Early and late markers for the detection of early-onset neonatal sepsis

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Dan Med Bull 2008;55:219-23

ABSTRACT

Introduction: In this study we tested how a combination of early and late paraclinic markers could predict early onset neonatal sepsis (EONS).

Methodology: The first 24 hours after the suspicion of EONS, we measured interleukine (IL)-6, IL-8, IL-10, IL-18, tumor necrosis factor-alpha (TNF-α), interferon gamma (INF-γ), procalcitonin (PCT) and C-reactive protein (CRP) at 8-hour intervals on 123 neonates clinically suspected for EONS. The neonates were divided into two groups. The sepsis group: 1A with blood culture verified bacteraemia and 1B strongly suspected sepsis (29 patients). The no sepsis group: 2A treated with antibiotics (37 patients) and 2B not treated with antibiotics (57 patients).

Results: Combined evaluation of each of the early markers with PCT >25 ng/ml for prediction of EONS at time 0, gave the following sensitivities and specificities: IL-6 >250 pg/ml: 71% and 88%; IL-8 >900 pg/ml: 50% and 88%; IL-10 >40 pg/ml: 43% and 87%; and immature/total (I/T) ratio >0.35: 59% and 88%. The results of IL-18, TNF-α and IFN-γ did not predict EONS.

Conclusion: IL-6 combined with PCT values is a fair way to evaluate EONS at the time of suspicion of infection. The "old" early marker, I/T ratio, is almost as efficient as IL-6. By combining an early and a late marker it may be possible to reduce the diagnostic "non-conclusive" period of paraclinic values.

Early onset neonatal sepsis (EONS) is often suspected in a neonatal intensive care unit (NICU). In general the percentage of fatal neonatal infections is highest in the neonates with the lowest gestational ages [1]. Many studies have tried to find reliable early reacting cytokines for detection of EONS [2]. Because of variable degrees of unspecific inflammation during the first three days of life [3-5], evaluation of possible infection is sometimes extremely difficult in the EONS. Therefore antibiotics may be used more often than necessary in the NICU, which increases the risk of antibiotic resistance [6].

The aim of the study was to analyse the dynamics of a number of paraclinic markers during the first 24 hours after the suspicion of EONS, and on the basis of the results to test how well a combination of an early and a late marker could predict EONS.

The inflammatory process in sepsis is biochemically very complex. From laboratory and clinical studies it is known that some proinflammatory cytokines peak very fast within one to four hours after a sepsis stimulus [2, 7-9]. C-reactive protein (CRP) rises to a maximum 12-24 hours and procalcitonin (PCT) 8-16 hours after the septic stimulus [4, 10]. CRP is induced by proinflammatory cytokines [11].

We evaluated seven early markers: Three proinflammatory cytokines, interleukine (IL)-6, tumor necrosis factor-alpha (TNF-α) and the chemokine IL-8; interferon gamma (IFN-γ) and one of the IFN-γ inducers, IL-18; the antiinflammatory cytokine IL-10; the ratio of immature to total number of neutrophiles (I/T-ratio) and finally a late marker, PCT.

Only high values of the markers are conclusive. We hypothesized that with suspected EONS either a high early or a high late marker strongly would support the diagnosis of EONS.

MATERIAL AND METHODS

The data of the study was collected in the NICU of Aalborg Hospital, University of Aarhus, between September 1999 and September 2001. Altogether, 123 neonates were consecutively included. Inclusion criteria were: clinical signs of EONS with at least one significant symptom indicating possible EONS (Table 1); age less than 72 hours at admission and a birth weight more than 1200 g. We recommended the weight limit of 1200 g based on our standards for the maximum sizes of individual blood samples related to the body mass. The study was approved by the local ethical committee. Both verbal and written informed consent was given by the parents.

The patients were retrospectively allocated into a sepsis and no sepsis group before the cytokine and PCT analyses were done:

1A

Sepsis. Infants with blood culture verified bacteraemia (four infants: one with Group B *Streptococcus* (GBS), two with *Escherichia coli* and one with *Staphylococcus aureus*).

1B

Strongly suspected sepsis (25 infants). Significant symptoms and inflammatory response defined by CRP >50 µg/ml at any time point, in conformity with other studies on EONS (12-14). This strategy was chosen as it is known that many true bacteraemia are not found by the standard blood culture [15, 16].

