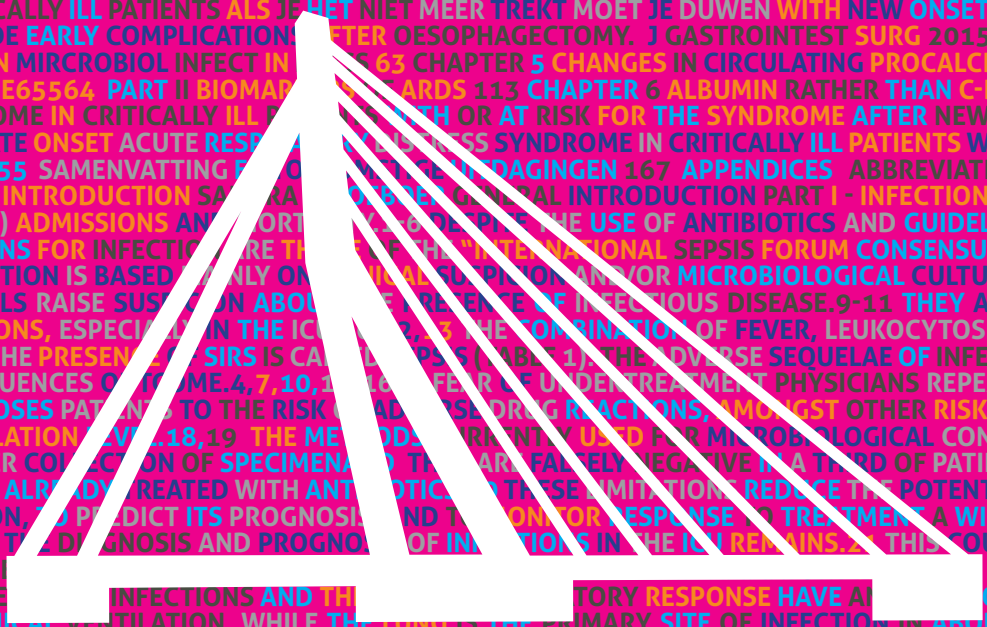

Sandra Helena Hoeboer

# Biomarkers of infection and its complications in the critically ill

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# BIOMARKERS OF INFECTION AND ITS COMPLICATIONS IN THE CRITICALLY ILL

**Biomarkers van infectie en infectieuze complicaties in intensive care patiënten**

Thesis

to obtain the degree of Doctor from the Erasmus University Rotterdam and VU University Amsterdam by command of the rector magnifici Prof.dr. H.A.P. Pols and Prof.dr. F.A. van der Duyn Schouten

and in accordance with the decision of the Doctorate Board
The public defence shall be held on Tuesday 9 June 2015 at 15:30 hours

by

Sandra Helena Hoeboer

ERASMUS UNIVERSITEIT ROTTERDAM

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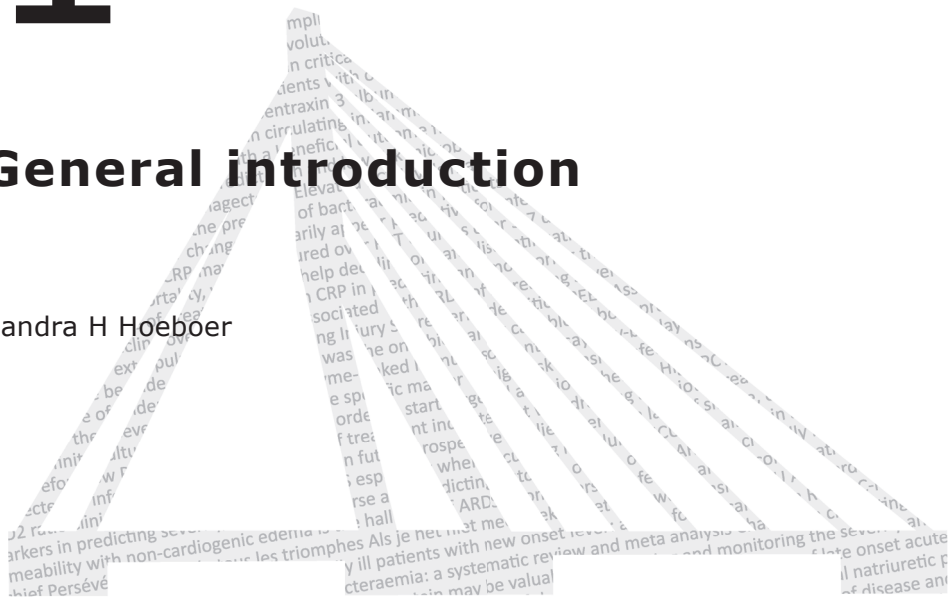
# 1

# General introduction

Sandra H Hoeboer



# GENERAL INTRODUCTION

## Part I - Infections in the critically ill

Microbial infections, and associated complications, are still an important cause of intensive care (ICU) admissions and mortality.[1-6] Despite the use of antibiotics and guidelines for supportive care mortality rates are up to 50% depending on disease severity.[1-7] The most widely accepted definitions for infection are those of the "International Sepsis Forum Consensus Conference on Definitions of Infection in the Intensive Care Unit" (ISFCC).[8] According to ISFCC criteria the likelihood of infection is based mainly on clinical suspicion and/or microbiological cultures.[8]

In clinical practice new onset fever, leukocytosis, tachypnea, tachycardia and elevated C-reactive protein (CRP) levels raise suspicion about the presence of infectious disease.[9-11] They are, however, markers of host inflammation and their value for the definite diagnosis of infection has considerable limitations, especially in the ICU.[10, 12, 13] The combination of fever, leukocytosis, tachypnea and tachycardia is considered the systemic inflammatory response syndrome to infection (SIRS). An infection in the presence of SIRS is called sepsis (Table 1). The adverse sequelae of infection: sepsis, septic shock, and organ failure, are partly caused by this host inflammatory response and each negatively influences outcome.[4, 7, 10, 14-16] In fear of undertreatment physicians repeatedly order cultures and start broad spectrum, empiric antibiotic treatment.[17] However, overtreatment unnecessarily exposes patients to the risk of adverse drug reactions, amongst other risks. Prolonged antibiotic therapy also results in bacterial selection in individual patients and microbial resistance on a population level.[18, 19]

The methods currently used for microbiological confirmation of infection have considerable limitations. The reporting of microbiological results takes at least 1 or 2 days after collection of specimenand they are falsely negative in a third of patients suspected of infection.[6, 9, 16] Cultures can also be falsely positive due to contaminants and may be insensitive in patients already treated with antibiotics.[20] These limitations reduce the potential of microbiological cultures to monitor the response to antibiotic treatment.

To support the early diagnosis of infection, to predict its prognosis, and to monitor response to treatment a wide variety of inflammatory biomarkers have been studied.[21] Nevertheless, controversy regarding the use of biomarkers for the diagnosis and prognosis of infections in the ICU remains.[21] This could be the result of heterogeneous study populations and endpoints. Another explanation is that these biomarkers have been used to diagnose sepsis, the unspecific host inflammatory response to infection, and less often to diagnose microbiologically proven infection.

**Table 1.** Definitions and criteria for the diagnosis of SIRS, sepsis and septic shock. [7]

| |
|---|
| **Systemic inflammatory response syndrome (SIRS)** |
| The clinical syndrome that results from a deregulated inflammatory response or to a non-infectious insult. The presence of at least 2 criteria are required for the diagnosis: |
| • Hyperthermia >38.3°C or Hypothermia <36°C |
| • Tachycardia >90 bpm |
| • Tachypnea >20 bpm |
| • Leukocytosis (>12 *$10^9$/L) or Leukopoenia (<4 *$10^9$/L) or >10% bands. |
| **Sepsis** |
| SIRS secondary to clinically diagnosed infection. Positive cultures add to the validity but are not required for the diagnosis. |
| **Severe Sepsis** |
| Sepsis and at least one sign of hypoperfusion or organ dysfunction not explained by another known aetiology of organ dysfunction: |
| • Hypotension (SBP <90 mmHg or MAP <65 mmHg) |
| • Lactate >2 mmol/L |
| • Areas of mottled skin or capillary refill >3 seconds |
| • Creatinine >2.0 mg/dl |
| • Disseminated intravascular coagulation (DIC) |
| • Platelet count <100 *$10^9$/L |
| • Acute renal failure or urine output <0.5 ml/kg/hr for >2 hours |
| • Hepatic dysfunction as evidenced by Bilirubin >2 or INR >1.5 |
| • Cardiac dysfunction |
| • Acute lung injury or ARDS |
| **Septic Shock** |
| Severe sepsis associated with hypotension (systolic blood pressure <90 mmHg or mean arterial pressure <60 mmHg) despite adequate fluid resuscitation and/or a serum lactate level >=4.0 mmol/L. |

## Part II - The Acute Respiratory Distress Syndrome

Severe infections and the host inflammatory response have an effect on individual organ systems as well. Around 75% of septic patients in the ICU develop respiratory failure requiring mechanical ventilation, while the lung is the primary site of infection in about 40-60% of cases.[1-4, 6, 14, 16] About half of the patients with sepsis fulfill the acute respiratory distress syndrome (ARDS) criteria.[1, 4, 14, 16] Mortality rates in ARDS patients vary between 20-50%, depending on disease severity.[22, 23] ARDS is caused by an insult to the alveolocapillary membrane that results in alveolocapillary inflammation and permeability that leads to formation of pulmonary oedema.[22, 24, 25] There can be a direct insult to the alveolocapillary membrane such as pneumonia or an indirect insult due to the host inflammatory response. Infections are the main cause of ARDS.[22, 24-26] The main symptom of ARDS is hypoxemia resulting from the generalised pulmonary oedema and reduced lung compliance.[22, 27, 28] Besides the laborious, invasive, direct measurement of

alveolocapillary permeability there is no true reference standard for diagnosis and monitoring ARDS at the bedside.[27] To diagnose ARDS various clinical scoring systems have been developed.[23, 26, 29] The recently developed Berlin definition (Table 2) is currently the preferred diagnostic standard in research[23], but controversy regarding its diagnostic value remains.[23, 30-33] A limitation of the Berlin definition is its dependency on ventilator settings. The level of positive end-expiratory pressure (PEEP) affects the oxygenation ratio and chest radiograph in mechanically ventilated patients. Moreover, the Berlin definition lacks a specific index of severity such as lung compliance. In contrast, the more extensive lung injury score (LIS, Table 2) gradually includes PEEP and lung compliance.[29] Finally, chest radiographs, an important feature of both systems, are subject to considerable

**Table 2.** Clinical classification systems of ARDS.

| **Berlin definition of ARDS.[23]** | |
|---|---|
| **Preconditions** | |
| Timing | Onset within 1 week of a known clinical insult or worsening of respiratory symptoms. |
| Imaging | Bilateral opacities on chest radiograph or computed tomography not fully explained by effusions, lobar/lung collapse, or nodules. |
| Origin of oedema | Respiratory failure not fully explained by cardiac failure of fluid overload (Need objective assessment to exclude hydrostatic oedema if no risk factor present (e.g. echocardiography). |
| **Oxygenation** | Berlin 1: Mild ARDS: 200 < $P_aO_2/F_iO_2$ mmHg ≤300 with PEEP or CPAP ≥5 $cmH_2O$ |
| | Berlin 2: Moderate ARDS: 100 < $P_aO_2/F_iO_2$ mm Hg ≤200 with PEEP ≥5 $cmH_2O$ |
| | Berlin 3: Severe ARDS: $P_aO_2/F_iO_2$ ≤100 mmHg with PEEP ≥5 $cmH_2O$ |

| **Lung injury score. [29]** | |
|---|---|
| **Anterior-posterior chest radiograph score** | **Hypoxemia severity score** |
| 0= no alveolar consolidations | 0= $P_aO_2/F_iO_2$ = >300 mmHg |
| 1= alveolar consolidations in 1 quadrant | 1= $P_aO_2/F_iO_2$ = 225-299 mmHg |
| 2= alveolar consolidations in 2 quadrants | 2= $P_aO_2/F_iO_2$ = 175-224 mmHg |
| 3= alveolar consolidations in 3 quadrants | 3= $P_aO_2/F_iO_2$ = 100-174 mmHg |
| 4= alveolar consolidations in all quadrants | 4= $P_aO_2/F_iO_2$ = <100 mmHg |
| **PEEP score (when ventilated)** | **Pulmonary compliance score** |
| 0= PEEP ≤5 $cmH_2O$ | 0= Compliance ≥80 mL/$cmH_2O$ |
| 1= PEEP 6-8 $cmH_2O$ | 1= Compliance 60-79 mL/$cmH_2O$ |
| 2= PEEP 9-11 $cmH_2O$ | 2= Compliance 40-59 mL/$cmH_2O$ |
| 3= PEEP 12-14 $cmH_2O$ | 3= Compliance 20-39 mL/$cmH_2O$ |
| 4= PEEP >15 $cmH_2O$ | 4= Compliance ≤19 mL/$cmH_2O$ |
| The final lung injury score is obtained by calculating the average of all four categories. | |
| No lung injury ≤1 mild ARDS 1-2.5 severe ARDS >2.5 | |

Abbreviations: ARDS- acute respiratory distress syndrome, $P_aO_2/F_iO_2$ - arterial $O_2$ pressure over inspiratory $O_2$ fraction, PEEP- positive end-expiratory pressure; pulmonary compliance=(tidal volume/(peak inspiratory pressure-PEEP).

interobserver variability.[34] The correlation between both clinical diagnostic systems and diffuse alveolar damage on autopsy is limited.[26, 35] Particularly when occurring late in the intensive care unit (ICU) clinicians may underdiagnose ARDS and may be poorly able to quantify its severity and course, since clinical classification systems are not commonly used in daily practice.[22, 26, 31, 35] Availability of biomarkers that are associated with the severity and course of ARDS in the critically ill could simplify diagnosis, monitoring and therefore management of the syndrome in daily clinical practice.[36, 37]

### Biomarkers

Ideally, a biomarker is an objective indicator of a physiologic or pathologic process that can be used for diagnosis, prognosis of disease and/or monitoring of response to treatment.[38] In recent years much effort has been invested into research on biomarkers of infection and organ failure. The biomarkers under evaluation in this thesis represent markers of inflammation, circulatory homeostasis and endothelial barrier function (Table 3). Whether these biomarkers are useful for the monitoring of infections and organ failure is not known or still under debate.

### Aim and outline of the thesis

**Part I -** We hypothesised that the increase in circulating inflammatory biomarkers during ICU-acquired infections depends on invasiveness and severity of disease. Therefore, the first goal is to find a single biomarker for discriminating between patients with and without microbial infection and to discriminate between those at low or high risk of developing infectious complications (i.e. bacteraemia, septic shock, death). The second is to determine its optimal cutoff value for biomarker-guided diagnostics and therapy in clinical practice and for future studies. We study the diagnostic accuracy and optimal cutoff of these biomarkers in 101 critically ill patients with new onset fever (**chapter 2**), 45 patients after elective esophagectomy (**chapter 3**), and perform a systematic review and meta-analysis of the literature on patients suspected of infection or sepsis (**chapter 4**). In addition, we hypothesised that the one-week course of biomarkers can be used to distinguish resolving microbial infection with a beneficial outcome from non-resolving or developing infections with a detrimental outcome associated with bacteraemia, septic shock, organ failure and death. In **chapter 5** we try to define values at which antibiotic treatment can be decided as appropriate and might allow safe discontinuation in 72 critically ill patients one-week after new onset fever.

**Part II** - We aim to determine the association of routine biochemical variables (**chapter 6**) and potentially more specific biomarkers (**chapter 7**) with the severity and one-week course of late onset ARDS in 101 at risk critically ill patients after new onset fever. We hypothesised that biomarkers directly associated with inflammation (CRP, ANG2; PCT, IL6) or vasculary leakage (ANG2, albumin) would be more accurate than those indirectly

associated with inflammation (PTX3) or vascular/circulatory homeostasis (proADM), independent of underlying ARDS risk factor.

**Table 3.** Biomarkers studied in this thesis.

| | |
|---|---|
| **C-reactive protein (CRP)** | CRP is released from the liver in response to stimulation by IL6. It can bind to molecules on dead or dying cells and certain bacteria. It promotes phagocytosis by macrophages. |
| **Procalcitonin (PCT)** | PCT is released from all parenchymal cells after direct stimulation of endotoxins or indirectly through inflammatory mediators. Its biological function is unclear. PCT may discriminate between infectious and non-infectious inflammation and possibly between infections of bacterial and viral origin. |
| **Interleukin 6 (IL6)** | IL6 is a cytokine with pro- and anti-inflammatory properties. Released early in the inflammatory cascade it is a mediator of fever, the acute phase response, and production of neutrophils. IL6 can be elevated in many non-infectious inflammatory states as well. |
| **Midregional pro-Adrenomedullin (proADM)** | ProADM is the precursor hormone of ADM. ADM release is stimulated by a variety of hormones, cytokines, and physical stress. ADM is a strong vasodilator that maintains blood flow to individual organs. On top of that, ADM regulates and modulates complement activity, is bactericidal and has metabolic properties. |
| **Midregional pro-Atrial Natriuretic Peptide (proANP)** | ProANP is the precursor hormone of ANP. ANP has well known natriuretic, kaliurietic, diuretic, vasodilative effects but also less known immune modulating properties. ANP is secreted as a resultant of atrial stretch mainly, but also by stimulation of pro-inflammatory cytokines. The reduced ejection fraction, increased ventricular diastolic volume and pressure observed in severe sepsis may explain the increase in ANP levels. |
| **Copeptin** | Copeptin is the precursor hormone of arginin vasopressin (AVP). AVP is important for maintaining circulatory homeostasis by regulating fluid balance and vascular tone in response to osmotic and hemodynamic stimuli. Increased levels are reported in the early phase of septic shock, while a relative AVP deficiency is seen in patients late during septic shock. |
| **Angiopoietin-2 (ANG2)** | ANG2 is released from the weibel palade bodies of endothelial cells after direct or indirect stimuli. Angiopoietin-2 dysregulates the endothelial barrier function in almost all organs promoting interstitial oedema and inflammation. |
| **Pentraxin-3 (PTX3)** | PTX3, is produced primarily in endothelial cells, macrophages and dendritic cells in response to stimulation by IL1 and Tumor Necrosis Factor-α, but not IL6. PTX3 has a role in inflammation and innate immunity. |
| **Albumin** | An important molecule to maintain plasma colloid oncotic pressure that can behave as a negative acute phase protein. In disease states with increased vascular permabliity the extravasation of albumin and the resultant low plasma colloid oncotic pressure promotes the formation of oedema. |
| **Lactate dehydrogenase (LDH)** | LDH is present in most cells but its physiologic levels are low. During cell injury large amounts of LDH can be released systemically. |

## REFERENCES


1. Angus DC, Linde-Zwirble WT, Lidicker J, et al. Epidemiology of severe sepsis in the United States: analysis of incidence, outcome, and associated costs of care. Crit Care Med 2001;29:1303-10.
2. Goncalves-Pereira J, Pereira JM, Ribeiro O, et al. Impact of infection on admission and of the process of care on mortality of patients admitted to the Intensive Care Unit - The INFAUCI study. Clin Microbiol Infect. 2014;20:1308-153.
3. Tromp M, Tjan DH, van Zanten AR, et al. The effects of implementation of the Surviving Sepsis Campaign in the Netherlands. Neth J Med 2011;69:292-8.
4. van Gestel A, Bakker J, Veraart CP, et al. Prevalence and incidence of severe sepsis in Dutch intensive care units. Crit Care 2004;8:R153-62.
5. van Zanten AR, Brinkman S, Arbous MS, et al. Guideline Bundles Adherence and Mortality in Severe Sepsis and Septic Shock. Crit Care Med. 2014;42:1890-8.
6. Vincent JL, Sakr Y, Sprung CL, et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006;34:344-53.
7. Dellinger RP, Levy M, Rhodes A, et al. Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock, 2012. Intensive Care Med 2013;39:165-228.
8. Calandra T, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. Crit Care Med 2005;33:1538-48.
9. Niven DJ, Leger C, Stelfox HT, et al. Fever in the critically ill: a review of epidemiology, immunology, and management. J Intensive Care Med 2012;27:290-7.
10. Sprung CL, Sakr Y, Vincent JL, et al. An evaluation of systemic inflammatory response syndrome signs in the Sepsis Occurrence In Acutely Ill Patients (SOAP) study. Intensive Care Med 2006;32:421-7.
11. Vincent JL, Donadello K, Schmit X. Biomarkers in the critically ill patient: C-reactive protein. Crit Care Clin 2011;27:241-51.
12. Marik PE. Definition of sepsis: not quite time to dump SIRS? Crit Care Med 2002;30:706-8.
13. Vincent JL. Dear SIRS, I'm sorry to say that I don't like you. Crit Care Med 1997;25:372-4.
14. Blanco J, Muriel-Bombin A, Sagredo V, et al. Incidence, organ dysfunction and mortality in severe sepsis: a Spanish multicentre study. Crit Care 2008;12:R158.
15. Gustot T. Multiple organ failure in sepsis: prognosis and role of systemic inflammatory response. Curr Opin Crit Care 2011;17:153-9.
16. Martin CM, Priestap F, Fisher H, et al. A prospective, observational registry of patients with severe sepsis: the Canadian Sepsis Treatment and Response Registry. Crit Care Med 2009;37:81-8.
17. Niven DJ, Stelfox HT, Shahpori R, et al. Fever in adult ICUs: an interrupted time series analysis*. Crit Care Med 2013;41:1863-9.
18. Cassell GH, Mekalanos J. Development of antimicrobial agents in the era of new and reemerging infectious diseases and increasing antibiotic resistance. JAMA 2001;285:601-5.

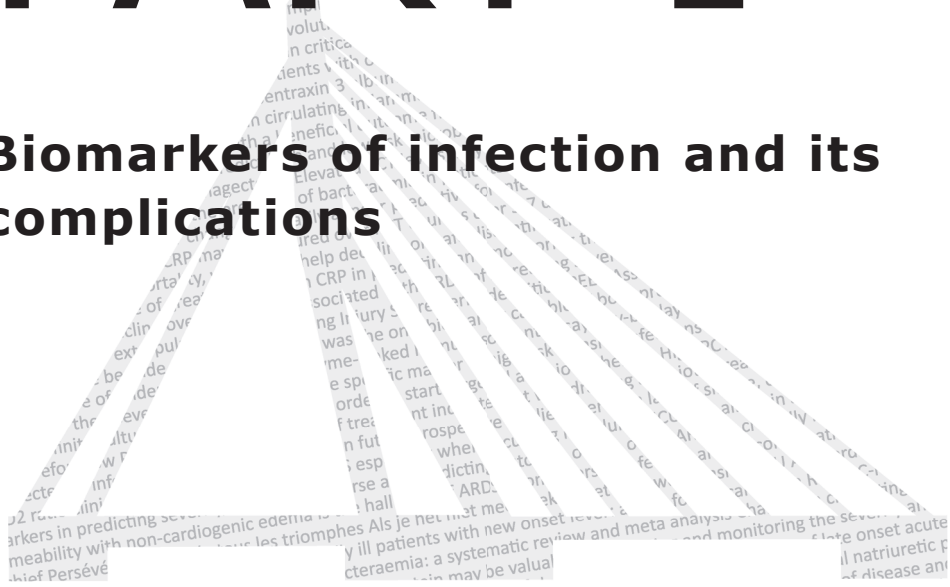
19. Rappuoli R. From Pasteur to genomics: progress and challenges in infectious diseases. Nature medicine 2004;10:1177-85.
20. Flayhart D, Borek AP, Wakefield T, et al. Comparison of BACTEC PLUS blood culture media to BacT/Alert FA blood culture media for detection of bacterial pathogens in samples containing therapeutic levels of antibiotics. J Clin Microbiol 2007;45:816-21.
21. Pierrakos C, Vincent JL. Sepsis biomarkers: a review. Crit Care 2010;14:R15.
22. Vincent JL, Sakr Y, Groeneveld J, et al. ARDS of early or late onset: does it make a difference? Chest 2010;137:81-7.
23. Force ADT, Ranieri VM, Rubenfeld GD, et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012;307:2526-33.
24. Wind J, Versteegt J, Twisk J, et al. Epidemiology of acute lung injury and acute respiratory distress syndrome in The Netherlands: a survey. Respir Med 2007;101:2091-8.
25. Tulapurkar ME, Almutairy EA, Shah NG, et al. Febrile-range hyperthermia modifies endothelial and neutrophilic functions to promote extravasation. Am J Respir Cell Mol Biol 2012;46:807-14.
26. Ferguson ND, Frutos-Vivar F, Esteban A, et al. Acute respiratory distress syndrome: underrecognition by clinicians and diagnostic accuracy of three clinical definitions. Crit Care Med 2005;33:2228-34.
27. Aman J, van der Heijden M, van Lingen A, et al. Plasma protein levels are markers of pulmonary vascular permeability and degree of lung injury in critically ill patients with or at risk for acute lung injury/acute respiratory distress syndrome. Crit Care Med 2011;39:89-97.
28. McCaffree DR, Gray BA, Pennock BE, et al. Role of pulmonary edema in the acute pulmonary response to sepsis. J Appl Physiol 1981;50:1198-205.
29. Murray JF, Matthay MA, Luce JM, et al. An expanded definition of the adult respiratory distress syndrome. The American review of respiratory disease 1988;138:720-3.
30. Villar J, Blanco J, Kacmarek RM. Acute respiratory distress syndrome definition: do we need a change? Curr Opin Crit Care 2011;17:13-7.
31. Costa EL, Amato MB. The new definition for acute lung injury and acute respiratory distress syndrome: is there room for improvement? Curr Opin Crit Care 2013;19:16-23.
32. Frohlich S, Murphy N, Boylan JF. ARDS: progress unlikely with non-biological definition. Br J Anaesth 2013;111:696-9.
33. Phillips CR. The Berlin definition: real change or the emperor's new clothes? Crit Care 2013;17:174.
34. Figueroa-Casas JB, Brunner N, Dwivedi AK, et al. Accuracy of the chest radiograph to identify bilateral pulmonary infiltrates consistent with the diagnosis of acute respiratory distress syndrome using computed tomography as reference standard. J Crit Care 2013;28:352-7.
35. Thille AW, Esteban A, Fernandez-Segoviano P, et al. Comparison of the Berlin definition for acute respiratory distress syndrome with autopsy. American journal of respiratory and Crit Care Med 2013;187:761-7.
36. Barnett N, Ware LB. Biomarkers in acute lung injury—marking forward progress. Crit Care Clin 2011;27:661-83.

37. Terpstra ML, Aman J, van Nieuw Amerongen GP, et al. Plasma biomarkers for acute respiratory distress syndrome: a systematic review and meta-analysis. Crit Care Med 2014;42:691-700.

38. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89-95.

# PART I

## Biomarkers of infection and its complications



# 2

# Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin

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Annelies N Tacx, Yvette J Debets-Ossenkopp, AB Johan Groeneveld

*J Infect 2012;64:484-93*

## ABSTRACT

O**bjective** Fever suggests the presence of microbial infection in critically ill patients. The aim was to compare the role of old and new biomarkers in predicting absence or presence of microbial infection, its invasiveness and severity in critically ill patients with new onset fever.

**Methods** We prospectively studied 101 patients in the intensive care unit with new onset fever (>38.3 °C). Routine infection parameters, lactate, procalcitonin (PCT), midregional pro-adrenomedullin (proADM), midregional pro-atrial natriuretic peptide (proANP) and copeptin (COP) were measured daily for three days after inclusion. Likelihood, invasiveness (by bloodstream infection, BSI) and severity of microbial infection were assessed by cultures, imaging techniques and clinical courses.

**Results** All patients had systemic inflammatory response syndrome; 45% had a probable or proven local infection and 12% a BSI, with 20 and 33% mortality in the ICU, respectively. Only peak PCT (cutoff 0.65 ng/mL at minimum) was of predictive value for all endpoints studied, i.e. BSI, septic shock and mortality (high risk infection) and infection without BSI, shock and mortality (low risk infection), at areas under the receiver operating characteristic curves varying between 0.67 ($P=0.003$) and 0.72 ($P<0.001$). In multivariable analysis, the combination of C-reactive protein and lactate best predicted high risk infection, followed by PCT. For low risk infection, PCT was the single best predictor.

**Conclusions** In critically ill patients with new onset fever, plasma PCT as a single variable, among old and new biomarkers, best helps, to some extent, to predict ICU-acquired, high risk microbial infection when peaking above 0.65 ng/mL and low risk infection when peaking below 0.65 ng/mL.

## INTRODUCTION

New onset fever in the critically ill raises the suspicion of microbial infection that may lead to sepsis and other harmful sequelae.[1,2] Fear of undertreatment contributes to ordering tests and prescribing antibiotics, before results of cultures become available, while overtreatment carries the risk of bacterial selection and overgrowth by induction of resistance.[3] The systemic inflammatory response syndrome (SIRS) criteria, including elevated white blood cell counts (WBC) may not accurately predict microbial infection and the common use of C-reactive protein (CRP) to predict infection, severity and outcome in critically ill patients is controversial.[4-11] Even minimally elevated lactate levels may predict a dismal outcome of infection during critical illness, relatively independent of sepsis and shock.[12-14]

The use of procalcitonin (PCT) for predicting microbial infection and its severity in the critically ill patient, rather than the general hospitalised patient,[15,16] is fraught with difficulty, because of varying sensitivity and specificity, even if potentially higher than of C-reactive protein (CRP).[1,5-7,9-11,17-21] This might relate to varying study populations and endpoints. PCT has been used to predict sepsis, i.e. the host response to either proven or suspected microbial infection and its severity, and rarely of microbiologically proven infection or bacteraemia[5,9-11,22-24] whereas the latter would be more helpful when considering potentially life-saving antibiotics in the critically ill.[25,26]

Studies on PCT in critically ill patients were either small, up to 50 patients,[1,5] confined to medical[5-7] or surgical patients, which may differ in infection and biomarker profiles,[9,11,18,21,27] and only rarely included both.[10,17,22,28]

They mostly included patients at admission to the intensive care unit (ICU)[4-7,18,21,22] and were mostly not designed, with exceptions,[1,10,21] to predict ICU-acquired microbial infection and associated risks. Studies evaluated PCT as an isolated biomarker[28,29] or compared it with a variety of others.[1,5-7,9-11,17,18,21,22,27] Single, but different,[1,5,9,11] or multiple endpoints as organ failures and mortality have been studied.[6,7,10,18,22,28] The heterogeneity among studies may explain, in part, why meta-analyses may contradict each other.[15,19,20] In any case, a low or decreasing PCT in the ICU has been used to help deciding on antibiotics, thereby reducing potentially harmful antibiotic exposure.[30,31] In the critically ill, however, an association of a low or decreasing PCT with a low risk has not been documented, the cutoff PCT values used have not been validated, and a small increase in mortality by PCT-guided antibiotics cannot be excluded.[30,31]

Novel biomarker prohormones for sepsis and its severity include midregional (MR) pro-adrenomedullin (proADM), MR pro-atrial natriuretic peptide (proANP) and copeptin (COP), precursors of adrenomedullin, atrial natriuretic peptide and vasopressin, respectively. They are secreted by vascular endothelium, heart and pituitary, respectively, and are involved in circulatory homeostasis, also during microbial infection.[33-35]

They could particularly predict the sequelae of severe microbial infection, i.e. the development of septic shock and associated mortality, but evaluation in the critically ill is scarce and inconclusive up till now.[33-36]

In the hypothesis that ICU-acquired infection differently increases circulating biomarkers, depending on invasiveness and severity of disease, we compared predictive values for microbial infection, bloodstream infection (BSI), septic shock or mortality, i.e. high risk infection, and in predicting infection without these complications (low risk infection), in patients with new onset fever in a mixed medical/surgical ICU. The goal was to find the ideal, single biomarker for prediction of high and low risk infection and to determine the associated cutoff value, for future studies on biomarker-guided antibiotics in the ICU.

## PATIENTS AND METHODS

In this prospective study, approved by the ethical committee of the VU University Medical Centre, Amsterdam, 101 consecutive patients presenting with new onset fever in the 24-bed mixed medical/surgical ICU were included. The department has about 1200 admissions annually. Limited by available research staff and office hours, we included 21 patients from August-December 2003, 33 patients from January-December 2004, 23 patients January-December 2005, 10 patients from January-December 2006 and 14 patients from January-August 2007. We did not perform a power analysis since we could not estimate the expected results and relatively arbitrarily stopped inclusion after reaching 100 patients. The Consort diagram details eligible and included patients (Fig. 1). All included patients or closest relatives gave informed consent prior to the study. Inclusion criteria were as follows. New onset fever was defined as a body

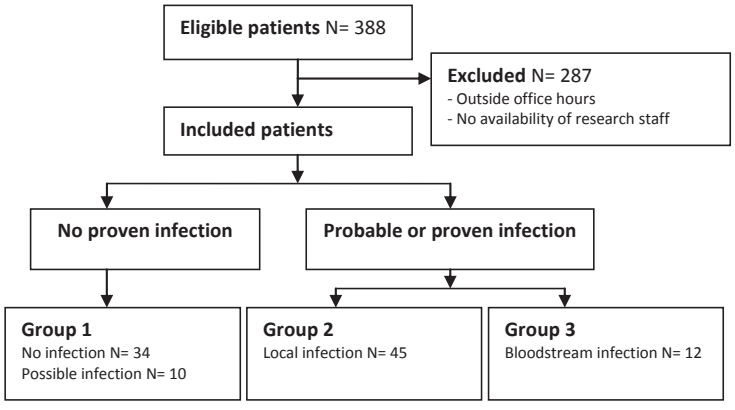


**Figure 1.** Consort diagram of patient inclusion.

temperature of 38.3 °C, measured rectally while in the ICU, preceded by a period of at least 24 h in the absence of fever (<37.5 °C). Exclusion criteria were absence of informed consent, pregnancy and a life expectancy of less than 24 h. Enrolment in the study followed within 12 h after inclusion criteria were met. At inclusion (Day 0), demographic and historical variables were recorded, such as age, gender, prior use of antibiotics, including selective decontamination of the digestive tract (SDD), steroids, immune status (active malignancy or other causes of an immunocompromised state) and reasons of admission. SDD was introduced for routine use on July 17 2006 and consisted of 4x daily administration of an oral paste and of a suspension via the nasogastric tube, containing the non-absorbable antibiotics tobramycin, amphotericin-B and colistin, in patients longer than 48 h on mechanical ventilation and 72 h in the ICU. Assessment of disease severity on ICU admission was done according to the Simplified Acute Physiology Score II (SAPS II). Parameters to calculate the Sequential Organ Failure Assessment Score (SOFA) were collected on the study days, at inclusion (Day 0) and on Days 1 and 2. From Day 0e2 clinical data were recorded, such as temperature, heart rate, respiratory parameters taken from the ventilator, white blood cell counts (WBC) using a Sysmex SE-9000 analyzer (Toa Medical Instruments, Kobe, Japan), C-reactive protein (CRP, Immunoturbidimetric assay, Modular analytics <P> Roche diagnostics, Mannheim, Germany) and lactate (Enzymatic method, Modular analytics <P> Roche diagnostics, Mannheim, Germany).

Blood samples were obtained on Days 0 (at time of inclusion) and daily at 7:00 h AM of each of the following Days 1 and 2. Samples were collected from an arterial catheter in standard Vacutainer tubes (Becton, Dickinson and Company, Erembodegem, Belgium) with ethylenediaminetetraacetic acid (EDTA), benzamidine and soybean trypsin inhibitor added. After tubes were centrifuged for 10 min at 1300 g, the plasma was aliquoted and stored at 80 C until further handling. Chest and sinus radiographs were obtained on Day 0, but other imaging was at the discretion of treating physicians. Blood was taken from routinely placed arterial catheters and collected in delayed vial entry bottles for aerobic and anaerobic cultures and processed with help of the BACTEC FX automatic analyzers (Becton, Dickinson and Company, Erembodegem, Belgium). Bottles were incubated for a maximum of five days. If the analyzers showed growth, Gram stains were prepared and identification and sensitivity cultures were processed. Other local specimens for microbial investigation were collected and sent to the microbiological laboratory, depending on the clinical infection, as judged by treating clinicians not involved in the study and unaware of PCT and novel biomarker results. Further investigations on infection e.g. fungal, viral or Chlamydia cultures, were left at the treating clinicians discretion. All collected specimens were handled using standardised procedures. All culture and staining results from specimens collected from Day 0-2 were evaluated. Only positive cultures that were not considered

to reflect colonization were used for analysis. For example blood cultures containing coagulase-negative staphylococci were considered contaminated if only one bottle revealed growth. The microbial agents isolated during microbiological evaluation were grouped into major species classes. Patients were followed until discharge from ICU or hospital or death.

**Definitions**

The SIRS criteria according to the ACCP/SCCM consensus conference criteria of: a body temperature >38 °C; a heart rate of >90 beats/min; a respiratory rate of >20 breaths/min or mechanical ventilation or white cell count (WBC) of <4.0 $10^9$/L or >12.0 $10^9$/L, were used. When SIRS and a probable/proven infection or BSI was present, patients were classified as having sepsis. On the basis of the collected data two investigators, blinded to the study results (SHH, ABJG), decided after completion of the study whether a possible, probable or proven infection was present from Day 0-2 after inclusion. In case of disagreement a third party was consulted. Source and likelihood of infection were based on criteria defined at the International Sepsis Forum Consensus Conference.[37] Patients were divided into groups of increasing likelihood of infection and invasiveness of associated micro-organisms, suggestive of increasing severity: Group 1 without infection or with possible infection but negative cultures, Group 2 with probable or proven local infection without BSI and Group 3 with BSI irrespective of local infection. Shock was defined by a systolic arterial pressure <90 mmHg or mean arterial pressure (MAP) <70 mmHg for at least 1 h despite adequate fluid resuscitation or requirement of vasopressor support to maintain MAP, from Day 0-7. In the presence of sepsis, shock was considered septic shock. All-cause mortality refers to Day 28 (within ICU or hospital) mortality after inclusion and ICU mortality (also beyond 28 days). While presence of either BSI, septic shock or mortality after inclusion in the disease course was considered indicative of high risk microbial infection, a low risk was defined by a probable or proven infection without BSI Day 0-2 and septic shock Day 0-7 and with survival up to 28 days. We evaluated a change of antibiotics during Day 0-7 and defined a change as starting or discontinuing one or more antibiotics of a different class. Tracheobronchitis was defined by fever with purulent sputum, acquired by tracheobronchial aspiration and yielding a positive culture of a potential pathogen, but no indication of infiltrates on chest imaging.

**Biomarker assays**

Biomarkers were measured using the Kryptor$^R$ compact system (Brahms Diagnostica, Henningsdorf, Germany) which uses Time Resolved Amplified Cryptate Emission (TRACE) technology. Assays were performed according to manufacturer's instructions. PCT was measured by use of the PCT sensitive, the lower detection limit being 0.02

ng/mL while the upper limit in healthy subjects is 0.05 ng/mL. The functional assay sensitivity (FAS) of the test is 0.06 ng/mL, with an intra-assay coefficient of variation (CV) and inter-assay CV of <6% in samples containing >0.3 ng/mL. Test specifics of proADM: lower detection limit 0.05 nmol/L, upper limit of normal 0.55 nmol/L. The FAS is 0.25 nmol/L, with an intra-assay CV of <4% and an inter-assay CV of <11% in samples containing 0.5e2.0 nmol/L. Test specifics of proANP: lower detection limit 2.1 pmol/L, upper limit in healthy controls 85.2 pmol/L. A FAS of 10 pmol/L and an intra-assay CV of <2.5% and inter-assay CV of <6.5% in samples containing 20 pmol/L. Test specifics for COP: lower detection limit 4.8 pmol/L, upper limit in healthy subjects m17.4 pmol/L. The FAS is <12 pmol/L, with an intra-assay CV of <12% and an inter-assay CV of <13% in samples containing >20 pmol/L.

**Statistical analysis**

This was performed using SPSS version 15 (SPSS inc., Chicago, Ill., USA). Data are expressed as median (range) or as number of patients (percentage) where appropriate, with median interquartile range in figures. All test were two-sided and a $P<0.05$ was considered statistically significant. Exact P's above 0.001 are given. Group differences were evaluated by use of the Kruskal-Wallis test or $X^2$ test, where appropriate. Non-Gaussian distributed data were logarithmically transformed, when appropriate, and generalised estimating equations (GEE) were done to evaluate group effects on variables, taking repeated measures in the same patients into account. Areas under the receiver operating characteristic curves (AUROC) were calculated to evaluate predictive values. We evaluated predictive values of Day 0 and peak levels (Day 0-2). Since reporting microbiological results takes at least 1e2 days after sending specimens for culture, it is hypothesised that the highest biomarker level reached within D0-2 would precede culture results and is therefore appropriate for predicting likelihood of infection. The peak value is the highest biomarker value measured on either D 0,1 or 2 for each individual patient. The optimum cutoff value was calculated on the basis of the highest sensitivity and specificity combined. Positive and negative predictive values and likelihood ratios were calculated. Backward multiple logistic regression was done, including all logarithmic transformed biomarker levels and selecting on the basis of the likelihood ratio, to find the smallest set of best predictors for high and low risk microbial infection. To this end, high risk infection was defined by either BSI, septic shock or 28-day mortality. The Hosmer Lemeshow test was done to evaluate goodness of fit.

## RESULTS

Patient characteristics are shown in Table 1, grouped according to likelihood and invasiveness of microbial infection. All patients had SIRS either at inclusion (99%) or on day 1 (1%), so that the 73 patients with probable/proven microbial infection (Groups 2 and 3) had sepsis. Females had higher risk for BSI than males, possibly explained by higher frequency of immunodepression in females than males (females 5 (83%) vs. males 1 (17%), P=0.005). Table 2 shows the sources of microbial infection and the organisms involved. The mortality rates did not differ between patients without or with BSI (22 (25%) vs. 4 (33%), P=0.52), nor did they differ between patients with or without septic shock (16 (24%) vs. 10 (29%), P=0.55), even though BSI predisposed to septic shock (Table 1).

### Biomarkers of infection and its invasiveness

Fig. 2 shows plasma levels in the course of time, according to likelihood and invasiveness of infection. Peak levels occurred at Day 0 in 61% of patients for PCT, 60% for COP, 55% for proADM, 48% for proANP, 46% for WBC, 40% for lactate and 35% for CRP, so that PCT was the first to peak. At Day 0, patients with BSI (Group 3) had higher CRP (in BSI 208 (52-421) vs. without BSI 113 (5-440) mg/L, P=0.043), higher lactate levels (1.6 (0.8-3.7) vs. 1.15 (0.4-2.2) mmol/L, P=0.018), and higher PCT values than patients without BSI (2.40 (0.84-73.2) vs. 0.60 (0.07-37.1) ng/mL, P=0.030). Peak levels are shown in Table 3. Peak CRP in BSI measured 231 (71-436) and without BSI 158 (5-454) mg/L (P=0.008). Lactate was higher in BSI with 1.9 (1.1e3.9) vs. 1.4 (0.5-13.1) mmol/L without BSI (P=0.006). Peak PCT was higher in BSI with 2.92 (0.09e75.29) than with 0.65 (0.08-37.14) ng/mL in the absence of BSI (P=0.021). Peak proADM was higher in BSI with 3.60 (0.82-18.57) vs. 1.60 (0.37-9.96) nmol/L in the absence of BSI (P=0.012).

### Septic shock

Day 0 CRP values were raised in patients with vs. without septic shock (174 (5-440) mg/L vs. 101 (5-279) mg/L, respectively, P=0.001). Day 0 PCT values were higher in septic shock (1.09 (0.07-73.2) ng/mL than without septic shock (0.35 (0.08-37.1) ng/mL (P<0.001). The Fig. 3 shows the course in time and Table 3 the peak values.

### Mortality

Non-survivors had higher proADM and proANP on Day 0 (2.93 (0.63-12.62) nmol/L and 396 (58-1684) pmol/L) than survivors (1.33 (0.05-9.47) nmol/L, P<0.001, and 202 (22-1613) pmol/L, P=0.001, respectively). PCT on Day 0 was also increased in non-survivors vs. survivors (0.87 (0.14-73.18) ng/mL vs. 0.56 (0.07-45.06) ng/mL, P=0.040). Fig. 4 shows the course in time and Table 3 the peak values.

**Table 1** Patient characteristics.

| | Group 1 N=44 | Group 2 N=45 | Group 3 N=12 | P |
|---|---|---|---|---|
| Age (year) | 63 (22-77) | 61 (19-81) | 67 (19-81) | 0.69 |
| Gender (male) | 32 (73) | 34 (76) | 3 (25) | 0.003 |
| SAPS II on admission | 47 (19-85) | 46 (23-78) | 54 (21-78) | 0.10 |
| SOFA at Day 0 | 8 (2-13) | 7 (2-14) | 10 (3-13) | 0.47 |
| Days from admission to Day 0 | 7 (1-78) | 7 (1-77) | 6 (1-45) | 0.91 |
| Temperature, °C D0 | 38.9 (38.4-40.0) | 38.9 (38.4-40.8) | 40.8 (39.3-40.3) | 0.06 |
| D1 | 38.4 (36.3-40.0) | 38.1 (35.9-40.4) | 38.5 (37.1-40.1) | 0.68 |
| D2 | 38.1 (36.4-39.9) | 38.2 (36.6-39.5) | 38.1 (36.3-39.4) | 0.01 |
| SIRS D0 | 44 (100) | 45 (100) | 11 (92) | 0.02 |
| D1 | 41 (93) | 43 (96) | 11 (92) | 0.83 |
| D2 | 36 (86) | 38 (56) | 11 (100) | 0.42 |
| Septic shock D0 | 0 | 20 (44) | 8 (67) | <0.001 |
| D1 | 0 | 20 (44) | 8 (67) | <0.001 |
| D2 | 0 | 19 (43) | 8 (67) | <0.001 |
| D7 | 0 | 25 (56) | 9 (75) | <0.001 |
| ICU length of stay, days | 29 (5-126) | 23 (4-95) | 35 (11-77) | 0.38 |
| 28-day mortality | 13 (30) | 9 (20) | 4 (33) | 0.48 |
| ICU mortality | 13 (30) | 9 (20) | 4 (33) | 0.48 |
| **Immunocompetence** | | | | |
| Active malignancies | 2 (5) | 4 (9) | 3 (25) | 0.09 |
| Immunocompromised state | 2 (5) | 2 (4) | 2 (17) | 0.25 |
| **Admission category** | | | | |
| General surgical | 24 (55) | 26 (58) | 4 (33) | 0.32 |
| Trauma | 6 (14) | 6 (13) | 1 (8) | 0.88 |
| Cardiac | 4 (9) | 2 (4) | 0 | 0.42 |
| Vascular | 6 (14) | 4 (9) | 0 | 0.36 |
| Respiratory insufficiency | 17 (39) | 20 (44) | 8 (67) | 0.22 |
| Sepsis | 9 (21) | 16 (36) | 6 (50) | 0.09 |
| Shock | 7 (16) | 8 (18) | 5 (42) | 0.13 |
| Post-CPR | 5 (11) | 3 (7) | 2 (17) | 0.54 |
| Neurological | 5 (11) | 8 (18) | 1 (8) | 0.57 |
| Other | 3 (7) | 5 (11) | 1 (8) | 0.78 |
| **Treatment up to 7 days prior to inclusion** | | | | |
| Antibiotics | 37 (84) | 40 (89) | 10 (83) | 0.77 |
| Steroids | 20 (46) | 19 (42) | 6 (50) | 0.34 |
| SDD | 21 (48) | 10 (22) | 5 (42) | 0.04 |

**Table 1** Patient characteristics. (continued)

| | Group 1 N=44 | Group 2 N=45 | Group 3 N=12 | P |
|---|---|---|---|---|
| **Treatment during study Day 0-7** | | | | |
| Therapeutic hypothermia | 4 (9) | 2 (4) | 2 (17) | 0.23 |
| Antibiotics | 41 (93) | 42 (93) | 12 (100) | 0.65 |
| Change in antibiotics | 24 (55) | 29 (64) | 9 (75) | 0.37 |
| Steroids | 19 (43) | 23 (51) | 8 (67) | 0.88 |
| SDD | 23 (52) | 9 (20) | 5 (42) | 0.006 |
| Mechanical ventilation | 43 (98) | 40 (89) | 12 (100) | 0.14 |
| duration, days | 24 (3-123) | 17 (3-82) | 29 (7-77) | 0.09 |
| Inotropic/vasopressor | 29 (67) | 24 (55) | 9 (82) | 0.18 |
| Renal replacement | 7 (16) | 0 | 1 (8) | 0.02 |
| Surgery | 9 (21) | 7 (16) | 1 (8) | 0.58 |

Median (range), or number (percentage), where appropriate; CPR= cardiopulmonary resuscitation. SAPS= simplified acute physiology score; SOFA= sequential organ failure assessment score; ICU= intensive care unit; SDD= selective decontamination of the digestive tract. Group 1= no or possible infection; Group 2= probable or proven infection; Group 3= bloodstream infection.

### Low risk infection

A change of antibiotics was as frequent in patients with a low risk (67%) as in patients with high risk infection (53%, P=0.62). Fig. 5 shows the course in time and Table 3 the peak values. Day 0 CRP was lower (87 (5-279) vs. 125 (5-440)) in low risk infection (P=0.02); the same applied for PCT (0.32 (0.10-37.1) vs. 0.69 (0.07-73.2), P=0.008) and proADM (1.26 (0.05-2.41) vs. 1.72 (0.05-12.62), P=0.008).

### Predictive values

Statistically significant predictions by peak values are depicted in Table 4. At a cutoff of 0.65 ng/mL, peak PCT carried sensitivities, specificities, positive and negative predictive values for BSI, septic shock and mortality of 67-77, 51-57, 14-44 and 78-92%, respectively. This indicates high negative predictive values for all four endpoints studied. Plasma PCT as a single variable best helps to predict ICU-acquired, high risk microbial infection when peaking above 0.65 ng/mL ands low risk infection when peaking below 0.65 ng/mL. At Day 0, the predictive value for BSI was highest for lactate and PCT (AUROC 0.72 and 0.69, P=0.03 or less, respectively), for septic shock highest for CRP and PCT (AUROC 0.72 and 0.68, P=0.002 or lower, respectively), and for 28-day mortality highest for proADM and PCT (AUROC 0.74 and 0.64, P=0.04 or lower, respectively). At Day 0, PCT was most predictive for low risk infection, followed by proADM (AUROC 0.77 and 0.70, P=0.001, respectively) and CRP (AUROC 0.69, P=0.003). Otherwise, SAPS II score at admission predicted at an AUROC of 0.63 (P=0.044) and SOFA score at Day 0 at 0.73 (P=0.001).

