CRP and leukocyte counts in differentiating between viral and bacterial infections, a group of children with bacterial infection from the same cohort is needed. During the study period, we were only able to include one patient with a - non-proven - bacterial infection. However, many earlier studies have shown that values of PCT during bacterial infection in children can be elevated[6-8]. Because of the small sample size, analysis of the acute phase response between patients infected with different viruses is difficult. A future study should be powered to discover possible differences in

levels of biomarkers between various viral infections. Another limitation is that diagnostic possibilities for bacterial infection in children are limited. To obtain culture materials may be difficult; moreover, a negative culture result does not exclude the presence of bacteria[15]. Although we confirmed the presence of virus in a large majority of our patients, a concomitant bacterial infection may be present in some. However, all of these patients recovered without antibiotics, suggesting that this possible presence of bacterial co-infection is of little clinical importance.

The clinical presentation of children with flu-like symptoms is aspecific. There is a need for accurate discriminative diagnostic tools to guide therapeutic management. Besides optimalization of microbiological and molecular diagnostic tests, the role and the use of a selection of biomarkers may be helpful in the clinical setting. PCT in combination with clinical symptoms and routinely used markers may be of use in this context, directing the clinician in choosing antiviral or antimicrobial therapy, but also guiding the selection of those patients at risk of developing severe disease and need for intensive monitoring.

In children with flu-like symptoms, a rapid and adequate diagnosis is of great importance. Reliable biomarkers that are readily available may enhance and quicken the diagnostic process. Based on our findings, PCT may be of added value when the etiology of flu-like symptoms is unclear and the treating physician is uncertain whether to initiate or withhold antibiotics.

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PTX3 predicts severe disease in febrile patients at the emergency department

Authors:
M. Limper*, MD; M.D. de Kruif*, MD, PhD; K. Sierhuis, MD; J.F.P. Wagenaar, MD, PhD; C.A. Spek, PhD; C. Garlanda, PhD; A. Cotena, PhD; A. Mantovani, PhD, H. ten Cate, MD, PhD; P.H. Reitsma, MD, PhD; and E.C.M. van Gorp, MD, PhD

**ML and MdK contributed equally to this study*

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ABSTRACT

The long pentraxin PTX3 is a promising marker of disease severity in infection and inflammation. Previous studies showed a strong correlation between mortality and high plasma levels of PTX3 in severely ill patients. In this study, we hypothesized that PTX3 may also be a predictive biomarker for disease severity in patients presenting with fever at an emergency department. Therefore, levels of PTX3 were measured in 211 febrile patients at the emergency and the levels were linked to markers of disease severity including admittance to a special care unit, bloodstream infection and congestive heart failure. In comparison to median baseline levels of 2.30 ng/ml (interquartile range 1.66-3.67 ng/ml), levels of PTX3 were significantly elevated in patients admitted to the intensive-/medium care unit (median value 44.4 ng/ml, interquartile range 13.6-105.9 ng/ml) and in patients referred to the ward (median value 14.2 ng/ml, interquartile range 7.01-25.1 ng/ml); as such, PTX3 was a significant predictor of admission to the ICU/MC. In addition, PTX3 was a significant predictor of duration of hospital stay and a weak correlation was found with mortality, but this association was no longer significant after correction for acute congestive heart failure. Furthermore, this study showed that PTX3 was a significant predictor for bloodstream infection (AUC =0.71; 95% CI 0.62-0.81) and acute congestive heart failure. Taken together, these data show that PTX3 may be a useful marker for differentiation of patients with severe disease in patients presenting with fever to the emergency department.

INTRODUCTION

Fever is characteristic for many inflammatory conditions, yet it is particularly associated with bacterial infections. At the emergency department, a quick diagnosis is essential in patients with fever to define patients at risk for severe sepsis as soon as possible, as it has implications for treatment and prognosis; this has been referred to as 'the golden hour of sepsis'[1, 2]. However, current diagnostic tools lack either speed, e.g. when taking bacterial cultures, or diagnostic value in terms of sensitivity and specificity[3, 4]. Plasma derived biomarkers may be helpful to solve this issue. A promising biomarker for differentiation of patients with fever is the acute phase protein pentraxin 3 (PTX3)[5].

The long pentraxin PTX3 belongs to an evolutionary conserved superfamily of pentraxins, which has been categorized into short and long pentraxins based on the structure of their subunits[6-8]. Other key members of this family include the short pentraxins C-reactive protein (CRP) and serum amyloid P component (SAP). Although the short and long pentraxins share common sequences, they are encoded by different genes and are differentially regulated[5]. The function of PTX3 is not completely clear, but involvement in inflammatory pathways is considered likely since mice lacking the gene encoding for PTX3 were shown to be highly susceptible for Aspergillus infections[9].

Increased levels of PTX3 have been reported in multiple inflammatory conditions[10-13]. In critically ill patients with sepsis, highly increased levels were detected, which correlated with disease severity, ranging from the systemic inflammatory response syndrome (SIRS) to sepsis and septic shock[14]. Moreover, PTX3 was found to correlate significantly with mortality[12, 14]. Furthermore, PTX3 has also been linked to severity of disease in patients with cardiovascular disease including acute myocardial infarction, unstable angina pectoris and heart failure[15-20].

Considering the potential of PTX3 as a biomarker in inflammatory disease, the marker is of interest to the relatively large group of patients presenting with fever to the emergency department. Therefore, we here aimed to determine levels of PTX3 in a cohort of 211 patients who presented to the emergency department with fever. In addition, the relationship was investigated between PTX3 and clinical markers linked to severity of disease, including admission to a special care unit, presence of bloodstream infection and congestive heart failure.

MATERIALS AND METHODS

Study design and definitions

The study was conducted at the emergency department of the Slotervaart hospital, Amsterdam, The Netherlands, between April 2004 and October 2006; the study protocol has been described in detail elsewhere[21]. The study was approved by the institutional scientific

and ethics committee of the Slotervaart hospital, Amsterdam, the Netherlands. Written informed consent was obtained from all subjects. Adult patients, 18-85 years old, presenting with fever to the emergency department were included. Fever was defined as an ear temperature of 38.0°C or higher. For diagnosis of bacteraemia, a total of 3 blood cultures were taken. Other, local bacterial and viral cultures were taken from the suspected focus of infection as judged by the treating physician. Mortality and admission to the intensive care unit or medium care (ICU/MC) were determined for a period of 30 days after admission. Bacteraemia was defined as a positive blood culture with a likely pathogen, considering the underlying disease, within 7 days of admission. According to the results, pathogens were classified into gram-negative or gram-positive organisms. Congestive heart failure was diagnosed by the treating physician based upon symptoms of dyspnea, radiologic findings and response to diuretic therapy. A medical history of heart failure in patient records was considered positive only in combination with current use of heart failure medication.

Laboratory methods

Blood samples were obtained by venapuncture at inclusion and follow-up. The samples were centrifuged within 15 minutes (2x 3000 rpm at 5 °C for ten minutes), aliquoted and stored at -80 °C. Plasma levels of PTX3 were measured using a sandwich enzyme-linked immunosorbent assay as previously described[22]. Plasma PTX3 levels are expressed as ng/ml. Other laboratory investigations were routinely performed by the clinical laboratory and the department of microbiology of the Slotervaart Hospital, Amsterdam, The Netherlands.

