# GENERAL INTRODUCTION

# CHAPTER 1

## INTRODUCTION AND OUTLINE OF THESIS

Yuri van der Does MD

**A revolution in medicine**

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

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

**Resistance**

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

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

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

**Antimicrobial stewardship**

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

**The emergency department**

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

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

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

**Biomarkers**

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

**Procalcitonin**

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

**Biomarkers for viral disease**

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

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

**Sepsis and severity of disease**

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

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

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

**Aims of this thesis**

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

**Outline of thesis**

*Part I PCT-guided therapy*

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

*Part II Additional biomarker strategies*

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

*Part III General discussion*

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

## REFERENCES


1. Brown K. Penicillin Man: Alexander Fleming And The Antibiotic Revolution. Stroud: Sutton Publishing Ltd; 2004.
2. Fleming A. On the Antibacterial Action of Cultures of a Penicillium, with Special Reference to their Use in the Isolation of B. influenzæ. British journal of experimental pathology 1929;10:226-36.
3. Gaynes R. The Discovery of Penicillin—New Insights After More Than 75 Years of Clinical Use. Emerging Infectious Diseases 2017;23:849-53.
4. Chain E, Florey HW, Adelaide MB, et al. Penicillin as a chemotherapeutic agent. 1940. Clinical orthopaedics and related research 1993:3-7.
5. Abraham EP, Chain E, Fletcher CM, et al. Further observations on penicillin. 1941. European journal of clinical pharmacology 1992;42:3-9.
6. Armstrong GL, Conn LA, Pinner RW. Trends in infectious disease mortality in the United States during the 20th century. JAMA : the journal of the American Medical Association 1999;281:61-6.
7. Davies J, Davies D. Origins and Evolution of Antibiotic Resistance. Microbiology and Molecular Biology Reviews : MMBR 2010;74:417-33.
8. Laxminarayan R, Duse A, Wattal C, et al. Antibiotic resistance-the need for global solutions. The Lancet Infectious diseases 2013;13:1057-98.
9. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nature medicine 2004;10:S122-9.
10. Fleming A, Nobel Lecture. december 11, 1945. (Accessed december 5, 2017, at https://www.nobelprize.org/nobel_prizes/medicine/laureates/1945/fleming-lecture.pdf.)
11. Darwin C. On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life. London: John Murray, Albemarle street; 1859.
12. Alekshun MN, Levy SB. Molecular mechanisms of antibacterial multidrug resistance. Cell 2007;128:1037-50.
13. Bronzwaer SL, Cars O, Buchholz U, et al. A European study on the relationship between antimicrobial use and antimicrobial resistance. Emerging Infectious Diseases 2002;8:278-82.
14. Goossens H, Ferech M, Vander Stichele R, Elseviers M. Outpatient antibiotic use in Europe and association with resistance: a cross-national database study. Lancet 2005;365:579-87.
15. Lopez Sastre JB, Perez Solis D, Roques Serradilla V, et al. Procalcitonin is not sufficiently reliable to be the sole marker of neonatal sepsis of nosocomial origin. BMC Pediatr 2006;6.
16. EMEA. The bacterial challenge: time to react. A call to narrow the gap between multidrug-resistant bacteria in the EU and the development of new bacterial agents. 2009.
17. Fowler T, Walker D, Davies SC. The risk/benefit of predicting a post-antibiotic era: is the alarm working? Ann N Y Acad Sci 2014;1323:1-10.
18. Shlaes DM, Gerding DN, John JF, Jr., et al. Society for Healthcare Epidemiology of America and Infectious Diseases Society of America Joint Committee on the Prevention of Antimicrobial Resistance: guidelines for the prevention of antimicrobial resistance in hospitals. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 1997;25:584-99.
19. Dellit TH, Owens RC, McGowan JE, Jr., et al. Infectious Diseases Society of America and the Society for Healthcare Epidemiology of America guidelines for developing an institutional program to enhance antimicrobial stewardship. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2007;44:159-77.
20. Cosgrove SE, Carmeli Y. The impact of antimicrobial resistance on health and economic outcomes. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2003;36:1433-7.
21. Spellberg B, Bartlett J, Wunderink R, Gilbert DN. Novel approaches are needed to develop tomorrow's antibacterial therapies. American journal of respiratory and critical care medicine 2015;191:135-40.
22. May L, Cosgrove S, L'Archeveque M, et al. A call to action for antimicrobial stewardship in the emergency department: approaches and strategies. Ann Emerg Med 2013;62:69-77.e2.
23. Davey P, Marwick CA, Scott CL, et al. Interventions to improve antibiotic prescribing practices for hospital inpatients. The Cochrane database of systematic reviews 2017;2:Cd003543.