**Table 2** Infection, sources and associated micro-organisms.

| | Group 1 N=44 | Group 2 N=45 | Group 3 N=12 | P |
|---|---|---|---|---|
| **Local infection** | | | | |
| Tracheobronchitis | 1 (2) | 18 (40) | 2 (17) | <0.001 |
| VAP | 1 (2) | 7 (16) | 1 (8) | 0.09 |
| HAP | 0 | 2 (4) | 0 | 0.28 |
| AP | 2 (5) | 1 (2) | 0 | 0.66 |
| Pleurisy/empyema | 0 | 3 (7) | 0 | 0.15 |
| Sinusitis | 2 (5) | 6 (13) | 2 (17) | 0.27 |
| Catheter infection | 3 (7) | 4 (9) | 4 (33) | 0.03 |
| Endocarditis | 0 | 1(2) | 0 | 0.53 |
| Peritonitis | 0 | 6 (13) | 0 | 0.02 |
| Pancreatitis | 0 | 1 (2) | 2 (17) | 0.01 |
| Skin and soft tissue | 2 (5) | 5 (11) | 1 (8) | 0.52 |
| Meningitis | 0 | 1 (2) | 0 | 0.53 |
| Primary bacteraemia | - | - | 3 (25) | - |
| **Local microbiology** | | | | |
| Enterobacteriaceae | 2 (22) | 13 (31) | 4 (33) | 0.84 |
| Staphylococci | 1 (11) | 11 (26) | 5 (42) | 0.29 |
| Pseudomonadaceae | 0 | 10 (24) | 1 (8) | 0.15 |
| Enterococci | 2 (22) | 4 (10) | 1 (8) | 0.52 |
| Xanthomonadaceae | 1 (11) | 6 (14) | 0 | 0.38 |
| Yeasts | 0 | 8 (19) | 2 (17) | 0.36 |
| Herpesviridae | 1 (11) | 2 (5) | 0 | 0.50 |
| Miscellaneous | 3 (33) | 28 (66) | 2 (17) | 0.004 |
| **Blood microbiology** | | | | |
| Enterobacteriaceae | - | - | 3 (25) | na |
| Staphylococci | - | - | 4 (33) | na |
| Pseudomonadaceae | - | - | 1 (8) | na |
| Enterococci | - | - | 2 (17) | na |
| Yeasts | - | - | 2 (17) | na |
| Miscellaneous | - | - | 1 (8) | na |

Median (range) or number (percentage) where appropriate; Abbreviations: D= day; SIRS= systemic inflammatory response syndrome. na= not applicable; VAP= ventilator-associated pneumonia; HAP= hospital-acquired pneumonia; AP= aspiration pneumonia

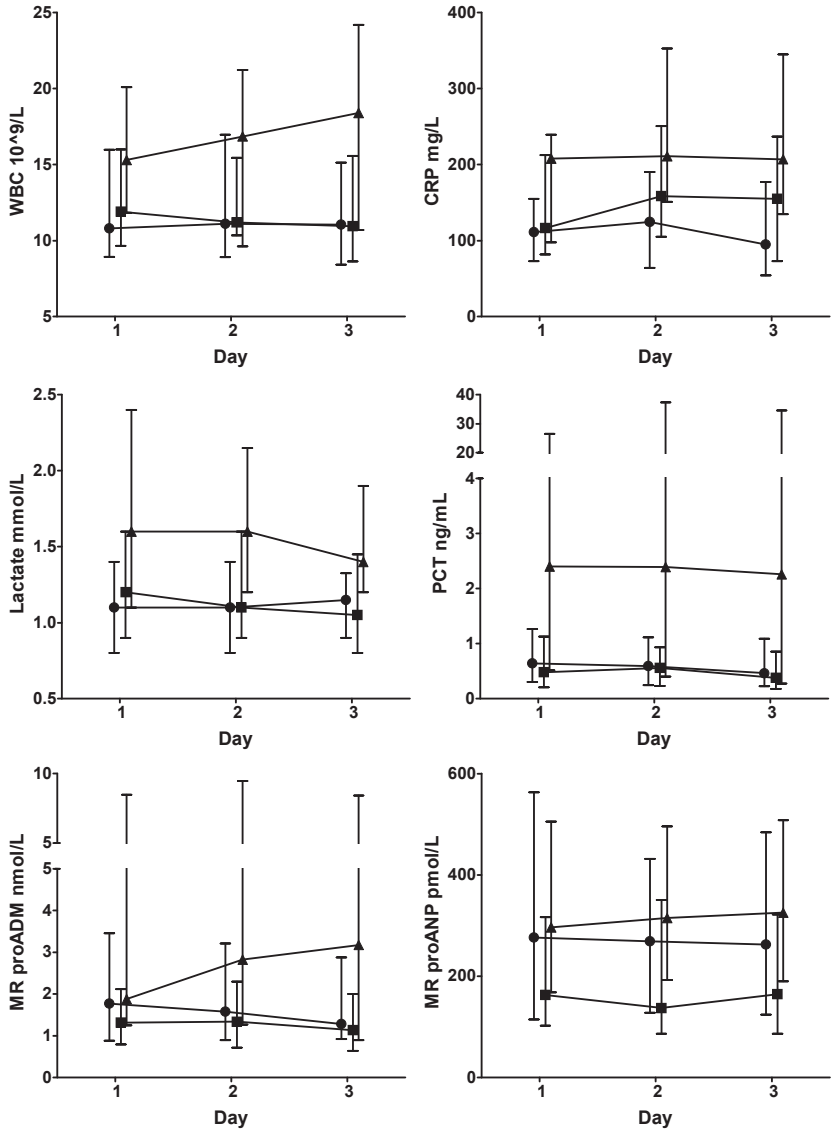


**Figure 2.** Course of plasma levels (median and interquartile range) in febrile critically ill patients with or without local infection or bloodstream infection.
Symbols: ● no/possible infection (Group 1, N=44); ■ local infection (Group 2, N=45); ▲ blood stream infection (Group 3, N=12). In generalised estimating equations (GEE) evaluating BSI vs. non-BSI: for CRP P=0.003, lactate P<0.001, PCT P=0.011, and proADM P=0.007.

**Table 3** Peak values of biomarkers in the groups.

| Day 0-2 Infection | Group 1 | Group 2 | Group 3 | P |
|---|---|---|---|---|
| | N=44 | N=45 | N=12 | |
| WBC, x10$^9$/L | 13.2 (5.5-38.5) | 12.8 (0.2-25.7) | 20.6 (2.5-81.7) | 0.08 |
| CRP, mg/L | 142 (27-440) | 153 (5-484) | 231 (71-436) | 0.004 |
| Lactate, mmol/L | 1.3 (0.5-2.3) | 1.4 (0.5-13.1) | 1.9 (1.1-3.9) | 0.02 |
| PCT, ng/mL | 0.72 (0.09-13.9) | 0.56 (0.08-37.1) | 2.92 (0.09-75.3) | 0.058 |
| proADM, nmol/L | 1.93 (0.50-9.80) | 1.52 (0.37-9.96) | 3.60 (0.82-18.57) | 0.03 |
| proANP, pmol/L | 293 (77-2,037) | 187 (23-823) | 342 (47-874) | 0.09 |
| COP, pmol/L | 34.4 (5.0-157.9) | 27.3 (5.0-97.4) | 33.7 (5.9-154.4) | 0.44 |
| Day 0-7 | No septic shock | Septic shock | | P |
| | N=67 | N=34 | | |
| WBC, x10$^9$/L | 12.9 (4.8-38.5) | 15.0 (0.2-81.7) | | 0.16 |
| CRP, mg/L | 146 (5-440) | 243 (5-484) | | <0.001 |
| Lactate mmol/L | 1.4 (0.5-2.5) | 1.6 (0.8-13.1) | | 0.07 |
| PCT, ng/mL | 0.57 (0.09-37.1) | 1.28 (0.08-75.3) | | 0.005 |
| proADM, nmol/L | 1.75 (0.37-9.80) | 1.89 (0.39-18.57) | | 0.23 |
| proANP, pmol/L | 296 (47-2,037) | 210 (23-874) | | 0.28 |
| COP, pmol/L | 31.5 (5.0-157.9) | 29.5 (5.0-154.4) | | 0.46 |
| Day 0-28 | Survivors | Non-survivors | | P |
| | N=75 | N=26 | | |
| WBC, x10$^9$/L | 12.5 (2.5-27.5) | 16.8 (0.2-81.7) | | 0.08 |
| CRP, mg/L | 177 (5-440) | 201 (38-484) | | 0.30 |
| Lactate, mmol/L | 1.3 (0.5-3.5) | 1.8 (0.9-13.1) | | 0.002 |
| PCT, ng/mL | 0.57 (0.08-45.1) | 1.10 (0.23-75.3) | | 0.009 |
| proADM, nmol/L | 1.52 (0.37-9.47) | 3.30 (0.63-18.57) | | 0.001 |
| proANP, pmol/L | 240 (23-1,613) | 385 (61-2,037) | | 0.006 |
| COP, pmol/L | 27.3 (5.0-154.4) | 38.1 (5.0-157.9) | | 0.04 |
| Day 0-28 low risk | No | Yes | | P |
| infection | N=84 | N=17 | | |
| WBC, x10$^9$/L | 14.2 (0.2-81.7) | 11.9 (4.8-21.6) | | 0.11 |
| CRP, mg/L | 198 (5-484) | 155 (5-279) | | 0.12 |
| Lactate, mmol/L | 1.5 (0.5-13.1) | 1.3 (0.5-2.1) | | 0.23 |
| PCT, ng/mL | 0.90 (0.08-75.3) | 0.32 (0.11-37.14) | | 0.004 |
| proADM, nmol/L | 1.97 (0.39-18.6) | 1.35 (0.37-2.98) | | 0.02 |
| proANP, pmol/L | 269.6 (23.2-2037.0) | 194.4 (70.3-654.3) | | 0.22 |
| COP, pmol/L | 33.7 (5.0-157.9) | 21.7 (5.0-73.4) | | 0.19 |

Median (range); Abbreviations: CRP= C-reactive protein, WBC= white blood cell count, PCT= procalcitonin, proADM= midregional pro-adrenomedullin, proANP= midregional pro-atrial natriuretic peptide, COP= copeptin. Group 1= no or possible infection; Group 2= probable or proven infection; Group 3= bloodstream infection.

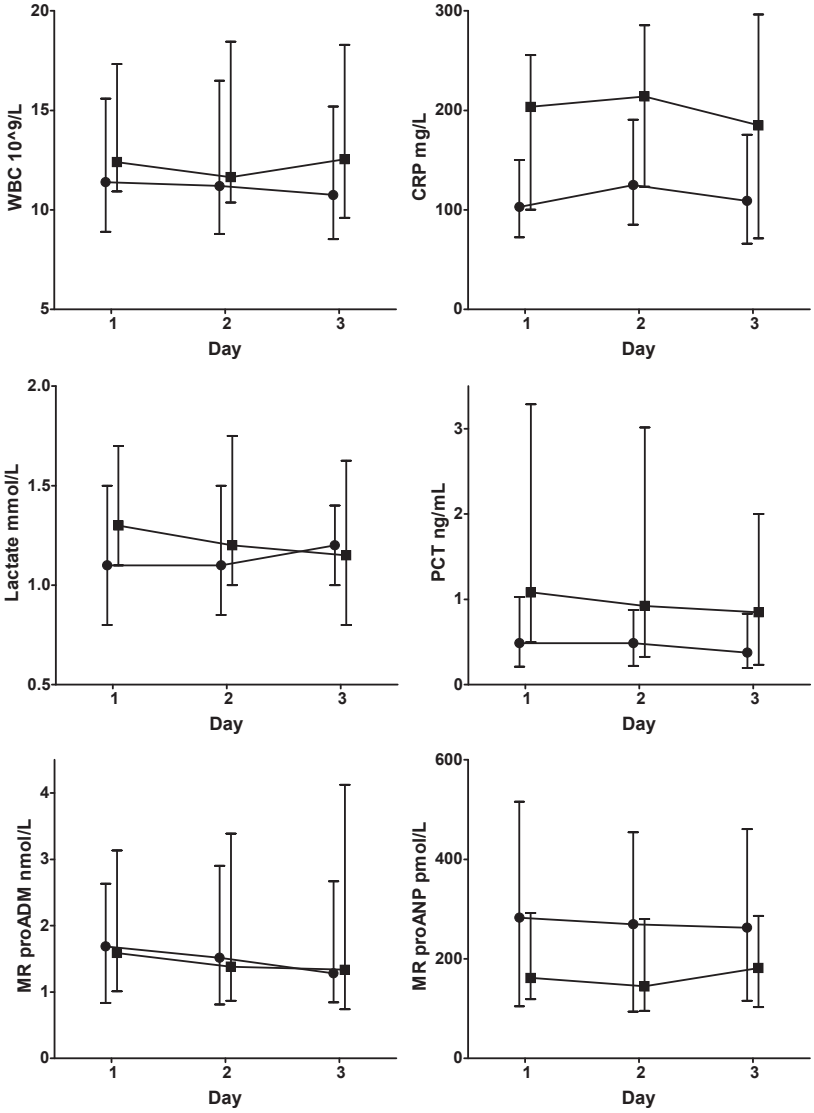


**Figure 3.** Course of plasma levels (median and interquartile range) in febrile critically ill patients with or without septic shock.
Symbols: ● no septic shock (N=67), ■ septic shock (N=34). In generalised estimating equations (GEE): for CRP P=0.009, lactate P=0.044 and PCT P=0.006.

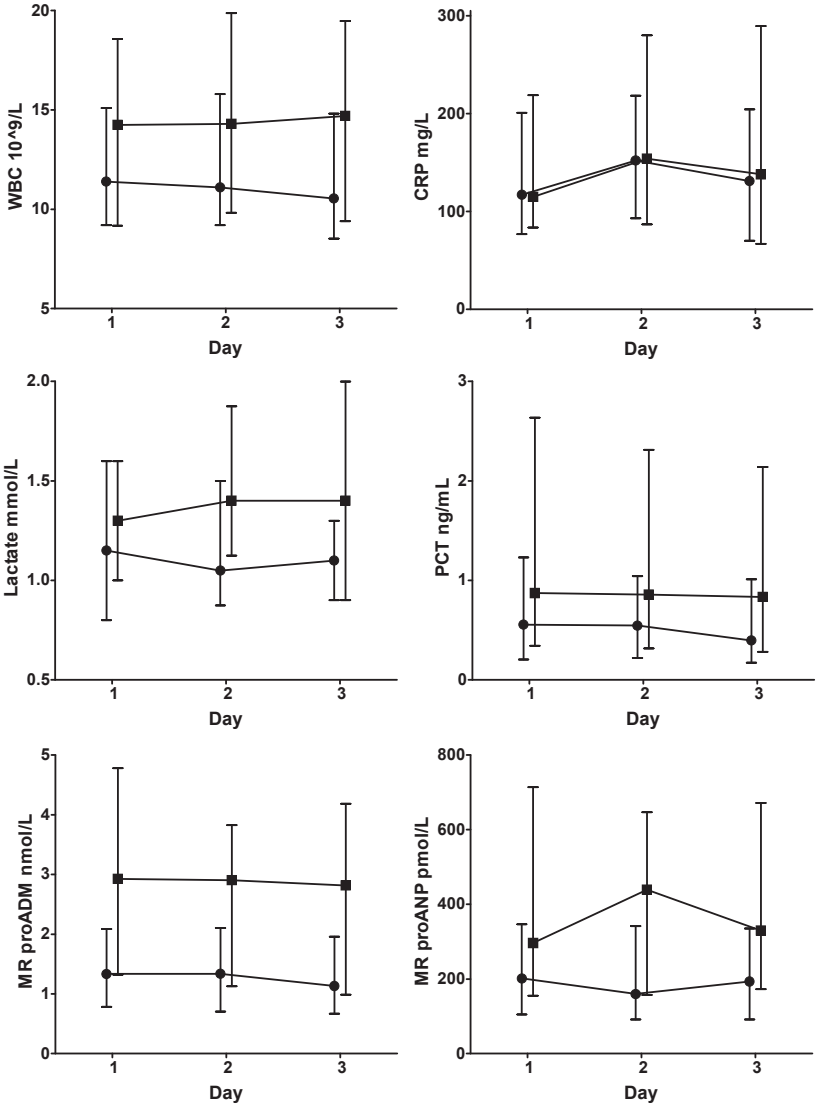


**Figure 4.** Course of plasma levels (median and interquartile range) in febrile critically ill patients, surviving or non surviving.
Symbols: ● survivors (N=75) and ■ non-survivors (N=26). In generalised estimating equations (GEE): for lactate P=0.001, for PCT P=0.012, for proADM P<0.001, for proANP P=0.006 and for COP P=0.027.

### Multivariable analysis

For high risk infection, the combination of peak CRP and lactate predicted best (P=0.033 and 0.001, respectively; Hosmer Lemeshow $X^2$ 8.3, df8, P=0.40), followed by PCT as

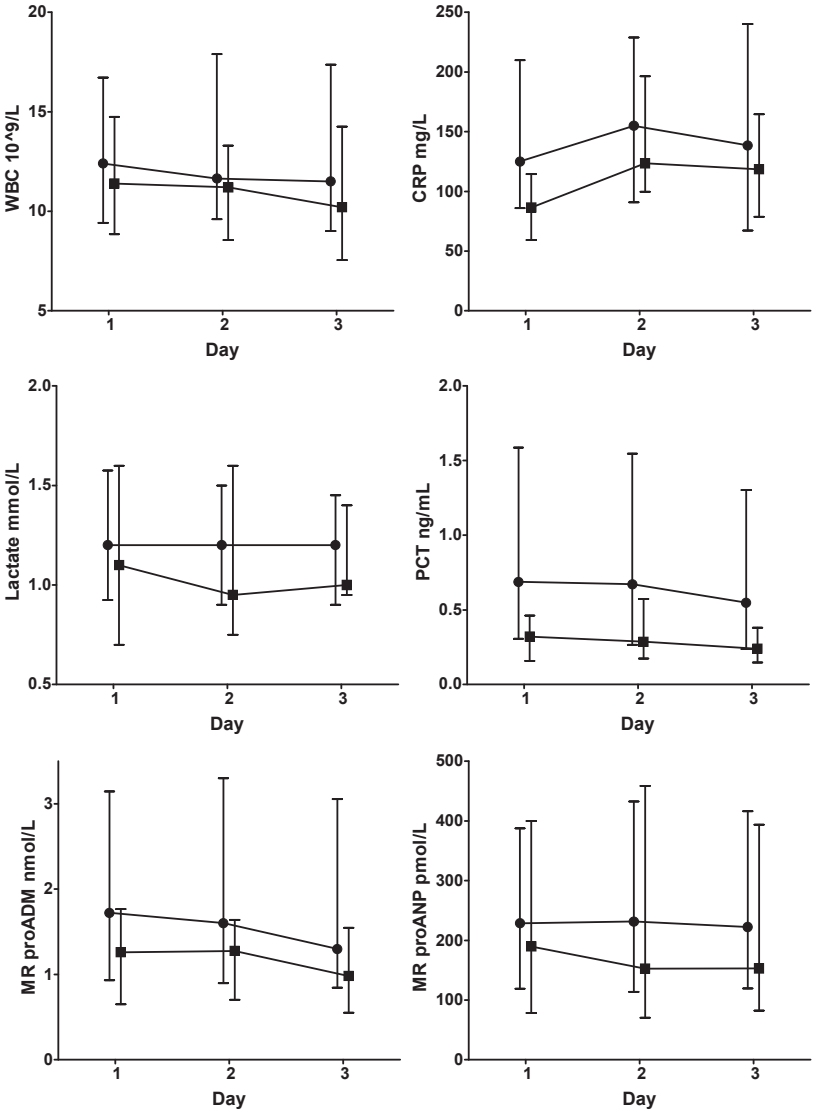


**Figure 5.** Course of plasma levels (median and interquartile range) in febrile critically ill patients, with low or high risk microbial infection.
Symbols: ● low risk infection (N=17) and ■ high risk infection (N=84). In generalised estimating equations (GEE): for PCT P=0.01 an proADM P=0.001)

**Table 4** Prediction by peak values of biomarkers.

| | WBC | CRP | Lactate | PCT | proADM | proANP | COP |
|---|---|---|---|---|---|---|---|
| | x10$^9$/L | mg/L | mmol/L | ng/mL | nmol/L | nmol/L | pmol/L |
| Bloodstream infection Day 0-2 | | | | | | | |
| Cutoff | 20.3 | 196 | 1.5 | 2.44 | 4.3 | - | - |
| AUROC | 0.70 | 0.74 | 0.75 | 0.71 | 0.72 | - | - |
| P | 0.02 | 0.006 | 0.004 | 0.02 | 0.01 | - | - |
| Sn | 58 | 92 | 83 | 58 | 50 | - | - |
| Sp | 84 | 60 | 61 | 85 | 91 | - | - |
| PPV | 33 | 23 | 23 | 35 | 43 | - | - |
| NPV | 94 | 98 | 96 | 94 | 93 | - | - |
| LHR | 3.7 | 2.3 | 2.2 | 4.0 | 5.6 | - | - |
| Septic shock Day 0-7 | | | | | | | |
| Cutoff | - | 208 | - | 1.98 | - | - | - |
| AUROC | - | 0.75 | - | 0.67 | - | - | - |
| P | - | <0.001 | - | 0.003 | - | - | - |
| Sn | - | 71 | - | 44 | - | - | - |
| Sp | - | 78 | - | 88 | - | - | - |
| PPV | - | 62 | - | 65 | - | - | - |
| NPV | - | 84 | - | 76 | - | - | - |
| LHR | - | 3.2 | - | 3.7 | - | - | - |
| Mortality Day 0-28 | | | | | | | |
| Cutoff | - | - | 1.7 | 0.65 | 2.79 | 565 | 31.5 |
| AUROC | - | - | 0.71 | 0.67 | 0.73 | 0.68 | 0.63 |
| P | - | - | 0.001 | 0.007 | <0.001 | 0.005 | 0.04 |
| Sn | - | - | 60 | 77 | 62 | 46 | 69 |
| Sp | - | - | 75 | 57 | 81 | 93 | 57 |
| PPV | - | - | 44 | 39 | 53 | 71 | 36 |
| NPV | - | - | 85 | 88 | 86 | 83 | 84 |
| LHR | - | - | 2.4 | 1.8 | 3.3 | 6.9 | 1.6 |
| Low risk infection Day 0-28 | | | | | | | |
| Cutoff | - | - | - | <0.65 | <1.91 | - | - |
| AUROC | - | - | - | 0.72 | 0.67 | - | - |
| P | - | - | - | <0.001 | 0.005 | - | - |
| Sn | - | - | - | 88 | 88 | - | - |
| Sp | - | - | - | 60 | 51 | - | - |
| PPV | - | - | - | 31 | 27 | - | - |
| NPV | - | - | - | 96 | 96 | - | - |
| LHR | - | - | - | 2.2 | 1.8 | - | - |

Abbreviations: D= day; WBC= white blood cell count; CRP= C-reactive protein, PCT= procalcitonin, proADM= midregional pro-adrenomedullin, proANP= midregional pro-atrial natriuretic peptide, COP= copeptin, AUROC= area under the receiver operating characteristic curve, P= p value, Sens= sensitivity, Spec= specificity, PPV= positive predictive value, NPV= negative predictive value, LHR= likelihood ratio; non-significant data have been omitted.

the best single predictive variable (P=0.017; Hosmer Lemeshow $X^2$ 5.1, df 8, P=0.75). For low risk infection, PCT was the single best predictive variable (P=0.015; Hosmer Lemeshow $X^2$ 9.8, df8, P=0.28).

## DISCUSSION

This study suggests that old and new biomarkers in critically ill patients with new onset fever differ in their predictive value according to invasiveness and severity of microbial infection, and that PCT may be of value as a single predictor of both high and low risk ICU-acquired infection.

We found that 57 of 101 (56%) febrile, critically ill patients had a probable/proven local infection or BSI, which agrees with the literature on comparable patient populations, in spite of female preponderance of patients with BSI's in our study.[2,21,26] Sources, micro-organisms and mortality rates are also in agreement with other studies, including those on ICU-acquired bacteraemia.[2,10,25,26]

The associations between invasiveness of microbial infection and development of septic shock is not beyond expectations either. Since all patients had SIRS, the syndrome had no predictive value for infection and all patients with infection thus suffered from new onset sepsis in the ICU. Nevertheless, the (peak) WBC count had some predictive value for likelihood and invasiveness of microbial infection, in contrast to the literature[8], but was of no predictive value for septic shock and mortality, in agreement with the literature.4 Peak CRP predicted, to a certain extent, both BSI and septic shock but not mortality in our patients, the latter again in agreement with the literature.[4,6-9,18] The value of these two commonly applied surrogate indicators of microbial infection and its severity in the critically ill is thus limited. In contrast, minimally elevated lactate levels were of some predictive value for BSI and ICU mortality, in line with previous studies for the latter.[12-14] The predictive value of lactate for BSI is a novel finding. Early lactate production even before onset of shock in bacteraemic patients might be explained by an increase in cellular $Na^+$-$K^+$ ATP-ase activity, among others.[38]

We found PCT to be helpful in discriminating BSI from infections without BSI, as reported before.[1,7,10,24] though predictive values of PCT were somewhat lower than in other patient populations. In these studies, PCT on admission appeared helpful in predicting, irrespective of localisation, a suspected infectious cause of SIRS (sepsis) and its severity, as compared to non-infected, critically ill patients with SIRS.[5,6,9,18,21,22,28] This can be explained by our inclusion criteria of ICU-acquired fever in patients with prior infection, surgery or other conditions that may confound PCT. Few studies that specifically addressed new onset fever in the ICU yielded highly variable results for PCT.[1,10,20,21] Nevertheless, for the 4 major endpoints, i.e. BSI, septic shock, mortality

and low risk infection, PCT was superior to lactate and our results thus agree with improved prognostication in septic shock by PCT over lactate.[6,32] Combining biomarkers[23,32] has only rarely been done but may improve prediction.[16] In multivariable analysis of high risk infection in our critically ill patients with new onset fever, the combination of CRP and lactate proved superior, directly followed by PCT. For low risk infection also, PCT proved the single best predictive variable. Finally, PCT peaked earlier than the other markers. The superior predictive value of PCT over the other biomarkers for all endpoints studied can be explained, among others, by the kinetics in response to infection, in parallel with its invasiveness and severity.[39] In contrast to PCT, other prohormones may only transiently increase upon microbial products, whereas the response of WBC and CRP may be relatively slow.[39]

PCT values above 0.25-0.5 ng/mL have been used to guide starting or continuing antibiotics in the ICU[30,31] and our study suggests a higher cutoff at 0.65 ng/mL to discriminate between high and low risk microbial infection in ICU- acquired fever, for which empiric antibiotics could be instituted or withheld, respectively, in future studies.[25,26] Many patients were on antibiotics even when fever was unlikely associated with microbial infection, representing potential overtreatment. We could not identify an effect of changing antibiotics on outcome but did not evaluate appropriateness in the absence of uniformly accepted criteria.[26]

Whereas other investigators found a difference in proADM or COP between infected and non-infected and non-surviving and surviving critically ill patients,[33-35] we found varying predictive values of the novel biomarker prohormones. However, proADM was predictive in 3 of 4 endpoints evaluated and thereby directly ranked behind PCT. The greater predictive value than that of PCT for outcome is in accordance with other studies.[36] Nonetheless, proADM levels did not supplement predictive values of PCT in multivariable analyses. We observed only minor predictive value of COP in our study in agreement with prior observations.[35]

Limitations of the study include the evaluation of new onset fever only, the heterogeneity of the study population and the persistently imperfect predictions by biomarkers of (severity of) infection. Heterogeneity could also be regarded as an advantage, however, concerning generalisibility of results. We separately studied medical and surgical patients and found no difference. The introduction of SDD did not change the predictive values of the biomarkers in this study, which may help in deciding on the use of biomarkers during SDD. That the percentage of fever from infectious vs. non-infectious causes decreased after introduction of SDD is in line with expectations. Another advantage of our study is the rigid documentation and classification of infection as an endpoint.

## CONCLUSIONS

In conclusion, our study in critically ill patients with new onset fever suggests that plasma PCT best serves as a single variable, among old and new biomarkers, to predict high and low risk microbial infection, although this prediction remains imperfect. The study may support evaluation of clinical decision making on starting or postponing empiric antibiotics at a cutoff of 0.65 ng/mL for peak PCT in ICU- acquired fever, in future studies.

## REFERENCES


1. Fraunberger P, Wang Y, Holler E, et al. Prognostic value of interleukin 6, procalcitonin, and C-reactive protein levels in intensive care unit patients during first increase of fever. Shock 2006;26:10-2.
2. Laupland KB, Shahpori R, Kirkpatrick AW, et al. Occurrence and outcome of fever in critically ill adults. Crit Care Med 2008;36:1531-5.
3. World Health Organization (WHO). WHO report on infectious disease: removing obstacles to healthy development 1999.
4. Clyne B, Olshaker JS. The C-reactive protein. J Emerg Med 1999;17:1019-25.
5. Selberg O, Hacker H, Martin M, et al. Discrimination of sepsis and systemic inflammatory response syndrome by determination of circulating plasma concentrations of procalcitonin, protein commplement 3a, and interleukin-6. Crit Care Med 2000;28:2793-8.
6. Linscheid P, Seboek D, Nylen ES, et al. In vitro and in vivo calcitonin I gene expression in parenchymal cells: a novel product of human adipose tissue. Endocrinology 2003;144:5578-84.
7. Gibot S, Cravoisy A, Kolopp-Sarda MN, et al. Timecourse of sTREM (soluble triggering receptor expressed on myeloid cells)-1, procalcitonin, and C-reactive protein plasma concentrations during sepsis. Crit Care Med 2005;33:792-6.
8. Povoa P, Coelho L l, Almeida E, et al. C-reactive protein as a marker of infection in critically ill patients. Clin Microbiol Infect 2005;11:101-8.
9. Rau BM, Kemppainen EA, Gumbs AA, et al. Evaluation of procalcitonin for predicting septic multiorgan failure and overall prognosis in secondary peritonitis. Arch Surg 2007;142:134-42.
10. Nakamura A, Wada H, Ikejiri M, et al. Efficacy of procalcitonin in the early diagnosis of bacterial infections in a critical care unit. Shock 2009; 31:586-91.
11. Tschaikowsky K, Hedwig-Geissing M, Braun GG, et al. Predictive value of procalcitonin, interleukin-6, and C-reactive protein for survival in postoperative patients with severe sepsis. J Crit Care 2011;26:54-64.
12. Howell MD, Donnino M, Clardy P, et al. Occult hypoperfusion and mortality in patients with suspected infection. Intensive Care Med 2007;33:1892-9.
13. Trzeciak S, Dellinger RP, Chansky ME, et al. Serum lactate as a predictor of mortality in patients with infection. Intensive Care Med 2007;33:970-7.
14. Nichol AD, Egi M, Pettila V, et al. Relative hyperlactatemia and hospital mortality in critically ill patients: a retrospective multi-center study. Crit Care 2010;14:R25.
15. Simon L, Gauvin F, Amre DK, et al. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis 2004;39:206-17.
16. Kofoed K, Andersen O, Kronborg G, et al. Use of plasma C-reactive protein, procalcitonin, neutrophils, macrophage migration inhibitory factor, soluble urokinase-type plasminogen activator receptor, and soluble triggering receptor expressed on myeloid cells-1 in combination to diagnose infections: a prospective study. Crit Care 2007;11:R38.
17. Luzzani A, Polati E, Dorizzi R, et al. Comparison of procalcitonin and C-reactive protein as markers of sepsis. Crit Care Med 2003;31:1737-41.

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

19. Uzzan B, Cohen R, Nicolas P, et al. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. Crit Care Med 2006;34:1996-2003.

20. Tang BMP, Eslick GD, Craig JC, et al. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. Lancet 2007;7:210-7.

21. Castelli GP, Pognani C, Cita M, et al. Procalcitonin as a prognostic and diagnostic tool for septic complications after major trauma. Crit Care Med 2009;37:1845-9.

22. Harbarth S, Holeckova K, Froidevaux C, et al. Diagnostic value of procalcitonin, interleukin- 6, and interleukin-8 in critically ill patients admitted with suspected sepsis. Am J Respir Crit Care Med 2001; 164:396-402.

23. Bell K, Wattie M, Byth K, et al. Procalcitonion: a marker of bacteraemia in SIRS. Anaesth Intensive Care 2003;31:629-36.

24. Jones AE, Fiechtl JF, Brown MD, et al. Procalcitonin test in the diagnosis of bacteremia: a meta-analysis. Ann Emerg Med 2007;50:34-1.

25. Laupland KB, Zygun DA, Davies D, et al. Population-based assessment of intensive care unit- acquired bloodstream infections in adults: incidence, risk factors, and associated mortality rate. Crit Care Med 2002;30:2462-7.

26. Corona A, Bertolini G, Lipman J, et al. Antibiotic use and impact on outcome from bacteraemic critical illness: the BActeraemia Study in Intensive Care (BASIC). J Antimicrob Chemother 2010;65:1276-85.

27. Hensler T, Sauerland S, Lefering R, et al. The clinical value of procalcitonin and neopterin in predicting sepsis and organ failure after major trauma. Shock 2003;20:420-6.

28. Clec'h C, Fosse J-P, Karoubi P, et al. Differential diagnostic value of procalcitonin in surgical and medical patients with septic shock. Crit Care Med 2006; 34:102-7.

29. Charles PE, Kus E, Aho S, et al. Serum procalcitonin for the early recognition of nosocomial infection in the critically ill patients: a preliminary report. BMC Infect Dis 2009;9:49.

30. Bouadma L, Luyt C-E, Cracco C, et al. PRORATA trial group: use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units (PRORATA trial): a multicenter randomised controlled trial. Lancet 2010;375:463-74.

31. Heyland DK, Johnson AP, Reynolds SC, et al. Procalcitonin for reduced antibiotic exposure in the critical care setting: a systematic review and an economic evaluation. Crit Care Med 2011;39:1792-9.

32. Phua J, Koay ESC, Lee KH. Lactate, procalcitonin, and aminoterminal pro-B-type natriuretic peptide versus cytokine measurements and clinical severity scores for prognostication in septic shock. Shock 2008;29:328-33.

33. Schuetz P, Christ-Crain M, Morgenthaler NG, et al. Circulating precursor levels of endothelin-1 and adrenomedullin, two endothelium-derived, counteracting substances, in sepsis. Endothelium 2007;14: 345-51.

34. Guignant C, Voirin N, Venet F, et al. Assessment of pro-vasopressin and pro-adrenomedullin as predictors of 28-day mortality in septic shock patients. Intensive Care Med 2009;35:1859-67.

35. Jochberger S, Dorler J, Luckner G, et al. The vasopressin and copeptin response to infection, severe sepsis, and septic shock. Crit Care Med 2009;37:476-82.

36. Kru€ger S, Ewig S, Giersdorf S, et al. The German Competence Network for the Study of Community Acquired Pneumonia (CAPNETZ) Study Group. Cardiovascular and inflammatory biomarkers to predict short- and long-term survival in community-acquired pneumonia. Am J Respir Crit Care Med 2010;182:1426-34.

37. Calandra T, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. Crit Care Med 2005;33:1538-48.

38. Bundgaard H, Kjeldsen K, Suarez Krabbe K, et al. Endotoxemia stimulates skeletal muscle Na-K-ATPase and raises blood lactate under aerobic conditions in humans. Am J Physiol Heart Circ Physiol 2003;284:H1028-34.

39. De Kruif MD, Lemaire LC, Giebelen IA, Struck J, et al. The influence of corticosteroids on the release of novel biomarkers in human endotoxemia. Intensive Care Med 2008;34:518-22.

# 3

# Rising C-reactive protein and procalcitonin levels precede early complications after oesophagectomy

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## ABSTRACT

**Objective** Elective oesophagectomy with gastric-tube reconstruction carries a high risk for complications. Early and accurate diagnosis could improve patient management. Increased C-reactive protein (CRP) levels may be associated with any, surgical or infectious, complication and procalcitonin (PCT) specifically with infectious complications.

**Methods** We measured CRP and PCT on postoperative days 0, 1, 2 and 3 in 45 consecutive patients. Complications were recorded up to 10 days post-oesophagectomy.

**Results** Twenty-eight patients developed a postoperative complication (5 surgical, 14 infectious, 9 combined surgical/infectious, including anastomotic leakage), presenting on day 3 or later. Elevated day 2, 3 and a rise in CRP preceded the diagnosis of general or combined surgical/infectious complications (minimum AUROC 0.75, P=0.006). Elevated day 3 PCT preceded combined complications (AUROC 0.86, P<0.001). High day 1 and 3 PCT levels preceded anastomotic leakage (minimum AUROC 0.76, P=0.005), as did the day 3 CRP levels and their increases (minimum AUROC 0.78, P=0.002).

**Conclusions** This small study suggests that high or increasing CRP levels may precede the clinical diagnosis of general or surgical/infectious complications after oesophagectomy. Elevated PCT levels may more specifically and timely precede combined surgical/infectious complications mainly associated with anastomotic leakage.

## INTRODUCTION

Early complications after elective oesophagectomy and gastric-tube reconstruction are associated with increased morbidity and mortality.[1-5] Recognition of patients at risk for complications before presentation of full blown symptoms could lead to early diagnosis and treatment which may improve outcome. However, the early recognition of complications by clinical characteristics and parameters in individual patients remains difficult, except perhaps for pulmonary complications.[2,3,6] Oesophagectomy in itself induces a strong inflammatory response and the value of systemic inflammatory response syndrome (SIRS) criteria fever, leukocytosis, tachypnea and tachycardia for the early diagnosis of complications is limited.[6-8] On the other hand, inflammatory biomarkers like C-reactive protein (CRP) and procalcitonin (PCT) might be useful in the early diagnosis of not yet clinically symptomatic postoperative complications. Previous studies reported an association between elevated CRP levels and (infectious) complications, sepsis, and mortality after esophagectomy.[8-12] However, CRP levels did not discriminate between surgical and infectious complications, requiring different therapeutic management strategies.[6,8-10,12,13] PCT is an allegedly more specific marker of severe infection and complications after surgery than CRP,[14-16] but the literature is inconclusive in this respect.[17,18] So far, only five studies reported on PCT levels post-esophagectomy,[1,11,13,19,20] of which only two focused on postoperative infectious complications.[11,13] The latter studies suggested that PCT is useful for the diagnosis of infectious complications and discriminating sepsis from SIRS post-oesophagectomy. The discriminating ability of PCT for complication subtypes is unknown, however.

We hypothesized that CRP is a sensitive but non-specific marker of developing complications after oesophagectomy, while PCT is a more specific marker of developing severe postoperative infections. We thus compared the use of CRP and PCT for early diagnosis of surgical and infectious complications.

## PATIENTS AND METHODS

This prospective observational study, approved by the medical ethical committee of the Erasmus Medical Centre (MEC-2010-199), was conducted between September 2011 and December 2012. Forty-five consecutive adult patients were included after giving written informed consent prior to surgery. We did not perform a power analysis for this proof of principle study. Because of competing studies and activities this proof of principle study was limited in time and therefore we could include only 45 patients in the time interval indicated. Oesophagectomy and gastric-tube reconstruction was performed by the transthoracic or transhiatal approach.[21] The gastric-tube reconstruc-

tion was performed by handsewn end-to-end or semimechanical end-to-side anastomosis.[22] After admission to the intensive care unit (ICU), patients were taken care of by board-certified intensivists unaware of biomarker results.

**Study protocol**

Upon ICU admission (day 0), baseline patient characteristics were recorded. Disease severity was estimated using the acute physiology and chronic health evaluation II (APACHE II)-score and organ failure was calculated by the sequential organ failure assessment (SOFA)-score. The preoperative risk assessment was done by using the American Society of Anaesthesiology (ASA) classification and Portsmouth predictor modification of the physiological and operative severity score for the enumeration of mortality and morbidity (P-POSSUM). Clinical parameters and blood samples for routine laboratory parameters, leukocyte counts, CRP and PCT levels were collected directly postoperatively on ICU admission (day 0), and in the morning of postoperative day 1, 2, 3. Leukocyte counts were measured using the Sysmex SE-9000 analyzer (Toa Medical Instruments, Kobe, Japan), normal values are 3.5-10*$10^9$/L. CRP was measured by an Immunoturbidimetric assay (Modular analytics <P> Roche diagnostics, Mannheim, Germany), normal values are <9 mg/L. PCT was measured using the PCT-sensitive for the Kryptor compact system (Brahms diagnostica, Henningsdorf, Germany). Assays were performed according to manufacturer's guidelines, the lower detection limit being 0.02 ng/mL, with an upper limit in healthy volunteers of 0.05 ng/mL. The functional assay sensitivity (FAS) of this test is 0.06 ng/mL, with an intra-assay coefficient of variation (CV) and inter-assay CV of <6% in samples containing >0.3 ng/mL.

**Definitions**

All complications up to 10-days post-oesophagectomy as decided by attending physicians were recorded, only if additional medical or surgical treatment was required, notably grade 2 or higher on the Accordion severity grading system.[4,5] The definitions of complications used in this study are depicted in Table 1. The 10-day cutoff was chosen based on a previous study from this group.[12] Infections were defined according to the International Sepsis Forum consensus conference criteria,[23] as agreed upon by the attending intensivists. Diagnostic imaging and collection of specimens and blood for microbial culture were left at the attending intensivists discretion. Specimen were processed according to standardized culture protocols and Gram stains were prepared. Cultures reflecting colonization rather than infection were excluded from final analysis. For example, blood cultures containing coagulase-negative staphylococci were considered contaminated if only one bottle showed growth. Because reporting of definite culture results can take several days the day of specimen collection was considered

**Table 1.** Definition of complications.

| Complication | Definition |
|---|---|
| Surgical | |
| Anastomotic leakage | Esophagoenteric leak confirmed by endoscopy or esophageal contrast videography that requires local treatment, surgical treatment, or removal of conduit. |
| Pleural effusion | Pleural effusion confirmed by radiology that requires drainage. |
| Chyle leak | Chylomicrons in pleura aspirate or milky discharge from chest tube at initiation of enteral feeding. |
| Laryngeal nerve palsy | Clinically suspected vocal cord paralysis confirmed by laryngoscopy. |
| Conduit ischemia/necrosis | Circular conduit ischemia/necrosis confirmed by endoscopy and/or surgically that requires local treatment or removal of conduit. |
| Thromboembolic disease | Deep venous thrombosis or pulmonary embolus. |
| Infectious | |
| Pneumonia | New infiltrate on chest radiograph and positive tracheal aspirate cultures that requires antibiotic treatment. |
| Empyema | Pleural effusion on chest radiograph and positive culture of aspirated specimen that requires antibiotic and radiological or surgical treatment. |
| Abscess | Intra-thoracic (mediastinal) or intra-abdominal abscess confirmed by radiology with positive culture of aspirated specimen that requires antibiotic, radiological, or surgical treatment. |
| Wound infection | Erythematous wound, with effluent of pus and/or positive culture that requires opening of wound and antibiotics. |
| Gastrointestinal | Stool culture positive for microbial pathogens that requires antibiotic treatment. |
| Urinary tract | Positive urine culture and urine sediment that require antibiotic treatment. |
| Bacteraemia | Blood samples showing positive growth and/or positive Gram stain, not reflecting colonization that requires antibiotic treatment. |

Patients could suffer from multiple complications simultaneously

the day of infection diagnosis. Patients were considered to have sepsis when presenting at least two SIRS criteria: body temperature <36 °C or >38.3 °C, heart rate >90 beats/min, respiratory rate >20 breaths/min or mechanical ventilation, a leukocyte count of either <4.0 $*10^9$/L or >12.0 $*10^9$/L, in the presence of a probable or proven infection, according to American College of Chest Physicians/Society of Critical Care Medicine guidelines.[24] Shock was defined by a systolic pressure <90 mmHg or a mean arterial pressure <60 mmHg for at least 1 hour, despite adequate fluid resuscitation, or requirement of vasopressor support to maintain mean arterial pressure. Shock in the presence of sepsis was considered septic shock. We report 30-day mortality.

### Statistical analysis

Patients were categorized into two groups, i.e. patients developing complications and without complications. In addition, to translate the results to clinical recommendations and to reflect complication severity, patients with postoperative complications were

categorized into 3 mutually exclusive complication groups. Patients could either have purely surgical complications, purely infectious complications, or combined surgical and infectious complications. We studied biomarker levels at day 0-3 and their fractional change (Δ) at day 3, i.e. day 3 divided by day 0 biomarker levels. Since most symptoms of complications appear after postoperative day 3, the levels measured between day 0-3 were considered early diagnostic for complications presenting between days 4-10. We used IBM SPSS statistics for Windows version 20 (IBM SPSS, Chicago, IL, USA) to analyze the data, except for analyzing the area under the receiver operating characteristic curve (AUROC). We present data as median (interquartile range) since many continuous data were non-normally distributed (Kolmogorov-Smirnov test $P<0.05$). We used a Kruskal-Wallis and Mann-Whitney U test to study group differences in continuous variables and the $X^2$ or Fisher exact test for categorical variables. To evaluate the early diagnostic value of biomarker levels for groups we calculated the area under the receiver operating characteristic curves (AUROC), for which non-Gaussian data were logarithmically transformed. Only the AUROC analyses were performed using MedCalc for Windows, version 13 (MedCalc Software, Ostend, Belgium). We considered an AUROC ≥0.70 as clinically relevant. The optimal diagnostic cutoff value was calculated as suggested by Zweig and Campbell.[25] To calculate the optimal criterion this method takes the disease prevalence and cost of true and false positive and negative decisions into account.[25] The Holm-Bonferroni method was used to correct for multiple testing.[26] We used multiple logistic regression with backward selection of logarithmically transformed biomarker levels to study their interdependency for the diagnosis of postoperative complications in general, complication subtypes and anastomotic leakage. We performed the Hosmer Lemeshow test to evaluate the goodness of fit. All tests were two-sided and P-values <0.05 were considered statistically significant, exact P-values are given unless <0.001.

## RESULTS

Twenty-eight patients (62%) suffered from a postoperative complication, of whom 5 had a surgical complication, 14 an infectious complication, and 9 combined surgical/infectious complications (Table 2). The manifestation of postoperative complications was on day 3 or later in all patients and in 92% of cases complications presented on day 4 or later. Patients developing combined surgical/infectious complications had more complications than patients in the other complication groups (3 vs. 1 complication). Table 3 shows baseline characteristics for patients developing complications and without complications. The number of female patients was higher in the infectious and combined surgical/infectious than in the surgical complication group. Almost all

**Table 2.** Complications up to 10 days post esophagectomy.

| Surgical complication (N=5) | Infectious complication (N=14) | Combined s/i complications (N=9) |
|---|---|---|
| Anastomotic leak | Pneumonia, wound infection | Anastomotic leak, chyle leak, wound infection |
| Anastomotic leak | Pneumonia, wound infection | Anastomotic leak, pneumonia, wound infection |
| Pleural effusion, chyle leak | Pneumonia | Anastomotic leak, pneumonia |
| Chyle leak | Pneumonia | Anastomotic leak, abscess, wound infection |
| Chyle leak | Pneumonia | Anastomotic leak, pleural effusion, abscess, wound infection |
| | Pneumonia | Anastomotic leak, pneumonia |
| | Pneumonia | Anastomotic leak, wound infection, pneumonia, empyema |
| | Pneumonia | Anastomotic leak, pneumonia, empyema |
| | Pneumonia | Chyle leak, abscess, pneumonia |
| | Wound infection | |
| | Wound infection | |
| | Wound infection | |
| | Urinary tract infection | |
| | Urinary tract infection | |

Complications presented no sooner than day 3; in 92% of cases, complications presented on day 4 or after. Abbreviations: s/i surgical/infectious.

patients suffered from SIRS at some point during the first 10-days postoperatively. Patients developing infectious complications had received antibiotics less often than the other patient groups. Patients suffering surgical or combined complications had a longer hospital stay than patients with an uncomplicated recovery (P=0.03 and P=0.02, respectively). All patients survived until 30 days postoperatively. The preoperative World Health Organization (WHO) performance score and pulmonary function tests were not predictive of postoperative complications. To avoid major overlap we do not separately report the baseline characteristics of patients with versus without anastomotic leak.

### Biomarker levels prior to diagnosis of complications

Figure 1 shows the data (days 0-3) for patients developing any complication and those without complications. Only statistically significant AUROC values are presented in Table 4. The day 3 leukocyte counts were higher in patients developing any complication than in those without, but the optimal cutoff value in AUROC was below the upper limit of the normal range. The day 2 and 3 CRP levels and their rise were higher in patients developing complications than in those without and had diagnostic value with

**Table 3.** Baseline characteristics.

| | Uncomplicated | | | Complicated | | | |
|---|---|---|---|---|---|---|---|
| | (N = 17) | (N = 28) | P[1] | Surgical (N = 5) | Infectious (N = 14) | Combined s/i (N = 9) | P[2] |
| Sex (M) | 16 (94) | 23 (82) | 0.39 | 2 (40) | 12 (86) | 9 (100) | 0.009 |
| Age (years) | 62 (14) | 63 (17) | 0.52 | 60 (15) | 65 (14) | 63 (20) | 0.89 |
| BMI ($cm^2$/kg) | 27.8 (4.7) | 23.8 (5.0) | 0.05 | 22.7 (8.3) | 25.3 (6.1) | 23.5 (2.9) | 0.12 |
| WHO performance score | | | | | | | |
| 0 | 7 (41) | 14 (50) | 0.57 | 7 (50) | 2 (40) | 5 (56) | 0.57 |
| 1 | 10 (59) | 14 (50) | | 7 (50) | 3 (60) | 4 (44) | |
| Preoperative pulmonary function | | | | | | | |
| FEV1 (% predicted) | 113 (27) | 98 (21) | 0.04 | 96 (27) | 100 (1) | 94 (22) | 0.19 |
| VC (% predicted) | 112 (28) | 111 (16) | 1.00 | 115 (17) | 108 (2) | 106 (22) | 0.58 |
| ASA class | | | | | | | |
| I | 2 (12) | 10 (11) | 0.86 | 2 (40) | 0 | 1 (11) | 0.08 |
| II | 13 (77) | 20 (71) | | 1 (20) | 11 (79) | 8 (89) | |
| III | 2 (12) | 5 (18) | | 2 (40) | 3 (21) | 0 | |
| P-POSSUM score | 35 (10) | 34 (5) | 0.50 | 34 (3) | 33 (8) | 35 (3) | 0.70 |
| Cell type | | | | | | | |
| Squamous cell carcinoma | 2 (12) | 9 (32) | 0.28 | 4 (29) | 2 (40) | 3 (33) | 0.85 |
| Adenocarcinoma | 13 (77) | 18 (64) | | 6 (64) | 3 (60) | 6 (67) | |
| Small cell neuroendocrine carcinoma | 1 (6) | 1 (4) | | 1 (6) | 0 | 0 | |
| Miscellaneous | 1 (6) | 0 | | 0 | 0 | 0 | |
| Clinical stage | | | | | | | |
| T | | | | | | | |
| 1 | 1 (6) | 0 | 0.10 | 0 | 0 | 0 | 0.26 |
| 2 | 2 (12) | 5 (18) | | 2 (14) | 1 (20) | 2 (22) | |
| 3 | 11 (65) | 19 (68) | | 12 (86) | 3 (60) | 4 (44) | |
| 4 | 3 (20) | 0 | | 0 | 0 | 0 | |
| Unknown | 0 | 4 (14) | | 0 | 1 (20) | 3 (33) | |
| N | | | | | | | |
| 0 | 5 (29) | 9 (32) | 0.67 | 5 (36) | 2 (40) | 2 (22) | 0.53 |
| 1 | 5 (29) | 9 (32) | | 3 (21) | 2 (40) | 4 (44) | |
| 2 | 6 (35) | 9 (32) | | 6 (43) | 1 (20) | 2 (22) | |
| 3 | 1 (6) | 0 | | 0 | 0 | 0 | |
| Unknown | 0 | 1 (4) | | 0 | 0 | 1(11) | |
| M | | | | | | | |
| 0 | 17 (100) | 26 (93) | 0.52 | 14 (100) | 5 (100) | 7 (78) | na |
| Unknown | 0 | 2 (7) | | 0 | 0 | 2 (22) | |
| Neoadjuvant chemoradiotherapy | 14 (82) | 26 (93) | 0.35 | 5 (100) | 13 (93) | 8 (89) | 0.66 |

**Table 3.** Baseline characteristics. (continued)

| | Uncomplicated | | | Complicated | | | |
|---|---|---|---|---|---|---|---|
| | (N = 17) | (N = 28) | P[1] | Surgical (N = 5) | Infectious (N = 14) | Combined s/i (N = 9) | P[2] |
| Surgical approach | | | | | | | |
| TH | 6 (35) | 10 (36) | 1.00 | 2 (40) | 5 (36) | 3 (33) | 1.00 |
| TT | 11 (65) | 18 (64) | | 3 (60) | 9 (64) | 6 (67) | |
| Open procedure | 16 (94) | 25 (59) | 1.00 | 12 (86) | 5 (100) | 8 (89) | 0.74 |
| Laparoscopic procedure | 1 (6) | 3 (11) | | 2 (14) | 0 | 1 (11) | |
| Hand sewn end-to-end | 7 (41) | 17 (61) | 0.23 | 8 (57) | 3 (60) | 6 (67) | 0.61 |
| Semimechanical side-to-end | 10 (59) | 11 (39) | | 6 (43) | 2 (40) | 3 (33) | |
| Operation duration (min) | 414 (186) | 383 (136) | 0.40 | 383 (171) | 382 (140) | 410 (156) | 0.79 |
| Blood loss (mL) | 1000 (800) | 675 (869) | 0.33 | 600 (765) | 725 (794) | 700 (960) | 0.33 |
| APACHE II score | 8 (5) | 8 (3) | 0.47 | 8 (2) | 7(6) | 9 (4) | 0.82 |
| SOFA score | | | | | | | |
| Day 0 | 7 (2) | 6 (4) | 0.36 | 4 (3) | 5 (3) | 5(2) | 1.00 |
| Day 1 | 5 (3) | 4 (5) | 0.18 | 5 (2) | 4 (6) | 4 (2) | 0.47 |
| Day 2 | 3 (1) | 3 (4) | 0.92 | 3 (4) | 2 (5) | 4 (2) | 0.97 |
| Day 3 | 1 (2) | 1 (4) | 0.15 | 3 (2) | 2 (3) | 3 (5) | 0.26 |
| SIRS (days 0–10) | 13 (77) | 27 (96) | 0.06 | 5 (100) | 14 (100) | 8 (89) | 0.17 |
| Sepsis (days 0–10) | 0 | 17 (64) | <0.001 | 0 | 11 (79) | 6 (67) | <0.001 |
| Septic shock (days 0–10) | 0 | 6 (21) | 0.07 | 0 | 3 (21) | 3 (33) | 0.06 |
| Prophylactic antibiotics i.o. | 17 (100) | 22 (100) | na | 5 (100) | 14 (100) | 9 (100) | na |
| Antibiotics received (days 0–10) | 4 (24) | 20 (71) | 0.002 | 10 (71) | 3 (66) | 7 (78) | 0.02 |
| Microbiology | | | | | | | |
| *Enterobacteriaceae* | 0 | 7 (25) | 0.03 | 0 | 5 (36) | 2 (22) | 0.03 |
| *Pseudomonaceae* | 0 | 5 (18) | 0.14 | 0 | 3 (21) | 2 (22) | 0.15 |
| *Staphylococcaceae* | 0 | 1 (4) | 1.00 | 0 | 1 (7) | 0 | 0.50 |
| *Streptococcaceae* | 0 | 1 (4) | 1.00 | 0 | 0 | 1 (11) | 0.25 |
| Miscellaneous | 0 | 6 (21) | 0.07 | 0 | 3 (21) | 3 (33) | 0.06 |
| Vasopressor need (days 0–10) | 7 (40) | 12 (43) | 0.91 | 2 (40) | 5 (36) | 5 (56) | 0.82 |
| ICU days | 3 (1) | 3 (1) | 0.64 | 3 (2) | 4 (2) | 3 (5) | 0.43 |
| In hospital days | 12 (6) | 16 (10) | 0.007 | 20 (12) | 15 (6) | 19 (12) | 0.02 |
| 30-day mortality | 0 | 0 | na | 0 | 0 | 0 | na |

Median (inter-quartile range), number (percentage), where appropriate; $P^1$ comparison of uncomplicated vs. complicated patients by Mann-Whitney*U* or Fisher's exact test where appropriate. $P^2$ comparison of uncomplicated patient and all three complication groups by Kruskal-Wallis *H* or $X^2$test, where appropriate.

