Nuance of nucleated rbcs (normoblastemia) in peripheral blood film

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Abstract:

Nucleated RBCs (NRBCs) are immature RBCs normally they are not seen in the peripheral blood after the neonatal period. Their presence in peripheral blood of children and adults signifies bone marrow damage or stress and potentially serious underlying disease. The presence of numerous NRBCs increases the WBC count in automated hematology analyzers. Most analyzers generate suspect flags for identifying abnormal cells, and the samples involved should be reviewed manually. Unfortunately, analyzers may not detect low levels of NRBCs. We recommend correcting the WBC count with even 1 NRBC/100 WBCs and reporting "occasional NRBC seen." This alerts clinicians for the significance of unexplained normoblastemia.

Keywords: Nucleated RBCs, Peripheral blood, Normoblastemia.

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Introduction:

Usually Nucleated RBCs are present in the peripheral blood of normal infants up to the fifth day of life. At birth, 3 to 10 NRBCs per 100 WBCs are present (1, 2). Premature birth and fetal hypoxia can cause increase in NRBC's number (3). After the neonatal period, the presence of NRBCs in the peripheral blood is usually associated with malignant neoplasm's, bone marrow diseases, and other serious disorders (2, 4, 5). The bone marrow has a special architecture its disruption leads to obvious changes. Normal mature bone marrow cells are deformable, so they can squeeze through small "portholes" in the endothelium to enter the peripheral circulation (6). Normoblasts and immature granulocytes, however, are less deformable and rarely enter the circulation. Their presence in the peripheral blood indicates that the mechanism of bone marrow barrier has been disrupted or extramedullary hematopoietic mechanism has been activated.

There are various mechanism associated with this conditions i.e. NRBCs in peripheral blood (Normoblastemia) and importantance to report their existence.

Mechanisms:

The mechanisms of normoblastemia are not completely obscure but may be classified as shown in the (Table: I) it will helpful to attribute the condition to a single process, it is important to understand that multiple interrelated mechanisms are frequently involved.

Hyposplenism and Asplenia:

Hyposplenism reflects the developmental immaturity of the reticuloendothelial system and is a reported cause of physiologic normoblastemia of neonates (6, 7) because normoblasts that escape from the marrow are normally cleared by the spleen, their presence in the peripheral blood suggests a hyposplenic state. In patients with myeloproliferative disorders, cellular overload may incapacitate splenic function (2, 5) the same phenomenon develops in patients with sickle cell disease in which abnormal RBCs flood the splenic machinery. Moreover, marrow stress and release of many normoblasts can overcome the ability of a normal spleen to clear them from circulation. This occurs with hypoxia, hemolytic anemia, anemia under treatment, megaloblastic anemia, ineffective erythropoiesis, collagen vascular diseases, malignant neoplasms, and chemotherapy treatment (4) the most useful and sensitive indicator of splenic function, however, the presence of Howell- Jolly bodies (RBC inclusions) in the peripheral blood film. The presence of normoblasts, although useful, may be nonspecific. Acanthocytes; target cells, stippled cells, and fragments are also nonspecific findings (8).

Anemia and Compensatory Erythropoietin:

In every types of severe anemia i.e. hemolytic, nutritional or anemia of blood loss normoblastemia is caused by hypoxic erythropoietin-induced compensatory erythropoiesis.8 The reduced oxygen carrying capacity of anemic blood causes tissue hypoxia, the main stimulus for RBC production. When hypoxia occurs, the kidneys produce erythropoietin if

it increased markedly, results in intense marrow erythropoietic activity. Whether marrow erythropoiesis is effective or ineffective depends on the underlying cause of the anemia. With effective erythropoiesis, the resulting accelerated compensatory activity produces prominent reticulocytosis, polychromasia, immature granulocytes (at times), and occasional NRBCs in the peripheral blood. These cells lineage and quantity or conditions are depends on the severity of the anemia and marrow response. If the marrow response is exaggerated, NRBCs are abundant with many "stress" reticulocytes, causing pseudomacrocytosis.When erythropoiesis is ineffective, and NRBCs may be prematurely released into the peripheral blood without reticulocytosis.Dysplastic RBC changes may occur, as shown by the appearance of macro-ovalocytes and teardrop cells in the peripheral blood(5-6,8-10).

