Chapter 11

Challenges in the Diagnosis of Sepsis of the Neonate

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1. Introduction

Despite the advances in neonatal care early onset neonatal sepsis remains a serious and potentially life-threatening disease with a mortality rate ranging from 1.5% in term to almost 40% in very low birth weight infants. [1,2] The signs and symptoms of neonatal sepsis may be subtle and nonspecific being clinically indistinguishable from various noninfectious conditions such as respiratory distress syndrome or maladaptation. The current practice of starting empirical antibiotic therapy in all neonates showing infection-like symptoms results in their exposure to adverse drug effects, nosocomial complications, and in the emergence of resistant strains. [3] Accurate and quick diagnosis is therefore essential for both protecting the infant from the consequences of the bacterial invasion and preventing damages deriving from the unnecessary use of antibiotics.

During the last decades efforts have been made to improve the laboratory diagnosis of neonatal sepsis by studying a large variety of inflammatory markers with diverse success. Some, like procalcitonin (PCT) and interleukins, especially interleukin-6 (IL-6), have demonstrated their benefit in clinical practice and are more and more implemented in neonatal wards and neonatal intensive care units. Others, like cell surface markers, show promising results in the research setting but in the clinical everyday practice their use is hindered by the need for sophisticated equipment, trained laboratory staff, and for rapid sample processing that cannot be delayed.

In this review we aim to give a comprehensive overview on laboratory parameters and microbiological techniques for the diagnosis of sepsis in the neonate.

2. Clinical signs of bacterial infection in the neonate

One of the most recent studies on clinical signs associated with neonatal sepsis was published by Modi et al. [4] in 2009 utilizing data from 26 UK neonatal units. The

population prevalence of 12 predefined clinical signs of infection captured daily for 28 days including the acute onset of increased oxygen requirement or ventilatory support, increase in apnoea/bradycardia, hypotension, glucose intolerance, impaired peripheral perfusion (capillary refill time >3 s/ pallor/ mottling/ core-peripheral temperature gap >2°C), lethargy/ irritability/ poor handling, temperature instability, ileus/ onset of feed intolerance, increase in serum bilirubin, fall in urine output, metabolic acidosis/base deficit <−10 mmol/l, and anticonvulsant therapy was evaluated. Three or more clinical signs had the best predictive accuracy for a positive blood culture with 76.2% specificity, and 61.5%, 46.9% and 78.2% sensitivity for all positive cultures, cultures yielding coagulase-negative staphylococci, or a recognised pathogen, respectively.

A more simplified but nevertheless widely used approach is to focus on the presence of three or more of the following categories of clinical signs [5]: apnoea/tachypnoea (>60/ min)/nasal flaring/retractions/cyanosis/respiratory distress; bradycardia (<100/ min) or tachycardia (>180/min); hypotonia or seizures; poor skin colour or capillary refilling time >2 seconds; irritability or lethargy. Together with historical factors associated with increased risk for infection including premature rupture of the membranes (PROM) (in term infants >18 hours), maternal fever during labour, and intraamniotic infection/ chorioamnionitis and two or more abnormal values of the so-called sepsis screen (white blood cell count, absolute neutrophil count, immature to total neutrophil – IT – ratio and CRP) findings are supportive of a diagnosis of bacterial infection of the neonate.

A recent review of criteria used for the classification of neonatal sepsis found a high disparity with exact specification in only a quarter of studies [6]. This inconsistency makes comparisons difficult and meta-analysis hardly possible. In 2002 the International Pediatric Sepsis Consensus Conference was held with the aim to create clear definitions for the systemic inflammatory response syndrome (SIRS) and different stages of sepsis for children adaptable for different age groups, ranging from term neonates to adolescents. The definitions should help researchers by creating standardized entry criteria for clinical trials and, thus, making studies comparable [7] following the successful and quickly applied definitions for adults from the American College of Chest Physicians/Society of Critical Care Medicine published in 1992. [8,9] SIRS was diagnosed when at least two of the following four criteria were positive (a or b obligatory): a) core temperature >38.5 °C or <36.0 °C; b) white blood cell count elevated or depressed [10], or >10% immature neutrophils; c) tachycardia >180/min or bradycardia <100/min over a >0.5 hour period without external stimulus or drug therapy; d) tachypnea >60/min or mechanical ventilation for an acute process. Sepsis was defined as SIRS in the presence of or as a result of proven or suspected infection.

The definitions of SIRS and sepsis correlated insufficiently with the diagnosis of culture proven early-onset sepsis, especially in term newborns, in a retrospective cohort study including all newborns with hospitalization within the first 72 hours of life and infants with episodes of suspected late-onset sepsis at our centre between 2004 and 2008. [11] In this age group the criteria showed low sensitivity and low predictive value. Postnatal rather than gestational age seemed to positively influence the correlation with culture proven infections demonstrating a good correlation with late-onset sepsis independent on the gestational age at onset.

