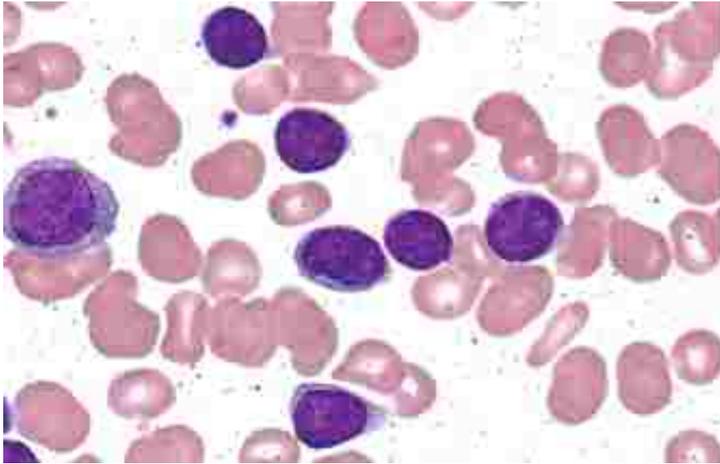


LYMPHOCYTOSIS

Causes and approach to evaluation in adults

General

Lymphocytosis refers to an increase of peripheral blood lymphocytes, which for adults is defined as an absolute lymphocyte count (ALC) $> 5.0 \times 10^9/L$.



Lymphocytosis

Lymphocytes generally constitute 8-33% of white blood cell count (WBC) in peripheral blood. The normal number and distribution of lymphocyte subsets vary with age.

The major lymphocyte subsets are:

1. T-cells: thymus-derived, important for Antigen recognition and binding 60 to 80% of the ALC
2. B-cells: bone marrow-derived, produces immunoglobulins ("antibodies"), 10 to 20% of the ALC
3. Natural Killer (NK)-cells: cytotoxic cells (large granular lymphocytes – LGL) that are part of the Innate resistance/ first line defence, 5 to 10% of the ALC

Lymphocyte differentiation and maturation are life-long processes that take place in the bone marrow and secondary lymphoid organs (lymph nodes, spleen, and thymus).

Lymphocytosis can be categorized as either polyclonal or monoclonal. Polyclonal lymphocytosis is usually secondary to stimulation or reaction to factors extrinsic to lymphocytes, generally infection and/or inflammation. Monoclonal lymphocytosis generally reflects an underlying lymphoproliferative disorder (malignant process) because of an intrinsic defect in the expanded lymphocyte population.

Causes of Lymphocytosis:

A. Polyclonal

These conditions are associated with an increase in the ALC secondary to a physiologic or pathophysiologic response to infection, toxins, cytokines or unknown factors.

1. Infectious Mononucleosis (IM): This is the most common reactive cause - usually Epstein Bar Virus (EBV) (15 - 25 years of age), Cytomegalovirus (CMV) (30 – 60 years of age) and less commonly HIV and Toxoplasma Gondii. The B-cell is infected in EBV IM and the macrophage in CMV IM. The lymphocytosis is caused by an increase in cytotoxic T-cells.

2. Bordetella Pertussis: Pertussis toxin blocks migration of lymphocytes from the blood stream into the lymph nodes because of inhibition of chemokine receptors. All the lymphocyte subsets are affected.
3. Stress: This includes conditions such as trauma, septic shock, myocardial infarction, sickle cell crisis, status epilepticus, acute cardiac failure. $ALC > 5 \times 10^9/L$; the lymphocytosis is usually transient, reverting back to normal within 24-48hrs. The mechanism is most likely due to lymphocyte redistribution, affecting all the subsets and can be induced by the adrenalin released or administered in response to the acute medical episode.
4. Hypersensitivity reactions: Delayed hypersensitivity reactions to insect bites may be associated with an increase in large granular lymphocytes. An infective mononucleosis-like syndrome can be induced by salazosulfapyridine or sulfasalazine.
5. Immune disorders:
 - a) Rheumatoid Arthritis(RA) may be associated with LGL lymphocytosis. These patients (0.6% of RA) may have neutropenia in the absence of splenomegaly and may represent a subset of Felty's syndrome.
 - b) Autoimmune pure red cell aplasia or Immune Thrombocytopenia and Aplastic Anaemia may also have LGL lymphocytosis.
6. Persistent lymphocytosis: This entails a chronic lymphocytosis associated with a variety of clinical conditions:
 - a) Cancer eg. thymoma (increased polyclonal T-cells), Acute Myeloid Leukaemia (AML)
 - b) Cigarette smoking – polyclonal CD4+ T-cells and B-cells
 - c) Post-splenectomy lymphocytosis – ALC ranges from $4-7 \times 10^9/l$ with increased LGL and expansion of the NK-cells
 - d) Chronic infection eg. leprosy, leishmaniasis, strongyloidiasis
7. Persistent Polyclonal B-cell lymphocytosis: A rare disorder with accumulation of polyclonal B-cells that have an unusual binucleate appearance on peripheral smear, more common among smokers and most often seen in young to middle- aged women. It is typically associated with the HLA-DR7 haplotype. Although the lymphocytosis is not progressive, many patients will have chromosomal abnormalities and a few will develop B-cell lymphoproliferative disorders.
8. Monoclonal:

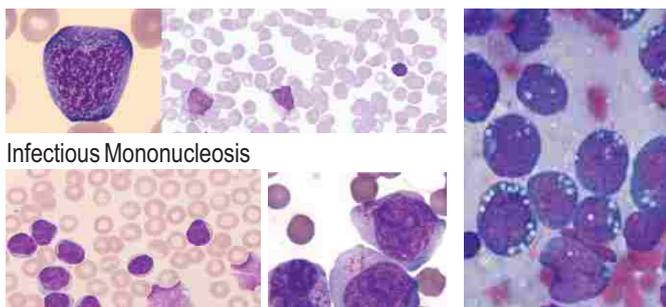
Clonal lymphocytosis exist on a spectrum that ranges from premalignant disorders to a variety of lymphomas and leukaemias, involving all the different lymphocyte subsets.