2A

No sepsis (treated with antibiotics) (37 infants). This group included infants where antibiotic treatment was initiated because of clinical symptoms but the inflammatory response was low to moderate (CRP ≤50 mg/l). The antibiotic treatment was withdrawn after few days (median length of treatment was three days) because dynamics and severity of symptoms never confirmed clinical EONS. Antibiotic treated infants routinely started up with ampicillin (200 mg/kg/day) and gentamycin (3-4 mg/kg/day) intravenously.

2B

No sepsis (not treated) (57 infants). These infants were observed closely because of sepsis relevant symptoms. The severities of symptoms were not, though, considered relevant for initial treatment. The following dynamics of the paraclinic and clinical symptoms supported this strategy. All infants recovered without need of antibiotics.

Blood sampling was performed 0, 8, 16, 24, 48 and 72 hours after the neonate was suspected of EONS. At time 0 hours a blood culture and leukocyte count including differential counts were made. CRP

Table 1. Percentage of infants with the specified clinical symptoms at inclusion.

	Sepsis (n=29) %	No sepsis (n=94) %
Respiratory: apnoea, respiratory distress, oxygen dependence	86	56
Circulatory: tachycardia, bradycardia, poor peripheral circulation, hypotension	45	21
Central nervous system: hypotonia, lethargy, irritability, seizures	55	45
Gastrointestinal: vomiting, aspirates, abdominal distension	14	32
Abnormal temperature: fever, hypothermia	10	3

was immediately analysed at all sampling times. At times 0, 8, 16 and 24 hours, 300 µl plasma was separated for later PCT and cytokine analysis. The capillary blood sample was taken using heel prick. The blood was then transported on ice, centrifuged, and the plasma was frozen at –70 °C.

The leukocyte differential counts were done manually by microscope.

CRP was routinely analysed by fixed-point immuno-rate colorimetri (Vitros 950, Ortho-Clinical Diagnostics, Rochester, USA). The detection limit was 10 µg/ml.

PCT was analysed using an immunoluminometric assay (LUMItest R PCT; BRAHMS Diagnostica, Berlin, Germany). The kit was used according to manufacturer´s recommendations. Detection limit was 0.08 ng/ml.

For the evaluation of IL-6, IL-8, IL-10, IL-18, IFN-γ and TNF-α, we used an in-house test based on the Luminex platform [17, 18]. The following antibody pairs were used: IL-6 (R&D Systems cat. nr. DY206 incl. standard), IL-8 (CLB Sanquin cat. nr. M9318. The standard was from NIBSC cat. nr. 89/520), IL-10 (CLB Sanquin cat. nr. M9310 incl. standard), IL-18 (Medical & Biological Laboratories Co. cat. nr. D044-3/D045-6 standard B001-5), IFN-γ (R&D Systems cat. nr. DY285 incl. standard) and TNF-α (R&D Systems cat. nr. DY210 incl. standard). 12.5 µl patient serum was used for the assay. Detection limits for the cytokines were: IL-6 4.6 pg/ml; IL-8 13.7 pg/ml; IL-10 13.7 pg/ml; IL-18 13.7 pg/ml; IFN-γ 7.8 pg/ml and TNF-α 13.7 pg/ml.

STATISTICAL METHODS

In the evaluation of sensitivity and specificity of the different cut-off values, we compared the results in the sepsis group with the results found in the group with no sepsis.

Differences between groups were analysed with Kruskal-Wallis One-Way ANOVA on Ranks – not corrected for ties. Differences were considered significant at $p<0.05$.

The statistical calculations were made by the NCSS statistical program.

RESULTS

DEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Except for GA (p=0.01), the two patient groups were not statistically significantly different from each other concerning background characteristics (p=0.14-0.87 One-Way ANOVA on ranks) (Table 2). The median gestational age was 38 weeks, the median birth weight 3170 g and the median age when included in the study was eight hours.

SYMPTOMS

When the clinical symptoms at inclusion were categorized as given in Table 1, the clinical score per patient (maximum one point from each of the five groups of symptoms) was 2.1 (0.94) (mean (SD)) for the sepsis group and 1.56 (0.82) for those with no sepsis.

DYNAMIC ANALYSIS

The dynamics of the markers are illustrated in Table 3; 50, 90 and 100 percentiles are shown, as we find these values are the most interesting when high specificity is wanted.

IL-6, IL-8 and IL-10 median concentrations peaked at the time of the initial blood sampling. PCT and CRP median values in group 1 created a characteristic "dome" peaking at 8-16 hours and 16-24 hours, respectively.

