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**Reduced Mortality and Increased BPD with Histologic**

**Chorioamnionitis and Leukocytosis in Very Low Birth Weight Infants**

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**Short title:** BPD and leukocytosis

**Abbreviations:** BPD (bronchopulmonary dysplasia), CLD (chronic lung disease), VLBW (very low birth weight), WBC (white blood cell), ANC (absolute neutrophil count).

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### **Abstract**

*Objective:* To investigate the association between leukocytosis, mortality, and bronchopulmonary dysplasia (BPD) in very low birth weight infants (VLBW) born to mothers with histologic chorioamnionitis.

*Study Design:* Retrospective cohort study from a single level 3 NICU. Study sample included infants born to mothers with histologic chorioamnionitis, n=252. Total white blood cells following birth were measured. Leukocytosis was defined as a total WBC count  $>30,000/\text{mm}^3$  in the 1<sup>st</sup> two days of life. Outcomes investigated included BPD, and death. Both unadjusted and multivariable analyses were performed.

*Results:* After controlling for potential confounding variables, infants who developed a leukocytosis following birth had an increased odds of BPD (4.6, 95% CI: 2.0-10.3), but a decrease odds of death (0.3, 95 % CI: 0.1-.90).

*Conclusions:* In our population of VLBW infants born to mother with histologic chorioamnionitis, leukocytosis following birth is associated with a decrease in mortality but an increase in BPD.

**Key words:** bronchopulmonary dysplasia, chronic lung disease, mortality, chorioamnionitis, premature birth, neutrophils

## Introduction

Bronchopulmonary dysplasia (BPD) remains one of the principle morbidities of premature birth. The pathophysiology of BPD has traditionally been thought to involve both exposure to supplemental oxygen and ventilator induced lung injury<sup>1</sup>. In addition to oxygen and ventilator induced lung injury, perinatal inflammation has emerged as an important contributing factor to the pathophysiology of BPD<sup>2-4</sup>. Specifically, chorioamnionitis, among other factors, has been associated with BPD in premature infants<sup>5-8</sup>. The role of inflammatory mediators including neutrophils and other white blood cells (WBC) in the development of BPD has yet to be fully defined. Neutrophils play an important role in the host response to bacterial infection. However, neutrophils may also damage host tissues by producing reactive oxygen metabolites, proteases, and elastases and have been associated with lung injury in adults<sup>9,10</sup>. We have previously demonstrated an association between intraventricular hemorrhage, another common morbidity of prematurity, and elevated neutrophils in very low birth weight infants<sup>11</sup>.

In this study we investigated the association between leukocytosis, mortality, and BPD in very low birth weight infants (VLBW) born to mothers with histologic chorioamnionitis. We hypothesized that among VLBW infants exposed to histologic chorioamnionitis, those babies who develop a leukocytosis following birth would be more likely to develop BPD compared to those infants without leukocytosis.

## Methods

After approval from the Institutional Review Board for the Christiana Care Health System, we performed a retrospective cohort investigation of infants <1500 grams birth weight admitted to the Christiana Hospital from July 1, 2002 to July 1, 2006. The Neonatal Intensive Care Unit at Christiana Hospital is a tertiary care nursery caring for both inborn (90%) and outborn (10%) infants. All data were obtained from a database of VLBW infants and from review of the medical record. The only exclusion criteria were infants with major congenital anomalies and outborn births.

VLBW infants were included in this investigation if they were inborn, had a placenta sent for pathologic examination, and had the diagnosis of histologic chorioamnionitis, n=252. Placental pathology is routinely reviewed in all infants born prematurely at Christiana Hospital. Only those infants born to a mother with histologic chorioamnionitis were included in the present investigation. The placentas were examined by one of nine attending pathologists at Christiana Health Care System who were blinded to the clinical and hematologic outcomes of the infants. The placentas were systematically reviewed according to the Placental Pathology Practice Guideline Development Task Force<sup>12</sup>. The diagnosis of histologic chorioamnionitis was abstracted from the clinical pathologic report made by the attending pathologist. The diagnosis of funisitis, inflammatory infiltration of vascular portion of the umbilical cord or Wharton's jelly, was also abstracted.

Data from the complete blood counts on admission, day 1, and 2 of postnatal life were

also obtained. Admission blood counts are routinely obtained on VLBW infants in order to screen for infection, changes in hematocrit, white blood cell or platelet counts which are often associated with preterm labor, preeclampsia or other conditions associated with prematurity. The decision to obtain a complete blood count on subsequent days following admission was made by the medical team caring for the baby. For the purposes of this study, we chose to investigate complete blood cell counts from the birth to day of life two in order to study the relationship between perinatal events on WBC, and minimize the effects of later postnatal events such as late onset sepsis and necrotizing enterocolitis on WBC.