Hypoxia:

The condition which reduces the quantity of oxygen transported to the tissues causes an increase in the rate of RBC production. As normoblastemia occurs in response to hypoxia in both anemia and cardiopulmonary disorders, the cause of hypoxia may differ in these conditions. In anemia, hypoxia results when the reduced hemoglobin concentration causes a corresponding decline in the oxygen carrying capacity of the blood (11-12). Cardiopulmonary hypoxia, however, may involve numerous mechanisms, including failure of the blood to absorb oxygen from the lungs, inadequate ventilation of alveoli, or right-to-left intrapulmonary shunting of the blood. Impaired cardiovascular circulation leading to an inadequate supply of oxygenated blood to the tissues may also cause hypoxia (13-14). Because some patients with cardiopulmonary disorders have pulmonary emboli or coronary thrombosis complications, the presence of normoblasts in these disorders may indicate unfavorable prognosis (8).

The concentration of hemoglobin also differs in these hypoxic situations. In cardiopulmonary hypoxia, the hemoglobin level is either high or within the reference interval, whereas in anemic hypoxia, it is much lower (9). The rate of RBC production, however, is not controlled by hemoglobin concentration; it appears to vary with the ability of the cells to transport oxygen to the tissues in response to a demand. Thus, if the oxygen supply is less than the tissues demand, more RBCs are produced, which, in turn, results in a higher hemoglobin level until the supply of oxygen meets the demand. In cases of transient increases in oxygen demand (a hypoxic stimulus), normoblastemia, if present, disappears with relief of the hypoxia. Accordingly, a hypoxic stimulus should be suspected whenever normoblastemia is accompanied by a normal to high hemoglobin level and mild to moderate polychromasia (13-15).

Bone Marrow Invasion and replacement:

Marrow replacement can occur in association with a primary hematologic disease such as leukemia, myeloma, or lymphoma. It can also be the result of secondary injury invading tumor cells, the presence of sarcoidosis, or infectious agents such as mycobacterium and fungi (6). Both primary and secondary reactions can produce marrow fibrosis (myelofibrosis), which changes the normal marrow micro architecture. This disruption may break down the marrow-blood barrier, causing untimely and disorderly release of NRBCs and progenitor cells into the circulation (10). Similarly, extensive marrow infiltration and replacement may cause mechanical "crowding out" of normal hematopoietic cells, leading to their escape into the peripheral blood and lodgment in other organs such as the spleen, liver, and lymph nodes. This process may contribute to extramedullary hematopoiesis. The initial peripheral blood picture may present unexplained normoblastemia, mild macrocytosis, giant platelets, myelocytes, thrombocytopenia, and, possibly, leucopenia with or without teardrop cells or blast cells (9).

Extra-medullary Hematopoiesis:

Recognized clinically as splenomegaly or hepatomegaly, extramedullary hematopoiesis appears to be caused by anemia, marrow replacement associated with acute leukemia, or other nonhematopoietic infiltrative processes (myelophthisis) (11). Presumably, hematopoietic stem cells are displaced from the marrow into the spleen or liver where they proliferate to cause hepatomegaly and splenomegaly. Splenomegaly also occurs when the marrow has been dispossessed by fibrosis. Because the spleen does not retain immature cells as efficiently as normal marrow, it may release NRBCs, peripheral blood smear from a patient with myelofibrosis shows many teardrop RBCs and a myelocyte. Chronic Hematopoietic Malignancies (5) reveal always immature granulocytes, megathrombocytes, and occasional blast cells into the peripheral blood, resulting in leukoerythroblastosis or the coexistence of myeloid precursors and NRBCs in the peripheral blood (12). Teardrop cells may be present. A leukoerythroblastic reaction may also be seen without marrow infiltration in normal newborns as well as in patients with thalassemia major with severe hemolytic crisis, hemorrhage, postsplenectomy, septicemia, [16] and therapy with granulocyte colony-stimulating factor (G-CSF). In addition, when bone marrow reserve is unable to meet the demand for accelerated erythropoiesis (as in chronic hemolytic anemia or longstanding anemia), blood cells may form in tissues