Abbreviations: *APACHE* Acute Physiology and Chronic Health Evaluation, *ASA class* American Society of Anesthesiology physical status classification, *BMI*body mass index, *FEV1* forced expiratory volume in 1 s, *VC* vital capacity, *ICU* intensive care unit, *i.o.* intra-operatively, *m* male, *na* not applicable, *P-POSSUM* Portsmouth Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity, s/i surgical/infectious, *SIRS* Systemic Inflammatory Response Syndrome, *SOFA* Sequential Organ Failure Assessment, *TH* transhiatal, *TT* transthoracic, *WHO* World Health Organization.

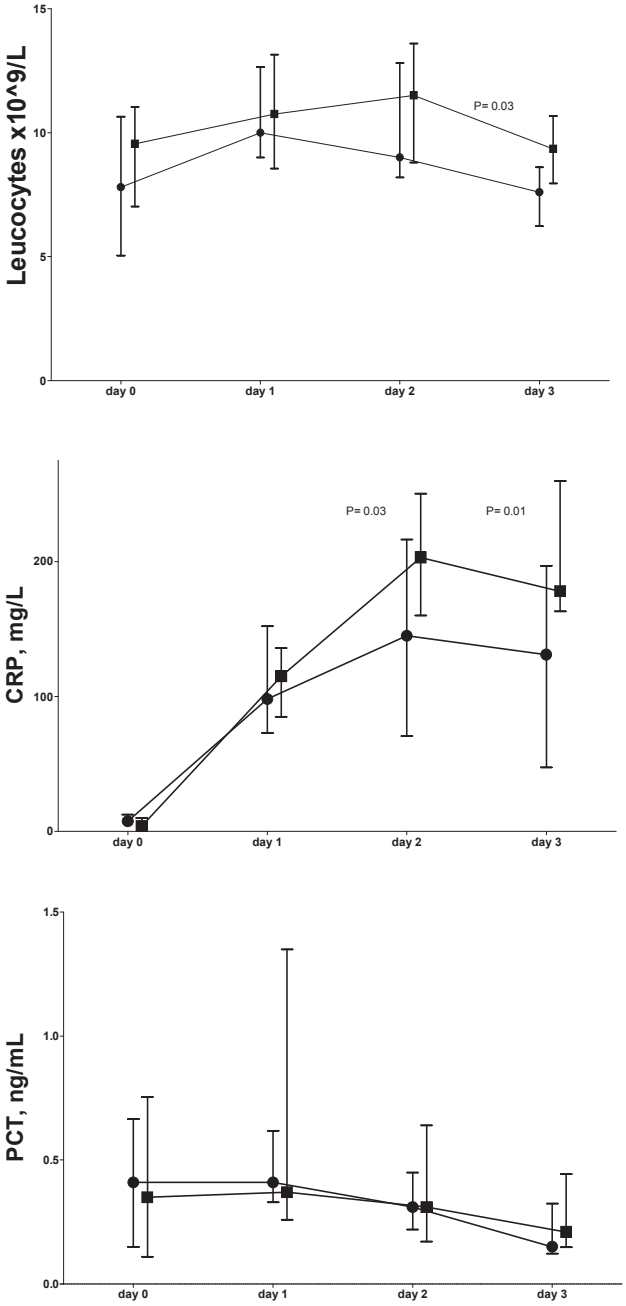


**Figure 1.** Early leukocyte and plasma biomarker levels (median and interquartile range) for complications up to 10 days after elective esophagectomy.
● without complications (N=17), ■ with complications (N=28). Abbreviations: CRP- C-reactive protein, PCT- procalcitonin. P values refer to Mann-Whitney U test.

**Table 4.** Diagnostic values of biomarkers (day 0-3) for complications (up to day 10).

| | Cutoff | AUROC | P value | SN | SP | PPV | NPV |
|---|---|---|---|---|---|---|---|
| **Diagnostic values for any complication** | | | | | | | |
| Leukocytes day 3 | $7.9 \times 10^9$/L | 0.71 | 0.02 | 75 | 64 | 78 | 60 |
| CRP day 2 | 100 mg/L | 0.71 | 0.04 | 100 | 36 | 74 | 100 |
| CRP day 3 | 68 mg/L | 0.75 | 0.006 | 100 | 43 | 75 | 100 |
| Δ CRP days 0–3 | 23 | 0.75 | 0.01 | 75 | 78 | 86 | 64 |
| **Diagnostic values for combined surgical/infectious complications** | | | | | | | |
| CRP day 3 | 316 mg/L | 0.80 | <0.001 | 0 | 100 | - | 84 |
| Δ CRP days 0–3 | 81 | 0.77 | 0.008 | 40 | 90 | 50 | 86 |
| PCT day 3 | 1.15 ng/mL | 0.86 | <0.001 | 38 | 100 | 100 | 81 |
| **Diagnostic values for anastomotic leak** | | | | | | | |
| CRP day 3 | 229 mg/L | 0.78 | 0.002 | 71 | 84 | 50 | 93 |
| Δ CRP days 0–3 | 55 | 0.82 | <0.001 | 80 | 80 | 50 | 94 |
| PCT day 1 | 1.82 ng/mL | 0.76 | 0.005 | 22 | 100 | 100 | 83 |
| PCT day 3 | 0.35 ng/mL | 0.86 | <0.001 | 67 | 80 | 55 | 87 |

Abbreviations: AUROC - Area Under the Receiver Operating Characteristics curve, CRP - C-reactive protein, NPV - Negative Predictive Value, PCT- Procalcitonin, PPV - Positive Predictive Value, SN - Sensitivity, SP – Specificity, Δ - fractional change (day 3 divided by day 0 value).

high sensitivities of optimal cutoff values. The fractional change of CRP levels on day 3 vs. day 0 in patients developing complications was 46 (91) and in patients without complications 19 (27), P=0.04. PCT levels could not discriminate between patients developing any type of complication and those without.

### Biomarker levels prior to diagnosis of complication subtypes

Figure 2 shows the data (days 0-3) for complication subtypes. On day 3, leukocyte counts were higher in patients with combined surgical/infectious complications than those without complications (P=0.01). The CRP levels on day 2 and 3 were higher in patients with infectious complications than in those without complications (P=0.03), whereas day 3 CRP levels were higher in patients with combined complications than in those without (P=0.01). The fractional increase in CRP was higher in patients developing combined complications, by 76 (41), than in patients without complications, by 19 (27), P=0.02. On day 3, PCT levels were higher in patients developing combined surgical/infectious complications than in those without complications and developing surgical or infectious complications (P=0.009).

Figure 3 shows the data (day 0-3) for patients with anastomotic leakage versus patients with other complications or without complications. On day 2 CRP levels were higher in patients developing other complications than anastomotic leakage compared to those without complications (P=0.02). However, on day 3 the CRP levels were

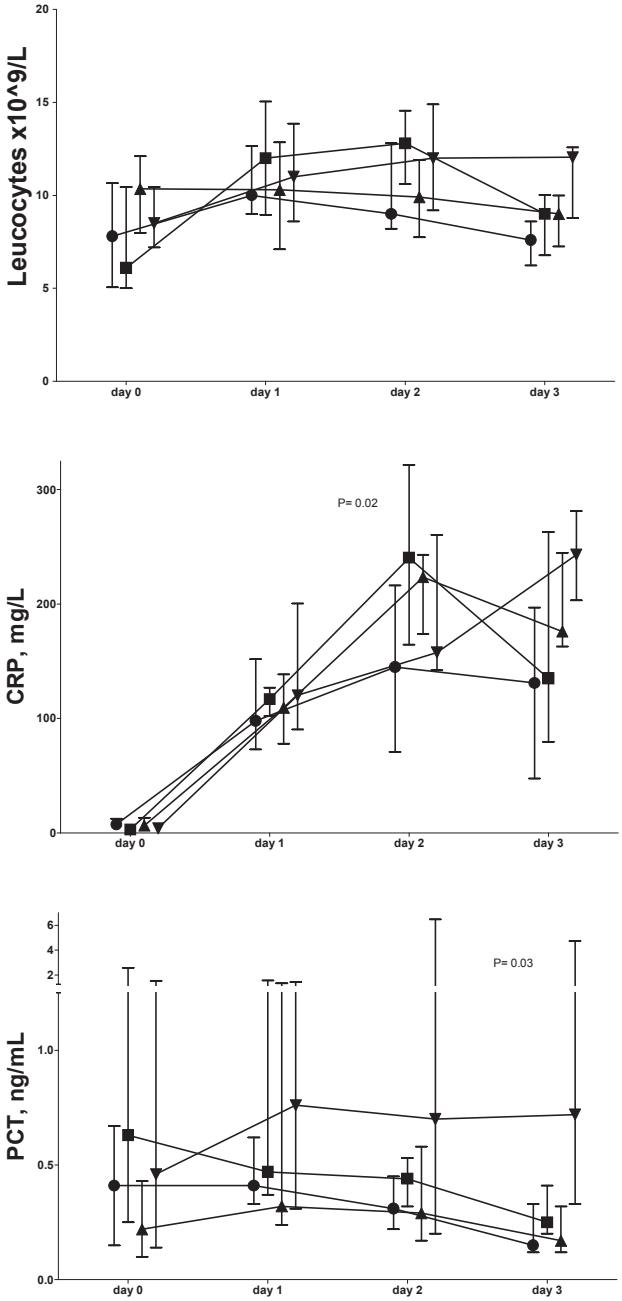


**Figure 2.** Early leukocyte and plasma biomarker levels (median and interquartile range) complications up to 10 days after elective esophagectomy.
● without complications (N=17), ■ surgical complications (N=5), ▲ infectious complications (N=14), ▼ combined surgical/infectious complications (N=9). Abbreviations: CRP - C-reactive protein, PCT - procalcitonin. P-values refer to Kruskal-Wallis test.

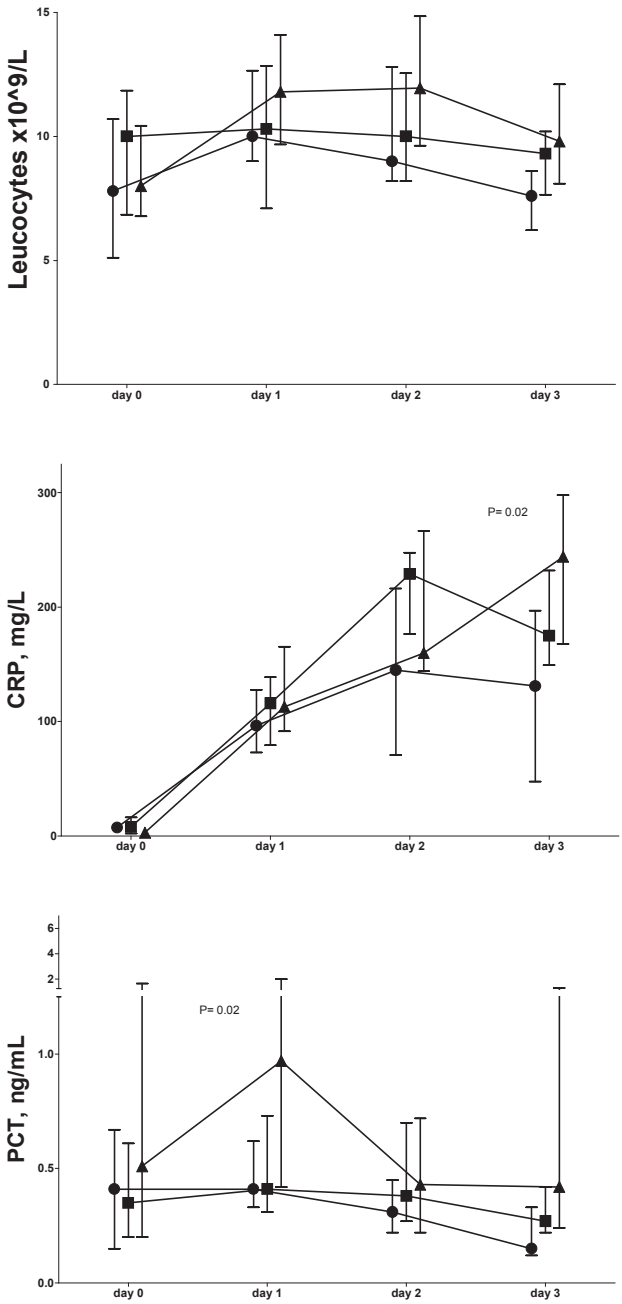


**Figure 3.** Early leukocyte and plasma biomarker levels (median and interquartile range) complications up to 10 days after elective esophagectomy.
● without complications (N=17), ■ with other complications (N=18), ▲ with anastomotic leakage (N= 10), Abbreviations: CRP- C-reactive protein, PCT- procalcitonin. P-values refer to Kruskal-Wallis test.

higher in patients developing anastomotic leak compared to all patients (N=35) without leakage (P=0.02). The PCT levels on day 1 and 3 were higher in patients with anastomotic leakage compared to all patients (N=35) without leakage (P=0.02 and P=0.03, respectively). Furthermore, the day 1 PCT levels were higher in patients with anastomotic leakage vs. other complications (P=0.02).

The diagnostic value of elevated day 3 PCT preceded the clinical diagnosis of combined complications and anastomotic leakage, as did the day 3 CRP levels and their fractional changes (Table 4). The diagnostic value of high day 1 PCT levels already preceded anastomotic leakage however.

### Multiple logistic regression

On day 2, CRP was diagnostic for developing complications independently from leukocytes and PCT (P=0.038, Hosmer Lemeshow $X^2$ 5.22, df8, P=0.734). On day 3 CRP levels were diagnostic for developing anastomotic leakage independently form leukocytes and PCT (P=0.032, Hosmer Lemeshow $X^2$ 8.8, df7, P=0.268). On day 1, PCT was diagnostic for developing anastomotic leak independently from leukocytes and CRP (P=0.016, Hosmer Lemeshow $X^2$ 8.064, df8, P=0.427).

## DISCUSSION

This relatively small study suggests that elevated CRP levels are a sensitive marker of complications developing post-oesophagectomy, whereas elevated PCT levels may specifically indicate the development of more severe combined surgical/infectious complications, mainly associated with anastomotic leakage, within 3 to 10 days post-oesophagectomy.

Even though all patients had low ASA-classification, P-POSSUM and APACHE II scores, 62% had early postoperative complications. There were no fatalities within 30 days postoperatively. The preoperative risk-assessment scores were comparable between groups and thus unsuitable for indicating development of a complicated postoperative clinical course. Although the complication rate appears relatively high, the rate and type are in line with the literature.[2-9,11-13,22,27,28] Up till now there are no uniformly accepted guidelines for reporting of postoperative complications, and a recent systematic review has shown a wide range in definitions hampering interpretation of study results.[4] The difficulty in uniform, mutually exclusive complication categories makes interpretation and comparison of studies difficult. We grouped complications since they represent different conditions and associated severities, whereas the group was too small to attempt to discriminate between individual complications. Patients who developed combined surgical/infectious complications had more complications

simultaneously than patients in the other complication groups. Furthermore, their hospital stay was longer than of patients with infectious complications or without complications.

This is the first study trying to discriminate among early postoperative complication types by using CRP and PCT. All complications presented on day 3 or later and in 92% of cases complications presented on day 4 or later. We may argue that since the cutoff values of day 2 and 3 biomarker levels precede the clinical symptoms and diagnosis of complication they are predictive in time. The elevation of CRP levels in patients without complications is also comparable to that reported before.[6,8,9,11-13] Studies reported high PCT levels, as in our study, after oesophagectomy or other extensive gastrointestinal surgeries irrespective of complications,[1,15,19] and high PCT levels, albeit not more elevated than CRP, in major anastomotic leakage after colorectal surgery.[16,18] Based on our observations and those of others,[6,7,9,12,15,17] one may thus hypothesize that both CRP and PCT increase following a surgical host response, but that PCT follows a more severe manifestation of this response, particularly when associated with surgical/infectious complications. Indeed, we could not discriminate infectious complications from surgical complications by use of PCT or CRP, but PCT rather than CRP was able to identify patients at risk for more severe combined complications after oesophagectomy.

In detail, CRP levels on day 2 and 3 were diagnostic for any complication presenting between days 3-10, independent of preoperative risk assessment score and SIRS criteria. The calculated sensitivity and specificity are similar to those reported in some previous studies,[9,10] but in slight contrast to others who found a diagnostic value of CRP no sooner than on postoperative day 4,[8,11,12] or no diagnostic value at all for anastomotic leakage or infectious complications.[6,13] In our study, CRP levels nor fractional increases could differentiate between complication groups, limiting the use of CRP levels for early recognition of complication subtypes. The low specificity and modest positive predictive value calculated from the AUROC suggest that the use of an elevated CRP alone as an indicator of developing complications post-oesophagectomy may lead to antibiotic overtreatment, amongst others, if considered specific for infection.

Plasma PCT levels have been studied and compared with CRP in patients after major surgery and trauma, but the results are inconclusive.[14-18,20] So far, one study on post-oesophagectomy showed a diagnostic value of PCT for development of sepsis,[11] and another one for infectious complications.[13] We found an early diagnostic value of day 3 PCT levels for combined surgical/infectious complications presenting between days 3-10 independently from preoperative risk-assessment scores, but not of infectious complications alone. PCT was the only marker of help in the early diagnosis of more severe complications and the earliest one to recognize anastomotic leakage, the most

common combined surgical/infectious complication. Even though the AUROC of day 3 CRP was statistically significant for combined complications the marker level had little positive predictive value. The positive predictive value of PCT levels is higher and PCT is therefore preferred over CRP for diagnosis of combined complications. As a result, elevated PCT levels at the cutoff levels presented could guide additional diagnostics and start of empirical antibiotics before full blown presentation of complications post-oesophagectomy.

The leukocyte counts peaked around the upper limit of normal on day 2 in agreement with some studies.[6,8,10,11] This relatively low leukocyte peak count could be explained by neo-adjuvant chemotherapy in the majority of patients. Some investigators found a moderately elevated leukocyte count on day 2 to 5 to predict anastomotic leak and infectious complications.[8,10,11] The leukocyte count in our study did not discriminate between surgical, infectious or combined complications and is therefore not useful for this purpose, as in other studies.[6,13] We included this SIRS criterion for reasons of comparison with CRP and PCT.

The limitations of this proof of principle study include its relatively small and heterogeneous sample size. Furthermore, little is known about the effects of neo-adjuvant chemo-radiotherapy on biomarker release and kinetics. However, almost all patients in our study received such treatment and predictive values of biomarkers were maintained. There is no difference in effect on postoperative CRP and PCT values reported between laparoscopic versus open surgery or between the transhiatal and transthoracic approach, respectively.[1,29]

## Conclusion

An increasing or high CRP level within 3 days after elective oesophagectomy may contribute to the early diagnosis of any postoperative complications presenting between postoperative days 3 and 10, independent of the preoperative risk assessment scores. Elevated PCT levels may specifically indicate severe combined surgical/infectious complications, mainly associated with anastomotic leakage, but may not recognize infectious complications alone. Nevertheless, PCT rather than CRP might be used for decisions on additional diagnostics and empirical antibiotic treatment in these patients.

## REFERENCES


1. Márton S, Szakmány T, Papp A, et al. Does transthoracic compared to transhiatal resection alter the early postoperative course of oesophagectomy? Dis Esophagus 2005;18:155-9.
2. Grotenhuis BA, van Hagen P, Reitsma JB, et al. Validation of a nomogram predicting complications after oesophagectomy for cancer. Ann Thorac Surg 2010;90:920-5.
3. Ferguson MK, Celauro AD, Prachand V. Prediction of major pulmonary complications after oesophagectomy. Ann Thorac Surg 2011;91:1494-1500.
4. Blencowe NS, Strong S, McNair AG, et al. Reporting of short-term clinical outcomes after oesophagectomy: a systematic review. Ann Surg 2012;255:658-66.
5. Carrott PW, Markar SR, Kuppusamy MK, et al. Accordion severity grading system: assessment of relationship between costs, length of hospital stay, and survival in patients with complications after oesophagectomy for cancer. J Am Coll Surg 2012;215:331-6.
6. Tsujimoto H, Ono S, Takahata R, et al. Systemic inflammatory response syndrome as a predictor of anastomotic leakage after oesophagectomy. Surg Today 2012;42:141-6.
7. Haga Y, Beppu T, Doi K, et al. Systemic inflammatory response syndrome and organ dysfunction following gastrointestinal surgery. Crit Care Med 1997;25:1994-2000.
8. Noble F, Curtis N, Harris S, et al. South Coast Cancer Collaboration–Oesophago-Gastric (SC-OG). Risk assessment using a novel score to predict anastomotic leak and major complications after oesophageal resection. J Gastrointest Surg 2012;16:1083-95.
9. van Genderen ME, Lima A, de Geus H, et al. Serum C-reactive protein as a predictor of morbidity and mortality in intensive care unit patients after oesophagectomy. Ann Thorac Surg 2011;91:1775-9.
10. Dutta S, Fullarton GM, Forshaw MJ, et al. Persistent elevation of C-reactive protein following esophagogastric cancer resection as a predictor of postoperative surgical site infectious complications. World J Surg 2011;35:1017-1025
11. Durila M, Bronský J, Haruštiak T et al. Early diagnostic markers of sepsis after oesophagectomy (including thromboelastography). BMC Anesthesiol 2012;28:12.
12. Warschkow R, Tarantino I, Ukegjini K, et al. Diagnostic study and meta-analysis of C-reactive protein as a predictor of postoperative inflammatory complications after gastroesophageal cancer surgery. Langenbecks Arch Surg 2012;397:727-36.
13. Ito S, Sato N, Kojika M, et al. Serum procalcitonin levels are elevated in esophageal cancer patients with postoperative infectious complications. Eur J Surg Res 2005;37:22-28.
14. Mokart D, Merlin M, Sannini A, et al. Procalcitonin, interleukin 6 and systemic inflammatory response syndrome (SIRS): early markers of postoperative sepsis after major surgery. Br J Anaesth 2005;94:767-73.
15. Uzzan B, Cohen R, Nicolas P, et al. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. Crit Care Med 2006;34:1996-2003.

16. Garcia-Granero A, Frasson M, Flor-Lorente B, et al. Procalcitonin and C-reactive protein as early predictors of anastomotic leak in colorectal surgery: a prospective observational study. Dis Colon Rectum 2013;56:475-83.
17. Hoeboer SH, Alberts E, van den Hul I, et al. Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin. J Infect 2012;64:484-93.
18. Lagoutte N, Facy O, Ravoire A, et al. C-reactive protein and procalcitonin for the early detection of anastomotic leakage after elective colorectal surgery: pilot study in 100 patients. J Visc Surg 2012;149:e345-9.
19. Molnár Z, Szakmány T, Köszegi T, Tekeres M. Microalbuminuria and serum procalcitonin levels following oesophagectomy. Eur J Anaesthesiol 2000;17:464-5.
20. Bogar L, Molnar Z, Tarsoly P, et al. Serum procalcitonin level and leukocyte antisedimentation rate as early predictors of respiratory dysfunction after oesophageal tumour resection. Crit Care 2006;10:R110
21. Omloo JM, Lagarde SM, Hulscher JB, et al. Extended transthoracic resection compared with limited transhiatal resection for adenocarcinoma of the mid/distal esophagus: five-year survival of a randomized clinical trial. Ann Surg 2007;246:992-1000.
22. Nederlof N, Tilanus HW, Tran TC, et al. End-to-end versus end-to-side esophagogastrostomy after esophageal cancer resection: a prospective randomized study. Ann Surg. 2011;254:226-33.
23. Calandra T, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. Crit Care Med 2005;33:1538e48.
24. Levy MM, Fink MP, Marshall JC, et al. International Sepsis Definitions Conference. 2001 SCCM/ESICM/ACCP/ ATS/SIS international sepsis definitions conference. Intensive Care Med 2003;29:530–538.
25. Zweig MH, Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clinical Chemistry 1993;39:561-577.
26. McLaughlin MJ, Sainani KL. Bonferroni, Holm, and Hochberg corrections: fun names, serious changes to P values. PM R. 2014 Jun;6:544-6.
27. Atkins BZ, Shah AS, Hutcheson KA, et al. Reducing hospital morbidity and mortality following oesophagectomy. Ann Thorac Surg. 2004;78:1170-6; discussion 1170-6.
28. van Hagen P, Hulshof MC, van Lanschot JJ, et al. CROSS group. Preoperative chemoradiotherapy for esophageal or junctional cancer. N Engl J Med. 2012; 366:2074-84.
29. Scheepers JJG, Sietses C, Bos DG, et al. Immunological consequences of laparoscopic versus open transhiatal resection of the distal esophagus and gastroeophageal junction. Dig Surg 2008;25:140-147.

# 4

# The diagnostic accuracy of procalcitonin for bacteraemia: a systematic review and meta-analysis

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## ABSTRACT

**Objective** The diagnostic use of procalcitonin for bacterial infections remains a matter of debate. So far most studies used ambiguous outcome measures such as sepsis instead of infection. We performed a systematic review and meta-analysis to investigate the diagnostic accuracy of procalcitonin for bacteraemia, a proven bloodstream infection.

**Methods** We searched all major databases from inception to June 2014 for original, English written, research articles that studied the diagnostic accuracy between procalcitonin and positive blood cultures in adult patients. We calculated the area under the summary receiver-operating characteristic curves (SROC) and pooled sensitivities and specificities. To minimise potential heterogeneity we performed subgroup analyses.

**Results** In total 58 of 1,567 eligible studies were included in the meta-analysis and provided a total of 16,514 patients of whom 3,420 suffered from bacteraemia. In the overall analysis the SROC was 0.79. The optimal and most widely used procalcitonin cutoff value was 0.5 ng/mL with a corresponding sensitivity of 76% and specificity of 69%. In subgroup analyses the lowest SROC was found in immunocompromised/neutropenic patients (0.71), the highest SROC was found in intensive care patients (0.88), sensitivities ranging 66-89% and specificities 55-78%.

**Conclusions** In spite of study heterogeneity, procalcitonin had a fair diagnostic accuracy for bacteraemia in adult patients suspected of infection or sepsis. In particular low procalcitonin levels can be used to rule out the presence of bacteraemia. Further research on the safety and efficacy of procalcitonin as a single diagnostic tool to withhold taking blood cultures is needed.

## INTRODUCTION

Infection and the subsequent sepsis syndrome are associated with morbidity and mortality.[1,2] The fear of undertreatment leads to the routine collection of specimen for microbiological culture and initiation of empiric antibiotic therapy.[3] On the other hand, antibiotic overuse increases microbial selection and resistance and can cause adverse drug reactions.[4] To assist the diagnosis of infection in clinical practice its symptoms have been grouped into the systemic inflammatory response syndrome (SIRS).[5] A clinically suspected or proven infection in the presence of SIRS is termed sepsis.[5] In recent years authors have studied the use of biomarkers, like procalcitonin, to improve the diagnosis of the sepsis syndrome rather than of proven infection.[6-9] The use of the sepsis syndrome as a surrogate for proven infection as an outcome parameter may be too sensitive and nonspecific. This could partially explain the contradicting results in previous studies[6-9,10] and meta-analyses on the diagnostic use of procalcitonin for sepsis.[11-18]

The definition of proven local infection remains matter of debate and we therefore study the more robustly defined proven bloodstream infections, i.e. bacteraemia. Bacteraemia can be identified in about 30% of septic patients and necessitates further diagnostic evaluation.[19] However, culture results take several days and can be falsely negative in patients on antibiotic treatment.[20-22] Recent studies demonstrated that procalcitonin can accurately predict bacteraemia in patients with community-acquired pneumonia,[23] acute fever,[24] and in elderly patients suspected of infection.[25] Procalcitonin can also accurately discriminate between true bacteraemia and coagulase negative staphylococci-contaminated blood cultures.[26] Another study demonstrated that bacteraemia is unlikely when procalcitonin levels are low.[27] Some meta-analyses focused on the diagnostic value of procalcitonin for microbiologically confirmed local infection[28-39] or bacteraemia.[40] However, the number of included studies was small, specific patient subgroups were analysed or studies concerning sepsis were included as well.[28-40]

We therefore performed a systematic review and meta-analysis to investigate the diagnostic accuracy of procalcitonin for bacteraemia. Our hypothesis is that in adult patients suspected for infection or sepsis procalcitonin is a useful biomarker of bacteraemia.

## METHODS

### Search strategy and study selection

We used the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement for reporting this systematic review and meta-analysis.[41] A flow-

chart of the literature search can be found in Figure 1. All prospective and retrospective, original, observational (case-control, cross sectional, cohort and longitudinal) studies published in English from inception until June 2014 were considered eligible for inclusion. Studies were screened by title and abstract and definite inclusion was decided upon after full text review.

We included studies on adult hospitalised patients suspected of infection or sepsis, in which bacteraemia with a known pathogen was confirmed by blood culture and measurement of procalcitonin levels was performed within 24 hours of inclusion. Studies had to give a detailed description of patient groups and demographic variables. The comparison of procalcitonin levels had to be between hospitalised patients with

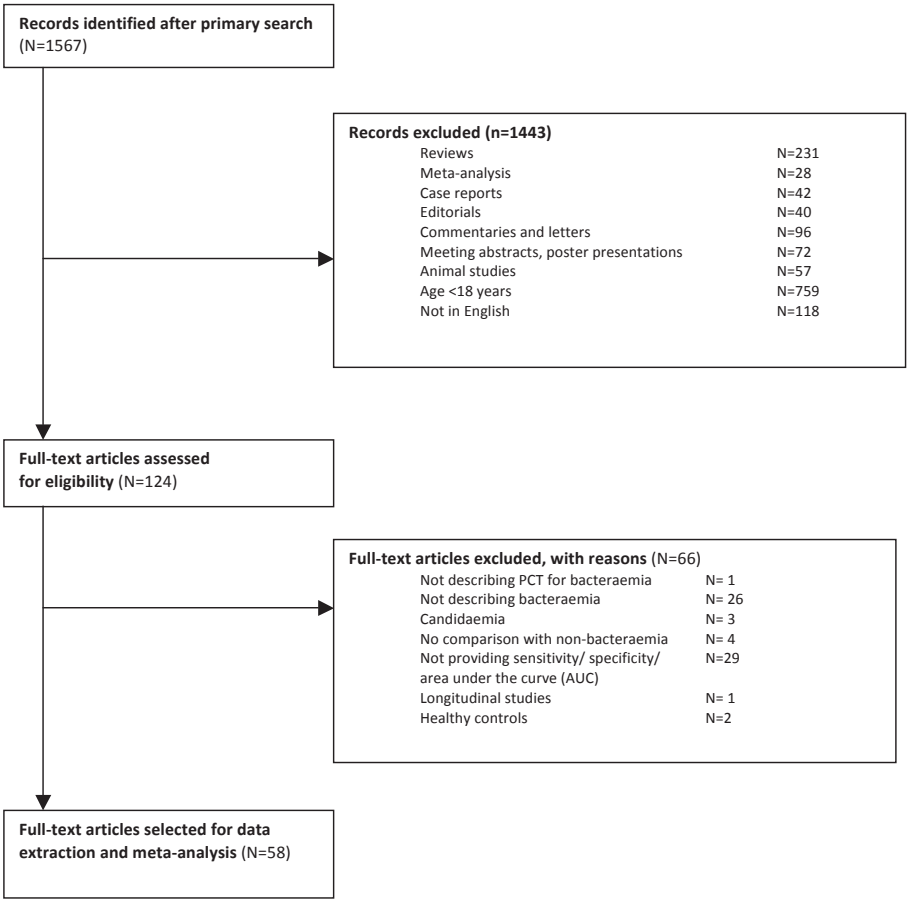


**Figure 1.** Flow chart of literature search.
Searched databases: PubMed, Medline, Embase, ISI Web of Knowledge, the Cochrane Library, Scopus, BioMed Central, and Science Direct.
Search strategy: (procalcitonin OR PCT) AND (bacterial infection OR bacteraemia OR bloodstream infection).

and without bacteraemia, regardless of clinical symptoms. To be included for analysis studies had to report the diagnostic accuracy estimates of procalcitonin for bacteraemia; knowingly area under the curve (AUC), sensitivity, specificity and corresponding P-values. The corresponding authors of eligible studies that did not provide sufficient data for meta-analysis were contacted to retrieve additional data. We excluded case-control studies were controls were healthy subjects, reviews, meta-analyses, case reports, editorials, commentaries, letters, meeting abstracts, poster presentations, animal studies and research performed in children (<18 years old). Two investigators (SHH and PJG) independently evaluated all eligible studies for inclusion and extracted the data. In case of disagreement a third investigator (ABJG) was consulted.

**Quality assessment**

We used the Quality Assessment of Diagnostic Accuracy Studies (QUADAS) tool,[42] scores range from 0 to 14, to assess the methodological quality of included studies.

**Statistical methods**

To avoid double inclusion of the same patient group we only included one sensitivity and specificity from each article, unless results clearly came from different patient groups. We used the bivariate random-effects regression model for pooling the sensitivity and specificity estimates, as recommended by the Cochrane Diagnostic Test Accuracy Working Group.[43] The bivariate model takes into account the potential trade-off between sensitivity and specificity by explicitly incorporating this negative correlation in the analysis.[44, 45] Cutoff values differed among the included studies, the cutoff value closest to 0.5 ng/mL was used for the analysis if multiple cutoff values were given. The 0.5 ng/ml cutoff was chosen based on recommendations of the manufacturer, current literature[46-49] and was the cutoff used most often in the included studies (Table 1). Summary receiver-operating characteristics curves (SROC) were drawn using the bivariate model. The closer the curve is to the upper left-hand corner of the SROC curve plot, the better the overall accuracy of the test. An area under the SROC curve between 0.90-1.0 is considered as excellent diagnostic accuracy, 0.80-0.90 as good, 0.70-0.80 as fair, 0.60-0.70 as poor and 0.50-0.60 as fail.[50] We expected substantial heterogeneity in the of the overall analysis and in order to obtain more homogenous results subgroup analysis were performed. First, we calculated the diagnostic accuracy in specific patient subgroups based on their underlying disease. We calculated the diagnostic accuracy in studies comparing bacteraemia vs. non-bacteraemia in patients with SIRS and comparing bacteraemia vs. non-bacteraemia in patients with SIRS developing localised infections. When a specific subgroup for the controls could not be identified we categorised the study in the category non-bacteraemia. We studied the diagnostic accuracy of procalcitonin for bacteraemia in immunocompromised/neutro-

**Table 1.** Study characteristics.

| Reference | Country | Inclusion criteria | Study population (N) | With bacteraemia (N) | Male (%) | Age (years) | Department | Type of patients | Immunocompromised | Assay type | Cut-off values, ng/mL | Quadas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Aalto 2004 [58] | Finland | Suspicion of systemic infection | 92 | 13 | 48 | 52 | ED | medical | no | 1 | 0.4 | 12 |
| Albrich 2011 [59] | Switzerland | Performing blood cultures[R] | 295 | 16 | - | 48 | ED | medical | no | 2 | 0.15 | 12 |
| Bell 2003[60] | Australia | SIRS and suspicion of infection | 123 | 12 | 66 | 61 | ICU | mixed | no | 1 | 3.03 | 11 |
| Bogar 2006 [61] | Hungary | New onset fever[*] | 39 | 23 | 71 | 56 | ICU | mixed | yes | 1 | N.A. | 12 |
| Bossink 1999 [62] | Netherlands | New onset fever | 300 | 53 | 51 | 60 | ward | medical | no | 1 | N.A. | 12 |
| Caterino 2004 [63] | USA | >65 years and performing blood cultures[*] | 108 | 14 | 50 | 76 | ED | medical | no | 1 | 0.5 | 13 |
| Charles 2008 [64] | France | Critically ill with BSI and VAP[R] | 161 | 117 | 58 | 65 | ICU | mixed | no | 2 | 0.5 | 13 |
| Chen 2011 [65] | China | Suspicion of catheter-related BSI | 55 | 25 | 65 | 53 | mixed | medical | no | 1 | 3.1 | 12 |
| Cheval 2000 [66] | France | Sepsis/ septic shock and no infection | 60 | 9 | 57 | 58 | ward | mixed | no | 1 | 0.55 | 10 |
| Chirouze 2002 [67] | France | Acute fever | 165 | 22 | 58 | 58 | ward | medical | no | 1 | 0.12 | 12 |
| Dwolatzky 2005 [68] | Israel | Proven microbial infection | 187 | 16 | 30 | 83 | ED | medical | no | 1 | 0.5 | 13 |
| Engel 1999 [69] | Germany | Febrile neutropenia | 44 | 15 | - | 47 | ward | medical | yes | 1 | 0.51 | 11 |
| Gac 2011 [70] | France | Febrile neutropenia[*] | 29 | 10 | 53 | 56 | ward | medical | yes | 3 | 0.5 | 11 |
| Gaini 2007 [71] | Denmark | Suspicion of severe infection | 154 | 34 | 50 | 61 | ward | medical | no | 2 | 2.19 | 13 |
| Giamarellos 2001 [72] | Greece | Febrile neutropenia. | 115 | 28 | 70 | 56 | ward | medical | yes | 1 | 0.5 | 11 |
| Giamarellou 2004 [73] | Greece | Febrile neutropenia. | 158 | 52 | 56 | 52 | ward | medical | yes | 2 | 1 | 11 |

**Table 1.** Study characteristics (continued)

| Reference | Country | Inclusion criteria | Study population (N) | With bacteraemia (N) | Male (%) | Age (years) | Department | Type of patients | Immunocompromised | Assay type | Cut-off values, ng/mL | Quadas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Guinard-Barbier 2011 [53] | France | Acute pyelonephritis* | 347 | 58 | 8 | 33 | ED | medical | no | 2 | 0.3 | 13 |
| Ha 2013 [74] | South Korea | Acute pyelonephritis[R] | 147 | 84 | 15 | 61 | ED | medical | no | 4 | 0.5 | 12 |
| Hoeboer 2012 [75] | Netherlands | New onset fever | 101 | 12 | 68 | 64 | ICU | mixed | no | 2 | 2.44 | 12 |
| Hoenigl 2013 [54] | Austria | SIRS and suspicion of infection* | 132 | 55 | 48 | 69 | ED | medical | no | 4 | N.A. | 13 |
| Hoenigl 2014 [76] | Austria | SIRS and performing blood cultures* | 898 | 666 | 58 | 67 | ED | medical | no | 3 | 0.5 | 12 |
| Jeong 2012 [77] | South Korea | Suspicion of bacteraemia [R] | 3343 | 331 | 59 | 65 | mixed | medical | no | 1 | 0.35 | 12 |
| Jimeno 2004 [78] | Spain | Febrile neutropenia. | 104 | 15 | 38 | 58 | ward | medical | yes | 1 | 0.5 | 11 |
| Kallio 2000 [79] | Finland | Cancer and suspicion for infection | 56 | 8 | 63 | 57 | ward | medical | yes | 1 | 0.36 | 12 |
| Karlsson 2010 [80] | Finland | Severe sepsis or septic shock | 160 | 69 | 68 | 60 | ICU | mixed | no | 3 | 1.2 | 14 |
| Kim D 2011 [81] | South Korea | Febrile neutropenia. | 286 | 38 | 57 | 39 | ED | medical | yes | - | 0.5 | 7 |
| Kim M 2011 [24] | South Korea | Fever and performing blood cultures | 252 | 31 | 44 | 54 | ED | medical | no | 4 | 0.5 | 7 |
| Koivula 2011 [82] | Finland | Febrile neutropenia. | 90 | 21 | 66 | 56 | ward | medical | yes | 2 | 0.5 | 11 |
| Lai 2010 [25] | Taiwan | SIRS and suspicion of infection | 155 | 48 | 60 | 77 | ED | medical | no | 2 | 0.38 | 13 |
| Lee 2013 [83] | South Korea | PCT measurements [R] | 357 | 199 | 53 | 66 | mixed | medical | no | 4 | 0.55 | 13 |
| Liaudat 2001[84] | Switzerland | Performing blood cultures | 200 | 50 | 52 | 60 | mixed | mixed | no | 1 | 0.5 | 12 |
| Loonen 2014 [85] | Netherlands | SIRS and suspicion of infection[R] | 125 | 27 | 60 | 65 | ED | medical | no | 3 | 2.0 | 14 |

**Table 1.** Study characteristics (continued)

| Reference | Country | Inclusion criteria | Study population (N) | With bacteraemia (N) | Male (%) | Age (years) | Department | Type of patients | Immunocompromised | Assay type | Cut-off values, ng/mL | Quadas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Mencacci 2012 [86] | Italy | Fever and suspicion of sepsis | 1009 | 133 | 55 | 69 | mixed | mixed | no | 4 | 0.37 | 13 |
| Menendez 2012 [55] | Spain | Pneumonia* | 685 | 48 | 59 | 64 | ward | medical | no | 6 | 0.36 | 12 |
| Muller 2010 [23] | Switzerland | Pneumonia* | 925 | 73 | 59 | 73 | ED | medical | no | 2 | 0.5 | 13 |
| Munoz 2004 [87] | Spain | Fever | 103 | 23 | 31 | 59 | ward | mixed | no | 1 | 0.1 | 11 |
| Nakamura 2009 [88] | Japan | High fever suspicion of bacteraemia | 116 | 65 | 65 | 59 | ICU | mixed | no | 4 | 0.38 | 10 |
| Nieuwkoop van 2010 [89] | Netherlands | Fever and urinary tract infection | 581 | 131 | 38 | 66 | mixed | mixed | no | 2 | 0.5 | 13 |
| Pereira 2013 [90] | Portugal | Pneumonia* | 108 | 15 | 63 | 61 | ICU | medical | no | 4 | 17 | 12 |
| Persson 2004 [91] | Sweden | Febrile neutropenia. | 94 | 21 | 41 | 54 | ward | medical | yes | 6 | 0.5 | 11 |
| Prat 2008 [92] | Spain | Febrile neutropenia. | 61 | 19 | 51 | 47 | ward | medical | yes | 2 | 0.5 | 8 |
| Ratzinger 2014 [93] | Austria | Suspicion of infection and performing blood cultures | 298 | 75 | 58 | 58 | ward | mixed | no | - | 0.35 | 13 |
| Riedel 2011 [27] | USA | Signs of infection and performing blood cultures[R] | 367 | 19 | - | 48 | ED | medical | no | 2 | 0.15 | 14 |
| Rintala 2001 [94] | Finland | Fever and a proven microbial infection | 29 | 13 | 52 | 49 | mixed | medical | no | 1 | <0.5 | 11 |
| Robinson 2011 [95] | Switzerland | Febrile neutropenia | 194 | 33 | 61 | 57 | ward | medical | yes | 2 | 0.5 | 12 |
| Romualdo 2014 [96] | Spain | SIRS and suspicion of infection | 226 | 37 | 58 | 69 | ED | medical | no | 3 | 0.45 | 13 |
| Schuetz 2007 [26] | Switzerland | Positive blood cultures | 19 | 7 | 65 | 63 | mixed | medical | no | 5 | 0.1 | 12 |
| Schuetz 2008 [97] | Switzerland | Pneumonia | 281 | 34 | 62 | 74 | ward | medical | no | 2 | 1.34 | 13 |

**Table 1.** Study characteristics (continued)

| Reference | Country | Inclusion criteria | Study population (N) | With bacteraemia (N) | Male (%) | Age (years) | Department | Type of patients | Immunocompromised | Assay type | Cut-off values, ng/mL | Quadas |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Shi 2013 [98] | China | New onset fever | 106 | 60 | 67 | 64 | ICU | mixed | no | 4 | N.A. | 12 |
| Shomali 2012 [99] | USA | Cancer and new fever | 248 | 30 | 57 | 56 | ward | medical | no | 2 | 0.5 | 13 |
| Su 2011 [100] | Taiwan | Performing blood cultures* | 558 | 84 | 57 | 61 | ED | medical | no | 1 | 0.5 | 12 |
| Suarez-Santamaria 2010 [101] | Spain | Proven microbial infection | 205 | 36 | 58 | 65 | ED | mixed | no | 6 | N.A. | 13 |
| Theodorou 2012 [57] | Greece | Suspicion of catheter related BSI+ | 46 | 26 | 61 | 48 | ICU | mixed | no | 1 | 0.7 | 13 |
| Tromp 2012 [102] | Netherlands | SIRS and suspicion of infection | 342 | 55 | 56 | 59 | ED | mixed | no | 2 | 0.5 | 13 |
| Tsalik 2012 [103] | USA | SIRS and suspicion of infection | 336 | 55 | 52 | 52 | ED | medical | no | 3 | 0.5 | 12 |
| Vanska 2012 [104] | Finland | Febrile neutropenia. | 100 | 19 | 61 | 66 | ward | medical | yes | 3 | 0.13 | 10 |
| von Lilienfeld-Toal 2004 [105] | Germany | Febrile neutropenia. | 53 | 18 | 48 | 57 | ward | medical | yes | 1 | 0.62 | 12 |
| Wang 2013 [10] | China | SIRS and performing blood cultures R* | 586 | 120 | 65 | 54 | mixed | mixed | no | 4 | 0.5 | 12 |
| **Total** | | | **16,514** | **3,420** | | | | | | | | **12 (7-14)** |

The mean or median age is provided, if mean/median was not provided, the mean age was manually calculated of the subgroups. Studies in which patients were excluded because of antibiotic use prior to PCT measurement are marked with an *. All studies have a prospective study design, retrospective studies are marked with R. Assay type: 1 = Lumitest Brahms, 2 = Kryptor Brahms, 3 = Elecsys Brahms Cobas Analyzer, 4 = Vidas Biomereux, 5= PCT sensitive Lia Brahms, 6 = Liason Brahms PCT. ED = emergency department; ICU = intensive care unit; USA = United States of America; QUADAS = quality assessment of diagnostic accuracy studies. BSI = bloodstream infection; SIRS = systemic inflammatory response syndrome; VAP = ventilator associated pneumonia.

penic and immunocompetent patients separately. We categorised all studies according to department of inclusion. Finally, we also studied retrospective studies separately from prospective studies. We tested for a threshold effect by adding a covariate for threshold to the bivariate model.

We used IBM statistics 21.0 (IBM SPSS, Chicago, IL, USA) and R 3.1.1 (Vienna, Austria XX) to analyse the data. The R package mada was used to perform the pooling of sensitivity and specificity and generating of SROC-curves. Pooled sensitivity and specificity estimates were generated, with their 95% confidence interval (CI). To assess heterogeneity among studies $I^2$ and $X^2$/cochrane Q statistics were performed. We used the Deeks funnel plot asymmetry test to evaluate potential publication bias.[51] $P<0.10$ for the slope coefficient is considered as significant asymmetry, which indicates potential publication bias. All other tests were two-sided and a $P<0.05$ was considered statistically significant; exact P-values >0.001 are given.

## RESULTS

### Literature search

The literature search resulted in a total of 1,567 articles of which 1,443 studies were excluded because of: written language other than English (N=118), age <18 years (N=759), in vitro/animal studies (N=57) or lack of original data (reviews, meta-analysis, case reports, editorials, commentaries and letters, meeting abstract, poster presentations, N=509). We performed a full text review of the 124 articles considered eligible for inclusion, which resulted in the exclusion of another 66 studies whom did not provide AUC values/ sensitivity/ specificity (N=29), did not study bacteraemia (N=26), did not compare to non-bacteraemia (N=4), studied candidaemia (N=3), used healthy controls (N=2), did not provide the procalcitonin level for bacteraemia (N=1), or used a longitudinal study design and analysis (N=1). The remaining 58 articles were used in the meta-analysis. The complete reference list containing all in- and excluded studies is presented in the supplemental material. Table 2 depicts the 66 studies excluded after full text review.

### Study characteristics and quality assessment

Table 1 provides some details of the included studies. In total, 16,514 patients of whom 3,420 suffered from bacteraemia were included. There was a slight tendency towards male preponderance. The average age ranged from 33 to 77 years. Eight studies had a retrospective and 50 a prospective study design. All 58 studies provided AUC values, but only 49 studies provided sensitivity and specificity. The cutoff values varied between 0.10 and 17 ng/mL. All samples for blood culture and procalcitonin

**Table 2.** Excluded studies.

| Reason of exclusion | Excluded study | |
|---|---|---|
| Not providing PCT | Pettila 2002 [6] | |
| Not studying bacteraemia | Adamzik 2010 [106] | Pizzolato 2014 [119] |
| | Barati 2008 [107] | Quiroga 2014 [120] |
| | Bele 2011[108] | Reynolds 2012 [121] |
| | Bugden 2004 [109] | Rowther 2009 [122] |
| | Delevaux 2002 [110] | Sakr 2008 [123] |
| | Fluri 2012 [111] | Stankovic 2010 [124] |
| | Freund 2012 [112] | Steichen 2009 [125] |
| | Hettwer 2010 [113] | Uusitalo 2011 [126] |
| | Jereb 2009 [114] | Viallon 2008 [127] |
| | van Langevelde 2000 [115] | Wang 2014 [1] |
| | Magrini 2013 [116] | Wunderink 2012 [128] |
| | Oberhoffer 2000 [117] | Yan 2014 [129] |
| | Patil 2012 [118] | Zhu 2014 [130] |
| Studying candidaemia | Charles 2006 [131] | Martini 2010 [133] |
| | Charles 2009 [132] | |
| No comparison to non-bacteraemia | Charles 2008 [134] | Mueller 2004 [136] |
| | Knudsen 2010 [135] | Shomali 2013 [137] |
| Not providing sensitivity or specificity | Ahn 2010 [138] | Lee 2014 [139] |
| No AUC values of PCT for bacteraemia | Al Shuaibi 2013 [140] | Lehmann 2010 [154] |
| | Aouifi 2000 [141] | Lodes 2012 [155] |
| | Bloos 2012 [142] | Mauro 2012 [156] |
| | Boussekey 2005 [143] | Park 2012 [56] |
| | Cuculi 2008 [144] | Peters 2006 [157] |
| | Endo 2012 [145] | Previsdomini 2012 [158] |
| | Feld 2008 [146] | Sandri 2008 [159] |
| | Foushee 2012 [147] | Scott 2003 [160] |
| | Gille johnson 2012 [148] | Su 2012 [161] |
| | Groeneveld 2008 [149] | Svaldi 2001 [162] |
| | Guven 2002 [150] | Ugarte 1999 [163] |
| | Juutilainen 2011 [151] | von Lilienfeld 2009 [164] |
| | Kim 2010 [152] | Yilmaz 2011 [165] |
| | Kruif de 2008 [153] | |
| Longitudinal studies not providing PCT on inclusion | Lavrentieva 2012 [166] | |
| Healthy controls | Gaini 2008 [167] | Kocazeybek [168] |

measurement were collected on inclusion or within 24 hours at the emergency department, ward and/or intensive care unit. The median QUADAS score was 12 (range 7-14) the per item QUADAS scores are presented in Table 3. Problematic QUADAS items were: the description of selection criteria and description of the execution of the reference standard, whether the index test results were interpreted without knowledge of the results of the reference standard and vice versa, reporting of uninterpretable/ intermediate test results and the explanation of withdrawals.

**The diagnostic accuracy of procalcitonin for bacteraemia**

In the overall analysis the area under the SROC was 0.79 (Figure 2 and Table 4). The optimal and most widely used procalcitonin cutoff value was 0.5 ng/mL (Table 5) and corresponded with a 76% sensitivity and 69% specificity (Table 4). In Figure 3, the sensitivity and specificity per study are given. The lowest SROC was found in immunocomprised/neutropenic patients (0.71), the highest SROC (0.88) in ICU patients. The lowest sensitivity was found in immunocomrpomised/neutropenic patients (66), the highest in ICU patients (89). The lowest specificity was found in patients with localised infections (55) and the highest in immunocompromised/neutropenic patients (78). Table 6 shows the 2x2 tables with low positive predictive values (17-28%) and high negative predictive values (95-98%) for different hospital settings at the 0.5 ng/ mL procalcitonin cutoff. There was significant heterogeneity in the overall analysis and in most subgroups (Table 4). However, there was no indication of a threshold-effect.

**Evaluation of publication bias**

Figure 4 displays the Deeks funnel plot asymmetry test of this meta-analysis. The Deeks test was not statistically significant (P=0.13) indicating that there is no direct evidence for publication bias.

