Original Article

Decrease In Absolute Monocyte Count As A Predictor Of NEC In Preterm Neonates With Gestational Age < 32 Weeks

Asif Ahmed, Qazi Iqbal Ahmad, Mudasir Nazir

Abstract:

Background: NEC, an inflammatory bowel necrosis of premature infants, is one of the leading cause of morbidity and mortality in infants born prior to 32 weeks' gestation and with a birth weight less than 1500 grams. Symptoms of NEC can present either as insidious like lethargy, poor feeding, temperature instability, and bilious emesis/aspirates or as catastrophic entity like hypotension, abdominal distention, respiratory depression, shock, and death. The effectiveness of any NEC treatment relies on how early and accurately it is diagnosed.

Methods: This study was a prospective observational study conducted over a period of two years. 60 patients were followed during their course of hospitalization. A complete history and physical examination was done at the time of admission. All the base line investigations including CBC for monocyte count were done at the time of admission. Serial CBCs were done in the study on Day 1st, 7th, 14th and on 28th day or at signs of deterioration. Enrolled patients were monitored daily for signs of NEC. Those patients who developed NEC were labeled as cases and those who didn't developed signs of NEC were labeled as controls. Monocytopenia was defined as a drop in blood monocyte concentration from the lower referential range of 2. Serial monocyte counts were compared with the development of NEC as well as with its severity.

Results: Out of 60 neonates, 15(25%) neonates developed NEC. There was equal prevalence of this disorder in both sexes with no difference in disease patterns or survival. Highest incidence was seen in the age group of 28-30 weeks of gestation, while the severest form of disease was seen in the gestational age group of 25-27 weeks corresponding to lowest birth weights. Higher yield on blood culture was found amongst the cases as compared to the controls. An absolute decrease in monocyte count was documented in the cases irrespective of the disease severity. An overall mortality of 40% was documented through the study among the cases.

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INTRODUCTION

Necrotising Enterocolitis (NEC), an inflammatory bowel necrosis of premature infants, is one of the leading cause of morbidity and mortality in infants born prior to 32 weeks' gestation and with a birth weight less than 1500 grams [1,2]. NEC has been defined as an acute inflammatory necrosis of the intestinal tract and it is the most common acquired gastrointestinal and surgical emergency for preterm very low birth weight (VLBW) infants in the neonatal intensive care unit (NICU)[3]. Children's hospital Los Angeles puts NEC as a devastating disease that affects mostly the intestine of premature infants. The wall of the intestine is invaded by bacteria, which cause local infection and inflammation that can ultimately destroy the wall of the bowel. Such bowel wall destruction can lead to perforation of the intestine and spillage of stool into the infant's abdomen, which can result in an overwhelming infection and death [4]. NEC may occur without warning and all too often rapidly evolves into a condition requiring resection of bowel and/or death. NEC began since the beginning of modern neonatal intensive care over 60 years ago. Even after fostering efforts for its understanding and eradication, NEC has persisted [5]. Pneumatosis is

Author Affiliations

Asif Ahmed MD, Assistant Professor Pediatrics, SKIMS Medical College Srinagar.Qazi Iqbal Ahmad MD[2] Professor Pediatrics, SKIMS Soura Srinagar., Mudasir Nazir MD, Senior Resident Pediatrics, SKIMS Soura Srinagar.

Correspondence

Mudasir Nazir MD, Senior Resident Pediatrics, SKIMS Soura Srinagar.E-Mail: arasijaskims@gmail.com

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seen as a hallmark of this disease. In the late 1970s Dr. Martin Bell proposed a staging system to help evaluate which NEC patients would best benefit from surgery. NEC is clearly more complicated than Bell originally envisioned with his staging and our current methods of clinical data gathering do not have sufficient granularity to carry us into the future [6].

Although prenatal factors like placental insufficiency, prolonged/premature rupture of membranes, chorioamnionitis, have also been attributed for its etiology, NEC in itself is a postnatal condition. Although our understanding of the disease process remains incomplete, the currently accepted unifying model for the pathogenesis of NEC includes contribution and interaction of four key components: i. Prematurity. ii. Aggressive Enternal Feedings. iii. Infectious Agents & Gut Bacteria. iv. Hypoxic-Ischemic insults [7].