The median concentrations of IL-6, IL-8, IL-10, I/T ratio and PCT (Table 3) were significantly different between the two groups at time 0, 8, 16 and 24 hours (p= 0-0.03), except the IL-8 values at 24 hours (p=0.68) and Il-10 at 16 and 24 hours (p=0.11 and 0.85).

IFN-γ only exceeded detection level in five patients and two of these patients were in the group of no sepsis (one treated and one not treated). TNF-α was measured above detection level in only seven patients, five from the sepsis group and two from the group of no sepsis (treated). For IL-18, IFN-γ and TNF-α there were no significant differences between the two groups (p= 0.10-0.66).

CUT-OFF VALUES

Using receiver operating characteristic (ROC) curves, the area under the curve (AUC) was greatest at time 0 hours for the cytokines IL-6 (0.77), IL-8 (0.70) and IL-10 (0,65). For PCT it was largest at 8 hours (0.83). AUC value for I/T ratio at time 0 was 0.81.

Table 2. Clinical and demographic characteristic of the infants.

	Sepsis (n=29)	No sepsis (n=94)
Gestational age, weeks*	39 (31-42)	37 (32-42)
Birth weight, g*	3.3 (1.7-3.9)	3.1 (1.9-4.2)
Age at examination, hours*	5 (1-33)	10 (1-46)
Prenatal antibiotics, %	28	26
Vaginal delivery, %	79	64
% apgar scores 5 minutes ≤ 7	3	11
Gender, % girls	52	43

*) Median (10-90 percentiles)

Table 3. Sampling time (hours), percentiles for each group on the early and late markers of infection.

	Sepsis (n=29) percentiles			No sepsis (n=94) percentiles		
Sampling time	50	90	100	50	90	100
CRP, µg/ml						
0 hour	19	78	169	<10	31	44
8 hours	52	81	165	11	35	46
16 hours	61	103	109	13	29	39
24 hours	65	117	197	12	30	36
48 hours	36	70	252	11	24	33
72 hours	21	55	270	<10	17	27
PCT, ng/ml						
0 hour	9	61	105	2	23	104
8 hours	33	120	160	7	33	111
16 hours	35	179	302	6	55	102
24 hours	27	169	282	5	42	82
IL-6, pg/ml						
0 hour	460	73,635	305,031	<4.6	96	25,214
8 hours	82	29,016	36,228	<4.6	12	4678
16 hours	<4.6	6126	10,732	<4.6	<4.6	187
24 hours	<4.6	626	1489	<4.6	<4.6	143
IL-8, pg/ml						
0 hour	319	37,257	67,433	<13.7	567	6351
8 hours	254	7405	14,028	<13.7	399	1784
16 hours	25	1203	2055	<13.7	520	2857
24 hours	<13.7	2612	3835	<13.7	901	6122
IL-10, pg/ml						
0 hour	26	590	4453	<13.7	52	1180
8 hours	<13.7	84	458	<13.7	<13.7	3917
16 hours	<13.7	38	210	<13.7	<13.7	230
24 hours	<13.7	<13.7	92	<13.7	<13.7	85
IL-18, pg/ml						
0 hour	21	118	181	23	157	361
8 hours	38	171	265	19	145	338
16 hours	54	140	169	22	128	353
24 hours	57	118	230	30	129	348
IFN-γ pg/ml						
0 hour	<7.8	9	22	<7.8	<7.8	39
8 hours	<7.8	<7.8	40	<7.8	<7.8	40
16 hours	<7.8	<7.8	42	<7.8	<7.8	17
24 hours	<7.8	<7.8	152	<7.8	<7.8	33
TNF-α, pg/ml						
0 hour	<13.7	29	48	<13.7	<13.7	<13.7
8 hours	<13.7	<13.7	34	<13.7	<13.7	90
16 hours	<13.7	16	29	<13.7	<13.7	84
24 hours	<13.7	<13.7	24	<13.7	<13.7	48
IT-ratio						
0 hour	0.31	0.62	0.76	0.12	0.31	0.55

Table 4. Cut-off values for prediction of early neonatal sepsis at time 0 hours. A: Cut-off values representing the situations in which the specificities are about 95%. B: Cut-off values representing the situations, in which sensitivity and specificity are almost equal, i.e. lower cut-off values. The corresponding likelihood ratio, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are listed for each of the markers.

