Reliability of Procalcitonin Concentrations for the Diagnosis of Sepsis in Critically Ill Neonates

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We evaluated the reliability of serum concentrations of procalcitonin for the diagnosis of early- and late-onset sepsis in a neonatal intensive care unit (NICU) setting. Timed procalcitonin determinations were prospectively obtained during two postnatal periods: 0–48 hours of age (period 1) and 3–30 days of age (period 2). In period 1, we measured procalcitonin concentrations in 83 healthy newborns (group 0) and in 120 NICU patients (14 with culture-proven sepsis, group 1A; 14 with clinical septicemia, group 1B; 75 with no evidence of infection, group 2; and 17 with uncertain findings, group 3). After we established 95% hour-specific reference ranges for group 0, we performed multiple linear regression analyses to determine which maternal, intrapartum, and neonatal complications would affect normal procalcitonin values. Maternal diabetes was the only variable identified in group 2 patients that induced a significant deviation from procalcitonin reference ranges. Analyses of the pooled procalcitonin values obtained for group 1 patients over the 48-hour period after birth yielded a sensitivity of 92.6% and a specificity of 97.5% for procalcitonin concentrations in the detection of early-onset sepsis. In period 2, blood samples from 23 cases with systemic infections were analyzed for procalcitonin concentrations at the onset of signs of infection. The control group was formed by matching four uninfected NICU patients to each infected case. None of the procalcitonin values for the 92 controls overlapped those for the cases (sensitivity and specificity, 100%). Procalcitonin is a promising marker for the diagnosis of early- and late-onset sepsis in neonates at high risk for this infection.

In contrast to the low incidence of infection in nurseries, the incidence of infections in neonatal intensive care units (NICUs) is often high and surpasses that reported for other hospital services and intensive care units [1]. Sepsis is a major problem in NICUs throughout the world, which is not surprising because a select group of very sick patients are cared for in these units.

Early detection of neonatal sepsis is difficult because the first signs of this disease may be minimal and are similar to those of various noninfectious processes, and definitive blood culture results are not immediately available. Furthermore, the results of recent studies have underlined the concern about possible culture-negative clinical sepsis, particularly in the setting of increasing maternal antibiotic use [2]. The availability of a laboratory test to accurately and more rapidly identify septic neonates than is done by the isolation of microorganisms from body fluid specimens would therefore be of considerable value in improving the outcome of this challenging clinical

problem and in minimizing unnecessary treatment of uninfected NICU patients. Several indicators have been evaluated for the diagnosis of neonatal sepsis and have included various leukocyte indices and acute-phase protein levels. However, the inability of any single laboratory test to date to provide rapid, reliable, and early identification of infected (and, as importantly, noninfected) neonates has led to a search for other diagnostic markers [3].

It has recently been reported that the concentration of procalcitonin, a propeptide of calcitonin that undergoes posttranslational proteolysis into its hormonal derivative, increases markedly in children [4–6] and adults [7–10] with sepsis or severe infections, as compared with controls, and has appeared to be a good predictor of infection severity and therapeutic outcome. Although the cellular source and mechanisms underlying the induction of procalcitonin during septic conditions have not yet been elucidated, the increase in procalcitonin concentrations has not been associated with an increase in calcitonin concentrations [4, 11]. Furthermore, the finding that procalcitonin is released into the circulation of healthy volunteers within 4 hours after a single injection of endotoxin, plateaus at 6–8 hours, and remains elevated for at least 24 hours makes procalcitonin a promising new marker for the early and sensitive identification of infected patients [11].

The results of two recent studies have suggested that procalcitonin concentrations may also be useful indices of neonatal sepsis. In an investigation by Gendrel et al. [12], which was performed in a selected population of nonpremature neonates

with wide-ranging differences in age, procalcitonin levels were significantly higher in neonates with sepsis than in uninfected symptomatic neonates and healthy newborns [12]. In the subsequent investigation by Monneret et al. [13], which was performed in a heterogenous population that included both neonates admitted to the NICU and those admitted to the regular nurseries, procalcitonin concentrations obtained before or after 72 hours of age were significantly higher in infected neonates than in uninfected neonates. These observations needed to be confirmed and extended to critically ill neonates admitted to the NICU, particularly during the first 48 hours of life, when the majority of diagnostic problems are encountered.

We conducted the present prospective study to define normal procalcitonin ranges for a group of healthy neonates during the first 48 hours after birth in order to investigate whether noninfectious neonatal and perinatal events alter procalcitonin values and to determine the sensitivity and specificity of procalcitonin concentrations for the diagnosis of early-onset sepsis among patients admitted to the NICU over the first several hours after birth. During the same study period, a case-control study was begun to evaluate whether noninfectious complications of extended hospitalization in the NICU would affect the accuracy of procalcitonin concentrations for diagnosing late-onset infection.