**Table 3.** Raw QUADAS scores.

| QUADAS item | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | QUADAS SCORE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Reference** | | | | | | | | | | | | | | | |
| Aalto 2004 [58] | y | y | y | y | y | y | y | y | y | y | u | y | n | y | 12 |
| Albrich 2011 [59] | y | y | y | y | y | y | y | y | y | y | n | y | n | y | 12 |
| Bell 2003 [60] | y | y | y | n | y | y | y | y | y | y | y | y | u | n | 11 |
| Bogar 2006 [61] | y | y | y | y | y | y | y | y | y | n | y | y | n | y | 12 |
| Bossink 1999 [62] | y | y | y | y | y | y | y | y | y | n | y | y | n | y | 12 |
| Caterino 2004 [63] | y | y | y | y | y | y | y | y | y | y | n | y | y | y | 13 |
| Charles 2008 [64] | y | n | y | y | y | y | y | y | y | y | y | y | y | y | 13 |
| Chen 2011 [65] | y | y | y | y | y | y | y | y | y | y | y | y | u | u | 12 |
| Cheval 2000 [66] | y | n | y | y | y | y | y | y | n | y | y | y | u | u | 10 |
| Chirouze 2002 [67] | n | y | y | y | y | y | y | y | y | y | u | y | y | y | 12 |
| Dwolatzky 2005 [68] | y | n | y | y | y | y | y | y | y | y | y | y | y | y | 13 |
| Engel 1999 [69] | y | y | y | y | y | y | y | y | n | y | u | y | y | n | 11 |
| Gac 2011 [70] | y | n | y | y | y | y | y | y | n | u | y | y | y | y | 11 |
| Gaini 2007 [71] | y | y | y | y | y | y | y | y | y | y | n | y | y | y | 13 |
| Giamarellos 2001 [72] | y | y | y | y | n | n | y | y | y | y | n | y | y | y | 11 |
| Giamarellou 2004 [73] | y | n | y | y | y | y | y | y | n | y | y | y | y | n | 11 |
| Guinard-Barbier 2001 [53] | y | y | y | y | y | y | y | y | y | u | y | y | y | y | 13 |
| Ha 2013 [74] | y | n | y | y | y | y | y | y | y | y | y | y | y | n | 12 |
| Hoeboer 2012 [75] | y | y | y | y | y | y | y | y | y | n | y | y | n | y | 12 |
| Hoenigl 2013 [54] | y | y | y | y | y | y | y | y | y | u | y | y | y | y | 13 |
| Hoenigl 2014 [76] | y | y | y | y | y | y | y | y | y | y | y | y | n | n | 12 |
| Jeong 2012 [77] | y | n | y | y | y | y | y | y | y | y | y | y | y | n | 12 |
| Jimeno 2004 [78] | y | y | y | n | n | y | y | y | y | y | n | y | y | y | 11 |
| Kallio 2000 [79] | y | n | y | y | y | y | y | y | n | y | y | y | y | y | 12 |
| Karlsson 2010 [80] | y | y | y | y | y | y | y | y | y | y | y | y | y | y | 14 |
| Kim D 2011 [81] | y | n | y | y | y | y | y | n | n | u | u | y | u | n | 7 |
| Kim M 2011 [24] | y | n | y | y | y | y | y | u | u | u | u | y | u | u | 7 |
| Koivula 2011 [82] | y | n | y | y | y | y | y | y | y | n | n | y | y | y | 11 |
| Lai 2010 [25] | y | y | y | y | y | y | y | y | y | y | y | y | y | n | 13 |
| Lee 2013 [83] | y | y | y | y | y | y | y | y | y | y | y | y | n | y | 13 |
| Liaudat 2001 [84] | y | u | y | y | y | y | y | y | y | y | n | y | y | y | 12 |
| Loonen 2014 [85] | y | y | y | y | y | y | y | y | y | y | y | y | y | y | 14 |
| Mencacci 2012 [86] | y | y | y | y | y | y | y | y | y | y | y | y | y | n | 13 |
| Menendez 2012 [55] | y | y | y | y | y | y | y | y | y | y | y | y | n | n | 12 |
| Muller 2010 [23] | y | y | y | y | y | y | y | y | y | y | y | y | n | y | 13 |
| Munoz 2004 [87] | y | y | y | n | y | y | y | y | y | y | y | y | u | u | 11 |
| Nakamura 2009 [88] | y | n | y | y | y | y | y | n | n | y | y | y | y | n | 10 |
| Nieuwkoop van 2010 [89] | y | y | y | y | y | y | y | y | y | y | y | y | n | y | 13 |

**Table 3.** Raw QUADAS scores. (Continued)

| QUADAS item | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | QUADAS SCORE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Reference** | | | | | | | | | | | | | | | |
| Pereira 2013 [90] | y | y | y | y | y | y | y | y | y | u | y | y | y | n | 12 |
| Persson 2004 [91] | y | n | y | y | y | y | y | y | y | n | y | y | y | n | 11 |
| Prat 2008 [92] | y | u | y | y | y | y | y | y | u | n | u | y | u | u | 8 |
| Ratzinger 2014 [93] | y | y | y | y | y | y | y | y | y | y | y | y | u | y | 13 |
| Riedel 2011 [27] | y | y | y | y | y | y | y | y | y | y | y | y | y | y | 14 |
| Rintala 2001 [94] | y | n | y | y | y | y | y | y | y | y | y | y | u | n | 11 |
| Robinson 2011 [95] | y | y | y | y | y | y | y | y | y | n | y | y | y | n | 12 |
| Romualdo 2014 [96] | y | y | y | y | y | y | y | y | y | y | y | y | n | y | 13 |
| Schuetz 2007 [26] | y | y | y | y | y | y | y | y | n | y | y | y | y | n | 12 |
| Schuetz 2008 [97] | y | y | y | y | y | y | y | y | y | y | y | y | y | n | 13 |
| Shi 2013 [98] | y | y | y | y | y | y | y | y | y | n | y | y | y | n | 12 |
| Shomali 2012 [99] | y | y | y | y | y | y | y | y | y | y | y | y | y | n | 13 |
| Su 2011 [100] | y | y | y | y | y | y | y | y | y | y | n | y | y | n | 12 |
| Suarez-Santamaria 2010 [101] | y | y | y | y | y | y | y | y | y | y | y | y | y | n | 13 |
| Theodorou 2012 [57] | y | y | y | y | y | y | y | y | y | y | y | y | n | y | 13 |
| Tromp 2012 [102] | y | y | y | y | y | y | y | y | y | y | y | y | n | y | 13 |
| Tsalik 2012 [103] | y | y | y | y | y | y | y | y | y | u | u | y | y | y | 12 |
| Vanska 2012 [104] | y | y | y | y | y | y | y | y | y | u | u | y | u | u | 10 |
| Von Lillienfeld-Toal 2004 [105] | y | y | y | y | y | y | y | y | n | y | y | y | y | n | 12 |
| Wang 2013 [10] | y | n | y | y | y | y | y | y | y | y | y | y | y | n | 12 |

The quality assessment of studies of diagnostic accuracy checklist. Item 1: Was the spectrum of patients representative of the patients who will receive the test in practice?; 2: Were selection criteria clearly described?; 3: Is the reference standard likely to correctly classify the target condition?; 4: Is the time period between reference standard and index test short enough to be reasonably sure that the target condition did not change between the two tests?; 5:Did the whole study population or a random selection of the sample, receive verification using a reference standard for diagnosis?; 6: Did patients receive the same reference standard regardless of the index test result?; 7: Was the reference standard independent of the index test?; 8: Was the execution of the index test described in sufficient detail to permit replication of the test?; 9: Was the execution of the reference standard described in sufficient detail to permit its replication?; 10: Were the index test results interpreted without the knowledge of the results of the reference standard?; 11: Were the reference standard results interpreted without knowledge of the index test results?; 12: Were the same clinical data available when test results were interpreted as would be available when the test is used in practice?; 13: Were uninterpretable / intermediate test results reported?; 14: Were withdrawals from the study explained? Each item can be answered with yes (Y), no (N) or unknown (U)

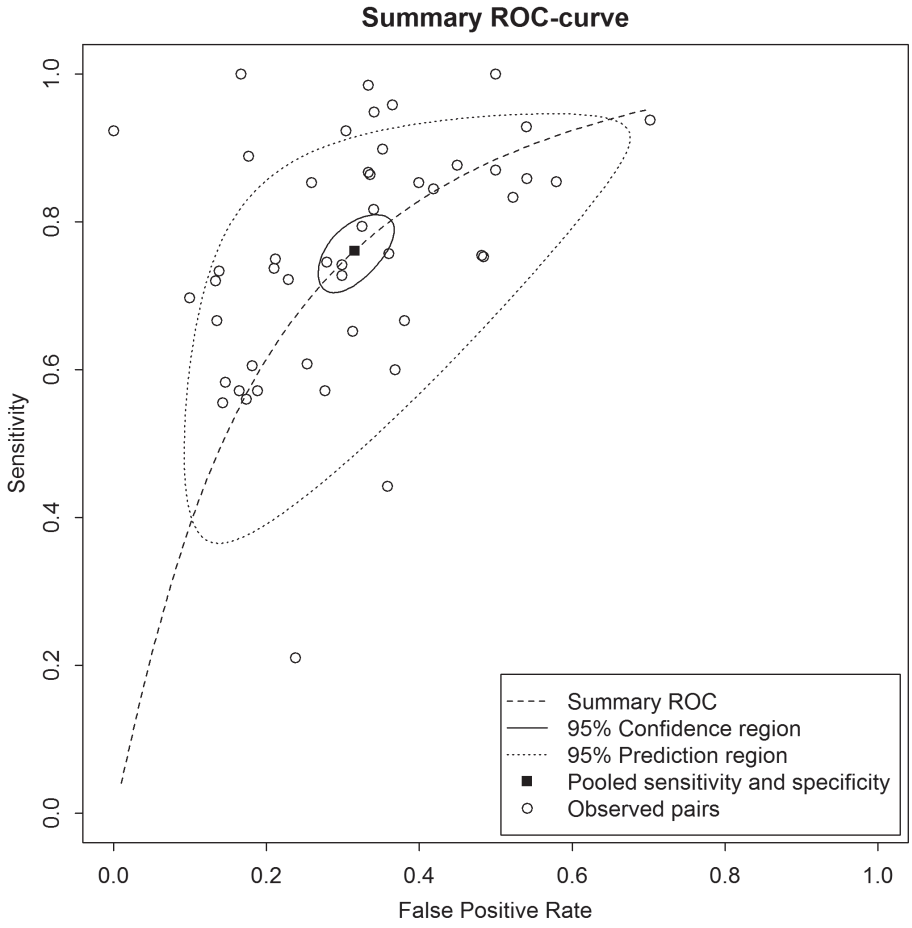


**Figure 2.** Summary receiver-operating characteristic (SROC) curve plot of procalcitonin for the diagnosis of bacteraemia, including all studies (N=58).
Individual studies are shown as open circles. Summary point is shown as a closed square, representing sensitivity estimates pooled by using bivariate random-effects regression model. The area under the SROC curve (dashed line) is 0.79, pooled sensitivity 76% and specificity 69%. The 95% confidence region displays the 95% confidence interval of the pooled sensitivity and specificity. The 95% prediction region is the region for a forecast of the true sensitivity and specificity in a future study.

**Table 4.** Accuracy estimates.

| Analysis | AUC | Pooled sensitivity (95% CI) | Pooled specificity (95% CI) | Heterogeneity (%) $I^2$ | $X^2$/ Q | *p* |
|---|---|---|---|---|---|---|
| **Overall** (N=3,420 ) | 0.79 | 76 (72-80) | 69 (64-72) | 86% | 1397 | <0.001 |
| **Control group** | | | | | | |
| Non-bacteraemia (N=1,884 ) | 0.78 | 72 (66-78) | 74 (69-76) | 88% | 1070 | <0.001 |
| SIRS (N=931) | 0.78 | 76 (60-87) | 66 (44-82) | 83% | 114 | <0.001 |
| Local infection and/or sepsis (N=605) | 0.84 | 84 (80-87) | 55 (47-63) | 71% | 162 | <0.001 |
| **Immunocompromised/ neutropenic** | | | | | | |
| Yes (N=320) | 0.71 | 66 (54-76) | 78 (71-83) | 76% | 120 | <0.001 |
| No (N=3,100) | 0.79 | 79 (75-83) | 65 (60-65) | 81% | 926 | <0.001 |
| **Department** | | | | | | |
| ICU (N=399) | 0.88 | 89 (79-94) | 68 (57-77) | 77% | 54 | <0.001 |
| Mixed (N=1,009) | 0.77 | 76 (65-85) | 66 (57-76) | 31% | 501 | <0.001 |
| Ward (N=587) | 0.76 | 71 (63-78) | 71 (64-77) | 90% | 433 | <0.001 |
| ED (N=1,425) | 0.78 | 76 (69-82) | 68 (61-75) | 77% | 285 | <0.001 |
| **Study type** | | | | | | |
| Prospective (N=2,507) | 0.79 | 76 (71-80) | 69 (64-73) | 86% | 721 | <0.001 |
| Retrospective (N=913) | 0.79 | 78 (66-86) | 68 (56-78) | 79% | 636 | <0.001 |

X2/Q = X2/cochrane Q, CI = confidence interval; ED = emergency department; ICU = intensive care unit; mixed = ICU/ ED/ ward together; SIRS = systemic inflammatory response syndrome

**Table 5.** Accuracy estimates for different cut-off values.

| Analysis | AUC | Pooled sensitivity (95% CI) | Pooled specificity (95% CI) | Heterogeneity (%) $I^2$ | $X^2$/ Q | *p* |
|---|---|---|---|---|---|---|
| **Cut-off 0.1** | 0.73 | 91 (82-96) | 35 (22-51) | 88% | 385 | <0.001 |
| **Cut-off 0.5** | 0.77 | 74 (66-81) | 68 (61-75) | 75% | 478 | <0.001 |
| **Cut-off 1.0** | 0.76 | 67 (52-78) | 74 (67-80) | 85% | 154 | <0.001 |
| **Cut-off 2.0** | 0.63 | 50 (31-69) | 83 (64-94) | 92% | 313 | <0.001 |

X2/Q = X2/cochrane Q, CI = confidence interval.

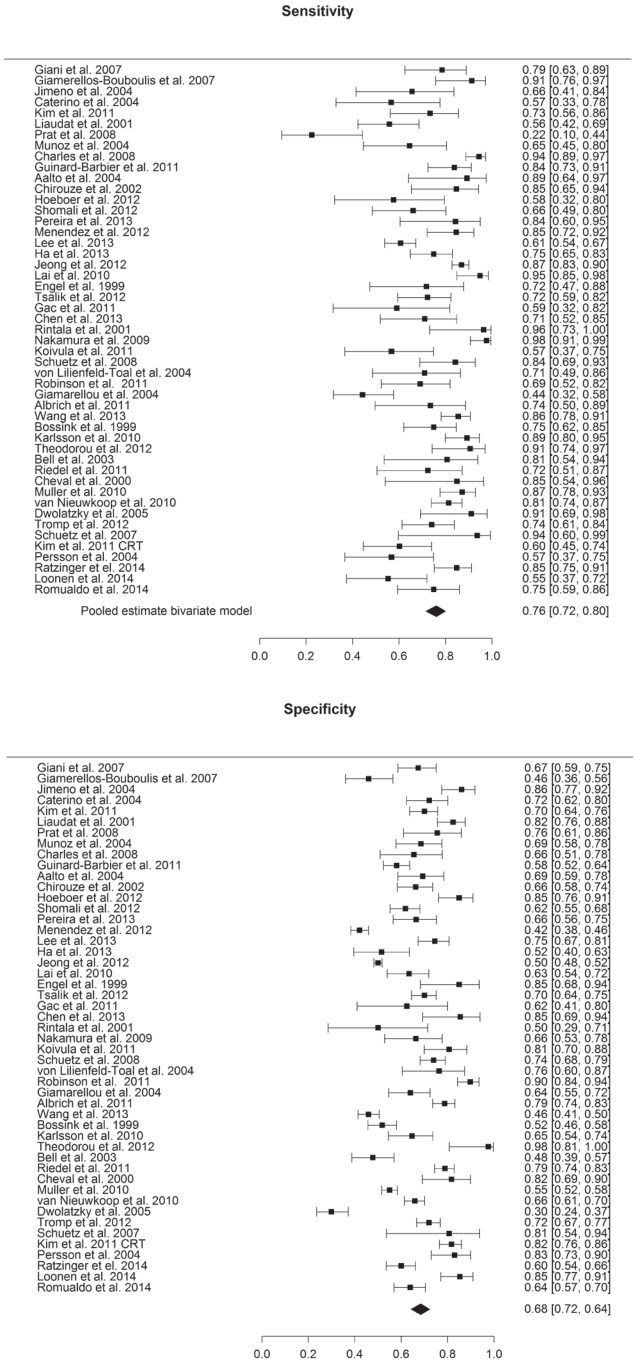


**Figure 3.** Accuracy estimates analysis for bacteraemia versus non-bacteraemia. including all studies (N=49).

**Table 6.** 2x2 tables with corresponding sensitivity, specificity, PPV and NPV.

| | Ward | | | Emergency Department | | | Intensive Care Unit | | |
|---|---|---|---|---|---|---|---|---|---|
| | BSI+ | BSI- | | BSI+ | BSI- | | BSI+ | BSI- | |
| PCT+ | 7 | 26 | 33 | 6 | 29 | 35 | 11 | 28 | 39 |
| PCT- | 3 | 64 | 67 | 2 | 63 | 65 | 1 | 60 | 61 |
| | 10 | 90 | 100 | 8 | 92 | 100 | 12 | 88 | 100 |
| Prevalence | | | 10% | | | 8% | | | 12% |
| Sensitivity | | | 71% | | | 76% | | | 89% |
| Specificity | | | 71% | | | 68% | | | 68% |
| PPV | | | 21% | | | 17% | | | 28% |
| NPV | | | 95% | | | 97% | | | 98% |

BSI = blood stream infection; NPV = negative predictive value; PCT = procalcitonin; PPV = positive predictive value, += positive test, -= negative test.

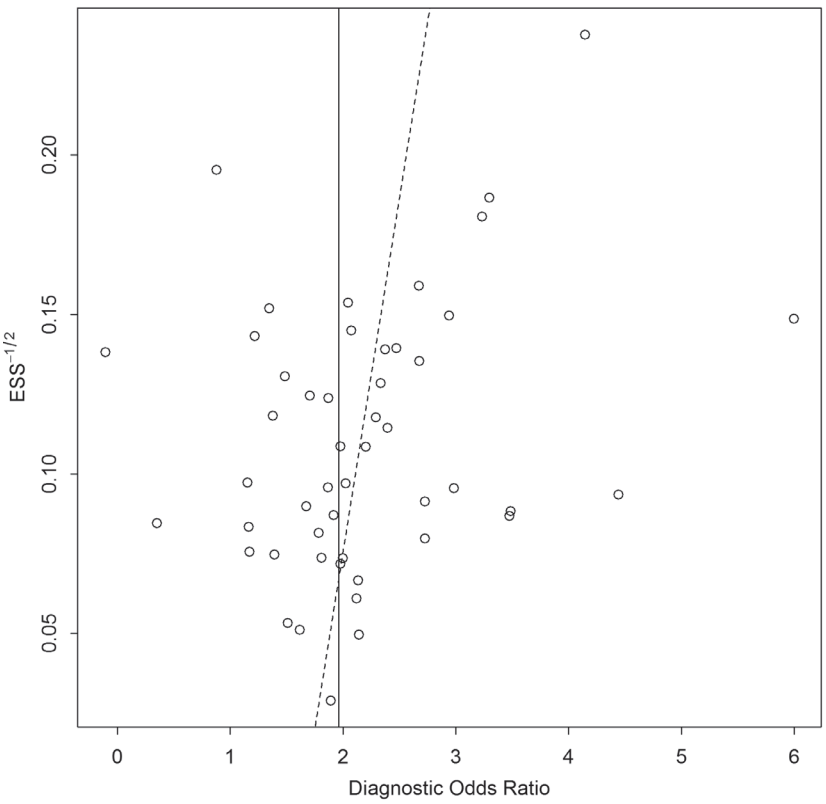


**Figure 4.** Evaluation of publication bias.
The Deeks funnel plot assymetry test was non-significant (P=0.13). Individual studies are shown as open circles and the interrupted line represents the regression line. ESS= effective sample size.

## DISCUSSION

This study evaluates the diagnostic accuracy of procalcitonin for bacteraemia in different subgroups of adult hospitalised patients suspected of infection or sepsis. Overall, at a cutoff level of 0.5 ng/mL, procalcitonin had a fair diagnostic accuracy for bacteraemia with an SROC of 0.79. The pooled AUC values of procalcitonin for the diagnosis bacteraemia in subgroups ranged from 0.71-0.88, with sensitivities ranging from 66% in immunocompromised/neutropenic patients to 89% in ICU patients and specificities ranging from 55% in bacteraemia vs. local infections to 78% in immunocompromised/neutropenic patients. Based on these results low procalcitonin levels in particular can be used to rule out the presence of bacteraemia.

Two previous meta-analyses on the diagnostic accuracy of procalcitonin for sepsis had contradicting conclusions while having comparable results.[12,18] Tang et al. concluded that there was no clear use for procalcitonin in diagnosing sepsis (area under the SROC of 0.78, sensitivity of 71% and specificity of 70%).[12] However, their inclusion may be biased by specifically excluding sepsis originating from certain types of common infection sites.[12] In contrast, Wacker et al. concluded that procalcitonin was useful for the diagnosis of sepsis (area under the SROC 0.85, sensitivity 77%, specificity 79%).[18] They included studies on adult and paediatric patients comparing sepsis to SIRS. Sepsis, however, was defined as clinically suspected or microbiologically proven infection.[18] Two other meta-analyses studying the diagnostic use of procalcitonin for bacterial infection found an area under the SROC curve ranging from 0.82-0.89, sensitivity 83-88%, specificity 81-83%.[28,34] Both analyses had comparable results but again contradicting conclusions. Simon et al. compared CRP and procalcitonin in a meta-analysis on the diagnostic accuracy in either proven or suspected bacterial infection, favouring PCT to be used in clinical practice.[28] In contrast, Lee et al. contented that PCT should not be used as single diagnostic tool for infection.[34] However their conclusion was based on only four studies on the diagnostic accuracy of procalcitonin for bacterial infection in elderly patients.[34] As far as we know there is only one previous meta-analysis on the diagnostic accuracy of procalcitonin for bacteraemia with an area under the SROC of 0.84, sensitivity 76%, specificity 70%.[40] This study concluded that widespread use of procalcitonin is not recommended because of the moderate diagnostic accuracy of PCT to predict bacteraemia.[40] This conclusion was based on 17 included studies of which not all contained bacteraemia as primary endpoint. Even though previous meta-analyses showed similar results their conclusion differ, possibly due to differences in interpretation of clinically useful AUC values. In contrast to our study, the above-mentioned meta-analyses only used a small selection of the available literature or used sepsis syndrome and not microbiologically documented infection as their endpoint. Our study shows that procalcitonin can be used in the diagnostic

process of bacteraemia regardless of its clinical symptoms. As shown in Table 6 low procalcitonin levels can be used to rule out the presence of bacteraemia in different clinical settings.

This meta-analysis has several limitations. There is some evidence for a concentration-response relation between procalcitonin levels and probability of infection and disease severity.[52] The definition of our primary outcome measure, bacteraemia, does not acknowledge such a concentration-response relation. Only a minority of the studies in this meta-analysis formally excluded patients treated with antibiotics prior to inclusion.[23,24,53-57] We cannot be certain that false negative results, due to possible antibiotic treatment prior to inclusion, led to underestimation of the effect. Even though the effect size is only fair (area under the SROC 0.79) its direction is positive in almost all studies, in spite of heterogeneity. High I-squares are to be expected because of the variation in cutoffs used in the different included studies and sensitivity and specificity both depend on cutoffs. To homogenise the results we attempted to use the sensitivities and specificities corresponding with the cutoff value closest to 0.5 ng/mL if multiple cutoff values were given. Other potential factors that could have contributed to heterogeneity are variety in inclusion criteria, underlying diseases, co-morbidities, clinical course and treatment prior to inclusion, variety in the control groups used for comparison against bacteraemia, department of sample collection, and differences in test performance of the various procalcitonin assays. In order to reduce the influence of these factors on heterogeneity we performed analyses in the supposedly more homogeneous patient subgroups. As to be expected, substantial heterogeneity remained in most subgroups. A Funnel plot analysis based on the standard error of the lnDOR can be misleading, therefore we evaluated potential publication bias using the recommended effective sample size-based funnel plots and associated regression tests of asymmetry according to Deeks.[51]

## Conclusions

In conclusion, this systematic review and meta-analysis shows that procalcitonin has a fair diagnostic accuracy for bacteraemia in adult, hospitalised patients suspected of infection or sepsis. In particular low procalcitonin levels can be used to rule out the presence of bacteraemia. Further research on the safety and efficacy of using procalcitonin as a single diagnostic tool to withhold taking blood cultures remains to be proven.

## REFERENCES


1. Kumar A, Roberts D, Wood KE, et al. Duration of hypotension before initiation of effective antimicrobial therapy is the critical determinant of survival in human septic shock. Crit Care Med 2006;34:1589-96.
2. Wang HE, Szychowski JM, Griffin R, et al. Long-term mortality after community-acquired sepsis:a longitudinal population-based cohort study. BMJ Open 2014;4:e004283.
3. Dellinger RP, Levy MM, Rhodes A, et al. Surviving Sepsis Campaign: International Guidelines for Management of Severe Sepsis and Septic Shock, 2012. Intensive Care Med 2013;39:165-228.
4. Kopterides P, Siempos II, Tsangaris I, et al. Procalcitonin-guided algorithms of antibiotic therapy in the intensive care unit: a systematic review and meta-analysis of randomized controlled trials. Crit Care Med 2010;38:2229-41.
5. Bone RC, Balk RA, Cerra FB, et al. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. Chest 1992;101:1644-55.
6. Harbarth S, Holeckova K, Froidevaux C, et al. Diagnostic value of procalcitonin, interleukin-6, and interleukin-8 in critically ill patients admitted with suspected sepsis. Am J Respir Crit Care Med 2001;164:396-402.
7. Pettila V, Hynninen M, Takkunen O, et al. Predictive value of procalcitonin and interleukin 6 in critically ill patients with suspected sepsis. Intensive Care Med 2002;28:1220-5.
8. Hensler T, Sauerland S, Lefering R, et al. The clinical value of procalcitonin and neopterin in predicting sepsis and organ failure after major trauma. Shock 2003;20:420-6.
9. Riedel S. Procalcitonin and the role of biomarkers in the diagnosis and management of sepsis. Diagn Microbiol and Inf Dis 2012;73:221-227.
10. Wang H, Yin F, Shen DX, et al. Predictive value of procalcitonin for excluding bloodstream infection: results of a retrospective study and utility of a rapid, quantitative test for procalcitonin. J Int Med Res 2013;41:1671-81.
11. Uzzan, B, Cohen R, Nicolas P, et al. Procalcitonin as a diagnostic test for sepsis in critically ill adults and after surgery or trauma: a systematic review and meta-analysis. Crit Care Med 2006;34:1996-2003.
12. Tang BM, Eslick GD, Craig JC, et al. Accuracy of procalcitonin for sepsis diagnosis in critically ill patients: systematic review and meta-analysis. Lancet Infect Dis 2007;7:210-7.
13. Sakr Y, Sponholz C, Tuche F, et al. The role of procalcitonin in febrile neutropenic patients: review of the literature. Infection 2008;36:396-407.
14. Yu Z, Liu J, Sun Q, et al. The accuracy of the procalcitonin test for the diagnosis of neonatal sepsis: a meta-analysis. Scand J Infect Dis 2010;42:723-33.
15. Mann EA, Wood GL, Wade CE. Use of procalcitonin for the detection of sepsis in the critically ill burn patient: a systematic review of the literature. Burns 2011;37:549-58.
16. Vouloumanou EK, Plessa E, Karageorgopoulos DE, et al. Serum procalcitonin as a diagnostic marker for neonatal sepsis: a systematic review and meta-analysis. Intensive Care Med 2011;5:2174-2178.

17. Haeulser GM, Carlesse F, Philips RS. An updated systematic review and meta-analysis of the predicitve value of serum biomarkers in the assessment of fever during neutropenia in children with cancer. Pediatr Infect Dis J 2013;10:e390-396.

18. Wacker C, Prkno A, Brunkhorst FM, et al. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. Lancet Infect Dis 2013;13:426-35.

19. Bates DW, Sands K, Miller E, et al. Predicting bacteremia in patients with sepsis syndrome. Academic Medical Center Consortium Sepsis Project Working Group. J Infect Dis 1997;176:1538-51.

20. Sands KE, Bates DW, Lanken PN, et al. Epidemiology of sepsis syndrome in 8 academic medical centers. JAMA 1997;278:234-40.

21. Magadia RR. Weinstein MP. Laboratory diagnosis of bacteremia and fungemia. Infect Dis Clin North Am 2001;15:1009-24.

22. Flayhart D, Borek AP, Wakefield T, et al. Comparison of BACTEC PLUS blood culture media to BacT/Alert FA blood culture media for detection of bacterial pathogens in samples containing therapeutic levels of antibiotics. J Clin Microbiol 2007;45:816-21.

23. Muller F, Christ-Crain M, Bregenzer T, et al. Procalcitonin levels predict bacteremia in patients with community-acquired pneumonia: a prospective cohort trial. Chest 2010;138:121-9.

24. Kim MH, Lim G, Kang SY, et al. Utility of procalcitonin as an early diagnostic marker of bacteremia in patients with acute fever. Yonsei Med J 2011;52:276-81.

25. Lai CC, Chen SY, Wang CY, et al. Diagnostic value of procalcitonin for bacterial infection in elderly patients in the emergency department. J Am Geriatr Soc 2010;58:518-22.

26. Schuetz P, Mueller B, Trampuz A. Serum procalcitonin for discrimination of blood contamination from bloodstream infection due to coagulase-negative staphylococci. Infection 2007;35:352-5.

27. Riedel S, Melendez JH, An AT, et al. Procalcitonin as a marker for the detection of bacteremia and sepsis in the emergency department. Am J Clin Pathol 2011;135:182-9.

28. Simon L, Gauvin F, Amre DK, et al. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis 2004;39:206-17.

29. Van den Breul, Thompson MJ, Haj-Hassan T, et al. Diagnostic value of laboratory tests in identifying serious infections in febrile children: systematic review. BMJ 2011;342:d3082.

30. Lin SG, Hou TY, Huang DH, et al. Role of procalcitonin in the diagnosis of severe infection in pediatric patients with fever and Neutropenia—a systemic review and meta-analysis. Pediatr Infect Dis J 2012;31: e182-8.

31. Wu JY, Lee SH, Shen CJ, et al. Use of serum procalcitonin to detect bacterial infection in patients with autoimmune diseases: a systematic review and meta-analysis. Arthritis Rheum 2012;64:3034-42.

32. Yo CH, Hsieh PS, Lee SH, et al. Comparison of the test characteristics of procalcitonin to C-reactive protein and leukocytosis for the detection of serious bacterial infections in children presenting with fever without source: a systematic review and meta-analysis. Ann Emerg Med 2012;60:591-600.

33. Dou YH, Du JK, Liu HL, et al. The role of procalcitonin in the identification of invasive fungal infection-a systemic review and meta-analysis. Diagn Microbiol Infect Dis 2013;76:464-9.

34. Lee SH, Chan RC, Wu JY, et al. Diagnostic value of procalcitonin for bacterial infection in elderly patients – a systemic review and meta-analysis. Int J Clin Pract 2013;67:1350-1357.

35. Shen CJ, Wu MS, Lin KH, et al. The use of procalcitonin in the diagnosis of bone and joint infection: a systemic review and meta-analysis. Eur J Clin Microbiol Infect Dis 2013;32:807-814.

36. Yu CW, Juan LI, Hsu SC, et al. Role of procalcitonin in the diagnosis of infective endocarditis: a meta-analysis. Am J Emerg Med 2013;31:935-41.

37. Huang SL, Lee HC, Yu CM, et al. Value of procalcitonin in differentiating pulmonary tuberculosis from other pulmonary infections: a meta-analysis. Int J Tuberc Lung Dis 2014;18:470-477.

38. Pfister R, Kochanek M, Leygeber T, et al. Procalcitonin for diagnosis of bacterial pneumonia in critically ill patients during 2009 H1N1 influenza pandemic: a prospective cohort study, systematic review and individual patient data meta-analysis. Crit Care 2014;10:R44.

39. Lin KH, Wang FL, Wu MS, et al. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection in patients with liver cirrhosis: a systematic review and meta-analysis. Diagn Microbiol Infect Dis 2014;21:S0732-8893.

40. Jones AE, Fiechtl JF, Brown MD, et al. Procalcitonin test in the diagnosis of bacteremia: a meta-analysis. Ann Emerg Med 2007;50:34-41.

41. Liberati A, Altman DG, Tetzlaff J, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: explanation and elaboration. BMJ 2009;339:b2700.

42. Whiting P, Rutjes AW, Reitsma JB, et al. The development of QUADAS: a tool for the quality assessment of studies of diagnostic accuracy included in systematic reviews. BMC Med Res Methodol 2003;3:25.

43. Leeflang MM, Deeks JJ, Gatsonis C, et al. Systematic reviews of diagnostic test accuracy. Ann Intern Med, 2008. 149:889-97.

44. Harbord RM, Whiting P. Metandi: Meta-analysis of diagnostic accuracy using hierarchical logistic regression. Stata Journal 2009;9:211-229.

45. Reitsma JB, Glas AS, Rutjes AW, et al. Bivariate analysis of sensitivity and specificity produces informative summary measures in diagnostic reviews. J Clin Epidemiol 2005;58:982-90.

46. Lai CC, Tan CK, Chen SY, et al. Diagnostic value of procalcitonin for bacteremia in patients with bacterial infection at the emergency department. J Infection 2010;61:512-5.

47. Mueller F, Christ-Crain M, Bregenzer T, et al. Procalcitonin levels predict bacteremia in patients with community-acquired pneumonia: a prospective cohort trial. Chest 2010;138:121-9.

48. Fazili T, Endy T, Javaid W, et al. Role of procalcitonin in guiding antibiotic therapy. Am J Health Syst Pharm 2012;69:2057-2061.

49. Quenot JP, Luyt CE, Roche N, et al. Role of biomarkers in the management of antibiotic therapy: an expert panel review II: clinical use of biomarkers for initiation or discontinuation of antibiotic therapy. Ann Int Care 2013;3:21.
50. Tape TG. Interpreting diagnostic tests. http://gim.unmc.edu/dxtests/Default.htm. Accessed October 12, 2014.
51. Deeks JJ, Macaskill P, Irwig L. The performance of tests of publication bias and other sample size effects in systematic reviews of diagnostic test accuracy was assessed. J Clin Epidemiol 2005;58:882-893.
52. Schuetz P, AlbrichW, Mueller B. Procalcitonin for diagnosis of infection and guide to antibiotic decisions: past, present and future. BMC Med 2011;9:107.
53. Guinard-Barbier S, Grabar S, Chenevier-Gobeaux C, et al. Is mid-regional pro-atrial natriuretic peptide (MRproANP) an accurate marker of bacteremia in pyelonephritis? Biomarkers 2011;16:355-63.
54. Hoenigl M, Raggam RB, Wagner J, et al. Diagnostic accuracy of soluble urokinase plasminogen activator receptor (suPAR) for prediction of bacteremia in patients with systemic inflammatory response syndrome. Clin Biochem 2013;46:225-9.
55. Menendez R, Sahuquillo-Arce JM, Reyes S, et al. Cytokine activation patterns and biomarkers are influenced by microorganisms in community-acquired pneumonia. Chest 2012;141:1537-45.
56. Park JH, Wee JH, Choi SP, et al. The value of procalcitonin level in community-acquired pneumonia in the ED. Am J Emerg Med 2012;30:1248-54.
57. Theodorou VP, Papaioannou VE, Tripsianis GA, et al. Procalcitonin and procalcitonin kinetics for diagnosis and prognosis of intravascular catheter-related bloodstream infections in selected critically ill patients: a prospective observational study. BMC Infect Dis 2012;12:247.
58. Aalto H, Takala A, Kautiainen H, et al. Laboratory markers of systemic inflammation as predictors of bloodstream infection in acutely ill patients admitted to hospital in medical emergency. Eur J Clin Microbiol Infect Dis 2004;23:699-704.
59. Albrich WC, Mueller B. Predicting bacteremia by procalcitonin levels in patients evaluated for sepsis in the emergency department. Expert Rev Anti Infect Ther 2011;9:653-6.
60. Bell K, Wattie M, Byth K, et al. Procalcitonin: a marker of bacteraemia in SIRS. Anaesth Int Care 2003;31:629-36.
61. Bogar L, Molnar Z, Kenyeres P, et al. Sedimentation characteristics of leucocytes can predict bacteraemia in critical care patients. J Clin Pathol 2006;59:523-5.
62. Bossink AW, Groeneveld AB, Thijs TG. Prediction of microbial infection and mortality in medical patients with fever: plasma procalcitonin, neutrophilic elastase-alpha1-antitrypsin, and lactoferrin compared with clinical variables. Clin Infect Dis 1999;29:398-407.
63. Caterino JM, Scheatzle MD, Forbes ML, et al. Bacteremic elder emergency department patients: procalcitonin and white count. Acad Emerg Med 2004;11:393-6.
64. Charles PE, Ladoire S, Snauwaert A, et al. Impact of previous sepsis on the accuracy of procalcitonin for the early diagnosis of blood stream infection in critically ill patients. BMC Infect Dis 2008;8:163.
65. Chen J, Wang Y, Shen Z, et al. Early diagnostic value of plasma PCT and BG assay for CRBSI after OLT. Transplant Proc 2011;43:1777-9.

66. Cheval C, Timsit JF, Garrouste-Orgeas M, et al. Procalcitonin (PCT) is useful in predicting the bacterial origin of an acute circulatory failure in critically ill patients. Intensive Care Med 2000;26 (Suppl 2):S153-8.

67. Chirouze C, Schuhmacher H, Rabaud C, et al. Low serum procalcitonin level accurately predicts the absence of bacteremia in adult patients with acute fever. Clin Infect Dis 2002;35:156-61.

68. Dwolatzky T, Olshtain-Pops K, Yinnon AM, et al. Procalcitonin in the elderly: normal plasma concentrations and response to bacterial infections. Eur J Clin Microbiol Infect Dis 2005;24:763-5.

69. Engel A, Steinbach G, Kern P, Kern WV. Diagnostic value of procalcitonin serum levels in neutropenic patients with fever: comparison with interleukin-8. Scand J Infect Dis 1999;31:185-9.

70. Gac AC, Parienti JJ, Chantepie S, et al. Dynamics of procalcitonin and bacteremia in neutropenic adults with acute myeloid leukemia. Leuk Res 2011;35:1294-6.

71. Gaini S, Koldkjaer OG, Møller HJ, et al. A comparison of high-mobility group-box 1 protein, lipopolysaccharide-binding protein and procalcitonin in severe community-acquired infections and bacteraemia: a prospective study. Crit Care 2007;11:R76.

72. Giamarellos-Bourboulis EJ, Grecka P, Poulakou G, et al. Assessment of procalcitonin as a diagnostic marker of underlying infection in patients with febrile neutropenia. Clin Infect Dis 2001;32:1718-25.

73. Giamarellou H, Giamarellos-Bourboulis EJ, Repoussis P, et al. Potential use of procalcitonin as a diagnostic criterion in febrile neutropenia: experience from a multicentre study. Clin Microbiol Infect 2004;10:628-33.

74. Ha YE, Kang CI, Wi YM, et al. Diagnostic usefulness of procalcitonin as a marker of bacteremia in patients with acute pyelonephritis. Scand J Clin Lab Invest 2013;73:444-8.

75. Hoeboer SH, Alberts E, van den Hul I, et al. Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin. J Infect 2012;64:484-93.

76. Hoenigl M, Raggam RB, Wagner J, et al. Procalcitonin fails to predict bacteremia in SIRS patients: a cohort study. Int J Clin Pract 2014;doi 10.1111:12474.

77. Jeong S, Park Y, Cho Y, et al. Diagnostic utilities of procalcitonin and C-reactive protein for the prediction of bacteremia determined by blood culture. Clin Chim Acta 2012;413:1731-6.

78. Jimeno A, García-Velasco A, del Val O, et al. Assessment of procalcitonin as a diagnostic and prognostic marker in patients with solid tumors and febrile neutropenia. Cancer 2004;100:2462-9.

79. Kallio R, Surcel HM, Bloigu A, et al. C-reactive protein, procalcitonin and interleukin-8 in the primary diagnosis of infections in cancer patients. Eur J Cancer 2000;36:889-94.

80. Karlsson S, Heikkinen M, Pettilä V, et al. Predictive value of procalcitonin decrease in patients with severe sepsis: a prospective observational study. Crit Care 2010;14:R205.

81. Kim DY, Lee YS, Ahn S, et al. The usefulness of procalcitonin and C-reactive protein as early diagnostic markers of bacteremia in cancer patients with febrile neutropenia. Cancer Res Treat 2011;43:176-80.

82. Koivula I, Hämäläinen S, Jantunen E, et al. Elevated procalcitonin predicts Gram-negative sepsis in haematological patients with febrile neutropenia. Scand J Infect Dis 2011;43:471-8.

83. Lee CH, Kim J, Park Y, et al. Delta neutrophil index discriminates true bacteremia from blood culture contamination. Clin Chim Acta 2014;427:11-4.

84. Liaudat S, Dayer E, Praz G, et al. Usefulness of procalcitonin serum level for the diagnosis of bacteremia. Eur J Clin Microbiol Infect Dis 2001;20:524-7.

85. Loonen AJM, De Jager CPC, Tosserams J, et al. Biomarkers and molecular analysis to improve bloodstream infection diagnostics in an emergency care unit. PLoS One 2014;9:e87315.

86. Mencacci A, Leli C, Cardaccia A, et al. Procalcitonin predicts real-time PCR results in blood samples from patients with suspected sepsis. PLoS One 2012;7:e53279.

87. Munoz P, Simarro N, Rivera M, et al. Evaluation of procalcitonin as a marker of infection in a nonselected sample of febrile hospitalized patients. Diagn Microbiol Infect Dis 2004;49:237-41.

88. Nakamura A, Wada H, Ikejiri M, et al, Efficacy of procalcitonin in the early diagnosis of bacterial infections in a critical care unit. Shock 2009;31:586-91.

89. van Nieuwkoop C, Bonten TN, van't Wout JW, et al. Procalcitonin reflects bacteremia and bacterial load in urosepsis syndrome: a prospective observational study. Crit Care 2010;14:R206.

90. Pereira JM, Teixeira-Pinto A, Basílio C, et al. Can we predict pneumococcal bacteremia in patients with severe community-acquired pneumonia? J Crit Care 2013;28:970-4.

91. Persson L, Engervall P, Magnuson A, et al. Use of inflammatory markers for early detection of bacteraemia in patients with febrile neutropenia. Scand J Infect Dis 2004;36:365-71.

92. Prat C, Sancho JM, Dominguez J, et al. Evaluation of procalcitonin, neopterin, C-reactive protein, IL-6 and IL-8 as a diagnostic marker of infection in patients with febrile neutropenia. Leuk Lymphoma 2008;49:1752-61.

93. Ratzinger F, Schuardt M, Eichbichler K, et al. Utility of sepsis biomarkers and the infection probability score to discriminate sepsis and systemic inflammatory response syndrome in standard care patients. PLoS One 2013;8:e82946.

94. Rintala EM, Aittoniemi J, Laine S, et al. Early identification of bacteremia by biochemical markers of systemic inflammation. Scand J Clin Lab Invest 2001;61:523-30.

95. Robinson JO, Lamoth F, Bally F, Knaup M, et al. Monitoring procalcitonin in febrile neutropenia: what is its utility for initial diagnosis of infection and reassessment in persistent fever? PLoS One 2011;6:e18886.

96. Romualdo LG, Torrella PE, Gonzalez MV, et al. Diagnostic accuracy of prepepsin (soluble CD 14 subtype) for prediction of bacteremia in patients with systemic inflammatory response syndrome in the emergency department. Clin Biochem 2014;47:505-508.

97. Schuetz P, Stolz D, Mueller B, et al. Endothelin-1 precursor peptides correlate with severity of disease and outcome in patients with community acquired pneumonia. BMC Infect Dis, 2008. 8:22.

98. Shi Y, DU B, Xu YC, et al. Early changes of procalcitonin predict bacteremia in patients with intensive care unit-acquired new fever. Chin Med J 2013;126:1832-7.

99. Shomali W, Hachem R, Chaftari AM, et al. Can procalcitonin distinguish infectious fever from tumor-related fever in non-neutropenic cancer patients? Cancer 2012;118:5823-9.

100. Su CP, Chen TH, Chen SY, et al. Predictive model for bacteremia in adult patients with blood cultures performed at the emergency department: a preliminary report. J Microbiol Immunol Infect 2011;44:449-55.

101. Suarez-Santamaria M, et al. Prognostic value of inflammatory markers (notably cytokines and procalcitonin), nutritional assessment, and organ function in patients with sepsis. Eur Cytokine Netw 2010;21:19-26.

102. Tromp M, Lansdorp B, Bleeker-Rovers CP, et al. Serial and panel analyses of biomarkers do not improve the prediction of bacteremia compared to one procalcitonin measurement. J Infect 2012;65:292-301.

103. Tsalik EL, Jaggers LB, Glickman SW, et al. Discriminative value of inflammatory biomarkers for suspected sepsis. J Emerg Med 2012;43:97-106.

104. Vanska M, Koivula I, Jantunen E, et al. IL-10 combined with procalcitonin improves early prediction of complications of febrile neutropenia in hematological patients. Cytokine 2012;60:787-92.

105. von Lilienfeld-Toal M, Dietrich MP, Glasmacher A, et al. Markers of bacteremia in febrile neutropenic patients with hematological malignancies: procalcitonin and IL-6 are more reliable than C-reactive protein. Eur J Clin Microbiol Infect Dis 2004;23:539-44.

106. Adamzik M, Eggmann M, Frey UH, et al. Comparison of thromboelastometry with procalcitonin, interleukin 6, and C-reactive protein as diagnostic tests for severe sepsis in critically ill adults. Crit Care 2010;14:R178.

107. Barati M, Alinejad F, Bahar MA, et al. Comparison of WBC, ESR, CRP and PCT serum levels in septic and non-septic burn cases. Burns 2008;34:770-4.

108. Bele N, Darmon M, Coquet I, et al. Diagnostic accuracy of procalcitonin in critically ill immunocompromised patients. BMC Infect Dis 2011;11:224

109. Bugden SA, Coles C, Mills GD. The potential role of procalcitonin in the emergency department management of febrile young adults during a sustained meningococcal epidemic. Emerg Med Australas 2004;16:114-9.

110. Delevaux I, André M, Colombier M, et al. Can procalcitonin measurement help in differentiating between bacterial infection and other kinds of inflammatory processes? Ann Rheum Dis 2003;62:337-40.

111. Fluri F, Morgenthaler NG, Mueller B, et al. Copeptin, procalcitonin and routine inflammatory markers-predictors of infection after stroke. PLoS One 2012;7:e48309.

112. Freund, Y, Delerme S, Goulet H, et al. Serum lactate and procalcitonin measurements in emergency room for the diagnosis and risk-stratification of patients with suspected infection. Biomarkers 2012;17:590-6.

113. Hettwer S, Wilhelm J, Schürmann M, et al. Microbial diagnostics in patients with presumed severe infection in the emergency department. Med Klin Intensivmed Notfmed 2012;107:53-62.

114. Jereb M, Kotar T, Jurca T, et al. Usefulness of procalcitonin for diagnosis of infective endocarditis. Intern Emerg Med 2009;4:221-6.

115. van Langevelde P, Joop K, van Loon J, et al. Endotoxin, cytokines, and procalcitonin in febrile patients admitted to the hospital: identification of subjects at high risk of mortality. Clin Infect Dis 2000;31:1343-8.

116. Magrini L, Travaglino F, Marino R, et al. Procalcitonin variations after Emergency Department admission are highly predictive of hospital mortality in patients with acute infectious diseases. Eur Rev Med Pharmacol Sci 2013;17:133-42.

117. Oberhoffer M, Russwurm S, Bredle D, et al. Discriminative power of inflammatory markers for prediction of tumor necrosis factor-alpha and interleukin-6 in ICU patients with systemic inflammatory response syndrome (SIRS) or sepsis at arbitrary time points. Intensive Care Med 2000;26:S170-4.

118. Patil VK, Morjaria JB, De Villers F, et al. Associations between procalcitonin and markers of bacterial sepsis. Medicina (Kaunas) 2012;48:383-7.

119. Pizzolato E, Ulla M, Galluzzo C, et al. Role of presepsin for the evaluation of sepsis in the emergency department. Clin Chem Lab Med 2014;10.1515;2014-0199.

120. Quiroga B, Villaverde M, Vega A, et al. Procalcitonin as an early predictor of acute infection in hemodialysis patients. Nefrologia 2014;34:341-6.

121. Reynolds SC, Shorr AF, Muscedere J, et al. Longitudinal changes in procalcitonin in a heterogeneous group of critically ill patients. Crit Care Med 2012;40:2781-7.

122. Rowther FB, Rodrigues CS, Deshmukh MS, et al. Prospective comparison of eubacterial PCR and measurement of procalcitonin levels with blood culture for diagnosing septicemia in intensive care unit patients. J Clin Microbiol 2009;47:2964-9.

123. Sakr Y, Burgett U, Nacul FE, et al. Lipopolysaccharide binding protein in a surgical intensive care unit: a marker of sepsis? Crit Care Med 2008;36:2014-22.

124. Stankovic Stojanovic K, Steichen O, Lionnet F, et al. Is procalcitonin a marker of invasive bacterial infection in acute sickle-cell vaso-occlusive crisis? Infection 2011;39:41-5.

125. Steichen O, Bouvard E, Grateau G, et al. Diagnostic value of procalcitonin in acutely hospitalized elderly patients. Eur J Clin Microbiol Infect Dis 2009;28:1471-6.

126. Uusitalo-Seppala R, Koskinen P, Leino A, et al. Early detection of severe sepsis in the emergency room: diagnostic value of plasma C-reactive protein, procalcitonin, and interleukin-6. Scand J Infect Dis 2011;43:883-90.

127. Viallon A, Guyomarc'h S, Marjollet O, et al. Can emergency physicians identify a high mortality subgroup of patients with sepsis: role of procalcitonin. Eur J Emerg Med 2008;15:26-33.

128. Wunderink RG, Diederich ER, Caramez MP, et al. Rapid response team-triggered procalcitonin measurement predicts infectious intensive care unit transfers. Crit Care Med 2012;40:2090-5.

129. Yan L, Liao P, Xu LL, et al. Usefulness of procalcitonin in elderly patients with bacterial infection. Clin Lab 2014;60:139-42.

130. Zhu Y, Cao X, Zhang K, et al. Delta Mean Neutrophil Volume (DeltaMNV) is Comparable to Procalcitonin for Predicting Postsurgical Bacterial Infection. J Clin Lab Anal 2014;doi 10.1001:21684.

131. Charles PE, Dalle F, Aho S, et al. Serum procalcitonin measurement contribution to the early diagnosis of candidemia in critically ill patients. Intensive Care Med 2006;32:1577-83.

132. Charles PE, Castro C, Ruiz-Santana S, et al. Serum procalcitonin levels in critically ill patients colonized with Candida spp: new clues for the early recognition of invasive candidiasis? Intensive Care Med 2009;35:2146-50.

133. Martini A, Gottin L, Menestrina N, et al. Procalcitonin levels in surgical patients at risk of candidemia. J Infect 2010;60:425-30.

134. Charles PE, Ladoire S, Aho S, et al. Serum procalcitonin elevation in critically ill patients at the onset of bacteremia caused by either Gram negative or Gram positive bacteria. BMC Infect Dis 2008;8:38.

135 Knudsen JB, Fuursted K, Petersen E, et al. Procalcitonin in 759 patients clinically suspected of infective endocarditis. Am J Med 2010;123:1121-7.

136. Mueller C, Huber P, Laifer G, et al. Procalcitonin and the early diagnosis of infective endocarditis. Circulation 2004;109:1707-10.

137. Shomali W, Hachem R, Chaftari AM, et al. Can procalcitonin differentiate Staphylococcus aureus from coagulase-negative staphylococci in clustered gram-positive bacteremia? Diagn Microbiol Infect Dis 2013;76:158-61.

138. Ahn S, Lee YS, Chun YH, et al. Predictive factors of bacteraemia in low-risk patients with febrile neutropenia. Emerg Med J 2012;29:715-9.

139. Lee H, Lee YS, Jeong R, et al. Predictive factors of bacteremia in patients with febrile urinary tract infection: an experience at a tertiary care center. Infection 2014;42:669-674.

140. Al Shuaibi M, Bahu RR, Chaftari AM, et al. Pro-adrenomedullin as a novel biomarker for predicting infections and response to antimicrobials in febrile patients with hematologic malignancies. Clin Infect Dis 2013;56:943-50.

141. Aouifi A, Piriou V, Bastien O, et al. Usefulness of procalcitonin for diagnosis of infection in cardiac surgical patients. Crit Care Med 2000;28:3171-6

142. Bloos F, Sachse S, Kortgen A, et al. Evaluation of a polymerase chain reaction assay for pathogen detection in septic patients under routine condition: an observational study. PLoS One 2012;7:e46003.

143. Boussekey N, Leroy O, Georges H, et al. Diagnostic and prognostic values of admission procalcitonin levels in community-acquired pneumonia in an intensive care unit. Infection 2005;33:257-63.

144. Cuculi F, Toggweiler S, Auer M, et al. Serum procalcitonin has the potential to identify Staphylococcus aureus endocarditis. Eur J Clin Microbiol Infect Dis 2008;27:1145-9.

