Summary and future perspectives

The result of the microbiological culture remains the gold standard to distinguish between SIRS and sepsis. However, the incubation time of bacteria requires a few days, whereas antimicrobial therapy should be started as soon as possible in septic patients. Unfortunately, blood cultures have only moderate sensitivity.[1] Nowadays it is known from newer modern, automated, continuous-monitoring blood culture systems (CMBCSs) two blood cultures detect only 80% of bloodstream infections and three blood cultures detect 96% of infection episodes.[2] Apparently, the gold standard of infection isn't that "gold" at all. Thus, there is need for reliable and early markers for infection in critically ill patients. This is the basic research question of this thesis: to analyze and evaluate markers and indices in the septic critically ill patient.

Part I of this thesis consists of the search for clinical indices for infection. Especially in the critically ill it is of utmost importance to detect the pathogen as soon as possible, knowing that delay with appropriate therapy, increases mortality by the hour.[3] In **chapter 2** we closely study pneumonia, a potential life-threatening complication after drowning. Bacteria causing pneumonia in drowning victims are often waterborne or from oropharyngeal origin and the bacteria found vary dependent on area and type of water. A submersion event in contaminated water likely increases the risk of pneumonia. The temperature of the water is also a risk factor, the warmer the water, the more increase of division rate of micro-organisms is present. Gram-negative bacteria are most commonly described, but gram-positive bacteria and anaerobes are also found.[4,5] Furthermore, fungi have also been described in the literature as a cause of pneumonia in drowning victims.[4,6-9] Being able to predict which patients will develop a pneumonia might have huge clinical benefits. We investigated risk factors for non-survival after a drowning accident and tried to obtain insights in the microbiology of drowning-associated pneumonia, by comparing causative micro-organisms to those cultured from locally retrieved water samples. Our cohort of adult drowning victims is characterized by a high mortality rate reflecting the severity of a drowning incident. Most prominent early risk factors for non-survival in a drowning accident were age, a low base-line pH, a high lactate on arrival in the emergency room (ER) and the need for mechanical ventilation. A lower body temperature did not seem to have a protective role with regard to survival. *Aeromonas spp*. and *Staphylococcus aureus* were the micro-organisms predominantly cultured in patients developing drowning-associated pneumonia. The predominantly used initial antibiotic regimen was adequately covering cultured micro-organisms. From all three water samples *Aeromonas spp*. was cultured. Three different species of *Aspergillus* were cultured from the water sites, without any resistances for azoles. However, distinguishing between colonization and infection remains difficult and the question if prophylactic antibiotics are superior to targeted therapy is still not

answered. Ideally, a randomised controlled trial will be performed with prophylactic antibiotics versus placebo in drowning victims with pneumonia as primary outcome. However, such a trial will not be feasible considering the low number of drowning victims. Certain biomarkers, like procalcitonin may help to differentiate between colonisation and true infection in these patients. However, when pneumonia is suspected and empirical antimicrobials are started these should, at least, be directed against micro-organisms that are commonly found in surface water. The same bacteria cultured form patients, *Aeromonas spp*. and *Staphylococcus aureus* were also the prominent micro-organisms in the water.

In **chapter 3** we predicted that the use of an Electronic Surveillance System (ESS) with decision making capabilities in the Intensive Care Unit (ICU), a so-called trigger screening, would allow more time to implement evidence-based processes aimed at reducing infections.[10] Catheter related sepsis (CRS) and ventilator associated pneumonia (VAP) were chosen as outcome parameters. The trigger screening method showed a high sensitivity and a high negative predictive value both for VAP (92.3% resp. 99.8%) and CRS (91.3% resp. 99.6%). It should be noticed that CDC-definitions were adjusted to allow automatic assessment. To meet to the VAP-criteria for both definitions two or more positive serial chest imaging results had to be present. In addition the CDC-criteria required also fever, reduced/increased leukocytes **and** at least one of the following: new onset of purulent sputum (or change in character of sputum or increased respiratory secretions, or increased suctioning requirements) **or** new onset or worsening cough **or** dyspnea or rales or bronchial breath sounds or worsening gas exchange. For our assessment more liberal definition were used, fever **or** reduced/increased leukocytosis **or** purulent sputum. This could have resulted in an overestimation of the amount of VAPs. However, despite this possible overestimation a very low incidence (13 out of 422) of VAP was seen. We have to acknowledge that the lower the incidence, the less frequent the trigger will be activated and thus the ESS will easily be highly specific. So with a high incidence of the outcome parameter it is possible the trigger surveillance system will be less specific.