other than the bone marrow.8 This extramedullary hematopoiesis represents a reversion of the involved tissues to their fetal blood-forming function, although this compensatory activity may also occur in myelophthisic anemia with fibrosis. Thus, differentiating between chronic hemolytic anemia and myelophthisic anemia with fibrosis is difficult without the patient's clinical history. Leukoerythroblastosis is evident in peripheral blood films, and teardrop cells may or may not be present. Teardrop cells are not usually seen in leukoerythroblastic reactions associated with hemorrhage, infection, or G-CSF therapy. They can be found in severe iron deficiency anemia, thalassemia, megaloblastic anemia, hemolytic anemia, leukemia, myelofibrosis, and drug-induced Heinz body formation (16). Teardrop cells may reflect dyspoiesis and thus are not specific for a single condition. Although the exact mechanism of teardrop cell formation is unclear, the formation of these cells from inclusion-containing RBCs is well documented. As cells with large rigid inclusions try to pass through the small splenic sinus openings, parts with large inclusions get pinched, causing the cells to stretch with irreversible loss of their shape. The result is teardrop cells. Moreover, teardrop cell formation represents the cells tortuous circulation through deformed marrow sinuses and diseased splenic cords. The presence of teardrop cells in the peripheral blood is thus significant and should alert morphologists to search for occasional NRBCs, megathrombocytes, stippled cells, immature granulocytes, or blast cells that would constitute conclusive evidence of myeloid metaplasia or leukoerythroblastosis (9, 16).

Other Mechanisms:

Why normoblastemia occurs with these disorders remains mysterious. Although the marrow-blood barrier appears to break down, the cause of the breakdown is unknown (9). Most of the disorders involved complex conditions that causes systemic diseases and influence bone marrow response. These diseases include uremia, sepsis, liver disease, and thermal injury (12).

Role of the Laboratory:

Laboratory professionals play an important role in detecting NRBCs when they review CBC and
WBC differential results obtained by automated hematology analyzers. Most analyzers generate suspect flags, (e.g., WBC*R, NRBC, Review Slide, Blasts) to help identify abnormal WBCs, and samples with flags should be microscopically examined. Although most instruments have >80% specificity for NRBC flags, they cannot consistently detect <5% NRBCs (12). The number of NRBCs in a 100- or 200-WBC differential count are reported as the number of NRBCs per 100 WBCs. In addition, the corrected WBC count is reported. It is also good practice to manually scan all blood films of new patients (without a diagnosis) for abnormalities that may not have been flagged (4, 6, 9).

Correction of WBC Count in normoblastemia :
Recognizing NRBCs is an important role as their presence affects the WBC count, because only a few NRBCs can have ominous implications in some patients. We therefore recommend that WBC counts with even 1 NRBC/100 WBCs be corrected and reported. This will alerts clinicians for the significance of unexplained normoblastemia. The correction can be made with a simple formula: Corrected WBC count, x 10^{9} /L= WBC count, x 10^{9} / L / (1+ (NRBC/100WBCs))
Computerized laboratory systems do these calculations automatically. Recent advances in hematology analyzers and their widespread use will improve manually correcting WBC counts (17-18). Therefore, setting a threshold of more than 4 or 5 NRBCs/100 WBCs before correcting the WBC count does not make clinical sense. In addition, many authors (16) advocate different correction formulae and cutoff values. We recommend correcting the WBC count with even 1 NRBC/100 WBCs and reporting "occasional NRBC seen" with <1 NRBC/100 WBCs.

Comments:

The presence of NRBCs in blood does not offer a diagnosis of disease; it may give invaluable clues to the presence of a serious condition. Homeostatically, NRBCs in blood symbolize a compensatory response to an excessive demand on the blood-forming organs (marrow stress) such as in severe anemia or hypoxia. Clinically, NRBCs may represent marrow fibrosis, marrow replacement by leukemic cells or metastatic tumor cells, or extramedullary hematopoiesis. Their presence indicates the extent to which bone marrow reacts to stress and disease. A recent technological breakthrough enables new hematology analyzers to identify NRBCs separately from WBCs (17-18) with this technology, more cells per specimen are characterized, and the new data might show that "rare" NRBCs occur more frequently than previously thought and that the significance of this should be revisited. Moreover, except for grossly abnormal results, manual correction of WBC counts may become unnecessary.

