ATYPICAL LYMPHOCYTES

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Our bodies are constantly exposed to pathogens present in the environment, including viruses, bacteria, parasites, and fungi. The immune response is responsible for defending us against them through a wide range of mechanisms. It is mediated by white blood cells (WBCs), also called leukocytes, which include neutrophils, eosinophils (acidophiles), basophils, lymphocytes, and monocytes. Innate and adaptive immunities work together to protect us (1).

Phagocytic WBCs (in particular, neutrophils) are part of the innate branch of the immune system, along with tissue macrophages, that have a number of different functions such as triggering the immune response and cell to cell communication. Lymphocytes mediate the adaptive immune response via antigen-specific activities.

Plasma cells, not normally seen in the peripheral blood, are differentiated B-lymphocytes. They are non-proliferating and much larger than B cells and can secrete large amounts of antibodies (2).

Blood Smear

Seen microscopically on a blood smear, lymphocytes have a regular, spherical nucleus and a high nucleo-cytoplasmic ratio. The cytoplasm, which varies in volume, is stained a pale blue with Romanowski staining and is either clear or with scanty, small pink granules. The presence of granules in a larger cell with more cytoplasm may indicate that the cell is a T-lymphocyte but immunophenotyping is required to further differentiate the different lymphocyte subtypes (3).

Leukocyte differential count

A complete blood count (CBC) is a highly automated laboratory test which generates data for all blood cells. Part of the CBC is the WBC differential which identifies and counts the different types of normal blood cells and also provides additional measurements and flags regarding atypical, abnormal and immature WBCs.

In the Yumizen hematology analyzers, white blood cells can be visualized on the Diff scattergram or LMNE matrix (Figure 1).In this matrix optical extinction (Y-axis) is plotted against volumes (X-axis) to form an image with several clusters of cellular events. The matrix has thresholds, some fixed and some mobile, which allows the different populations to be determined and to detect abnormalities.

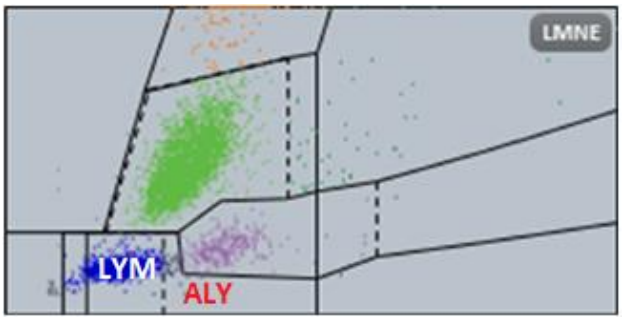


Figure 1 : Lymphocytes (LYM) and ALYs on LMNE matrix

This precise technology allows Yumizen hematology analyzers to define two additional leukocyte populations of relevance to lymphocytic cells: Large Immature Cells (including immature lymphoid cells – IML) and Atypical lymphocytes (ALY), reported in percentage as well as absolute counts.

Keywords: White Blood Cells, Lymphocyte, Plasma Cell, LMNE Matrix.

What are Atypical Lymphocytes?

Atypical lymphocytes are lymphocytes that have been activated to respond to a viral infection (occasionally a bacterial or parasitic infection). These cells are characterized by their increased size and often the presence of active protein synthesis which results in a deepening of the blue coloration of the cytoplasm by Romanowski staining. The increased activity can also give rise to changes in the nucleus, giving it a more open appearance with the possible presence of nucleoli (4). They vary in morphologic detail as well as surface marker characteristics, indicating that they comprise a heterogeneous mixture of cell types with a polyclonal immune response to antigenic stimulation. Lymphocytes appear on blood films in many varieties. Pathologically altered lymphocytes are associated with numerous diseases. For instance, small lymphocytes and a population of medium-sized lymphoid cells with high proliferative and apoptotic activity can be observed in Burkitt lymphoma (Figure 2).

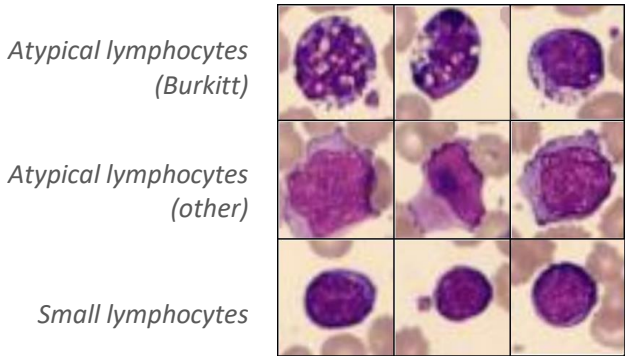


Figure 2: Different atypical lymphocytes identified on MGG-stained blood smear in a case of Burkitt lymphoma

ALY parameter on Yumizen hematology analyzers

On Yumizen hematology analyzers, the ALY parameter is provided for research purposes only (RUO). The different types of cells found in this area of the LMNE matrix and individual specificities make it difficult to provide definitive cut-off values for ALYs. Depending on the cells present, there may be an overlap between ALY and the IML of the large Immature cell (LIC) population.