To conclude, the trigger based ESS in this study was effective and theoretically time-saving. For CRS and VAP screening, manual labor time for screening and determination of infection was reduced by 84% from an original 4, 4 hours per week to 42 minutes per week. However, this trigger based ESS is developed for retrospective surveillance and not for real-time surveillance, because of the interpretation of x-rays and blood cultures are needed. Studies which evaluated natural language processing to search for the word ‘pneumonia’ in radiographic reports have been performed and with faster technologies like polymerase chain reaction (PCR) and matrix assisted laser desorption/ionization (MaldiTof) a complete trigger based system, which will alert the attending physician the same day will be the near future.[11]

Part II of this thesis describes observational cohorts of critically ill patients suspected of viral pathogens in the respiratory tract and tries to assess the pulmonary pathogenicity of these viruses. In **Chapter 4** three objectives were evaluated. At first, we studied how many patients admitted to ICU with symptoms of an acute respiratory tract infection have evidence for a respiratory virus infection. During the study period 82 samples were analyzed and 29 patients were found to be positive for viral detection from BAL fluid specimens or throat swab of a virus, which is equivalent to 35%, which was relatively high in comparison to previous literature.[12-14] The major limitation of this retrospective study is that all samples were obtained when patients were suspected of a viral respiratory tract infection at any moment during admission, which is illustrated by the fact that during a rather long period (18 months including 2 winter periods) only 99 patients were sampled. If this study was performed prospectively, a swab of every patient suspected of a viral respiratory tract infection should obtain and repeated periodically. This would have been a more optimal systemic approach with more realistic incidence number for community- and acquired respiratory tract infections. The second objective was to analyze clinical characteristics between the virus infected as defined by a positive viral PCR and non-virus-infected patients and nosocomial versus community acquired acquisitions. Neither pulmonary pathogenicity nor mortality attributable to viral detection was seen in this study, regarding LIS, CPIS and SOFA-scores. In contrast to bacterial pneumonia, there is no existing cut-off and the incubation time for each virus may be different. Therefore, all medical records were reviewed for the onset of symptoms and incubation-time for each virus, if the PCR-test became positive after 3 days of admission.

The third objective was to evaluate how many of these patients viruses were acquired nosocomial. This was seen in 17% (5 of 29 patients) of the virus infected patients, with a mortality rate of 80%. Since all of the patients with a hospital-acquired viral pneumonia were immunocompromised, it remains unclear if non-survivors suffered from viral carriage or from invasive viral infection. Our hypothesis is that viral detection serves as a marker for severity of disease rather than playing a damaging role in the respiratory tract. [15,16]

In **chapter 5** we investigated the hypothesis that a high HSV-1 load in the lower respiratory tract in critically ill patients relates to the severity of the underlying disease and/ or clinical pulmonary infection. Therefore, tracheal aspirates and Broncho alveolar fluids were analyzed by quantitative real-time PCR assay for HSV-1. Clinical and infectious pulmonary courses were studied according to HSV-1 loads and risk factors were examined. The results indicate that HSV-1 status was not associated with any differences in pulmonary or systemic variables: the load did not relate to LIS, CPIS, SIRS or SOFA-scores. Only, a direct relation between SAPS II score (first 24h on admission) and HSV-1 load in the first specimen was present ($r_s = 0.41$), suggesting that the presence of HSV-1 in the lower respiratory tract in critically ill, does not

correlate with indicators of pulmonary injury but only with severity of underlying disease at admission. The latter may have led to immune suppression, viral reactivation and shedding. No evidence was found for pulmonary pathogenicity or mortality attributable to HSV-1. Thus, the HSV-load correlates with the severity of the disease, but not with scores that indicate any form of lung damage. It seems, therefore, the more morbidity and severity of illness the higher the HSV load without increasing pathogenicity to the lung. No attributable mortality was seen in patients with HSV in the lower respiratory tract, which was in accordance with previous studies.[17,18]

The question arises whether it could be beneficial for these patients to start treatment with an antiviral agent? Although there is no level 1 evidence for treatment of an active herpes simplex, a retrospective study by Traen et al indicates that acyclovir therapy might reduce ICU and hospital mortality in ICU patients with HSV in BALF.[19] Our results do not provide this information. However, withholding antiviral treatment in critically ill patients seems injudicious.

So in theory this means HSV is not a good marker for infection in general but might be for severity of illness or the degree of immunoparalysis? However, there are much more bedside clinical- and laboratory parameters to give an indication of severity of illness than to determine a cost- and time consuming HSV-load in broncheo-alveolar lavage or trachea-aspirate. So the search for a good marker for infection continues….