Materials and Methods

This prospective study was conducted between February 1996 and February 1997 at ''La Sapienza'' University Hospital (Rome). During the study period, timed procalcitonin determinations were prospectively obtained according to two postnatal periods: 0–48 hours of age (period 1) and 3–30 days of age (period 2). These times were chosen to coincide with those previously reported for a strict differentiation between early- and late-onset neonatal infections [1].

Institutional review board approval is not required in our hospital for studies involving blood sampling, but permission to perform the investigation was obtained from the study subjects' parents or guardians.

Period 1 and study population.   Two distinct populations were sampled for procalcitonin concentrations. The first group included a group of healthy neonates who were admitted to the well-baby nursery service of the obstetrics unit, while the second comprised all symptomatic preterm and term neonates who were admitted to the NICU and were evaluated for sepsis before 49 hours of age. The NICU is the major tertiary care referral center for high-risk neonates in the Rome area. All procalcitonin determinations performed between 0 and 48 hours of age were recorded and timed for both study populations according to hours after birth.

Healthy neonates (group 0) were defined as those who were born after uncomplicated pregnancy and labor and who had normal postnatal courses until day 3 of life, when they were discharged home. For this group of neonates, procalcitonin

sampling coincided with routine blood collection performed on one or more occasions.

NICU infants were enrolled in the study at the time of admission, and serum samples for procalcitonin determination were obtained after routine sepsis studies had been completed. Whenever possible, subsequent procalcitonin values over period 1 were determined in samples collected at the time of routine blood sampling. At the time of initial evaluation, chest radiographs and blood samples from peripheral veins were obtained for every NICU patient for routine culture of aerobic and anaerobic bacteria and mycoplasmas. Cultures of CSF and urine were performed when appropriate. In addition, tracheal samples from intubated patients were cultured for mycoplasmas and, when obtained within 8 hours after birth, for aerobic and anaerobic bacteria [14]. Screening for IgM antibodies to *Toxoplasma,* rubella virus, cytomegalovirus, herpes simplex virus, and *Treponema pallidum* was done in the first few postnatal days if congenital infection seemed likely.

Sepsis screening, consisting of a WBC count, absolute neutrophil count, immature/total neutrophil ratio, and C-reactive protein (CRP) level, was performed for all infants at the time of initial evaluation; further blood samples for the sepsis screen were obtained daily following the patient's clinical course. Pathological leukocyte indices were defined according to the criteria of Manroe et al. [15] and Lloyd and Oto [16]. A serum CRP value of ≥ 1.0 mg/dL, as determined by the rate nephelometry method (Beckman Instruments, Brea, CA), was defined as abnormally elevated [17]. A sepsis screen in which any two or more of the four test results were abnormal was considered supportive of a diagnosis of infection [18, 19].

Demographic, pregnancy, and delivery data were collected shortly after birth, and neonatal and outcome data were prospectively collected until discharge or death.

Patients were classified as follows: group 1A, early-onset documented infection; group 1B, strong evidence of infection (or ''clinical septicemia''); group 2, no infection; and group 3, uncertain. Infection status was designated retrospectively because all neonates were considered at risk for, or demonstrated clinical evidence of, infection. Those of us (C. C., A. P., and L. P.) responsible for classifying the infants as septic vs. nonseptic were completely blinded to the procalcitonin values.

Group 1A included neonates with a positive culture of one or more blood specimens drawn within the first 48 hours of life, in association with clinical signs of infection. Cases of proven sepsis with associated clinical and radiographic findings of pneumonia were included in this category.

Group 1B included neonates with negative body-fluid cultures who met all of the following criteria: clinical signs of sepsis and/or radiographic findings consistent with pneumonia, a positive sepsis screen, and certain historical and clinical factors associated with increased risk for infection [20]. Clinical signs of infection were defined as the presence of three or more of the following categories of clinical signs: apnea/tachypnea/cyanosis/respiratory distress; bradycardia/tachycardia; hypoto-

nia/seizures; poor perfusion or hypotension; irritability/lethargy or poor feeding; or hepatosplenomegaly/jaundice/abdominal distension [21].

Group 2, designated as ''no infection,'' included neonates who presented with various types of distress and nonspecific abnormal clinical signs but were apparently well within 48–72 hours after birth.

Group 3 included newborns who could not be included in group 1 or 2. All of these infants had negative body-fluid cultures, had fewer than three clinical signs of infection, and had abnormal values for only one or none of the four sepsis screening tests.

