Early procalcitonin kinetics and adequate empiric antibiotic therapy in critically ill

PhD Thesis

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III. Ildikó László, **Domonkos Trásy**, Zsolt Molnár, János Fazakas: Sepsis: From Pathophysiology to Individualized Patient Care. *J Immunol Res*. 2015;2015:510436. **IF: 2.934**

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I. János Fazakas, **Domonkos Trásy**, Zsolt Molnár: Interpreting procalcitonin at the bedside. Jean-Louis Vincent (ed.) *Annual Update in Intensive Care and Emergency Medicine 2016*. Springer Verlag, Berlin-Heidelberg, 2016. pp. 3-15.

LIST OF ABSTRACTS RELATING TO THE SUBJECT OF THE THESIS

I. **Trásy Domonkos:** Korai prokalcitonin (PCT) kinetika és adekvát antibiotikum terápia szepszisben. *Aneszteziológia és Intenzív Terápia* 2013-43 (Suppl. 1) 5.

II. **Domonkos Trásy**, Márton Németh, Angelika Osztroluczki, Krisztián Tánczos, Nándor Öveges, Péter Hankovszky, Zsolt Molnár: Early procalcitonin kinetics may indicate effective empirical antibiotic therapy within hours after starting treatment (a pilot study). *Intensive Care Med*. 2013; 39 (S2)78.

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V. **Domonkos Trásy**, Márton Ferenc Németh, Krisztián Tánczos, Angelika Osztroluczki, János Fazakas, Péter Hankovszky, Edit Hajdú, Zsolt Molnár: Early procalcitonin kinetics may indicate effectiveness of the empirical antibiotic therapy in sepsis within hours. *Intensive Care Medicine* Supplement 1, Volume 40 October 2014; 0463.

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ABBREVATIONS

AB – antimicrobial therapy
ACTH – adrenocorticotroph hormone
ADM – adrenomedullin
ARDS – acute respiratory distress sy.
AUC – area under the curve
AVP – vasopressin
BSI – blood stream infection
BT – body temperature
CGRP – calcitonin gene related peptide
CI – confidence interval
CRP – C-reactive protein
DAMP –damage-associated mol. pattern
DO_2–oxygen delivery
EC – extra corporeal
ECLIA – electrochemiluminescence immunoassay
GH – growth hormone
HMG-1 – high-mobility group protein 1
HS – high sensitivity protein
ICU – intensive care unit
IFN – interferon
IL –interleukin
IPPV – intermittent pos. pressure ventilation
I-R – ischemia-reperfusion
LOS – length of stay
LPS – lipoprotein
LT – leukotriene
M/F – male/female
MBL – mannose-binding lectin
MET – measure, evaluate and treat
MIF – macrophage migration inhibitory fact.
NALP – type of a NOD like receptor
NO – nitric oxide
NOD –nucl.-binding oligomerization domain
NPV – negative predictive value
PAMP –pathogen-associated mol. pattern
PaO_2/FiO_2 – ratio of arterial oxygen partial pressure to fractional inspired oxygen
PCT –procalcitonin
PG – prostaglandin
PM –predicted mortality
PPV – positive predictive value
ROC – receiver operating characteristic
ROI –reactive oxygen intermediates
SAA – serum almyloid A
SAPS – simplified acute physiology score
SIRS – systemic inflammatory response sy.
soluble CD14 subtype–presepsin
SPFA – sequential organ failure assessment
SPSS–Stat. Package for the Social Sciences
SSCG–surviving sepsis campaign guideline
suPAR –soluble urokinase-type plasminogen activator receptor
TGF –transforming growth factor
TNF – tumor necrosis factor
UTI – urinary tract infection
VO_2–oxygen consumption
WBC – white blood cell

SUMMARY OF THE THESIS

Sepsis has become a major health economic issue, with more patients dying in hospitals due to sepsis related complications compared to breast and colorectal cancer together. Despite extensive research in order to improve outcome in sepsis over the last few decades, results of large multicenter studies were by-and-large very disappointing. This fiasco can be explained by several factors, but one of the most important reasons is the uncertain definition of sepsis resulting in very heterogeneous patient populations, and the lack of understanding of pathophysiology, which is mainly based on the imbalance in the host-immune response. However, this heroic research work has not been in vain. Putting the results of positive and negative studies into context, we can now approach sepsis in a different concept, which may lead us to new perspectives in diagnostics and treatment. While decision making based on conventional sepsis definitions can inevitably lead to false judgment due to the heterogeneity of patients, new concepts based on currently gained knowledge in immunology may help to tailor assessment and treatment of these patients to their actual needs.

Early diagnosis of sepsis is crucial in treating septic patients and in these cases starting appropriate antibiotic therapy in time have a significant effect on survival. However, there is very little to help the clinician during the first 24 hours whether the administered empirical antibiotics treatment is effective or not. Procalcitonin is a reliable sepsis marker with short half life but its role in predicting bacterial infection and early decision making in assisting antibiotic therapy is undiscovered.

Therefore we investigated the value of procalcitonin levels and changes from the day before (t_{-1}) to the day when infection was suspected (t_0), and after starting empirical antibiotic therapy 8 hourly (t_8, t_{16}, t_{24}) then daily ($day_{2\text{-}5}$) in predicting infection and if the antibiotic treatment is effective or ineffective in intensive care patients.

Our results showed significant difference in procalcitonin kinetics in patients with and without bacterial infection and whom empiric antibiotic treatment is turned out afterwards to be effective or ineffective.

There are three fundamental questions to be answered when treating patients with suspected or proven infections on the ICU: 1) is there infection, in other words should we start empirical antibiotic therapy; 2) is the commenced antibiotic effective; and finally 3) when should we stop antibiotic treatment? Our research team decided to give exact answers to these questions with the help of procalcitonin as it is a fundamental problem in our daily practice in the ICU.

1 INTRODUCTION

One of the most challenging tasks in critical care medicine is the treatment of serious infection related multiple organ dysfunction, termed in general as sepsis, severe sepsis, and septic shock. Despite improved awareness of critical care practitioners and the implementation of international guidelines, the mortality ranges between 28-41% in North America and Europe alike and still the leading cause of death among critically ill patients worldwide [1]. However, sepsis means a very heterogeneous patient population, which varies in etiology and severity; therefore, universally applicable diagnostic criteria and treatment algorhythms are difficult to be defined. This heterogeneity proved to be one of the most difficult hurdles that most prospective randomized trials could not concur; hence, they failed to show either clear survival benefit or positive results of single center studies that were later contradicted by large multicenter trials [2]. Nevertheless, sepsis has become a very important health economic issue all around the world.

Furthermore, treating sepsis is a multidisciplinary task. Early recognition and immediate start of initial steps which stand on three main pillars of: resuscitation parallel with source control and adequate antimicrobial therapy [3] undoubtedly are inevitable to give the best possible chance for survival received strong recommendation by the Surviving Sepsis Campaign guidelines [1], which has to be started on the primary care level: outside the hospital, in the emergency department or on the wards. In the absence of adequate initial management, providing even the highest level of intensive care would be in vain (Figure 1). There is firm evidence that delaying appropriate antibiotic treatment increases mortality regardless from the severity of organ dysfunction [4, 5].

However, in almost 30% of cases empirical antibiotics proved to be inadequate in the hospitals in general and on intensive care units (ICU) as well [6]. To improve efficacy, empirical antibiotic therapy is guided by local protocols based on international guidelines in most centers.

However, while recognizing organ failure via objective signs is relatively easy, diagnosing infection as possible underlying cause remains a challenge. Due to the non-specific properties of conventional signs of infection, such as body temperature and white blood cell count (WBC), biomarkers have been searched to aid diagnosis for decades.

One of the most studied biomarkers is procalcitonin (PCT) [7]. Its role in assisting antibiotic therapy has been studied extensively, with contradicting results. There are positive studies [8, 9] showing that a PCT-guided patient management reduced antibiotic exposure and length of antibiotic

therapy without affecting patient outcomes. There are also negative studies, which could not show this benefit at all [10–12].

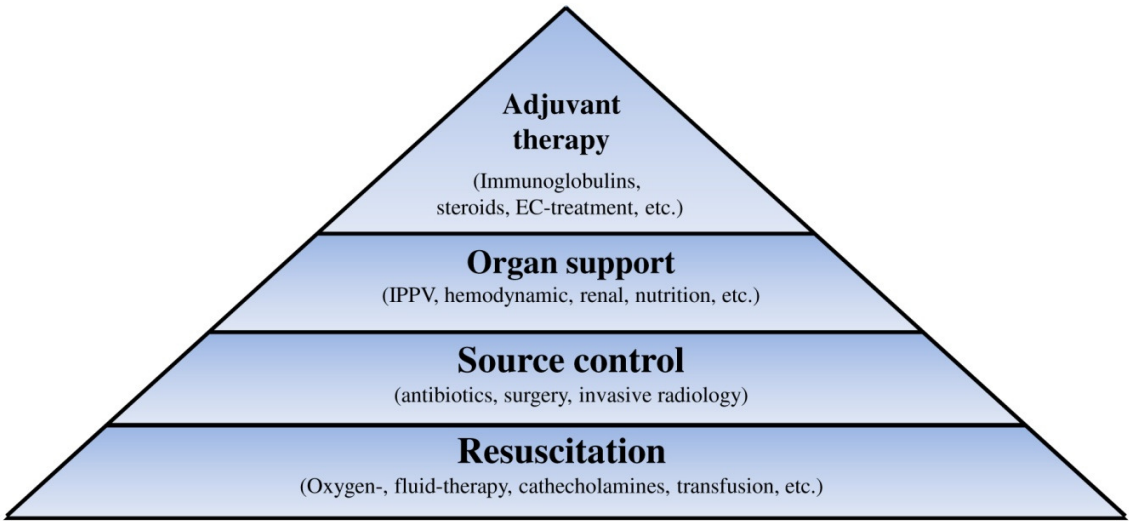


Figure 1 "Sepsis-triangle" treatment. EC - extra corporeal and IPPV - intermittent positive pressure ventilation

Although the results of prospective randomized clinical trials may be disappointing as far as survival is concerned, it is beyond doubt that we have learned a lot about the pathophysiology of sepsis during performing these studies over the last few decades understanding the values and limitations of inflammatory biomarkers it is inevitable to understand the immunological background of critical illness determined mainly by the host response. Moreover, putting the results of these studies in the context, based on new insights of the pathomechanism of the clinical picture of sepsis and systemic inflammation generated mainly by the individuals' host response, may explain the differences between the reported results and help the clinician to interpret results of diagnostic tests and rationalize treatment modalities with more confidence in the most appropriate way at the bedside.

1.1 Sepsis is not a "definitive" disease

In medical school we were brought up in the world of "definitive diagnoses." This means that patients come in with a certain complaint, the physician after taking medical history, performing physical examination and diagnostic tests, defines the diagnosis and treat the patient accordingly. In the

case of a well-defined disease more-or-less the same or similar diagnostic tests and therapeutic interventions are performed all around the world. This holds true for most diseases in classical medicine and surgery as examples, in stroke, myocardial infarction, bone fractures, intracranial haemorrhage, etc., we have diagnostic tests with very high sensitivity and specificity. However, defining sepsis is not that simple.

The term we call “sepsis syndrome” was conceived in a hotel room in Las Vegas in 1980, during the designing of the protocol writing of one of the first prospective randomized trials in sepsis, performed by a group of scientists led by the late Roger Bone [13]. The study ended with non-significant results, but based on the inclusion criteria of this study a statement paper was later published by the same authors titled “Sepsis Syndrome: A Valid Clinical Entity” [14], after which the medical society started to deal with sepsis as with a definitive disease. As a definitive disease, physicians wanted one single test with high sensitivity and specificity to diagnose sepsis, and there was an urge to find an “anti-sepsis magic bullet”. Neither of these wishes have and will never ever come true.

Regarding the definition and diagnosis of sepsis, the classical signs of the “sepsis syndrome” such as fever/hypothermia, leukocytosis/leukopenia, tachycardia and hypotension, meant a very large and non-specific/non-infectious cohort of patients. For this reason, a few years later a consensus conference was brought together and defined the so called “consensus criteria” of sepsis which has been used for decades in research and clinical practice alike [15]. In the most current SSCG a more robust, more detailed definition has been created, in order to “save” the previous concept of the Bone criteria [1] but it has almost immediately been questioned and criticized by experts, also taking part in the Surviving Sepsis Campaign process [16].

These efforts clearly show that finding the appropriate definition of sepsis has been a continuous challenge for more than 30 years. The difficulty in defining sepsis originates from its pathophysiology, which is affected by numerous individual variations of the host response. This has been recognized by international societies and currently an international Task Force has been working on a new, pathophysiology based sepsis definition. Nevertheless, in most specialties diagnostic laboratory or radiological tests have very high sensitivity and specificity often reaching almost 95-100% [17]. However, in the case of sepsis it is different, which makes not just the diagnosis but the interpretation of the results of clinical trials and also epidemiological data very difficult.

1.2 Epidemiology

According to recent surveys we treat several folds more critically ill patients on the intensive care units worldwide these days as compared to the figures from more than 10 years ago [18]. There seems to be an increase in the incidence of sepsis, with mortality rates of 20–50%, and according to recent data from the United States, sepsis is the single most expensive reason for hospitalization at present [19, 20]. However, it is important to note that reported mortality shows considerable variation across the globe. A recent retrospective analysis from Australia and New Zeeland showed an increase in the number of critically ill and septic patients over the last 12 years, with a mortality reduction from more than 30% to less than 20% [18]. In the PROCESS trial from the United States mortality was around 20% [21]. According to these data outcome has improved dramatically over the years. However, results from Europe, both retrospective and prospective, indicate greater mortality of 45–55%, which was also accompanied by a 2- to 3-fold longer ICU and hospital stay [22, 23], as compared to that reported by the two previously mentioned studies. This raises the question of whether the care is better in those countries which reported lower mortality rate or is it the patient selection that causes this difference? Although it is difficult to give a definitive answer, referring to our previous chapter, due to the difficulties in defining sepsis, severe sepsis, and septic shock, one cannot exclude that this difference can be the result of the uncertainties in patient selection, and, in those countries reporting higher mortality rates, sicker patients were included in the "septic shock" cohort.

