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Relationship between the neonatal white blood cell count and histologic chorioamnionitis in preterm newborns

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***Objective*: The aim was to examine the relationship between neonatal white blood cell (WBC) count and the diagnosis of histologic chorioamnionitis (HCA). *Design*: We measured WBC, a widely used marker of inflammation, to evaluate whether the values at birth were associated with HCA. *Setting*: NICU, Department of Pediatrics of Padua University, Padua, Italy. *Subjects*: WBC count was evaluated in 71 preterm neonates (<32 weeks of gestation) with HCA and in a control group without HCA on day 1, 3, and 6 after delivery. Logistic regression analysis and diagnostic accuracy analysis were used to assess the association between WBC counts and HCA. *Main results*: WBC levels were significantly higher in infants with HCA than in those without HCA (Median IQR, WBC (x10^9/l): day 1, 13.2 (6.2–21.8) vs 8.1 (6–11.4), $p < 0.001$; day 3, 17.4 (11.4–26.9) vs 6.3 (5.2–8.3), $p < 0.001$; day 6, 18.4 (11.1–31) vs 6.5 (4.4–9), $p < 0.0001$). The neonatal WBC count on the third day of life was the most sensitive parameter associated with HCA (sensitivity: 0.80; specificity: 0.88). The cut-off value based on the ROC curve was 10 (x10^9/l). *Conclusions*: WBC count in the third day of life is strongly associated with HCA.**

Keywords: Preterm birth, histologic chorioamnionitis, HCA, fetal inflammatory response syndrome, white blood cell count

Introduction

Intra-amniotic infection (IAI) is an important and potentially preventable cause of preterm birth and adverse neonatal sequelae [1–4]. Fetal microbial invasion can result in a systemic fetal inflammatory response syndrome (FIRS) that can progress toward multiple organ dysfunction, septic shock, and perhaps death in the absence of timely delivery [4]. Overt or subclinical IAI is present in at least 50% of extremely premature births; an inverse relationship has been demonstrated between gestational age at birth and both the frequency of microorganisms recovered from the chorioamnion and chorioamnionitis [5–7]. Anaerobic, aerobic and atypical bacteria contribute to the list of microbes associated with chorioamnionitis [8]. An early diagnosis of IAI would facilitate appropriate interventions [9–11].

Early diagnosis of IAI is challenging, however, because clinical signs and symptoms tend to be late manifestations of this condition [12]. Regrettably, histological evaluation of the chorioamniotic membranes, frequently used standard for the diagnosis of IAI, with the accumulation of neutrophils representing the first line of fetal defense, is impossible prior to delivery [13]. In addition, the available maternal noninvasive diagnostic tests have limited predictive value and amniocentesis or cordocentesis is required [8].

Identification of other tests in fetal life and in the early neonatal life predictors of histological chorioamnionitis (HCA) is also desirable. Because the hematologic response of the human fetus with IAI is characterized by an increase in the granulocyte lineage, including amniotic fluid white blood cell count increase and neutrophilia at the cordocentesis were successfully used as an indirect index of IAI [14,15], we hypothesized that WBC count obtained in early life may be associated with IAI. Thus, the aim of the present study was to evaluate if measurable changes in WBC count are indeed detected in preterm neonates presenting with HCA and if there is a relationship with that condition.

Materials and methods

This was a case-control study designed to examine the relationship between the neonatal WBC count and the diagnosis of histologic chorioamnionitis among neonates born at <32 completed weeks who were consecutively admitted to the NICU at the Department of Pediatrics of Padua University from January 2003 to December 2010 identified from a prospectively managed database. Preterm infants were classified on the basis of the results of the placental histology (present HCA *vs* absent HCA). 479 NICU admitted premature infants were eligible, 101 (21%) of them presenting with HCA. Therefore, 71 of the neonates with HCA were considered the study group and 71, matched for gestational age but without HCA were considered the control group. The infants' gestational age (GA) was determined by obstetrical dating, ultrasound study, and Ballard score. Infants with chromosomal abnormalities and/or severe complex congenital anomalies and those lacking of a timely WBC count were excluded. The study was performed with the approval of the Medical Faculty's Ethics Committee and informed consent forms, in accordance with the Declaration of Helsinki, were obtained from all participating mothers.

