PART III
CONCLUSIONS

CHAPTER 9

GENERAL DISCUSSION

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Aims of this thesis

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

Main results

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

PCT-guided therapy

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

Initiation of antibiotics

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

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

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

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

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

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

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

Additional biomarker strategies

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

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

Severity of disease

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

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

Next steps

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

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

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

Future diagnostic modalities

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

Future treatments

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

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

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

Putting it in perspective

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

Closing comments

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

REFERENCES

1. Assicot M, Gendrel D, Carsin H, Raymond J, Guilbaud J, Bohuon C. High serum procalcitonin concentrations in patients with sepsis and infection. Lancet 1993;341:515-8.
2. 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.
3. Soni NJ, Samson DJ, Galaydick JL, et al. Procalcitonin-guided antibiotic therapy: a systematic review and meta-analysis. Journal of hospital medicine 2013;8:530-40.
4. Croskerry P. Achieving quality in clinical decision making: cognitive strategies and detection of bias. Acad Emerg Med 2002;9:1184-204.
5. van der Does Y, Rood PP, Haagsma JA, Patka P, van Gorp EC, Limper M. Procalcitonin-guided therapy for the initiation of antibiotics in the ED: a systematic review. The American journal of emergency medicine 2016;34:1286-93.
6. 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.
7. Schuetz P, Christ-Crain M, Thomann R, et al. Effect of procalcitonin-based guidelines vs standard guidelines on antibiotic use in lower respiratory tract infections: The ProHOSP randomized controlled trial. JAMA - Journal of the American Medical Association 2009;302:1059-66.
8. Limper M, Gerstenbluth I, Duits AJ, van Gorp EC. Epidemiology of febrile diseases in the emergency department of a Caribbean island: the Curacao experience. The West Indian medical journal 2012;61:76-80.
9. Limper M, van der Does Y, Brandjes DP, De Kruif MD, Rood PP, van Gorp EC. Procalcitonin guided antibiotic therapy in patients presenting with fever in the emergency department. The Journal of infection 2014;69:410-2.
10. van der Does Y, Limper M, Schuit SC, et al. Higher diagnostic accuracy and cost-effectiveness using procalcitonin in the treatment of emergency medicine patients with fever (The HiTEMP study): a multicenter randomized study. BMC emergency medicine 2016;16:17.
11. Yealy DM, Fine MJ. Measurement of serum procalcitonin: a step closer to tailored care for respiratory infections? JAMA : the journal of the American Medical Association 2009;302:1115-6.
12. van der Does Y, Limper M, Jie KE, et al. Procalcitonin-guided antibiotic therapy in patients with fever in a general emergency department population: a multicenter noninferiority randomized clinical trial (HiTEMP study). (submitted for publication) 2018.
13. Tversky A, Kahneman D. Judgment under Uncertainty: Heuristics and Biases. Science (New York, NY) 1974;185:1124-31.
14. 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.
15. 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.
16. 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.
17. Vouloumanou EK, Karageorgopoulos DE, Kazantzi MS, Kapaskelis AM, Falagas ME. Antibiotics versus placebo or watchful waiting for acute otitis media: a meta-analysis of randomized controlled trials. The Journal of antimicrobial chemotherapy 2009;64:16-24.
18. 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.
19. Srugo I, Klein A, Stein M, et al. Validation of a Novel Assay to Distinguish Bacterial and Viral Infections. Pediatrics 2017;140.
20. van der Does Y, Rood PPM, Ramakers C, et al. Identifying patients with bacterial infections using a combination of biomarkers in the emergency department. (unpublished manuscript).

21. Fontana JM, Alexander E, Salvatore M. Translational research in infectious disease: current paradigms and challenges ahead. Translational research : the journal of laboratory and clinical medicine 2012;159:430-53.
22. Jean Beltran PM, Federspiel JD, Sheng X, Cristea IM. Proteomics and integrative omic approaches for understanding host-pathogen interactions and infectious diseases. Molecular systems biology 2017;13:922.
23. Nylen ES, Whang KT, Snider RH, Jr., Steinwald PM, White JC, Becker KL. Mortality is increased by procalcitonin and decreased by an antiserum reactive to procalcitonin in experimental sepsis. Critical care medicine 1998;26:1001-6.
24. Steinwede K, Henken S, Bohling J, et al. TNF-related apoptosis-inducing ligand (TRAIL) exerts therapeutic efficacy for the treatment of pneumococcal pneumonia in mice. The Journal of experimental medicine 2012;209:1937-52.
25. Wittebole X, De Roock S, Opal SM. A historical overview of bacteriophage therapy as an alternative to antibiotics for the treatment of bacterial pathogens. Virulence 2014;5:226-35.