Statistical analysis

Data analyses were performed using SPSS version 15.0. Group differences were calculated using Mann-Whitney U test. To determine correlations between plasma levels of PTX3 and relevant clinical parameters, logistic regression analysis was performed for binominal variables and Spearman's ranked correlation test for continuous variables. Data are presented as medians with corresponding interquartile ranges (IQR) or as numbers with percentages; odds ratios (OR) with 95 % confidence intervals (CI) and Spearman's rho coefficients are shown when appropriate. A p value <0.05 was considered significant.

RESULTS

Patients

In total, 211 patients were included. From these, a number of 15 patients was admitted to the intensive care unit (n =10) or medium care unit (n =5); these patients are further referred to as the ICU/MC group versus the 'ward' group (n =194). Patient characteristics are shown in table 1. Median age was 67 years (IQR 61-72 years) and 63 (IQR 46-74 years) in the ICU/MC-

and ward group, respectively. Five patients died. At a one-month follow-up visit, samples were collected from 55 people who returned after full recovery of their illness.

Table 1. Patient characteristics

	Non-ICU/MCU N=194 n (%) / median (IQR)	ICU/MCU admission N=15 n (%) / median (IQR)
Age, years	62 (45-74)	69 (62-77)
Sex, male n (%)	106 (54%)	9 (60%)
Underlying morbidity		
malignancy, n (%)	17 (9%)	0 (0%)
HIV, n (%)	9 (5%)	1 (7%)
diabetes mellitus, n (%)	32 (16%)	5 (33%)
Location		
cardiovascular, n (%)	1 (1%)	0 (0%)
respiratory, n (%)	94 (48%)	7 (47%)
gastro-intestinal, n (%)	31 (16%)	3 (20%)
Musculo-skeletal, n (%)	3 (2%)	0 (0%)
urogenital, n (%)	27 (14%)	4 (27%)
skin, n (%)	12 (6%)	0 (0%)
other, n (%)	12 (6%)	0 (0%)
unknown, n (%)	16 (8%)	1 (7%)
SIRS, n (%)	151 (77%)	15 (100%)
Sepsis, n (%)	53 (27%)	10 (67%)
Positive culture, n (%)	64 (33%)	10 (67%)
Positive blood culture, n (%)	31 (48%)	4 (40%)
Gram-positive, n (%)	29 (45%)	5 (50%)
History of congestive heart failure, n (%)	19 (10%)	7 (47%)
Acute congestive heart failure, n (%)	13 (7%)	7 (47%)
C-reactive protein, ng/ml	139 (68-246)	169 (66-284)
Hospital stay, days	7 (4-12)	22 (18-32)
30-day mortality, n (%)	2 (1%)	3 (20%)

PTX3 levels and disease severity

Levels of PTX3 are shown in figure 1. At baseline, median PTX3 levels were 2.30 ng/ml (IQR 1.66-3.67 ng/ml). The levels were increased in ward patients (14.2 ng/ml; IQR 7.01-25.1 ng/ml; p =0.0009), whereas highly elevated levels were observed in patients admitted to ICU/MC (44.4 ng/ml; IQR 13.6-105.9 ng/ml; p <0.0001).

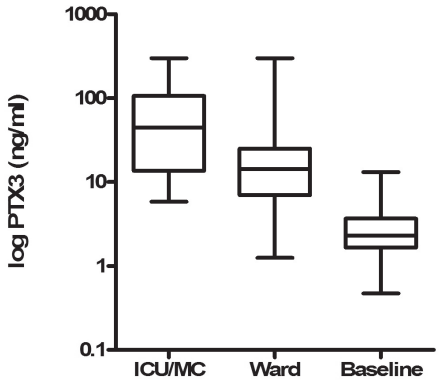

Figure 1: Levels of PTX3. PTX3 was measured in 211 patients presenting with fever to the emergency department. Patients were admitted either to the ward or to the intensive care- or medium care unit (ICU/MC) and baseline samples were collected at a 1-month follow-up visit.

Using logistic regression analysis, PTX3 was a significant predictor of ICU/MC referral (OR =1.014; 95% CI 1.006- 1.021; p <0.0001). After adjustment of this association for congestive heart failure, the OR was 1.013 (95% CI 1.005-1.020; p =0.001). PTX3 levels showed a

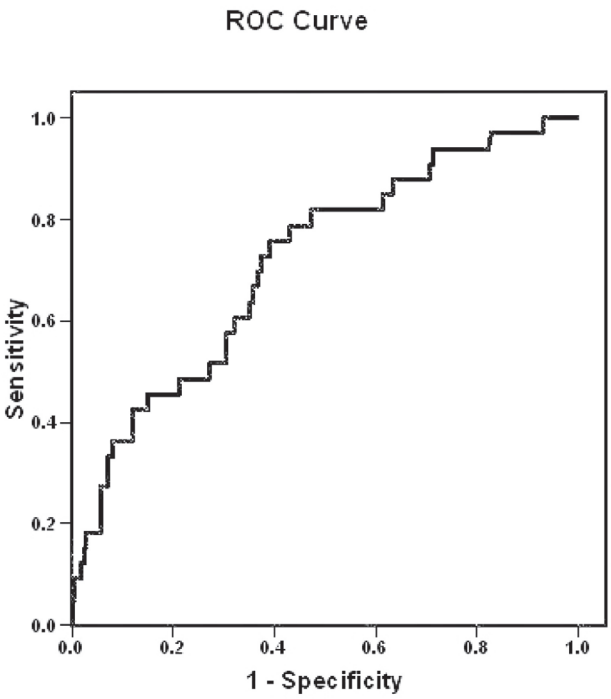

Figure 2: ROC curve for prediction of bacteraemia by PTX3. Levels of PTX3 and blood culture results were determined in 211 patients presenting with fever to the emergency department.

weak association with mortality (OR 1.010; 95% CI 1.002-1.019; p =0.018). After adjustment for congestive heart failure, the association was no longer significant (p =0.120). The total duration of hospital stay correlated with PTX3 values at admission (Spearman's rho 0.191; p =0.006). Levels of PTX3 correlated with levels of CRP (Spearman's rho 0.355; p <0.0001).

Bloodstream infection

A significant correlation between PTX3 levels and presence of bloodstream infection was observed (OR 1.012; 95% CI 1.005-1.020; p =0.002). The Receiver Operating Characteristic curve is presented in figure 2. The area under the curve was 0.71 (95% CI 0.62-0.81).

At a previously set 'normal' cut-off value of PTX3 of 2.0 ng/ml, our data showed a sensitivity of 100% with a specificity of 2%. The optimum cut-off value was 16.1 ng/ml, which showed a sensitivity and specificity for prediction of bloodstream infection of 76% and 61%, respectively. Other cut-off points are presented in table 2. The levels of PTX3 were not differentially associated with infection by either gram-negative or gram-positive organisms.