Symptoms of Necrotising Enterocolitis (NEC) can present either as insidious like lethargy, poor feeding, temperature instability, and bilious emesis/aspirates or as catastrophic entity like hypotension, abdominal distention, respiratory depression, shock, and death, and abnormal laboratory levels are also reported including metabolic acidosis, hypo- or hyperglycemia, anemia, thrombocytopenia, and electrolyte derangements. Intestinal symptoms include abdominal distention, abdominal tenderness, delayed gastric emptying, feeding intolerance, bilious aspirates, and gross or occult blood in the stool [8]. The effectiveness of any NEC treatment relies on how early and accurately it is diagnosed, which is usually achieved on the basis of readily available clinical, radio-graphic and laboratory data. Abdominal ultrasonography is a newer technique to aid in the diagnosis of NEC and may be more sensitive than abdominal radiography in detecting bowel necrosis and alterations in bowel wall perfusion as confirmed at laparotomy. The sonographic findings include central echogenic focus of bowel wall and a hypoechoic rim (the pseudo-kidney sign) that indicates necrotic bowel and imminent perforation.

The normal values of hematological parameters are not generally available for neonates because blood values are not recorded in normal neonates, hence 'reference ranges' are used in neonatal hematology. Even in reference ranges, variability is seen from the works of Kato (1340 to 2200 μl^{-1}) to Rajadurai et al[9], who notes monocytosis >1700 μl^{-1} . This discussion pertains to the database of CBCs of neonates collected from 18 hospitals in US with gestational age 22 to 42 weeks and time period of 1-28 days of life. The ranges consist of the 5th to 95th percentile values assembled from large numbers of neonates with minimal pathology or with pathology not thought to be relevant to the laboratory parameter under study. The mean values for monocytes increased approximately linearly over this interval with a reference range at 40 weeks of 300 to 3300 μl^{-1} (mean 1400 μl^{-1}).

Monocytosis has been observed in critically ill preterm infants, although the exact mechanism underlying this response remains unclear. The presence of monocytosis in the works of "Rajadurai et al." [9], was associated with lower mean birth weight and gestational age, leukocytosis, multiple transfusions, albumin infusions and theophylline therapy, but not with maternal risk factors (pre-eclampsia, diabetes and chorioamnionitis) or specific neonatal variables such as birth asphyxia, respiratory disease and sepsis. An association with maternal steroid therapy was shown. The researchers speculated that monocytosis represented a physiological, although immature, response of the marrow of small premature infants to a variety of exogenous stimuli, including drugs and foreign protein infusions. Monocytosis has been reported in neonates with intrauterine infections such as candidiasis and syphilis, although many of these infants had an increase in all leukocyte lineages. Monocytosis is occasionally noted in neutropenic infants. In some of the early studies, enumeration of these immature cells as monocytes may also have contributed to the marked but transient monocytosis noted during the early neonatal period. In addition to any clinical utility of these reference ranges, a gradual increase in blood concentration of monocytes between week 22 and term will interest developmental biologists. Surely, the gradual but steady increase in the circulating concentration of monocytes indicates the relevance of roles that they have in human developmental biology [9,10]. The systemic inflammatory response during NEC is characterized by elevated circulating cytokine levels and hematological abnormalities such as thrombocytopenia, increased or decreased neutrophil counts, low monocyte counts, and anemia.

METHODS

This study was a prospective observational study which was conducted in the postgraduate department of Paediatrics and Neonatology of a tertiary care teaching hospital over a period of two years.

Inclusion criteria:

1. Preterm baby with gestation age < 32 weeks
2. No evidence of early onset-sepsis.

Exclusion criteria:

1. Neonatal Sepsis.
2. Surgical abdominal conditions; like omphalocele & gastroschisis, CDH, intussception or any other condition that would warrant a differential diagnosis.

A total of 60 patients were followed during their course of hospitalization. A complete history and physical examination was done at the time of admission. All the base line investigations including CBC for monocyte count were done at the time of admission. Serial CBCs were done in the study on Day 1st, 7th, 14th and on 28th day or at signs of deterioration. Enrolled patients were monitored daily for signs of NEC.

The diagnosis of NEC was based on Bell's criteria which classifies NEC as Stage IA (Suspected NEC), Stage IB(Suspected NEC), Stage IIA(Definite NEC, Mildly ill), Stage IIB(Definite NEC Moderately ill), Stage IIIA (Advanced NEC Severely ill with bowel intact) and Stage IIIB (Advanced NEC Severely ill with bowel perforated).

