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Review Article

Neutropenia in Premature Infants

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Abstract

Neutropenia is a self-resolving condition in many newborn infants. However, the formation and the role of the neutrophils are considered to be slightly different in the premature infants. The neutrophils act as a vital component in the innate immunity so the neonatal neutropenia in premature infants must be identified promptly and treated depending on the risk factors, especially of the clinical infections. Neutropenia in premature infants has various causes, such as maternal and pre-natal conditions, congenital syndromes, immune-mediated processes, nosocomial infections and idiopathic. However, not all neutropenia in the premature infants is clinically relevant and often does not add to the danger of infection. In this review article, we will discuss the development of neutrophils, the causes of neonatal neutropenia, and the relationships of neutropenia with premature infants and some viable management of neutropenia in premature infants.

Keywords: Premature Infants; Neutropenia; Absolute Neutrophils Count (ANC)

Abbreviations: ANC-absolute neutrophils count; G-CSF-granulocyte colony-stimulating factor; GM-CSF-granulocyte macrophage colony-stimulating factor; IVIG-intravenous immunoglobulin; µL-microliter; NICU-neonatal intensive care unit; RCTs-randomized controlled trials; rG-CSF-recombinant granulocyte colony-stimulating factor; rGM-CSF-recombinant granulocyte macrophage colony-stimulating factor; WBC-white blood cells

1. Background

Neutrophils, a type of leukocytes, consist of more than fifty percentage of the total leukocytes count [1]. In 1880, a staining technique was developed by Ehrlich that simplified the identification of the developing phagocytes in the bone marrow, blood, and tissues. Because of the Erhlich's stains, the possibility of the microscopic observations of

the blood cells turned out to be common that resulted in the clear definitions of the normal counts, leukopenia, leukemia, neutropenia, and agranulocytosis [2]. Neutrophils are the main type of effector cells in the innate immune system [3, 4], which circulate in the blood and engulf invading microorganisms such as bacteria and fungi by phagocytosis. They are comparatively short-lived having a circulating half-life of 6 to 8 hours and are produced at the rate of 5×10^{10}–10×10^{10} cells/day [5]. Although in pediatric populations, normal ranges vary depending mainly on ages, normal neutrophil counts in premature infants vary by gestational age at birth and birth weight [1]. Therefore, it is utmost important to carefully evaluate the neutrophils count in premature and critically-ill infants. Although neutropenia is usually a benign condition that runs a self-limited course in the majority of the infants, it can be persistent and constitute a crucial deficit in the antimicrobial defense in certain premature infants. An orderly progression of proliferation, differentiation, and maturation of the stem cell from myeloblast to fully mature segmented cell results in the development of mature neutrophils [6]. The development of neutrophils involves three stages: multiplication, maturation and functional. On an average, the first stage takes 14 days, where a mitotic period is of 7.5 days, the second stage 6.5 days and the final stage 2.5 days [6]. The first granulocyte-committed progenitor is the myeloblast (found in bone marrow), which further differentiates/matures into promyelocyte. Later, promyelocytes mature into myelocytes [7]. The cells in these three stages of development are able to proliferate. The result of the mitotic division is supposed to be symmetrical ones producing two cells which either proliferate or go into further maturation [8]. The mitotic pool denotes to committed granulocytic progenitor cells undergoing proliferation and differentiation, whereas, fully differentiated mature neutrophils comprise the post-mitotic pool that forms the bone marrow reserve and are available for release [5]. The segmented cells of the bone marrow are initially triggered when there is a higher demand of polymorphonuclear cells (PMNs) so that the inactive population becomes partly an active one and produces new segmented cells. Due to this renewal system, a continuous output of more polymorphonuclear cells (PMNs) is possible [8].