In 32 neonates with blood culture proven early-onset bacterial infection using the definition of SIRS two thirds of term newborns and one third of preterm newborns would have been missed in the diagnosis of culture proven bacterial infection. [11] Sensitivity, specificity, positive and negative predictive value of either culture or clinical positive sepsis is shown in table 1. [11,12]

Weeks Gestational Age					
	Total	**<28**	**28-32**	**33-37**	**>37**
Culture proven early-onset bacterial infection					
Sensitivity	50 (32-68)	67 (30-93)	67 (22-96)	50 (7-93)	31 (9-61)
Specificity	80 (76-84)	66 (46-82)	68 (60-76)	84 (77-89)	90 (84-94)
PPV	15 (9-23)	38 (15-65)	9 (2-20)	7 (1-24)	20 (6-44)
NPV	96 (94-98)	86 (65-97)	98 (93-100)	98 (95-100)	94 (89-97)
Culture proven and clinical early-onset bacterial infection					
Sensitivity	66 (59-74)	77 (56-91)	85 (71-94)	63 (46-78)	48 (34-62)
Specificity	80 (76-84)	66 (46-82)	68 (60-76)	84 (77-89)	90 (84-94)
PPV	54 (47-61)	67 (47-83)	48 (36-59)	49 (34-64)	62 (46-76)
NPV	87 (84-90)	76 (55-91)	93 (86-97)	90 (84-95)	84 (77-89)

Abbreviations: SIRS, systemic inflammatory response syndrome; PPV, positive predictive value; NPV, negative predictive value

Table 1. Sensitivity, specificity, positive and negative predictive value (95% confidence interval) of the definitions of SIRS in the diagnosis of culture proven and clinical early-onset bacterial infection according to different gestational ages (11,12).

In a retrospective cohort study set in our level III neonatal intensive care unit, we aimed to evaluate the role of fever, hypothermia, and temperature instability in term and preterm newborns during the first three days of life and to identify risk factors for early onset sepsis among newborns presenting with these temperature symptoms. [13] Between 2004 and 2007 we included 851 newborns of whom 127 (15%) presented with temperature symptoms during the first three days of life. Sixty-nine (8% of the total cohort) of them had fever (rectal temperature >38.5 °C), 69 (8%) had hypothermia (rectal temperature <36.0 °C), and 55 (6%) had temperature instability, defined as an increase or decrease of rectal temperature of >1.5 °C within three hours. Fourteen of the 127 newborns presenting with temperature symptoms had culture proven early-onset sepsis/pneumonia (33% of all 42 newborns with culture proven sepsis/pneumonia), 67 had clinical sepsis (30% of all 209 newborns with clinical sepsis) and 46 were diagnosed as being sepsis negative (8% of all 600 sepsis negatives). Factors associated with culture proven sepsis/pneumonia in newborns presenting with temperature symptoms were maternal fever (p=.009), chorioamnionitis (p<.001), antibiotic therapy of the mother (p=.04), poor skin colour (p=.001) and syndrome of persistent fetal circulation (p=.01). Thus, every seventh newborn hospitalized at our neonatal intensive care unit developed fever, hypothermia and/or temperature instability during the first three days of life. Two thirds of them had culture proven or clinical sepsis. Despite low sensitivity temperature symptoms were highly specific for bacterial infection in preterm and term newborns.

3. Gold standard blood culture

The isolation of an organism confers many advantages, including the optimal choice and duration of antibiotic treatment. [14] Blood cultures are still the gold standard in the diagnosis of neonatal sepsis. However, obtaining cultures from neonates can be difficult as sample volumes are small and a substantial number of cultures turn out to be contaminated or negative. [15] The minimum volume required for a reliable culture result has been estimated as 1.0 ml. [14] In clinical practice samples often contain less; in a prospective study of 298 sets of blood cultures from critically ill neonates 55% of aerobic culture vessels contained not as much as 0.5 ml of blood. [14] False negative results may arise from insufficient or missing living bacteria in the sample resulting from low specimen volume, only transient bacteremia, or administration of antibiotics prior to sampling including administration of intrapartum antibiotics to the mother. The microbiological results are not available until 24 to 48 hours after sampling and thus have no influence on the initial choice on whether to initiate or withhold antibiotic therapy. Furthermore, obtaining adequate samples from premature infants can be challenging in view of the concerns about blood volume depletion in these infants. Therefore, we are challenged to think beyond the current paradigm of basing sepsis diagnosis in neonates entirely on blood culture results.

4. Polymerase chain reaction and hybridization methods in the detection of bacterial genomes

More recently, an increasing number of reports on the use of molecular methods including polymerase chain reaction (PCR) and hybridization methods in the detection of bacterial genomes in blood samples have appeared. Molecular assays have the advantage of direct pathogen detection in a more rapid turnover time compared to blood cultures with results being available within a few hours. The sensitivity of molecular methods in the diagnosis of sepsis may be higher compared to blood cultures and ranges from 41 to 100% with the majority of studies reporting values between 90 and 100%, with a specificity ranging from 78 to 100%. [16] Molecular assays might eventually replace blood cultures, but will continue as a supplement to blood cultures until they are adequately evaluated. [16] Microarray hybridization and next-generation sequencing techniques can lead not only to rapid identification of organisms, but also to evaluation of organism characteristics such as virulence and antibiotic susceptibility. Another exciting prospect is the ability to quantify bacterial loads (analogous to viral loads), which can then be followed during therapy to assess response. [16]

5. White blood cell count

In a recent paper Murphy and Weiner [17] reported on 100% sensitivity and 100% negative predictive value of two normal white blood cell counts (WBC) within 8 to 12 hours and a negative blood culture at 24 hours for ruling out early-onset sepsis in the neonate. A normal WBC was defined as values between 6.000 and 30.000 per μL, and an IT-ratio as less than

0.2. The strength of the study was the high number of 3213 patients included that retrospectively was identified by an electronic database over a 10 years time period. Data revealed an overall low culture proven sepsis rate of 0.73%. Nevertheless, we would argue not to solely rely on these negative results besides the neonate is asymptomatic.