Period 2 and study population. Patients between 3 days and 30 days of age who presented with systemic infectious conditions during their NICU stay had blood drawn for procalcitonin determinations at the onset of signs of infection and after complete recovery. Neonates who had had a documented episode of systemic infection within the previous 7 days were excluded. A control group was selected from all NICU patients who had no clinical and laboratory evidence of infection at the time of procalcitonin sampling during the same study period. Four controls were chosen for each case. The controls were matched to the individual cases as closely as possible for number of days in the hospital (i.e., until the index case occurred) and postnatal age.

Procalcitonin determination. Blood samples were obtained by venipuncture and centrifuged within 30 minutes of collection. Serum (40 µL, allowing a double determination) was stored at −70°C before analysis.

We used the LUMItest procalcitonin kit (Brahms Diagnostica; GmbH, Berlin), an immunoluminometric assay for the specific measurement of procalcitonin in serum (limit of detection, 0.08 ng/mL). Two antigen-specific monoclonal antibodies that bind procalcitonin (the antigen) at two different binding sites, e.g. the calcitonin and katacalcin segments, are used for this assay. The assay was performed according to the recommended procedure. Luminescence was measured automatically in a Berilux Analyzer 250 (Behring Diagnostics, Marburg, Germany). Results of the LUMItest were calculated with the assistance of the software built into the analyzer. Interassay and intra-assay variations at both low and high concentrations were <8% and 7%, respectively. The assay could be completed within 2 hours.

Statistical analysis. This study had four objectives. First, to determine the reference ranges for procalcitonin across the range of postnatal hours from 0 hours to 48 hours, we constructed 95% hour-specific reference ranges for healthy neonates by following the sequence of six steps described by Royston [22] (see Statistical Appendix).

Our second objective was to compare procalcitonin levels obtained for NICU patients with noninfectious perinatal and/or neonatal complications to the established reference ranges. We then performed multiple linear regression analyses to explore the association between the procalcitonin response during the first 48 hours after birth and the following variables identified for the symptomatic uninfected group of neonates: age (e.g., hours), sex, birth weight, gestational age, birth asphyxia (e.g., Apgar score of ≤5 at 5 minutes of life), mode of delivery, pregnancy-induced hypertension, gestational diabetes, stressful labor (e.g., prolonged second stage, breech presentation, or placental abnormalities), prenatal steroid exposure, prolonged rupture of membranes (>24 hours; PROM), hyaline membrane disease, intraventricular hemorrhage (IVH), hemolytic disease, and hypocalcemia [23].

Our third objective was to assess the reliability of the procalcitonin concentration for the diagnosis of early-onset sepsis. The sensitivity and specificity of this marker were calculated by using the upper limits (or 97.5th percentile) of the reference sample for the first 48 hours of life as a cutoff point.

Our final objective was to determine the reliability of the procalcitonin concentration for the diagnosis of sepsis from 3 days to 30 days after birth. In this respect, sensitivities and specificities were calculated by receiver operating characteristics.

All statistical tests were based on a significance level of <.05.

Results

Period 1

Study population. The study population comprised 83 healthy newborns and 126 newborns admitted to the NICU. Six of the 126 neonates admitted to the NICU were excluded from the analysis because they had serious noncardiac congenital malformations (n = 2) or cardiac malformations (n = 4); thus, 120 patients were available for analysis.

Group 1A included 14 neonates with culture-proven sepsis (table 1). The two cases with initial blood cultures positive for *Staphylococcus epidermidis* were considered to have true septicemia on the basis of their clinical courses and results of ancillary laboratory studies [2, 24]. Of the 14 infants with proven sepsis, five also had associated radiographic evidence of pneumonia together with tracheal aspirates positive for group B *Streptococcus* (one), *Ureaplasma urealyticum* (two) [25], or *Staphylococcus aureus* (two) within 8 hours after birth.

Group 1B was composed of 14 neonates with negative blood cultures but definite clinical signs of sepsis and positive sepsis screens. These 14 neonates all were delivered of mothers who had received antibiotics before delivery because of clinical (n = 8) and/or histopathologic (n = 3) evidence of chorioamnionitis [26], vaginal cultures persistently positive for group B *Streptococcus* (n = 4), or preterm PROM (n = 2). Four of the 14 group 1B patients had radiographic evidence of pneumonia. All 14 neonates survived.

Group 2 included 75 neonates who presented with one or more conditions, including birth asphyxia (41), hyaline membrane disease (33), transient tachypnea (6), pneumothorax (3),

Table 1. Serial procalcitonin determinations for neonates with early-onset culture-proven septicemia.