Those patients who developed NEC were labeled as cases and those who didn't developed signs of NEC were labeled as controls. CBC was done in automated 5 part SYSMEX CBC analyzer. Monocytopenia was defined as a drop in blood monocyte concentration from the lower referencial range of 2. Serial monocyte counts were compared with the development of NEC as well as with its severity in all the enrolled patients in order to study the predictive value of serial monocyte count for the development of NEC.

The data was tabulated and graphical representation was depicted wherever necessary. This data analysis was followed by statistical analysis by using SPSS software.

RESULTS

We did a prospective observational study with a follow up of 28 days in NICU in the department of Pediatrics and Neonataology. Absolute Monocyte Counts were studied in subjects vis-a-vis their clinical course, disease progression and finally statistical analysis was done. Out of 60 neonates, 15(25%) neonates developed NEC. There was equal prevalence of this disorder in both sexes with no difference in disease patterns or survival. Highest incidence was seen in the age group of 28-30 weeks of gestation, while the severest form of disease was seen in the gestational age group of 25-27 weeks corresponding to lowest birth weights. Higher yield on blood culture was found amongst the cases as compared to the controls. An absolute decrease in monocyte count was documented in the cases irrespective of the disease severity. An overall mortality of 40% was documented through the study among the cases.

Out of 15 confirmed NEC neonates, 11 (73%) cases were of NEC category and 4 (27%) were of Advanced NEC category. In a total of 15 patients; 7 (46%) babies were males & 8 (53%) were females (P=0.94). Out of the total 60 patients, one case of NEC-III occurred in babies with gestational age of <27 Weeks. Two cases of NEC and one case of Advanced NEC occurred in babies with gestational age of 27-28 weeks. Seven cases of NEC & one case of Advanced NEC had a gestational age of 29-30 weeks. Two cases of NEC & one case of advanced NEC occurred in babies with gestational age of 31-32 NEC. The same reflects a significant incidence of NEC in smaller gestational age (Table 1). **p (<0.0001).**

We analysed the relation between low birth weight and NEC prevalence. In weight group of <1Kg, 75% prevalence was seen. In a total of four Advanced NEC studied, three of them belonged to this weight group. No NEC-II case was witnessed in this weight group.

Table1 Association of gestational age with Necrotising Enterocolitis (NEC) incidence.

Gestational Age	NEC	Advanced NEC	No NEC
<27 weeks	0	1	0
27-28 weeks	2	1	1
29-30 weeks	7	1	10
31-32 weeks	2	1	34

In weight group 1.01- 1.50 Kg, 08 NEC and 01 Advanced NEC was witnessed. In weight group of 1.51-2.0Kg, 03 NEC cases were witnessed. In the weight group of 2.1-2.5Kg none of the cases of NEC or Advanced NEC were witnessed. The same clearly reflects a direct relation between low birth weight and NEC incidence as shown in Table2. (**p.value was <0.0001**).

Table 2 Association between NEC and Birth Weight.

Weight Group	No NEC	NEC	Advanced NEC
<1 Kg	3	0	3
1-1.5 Kg	20	8	1
1.5-2 Kg	16	3	0
2-2.5Kg	9	0	0

Amongst the 60 enrolled subjects; 14 patients had a positive blood culture with 7 patients from NEC) group; 1 patients from Advanced NEC group and 6 patients from No NEC group.46 patients had a negative blood culture with 4 patients from NEC group; 3 from Advanced NEC group and 39 from No NEC group. (p.value was 0.002)

In a total of 8 NEC patients with positive culture report, one of them grew Candida, two of them grew Enterobacter aerogenes, another two grew Klebseilla Pneumoniae, and three of them grew Staphyllococus Epidermidis.

We analysed the total leucocyte counts in cases & controls. In control group mean Total Leukocyte Counts were 2346.66/µl, in NEC group mean Total Leukocyte Counts were 6370.00/µl and in Advanced NEC group mean Total Leukocyte Counts were 7300.00/µl. A proportional increase in Total Leukocyte Counts was witnessed with Absolute Monocyte Count (AMC) drop and with disease severity (p.value <0.0001).

