IMMATURE GRANULOCYTE (2023)

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Complete Blood Count (CBC) or Full Blood Count (FBC) analysis is a pivotal starting point in health screening, diagnosis & monitoring of disease progression or therapy. Traditional complete blood count (CBC) indices provide a snapshot of the current state of the hematologic system. But CBC analysis alone does not allow for a final diagnosis. Its combination with other tests from biochemistry, microbiology, protein electrophoresis, immuno-phenotyping or other tests would be required along with clinical presentation and history of the patient for an accurate diagnosis.

Nevertheless, information from the CBC parameters give good idea about the pathology of the patient and can point out critical situations requiring urgent clinical decisions & management.

CBC analysis includes several parameters, some of them are counted, and others are measured or calculated. Among them, white blood cell (WBC) analysis is very useful for assessing the immune system status and for detection of various types of infections.

Leukocyte differential count

Leukocytes, also called White blood cells (WBC), represent all the cells of the immune system circulating in the peripheral blood that take part in both innate and adaptative immunity.

They protect their host against pathogens or abnormal physiological processes. The screening of the various cellular components of WBC is called a differential WBC count or DIFF (Differential).

Immature forms, morphological abnormalities or presence of unexpected cells would be investigated through WBC differential count.

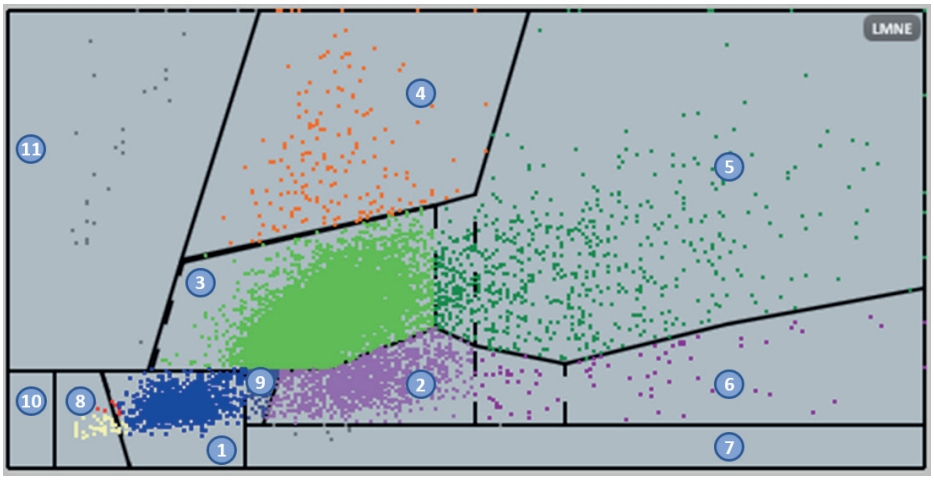
The DIFF analysis associated parameters are always reported in both absolute counts and percentages.

HORIBA Medical hematology analysers offers a DIFF analysis with a full coverage of the five types of leukocytes (Lymphocytes, Monocytes, Neutrophils, Eosinophils and Basophils). DIFF analysis also include Immature cells like Immature lymphocytes (IML), Immature monocytes (IMM) and immature granulocytes (IMG).

Lymphocytes comprises two populations: B cells and T cells. The B cells produce antibodies that are used to attack invading bacteria, viruses, and toxins. The T cells destroy the body's own cells that have themselves been taken over by viruses or become cancerous[1]. A monocyte is a cell that differentiates into populations of macrophages and dendritic cells to regulate cellular homeostasis, especially in the setting of infection and inflammation[2].

Neutrophils are the most abundant leukocyte in circulating blood and these short-lived cells play a key role in immune defence from bacterial infections. Eosinophils are responsible for fighting multicellular parasites and certain infections. They also control mechanisms associated with allergy and asthma. Basophils are responsible for inflammatory reactions during immune response, producing compounds like histamine and serotonin that induce inflammation, but also heparin that prevents blood clotting. They can be involved in acute and chronic allergic diseases.