145. Endo S, Suzuki Y, Takahashi G, et al. Usefulness of presepsin in the diagnosis of sepsis in a multicenter prospective study. J Infect Chemother 2012;18:891-7.

146. Feld R. Bloodstream infections in cancer patients with febrile neutropenia. Int J Antimicrob Agents 2008;32:S30-3.

147. Foushee JA, Hope NH, Grace EE. Applying biomarkers to clinical practice: a guide for utilizing procalcitonin assays. J Antimicrob Chemother 2012;67:2560-9.

148. Gille-Johnson P, Hansson KE, Gardlund B. Clinical and laboratory variables identifying bacterial infection and bacteraemia in the emergency department. Scand J Infect Dis 2012;44:745-52.

149. Groeneveld AB, Hack CE. The role of the innate immune response in hospital- versus community-acquired infection in febrile medical patients. Int J Infect Dis 2008;12:660-70.

150. Guven H, Altintop L, Baydin A, et al. Diagnostic value of procalcitonin levels as an early indicator of sepsis. Am J Emerg Med 2002;20:202-6.

151. Juutilainen A, Hämäläinen S, Pulkki K, et al. Biomarkers for bacteremia and severe sepsis in hematological patients with neutropenic fever: multivariate logistic regression analysis and factor analysis. Leuk Lymphoma 2011;52:2349-55.

152. Kim KE, Han JY. Evaluation of the clinical performance of an automated procalcitonin assay for the quantitative detection of bloodstream infection. Korean J Lab Med 2010;30:153-9.

153. de Kruif MD, Lemaire LC, Giebelen IA, et al. The influence of corticosteroids on the release of novel biomarkers in human endotoxemia. Intensive Care Med 2008;34:518-22.

154. Lehmann LE, Hunfeld KP, Steinbrucker M, et al. Improved detection of blood stream pathogens by real-time PCR in severe sepsis. Intensive Care Med 2010;36:49-56.

155. Lodes U, Bohmeier B, Lippert H, et al. PCR-based rapid sepsis diagnosis effectively guides clinical treatment in patients with new onset of SIRS. Langenbecks Arch Surg 2012;397:447-55.

156. Mauro MV, Cavalcanti P, Perugini D, et al. Diagnostic utility of LightCycler SeptiFast and procalcitonin assays in the diagnosis of bloodstream infection in immunocompromised patients. Diagn Microbiol Infect Dis 2012;73:308-11.

157. Peters RP, Twisk JW, van Agtmael MA, et al. The role of procalcitonin in a decision tree for prediction of bloodstream infection in febrile patients. Clin Microbiol Infect 2006;12:1207-13.

158. Previsdomini MG, Cerutti B, Dolina M, et al. Predictors of positive blood cultures in critically ill patients: a retrospective evaluation. Croat Med J 2012;53:30-9.

159. Sandri MT, Passerini R, Leon ME, et al. Procalcitonin as a useful marker of infection in hemato-oncological patients with fever. Anticancer Res 2008;28:3061-5.

160. Scott LK, Grier LR, Arnold TC, et al. Serum procalcitonin concentration as a negative predictor of serious bacterial infection in acute sickle cell pain crisis. Med Sci Monit 2003;9:CR426-31.

161. Su L, Han B, Liu C, et al. Value of soluble TREM-1, procalcitonin, and C-reactive protein serum levels as biomarkers for detecting bacteremia among sepsis patients with new fever in intensive care units: a prospective cohort study. BMC Infect Dis 2012;12:157.

162. Svaldi M, Hirber J, Lanthaler AI, et al. Procalcitonin-reduced sensitivity and specificity in heavily leucopenic and immunosuppressed patients. Br J Haematol 2001;115:53-7.

163. Ugarte H, Silva E, Mercan D, et al. Procalcitonin used as a marker of infection in the intensive care unit. Crit Care Med 1999;27:498-504.

164. von Lilienfeld-Toal M, Lehmann LE, Raadts AD, et al. Utility of a commercially available multiplex real-time PCR assay to detect bacterial and fungal pathogens in febrile neutropenia. J Clin Microbiol 2009;47:2405-10.

165. Yilmaz G, Köksal I, Karahan SC, et al. The diagnostic and prognostic significance of soluble urokinase plasminogen activator receptor in systemic inflammatory response syndrome. Clin Biochem 2011;44:1227-30.

166. Lavrentieva A, Papadopoulou S, Kioumis J, et al. PCT as a diagnostic and prognostic tool in burn patients. Whether time course has a role in monitoring sepsis treatment. Burns 2012;38:356-63.

167. Gaini S, Pedersen SS, Koldkaer OG, et al. New immunological serum markers in bacteraemia: anti-inflammatory soluble CD163, but not proinflammatory high mobility group-box 1 protein, is related to prognosis. Clin Exp Immunol 2008;151:423-31.

168. Kocazeybek B, Kucukoglu S, Oner YA. Procalcitonin and C-reactive protein in infective endocarditis: correlation with etiology and prognosis. Chemotherapy 2003;49:76-84.

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# Changes in circulating procalcitonin versus C-reactive protein in predicting evolution of infectious disease in febrile, critically ill patients

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## ABSTRACT

**Objective** Although absolute values for C-reactive protein (CRP) and procalcitonin (PCT) are well known to predict sepsis in the critically ill, it remains unclear how changes in CRP and PCT compare in predicting evolution of: infectious disease, invasiveness and severity (e.g. development of septic shock, organ failure and non-survival) in response to treatment. The current study attempts to clarify these aspects.

**Methods** In 72 critically ill patients with new onset fever, CRP and PCT were measured on Day 0, 1, 2 and 7 after inclusion, and clinical courses were documented over a week with follow up to Day 28. Infection was microbiologically defined, while septic shock was defined as infection plus shock. The sequential organ failure assessment (SOFA) score was assessed.

**Results** From peak at Day 0-2 to Day 7, CRP decreased when (bloodstream) infection and septic shock (Day 0-2) resolved and increased when complications such as a new (bloodstream) infection or septic shock (Day 3-7) supervened. PCT decreased when septic shock resolved and increased when a new bloodstream infection or septic shock supervened. Increased or unchanged SOFA scores were best predicted by PCT increases and Day 7 PCT, in turn, was predictive for 28-day outcome.

**Conclusion** The data, obtained during ICU-acquired fever and infections, suggest that CRP may be favoured over PCT courses in judging response to antibiotic treatment. PCT, however, may better indicate the risk of complications, such as bloodstream infection, septic shock, organ failure and mortality, and therefore might help deciding on safe discontinuation of antibiotics. The analysis may thus help interpreting current literature and design future studies on guiding antibiotic therapy in the ICU.

## INTRODUCTION

In critically ill patients, new onset fever often prompts clinicians to search for nosocomial microbial infection and to start empiric antibiotics in attempts to diminish morbidity and mortality.[1-3] Plasma levels of C-reactive protein (CRP) or procalcitonin (PCT) are often used to increase the a priori probability of microbial infection and sepsis in the intensive care unit (ICU).[2-12] In a previous study on absolute levels within the first days after new fever onset in critically ill patients, we found that CRP may particularly help in predicting local microbial infection and PCT in predicting bloodstream infection (BSI) and a downhill clinical course.[3] The clinical relevance of changes in the markers is less clear, however. Changes in CRP and PCT over 2-7 days have been described in non-critically ill patient populations,[13-20] in relatively small studies, about 50 patients or less,[4,9,13,15,16,19,21-25] in specific conditions [2,8,10,13,15-17,19,20,22,24,26-29] or in heterogeneous conditions in the ICU, [4-7,9,11,21,23,30-32] to judge the course of infection and its sequelae. CRP decreases of more than about 25% per day within the first week of treatment of (bloodstream) infections or sepsis have been suggested to help predict a beneficial response and disease course, while slower decreases or increases have been associated with persistent infection, organ failure or mortality, also in the ICU. [7,8,11,17-19,21-,23,26-29,32] A relatively rapid fall of PCT may be associated with a beneficial outcome of pneumonia, meningitis, burn-associated or other infections, whereas a rise may be associated with organ failure and mortality and thus might have predictive value. [5,6,8-10,15,16,20,24,26,27,30,31] A fall in PCT below 0.5 ng/mL, a threshold suggested in studies on non-critically ill patients, has been used to safely and effectively shorten the duration of administration of antibiotics for infections in the ICU.[33] CRP and PCT changes have been compared in their relative ability to detect the evolution of infection or sepsis, within[4-6,10,24-27,30,31] or outside the ICU.[8,14,15,18,19] CRP may display slower kinetics than PCT and in some, but not all studies, decreases or increases of the latter may better predict a beneficial or downhill course with resolving or aggravating organ failure, respectively.[2,4,6,8,10,14-16,19,24-27,30,31,34] Nevertheless, the relative value of marker changes in predicting response to antibiotic treatment of ICU-acquired infections is only rarely addressed.[10,15,24,26,27,31] Hence, general conclusions on the relative superiority of the markers for specific endpoints in the critically ill are hard to draw from the literature and the controversy is ongoing.

In order to further help clinical decision making on the basis of changes in CRP and PCT in the ICU, we hypothesised for the current study that, in general critically ill patients with new onset fever, the 1-week course of CRP and PCT levels can be used to distinguish resolving microbial infection with a beneficial outcome from non-resolving or developing infection with a detrimental outcome associated with BSI, septic shock, organ failure and death. We also hypothesised that CRP and PCT differ in this respect,

so that CRP primarily predicts the course of local infection and PCT that of systemic infection and its adverse sequelae. This would also help to define values at which antibiotic treatment can be decided as appropriate or to allow safe discontinuation in the ICU.

## PATIENTS AND METHODS

This is a prospective observational study on causes and consequences of ICU-acquired fever, approved by the medical ethical committee of the VU University Medical Centre, Amsterdam, conducted between 2003 and 2007. All patients or closest relatives gave their written informed consent, leading to inclusion of 101 consecutive patients with new onset fever, admitted to a mixed medical/surgical ICU, and the complete protocol has been described elsewhere.[3] The current analysis is on the 72 patients having completed a follow up of at least 7 days in the ICU (Consort diagram Figure 1). To briefly reiterate, a body temperature >38.3 °C measured rectally was the main inclusion criterion, while admitted to the ICU for at least 24 hours without fever (body temperature <37.5 °C). Exclusion criteria were: age under 18 years, pregnancy and life expectancy of <24 hours. Enrolment had to be completed within 12 hours of meeting inclusion criteria and was marked Day 0 (D0). Demographic data were collected. Disease severity was expressed through the simplified acute physiology scores (SAPS) II and monitored using sequential organ failures assessment (SOFA)-scores. On Days (D) 0, 1, 2 and 7 clinical data were recorded and blood was drawn for determination of routine parameters and markers. Antibiotic treatment and changes within the study period were recorded.

Routine chest- and sinus-radiographs were taken on D0 and 7, all other diagnostic imaging were ordered by treating physicians, blinded to results, as considered necessary. Blood samples for microbiological culture were taken from indwelling arterial

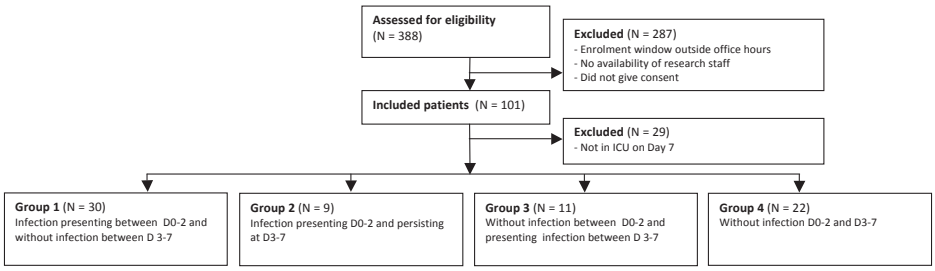



**Figure 1.** Consort diagram

catheters using delayed vial entry bottles for aerobic and anaerobic cultures and processed according to protocol. Depending on suspicion of local infection, specimens for microbial culture were collected. Investigation of fungal, viral or atypical microorganisms was left at the treating physician's discretion. All culture and staining results from specimens drawn between D0-7 were evaluated. Positive cultures considered to represent colonization were not considered to represent infection and microorganisms are grouped according to genus. The presence of infection was determined by researchers (SHH and ABJG) blinded to study results and classified by likelihood into possible, probable or proven infection, according to criteria defined at the International Sepsis Forum Consensus Conference.[35] Infections are only considered when probable or proven.[3] Sepsis was defined as infection in the presence systemic inflammatory response syndrome (SIRS) criteria, according to ACCP/SCCM (American Society of Chest Physicians/ Society of Critical Care Medicine).[36] Criteria of shock criteria were a systolic arterial pressure <90 mmHg or a mean arterial pressure (MAP) <70 mmHg for at least one hour, despite fluid resuscitation, or need of vasopressor treatment. Shock in the presence of sepsis was marked septic shock. Organ failure was assessed by the sequential organ failure assessment (SOFA) score on Day 0, 1, 2 and 7.

Patients were divided into four categories of infectious course: Group 1 with infection presenting at D0-2 and without evidence of infection at D3-7, thus having a response to treatment and resolving infection, Group 2 with infection presenting D0-2 and persisting positive cultures from the same infection site and/or persisting positive cultures with the same microorganism at D3-7, thus having treatment failure, Group 3 without infection D0-2 and with infection D3-7, thus having a new infection, and Group 4 without infection D0-2 and D3-7. Complications of infection were considered BSI, septic shock and outcome. Therefore a similar division was done for BSI, irrespective of local cultures. Group 1a with BSI presenting D0-2 and without BSI D3-7, thus resolving BSI in response to treatment. Group 2a with BSI presenting D0-2 and with persisting BSI D3-7, i.e. treatment failure, Group 3a without BSI D0-2 and with infection D3-7, having a new BSI and Group 4a without BSI D0-2 and D3-7. Septic shock was defined by the presence of shock within 12 hours prior or 12 hours after the determination of infection in either one of the respective study intervals. Group 1b represents presence of septic shock in D0-2 but without septic shock in D3-7, Group 2b presents septic shock both in D0-2 and D3-7, Group 3b absence of septic shock in D0-2 but presence of septic shock D3-7, and Group 4b absence of septic shock in both study intervals. Analogously, we divided patients with decreasing SOFA scores (peak D0-2 to 7; Group 1c), unchanged SOFA scores (Group 2c) and increasing SOFA scores (Group 3c). Outcome is 28-day survival or all-cause mortality.

Routine parameters measured were white blood cell count (WBC) (Sysmex SE-9000 analyzer, Toa Medical Instruments, Kobe, Japan, normal values 4.5-10 $x10^9$/L), lactate

(Enzymatic method, Modular analytics <P> Roche diagnostics, Mannheim, Germany, normal values <1.8 mmol/L) and C-reactive protein (CRP, Immunoturbidimetric assay, Modular analytics <P> Roche diagnostics, Mannheim, Germany, normal values <5 mg/L). Procalcitonin was measured through the Kryptor compact system (Brahms Diagnostica, Henningsdorf, Germany, normal values <0.08 ng/L) using time resolved amplified cryptate emission (TRACE) technology.

### Statistical analysis

CRP and PCT courses were expressed as fractional changes at D7 vs. peak values at D0-2. To further separate differences in absolute levels and changes, we used the Kruskal-Wallis test to evaluate group differences in the respective values. Area under the receiver operating characteristic curves (AUROC) were used to evaluate predictive values, such as sensitivity and specificity of optimal cut off values, defined at highest combined sensitivity and specificity, and their statistical significance. Exact P values are given unless <0.001, and values <0.05 were considered statistically significant. Data are expressed as number (percentage) or median (range) in tables and median, interquartile range in figure 2.

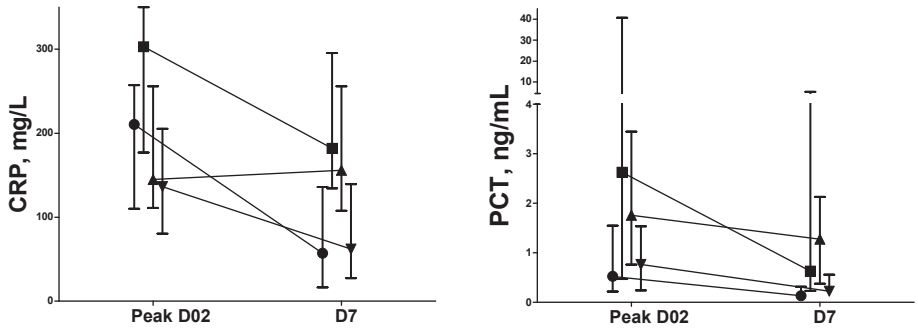



**Figure 2.** Evolution of C-reactive protein and procalcitonin according to evolution of infection (i) in febrile critically ill patients: CRP and PCT levels presented as median (interquartile range). ● Group 1=i Day (D)0-2 no i D3-7; ■ Group 2=i D0-2 and i D3-7; ▲ Group 3=no i D0-2 but i D3-7; ▼ Group 4=no i D0-2 nor D3-7. For CRP D0-2 P=0.009, for CRP D7 P=0.002, for change P= 0.004; for PCT D0-2 P=0.054, PCT D7 P<0.001, for change P=0.23, among groups.

## RESULTS

Table 1 represents patient characteristics and Table 2 infection characteristics, grouped according to course of infection. Fifty-two patients with new onset fever had resolving (Group 1) or no (Group 4) infection and only twenty had non-resolving (Group 2) or new (Group 3) infection, but (change of) treatment was mostly successful and ultimate mortality did not differ among groups. Most patients with infections had SIRS and therefore sepsis. The number of days from ICU admission until study inclusion was lowest in Group 2 and 3 and the need for vasopressor and renal replacement therapy was highest in Group 3. Infections with yeast were more persistent than infections with other microorganisms. 28-Day mortality in BSI was for Group 1a-4a 13, 100, 40 and 16%, respectively (P=0.02), and in septic shock for Group 1b-4b 19, 40, 20 and 17%, respectively (P=0.68).

### Course of infectious disease, CRP and PCT

Figure 1 shows the differences in marker levels in time for the infection groups. Table 3 shows that, among changes, those in CRP, rather than PCT, differed between groups according to infectious status, with a large decrease in Group 1 and persistently high values in Group 2 and 3, whereas absolute values of both CRP and PCT differed among groups. Table 4 shows that, among changes, those in PCT best discriminated between BSI groups, whereas absolute values of WBC, PCT and lactate at D7 differed among groups. Table 5 displays course in time for septic shock showing that, among changes, those in PCT better discriminated between groups than changes in CRP, whereas absolute values of WBC (D7), CRP, PCT also differed among groups. Table 6 shows that changes in SOFA score were particularly associated with changes in PCT.

### Predictive values for evolving infectious disease

Table 7 summarises the predictive values of changes in markers for resolving (Group 1, a, b or c) or new (Group 3, a, b, or c) infection, BSI, septic shock or organ failure vs. other groups. Most AUROC's were above 0.70, with high specificities of optimal cut off values. The table shows that CRP changes particularly predict changes in the status of infections and their complications knowingly BSI and septic shock, whereas PCT changes primarily predict the latter complications and the course of organ failure. Optimal cut off values are shown with decreases in markers by 86% or more and increases by 23% or more, and higher sensitivities of PCT than of CRP.

### D7 values: group differences and outcome prediction

At D7, CRP had a cut off <20.9 mg/L predicting Group 1 (at AUROC 0.67, P=0.01, sensitivity 31 and specificity 93%). For PCT, the cut off was <0.18 ng/mL (at AUROC

**Table 1.** Patient characteristics.

| | | Group 1 N=30 | Group 2 N=9 | Group 3 N=11 | Group 4 N=22 | P |
|---|---|---|---|---|---|---|
| **General** | | | | | | |
| Age (year) | | 62 (19-79) | 63 (36-71) | 68 (22-76) | 66 (26-77) | 0.91 |
| Gender (male) | | 22 (73) | 6 (68) | 8 (73) | 16 (73) | 0.98 |
| SAPS II on admission | | 48 (21-72) | 49 (29-78) | 50 (24-84) | 45 (27-85) | 0.99 |
| Peak SOFA at D0-2 | | 8 (3-13) | 8 (3-13) | 10 (4-14) | 8 (3-16) | 0.31 |
| SOFA at D7 | | 5 (0-15) | 5 (4-14) | 7 (5-13) | 5 (2-21) | 0.28 |
| Temp ºC | D0-2 | 39.2 (38.5-40.8) | 39.3 (38.6-40.0) | 39.0 (38.7-40.0) | 39.0 (35.8-39.8) | 0.80 |
| | D7 | 37.6 (36.5-39.0) | 39.2 (36.7-40.0) | 38.1 (37.3-39.7) | 37.8 (36.5-40.0) | 0.03 |
| Change | | 0.96 (0.89-1.00) | 1.00 (0.93-1.02) | 0.98 (0.94-1.03) | 0.96 (0.93-1.00) | 0.05 |
| SIRS. | D0-2 | 30 (100) | 9 (100) | 11 (100) | 22 (100) | 1.0 |
| | D7 | 23 (85) | 9 (100) | 9 (82) | 17 (90) | 0.65 |
| Sepsis, | D0-2 | 30 (100) | 9 (100) | 0 (0) | 0 (0) | 1.00 |
| | D7 | 0 (0) | 9 (100) | 9 (82) | 0 (0) | 0.19 |
| Septic shock, | D0-2 | 15 (50) | 6 (67) | 0 (0) | 0 (0) | 0.39 |
| | D7 | 0 (0) | 7 (78) | 8 (73) | 0 (0) | 0.80 |
| Days from admission to D0 | | 7 (1-77) | 4 (1-12) | 5 (1-16) | 12 (2-78) | 0.01 |
| Duration mechanical ventilation, days | | 22 (5-82) | 26 (4-42) | 16 (11-82) | 35 (10-123) | 0.15 |
| ICU length of stay, days | | 28 (10-95) | 26 (12-44) | 26 (12-85) | 37 (11-126) | 0.41 |
| 28-day mortality | | 5 (17) | 2 (22) | 2 (18) | 5 (23) | 0.95 |
| ICU mortality | | 7 (23) | 1 (11) | 2 (18) | 5 (23) | 0.87 |
| **Admission category** | | | | | | |
| Trauma | | 4 (13) | 1 (11) | 2 (18) | 4 (18) | 0.94 |
| Surgery | | | | | | |
| general | | 17 (57) | 6 (67) | 9 (82) | 13 (59) | 0.50 |
| cardiac | | 1 (3) | 1 (11) | 1 (9) | 3 (14) | 0.60 |
| vascular | | 2 (7) | 1 (11) | 4 (36) | 9 (2) | 0.08 |
| Neurologic | | 5 (17) | 2 (22) | 1 (9) | 3 (14) | 0.86 |
| Respiratory insufficiency | | 17 (57) | 2 (22) | 4 (36) | 7 (32) | 0.16 |
| post CPR | | 3 (10) | 0 (0) | 2 (18) | 0 (0) | 0.18 |
| Sepsis | | 12 (40) | 3 (33) | 1 (9) | 7 (32) | 0.32 |
| Shock | | 9 (30) | 1 (11) | 0 (0) | 7 (32) | 0.13 |
| **Treatment up to 7 days prior to inclusion** | | | | | | |
| Corticosteroids | | 14 (47) | 3 (33) | 4 (36) | 12 (55) | 0.65 |
| SDD | | 8 (27) | 2 (22) | 4 (36) | 12 (55) | 0.65 |
| Surgery | | 4 (13) | 2 (22) | 1 (9) | 2 (9) | 0.77 |

**Table 1.** Patient characteristics. (Continued)

| | Group 1 N=30 | Group 2 N=9 | Group 3 N=11 | Group 4 N=22 | P |
|---|---|---|---|---|---|
| **Treatment during study D0-7** | | | | | |
| Antibiotics | 30 (100) | 9 (100) | 11 (100) | 19 (86) | 0.07 |
| Change in antibiotics | 20 (67) | 8 (89) | 7 (64) | 13 (59) | 0.46 |
| Corticosteroids | 19 (63) | 4 (44) | 4 (36) | 12 (55) | 0.65 |
| SDD | 8 (27) | 2 (22) | 4 (36) | 12 (55) | 0.65 |
| Mechanical ventilation | 29 (97) | 8 (89) | 11 (100) | 22 (100) | 0.35 |
| Inotropic/vasopressors | 16 (57) | 8 (89) | 11 (100) | 13 (59) | 0.03 |
| Renal replacement therapy | 1 (3) | 0 (0) | 4 (36) | 2 (9) | 0.01 |
| Surgery | 3 (10) | 1 (11) | 2 (18) | 3 (14) | 0.91 |

Median (range), or number (percentage); SAPS=simplified acute physiology score; SOFA=sequential organ failure assessment score; ICU=intensive care unit; CPR=cardiopulmonary resuscitation; SDD=selective decontamination of the digestive tract. Group 1= infection (I) Day (D)0-2 not D3-7; Group 2= I D02 and I D3-7; Group 3=no I D0-2 but D3-7; Group 4=no I D0-2 nor D3-7.

0.76, P<0.001, sensitivity 63 and specificity 93%). For a PCT cut off <0.5 ng/mL sensitivity was 87 and specificity 45%. PCT at D7 also predicted, at a cut off >7.8 ng/mL (AUROC 0.67, P=0.03, sensitivity 13 and specificity 96%), no decrease in SOFA score (Group 3c+2c). On D7, only WBC and PCT were lower in 28-day survivors than in non-survivors (P=0.003 and 0.02, respectively). The AUROC for non-survival for WBC at D7, at an optimal cut off >33.1 $x10^9$/L, was 0.76 (P<0.001, with sensitivity of 0 and specificity of 100%) and for PCT at D7 0.70 (P=0.005, with sensitivity 29 and specificity 95%), at an optimal cut off >2.6 ng/mL.

## DISCUSSION

This prospective, medium-sized study shows that changes in CRP and PCT, rather than changes in WBC, in general critically ill patients with new onset fever predict infectious courses in response to treatment, but in a different manner. CRP changes appear most predictive for changes in infectious status and complications such as BSI and development of septic shock. Whereas PCT changes primarily appear predictive for infectious complications BSI, development of septic shock, associated organ failure and death.

Our study is the third one comparing CRP and PCT and the second one comparing their courses in the evolution of nosocomial infection in the general critically ill patient with new onset fever.[9,12] Our data do not suggest different kinetics per se over a 5-7 day course, in contrast to the slower kinetics of CRP than of PCT in the critically ill suggested by others.[4,6,8,10,12,15,27,31,34] This study however, suggests that CRP

**Table 2.** Infection characteristics.

| | Group 1 N=30 | Group 2 N=9 | Group 3 N=11 | P |
|---|---|---|---|---|
| **Infection D0-2** | | | | |
| Tracheobronchitis | 8 (27) | 5 (55) | - | 0.11 |
| CAP | 1 (3) | 0 (0) | - | 0.58 |
| VAP | 6 (20) | 1 (11) | - | 0.54 |
| Aspiration pneumonia | 1 (3) | 0 (0) | - | 0.58 |
| Pleurisy/empyema | 1 (3) | 1 (11) | - | 0.35 |
| Sinusitis | 5 (17) | 1 (11) | - | 0.19 |
| Catheter infection | 2 (7) | 2 (22) | - | 0.18 |
| Peritonitis | 2 (7) | 1 (11) | - | 0.43 |
| Pancreatitis | 2 (7) | 0 (0) | - | 0.43 |
| Skin and soft tissue | 7 (23) | 0 (0) | - | 0.15 |
| **Infection D3-7** | | | | |
| Tracheobronchitis | - | 3 (33) | 2 (18) | 0.44 |
| VAP | - | 1 (11) | 4 (36) | 0.19 |
| Aspiration pneumonia | - | 0 (0) | 0 (0) | 1.0 |
| Pleurisy/empyema | - | 1 (11) | 0 (0) | 0.26 |
| Sinusitis | - | 1 (11) | 2 (18) | 0.66 |
| Catheter infection | - | 1 (11) | 1 (9) | 0.88 |
| Peritonitis | - | 1 (11) | 0 (0) | 0.26 |
| Skin and soft tissue | - | 0 (0) | 4 (36) | 0.04 |
| Meningitis | - | 1 (11) | 0 (0) | 0.26 |
| **Local microbiology D0-2** | | | | |
| Enterobacteriaceae | 9 (30) | 4 (44) | - | 0.85 |
| Staphylococci | 10 (33) | 3 (33) | - | 1.00 |
| Pseudomonadaceae | 5 (17) | 0 (0) | - | 0.19 |
| Enterococci | 4 (13) | 0 (0) | - | 0.25 |
| Xantomonadaceae | 3 (10) | 2 (22) | - | 0.34 |
| Yeasts | 5 (17) | 3 (33) | - | 0.28 |
| Miscellaneous | 12 (40) | 3 (33) | - | 0.72 |
| **Local microbiology D3-7** | | | | |
| Enterobacteriaceae | - | 4 (44) | 4 (36) | 0.71 |
| Staphylococci | - | 3 (33) | 3 (27) | 0.80 |
| Pseudomonadaceae | - | 0 (0) | 1 (9) | 0.35 |
| Enterococci | - | 0 (0) | 2 (18) | 0.18 |
| Xantomonadaceae | - | 2 (2) | 2 (18) | 0.82 |
| Yeasts | - | 3 (33) | - | 0.04 |
| Miscellaneous | - | 3 (33) | 2 (18) | 0.82 |

**Table 2.** Infection characteristics. (Continued)

| | Group 1 N=30 | Group 2 N=9 | Group 3 N=11 | P |
|---|---|---|---|---|
| **Blood stream infection D0-2** | | | | |
| Enterobacteriaceae | 1 (3) | 1 (11) | - | 0.35 |
| Staphylococci | 4 (13) | 0 (0) | - | 0.25 |
| Enterococci | 1 (3) | 1 (11) | - | 0.35 |
| Yeasts | 0 (0) | 2 (22) | - | 0.008 |
| Miscellaneous | 1 (3) | 0 (0) | - | 0.58 |
| **Blood stream infection D3-7** | | | | |
| Enterobacteriaceae | - | 1 (11) | 1 (9) | 0.88 |
| Staphylococci | - | 0 (0) | 2 (18) | 0.18 |
| Enterococci | - | 1( 11) | 2 (18) | 0.66 |
| Yeasts | - | 2 (22) | 0 (0) | 0.10 |

Number (percentage); CAP=community-acquired pneumonia; VAP=ventilator-acquired pneumonia; Group 1= infection (I) Day (D)0-2 not D3-7; Group 2= I D0-2 and I D3-7; Group 3= no I D0-2 but D3-7.

**Table 3.** Evolution of infection.

| | Group 1 N= 30 | Group 2 N = 9 | Group 3 N = 11 | Group 4 N = 22 | p |
|---|---|---|---|---|---|
| WBC D0-2, $x10^9$/L | 13.9 (2.5-24.4) | 12.8 (7.9-81.7) | 16.2 (9.0-24.8) | 11.6 (7.8-23.9) | 0.22 |
| WBC D7, $x10^9$/L | 11.5 (4.9-23.2) | 16.9 (8.3-30.2) | 16.2(6.4-33.0) | 11.9 (5.3-29.2) | 0.06 |
| WBC change | 0.80 (0.47-3.20) | 0.75 (0.37-1.41) | 1.16 (0.40-2.95) | 0.94 (0.50-1.65) | 0.55 |
| CRP D0-2, mg/L | 210 (5-397) | 303 (102-421) | 145 (38- 440) | 137 (27-248) | 0.009 |
| CRP D7, mg/L | 57 (2-267) | 182 (22-416) | 156 (49-304) | 62 (6-265) | 0.002 |
| CRP change | 0.40 (0.02-1.15) | 0.68 (0.07-1.82) | 0.93 (0.44-6.97) | 0.58 (0.11-2.58) | 0.004 |
| PCT D0-2, ng/mL | 0.5 (0.08-45.1) | 2.6 (0.08-75.3) | 1.7(0.3-6.3) | 0.8 (0.1-2.8) | 0.054 |
| PCT D7, ng/mL | 0.1 (0.06-38.5) | 0.6 (0.1-24.3) | 1.3 (0.3-20.8) | 0.2 (0.08-4.3) | <0.001 |
| PCT change | 0.30 (0.05-1.57) | 0.42 (0.04-2.97) | 0.52 (0.08-68.3) | 0.44 (0.11-5.88) | 0.23 |
| Lactate D0-2, mmol/L | 1.6 (0.5-3.5) | 1.5 (0.9-3.5) | 1.3 (1.0-2.3) | 1.4 (0.5-2.0) | 0.60 |
| Lactate D7 mmol/L | 1.1 (0-4.3) | 1.1 (0.9-3.1) | 1.1 (0.7-1.8) | 1.0 (0.5-2.2) | 0.67 |
| Lactate change | 0.77 (0-2.08) | 0.95 (0.48-1.72) | 0.85 (0.43-1.60) | 1.06 (0.42-1.50) | 0.32 |

Median (range) for WBC=white blood cell count; CRP=C-reactive protein; PCT=procalcitonin; Group 1=infection (I) Day (D)0-2 not D3-7; Group 2=I D0-2 and I D3-7; Group 3=no I D0-2 but D3-7; Group 4=no I D0-2 nor D3-7.

changes are more associated with evolution of infection and PCT more with evolution of adverse infectious sequelae. Although the AUROC's of changes in markers seem higher than those for absolute levels, our current findings on absolute levels are in line with those reported earlier[3], suggesting that during nosocomial fever in the critically ill CRP is more likely to contribute to infection diagnosis whereas PCT has better

**Table 4.** Evolution of bloodstream infection.

| | Group 1a N=8 | Group 2a N=2 | Group 3a N=5 | Group 4a N=57 | p |
|---|---|---|---|---|---|
| WBC D0-2, x10$^9$/L | 18.2 (2.5-27.5) | 53.2 (24.7-81.7) | 13.9 (9.0-19.8) | 12.8 (7.8-24.8) | 0.08 |
| WBC D7, x10$^9$/L | 15.5 (8.0-23.2) | 24.2 (18.3-30.2) | 23.1 (10.5-33.0) | 12.3(4.9-29.2) | 0.007 |
| WBC change | 0.76 (0.55-3.20) | 0.56 (0.37-0.74) | 1.17 (1.14-2.95) | 0.88(0.40-2.33) | 0.02 |
| CRP D0-2, mg/L | 220 (71-397) | 362 (303-421) | 139 (38-257) | 183 (5-440) | 0.07 |
| CRP D7, mg/L | 57 (3-267) | 205 (22-389) | 54 (101-304) | 85 (2-416) | 0.20 |
| CRP change | 0.14 (0.04-1.01) | 0.50 (0.07- 0.92) | 1.07 (0.73-6.97) | 0.55 (0.02-2.93) | 0.02 |
| PCT D0-2, ng/mL | 1.6 (0.09-45.1) | 74.2 (73.2-75.3) | 0.8 (0.3-3.4) | 0.6 (0.08-37.2) | 0.07 |
| PCT D7, ng/mL | 0.2 (0.06-7.8) | 13.6 (2.9-24.3) | 2.1 (1.3-20.8) | 0.2 (0.06-38.5) | 0.002 |
| PCT change | 0.32 (0.05-2.97) | 0.19 (0.04-0.33) | 2.80 (0.45-68.3) | 0.43 (0.05-5.88) | 0.01 |
| Lactate D0-2, mmol/L | 1.7 (1.1-3.5) | 2.6 (1.8-3.5) | 1.2 (1.0-1.5) | 1.4 (0.5-2.3) | 0.05 |
| Lactate D7, mmol/L | 2.1 (1.2-4.3) | 2.5 (1.8-3.1) | 1.3 (1.1-1.6) | 1.0 (0 -2.2) | 0.002 |
| Lactate change | 1.15 (0.50-2.08) | 1.12 (0.51-1.72) | 1.01(0.73-1.60) | 0.79 (0-1.50) | 0.20 |

Median (range) for WBC=white blood cell count; CRP=C-reactive protein; PCT=procalcitonin. Group 1b=septic shock (SS) Day (D) 0-2 not D3-7; Group 2b= SS D0-2 and SS D3-7; Group 3b= no SS D0-2 but D3-7; Group 4b=no SS D0-2 nor D3-7.

**Table 5.** Evolution of septic shock.

| | Group 1b N = 16 | Group 2b N = 5 | Group 3b N = 10 | Group 4b N = 41 | p |
|---|---|---|---|---|---|
| WBC D0-2, x10$^9$/L | 13.9 (2.5-24.4) | 19.8 (7.9-81.7) | 17.5 (9.0-27.5) | 12.3 (7.8-23.9) | 0.17 |
| WBC D7, x10$^9$/L | 14.5 (4.9-23.2) | 17.1 (9.3-30.2) | 19.0 (10.5-33.0) | 10.7 (5.3-29.2) | 0.001 |
| WBC change | 0.93 (0.47-3.20) | 0.74 (0.37-1.34) | 1.14 (0.60-2.95) | 0.81(0.40-2.33) | 0.25 |
| CRP D0-2, mg/L | 243 (5.0-397) | 306 (102-421) | 142 (38-257) | 181 (5-440) | 0.004 |
| CRP D7, mg/L | 57 (3.0-416) | 182 (22-389) | 156 (101-304) | 61 (2-265) | 0.01 |
| CRP change | 0.31 (0.04-1.12) | 0.56 (0.07-1.82) | 1.03 (0.48-6.97) | 0.51 (0.02-2.58) | 0.003 |
| PCT D0-2, ng/mL | 1.1 (0.08-45.1) | 8.2 (0.3-75.3) | 1.3 (0.08-6.3) | 0.5 (0.09-37.1) | 0.02 |
| PCT D7, ng/mL | 0.2 (0.06-2.6) | 0.6 (0.2-24.3) | 1.6 (0.2-20.8) | 0.2 (0.06-3.5) | 0.001 |
| PCT change | 0.18 (0.05-2.00) | 0.18 (0.04-0.64) | 1.73 (0.19-68.3) | 0.43 (0.08-5.88) | <0.001 |
| Lactate D0-2, mmol/L | 1.6 (1.0-3.5) | 1.5 (1.0-3.5) | 1.2 (0.9-2.3) | 1.4 (0.5-2.2) | 0.32 |
| Lactate D7, mmol/L | 1.2 (0-4.3) | 1.3 (0.9-3.1) | 1.1 (0.7-2.4) | 1.0 (0.5-2.7) | 0.21 |
| Lactate change | 0.86 (0-1.23) | 0.83 (0.51-1.72) | 1.00 (0.43-1.60) | 0.80 (0.38-2.08) | 0.69 |

capability of predicting risks of infections. PCT has also been reported to be capable, more than CRP, of early discrimination between severe infection or sepsis on the one hand and non-infectious SIRS or uncomplicated infection on the other.[2,4-6,8-10,12] PCT increases predicted bloodstream invasion, septic shock and organ failure and carried greater prognostic significance in the course of infectious disease than CRP on D7 thus supporting that PCT is more useful in predicting infectious complications, also in

**Table 6.** Evolution of SOFA scores.

| | Group 1c N = 52 | Group 2c N = 8 | Group 3c N = 8 | p |
|---|---|---|---|---|
| WBC D0-2, x10$^9$/L | 13.5 (2.5-27.5) | 13.2 (9.2-19.8) | 16.1(8.0-81.7) | 0.76 |
| WBC D7, x10$^9$/L | 13.0 (4.9-33.0) | 13.2 (8.5-27.2) | 15.5 (6.9-30.2) | 0.37 |
| WBC change | 0.83 (0.40-3.20) | 1.09 (0.67-1.96) | 0.82 (0.37-2.56) | 0.30 |
| CRP D0-2, mg/L | 202 (5-440) | 173 (38-290) | 157 (59-421) | 0.87 |
| CRP D7, mg/L | 85 (2-416) | 160 (41-304) | 92 (18-389) | 0.13 |
| CRP change | 0.51 (0.02-2.93) | 0.95 (0.20-6.97) | 0.72 (0.17-1.03) | 0.11 |
| PCT D0-2, ng/mL | 0.67 (0.08-75.3) | 0.68 (0.08-1.98) | 1.42 (0.14-73.2) | 0.37 |
| PCT D7, ng/mL | 0.23 (0.06-38.5) | 0.49 (0.15-10.4 | 1.36 (0.12-24.3) | 0.13 |
| PCT change | 0.39 (0.04-68.3) | 1.73 (0.18-5.88) | 0.67 (0.18-1.79) | 0.01 |
| Lactate D0-2, mmol/L | 1.4 (0.5-3.5) | 1.2 (0.50-1.8) | 1.7 (1.2-3.5) | 0.03 |
| Lactate D7, mmol/L | 1.1 (0.5-2.7) | 1.1 (0.7-1.9) | 1.0 (0.6-4.3) | 0.96 |
| Lactate change | 0.80 (0.38-2.08) | 1.17 (0.77-1.40) | 0.63 (0.38-1.72) | 0.15 |

Median (range) for WBC=white blood cell count; CRP=C-reactive protein; PCT=procalcitonin. Group 1c decreasing SOFA scores between D0-2 and D7. Group 2c unchanged SOFA scores, Group 3c increase in SOFA scores.

the ICU, even when not predicting new BSI.[2,4-6,8-10,12,14,20,24-27,30,31] In leptospirosis, PCT normalises upon treatment in 4 days and CRP within 7 days in non-severe cases and both return to normal in 7 days in severe infections[19], suggesting greater sensitivity of PCT than of CRP to infection severity, in line with our data. Conversely, we can assume in line with others,[10,15,17,21-23,28,29,31,32] that the decrease in CRP in Group 1 with resolving infection resulted from appropriate antibiotic treatment, so that we cannot exclude that the persistent infection in Group 2 with less decreases was caused by treatment failure or slow response, even though not associated with increased mortality. The threshold for CRP at D7 to decide on the resolution of infection only is about 21 mg/L. Our study does not agree with the relation between rate of decline in CRP up to D7 during treatment for infection in the ICU and survival,[22,24,26-29,31] since the change of CRP did not predict outcome. The difference with our study may relate to differences in inclusion criteria, among others. Our study suggesting greater value of decreases in CRP than of PCT in resolving nosocomial infection in the ICU, does also not agree with the reported superior value of PCT decreases in predicting response of infections to treatment.[10,15,26,31] A decrease of PCT to 0.5-1.0 ng/mL or lower has otherwise been used for allegedly safe discontinuation of antibiotics in patients with presumed infection given antibiotics with high likelihood for survival in the ICU.[8-10,12,16,24,26,27,31,33] However, our data are not in line with this threshold and suggest a lower value of about 0.2 ng/mL, after one week treatment, since the values associated with non-resolving infection, increasing SOFA and mortality are higher.

**Table 7.** Predictive values for changes of markers.

| Infection | Resolving (Group 1) | | | | | | | New (Group 3) | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Cut off | AUROC | P | Sens | Spec | PPV | NPV | Cut off | AUROC | P | Sens | Spec | PPV | NPV |
| WBC change | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| CRP change | <0.14 | 0.72 | <0.001 | 31 | 93 | 75 | 65 | >2.57 | 0.76 | <0.001 | 20 | 100 | 100 | 88 |
| PCT change | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| Bloodstream infection | | | | | | | | | | | | | | |
| | Resolving (Group 1a) | | | | | | | New (Group 3a) | | | | | | |
| | Cut off | AUROC | P | Sens | Spec | PPV | NPV | Cut off | AUROC | P | Sens | Spec | PPV | NPV |
| WBC change | - | - | - | - | - | - | - | >2.57 | 0.87 | <0.001 | 20 | 98 | 50 | 94 |
| CRP change | <0.04 | 0.73 | 0.04 | 29 | 57 | 50 | 92 | >2.95 | 0.84 | <0.001 | 20 | 100 | 100 | 94 |
| PCT change | - | - | - | - | - | - | - | >2.00 | 0.89 | <0.001 | 60 | 97 | 60 | 97 |
| Septic shock | Resolving (Group 1b) | | | | | | | New (Group 3b) | | | | | | |
| | Cut off | AUROC | P | Sens | Spec | PPV | NPV | Cut off | AUROC | P | Sens | Spec | PPV | NPV |
| WBC change | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| CRP change | <0.06 | 0.70 | 0.01 | 31 | 98 | 83 | 83 | >2.57 | 0.83 | <0.001 | 20 | 100 | 100 | 81 |
| PCT change | <0.13 | 0.72 | 0.007 | 31 | 93 | 56 | 83 | >1.78 | 0.82 | <0.001 | 50 | 97 | 71 | 92 |
| SOFA scores | Not increasing (Group 1c+2c) | | | | | | | Not decreasing (Group 2c+3c) | | | | | | |
| | Cut off | AUROC | P | Sens | Spec | PPV | NPV | Cut off | AUROC | P | Sens | Spec | PPV | NPV |
| WBC change | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| CRP change | - | - | - | - | - | - | - | >2.95 | 0.67 | 0.02 | 6 | 100 | 100 | 78 |
| PCT change | - | - | - | - | - | - | - | >1.23 | 0.73 | 0.001 | 38 | 92 | 60 | 83 |

WBC=white blood cell count; CRP=C-reactive protein; PCT=procalcitonin; AUROC=area under the receiver operating characteristic curve; Sens=sensitivity; Spec=specificity at optimal cut off values. PPV=positive predictive value; NPV=negative predictive value. Statistically significant AUROC's are given only. A value less than 1 denotes a fractional decrease from Day 0-2 to 7 and a value above 1 a fractional increase.

Some limitations of this study should be addressed. A fair number of patients received corticosteroids, including so called low dose steroids for treatment of relative adrenal insufficiency during sepsis, prior and during inclusion, but this may hardly affect marker levels as related to the course of infection as our data in line with those of others suggest.[13,17,37] We used selective decontamination of the digestive tract by non-absorbable antibiotics for infection prevention in many of our patients but the use apparently did not confound the value of CRP and PCT changes. We cannot exclude that Group 4 patients without infections had benefited from this type of infection prevention and had received overtreatment by empiric antibiotics, in the absence of demonstrable microbial infection. We chose to classify all groups similar to our primary outcome variable: course of infectious disease, thus always entailing four groups per outcome measure, except for SOFA score. This resulted in uneven numbers per patient group, and the small number of patients in group 2a is a consequence of this uniform categorisation. However, small groups may not invalidate statistical significance. The study carries the advantage over many others[2,4-7,17,30-32] of documentation of microbial infection and definitions of infectious complications rather than stages of 'sepsis' in the critically ill. Finally, our study lacks daily measurements, as other have done in spite of increasing costs,[2,5-11,14,15,18,20,21-23,25,31,32] so that we cannot conclude on rapid time courses of the infection markers. However, our data suggest clinical usefulness of the sampling regimen followed. We did not evaluate biomarker changes over shorter periods since the kinetics of resolving and developing infections may differ according to infectious focus and causative microorganism, among others.

## CONCLUSIONS

In conclusion, our study on ICU-acquired fever and infections suggests that CRP may be favoured over PCT courses over 5-7 days in judging response to antibiotic treatment, whereas the latter may better indicate the risk of complications, such as bloodstream infection, septic shock, organ failure and mortality, which may help deciding on safe discontinuation of antibiotics. The analysis may thus help interpreting current literature and design future studies on guiding antibiotic therapy in the ICU.

## REFERENCES


1. Laupland KB, Shahpori R, Kirkpatrick AW, et al. Occurrence and outcome of fever in critically ill adults. Crit Care Med 2008;36:1531-1535.
2. Castelli GP, Pognani C, Cita M, et al. Procalcitonin as a prognostic and diagnostic tool for septic complications after major trauma. Crit Care Med 2009;37:1845-1849.
3. Hoeboer SH, Alberts E, van den Hul I, et al. Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin. J Infect 2012;64:484-493.
4. Meisner M, Tschaikowsky K, Palmaers T, et al. Comparison of procalcitonin (PCT) and C-reactive protein (CRP) plasma concentrations at different SOFA scores during the course of sepsis and MODS. Crit Care 1999;3:45-50.
5. Luzzani A, Polati E, Dorizzi R, et al. Comparison of procalcitonin and C-reactive protein as markers of sepsis. Crit Care Med 2003;31:1737-1741.
6. Castelli GP, Pognani C, Meisner M, et al. Procalcitonin and C-reactive protein during systemic inflammatory response syndrome, sepsis and organ dysfunction. Crit Care 2004;8:R234-R242.
7. Povoa P, Coelho L, Almeida E, et al. Early identification of intensive care unit-acquired infections with daily monitoring of C-reactive protein: a prospective observational study. Crit Care 2006;10:R63.
8. Rau BM, Frigerio I, Buchler MW, et al. Evaluation of procalcitonin for predicting septic multiorgan failure and overall prognosis in secondary peritonitis: a prospective, international multicenter study. Arch Surg 2007;142:134-142.
9. Tsangaris I, Plachouras D, Kavatha D, et al. Diagnostic and prognostic value of procalcitonin among febrile critically ill patients with prolonged ICU stay. BMC Infect Dis 2009;9:213.
10. Lavrentieva A, Papadopoulou S, Kioumis J, et al. PCT as a diagnostic and prognostic tool in burn patients. Whether time course has a role in monitoring sepsis treatment. Burns 2012;38:356-363.
11. Reynolds SC, Shorr AF, Muscedere J, et al. Longitudinal changes in procalcitonin in a heterogeneous group of critically ill patients. Crit Care Med 2012;40:2781-2787.
12. Su L, Han B, Liu C, et al. Value of soluble TREM-1, procalcitonin, and C-reactive protein serum levels as biomarkers for detecting bacteremia among sepsis patients with new fever in intensive care units: a prospective cohort study. BMC Infect Dis 2012;12:157.
13. Confalonieri M, Urbino R, Potena A, et al. Hydrocortisone infusion for severe community-acquired pneumonia: a preliminary randomized study. Am J Respir Crit Care Med 2005;171:242-248.
14. Persson L, Soderquist B, Engervall P, et al. Assessment of systemic inflammation markers to differentiate a stable from a deteriorating clinical course in patients with febrile neutropenia. Eur J Haematol 2005;74:297-303.
15. Viallon A, Guyomarc'h P, Guyomarc'h S, et al. Decrease in serum procalcitonin levels over time during treatment of acute bacterial meningitis. Crit Care 2005;9:R344-R350.

16. von Lilienfeld-Toal M, Schneider A, Orlopp K, et al. Change of procalcitonin predicts clinical outcome of febrile episodes in patients with hematological malignancies. Support Care Cancer 2006;14:1241-1245.

17. Bruns AHW, Oosterheert JJ, Hak E, et al. Usefulness of consecutive C-reactive protein measurements in follow-up of severe community-acquired pneumonia. Eur Respir J 2008;32:726-732.

18. Lannergård A, Viberg A, Cars O, et al. The time course of body temperature, serum amyloid A protein, C-reactive protein and interleukin-6 in patients with bacterial infection during the initial 3 days of antibiotic therapy. Scand J Infect Dis 2009;41:663-671.

19. Crouzet J, Faucher JF, Toubin M, et al. Serum C-reactive protein (CRP) and procalcitonin (PCT) levels and kinetics in patients with leptospirosis. Eur J Clin Microbiol Infect Dis 2011;30:299-302.

20. Lacoma A, Rodriguez N, Prat C, et al. Usefulness of consecutive biomarkers measurement in the management of community-acquired pneumonia. Eur J Clin Microbiol Infect Dis 2012;31:825-833.

21. Yentis SM, Soni N, Sheldon J. C-reactive protein as an indicator of resolution of sepsis in the intensive care unit. Intensive Care Med 1995;21:602-605.

22. Coelho L, Povoa P, Almeida E, et al. Usefulness of C-reactive protein in monitoring the severe community-acquired pneumonia clinical course. Crit Care 2007;11:R92.

23. Schmit X, Vincent JL. The time course of blood C-reactive protein concentrations in relation to the response to initial antimicrobial therapy in patients with sepsis. Infection 2008;36:213-219.

24. Hillas G, Vassilakopoulos T, Plantza P, et al. C-reactive protein and procalcitonin as predictors of survival and septic shock in ventilator-associated pneumonia. Eur Respir J 2010;35:805-811.

25. Theodorou VP, Papaioannou VE, Tripsianis GA, et al. Procalcitonin and procalcitonin kinetics for diagnosis and prognosis of intravascular catheter-related bloodstream infections in selected critically ill patients: a prospective observational study. BMC Infect Dis 2012;12:247.

26. Luyt CE, Guerin V, Combes A, et al. Procalcitonin kinetics as a prognostic marker of ventilator-associated pneumonia. Am J Respir Crit Care Med 2005;171:48-53.

27. Seligman R, Meisner M, Lisboa TC, et al. Decreases in procalcitonin and C-reactive protein are strong predictors of survival in ventilator-associated pneumonia. Crit Care 2006;10:R125.

28. Lisboa T, Seligman R, Diaz E, et al. C-reactive protein correlates with bacterial load and appropriate antibiotic therapy in suspected ventilator-associated pneumonia. Crit Care Med 2008;36:166-171.

29. Coelho L, Salluh J, Soares M, et al. Patterns of c-reactive protein RATIO response in severe community-acquired pneumonia: a cohort study. Crit Care 201216:R53.

30. Gibot S, Cravoisy A, Kolopp-Sarda MN, et al. Time-course of sTREM (soluble triggering receptor expressed on myeloid cells)-1, procalcitonin, and C-reactive protein plasma concentrations during sepsis. Crit Care Med 2005;33:792-796.

