Summary and future perspectives

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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.\textsuperscript{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 patient’s 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\textsubscript{a}O\textsubscript{2}/F\textsubscript{I}O\textsubscript{2} ratio) or by other pulmonary pathology (chest radiographs, P\textsubscript{a}O\textsubscript{2}/F\textsubscript{I}O\textsubscript{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.\textsuperscript{8} This is reflected by the limited association between clinical ARDS and diffuse alveolar damage at autopsy.\textsuperscript{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. 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. 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 inability to prove causality when a pathogen is detected. 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.\textsuperscript{20-23} However, on its own direct measurement of microbial DNA may be too sensitive and provides no information on clinical relevant bacterial load.\textsuperscript{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.\textsuperscript{24,25} The comparison of gene expression profiles of for instance peripheral blood leukocytes could be used to differentiate between inflammation and infection.\textsuperscript{24-26} In vivo studies have shown that microarray profiles were capable of discriminating between healthy controls, patients with bacterial infection, viral infection and a co-infection, amongst others.\textsuperscript{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.\textsuperscript{24,25,27} Microarray 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.\textsuperscript{24-31} There are some limitations to the use of microarrays as well. Microarrays 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.\textsuperscript{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.\textsuperscript{5,6} The next step may be the complete sequencing of human and microbial DNA which may prove to be a more reliable method.\textsuperscript{32-37} But this method will not resolve the limitations mentioned for microarray techniques.\textsuperscript{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