Table 2: Cut-off values of PTX3 for prediction of bloodstream infection in patients presenting with fever to the emergency department.

Cut-off value (ng/ml)	Sensitivity	Specificity	Positive predictive value	Negative predictive value
2.00	100%	2%	16%	100%
7.96	90%	29%	20%	94%
16.1	76%	61%	27%	93%
43.8	36%	90%	40%	88%
100	15%	98%	56%	86%

Congestive heart failure

PTX3 was associated with acute congestive heart failure (OR 1.008; 95% CI 1.002-1.015; p =0.016). In addition, PTX3 levels at admission were related to a positive medical history of heart failure (OR 1.007; 95% CI 1.000-1.013; p =0.047); however, after adjustment for acute congestive heart failure this association was not significant. Baseline levels of PTX3 did not correlate with a medical history of heart failure.

DISCUSSION

In this study, we investigated the potential use of the long pentraxin PTX3 as a biomarker in patients presenting with fever to the emergency department. At admission, levels of PTX3 in febrile patients were significantly elevated in comparison to samples collected after full recovery at a one-month follow-up visit. Moreover, in patients admitted to the ICU/MC, the

levels of PTX3 were higher in comparison to levels of patients referred to the ward; as such, PTX3 was shown to be a significant predictor of admission to the ICU/MC. In addition, PTX3 was a significant predictor of duration of hospital stay and a weak correlation was found with mortality, though this association was no longer significant after correction for acute congestive heart failure. Furthermore, this study showed for the first time that PTX3 was a significant predictor for bloodstream infection and that, in agreement with data from others[16, 17], the levels correlated with acute congestive heart failure. Taken together, these data show that PTX3 may be a useful marker for differentiation of patients with severe disease in patients presenting with fever to the emergency department.

Fever is a presentation symptom which is more than any other symptom suggestive for microbial infection and sepsis. In recent years, it has become increasingly clear that in patients with sepsis, early recognition of severe disease is of major importance to therapy and prognosis, as it was demonstrated that early, aggressive treatment may reduce mortality and that the efficacy of many sepsis intervention strategies appears to depend on the prognosis of the patient[23, 24]. Within this context, there is a need for biomarkers to predict severe disease such as PTX3. The findings in our study, which showed that PTX3 correlated with ICU/MC admission and duration of hospital stay, are consistent with findings in patients with SIRS, sepsis or septic shock at the ICU and in patients who suffered from severe leptospirosis or dengue virus infections[12, 14]. Of note, associations with disease severity were also reported recently in other conditions such as renal failure, acute myocardial infarction or psoriasis[10, 15, 19, 20, 25]. As such, PTX3 is a stable, promising predictor of disease severity in different patient populations with inflammatory disease.

The plasma levels of PTX3 that were found in this study in patients with fever were 14.2 ng/ml in the ward group and 44.4 ng/ml in the ICU/MC patients. These levels are higher than a previously set 'normal' value of 2.0 ng/ml[15], though, like in the current study, also slightly higher baseline levels have been reported[12]. The levels found in the current study are relatively high as compared to levels in moderately ill patients showing median values of 2.84 ng/ml and 6.20 ng/ml in patients with psoriasis and unstable angina pectoris, respectively[10, 18]. However, comparable levels of 28.0 ng/ml were found in patients with SIRS, whereas in septic shock much higher levels were observed with a median of 251 ng/ml[14]. These results indicate that not only within studies, but also between studies, PTX3 levels associate with the severity of the immunological stimulus.

Bloodstream infection is associated with a higher mortality than localized infection; thus, it must be regarded as a marker of disease severity[3, 26, 27]. Studies showed that other biomarkers such as procalcitonin, CRP or coagulation markers were able to predict bloodstream infection[28-30]. The data from the current study show that PTX3 may be an additional marker for bloodstream infection. The optimum cut-off value in our study was 16.1 ng/ml, which showed a sensitivity of 76% and specificity of 61%. Though, like most current biomarkers in this setting, these figures are not sufficient for diagnosis and clinical decision making based upon the value of PTX3 alone, these predictive properties of PTX3 are of interest to current,

promising efforts aiming to combine various biomarkers into one single, optimal predictive model[21, 30, 31].

The association found in the current study between acute congestive heart failure and PTX3 is consistent with earlier research showing higher levels of PTX3 in patients suffering from myocardial infarction, unstable angina pectoris or heart failure[15-19]. The underlying mechanism may be related to activation of inflammatory pathways induced by myocardial stress[32]. Also coagulation pathways may be involved, as was suggested after finding enhanced expression of tissue factor, which is a major initiator of the coagulation cascade, in monocytes and endothelial cells stimulated by PTX3[33, 34].

The precise pathophysiological role of PTX3 remains to be elucidated. Like other pentraxins, PTX3 is not only an acute phase protein, with elevated levels as a result of inflammation, but also acts as a component of innate immunity[5, 8]. A protective role of PTX3 was demonstrated in PTX3 knock-out mice with fungal infections, displaying a beneficial effect on immunity and clinical outcome[9]. PTX3 appears to be stored in specific granules in neutrophils and can be secreted to neutrophil extracellular taps in response to immunological stimuli[35]. Further mechanisms include regulation of complement activation via binding of PTX3 to complement factors[36] and a role in the regulation the recognition of apoptotic cells by inflammatory cells[37].

Investigators have suggested previously that PTX3 may be selectively induced by various pathogens[13]. It was demonstrated that PTX3 binds to *Pseudomonas aeruginosa*, *Salmonella typhimurium*, and *Paracoccidioides brasiliensis*, but not to *Escherichia coli*, *Burkholderia cepacia*, and *Listeria monocytogenes*[9]. In fungi, PTX3 was bound selectively to *Aspergillus fumigatus*, *Aspergillus flavus* and *Aspergillus niger*, but not to *Candida albicans*[9]. Because of these results and because differential associations were found between the short pentraxin CRP and gram-negative bacteraemia[38], we tested this hypothesis for the difference between gram-negative and gram-positive organisms and PTX3; yet we found no association.

Observational studies are limited by their inability to distinguish between primary and secondary effects and by difficulties to control for confounding factors[39, 40]. Nevertheless, they have often proven useful to find patterns of associations helping to elucidate mechanisms of clinical disease. With these limitations in mind, the current study shows that in patients presenting with fever to the emergency department, levels of PTX3 were elevated and correlated with disease severity parameters including admission a special care unit, bloodstream infection and congestive heart failure. As such, alone or in combination with other markers, PTX3 may be a valuable biomarker for disease severity at the emergency department.

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PART III: SUMMARY AND APPENDICES

Summary and discussion

Authors:
M. Limper, MD; A.J. Duits, PhD and E.C.M. van Gorp, MD, PhD

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Fever as a symptom of disease remains a challenge for clinicians. Fever, often associated with bacterial infection, can be the result of a variety of pathological conditions. Even though relatively new diagnostic tools, such as polymerase chain reaction (PCR), have been successfully introduced in daily clinical practice, a fast and reliable diagnosis of diseases causing fever is still difficult to obtain. Commonly used tests for this purpose have low sensitivity and specificity; as a result, patients are frequently treated solely based on circumstantial evidence. It is important to improve diagnostics in the field of fever. Not only for patients - who will receive the appropriate treatment faster and will suffer less unnecessary side effects of drugs that have been wrongfully prescribed -, but also for society in general, aiding the combat against antibiotic resistance and reducing health care costs.