Indeed, patients with the same diagnosis of "septic shock" could have completely different severity and prognosis. The same holds true for every potential "insult" in critical care, such as trauma, sterile inflammation (acute pancreatitis), ischemia-reperfusion injury, major surgery, burns, and infection. These conditions share the same feature in their pathophysiology, namely, that it is not the insult per se, but the host's response, especially the immune response, which determines severity and outcome (Figure 2).

1.3 Pathophysiology

1.3.1 From Localized Insult to dysregulated host response caused Cytokine Storm

The immune system is a complex network that involves many different players interacting with each other as an orchestra, a dynamic balance between the pro-, and anti-inflammatory forces. The immune response to pathogens relies on both innate and adaptive components. The first line of defense

against invaders consists of physical barriers such as the skin [24, 25], the mucous membranes of our gastrointestinal [26], and respiratory [27] and genitourinary [28] tracts. The second line is the rapid defense, eradication of the invaders by the innate immune system (including complement proteins, sentinel phagocyte cells, and natural killer cells), which plays an activator and a controller role of the adaptive immune system [29]. The innate system acts by broad recognition of antigens, mainly by sensing pathogen-associated molecular patterns (PAMP) of carbohydrates and fatty acids located on the surfaces of common pathogens. While the adaptive immune system's role is to control the process and keep it localized to the site of the insult [30]. By-and-large when there is an imbalance due to the dysregulation of the pro-, and anti-inflammatory forces, the local response spread systemically the activation of several classes of pattern recognition receptors will generate a "cytokine-chemokine storm" [31, 32].

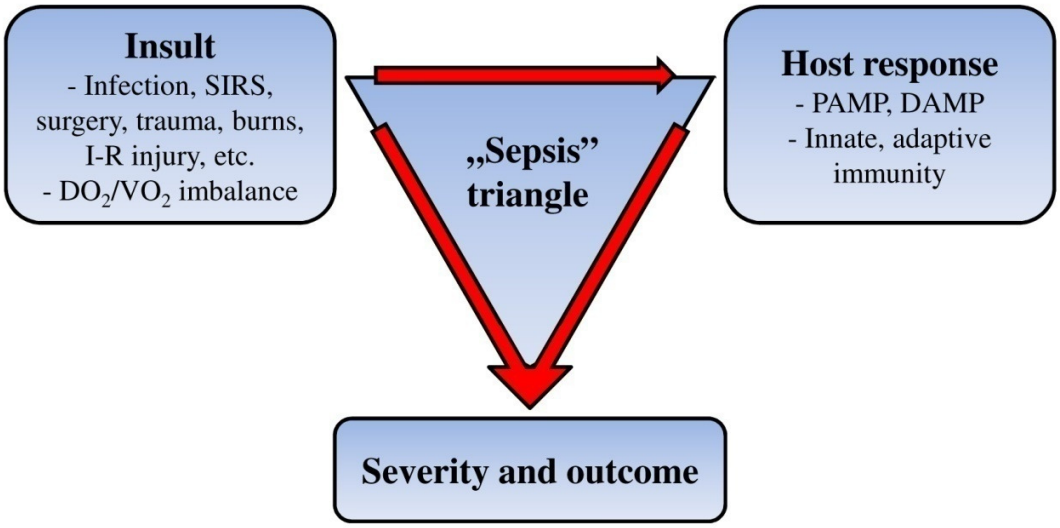


Figure 2 "Sepsis-triangle": pathomechanism. SIRS - systemic inflammatory response syndrome; I-R - ischemia-reperfusion; DO_2 - oxygen delivery; VO_2 - oxygen consumption; PAMP - pathogen-associated molecular patterns; DAMP - damage-associated molecular patterns

However, very similar molecules are released due to cell injury after trauma, burns, ischemia-reperfusion, pancreatitis, major surgery, and so forth, derived mainly from the mitochondria of the

injured or stressed cells that found during PAMPs, and can also cause a cytokine storm. This process accompanying tissue injury is called “damage-associated molecular patterns” (DAMP).This similarity is due to the fact that the bacteria and the mitochondria (which is more-or-less an encapsulated bacterium) share very similar genetic background. This explains why tissue injury induced DAMP and bacterial infection induced PAMP manifest in similar host responses and clinical manifestations [33].

This highlights the fact that the Bone-concept inevitably mixed patients who suffered insults due to PAMP, DAMP or the mixture of the two.

Activation of neutrophils, macrophages, and monocytes by costimulatory molecules at the site of infection will turn the local adaptive immune system on and give “permission” to the adaptive system to respond to an infectious insult. The aim of the innate response is the eradication of the DAMP and PAMP, which is followed by the adaptive response with the resolution of the immunological process. The adaptive immune response is based on maturation and proliferation, both influenced by the “cytokine signature” of the innate response. In other words, every host has its own “cytokine signature” for a certain insult. Under normal circumstances these processes are well regulated maintaining an even balance between counteracting forces, hence keeping the inflammatory response localized.

However, in the case of an unbalanced (pro-inflammatory and anti-inflammatory), dysregulated (maturation and proliferation) response, the localized process goes out of control and becomes systemic, in other words the disease of the whole body; hence, it gives way for impairing the function of distant vital organs. This makes the clinical manifestation of critical illness so similar regardless of the insult. To give an example, the same gravity of acute respiratory distress syndrome (ARDS), shock, or deterioration in mental function can occur in pancreatitis, just as well as after major surgery, or due to any type of infection (Figure 3). The adaptive immune system as the third level of defense is based on its memories. It can adapt and protect us against almost any invader.

In brief the “cytokine signature” of neutrophils and macrophages will give signals to the T and B lymphocytes via the dendritic cells, which after proliferation by maturation will express different cell surface receptors in soluble ormembrane bound forms. The adaptive immune response is a soluble matrix, which consists of the cascade-type activation of cytokines, coagulation factors, the release of acute phase proteins, stress hormones, and different chemokines and hormokines, forming a complex network. The key factor of immune resolution is the balance between pro-inflammatory and anti-inflammatory forces, which is mainly determined by the balance between the relationship of Th1, Th2, Th17, and $\gamma\Delta$T to each other, namely, the maturation, magnitude, and the duration of their activity [34].

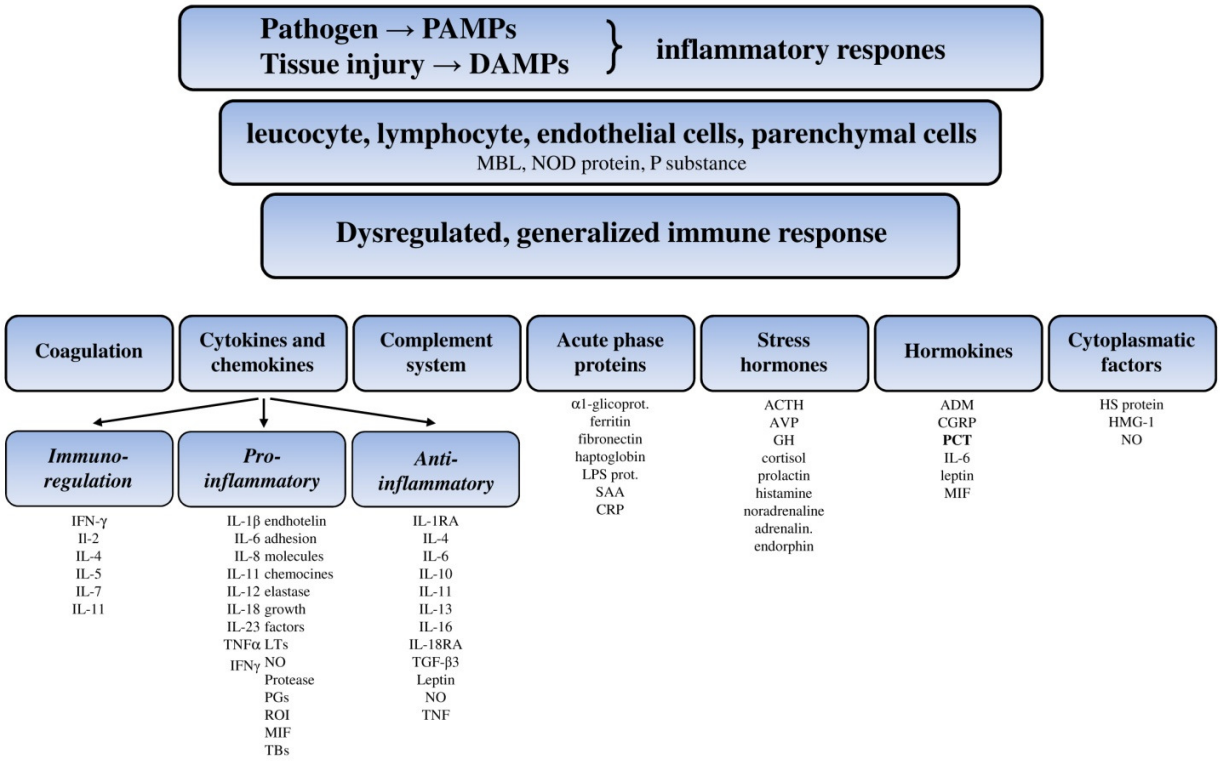


Figure 3 The main pillars of systemic inflammatory response. PAMPs - pathogen-associated molecular patterns; DAMPs - damage-associated molecular patterns; MBL - mannose-binding lectin; NOD protein - nucleotide-binding oligomerization domain protein; and NALP - a type a NOD like receptor

1.3.2 From Systemic Inflammatory Response Syndrome (SIRS) to dysregulated host response and Immunoparalysis

Based on the Bone-criteria, systemic inflammatory response syndrome, invented on the Consensus Conference in 1991 [15], initially meant the classical "sepsis syndrome" criteria, without proven infection. The SIRS-criteria have also been criticized for similar reasons as the "sepsis syndrome" definition, but nevertheless this "SIRS concept" assumed that systemic inflammatory response can occur for an insult without infection.

In the past SIRS was mainly thought to be related to the imbalance between the pro-inflammatory and anti-inflammatory responses. However, it is more complex. In the context of the innate and adaptive immune responses both pro-inflammatory and anti-inflammatory processes take place in a

parallel fashion. Normally, an infectious insult is followed by the activation of both the pro-, and anti-inflammatory forces. In general we can say that there is a short delay of the anti-inflammatory response as compared to the pro-inflammatory. Nevertheless, the role of pro-inflammation, mainly regulated by the innate immune system, is to kill the invaders, while the adaptive immune system regulated anti-inflammation's task is to keep the process localised, and apart from general symptoms as being weak, suffering from fever, there is no other harm done, and after a few days the fight against the pathogens is over, the immunity return to baseline activity. However, in septic patients an oversized pro-inflammatory response may take place, via which the localized insult becomes systemic, and will lead to different degree of tissue damage, shock, and eventually organ failure [30]. These patients can die within hours or days due to this fulminant septic shock. At a later stage a dysregulated, systemic form of the adaptive response, which means an overwhelming anti-inflammatory response, could induce immunoparalysis, jeopardizing the body's defense, hence leaving it prone to further, even opportunistic infections [32]. These patients usually suffer from recurrent septic insults and may also die due to this acquired immune suppression.

1.4 Diagnostic Challenges

The question "Is this patient septic?" is frequently asked on intensive care unit ward rounds. However, this may be an irrelevant issue. Why? Because on the one hand, we should recognize a critically ill patient via objective signs of organ dysfunction such as hypotension, hypoperfusion, altered mental status, acid-base imbalance, hypoxemia, lactate levels, renal and liver dysfunction, and thrombocytopenia, which renders the immediate start of basic and organ specific supportive therapy, regardless of the actual diagnosis. On the other hand, what is of pivotal importance is not that the patient is septic or not, but whether the onset of critical illness is due to infection or not? If there is any suspicion of the possibility of an infection, after the fist level of resuscitation, empirical antibiotic therapy should be started immediately and also other levels should be performed of the sepsis triangle like surgical source control (Figure 1) [27]. But if it is not related to a bacterial infection, antibiotics are not just a waste of time and money, but they may also do harm in short and long term, due to its several undesired effects. Therefore, it is not "sepsis" what we treat, but organ dysfunction and infection.

Diagnosing infection on the ICU is not easy and requires a multimodal approach. One of the most common misconceptions in sepsis diagnosis is that we have been searching for specific "marker(s) of

sepsis." However, there is not and there will never be one single marker which is able to diagnose sepsis, mainly due to the very colourful manifestation of sepsis and due to the heterogeneity of patients.

1.4.1 Conventional Markers of Infection

Clinical signs are the most important in recognizing critical illness and suspecting infection and even the source of infection, but they cannot prove infection on their own. Conventional (fever/hypothermia, leukocytosis/leukopenia, tachypnoe, tachycardia and hypotension) indicators, also listed in the classical "sepsis-syndrome" criteria, are very nonspecific, in fact poor indicators of infection [35]. For microbiological proof of infection, although the gold standard of the diagnosis, unfortunately results often only become available 24–48 hours at the earliest after sending the specimen to the laboratory, and negative result do not necessarily rule out infection. According to our current concept, it is of utmost importance to start adequate antibiotic therapy as soon as possible, but at least within an hour after the onset of infection caused hypotension; otherwise chances for survival are reducing by the hour [4].

Neutrophil-lymphocyte count ratio is a cheap, fast, and easily available tool to diagnose bacteremia and was found to improve bloodstream infection diagnostics in a recent study on the emergency ward [36]. This simple test may also have a potential in the future.

Finally, new molecular biology techniques are now available to define the presence of bacterial or fungal DNA within the bloodstream of patients [37, 38]. Highly sophisticated molecular biology based tests such as polymerase chain reaction (PCR), matrix assisted laser desorption/ionization (Maldi/Tof), and peptide nucleic acid fluorescence in situ hybridization (PNAFISH) based pathogen detection can theoretically shorten the recognition of the underlying pathogen to about 8 hours [39]. However, these cannot differentiate between colonization and clinically significant infection. To fill this gap inflammation indicator laboratory measurements have been developed, which are sensitive and specific enough to show the onset and magnitude of bacterial invasion caused inflammatory response as soon as possible and may also be able to follow the progress of the disease within hours. These biologically active substances are called biomarkers.