The ideal biomarker is Specific (as well as Sensitive), Measurable with a high degree of precision, Available (and Affordable), Responsive and Reproducible, with results available in a Timely fashion to guide therapy (SMART).[20] In addition, it could be of great value when a biomarker can be used to optimize antibiotic treatment and assist in monitoring of the patient during the selected therapy. Furthermore a sepsis biomarker can discriminate between infection and inflammation and is an indicator for disease severity.

With this in mind we explored two different biomarkers: CD64 and PCT. The expression of CD64 on neutrophils was investigated in **chapter 6** as a potential indicator for bacterial infection in adult critically ill patients as a subgroup analysis of the SAPS-study. Our analysis showed a slight difference in CD64 expression between septic patients with a positive culture and patients with a negative culture in the first two days of their antibiotic treatment. No difference in expression was seen between survivors and non-survivors, however, CD64 expression correlated with disease severity expressed at day one. Secondly, we studied longitudinal expression patterns of CD64 on neutrophils with regard to outcome and sepsis severity. Here, we have shown that uncorrected analysis showed no significant difference in the longitudinal course of CD64 index during the first 14 days of follow-up

between survivors versus non-survivors, however mean CD64 index showed a stronger decrease over time in the survivor group. A difference was seen in the longitudinal course of mean CD64 index for patients with sepsis, severe sepsis and septic shock during the 14 day period, however, when corrected for intervention group, age, gender and apache-score, the only significant difference in mean CD64 index was found to be at day 1.

So there appears to be limited value of longitudinal CD64 measurements, nor in guidance for therapy or the possibility to discriminate between patients with or without septic shock. Furthermore, the CD64 assay is a time-consuming flowcytometry, which limits its application the septic critically ill patient.

PCT has superior kinetics in comparison to eg. CRP and we evaluated in **chapter 7**, whether PCT-measurements are more useful than CRP in predicting the presence of an infection in patients with SIRS admitted to the ICU.[21] Our data indicate that CRP is not a good biomarker to distinguish SIRS from sepsis in a cohort of general ICU patients with a moderate *a priori* chance of infection.[3] Surprisingly, PCT did not perform better than CRP. The high predictive values of PCT as shown in many previous studies could not be confirmed in our heterogeneous ICU population with a low prevalence of "proven infection".[22,23] In our cohort and various other trials PCT failed to discriminate between SIRS and sepsis and therefore its diagnostic capacity as a single-measurement biomarker is inadequate.[24-26] We, therefore, conclude that a single PCT value should not guide the physician in whether to start or withhold antibiotics in a critically ill patient. Starting or withholding antibiotics in the critically ill patient should never rely on one biomarker, especially not with something as crucial and lifesaving as with antibiotics. Such a decision can only be made after integration of all available parameters like physical examination, all other laboratory values and clinical parameters.
Previous randomized controlled trials with patients with severe sepsis and septic shock (n= 1075) have shown that PCT kinetics can adequately guide the duration and individualize antibiotic therapy.[27-31] All studies so far were performed in a setting of high consumption of antibiotics, unlike the Netherlands. Therefore, a large a large prospective, open-label, multi-center, randomized Dutch PCT-guidance trial was designed. The SAPS trial targets a heterogeneous real-life population, is the largest trial so far, involves over 15 centers, allows SDD/SOD and anticipates an already low use of antibiotics in the control arm Chapter 8 of this thesis exposed the design of the study. Chapter 9 depicts the Stop Antibiocs on guidance op Procalcitonin Study (SAPS) based on the following hypothesis: could PCT guidance in comparison to standard-of-care reduce the duration of antibiotic treatment and thus the amount of antibiotics administered, without increasing mortality or recurrent infections. The SAPS-study revealed a shorter median duration of prescribed antibiotics in the first 28 days for the PCT guided group (5 days; IQR 3-8 days) versus the standard-of-care group (7 days; IQR 4-10 days) $P<0.001$.

At 28 days after randomization 149/761 (19.6%) patients had died in the PCT-guided group versus 196/785 (25.0%) in the standard-of-care group (P=0.006). One year after randomization this remarkable mortality difference remained with 265/761 (34.8%) deaths in the PCT-guided group versus 321/785 (40.9%) in the standard-of-care group (log-rank test P=0.006). We anticipated a mortality rate which was similar in both groups, so this was a very unexpected result which we could not easily explain. We hypothesized that the reduced mortality in the PCT-group could, at least partly, be the result of an incentive for the treating physician to search for another focus or an alternative diagnosis if PCT-levels remained low. Similarly, persistently high levels of PCT may be seen by the treating physician as a need to critically review antimicrobial treatment.