In this thesis, we aimed to study biomarkers in inflammation and infection, with a special focus on the distinction between infectious and non-infectious fever. Epidemiological knowledge is the first step in the process of finding better biomarkers for diagnosing febrile patients. Therefore, after a general introduction in *chapter 1*, in *chapter 2* the epidemiology of patients presenting with fever at an Emergency Department (ED) in Amsterdam, the Netherlands and Curaçao, Netherlands Antilles is presented. We show that both in the Netherlands and in Curaçao, mortality in these patient groups is considerably high (4.2 and 7.9 %, respectively) and most patients suffer from bacterial infections. However, approximately 1 out of 20 febrile patients at the ED is eventually diagnosed with a non-infectious cause of fever, with malignancy as most notable underlying cause. Although no data on incidence of non-infectious fever at the ED have been published before, the incidence of infectious fever we find is comparable to that found by others; therefore, our findings can be extrapolated to other hospitals worldwide.

Proving the absence of bacterial infection may be worth as much as demonstrating the presence of bacterial infection and may in some situations be easier and enough to guide therapy. In *chapter 3*, we therefore review the literature investigating the diagnostic value of biomarkers during episodes of non-infectious fever. Among many possible markers, for now procalcitonin (PCT) seems to be the most sensitive and specific laboratory marker for this purpose, particularly in autoimmune, autoinflammatory and malignant diseases.

In Part II, we describe our studies in different patient cohorts with focus on biomarkers, suitable for discriminating between – bacterial - infection and non-infectious inflammation.

In *chapter 4,* we focus on the predictive value of biomarkers in patients with arthralgia, with some of these patients progressing to rheumatoid arthritis, a non-infectious, inflammatory disease with a marked acute phase response. We observed a trend towards higher levels of several markers of the acute phase response in those patients who progressed to rheumatoid arthritis; these higher levels may be of use for research purposes, but do not possess enough diagnostic power to have clinical consequences on the individual level.

In *chapter 5, 6 and 7* we compare sensitivity and specificity of different commonly used and experimental biomarkers in different febrile patient groups. *Chapter 5* describes the development of a clinical decision rule for the prediction of bacterial infection in febrile patients at an emergency department in Amsterdam. We show that adding PCT to a

model including a clinical variable (cold chills) and a commonly used laboratory marker (C-reactive protein (CRP)) leads to superior predictive power. In *Chapter 6* we demonstrate for the first time that PCT is a valuable biomarker of bacterial infection in a febrile Afro-Caribbean population. *Chapter 7* focuses on febrile children during the 2009 influenza A (H1N1) pandemic. In a small cohort study, we show that regular diagnostic tests such as white blood cell count and CRP are unreliable for the diagnosis of viral disease and that PCT may be of added value when the treating physician is uncertain whether to withhold antibiotics.

Not every bacterial infection requires treatment with antibiotics. Knowing which patient needs to be treated without any further delay and which patient can be safely observed without treatment, would be very helpful in daily practice. In *chapter 8* we investigate the correlation of several biomarkers with severity of disease. We conclude that the long pentraxin-3 (PTX-3), a relatively new and unknown marker of the acute phase response, may serve as a good indicator of severity of disease and mortality in patients with fever at the emergency department.

Discussion and recommendations for future research

Epidemiology of infectious diseases is continuously changing with place and time. Pathogens are evolving and (re-)emerging, climate change brings new diseases and eliminates others, medicines cure patients but may create new diseases. New diagnostic tools give rise to the discovery of completely new and unexpected pathogens. It is important to systematically investigate and document the pathogens involved in febrile disease in different regions worldwide. With better knowledge of which pathogens to expect while examining a febrile patient, the pre-test chance of a positive finding with an appropriately chosen test will be higher, enabling physicians to diagnose patients more accurately. Systematic in- and out-hospital surveillance will help to detect possible infectious threats more effectively. With a better understanding of the epidemiology of disease, better preventive measures can be taken.

For optimal diagnostics in febrile disease, one should be able to prove either the presence of a pathogen (at the same time excluding other pathogens) or non-infectious disease with certainty. Given the enormous amount of pathogens and non-infectious febrile conditions, this seems far out of reach. On the other hand, the introduction of PCR as a diagnostic tool into clinical practice already has led to much more possibilities for achieving this objective. Further research should focus on the impact of PCR and other molecular techniques that give us the opportunity to demonstrate or exclude many pathogens with one fast and reliable test. Until better direct diagnostic tests have been validated, the results for the biomarkers as presented in this thesis are of much added value in the diagnostic work-up of a febrile patient. For now, PCT seems the most reliable and effective of the newly introduced markers. With a vast amount of clinical data from retrospective and prospective studies, use of PCT now becomes more and more accepted in daily clinical practice.

Despite many clinical studies with promising results, the (patho-)physiological role of PCT remains unclear. Whereas CRP has been well-characterized in many studies - both from the point of view of production, which almost uniformly takes place in the liver after stimulation by IL-6, as from its function, the binding to phosphocholine expressed on certain microbes and on necrotic or apoptotic cells, thus activating the complement system via C1q and enabling opsonization[1] – to date, it is not yet elucidated what the source of 'inflammatory PCT' is. Under physiological circumstances, PCT is produced in the C-cells of the thyroid gland and practically all is converted into calcitonin, resulting in very low levels of circulating PCT. Whereas thyroid C-cells react to several stimuli, such as hypercalcemia, glucocorticoids, glucagon and β-adrenergic stimulation, by releasing more PCT, no link between 'inflammatory PCT' and these stimuli can be shown.[2] A strong link between bacterial endotoxins, as well as TNF, and elevated PCT levels has been established.[3] It has been hypothesized that PCT is involved in modulation of NO synthesis, and, as such, should be classified as a pro-inflammatory cytokine. Also, PCT has been proposed to have an analgesic effect, with an association between lower levels of nocireceptors and higher PCT levels.[4] Lastly, it was shown that exogenous administration of PCT in septic animals led to an increase in mortality; as such, PCT may not only be an indicator of severity of disease, but may also increase mortality via an unknown pathway.[5] Given the fact that during bacterial infection the human body spends its energy on the production of large quantities of PCT, one would assume that there must be a pathophysiological role for PCT. Future research is needed to further clarify this. Despite the uncertainty about the source of PCT and its function, it is the first biomarker since the introduction of CRP that literally found its way from bench to bedside and provides a welcome addition to the current diagnostic panel.

However, a well-designed randomized prospective cohort study, evaluating PCT as a guide for antibiotic therapy in febrile patients visiting the emergency department is still lacking.