Case no.	Blood isolate	First sample			Second sample			Outcome
		Age (h)	PCT level (ng/mL)*	CRP level (mg/dL)	Age (h)	PCT level (ng/mL)*	CRP level (mg/dL)	
1	GBS	3	500	2.1	27	500	10.5	Died
2	GBS	2	5.0	0.1	28	692.3	7.5	Survived
3	GBS	21	42.9	1.1	45	17.1	1.9	Survived
4	*Escherichia coli*	6	12.8	0.3	48	89.3	4.7	Survived
5	*E. coli*	20	70.1	1.7	44	42.7	2.9	Survived
6	*E. coli*	12	76.7	0.5	37	37.9	4.5	Survived
7	*E. coli*	24	41.7	1.8	ND	. . .	. . .	Died
8	*E. coli*	24	98.2	3.5	ND	. . .	. . .	Survived
9	*Staphylococcus aureus*	12	60.5	0.4	48	20.9	2.3	Survived
10	*S. aureus*	5	4.3	0.1	24	40.8	0.7[†]	Survived
11	*Staphylococcus epidermidis*	1	0.4 (≠)	0.2	25	40.2	0.6[†]	Survived
12	*S. epidermidis*	21	1.3 (≠)	0.1	45	28.9	1.8	Survived
13	*Ureaplasma urealyticum*	12	63.1	0.4	30	12.8	3.1	Survived
14	*U. urealyticum*	12	79.3	1.5	48	39.2	3.9	Survived

NOTE.   CRP = C-reactive protein; GBS = group B streptococcus; ND = not done; PCT = procalcitonin.

* Values were outside upper limits of the reference range, unless specified (≠).

[†] Abnormal CRP response was demonstrated in cases 10 (1.9 mg/dL) and 11 (2.1 mg/dL) by third determinations obtained at postnatal hours 48 and 47, respectively.

IVH (2), hemolytic disease (1), hypoglycemia (10), hypocalcemia (14), and meconium aspiration syndrome (3). Seventy-one (94.6%) of these 75 neonates were born after pregnancies complicated by diabetes (5 neonates), pregnancy-induced hypertension (10), stressful labor (17), preterm PROM (7), preterm gestation (51), or intrauterine growth retardation (7). A history of prenatal steroid exposure was present in 28 group 2 patients. All 75 of these neonates, none of whom had clinical or microbiological evidence of infection over their postnatal courses, received antibiotic therapy for ≤3 days. Nonetheless, seven of the 75 neonates had abnormal values for one ($n = 3$) or more ($n = 4$) of the four sepsis screening tests. These seven neonates included four who had abnormal CRP results (specificity, 94.6%).

Group 3 included 17 neonates in whom systemic infection could neither be confirmed nor excluded on the basis of the clinical, microbiological, and laboratory findings. Antibiotics were administered to these patients for ≥6 days.

There were no significant differences among the three patient groups with respect to gestational age (group 1, mean [±SD] gestational age, 34.6 ± 4.7 weeks; group 2, 33.7 ± 4.1 weeks; group 3, 35.0 ± 3.4 weeks); however, the patients in group 2 had significantly lower birth weights (mean [±SD] birth weight, 2.0 ± 0.8 kg; $P < .01$, analysis of variance) than those in groups 1 (2.5 ± 0.9 kg) and 3 (2.5 ± 0.8 kg). The healthy controls included neonates who were significantly larger (mean [±SD] birth weight, 3.2 ± 0.4 kg; $P < .001$, analysis of variance) and more mature (mean [±SD] gestational age, 39.1 ± 1.2 weeks; $P < .001$, analysis of variance) than those in all three patient groups.

Procalcitonin values among study groups.   A total of 124 procalcitonin determinations were obtained for group 0 to construct procalcitonin reference ranges from 0 hours to 48 hours after birth (figure 1). The predicted lower limits were <0.08 ng/mL soon after birth, with peak levels of 0.6 ng/mL from 21 hours to 24 hours after birth and a return to the initial values (<0.08 ng/mL) at 47–48 hours of life. The predicted upper
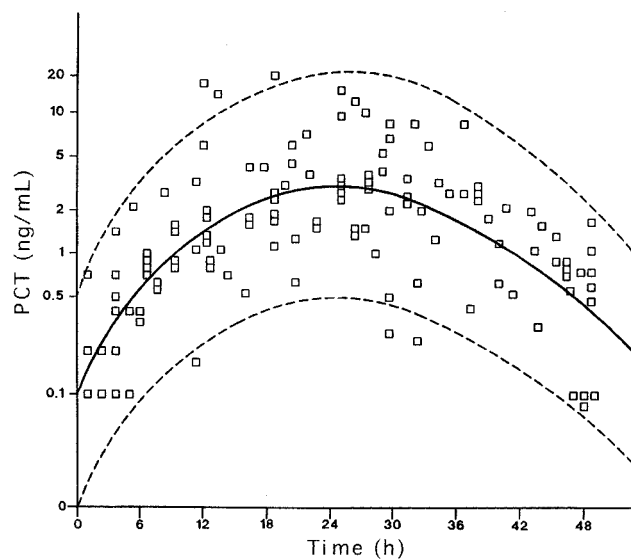