The decision to initiate mechanical ventilation was made based on clinical grounds by the attending neonatologist caring for the infant. The diagnosis of BPD was based on the presence of a requirement for supplemental oxygen at 28 days of life <sup>1</sup>. Infants were given the diagnosis of chronic lung disease (CLD) if they required supplemental oxygen at 36 weeks post-conceptual age. Gestational age was based on best obstetrical estimate. Modified Ballard exam was used only if an obstetrical estimate was not available. Patients were given the diagnosis of sepsis only if they had a positive blood culture.

The diagnosis of preeclampsia was determined by the Attending Obstetrician as per the American College of Obstetricians and Gynecologist guidelines <sup>13</sup>. Mothers were classified as having clinical chorioamnionitis if diagnosed by the attending obstetrician along with the presence of maternal fever, uterine tenderness, or positive culture of the

amniotic fluid. Prolonged rupture of membranes was defined as being > 18 hours in duration. Infants were classified as receiving antenatal steroids if their mothers received any glucocorticoids prior to birth. The preferred antenatal glucocorticoid at our institution is betamethasone with a single course considered two doses given 12 hours apart. Repeated doses of antenatal glucocorticoids were not given at our institution during the study time period.

The majority of blood specimens for complete blood counts were obtained from indwelling arterial lines, or from an arterial puncture. Specimens obtained from a warmed heel or venipunctures were also used. Specimens were not corrected for sight of sampling as this is not done clinically. Blood counts were obtained within 1 hour of admission to the Neonatal Intensive Care Unit and then daily on day of life 1-2. Following admission, blood specimens were routinely obtained in the morning. If an infant had more than one blood specimen on days 1-2 the morning blood count was used. Peripheral white blood cell counts were measured in standard fashion using a Coulter Counter (Hialeah, FL) with manual cell differentiation. The absolute neutrophil count was calculated from the manual cell differential. Absolute neutrophil count was calculated by multiplying the percentage of neutrophils and the percentage of bands by the total white blood cell count. Reported white blood cell counts are adjusted values after correcting for the number of nucleated red blood cells. For the purposes of this study, leukocytosis was defined as a WBC count  $\geq 30,000/\text{mm}^3$  at any time during the 1<sup>st</sup> 2 days of life.

### *Statistics*

All statistical calculations were done on commercially available software (Statistica, Tulsa, OK). One-way ANOVA,  $\chi^2$ , and Mann-Whitney U test, as appropriate, were used for analysis. Repeated measures two-way ANOVA was performed to measure the effect of peripheral blood count over time on BPD and CLD. Multivariable logistic regression was also used to assess the relationship between BPD, CLD, mortality, and leukocytosis. Independent variables were added to the model if they reached statistical significance on unadjusted analysis or are standard potential confounders. A p value of  $< .05$  was considered significant. Data are expressed as mean  $\pm$  SD unless otherwise specified.

## Results

During the 4 year study period 675 inborn VLBW infants were cared for in the NICU at Christiana Hospital who had available report of placental pathology. The study sample consisted of 252 (37%) infants with the diagnosis of histologic chorioamnionitis.

Of the infants born to mothers with histologic chorioamnionitis, 56 (22%) developed a leukocytosis during the 1<sup>st</sup> two days following birth. Those infants with a leukocytosis were of lower gestational age and birth weight compared to infants who did not develop a leukocytosis (Table 1). There were no differences in race or gender in the infants with leukocytosis compared to those infants without leukocytosis. Those infants with a leukocytosis had a lower Apgar score at 5 minutes compared to those infants without a leukocytosis. There were no differences in 1 minute Apgar scores, proportion of infants receiving mechanical ventilation, proportion of infants receiving surfactant, or proportion of infants with early sepsis in those infants with leukocytosis compared to those without a leukocytosis.

Infants with leukocytosis were less likely to be born to mothers with preeclampsia, be multiple gestation birth, or be born by cesarean delivery, compared to those infants without leukocytosis. There were no differences in the diagnoses of prolonged rupture of membranes, clinical chorioamnionitis, proportion of mothers receiving antenatal steroids, or antibiotics between groups (Table 2).

The rate of associated funisitis was higher in infants with leukocytosis compared to those

infants without leukocytosis (65% vs. 36%,  $p=.001$ ) respectively. Infants with leukocytosis had a higher absolute neutrophil count on admission, as well as day of life number one, and day of life two compared to infants without leukocytosis (Table 3.) In addition, those infants who developed CLD had a higher ANC on admission, day of life one, and day of life two, compared to those infants who did not develop CLD (Table 3.)