We recorded the lowest percentage wise drop in mean Absolute Monocyte Count in our subjects. Taking 8% as upper referential range and 2% as the lower referential range, in a total of 60 patients, a maximum mean drop of -0.28% was seen in control group. In NEC patients a maximum mean drop of -2.94% was seen and in Advanced NEC cases, a maximum drop of -3.94% was seen. (**Table 3**) The same reflects a decrease in Absolute Monocyte Count in cases, with a significant p.value of < 0.001.

Table 3. Percentage wise drop in Absolute Monocyte Count in studied Patients

Group	Number of patients	Lower Referencial Range	Mean% Drop in Absolute Monocyte Count in NEC cases	Standard Deviation	*p.value*
NEC	11	2	-2.94	1.800	0.0001
Advanced NEC	4	2	-3.30	0.989	
No NEC	45	2	-0.28	2.04	

We also recorded number wise Absolute Monocyte Count in studied patients. Taking 8% as upper referential range and 2% as the lower referential range, in a total of 60 patients, mean numerical value of +135.89 was seen in control group. In NEC patients, a negative value of -190.95 was seen and in Advanced NEC patients, a negative value of-338.35 was seen. The same reflects a number wise decrease in Absolute Monocyte Count in cases with a significant p.value of < 0.001.

In a total of 60 patients, 10(17%) patients deteriorated during the disease course, 5(8.3%) patients improved and 45(75%) patients remained stable (p.value=0.0001).

DISCUSSION

NEC has been defined as an acute inflammatory necrosis of the intestinal tract and the most common acquired gastrointestinal and surgical emergency for preterm very low-birth weight infants in the neonatal intensive care unit[3]. The wall of the intestine gets invaded by bacteria, which cause local infection and inflammation that can ultimately destroy the wall of the bowel. Such bowel wall destruction can lead to perforation of the intestine and spillage of stool into the infant's abdomen, which can result in an overwhelming infection and death[4]**.** The risk of developing NEC and the severity of the disease is inversely related to gestational age and weight at birth and the currently accepted unifying model for the pathogenesis of NEC includes contribution and interaction of prematurity, aggressive enteral feedings, infectious agents & gut bacteria, hypoxic-ischemic insults[7]. The diagnosis with the help of radiological findings vary by gestational age; intramural gas has been detected in infants of ≥37 weeks gestational age with NEC, but a diminished picture of only about 29% in infants with ≤26 weeks' gestational age. Abdominal ultrasonography is a newer technique to aid in the diagnosis of NEC and may be more sensitive than abdominal radiography in detecting bowel necrosis and alterations in bowel wall perfusion as confirmed at laparotomy. Monocytosis has been reported in neonates with intrauterine infections such as candidiasis and syphilis, although many of these infants had an increase in all leukocyte lineages. Monocytosis is occasionally noted in neutropenic neonates [10].

We performed a prospective observational study on preterm neonates with gestational age of <32 weeks, admitted in the department of Pediatrics and Neonatology over a period of two years, primarily taking into account the CBC of premature babies with a gestational age of <32 weeks. We carried out the CBC of studied subjects on day 1st, day 7th, day 14th and day 28th or at any time of clinical deterioration. In our final analysis, we included 60 preterm neonates with gestational age <32 weeks, out of those 60 patients during the course of hospitalization, 15 neonates developed NEC and 45 neonates remained stable with no signs of NEC.

Unlike previous works we performed CBC on subjects in anticipation or on deterioration as well as on dates specified. In our cases group, compared to the control asymptomatic group, monocyte counts were significantly decreased in patients with both NEC and Advanced NEC before the onset of NEC and in the 1st follow-up CBC. In patients with NEC, Absolute Monocyte Count on percentage basis decreased from lower referencial range to a mean of -2.94, whereas in those with Advanced NEC, the Absolute Monocyte Count decreased from lower referencial range to a mean of -3.30, with no significant changes in Absolute Neutrophillic Counts, and Absolute Leukocyte Counts. In the control group, there were no significant changes in the Absolute Monocyte Counts. We also recorded the number wise counts of Absolute Monocyte Count (AMC) in NEC, Advancedd NEC and in No NEC groups. We witnessed a steady decrease in number wise Absolute Monocyte Count (AMC) in studied groups with the control group having a mean value of +135.89, in NEC patients, a negative value of -190.95 was seen and in Advanced NEC a negative value of -338.35 was seen; but the same could not be justified due to different Total Leukocyte counts exhibited by individual patients.