We conducted a pilot-study at the Slotervaart Hospital in Amsterdam, the Netherlands, showing that use of PCT leads to a reduction of antibiotic use and is safe, with no difference in mortality or length of hospital stay between a PCT-guided antibiotic therapy group and the control group. In this study, all patients at the Emergency Department with fever, defined as a temperature > 38.2 ˚C, were included and randomized to the PCT-guided antibiotic therapy-arm or the standard-of-care arm. Routine clinical work-up was performed in all patients, including routine laboratory analysis and necessary supplementary diagnostics as judged by the attending physician. After finishing the work-up, the attending physician was required to give a provisional diagnosis, discerning between bacterial- and non-bacterial disease; furthermore, the physician was asked to state whether antibiotics were deemed necessary. After this, for patients in the PCT-guided arm, the PCT-value was visible for the treating physician. Based on pre-defined cut-off values, an advise on antibiotics was given (PCT < 0.5 ng/mL; no antibiotics advised – PCT > 0.5 ng/mL; antibiotics advised). The treating physician could ignore the PCT-based advise at all times, but was in that case asked for a motivation.

One hundred and eight patients were included. PCT was a better predictor of bacterial infection than other laboratory measurements, including CRP. In the PCT-guided group, fewer antibiotics were prescribed as compared to the standard-of-care group. There was no difference in length of hospital stay or mortality between groups.

From this small pilot study, we can conclude that PCT-guided antibiotic therapy seems useful and safe in patients with undifferentiated fever at the Emergency Department. We are currently starting up a large trial to further confirm these findings.

As mentioned above, biomarkers are able to guide us through the work-up of febrile patients, providing us with important information that adds up to other clinical findings, resulting in a treatment plan with or without antibiotics. However, after the very start of infection or inflammation, it takes substantial time for biomarkers to be produced. Moreover, the measurement of circulating biomarkers is a 'downstream'-measurement, and as infection and inflammation share many common immunological pathways, important differentiating information will not be available. Ideally, one should be able to measure biomarker production at the source, that is, at the site of RNA-production.

In clinical practice, the introduction of new and very sensitive molecular techniques such as PCR has contributed to easier diagnosis of viral disease.[6] To date, however, growth of bacteria remains the gold standard for the confirmation of bacterial infections. As cultivation of bacteria is not straight-forward, with many species growing slowly and requiring special media, it is often difficult or even impossible to confirm bacterial etiology, resulting in overuse of antibiotics and uncertainty about definite diagnosis. To overcome the problem of cultivation, the host response to a certain pathogen could be used as a diagnostic tool. It has been extensively shown that host gene signatures differ with different pathogens. Specific pattern-recognition receptors are expressed on leukocytes and can be measured *in vivo*.[7,8] It has not only been shown that comparison of global gene expression profiles reveals specific signatures for bacteria; also, gene expression profiling has been shown to be a valuable tool to distinguish auto-immune disease from viral disease, and to distinguish one virus from another.[8,9]

Micro-arrays, such as MLPA as described in *chapter 4*, measure the differences in expression of thousands of genes during physiological and pathological states. By measuring gene expression in a parallel way, micro-array based techniques allow for different molecular pathways in the cell to be studied and insight is provided in the dynamics of physiological and pathological processes. Furthermore, subtle differences in gene expression can be determined, as micro-arrays are very sensitive. Micro-arrays can be used both for genotyping and gene expression measurements. Genotyping can be used to analyze the genomic DNA content of a pathogen; gene expression can be measured in either pathogen or host, thus enabling investigation of host-pathogen interactions.

Micro-arrays can thus be used as valuable tools for diagnosis of infection by means of organism detection or discovery of novel pathogens, can be used for epidemiological investigation and can give insight in virulence of pathogens. Furthermore, the host response can be measured dynamically and susceptibility to certain pathogens can be evaluated.[10, 11]

One of the clinical shortcomings of micro-arrays is the enormous amount of information they provide. In a hospital setting, most of this information is too detailed and as such irrelevant. For patient care, presence or absence of specified pathogens should be confirmed in a fast and reliable way. It has been shown that a relatively small selection of gene expression in the host is sufficient to answer these basic questions, by measuring pathogen-specific gene expression signatures or patterns.[8] These patterns do not only indicate the presence of different types of bacterial or viral infections; by comparing over- and under-expression of RNA with the mean expression in healthy controls, a relative 'distance to health' can be estimated, thus indicating severity of disease.[12]

If the use of micro-arrays as diagnostics in febrile disease becomes more practically feasible, diagnostic procedures will be faster and more reliable. The dynamics of the acute phase response during different pathological conditions will be unraveled from a 'host perspective', not only resulting in more reliable diagnostics, but potentially also in new targets for therapy. As the use of micro-arrays is becoming easier and cheaper, micro-arrays will find their way to hospital use and will probably replace biomarker testing in the upcoming decades.

Further biomarker studies in febrile patient populations should not only prospectively evaluate if PCT can indeed be used as a diagnostic tool in a larger population with undifferentiated fever, but also if a PCT-guided therapy is safe and cost-effective. Another potential strength of PCT, which has not been evaluated in this thesis but has been shown mostly in Intensive Care studies[13, 14], is its ability to guide termination of antibiotics after treatment has been initiated. Despite earlier suggestions that the average duration of treatment with antibiotics might be too long[15], clinicians still tend to treat infections for at least seven days. Declining PCT values may be used as a guide for shorter treatment. Again, a well-designed prospective non-ICU study needs to be undertaken.

In our opinion, gene profiling studies will be the focus of biomarker research in the next decade. It will be very interesting to perform sequential gene expression during infection and inflammation, mapping the dynamics of gene pathways in health and disease. With better applied bioinformatics, future studies will enable the streamlining of the massive amount of information gene profiling techniques provide, and hopefully result in new sets of biomarkers - from bench to bedside.

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CHAPTER 11

Nederlandse samenvatting en discussie

Koorts blijft voor artsen een lastig te interpreteren symptoom van ziekte. Bij koorts wordt vaak gedacht aan bacteriële infectie, maar koorts kan ontstaan door een veelheid aan ziektebeelden. Ondanks de recente klinische introductie van nieuwe diagnostische hulpmiddelen, zoals de *polymerase chain reaction* (PCR), is het nog altijd moeilijk om een snelle en betrouwbare diagnose te stellen bij patiënten met koorts. De doorgaans gebruikte diagnostische onderzoeken hebben een lage sensitiviteit en specificiteit; dientengevolge worden patiënten vaak slechts behandeld op basis van indirecte aanwijzingen. Het is belangrijk om de diagnostiek in het 'koortsveld' te verbeteren; niet alleen voor patiënten, die met snellere en betere diagnostiek eerder de juiste therapie zullen krijgen en daardoor minder last zullen hebben van onnodige bijwerkingen door onterecht voorgeschreven medicatie, maar ook voor de maatschappij, om antibiotica-resistentie terug te dringen en kosten van de gezondheidszorg te reduceren.