These populations apart from basophils, can easily be visualized on the LMNE Matrix of Yumizen hematology analysers (Figure 1). In this matrix, optical extinction (Y-axis) is plotted against volumes (X-axis) to form a representation with several clusters of cells. Two additional parameters are derived from the LMNE matrix, atypical lymphocytes (ALY) and Large Immature Cells (LIC) the latter grouping together immature lymphocytic, granulocytic and monocytic cells zones respectively.



1. Lymphocytes (LYM)
2. Monocytes (MON)
3. Neutrophils (NEU)
4. Eosinophils (EOS)
5. Immature Granulocytes (IMG)
6. Immature Monocytes (IMM)
7. Immature Lymphocytes (IML)
8. Erythroblasts or Platelet aggregates
9. Atypical Lymphocytes (ALY)
10. Background Noise Low
11. Background Noise High

Figure 1: LMNE matrix and name of the corresponding zones

What are Immature granulocytes?

The immune system comprises three types of granulocytic cells: Eosinophils, Basophils and Neutrophils. These cells result from a 6-stage differentiation process in the bone narrow call granulopoiesis. The first recognizable cell of this differentiation process is the myeloblast. The other stages in the order of differentiation are promyelocytes, myelocytes, metamyelocytes and band cell respectively (Figure 2).

Immature granulocyte represents three population of cells: promyelocytes, myelocytes and metamyelocytes. Under physiological conditions, these populations are absent from the peripheral blood except for new-borns and significant amount should lead to further investigation. Indeed, in some cases, a high immature granulocyte population in the peripheral blood also called “left shift” can be observed. It usually reflects an increased myeloid cell production under pathological conditions that could be an inflammatory infection, severe haematological disease, cancer, tissue necrosis, acute transplant rejection, surgical or orthopedic trauma, myeloproliferative diseases, steroid use, and also in pregnancy [3].

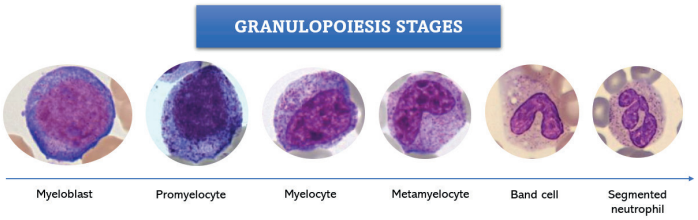


Figure 2: Optical microscope pictures of the neutrophil lineage cells during each stage of granulopoïesis

Immature granulocyte measurement state of the art

To date, **manual microscopy count** has been for a long time the only way for Immature granulocyte analysis. Stained blood smear examination allows to compute the Immature granulocytes in percentages and detect many morphological abnormalities. **Digital microscopy** (CellaVision) can save slide reading time, but the accuracy of the classifying software still requires a human operator to check and correct misclassified cells so nowadays leukocytes counting is done by automated analyzers.

Last, IMG count can be done by **multi-color flow cytometry** using dyes or antibodies for labelling. This can be done by targeting the expression of CD45, CD11b, CD15 and CD16 as their expression differs during each level of granulopoiesis differentiation stages. However, flow cytometry is expensive, requires experienced specialists not available 7/24, so it is mainly used for further exploration when hemopathies are suspected.

According to studies, manual microscopy count methods do not allow accurate and precise count because it consistently underestimated the Immature Granulocytes at low counts[3]. Automated flow cytometry count can be considered as a reference method as it is superior to manual microscopic morphological count[4].

Immature granulocyte (IMG) parameter on Yumizen hematology analysers

HORIBA Medical Haematology analysers provide a parameter called Immature Granulocyte (IMG) for a full coverage of immature granulocyte population when running a WBC differential count. The IMG parameter allows clinical laboratories to screen for abnormal cells in the peripheral blood, faster and more specific approach way Immature granulocyte population can be easily distinguished on the LMNE Matrix with deep green dots (Figure 3).