The matrix employs mobile thresholding to optimize the capture of the ALY population. While this area generally represents large activated and reactive lymphocytes, it may also contain some small blasts, plasma cells and the abnormal cells of lymphoproliferative disorders such as CLL, Sezary cells and tricholeucocytes (hairy cells).

Where populations are less easily separated: Lymphocytes (LYM), Monocytes (MON), Neutrophils (NEU) and ALY (#, %) are flagged with a suspicion when the separation threshold is not found between the lymphocytes and monocytes areas (SepLymMon alarm), (Figure 3) allowing the reviewer to take further action such as blood film examination.

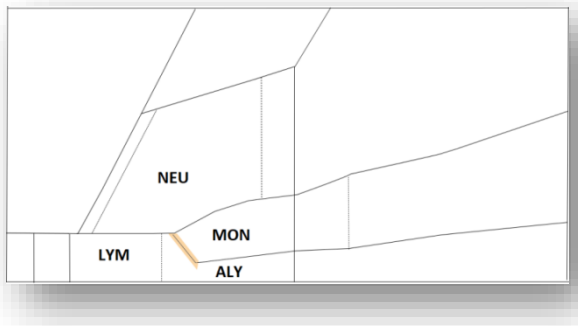


Figure 3: Separation threshold triggering the ALY alarm on LMNE matrix

Although ALYs are unusual for healthy individuals, clinical test results are commonly interpreted against population-based reference intervals. Therefore, a reference interval study according to the CLSI C28-A3 guideline (Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory) was performed for the ALY parameter on Yumizen H2500 and H1500 analyzers. A total of 240 (120 males and 120 females, > 18 years of age) whole blood samples collected in EDTA from apparently healthy Caucasian donors were analyzed in duplicate on three instruments, two Yumizen H2500 and one Yumizen H1500. Samples were kept at room temperature between the sampling and the test. The reference interval was determined in order to include the lower and upper reference limits, which enclose 95% of the values from the reference population subjects. The results are given in Table 1.

Keywords: Infection, Romanowski staining, mobile tresholding.

	Female	Male	**Bibliographic data** (male and female)
Lower reference limit (**ALY%**)	0.20	0.20	0.00
Upper reference limit (**ALY%**)	1.90	1.70	2.50
Lower reference limit (**ALY#**)	0.01	0.01	0.00
Upper reference limit (**ALY#**)	0.14	0.09	0.20

Table 1: Lower and upper reference limits determined by the study for ALY parameter compared to values from bibliographic data (5, 6).

What is their clinical significance?

The presence of few atypical lymphocytes in most blood samples is probably of little clinical significance. However, the ALY parameter can help the clinician to consolidate a diagnosis or to use it for a prognostic purpose. Atypical lymphocytes are most commonly found in Infectious Mononucleosis, Toxoplasmosis, Hepatitis and other viral infections.

Atypical lymphocytes have also been observed in the peripheral blood of patients in a large number of other clinical situations, including immune reactions to transplantation and immunization, collagen diseases and other autoimmune disorders, malignant disease, drug reactions, certain bacterial infections, medications and stress (7). In Acute Lymphoid Leukemia (ALL) with small blasts, these lymphoblasts are frequently seen in the ALY area. Figure 4 shows an example of T-cell ALL where the lymphoblast population is especially present in the atypical lymphocyte area of the LMNE matrix.

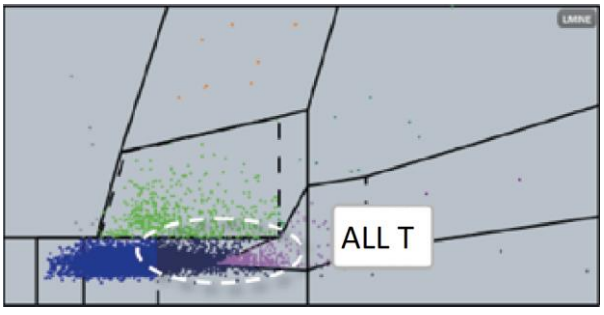


Figure 4: Lymphoblast population in acute lymphoid leukemia T (ALL T)

In chronic lymphoid leukemia, atypical lymphocytes and immature lymphocytic cells also can be observed on the ALY area of the DIFF scattergram (8).

Small compact blast cell population from acute myeloid leukemia can feature in this region owing to their smaller size. In this case, the compact blast population can be observed on the LMNE matrix between the lymphocyte, atypical lymphocyte and monocyte areas and are flagged accordingly. For monitoring the progress of a given infection, ALYs can be used as a prognostic tool. For example, atypical lymphocytes circulating in blood have been reported in COVID-19 patients. COVID-19 reactive lymphocytes were found to be related to a better evolution and prognosis of the disease. Their presence suggests an abundant production of virus-specific T cells, thus indicating the better prognosis of patients with these cells in the circulation (9, 10).