In dit proefschrift hebben wij biomarkers bij ontsteking en infectie onderzocht, waarbij vooral gekeken is naar het onderscheid tussen infectieuze en niet-infectieuze koorts. De eerste stap op weg naar betere biomarkers voor diagnostiek bij koortspatiënten bestaat uit gedegen kennis van epidemiologie van koortsende ziektes. Om die reden wordt - na een algemene introductie in hoofdstuk 1 – in hoofdstuk 2 de epidemiologie van patiënten die zich met koorts op de Spoedeisende Eerste Hulp (SEH) in Amsterdam en Curaçao presenteren, beschreven. We laten zien dat in Nederland en op Curaçao mortaliteit in deze patiëntengroep aanzienlijk is (respectievelijk 4.2 % en 7.9 %) en dat de meerderheid van patiënten een bacteriële infectie heeft. Ongeveer 1 op de 20 patiënten met koorts op de SEH blijkt echter niet-infectieuze koorts te hebben, met maligniteiten als meest voorkomende onderliggende ziekte. Incidentie-data van niet-infectieuze koorts op de SEH zijn niet eerder gepubliceerd; aangezien de door ons gevonden incidentie van infectieuze koorts vergelijkbaar is met de incidentie zoals door anderen gevonden, lijkt extrapolatie van onze data naar andere ziekenhuizen wereldwijd gerechtvaardigd.

Het bewijzen van afwezigheid van bacteriële infectie, in plaats van het aantonen van de áánwezigheid ervan, kan in klinische situaties evenveel waard zijn, is soms eenvoudiger en kan voldoende zijn om therapeutische beslissingen te sturen. In hoofdstuk 3 presenteren we een overzicht van de literatuur waarin de diagnostische waarde van biomarkers tijdens episodes van niet-infectieuze koorts is onderzocht. Op dit moment lijkt procalcitonine (PCT), naast andere mogelijke markers, de meest geschikte laboratoriumtest voor het onderscheid tussen infectieuze en niet-infectieuze koorts, met name bij auto-immuun, auto-inflammatoire en maligne ziektes.

In Deel II ligt de nadruk op biomarkers en hun rol in het onderscheid tussen (bacteriële) infecties en niet-infectieuze ontsteking. We lichten in hoofdstuk 4 de voorspellende waarde van biomarkers toe bij patiënten met arthralgie, waarbij sommige patiënten reumatoïde arthritis ontwikkelen, een niet-infectieuze ontstekingsziekte met een uitgesproken acute fase-reactie. We observeerden een trend richting hogere waardes van verschillende markers van de acute fase-reactie in die patiënten die uiteindelijk reumatoïde arthritis ontwikkelden;

deze hogere waardes kunnen van waarde zijn voor verder onderzoek op groepsniveau, maar de verschillen zijn te klein om klinische consequenties te hebben voor de individuele patiënt.

In hoofdstuk 5, 6 en 7 vergelijken wij de sensitiviteit en specificiteit van verscheidene veelgebruikte en experimentele biomarkers in verschillende patiëntengroepen met koorts. Hoofdstuk 5 beschrijft de ontwikkeling van een klinische beslisregel die de aanwezigheid van bacteriële infectie voorspelt bij patiënten met koorts op de SEH in Amsterdam. We laten zien dat een model bestaand uit PCT, een klinische variabele (koude rillingen) en een veelgebruikte laboratoriumtest (C-reactive protein (CRP)) de grootste voorspellende waarde voor dit doel geeft. In hoofdstuk 6 tonen wij voor het eerst aan dat PCT een waardevolle biomarker is voor bacteriële infectie in een Afro-Caribische populatie. Hoofdstuk 7 beschrijft een cohort van kinderen met koorts tijdens de 2009 influenza A (H1N1) pandemie. In een kleine cohortstudie tonen wij aan dat reguliere laboratoriumdiagnostiek naar virusinfectie met behulp van CRP en hoeveelheid witte bloedcellen onbetrouwbaar is en dat PCT bij deze kinderen van toegevoegde waarde kan zijn als de behandelend arts twijfelt over het al dan niet geven van antibiotica.

Niet iedere bacteriële infectie hoeft antibiotisch te worden behandeld. Voor de dagelijkse praktijk is het belangrijk te weten welke patiënt met spoed moet worden behandeld met antibiotica en welke patiënt veilig geobserveerd kan worden zonder behandeling. In hoofdstuk 8 onderzoeken wij de correlatie van verscheidene biomarkers met ernst van ziekte. We concluderen dat het long pentraxin-3 (PTX-3) – een relatief nieuwe en onbekende marker voor acute fase-reactie – een goede voorspeller van ernst van ziekte en van mortaliteit kan zijn bij mensen met koorts op de SEH.

Discussie en aanbevelingen voor verder onderzoek

Epidemiologie van infectieziekten is locatie- en tijdsgebonden. Pathogenen evolueren, klimaatverandering staat aan de wieg van nieuwe ziektes en elimineert andere; medicijnen genezen patiënten, maar kunnen op hun beurt weer nieuwe ziektes induceren. Nieuwe diagnostische hulpmiddelen dragen bij aan de ontdekking van volledig nieuwe en onvermoede pathogenen. Het is en blijft belangrijk om de pathogenen, betrokken bij het ontstaan van koortsende ziektes in de gehele wereld, systematisch in kaart te brengen. Door meer kennis kan een behandelend arts beter anticiperen op de aanwezigheid van bepaalde pathogenen bij een patiënt met koorts, waardoor de pre-test kans op een positieve bevinding bij een juist gekozen test toeneemt en de diagnostiek nauwkeuriger wordt. Systematische *surveillance* binnen en buiten het ziekenhuis zal bijdragen tot eerdere detectie van mogelijke infectieuze dreiging. Door grotere kennis van verspreiding van ziekte kunnen betere preventieve maatregelen worden genomen.

Voor optimale diagnostiek van koortsende ziektes zou men enerzijds de aanwezigheid van een pathogeen met zekerheid moeten kunnen aantonen en tegelijkertijd andere pathogenen of niet-infectieuze koortsende ziektes uit moeten kunnen sluiten. Gezien de overweldigende hoeveelheid pathogenen en niet-infectieuze koortsende ziektebeelden, lijkt dit te hoog

gegrepen. Daar staat tegenover dat het aantonen van verwekkers, sinds de klinische introductie van PCR- en andere moleculaire technieken, makkelijker is geworden. Toekomstig onderzoek moet laten zien of deze technieken ons in staat stellen om pathogenen met een simpele en robuuste test aan te tonen of uit te sluiten. Totdat betere diagnostische testen zijn gevalideerd, is het 'indirecte bewijs' dat verkregen wordt door gebruik van biomarkers bij het aanvullend onderzoek van patiënten met koorts van grote waarde. Op dit moment lijkt PCT de meest betrouwbare en effectieve nieuwe marker. Mede dankzij een grote hoeveelheid klinische data, verkregen uit retrospectieve en prospectieve, goed-opgezette en grote studies, valt wordt de PCT-test steeds belangrijker in de dagelijkse klinische praktijk.

