C reactive protein and procalcitonin: Reference intervals for preterm and term newborns during the early neonatal period

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A B S T R A C T

Background: There is still no study evaluating the influence of gestational age (GA) per se on C reactive protein (CRP) and procalcitonin (PCT) reference intervals. We therefore investigated how length of gestation, age (hours), and prenatal and perinatal variables might influence the levels of CRP and PCT. We also determined 95% age-specific reference intervals for CRP and PCT in healthy preterm and term babies during the early neonatal period.

Methods: One blood sample (one observation per neonate) was taken for CRP and PCT from each newborn between birth and the first 4 (for term), or 5 days (for preterm newborns) of life by using a high-sensitive CRP and PCT assays.

Results: Independently of gender and sampling time, GA had a significantly positive effect on CRP, and a significantly negative effect on PCT. Compared with healthy term babies, healthy preterm babies had a lower and shorter CRP response, and, conversely, an earlier, higher, and longer PCT response. CRP reference intervals were affected by a number of pro-inflammatory risk factors.

Conclusions: Age- and GA-specific reference ranges for both CRP and PCT should be taken into account to optimize their use in the diagnosis of early-onset neonatal sepsis.

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1. Introduction

The utility of C reactive protein (CRP) for the diagnosis of early-onset neonatal infection has been the subject of controversy because of its unsatisfactory sensitivity. The CRP concentration increases physiologically in newborns within the first days after birth. This dynamic behavior may in part account for the low diagnostic accuracy of CRP measurements in neonatal infection, particularly when measured shortly after birth. Similarly, other markers of inflammation including procalcitonin (PCT) demonstrate a natural increase within a few days after birth, necessitating careful adjustments to the normal ranges. Thus, in estimating the sensitivities and specificities of these neonatal markers for the diagnosis of sepsis throughout the first days of life, it is important to consider their normal kinetics and their pattern(s) of response in the healthy neonate.

Data pertaining to reference intervals for CRP during the neonatal period are limited. In the majority of published reports, CRP upper limits have been obtained from symptomatic uninfected patients [1–11]. There are few studies of upper limits for CRP in the healthy newborn [12–15]. Furthermore, most of these were based on relatively small sample sizes with wide-ranging postnatal ages [12–14]. More important, although advances in neonatal intensive care have led to increasing preterm birth rates, to our knowledge, there is still no study evaluating the influence of gestational age (GA) per se in the development of CRP reference intervals during the neonatal period. Finally, though high-sensitivity assay for CRP has become available over the last decade in standard clinical laboratories, there is no study assessing the CRP postnatal changes in the healthy preterm and term neonates using this analytic method.

Data pertaining to reference intervals for PCT are also limited. In a previous cross-sectional study, we showed that in the healthy term neonates circulating concentrations of PCT increase gradually from birth to reach peak values at about 24 h of age and then decrease gradually by 48 h of life [16]. No similar normogram exists for the healthy preterm infants even though they may have different pharmacokinetics.

Abbreviations: CRP, C reactive protein; PCT, procalcitonin; GA, gestational age; BW, birth weight; CRPHS, C reactive protein high sensitive.

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For these reasons, we sought to describe the reference ranges for both CRP and PCT in a large sample of healthy preterm and term newborns during the first days of life by using more sensitive CRP and PCT assays than those previously reported [1–18].

2. Materials and methods

2.1. Subjects

Our population consisted of healthy preterm and term infants who, from birth, were prospectively recruited over an 18-month period to the Neonatal Unit of Policlinico Umberto I’ Hospital, Sapienza University of Rome. The study protocol was approved by the clinical research ethics committee of the Hospital. Eligible for enrollment were neonates who were delivered from singleton pregnancies with birth weights (BWs) appropriate for GA and with normal results on physical examination at birth that implied no need for empiric management. We excluded babies born to mothers with: (a) multiple preexistent or pregnancy-related noninfectious complications; or (b) clinically evident intra-amniotic infection. The neonate was included in the study if all of the following criteria were met: a) one peripheral blood sample (corresponding to one observation per neonate) had been obtained after maternal consent for simultaneous measurement of both CRP and PCT between birth and the first 4 (for term), or 5 days (for preterm newborns) of life at the time of a routine blood collection or routine newborn screening for inborn errors of metabolism; (b) the neonate had a continuous uncomplicated postnatal hospital stay and was discharged as healthy from the Hospital; and (c) the neonate had normal assessments at the 2–wk (for term and preterm) and 4–wk (for preterm neonates) follow-up visits. The time limit of sample collection was extended in the preterm babies to postnatal day 5 because a previous study in symptomatic uninfected preterm babies suggested a return to initial PCT values after 4 days of life, implying therefore a longer PCT rise in preterm than in term infants [17]. A total of 421 newborns qualified for analysis of CRP and PCT reference intervals.