Several limitations of the SAPS study should be stated. Of all patients in the PCT-guided strategy 30% were discharged from the ICU before reaching the stopping advice. Further reduction of antibiotics would have been achieved if PCT-guidance would have been continued on the wards, we considered not feasible for the SAPS study. Furthermore, the standard-of care group did not receive at some point a warning to stop or continue antibiotics. Therefore, we don't know the effect of a daily warning call alone.

Overall daily PCT-measurements facilitated a safe reduction in antibiotic duration, but we have to be careful. In some severely ill patients PCT remained low, although there was a clear bacterial cause of sepsis, e.g. secondary meningitis with gram-positive bacteria. During the trial only a stopping advice was generated when the stopping rules were reached, however it was up to the intensivist whether or not to actually discontinue antibiotics. Thus, in these patients antibiotics were continued, but it indicates we should never trust one biomarker by itself and further analyses are needed in these subgroups. Another point of interest will be to analyze the patients that died early, and caused the difference in mortality rate between PCT- and the standard-of care group in the first two weeks. What are the causes of death and what were the PCT-values. Were there any additional examinations performed for patients with an extremely high or low PCT-value?

The SAPS-trial clearly showed that PCT measurements can safely reduce AB consumption and we believe that the potential in reducing and optimizing antibiotic use in the ICU is large. Apparently, physicians were often afraid to stop antibiotics in the critically ill patient as reflected by the low protocol adherence. Many patients in the standard of care group e.g. CAP or intra-abdominal infections, were treated even longer than the national guidelines recommended. PCT might be supportive in stopping antibiotics in these severely ill patients, which is a topic for further (subgroup) analysis. In most patients 5 days of antibiotic treatment may be sufficient, even in these complicated infections. Given the global health threats posed by emerging antibiotic resistance, this is a fact which should not be ignored.[32]

Future perspectives

Nowadays more patients are dying from sepsis related complications than breast- and colorectal cancer together, making sepsis a major health economic issue. Early recognition of sepsis and thereby instantaneous initiation of resuscitation and administration of appropriate antibiotics are pivotal for a better chance of survival. Therefore, prompt recognition of sepsis by indices and markers of infection and thereby fast prescribing of adequately antibiotics is pivotal and represents a major challenge of current research. Clinical signs will remain the most important in identifying the septic patient, however, they the infection itself still needs to be proved.[33] Conventional microbiological standard procedures provide valuable information on bacterial or fungal species of sepsis-relevant microbes, however, these techniques perform poorly in the first relevant two days of the septic patient. Newer techniques to define bacterial DNA in the bloodstream of patients like polymerase chain reaction (PCR) and matrix assisted laser desorption/ionization (MaldiTof) are widely available and can potentially reduce identification around 8 hours. To identify sepsis in an early stage specific laboratory tests and biomarkers are used. To date, controversy exists about the most optimal biomarker concerning sepsis. Naturally, each biomarker has its advantages and limitations. In this thesis we showed the physician cannot depend on one biomarker to start or withhold antibiotics in the critical ill patient, but based on our study, it is reasonable to assume that there is a role for procalcitonin in guidance of antibiotic therapy in the septic critically ill patient using a simple algorithm with stopping rules.

In future research we should focus on subgroups which were excluded now e.g. immunocompromised patients or patients treated for illnesses requiring prolonged antibiotic treatment. There are basically no reasons why measuring PCT would not be useful in reducing duration of antibiotic in these infections as well, although probably with a longer follow-up and a stricter threshold. First, more observational research with PCT-measurements in these kinds of patients is necessary, in order to define which threshold is safe for a stopping advice of antibiotics. Furthermore, future research has to be focused not only on de-escalation of antibiotics, but also on individualizing antibiotic treatment. In most patients duration of antibiotics probably can be shortened, however, some might need a longer duration of antibiotic therapy. Ideally, research should be focused on custom made treatment for every patient based on diagnostic algorithms and antibiotic stewardship (ABS) programs. Such strategies should be based on fast identification of the septic patient, aggressive microbiologic testing, optimal selection of antimicrobial agents and individualized dosing based on pharmacokinetics and pharmacodynamics, antibiotic de-escalation and optimization of duration of antibiotic therapy together with provider education and feedback. [34] Optimizing antibiotic treatment should be combined with various tools such as computerized decision support systems in combination with biomarkers and rapid testing like MaldiTof and infectious disease experts as part of

antibiotic stewardship.[35] This will likely lead to more rapid diagnosis of bacterial and viral infections and therefore swift treatment and isolation measurements.[33] Finally a link with a data management system is needed, locally and nationally, which will allow an effective epidemiological surveillance. From this we will have more knowledge with respect to antibiotic selection. There will be a better control of disease outbreaks and hopefully ultimately put an end of the increasing antibiotic resistance.[35]