Ondanks een veelheid aan klinische studies met veelbelovende resultaten, blijft de (patho-)fysiologische rol van PCT onopgehelderd. Waar de fysiologie van CRP duidelijk omschreven is – met enerzijds de productie, die vrijwel volledig plaatsvindt in de lever na stimulatie door IL-6, en anderzijds de functie, het binden van fosfocholine dat tot expressie wordt gebracht door bepaalde micro-organismen en door necrotische of apoptotische cellen, waardoor het complementsysteem via C1q wordt geactiveerd en opsonisatie mogelijk wordt[1] – is tot op heden niet duidelijk waar het 'ontstekings-PCT' geproduceerd wordt. Onder fysiologische omstandigheden wordt PCT geproduceerd door de C-cellen van de schildklier en wordt vrijwel al het PCT direct omgezet in calcitonine, waardoor het circulerend PCT erg laag is. Ondanks het feit dat de C-cellen van de schildklier na verscheidene stimuli, zoals hypercalciëmie, glucocorticoïden, glucagon en β-adrenerge prikkels, reageren met het afgeven van meer PCT, kan er geen relatie tussen deze stimuli en het 'ontstekings-PCT' worden gevonden.[2] Er is sprake van een duidelijke relatie tussen bacteriële endotoxines en TNF met hoge waardes van circulerend PCT.[3] Er wordt gesteld dat PCT betrokken is bij de modulatie van NO-synthese en op die manier als pro-inflammatoir cytokine geclassificeerd zou moeten worden. Tevens zou PCT mogelijk een pijnstillend effect hebben, waarbij een associatie tussen de aanwezigheid van minder noci-receptoren bij hogere PCT-waardes is aangetoond.[4] Als laatste is verder gebleken dat de experimentele toediening van exogeen PCT aan septische dieren een hogere mortaliteit gaf; op die manier zou PCT niet alleen een indicator zijn voor ernst van ziekte, maar mogelijk ook direct de mortaliteit kunnen verhogen via een vooralsnog onbekend mechanisme.[5] Vanwege het feit dat het menselijk lichaam tijdens een bacteriële infectie aanzienlijke hoeveelheden energie spendeert aan de productie van PCT, lijkt het aannemelijk dat PCT een pathofysiologische rol moet spelen. Verder onderzoek is nodig om dit te verduidelijken. Ondanks de onduidelijkheid over de herkomst van PCT tijdens infectie en de functie, is PCT de eerste biomarker sinds de introductie van CRP die zijn weg van het lab naar het ziekenhuisbed heeft weten te vinden, en vormt het een welkome aanvulling op de reeds bestaande diagnostische markers.

Een goed-opgezette, gerandomiseerde, prospectieve cohortstudie die PCT gebruikt als leidraad voor antibiotische therapie bij patiënten met koorts op de SEH, ontbreekt nog.

Wij hebben een *pilot-study* uitgevoerd in het Slotervaartziekenhuis te Amsterdam, waarin we aantonen dat het gebruik van PCT leidt tot minder antibioticagebruik en veilig is. In deze studie werden alle SEH-patiënten met koorts, gedefinieerd als een temperatuur

> 38.2 ˚C, geïncludeerd en gerandomiseerd, waarna behandeling ofwel plaats vond binnen de 'PCT-geleide antibiotische therapie-arm', ofwel binnen de *standard-of-care* arm. De gebruikelijke klinische diagnostiek werd bij alle patiënten uitgevoerd, inclusief routinebloedonderzoek en al het verdere aanvullend onderzoek dat volgens de behandelend arts geïndiceerd was. Na afronden van de diagnostiek werd de behandelend arts gevraagd om een werkdiagnose, met een onderscheid tussen bacteriële- en niet-bacteriële ziektes; daarnaast werd de behandelend arts gevraagd een uitspraak te doen over de noodzaak van het geven van antibiotica. Hierna werd de PCT-uitslag van patiënten binnen de PCT-geleide arm vrijgegeven. Gebaseerd op tevoren vastgestelde afkapwaarden werd op basis van de uitslag een antibiotica-advies gegeven (PCT < 0.5 ng/mL; geen antibiotica geadviseerd – PCT > 0.5 ng/mL; antibiotica geadviseerd). De behandelend arts kon dit advies te allen tijde negeren; wel werd dan om een motivatie gevraagd.

108 Patiënten werden geïncludeerd. PCT was een betere voorspeller van bacteriële infectie dan andere laboratoriumbepalingen, inclusief CRP. In de PCT-geleide groep werden minder antibiotica voorgeschreven in vergelijking met de controlegroep. Er was geen verschil in duur van ziekenhuisopname of in mortaliteit tussen de groepen.

Uit deze kleine *pilot-study* kunnen we concluderen dat PCT-geleide antibiotische therapie zinvol en veilig lijkt te zijn bij patiënten met ongedifferentieerde koorts op de SEH. Op dit moment starten wij een grote studie om dit nader te bekijken.

Zoals hierboven beschreven, vormen biomarkers een goede aanvulling op de diagnostiek bij koortspatiënten; mede aan de hand van biomarkers wordt in de praktijk een behandelplan met of zonder antibiotica opgesteld. Echter, tussen het begin van infectie of inflammatie en de productie van biomarkers gaat een aanzienlijke hoeveelheid tijd voorbij. Het meten van biomarkers is een '*downstream*'-meting, en aangezien infectie en inflammatie veel overlap kennen in de immunologische '*pathways*', gaat stroomafwaarts potentieel onderscheidende informatie verloren. In de ideale situatie zou biomarkerproductie gemeten moeten worden bij de bron, i.e. op de plek van RNA-productie.

In de klinische praktijk heeft de introductie van nieuwe en erg gevoelige moleculaire technieken als PCR geleid tot eenvoudigere diagnostiek naar virusziektes.[6] Tot nu toe blijft echter het kweken van bacteriën de gouden standaard voor het bevestigen van bacteriele infectie. Aangezien het kweken van bacteriën niet altijd even eenvoudig is, met soms langzame groei en de noodzaak tot kweken op speciale media, is het in de praktijk veelal moeilijk of zelfs onmogelijk om bacteriële etiologie te bewijzen. Hierdoor wordt vaak onnodig antibiotisch behandeld en blijft er onzekerheid over definitieve diagnoses bestaan. Om dit probleem op te lossen, zou de reactie van de gastheer tegen een bepaald pathogeen als diagnosticum kunnen worden gebruikt. Het is veelvuldig aangetoond dat er bij infectie met specifieke pathogenen kenmerkende gen-expressiepatronen meetbaar zijn bij de gastheer. Specifieke '*pattern-recognition receptors*' worden op witte bloedcellen tot expressie gebracht en kunnen *in vivo* worden gemeten.[7,8] Niet alleen is bewezen dat door vergelijking van gen-expressieprofielen gedifferentieerd kan worden tussen verschillende bacteriën; gen-expressieprofielen kunnen tevens gebruikt worden als middel om auto-immuunziektes

van virusinfecties te onderscheiden, en om verschillende virussen onderling te kunnen onderscheiden.[8, 9]

Micro-arrays - zoals de MLPA, beschreven in hoofdstuk 4 – meten patronen van verschillen in genexpressie van duizenden genen tegelijk in fysiologische en pathologische omstandigheden. Door genexpressie parallel te meten, kunnen met behulp van micro-arrays verschillende intracellulaire cascades worden bestudeerd en kan meer inzicht worden verkregen in de dynamiek van fysiologische en pathologische processen. Aangezien micro-arrays erg sensitief zijn, kunnen subtiele verschillen in genexpressie worden vastgesteld. Micro-arrays kunnen worden gebruikt voor genotypering en voor het meten van genexpressie. Genotypering kan worden gebruikt om het genomisch DNA van een pathogeen te analyseren; genexpressie kan in het pathogeen of in de gastheer worden gemeten, waardoor het mogelijk is om interacties tussen pathogeen en gastheer te onderzoeken.