Neutropenia is termed as an absolute neutrophils count (ANC) less than two standard deviations below the mean value for age statistically [9] or alternatively, below the 5th percentile for an age-defined population [3, 10]. Here, the ANC will be calculated as the automated value of white blood cells count × (%segmented neutrophils+%bands)/100 [11]. The first study conducted by the Manroe et al. [10] using the cohort of 434 infants (38.9 ± 2.4 weeks of gestation) compiled the reference ranges for blood neutrophil concentrations using the data obtained from that study. The study showed that the neutrophil counts peaked at 12-14 hours with a minimum of 7,800 cells/mm^3 and a maximum of 14,500 cells/mm^3 and then stabilized at a lower value of 1,750 total neutrophils/mm^3 by 72 hours of life. A stable maximum value of 5,400 neutrophils/mm^3 reached at 5 days. Since the reference ranges were more appropriate for the term and late preterm infants, Mouzinho et al. [12] later on carried another study. According to the study, the upper limits of blood neutrophil concentrations were almost similar to Manroe et al. but the lower limit showed greater variations. Most recently, Schmutz et al. [11] conducted a study in infants born at 23-42 weeks of gestation, using 30,354 complete blood counts records. In his study, the usual range of ANC was between 2700-13,000/μL (5^{th}-95^{th} percentile) for infants more than 36 weeks of gestation, between 1000-12,500/μL for infants 28-36 weeks of gestation and 1300-15,300/μL for infants lower than 28 weeks of

gestation during 72 to 240 hours after birth. The upper limits of ANC were significantly higher than the ranges reported by both Manroe and Mouzinho.

2. Neutropenia in Premature Infants

Premature birth is defined as birth prior to 37 completed weeks of gestation [13]. Premature births account for 11% of deliveries worldwide [14]. China comes in the second rank after India with 1,172,300 premature infants among the 10 countries having the majority of premature births [15]. Similarly, neutropenia is amongst the most frequently identified abnormalities in neonatal wards and neonatal intensive care units (NICUs). Among the total NICU admissions, approximately 8% of the newborn infants are detected with blood neutrophil counts <1000/μL and 6-58% of premature infants are known to have decreased blood neutrophil counts on at least one occasion [16]. The incidence of neutropenia varies by ethnicity, gestational age, and growth patterns. It is inversely proportional to birth weight and gestational age [17], whereas directly proportional to the grade of hypertension in mothers [12, 18, 19]. Female sex, high-altitude delivery, and mode of delivery of the newborn infants are other associated risk factors [17]. Since the immune systems of premature infants have a relatively smaller pool of neutrophils and monocytes, the potential of these cells to destroy pathogens are impaired and also T-cell activation is limited because of the lower production of cytokines. Consequently, this leads to a decrease in the ability of these cells to combat bacteria and detect viruses in cells compared to mature infants [20-22]. Though a range of common prenatal and postnatal events accompanying premature birth possess an ability to modulate immunity [20], exposure to labor and vaginal delivery have been associated with improved neutrophil function possibly because of immune priming in premature infants [17].

2.1 Causes of neonatal neutropenia

Neutropenia can be due to varied reasons. The three mechanisms-decrease in the production rate of neutrophils, excessive margination of neutrophils, and increase in destruction rate or combinations of these are considered to be the main reasons [23].

1. Decreased neutrophil production
    - Maternal and pre-natal conditions-maternal hypertension, pre-eclampsia, pregnancy-induced hypertension, intrauterine growth restriction
    - Donors of twin-twin transfusions
    - Infants with Rh hemolytic disease
    - Chronic neutropenia in bone marrow failure syndromes (Kostmann syndrome, Reticular dysgenesis, Cyclic neutropenia, Barth syndrome, Schwachman-Diamond syndrome, Cartilage-hair hypoplasia)
    - Inborn errors of metabolism
    - Glycogen storage disease type 1b, Organic acidemias
    - Viral infections
    - Rubella, Cytomegalovirus (Intrauterine infections)

- Copper deficiency
- Alloimmune neutropenia associated with anti-NB1 antibodies**.**