1.4.2 The Role of Biomarkers at the Bedside

There have been several biomarkers developed so far [2], but neither is suitable for all purposes. Every marker has its own merit and limitations. They inevitably can support decision making but they will never be able to differentiate "sepsis" from "SIRS" with a100% sensitivity and specificity, mainly

due to the problems we discussed earlier in details regarding the problems of defining sepsis, and also due to the complex, overlapping pathomechanism of PAMP and DAMP. This is in sharp contrast with the diagnostic power of certain biomarkers used in the world of “definitive” diseases, where several laboratory parameters have this ability. Nevertheless, there is still an ongoing search for better, new markers of inflammatory response and infection, with promising preliminary results [40].

There are almost 200 so-called sepsis markers [7]; therefore, discussing the features of those cannot be integrated into my current thesis. I will mainly focus on the two most commonly used markers in infection/sepsis diagnostics and for guiding therapeutic interventions: procalcitonin (PCT) and C-reactive protein (CRP) [7]. However, briefly mentioning the main features of a few of other new markers already applied in daily practice, such as soluble CD14 subtype (presepsin) and soluble urokinase-type plasminogen activator receptor (suPAR), maybe worthwhile. Higher presepsin concentrations in septic patients were associated with ICU mortality in a recent large multicenter trial [41]. It was also suggested that changes in plasma concentrations may reflect the appropriateness of antibiotic therapy, but this have to be confirmed by future studies [41]. Regarding the suPAR molecule it has been shown to be a very good indicator of severity of the acute disease and shows good correlation with the degree of organ dysfunction in the critically ill but cannot be regarded as a “sepsis marker” due to its low specificity [42].

Any condition inducing DAMP [43] or PAMP could shed the endothelial glycocalyx layer. It has been confirmed in several experimental studies in different septic models that damage of the endothelial glycocalyx layer is reflected in elevated serum syndecan-1 and syndecan-4 levels [44–47] which may be potentially a very interesting marker in the future, but again, it may be nonspecific for bacterial infection only.

Nevertheless, the two most commonly used markers in infection/sepsis diagnostics and for guiding therapeutic interventions are PCT and CRP [7]. Despite their popularity, there are still many pros and cons without clear answers regarding their usefulness and interpretation in guiding patient management.

Procalcitonin is detectable in the serum within a few (4–6) hours after the onset of bacterial infection. During the “normal” course of an infection it reaches its peak within 24 hours and then starts its decline in the case of adequate treatment with levels reducing by roughly 50% daily according to its half-life [48]. In contrast, CRP moves “slowly”, and under similar circumstances it reaches its maximum value usually within 48 hours. This is in general unacceptable on the ICU, as every hour delay in starting for example appropriate antibiotic treatment can affect mortality as indicated by the

study of Kumar et al. [4]. However, levels are generally elevated in most ICU patients, making interpretation of CRP very difficult [49]. The other major problem with CRP on the ICU is that it is lagging way behind the actual events of the inflammatory process. The most important differences between the two markers are summarized in Table 1.

Table 1 Comparison of CRP vs. PCT (advantages and disadvantages)

	CRP	PCT
Differentiating bacterial infection from SIRS	-	specific for bacteria
Response to infection	slower (days)	2-6 hours
Peak response after infection	2-3 days	12-48 hours
Half life	several days	20-35 hours
Plasma kinetic	slow	rapid
Price	+	++++
Correlates disease severity, and progression	slightly	+++
Correlates effective therapy	+	+++
Prognostic factor for mortality	weak or nonexistent	good predictor
Differentiating G+ from G-	-	++
Response to other factors	virus, autoimmune diseases, local infections, surgery, trauma	surgery, trauma, burn, cardiogen shock, liver chirrosis
Fungal infection	same as bacterial	slightly elevated
Immunsuppression	formation can be changed	the induction is reduced
Biological effect	opsonin for phagocytosis	chemokine
Sensitivity/specificity	sensitive but nonspecific	sensitive and specific
General use	outpatient care	intensive care

Procalcitonin differentiates bacterial infections from systemic inflammatory response of other etiologies with higher sensitivity and specificity compared to CRP [50] and also have a good prognostic value regarding survival [51]. There is considerable evidence that PCT supported decision making during antibiotic treatment has several beneficial effects. It considerably reduced antibiotic use in lower respiratory tract infections without compromising survival [8], and it may also shorten the duration of antibiotic treatment on the ICU [9].There are many studies reporting that PCT values correlate with severity and differ significantly in patients with SIRS, sepsis, severe sepsis and septic shock [52].It must be considerated that the same absolute values of PCT cannot be used in all circumstances. It has

been reported that PCT levels are higher in surgical compared to medical patients [53], and elevated PCT can also be present without infection, in conditions such as trauma [54], surgery [55] or after cardiac arrest [56]. There is some evidence that evaluating PCT kinetics may be superior to absolute values [53, 57]. However, learning how to use biomarkers and interpreting PCT values correctly on admission or after the onset of an acute insult, let it be infectious or not, is not simple.

2 AIMS AND SCOPES

There are three fundamental questions to be answered during our ward rounds when treating patients with suspected or proven infections on the ICU: 1) is there infection, in other words should we start empirical antibiotic therapy; 2) is the commenced antibiotic effective; and finally 3) when should we stop antibiotic treatment? Our research team decided to give exact answers to these questions as it is a fundamental problem in our daily practice in the ICU.

Starting with the second question, during the initial phase of sepsis treatment before the first microbiological results physicians have little help to confirm adequacy of the commenced antibiotics. As PCT is a fast reacting biomarker with a half-life of 24 hours, theoretically it is possible that the early kinetics of PCT within this first 24 hours after commencing empirical antibiotic therapy may reflect the efficacy of the treatment. As this has not been investigated before, we decided to perform a prospective observational study with 3 goals: a) to describe early kinetics of PCT measured 8 hourly after starting empirical antibiotic therapy within the first 24 hours, b) to investigate if the observed kinetics shows any difference in patients in whom the antibiotic treatment eventually proved to be appropriate *versus* those in whom it proved to be inappropriate, and c) to determine the cut-off value of PCT with highest discriminatory power.

Regarding the first question, as a spin off study after the original EProK-study has been completed, in a *post hoc* analysis those patients in whom PCT and CRP values were available from the previous day (t_{-1}) were included in a second analysis. Our aim was to investigate whether the absolute value of PCT measured in critically ill patients on the day when infection was suspected, or the change in PCT (delta PCT) from the day before to the day when infection was suspected, was a better indicator of infection.

However concerning the third question we found to be answered by Boudama et al. in the PRORATA trial [9], as our daily practice is accepted applying that algorithm so our study has not been extended for investigating the duration of the antibiotic therapy.

3 METHODS

3.1 Patient selection

This prospective observational study was undertaken between October 2012 and October 2013 and was approved by the Regional and Institutional Human Medical Biological Research Ethics Committee, University of Szeged, Hungary (WHO-3005; 19.04.2012, Chairperson Prof. T. Wittmann). The investigation was performed at the University of Szeged (Szeged, Hungary), Albert Szent-Györgyi Health Center in a 27 bed multidisciplinary tertiary intensive care unit. The study was registered at ClinicalTrials.gov with the registration number: NCT02294695. Written informed consent was obtained from all subjects or from their relatives.

Inclusion criteria: All patients over 18 years with suspected infection on admission or during their stay on the intensive care unit were screened for eligibility. Patients were enrolled when the attending intensive care specialist suspected infection (for explanation see below) and decided to start empirical antibiotic therapy.

Exclusion criteria: age <18 years; antimicrobial therapy within 48 hours; conditions that have been shown to interfere with the inflammatory response such as: acute renal replacement therapy in the first 24 hours [9], cardiopulmonary resuscitation[58], patients with end stage diseases and immunocompromised patients.

3.2 Subgroups and definitions

Diagnosis of infection and appropriateness of the empirical antimicrobials were established as a retrospective analysis based on recommendations [58], clinical parameters, biochemical and microbiological results, evaluated by two experts blinded for the PCT data apart from the first PCT result: an infectologist and an intensivist, who also took into consideration the recommendations of the International Sepsis Forum Consensus Conference [58]. Based on these results, patients were then grouped into Infectious-, and Noninfectious-groups. Patients with suspected infection but negative microbiology were also excluded from the final analysis.

Antimicrobial therapy was also evaluated, and it was considered appropriate, if a) the isolated pathogens were susceptible to at least one of the commenced antimicrobials [59] administered at the onset of sepsis according to the corresponding susceptibility testing report, and the appropriate dosage

as recommended by our local protocols. Based on these results patients were grouped *"post hoc"* into appropriate (A-group) and inappropriate (IA-group) antimicrobial treatment groups.

For subgroup analysis, patients were also divided further into 'Medical' and 'Surgical' groups. Medical-group represents patients, who had no surgical intervention before and during the study period, and for source control they did not require surgery. In the Surgical-group infection was either related to an operation, or required surgery for source control [60]. These groups were also further divided *post hoc* into appropriate and inappropriate antibiotic groups: $A_{m(edical)}$-, and $A_{s(urgical)}$-group; IA_m-, and IA_s-group.

3.3 Protocol and data collection

Whenever infection was suspected by the attending physician, the signs of infection and the suspected source were recorded, which were: high/low temperature (<36°C; >38°C), white blood cell count (<4.000; >12.000 million/ml), acute worsening of the clinical picture (hemodynamic instability, worsening PaO_2/FiO_2 ratio, deterioration in mental status or any other clinical sign indicating infection) and elevated or increasing (as compared to the previous day) PCT levels. Specimens were then sent for microbiology and antibiotic therapy was commenced. The choice of antimicrobials was determined by local protocols based on international guidelines [61–63].

3.3.1 Data collection

After enrollment demographic data and parameters of vital organ function were registered. Patients were followed up for 6 days. In addition to detailed collection of physiological and laboratory data during those 6 days, the length of intensive care unit and hospital stay, 28 day-, and the overall mortality were also documented.

3.3.2 Procalcitonin measurement

Procalcitonin levels were determined immediately before the initiation of antimicrobials (t_0), 8 hourly (t_8, t_{16}, t_{24}) during the first 24 hours and then daily (day_2-day_6). The flow chart of the data collection is summarized in Figure 4.

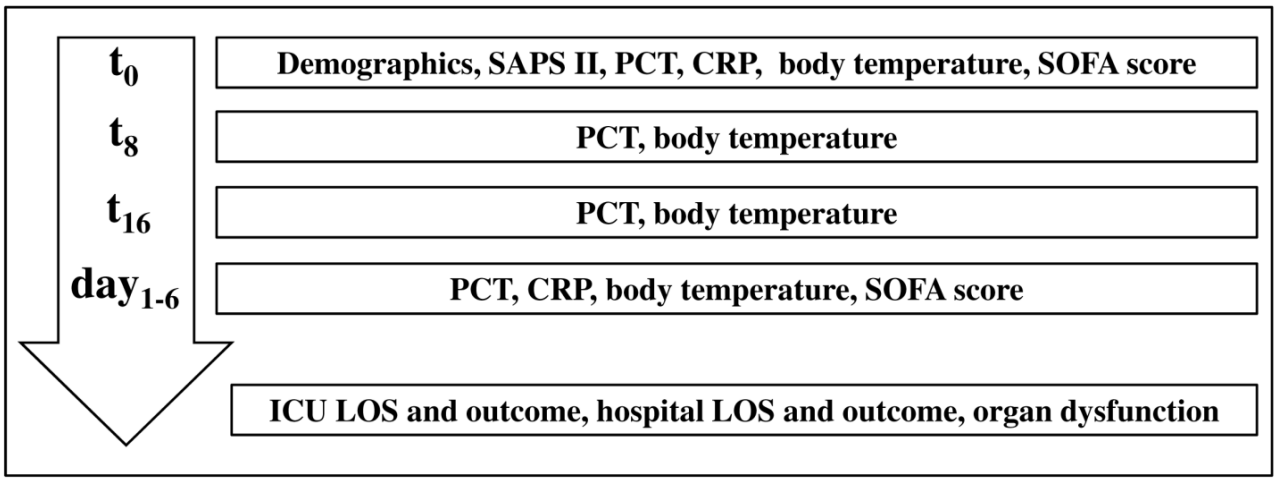


Figure 4 Flowchart: $t_{0\text{-}24}$ indicates sampling within the first 24 hours after commencement of empirical antimicrobials; SAPS II - Simplified Acute Physiology Score II; SOFA - Sequential Organ Failure Assessment; LOS - length of stay

Serum PCT levels were measured with Cobas 6000 analyzer (Hitachi High-Technologies Corporation, Tokyo, Japan). Analyzer reagents (Elecsys® B·R·A·H·M·S PCT assay) were developed in collaboration with B·R·A·H·M·S corporation (Hennigsdorf, Germany) and Roche Diagnostics (Mannheim, Germany). Procalcitonin was determined by electrochemiluminescence immunoassay (ECLIA) serum on the automated Roche Elecsys and Cobas immunoassay analyzers.

3.3.3 Microbiological staining and antibiograms

Microbiological tests were performed and sent at t_0, before the first antimicrobial dose was administered and if necessary repeated on the following days, to identify microorganisms and their resistance. The type of antimicrobials, the dosage, the bacterial strains and their antibiogram profile were recorded daily or whenever the results were available.

3.4 Statistical analysis

The primary end point of the study was the difference in PCT kinetics after 24 hours of starting the antimicrobial treatment. According to our former pilot study [64] a PCT increase of <70% within the first 24 hours, compared to the baseline value (t_0) had an 84% positive predictive value with 80% sensitivity and 41% specificity (p=0.059), indicating appropriate antimicrobial treatment. Therefore, for the study to have 80% power to show the smallest clinically relevant difference of 15% , an increase of

PCT between the A-, and IA-groups (i.e.: 70% increase in the IA-, and 55% increase of PCT in the A-group from t_0 to t_{24}) with a $p<0.05$, the required sample size was at least 161 patients. Based on this calculation we decided to enroll patients for at least 12 months.

Data were analyzed using IBM SPSS Statistics Version 20 (Armonk, NY, USA) and SystatSoftware IncSigmaPlot 12.5 (London, UK) software. For continuous data, the Shapiro-Wilk tests were performed to assess normal distribution. Demographic data were analyzed between groups with the Student's t-test or non-parametric data with the Mann–Whitney U-test as appropriate. Biomarkers were analyzed by using Two Way Repeated Measures Analysis of Variances (All Pairwise Multiple Comparison Procedures: Holm-Sidak method). Categorical data were compared using χ^2 tests. Receiver operating characteristic (ROC) curve and the respective areas under the curves (AUC) were calculated for PCT and CRP levels. The best cut off values were determined to maximize the Youden index (J = max [Sens + Spec – 1]. The test parameters (sensitivity, specificity, positive and negative predictive values) were compared by their 95% confidence intervals. A level of $p<0.05$ was defined as statistically significant. Data are given in mean ± standard deviation or median (25-75% interquartile range) as appropriate.