All antepartum and intrapartum data were collected prospectively, and included maternal age, preexistent or pregnancy-related diseases, prenatal steroid exposure, mode of delivery, duration of active labor, interval between rupture of membranes and delivery, intrapartum antimicrobial administration, and intrapartum fetal distress [19]. The institutional policy was to give penicillin or broad-spectrum antibiotics to all women identified as group B streptococcus (GBS) carriers at 35–37 weeks of gestation [20]. If the results of GBS cultures were not known at the onset of labor or rupture of membranes, intrapartum antimicrobial prophylaxis was administered if either or both of the following risk factors were present: preterm labor at <37 weeks of gestation, and rupture of membranes ≥18 h before delivery [20]. Neonatal data included GA, BW, and gender. GA was established on the basis of best obstetric estimates, including last menstrual period and first or second trimester ultrasonography.

2.2. CRP and PCT determinations

Serum samples for duplicate CRP and PCT determinations were stored in small aliquots at −80 °C until assayed. CRP concentrations were measured on a COBAS 6000 analyzer (Roche Diagnostics) with a particle-enhanced immunoturbidimetric method using latex particles coated with monoclonal antibodies anti-CRP antibodies and turbidity reading of the precipitate at 552 nm [CRP high sensitive (CRPHS) assay; Roche Diagnostics]. The limit of quantification for CRPHS assay was 0.3 mg/L, and the day-to-day imprecision (50 determinations) was 2.9% at 0.4 mg/L and 1.5% at 12.6 mg/L. PCT was estimated by an immuno-time-resolved amplified cryptate technology assay (Kryptor PCT; BRAHMS). This assay is based on a sheep polyclonal antibody against calcitonin and a monoclonal antibody against katacalcin, which bind to the calcitonin and katacalcin sequence of calcitonin precursor molecules. The assay is very sensitive, with a limit of quantification of 0.06 μg/L. According to the manufacturer the inter-assay CV is 3% on the whole PCT concentration range. CRP and PCT determinations were performed without knowledge of the antepartum, intrapartum, and neonatal data.

2.3. Statistical analysis

This study had two broad objectives. The first was to investigate how length of gestation, age (hours) and other characteristics of the mothers and babies might influence the levels of CRP and PCT. The second was to determine 95% age-specific reference intervals for CRP and PCT for postnatal ages up to a maximum of 120 h after birth.

From previous studies [15,16,18] it was expected that the levels of CRP and PCT would be approximately log-normally distributed, and when the data confirmed this, the natural logarithms of all observed values of CRP and PCT were calculated and used in all subsequent analyses. In order to investigate how the length of gestation, age (hours) and other characteristics of the mothers and babies might influence the levels of ln CRP and ln PCT, GA was classified as preterm and term, and the multiple log-linear regression of CRP and PCT on the independent variables was calculated. The results of these regression analyses showed that the relationships between ln CRP and age and ln PCT and age were not linear and this implied that the reference intervals should be calculated using polynomial regression. The strong effect of GA suggested that reference intervals of CRP and PCT would be necessary for preterm and term babies separately.

The four 95% age-specific reference ranges were constructed following the sequence of six steps described by Royston [21]. The six steps are: (1) plot the data, (2) fit a polynomial curve, (3) assess the residuals, (4) transform the data, (5) check the distribution throughout the range of the independent variable and (6) calculate the reference range. For both preterm and term babies, the plots of the data of PCT and CRP, against age (hours) showed no outliers or peculiarities and there was a clear tendency for the values to increase immediately after birth reaching a peak, after which the PCT values declined while the CRP values also decreased but more slowly. The individual points on the scatter diagrams were not normally distributed about the trend, but positively skewed and the variability was greater where the mean was higher. These two observations, together with the evidence provided by earlier studies [15,16,18], confirmed that the natural logarithms of the PCT and CRP levels should be used for the calculation of the reference intervals in order to normalize the distributions and stabilize the variance of the residuals. This was checked by inspecting histograms of the residuals, plots of the residuals against age in hours, and normal probability plots from which the Shapiro–Francia statistic was calculated.