Figure 1. Age-specific 95% reference range for procalcitonin (PCT) in healthy neonates (group 0; see text) from birth to 48 hours of life. The squares represent single values; the dotted lines represent lower and upper limits; the bold line represents the geometric mean.
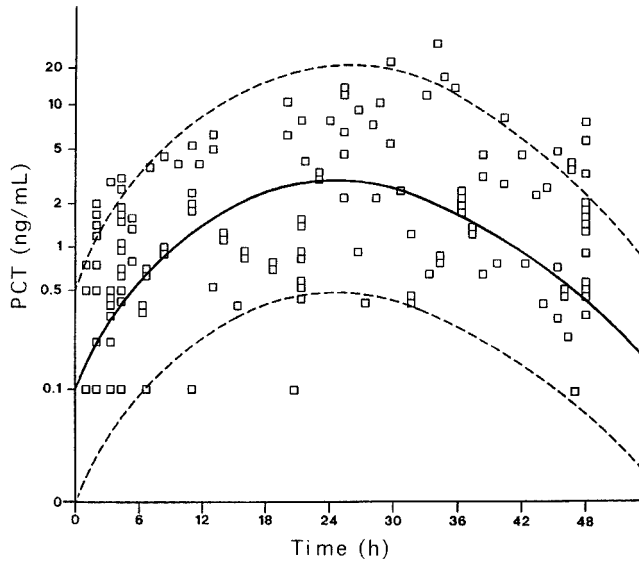
Figure 2. Distribution of procalcitonin (PCT) values obtained from uninfected patients (group 2; see text) between birth and 48 hours of age. Squares represent single values. The dotted lines represent lower and upper limits of the reference range; the bold line represents the geometric mean.

limits were observed soon after birth at 0.7 ng/mL, increased gradually to reach the peak values of ∼21 ng/mL between 21 hours and 24 hours of age, and then decreased gradually to 2.0 ng/mL by 48 hours of life. Of the 124 values used to construct the reference range, three (2.4%) were higher than the upper limit and three (2.4%) were less than the lower limit.

Group 2 (uninfected patients) provided 137 procalcitonin determinations (figure 2). Of these, 22 (16.0%) fell outside the established reference ranges, and 17 (12.4%) were above the 97.5th percentile. We performed multiple linear regression analysis to identify variables that might contribute to an increase in procalcitonin values. Apart from age (hours), none of the variables identified from the maternal and neonatal histories (see statistical methods) had statistically significant effects on the values for procalcitonin, although the variable diabetes mellitus approached significance (P = .10). Inclusion of only the age and gestational diabetes variables indicated that procalcitonin values were significantly (P < .05) higher for neonates born to diabetic mothers.

Group 1 provided a total of 54 procalcitonin determinations (figure 3). Of these, 50 were above the upper limits of the reference range, whereas four were below, yielding an overall sensitivity of 92.6%, a specificity of 97.5%, a positive predictive value of 94.3%, and a negative predictive value of 96.8%. It is noteworthy that none of the infants with early-onset sepsis had been delivered of diabetic mothers. Thus, all abnormal values could be accepted as indicating the presence of infection.

Group 3 provided a total of 20 procalcitonin determinations. Of these, 11 (55.0%) fell outside the upper limits of the normal

range. Because the diagnosis of neonatal infection in this group could be neither confirmed nor excluded, the sensitivity and specificity of the procalcitonin concentrations were not calculated.

Procalcitonin and CRP sensitivities. Of the 28 group 1 patients, 24 (sensitivity, 85.7%) had abnormal procalcitonin values on initial evaluations (table 1). Three (one in group 1A and two in group 1B) of the four initial false-negative procalcitonin results were obtained within the first 3 hours of life, while the last one (group 1A) was obtained 21 hours after birth. Abnormal procalcitonin responses were demonstrated in the four patients within the subsequent 18–24 hours. In contrast, 13 (sensitivity, 46.4%; P = .004, Fisher's exact test) of the 28 group 1 patients had abnormal CRP levels on initial determinations (table 1). Most (14 of 15) initial false-negative CRP results (seven in group 1A and seven in group 1B) occurred within the first 12 hours (median, 7 hours) after birth. All 15 infants with initial false-negative CRP results showed subsequent abnormal CRP responses within the first 24 (n = 12) or 48 (n = 3) hours after the initial CRP investigation.