Infants with leukocytosis had increased occurrence of BPD and CLD compared to infants without leukocytosis (Table 4). There were no differences in the rate of death, but infants with leukocytosis had an increased occurrence of the combined outcome of death and/or CLD compared to those without leukocytosis (Table 4). After multivariable analysis, the odds of developing both BPD and CLD remained increased in infants with leukocytosis (Table 4). After adjusting for potential confounding variables, infants with leukocytosis had decreased odds of death, but there were no differences in the odds of the combined outcome of death and or CLD in infants with or without leukocytosis (Table 4). Two different multivariable models were created. The first multivariable models controlled for gestational age, 5-minute Apgar scores, multiple gestation, antenatal antibiotics, and caesarean delivery. Although mechanical ventilation is known to be an important factor in the pathophysiology of BPD, it was not added to Model 1 because mechanical ventilation may be an important variable in the causal pathway between perinatal inflammation and the development of BPD or CLD. In model 2, mechanical ventilation was added as an independent variable but the results were unchanged.

## Discussion

The major finding of the present investigation is that, in VLBW infants exposed to histologic chorioamnionitis, infants who develop a leukocytosis following birth have increased odds of BPD and CLD but decreased odds of death. Our data support other research indicating an association between BPD and leukocytosis in preterm infants<sup>14-16</sup>. To our knowledge, this is the first study limited to infants exposed to histologic chorioamnionitis, and the first report of an increase in survival with an associated leukocytosis.

From our data we can not determine why mortality may be reduced with leukocytosis following fetal exposure to histologic chorioamnionitis. The rate of early onset culture proven sepsis was low in our study sample but, as our study included only those babies exposed to histologic chorioamnionitis, risk of sub-clinical bacterial infection was likely high. As histologic chorioamnionitis frequently accompanies premature birth<sup>17</sup>, and may be indicative of exposure to multiple microorganisms<sup>18</sup>, a robust inflammatory response may be important in preventing more systemic bacterial infection thus decreasing risk of mortality. Bacterial invasion of the umbilical cord has been associated with increased rate of microorganism recovery in the placenta<sup>18</sup>. In support of this finding, infants in our study sample with funisitis had an increased occurrence of leukocytosis. Neutrophils and other white blood cells are an important component of host defense. However, a consequence of a robust post-natal inflammatory response may be the associated development of BPD. Alternatively, clinical chorioamnionitis has been associated with

cortisol deficiency<sup>19</sup>. As steroids are known to increase peripheral WBC the survival advantage associated with leukocytosis may be indicative of other associated factors such as adequate cortisol levels.

The pathophysiology of BPD, one of the major morbidities of prematurity, is complex and has been associated with chorioamnionitis and antenatal inflammation<sup>7,8,19-21</sup>. For that reason we sought to explore some of the factors associated with histologic chorioamnionitis which may lead to the development of BPD. The importance of fully understanding the causes of BPD is highlighted by the association with poor neurodevelopmental outcome<sup>22</sup>. Although our data show an association between BPD and an elevation in neutrophils, we are unable to determine whether leukocytosis is causal of BPD or is simply an associated factor. There is however biologic plausibility for a potential causal role of increased neutrophils leading to BPD. Neutrophils release factors such as elastases, metalloproteinases, and reactive oxygen species which can damage alveoli or airway epithelium<sup>10,20</sup>. Neutrophils have been shown to play an important role in reperfusion following hypoxia-ischemia brain injury and accumulate in areas of the brain exposed to an ischemic insult<sup>23,24</sup>. We have previously demonstrated an association between IVH, another common morbidity of prematurity, and leukocytosis<sup>11</sup>. Elevated neutrophils following chorioamnionitis may worsen lung injury by altering the microvascular circulation, leading to oxidant injury, reperfusion injury, or by direct inflammatory alveolar injury. There are also a number of other alternative explanations. Histologic chorioamnionitis has been associated with elevation in cytokines such as TNF- $\alpha$  and IL-6 among other factors<sup>25,26</sup>. The observed leukocytosis may be a marker for

infants with a more intense inflammatory response, and lung injury may be caused by other associated factors rather than neutrophils. Susceptibility to chorioamnionitis has also been associated with certain TNF- $\alpha$  genotypes, DNA polymorphisms for interleukin-6, and A blood type<sup>27-29</sup>. Therefore leukocytosis may be a marker for genes which predispose to elevation in WBC and lung injury. Leukocytosis may have also have resulted from sub-clinical maternal or neonatal infection, or hypoxic stress which may have had direct detrimental pulmonary effects.

Our study provides further support to the findings of previous research showing an association between elevated WBC and bronchopulmonary dysplasia<sup>14, 16</sup> in premature infants exposed, and unexposed, to histologic chorioamnionitis. Hsiao et al showed an increased risk of BPD with total WBC  $>30,000/\text{mm}^3$ , in infants 1000 grams birthweight<sup>16</sup>. Zanardo et al showed an increased risk of BPD with WBC  $>40,000/\text{mm}^3$  in infants  $<31$  weeks gestation<sup>14</sup>. Our study differed from both of these investigations by investigating only infants exposed to histologic chorioamnionitis. Therefore our data must be interpreted with caution and our findings of increased odds of BPD associated with leukocytosis may only be applied to VLBW infants exposed to histologic chorioamnionitis.