	Cut-off	Likelihood ratio	Sensitivity, %	Specificity, %	PPV, %	NPV, %
A						
IL-6	250 pg/ml	10	57	94	76	88
IL-8	900 pg/ml	8	36	96	71	83
IL-10	100 pg/ml	5	29	94	62	81
I/T ratio	0.35	10	48	95	76	84
PCT	25 ng/ml	3	21	92	46	79
B						
IL-6	12 pg/ml	2	71	71	43	89
IL-8	130 pg/ml	2	64	70	40	86
IL-10	15 pg/ml	3	64	79	47	86
I/T ratio	0.2	3	70	75	49	88
PCT	5.75 ng/ml	2	68	67	39	87

At time 0 hours we found cut-off values with specificities around 95% using ROC curves and tables. These cut-off values had rather low sensitivities ranging from 21 to 57% (Table 4A). The highest sensitivity was seen for IL-6 and the second highest for I/T ratio.

COMBINATION OF EARLY AND LATE MARKERS AT TIME 0 HOURS

Combining IL-6 with PCT gave the highest sensitivity: sensitivity 71%, specificity 88% (I/T ratio combined with PCT was 59% and 88%) (Table 5A).

ALTERNATIVE CUT-OFF VALUES OFTEN USED IN OTHER STUDIES

Cut-off values for each marker were calculated using the points closest to the upper left corner of the ROC curves (where sensitivity and specificity are approximately identical). This strategy is often applied in other studies [2, 19-21]. In our study, sensitivities then varied from 64 to 71% and specificities from 61 to 79% (Table 4B). When early markers were combined with PCT the sensitivity rose, but as expected, the specificity dropped drastically. The values for IL-6 combined with PCT now were 93% and 46% (Table 5B).

Table 5. Combination of an early and a late marker at time 0 hours for prediction of early neonatal sepsis. Cut-off values are those listed in Table 4. A: Cut-off valuws representing the situation in which the specificity of the single marker is around 95%. B: Cut-off values representing the situation in which sensitivity and specificity of the single marker are almost equal. The corresponding likelihood ratio, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) are listed for each of the markers.

	Sensitivity, %	Specificity, %	PPV, %	NPV, %
A				
PCT+ IL-6	71	88	65	91
PCT+ IL-8	50	88	56	85
PCT+ IL-10	43	87	50	83
PCT+ I/T ratio	59	88	62	86
B				
PCT+ IL-6	93	46	35	95
PCT+ IL-8	89	44	33	93
PCT+ IL-10	93	48	36	96
PCT+ I/T ratio	85	60	35	92

PCT

We found that PCT >25 ng/ml was a fair late marker of EONS at blood sampling at time 8 hours (AUC 0.83, Likelihood Ratio 4, sensitivity 70% and specificity 86%), table not shown.

INDIVIDUAL MARKER CURVES

To illustrate the dynamics of the markers in verified EONS, the data from the four infants are shown in Figure 1.

DISCUSSION

We found that IL-6, IL-8 and IL-10 were reacting with maximum plasma concentrations at the time when the infants were suspected of having sepsis. PCT and CRP were late reacting with maximum concentrations at 8-16 and 16-24 hours, respectively. Especially we noted that when the cytokine values were decreasing, PCT and CRP had just started to increase.

When we looked at the high cut-off values, i.e. with specificities around 95%, of the single early markers at time 0 hour, IL-6 was the best marker followed by the I/T-ratio; however, the sensitivity was only 57% and 48%, respectively.

To improve the effectiveness of the tests we combined an early and a late marker. The combined value of IL-6 and PCT at the first blood sample was a fair EONS predictor (sensitivity 71% and specificity 88%). Observe that I/T ratio is almost as efficient as IL-6. By introduction of these markers it may be possible to reduce the diagnostic "non-conclusive" period of paraclinical values.

In the sepsis group 26 of 29 infants had significant paraclinic values for EONS at time 0. In the no sepsis group 21 of 94 infants (37 were treated in the study) had paraclinic values significant for EONS within the first 16 hours. So using paraclinic values alone for the evaluation of EONS, 16 infants less would need to be treated. This way the total days of antibiotic treatment in the no sepsis group might be reduced from 111 to 63 days.