References

1. Peters RP, van Agtmael MA, Danner SA, Savelkoul PH, Vandenbroucke-Grauls CM. New developments in the diagnosis of bloodstream infections. Lancet Infect Dis. 2004;4:751–60
2. Cockerill FR 3rd1, Wilson JW, Vetter EA, Goodman KM, Torgerson CA, Harmsen WS, Schleck CD, Ilstrup DM, Washington JA 2nd, Wilson WR. Optimal testing parameters for blood cultures. Clin Infect Dis. 2004 Jun 15;38(12):1724-30
3. Rivers E1, Nguyen B, Havstad S, Ressler J, Muzzin A, Knoblich B, Peterson E, Tomlanovich M; Early Goal-Directed Therapy Collaborative Group. Early goal-directed therapy in the treatment of severe sepsis and septic shock. N Engl J Med. 2001 Nov 8;345:1368-77
4. Ender PT, Dolan MJ. Pneumonia associated with near-drowning. Clin Infect Dis 1997; 25;4:896-907
5. Tadié JM, Heming N, Serve E, Weiss N, Day N, Imbert A, Ducharne G, Faisy C, Diehl JL, Safran D, Fagon JY, Guérot E. Drowning associated pneumonia: a descriptive cohort. Resuscitation 2012; 83;3:399-401
6. Modell JH. Drowning (1993) N Engl J Med. 28;328:253-6
7. Katragkou A, Dotis J, Kotsiou M, Tamiolaki M, Roilides E. Scedosporium apiospermum infection after near-drowning. Mycoses. 2007; 50;5:412-21
8. Leroy P, Smismans A, Seute T. Invasive pulmonary and central nervous system aspergillosis after near-drowning of a child: case report and review of the literature. Pediatrics.2006; 118;2:509-13
9. van Dam AP, Pruijm MT, Harinck BI, Gelinck LB, Kuijper EJ. Pneumonia involving Aspergillus and Rhizopus spp. after a near-drowning incident with subsequent Nocardia cyriacigeorgici and N. farcinica coinfection as a late complication. Eur J Clin Microbiol Infect Dis 2005; 24;1:61–4
10. Atreja A, Gordon SM, Pollock DA, Olmsted RN, Brennan PJ. Opportunities and challenges in utilizing electronic health records for infection surveillance, prevention, and control. Am J Infect Control 2008;36:S37-S46
11. Haas JP, Mendonca EA, Ross B, Friedman C, Larson E. Use of computerized surveillance to detect nosocomial pneumonia in neonatal intensive care unit patients. Am J Infect Control. 2005;33:439–443
12. Vincent J.-L. and Teixeira L. Sepsis biomarkers. Values and limitations. Am J Resp Crit Care Med 2014 Nov 15;190:1081-2.
13. Choi SH, Hong SB, Ko GB, et al. Viral infection in patients with severe pneumonia requiring intensive care unit admission. Am J Respir Crit Care Med. 2012 Aug 15;186:325-32.