By means of a retrospective cohort analysis of preterm and term neonates admitted to our NICU between 2004 and 2007, including 737 of a total of 1301 neonates who had at least one WBC count determination during the first 72 hours of life, we sought to proof the usefulness of leukocyte counts in the evaluation of early-onset sepsis. [18] WBC counts were done in 1 to 9 times per case (mean 1.65). Median gestational age was 34 weeks and median birth weight 2137 grams, and preterm to term born ratio was 236 (32%) to 501 (68%). Culture proven EOS was diagnosed in 39 neonates (5.3%), and pathogens yielded Group B streptococci in 51%, Ureaplasma urealyticum in 26%, Escherichia coli in 10%, Staphylococcus aureus in 5%, and single cases with Enterococci (3%), Chlamydia (3%), and Klebsiella (3%) infection. Defining normal WBC counts between 9.000 and 34.000 per μl revealed 39% of cases with culture proven EOS having abnormal values. By a second approach defining normal WBC counts between 8.500 and 21.500 per μl calculated using the Youden index (0.29 for optimal cut-off values, sensitivity 64% and specificity 66%) data revealed 59% of cases having abnormal values. Sensitivity of WBC counts decreased from 0 - 24 hours to 48 - 72 hours of age. The IT-ratio showed sensitivity, specificity, positive predictive and negative predictive value (95% confidence interval) of 14 (5-29), 97 (95-99), 36 (13-65), and 92 (89-94) percent, respectively.

Measurement of immature neutrophil granulocytes has been considered to be a helpful early indicator of various infectious conditions [19,20] and has a long clinical tradition in the diagnosis of bacterial sepsis in neonates. [21] The detection of immature granulocytes (IGs) by microscopic count necessitates experienced laboratory staff; furthermore morphological classification of IGs are subject to a considerable reader bias and interpretative errors; especially in neonates, when leucocytosis occurs frequently during the first days of life, this method seems to provide only limited reproducibility. [22,23] Contrariwise in performing a standard 100-cell manual differential small numbers of IG can often be overlooked in samples of leukopenic patients. Hence, automated measurement of IG counts could represent a reliable and utile method in the prediction of bacterial infection in neonates. Automated hematology analyzers enable a fast, accurate and less-labor intensive method for the detection of IGs and could improve screening and monitoring for neonatal septicemia. [24-26] The detection limit of IGs has been described to be 0.1% which is considerably lower than in a manual smear. The assessment of a complete blood count with white blood cell differential is usually performed as a routine method to evaluate newborns at risk; the automated simultaneous enumeration of IGs provides additional information without the need of further costs and blood sampling, which might be of special importance in preterm babies.

The Sysmex XE-2100 [27] - a multiparameter automated hematology analyzer - offers the possibility to detect IGs including metamyelocytes, myelocytes, and promyelocytes by the measurement of white blood cell differential counts by flow cytometry in the DIFF-channel. In a separate channel, called the IMI-channel not only IGs, but also bands, blasts, and

hematopoietic progenitor cells are detected. The reaction principle is based on differences in membrane composition between mature and immature cells. The superiority of the flow cytometric IG count performed by the Sysmex XE-2100 compared to the manual morphology count as a reference method for IG counting has been demonstrated in several studies. [25,28]

To determine the predictive value of the immature granulocyte number and the immature myeloid information in neonatal early-onset sepsis we examined 133 blood samples of patients admitted to our neonatal intensive care unit. [29] The number of immature granulocytes and the immature myeloid information were significantly elevated in 21 neonates with early-onset sepsis compared to 112 controls (median 0.28 vs. 0.05 x 109/L, $p=0.049$ and 639 vs. 89, $p<0.0001$, respectively).

6. C-reactive protein

CRP is one of the most widely available; most studied, and most used laboratory tests for neonatal bacterial infection. It is well known that it provides limited sensitivity when determined during the early phases of the disease, especially at the initial presentation, but provides very high negative predictive values and is thus useful for identifying infants unlikely to be infected or monitoring the response to treatment. [30-33] However, the current literature provides a growing body of evidence suggesting the so far reported characteristics of CRP may not be as suitable for the use in preterm as in term newborns. [34-36] Furthermore, the use of CRP in neonatal sepsis is complicated by a nonspecific rise that starts shortly after birth. [37,38]

Any elevation of serum CRP in the neonate always represents endogenous synthesis, since it passes the placenta in exceedingly low quantities. [39] De novo hepatic synthesis starts very rapidly after a single stimulus with serum concentrations rising above 5 mg/l by about 6 hours and peaking around 48 hours. [40]