4 RESULTS

Over the study period 209 patients were enrolled. Demographics are summarized in Table 2. From the 209 patients, 141 (67%) had proven infection, with infection unproven in 44 (21%). In 24 patients,

Table 2 Demographics and infection sources in the entire cohort

		Total (n=209)	Appropriate AB (n=108)	Inappropriate AB (n=33)	p value
Age (years)		68 (19)	68 (19)	69 (20)	0.842
Male, n (%)		117 (56)	60 (55)	16 (48)	0.476
Body height (cm)		170 (12)	169 (14)	165 (19)	0.422
Body weight (kg)		76 (25)	70 (18)	80 (27)	0.218
SAPS II points		67 ± 19	68 ± 19	72 ± 16	0.333
SAPS II PM		78 (47)	81 (32)	88 (27)	0.298
SOFA score points at t_0		14 (5)	14 (4)	15 (6)	0.298
delta SOFA score points (t_0-t_{24})		0 (2)	0 (2)	0 (2)	0.568
ICU days before enrollment		1(2)	0 (2)	1 (2)	0.798
ICU LOS (day)		8 (9)	8 (10)	9 (11)	0.263
ICU survival, n (%)		151 (72)	84 (78)	14 (42)	<0.001
Hospital LOS (day)		15 (17)	16 (22)	17 (16)	0.444
Hospital survival (%)		126 (60)	68 (63)	13 (39)	0.017
Mechanical ventilation (day)		4 (8)	4 (9)	7 (8)	0.011
Vasopressor therapy (day)		3 (4)	3 (3)	5 (4)	0.004
Renal replacement therapy, n (%)		65 (31)	33 (31)	19 (57)	0.005
Nosocomial infecion, n (%)			53 (49)	16 (48)	0.953
Source of infection, n (%)	Respiratory		54 (50)	25 (76)	0.007
	Abdominal		19 (18)	5 (15)	0.744
	Soft tissue		15 (14)	2 (6)	0.227
	UTI		10 (9)	2 (6)	0.564
	BSI		7 (6)	0	0.134
	Meningitis		5 (5)	0	0.208
	Other		1 (1)	1 (3)	0.371

Data are given as median (interquartile range) or mean ± standard deviation as appropriate. Total = infection (appropriate and inappropriate) + no infection group; Regarding the source of a patient may have more than one infections at the same time. AB - antimicrobial therapy; n – number of patients; M - male; SAPS - Simplified Acute Physiology Score; PM - predicted mortality; ICU - intensive care unit; LOS - length of stay; UTI - urinary tract infection; BSI - bloodstream infection.

although infection was highly likely, microbiology did not reveal pathogens, hence these subjects were excluded from the final analysis. Procalcitonin at t_0 was significantly higher in the Infectious-group compared to Noninfectious-group: 4.53 (1.76-16.30) *vs.* 1.0 (0.13-2.98) ng/ml, p=0.024, respectively. In the Infectious-group (n=141) 108 (77%) patients received appropriate antimicrobial therapy (A-group) and in 33 (23%) antimicrobials proved to be inappropriate (IA-group).

Regarding demographics there were no differences between the A-, and IA-groups but ICU-, and hospital-survival was significantly higher in the A-group (Table 2). These patients also required less vasopressors and renal replacement therapy compared to the IA-group. From the 33 patients in the IA-group, antimicrobials were changed in 20 patients on day 2 or 3, without any significant effect on PCT kinetics, compared to the other 18 patients (data not shown).

4.1 Procalcitonin kinetics

In both groups the increase in PCT levels continued after the initiation of empirical antimicrobial treatment (t_0) until 16 hours (t_{16}) (Figure 5-A). In the IA-group there was a significant increase from t_{16} to t_{24}, while in the A-group there was a significant decrease from t_{16} to t_{24}. By t_{24} the PCT reached significantly higher levels in the IA-group and remained higher the following day compared to the A-group. In the A-group PCT levels peaked at t_{16}, while in the IA-group the peak was at t_{24}. From t_{24} until the 5th day PCT levels decreased in both groups (Figure 5-A).

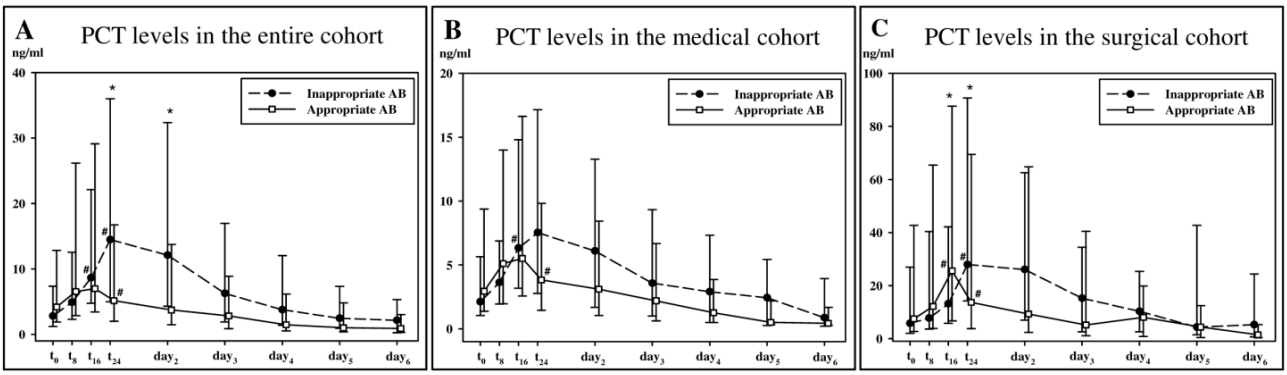


Figure 5 PCT absolute values in the entire cohort and in the medical and surgical cohort. Data are presented as median and interquartile range. AB indicates antimicrobial therapy. #p< .05 within groups; * p< 0.05 between groups.

There was a non-significant increase in CRP from t_0 to t_{24} in both groups. In the A-group CRP peaked at $t_{24.}$, In the IA-group CRP remained high on day_2 with levels remaining higher compared to the A-group on $days_{3\text{-}5}$. After day_2 CRP levels decreased in both groups (Figure 6-A). Body temperature showed no significant change over time (Figure 6-B).

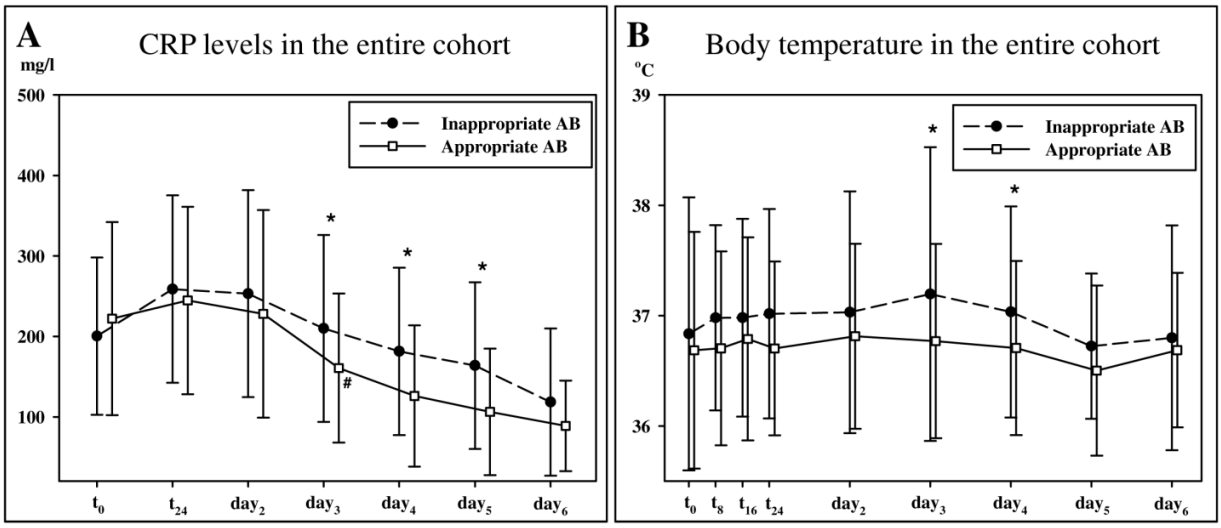


Figure 6 CRP and body temperature in the entire cohort

Data are presented as mean ±standard deviation; AB – antimicrobial therapy; $t_{0\text{-}24}$ - sampling within the first 24 hours after commencement of empirical antimicrobials; # $p<0.05$ within groups; * $p<0.05$ between groups

4.2 PCT kinetics in medical and surgical patients

In patients with infection, PCT followed similar kinetics in medical (n=91) and surgical (n=50) patients, with substantial differences in the absolute values at t_0 (median: 2.74 [25-75% interquartile range: 1.30-7.72] *vs*. 6.46 [2.61-40.07], p=0.002) and at t_{24} (4.41 [1.52-13.55] *vs*. 17.02 [6.74-69.45] ng/ml, $p<0.001$, respectively). Medical and surgical patients were further divided into appropriate: A_m-(n=70), A_s-(n=38), and inappropriate: IA_m-(n=21), IA_s-(n=12) subgroups. Kinetics in all subgroups followed the same pattern as described for the whole sample (Figure 5-B, C).

Kinetics of CRP where similar in both groups, with no significant differences within and between groups during the study period (Figure 7-A, B). The same holds true for body temperature, with the only difference being that in the medical cohort at t_{24}, temperature was higher for the next 3 days (Figure 7-C, D).

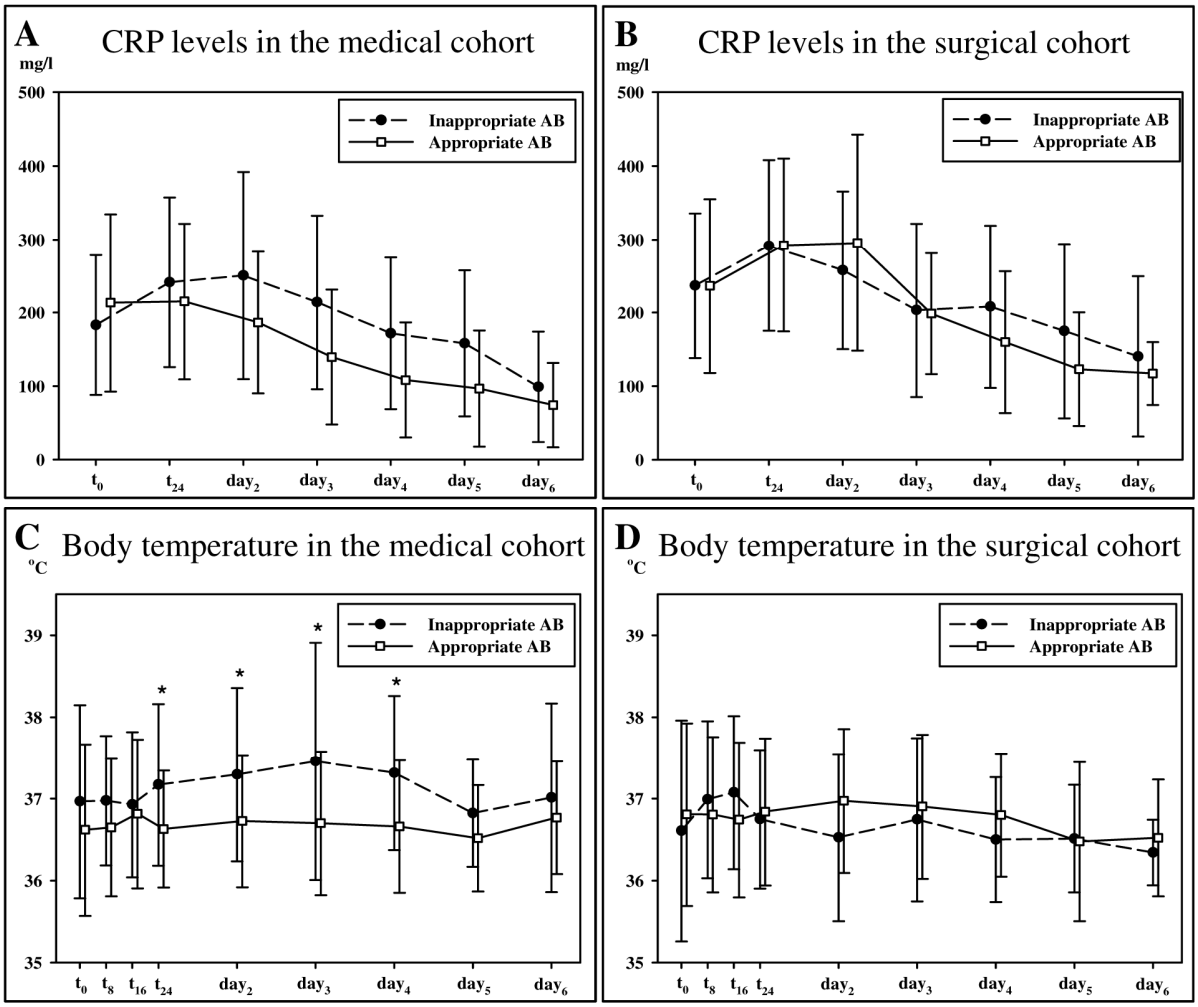
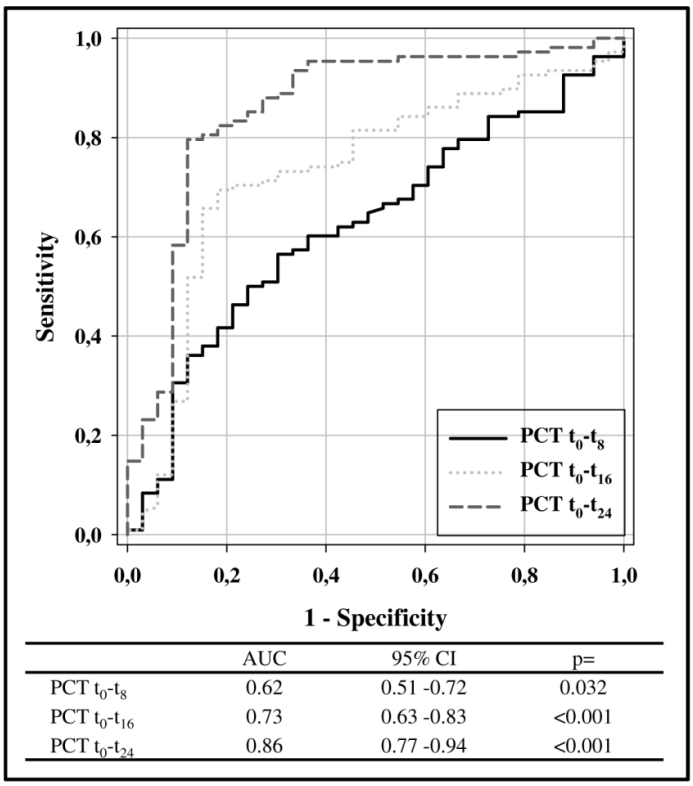


Figure 7 CRP and body temperature in medical and surgical patients

Data are presented as mean ±standard deviation; AB – antimicrobial therapy; $t_{0\text{-}24}$ - sampling within the first 24 hours after commencement of empirical antimicrobials; * $p<0.05$ between groups

4.3 Predictive value of PCT for indicating appropriate antimicrobial treatment

The ROC analysis revealed that a PCT elevation from t_0 to t_{16} had an AUC of 0.73 (95% CI, 0.63-0.83; $p<0.001$), from t_0 to t_{24} 0.86 (95% CI, 0.77-0.94; $p<0.001$) (Figure 8). According to the Youden-index, the best cut off for PCT increase from t_0 to t_{16} was 69.2%, and from t_0 to t_{24} it was 73.5% (Table 3).