Placentas were routinely examined in neonates born before 32 weeks of gestation. Placental histological examination was performed by a single pathologist (S.C.) who was unaware of their clinical course and outcomes and issued the report according to the Guidelines of the College of American Pathologists [16], and

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the classification of histological chorioamnionitis of Redline et al. [17], as previously described [18].

For each premature infant, clinical characteristics, laboratory data, and the initial hospital course were recorded.

Blood samples were routinely collected from the umbilical artery catheter or from a peripheral vein on day 1, before the first 2 hours after birth and before antibiotic treatment, for hematologic parameters baseline data and as possible on days 3 and 6, in a non interventional way as part of sepsis screening and/or hematologic data monitoring.

WBC counts were determined using standard laboratory techniques, using a Sysmex XE-2100 as urgent or a Bayer ADVIA 120 counter for programmed hematologic controls, including the absolute segmented neutrophil, eosinophil, and basophil together with lymphocyte and monocyte differential counts. Consequently, differential counts comparison on day one were omitted, after computing the power (0.29) of the test.

Antibiotics were prophylactically administered in all NICU admitted premature infants and stopped according to negativity of sepsis screen, based on total leukocyte count, absolute neutrophil count, C-reactive protein levels, and in the presence of negative bacterial cultures. Neonates were considered to have proven early onset sepsis if a culture yielded pathogenic bacteria. Bacteria recovered in cultures were considered to be pathogenic unless they were normal skin or upper respiratory flora, all other laboratory studies were normal, and the infant either had no clinical signs of infection.

Transfusion with fresh plasma or red blood cells and platelets was given as necessary, depending on the clinical course, as determined by the individual attending physician. None of the patients received transfusions with WBCs during the study.

Statistical Analysis. Data are presented as the mean (SD, standard deviations) or median (IQR, interquartile range). We compared continuous variables using Wilcoxon's test and categorical variables using the Fischer's exact test. Sensitivity and specificity were calculated. Receiver operating characteristic (ROC) curves were constructed for total WBC count on day 1, 3, and 6 with respect to prediction of HCA. Areas under ROC curves and their standard errors were determined and compared using the normal distribution, with correction for correlation of observations derived from the same cases. A larger area under a ROC curve (AUC) indicates superior test performance, with 1 representing 100% sensitivity and specificity and 0.5 representing no discriminatory utility. Confidence intervals (CIs) for sensitivity and specificity were calculated using the normal or binomial distributions. Statistical significance was assumed at $p < 0.05$. All analyzes were performed using R 2.5 software.

Results

Clinical parameters of preterm neonates with and without HCA are summarized in Table I. The characteristics of the two groups, matched for gestational age, were similar with regard to maternal age at delivery, prenatal steroids, PROM, delivery route, sex, and Apgar score at 1 and 5 minutes. Mothers of premature infants with HCA were more frequently treated with antibiotics during pregnancy. Mothers of premature infants without HCA were, instead, more frequently affected by pre-eclampsia and intrauterine growth restriction (IUGR), which was associated with a negative effect on birth weight. Comparisons of the WBC counts between premature infants with present HCA and absent HCA are reported in Table II. The WBC count in the HCA group was significantly higher beginning on day 1 and continued to increase until day 6. Much of the increase was due to the high number of neutrophils, significantly higher on days 3 and 6 in comparison to the no HCA group (Table II).

Table I. Clinical characteristics of mothers and neonates with and without histological chorioamnionitis.