For the diagnosis of early onset sepsis in clinical practice the sensitivity is more important compared to the specificity, as the consequences of unnecessarily treating an uninfected infant bear fewer complications than not treating an infected child. Up to date the most used cut-off value is 10 mg/l irrespective of the gestational and postnatal age of the neonate. In view of the physiologic dynamics of CRP during the first days after birth and the influence of gestational age on its response to infection, it appears reasonable to reconsider this static cut-off value and evaluate the possible advantages of the introduction of dynamic reference values. [41] However, the current literature lacks sufficient evidence to make recommendations for the use in clinical practice. CRP reaches the best diagnostic accuracy when combined to another infection marker that compensates for its diagnostic weakness and provides reliable sensitivity during the early phases of sepsis. Suitable markers include but are not limited to PCT, IL-6, and IL-8. Many further parameters may provide similar good results but are not yet sufficiently examined to be applied to clinical practice. CRP is particularly useful for monitoring the response to treatment and for ruling out an infection: A repeated determination of CRP 24 to 48 hours after the initiation of antibiotic therapy has been reported to carry a 99% negative predictive value in accurately identifying uninfected

neonates though nothing replaces the clinical impression. CRP values undergo a physiological 3-day-rise after birth. [37,38] This physiologic dynamics as well as certain maternal and perinatal factors may affect interpretation of what constitutes "normal" CRP values in healthy neonates. Furthermore, some reports suggest non-infectious confounders such as meconium aspiration syndrome and perinatal maternal risk conditions may significantly elevate CRP values in symptomatic or at risk neonates and thus confound interpretation of CRP values in the diagnosis of sepsis. [34]

7. Inflammatory cytokines and other inflammatory indices

Sepsis is a pathogen initiated but a cytokine-mediated condition in which immune, inflammatory, and coagulation homeostasis is disturbed. [42] After contact to bacterial antigens inflammatory cytokines and growth factors, and their secondary mediators, which include nitric oxide, thromboxanes, leukotrienes, platelet-activating factor, prostaglandins, and complement, cause activation of the coagulation cascade, the complement cascade, as well as the production of acute-phase reactants. The amount of mediators involved in the inflammatory response to bacterial invasion represents additional opportunity for further improving the diagnosis of sepsis, provided that the right parameter is determined by the right technique at the right time. In fact, each parameter, e.g. acute phase reactants, chemokines and cytokines, has distinct characteristics and a different set of indications and restrictions when applied to different types of infections or even different phases of the infective process.

In 2003 a systematic review of modern diagnostic tests for neonatal sepsis [43] highlighted the following problems by use or recommendation of certain parameters: 1) the cut-off laboratory values that were chosen to distinguish between the presence and absence of infection appear to be unique to each study making any comparisons difficult, 2) the range of test sensitivity and specificity, both calculated and reported, was large, and sensitivities ranged from 57% to 100%, specificities from 43% to 100%, and, similarly, positive likelihood ratios from 1.5 to ∞, 3) the authors also assessed the accuracy of combinations of tests (evaluated in 3 studies) and none of these test combinations had a positive likelihood ratio of more than 10.

Focussing on the early detection of neonatal early-onset sepsis we studied the reliability of procalcitonin (PCT) and interleukin-6 (IL-6) compared with CRP and IT-ratio - used as routine parameter for the diagnosis of bacterial infection - at the age of the first 12 hours of life. [5] In this age-group PCT showed the highest sensitivity followed by CRP, IL-6, and IT-ratio when using ROC analysis with the Youden´s index for optimal cut-off values. The combination of the diagnostic tests revealed the best results for the prediction of bacterial infection within 12 hours of age. The combination of PCT and IL-6 yielded a sensitivity of 89%, a specificity of 91%, a positive predictive value of 94% and a negative predictive value of 84%, the combination of CRP and IT-ratio a sensitivity of 82%, a specificity of 96%, a positive predictive value of 97% and a negative predictive value of 78%. Differences were not significant. The single test results are shown in figure 1 – 4, and the ROC-analysis of all four inflammatory indices is shown in figure 5.