14. Wiemken T, Peyrani P, Bryant K, Kelley RR, Summersgill J, et al. Incidence of respiratory viruses in patients with community-acquired pneumonia admitted to the intensive care unit: results from the Severe Influenza Pneumonia Surveillance (SIPS) project. Eur J Clin Microbiol Infect Dis. 2013 May;32(5):705-10.
15. Østby A-C, Gubbels S, Baake G, Nielsen LP, Riedel C, et al. Respiratory virology and microbiology in intensive care units: a prospective cohort study. APMIS. 2013 Nov;121:1097-108
16. Van den Brink J-W, Simoons-Smit AM, Beishuizen A, Girbes ARJ, Strack van Schijndel RJM, Groeneveld ABJ. Respiratory herpes simplex virus type 1 infection/colonisation in the critically ill: marker or mediator ? J Clin Virol 2004;30:68-72
17. Scheithauer S, Manemann AK, KrugerS, Hausler M, Kruttgen A, Lemmen SW et al. Impact of herpes simplex virus detection in respiratory specimens of patients with suspected viral pneumonia. Infection 2010;38:401-5
18. De Vos N, van Hoovels L, Vankeerbergen A, Van Vaerenbergh K, Boel A, Demeyer I et al. Monitoring of herpes simplex virus in the lower respiratory tract of critically ill patients using real-time PCR: a prospective study. Clin Microbiol Infect 2009;15:358-63
19. Traen S, Bochanen N, Ieven M, Schepens T, Bruynseels P, Verbrugghe W, et al. Is acyclovir effective among critically ill patients with herpes simplex in the respiratory tract? J Clin Virol. 2014;60:215-21.
20. Pro/Con debate: Is procalcitonin useful for guiding antibiotic decision making in critically ill patients? Shehabi Y, Seppelt I. Critical Care 2008;12:211
21. Meynaar IA, Droog W, Batstra M, Vreede R, Herbrink P. In Critically Ill Patients, Serum Procalcitonin Is More Useful in Differentiating between Sepsis and SIRS than CRP, Il-6, or LBP. Crit Care Res Pract 2011;2011:594645
22. Müller F, Christ-Crain M, Bregenzer T, Krause M, Zimmerli W, Mueller B, Schuetz P; ProHOSP Study Group. Procalcitonin levels predict bacteremia in patients with community-acquired pneumonia: a prospective cohort trial. Chest. 2010 Jul;138:121-9
23. Ruiz-Alvarez MJ, García-Valdecasas S, De Pablo R, Sanchez García M, Coca C, Groeneveld TW, Roos A, et al. Diagnostic efficacy and prognostic value of serum procalcitonin concentration in patients with suspected sepsis. J Intensive Care Med. 2009;24(1):63-71.
24. Layios N, Lambermont B, Canivet JL, Morimont P, Preiser JC, Garweg C, et al. Procalcitonin usefulness for the initiation of antibiotic treatment in intensive care unit patients. Crit Care Med. 2012;40:2304-9.
25. Masson S, Caironi P, Spanuth E, Thomae R, Panigada M, Sangiorgi G, Fumagalli R, Mauri T, Isgrò S, Fanizza C, Romero M, Tognoni G, Latini R, Gattinoni L; ALBIOS Study

Investigators. Presepsin (soluble CD14 subtype) and procalcitonin levels for mortality prediction in sepsis: data from the Albumin Italian Outcome Sepsis trial. Crit Care. 2014 Jan 7;18:R6.

26. Anna Prkno, Christina Wacker, Frank M Brunkhorst and Peter Schlattmann. Procalcitonin-guided therapy in intensive care unit patients with severe sepsis and septic shock – a systematic review and meta-analysis. Critical Care201317:R291
27. Brechot N, Hékimian G, Chastre J, Luyt C-E. Procalcitonin to guide antibiotic therapy in the ICU. International Journal of Antimicrobial Agents 2015;46:S19-S24
28. Nobre V, Harbarth S, Graf JD, Rohner P, Pugin J. Use of procalcitonin to shorten antibiotic treatment duration in septic patients: a randomized trial. Am J Respir Crit Care Med. 2008;177:498-505.
29. 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 2009;302:1059-1066
30. Hochreiter M, Köhler T, Schweiger AM, et al. Procalcitonin to guide duration of antibiotic therapy inintensive care patients: a randomized prospective controlled trial. Crit Care 2009;13:R83.
31. Bouadma L, Luyt CE, Tubach F, et al. Use of procalcitonin to reduce patients' exposure to antibiotics in intensive care units (PRORATA trial): a multicentre randomised controlled trial. Lancet. 2010;375:463-74.
32. Philipp Schuetz, Beat Müeller. Procalcitonin in critically ill patients: time to change guidelines and antibiotic use in practice. The Lancet Infectious Diseases, Vol. 16, No. 7, p758–760
33. László I, Domonkos T, Molnár Z, Fazakas J. Sepsis: From pathophysiology to individualized Patient Care. Journal of Immunology Research 2015;
34. Bassetti M, De Waele JJ, Eggimann P, Garnacho-Montero J, Kahlmeter G, Menichetti F, Nicolau DP et al. Preventive and therapeutic strategies in critically ill patients with highly resistant bacteria. Intensive Care Med 2015: 41;776-795
35. Zhang YZ, Singh S. Antibiotic stewardship programmes in intensive care units: why, how, and where are they leading us. World J Crit Care Med 2015:4;13-28