Period 2

Study population. During the study period, 23 cases with late-onset infection (mean [±SD] postnatal age, 14.7 ± 9.1 days) and 92 controls (mean [±SD] postnatal age, 14.5 ± 9.1 days) were available for determining the sensitivities and specificities of procalcitonin concentrations across the range 3–30 days of age. There were no significant differences be-
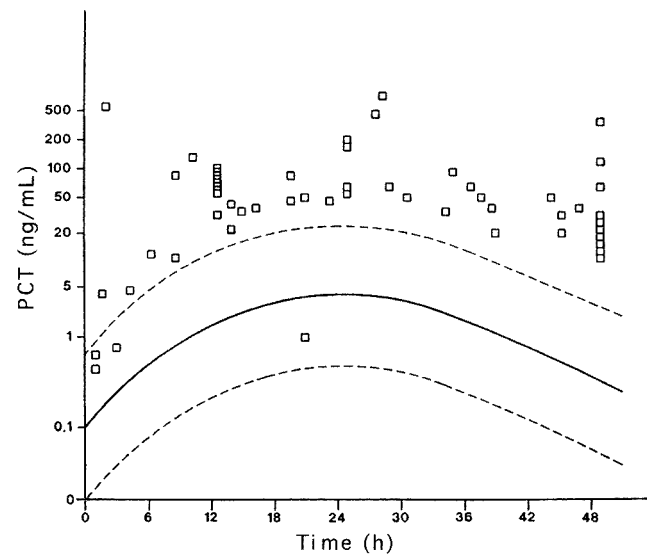

Figure 3. Distribution of procalcitonin (PCT) values obtained for patients with early-onset infection (group 1; see text) between birth and 48 hours of age. The squares represent single values; the dotted lines represent lower and upper limits of the reference range; the bold line represents the geometric mean.

Table 2. Clinical and laboratory findings for newborns with late-onset infection.

Case no.		Blood isolate	Times of sampling (postnatal d)	PCT level (ng/mL)	CRP level (mg/dL)	Outcome
1	Sepsis	*Staphylococcus aureus*	3	12.4	1.8	Survived
2	Sepsis	*Staphylococcus epidermidis*	4	4.3	1.6	Survived
3	Sepsis	*Klebsiella pneumoniae*	6	249.1	5.9	Survived
4	NEC (IIIa)*	. . .	6	50.5	4.5	Died
5	Sepsis	*Staphylococcus haemolyticus*	7	8.8	0.2	Survived
6	Sepsis	*S. epidermidis*	7	2.0	1.4	Survived
7	NEC (IIa)*	. . .	7	8.1	2.8	Survived
8	Sepsis	*K. pneumoniae*	7	59.7	1.9	Survived
9	Sepsis	*S. aureus*	8	57.3	5.0	Survived
10	Sepsis	*S. epidermidis*	9	5.0	0.4	Survived
11	Sepsis	*S. epidermidis*	10	2.0	0.1	Survived
12	Sepsis	*S. epidermidis*	12	2.7	1.6	Survived
13	Sepsis	*S. haemolyticus*	14	235.7	3.2	Died
14	Sepsis	*S. epidermidis*	15	3.3	0.1	Survived
15	Sepsis	*Candida albicans*	17	15.6	3.9	Survived
16	Sepsis	*S. aureus*	19	62.9	8.6	Survived
17	Sepsis	*S. epidermidis*	20	6.7	0.3	Survived
18	Sepsis	*S. epidermidis*	21	2.4	0.3	Survived
19	Sepsis and meningitis	*Pseudomonas aeruginosa*[†]	25	101.4	4.2	Survived
20	Sepsis	*K. pneumoniae*	27	39.7	10.4	Survived
21	Sepsis	*S. epidermidis*	29	2.2	7.8	Survived
22	NEC (IIb)*	. . .	30	22.8	20.9	Survived
23	Sepsis	*S. epidermidis*	30	9.4	0.5	Survived

NOTE. CRP = C-reactive protein; NEC = necrotizing enterocolitis; PCT = procalcitonin.

 * Staging criteria according to Walsh et al. [27].

 [†] The organism was also recovered from the CSF of the neonate.

tween cases and controls with respect to gestational age (mean [±SD] gestational age, 31.5 weeks ± 4.5 weeks vs. 32.8 ± 4.6 weeks) and birth weight (mean [±SD] birth weight, 1.5 ± 0.9 kg vs. 1.7 ± 0.7 kg). Twenty of the 23 cases had culture-proven sepsis and/or meningitis, and three had necrotizing enterocolitis but negative blood cultures (table 2). All cases with blood cultures positive for coagulase-negative staphylococci had clinical signs of septicemia, and the positive culture results were associated with positive sepsis screens of initial or later samples. Four of the 12 neonates with late-onset sepsis due to coagulase-negative staphylococci had more than one blood culture positive for the same species of coagulase-negative staphylococcus.