We found that IL-18, IFN-γ and TNF-α were of no use in the diagnosis of the EONS. To our knowledge IL-18 has not previously been studied in infected infants. Lauw et al [22] found that IL-18 was raised in infected adults. Ng et al [18] found that IFN-γ was increased in septic neonates. In five out of six studies summarized by Mehr and Doyle [2] TNF-α was increased in infected infants, especially if they had septic shock. In our study very few infants had septic shock, which might be the reason for the difference between the results.

There are no rules concerning how to choose cut-off values from an ROC curve. The values at which sensitivity and specificity are equal are often used. Others prefer a high sensitivity to avoid missing a relevant patient [23]. In our study we chose cut-off values with high specificity (approximately 95%) for the detection of sepsis. But to be able to compare our study with others [2, 19-21] we calculated the diagnostic power when combining low cut-off values on the markers, i.e. the situation where sensitivity and specificity were almost equal. This strategy resulted in too many false positive results (specificity 44-60% in the group of no sepsis).

So if combinations of early and late markers are used at time 0 it is important to use high cut-off values. This way a restrictive antibiotic politic is supported. Remembering though, that when the clinical symptoms are considered serious, treatment should always be initiated without waiting for initial paraclinic values.

THE STRENGTH OF THIS STUDY

1. We analysed the dynamics of the markers every eight hour in the first 24 hours. This way we could show that the values of the cytokines fell rapidly, while the late markers peaked 16-24 hours after the suspicion of EONS.
2. Therefore our high cut-off technique focusing on peak values of early and late markers is valuable. Screening for a high early or