The classification of mechanisms associated with normoblastemia (see Table-I), although useful, is oversimplified because it emphasizes only the predominant cause of the disorders listed. In many conditions, more than one mechanism is operative

(Fig.1 & Fig. 2). The severe hemolytic anemia of the newborn (erythroblastosis fetalis) in which the severe stress of anemia and hypoxia on marrow erythropoiesis is the primary cause of normoblastemia (19). The combination of marrow stress with the immaturity (hyposplenism) of the reticuloendothelial system and the availability of extramedullary hematopoiesis probably accounts for the extreme normoblastemia or leukoerythroblastosis.Similarities to this are evident in leukemia, myelophthisic anemia, or myelofibrosis; although the primary cause of normoblastemia in these conditions is the crowding out of hematopoietic cells or the disruption of marrow architecture, concurrent anemia,hyposplenism, or extramedullary hematopoiesis may also contribute to normoblastemia.

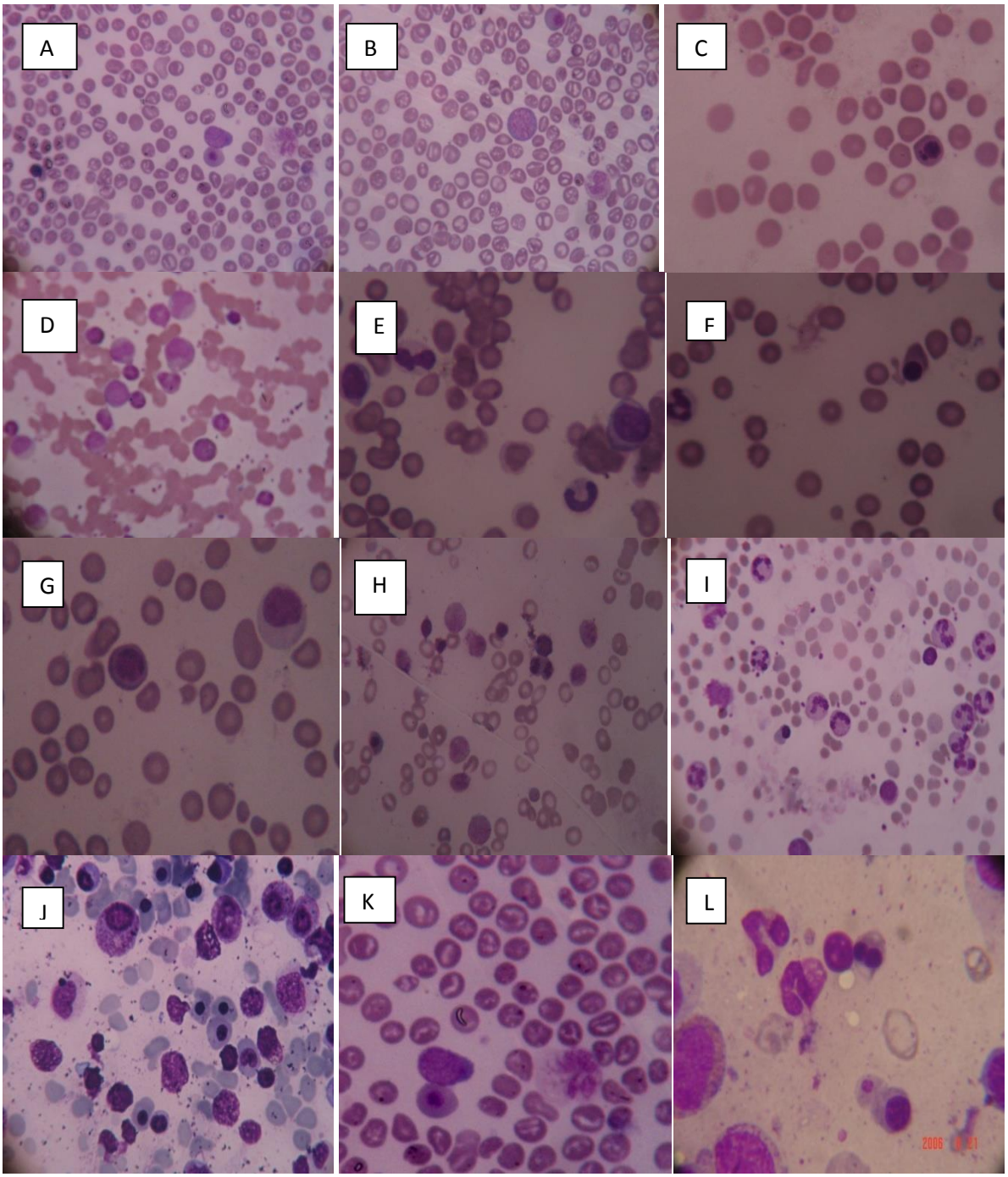

Fig-1: Leishman stained peripheral smear showing NRBCs (Normoblastemia) and various associated conditions e.g, A-Malaria,B-Thalaseamia,C-Macrocytic anemia,D-Leucoerythroblastic reaction,E-Leukemia,F-Microangiopathic haemolytic anemia,G-Haemolytic anemia,H-Iron def.anaemia,I-Myelofibrosis,J-BM Myelodysplasia,K-Megalolastic anemia,L-Preleukemia,