	AUC	95% CI	p=
PCT t_0-t_8	0.62	0.51 -0.72	0.032
PCT t_0-t_{16}	0.73	0.63 -0.83	<0.001
PCT t_0-t_{24}	0.86	0.77 -0.94	<0.001

Figure 8 ROC curve

ROC - receiver operating characteristic curve; PCT – proalcitonin; $t_{0\text{-}24}$ - sampling within the first 24 hours after commencement of empirical antimicrobials; AUC - area under the ROC curve; CI - confidence interval

Table 3 Cut off values for appropriate and inappropriate antimicrobial treatment in the entire cohort

	Cut off value	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	p
PCT t_0-t_8	≥45.6%	56.5% (0.46-0.66)	69.7% (0.51-0.84)	31.7% (0.28-0.54)	85.4% (0.78-0.92)	0.090
PCT t_0-t_{16}	≥69.2%	65.7% (0.56-0.74)	84.8% (0.68-0.94)	42.1% (0.33-0.60)	92.8% (0.82-0.95)	0.048
PCT t_0-t_{24}	≥73.5%	79.6% (0.70-0.86)	87.8% (0.71-0.96)	53.5% (0.41-0.70)	95.2% (0.84-0.98)	<0.001

PCT – procalcitonin; CI – confidence interval; PPV – positive predictive value; NPV – negative predictive value; PCT – procalcitonin; The best cut off value was determined using the Youden index.

5 METHODS OF THE SPIN OFF SUTDY

This prospective observational study, as part of the Early Procalcitonin Kinetics (EProK)-study, was registered at ClinicalTrials.gov with the registration number: NCT02311816.

The analysis was performed from the same database with those patients in whom PCT and CRP values were available from the previous day (t_{-1}), otherwise the inclusion and exclusion criteria were the same as in the EProK-study, detailed in the methods chapter earlier.

5.1 Subgroups and definitions

Diagnosis of infection was based on a *post hoc* analysis of mainly microbiological results but also clinical parameters and biochemical results which were evaluated by our two experts blinded for the PCT data apart from the first PCT measurement (t_0, see below). The experts also took into consideration the recommendations of international guidelines [15, 58]. Based on these results, patients were grouped into ”infection” (I)-, and ”no infection” (NI)-groups.

For subgroup analysis, patients were divided into ”medical” and ”surgical” groups. The medical-group represented patients who had had no surgical intervention before and during the study period and for source control they did not require surgery. In the surgical-group infection was either related to an operation, or required surgery for source control [53].

5.2 Protocol and data collection

The same protocol was followed as described before including the data collection, the PCT measurements, the microbiology and the statistical methods too. The flow chart of the data collection is summarized in Figure 9.

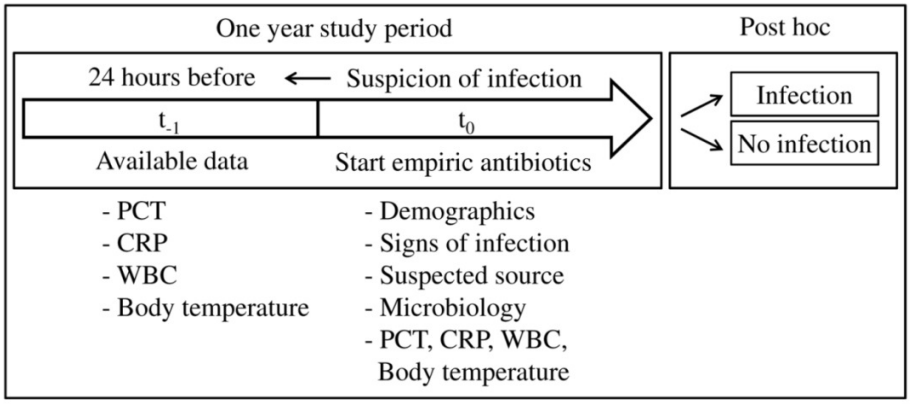


Figure 9 Flow chart

6 RESULTS OF THE SPIN OFF STUDY

Over the one year study period all ICU patients were screened for eligibility and 209 patients were recruited into the EProK-study. Out of the 209 patients in the current post hoc analysis we include 114 cases where PCT values were available from the previous day. Demography and outcomes characteristics for the entire cohort are summarized in Table 4. Out of the 114 patients, 85 (75%) patients were identified as having proven infection and in 29 (25%) patients the presence of infection was highly unlikely. Disease severity scores and outcomes were similar in the two groups, but the NI-group required less organ support.

Table 4 Demographics, organ support and outcome in the entire cohort

	Total	NI-group	I-group	p value
Age (years)	65 (22.5)	67 (25.5)	65 (22)	0.772
Gender (M/F)	69/45	15/14	54/31	0.261
Body height (cm)	170 (12)	167 (19)	170 (11)	0.766
Body weight (kg)	73 (25)	80 (25)	70 (20)	0.345
SAPS II points	62.2 ± 20.5	62.7 ± 25.5	66.1 ± 18.6	0.513
SAPS II PM (%)	77.2 (52.1)	64.0 (75.9)	78.5 (42.1)	0.437
ICU days before enrollment	1 (3)	1 (3)	1 (3)	0.669
Mechanical ventilation	80 (70.2%)	12 (41.4%)	68 (80.0%)	<0.001
Vasopressor therapy	69 (60.5%)	13 (44.8%)	56 (65.9%)	0.045
ICU LOS (day)	9 (12)	8 (8)	9 (12)	0.089
ICU survival	84 (73.7%)	24 (82.8%)	60 (70.6%)	0.199
Hospital LOS (day)	17 (20)	14 (17)	19 (22)	0.050
Hospital survival	67 (58.8%)	20 (68.9%)	47 (55.3%)	0.197
28 day survival	64 (56.1%)	19 (65.5%)	45 (52.9%)	0.239

Data are given as mean ± standard deviation or median (interquartile range) as appropriate. M - male; F - female; SAPS - Simplified Acute Physiology Score; PM - predicted mortality; ICU - intensive care unit; LOS - length of stay; Mechanical ventilation and vasopressor therapy represents data at the day of enrollment.

The clinical and laboratory signs of infection on which the clinicians suspected infection at the time of inclusion (t_0) are summarized in Table 5. Although all indices were higher in the I-group, but

only the altered level of consciousness, hemodynamic instability and the PCT was significantly different between the two groups.

Table 5 Clinical signs and suspected source of infection at enrollment (t_0)

	Total n=114	NI-group n=29	I-group n=85	p value
Fever (<36°C; >38°C)	55 (48.2%)	13 (44.8%)	42 (49.4%)	0.670
WBC (>12 or<4 x 10^9/L)	82 (71.9%)	22 (75.9%)	60 (70.6%)	0.585
Impaired gas exchange	82 (71.9%)	18 (62.1%)	64 (75.3%)	0.171
Impaired consciousness	59 (51.8%)	9 (31.0%)	50 (58.8%)	0.010
Hemodynamic instability	74 (64.9%)	13 (44.8%)	61(71.8%)	0.009
PCT (ng/ml)	3.37 (9.22)	1.12(1.36)	4.62 (10.72)	0.018
CRP (mg/l)	182.75 (158.5)	147.60(156.50)	208.80(140.60)	0.301
Respiratory	72 (63.2%)	17 (58.6%)	55 (64.7%)	0.557
Soft tissue	13 (11.4%)	2 (6.9%)	11 (12.9%)	0.377
Abdominal	14 (12.3%)	7 (24.1%)	7 (8.2%)	0.024
Urinary tract	5 (4.4%)	0	5 (5.9%)	0.182
Bloodstream	6 (5.3%)	2 (6.9%)	4 (4.7%)	0.648
Central nervous system	4 (3.5%)	1 (3.4%)	3 (3.5%)	0.984

WBC - white blood cell count, PCT - procalcitonin, CRP - C-reactive protein, The PCT and CRP values are presented as: median (interquartile range).

Regarding the suspected source of infection, generally there was non-significant difference between the groups, but significantly more patients were suspected of having abdominal related infection in the NI-group.

6.1 PCT, CRP, WBC and temperature values at t_{-1} and t_0

6.1.1 Total sample

Measurement results at t_{-1} and t_0 in the I-, and NI-groups are shown in Figure 10. PCT absolute values were similar at t_{-1}, but by t_0 in the I-group levels were significantly higher compared to the NI-group and there was also a significant increase from t_{-1}, while there was no such change in the NI-group. There was no significant difference in CRP and WBC count between the two groups and nor could we find significant changes from t_{-1} to t_0. There was no difference between the groups for body

temperature but there was a statistically significant increase in the NI-group by t_0. It is of note that body temperature remained <38°C in almost all patients.

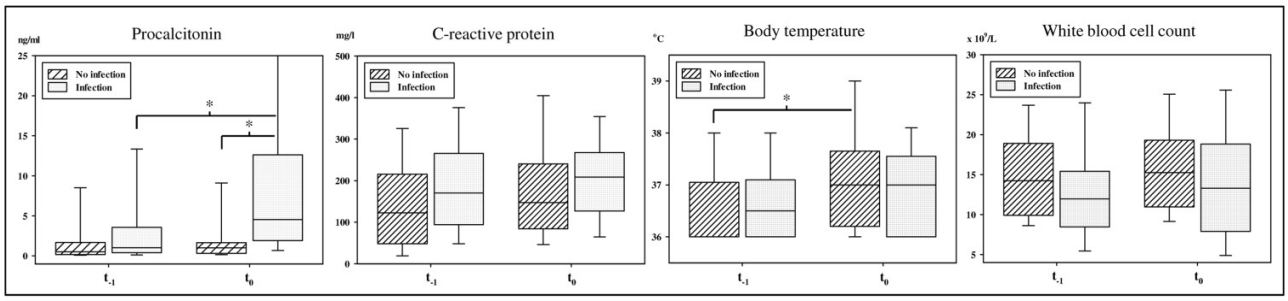


Figure 10 PCT, CRP, body temperature and WBC count absolute values in the total cohort Boxplots presents median (interquartile range) 10th and 90th percentile.* indicates p <0.05

6.1.2 Medical and surgical patients

Measurement results in medical (n=80) and surgical (n=34) patients are summarised in Table 6. In the surgical subgroup PCT absolute values were significantly higher than in the medical cohort, but the pattern of change was similar.

Table 6 PCT, CRP, body temperature and white blood cell count in medical and surgical patients with and without infection

		NI-group		I-group	
		t_{-1}	t_0	t_{-1}	t_0
Medical	PCT (ng/ml)	0.26 (0.57)	0.54 (1.16)*	0.89 (1.52)	3.17 (5.9)*#
	CRP (mg/l)	136.7 (159.1)	141 (125.9)	150 (184.3)	164.2 (145.3)
	BT (°C)	36 (1.02)	37 (0.82)*	36.9 (1.23)	37 (1.6)
	WBC (x10^9/l)	14.32 (8.9)	15.4 (8.64)	12.06 (6.36)	13.76 (10.16)
Surgical	PCT (ng/ml)	3.5 (9.91)	2.89 (9.33)	3.83 (22.55)	14.9 (58.06) *#
	CRP (mg/l)	95 (342.5)	163 (327.4)	199.5 (130.1)	243.2 (112.7)
	BT (°C)	36.5 (2)	36.5 (2.4)	36 (1)	36.9 (1.1)
	WBC (x10^9/l)	8.99 (7.37)	14.56 (9.65)	11.9 (10.06)	10.91 (9.9)

Data are presented as median (interquartile range).PCT = procalcitonin, CRP = C-reactive protein, BT = body temperature, WBC = white blood cell count; * <0.05 within groups, # <0.05 between groups

In the NI-group there was a slight, but statistically significant increase in medical patients from t_{-1} to t_0, while there was no significant change in surgical patients, where levels actually decreased slightly. However, in the I-group there was an almost 3-fold increase in the PCT levels.

Regarding the CRP, body temperature and WBC count, there was no significant changes over time and no differences between medical and surgical patients.

6.2 Predictive value for indicating infection

The predictive value for infection for the absolute values of PCT, CRP, temperature and WBC count can be seen in Figure 11, and are summarised in Table 7. Only PCT had a significant predictive value, but with a poor AUC (Figure 11). However, regarding the percentage and delta changes CRP, temperature and WBC counts diagnostic value did not change, while PCT's AUC for both percentage and delta changes had a significantly better performance for predicting infection. Similar patterns were observed in the medical and surgical subgroups (Table 7).