Our data have a number of important limitations. Our finding of an association of leukocytosis and BPD can only be applied to VLBW infants exposed to histologic chorioamnionitis. Other limitations of our study may include the possibility of inter-

observer variability and lack of consensus on the diagnosis of histologic chorioamnionitis. However, because all patients are from a single institution, adhering to methodical practice guidelines and standard definitions of histologic chorioamnionitis likely limited this variability. As we wanted to study the association of histologic chorioamnionitis and leukocytosis, our study was also limited to white blood cell counts in the first 2 days following birth. We therefore can not comment on any association between leukocytosis beyond this time period and the development of BPD.

In summary, our study is important in showing an association between leukocytosis following birth and increased odds of BPD, and CLD, and decreased odds of death, in those VLBW infants exposed to histologic chorioamnionitis. As histologic chorioamnionitis has been shown to be associated with lung injury in premature infants, our data are important in helping to elucidate the pathophysiology of lung injury in those infants exposed to histologic chorioamnionitis. Our data are important for hypothesis generation and add to the body of evidence showing an association between antenatal inflammation and postnatal outcomes.

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**Table 1. Study demographics**

	<u>Leukocytosis (n=56)</u>	<u>No Leukocytosis (n=196)</u>	<u>p</u>
Gestational Age (weeks)	25.6 ± 1.6	27.2 ± 2.5	<.01
Birth weight (grams)	834 ± 216	1012 ± 277	<.01
Male gender	54%	48%	.42
Race (Caucasian/ African- American/Hispanic)	41%/41%/16%	41%/48%/7%	.22
Apgar 1 minute (median)	4	5	.08
Apgar 5 minute (median)	7	8	.03
Any mechanical ventilation	91%	75%	.11
Surfactant replacement	84%	74%	.13
Sepsis, culture proven <72 hours of life	4%	2%	.40

**Table 2. Maternal diagnoses**

	<u>Leukocytosis (n=56)</u>	<u>No Leukocytosis (n=196)</u>	<u>p</u>
Preeclampsia	2%	12%	.02
Multiple gestation	9%	26%	.01
Prenatal Steroids	82%	80%	.73
Antenatal antibiotics	56%	47%	.11
Clinical Chorioamnionitis	34%	18%	.45
Prolonged Rupture of Membranes	30%	30%	.9
Cesarean birth	41%	48%	.02

**Table 3. Absolute neutrophil counts in those infants with leukocytosis compared to those without leukocytosis and those infants with CLD compared to those without CLD.**

	<u>Leukocytosis</u> (n=56)	<u>No Leukocytosis</u> (n=196)	p	<u>CLD</u> (n=66)	<u>No CLD</u> (n=186)	p
ANC admission (x 1000/mm <sup>3</sup> )	18.0 ± 12.5	4.7 ± 9.1	<.01	11.2 ± 11.2	6.5 ± 7.5	<.01
ANC day 1 (x 1000/mm <sup>3</sup> )	31.4 ± 15.1	7.9 ± 4.8	<.01	18.3 ± 11.8	11.8 ± 12.2	<.01
ANC day 2 (x 1000/mm <sup>3</sup> )	28.5 ± 15.4	7.9 ± 4.6	<.01	17.4 ± 13.9	10.7 ± 11.5	<.01

**Table 4.** Bivariable and Multivariable analysis of BPD and Death.

Multivariable model 1 controlled for gestational age, 5-minute Apgar Score, antenatal antibiotics, multiple gestation birth, and mode of delivery. Model 2 controlled for mechanical ventilation as well as all variables in Model 1.

	Leukocytosis Present (n=56)	Leukocytosis Absent (n=196)	Unadjusted Odds (95% CI) with Leukocytosis	Multivariable Model 1 Adjusted odds (95% CI) with Leukocytosis	Multivariable Model 2 Adjusted odds (95% CI) with Leukocytosis
CLD	46% (n=26)	19% (n=37)	3.7 (2.0-7.1)	4.0 (1.9-8.5)	3.7 (1.7-7.9)
BPD	71% (n=40)	42% (n=82)	3.5 (1.8-6.7)	4.6 (2.0-10.3)	4.5 (1.9-10.1)
Death	14% (n=8)	16% (n=31)	0.9 (0.4-2.2)	0.3 (0.1-.90)	0.3 (0.1-.92)
Death and or CLD	59% (n=33)	34% (n=67)	2.8 (1.5-5.1)	2.2 (0.9-5.1)	2.0 (0.8-4.7)