Procalcitonin and CRP sensitivities. At the time of initial evaluation, procalcitonin concentrations were significantly higher in the 23 cases (mean [±SE] concentration, 41.9 ± 14.3 ng/mL; $P < .0001$, Student's t test) than in the 92 controls (mean [±SE] concentration, 0.2 ± 0.02 ng/mL). In cases, procalcitonin levels ranged from a minimum of 2.0 ng/mL to a maximum of 249.1 ng/mL, while in controls procalcitonin levels ranged from 0.08 ng/mL to 1.0 ng/mL (sensitivity and specificity, 100%). Two of the 23 cases died, and 21 survived. There was no decline in procalcitonin concentrations in the two nonsurvivors until death. Procalcitonin levels returned to normal (<1.0 ng/mL) in the 21 survivors 3–7 days after initiation of appropriate antibiotic therapy.

Considering only survivors, the mean (±SE) procalcitonin levels in cases with sepsis caused by coagulase-negative staphylococci ($n = 11$) were lower (4.4 ± 0.8 ng/mL; $P < .0001$) than in those ($n = 10$) with other systemic infectious conditions (62.9 ± 22.6 ng/mL).

When CRP levels were considered alone as initial indicators of late-onset infection (table 2), seven of the 23 cases were missed (sensitivity, 69.5%). These cases had sepsis due to coagulase-negative staphylococci, with abnormal increases in CRP levels that were apparent 24–48 hours after onset of sepsis.

Discussion

Early-onset infection. Our study on the reliability of procalcitonin concentrations for diagnosing early-onset sepsis was performed in the clinical setting of a referral NICU, where a population of critically ill infants with a wide spectrum of clinical problems is routinely evaluated for possible sepsis at different, unselected times over the first several hours after birth. In this context, we have delineated a clinically useful set of procalcitonin reference ranges that allow a considered approach to the identification of infected and uninfected neonates at high risk throughout the initial 48 hours of life.

Our data demonstrate that during the 48-hour period after birth, normal procalcitonin values change hourly. This finding contrasts with that of Gendrel et al. [12], who reported that in

healthy neonates (procalcitonin value, <0.7 ng/mL), the mean value did not differ significantly from those for healthy children and adults. In that study, however, the ages of healthy neonates ranged from 1 day to 5 days, with the mean age unspecified. Our results confirm and extend the findings in a previous report by Monneret et al. [13], in which the authors mentioned the relative procalcitonin peak on the first day of life among uninfected infants. In the present study, we report a previously uninvestigated feature, the kinetics of procalcitonin during the first 2 days of life in healthy full-term newborns. Although the cellular origin and metabolic pathways of the increase in the procalcitonin concentration seen during the immediate postnatal period in infants who are apparently healthy are not known, this increase seems to reflect a physiological mechanism (independent of any stimulus due to infection status or other abnormal clinical states).

Of the various maternal, intrapartum, or neonatal complications that might affect the interpretation of procalcitonin values during the 48-hour postnatal period, we identified gestational diabetes as the only variable that induced a significant deviation from procalcitonin reference ranges. Birth weight, length of gestation, or other factors inherently due to either prematurity or low birth weight did not significantly affect normal procalcitonin values, demonstrating therefore that the level of development had no effect on the procalcitonin response. Consequently, it appears that the finding of an abnormal procalcitonin value during this postnatal period, in the absence of gestational diabetes, may be a useful diagnostic marker for separating early-onset infection from other causes of neonatal distress.

In our clinical setting the overall sensitivity of the procalcitonin concentration for the diagnosis of early-onset sepsis was high. Nonetheless, as our report demonstrates, a normal procalcitonin value might actually be most expected during the first few hours of life for some infants. Possibly related to this phenomenon were the longer induction periods for neonatal response after exposure to etiologic agents such as coagulase-negative staphylococci or, in some instances, to transplacentally passed antibiotics, which have an unpredictable effect on culture results as well as on the responses of neonates. However, from a practical perspective, when procalcitonin values were compared at the corresponding time with CRP values, the most commonly used reference markers—the sensitivities of procalcitonin and CRP—were 85.7% and 46.4%, respectively, in initial determinations; these sensitivities rose to 100% and 89.2%, respectively, by the time of the next determinations. The findings concerning the CRP response correlate well with those recently reported by Messer et al. [28]. These investigators found that the sensitivity of the CRP level in initial determinations was only 45% for patients with documented early-onset septicemia and 44% for those with clinical septicemia; the specificity of this level was 96%. In addition, Messer et al. found that the overall sensitivity of the CRP level had risen to 83% 24 hours later.

Ideally, to be useful in establishing the diagnosis of neonatal sepsis, any diagnostic test should have maximum sensitivity (i.e., not miss any cases of sepsis) and maximum specificity (i.e., exclude sepsis when the test is negative). Our data show that the procalcitonin concentration appears to be a highly specific and sensitive marker of early-onset neonatal sepsis, provided that whenever clinical evidence conflicts with a normal procalcitonin result during the early postnatal period, procalcitonin determinations are repeated within the next 24 hours to maximize identification of the infected neonate. If these results are confirmed for larger groups of patients, the use of this marker should offer the possibility of reducing the frequency of unnecessary antibiotic therapy.