To calculate the polynomial regression, the response (ln CRP or ln PCT) was regressed first on age, x, then x and x^2, then x, x^2, x^3 and so on until the addition of further powers of age did not produce statistically significant coefficients. To calculate the predicted values of ln CRP and ln PCT, the values of age measured in hours were inserted into the polynomial regression equation. The upper and lower limits were calculated as the predicted value plus or minus twice the standard deviation of the residuals.

3. Results

3.1. Subject characteristics

Table 1 presents maternal and neonatal characteristics of the study population, of whom 221 were born at term (range 37.0–39.0 weeks) while 200 were preterm (range 30.0–36.0 weeks), with 27 out of the 200 preterm infants (13.5%) below 33 weeks of gestational age. Most babies (94.8%) were born to Caucasian mothers.
3.2. C reactive protein

Using multiple log-linear regression analysis, it was found that GA had a significant, positive effect on CRP independently of gender and sampling time. Babies’ mean CRP increased by 6.0% (95% CI, 1.1–11.2%; P < 0.01) per week of GA at delivery. When BW was included instead of GA (because of collinearity), the mean CRP increased by 2.4% (95% CI, 0.7–4.2%; P < 0.01) per 100 g of BW independently of gender and neonatal age. Babies’ CRP was not associated with gender. This prompted us to assess separate CRP reference intervals for the term and preterm neonates.

For the term neonates, the predicted CRP levels (lower and upper limits) were, at birth, 0.1 (0.01–0.65) mg/L, increased gradually to 1.5 (0.2–10.0) mg/L at 21 h of age, after which they slightly increased to reach 1.9 (0.3–13.0) mg/L at 56–70 h, and then declined to 1.4 (0.2–9.0) mg/L at 96 h (Fig. 1). For the preterm infants, the predicted CRP levels were 0.1 (0.01–0.64) mg/L at birth, with peak levels of 1.7 (0.3–11.0) mg/L at 27–36 h, and then declined to 0.7 (0.1–4.7) mg/L at about 90 h (Fig. 2). There appeared to be a tendency for the values to increase again after about 100 h reaching 4.9 (0.7–32.0) mg/L at 120 h but this should be interpreted cautiously given that there are few observations at these ages. Of the 221 values used to construct CRP reference intervals in the term neonate, seven (3.2%) were higher than the upper limit and one (0.5%) was less than the lower limit. Of the 200 values used to construct CRP reference intervals in the preterm neonate, five (2.5%) were higher than the upper limit and five (2.5%) were less than the lower limit.

The variance of the residuals did not change with age, and they were normally distributed. The Shapiro–Francia statistic was 0.983 for the term babies and 0.980 for the preterm babies.

Of the antenatal and perinatal variables, duration of active labor, prenatal steroids, time of ruptured membranes, and intrapartum antimicrobial prophylaxis had a significant effect on CRP concentrations when adjusted for GA, gender, and sampling time. The mean CRP concentration was increased by 0.4% (95% CI, 0.15–0.67%; P < 0.05) per hour of ruptured membranes, and by 14.5% (95% CI, 5.2–25.0%; P < 0.01) per hour of active labor. The babies’ mean CRP concentration was increased by 40% (95% CI, 0.0%–95.5%; P < 0.05) and by 28% (95% CI, 0.0%–64.0%; P < 0.05) if the mother had a history of prenatal steroid exposure and intrapartum antimicrobial prophylaxis, respectively. CRP concentrations were also affected by the mode of delivery. After adjustment for GA, gender, and sampling time, the mean geometric CRP concentrations were significantly higher in babies born by vaginal delivery than in those born by cesarean section [1.06 (95% CI, 1.1–1.23) vs 0.56 (0.50–0.62) mg/L; P < 0.05]. The significance was much greater when babies vaginally delivered were compared with those born by elective cesarean section (P < 0.01). Finally, the variable intrapartum fetal distress approached significance (P = 0.08).
3.3. Procalcitonin

In regression analysis, GA had a significant, negative effect on PCT independently of gender and sampling time. Babies’ mean PCT decreased by 11.4% (95% CI, 6.4%–16.1%; \( P < 0.0001 \)) per week of GA at delivery. When BW was included instead of GA, mean PCT decreased by 2.2% (95% CI; 0.2%–4.1% \( P<0.05 \)) per 100 g of BW independently of gender and sampling time. Babies’ PCT was not associated with gender. We therefore chose to construct separate PCT reference intervals for the term and preterm neonates.