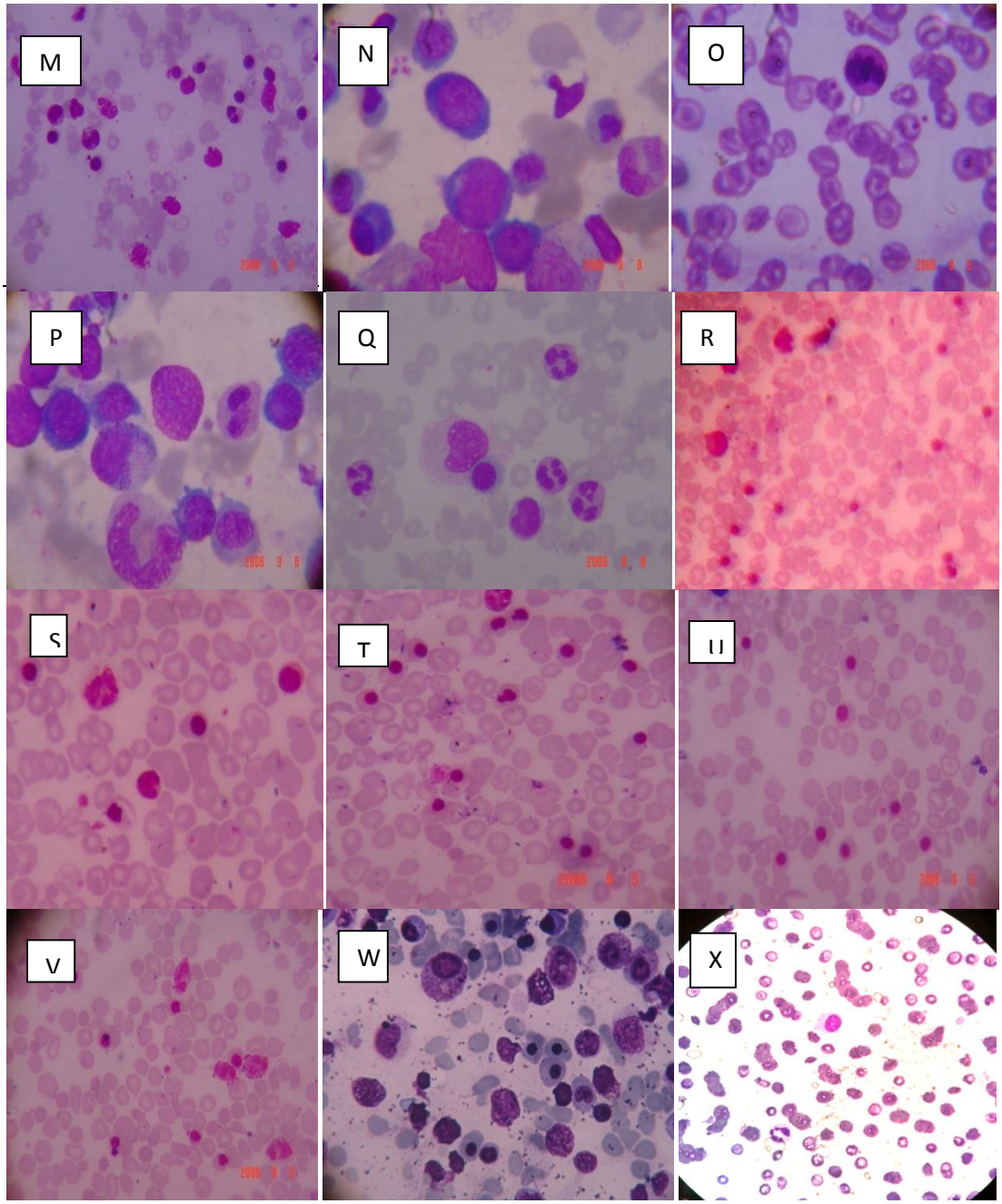

Fig-2: Leishman stained peripheral smear showing NRBCs (Normoblastemia) and various associated conditions e.g, M-Haemorrhage,N-Myeloproliferative deasease,O-Thalsaemia,P-BM Megaloblastic anemia,Q-Polycythemia vera ,R-Anaemia of chronic desease,S-New born,T-Dyserythropoitic desease ,U-severe pulmonary deases,V-Anaemia under treatment,W-BM Dyserythropoitic desaese ,X-Plasma cell in myeloma .

Furthermore, in cardiopulmonary disorders, normoblastemia is more pronounced when anemia is also present. Therefore, it should be easier to differentiate one disorder (mechanism) from the other by considering the total clinical picture. Although studies show that even 1 NRBC in the peripheral blood of adults may indicate a serious disease, (4,20,21) clinicians and laboratory professionals do not agree on its clinical significance, especially when unsupported by other data. The differing views are probably due more to perception than reality. In primary health care units, normoblastemia is rare and may signify a pathologic condition. In contrast, ormoblastemia is a common finding in an acute care hospital. As a result, NRBCs may be perceived as ordinary cells of questionable clinical significance; in these cases, the importance of normoblastemia is relative and depends on the type of hospital and patient population. We believe that unexplained normoblastemia is important because it offers invaluable insight into disease processes or progressions that occur in conditions such as metastatic carcinoma, bone marrow conditions, systemic infections,