Late-onset infection.   In the present study, the procalcitonin concentration after the first 48 hours of life proved to be an ideal marker for identifying in the early course neonates in the NICU who developed infectious complications. This finding stems from the fact that none of the procalcitonin values for controls overlapped those for patients with systemic infectious conditions.

After excluding the subpopulation of nonsurvivors whose elevated procalcitonin levels were also correlated with outcome, we noted distinct elevations of procalcitonin in infants with late-onset infection due to coagulase-negative staphylococci, and we suspected that these levels, which were distinctly lower than those found in cases with other systemic infectious conditions, might reflect a systemic response to the typically indolent course of infection with these organisms [29]. Nosocomial sepsis due to coagulase-negative staphylococci is currently an important and frequent event in NICUs [30].

Although our study included small numbers of patients and more stringent data are needed, our finding is encouraging. DaSilva et al. [31] studied the diagnostic value of leukocyte indices in late neonatal sepsis and found that these indices, interpreted either independently or in combination, were of low diagnostic accuracy for evaluating neonates with suspected late sepsis caused by coagulase-negative staphylococci. Schmidt et al. [32] studied the pathogenicity of coagulase-negative staphylococci in newborns and found that CRP levels were abnormal at the time of initial evaluation in 64% of subjects (mean postnatal age, 25 days) with sepsis due to these pathogens. This finding is in keeping with our finding that when CRP levels are used alone for diagnosis, a significant proportion of patients with nosocomial systemic disease caused by coagulase-negative staphylococci will be missed in the early phase.

In the NICU, considerable overhospitalization and overuse of antibiotics, with possible emergence of multiresistant organisms, have a substantial impact on the morbidity and mortality associated with late-onset infection [33]. Therefore, the increasing pressure for cost containment and more efficient medical care has sparked renewed interest in devising improved diagnostic strategies that enable clinicians to identify neonates with late-onset infection early and to target them for antibiotic therapy. Fortunately, we can conclude that in addition to the observed favorable clinical experience with the use of procalcitonin levels for diagnosing early-onset infection, procalcitonin

appears to be an effective laboratory marker for accurately identifying NICU patients early in the course of late-onset infections and for monitoring the clinical courses of these patients. The amount of blood needed for determination of procalcitonin levels is small, the results can be available within 2 hours, and the cost is moderate.

Statistical Appendix

Reference ranges were constructed following the strategy described in a detailed paper by Royston [22] in which it is recommended that the following steps be followed: (1) plot data; (2) fit a polynomial curve; (3) assess the residuals; (4) transform the data, if necessary; (5) check the distribution throughout the range of the independent variable; and (6) calculate the reference range.

For the data on procalcitonin values according to the age of healthy neonates, there were no outliers or peculiarities, and there was a clear tendency for the mean values of procalcitonin to increase to a peak after birth and then to decrease to approximately the level observed at birth. The points were not normally distributed about the trend but positively skew, and the variability was greater where the mean was higher. These latter two observations suggested that it might be useful to analyze the natural logarithm (ln) of the procalcitonin levels. In the polynomial regression of ln procalcitonin on x (hours after birth of the neonate), the cubic term was significant (a quartic term was tried but was not statistically significant).

The polynomial regression of ln procalcitonin on age x age^2 and age^3 was: ln procalcitonin = $-2.3330 + 0.36563x - 0.010748x^2 + 7.7145 \times 10^{-5}x^3$, and the standard deviation of the residuals was 0.88550. To calculate the predicted values of ln procalcitonin, the values of age in hours were inserted in the equation in place of x. The upper and lower limits of the normal range were calculated as the predicted value plus or minus twice the standard deviation. The standard error of the values of the lower and upper limits of ln procalcitonin could be calculated to be 0.136 by using the formula of Royston, which corresponded to 14.5% of the value of the limit.

To investigate the possible bias introduced by including all the 124 observations made on 83 healthy neonates, we took a random sample of one observation per patient, and the calculations of the normal ranges were repeated. The polynomial regression equation for the random sample of 83 single observations on the 83 neonates (ln procalcitonin = $-2.2562 + 0.35971x - 0.010461x^2 + 7.3331 \times 10^{-5}x^3$, with the standard deviation of the residuals = 0.88087) was very similar to that derived from all the 124 observations (see above). The predicted values of procalcitonin derived from the two equations never differed by more than 7%, and this maximum difference occurred at 1 hour after birth. Since a difference of 7% can have little clinical relevance, we chose to present the normal ranges on the basis of all 124 observations in order to maximize their precision.

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