For the term neonates, the predicted PCT and normal range were, at birth, 0.08 (0.01–0.55) \( \mu \text{g/L} \), rising to peak levels of 2.9 (0.4–18.7) \( \mu \text{g/L} \).
μg/L at 24 h after birth, and reducing slowly to a minimum of 0.3 (0.04–1.8) μg/L at about 80 h (Fig. 3). Thereafter, the values tended to increase slightly reaching 0.6 (0.1–4.2) μg/L at 96 h but, due to the small number of observations at these higher ages, this trend may be imprecise. For the preterm babies, the values were 0.07 (0.01–0.56) μg/L at birth, rising rapidly to 6.5 (0.9–48.4) μg/L at 21 to 22 h before declining gradually to 0.10 (0.01–0.8) μg/L at about 5 days (Fig. 4). Of the 221 values used to construct PCT reference intervals for the term neonates, two (0.9%) were higher than the upper limit and five (2.3%) were less than the lower limit. Of the 200 values used to construct the PCT reference intervals in the preterm neonate, five (2.5%) were higher than the upper limit and seven (3.5%) were less than the lower limit.

The variance of the residuals did not change with age, and they were normally distributed. The Shapiro–Francia statistic was 0.987 for the term babies and 0.984 for the preterm babies.

After adjustment for GA, gender, and neonatal age, none of the antenatal and perinatal variables had statistically significant effects on the values for PCT, although the variables time of ruptured membranes and gestational diabetes approached significance (P = 0.05, and P = 0.06, respectively).

4. Discussion

The main purpose of this study was to determine age-related reference intervals for CRP and PCT in the preterm and term baby during the early neonatal period. Our data show that clinical use of CRP over the first days of life requires the use of cutoff values specific not only to postnatal age, but also to GA. In fact, we found that healthy preterm babies have a lower and shorter CRP response compared with that in healthy term babies, demonstrating the effects of development per se on CRP dynamics. This may be related to possible immature liver function and the inability of the liver to produce CRP. Relevant to this, neonatal characteristics such as a lower GA (<38 weeks) and lower BW (<2500 g) have been reported by Ishibashi et al. to be associated with significantly smaller CRP increases compared with those in babies with a higher GA and higher BW [11]. However, in that study involving symptomatic uninfected babies, the independent effect of prematurity on CRP response was not assessed.

It is true that the sensitivity of CRP increases over time [22,23], making serial measurements useful for those situations where one needs to decide how long to treat [24]. However, by that point, most newborns will be asymptomatic and will have confirmed negative culture results. Indeed, the unsatisfactory sensitivity of CRP pattern recognition for neonatal infection might be related to the insensitive analytic method used to detect the CRP time course after the initial assessment. In 1987, in a prospective study involving 249 babies (GA, 28–43 weeks; BW, 830–4820 g; and neonatal age, between birth and 27 days), Mathers and Pohlandt conducted a diagnostic audit of CRP in neonatal infection using a method in which 6 mg/L was the lower limit of detection [6]. They found that only 3 out of 19 infants with proven sepsis had serum CRP over 6 mg/L on admission, and concluded that a “normal” CRP was virtually useless for exclusion of early septicaemia. However, three years later, in a prospective study involving a large group of preterm neonates, Wasunna et al. showed that none of the cord CRP levels of their infants with confirmed sepsis was over 6 mg/L, but most were over the 95th centile value of 1.42 mg/L, as established by a radiometric monoclonal antibody immunoassay in the cord of 104 preterm uninfected symptomatic babies [8]. Thus a more sensitive (and precise) CRP assay such as we have used here should be valuable during the very early neonatal period to improve the diagnostic accuracy of CRP in the earliest course of infection.

An unexpected finding in the preterm infant with sampling extended into the fifth postnatal day was the spontaneous apparent tendency to return to increased CRP levels. However, the sample sizes at these higher ages were small. Though the newborn is initially covered with a surface microbicidal shield to protect it during its transition to extra-uterine life [25], the neonatal skin and gut are then rapidly colonized with microbial flora. Thus while the birth process initiates an acute-phase reaction in the neonate, it is possible that the...
reactivation of this acute-phase response may be due in the preterm neonate to the markedly enhanced clearance and detoxification of microbes and microbial toxins that have translocated across mucous membranes during birth and/or initial colonization [26].