31. Charles PE, Tinel C, Barbar S, et al. Procalcitonin kinetics within the first days of sepsis: relationship with the appropriateness of antibiotic therapy and the outcome. Crit Care 2009;13:R38.

32. Povoa P, Teixeira-Pinto AM, Carneiro AH. C-reactive protein, an early marker of community-acquired sepsis resolution: a multi-center prospective observational study. Crit Care 2011;15:R169.

33. Schuetz P, Muller B, Christ-Crain M, et al. Procalcitonin to initiate or discontinue antibiotics in acute respiratory tract infections. Cochrane Database Syst Rev 2012;9:CD007498.

34. Nijsten MW, Olinga P, The TH, de Vries EG, et al. Procalcitonin behaves as a fast responding acute phase protein in vivo and in vitro. Crit Care Med 2000;28:458-461.

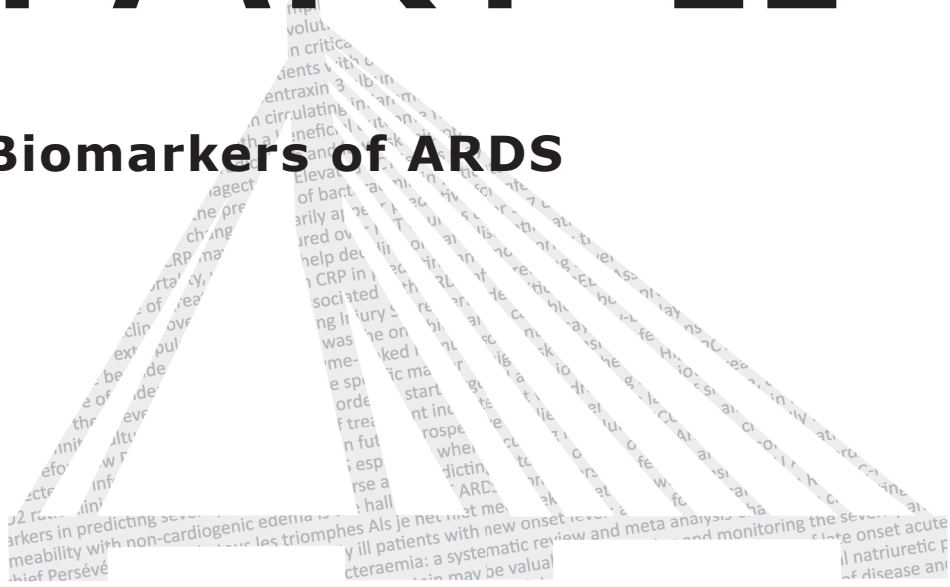
35. Calandra T, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. Crit Care Med 2005;33:1538-1548.

36. Levy MM, Fink MP, Marshall JC, et al; international sepsis definitions conference. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Intensive Care Med 2003;29:530-538.

37. de Kruif MD, Lemaire LC, Giebelen IA, et al. The influence of corticosteroids on the release of novel biomarkers in human endotoxemia. Intensive Care Med 2008;34:518-522.

# PART II

## Biomarkers of ARDS



# 6

# Albumin rather than C-reactive protein may be valuable in predicting and monitoring the severity and course of acute respiratory distress syndrome in critically ill patients with or at risk for the syndrome after new onset fever

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## ABSTRACT

**Objective** We studied the value of routine biochemical variables albumin, C-reactive protein (CRP) and lactate dehydrogenase (LDH) to improve prediction and monitoring of acute respiratory distress syndrome (ARDS) severity in the intensive care unit.

**Methods** In 101 critically ill patients, with or at risk for ARDS after new onset fever, data were collected on days (D) 0, 1, 2, and 7 after inclusion. ARDS was defined by the Berlin definition and lung injury score (LIS).

**Results** At baseline, 48 patients had mild to severe ARDS according to Berlin and 87 according to LIS (Rs=0.54, P<0.001). Low baseline albumin levels were moderately associated with maximum Berlin and LIS categories within 7 days; an elevated CRP level was moderately associated with maximum Berlin categories only. The day-by-day Berlin and LIS categories were inversely associated with albumin levels (P=0.01, P<0.001) and directly with CRP levels (P=0.02, P=0.04, respectively). Low albumin levels had monitoring value for ARDS severity on all study days (area under the receiver operating characteristic curve, AUROC, 0.62-0.82, P<0.001-0.03), whereas supranormal CRP levels performed less . When the Berlin or LIS category increased, albumin levels decreased ≥1 g/L (AUROC 0.72-0.77, P=0.001) and CRP increased ≥104 mg/L (only significant for Berlin, AUROC 0.69, P=0.04). When the LIS decreased, albumin levels increased ≥1 g/L (AUROC 0.68, P=0.02). LDH was higher in 28-day non-survivors than survivors (P=0.007).

**Conclusions** Overall, albumin may be of greater value than CRP in predicting and monitoring the severity and course of ARDS in critically patients with or at risk for the syndrome after new onset fever. Albumin levels below 20 g/L as well as a decline over a week are associated with ARDS of increasing severity, irrespective of its definition. LDH levels predicted 28-day mortality.

## INTRODUCTION

The acute respiratory distress syndrome (ARDS) is caused by alveolocapillary inflammation and increased permeability following a direct pulmonary or extrapulmonary insult. Many conditions, such as sepsis and trauma, which increase the risk for developing or worsening of ARDS are associated with fever. Fever , in turn, may aggravate alveolocapillary inflammation.[1,2] The recent Berlin definition and the old, more elaborate lung injury score (LIS)[2-8] are used to diagnose and classify ARDS. One of the drawbacks of the Berlin definition, even though moderately relating to lung edema,[9] is its dependency on ventilator settings in mechanically ventilated patients (with positive end-expiratory pressure, PEEP, affecting the oxygenation ratio) and lack of a specific index of severity as the total respiratory compliance.[5,7] PEEP and compliance are incorporated in the LIS,[3] which may therefore constitute a refined but more complex measure of clinical severity that correlates with alveolocapillary permeability and can be assessed at the bedside if the measurement technique is available.[10,11] The Berlin definition further includes preconditions and bilateral consolidations, even in the lowest class, while the lowest class of LIS may contain unilateral consolidation. Another limitation of these clinical classifications systems is their use of chest radiographs in the diagnostic work up. Interobserver agreement on chest imaging is poor, leading to frequent false positives and false negatives.[12,13] The systems have been compared and only partial overlap has been acknowledged.[1,4] Notably, agreement between clinical ARDS definitions and autopsy findings of diffuse alveolar damage is moderate.[4,8] Moreover, clinicians may underdiagnose ARDS, particularly when occurring late in the intensive care unit (ICU), and may be poorly able to quantify its severity and course, particularly when clinical classification systems are not commonly used.[4,6-8,14]

Therefore, the search for accurate biomarkers reflecting the severity and course of alveolocapillary inflammation and increased permeability underlying the non-cardiogenic pulmonary edema of ARDS is ongoing.[6,15] We and others described that circulating albumin levels, in cross-sectional studies, inversely relates to increased alveolocapillary permeability and that hypoalbuminemia predict ARDS and oedema formation in at risk patients.[9,11,16-19] Extravasation of albumin following increased permeability lowers albumin levels and the resultant low plasma colloid osmotic pressure promotes oedema formation. Inflammation and injury markers such as C-reactive protein (CRP)[18,20-25] and lactate dehydrogenase (LDH)[24,26] have been suggested to help predict early onset ARDS and its outcome in cross-sectional studies. Since both clinical classifications systems allow coincident ARDS and hydrostatic oedema, inflammatory markers such as CRP may be of value in separating non-hydrostatic from hydrostatic edema.[15,23] Meduri et al.[20] showed a decline in CRP and LIS in early ARDS patients responding to corticosteroids. However, the ARDS monitoring value of these routine

biochemical markers, often available on a daily basis in the intensive care unit (ICU), is unknown. Associations with the severity and course of ARDS, if any, could be of value in monitoring and therefore in the management of the syndrome at the bedside.

The aim of the present study is to determine whether albumin, CRP and LDH levels are associated with the severity and course of ARDS in critically ill patients after new onset fever with or considered at risk for the syndrome defined by the Berlin and the LIS criteria. The hypothesis was that decreasing albumin and increasing CRP and LDH reflect, accurately enough for clinical use, increasing severity of ARDS if judged by both clinical classification systems. Indeed, we reasoned that the overlap of systems would be a better reference standard for potential biomarkers than either system alone.

## PATIENTS AND METHODS

This was a prospective observational cohort study on the predictive and monitoring value of routine biochemical parameters for ARDS severity. The study was subsidiary to the original study on biomarkers of infection and subsequent organ failure in 101 consecutive critically ill patients with ICU-acquired fever.[27] Fever is a warning sign of inflammation. Many conditions associated with the development of ARDS, i,e, sepsis, trauma, burn injury, transfusion related lung injury amongst others, are accompanied by fever due to inflammation. The study was approved by the local Ethical Committee of the VU University Medical Centre, Amsterdam. All patients or closest relatives gave written informed consent and a full description of the protocol can be found in a previous publication on this cohort evaluating biomarkers of infection only.[27] To briefly summarise: the main inclusion criterion was new onset fever: a body temperature >38.3 °C measured rectally, while body temperature in the first 24 hrs of ICU stay was <37.5 °C. Exclusion criteria were: age under 18 years, pregnancy, and life expectancy of <24 hours. Patients were taken care of by intensivists unaware of test results according to international and local standards. Albumin infusion was no part of standard treatment.

### Protocol

The day of new onset fever was marked day 0 (D0). Within 12 hours of meeting inclusion criteria we recorded: demographic variables, risk factors, and baseline characteristics. Disease severity was expressed by the simplified acute physiology score (SAPS) II on admission. The sequential organ failure assessment (SOFA) scores were used to monitor organ failure. Mechanical ventilation was pressure guided (control or support) and protective according to standard of care in our hospital. Chest radiographs collected on study days were reviewed by two authors (SHH and ABJG) blinded to the study

results in an effort to exclude severe fluid overload or signs of congestive heart failure in classifying alveolar consolidations, In addition to the chest radiographs we used the central venous pressure (CVP), which was routinely measured in 83% of patients, to rule out severe fluid overload. The routine biochemical variables, albumin, CRP, LDH, and respiratory parameters like ventilator settings and daily chest radiographs were collected on D0, 1, 2, and 7. Total respiratory dynamic compliance was calculated from tidal volume/(plateau pressure-positive end-expiratory pressure), mL/cm$H_2O$. The need for additional imaging and collection of specimen for cultures was decided upon by treating physicians blinded to study results. In case culture and/or imaging results were positive we considered the day of their collection the day of diagnosis. Sepsis is the simultaneous presence of either clinically suspected or proven infection and the systemic inflammatory response syndrome. Patients were considered suffering shock when a systolic arterial pressure <90 mmHg or a mean arterial pressure (MAP) <65 mmHg was observed for at least one hour despite adequate fluid resuscitation and/or need of vasopressor administration. All definitions, including infections, are in line with American Society of Chest Physicians/ Society of Crit Care Med criteria.[28,29] For the sake of clarity, pneumonia is either community-, hospital- or ventilator-acquired. To define ARDS severity on study days, both the Berlin definition and the LIS were used. The Berlin definition divides patients into 4 categories that reflect the severity of the syndrome: no ARDS (Berlin 0, not fulfilling preconditions or $P_aO_2/F_IO_2$ >300 mmHg), mild ARDS (Berlin 1, 200 mmHg< $P_aO_2/F_IO_2$ ≤300 mmHg), moderate ARDS (Berlin 2, 100 mm Hg< $P_aO_2/F_IO_2$ ≤200 mm Hg), and severe ARDS (Berlin 3, $P_aO_2/F_IO_2$ ≤100 mmHg). Patients suffer from ARDS if its onset is within 1 week of a known clinical insult or worsening of respiratory symptoms, there are bilateral opacities on chest radiograph not fully explained by cardiac failure of fluid overload, and the PEEP level is ≥5 cm$H_2O$.[7] We also calculated the LIS [3]; an average based on classification of patients by the number of quadrants with alveolar consolidation on the anterior-posterior chest radiograph, severity of hypoxemia, pulmonary compliance (tidal volume/(peak inspiratory pressure-PEEP)), and PEEP level. We used the lowest $P_aO_2/F_IO_2$ measured on study days and recorded the corresponding PEEP levels and compliance at the time of sampling. Based on their LIS, patients were divided into three categories that reflect disease severity: no lung injury (LIS ≤1), mild ARDS (LIS 1-2.5), and severe ARDS (LIS >2.5).[6] Follow up was until day 28 and we checked the clinical state or date of death for all patients.

**Biochemistry**

Albumin was measured by using Albumin/BCP (Roche Diagnostics, Mannheim, Germany); normal values are 35-47 g/L. CRP was measured using an immunoturbidimetric assay by Modular analytics <P> Roche diagnostics (Mannheim, Germany) and normal

values are <5 mg/L. LDH was measured using lactate dehydrogenase optimised (Roche diagnostics, Mannheim, Germany); the normal range is 240- 480 U/L.

**Statistical analysis**

Data are expressed as median (interquartile range) or number (percentage) where appropriate. Non-normally distributed data were logarithmically transformed where appropriate. To study group differences in continuous variables we performed the Kruskal-Wallis test followed by a Mann-Whitney U test and for categorical variables we used the $X^2$ test. We used the Spearman's rank correlation for non-normally distributed data to indicate any overlap between the Berlin and LIS categories. First, to evaluate the diagnostic value of day 0 routine biochemical variable levels for the maximum ARDS severity within one week after inclusion, we calculated the area under the receiver operating characteristic curve (AUROC) and associated statistical predictive variables, such as optimal cutoff values, sensitivity, specificity, positive and negative predictive values. We performed the AUROC analyses using MedCalc for Windows, version 13 (MedCalc Software, Ostend, Belgium). The optimal diagnostic cutoff value was derived from the optimal Youden's index ( J= sensitivity + specificity-1; were J=1 represents perfect diagnostic test accuracy).[30] Prior to data-analysis and in line with the literature we decided that an AUROC >0.65 was clinically relevant and >0.70 of good discriminative value. Subsequently, to study the monitoring value of routine biochemical markers for ARDS longitudinally, we performed generalised estimating equations (GEE), taking repeated measures in the same patient and first order interactions into account. To further study the monitoring value of the biochemical markers for ARDS severity, we calculated the AUROCs on individual study days. Finally, we compared the change in biomarker levels (increase or decrease) over 7 days between patients with increasing, equal or decreasing ARDS severity. To study this association we calculated the day 0 to day 7 change in routine biochemical variables (Δ=D0-7) and the change in Berlin and LIS category and tested for differences between groups. We compared routine biochemical variable levels between 28-day survivors and non-survivors and between 28-day survivors and non-survivors with a maximum Berlin ≥1 or maximum LIS >1. Since LDH did not appear useful in diagnosing ARDS severity and course, associations with outcome are reported only. All tests were two-sided and P-values ≤0.05 were considered statistically significant. Exact P values are given, unless <0.001.

# RESULTS

## Patients

Baseline patient characteristics according to Berlin categories are presented in Table 1. Of the 101 patients, 53 (52%) had no ARDS on D0, 9 (9%) mild ARDS, 32 (32%) moderate, and 7 (7%) severe ARDS. In patients with severe ARDS (Berlin 3), SOFA scores were higher than in those without ARDS (Berlin 0, P= 0.02). The $P_aO_2/F_IO_2$ ratio in patients without ARDS (Berlin 0) was lower than in those with mild ARDS (Berlin 1, P=0.001), but higher than in Berlin categories 2 (P=0.05) and 3 (P<0.001) (Table 2). Despite the relatively low $P_aO_2/F_IO_2$ ratio in the Berlin 0 category these patients did not fulfil the other prerequisites for ARDS. Similar variables are presented for the LIS categories on D0 in Table 1. According to the LIS, 14 (14%) patients had no ARDS on D0, 69 (68%) mild, and 18 (18%) severe ARDS. In comparison to patients without lung injury, patients with mild (LIS >1.0) or severe ARDS (LIS >2.5) were more likely to need mechanical ventilation (P=0.001 and P=0.02), required more ventilator days (P=0.04 and P=0.03), and had a higher D0 SOFA score (P=0.02 and P= 0.001; Table 2). On the day of inclusion an ARDS risk factor (Table 3) was present in 93% of Berlin ARDS patients and 96% of LIS ARDS patients, while some patients suffered from more than one risk factor. The correlation between the Berlin and LIS categories was moderate (Rs=0.54, P<0.001) (Figure 1). Forty-one patients had a Berlin category <1 and 6 patients had a LIS ≤1 throughout the study.

## Association with ARDS severity

Table 4 shows some associative values of D0 albumin and CRP for the maximum Berlin and LIS categories within one week after inclusion. During the week, 42 patients reached a maximum Berlin <1 and 59 patients a maximum Berlin ≥1, whereas 6 patients reached a maximum LIS ≤1 and 95 patients a maximum LIS >1. Patients with a maximum Berlin ≥1 reached their maximum Berlin score after day 0 in 30% of cases. Patients with a maximum LIS >1 reached their maximum LIS score after day 0 in 26% of cases. The associative values of albumin ranged between (AUROC) 0.62 to 0.65 (P=0.04 or lower). An albumin level <20 g/L was associated with a maximum Berlin category ≥1 and albumin <22 g/L was associated with a maximum LIS >2.5. In contrast, CRP levels >138 mg/L were associated with a maximum Berlin category ≥2 while CRP levels >81 mg/L were associated with a maximum LIS >1.

## Monitoring ARDS severity

Figure 2 presents values according to Berlin categories and Figure 3 according to LIS categories in the course of time. Of note, changing numbers per day indicate that ARDS was deteriorating or improving over time in some patients. Albumin levels were lower

**Table 1.** Patient characteristics according to Berlin and LIS categories of ARDS at baseline.

| Berlin category | 0 | 1 | 2 | 3 | |
|---|---|---|---|---|---|
| | N = 53 | N = 9 | N = 32 | N = 7 | P-value |
| Age, years | 61 (30) | 71 (22) | 63 (24) | 69 (29) | 0.21 |
| Sex, male | 39 (74) | 5 (56) | 20 (63) | 5 (71) | 0.60 |
| SAPS II admission | 46 (20) | 59 (24) | 49 (16) | 44 (57) | 0.39 |
| SOFA D0 | 7 (4) | 8 (5) | 9 (5) | 10 (5) | 0.06 |
| ICU days until inclusion | 6 (12) | 7 (19) | 8 (12) | 9 (32) | 0.98 |
| CVP D0, mmHg | 9 (5) | 5 (2) | 6 (6) | 7 (3) | 0.26 |
| CVP D1, mmHg | 8 (5) | 6 (5) | 7 (4) | 6 (0) | 0.83 |
| CVP D2, mmHg | 7 (4) | 9 (2) | 6 (4) | 5 (0) | 0.25 |
| CVP D7, mmHg | 7 (4) | 9 (4) | 7 (5) | 9 (1) | 0.49 |
| Vasopressor use D0-7 | 28 (53) | 6 (67) | 23 (72) | 5 (71) | 0.20 |
| Renal replacement therapy D0-7 | 3 (6) | 1 (11) | 4 (13) | 0 | 0.57 |
| Albumin 20% administration (100 mL) D0-7 | 3 (6) | 2 (22) | 8 (25) | 0 | 0.03 |
| Corticosteroids use D -7-0 | 23 (43) | 5 (56) | 14 (44) | 3 (43) | 0.92 |
| Corticosteroid use D 0-7 | 23 (43) | 7 (78) | 16 (50) | 4 (57) | 0.28 |
| 28-day mortality | 9 (17) | 4 (44) | 10 (31) | 3 (43) | 0.15 |
| LIS category | LIS <1 | LIS 1.0-2.5 | LIS >2.5 | | |
| | N =14 | N = 69 | N =18 | P-value | |
| Age, years | 62 (28) | 63 (24) | 59 (28) | 0.92 | |
| Sex, man | 10 (71) | 47 (68) | 12 (67) | 0.96 | |
| SAPS II at admission | 49 (20) | 47 (20) | 45 (23) | 0.35 | |
| SOFA D0 | 5 (2) | 8 (5) | 10 (3) | 0.004 | |
| ICU days until inclusion | 6 (14) | 7 (9) | 6 (12) | 0.85 | |
| CVP D0, mmHg | 8 (6) | 7 (5) | 8 (5) | 0.79 | |
| CVP D1, mmHg | 7 (4) | 7 (6) | 7 (4) | 0.50 | |
| CVP D2, mmHg | 3 (0) | 7 (3) | 7 (6) | 0.32 | |
| CVP D7, mmHg | 6 (7) | 7 (5) | 8 (3) | 0.61 | |
| Vasopressor use D0-7 | 5 (39) | 4 (63) | 14 (82) | 0.05 | |
| Renal replacement therapy D0-7 | 0 | 7 (10) | 1 (6) | 0.40 | |
| Albumin 20% administration (100 mL) D 0-7 | 0 | 12 (17) | 1 (6) | 0.12 | |
| Corticosteroids use D -7-0 | 6 (43) | 31 (45) | 8 (44) | 0.99 | |
| Corticosteroid use D 0-7 | 4 (29) | 35 (51) | 11 (61) | 0.18 | |
| 28-day mortality | 2 (14) | 18 (26) | 6 (33) | 0.47 | |

Median (inter quartile range) or number (percentage), where appropriate. Abbreviations: ARDS- acute respiratory distress syndrome; CPR- cardiopulmonary resuscitation; CVP- central venous pressure; D- day; ICU- intensive care unit; $P_aO_2/F_IO_2$- arterial $O_2$ pressure over inspiratory $O_2$ fraction; PEEP- positive end-expiratory pressure; SAPS- simplified acute physiology score; SOFA- sequential organ failure assessment.

**Table 2.** Ventilator course between days 0 and 7 according to Berlin and LIS categories of ARDS.

| Berlin category | 0 | 1 | 2 | 3 | |
|---|---|---|---|---|---|
| | N = 53 | N = 9 | N = 32 | N = 7 | P-value |
| **Ventilator course D0-7** | | | | | |
| Mechanical ventilation D0 | 47 (89) | 9 (100) | 32 (100) | 7 (100) | 0.12 |
| duration, days | 22 (30) | 23 (27) | 22 (25) | 16 (26) | 0.85 |
| $P_aO_2/F_IO_2$ ratio D0 | 180 (76) | 226 (60) | 155 (49) | 89 (32) | <0.001 |
| $P_aO_2/F_IO_2$ ratio D1 | 191 (66) | 208 (64) | 156 (34) | 91 (0) | <0.001 |
| $P_aO_2/F_IO_2$ ratio D2 | 194 (105) | 252 (41) | 168 (35) | 68 (0) | <0.001 |
| $P_aO2/F_IO_2$ ratio D7 | 189 (110) | 239 (23) | 161 (48) | 73 (21) | <0.001 |
| PEEP D0, $cmH_20$ | 8 (7) | 8 (4) | 10 (4) | 10 (2) | 0.10 |
| PEEP D1, $cmH_20$ | 8 (7) | 8 (6) | 10 (4) | 13 (0) | 0.13 |
| PEEP D2, $cmH_20$ | 8 (7) | 10 (6) | 10 (4) | 8 (0) | 0.40 |
| PEEP D7, $cmH_20$ | 6 (6) | 11 (7) | 9 (4) | 13 (8) | 0.001 |
| Compliance D0, mL/$cmH_20$ | 32 (23) | 39 (13) | 38 (23) | 36 (27) | 0.88 |
| Compliance D1, mL/$cmH_20$ | 39 (18) | 35 (24) | 35 (18) | 21 (0) | 0.24 |
| Compliance D2, mL/$cmH_20$ | 35 (20) | 40 (30) | 35 (20) | 33 (0) | 0.78 |
| Compliance D7, mL/$cmH_20$ | 47 (34) | 31 (12) | 37 (24) | 19 (9) | 0.11 |
| Tidal volume D0, mL | 500 (217) | 500 (116) | 520 (206) | 530 (256) | 0.92 |
| Tidal volume D1, mL | 520 (120) | 500 (140) | 500 (210) | 530 (150) | 0.68 |
| Tidal volume D2, mL | 520 (123) | 500 (147) | 505 (250) | 550 (300) | 0.78 |
| Tidal volume D7, mL | 530 (192) | 463 (216) | 500 (150) | 450 (250) | 0.17 |
| Chest radiograph D0, quadrants | 1 (1) | 2 (0) | 2 (1) | 2 (1) | <0.001 |
| Chest radiograph D1, quadrants | 1 (1) | 2 (0) | 2 (1) | 2 | <0.001 |
| Chest radiograph D2, quadrants | 1 (1) | 2 (1) | 2 (1) | 3 | <0.001 |
| Chest radiograph D7, quadrants | 0 (1) | 2 (0) | 2 (2) | 3 (1) | <0.001 |
| LIS category | LIS <1 | LIS 1.0-2.5 | LIS >2.5 | | |
| | N =14 | N = 69 | N =18 | P-value | |
| **Ventilator course D0-7** | | | | | |
| Mechanical ventilation D0 | 10 (71) | 67 (97) | 18 (100) | 0.001 | |
| duration, days | 11 (20) | 22 (27) | 28 (21) | 0.07 | |
| $P_aO_2/F_IO_2$ ratio D0 | 214 (130) | 174 (68) | 112 (70) | <0.001 | |
| $P_aO_2/F_IO_2$ ratio D1 | 238 (157) | 184 (60) | 149 (40) | <0.001 | |
| $P_aO_2/F_IO_2$ ratio D2 | 284 (111) | 181 (72) | 156 (48) | <0.001 | |
| $P_aO_2/F_IO_2$ ratio D7 | 269 (162) | 177 (79) | 102 (78) | <0.001 | |
| PEEP D0, $cmH_20$ | 5 (2) | 9 (6) | 14 (3) | <0.001 | |
| PEEP D1, $cmH_20$ | 5 (1) | 9 (4) | 13 (4) | <0.001 | |
| PEEP D2, $cmH_20$ | 5 (1) | 9 (5) | 12 (6) | <0.001 | |
| PEEP D7, $cmH_20$ | 4 (3) | 8 (6) | 14 (5) | <0.001 | |
| Compliance D0, mL/$cmH_20$ | 44 (57) | 39 (20) | 27 (18) | <0.001 | |
| Compliance D1, mL/$cmH_20$ | 51 (25) | 37 (18) | 28 (21) | <0.001 | |

**Table 2.** Ventilator course between days 0 and 7 according to Berlin and LIS categories of ARDS. (continued)

| LIS category | LIS <1 | LIS 1.0-2.5 | LIS >2.5 | |
|---|---|---|---|---|
| | N =14 | N = 69 | N =18 | P-value |
| Compliance D2, mL/cm$H_20$ | 61 (105) | 35 (19) | 31 (21) | 0.01 |
| Compliance D7, mL/cm$H_20$ | 65 (75) | 37 (24) | 20 (11) | 0.007 |
| Tidal volume D0, mL | 409 (268) | 500 (176) | 550 (154) | 0.39 |
| Tidal volume D1, mL | 523 (177) | 500 (166) | 523 (95) | 0.92 |
| Tidal volume D2, mL | 490 (138) | 525 (133) | 535 (194) | 0.43 |
| Tidal volume D7, mL | 450 (150) | 500 (213) | 500 (138) | 0.71 |
| Chest radiograph D0, no quadrants | 0 (1) | 2 (1) | 2 (3) | <0.001 |
| Chest radiograph D1, no quadrants | 1 (1) | 1 (1) | 2 (2) | <0.001 |
| Chest radiograph D2, no quadrants | 0 (1) | 2 (1) | 2 (3) | <0.001 |
| Chest radiograph D7, no quadrants | 1 (0) | 2 (1) | 2 (1) | 0.005 |

Median (interquartile range) or number (percentage), where appropriate. Abbreviations: ARDS- acute respiratory distress syndrome; D- day; ICU- intensive care unit; $P_aO_2/F_IO_2$- arterial $O_2$ pressure over inspiratory $O_2$ fraction; PEEP- positive end-expiratory pressure.

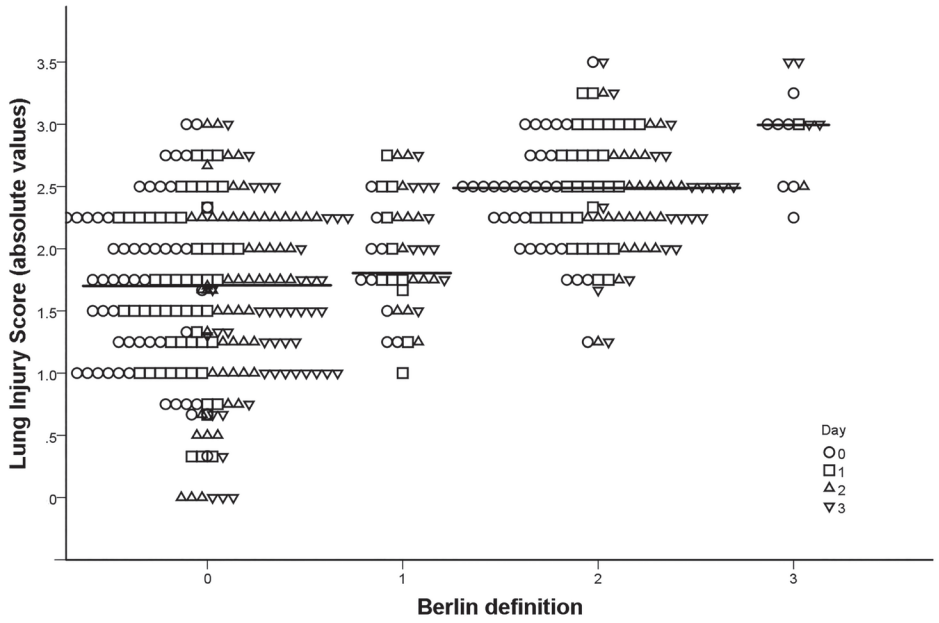


**Figure 1.** Scatterplot of the Berlin definition categories vs. the lung injury score of ARDS ($R_s$=0.54, P<0.001).

**Table 3.** ARDS risk factors on ICU admission and on study inclusion.

| Berlin category | 0 | 1 | 2 | 3 | |
|---|---|---|---|---|---|
| | N = 53 | N = 9 | N = 32 | N = 7 | P-value |
| **ARDS risk factors on ICU admission** | | | | | |
| Sepsis | 14 (26) | 4 (44) | 12 (38) | 1 (14) | 0.42 |
| Shock | 8 (15) | 3 (33) | 9 (28) | 0 | 0.18 |
| Trauma | 11 (21) | 0 | 2 (6) | 0 | 0.09 |
| General surgery | 30 (57) | 3 (33) | 16 (50) | 5 (71) | 0.43 |
| Vascular surgery | 4 (8) | 1 (1) | 3 (9) | 2 (29) | 0.38 |
| Cardiac surgery | 3 (6) | 0 | 2 (6) | 1 (14) | 0.69 |
| Intracranial bleeding | 10 (19) | 2 (22) | 1 (3) | 1 (14) | 0.19 |
| CPR | 6 (11) | 1 (11) | 2 (6) | 1 (14) | 0.86 |
| Other | 7 (13) | 0 | 2 (6) | 0 | 0.38 |
| **ARDS risk factors on D0** | | | | | |
| Sepsis | 29 (56) | 4 (44) | 13 (41) | 4 (57) | 0.66 |
| Shock | 14 (26) | 5 (56) | 14 (44) | 4 (57) | 0.13 |
| Pneumonia | 3 (6) | 0 | 4 (13) | 2 (29) | 0.14 |
| Aspiration pneumonia | 3 (6) | 0 | 0 | 0 | 0.43 |
| Peritonitis | 3 (6) | 1 (11) | 1 (3) | 0 | 0.71 |
| Infected pancreatitis | 2 (4) | 0 | 1 (3) | 0 | 0.89 |
| Miscellaneous infection | 20 (38) | 2 (22) | 10 (31) | 3 (43) | 0.75 |
| Surgery within 48 hrs prior to inclusion | 8 (15) | 1 (11) | 4 (13) | 0 | 0.73 |
| LIS category | LIS ≤1.0 | LIS 1.0-2.5 | LIS >2.5 | | |
| | N =14 | N = 69 | N =18 | P-value | |
| **ARDS risk factors on ICU admission** | | | | | |
| Sepsis | 3 (21) | 22 (32) | 6 (33) | 0.72 | |
| Shock | 2 (14) | 17 (25) | 1 (6) | 0.17 | |
| Trauma | 1 (7) | 10 (15) | 2 (11) | 0.73 | |
| General surgery | 8 (57) | 37 (54) | 9 (50) | 0.92 | |
| Vascular surgery | 0 | 8 (11) | 2 (11) | 0.41 | |
| Cardiac surgery | 0 | 4 (6) | 2 (11) | 0.42 | |
| Intracranial bleeding | 5 (36) | 7 (10) | 2 (11) | 0.04 | |
| CPR | 1 (7) | 7 (10) | 2 (11) | 0.93 | |
| Other | 3 (21) | 5 (7) | 1 (6) | 0.20 | |
| **ARDS risk factors on D0** | | | | | |
| Sepsis | 9 (64) | 34 (49) | 7 (39) | 0.36 | |
| Shock | 2 (14) | 27 (39) | 8 (44) | 0.16 | |
| Pneumonia | 1 (7) | 6 (9) | 2 (11) | 0.92 | |
| Aspiration pneumonia | 1 (7) | 1 (2) | 1 (6) | 0.41 | |
| Peritonitis | 1 (7) | 3 (4) | 1 (6) | 0.90 | |
| Infected pancreatitis | 2 (14) | 1 (1) | 0 | 0.03 | |
| Miscellaneous infection | 7 (50) | 25 (36) | 3 (17) | 0.13 | |
| Surgery within 48 hrs prior to inclusion | 1 (7) | 9 (13) | 3 (17) | 0.73 | |

Number (percentage). Abbreviations: ARDS- acute respiratory distress syndrome; CPR- cardio-pulmonary resuscitation; ICU- intensive care unit; hrs- hours; LIS- lung injury.

**Table 4.** Diagnostic values of D0 albumin and CRP for maximum Berlin and LIS categories within one week after new onset fever in critically ill patients.

| | AUROC | 95% CI | P-value | Optimal cutoff | SN | SP | PPV | NPV |
|---|---|---|---|---|---|---|---|---|
| **Maximum Berlin ≥1 (N=59)** | | | | | | | | |
| Albumin | 0.65 | 0.53-0.76 | 0.01 | <20 g/L | 71 | 58 | 71 | 58 |
| **Maximum Berlin ≥2 (N=50)** | | | | | | | | |
| Albumin | 0.63 | 0.52-0.74 | 0.02 | <20 g/L | 72 | 53 | 61 | 65 |
| CRP | 0.62 | 0.51-0.74 | 0.03 | >138 mg/L | 54 | 76 | 69 | 62 |
| **Maximum LIS >1.0 (N=95)** | | | | | | | | |
| CRP | 0.82 | 0.64-1.00 | 0.002 | >81 mg/L | 77 | 80 | 99 | 15 |
| **Maximum LIS >2.5 (N=34)** | | | | | | | | |
| Albumin | 0.62 | 0.51-0.73 | 0.04 | <22 g/L | 91 | 31 | 41 | 87 |

Abbreviations: AUROC- area under the curve; ARDS- acute respiratory distress syndrome; CI - confidence interval; CRP- C-reactive protein; LIS- lung injury score; NPV- negative predictive value; PPV- positive predictive value; SN- sensitivity; SP- specificity.

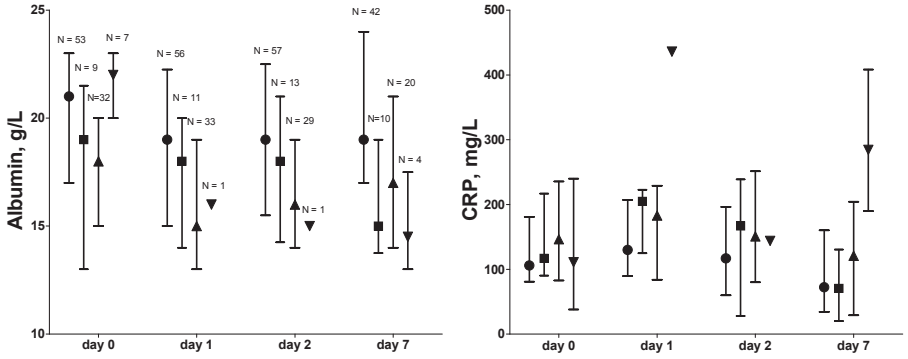


**Figure 2.** Median and interquartile range of albumin and C-reactive protein (CRP) for the Berlin definition on ARDS.

The Berlin categories are inversely associated with albumin levels (P=0.05) and directly with CRP levels (P=0.02) in generalized estimating equations. ● no acute respiratory distress syndrome (ARDS, Berlin 0), ■ mild ARDS (Berlin 1), ▲ moderate ARDS (Berlin 2) ▼ severe ARDS (Berlin 3). Numbers refer to numbers of patients.

and CRP levels were higher with increasing Berlin and LIS category. The albumin levels had a monitoring value, albeit moderate, on all study days and cutoff values generally decreased with increasing ARDS severity (AUROC between 0.62-0.82, P<0.001-0.03, Table 5). CRP levels had less frequent monitoring value for ARDS severity. Figure 4 depicts the change in albumin and CRP levels between D0 and 7 (ΔD0-7) in relation to the change in Berlin and LIS category: albumin levels inversely related to change in ARDS severity regardless of definition. Increasing CRP levels were associated with increasing Berlin definition only. A decrease in albumin of ≥1 g/L and an increase of

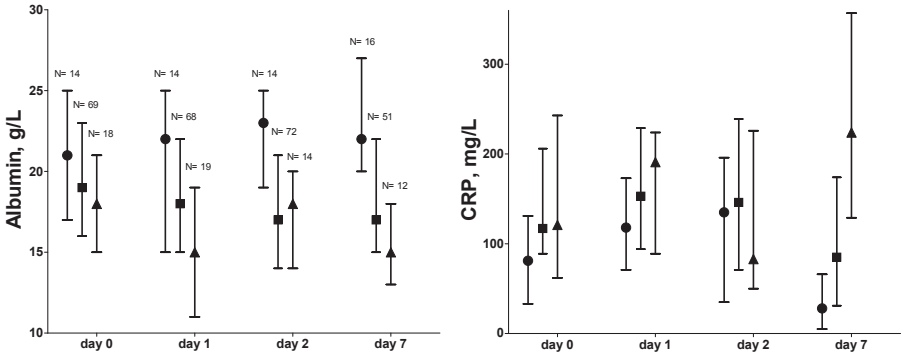


**Figure 3.** Median and interquartile range of albumin and C-reactive protein (CRP) for the lung injury score (LIS).

The LIS categories are inversely associated with albumin (P<0.001) and directly with CRP (P=0.04) in generalized estimating equations. ● no lung injury (LIS ≤1.0), ■ mild acute respiratory distress syndrome ARDS (LIS 1.0-2.5), ▲ severe ARDS (LIS >2.5). Numbers refer to numbers of patients.

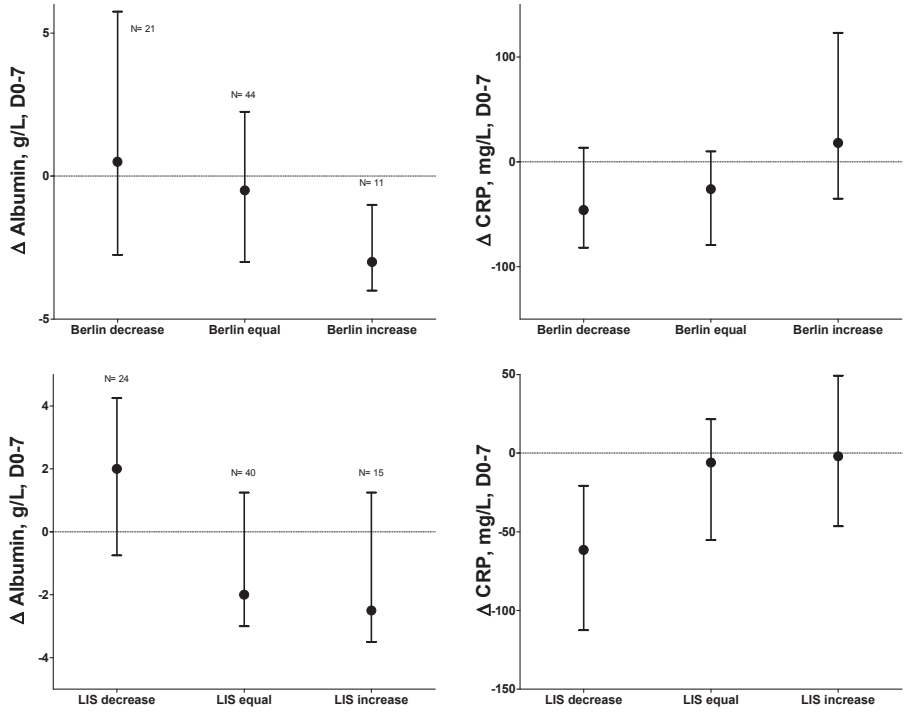


**Figure 4.** Changes of albumin and C-reactive protein (CRP) levels for changes in Berlin and lung injury score (LIS) categories between D0-7.

The change in albumin levels is associated with a change in Berlin and LIS category (P=0.05 and P=0.03, respectively). A change in CRP levels is associated with a change in LIS category only (P=0.03).

**Table 5.** Monitoring values for ARDS severity on study days.

| | | AUROC | 95% CI | P-value | Optimal cutoff | SN | SP | PPV | NPV |
|---|---|---|---|---|---|---|---|---|---|
| **Berlin ≥1** | **day 0** | | | | | | | | |
| | Albumin | 0.62 | 0.52-0.72 | 0.03 | <20 g/L | 71 | 51 | 58 | 65 |
| | **day 1** | | | | | | | | |
| | Albumin | 0.66 | 0.56-0.77 | 0.003 | <20 g/L | 84 | 44 | 55 | 77 |
| | **day 2** | | | | | | | | |
| | Albumin | 0.67 | 0.56-0.78 | 0.002 | <17 g/L | 67 | 65 | 58 | 73 |
| | **day 7** | | | | | | | | |
| | Albumin | 0.71 | 0.59-0.81 | <0.001 | <14 g/L | 38 | 92 | 81 | 63 |
| **Berlin ≥2** | **day 1** | | | | | | | | |
| | Albumin | 0.67 | 0.57-0.76 | 0.002 | <18 g/L | 73 | 54 | 44 | 80 |
| | **day 2** | | | | | | | | |
| | Albumin | 0.68 | 0.58-0.77 | <0.001 | <17 g/L | 73 | 62 | 46 | 84 |
| | **day 7** | | | | | | | | |
| | CRP | 0.65 | 0.53-0.76 | 0.045 | >105 mg/L | 67 | 64 | 47 | 80 |
| **Berlin ≥3** | **day 7** | | | | | | | | |
| | Albumin | 0.77 | 0.65-0.86 | 0.01 | <18 g/L | 100 | 49 | 10 | 100 |
| | CRP | 0.91 | 0.82-0.97 | <0.001 | >162 mg/L | 100 | 73 | 19 | 100 |
| **LIS >1** | **day 0** | | | | | | | | |
| | CRP | 0.70 | 0.60-0.79 | 0.01 | >81 mg/L | 78 | 54 | 92 | 27 |
| | **day 1** | | | | | | | | |
| | CRP | 0.65 | 0.55-0.75 | 0.04 | >182 mg/L | 42 | 92 | 97 | 20 |
| | **day 2** | | | | | | | | |
| | Albumin | 0.82 | 0.73-0.89 | <0.001 | <21 g/L | 86 | 64 | 94 | 43 |
| | **day 7** | | | | | | | | |
| | Albumin | 0.81 | 0.70-0.89 | <0.001 | <17 g/L | 56 | 92 | 97 | 31 |
| | CRP | 0.79 | 0.68-0.87 | <0.001 | >60 mg/L | 68 | 79 | 93 | 36 |
| **LIS >2.5** | **day 1** | | | | | | | | |
| | Albumin | 0.69 | 0.56-0.78 | 0.02 | <11 g/L | 39 | 96 | 70 | 88 |
| | **day 7** | | | | | | | | |
| | Albumin | 0.72 | 0.60-0.82 | 0.004 | <18 g/L | 83 | 53 | 26 | 94 |
| | CRP | 0.83 | 0.73-0.91 | <0.001 | >158 mg/L | 75 | 80 | 41 | 94 |

Abbreviations: AUROC- area under the receiver operating characteristics curve; CI - confidence interval; CRP- C-reactive protein; LIS- lung injury score; NPV- negative predictive value; PPV- positive predictive value; SN- sensitivity- SP- specificity.

CRP ≥104 mg/L were associated with an increase in ARDS severity by Berlin category (AUROC 0.72, P=0.001 with sensitivity 100, specificity (SP) 42, positive predictive value (PPV) 23 and negative predictive value (NPV) 100 %; AUROC 0.69, P=0.04, SN 27, SP 98, PPV 78 and NPV 88%, respectively). A decrease in albumin ≥1 g/L was

associated with an increase in LIS category (AUROC 0.77, P<0.001, SN 91, SP 54, PPV 26, NPV 97), and an increase in albumin ≥1 g/L with a decrease in LIS category (AUROC 0.68, P=0.02, SN 61, SP 73, PPV 42, NPV 85).

### Mortality

In 28-day non-survivors, D2 and peak LDH levels were higher (647 (5005) and 756 (409) U/L, respectively, P=0.003) than in survivors (435 (199) and 543 (362) U/L, respectively, P=0.007). In patients with ARDS according to the Berlin definition, LDH levels were higher in non-survivors (665 (421) U/L) than in survivors (458 (243) U/L) on D1 (P=0.03), in non-survivors (706 (621) U/L) than in survivors (452 (225) U/L) on D2 (P<0.001), and in non-survivors (618 (364) U/L) than in survivors (454 (258) U/L) on D7 (P=0.02). Peak LDH levels in non-survivors (876 (653) U/L) were higher than in survivors (581 (347) U/L, P=0.002). In patients with ARDS according to the LIS, peak LDH levels in non-survivors (756 (409) U/L) were higher than in survivors (548 (359) U/L, P=0.009). Albumin and CRP did not have prognostic significance.

## DISCUSSION

This longitudinal study in critically ill patients with or at risk for ARDS with new onset fever suggests that albumin rather than CRP levels are valuable in daily monitoring of ARDS severity and course at the bedside. Although the associative values were only moderate, a low albumin was a useful indicator on all study days, while a supranormal CRP cutoff was less frequently associated with ARDS severity. During the week, a change in albumin levels was inversely related to a change in ARDS severity regardless of definition. In contrast, increasing CRP levels were associated with increasing Berlin categories only. The LDH levels only predicted 28-day mortality.

Only partial overlap between Berlin and LIS categories has been observed before.[1,4] In the absence of a reference standard like autopsy or measurement of alveolar-capillary permeability, we cannot determine whether the Berlin categories underestimated or the LIS overestimated the severity of ARDS. A relatively high $P_aO_2/F_IO_2$ ratio, in the presence of relatively high PEEP, may not meet Berlin criteria if preconditions and bilaterality are absent, whereas PEEP adds to the LIS score.[3] The sensitivity of compliance, which is often the first parameter to deteriorate after initiation of lung injury, even before onset of edema, could also explain the higher frequency of ARDS by LIS than Berlin definitions.[31] The Berlin definition includes bilateral chest radiograph abnormalities, while the LIS includes quadrants. However, chest radiographs have high interobserver variability, leading to frequent false positives and negatives.[12,13] As such the LIS may constitute a more sensitive measure of the clinical severity of ARDS

correlating with alveolocapillary permeability than the Berlin definition, but thereby carries the risk of oversensitivity and overestimation,[1,5] In any case, the CVP was comparable between Berlin and LIS categories, so it is less likely that severe fluid overload explains the difference in ARDS rating between definitions. Otherwise, the rate and distribution of risk factors in this population with or at risk for late ARDS in the ICU is in agreement with the literature, showing ICU-acquired sepsis as the leading cause (Table 3).[1,14] The relatively high ARDS prevalence reflects the selection of critically ill patients with new onset fever, suggesting new onset sepsis or inflammation both important ARDS risk factors.

We reasoned that an association with both ARDS severity classifications would render a potential biomarker clinically valuable, in the absence of a true reference standard of ARDS. Albumin levels had monitoring value for ARDS defined by the Berlin definition and the LIS on all study days and cutoff values in AUROC's declined as disease severity increased. This agrees with the idea that a low albumin is indeed involved in ARDS pathogenesis, i.e. increased permeability oedema, as suggested before in cross-sectional studies.[9,11,16-19] Albumin levels did not prognosticate outcome as in other studies.[18] CRP levels had no consistent monitoring ability for ARDS. A supranormal CRP was mainly associated with severe ARDS on D7. Our data suggest that CRP is not useful as a marker of ARDS severity and course, in line with some studies .[22,24] However, in previous studies CRP had value in differentiating ARDS from cardiogenic pulmonary edema[23] and the CRP and LIS decline upon successful ARDS treatment by corticosteroids.[20] In our study, patients with cardiogenic oedema were excluded. The use of corticosteroids on clinical indication could have been a confounder but distribution between ARDS categories was comparable. CRP levels did not prognosticate outcome in our study in line with some,[22] but in contrast to reports on the association between elevated CRP levels and survival[21] or non-survival.[25] Even though ARDS can be considered an inflammatory response of the lung, numerous other factors can be responsible for elevated CRP levels in critically ill patients. The levels of LDH, a marker of cell damage, were not diagnostic of ARDS severity and course in line with some,[24] but in contrast to other observations suggesting elevated levels in sepsis patients progressing to ARDS.[26] The LDH levels were however associated with 28-day mortality, which has not been reported before.

A limitation of this study is its relatively small sample size and heterogeneous population. Considering generalisabilty of the results the latter might be an advantage as well. We included patients with the symptom fever rather than with specific conditions to focus on an inflammatory response as a major risk factor for developing or worsening ARDS. Few patients received corticosteroids or albumin as part of their treatment, but their distribution was equal between ARDS categories and therefore do not invalidate our conclusions. With exceptions, the AUROC's were generally not >0.75.

Low predictive capacity could also be related to the inclusion of high risk patients only. This must be weighed against the accessibility of these variables which are collected almost daily and routinely in many ICU's. Nevertheless, even though the associations between albumin levels and ARDS were modest, they were present on all individual study days and over the course of a week. Furthermore, albumin was inversely related to disease severity regardless of the clinical definition and its course predicted disease course (AUROC 0.68-0.77 respectively), while neither albumin nor CRP had any predictive value for 28-day mortality, possibly due to the limited power of this study. Our study suggests that albumin levels may have practical value in monitoring the severity of ARDS at the bedside of critically ill patients without the need for LIS calculations which are hardly done routinely. Assessing the $P_aO_2/F_IO_2$ ratio and chest radiograph may be insufficient to monitor ARDS, since both are treatment-dependent, for instance with higher $P_aO_2/F_IO_2$ ratios and more aerated chest radiographs with higher PEEP. Even though two authors reviewed clinical history, chest radiographs, and CVP to exclude severe fluid overload or congestive heart failure in classifying alveolar consolidations we cannot fully exclude a a component of hydrostatic oedema in some of our ARDS patients. Nevertheless, even when there is dilution due to fluid administration hypoalbuminemia leads to lowered oncotic pressure and in the presence of increased vascular permeability this leads to pulmonary oedema and ARDS. As shown by others low total protein and albumin levels, regardless of fluid state, are associated with the presence and development of ARDS.[11,16,17] Our study adds to the latter studies by focusing on the value of albumin in late ARDS (85-90% after 48 hours, depending on definition) in the ICU, a commonly underdiagnosed condition.[14]

## CONCLUSIONS

Overall, albumin rather than CRP may be valuable in predicting and monitoring the severity and course of ARDS in febrile critically patients with or at risk for the syndrome. Albumin levels below 20 g/L as well as a decline in albumin levels are associated with ARDS of increasing severity, irrespective of definition. LDH levels predicted 28-day mortality but had no monitoring value for ARDS severity.