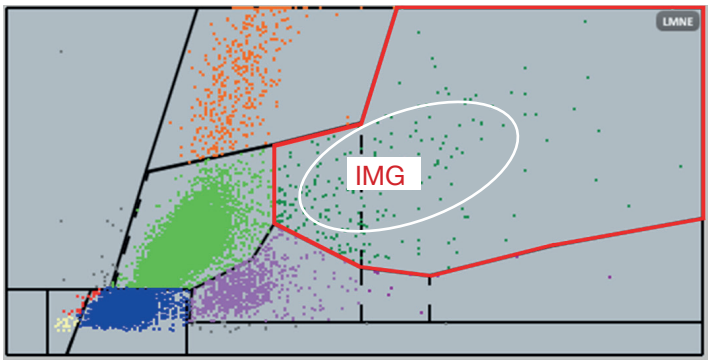


Figure 3: IMG zone on the LMNE matrix

The IMG detection principle is based on the Double Hydrodynamic Sequential System «DHSS» flowcytometry. Cells are detected, counted and measured using a combination of impedance and optical extinction. Immature granulocytes have a high potential of light scattering (complex intracellular content). Immature granulocytic cells (IMG) are detected by their larger volumes and by their increased quantity of granules which is detected by the optical extinction technology. The cell population thresholds (some of them mobiles and the others fixed) give the normal limits for the normal leukocyte morphologies.

Changes in the morphology of a specific population is indicated on the matrix by a shift in the corresponding population. HORIBA medical has tried to enhance this additional information given by the analyser by determining a reference interval for a given population (Table 1).

Parameters	Normal Range			
	IMG#		IMG%	
Reference Limits	Lower	Upper	Lower	Upper
Male	0,00	0,04	0,00	0,60
Female	0,00	0,05	0,00	1,20
Bibliographic data[6]	0,00	0,50	0,00	2,00

Table 1: Table of normal ranges for IMG parameter based on Horiba Medical study

These normal ranges were assessed by a study of 240 blood samples (120 males and 120 females, > 18 years of age) from a French population analysed in duplicate on one Yumizen H1500 and two Yumizen H2500 following CLSI C28-A3.[6] Males and females sampling was performed using EDTA anticoagulant. Samples were kept at room temperature between the sampling and the test. These normal ranges will vary with sample population and/or geographical location. HORIBA Medical highly recommends that each laboratory establishes its own normal ranges based upon its local population.

The evaluation of these normal ranges has allowed the installation of alarms on the system when the count exceeds the latter. The Yumizen LMNE matrix is able to give a visual rendering in condition of high IMG and low IMG (Figure 4).

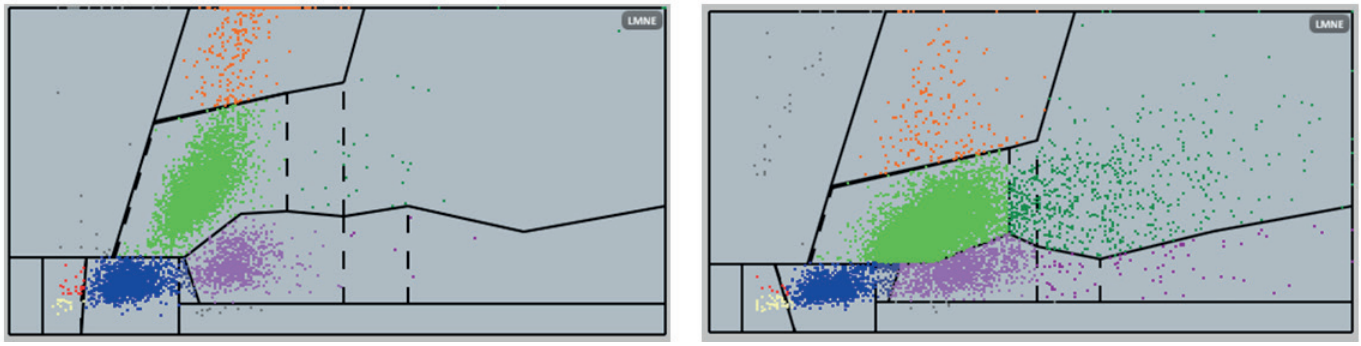


Figure 4: Low IMG (left) Vs High IMG (right) from HORIBA Yumizen LMNE matrix

What is the clinical significance of Immature Granulocytes?