Micro-arrays kunnen waardevolle middelen zijn bij de diagnostiek van infectie door de detectie van (nieuwe) pathogenen, kunnen gebruikt worden voor epidemiologisch onderzoek en kunnen meer inzicht geven in de virulentie van pathogenen. Daarnaast kan de reactie van de gastheer op pathogenen dynamisch gemeten worden en kan de gevoeligheid van de gastheer voor bepaalde pathogenen in kaart worden gebracht.[10, 11]

Eén van de klinische gebreken van micro-arrays is de enorme hoeveelheid informatie die ze genereren. In het ziekenhuis is het grootste deel van deze informatie te gedetailleerd en daarom irrelevant. Voor goede patiëntenzorg gaat het erom de aan- of afwezigheid van specifieke pathogenen op een snelle en betrouwbare manier aan te tonen. Eerder onderzoek heeft laten zien dat een relatief kleine selectie van genexpressie in de gastheer afdoende is om deze basale vragen te beantwoorden door het meten van pathogeen-specifieke 'genexpressie-handtekeningen' of patronen.[8] Deze patronen tonen niet alleen de aanwezigheid aan van verschillende soorten bacteriële of virale infecties; door het vergelijken van over- en onderexpressie van RNA met de gemiddelde expressie in gezonde controles, kan een relatieve 'afstand tot gezondheid' worden geschat, waardoor ernst van de ziekte gekwantificeerd kan worden.[12]

Als het gebruik van micro-arrays in de klinische praktijk beschikbaar komt, zullen diagnostische procedures sneller en betrouwbaarder worden. De dynamiek van de acuutfase reactie tijdens verschillende pathologische situaties zal ontrafeld worden vanuit een 'gastheerperspectief', wat niet alleen zal resulteren in betrouwbaarder diagnostiek, maar mogelijk ook in nieuwe aangrijpingspunten voor therapie. Nu micro-arrays goedkoper en eenvoudiger in gebruik worden, zullen zij hun weg naar het ziekenhuis vinden en de komende decennia hoogstwaarschijnlijk het reguliere testen van biomarkers vervangen.

Verdere biomarkerstudies in patiëntenpopulaties met koorts moeten niet alleen prospectief evalueren of PCT daadwerkelijk gebruikt kan worden als diagnostische test in een grotere populatie met ongedifferentieerde koorts; tevens zal moeten worden onderzocht of PCT veilig en kosten-effectief is. Een andere mogelijke kracht van PCT, die niet is geëvalueerd in dit proefschrift maar vooral in eerdere Intensive Care-studies is aangetoond[13, 14], is de mogelijkheid om PCT-geleid antibiotica te staken nadat therapie begonnen is. Ondanks eerdere suggesties dat de gemiddelde antibiotische behandeling bij infecties mogelijk te lang

is[15], neigen clinici er nog altijd naar infectie minimaal zeven dagen te behandelen. Dalende PCT-waardes zouden kunnen worden gebruikt als leidraad voor kortere behandeling. Ook hier is een goed-vormgegeven prospectieve niet-IC-studie nodig.

Naar onze mening zal het focus van biomarkerstudies het komende decennium liggen op genexpressiestudies. Het zal erg interessant zijn om sequentieel genexpressie tijdens infectie en inflammatie te meten, waarbij de dynamiek van gencascades tijdens ziekte en gezondheid in kaart kan worden gebracht. Met de ontwikkeling van betere toegepaste bioinformatica zullen toekomstige studies in staat zijn om de enorme hoeveelheid aan informatie die door genexpressie-technieken wordt gegenereerd, te stroomlijnen; dit zal hopelijk resulteren in een nieuwe set van biomarkers, vanuit het lab naar het ziekenhuisbed.

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Curriculum Vitae

Maarten Limper was born in 1980 in Amsterdam. After finishing high school at Nieuwer Amstel in Amstelveen, he started his medical training at the University of Amsterdam in 1998. Concurrently, he studied Philosophy at the same university, starting in 1999. In 2005, he received his Medical Doctor degree; subsequently he was working as a medical doctor in acute psychiatry (Crisisdienst Oost – AMC/de Meren Amsterdam), internal medicine (St. Elisabeth Hospital, Curaçao) and in clinical psychiatry (UMC Utrecht). As from 2007, he worked as a medical doctor and researcher at the department of internal medicine of the Slotervaart Hospital in Amsterdam and the St. Elisabeth Hospital in Curaçao; this thesis is the product of that period.

In 2011, he started specializing in Internal Medicine at the Academic Medical Center Amsterdam and Slotervaart Hospital Amsterdam, under supervision of prof. dr. J. Hoekstra and prof. dr. D.P.M. Brandjes. In 2013, he continued his training at the Erasmus MC Rotterdam, under supervision of prof. dr. J. van Saase. In 2015, he will continue his training as internist-infectiologist at the Erasmus MC, under supervision of dr. J. Nouwen, and as internist-immunologist, under supervision of prof. dr. P.M. van Hagen.

Maarten Limper werd in 1980 te Amsterdam geboren. Na het gymnasium op het Nieuwer Amstel te Amstelveen te hebben afgerond, begon hij in 1998 met de studie Geneeskunde aan de Universiteit van Amsterdam. Daarnaast studeerde hij vanaf 1999 Wijsbegeerte aan dezelfde universiteit. In 2005 werd het artsexamen behaald, waarna hij achtereenvolgens periodes als arts bij de acute psychiatrie (Crisisdienst Oost - AMC/de Meren), de interne geneeskunde (St. Elisabeth Hospitaal, Curaçao) en de klinische psychiatrie (UMC Utrecht) werkte. Vanaf 2007 was hij als arts en onderzoeker werkzaam op de afdeling interne geneeskunde van het Slotervaart Ziekenhuis te Amsterdam en van het St. Elisabeth Hospitaal te Curaçao; dit proefschrift is het resultaat van die periode.

In 2011 begon hij met de opleiding Interne Geneeskunde via het Academisch Medisch Centrum Amsterdam en het Slotervaartziekenhuis Amsterdam, met als opleiders prof. dr. J. Hoekstra en prof. dr. D.P.M. Brandjes. Per 2013 vervolgde hij de opleiding tot internist in het Erasmus MC, met als opleider prof. dr. J. van Saase. Per 2015 vervolgt hij zijn opleiding tot internist-infectioloog aan het Erasmus MC, met als opleider dr. J. Nouwen, en tot internist-immunoloog, met als opleider prof. dr. P.M. van Hagen.