and cardiopulmonary complications. The presence of normoblastemia with certain clinical conditions may indicate that a bone marrow examination is necessary to rule out hematologic malignant neoplasms or unsuspected blood disorders. When viewed in this context, one NRBC may lead to more timely medical intervention, thus increasing the chance of a positive outcome(2).

Conclusion:

The findings of the normoblastemia may be diagnostic and prognostic aid to physician in the above mentioned and discussed conditions of clinical circumstances.

Table 1: Mechanisms and Conditions Associated With Normoblastemia	
Hyposplenism, asplenia (4, 6-8)	Sickle cell anemia
	Newborn (physiologic)
	Splenectomy
	Essential thrombocytosis
	Hemolytic anemia
	Malaria
Anemia, compensatory erythropoiesis (5, 6, 8,9,10)	Severe anemia (any cause)
	Hemolytic anemia
	Iron deficiency anemia
	Megaloblastic anemia
	Hemorrhage
	Anemia under treatment
	Microangiopathic hemolytic anemia
	Thalassemia major
Hypoxia (8- 9, 11-16)	Severe pulmonary disease
	Congestive cardiac failure
	Cyanotic heart disease
Marrow invasion, replacement (6,9-10)	Preleukemia
	Leukemia
	Lymphoma
	Neuroblastoma
	Myelodysplasia
	Myelofibrosis
	Plasma cell myeloma
	Myeloproliferative disorder
	Gaucher and other storage disease
	Granuloma (ie, tuberculosis)
	Collagen vascular disease
	Fungal infection
	Histiocytosis
	Tumor cell presence
	Sarcoidosis
	Osteopetrosis
Extramedullary hematopoietic (5, 8, 9,11,12,16)	Myelophthisis
	Osteopetrosis
	Myeloid metaplasia
	Myelofibrosis
	Chronic hemolytic anemia
	Polycythemia vera
	Leukemia
Other (9, 12)	Uremia

	Sepsis
	Liver disease
	Diabetic ketoacidosis
	Inflammatory bowel disease
	Renal transplant
	Thermal injury
	Chemotherapy

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