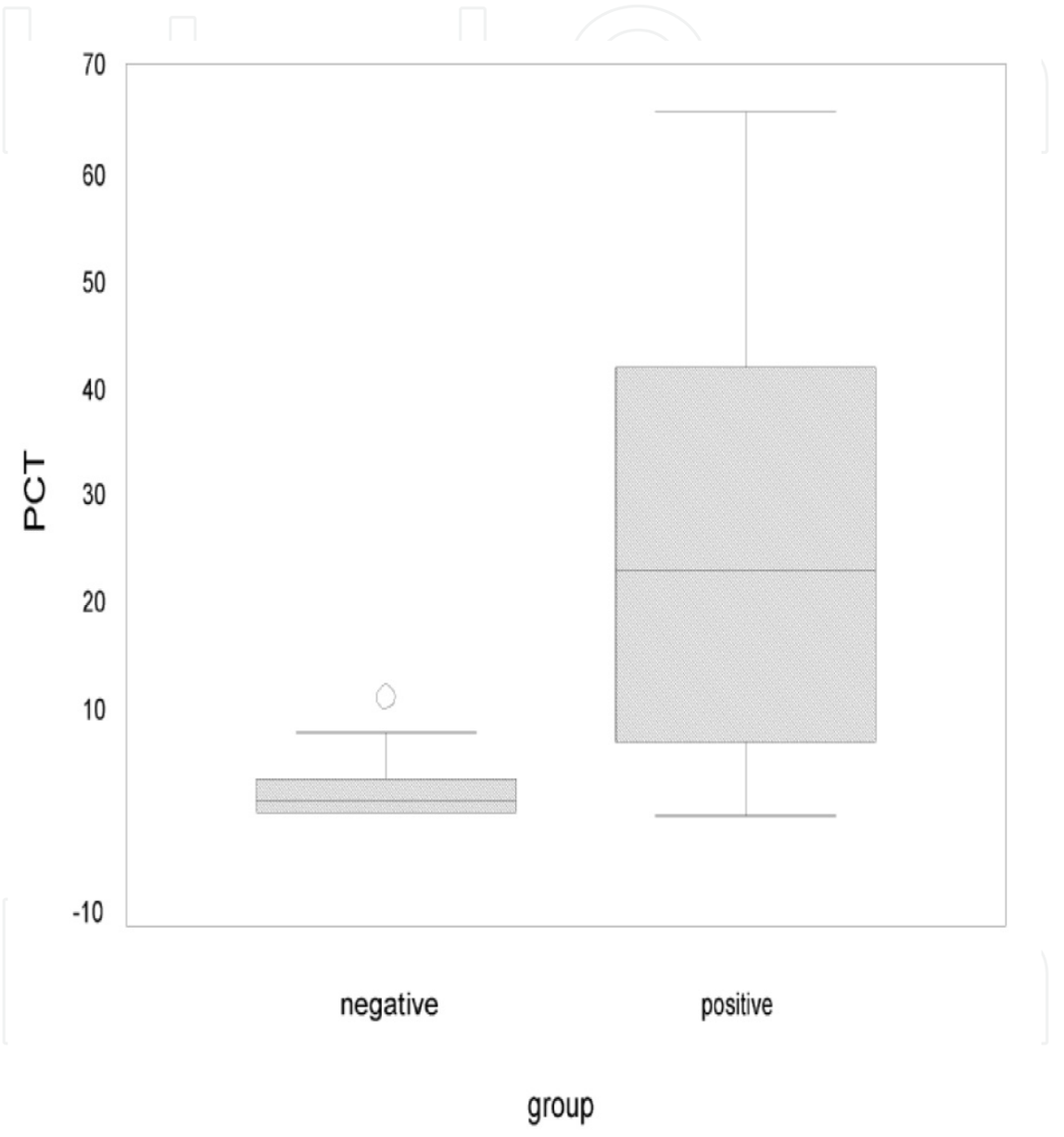


Figure 1. Procalcitonin values (ng/L) of 41 neonates with blood culture positive and clinical early-onset sepsis compared with 27 neonates without sepsis within 12 hours of life (p<0,001).