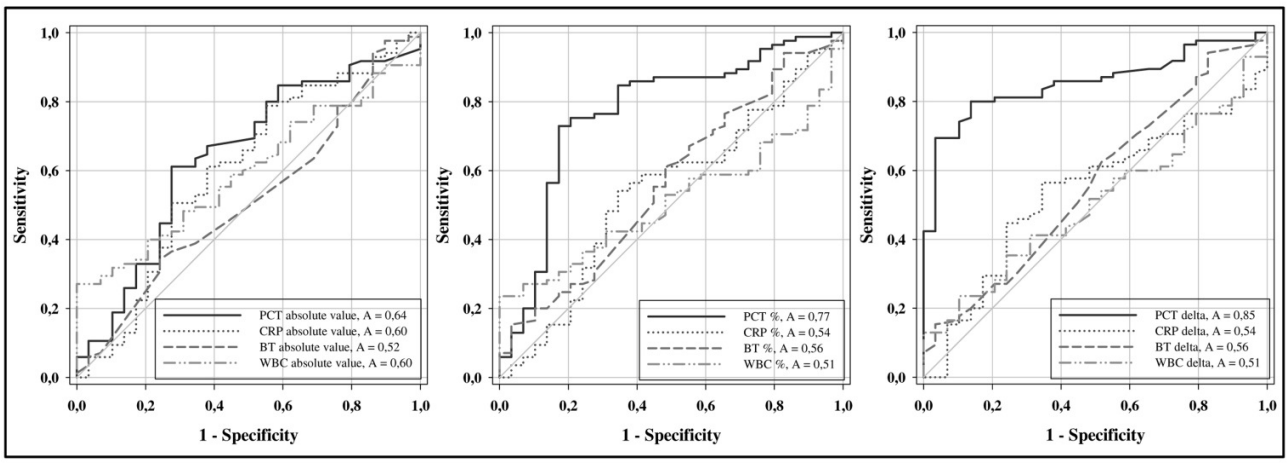


Figure 11 The predictive value of the absolute values, percentage and delta changes of PCT, CRP, temperature and WBC count for infection in the total cohort

6.3 Best cut-off value

The best cut-off values were defined for PCT only as there was no significant predictive value for the other parameters, as determined by the Youden index. For the PCT absolute value it was 0.84 ng/ml with a sensitivity of 61 % (95% CI: 50-72) and specificity 72 % (53-87) to indicate infection in the ICU. Regarding the percentage change a PCT increase of >88% from t_{-1} to t_0 had a

sensitivity of 75% (65-84) and specificity of 79% (60-92) and a PCT delta change of>0.76 ng/ml had a sensitivity of 80% (70-88) and specificity of 86% (68-96) to indicate infection.

Table 7 The predictive value of the absolute values, percentage and delta changes of PCT, CRP, temperature and WBC count for infection in the total cohort

		absolute value			percentage changes			absolute value changes		
		AUC	95% CI	p value	AUC	95% CI	p value	AUC	95% CI	p value
Total	PCT	0.64	0.52-0.76	0.022	0.77	0.66-0.87	<0.001	0.85	0.78-0.92	<0.001
	CRP	0.60	0.47-0.72	0.103	0.54	0.41-0.66	0.530	0.54	0.42-0.65	0.536
	BT	0.52	0.39-0.63	0.804	0.56	0.44-0.68	0.300	0.56	0.44-0.68	0.322
	WBC	0.60	0.48-0.70	0.125	0.51	0.40-0.61	0.852	0.51	0.39-0.61	0.924
Medical	PCT	0.67	0.54-0.80	0.016	0.76	0.63-0.88	<0.001	0.83	0.73-0.92	<0.001
	CRP	0.58	0.44-0.72	0.248	0.57	0.42-0.70	0.359	0.57	0.44-0.70	0.306
	BT	0.51	0.37-0.64	0.858	0.64	0.50-0.77	0.055	0.64	0.49-0.77	0.060
	WBC	0.57	0.44-0.70	0.329	0.56	0.43-0.68	0.441	0.57	0.43-0.69	0.365
Surgical	PCT	0.78	0.58-0.97	0.025	0.80	0.59-1.00	0.014	0.94	0.85-1.00	<0.001
	CRP	0.56	0.23-0.87	0.654	0.56	0.29-0.81	0.654	0.54	0.31-0.76	0.749
	BT	0.52	0.22-0.80	0.898	0.63	0.39-0.85	0.306	0.63	0.39-0.86	0.296
	WBC	0.63	0.44-0.82	0.277	0.67	0.47-0.86	0.166	0.71	0.49-0.90	0.108

AUC = area under the ROC curve, CI = confidence interval, PCT = procalcitonin, CRP = C-reactive protein, BT = body temperature, WBC = white blood cell count

Data were also analyzed using the logistic regression model for finding the best combination of these four parameters together to predict infection in the ICU. However, none of the combinations tested improved the performance for predicting infection (data not shown).

7 DISCUSSION

The main findings of the EProK study were that PCT kinetics during the first 24 hours after commencing empirical antimicrobial therapy show significant differences in patients on appropriate antimicrobial therapy compared to those on inappropriate antimicrobial therapy and that there were significantly higher absolute PCT values, but similar kinetics in surgical compared to medical patients with infections.

7.1 Diagnosing infection

Despite advances in critical care, diagnosing infection and sepsis has remained a major challenge in clinical practice. Appropriate decision making has paramount importance as the delay in adequate antibiotic treatment of sepsis and septic shock evokes worsening morbidity and mortality results [4, 58]. Although the classical definitions of 'sepsis syndrome' proposed by Bone et al [14], and the so called 'consensus criteria' [15] has been implemented worldwide for decades, it still remains one of the greatest challenges to differentiate between systemic inflammatory response from bacterial infection at the bedside. It has been shown for long that fever and leucocytosis on their own are poor indicators of bacterial infections [35], and the consensus criteria itself has also been criticised [65]. This uncertainty in the diagnosis may lead to unnecessary overuse of antibiotics resulting the emergence of multidrug-resistant bacteria [1, 66], complications related to the side effects of the antibiotics themselves and increased burden of healthcare expenses [67, 68]. Despite its importance, there is no gold standard for diagnosing/proving infection in the critical care setting.

In this diagnostic dilemma, there has been considerable interest in the use of infection/sepsis biomarkers. Among these biomarkers the two most commonly used are PCT and CRP. Several studies have shown that PCT has a better sensitivity and specificity for indicating bacterial infections than CRP [69]. This was confirmed by the results from this study, in which as compared to CRP, temperature and leukocytosis and where only PCT was able to differentiate patients with proven infection from patients with no infection. These results are similar to those reported by recent large randomized trials [8, 9].

Procalcitonin is also considered as a good prognostic marker [70] and due to its favourable kinetic profile PCT can potentially be a useful biomarker for guiding antibiotic therapy. However, the results are so far conflicting. In a landmark study by Christ-Crain et al., antibiotic exposure was reduced by

50% (44% versus 83%) with no adverse effect on outcome in patients with acute respiratory complaints and receiving antibiotics according to their admission PCT levels as compared to patients treated with antibiotics based on conventional signs of infection [8]. A subsequent multicenter trial also found a decrease in antibiotic exposure in patients treated on the ICU with community acquired pneumonia by 32%, exacerbation of COPD by 50% and acute bronchitis by 65% [71]. Similar results were reported in another large clinical trial, the PRORATA study [9]. The novelty of the PRORATA study was that not only the commencement, but the discontinuation of antibiotic treatment was assisted by PCT levels. The results of this study showed that for patients with suspected infections PCT-guided antibiotic treatment lowered antibiotic exposure by 23% (14.3 versus 11.6 antibiotic free days) and was non-inferior to standard care with respect to outcomes. Two German studies assessed the effect of PCT guidance in high risk surgical patients with suspected bacterial infections during the postoperative period in the ICU [72, 73]. PCT-guided therapy resulted in a significant reduction of antibiotic therapy and length of intensive care stay (15.5 versus 17.7 days) with similar other outcomes. Although the current study was an observational and not a randomised trial comparing PCT-based therapy to conventional measures, but nevertheless our results give further support to the findings above.

In our spin off study could be seen that an increase in PCT levels from the day before (t_{-1}) to the day when infection was suspected (t_0) predicted infection, while in patients with no proven infection PCT remained unchanged. Furthermore, regarding the conventional indicators of infection such as WBC, body temperature and CRP, neither the absolute values nor their change from t_{-1} to t_0 could predict infection.

In our study 75% of patients had proven infection. This complex post hoc analysis of all results, is fundamentally different from ”labelling” patients as septic, based solely on the Surviving Sepsis Guideline criteria at the time of initial assessment most often seen in several studies [74, 75]. Although our method also has some uncertainties, but provides a more robust approach with all data - clinical, biochemical and microbiology alike -, taken into account to aid the diagnosis of patients with bacterial infection. However, it is also important to acknowledge, that there is no gold standard to diagnose infection, therefore despite all our efforts, some patients in the NI-group may had culture negative infection.

In our investigation it was found that conventional indicators of infection, such as body temperature and white cell count had no value in diagnosing infection. Levels of the WBC count remained elevated on both days and there was no significant change in over time. This phenomenon can

be explained by the non-specific activation of the immune cascade often seen in ICU patients [12]. Although there was a statistically significant increase in body temperature in the NI-group, but levels by-and-large remained below 38°C in almost all patients. These results are in accord with recent findings that increased temperature alone did not predict infection [76].

Although microbiology remains the gold standard for confirming pathogens, but results come back late at least 24-48 hours after sampling. Furthermore, in several cases results remain negative, despite obvious signs of infection. In order to help the diagnostic process several novel biomarkers of infection have been developed [7]. However, all biomarkers share the same limitations that ”one size will not fit all”, which is due to the complex pathomechanism and the heterogeneity of patients.

The two most commonly used markers in infection/sepsis diagnostics are PCT and CRP [7]. Procalcitonin is detectable in the serum within a few (2-4) hours after the onset of bacterial infection. It reaches its peak within 24 hours and then starts its decline in the case of adequate treatment with roughly 50% daily decrease according to its half-life [48]. In contrast, CRP has a delayed response. It reaches its maximum value usually after 48 hours of an insult and in general it is lagging way behind the actual events of the inflammatory and clinical process. Furthermore, CRP levels are generally elevated in most ICU patients regardless of the etiology. In our study neither the absolute values of CRP, nor its delta changes could indicate new onset infection. Patients had elevated CRP values with median of almost 200 mmol/l for the whole cohort, which makes interpretation very difficult. Furthermore the kinetics did not show any significant change over time either. Therefore, our results questions the place of CRP measurements for diagnosing infection on the ICU.

The most important finding of the current study is to show the superiority of PCT kinetics over the absolute values to indicate new onset infection on the ICU. These are in accord with that of reported by Tsangaris et al. [57]. They observed a two-fold increase of PCT levels from the day before to the day when there was a sudden onset of fever in patients with proven infection, but no change in PCT was found in patients with no infection. They concluded that in patients treated chronically on the ICU, PCT values on the day of fever onset must be compared to values measured on the previous day in order to define whether this rise in temperature was due to infection or not. An important difference between their and our study is that in our patients, body temperature merely reached 38°C, in fact most of these patients were apyrexial, despite 75% had proven infection. Therefore, we recommend to evaluate PCT kinetics but not only in the case of fever onset, but whenever infection is suspected on the ICU. Based on the current results, the best cut off values were also determined for PCT change, which were >88%

and >0.76 ng/ml delta change from t_{-1} to t_0. The reasons why a given absolute value of any biomarker, not just PCT, may be of limited value as compared to its changes can be explained by the pathomechanism of systemic inflammation. It was a very important discovery that after trauma, burns, ischemia-reperfusion, pancreatitis, major surgery, etc., same or similar molecules are released mainly from the mitochondria, as after an infectious insult. Based on etiology these are called 'damage-associated molecular patterns' (DAMP), or 'pathogen associated molecular patterns' (PAMP). After similar mediators/proteins are released acting on the same receptors of monocytes inflicting similar inflammatory response, including PCT release and distant organ dysfunction [33, 77].

We observed significant difference in the PCT-levels between patients with and without proven infection in contrast to CRP and temperature, suggesting that PCT is superior as a marker for initiating antibiotic treatment in the critically ill as compared to CRP and temperature. However, the interpretation of PCT measurements, especially the absolute values on their own may be misleading. Therefore, evaluating PCT kinetics and an aetiology-based approach may prove more appropriate, which will be further discussed in the next paragraphs.

7.2 Appropriate antimicrobial therapy

Early identification and adequate antibiotic treatment of septic patients is of pivotal importance as any delay in starting effective antibiotic therapy is associated with increased in-hospital mortality [5, 78] although such therapy is a common feature in the ICU, reportedly as high as 25-30% [6, 59]. Our results are in accord with these findings as despite using international guidelines based local protocols, and regular consultation with clinical microbiologists and infectious disease specialist for years, 23% of patients with proven infection received inappropriate antibiotics initially. The main reason for this high rate of inadequacy reported worldwide may be due to the fact that reliable clinical and biochemical signs of bacterial infections are lacking, and the microbiological proof becomes available well after the antibiotic therapy was commenced. This is also supported by the fact, that apart from the respiratory origin of infection, we could not identify significant differences between the A-, and IA-groups neither in etiology, origin of infection (community or nosocomial) nor in demographics. Once inappropriate antimicrobials are initiated, it often takes days (until organism isolation and sensitivities are produced) to correct them. In our study 23% of patients received inappropriate antimicrobials. This high incidence may be due to the lack of fast and reliable diagnostic tests for bacterial infections, and to the subsequent

delay in microbiological results. Earlier recognition of potential inappropriate microbial therapy may allow an opportunity to substantially improve outcome.

To our knowledge, this is the first study to show that the early kinetics of PCT measured within the first 24 hours may help clinicians to evaluate the appropriateness of empirical antimicrobial therapy in critically ill patients. The rationale for measuring successive PCT levels within this timeframe came from the assumption that by giving appropriate antibiotics this may slow the inflammatory response within hours, and that this could be detected by serial measurements of PCT.

In this study, although PCT continued to increase after the initiation of empirical antimicrobial treatment during the first 16 hours in both the A-, and IA-groups, in the A-group there was a significant PCT decrease during the next eight hours (t_{24}), while in the IA-group PCT continued to increase and reached a significantly higher level by t_{24}, and remained higher the next day compared to the A-group. A PCT increase of ≥69.2% during the first 16 hours or a PCT increase ≥73.5% during the first 24 hours were the best cut off values to indicate inappropriate antimicrobial treatment. It is known that PCT increases within hours after an infectious insult and levels halve daily once the infection is controlled by the host immune system and/or by appropriate antibiotic therapy [79]. This feature explains the significant difference found in the PCT kinetics between the A-, and IA-groups during the first 24 hours.