Automated count of Immature Granulocyte has shown many clinical interests in the management of diseases and so in the improvement of patient clinical outcomes. IMG, act as a haematological biomarker that helps to establish severity of infection and risk of sepsis for patients not only in the emergency department but also patients showing early signs of infections and or bone marrow disorders at Physicians clinics.
According to study[7], Immature granulocyte count is an early marker for the diagnosis of **acute appendicitis (AA)**. AA is the most common abdominal pain treated by a surgical intervention called appendectomy. It exists two types of AA: **simple appendicitis (SA)** without complications and **complicated appendicitis (CA)** with the risk of post-operative complication. An early discrimination between SA and CA is essential to administrate an appropriate treatment to reduce postoperative morbidity rates and medical costs.

Unfortunately, current biomarker like neutrophil/lymphocyte ratio (NLR), C-reactive protein or bilirubin levels can't discriminate CA from SA. With this study, Yılmaz Ünal find out that immature granulocyte percentage can be used to discriminate CA and SA and absolute count to discriminate between AA and normal appendicitis.[7]
In another study[8], Güngör et al. found that Immature granulocyte count is an appropriate marker to predict **Serious Bacterial Infection (SBI)** and its severity in paediatric unit. The stakes are high, indeed, SBI can be life threatening for children with meningitis or urinary tract infection. Until now, great biomarkers were used for diagnosis like C-reactive protein (CRP) or absolute neutrophil count (ANC). However, IMG automated count is faster, is less expensive, is less labour intensive and does not require additional blood collection. In the study they reported that the mean immature granulocyte percentage was higher in the patient with SBI compared to non-SBI[8].

In addition to its usefulness in paediatric unit, van der Geest et al. showed that the prediction of the invasiveness and severity of **microbial infection** in critically ill patient hospitalised in intensive care unit can be assessed using Immature Granulocyte count[9].

Immature granulocyte count is a useful marker to predict inflammation. Karakulak et al. demonstrated that the severity and in-hospital mortality of **acute pancreatitis (AP)** may correlate with an high immature granulocyte percentage[10]. They found out that immature granulocyte percentage levels in patients during admission was higher in severe AP and hospital death compared to those with mild and moderate levels. The early diagnosis is major need for the administration of a suitable treatment as 1% of patients with AP die at the time of admission. In addition, another study[11] by Huang et al. says that "AP has been established as one of common precipitating factors for the incidence of **acute respiratory distress syndrome" (ARDS)**. Thus, they also showed that Immature granulocyte percentage is a useful biomarker to identify rapidly AP patients at high risk for ARDS in the early stages of the disease on admission[11].

Finally, study by Ayres et al.[12] showed that immature granulocyte percentage is able to rule out **Sepsis** when it is <2% Immature granulocyte in early state of the disease. Sepsis is a clinical syndrome of life-threatening organ dysfunction caused by a dysregulated response to infection[13]. Sepsis is characterized by a recruitment of immature neutrophiles into the bloodstream up to 10-fold, usually referred as the "left shift". Sepsis is the most prevalent cause of death in intensive care facilities according to this article making its early diagnosis a need in order to administrate a suitable antibiotic treatment. Blood culture is the "gold standard" method but it is more expensive, requires times compare to IMG count.[12]

In neonatal intensive care unit, **early onset sepsis (EOS)** is a major cause of morbidity and mortality according to an article by Cimenti et al.[14]. In the study, they conclude that "significantly higher percentages of Immature Granulocyte were observed in infected compared to non-infected neonates and in neonates with positive compared to negative blood cultures"[14]. Furthermore, this study showed that automated IMG count can replace "gold standard" techniques for the diagnosis of neonatal sepsis infection[5]. The automated count of immature granulocyte offers useful information for the diagnosis of neonatal early onset sepsis.

Conclusion

The introduction of WBC differential count using an association of impedance and optical extinction technologies has allowed the screening of abnormal leukocyte in the peripheral blood. Among them, with the IMG parameter, Yumizen analysers can flag abnormal Immature granulocyte levels in patient sample. Studies have demonstrated strong correlation between automated immature granulocyte and gold standard technique like manual microscopic count. Studies have also proven the clinical utility of such parameter and technology in the diagnosis and management of several diseases.

Immature granulocyte count using IMG parameter is accurate, reliable and can be obtained rapidly, without additional reagents, blood collection and without changing your current HORIBA Medical equipment. IMG values can be obtained without additional cost in WBC differential count.

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