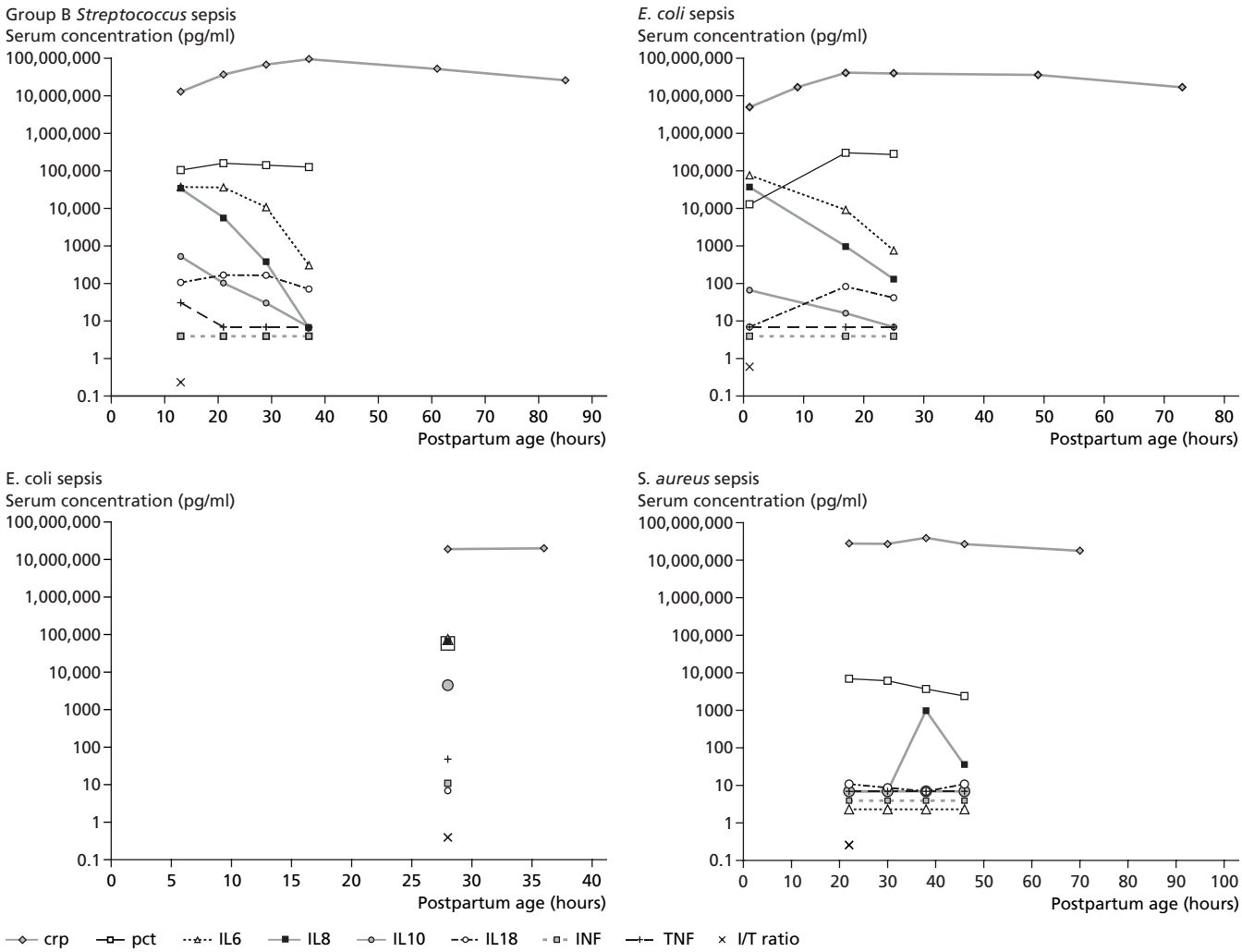

Figure 1. Marker dynamics in the sick infants with blood culture verified bacteraemia. Note that all concentrations have been transformed to pg/ml on a logarithmic scale. I/T ratio at time 0 were respectively 0.23, 0.6, 0.39 and 0.26 in the four infants. We only have few data on infant no. 3 as he was transferred to another hospital for operation of suspected NEC (he died 12 hours after admission). Note that the dynamics in infant nr 4 is very discrete.We have chosen to mark values under detection level using the half of the detectable value.

late marker minimises the risk of reacting on an insignificant raise in values caused by unspecific inflammation in the newborn.

3. Further, by using the LUMINEX flow cytometric technique, we could analyse many markers although we only had a small serum volume.

THE WEAKNESS OF THE STUDY

1. We only had very few infants with a positive blood culture.
2. Our paraclinic definition of sepsis was based on CRP values above 50 mg/l. Many studies have used other values and parameters [2, 19-21] but unfortunately no consensus exists on this subject. We find our method acceptable. Especially as the symptom scores on every infant has been evaluated by a senior neonatologist before the analyses of the new markers were made.
3. Markers related to CRP are biased to better results, as CRP are part of our definition of sepsis [10, 24].
4. We have not included the most vulnerable infants with a birth weight less than 1200 g.
5. The markers from the present study and the interpretation resulting from the chosen cut-off values ought to be tested in a prospective clinical study.

CONCLUSION

PCT combined with IL-6 (or I/T ratio) is fair for detecting EONS at the first blood sample. By the introduction of IL-6 and PCT it may be possible to reduce the number of infants treated with antibiotics by reducing the diagnostic "non–conclusive" period of the paraclinical values.

ACKNOWLEDGEMENTS

We thank our medical staff for their help in collecting the data, the laboratory staff of the Department of Clinical Biochemistry, Aalborg Hospital, the laboratory assistant Eva Poulsen, Department of Clinical Immunology, Aalborg Hospital, for setting up new tests for this study, and finally Henrik Toft Sørensen and Gunnar Lauge Nielsen, Department of Epidemiology, Aarhus University Hospital, for providing ideas about how to analyse the results.

This work was supported by grants from Aalborg Frivillige Bloddonoreres Fond, Den Obelske familiefond and Nordjyllands Lægekredsforenings Forskningsfond. The PCT kit was sponsored by BRAHMS Diagnostica, Berlin, Germany.

References

1. Kramer MS, Demissie K, Yang H, Platt RW, Sauve R, Liston R. The contribution of mild and moderate preterm birth to infant mortality. Fetal and Infant Health Study Group of the Canadian Perinatal Surveillance System. JAMA 2000;284:843-9.
2. Mehr S, Doyle LW. Cytokines as markers of bacterial sepsis in newborn infants: a review. Pediatr Infect Dis J 2000;19:879-87.
3. Manroe BL, Weinberg AG, Rosenfeld CR, Browne R. The neonatal blood count in health and disease. I. Reference values for neutrophilic cells. J Pediatr 1979;95:89-98.
4. Chiesa C, Panero A, Rossi N, Stegagno M, De GM, Osborn JF et al. Reliability of procalcitonin concentrations for the diagnosis of sepsis in critically ill neonates. Clin Infect Dis 1998;26:664-72.