The present study indicates that CRP reference intervals may be affected by a number of pro-inflammatory risk factors such as prolonged rupture of membranes, preterm labor and GBS colonization, as inferred by the need for intrapartum antimicrobial prophylaxis [20]. Yet, a novel finding observed is that neonates prenatally exposed to steroids had increased CRP concentrations. About 75% of women in preterm labor receive glucocorticoids to enhance fetal lung maturation [27]. In clinical practice, glucocorticoids are often administered in the presence of preterm rupture of membranes and histological chorioamnionitis [28,29]. Thus a large number of infants delivered prematurely are exposed to the combined effects of antenatal glucocorticoids and a pro-inflammatory stimulus. Further confounding variables associated with CRP increases in the healthy neonate were vaginal delivery, a longer duration of active labor, and possibly intrapartum fetal distress, thus confirming that CRP neonatal response may be also related to the physical stress on babies during delivery [11]. In contrast, our data indicate that interpretation of PCT reference intervals may be possibly hampered by specific confounders, such as prolonged rupture of membranes and gestational diabetes. These findings were not unexpected [16,18].

As observed with CRP, our study shows that the clinical utility of PCT in the diagnosis of early-onset sepsis will depend upon the establishment of reference ranges specific to both gestational and postnatal ages. Our earlier experience in a smaller sample of healthy full-term newborns suggested a physiologic increase in PCT levels over the first 2 days of life [16]. Our present results confirm and expand this. A novel finding is that, during the early neonatal period, the healthy preterm baby has an earlier, higher, and longer PCT response compared with that presently found in the healthy term baby, demonstrating an inverse relationship between stage of development and magnitude of neonatal PCT response. This is in contradiction with the report by Turner et al. [17] whose nomogram during the first 4 days of life of preterm symptomatic uninfected infants, stratified by gestational age (24–30 and 31–36 weeks gestation), suggested that PCT concentrations decrease with prematurity. However, PCT reference intervals were calculated in their cohort by repeated measurements on the same infant without checking the magnitude of the bias that this might introduce. Repeated measurements are not equivalent to independent observations. If, for example, the patients for whom repeated measurements are made, have particular characteristics, then to include the repeated measurements would introduce a bias into the estimates of the reference intervals. In particular, the standard deviation would be under-estimated and the reference intervals would tend to be too narrow. For this reason we chose to make only one observation for each healthy neonate.

Despite its potential clinical usefulness, surprisingly little is known about the structure of PCT, its biological properties and source of origin. The entire literature on PCT has long assumed a size of 116, a molecule with two additional aminoacids (Ala-Pro) at the N-terminal which can be cleaved by dipeptidyl peptidase IV leading to N-terminal truncated PCT 3–116. In 2001, Weglöhner et al. showed by mass-spectrometric analysis that in sera from septic patients with high PCT immune reactivity, the truncated form PCT 3–116 was the major circulating form [30]. Nonetheless, the function of serum PCT 3–116 in septic and healthy status as well as the importance of the N-terminal truncation are still unknown. More recently, with newly developed monoclonal antibodies against the aminoterminal of PCT 1–116 and PCT 3–116, Struck et al. comparatively assessed the kinetics of amino-terminal variants of PCT by inducing acute systemic inflammation in 22 healthy individuals [31]. At the earliest time point that PCT was detectable (4 h after stimulation), the initially synthesized PCT1-116 already was partially converted to PCT3-116. Between 4 and 6 h after stimulation, de novo PCT synthesis and proteolytic conversion apparently occurred at a comparable rate. However, when the systemic inflammation vanished, the conversion rate exceeded the rate of synthesis, and the concentrations of PCT 1–116 increased at lower rate than those of PCT3-116 or total PCT [31]. Thus, although the results of our study based on the total PCT cannot discriminate whether the different PCT kinetics between preterm and term babies is mediated by the effects of development and maturation on the synthesis of PCT1-116 as well as on the rate of N-terminal truncation, our results clearly emphasize the need of future studies to detail the role of both PCT species in the age-and GA-dependent PCT reference intervals during the early neonatal period, and therefore their usefulness in improving diagnosis and prognosis of neonatal infection.

Prospective studies are now needed to validate these reference intervals and to determine their predictability and usefulness in the diagnosis of early-onset infection in both preterm and term neonates. Supplementary materials related to this article can be found online at doi:10.1016/j.jca.2011.02.020.

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