Comments

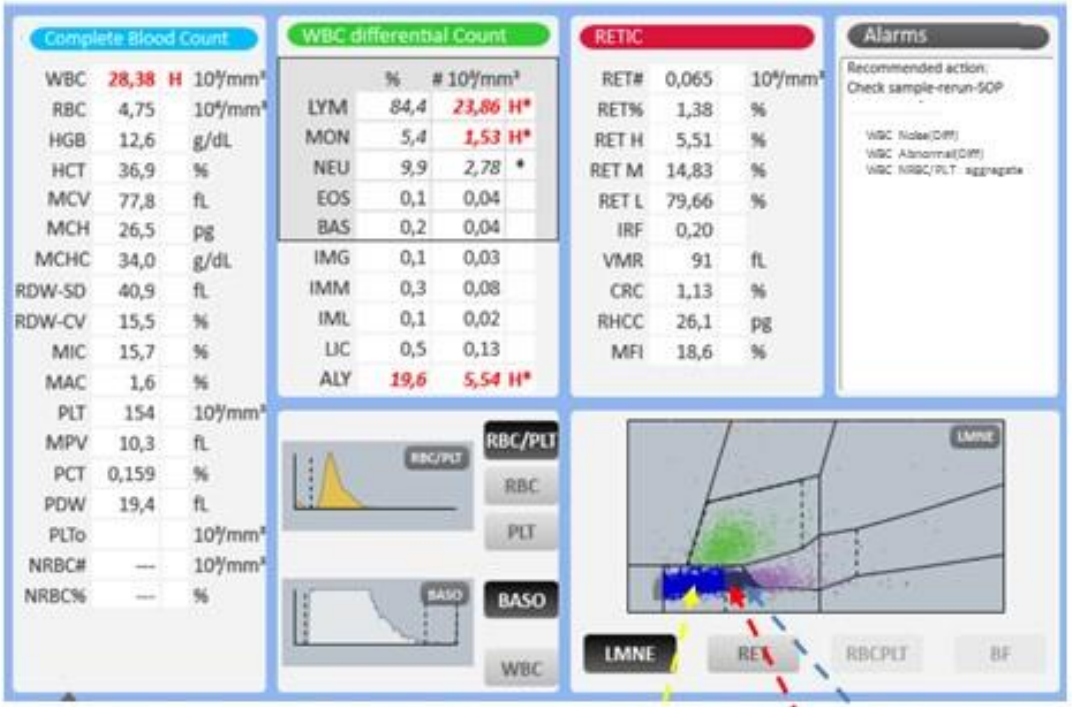
Today's high-throughput analyzers are designed to provide the most comprehensive information about a sample. This data is based on threshold values and complex algorithms, and it must be subsequently carefully interpreted by the clinician. In this context, the ALY parameter provides information that may complement the routine blood parameters and add to the patient's clinical picture. It may also be useful in disease management or for prognostic purposes.

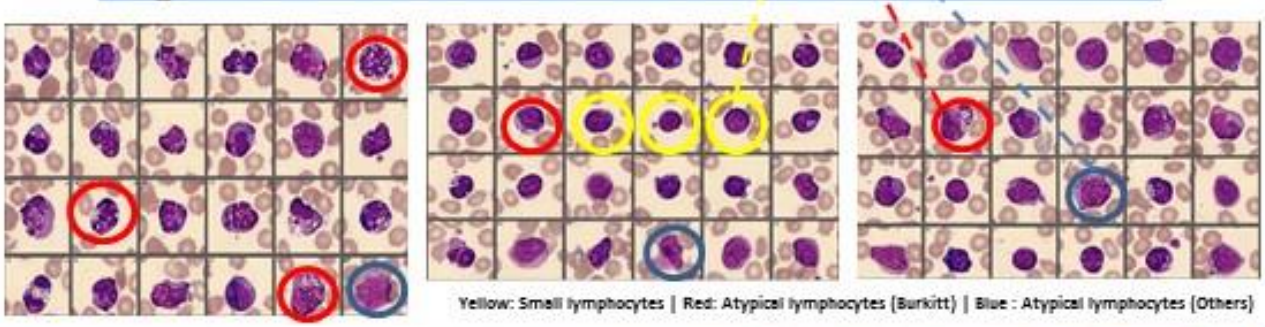
Keywords: Leukemia, lymphoblast, COVID-19

HORIBA
Medical

Yumizen
Chase the Case #2

Patient demography: 32 years, Male
Suspicion: Lymphoma suspected
Clinical Sign: Splenomegaly and enlarged lymph nodes
Other results: Immunophenotyping: The analyzed population corresponds to mature B lymphocytes with clonality for light chain immunoglobulin Lambda Immunophenotyping compatible with Chronic Lymphoproliferative Disease B.
Diagnosis: Burkitt's lymphoma





Yellow: Small lymphocytes | Red: Atypical lymphocytes (Burkitt) | Blue : Atypical lymphocytes (Others)

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Keywords: Burkitt lymphoma, Splenomegaly, Immunophenotyping

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Thanks, note:

“The authors thank Dr Parag Dharap, MD, Dr. Dharap's Diagnostic Centre, Mumbai, India for manuscript review and for general advice"