24. Seymour CW, Liu VX, Iwashyna TJ, et al. Assessment of Clinical Criteria for Sepsis: For the Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA : the journal of the American Medical Association 2016;315:762-74.
25. Limper M, Eeftinck Schattenkerk D, de Kruif MD, et al. One-year epidemiology of fever at the Emergency Department. The Netherlands journal of medicine 2011;69:124-8.
26. Ferrer R, Martin-Loeches I, Phillips G, et al. Empiric antibiotic treatment reduces mortality in severe sepsis and septic shock from the first hour: results from a guideline-based performance improvement program. Critical care medicine 2014;42:1749-55.
27. Rhodes A, Evans LE, Alhazzani W, et al. Surviving Sepsis Campaign: International Guidelines for Management of Sepsis and Septic Shock: 2016. Intensive care medicine 2017;43:304-77.
28. Biomarkers Definitions Working G. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther 2001;69:89-95.
29. Pierrakos C, Vincent JL. Sepsis biomarkers: a review. Critical care 2010;14:R15.
30. Volanakis JE. Human C-reactive protein: expression, structure, and function. Mol Immunol 2001;38:189-97.
31. Black S, Kushner I, Samols D. C-reactive Protein. J Biol Chem 2004;279:48487-90.
32. Matwiyoff GN, Prahl JD, Miller RJ, et al. Immune regulation of procalcitonin: a biomarker and mediator of infection. Inflamm Res 2012;61:401-9.
33. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 2004;39:206-17.
34. Wiley SR, Schooley K, Smolak PJ, et al. Identification and characterization of a new member of the TNF family that induces apoptosis. Immunity 1995;3:673-82.
35. Falschlehner C, Schaefer U, Walczak H. Following TRAIL's path in the immune system. Immunology 2009;127:145-54.
36. Liu M, Guo S, Hibbert JM, et al. CXCL10/IP-10 in infectious diseases pathogenesis and potential therapeutic implications. Cytokine Growth Factor Rev 2011;22:121-30.
37. Oved K, Cohen A, Boico O, et al. A novel host-proteome signature for distinguishing between acute bacterial and viral infections. PloS one 2015;10:e0120012.
38. van Houten CB, de Groot JAH, Klein A, et al. A host-protein based assay to differentiate between bacterial and viral infections in preschool children (OPPORTUNITY): a double-blind, multicentre, validation study. The Lancet Infectious diseases 2017;17:431-40.
39. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). JAMA : the journal of the American Medical Association 2016;315:801-10.
40. Wiersinga WJ, Leopold SJ, Cranendonk DR, van der Poll T. Host innate immune responses to sepsis. Virulence 2014;5:36-44.
41. Angus DC, van der Poll T. Severe sepsis and septic shock. The New England journal of medicine 2013;369:840-51.
42. Brain SD, Grant AD. Vascular actions of calcitonin gene-related peptide and adrenomedullin. Physiol Rev 2004;84:903-34.
43. Eto T, Kitamura K, Kato J. Biological and clinical roles of adrenomedullin in circulation control and cardiovascular diseases. Clin Exp Pharmacol Physiol 1999;26:371-80.
44. Schuetz P, Wolbers M, Christ-Crain M, et al. Prohormones for prediction of adverse medical outcome in community-acquired pneumonia and lower respiratory tract infections. Critical care (London, England) 2010;14:R106.
45. Levin ER. Endothelins. The New England journal of medicine 1995;333:356-63.
46. Schuetz P, Christ-Crain M, Morgenthaler NG, Struck J, Bergmann A, Muller B. Circulating precursor levels of endothelin-1 and adrenomedullin, two endothelium-derived, counteracting substances, in sepsis. Endothelium : journal of endothelial cell research 2007;14:345-51.
47. Tschaikowsky K, Sagner S, Lehnert N, Kaul M, Ritter J. Endothelin in septic patients: effects on cardiovascular and renal function and its relationship to proinflammatory cytokines. Critical care medicine 2000;28:1854-60.
48. Thuno M, Macho B, Eugen-Olsen J. suPAR: the molecular crystal ball. Dis Markers 2009;27:157-72.
49. Rasmussen LJ, Ladelund S, Haupt TH, et al. Soluble urokinase plasminogen activator receptor (suPAR) in acute care: a strong marker of disease presence and severity, readmission and mortality. A retrospective cohort study. Emergency medicine journal : EMJ 2016;33:769-75.

50. Koch A, Voigt S, Kruschinski C, et al. Circulating soluble urokinase plasminogen activator receptor is stably elevated during the first week of treatment in the intensive care unit and predicts mortality in critically ill patients. Critical care (London, England) 2011;15:R63.
51. Evidence-based medicine. A new approach to teaching the practice of medicine. JAMA : the journal of the American Medical Association 1992;268:2420-5.
52. Klein JG. Five pitfalls in decisions about diagnosis and prescribing. BMJ (Clinical research ed) 2005;330:781-3.