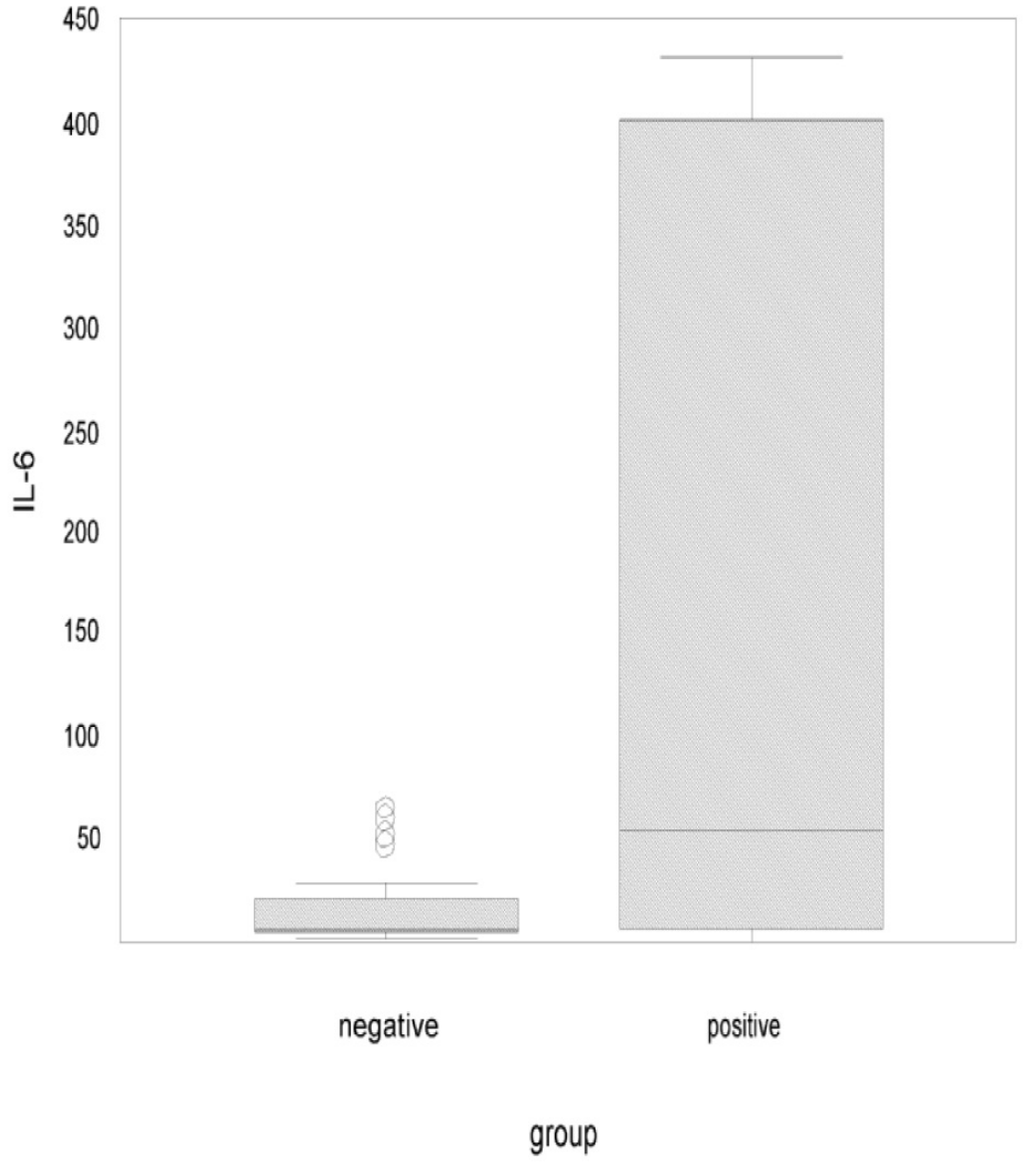


Figure 2. Interleukin-6 values (pg/L) of 41 neonates with blood culture positive and clinical early-onset sepsis compared with 27 neonates without sepsis within 12 hours of life (p<0,001). (5)

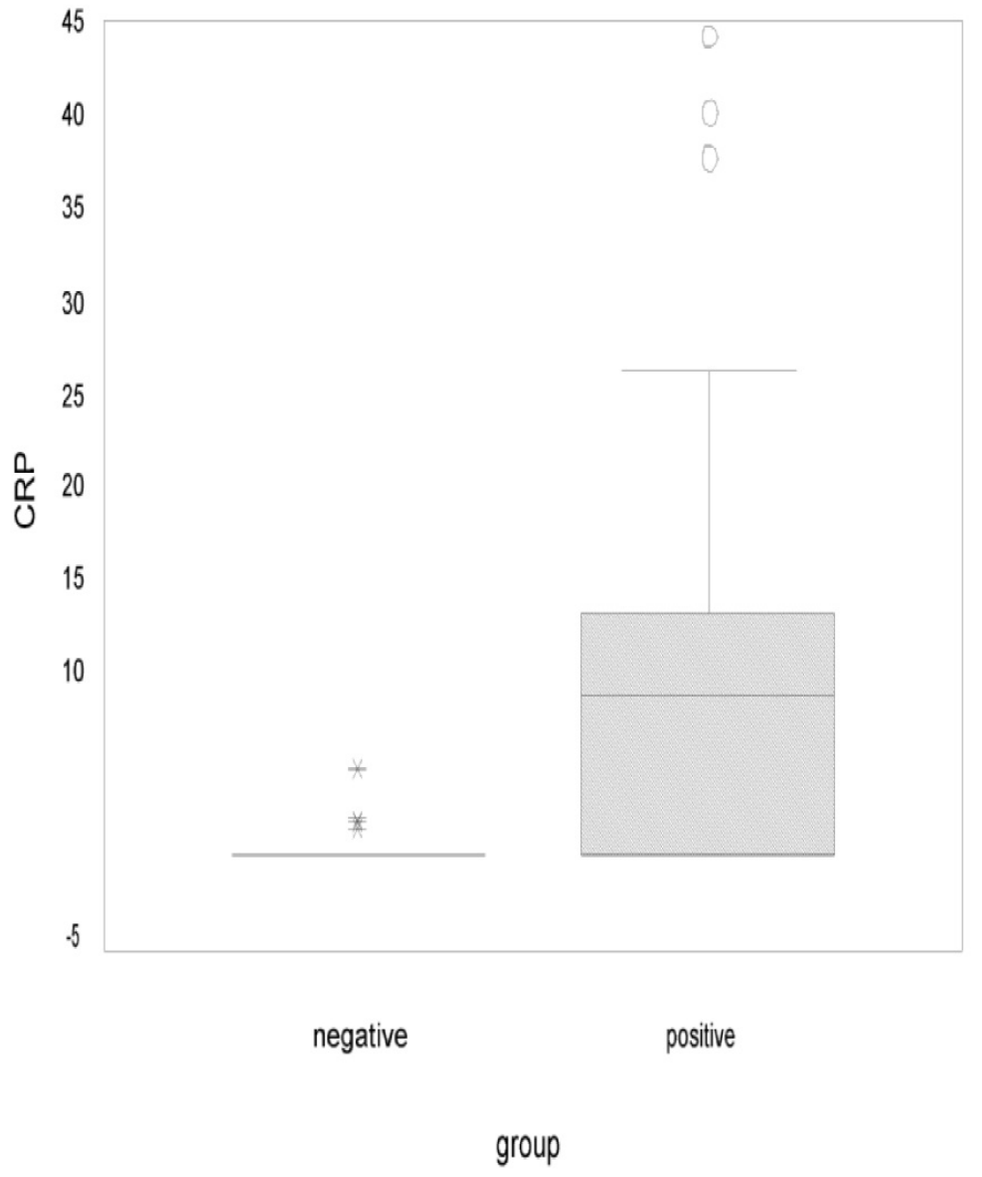


Figure 3. C-reactive protein values (mg/L) of 41 neonates with blood culture positive and clinical early-onset sepsis compared with 27 neonates without sepsis within 12 hours of life (p<0,001). (5)