Whether cases or controls, we observed an almost equal frequency of blood culture-positive subjects. Gender difference was non-significant in terms of NEC precedence or survivability. Moreover monocytosis has been noted in neonatal infections, we did not detect a significant difference in monocyte counts in neonates within the control group. Overall, the NEC group had a higher incidence of systemic signs, gastrointestinal bleeding, and mortality, indicating a higher acuity of illness than controls. A major limitation of our study is the limited number of subjects studied with possibility of also being biased due to mono-centric nature, limited infrastructure etc. Considering the limited sample size, our findings need further validation in larger/multi-centric cohorts. Further study is also needed to evaluate

maternal/neonatal covariates known to be associated with NEC such as chorioamnionitis, anemia, transfusions and infections.

In our study we witnessed a fall in Absolute Monocyte Count in NEC confirmed cases and this fall in Absolute Monocyte Count can be a useful early predictive marker of NEC especially in low birth weight neonates. At the time of onset of systemic and abdominal signs, a drop in Absolute Monocyte Count (from the last available test) correctly discriminated between NEC vs other causes of deterioration with an accuracy of 86% (we included 60 preterm neonates out of which 15 developed NEC and among them 13 [86%] patients showed decrease in Absolute Monocyte count, while in 2 [14%] patients there was no change in Absolute Monocyte count). Although this drop in Absolute Monocyte Count is readily available on CBC reports generated from hematology analyzers, yet most neonatologists currently do not evaluate monocyte counts routinely in their practice. Previous studies have identified several candidate biomarkers of Necrotising Enterocolitis (NEC), with "Hutter et al." **[11]** being the first to report hematolgoical abnormalities in NEC, particularly the low granulocyte count in severe NEC cases, followed by "Gorden et al", "Benkoe et al,"[6,12] Maheshwari et al [13] who evaluated biomarkers such as the white blood cells, circulating cytokines, IL-8, Inter-alpha etc. However, despite its modest diagnostic accuracy, the Absolute Monocyte Count is an attractive marker of NEC because the information is already available to the clinician at no extra cost and its high negative predictive value (88%) can help exclude the diagnosis of NEC in neonates who develop any systemic or abdominal signs. Because most clinical laboratories now use automated hematology counters, there are additional advantages of rapid turnaround times, a high degree of consistency and the ease of extrapolation of findings to other centers. Interestingly, decreased blood monocyte counts are likely to be a unique feature of NEC. Growth-restricted preterm neonates may have low monocyte counts but most of these neonates show a suppression of all leukocyte lineages and not isolated monocytopenia. Our findings are in concordance with the works of "Remon et al."[14]who noted Absolute Monocyte Count drop from median 1.7×10^9/L (interquartile range (IQR) 0.98–2.4) to 0.8 (IQR 0.62–2.1); p <0.05. In stage III NEC they reported monocyte counts decreased from median 2.1 × 109/L (IQR 0.1.5–3.2) to 0.8 (IQR 0.6–1.9); p <0.05 and with the works of "Desiraju et al."[15], recording Median Absolute Monocyte Count (AMC) changes of +0.5% (p = 0.56) in rule-out NEC, compared with −44.5% (p < 0.0001) in Stage 2 NEC and −81.9% (p < 0.0001) in Stage 3 NEC.

Absolute Monocyte Count as a novel biomarker for the prediction of NEC may be new but similar to our predecessors we also witnessed a significant percentage wise drop in Absolute Monocyte Count in patients with NEC and Advanced NEC. While low birth weight remains the most potent and lethal factor in the incidence and severity of NEC; prematurity, aggressive feeding and gut colonisation also contribute significantly in its incidence. Compared to the first available Absolute Monocyte Count from prior to onset of any abdominal and respiratory signs, an acute drop in blood monocyte concentration can identify NEC with 86% accuracy (*p.value* <0.001). In a preterm neonate with signs of NEC, a fall in Absolute Monocyte Count by -2.94 indicated NEC, with increased drop in severe cases by -3.30, the same will help to diagnose Necrotising Enterocolitis in its early stages to provide timely management and avoid any aggravation. Pertinently we could not justify drop in blood monocyte concentration in terms of numbers because of different total leukocyte counts in different patients.

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