5. Monneret G, Labaune JM, Isaac C, Bienvenu F, Putet G, Bienvenu J. Increased serum procalcitonin levels are not specific to sepsis in neonates. Clin Infect Dis 1998;27:1559-61.
6. Balslev T, Kristensen B, Ebbesen F, Schumacher H, Korsager B. Gentamicin-resistant Enterobacteriaceae in a neonatal intensive care unit. Acta Paediatr 1993;82:792-3.
7. Dandona P, Nix D, Wilson MF, Aljada A, Love J, Assicot M et al. Procalcitonin increase after endotoxin injection in normal subjects. J Clin Endocrinol Metab 1994;79:1605-8.
8. Kuster H, Weiss M, Willeitner AE, Detlefsen S, Jeremias I, Zbojan J et al. Interleukin-1 receptor antagonist and interleukin-6 for early diagnosis of neonatal sepsis 2 days before clinical manifestation. Lancet 1998;352: 1271-7.
9. Tavares E, Maldonado R, Ojeda ML, Minano FJ. Circulating inflammatory mediators during start of fever in differential diagnosis of gram-negative and gram-positive infections in leukopenic rats. Clin Diagn Lab Immunol 2005;12:1085-93.
10. Chiesa C, Pellegrini G, Panero A, Osborn JF, Signore F, Assumma M et al. C-reactive protein, interleukin-6, and procalcitonin in the immediate postnatal period: influence of illness severity, risk status, antenatal and perinatal complications, and infection. Clin Chem 2003;49:60-8.
11. Zhang D, Sun M, Samols D, Kushner I. STAT3 participates in transcriptional activation of the C-reactive protein gene by interleukin-6. J Biol Chem 1996;271:9503-9.
12. De Bont ES, Martens A, van RJ, Samson G, Fetter WP, Okken A et al. Diagnostic value of plasma levels of tumor necrosis factor alpha (TNF alpha) and interleukin-6 (IL-6) in newborns with sepsis. Acta Paediatr 1994;83:696-9.
13. Hatherill M, Tibby SM, Sykes K, Turner C, Murdoch IA. Diagnostic markers of infection: comparison of procalcitonin with C reactive protein and leucocyte count. Arch Dis Child 1999;81:417-21.
14. Martin H, Olander B, Norman M. Reactive hyperemia and interleukin 6, interleukin 8, and tumor necrosis factor-alpha in the diagnosis of early-onset neonatal sepsis. Pediatrics 2001;108:E61.
15. Schelonka RL, Chai MK, Yoder BA, Hensley D, Brockett RM, Ascher DP. Volume of blood required to detect common neonatal pathogens. J Pediatr 1996;129:275-8.
16. Squire E, Favara B, Todd J. Diagnosis of neonatal bacterial infection: hematologic and pathologic findings in fatal and nonfatal cases. Pediatrics 1979;64:60-4.
17. De JW, te VH, Prakken BJ, Kuis W, Rijkers GT. Simultaneous detection of 15 human cytokines in a single sample of stimulated peripheral blood mononuclear cells. Clin Diagn Lab Immunol 2003;10:133-9.
18. Ng PC, Li K, Wong RP, Chui K, Wong E, Li G et al. Proinflammatory and anti-inflammatory cytokine responses in preterm infants with systemic infections. Arch Dis Child Fetal Neonatal Ed 2003;88:F209-F213.
19. Franz AR, Kron M, Pohlandt F, Steinbach G. Comparison of procalcitonin with interleukin 8, C-reactive protein and differential white blood cell count for the early diagnosis of bacterial infections in newborn infants. Pediatr Infect Dis J 1999;18:666-71.
20. Blommendahl J, Janas M, Laine S, Miettinen A, Ashorn P. Comparison of procalcitonin with CRP and differential white blood cell count for diagnosis of culture-proven neonatal sepsis. Scand J Infect Dis 2002;34:620-2.
21. Laborada G, Rego M, Jain A, Guliano M, Stavola J, Ballabh P et al. Diagnostic value of cytokines and C-reactive protein in the first 24 hours of neonatal sepsis. Am J Perinatol 2003;20:491-501.
22. Lauw FN, Simpson AJ, Prins JM, Smith MD, Kurimoto M, van Deventer SJ et al. Elevated plasma concentrations of interferon (IFN)-gamma and the IFN-gamma-inducing cytokines interleukin (IL)-18, IL-12, and IL-15 in severe melioidosis. J Infect Dis 1999;180:1878-85.
23. Altman DG, Bland JM. Diagnostic tests 3: receiver operating characteristic plots. BMJ 1994;309:188.
24. Baumann H, Gauldie J. The acute phase response. Immunol Today 1994;15:74-80.