It is also important to note that despite the inappropriate treatment after day one PCT decreased in both groups, although PCT levels remained significantly higher in the IA-group. This is in line with the results from Charles et al. [59], who measured PCT during the first 4 days of treatment in 180 patients with documented sepsis on a medical ICU, and PCT time course was analysed according to the appropriateness of the first-line empirical antibiotic therapy. They found that PCT decreased from day 2 to day 3 in both, the appropriate and inappropriate groups, but the decrease was significantly greater in the appropriate group. This can be explained by the finding that adequate supportive therapy on its own may attenuate the inflammatory response [80]. Furthermore, monocytes become exhausted after a certain period of time, also affecting PCT production [81, 82].

The clinical importance of these findings is emphasised by the significant difference in hospital mortality in our study: 35% in the A-group and 66% in the IA-group. In the study by Charles et al., a PCT decrease from day 2 to day 3 less than 30% was associated with death, and a delta PCT day 2 – day 3 was also found to be an independent predictor of mortality [59]. This gives further support to previous findings that early adequate antibiotic therapy has a pivotal role on survival [5, 78].

It is also true by the study by Charles et al. [59], they observed a signal of lower PCT levels on day 2 in the appropriate group, but the difference wasn't significant. This may be due to the fact that in their study PCT was measured retrospectively, therefore the time elapsed between the PCT measurements and the antibiotic therapy was uncontrolled, unlike this study where investigating PCT kinetics as precisely as possible during the first 24 hours was a particular aim.

It may be of significance that the grouping was based on the initial antibiotic therapy, hence no patients were "crossed over" from the inappropriate to the appropriate group. However, from the 33 patients in the IA-group, antibiotics were changed on day 2 or day 3 in 18 cases. We analyzed and compared PCT kinetics in patients in whom we changed (n=18) as compared to patients in whom we did not change (n=15) antibiotics over the study period but found no significant differences (data not shown).

Translating the results of the current study into clinical practice means that measuring PCT on commencement of antimicrobials (t_0) and then at 16 and 24 hours, by evaluating the percentage change rather than the absolute values the clinicians may have some additional early help when there is nothing else to follow but the clinical picture. In those cases where there is a "large" increase within the first 16-24 hours (≥69.2-73.5%), may indicate inappropriate antimicrobial therapy, while a lower grade increase or a decreasing tendency after 16 hours would support appropriate antimicrobial therapy. Within the first 16 hours, followed by further increase after that indicates that antibiotic therapy may need adjusting, such as reevaluating dosage, escalating or changing treatment; in the case of a lower grade increase till 16 hours followed by a decreasing tendency may mean adequate empirical antibiotic therapy. However, it is important to note, that one should never treat one single parameter, and PCT also has to be dealt with in the context of the full clinical picture and other biochemical and microbiological results. The clinical importance of our findings is emphasized by the significant difference in hospital mortality between the A-group and the IA-group (37% versus 61%).

Another important finding of the current study is that CRP did not differentiate between the appropriate and inappropriate groups within the first 48 hours while changes in the PCT levels gave a significant signal within the first 24 hours regarding the adequacy of empirical antibiotic therapy. This is in accordance with previously published data indicating that CRP is a "slow" marker and not as reliable as PCT in the critically ill [83, 84]. The same holds true for body temperature, which as with other studies, highlights that its use for guiding antimicrobial therapy is questionable [35].

7.3 PCT kinetics in surgical as compared to medical patients

Our results also support that PCT is several times higher in surgical compared to medical patients in septic shock despite the similar clinical manifestation and severity of the clinical picture [53], but we also found that early kinetics were similar to that found in the whole sample. Our data also suggest that percentage changes of PCT may be a better, universally applicable approach for monitoring treatment progress rather than absolute values.

A recently published randomized, interventional study on antibiotic-escalation based on daily PCT measurements by Jensen et al., found that PCT-guided antimicrobial escalation did not improve survival (31.5 % *vs*. 32 %) and led to organ-related harm, such as increased rate of mechanical ventilation, worsening glomerular filtration rate and prolonged length of stay (by 1 day) in the intensive care unit [51]. In a subsequent single-centre, prospective study the authors tested the usefulness of PCT for the reduction of antibiotic consumption in a multidisciplinary (surgical and medical) intensive care unit. They concluded that PCT measurements for the initiation of antimicrobials did not appear to be helpful in a strategy aiming at decreasing the antibiotic consumption in intensive care unit patients [10]. The authors of the PASS study and also in the study by Layios et al., used the same PCT algorithm, as first reported by Müller and co-workers [50]. However, in both studies the threshold for intervention was a PCT of >1 ng/ml. As 40% of the patients in both trials were surgical, in whom this threshold for intervention may be too low, one cannot exclude that these patients may have had received antibiotics unnecessarily. This overuse of antibiotics could be one of the reasons of the worse outcome in the PCT-guided group in both studies.

There is strong evidence that, due to direct cellular damage as in severe trauma, major surgery, and after ischemia-reperfusion, also known as damage associated molecular patterns (DAMP), hence unspecific elevations of PCT levels can typically be seen in the absence of a bacterial infection [84, 85] that there is an inflammatory mediator release very similar to that following an infectious insult. In the case of infection, a similar inflammatory response, often referred to as pathogen associated molecular pattern (PAMP), will also be triggered [86]. Therefore, unspecific elevations in PCT levels can typically be seen in the absence of a bacterial infection [84, 85]. Theoretically, in surgical patients with sepsis DAMP and PAMP takes place at the same time leading to a pronounced inflammatory response, whilst in medical patients PAMP may occur on its own, resulting in a less extensive inflammatory response [60].This theory has nicely been confirmed by Clec'h et al., that the same severity of SIRS, sepsis and septic shock is accompanied by several times higher PCT levels in the surgical as compared

to the medical patients. Therefore, using a cut-off value of PCT of 1 ng/ml in a surgical population will inevitably give false positive results and lead to unnecessary overuse of antibiotics. This is what may have happened in the above mentioned two negative studies, which explains their results at least in part. This feature is the reason why the same absolute values of PCT may mean completely different information in a medical compared to a surgical patient, but as shown in our results, kinetics follow a uniform pattern.

Our results in the EProK study are in accord with the Clec'h et al., study and also add further important information. We found that although the absolute values of PCT showed substantial differences, with being higher in the surgical group as compared to the medical patients, but we also found similar kinetics in both patient populations. This indicates, that using the same threshold of PCT for surgical and medical patients should not be recommended, but following the kinetics, i.e.: the percentage changes of PCT over time may be a better monitoring tool for diagnosing new onset infection and monitoring treatment progress. It is also important to note, that PCT reached its peak value at t_8 in the medical group receiving appropriate antibiotics (A_m-group), whilst in the A_s-group the peak was reached 8 hours later, at t_{16}. This suggests, that in medical patients PCT may indicate efficacy of therapy several hours earlier as compared to surgical patients. However, due to the small sample size of these subgroups we cannot give the exact percentage changes of PCT within 8 (medical) and 16 (surgical) hours to predict appropriate antibiotic treatment. It is something what has to be investigated further in the future.

This phenomen could be seen in our spin off study as well what also explains why PCT levels were elevated in our surgical patient population without proven infections, with median values of around 3.5 (NI-group) and 3.8 (I-group) ng/ml at t-1. The corresponding PCT values in medical patients were substantially lower (0.26 and 0.89 ng/ml, respectively). Although levels were higher in the I-group at t-1, but this difference did not reach statistical significance, while there was a several fold increase in the I-group in both medical and surgical patients with no change in kinetics in the NI-groups.

Our studies provide further evidence that changes or kinetics of PCT may be superior to absolute values.

7.4 Limitations: important to note

The most important limitation for us during the analysis of the results was the lack of gold standard for diagnosing infection. Although we attempted to reduce the potential error in judgment, by allocating

patients into each group by two independent experts blinded for PCT-kinetics in a *post hoc* fashion taking all clinical and microbiology data into account, one cannot exclude that mistakes may have still appeared in the process. It remains uncertain, what was the reason of measuring PCT values on the previous day before starting empiric antibiotic therapy in more than 50% out of the 209 patients of the EProK study. Therefore, some selection bias cannot be excluded. Furthermore, the power analysis sample size could have been larger to detect a stronger signal, so a multicenter design would have been better especially so to draw firm conclusions regarding the medical, surgical subgroups, although the trend in our results is certainly promising. It may also be important to note the uneven proportion of patients in the appropriate and inappropriate groups (75% vs. 25%), which is a general limitation of every study in this field, which may also have affected our results. The median day of inclusion into the study from ICU admission was 1 day, indicating, that 50% of patients had PCT measurements on ward/Accident and Emergency unit, before admission. However, this may also reinforces the importance of measuring PCT values consecutively. Finally, the clinical impact of our findings will have to be tested in a prospective randomized trial in order to see whether tailoring empirical antimicrobial therapy to PCT-kinetics, has any effect on outcome.

7.5 Conclusion

In the ERroK study PCT kinetics within the first 24 hours after commencing empirical antimicrobial therapy showed a significant increase in patients in whom therapy proved to be inappropriate, while in the appropriate group, after a brief increase at 16 hours, there was a significant decrease by 24 hours (Figure 12). Applying this approach may be helpful in quickly tailoring antimicrobial therapy for the patient's specific needs. However, the clinical relevance of this "PCT kinetics-guided approach" should be confirmed in a prospective randomized fashion.

The main finding of the spin off observational study was that an increase in PCT levels from the day before (t_{-1}) to the day when infection was suspected (t_0) predicted infection, while in patients with no proven infection PCT remained unchanged. Based on the data presented spot PCT may not be adequate to differentiate infection from non-infectious inflammatory response. Furthermore, conventional indicators of infection such as white cell count, body temperature and CRP have limited use to predict infection. The clinical implication of these results is, that daily PCT measurements in patients with high risk of infection would give the opportunity to evaluate PCT kinetics, which may improve our diagnostic accuracy and rationalize antibiotic therapy on the ICU.

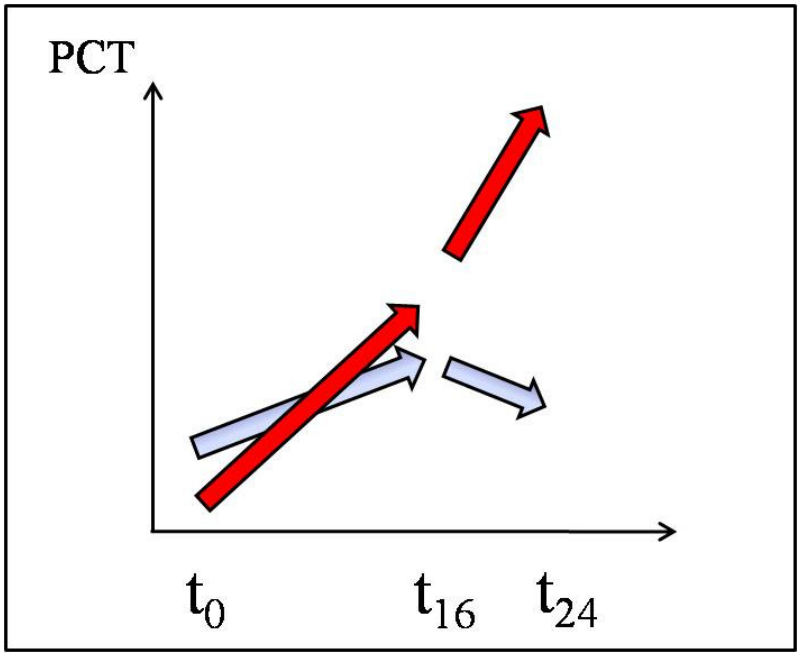


Figure 12 Procalcitonin kinetics in the first 24 hours in appropriate (blue arrows) and inappropriate (red arrows) empiric antibiotic therapy groups. The draft version of Figure 5 A.

8 PCT-ASSISTED ANTIBIOTIC THERAPY

There are three fundamental questions to be answered during our ward rounds when treating patients with suspected or proven infections on the ICU: 1) is there infection, in other words should we start empirical antibiotic therapy; 2) is the commenced antibiotic effective; and finally 3) when should we stop antibiotic treatment?

8.1 Diagnosing infection and starting antibiotics

The role of PCT in diagnosing infection has been discussed in details earlier. Several studies investigated the effects of PCT-guided antibiotic therapy on patient outcomes. In a landmark study by Christ-Crain et al., antibiotic exposure was reduced by 50% (44% versus 83%) in patients admitted to emergency wards with acute respiratory complaints, when antibiotic therapy was guided by admission PCT levels, as compared to conventional signs of infection only [8]. Two subsequent multicenter trials also found a decrease in antibiotic exposure in patients treated on the ICU with infections [9, 71]. The possible reasons why other studies [10, 12], could not find positive results were discussed earlier.

Patients treated on the ICU for a longer period of time may develop an imbalance between pro-, and anti-inflammatory forces in a way that the latter becomes prominent. These patients will become immunoparalyzed, making them prone to a series of recurrent infections. Detecting infection in these patients may prove even more difficult. In a recent study by Rau et al. [87], it was found that in patients with secondary peritonitis, PCT levels increased and indicated infection, but the peak values decreased significantly by each new insult. This was also supported by Charles et al. [59]. They found that during the first infectious insult the PCT mean was 55 ng/ml, but during the second infectious insult despite the same clinical gravity it was several fold less, 6.4 ng/ml. These data indicate that as we go along in time, patients become immunoparalized on the ICU and lower levels of PCT should be taken just as seriously as high levels at the early course of the disease. Furthermore, this provides further evidence, that PCT will respond to the same insult differently during the course of the disease, hence should be interpreted with special attention.

To put a bit of that into clinical practice, two very common scenarios will be discussed, which are summarized in two decision trees (Figure 13 and 14). Figure 13 shows how PCT can help in decision making when infection is suspected. If the patient is hemodynamically unstable and infection is likely, by definition he/she has septic shock or at least one cannot exclude it, hence antibiotic therapy

shouldn't be delayed but has to be commenced immediately, regardless of the PCT or any biomarker value [4]. However, if the patient is stable hemodynamically, and PCT is "low" or decreasing (based on the problems with absolute PCT values, discussed in detail above, we deliberately avoid giving exact numbers), then we can wait, observe the patient and reassess later (Figure 13) [8].