Number (%)	Present HCAM ($n = 71$)	Absent HCA ($n = 71$)	p
Mothers			
Age (years)	32,6 ± 5.6	32,9 ± 6.0	0.81
Cesarean section	56 (78.8)	64 (90.1)	0.06
PPROM >24 h	25 (35.2)	15 (21.1)	0.06
Pre-eclampsia	1 (1.4)	22 (30.9)	<0.0001
Preterm labor	41 (61.9)	23 (32.3)	0.0004
IUGR	9 (12.6)	34 (47.8)	0.001
Antibiotics	23 (32.3)	12 (16.9)	0.03
Neonates			
Prenatal steroids	63 (88.7)	63 (88.7)	1
Gestational age (wks)	26.7 ± 2.4	26.9 ± 2.3	0.2
Male	37(52.1)	35 (49.2)	0.1
Birth weigth (g)	1144 ± 496	989 ± 422	0.04
Length (cm)	36.3 ± 3.8	35.1 ± 4.2	0.1
Head circumference (cm)	25.5 ± 2.8	25.1 ± 2.8	0.37
Apgar Score <5			
at 1 min	28 (39.4)	22 (30.)	0.29
at 5 min	3 (4.2)	5 (7)	0.46
Early sepsis	5 (7)	3 (4.1)	0.49

The WBC changes were not correlated with changes in C-reactive protein levels (HCA+ vs HCA–, day1: HCA+ –0.10, $p = 0.42$; HCA– 0.008, $p = 0.95$; day 3: HCA+ 0–03, $p = 0.85$; HCA– –0.11, $p = 0.44$; day 6: HCA+ 0.18, $p = 0.18$; HCA– 0.10, $p = 0.42$, respectively). The absolute lymphocyte count was, moreover, higher in the HCA group from day 3 (Median, IQR: 2.8 (1.9–4.6) vs 1.6 (1.1–2.8), $p < 0.001$ to day 6; Median, IQR: 4.2 (3.2–6.1) vs 2.4 (1.6–3.1), $p < 0.00$), while both groups had comparable basophil, eosinophil, monocyte, and platelet counts. The presence of HCA was not associated with effects on hematocrit or hemoglobin levels, but these data were not included in the present analysis.

The diagnostic indices of WBC count on day 1, 3, and 6 in prediction of HCA are outlined in Table III. With regard to the three ROC curves analyzed, the one performed on day 3 was the most sensitive and most specific predictor of HCA (sensitivity: 0.80 and specificity: 0.88The cut-off value for WBC count determined by the ROC curve was 10 ($\times 10^9$/L).

Discussion

This study indicates that preterm infants with diagnosed HCA have a significant rise in WBC counts during the first 6 days after birth, with much of the elevation attributed to an increase in the absolute number of neutrophils. Using a $>10 \times 10^9$/l cut-off, WBC is, in fact, the most significant parameter associated with HCA on day 3 in premature neonates less than 32 weeks of gestation.

Since neutrophils are a key component of the inflammatory process, it is not surprising that they are involved in histologic chorioamnionitis. The diffused infiltration by neutrophils of the chorioamniotic membranes is a maternal inflammatory response–such neutrophils are derived from the decidua. A fetal inflammatory response is detected by the presence of funisitis

Table II. White blood cell count and absolute neutrophil count (×109/l) in preterm neonates with present and absent histological chorioamnionitis.

	I day			III day			VI day		
	HCA+	HCA-	*p*	HCA+	HCA-	*p*	HCA+	HCA-	*p*
WBCs	13.2 (6.2–25.8)	8.1 (6–11.4)	<0.04	17.4 (11.4–26.9)	6.3 (5.2–8.3)	<0.001	18.4 (11.1–31)	6.5 (4.4–9)	<0.001
Neutrophil count				11.9 (6.7–20.2)	3.5 (2.9 –4.8)	<0.001	9.8 (41–23.9)	2.1 (1.5–3.8)	<0.001