## REFERENCES


1. Wind J, Versteegt J, Twisk J, et al. AB: Epidemiology of acute lung injury and acute respiratory distress syndrome in the Netherlands: a survey. Respir Med 2007;101:2091-8.
2. Tulapurkar ME, Almutairy EA, Shah NG, et al. Febrile-range hyperthermia modifies endothelial and neutrophilic functions to promote extravasation. Am J Respir Cell Mol Biol 2012;46:807-14.
3. Murray JF, Mathay MA, Luce JM, et al. An expanded definition of the adult respiratory distress syndrome. Am Rev Respir Dis 1988;138:720-723.
4. Ferguson ND, Frutos-Vivar F, Esteban A, et al. Acute respiratory distress syndrome: underrecognition by clinicians and diagnostic accuracy of three clinical definitions. Crit Care Med 2005;33:2228–34.
5. Costa ELV, Amato MBP. The new definition for acute lung injury and acute respiratory distress syndrome: is there room for improvement? Curr Opin Crit Care 2013;19:16-23
6. Frohlich S, Murphy N, Boylan JF. ARDS: progress unlikely with non-biological definition. Br J Anaesth 2013;111:696–9.
7. Hernu R, Wallet F, Thiollière F, et al. An attempt to validate the modification of the American-European consensus definition of acute lung injury/acute respiratory distress syndrome by the Berlin definition in a university hospital. Intensive Care Med 2013;39:2161-70.
8. Thille AW, Esteban A, Fernández-Segoviano P, et al. Comparison of the Berlin definition for acute respiratory distress syndrome with autopsy. Am J Respir Crit Care Med 2013;187:761-7.
9. Kushimoto S, Endo T, Yamanouchi S, et al. the PiCCO Pulmonary Edema Study Group: Relationship between extravascular lung water and severity categories of acute respiratory distress syndrome by the Berlin definition. Crit Care 2013; 17:R132.
10. Groeneveld AB1, Raijmakers PG. The 67gallium-transferrin pulmonary leak index in patients at risk for the acute respiratory distress syndrome. Crit Care Med 1998;26:685-91.
11. Aman J, van der Heijden M, van Lingen A, et al. Plasma protein levels are markers of pulmonary vascular permeability and degree of lung injury in critically ill patients with or at risk for acute lung injury/acute respiratory distress syndrome. Crit Care Med 2011;39:89-97.
12. Rubenfeld GD, Caldwell E, Granton J, et al. Interobserver variability in applying a radiographic definition for ARDS. Chest 1999;116;5:1347-53.
13. Meade MO, Cook RJ, Guyatt GH, et al. Interobserver variation in interpreting chest radiographs for the diagnosis of acute respiratory distress syndrome. Am J Respir Crit Care Med 2000;161:85-90.
14. Vincent JL, Sakr Y, Groeneveld J, et al. ARDS of early or late onset: does it make a difference? Chest 2010;137:81-7.
15. Terpstra ML, Aman J, van Nieuw Amerongen GP, et al. Plasma biomarkers for acute respiratory distress syndrome: A systematic review and meta-analysis. Crit Care Med 2014;42:691-700.

16. Mangialardi RJ, Martin GS, Bernard GR, et al. Hypoproteinemia predicts acute respiratory distress syndrome development, weight gain, and death in patients with sepsis. Crit Care Med 2000;28:3137-45.

17. Arif SK, Verheij J, Groeneveld AB, et al. Hypoproteinemia as a marker of acute respiratory distress syndrome in critically ill patients with pulmonary edema. Intensive Care Med 2002;28:310-7.

18. Lee JH, Kim J, Kim K, et al. Albumin and C-reactive protein have prognostic significance in patients with community-acquired pneumonia. J Crit Care 2011;26:287-294.

19. Zhang Z. Lu, Ni H, Sheng X, et al. Predictions of pulmonary edema by plasma protein levels in patients with sepsis. J Crit Care 2012;27:623-9.

20. Meduri GU, Golden E, Freire AX, et al. Methylprednisolone infusion in early severe ARDS: results of a randomized controlled trial. Chest 2007;131:954-63.

21. Bajwa EK, Khan UA, Januzzi JL, et al. Plasma C-reactive protein levels are associated with improved outcome in ARDS. Chest 2009;136:471-80.

22. Lee YL, Chen W, Chen LY, et al. Systemic and bronchoalveolar cytokines as predictors of in-hospital mortality in severe community-acquired pneumonia. J Crit Care 2010;25:176.e7-13.

23. Komiya K, Ishii H, Teramoto S, et al. Diagnostic utility of C-reactive protein combined with brain natriuretic peptide in acute pulmonary edema: a cross sectional study. Respir Res 2011;12:83.

24. Osaka D, Shibata Y, Kanouchi K, et al. Soluble endothelial selectin in acute lung injury complicated by severe pneumonia. Int J Med Sci 2011;8:302–308.

25. Komiya K, Ishii H, Teramoto S, et al. Plasma C-reactive protein levels are associated with mortality in elderly with acute lung injury. J Crit Care 2012;27:524.e1-6.

26. Leff JA, Parsons PE, Day CE, et al. Serum antioxidants as predictors of adult respiratory distress syndrome in patients with sepsis. Lancet 1993;341:777–780.

27. Hoeboer SH, Alberts E, van den Hul I, et al. Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin. J Infect 2012;64:484-93.

28. Levy MM, Fink MP, Marshall JC, et al. International Sepsis Definitions Conference: 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Intensive Care Med 2003;29:530–538.

29. Calandra T, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. Crit Care Med 2005;33:1538–1548.

30. Youden WJ. Index for rating diagnostic tests. Cancer 1950;3: 32-35.

31. McCaffree DR, Gray BA, Pennock BE, et al. Role of pulmonary edema in the acute pulmonary response to sepsis. J Appl Physiol 1981;50:1198-205.

# 7

# Serial inflammatory biomarkers of the severity, course and outcome of late onset acute respiratory distress syndrome in critically ill patients with or at risk for the syndrome after new onset fever

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*Biomark Med in press*

## ABSTRACT

**Objective** Accurate biomarkers of the acute respiratory distress syndrome (ARDS) may help risk stratification and management. We assessed the relation between several biomarkers and the severity, course and outcome of late onset ARDS in 101 consecutive critically ill patients with new onset fever.

**Methods** On study days 0, 1, 2 and 7 we measured angiopoietin-2 (ANG2), pentraxin-3, interleukin-6 (IL6), procalcitonin (PCT), and midregional pro-adrenomedullin (proADM). ARDS was defined by the Berlin definition and by the lung injury score (LIS).

**Results** At baseline, 48% had ARDS according to the Berlin definition and 86% according to the LIS. Baseline markers poorly predicted maximum Berlin categories attained within 7 days, whereas ANG2 best predicted maximum LIS. Depending on the ARDS definition, the day-by-day area under the receiver operating characteristic curves suggested greatest monitoring value for IL6 and PCT, followed by ANG2. ANG2 and proADM predicted outcome, independently of disease severity.

**Conclusions** Whereas IL6 and PCT had some disease monitoring value, ANG2 was the only biomarker capable of both predicting the severity, monitoring the course and predicting the outcome of late onset ARDS in febrile critically ill patients, irrespective of underlying risk factor, thereby yielding the most specific ARDS biomarker among those studied.

## INTRODUCTION

The acute respiratory distress syndrome (ARDS) following sepsis, trauma, pancreatitis and other insults is caused by alveolocapillary inflammation and increased permeability and is frequently underdiagnosed, particularly when developing late (≥48 hours) in the intensive care unit (ICU).[1-3] Many underlying conditions of ARDS are associated with fever, such as sepsis, and fever itself may also aggravate alveolocapillary inflammation[4]; fever therefore denotes a risk factor. There are various clinical classification systems for ARDS, including the recent Berlin definition and the old, more elaborate

**Table 1.** Clinical classification systems of the Acute Respiratory Distress Syndrome.

| **Berlin definition of ARDS[6]** | |
|---|---|
| Preconditions: | |
| Timing | Onset within 1 week of a known clinical insult or worsening of respiratory symptoms. |
| Imaging | Bilateral opacities on chest radiograph or computed tomography not fully explained by effusions, lobar/lung collapse, or nodules. |
| Origin of oedema | Respiratory failure not fully explained by cardiac failure of fluid overload (Need objective assessment to exclude hydrostatic oedema if no risk factor present (e.g. echocardiography). |
| Oxygenation | Berlin 1: Mild ARDS: 200 < $P_aO_2/F_iO_2$ mmHg ≤300 with PEEP or CPAP ≥5 $cmH_2O$ |
| | Berlin 2: Moderate ARDS: 100 < $P_aO_2/F_iO_2$ mm Hg ≤200 with PEEP ≥5 $cmH_2O$ |
| | Berlin 3: Severe ARDS: $P_aO_2/F_iO_2$ ≤100 mmHg with PEEP ≥5 $cmH_2O$ |

| **Lung injury score[5]** | |
|---|---|
| Anterior-posterior chest radiograph score | Hypoxemia severity score |
| 0= no alveolar consolidations | 0= $P_aO_2/F_iO_2$ = >300 mmHg |
| 1= alveolar consolidations in 1 quadrant | 1= $P_aO_2/F_iO_2$ = 225-299 mmHg |
| 2= alveolar consolidations in 2 quadrants | 2= $P_aO_2/F_iO_2$ = 175-224 mmHg |
| 3= alveolar consolidations in 3 quadrants | 3= $P_aO_2/F_iO_2$ = 100-174 mmHg |
| 4= alveolar consolidations in all quadrants | 4= $P_aO_2/F_iO_2$ = <100 mmHg |
| PEEP score (when ventilated) | Pulmonary compliance score |
| 0= PEEP ≤5 $cmH_2O$ | 0= Compliance ≥80 mL/$cmH_2O$ |
| 1= PEEP 6-8 $cmH_2O$ | 1= Compliance 60-79 mL/$cmH_2O$ |
| 2= PEEP 9-11 $cmH_2O$ | 2= Compliance 40-59 mL/$cmH_2O$ |
| 3= PEEP 12-14 $cmH_2O$ | 3= Compliance 20-39 mL/$cmH_2O$ |
| 4= PEEP >15 $cmH_2O$ | 4= Compliance ≤19 mL/$cmH_2O$ |

The final lung injury score is obtained by calculating the average of all four categories.

No lung injury ≤1 mild ARDS 1-2.5 severe ARDS >2.5

Abbreviations: ARDS- acute respiratory distress syndrome, $P_aO_2/F_iO_2$ - arterial $O_2$ pressure over inspiratory $O_2$ fraction, PEEP- positive end-expiratory pressure; pulmonary compliance = (tidal volume / (peak inspiratory pressure - PEEP).

lung injury score (LIS, Table 1).[1,5-7] Although used as a clinical standard for diagnosis, the limitation of the Berlin definition is its dependency on ventilator treatment (with positive end-expiratory pressure affecting the oxygenation ratio and chest radiography in mechanically ventilated patients) and lack of a specific index of severity such as the total respiratory compliance, that is incorporated in the LIS.[5-8] The LIS score, however, does not include bilateral consolidations as a criterion. The LIS may therefore constitute a more refined but complex and not routine measure of the clinical severity of ARDS correlating with alveolocapillary permeability.[1,9] Nevertheless, the agreement of both classification systems with diffuse alveolar damage at autopsy is limited[1,10,11] and apart from the laborious direct measurement of alveolocapillary permeability, if available,[9] there is no in vivo reference standard for diagnostics and monitoring.[12] Moreover, two phenotypes of ARDS may prevail: one with hyperinflammatory sepsis, shock and a poor outcome and the other without such abnormalities.[13]

Because of clinical classification problems, amongst others, there is an active search for biomarkers that may accurately predict the development or presence of alveolocapillary inflammation of ARDS and would help in risk stratification and management in future studies.[14-16] We and others previously described that circulating angiopoietin-2, possibly derived from the pulmonary vessel wall, is associated with alveolocapillary permeability, development of clinical ARDS, positive fluid balance and mortality in the critically ill sepsis or trauma patients, even though sepsis and trauma may predispose to different ARDS phenotypes.[12,13,16,17-24] The biomarker value for ARDS of alternative molecules such as pentraxin-3, a pro-inflammatory acute phase mediator,[25-27] interleukin-6, a cytokine with both pro- and anti-inflammatory properties,[14-16,19,25,28-33] procalcitonin, a marker of inflammation,[15,16,34,35] and midregional pro-adrenomedullin, a stable fragment of adrenomedullin with immune modulating, metabolic and vasodilator actions and prognostic properties in pneumonia and sepsis,[36-39] remains unclear up till now. The literature on foregoing biomarkers reported on less than 60 patients[13,17,20,26,28,32,35] or on only one single marker or clinical classification system for ARDS.[14,15,17-24,26,28,31-35] Moreover, many studies were cross-sectional.[11,12,14,15,17-19,21,23,24,30-39] Since these studies focused mostly on early ARDS development or its outcome, the value of biomarkers in reflecting severity and course of late onset ARDS regardless of risk factors is largely unknown. Therefore, the goal of the present, longitudinal study was to investigate which biomarker would be most accurate in predicting and monitoring the severity, course and outcome of ARDS in critically ill patients after new onset fever who are at risk for the syndrome. The hypothesis was that biomarkers directly associated with inflammation, such as angiopoietin-2, interleukin-6 and procalcitonin would be more accurate than those indirectly associated with inflammation, such as pentraxin-3 and proadrenomedullin, independent of underlying ARDS risk factor.[13,15,16,18,29]

## PATIENTS AND METHODS

This was a prospective observational study on the accuracy of biomarkers to predict and monitor ARDS severity in critically ill patients with new onset fever. This study was ancillary to the original one evaluating the role of biomarkers to predict infection and resultant organ failure.[40] The original study was approved by the VU University Medical Ethical Committee, Amsterdam, the Netherlands. All patients or closest relatives gave their written informed consent prior to inclusion. For the original study we consecutively included 101 critically ill patients whom developed new-onset fever in the ICU. New onset fever was defined as a rectal temperature >38.3 °C while rectal temperature in the preceding 24 hours was <37.5 °C. Due to limited availability of the research staff and competing studies we only included during office hours. The main exclusion criteria were: lack of informed consent, age <18 years, pregnancy, or life expectancy of <24 hours. All patients were taken care of according to local guidelines by certified intensivists unaware of biomarker levels. Need of diagnostic imaging and sampling of specimen for culture was left at the treating physician's discretion, who was unaware of biomarker results.

### Study protocol

Day 0 (baseline) was the day of new onset fever. Demographic variables, potential ARDS risk factor on ICU admission and baseline characteristics were recorded within 12 hours of presenting new onset fever. To express disease severity, we used the simplified acute physiology score (SAPS) II on admission and the sequential organ failure assessment (SOFA) score on study days. In addition to clinical and radiological assessment, the central venous pressure (CVP, mmHg), which was routinely measured in 83% of patients, was registered to exclude severe fluid overload and severe heart failure. On study days 0, 1, 2, and 7 respiratory parameters, chest radiographs, and blood samples for biomarker measurement were collected.

### Definitions

The Berlin definition and LIS were used to diagnose ARDS and classify ARDS severity on study days. We reviewed all chest radiographs collected on study days unaware of biomarker results and attempted to exclude gross overhydration or signs of congestive heart failure in classifying alveolar consolidations, heart size, vascular pedicle, vessel distribution Kerley lines and pleural effusion. The Berlin definition consists of 4 categories that reflect ARDS severity: no ARDS (Berlin 0), mild ARDS (Berlin 1), moderate ARDS (Berlin 2), and severe ARDS (Berlin 3) according to the $P_aO_2/F_IO_2$ ratio, regardless of positive end-expiratory pressure, and fulfillment of other preconditions, such as a known clinical etiology, bilateral chest radiograph abnormalities and ab-

sence of a hydrostatic cause.[6] In contrast, the LIS consists of 3 categories that reflect ARDS severity: no lung injury/ARDS (LIS ≤1), mild (LIS 1-2.5) and severe ARDS (LIS >2.5).[5] The ratio incorporates the $P_aO_2/F_IO_2$ ratio, the PEEP, total respiratory dynamic compliance and number of quadrants with alveolar consolidations on chest radiography, in the presence of a known clinical etiology. Compliance is calculated from plateau inspiratory pressure-PEEP/tidal volume, in pressure-guided mechanically ventilated patients. In addition, the maximum Berlin category and maximum LIS during the 7-day course were registered. We used the lowest $P_aO_2/F_IO_2$ ratio per day and corresponding PEEP levels and compliance. Sepsis was defined according to American Society of Chest Physicians/ Society of Crit Care Med criteria[41] as the presence of either clinically suspected or proven infection and the systemic inflammatory response syndrome. We considered the day of specimen collection and/or imaging the day of diagnosis when results were positive. Shock was diagnosed when the systolic arterial pressure was <90 mmHg or the mean arterial pressure (MAP) was <65 mmHg for ≥1 hour in spite of adequate fluid resuscitation and/or need of vasopressor administration.[42] Septic shock was the presence of sepsis and shock. Mortality is 28-day mortality.

**Biomarker assay test specifics**

Angiopoietin 2 (ANG2) and pentraxin 3 (PTX3) were measured using the Quantikine Enzyme-Linked Immunosorbent A*ssay* (ELISA) Kit (R&D systems Inc. Minneapolis, United States). Test specifics for ANG2: lower detection limit 0.05 ng/mL, upper detection limit 3 ng/mL, normal value in healthy subjects around 0.1 ng/mL with an upper limit of 1.2 ng/mL [7,12]. The intra-assay coefficient of variation (CV) is 6.5% and inter-assay CV 10%, while the average recovery in serum is 100%. Test specifics for PTX3: lower detection limit 0.31 ng/mL, upper detection limit 20 ng/mL with an upper limit in healthy subjects <2 ng/mL. The intra-assay CV is <4.5% and inter-assay CV 6%, with average recovery in serum of 99%. Interleukin-6 (IL6) was measured using the Luminex performance Assay Fluorokine MAP (R&D systems Inc. Minneapolis, United States): lower detection limit 0.36 pg/mL, normal value of IL6 in healthy subjects around 1 pg/mL, intra-assay CV <5%, inter-assay CV <9%, and average recovery in serum 108%. Procalcitonin (PCT) and midregional pro-adrenomedullin (proADM) were measured using the Kryptor compact system (Brahms Diagnostica, Henningsdorf, Germany). For PCT: lower detection limit 0.02 ng/mL, upper limit in healthy subjects 0.05 ng/mL, and intra-assay and inter-assay CV <6%. Test specifics of pro-adrenomedullin (proADM): lower detection limit 0.05 nmol/L, upper limit in healthy subjects 0.55 nmol/L, intra-assay CV <4% and inter-assay CV <11%.

### Statistical analysis

Since biomarker values were distributed non-normally (Kolmogorov-Smirnov P<0.05), data are expressed as median (interquartile range) or number (percentage) where appropriate. To determine group differences at baseline we used the Kruskal-Wallis test for non-parametric continuous variables and for categorical variables the $X^2$ or Fisher exact test where appropriate. In order to perform generalized estimating equations (GEE), taking repeated measurements into account, non-normally distributed data where logarithmically transformed where appropriate. GEE was used to evaluate associations between biomarker levels with ARDS severity throughout the 7-day course. Furthermore, GEE were used to compare biomarker levels throughout the 7-day course between survivors and non-survivors. To study the diagnostic, monitoring and predictive value of baseline biomarkers for maximum ARDS categories, of day-to-day values of severity of ARDS and of both baseline and day-to-day values for mortality, the area under the receiver operating characteristic curve (AUROC) was calculated, after logarithmic transformation of biomarker values, optimal cut-off, sensitivity, specificity, positive and negative predictive values were calculated. The AUROC analyses were performed using MedCalc for Windows, version 13 (MedCalc Software, Ostend, Belgium). We considered an AUROC <0.70 poor, 0.70-0.80 fair, 0.80-0.90 good, and >0.90 excellent. The optimal diagnostic cutoff value was derived from the optimal Youden's index (J= sensitivity + specificity-1; were J=1 represents perfect diagnostic test accuracy).[43] To study the predictive value and interdependency of baseline biomarker levels, sepsis and SAPS II for 28-day mortality multivariate logistic regression was performed, with the Hosmer-Lemeshow test for goodness of fit, using stepwise backward elimination of predictors based on the likelihood ratio. Two sided P-values <0.05 were considered statistically significant unless specified otherwise; P-values are exact unless P<0.001. To correct for multiple testing only P-values ≤0.01 were considered statistically significant in the tables with AUROC's.

## RESULTS

### Patient characteristics

According to the Berlin definition, 53% had no ARDS (Berlin 0) at baseline, 9% mild (Berlin 1), 32% moderate (Berlin 2), and 7% severe ARDS (Berlin 3). In 90% of patients ARDS according to the Berlin definition developed 48 hours after ICU admission. Compared to patients without ARDS (Berlin 0), patients with ARDS (Berlin ≥1) had higher day 0 SOFA scores, more often suffered from shock, more likely needed vasopressors and had a higher 28-day mortality rate. The day 0 $P_aO_2/F_IO_2$ ratio in patients without ARDS (Berlin 0) was lower than in those with mild ARDS (Berlin 1,

P=0.001), but higher than in Berlin categories 2 (P=0.05), and 3 (P<0.001). According to the LIS categories 14% of patients had no ARDS (LIS ≤1) at baseline, 68% mild and 18% severe ARDS (Table 2). In 85.% of patients ARDS according to LIS developed 48 hours after ICU admission. Patients with ARDS according to LIS had higher day 0

**Table 2.** Patient characteristics by Berlin definition and LIS at baseline.

| | **Berlin 0** | **Berlin ≥1** | | **LIS ≤1** | **LIS >1** | |
|---|---|---|---|---|---|---|
| | **N=53** | **N=48** | **P** | **N=14** | **N=87** | **P** |
| **General characteristics** | | | | | | |
| Age, years | 61 (40-70) | 67 (52-75) | 0.04 | 62 (42-70) | 63 (48-73) | 0.70 |
| Sex, man | 39 (74) | 30 (63) | 0.23 | 10 (71) | 59 (68) | 0.79 |
| ICU days until inclusion | 6 (4-16) | 8 (3-15) | 0.69 | 6 (4-18) | 7 (3-15) | 0.93 |
| SAPS II | 46 (46-55) | 50 (38-57) | 0.22 | 49 (41-61) | 47 (35-55) | 0.16 |
| SOFA day 0 | 7 (5-9) | 9 (5-10) | 0.03 | 5 (5-7) | 8 (5-10 | 0.008 |
| SOFA day 1 | 6 (4-9) | 8 (7-10) | 0.01 | 5 (4-7) | 8 (5-10) | 0.02 |
| SOFA day 2 | 5 (4-9) | 8 (5-10) | 0.11 | 4 (3-6) | 7 (4-10) | 0.18 |
| SOFA day 7 | 5 (4-8) | 6 (4-9) | 0.60 | 5 (2-7) | 6 (4-8) | 0.28 |
| Shock day 1 | 14 (26) | 23 (48) | 0.04 | 3 (21) | 34 (39) | 0.25 |
| Shock day 2 | 12 (23) | 22 (46) | 0.02 | 3 (23) | 31 (36) | 0.53 |
| Shock day 7 | 9 (25) | 15 (41) | 0.21 | 4 (57) | 20 (30) | 0.21 |
| Vasopressor use day 0-7 | 28 (54) | 34 (74) | 0.04 | 5 (39) | 57 (67) | 0.05 |
| 28-day mortality | 9 (17) | 17 (35) | 0.03 | 2 (14) | 24 (28) | 0.29 |
| **Potential ARDS risk factor on ICU admission** | | | | | | |
| Sepsis | 14 (26) | 17 (35) | 0.39 | 3 (21) | 28 (32) | 0.54 |
| Shock | 8 (15) | 12 (25) | 0.23 | 2 (14) | 18 (21) | 0.73 |
| Trauma | 11 (21) | 2(4) | 0.02 | 1 (7) | 12 (14) | 0.69 |
| General surgery | 30 (57) | 24 (50) | 0.55 | 8 (57) | 46 (33) | 1.00 |
| Vascular surgery | 4 (8) | 6 (13) | 0.51 | 0 | 10 (12) | 0.35 |
| Cardiac surgery | 3 (6) | 3 (6) | 1.00 | 0 | 6 (7) | 0.59 |
| Intracranial bleeding | 10 (11) | 4 (8) | 0.16 | 5 (36) | 9 (10) | 0.02 |
| CPR | 6 (11) | 4 (8) | 0.74 | 1 (7) | 9 (10) | 1.00 |
| Other | 7 (13) | 2 (40 | 0.17 | 3 (21) | 6 (7) | 0.11 |
| **Potential ARDS risk factor on day 0** | | | | | | |
| Sepsis | 29 (56) | 21 (43) | 0.32 | 9 (64) | 41 (48) | 0.39 |
| Shock | 14 (26) | 23 (48) | 0.04 | 2 (14) | 35 (40) | 0.08 |
| Pneumonia | 3 (6) | 6 (13) | 0.30 | 1 (7) | 8 (9) | 1.00 |
| Aspiration pneumonia | 3 (6) | 0 | 0.24 | 1 (7) | 2 (2) | 0.36 |
| Peritonitis | 3 (6) | 2 (4) | 1.00 | 1 (7) | 4 (5) | 0.53 |
| Infected pancreatitis | 2 (4) | 0 | 1.00 | 2 (14) | 1 (1) | 0.05 |
| Miscellaneous infections | 20 (38) | 15 (31) | 0.54 | 7 (50) | 28 (32) | 0.23 |
| Surgery within 48 hrs prior to inclusion | 8 (15) | 5 (9) | 0.72 | 1 (7) | 12 (14) | 0.69 |

**Table 2.** Patient characteristics by Berlin definition and LIS at baseline. (continued)

| | Berlin 0 | Berlin ≥1 | | LIS ≤1 | LIS >1 | |
|---|---|---|---|---|---|---|
| | N=53 | N=48 | P | N=14 | N=87 | P |
| **Pulmonary indices day 0** | | | | | | |
| Mechanical ventilation, yes | 47 (87) | 48 (100) | 0.02 | 10 (71) | 85 (98) | <0.001 |
| duration, days | 22 (10-39) | 21 (13-37) | 0.46 | 11 (7-27) | 23 (12-40) | 0.03 |
| $P_aO_2/F_iO_2$ ratio | 180 (138-214) | 158 (115-183) | 0.03 | 214 (187-317) | 165 (125-191) | <0.001 |
| PEEP, $cmH_20$ | 8 (5-12) | 8 (8-12) | 0.18 | 5 (4-6) | 10 (7-12) | <0.001 |
| Compliance, $mL/cmH_20$ | 33 (25-49) | 38 (25-45) | 0.91 | 44 (25-82) | 36 (25-45) | 0.35 |
| Tidal volume, mL | 500 (412-631) | 520 (430-602) | 0.96 | 409 (350-618) | 520 (430-606) | 0.19 |
| Chest radiograph, no quadrants | 1 (0-1) | 2 (2-3) | <0.001 | 0 (0-1) | 2 (1-3) | <0.001 |
| CVP, mmHg | 9 (5-10) | 6 (5-9) | 0.08 | 8 (4-10) | 7 (5-10) | 0.90 |

Median (interquartile range) or number (percentage) where appropriate. Abbreviations: ARDS- acute respiratory distress syndrome; CPR- cardiopulmonary resuscitation; CVP- central venous pressure; day* - insult within the 3 days preceding inclusion; ICU- intensive care unit; LIS- lung injury; $P_aO_2/F_iO_2$ - arterial $O_2$ pressure over inspiratory $O_2$ fraction; PEEP- positive end-expiratory pressure; SAPS- simplified acute physiology score; SOFA- sequential organ failure assessment.

SOFA scores, were more likely to need vasopressors, respiratory support and required more mechanical ventilation days. In 93% of Berlin ≥1 patients and 96% of LIS>1 patients a potential ARDS risk factor was present on ICU documented and occasionally patients suffered two or more potential risk factors (Table 2). Forty-two patients never met the Berlin and 6 never the LIS definition of ARDS in the course of the study and severity in the other patients varied over time. In 30 and 26%, respectively, patients reached their maximum Berlin AND LIS category after day 0. Relatively low baseline CVP suggests that absence of overhydration.

### Baseline biomarker levels and maximum ARDS severity within 7 days

Only baseline PTX3 (P=0.03) and IL6 (P=0.04) differed across maximum Berlin categories, while baseline ANG2 (P=0.007), IL6 (P=0.02), and PCT (P=0.007) differed across maximum LIS categories (Table 3). However, in the sensitivity analysis the biomarker concentrations differed mainly at low Berlin and high LIS categories. Baseline ANG2 predicted maximum ARDS severity according to LIS (Table 4). Other biomarkers' predictive values were poor.

### Day-by-day biomarker levels for monitoring ARDS

The following associations were independent of including sepsis as a factor. The ANG2 and PCT levels were directly associated with Berlin and LIS categories, throughout the 7-day course (Fig .1 and 2). IL6 and ProADM levels had a direct association with LIS

**Table 3.** Sensitivity analysis of baseline biomarker levels for maximum late ARDS severity by Berlin and LIS categories.

| **maximum Berlin category day 0-7** | | | | | | | |
|---|---|---|---|---|---|---|---|
| | **0** | **1** | **2** | **3** | **P** | | |
| | **N=42** | **N=9** | **N=39** | **N=11** | **Berlin ≥1 vs 0** | **Berlin ≥2 vs ≤1** | **Berlin 3 vs ≤2** |
| ANG2, ng/mL | 2.3 (1.3-4.7) | 4.2 (1.8-7.2) | 4.0 (2.0-7.1) | 3.6 (2.3-7.7) | 0.03 | 0.06 | 0.44 |
| PTX3, ng/mL | 9.3 (4.7-19.3) | 14.6 (8.1-36.8) | 15.2 (9.6-27.9) | 13.8 (10.1-35.0) | 0.002 | 0.01 | 0.38 |
| IL6, pg/mL | 49.2 (17.8-88.4) | 72.5 (40.6-81.5) | 66.0 (25.5-184.3) | 232.6 (64.7-331.6) | 0.02 | 0.02 | 0.03 |
| PCT, ng/mL | 0.44 (0.15-1.13) | 1.36 (0.23-3.81) | 0.68 (0.32-2.04) | 0.65 (0.35-1.98) | 0.02 | 0.09 | 0.37 |
| proADM, nmol/L | 1.53 (0.76-2.02) | 2.04 (0.99-7.55) | 1.52 (1.04-3.13) | 2.46 (0.37-3.45) | 0.05 | 0.27 | 0.47 |
| **maximum LIS category day 0-7** | | | | | | | |
| | **≤1** | **1-2.5** | **>2.5** | | **P** | | |
| | **N=6** | **N=62** | **N=34** | | **LIS >1 vs ≤1** | **LIS >2.5 vs ≤2.5** | |
| ANG2, ng/mL | 1.1 (1.0-2.2) | 2.8 (1.5-4.9) | 4.2 (2.3-5.4) | | 0.02 | 0.01 | |
| PTX3, ng/mL | 4.9 (4.0-26.3) | 12.3 (5.9-25.7) | 14.3 (9.9-29.9) | | 0.18 | 0.06 | |
| IL6, pg/mL | 55.9 (9.5-133.3) | 49.5 (19.4-100.2) | 101.2 (62.0-240.7) | | 0.47 | 0.004 | |
| PCT, ng/mL | 0.32 (0.18-0.57) | 0.50 (0.17-1.24) | 0.78 (0.55-3.20) | | 0.15 | 0.003 | |
| proADM, nmol/L | 0.88 (0.66-1.66) | 1.62 (0.81-2.65) | 1.96 (1.26-3.47) | | 0.14 | 0.07 | |

Median (interquartile range), P- Mann Whitney U test. Abbreviations: ANG2- angiopoietin 2, ARDS- acute respiratory distress syndrome, IL6- interleukin 6, LIS- lung injury score, max- maximum ARDS severity within 7 days of new onset fever, PCT- procalcitonin, PTX3- Pentraxin 3, proADM- midregional pro-adrenomedullin

only. The day-by-day AUROC's (Table 5) suggested greatest monitoring value for IL6 and PCT, followed by ANG2 and then by proADM, depending on the ARDS definition.

### Mortality

Mortality of patients with maximum Berlin categories increased from 12% (Berlin 0) to 33 % (Berlin 1), 31% (Berlin 2) and 55% (Berlin 3, P=0.02). For maximum LIS category, mortality increased from 0 (LIS ≤1) to 24% (LIS 1-2.5) and 32% (LIS >2.5 P=0.27). Baseline values of ANG2, PCT, IL6 and proADM were higher in 28-day non-survivors than in survivors (Table 6). Mortality prediction was investigated in the entire cohort and in the subgroups of ARDS according to the Berlin criteria or the LIS score. In the entire cohort, multiple logistic regression showed that baseline ANG2 (P=0.001,

**Table 4.** Statistically significant prediction of baseline biomarker concentrations for maximum Berlin and LIS categories within one week after inclusion (day 0-7).

| | AUROC | P | Optimal cut off | SN | SP | PPV | NPV |
|---|---|---|---|---|---|---|---|
| **maximum Berlin ≥1 (N=59)** | | | | | | | |
| PTX3 | 0.68 | 0.001 | 6.0 ng/mL | 92 | 41 | 69 | 77 |
| IL6 | 0.64 | 0.01 | 61.7 pg/mL | 64 | 61 | 70 | 54 |
| PCT | 0.64 | 0.01 | 0.49 ng/mL | 68 | 56 | 69 | 55 |
| **maximum Berlin ≥2 (N=50)** | | | | | | | |
| PTX3 | 0.65 | 0.01 | 7.4 ng/mL | 88 | 42 | 60 | 78 |
| **maximum LIS >1 (N=95)** | | | | | | | |
| ANG2 | 0.80 | <0.001 | 2.5 ng/mL | 57 | 100 | 100 | 11 |
| **maximum LIS >2.5 (N=34)** | | | | | | | |
| ANG2 | 0.65 | 0.006 | 1.8 ng/mL | 97 | 32 | 42 | 96 |
| IL6 | 0.68 | 0.002 | 63.1 pg/mL | 76 | 59 | 49 | 83 |
| PCT | 0.68 | <0.001 | 0.52 ng/mL | 75 | 55 | 47 | 84 |

To correct for multiple testing an adjusted P-value ≤0.01 was considered statistically significant. Abbreviations: ANG2- angiopoietin 2, ARDS- acute respiratory distress syndrome, AUC- area under the receiver operating characteristics curve, IL6- interleukin 6, LIS- lung injury score, NPV- negative predictive value, PCT- procalcitonin, PPV- positive predictive value, PTX3- pentraxin 3- proADM- midregional pro-adrenomedullin, SN- sensitivity, SP- specificity.

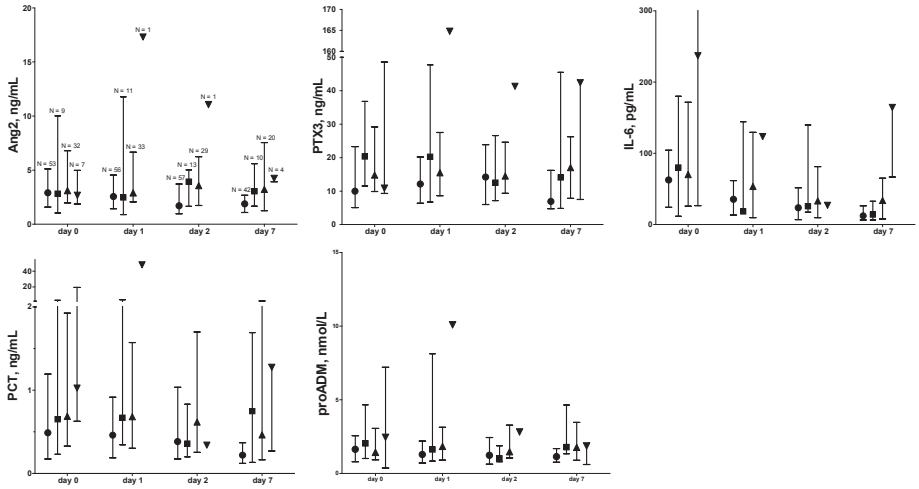



**Figure 1.** Monitoring value of plasma biomarker levels (median and interquartile range) per day for the Berlin definition on ARDS.

In critically ill patients with new onset fever, Berlin definition categories over one week are directly related with the levels of ANG2 (P=0.04) and PCT (P=0.006 ), independent of sepsis (P=0.77 and 0.57, respectively), in generalized estimating equations. ● no ARDS (Berlin 0), ■ mild ARDS (Berlin 1), ▲ moderate ARDS (Berlin 2), ▼ severe ARDS (Berlin 3). Abbreviations: ARDS- acute respiratory distress syndrome, ANG2- angiopoietin-2, PTX3- pentraxin-3, IL6- interleukin-6, PCT- procalcitonin, proADM- midregional pro-adrenomedullin.

**Table 5.** Statistically significant areas under the receiver operating characteristic curve and associated variables for day-to-day biomarker values.

| | | AUROC | P | Optimal cut off | SN | SP | PPV | NPV |
|---|---|---|---|---|---|---|---|---|
| **Berlin ≥1** | | | | | | | | |
| day 2 | ANG2 | 0.67 | 0.003 | 1.7 ng/ml | 76 | 53 | 55 | 74 |
| day 7 | ANG2 | 0.69 | 0.005 | 3.2 ng/ml | 50 | 92 | 84 | 67 |
| | PCT | 0.68 | 0.01 | 0.37 ng/ml | 58 | 78 | 69 | 68 |
| | proADM | 0.70 | 0.002 | 1.4 nmol/l | 68 | 72 | 68 | 72 |
| **Berlin ≥2** | | | | | | | | |
| day 7 | IL6 | 0.71 | 0.003 | 27.5 pg/ml | 68 | 80 | 63 | 84 |
| **Berlin ≥3** | | | | | | | | |
| day 7 | ANG2 | 0.79 | <0.001 | 3.7 ng/ml | 100 | 77 | 17 | 100 |
| | IL6 | 0.95 | <0.001 | 65.6 pg/ml | 100 | 86 | 25 | 100 |
| **LIS >1** | | | | | | | | |
| day 0 | ANG2 | 0.73 | 0.005 | 1.5 ng/ml | 86 | 57 | 93 | 40 |
| | IL6 | 0.71 | 0.005 | 12.6 pg/ml | 91 | 43 | 91 | 43 |
| | PCT | 0.71 | 0.002 | 0.49 ng/ml | 64 | 79 | 95 | 26 |
| | proADM | 0.73 | <0.001 | 0.91 nmol/l | 78 | 64 | 93 | 32 |
| day 2 | ANG2 | 0.74 | <0.001 | 3.2 ng/ml | 51 | 92 | 98 | 23 |
| | proADM | 0.75 | <0.001 | 1.02 nmol/l | 68 | 77 | 95 | 27 |
| day 7 | IL6 | 0.77 | <0.001 | 20.4 pg/ml | 53 | 100 | 100 | 31 |
| | PCT | 0.75 | <0.001 | 0.35 ng/ml | 49 | 100 | 100 | 29 |
| **LIS >2.5** | | | | | | | | |
| day 0 | PCT | 0.66 | 0.01 | 0.54 ng/ml | 89 | 51 | 29 | 96 |
| | proADM | 0.66 | 0.01 | 1.26 nmol/l | 59 | 46 | 27 | 95 |
| day 1 | PCT | 0.69 | 0.007 | 1.07 ng/ml | 58 | 79 | 41 | 89 |
| day 7 | IL6 | 0.84 | <0.001 | 29.5 pg/ml | 82 | 75 | 38 | 96 |
| | PCT | 0.73 | 0.003 | 0.23 ng/ml | 90 | 54 | 25 | 97 |

To correct for multiple testing an adjusted P-value ≤0.01 was considered statistically significant. Abbreviations: ANG2- angiopoietin-2, ARDS- acute respiratory distress syndrome, AUC- area under the receiver operating characteristics curve, IL6- interleukin-6, LIS- lung injury score, NPV- negative predictive value, max- maximum ARDS severity within 7 days of new onset fever, PCT- procalcitonin, PPV- positive predictive value, PTX3- pentraxin-3- proADM- midregional pro-adrenomedullin, SN- sensitivity, SP- specificity.

Hosmer-Lemeshow $X^2$ 11.4, df 8, P=0.18) and baseline proADM (P=0.001, Hosmer-Lemeshow $X^2$ 7.0, df 8, P=0.53) predicted 28-day mortality, both independently from SAPS II and sepsis. The associated AUROC was 0.74 (P<0.001), while inclusion of SAPS II and sepsis did not further contribute. Furthermore, throughout the 7-day course, day-by-day levels of ANG2 (P<0.001), PTX3 (P=0.03), PCT (P=0.03) and proADM (P<0.001) were higher in non-survivors (Fig. 3). In patients with a maximum Berlin ≥1 or maximum LIS category >1, ANG2 and proADM at baseline were higher in non-survivors than survivors (Table 6). In patients with a maximum Berlin ≥1, multiple logistic regression showed that baseline ANG2 (P=0.02, Hosmer-Lemeshow

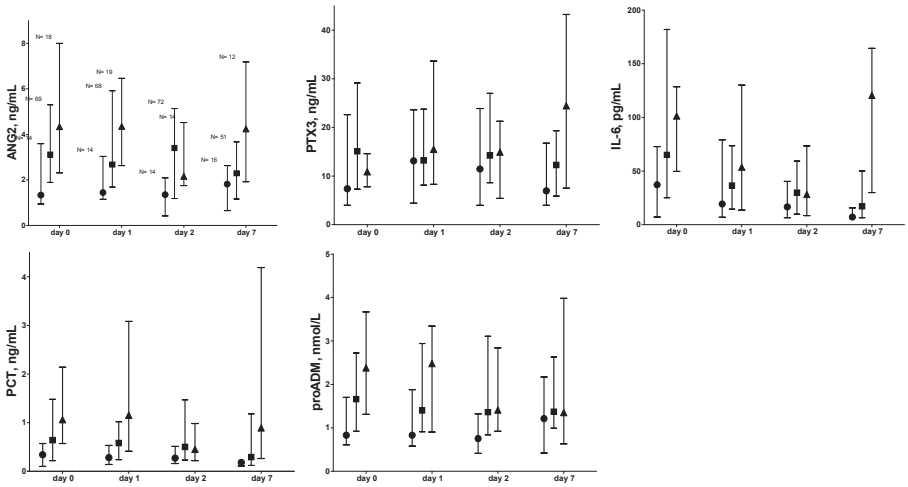



**Figure 2.** Monitoring value of plasma biomarker levels (median and interquartile range) per day for the lung injury score.
In critically ill patients with new-onset fever**,** LIS category over one week is directly related with the levels of ANG2 (P=0.03), IL6 (P<0.001), PCT (P<0.001) and proADM (P<0.001), independent of sepsis (P= 0.06, 0.25, 0.50 and 0.56, respectively), in generalized estimating equations. ● no ARDS, ■ mild ARDS, ▲ severe ARDS. Abbreviations: ARDS- acute respiratory distress syndrome, ANG2- angiopoietin-2, LIS- lung injury score, PTX3- pentraxin-3, IL6- Interleukin-6, PCT- procalcitonin, proADM- midregional pro-adrenomedullin.

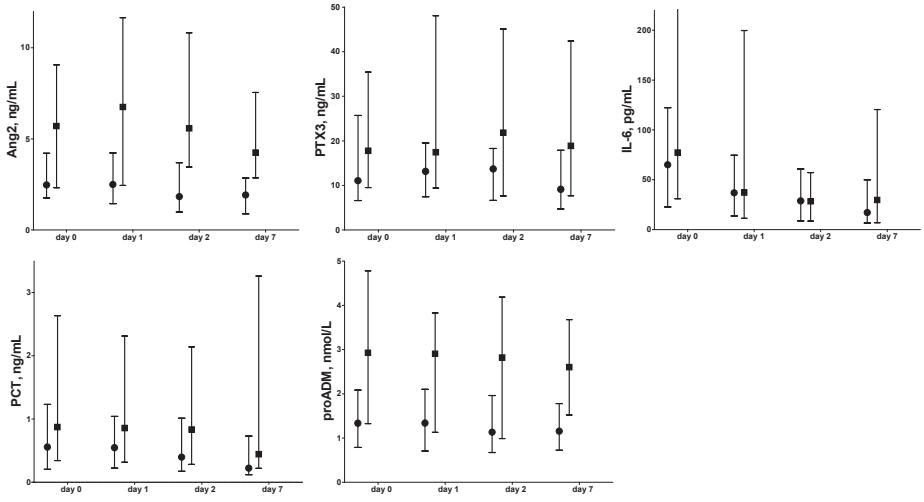



**Figure 3.** Monitoring value of plasma biomarker levels (median and interquartile range) per day in 28-day survivors and non-survivors.
Non-survival is directly related with ANG2 (P<0.001), PTX3 (P=0.01), PCT (P=0.006), and pro-ADM (P<0.001) levels in generalized estimating equations. ● Survivors (N=75), ■ non-survivors (N=26). Abbreviations: ARDS- acute respiratory distress syndrome, ANG2- angiopoietin-2, PTX3- pentraxin-3, IL6- interleukin-6, PCT- procalcitonin, proADM- midregional pro-adrenomedullin.

**Table 6.** Baseline biomarker values in survivors and non-survivors.

| **All patients** | **Survivors** | **Non-survivors** | |
|---|---|---|---|
| | **N = 75** | **N = 26** | **P** |
| ANG2, ng/mL | 2.5 (1.8-4.2) | 5.7 (2.3-9.1) | 0.001 |
| PTX3, ng/mL | 11.0 (6.6-25.7) | 17.7 (9.5-35.4) | 0.06 |
| IL6, pg/mL | 65.0 (22.8-122.3) | 77.2 (31.0-223.42) | 0.23 |
| PCT, ng/mL | 0.56 (0.21-1.23) | 0.87 (0.34-2.6) | 0.04 |
| proADM, nmol/L | 1.33 (0.79-2.08) | 2.93 (1.33-4.78) | <0.001 |
| **maximum Berlin >1** | **Survivors** | **Non-survivors** | |
| | **N = 38** | **N = 21** | **P** |
| ANG2, ng/mL | 2.8 (1.9-4.3) | 5.95 (2.5-9.2) | 0.004 |
| PTX3, ng/mL | 12.4 (9.2-28.1) | 21.4 (13.0-35.9) | 0.11 |
| IL6, pg/mL | 76.1 (26.6-233.7) | 80.0 (29.3-237.3) | 0.86 |
| PCT, ng/mL | 0.64 (0.23-1.94) | 0.85 (0.45-3.31) | 0.16 |
| proADM, nmol/L | 1.32 (0.87-2.21) | 3.15 (1.8-6.5) | 0.001 |
| **maximum LIS >1** | **Survivors** | **Non-survivors** | |
| | **N = 69** | **N = 26** | **P** |
| ANG2, ng/mL | 2.6 (1.9-4.3) | 5.7 (2.3-9.1) | 0.001 |
| PTX3, ng/mL | 11.1 (7.1-25.8) | 17.7 (9.5-35.4) | 0.09 |
| IL6, pg/mL | 65.6 (24.2-124.1) | 77.2 (31.0-223.4) | 0.27 |
| PCT, ng/mL | 0.57 (0.20-1.36) | 0.87 (0.34-2.63) | 0.06 |
| proADM, nmol/L | 1.35 (0.81-2.10) | 2.93 (1.33-4.78) | 0.001 |

Median (interquartile range). Abbreviations: ANG2- angiopoietin-2, ARDS- acute respiratory distress syndrome, proADM- midregional pro-adrenomedullin, IL6- interleukin-6, LIS- lung injury score, PCT- procalcitonin, PTX3- pentraxin-3.

$X^2$ 5.7, df 8, P=0.68) and baseline proADM (P=0.003, Hosmer-Hemeshow $X^2$ 8.6, df 8, P=0.38) predicted 28-day mortality independent from SAPS II and sepsis (AUROC 0.74 and 0.76 (P<0.001), respectively). In patients with maximum LIS >1, baseline ANG2 (P=0.003, Hosmer-Lemeshow $X^2$ 7.9, df 8, P=0.44) predicted 28-day mortality independently from sepsis but not SAPS II (P=0.03), AUROC 0.73 (P<0.001). Baseline proADM (P=0.001, Hosmer-Hemeshow $X^2$ 12.3, df 8, P=0.14) predicted 28-day mortality, independently from SAPS II and sepsis(AUROC 0.73, P<0.001).

## DISCUSSION

In this longitudinal study in critically ill patients with or at risk for late ARDS after new onset fever, ANG2 was of value in predicting the maximum severity, monitoring the course and predicting the outcome of the syndrome, whereas IL6 and PCT had value for daily monitoring of severity, irrespective of underlying risk factor. ProADM only

predicted 28-day mortality, independent of the SAPS II score. The data suggest ANG2 as the most specific ARDS biomarker, among those studied.

To the best of our knowledge, this is the first longitudinal study relating a panel of biomarkers to both Berlin and LIS-defined, late onset ARDS in the ICU. The differences between the classification systems may explain why the monitoring ability of biomarker levels better correlated with LIS than Berlin definitions. The Berlin definition requires bilateral radiographic consolidations and the severity of ARDS in the Berlin definition is expressed by gas exchange only, which is dependent of ventilator settings. Its focus is, therefore, more on diagnosis of ARDS than on monitoring of its severity.[7,8] In contrast, the LIS includes radiographic criteria, gas exchange, level of PEEP and compliance. As such, the LIS may better represent severity of lung injury than the Berlin definition, but carries the risk of oversensitivity and thus overestimation of the syndrome, since more patients may not have ARDS according to Berlin than LIS categories, as indicated by our current and previous studies.[2,8] Conversely, relatively low ANG2 levels already predicted a maximum LIS >1, but this association at low disease severity may be clinically less useful than those at high disease severity. Indeed, the optimal cutoffs using the Youden index[43] resulted in higher positive predictive values with less disease severity and higher negative predictive values with greater disease severity. We used both overlapping clinical systems to evaluate the uniform clinical value of biomarkers in the absence of a true reference standard, even though they may not accurately predict diffuse alveolar damage at autopsy.[1,10,11] In any case, the mortality of our ARDS patients that somewhat increased with increasing severity roughly agrees with the literature.[3,7,12-14,16,19,20,28,29] Of note, mortality increased across Berlin categories while the increase did not reach statistical significance for increasing LIS categories, possibly because of underpowering.

Even though the baseline predictive values of biomarkers for maximum Berlin and LIS categories within the study week were relatively poor, the day-to-day diagnostic value for severity and thus the monitoring value of these biomarkers were greater. This particularly applies to IL6, PCT and ANG2, irrespective of ARDS definitions. Indeed, alveolocapillary inflammation and increased vascular permeability with non-cardiogenic edema is the hallmark of ARDS, and ANG2 may be directly involved.[12,17,19] Increasing cutoff values for ANG2 with increasing severity of ARDS, according to LIS mainly, supports a pathophysiologic role. The literature reports varying predictive values of ANG2 for early ARDS,[12,16,19-24] while its monitoring value has not yet been reported. Furthermore, ANG2 predicted mortality independently of the SAPS II score and sepsis, in agreement with most,[12,19,20,22] but not with another, small study.[15] IL6 levels, which may reflect inflammation, were not predictive for 28-day mortality, in contrast to other studies.[14,15,28-31] The value and limitations of IL6 to diagnose and monitor ARDS severity, resulting from community-acquired pneumonia, for instance, have been described

before.[14,24,30,32,33] For PCT, we found no study to document its monitoring value for ARDS even though it may predict early ARDS development in community acquired pneumonia and a poor outcome.[34,35,37] In contrast, another study found no relation between PCT levels and mortality in ARDS,[15] in line with our results.

PTX3 behaves like an acute phase protein, is a marker of innate immunity and inflammation, and may have a role in the complement-mediated clearance of apoptotic cells.[25] PTX3 overexpression has been found in severe infections, mechanical ventilation and other risk factors for ARDS.[25,26,27] Unlike previous reports,[26,27] PTX3 in our study was poorly associated with ARDS severity and non-survival. Of note, our PTX3 levels were relatively low as compared to those in previous studies on sepsis and ARDS, which may relate to differences in patient mix.[26,27] As part of the calcitonin-gene family such as PCT, proADM levels rise during severe infections and inflammation and this has been associated with disease severity in pneumonia and sepsis in previous studies.[36-39] ProADM had fair predictive value for maximum ARDS severity and non-survival and correlated to LIS but not to Berlin categories throughout the 7-day course. In multiple logistic regression, proADM predicted 28-day mortality, independently from SAPS II. To our best knowledge, this is the first study evaluating the value of proADM in predicting and monitor ARDS severity, which appeared limited.

The study has several limitations including moderate size and heterogeneity of the study population. We used fever as the inclusion criterion to define a general symptom of conditions that would predispose patients to late onset ARDS in the ICU.[3] Moreover, fever may augment alveolocapillary inflammation.[4]. Potentially resultant patient heterogeneity can also be regarded as a factor favoring generalizability of results, which would have been less if a more specific condition as entry criterion had been chosen. We reviewed clinical symptoms, history, and the chest radiographs in combination with CVP levels in an attempt to exclude gross overhydration or congestive heart failure in classifying alveolar consolidations. However, we cannot fully exclude a cardiogenic component of edema in some of these ARDS patients. We also focused on the course of ARDS rather than its development since the study goal was to find biomarkers of value in predicting severity and monitoring of late onset ARDS rather than prediction of the development of the syndrome. In line with recent research that ARDS phenotypes may differ according to sepsis as an etiologic factor,[13] we examined the contribution of this factor to the associations reported, as done before,[15,29] which appeared negligible. The value of many biomarkers in our study was nevertheless imperfect with some AUROC <0.70, allowing future studies in search of better ARDS biomarkers. Nevertheless, the availability of rapid measurements could facilitate future studies on stratifying risks and monitoring treatment of ARDS by ANG2 for instance.

## CONCLUSIONS

In conclusion, ANG2 was the only biomarker capable of both predicting the severity, monitoring the course, and predicting the outcome of late onset ARDS in febrile critically ill patients, irrespective of underlying risk factor, thereby yielding the most specific ARDS biomarker among those studied. In contrast to IL6 and PCT which had some disease monitoring value only.