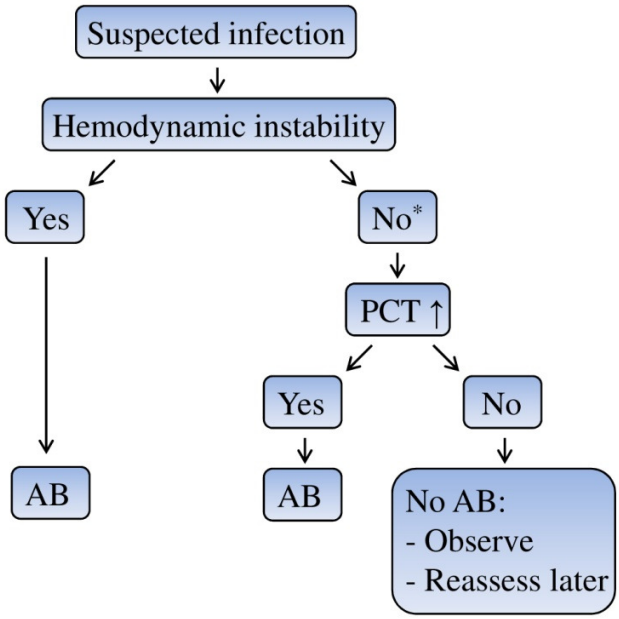


Figure 13 Guideline for staring empiric antibiotic therapy

The next scenario demonstrates the reevaluation of the situation when microbiological data becomes available, which usually takes place 2-3 days after specimen were sent off to the laboratory and antibiotics had been commenced (Figure 14). There are several issues to consider. Whether or not there was clinical improvement, combining it with both the microbiological and PCT results, this multimodal approach can assist us whether to continue, reconsider or change antibiotics and/or reassess organ support and most importantly, to stop antibiotics even on day 3 if they are considered unnecessary. (For more explanation see Figure 14.)

This multimodal evaluation could be called the MET-concept: Measure, Evaluating and Treat, which may help to individualize suspected infection management in the early course of sepsis on the ICU.

8.2 Evaluating antibiotic appropriateness

Once we decided to commence empirical antibiotic therapy, it is of utmost importance to confirm the appropriateness, type and dose, or change treatment if needed as soon as possible, because antibiotic therapy is a double edged sword. On the one hand we have some evidence that in septic shock every hour delay in starting adequate antibiotic therapy could have serious effect on survival [4]. On the other hand, unnecessary overuse of antibiotics can also cause harm, such as: increased bacterial resistance, the occurrence of multi-resistant strains, invasive fungal infections, side effects of the drug itself, and increased costs [68]. International guidelines based local protocols and antibiotic stewardship may help in choosing the right medication with the best possible chance. Unfortunately, no matter how hard we try, it seems that inappropriate empirical antibiotic therapy is a common feature on the ICU and in the hospital in general, and it can be as high as 25-30% [6, 59]. The gold standard for proving appropriateness of antibiotic therapy is the microbiological confirmation of the bacteria and its susceptibility. However, these results may come far too late, in reality days after the specimen had been sent, but treatment cannot be delayed. At present there is very little to help the clinicians at the early stage of patient care to confirm appropriate antibiotic treatment. In a recent pilot study we measured PCT before initiating antibiotics (t_0), and then 8 hourly (t_8, t_{16}, t_{24}) after commencing empirical antibiotic therapy, and found that there was a significant difference in the kinetics between patients receiving appropriate as compared to those getting inappropriate antibiotic therapy. Receiver Operating Characteristic analysis revealed that a PCT elevation ≥69% within the first 16 hours (i.e. from t_0-t_{16}) had an area under curve (AUC) for predicting inappropriate antibiotic treatment of 0.73 [95%CI: 0.63-0.83], $p<0.001$; from t_0-t_{24} a ≥73,5% increase had an AUC of 0.86 [0.77-0.94], $p<0.001$. These data suggest that early response of PCT within the first 24 hours of commencing empirical antibiotics in critically ill patients may help the clinician to evaluate the appropriateness of therapy, a concept which certainly will have to be tested in the future. Nevertheless, hospital mortality was 37% in the appropriate and 61% in the inappropriate group ($p=0.017$), which provides further evidence that choosing inappropriate antibiotic therapy seriously affects survival.

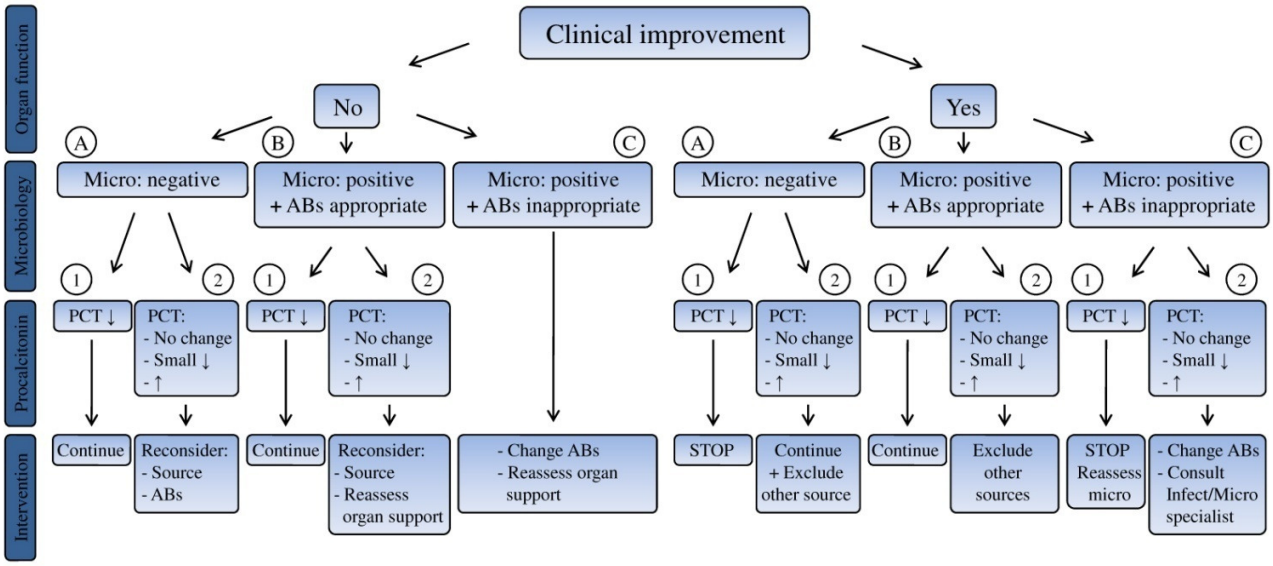


Figure 14 Multimodal reassessment of antibiotic therapy on day 2-3
ABs - antibiotics; Micro - microbiology; PCT - procalcitonin (↑ - increasing; ↓ - decreasing levels); Cont - continue; Infect -infectologist.
Once empirical antibiotics were commenced, a multimodal reassessment of the situation, once microbiological data is available usually on day 2 or 3, is strongly recommended.
No clinical improvement: Despite no clinical improvement a decrease in PCT (A-1) still may indicate that the infection is under control, but the patient needs more time to gain benefit from treatment. Therefore, ABs should be continued. On the contrary (A-2), if PCT is not decreasing or even increasing, these can be important signs that infection is not under control, hence source of infection and antibiotics (type, dose) should be reassessed. If antibiotics are appropriate, depending on PCT changes ABs should either be continued (B-1) or other sources of infection should be looked for (B-2). In case of inappropriate ABs and no clinical improvement, regardless of the PCT, therapy should be changed (C).
Clinical improvement: If there is no proof of infection (micro: negative), based on PCT changes (↓ or ↑) infection may be excluded and ABs stopped (A-1), or continued (A-2). Similar algorithms can be applied if ABs are appropriate (B-1,2). If ABs are inappropriate and PCT decreases, then one may consider the microbiology as false positive and stop ABs (C-1), because it is highly unlikely that there is clinical improvement and decreasing PCT if an infection is not under control due to inappropriate ABs. This scenario happens when there are pathogens (colonization for example), but no infection. Finally, in case of inappropriate ABs and unfavourable PCT changes (C-2), consultation with infectologists and microbiologists is recommended.

8.3 Stopping antibiotic therapy

Procalcitonin, mainly due to its favourable kinetic profile can potentially be a useful biomarker for also the cessation of antibiotic treatment [70]. In the PRORATA study [9], PCT-guided antibiotic management was tested in an ICU population. Similarly to the Christ-Crain study [8], antibiotics were encouraged in case of elevated PCT levels, and discouraged when levels were low. The novelty of this trial was, that investigators were encouraged to discontinue antibiotics when PCT concentration was less than 80% of the peak value or when absolute concentration of less than 0.5 ng/ml was reached. This approach shortened antibiotic exposure by 23%, and by almost 3 days in the PCT-group as compared to conventionally treated patients. Putting these results into clinical practice is also included in Figure 15.

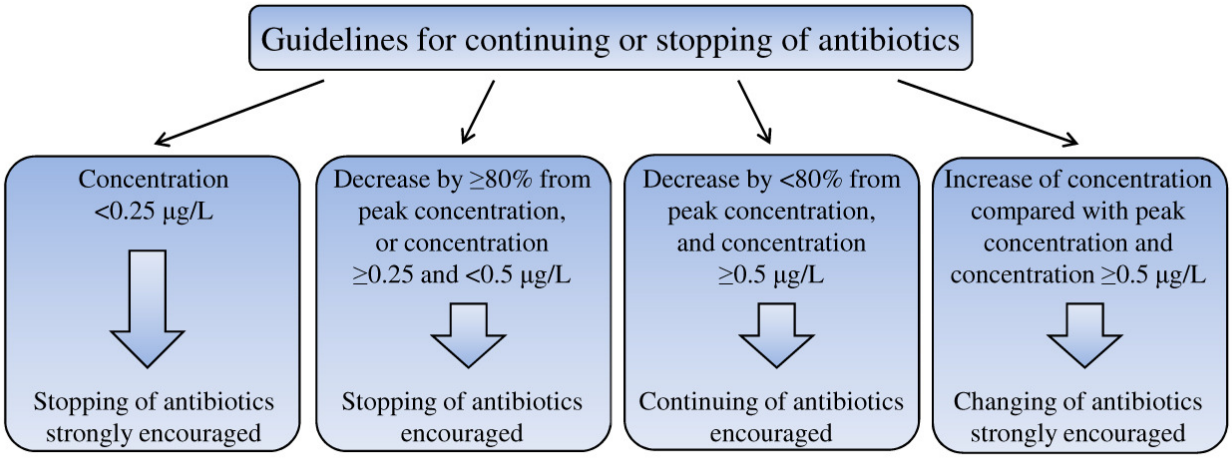


Figure 15 Guideline for stopping antibiotic by the PRORATA study

However, it is very important to acknowledge that patients in the PCT-group also received antibiotics for a shorter period of time as recommended by guidelines and their local protocols. Despite the significantly shorter antibiotic therapy, they were unable to show any difference in outcome between the groups, in other words patients did not suffer harm from not receiving antibiotics for the length of time recommended by guidelines. In two other studies on high risk surgical patients with suspected infection, PCT-guided therapy during the postoperative period in the ICU also resulted in significant reduction of antibiotic therapy and length of intensive care stay [72, 73].

In a recent large multicenter study by Shehabi et al., the authors could not find significant differences between the PCT-guided as compared to the conventional groups, although patients received a median of 2 days less (9 vs 11 day) antibiotics, which in fact was the main outcome measure [12]. However, as it was clearly stated by the authors, the study was powered for a very ambitious 25% reduction in the length of antibiotic treatment based on an estimated 9 days long antibiotic therapy, which in fact translated after the final analysis into an almost 4 days reduction in the study arm, with 11 antibiotic treatment days being the baseline in the control arm.

8.4 Final conclusion

In this deadly battle of fighting the burden of serious infections on the ICU, we often keep missing the point. Although sepsis exists, just like critical illness, but precisely defining it is probably impossible due its diversity in etiology, pathomechanism and clinical manifestation. Therefore, interpreting the results of sepsis studies is a daunting task. Procalcitonin is definitely one of the most reliable inflammatory markers in the critically ill to date, and there is also convincing evidence that its use to guide antibiotic therapy can rationalize starting, escalating and stopping antibiotic therapy. Furthermore, when the concept applied highlighted in this thesis, PCT may also become cost effective, by not starting at all, or stopping antibiotic therapy early. However, starting or stopping antibiotic treatment is more complex than just treating one single figure or even the kinetics of PCT values. A multimodal, individualized concept, consisting of a) recognizing organ dysfunction, b) identifying the possible source, c) following the clinical picture and d) taking PCT and PCT-kinetics into account, is necessary to make the most out of your PCT and to do the best of your patients in your everyday practice. And yes, it requires a well-trained, thinking physician, who dials in all information, “seasons” it with his/her experience and then makes a decision. And even if this decision turns out to be a wrong one retrospectively, it doesn’t matter. Because, these “wrong decisions” are what we call later as: “experience”.

KEY MESSAGE OF THE EPROK STUDY

- Early PCT kinetics within the first 24 hours after commencing empirical antimicrobials are different in patients receiving appropriate as compared to patients on inappropriate antimicrobial therapy.
- In case of appropriate therapy PCT peaks around 16 hours after commencing empirical antimicrobials and by 24 hours it already shows a decline.
- In patients on inappropriate antimicrobials PCT levels show a continuous increase within the first 24 hours during empirical antimicrobial therapy.
- Although absolute values showed several fold difference in medical as compared to surgical patients with suspected infection, but kinetics in those receiving appropriate as compared to those who received inappropriate antimicrobials were similar to that of reported in the whole sample.

KEY MESSAGE OF THE SPIN OFF STUDY

- Kinetics of procalcitonin values based on daily measurement are superior to absolute values only in diagnosing infection on the ICU.
- Absolute values of procalcitonin may be of limited use in diagnosing infection on the ICU.
- Both absolute values and kinetics of C-reactive protein and body temperature are poor indicators of infection on the ICU.

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