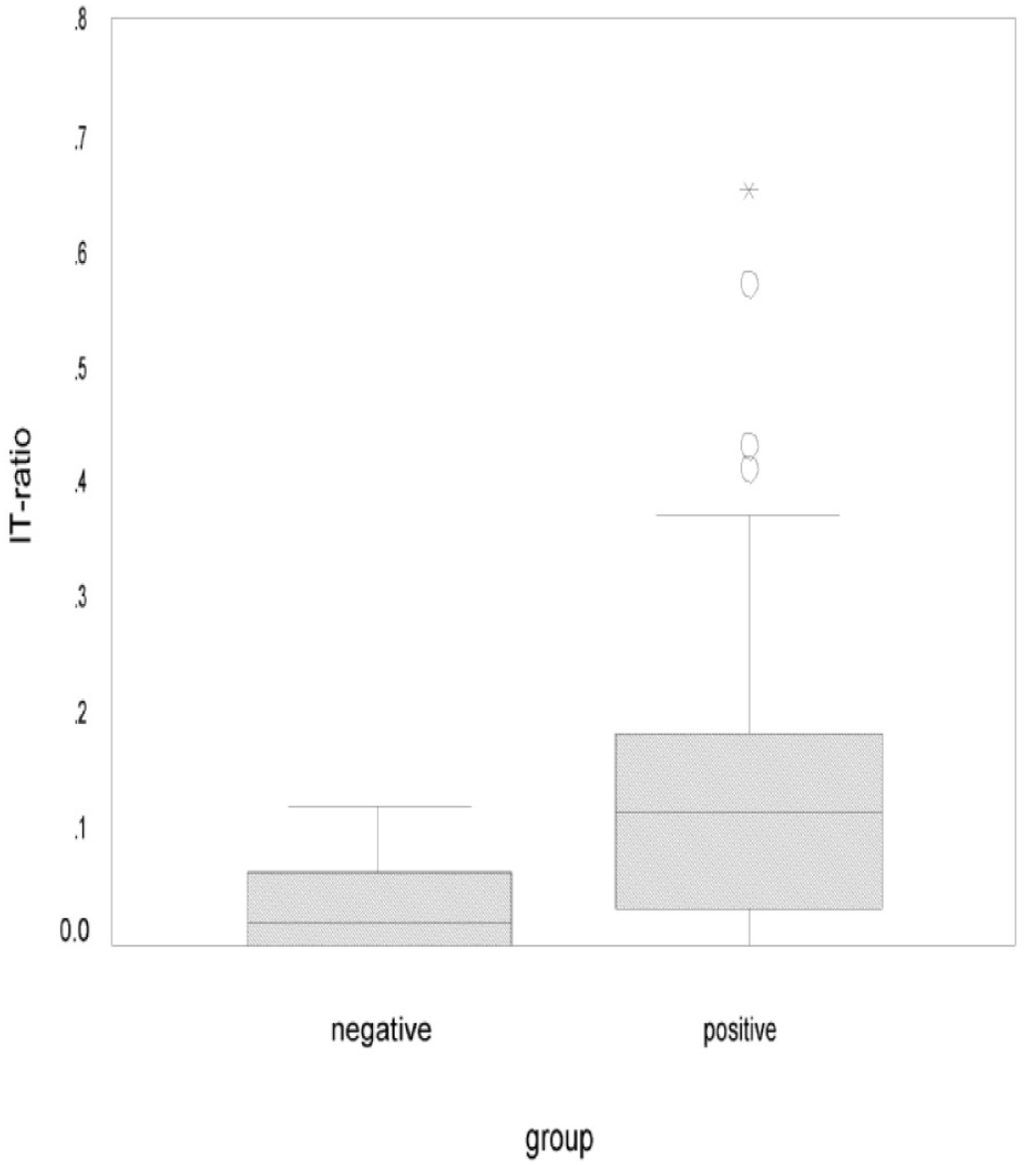
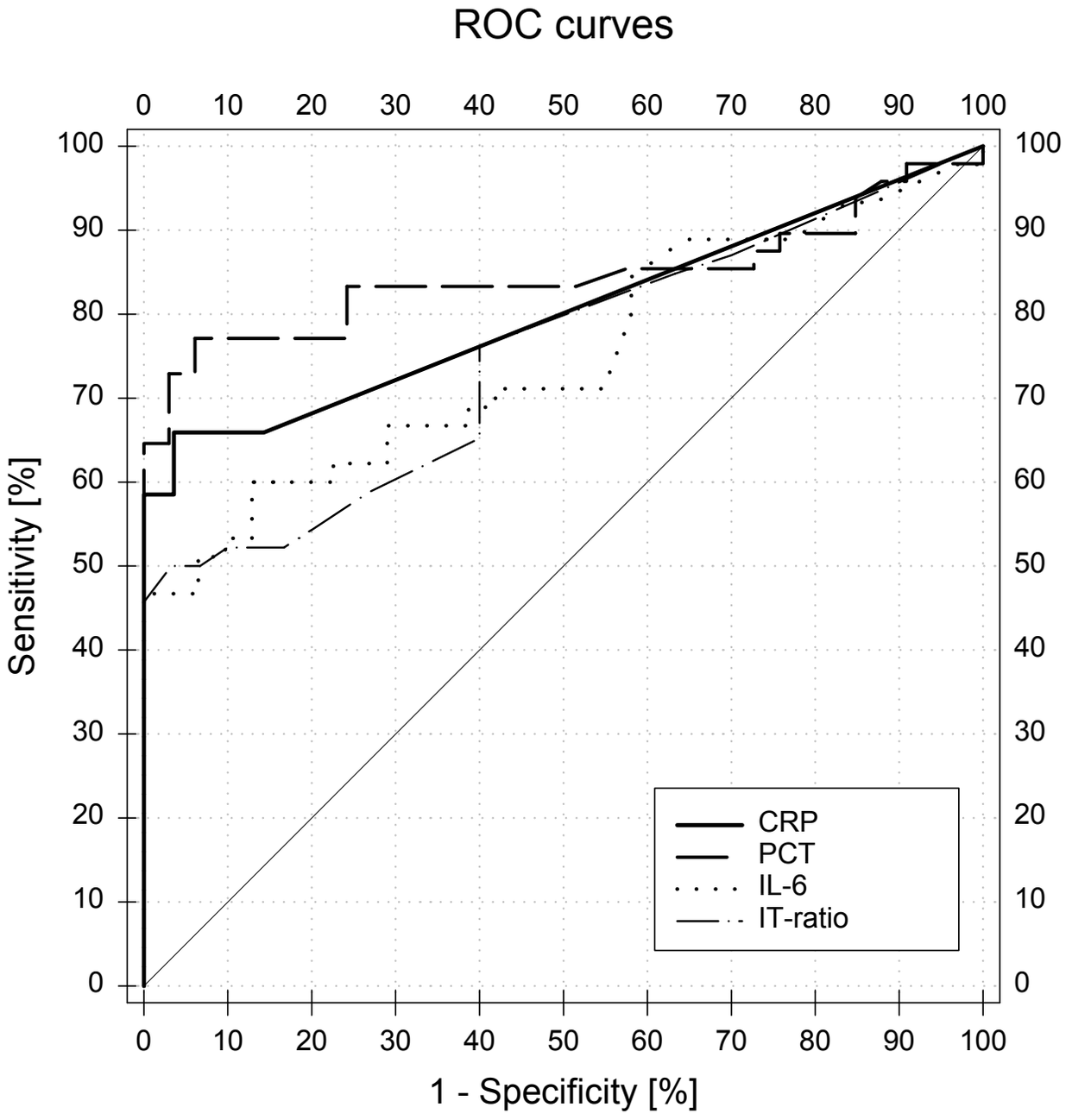