2. Increased neutrophil destruction (utilization)
   - Bacterial or fungal sepsis (including necrotizing enterocolitis)
   - Immune-mediated (alloimmune neutropenia of the newborn, autoimmune neutropenia of infancy, and isoimmune or neonatal autoimmune neutropenia)**.**
3. Excessive neutrophil margination
4. Drug-induced Neutropenia
5. Others
   - Idiopathic Neutropenia of Prematurity**.**

2.2 Clinical management of neutropenia in premature infants

Since the risk of infection is directly related to the duration and the severity of neutropenia in premature infants, any neutropenia that lasts more than five days or particularly has an ANC less than 500/μL should undergo thorough evaluation [17, 24]. If the patient survives, neutropenia resolves quickly in the majority of premature infants. For an example, in most cases of neutropenia accompanying PIH, the neutropenia generally resolves in 3 to 5 days. But an additional evaluation must be considered if neutropenia persists more than 2 or 3 days in an infant with sepsis. There is still a dilemma whether or not to use the prophylactic antibiotics in the neutropenic infants as it might lead to fungal sepsis or to sepsis with highly resistant bacteria [24]. However, sepsis should be considered as a part of the differential diagnosis and an appropriate antibiotic therapy should be initiated while awaiting culture reports in a critically-ill infant [25]. A variety of treatment modalities has been recommended to increase the neutrophils production and functions in the premature infants. Although some of the treatment regimens have not shown much beneficial effect to the premature infants, they are still being widely accepted for the treatment of neutropenia in premature infants and some newer drugs are under clinical trials.

2.2.1 Intravenous immunoglobulin (IVIG): Intravenous immunoglobulin (IVIG) is a widely used drug in pediatric practice for immune disorders. Numerous researches have been carried out to see the effectiveness of IVIG in the neutropenic premature infants. A Study conducted by Sandberg et al. [26] shows that there is no role of IVIG as prophylactic immunotherapy to improve the immune competence in premature infants for preventing severe neonatal infections. Similarly a systemic review carried out by Ohlsson et al. [27] concluded that there is no substantial reduction in the mortality during the hospital stay in infants with suspected or proven infection and the study does not recommend the routine administration of IVIG or IgM-enriched IVIG to prevent mortality in infants with suspected or proven neonatal infection. However, recently Liu et al. [28] stated that premature infants could be benefitted from a high dose IVIG (1-2 g/Kg) by avoiding increased inflammation and restoring the balance in the immune homeostasis.

2.2.2 Corticosteroids: Corticosteroids have been largely used in the management of immune-mediated neonatal neutropenia [29] but the inconsistent outcome does not reassure the use in neutropenic infants. However, according to the study conducted by Bux et al. [30], the response of corticosteroid therapy was better in primary autoimmune neutropenia (AIN). Dexamethasone administration prior to Cardiopulmonary bypass (CPB) results in the reduction in the inflammatory response but absolute neutrophil counts are not affected [31]. Generally, antenatal corticosteroid is recommended for women who are at risk of a premature delivery before 34 weeks of gestation for the fetal lung maturation [32, 33], but the usage of postnatal steroids in premature infants has been frequently associated with detrimental neurodevelopmental outcomes. Likewise, postnatal growth patterns of prematurely born infants are affected by corticosteroid treatment, more by dexamethasone than by hydrocortisone [34], so postnatal steroids should only be reserved for premature infants who remain ventilator-dependent after the first week of life and the dose and duration of the treatment should be the minimum possible required to achieve extubation [35].

2.2.3 Granulocyte Transfusions: The granulocyte concentrates transfusion has been proposed in the past for newborn infants with severe neutropenia with severe sepsis resistant to antibiotic treatment. However, at present, there are no defined indications in this concern [36]. Granulocyte transfusions may help in acute situations but the long-term benefit remains unclear [17]. According to the Cochrane database systemic review done in 2011, currently, there is insufficient evidence from randomized controlled trials (RCTs) to validate the routine use of granulocyte transfusions in neutropenic and septic infants, so more researchers are encouraged in multicenter trials [37]. Therefore, in view of the probable adverse effects (transmission of infections), it is more appropriate to use recombinant granulocyte growth factors (recombinant granulocyte colony-stimulating factor, recombinant granulocyte-monocyte colony-stimulating factor) [36].