(infiltration of neutrophils of the umbilical cord of the chorionic plate or vessels) [4]. As neutrophils in the amniotic fluid are predominantly of fetal origin, also the amniotic fluid WBC count has been used as an indirect index of fetal inflammation [15]. This amniotropic neutrophil migration is due to gradients of potent neutrophil chemokines, such as IL-8 and CXCL6 (GCP-2), whose concentrations markedly increase in response to microbial invasion of the amniotic cavity [19–21]. Moreover, two-thirds of fetuses with FIRS have neutrophilia, defined as a neutrophil blood count above the 95th percentile for the gestational age, while only 7.1% of these fetuses had neutropenia [14]. This is significant since investigators have already studied WBC, neutrophil counts, and various other markers, such as proinflammatory cytokines, C-reactive Protein, granulocyte elastase, ferritin or glycodelin, in maternal blood and attempted to use them to predict acute chorioamnionitis, but the results have been modest and limited in clinical significance. Indeed, analysing amniotic fluid leukocyte count appeared to be a better predictor of amniotic fluid infection than total WBC count levels [15,22].

The causes for the significantly higher WBC count observed in premature infants with HCA still need to be elucidated [22]. There are several possible mechanisms to account for this effect. First, an increased WBC count in neonates with chorioamnionitis, compatible with granulocyte colony stimulating factor (GCSF) precursor stimulation, has been described. Fetuses with FIRS have, in fact, a higher cord plasma concentration of GCSF than those without FIRS [23]. Second, neonatal leukemoid reaction, a recently not particularly rare documented phenomenon implicated in the sequence of FIRS [24,25] appears to be the kinetic result of a transient acceleration (8.5 ± 3.8 days) in neutrophil production by GCSF [24,26]. This is relevant since GCSF is one of the key cytokines of FIRS that orchestrate the granulopoiesis [25,27], mediate the inflammation-induced preterm labor [28], accelerate fetal lung maturity by inducing surfactant synthesis [29], and produce neonatal leukemoid reaction postnatally in sufficiently high doses [30].

Two major inferences arise from these findings. One is that that neutrophil infiltration of the placental membranes supports the frequently used standard diagnosis of chorioamnionitis and related WBC count of premature infants. The other is that the IAI may herald the primary physiologic regulators of neutrophil production both in fetal and early neonatal life [22,31]. Careful attention is thus needed when interpreting the hematologic values of sick preterm infants. An understanding of the influence of chorioamnionitis on these parameters is also essential.

However, this study has some limitations. Data from patients with diseases known to be characterized by a large proportion of either abnormally high (early onset bacterial sepsis) or abnormally low neutrophil counts (pregnancy-induced hypertension, early onset bacterial sepsis or congenital neutropenia) were not excluded from our analysis [32]. Future prospective studies are, moreover, needed to evaluate the cut-off in WBC levels identified by our ROC curve analysis and its use to predict alone or in combination with other biochemical and/or biophysical markers, HCA in preterm infants.

Table III. Diagnostic indices of white blood cell count associated with histological chorioamnionitis.

Variable (%)	AUC	Sensitivity	Specificity	Accuracy
WBC cut-off value				
Day I: 13,000/mm^3	0.64*	0.51	0.79	0.65
Day III:10,000/mm^3	0.89*	0.80	0.88	0.84
Day VI: 11,000/mm^3	0.88*	0.75	0.86	0–81

WBC, white blood cell count; AUC, area under curve.
*$p < 0.001$.

Conclusions

In conclusion, these findings indicate that WBC count is strongly associated with HCA in preterm infants. According to the ROC curve analysis, the WBC count on the third postnatal day is the most sensitive parameter associated with HCA compared to WBC at birth and on day 6. Although our study did not seek to determine the clinical and therapeutic implications of these changes, the potential alterations in blood values should be taken into consideration when evaluating NICU admitted preterm infants. Confirmation of this relationship and extension to more specific subgroups of IAI require further studies.

Declaration of Interest: The authors report no conflicts of interest.

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