Figure 4. Immature-to-total neutrophil ratio values of 41 neonates with blood culture positive and clinical early-onset sepsis compared with 27 neonates without sepsis within 12 hours of life (p<0,001). (5)



CRP = C-reactive protein, PCT = procalcitonin, IL-6 = interleukin-6, IT-ratio = immature- to-total neutrophil ratio

Figure 5. Receiver operating characteristic curves for values of Procalcitonin, Interleukin-6, CRP, and IT-ratio in 76 neonates within 12 hours of life. Area under the curve was 0,845 (CI 95% 0,741 – 0,949) for PCT; 0,763 (CI 95% 0,641 – 0,884) for IL-6; 0,812 (CI 95% 0,702 – 0,922) for CRP; and 0,770 (CI 95% 0,651 – 0,890) for IT-ratio. Differences were not significant. (5)

Vouloumanou et al. [44] recently analyzed 16 studies (involving 1,959 neonates and including our above mentioned study) that evaluated PCT in neonates with culture proven or clinically diagnosed sepsis in comparison with ill neonates with other conditions. The pooled (95% confidence interval) sensitivity and specificity were 81% (74–87%) and 79% (69–87%), respectively. The area under the ROC curve (AUC) was 0.87. The diagnostic accuracy of PCT seemed higher for neonates with late-onset sepsis (>72 h of life) than for those with early onset sepsis; the AUC for these analyses was 0.95 and 0.78, respectively. However, fewer data were available for late-onset sepsis. High statistical heterogeneity was observed for all analyses. In view of the marked observed statistical heterogeneity, along with the lack of a uniform definition for neonatal sepsis, the authors stated that interpretation of these findings should be done with appropriate caution.

8. Conclusions

At the moment none of the described current diagnostic markers are sensitive and specific enough to influence the judgment whether or not to withhold antimicrobial treatment independent of the clinical findings. Efforts were done to improve diagnostic accuracy by combining multiple markers in order to further enhance the diagnostic accuracy of these mediators in identifying infected neonates. Due to many physiologic changes of inflammatory parameters in the neonatal period and considering the differences between preterm and term infants diagnosis of bacterial infection might better be based on dynamic than static cut-off values during the first 48 to 72 hours of age.

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