2.2.4 Recombinant granulocyte colony-stimulating factor (rG-CSF) and recombinant granulocyte macrophage colony-stimulating factor (rGM-CSF): The hematopoietic colony-stimulating factors G-CSF (granulocyte colony stimulating factor) and GM-CSF (granulocyte-macrophage colony stimulating factor) are cytokines that increase the circulating neutrophils count by stimulating the neutrophils progenitor cells in the bone marrow, increase marrow reserve and reduce neutrophil apoptosis [38, 39]. Hypothetically rG-CSF and rGM-CSF should decrease the mortality rate in infants with septicemia. To test this hypothesis, a meta-analysis was carried out enrolling 5 studies, the result showed that although there was a slight reduction in the mortality rate in rG-CSF/rGM-CSF recipient infants with presumed septicemia but the result was more evident in the group of infants with neutropenia only [40]. Similarly, another study found that administration of rG-CSF in premature infants with the clinical diagnosis of early-onset sepsis was correlated with lower incidence of nosocomial infection over the succeeding three weeks period and the rise of neutrophil concentration in peripheral blood and bone marrow, but there was no difference in the overall mortality rate [41]. Another study conducted by Miura et al. [42] showed that five day period of G-CSF therapy in neutropenic premature infants with clinical sepsis is safe and also reduces the length of hospital stay but no improvement in the mortality rate. The prophylactic use of rG-CSF and rGM-CSF treatment in larger studies have not shown any evident reduction of infectious complications or an improved overall survival rate and almost identical results were observed in studies evaluating G-CSF and GM-CSF as intervention

therapy in infants with sepsis. So based on recent findings rG-CSF and rGM-CSF are not recommended for the routine use in premature as well as term infants [43, 44]. However, rG-CSF is highly efficient at correcting immune-mediated and also effective in cases of congenital neutropenia [16]. In cases of neutropenia in relation to maternal preeclampsia and idiopathic neutropenia of prematurity, the treatment preferences are in accordance with the standard protocol or an individual's decision following the duration and the severity of neutropenia [16, 43].

2.2.5 Pentoxifylline: The immunomodulatory property of pentoxifylline has a distinct immunological response in premature infants resulting in the quantitative and the qualitative differences on the levels of surface marker and cytokine production [45]. A cross-sectional study enrolling 18 very low birth weight (VLBW) premature infants with nosocomial sepsis shows a significant reduction in the immature-to-total neutrophil ratio (I/T) and C-reactive protein (CRP) following pentoxifylline therapy as an adjunct therapy to antibiotics regimens [46]. A Cochrane database analysis in 2015 also concluded that pentoxifylline used in addition to antibiotics decreases mortality in neonatal sepsis without any adverse effects. But due to the minimal quality evidence from relatively small studies, well-designed multicentre trials are still of prime importance to confirm the effectiveness of pentoxifylline in minimizing mortality and morbidity rates in infants with sepsis [47]. A large retrospective cohort study enrolling 311 premature infants concluded that pentoxifylline is compatible with common neonatal medications in NICU when infused via the same intravenous line [48]. Recently, numerous health centers have been using pentoxifylline as an adjuvant therapy to treat premature infants with illnesses, such as nosocomial infections, but no any serious adverse effects have been noticed. So it can be considered a safe drug but needs more clinical trials to recommend for the routine use.

3. Conclusion

The recent advances in neonatal medicine have a substantial contribution to the evident diminution in neonatal mortality. But, the increasing survival rate of premature infants has resulted in growing numbers of premature infants with illnesses and long-term disabilities. The scientific studies emphasizing on neutropenia in premature infants and the most appropriate treatment modalities of those premature infants are still limited and many of them are controversial. Hence, we strongly encourage further studies in this field that will address the issues in premature infants.

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