## REFERENCES


1. Ferguson ND, Frutos-Vivar F, Esteban A, et al. Acute respiratory distress syndrome: underrecognition by clinicians and diagnostic accuracy of three clinical definitions. Crit Care Med 2005;33:2228–34.
2. Wind J, Versteegt J, Twisk J, et al. Epidemiology of acute lung injury and acute respiratory distress syndrome in The Netherlands: a survey. Respir Med 2007;101:2091-8.
3. Vincent JL, Sakr Y, Groeneveld J, et al. ARDS of early or late onset: does it make a difference? Chest 2010;137:81-7.
4. Tulapurkar ME, Almutairy EA, Shah NG, et al. Febrile-range hyperthermia modifies endothelial and neutrophilic functions to promote extravasation. Am J Respir Cell Mol Biol 2012;46:807–814.
5. Murray JF, Matthay MA, Luce JM, et al. An expanded definition of the adult respiratory distress syndrome. Am Rev Respir Dis 1988;138:720-723.
6. The ARDS Definition Task Force. Acute respiratory distress syndrome: the Berlin definition. JAMA 2012;307:2526-2533.
7. Hernu R, Wallet F, Thiollie`re F, et al. An attempt to validate the modification of the American-European consensus definition of acute lung injury/acute respiratory distress syndrome by the Berlin definition in a university hospital. Intensive Care Med 2013;39:2161–2170.
8. Costa ELV, Amato MBP. The new definition for acute lung injury and acute respiratory distress syndrome: is there room for improvement? Curr Opin Crit Care 2013;19:16–23.
9. Groeneveld AB, Raijmakers PG. The $^{67}$gallium-transferrin pulmonary leak index in patients at risk for the acute respiratory distress syndrome. Crit Care Med 1998;26:685-91.
10. Frohlich S, Murphy N, Boylan JF. ARDS: progress unlikely with non-biological definition. Br J Anaesth 2013;111:696–9.
11. Thille AW, Esteban A, Fernández-Segoviano P, et al. Comparison of the Berlin definition for acute respiratory distress syndrome with autopsy. Am J Respir Crit Care Med 2013;187:761-7.
12. Van der Heijden M, Nieuw Amerongen van GP, Koolwijk P, et al. Angiopoietin-2, permeability oedema, occurrence and severity of ALI/ARDS in septic and non-septic critically ill patients. Thorax 2008;63:903-909.
13. Calfee CS, Delucchi K, Parsons PE, et al; the NHLBI ARDS Network. Subphenotypes in acute respiratory distress syndrome: latent class analysis of data from two randomised controlled trials. Lancet Respir Med 2014;2:611-20.
14. Agrawal A, Zhuo H, Brady S, et al. Pathogenetic and predictive value of biomarkers in patients with ALI and lower severity of illness from two clinical trials. Am J Physiol Lung Cell Mol Physiol 2012;303:L634-9.
15. Tsantes A, Tsangaris I, Kopterides P, et al. The role of procalcitonin and IL-6 in discriminating between septic and non-septic causes of ALI/ARDS: a prospective observational study. Clin Chem Lab Med 2013;10:1-8.
16. Terpstra M, Aman J, van Nieuw Amerongen GP, et al. Plasma biomarkers for acute respiratory distress syndrome: a systematic review and meta-analysis. Crit Care Med 2014;42:691-700.

17. Parikh SM, Mammoto T, Schultz A, et al. Excess circulating angiopoietin-2 may contribute to pulmonary vascular leak in sepsis in humans. PloS Med 2006;3; 0356-0370.
18. Calfee CS, Eisner MD, Ware LB, et al; Acute Respiratory Distress Syndrome Network, National Heart, Lung, and Blood Institute. Trauma-associated lung injury differs clinically and biologically from acute lung injury due to other clinical disorders. Crit Care Med 2007;35:2243-50.
19. Gallagher DC, Parikh SM, Balonov K, et al. Circulating angiopoietin 2 correlates with mortality in a surgical population with acute lung injury/adult respiratory distress syndrome. Shock 2008;29:656-61.
20. van der Heijden M, Pickkers P, van Nieuw Amerongen GP, et al. Circulating angiopoietin-2 levels in the course of septic shock: relation with fluid balance pulmonary dysfunction and mortality. Intensive Care Med 2009;35:1567-1574.
21. Fremont RD, Koyama T, Calfee CS, et al. Acute lung injury in patients with traumatic injuries: utility of a panel of biomarkers for diagnosis and pathogenesis. J Trauma 2010;68:1121–112.
22. Calfee CS, Gallagher D, Abbott J, et al; the NHLBI ARDS Network. Plasma angiopoietin-2 in clinical acute lung injury: prognostic and pathogenetic significance. Crit Care Med 2012;40:1731-1737.
23. Agrawal A, Matthay MA, Kangelaris KN, et al. Plasma angiopoietin-2 predicts the onset of acute lung injury in critically ill patients. Am J Respir Crit Care Med 2013;187:736-742.
24. Ware LB, Koyama T, Zhao Z, et al. Biomarkers of lung epithelial injury and inflammation distinguish severe sepsis patients with acute respiratory distress syndrome. Crit Care 2013;17:R253.
25. He X, Han B, Liu M. Long pentraxin 3 in pulmonary infection and acute lung injury. Am J Physiol Lung Cell Mol Physiol 2007;292:L1029-L1049.
26. Mauri T, Coppadoro A, Bellani G, et al. Pentraxin 3 in acute respiratory distress syndrome: an early marker of severity. Crit Care Med 2008;36:2302-8.
27. Mauri T, Bellani G, Patroniti N, et al. Persisting high levels of plasma pentraxin 3 over the first days after severe sepsis and septic shock onset are associated with mortality. Intensive Care Med 2010;36:621-9.
28. Meduri GU, Headley S, Kohler G, et al. Persistent elevation of inflammatory cytokines predicts a poor outcome in ARDS. Plasma IL-1 beta and IL-6 levels are consistent and efficient predictors of outcome over time. Chest 1995;107:1062-73.
29. Headley AS, Tolley E, Meduri GU. Infections and the inflammatory response in acute respiratory distress syndrome. Chest 1997;111:1306-21.
30. Lee YL, Chen W, Chen LY, et al. Systemic and bronchoalveolar cytokines as predictors of in-hospital mortality in severe community-acquired pneumonia. J Crit Care 2010;25:176.e7-13.
31. Ware LB, Koyama T, Billheimer DD, et al; NHLBI ARDS Clinical Trials Network. Prognostic and pathogenetic value of combining clinical and biochemical indices in patients with acute lung injury. Chest 2010;137:288-96.
32. Raymondos K, Martin MU, Schmudlach T, et al. Early alveolar and systemic mediator release in patients at different risks for ARDS after multiple trauma. Injury 2012;43:189-195.

33. Ware LB, Koyama T, Zhao Z, et al. Biomarkers of lung epithelial injury and inflammation distinguish severe sepsis patients with acute respiratory distress syndrome. Crit Care 2013;17:R253.

34. Boussekey N, Leroy O, Georges H, et al. Diagnostic and prognostic values of admission procalcitonin levels in community-acquired pneumonia in an intensive care unit. Infection 2005;33:257-63.

35. Tseng JS, Chan MC, Hsu JY, et al. Procalcitonin is a valuable prognostic marker in ARDS caused by community acquired pneumonia. Respirology 2008;13:505-509.

36. Christ-Crain M, Morgenthaler NG, Struck J, et al. Mid-regional pro-adrenomedullin as a prognostic marker in sepsis: an observational study. Crit Care 2005;9:R816-24

37. Christ-Crain M, Morgenthaler NG, Stolz D, et al. Pro-adrenomedullin to predict severity and outcome in community-acquired pneumonia (ISRCTN04176397). Crit Care 2006;10:R96.

38. Huang DT, Angus DC, Kellum JA, et al. Midregional proadrenomedullin as a prognostic tool in community-acquired pneumonia. Chest 2009;136:823-31.

39. Courtais C, Kuster N, Dupuy AM, et al. Proadrenomedullin, a useful tool for risk stratification in high Pneumonia Severity Index score community acquired pneumonia. Am J Emerg Med 2013;1:215-21.

40. Hoeboer SH, Alberts E, van den Hul I, et al. Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin. J Infect 2012;64:484-93.

41. Levy MM, Fink MP, Marshall JC, et al. International Sepsis Definitions Conference. 2001 SCCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. Intensive Care Med 2003;29:530–538.

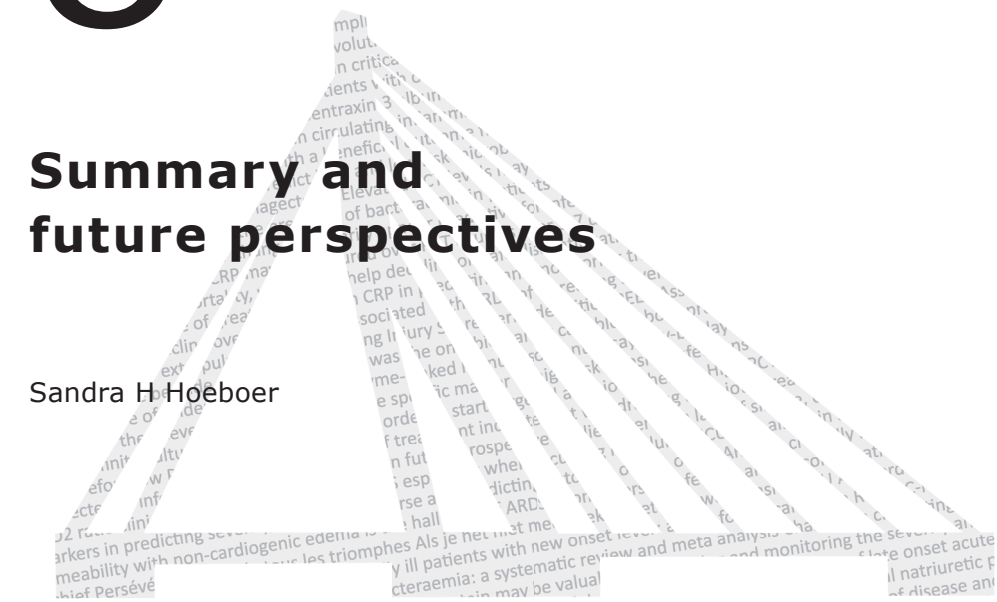
42. Calandra T, Cohen J. The international sepsis forum consensus conference on definitions of infection in the intensive care unit. Crit Care Med 200533:1538–1548.

43. Youden WJ. Index for rating diagnostic tests. Cancer 1950;3:32-35.

# 8

# Summary and future perspectives

Sandra H. Hoeboer



## SUMMARY

In this thesis we evaluated the use of biomarkers in critically ill patients with new onset fever for diagnosis, monitoring and prognosis of infection and its complications, with special focus on ARDS. We chose fever as our main inclusion criterion because it still is an important symptom for clinicians to consider the presence of infection in their patients.

**Part I** - Chapter 2 focuses on the prediction of the severity of infection invasiveness to the blood stream (bacteraemia), septic shock and survival in critically ill patients with new onset fever. A probable or proven local infection complicated by bacteraemia, septic shock or non-survival was considered as a high risk infection. We measured old (WBC, lactate and CRP) and new (PCT, proADM, proANP and COP) biomarkers for three days after fever onset in 101 critically ill patients. Fifty-seven patients had a probable or proven infection (45 only local infection and 12 bacteraemia). Our results suggest that elevated CRP levels (optimal cutoff >196 mg/L) are a sensitive indicator of the presence of microbial infection irrespective of its invasiveness or severity. High PCT levels, on the other hand, may be of value as an indicator of high risk ICU-acquired infection, (optimal cutoff >1.98 ng/mL). Low PCT values in particular (optimal cutoff <0.65 ng/mL) indicated the absence of bacteraemia, shock or mortality. Among the studied biomarkers, PCT had superior predictive value for all four major endpoints, it also peaked earlier than other markers.

The use of these inflammatory biomarkers early postoperative remains a matter of debate. Surgery itself, and oesophagectomy in particular, triggers an inflammatory response limiting the use of SIRS criteria for diagnosing early postoperative (infectious) complications. The value of PCT early postoperative remains uncertain. As a proof of principle, we measured CRP and PCT in 45 consecutive patients undergoing elective oesophagectomy with gastric-tube reconstruction (chapter 3). The results suggest that increasing or high CRP levels within the first 3 days post-oesophagectomy contribute to the early diagnosis of any postoperative complication presenting between postoperative days 3 and 10, independent of the preoperative risk assessment scores. Elevated PCT levels may specifically indicate the development of more severe combined surgical/infectious complications, mainly associated with anastomotic leakage, that required longer hospitalisation. PCT did not signal infectious complications alone. Elevated PCT rather than CRP indicates a certain degree of urgency warranting empirical (antibiotic) treatment while awaiting results from microbiological cultures and diagnostic imaging. Low PCT levels may reassure clinicians to await definite test results to initiate targeted antibiotic therapy.

In the majority of the literature PCT is used to diagnose sepsis and not proven infection. This may be one of the reasons why previous meta-analyses on the diagnostic use of PCT for sepsis and infection have been contradicting in their results. All through this thesis we have tried to study the use of biomarkers in diagnosing underlying disease rather than symptoms, or more precisely in diagnosing (culture) proven infection rather than sepsis. We performed a systematic review and meta-analysis to study the diagnostic accuracy of PCT for culture proven bacteraemia in patients suspected for infection or sepsis (chapter 4). The 58 included articles together study 17,155 patients of whom 3,420 suffered from bacteraemia Overall PCT at a cutoff value of 0.5 ng/mL had good diagnostic value for bacteraemia area under the hierarchical summary receiver operating characteristics curve (HSROC) 0.79, sensitivity 76, specificity 69. In an attempt to reduce heterogeneity of the study population we performed the same analysis in a variety of subgroups. The area under the HSROC ranged from 0.77-0.84, with sensitivities ranging from 66-89 and specificities ranging from 55-78. This meta-analysis shows that PCT has a good diagnostic accuracy for culture proven bacteraemia in adult patients suspected of infection or sepsis.

Finally in chapter 5 we focused on the monitoring value of CRP and PCT during a one week course. We studied fractional changes in CRP and PCT levels for predicting the evolution of microbial infection, its invasiveness (bacteraemia) and severity (septic shock, SOFA scores) in response to treatment. CRP levels decreased during the week when (bloodstream) infection and septic shock resolved (fractional change <0.14) and CRP levels increased when complications such as a new (bloodstream) infection or septic shock supervened (fractional change >2.57). PCT levels decreased when septic shock resolved (fractional change <0.13) and increased when a new bloodstream infection or septic shock supervened (fractional change >1.57). An increase in PCT levels also best predicted increasing or not declining SOFA scores (fractional change >1.23). We may conclude that CRP levels proved more sensitive for the evolution of (local) microbial infections than PCT levels. On the other hand, PCT increases predicted bloodstream invasion, septic shock, and organ failure and 28-day mortality, supporting the hypothesis that PCT is more useful in predicting infectious complications than CRP.

From the results generated in part I of this thesis we may conclude that CRP is a sensitive marker of infections irrespective of their severity, while PCT is a more specific marker of high risk invasive infections (bacteraemia) and its complications (septic shock, organ failure and mortality). The discriminative power of CRP levels between mild and life threatening infections was less than for PCT. To start empirical treatment based on CRP levels alone will lead to overtreatment if considered specific for infection. In the presence of elevated CRP levels (>196 mg/mL) low PCT levels (<0.5-0.65 ng/mL) could reassure clinicians that there is time to await definite culture and imaging results in order to start targeted (antibiotic) therapy. High PCT levels war-

rant empirical treatment. Withholding unnecessary (empiric) antibiotic treatment will aid in the prevention of emerging microbial resistance and unnecessary adverse drug reactions, amongst others.[1-7] Suffice to say that a grey area remains (PCT 0.5-2ng/mL) where a high risk infection cannot be proven or excluded based on PCT levels. The change in CRP over a one week course could contribute in the assessment of a patients response to treatment (fractional change <0.14). Again, increasing PCT levels indicate a dismal course or outcome (fractional change >1.57). Therefore low PCT levels (<0.25 ng/mL) after one week of treatment indicate that withdrawing antibiotic treatment is justifiable. The safety of PCT as a single decision tool to withhold cultures and additional imaging in patients suspected of infection remains to be proven in future prospective studies.

**Part II** – There is a lack of simple, objective, and precise tools for ARDS diagnosis and monitoring in clinical practice. The current clinical classification systems, such as the relatively simple Berlin criteria and more extensive LIS, use parameters with known interobserver variability (chest radiographs), that are influenced by ventilator settings (chest radiographs, $P_aO_2/F_IO_2$ ratio) or by other pulmonary pathology (chest radiographs, $P_aO_2/F_IO_2$ ratio, compliance, PEEP). In clinical practice it is especially difficult to discriminate ARDS from diffuse pulmonary infection. Furthermore, deterioration in gas exchange may also be due to sputum retention or diffuse (micro) atelectasis. As a result clinicians may underdiagnose ARDS especially when occurring late during ICU stay.[8] This is reflected by the limited association between clinical ARDS and diffuse alveolar damage at autopsy.[9]

In the second part of the thesis, we longitudinally evaluated the use of routine biochemical variables like albumin, CRP and LDH (chapter 6) and other potentially more specific biomarkers like ANG2, PCT, PTX3 and proADM (chapter 7) for diagnosing severity, monitoring course and predicting outcome in late onset ARDS. These new markers could also increase the pathophysiological understanding of ARDS. In the absence of a true reference standard we reasoned that the overlap between the Berlin definition and LIS would be a better reference standard for potential biomarkers than either system alone.

In chapter 6, overall, albumin but not CRP levels appeared valuable in daily monitoring of ARDS severity and course at the bedside. Although the associative values were only moderate, low albumin levels (<22 g/L) were inversely related to Berlin and LIS severity categories from day 0 onward, while elevated CRP levels (>60 mg/mL)were associated with severe ARDS on day 7 only. During the week, a change in albumin levels was inversely related to a change in ARDS severity regardless of its definition. In contrast, increasing CRP levels were associated with increasing Berlin definition only. Of all conventional markers, LDH levels predicted 28-day mortality.

The data in chapter 7 suggest that among the novel and more specific biomarkers ANG2 is the most specific and uniform ARDS biomarker. ANG2 was the only biomarker able to predict ARDS severity, to monitor its course and to predict mortality, irrespective of definitions and underlying risk factor. In contrast IL-6 and PCT had some disease monitoring value only. However, the predictive and monitoring values of ANG2 were not perfect (AUROC 0.65-0.80) and warrant future studies in search of ARDS biomarkers.

In conclusion, albumin and ANG2, both linked to alveolocapilary permeability, were the most consistent and therefore most valuable markers in predicting severity, monitoring course and predicting outcome of late onset ARDS in critically ill patients within one week after new onset fever. Indeed, alveolocapillary inflammation and increased vascular permeability with non-cardiogenic edema is the hallmark of ARDS. Hypoalbuminemia lowers oncotic pressure and in the presence of increased vascular permeability this can increase pulmonary oedema and ARDS severity. As shown in previous cross-sectional studies low total protein and albumin levels, regardless of fluid state, are associated with the presence or development of ARDS.[10-14] Whether this hypoalbuminemia is due to decreased synthesis, increased breakdown, leakage to the interstitium or fluid resuscitation we cannot conclude from this study. All have likely played a role. Up to now longitudinal data using albumin as a monitoring tool for ARDS severity are scarce. ANG2 may be directly involved in the activation of vascular endothelium through the angiopoietin-Tie2 system. The resultant modulation of the cell-cell junction stability, thrombin-induced cell contractility and gap formation lead to increased pulmonary vascular permeability.[15-17] The increasing cutoff values for ANG2 with increasing severity of ARDS, according to LIS, supports a pathophysiologic role.

## FUTURE PERSPECTIVES

Although biomarkers are of added value in identification of patients subject to high risk infection and its complications there are considerable challenges before further progress can be made. Some of the most prominent problems include the lack of easy access, unambiguous, objective diagnostic gold standards and definitions. Current microbiological detection relies on gram, stain and laboratory identification after culture. There are several factors that make this approach suboptimal amongst which slow growth, resistance to cultivation in vitro and unability to prove causality when a pathogen is detected.[5] Positive cultures do not discriminate with certainty between contamination, colonisation and infection. The wide variety of definitions currently used to diagnose infection and its severity, respectively, also complicate the interpre-

tation of results. These problems regarding diagnostic standards and definitions apply to ARDS as well.

**Increasing the sensitivity and specificity of the diagnosis infection**

The direct measurement of microbial DNA in blood by real-time polymerase chain reaction (PCR) in specimen has been suggested to improve the diagnostic process of infectious disease.[20-23] However, on its own direct measurement of microbial DNA may be too sensitive and provides no information on clinical relevant bacterial load.[20] Biomarkers of host inflammation could be used to judge the clinical relevance of the PCR findings. Another potential diagnostic tool is gene expression microarray.[24,25] The comparison of gene expression profiles of for instance peripheral blood leukocytes could be used to differentiate between inflammation and infection.[24-26] In vivo studies have shown that micro array profiles were capable of discriminating between healthy controls, patients with bacterial infection, viral infection and a co-infection, amongst others.[24-26] Furthermore, different microbes induce different gene expression profiles and these may be used to indicate the offending microbe, class, genus, species and even genetically distinct strains and their virulence specifically.[24,25,27,] Micro array can also be used to study the interactions between the host (i.e. inflammation) and pathogens and can thereby provide information on variability in disease severity and host susceptibility.[24-31] There are some limitations to the use of micro arrays as well. Micro arrays generate enormous amounts of data resulting in challenging and complicated data analyses. Also, there is no clear consensus on the optimal way of interpreting these data, they rely on large quantitative changes and may thereby overlook small changes in smaller biologically important genes (needle-in-a-haystack). Finally, they are technically sophisticated and not yet executable in smaller laboratories.[24,25] Furthermore, knowing that gene-expression is altered by certain microbes or as a response to microbes does not per se lead to understanding the mechanism.[5,6] The next step may be the complete sequencing of human and microbial DNA which may prove to be a more reliable method.[32-37] But this method will not resolve the limitations mentioned for micro array techniques.[32-37] Before these techniques can become the golden diagnostic standard in daily practice more research needs to be done. Furthermore, they have to become affordable and applicable on a wider scale.

Finally, in medicine, but also in life, we try to dichotomize and categorize complex problems. The transitions from colonisation to infection, from mild to severe local infection and from contained to systemic infection with multiple organ failure are gradual. Concluding from this thesis and the literature in general, we may have to consider a combination of biomarkers instead of looking for a single holy grail and be aware of the pathophysiological mechanism underlying the different biomarkers.

**Increasing the sensitivity and specificity of the diagnosis of ARDS**

The diagnosis of ARDS in clinical practice is challenged by its complex and multifactorial underlying pathophysiology and lack of a gold diagnostic standard in vivo. The current clinical diagnostic criteria are non-specific and many pulmonary and cardiac conditions can influence these criteria. Due to its diverse aetiology, a single diagnostic and monitoring biomarker may not exist. The biomarkers in this thesis reflect inflammation and capillary leak in general but may not be specific enough for pulmonary inflammation and leakage. Even more important, the many different possible ARDS risk factors suggest a final common inflammatory pathway with similar symptoms resulting from different provoking diseases. Microarray and DNA sequencing techniques may help in discriminating between these different aetiologies of ARDS and genetic susceptibility.[37-45] If we could reclassify patients with similar clinical symptoms into their underlying aetiological classes further targeted research on underlying pathophysiological mechanisms, clinical classification systems and therapy may again progress.

## REFERENCES


1. Livermore DM, Dudley MN. Antimicrobials: better use, better drugs, or both? Curr Opin Microbiol 2000;3: 487–488.
2. Seppälä H, Klaukka T, Vuopio-Varkila J, et al. The effect of changes in the consumption of macrolide antibiotics on erythromycin resistance in group A streptococci in Finland. Finnish Study Group for Antimicrobial Resistance. N Engl J Med 1997;337:441-6.
3. Patterson JE. Antibiotic utilization. Is there an effect on antimicrobial resistance? Chest 2001;119: 426S–430S.
4. Alanis AJ. Resistance to antibiotics: are we in the post-antibiotic era? Arch Med Res 2005;36:697-705.
5. Vanderkooi O, Low D, Green K, et al. Predicting antimicrobial resistance in invasive pneumococcal infections. Toronto invasive bacterial disease network. Clin Infect Dis 2005;40:1288–1297.
6. Fair RJ, Tor Y. Antibiotics and bacterial resistance in the 21st century. Perspect Medicin Chem 2014;28;6:25-64.
7. Michael CA, et al. The antimicrobial resistance crisis: Causes, consequences and management. Frontiers in Public Health 2014;2:145.
8. Vincent JL, Sakr Y, Groeneveld J, Zandstra DF, et al. ARDS of early or late onset: does it make a difference? Chest 2010;137:81-7.
9. Thille AW, Esteban A, Fernández-Segoviano P, et al. Comparison of the Berlin definition for acute respiratory distress syndrome with autopsy. Am J Respir Crit Care Med 2013;187:761-7.
10. Mangialardi RJ, Martin GS, Bernard GR, et al. Hypoproteinemia predicts acute respiratory distress syndrome development, weight gain, and death in patients with sepsis. Crit Care Med 2000;28:3137-45.
11. Arif SK, Verheij J, Groeneveld AB, et al. Hypoproteinemia as a marker of acute respiratory distress syndrome in critically ill patients with pulmonary edema. Intensive Care Med 2002;28:310-7.
12. Aman J, van der Heijden M, van Lingen A, et al. Plasma protein levels are markers of pulmonary vascular permeability and degree of lung injury in critically ill patients with or at risk for acute lung injury/acute respiratory distress syndrome. Crit Care Med 2011;39:89-97.
13. Lee JH, Kim J, Kim K, et al. Albumin and C-reactive protein have prognostic significance in patients with community-acquired pneumonia. J Crit Care 2011;26:287-294.
14. Zhang Z. Lu, Ni H, Sheng X, Jin N. Predictions of pulmonary edema by plasma protein levels in patients with sepsis. J Crit Care 2012;27:623-9.
15. van der Heijden M, van Nieuw Amerongen GP, van Hinsbergh VW, et al. The interaction of soluble ie2 with angiopoietins and pulmonary vascular permeability in septic and nonseptic critically ill patients. Shock 2010;33:263-8.
16. van der Heijden M, van Nieuw Amerongen GP, Chedamni S, et al. The angiopoietin-Tie2 system as a therapeutic target in sepsis and acute lung injury. Expert Opin Ther Targets 2009;13:39-53.
17. van der Heijden M, van Nieuw Amerongen GP, van Bezu J, et al. Opposing effects of the angiopoietins on the thrombin-induced permeability of human pulmonary microvascular endothelial cells. PLoS One 2011;6:e23448.

18. Sturn DH, Feistritzer C, Mosheimer BA, et al. Angiopoietin affects neutrophil migration. Microcirculation 2005;12:393-403.

19. Murdoch C, Tazzyman S, Webster S, et al. Expression of Tie-2 by human monocytes and their responses to angiopoietin-2. J Immunol 2007;178:7405-11.

20. Mackay IM. Real-time PCR in the microbiology laboratory. Clin Microbiol Infect 2004;10:190–212.

21. Chang SS, Hsieh WH, Liu TS, et al. Multiplex PCR system for rapid detection of pathogens in patients with presumed sepsis - a systemic review and meta-analysis. PloS one 2013;8:e623

22. Obara H, Aikawa N, Hasegawa N, et al. The role of a real-time PCR technology for rapid detection and identification of bacterial and fungal pathogens in whole-blood samples. J Infect Chemother 2011;17:327-33.

23. Yanagihara K, Kitagawa Y, Tomonaga M, et al. Evaluation of pathogen detection from clinical samples by real-time polymerase chain reaction using a sepsis pathogen DNA detection kit. Crit Care 2010;14:R159.

24. Bryant PA, Venter D, Robins-Browne R, et al. Chips with everything: DNA microarrays in infectious diseases. Lancet Infect Dis 2004;4:100-11.

25. Mejias A, Ramilo O. Transcriptional profiling in infectious diseases: ready for prime time? J infect 2014;68:S94-9.

26. Ramilo O, Allman W, Chung W, et al. Gene expression patterns in blood leukocytes discriminate patients with acute infections. Blood 2007;109:2066e77

27. Manger ID, Relman DA. How the host 'sees' pathogens: global gene expression responses to infection. Cur Opin Immunol 2000;12:215-8.

28. Toufeer M, Bonnefont CM, Foulon E, et al. Gene expression profiling of dendritic cells reveals important mechanisms associated with predisposition to Staphylococcus infections. PLoS One 2011;6:e22147.

29. Banchereau R, Jordan-Villegas A, Ardura M, et al. Host immune transcriptional profiles reflect the variability in clinical disease manifestations in patients with Staphylococcus aureus infections. PLoS One 2012;7:e34390.

30. Fittipaldi N, Beres SB, Olsen RJ, et al. Full-genome dissection of an epidemic of severe invasive disease caused by a hypervirulent, recently emerged clone of group A Streptococcus. Am J Pathol 2012;180:1522–1534.

31. Huang J, Sun Z, Yan W, et al. Identification of microRNA as sepsis biomarker based on miRNAs regulatory network analysis. Biomed Res Int 2014;2014: 594350.

32. den Bakker HC, Allard MW, Bopp D, et al. Rapid Whole-Genome Sequencing for Surveillance of Salmonella enterica Serovar Enteritidis. Emerg Infect Dis 2014;20:1306-14.

33. Koser CU, Bryant JM, Becq J, et al. Whole-genome sequencing for rapid susceptibility testing of M. tuberculosis. N Eng J Med 2013;369:290-2.

34. Koser CU, Fraser LJ, Ioannou A, et al. Rapid single-colony whole-genome sequencing of bacterial pathogens. J Antimicrob Chemother 2014;69:1275-81.

35. Reuter S, Ellington MJ, Cartwright EJ, et al. Rapid bacterial whole-genome sequencing to enhance diagnostic and public health microbiology. JAMA Intern Med 2013;173:1397-404.

36. Roetzer A, Diel R, Kohl TA, et al. Whole genome sequencing versus traditional genotyping for investigation of a Mycobacterium tuberculosis outbreak: a longitudinal molecular epidemiological study. PLoS Med 2013;10:e1001387.

37. Snitkin ES, Zelazny AM, Thomas PJ, et al. Tracking a hospital outbreak of carbapenem-resistant Klebsiella pneumoniae with whole-genome sequencing. Sci Transl Med 2012;4:148ra16.

38. Gao L, Barnes KC. Recent advances in genetic predisposition to clinical acute lung injury. Am J Physiol Lung Cell Mol Physiol 2009;296:L713-25.

39. Flores C, Pino-Yanes MM, Casula M, et al. Genetics of acute lung injury: past, present and future. Minerva Anestesiol 2010;76:860-4.

40. Matsuda A, Kishi T, Jacob A, et al. Association between insertion/deletion polymorphism in angiotensin-converting enzyme gene and acute lung injury/acute respiratory distress syndrome: a meta-analysis. BMC Med Genet 2012;13:76.

41. Tejera P, Meyer NJ, Chen F, et al. Distinct and replicable genetic risk factors for acute respiratory distress syndrome of pulmonary or extrapulmonary origin. J Med Genet 2012;49:671-80.

42. Meyer NJ, Feng R, Li M, et al. IL1RN coding variant is associated with lower risk of acute respiratory distress syndrome and increased plasma IL-1 receptor antagonist. Am J Respir Crit Care Med 2013;187:950-9.

43. Sun X, Ma SF, Wade MS, et al. Functional promoter variants in sphingosine 1-phosphate receptor 3 associate with susceptibility to sepsis-associated acute respiratory distress syndrome. Am J Physiol Lung Cell Mol Physiol 2013;305:L467-77.

44. Bhargava M, Becker TL, Viken KJ. Proteomic profiles in acute respiratory distress syndrome differentiates survivors from non-survivors. PLoS One 2014;9:e109713.

45. Shortt K, Chaudhary S, Grigoryev D, et al. Identification of novel single nucleotide polymorphisms associated with acute respiratory distress syndrome by exome-seq. PLoS One 2014;9:e111953.

46. Tejera P, O'Mahony DS, Owen CA, et al. Functional characterization of polymorphisms in the peptidase inhibitor 3 (elafin) gene and validation of their contribution to risk of acute respiratory distress syndrome. Am J Respir Cell Mol Biol 2014;51:262-72.

## SAMENVATTING

In dit proefschrift wordt de rol van biomarkers voor het diagnosticeren, monitoren, prognosticeren van infecties en infectieuze complicaties bij kritiek zieke patiënten met koorts beschreven. We besteden extra aandacht aan ARDS ls complicatie van infectie en ontsteking in kritiek zieke patiënten met koorts. Het belangrijkste inclusiecriterium was koorts omdat dit nog steeds een van de belangrijkste symptomen is voor artsen om de aanwezigheid van een infectie bij hun patiënt te overwegen. Het is belangrijk om een onderscheid te kunnen maken tussen koorts ontstaan door de aanwezigheid van bacteriën en koorts ontstaan door "steriele" ontsteking. De eerste moet behandeld worden met antibiotica, voor de tweede is de behandeling afhankelijk van de oorzaak van de steriele ontsteking.

**Deel I -** In het eerste hoofdstuk ligt de focus op het diagnosticeren en voorspellen van de ernst van een infectie bij kritiek zieke patiënten met koorts. Al heel lang gebruikt men witte bloedcellen en andere ontstekingswaarden (bijvoorbeeld CRP) om de aanwezigheid van een infectie waarschijnlijker te maken, maar deze parameters kunnen ook verhoogd zijn bij een steriele ontsteking. De gedachte is dat de nieuwe biomarkers beter dan de oudere ontstekingswaarden een onderscheid kunnen maken tussen infectie en steriele ontsteking. We vergeleken de diagnostische en voorspellende waarde van lang bekende ontstekingswaarden namelijk: witte bloedcellen, lactaat en CRP met nieuwere biomarkers (ontstekingsstoffen) zoals: PCT, proADM, proANP en COP. Er werden 3 groepen gedefinieerd: patiënten met koorts maar zonder infectie, patiënten met een lokale infectie (infectie van een weefsel of orgaan) en patiënten met bacteriën in de bloedbaan (bacteriëmie). Ernstige infecties werden gedefinieerd als een lokale infectie mét bacteriën in de bloedbaan (bacteriëmie) en/of septische shock (een lage bloeddruk ten gevolge van infectie) en/of overlijden binnen 28 dagen na het ontstaan van de koorts. In totaal werden er 101 kritiek zieke patiënten met koorts ontstaan op de intensive care geïncludeerd. Van hen hadden 45 een lokale infectie (infectie beperkt tot een orgaan of weefsel) en 12 een infectie met bacteriëmie. De resultaten suggereren dat verhoogde CRP waarden een gevoelige maat zijn voor de aanwezigheid van bacteriële infecties, onafhankelijk van hun ernst. Hoge PCT waarden, aan de andere kant, blijken een specifieke maat voor hoog risico infecties op de intensive care. Lage PCT waarden in het bijzonder duidden op de afwezigheid van bacteriëmie, septische shock of overlijden binnen 28 dagen na het ontstaan van koorts. Onder de bestudeerde biomarkers had PCT de beste voorspellende waarde voor eindpunten, daarnaast bereikte het eerder dan de andere markers zijn piekwaarde.

Het gebruik van biomarkers vroeg na een operatie is lastig omdat een operatie veel steriele ontsteking veroorzaakt. Vooral een zogenoemde electieve oesofagectomie met buismaagreconstructie (verwijderen van de slokdarm omdat er een slokdarm tumor

is) brengt veel steriele ontsteking teweeg, maar gaat ook regelmatig gepaard met complicaties van chirurgische (waardoor er nog meer steriele ontsteking ontstaat) of infectieuze aard. Hierdoor is de voorspellende waarde van de langer bekende ontstekingsparameters (witte bloedcellen en CRP) kort na een operatie vaak beperkt. De waarde van PCT na een operatie is echter nog steeds niet helder, maar men denkt dat deze superieur zou kunnen zijn aan de waarde van CRP. We vergeleken de voorspellende waarde van CRP en PCT voor het ontstaan van chirurgische en infectieuze complicaties in 45 patiënten na een electieve oesofagectomie met buismaagreconstructie. De resultaten laten zien dat in deze groep hoge CRP waarden binnen 3 dagen na een oesofagectomie bijdragen aan de vroege diagnose van postoperatieve complicaties, ongeacht of deze van chirurgische of infectieuze aard zijn en onafhankelijk van preoperatieve risico scores. Verhoogde PCT waarden waren een specifieke maat voor de meer ernstige gecombineerde chirurgische-infectieuze complicaties en gingen meestal gepaard met anastomose lekkage, deze patiënten verbleven dan ook langer in het ziekenhuis. PCT kon helaas niet geïsoleerde infectieuze complicaties diagnosticeren. Verhoogde PCT waarden en niet CRP wijzen op een bepaalde mate van ernst en noodzaak tot spoedige behandeling, ook al is de precieze bacterie of complicatie nog niet bekend. Lage PCT waarden zijn geruststellend en geven artsen de tijd om aanvullend onderzoek af te wachten zodat zij met gerichte therapie kunnen starten.

In het overgrote deel van de literatuur is PCT gebruikt om sepsis te diagnosticeren en niet een bewezen infectie. Sepsis is een klinisch beeld dat ontstaat door een heftige immuunreactie van het lichaam, regelmatig als gevolg van infectie, maar soms ten gevolge van steriele ontsteking. Wij denken dat de tegenstrijdige resultaten in eerdere onderzoeken voor een deel te maken hebben met het gebruik van deze niet robuuste uitkomstmaat. Gedurende dit proefschrift hebben wij geprobeerd om de biomarkers te gebruiken ter diagnose van ziekte (infectie) en niet ter diagnose van symptomen (sepsis). In hoofdstuk 4 bekijken en vergelijken we de literatuur waarin de PCT waarde van patiënten met een bewezen bacteriëmie vergeleken wordt met de PCT waarde van patiënten zonder bewezen infectie. In totaal werden er 58 artikelen geïncludeerd, waaraan 17.155 patiënten deelnamen, van wie 3.420 een bacteriëmie hadden. PCT had bij een afkapwaarde van 0.5 ng/mL een goede diagnostische waarde voor bacteriëmie in patiënten die werden verdacht van een infectie.

Tot slot onderzochten we in hoofdstuk 5 of CRP en PCT gebruikt kunnen worden om een uitspraak te doen over de ontwikkeling van het ziektebeeld in de week na het ontstaan van koorts. We onderzochten de voorspellende waarde van een relatieve verandering van CRP en PCT gedurende een week voor de ontwikkeling van een infectie, bacteriëmie en ziekte ernst. Uit de resultaten kunnen we concluderen dat CRP meer dan PCT een gevoelige marker is voor de evolutie van (lokale) infecties. Daartegenover staat dat een verhoogd PCT voorspellend is voor bacteriëmie en ziekte

ernst: septische shock, orgaanfalen en sterven binnen 28 dagen na het ontstaan van koorts. Dit sterkt de hypothese dat PCT waardevoller is voor het voorspellen van infectieuze complicaties dan CRP.

Samenvattend kunnen we naar aanleiding van de resultaten uit deel I van dit proefschrift concluderen dat CRP een gevoelige biomarker is voor infecties ongeacht de ernst van deze infectie. PCT daarentegen is een specifiekere marker van hoog risico infecties en infectieuze complicaties. Het onderscheidende vermogen van CRP tussen milde en levensbedreigende infecties was kleiner dan die van PCT. Indien je CRP gebruikt als diagnosticum voor het starten van antibiotica zal je een deel van de patiënten onnodig antibiotica geven. Lage PCT waarden zouden artsen kunnen ondersteunen in hun beslissing dat het veilig is om onderzoeksresultaten af te wachten zodat gerichte therapie gestart kan worden. Het verminderen van onnodige antibiotische behandelingen kan helpen in de preventie van resistente bacteriën en voorkomt onnodige allergische reacties bij patiënten. Er blijft echter wel een grijs gebied waarin de biomarker waarden wel verhoogd zijn, maar niet extreem verhoogd. De interpretatie van de biomarker waarden in dit grijze gebied blijft lastig. Een interessante bevinding is ook dat een daling van de CRP waarden gedurende een week gebruikt kan worden om het slagen van de behandeling te objectiveren, terwijl het stijgen van PCT waarden met name gebruikt kan worden om een achteruitgang/verslechtering van het ziektebeeld te objectiveren. Lage PCT waarden 1 week na het begin van koorts bij kritiek zieke patiënten zouden gebruikt kunnen worden om het stoppen van antibiotica te verdedigen of het veilig is om PCT te gebruiken als enige maat om het starten van antibiotica op te baseren, kunnen we uit dit proefschrift niet concluderen. Verder onderzoek zal nodig zijn.

**Deel II –** Het acute respiratory distress syndrome (ARDS) is longschade ontstaan door ontsteking van de kleine longblaasjes en de daaromheen gelegen bloedvaatjes. Hierdoor gaan de bloedvaatjes rondom de longblaasjes lekken en komt er ontstekingsvocht in de longblaasjes. Door dit ontstekingsvocht wordt het moeilijk om genoeg zuurstof ($O_2$) vanuit de longen naar het bloed te transporteren. Daarnaast worden de longen door de ontsteking stugger, waardoor de uitwisseling van zuurstof en koolzuur ($CO_2$) nog moeilijker is. ARDS is een ernstig ziektebeeld bij kritiek zieke patiënten waaraan mensen kunnen overlijden of beperkende restschade aan overhouden. Er is een gebrek aan simpele, objectieve methoden om ARDS te diagnosticeren aan bed van een kritiek zieke patiënt. Bij de huidige ARDS scoresystemen wordt gebruik gemaakt van onder andere het verschil tussen de hoeveelheid zuurstof in de beademingslucht en het bloed, röntgenfoto's van de longen, en elasticiteit van de longen. Het blijkt echter dat ook bij andere longproblemen deze parameters afwijkend kunnen zijn en dat er veel verschil is in interpretatie.

In het tweede deel van dit proefschrift hebben we geprobeerd om met behulp van reeds bekende langer gebruikte bloedwaarden zoals albumine, LDH en CRP (hoofdstuk 6) en nieuwe biomarkers zoals ANG2, PCT, PTX3 en proADM (hoofdstuk 7) de ernst, het verloop en de uitkomst van ARDS te diagnosticeren. Het blijkt dat met name albumine en ANG2, beide een maat voor lekkende bloedvaten ten gevolge van ontsteking, gebruikt konden worden voor het diagnosticeren, vervolgen en voorspellen van de uitkomst van ARDS. De diagnostische en voorspellende waarde van deze twee biomarkers was echter niet perfect. Er zal verder onderzoek nodig zijn naar de onderliggende mechanismen van ARDS voordat deze markers gebruikt kunnen worden in de dagelijkse praktijk.

## TOEKOMSTIGE UITDAGINGEN

Ondanks dat er een toegevoegde waarde is voor het gebruik van biomarkers bij het diagnosticeren van infecties en infectieuze complicaties liggen er verschillende uitdagingen in de toekomst. Zo is er nog winst te behalen in het vaststellen van de aanwezigheid van bacteriën. De huidige kweekmethodes zijn nog niet waterdicht. Zo kan het gebeuren dat er wel een bacterie aanwezig is maar dat je deze met de huidige kweekmethodes niet of pas relatief laat vindt. Een oplossing zou kunnen liggen in het screenen van DNA- en eiwitpatronen in bijvoorbeeld witte bloedcellen van patiënten verdacht voor een infectie. Dit soort methodes zijn echter bewerkelijk en kostbaar en kunnen nog niet in elk laboratorium worden uitgevoerd.

De technieken waarbij DNA- en eiwitpatronen in cellen bestudeerd kunnen worden zouden ook gebruikt kunnen worden om een beter begrip te krijgen van het zeer diverse ziektebeeld dat ARDS is. Misschien dat patiënten die we nu diagnosticeren met ARDS wel een heel ander onderliggend ziektebeeld hebben maar dat de symptomen erg op elkaar lijken.

Tot slot; in de geneeskunde maar ook in het dagelijks leven proberen wij complexe problemen in simpele categorieën in te delen, terwijl veel van deze problemen zich misschien meer op een continue schaal bevinden. Concluderend uit dit proefschrift, en de literatuur in het algemeen, zouden we moeten overwegen om de zoektocht naar de heilige graal der biomarkers te verschuiven naar het doorgronden van de mechanismen die ten grondslag liggen aan deze biomarkers.

# LIST OF ABBREVIATIONS

ARDS - Acute respiratory distress syndrome
AUROC - Area Under the Receiver Operator Characteristics curve
APACHE II score - Acute Physiology And Chronic Health Evaluation II score
ASA classification - American Society of Anesthesiologists classification
CI - Confidence interval
CPR - Cardiopulmonary resuscitation
CRP - C-reactive protein
CV- Coefficient of Variation
CVP - Central venous pressure
D - Day
ICU - Intensive Care Unit
LDH - Lactate dehydrogenase
LHR - Likelihood ratio
LIS - Lung injury score
NPV - Negative predictive value
$P_aO2/F_IO_2$ - Arterial $O_2$ pressure over inspiratory $O_2$ fraction
PCT - Procalcitonin
PEEP - Positive end-expiratory pressure
P-POSSUM - Portsmouth Physiological and Operative Severity Score for the enUmeration of Mortality and morbidity
PPV - Positive predictive value
SAPS - Simplified acute physiology score
SIRS - Systemic Inflammatory Response Syndrome
SN - Sensitivity
SP - Specificity
SOFA score - Sequential Organ Failure Assessment score
WHO - World Health Organization

# CURRICULUM VITAE

Sandra Helena Hoeboer was born on 21 June 1988 in Naarden, The Netherlands, to Cor and Jacqueline Hoeboer. She has two younger siblings, Nicky and Robert. After graduating from secondary school (VWO, S.G. Huizermaat, Huizen) she studied medicine between 2006 and 2012 at the VU University in Amsterdam. During her studies she participated in the Honours Programme and started her PhD trajectory at the VU Medical Center Department of Intensive Care Medicine under supervision of prof. dr. A.B.J. Groeneveld, and later also prof. dr. H.M. Oudemans-van Straaten. After graduating university she started her professional career at the Erasmus Medical Center Rotterdam as a junior doctor and PhD-student in Intensive Care Medicine. As of October 2014 she has started her specialty training in Internal Medicine at Tergooi Ziekenhuizen Hilversum and Blaricum.

| Course title | ECTS |
|---|---|
| BROK cursus | 1.5 |
| Biomedical English Writing and Communication | 3 |
| | |
| Other courses | |
| Courses and workshops of third parties | |
| Practical Biostatistics | 1.5 |
| Masterclass Medical Business 2013 | 0.6 |
| Masterclass Medical Business 2014 | 0.6 |
| MBE Summer Academy | 2 |
| FCCS cursus | 1.5 |
| | |
| Lectures | 0.1 |
| Refereerbijeenkomst fluids in the ICU | 0.1 |
| Symposia - Congresses | |
| ISICEM 2013, poster presentation | 1.5 |
| ESICM 2011, poster presentation | 1.8 |
| ISICEM 2010 | 1.2 |
| | |
| Supervision research student | |
| Noel Engels | 0.6 |

# LIST OF PUBLICATIONS

## Publications related to this thesis

1. Hoeboer SH, Alberts E, van den Hul I, Tacx AN, Debets-Ossenkopp YJ, Groeneveld ABJ. Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin. J Infect 2012 May;64:484-93.

2. Hoeboer SH, Groeneveld ABJ. Changes in circulating procalcitonin versus C-reactive protein in predicting evolution of infectious disease in febrile, critically ill patients. PLoS One 2013 Jun;8:e65564.

3. Hoeboer SH, Groeneveld ABJ, Engels N, van Genderen M, Wijnhoven BPL, van Bommel J. Rising C-reactive protein and procalcitonin levels precede early complications after oesophagectomy. J Gastrointest Surg in press.

4. Hoeboer SH, van der Geest PJ, Nieboer D, Groeneveld ABJ. The diagnostic accuracy of procalcitonin for bacteraemia: a systematic review and meta-analysis. Clin Mircrobiol Infect in press.

5. Hoeboer SH, Oudemans-van Straaten HM, Groeneveld ABJ. Albumin rather than C-reactive protein may be valuable in predicting and monitoring the severity and course of acute respiratory distress syndrome in critically ill patients with or at risk for the syndrome after new onset fever. BMC Pulm Med in press.

6. Hoeboer SH, Groeneveld ABJ, Oudemans-van Straaten HM. Serial inflammatory biomarkers of the severity, course and outcome of late onset acute respiratory distress syndrome in critically ill patients with or at risk for the syndrome after new onset fever. Biomark Med in press.

## ABSTRACTS

1. Old and new biomarkers for predicting high and low risk microbial infection in critically ill patients with new onset fever: a case for procalcitonin. Hoeboer SH, Alberts E, van den Hul I, Tacx AN, Debets-Ossenkopp YJ, Groeneveld ABJ. European Society of Intensive Care Medicine Annual Congress 2011.

2. Changes in circulating procalcitonin versus C-reactive protein in predicting evolution of infectious disease in febrile, critically ill patients. Hoeboer SH, Groeneveld ABJ. International Symposium on Intensive Care and Emergency Medicine, Brussels 2013.

3. The diagnostic accuracy of procalcitonin for bacteraemia: a systematic review and meta-analysis. Hoeboer SH, van der Geest PJ, Nieboer D, Groeneveld ABJ. Internistendagen, Nederlandse Internisten Vereniging, 2015.

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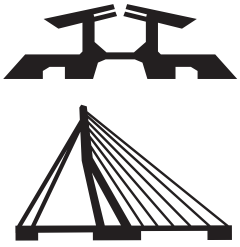
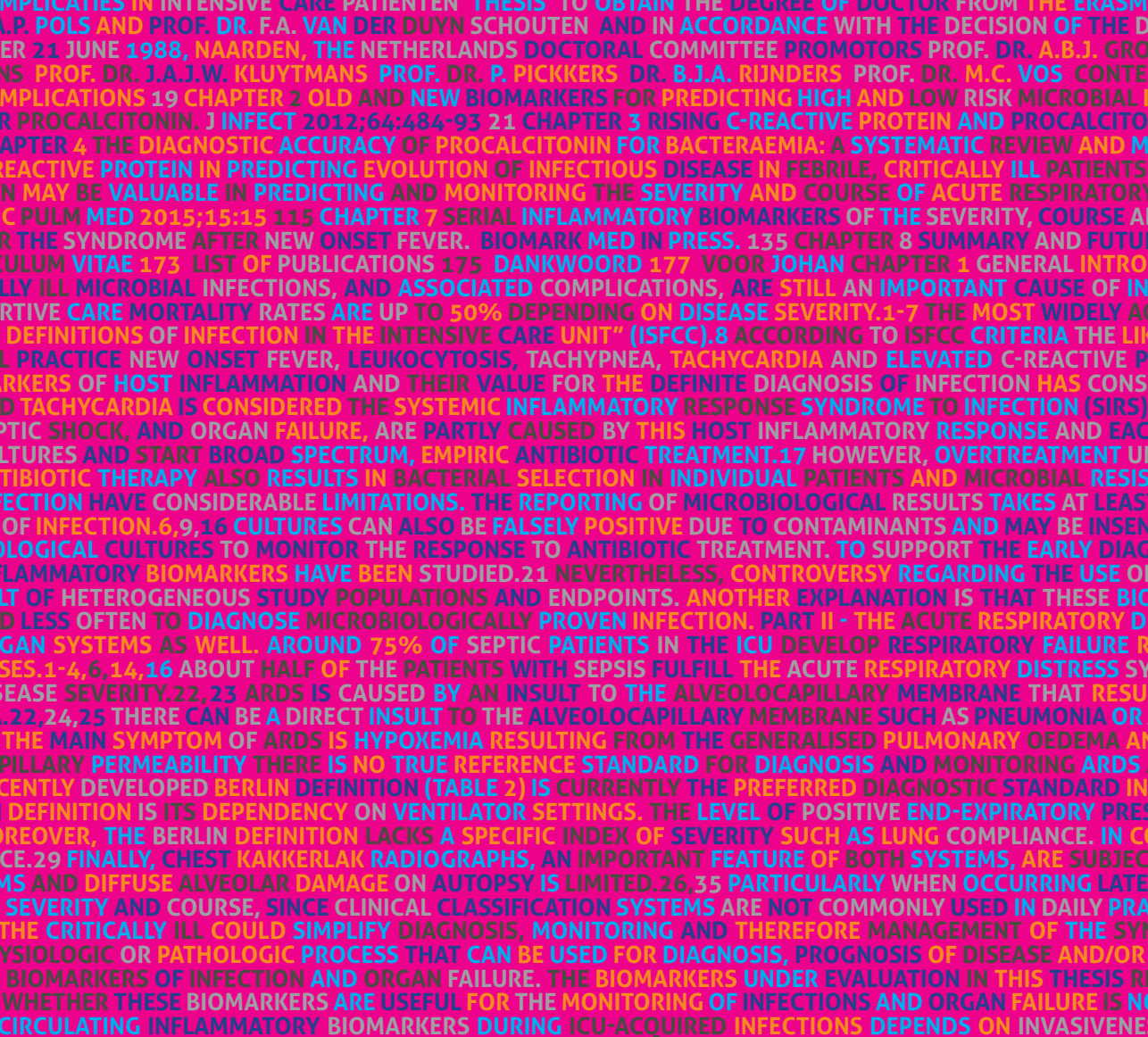
Lieve Sandra, omdat je trots mag zijn en het jezelf moet gunnen.





*Persévérer, secret de tous les triomphes.*

**-Victor Hugo-**