 1. Monoclonal B-cell lymphocytosis (pre-malignant): This condition is characterized by monoclonal B-cell count $< 5 \times 10^9/L$ in the peripheral blood, with no associated organomegaly or lymphadenopathy. The frequency increases with age, (50 - 75% of 90 year olds) and the higher the clonal lymphocyte count, the higher the frequency to CLL progression.
 2. Lymphoproliferative disorders: These comprise a wide spectrum of different morphologic and clinical syndromes and can arise from primitive lymphoid stem cells or from mature, differentiated lymphoid cells e.g. Chronic Lymphocytic Leukaemia (CLL), Follicular Lymphoma, Mantle Cell Lymphoma, Acute Lymphoblastic Lymphoma, etc.

Investigations:

The initial evaluation includes history, physical examination and FBC with peripheral smear review.

1. Full Blood Count (FBC): The FBC should be repeated to confirm the abnormality – the period will depend on the clinical state, the peripheral smear findings and the ALC. The higher the count, the greater the likelihood of a malignant disorder. Other abnormalities in the FBC include eosinophilia associated with allergic conditions, drugs or inflammatory disorders; neutrophilia/ monocytosis associated with infections or asplenia and anaemia (haemolytic anaemia or pure red cell aplasia) associated with CLL or LGL leukaemia and thrombocytopenia.
2. Peripheral smear: The peripheral smear examination should always be performed as distinctive lymphoid forms may be seen, being clues to the origin of the lymphocytosis.



Infectious Mononucleosis

Chronic Lymphocytic Leukaemia

Large Granular Lymphocytes

Burkitt's lymphoma

3. Other tests: Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), urea, creatinine and electrolytes (U+E/CR) and liver function tests (LFT).
4. Specialised tests: When indicated, specialized tests may help distinguish between reactive and malignant disorders and identify associated clinical findings. Features suggestive of malignancy include persistent lymphocytosis (ALC > 5X10⁹ /L) on repeat FBC after 4wks in the absence of viral infection, offending drugs or asplenia; cytopenias on FBC; worrisome findings on peripheral smear such as blasts or lymphocyte morphology suggestive of malignancy; lymphadenopathy or hepatosplenomegaly and B-symptoms (fever, weight loss and night sweats).

These tests include:

- a) Lymph node/ tissue biopsy
- b) Tests for Clonality:
 - i) Flow cytometry: This is the preferred technique to define clonality because it is rapid and more cost-effective.
 - ii) Molecular testing: PCR and DNA sequencing based on the rearrangements of the immunoglobulin genes (B-cells) or T-cell receptor genes (T-cells).
 - iii) Chromosomal analysis: FISH analysis is important in diagnosing clonal disorders that are not differentiated based on morphology and immunophenotype eg. t(11;14) in Mantle Cell Lymphoma.

These tests are expensive and should, preferably, be requested after consultation with a haematologist.

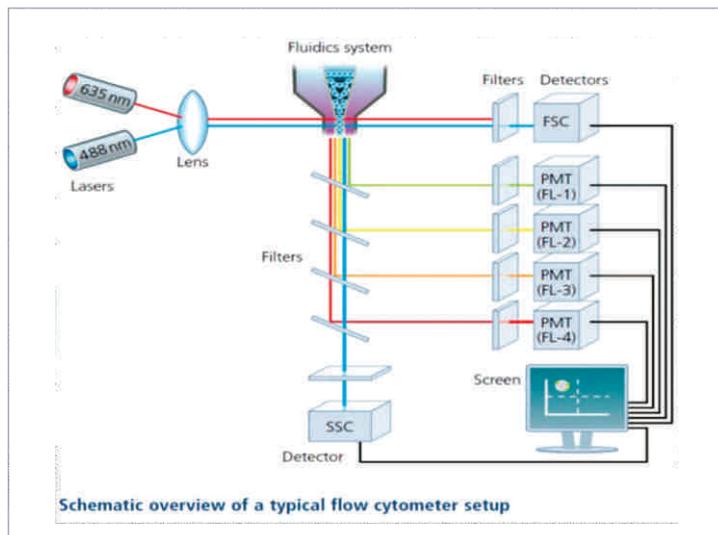
The value of Flowcytometry (FC):

FC plays a very important role in the diagnosis, subclassification and post-treatment monitoring of haematologic neoplasms.

Flow cytometry defines clonality and determines lineage (B-cell vs T-cell) and immunophenotype (marker profile) of the lymphocytes.

FC analysis requires fresh material samples (blood, bone marrow aspirate, effusions, CSF, fine needle aspirates). The samples are incubated with monoclonal antibodies directed towards specific surface, cytoplasmic or nuclear antigens. These antibodies are conjugated with fluorochromes

which are excited or stimulated by lasers in the flow cytometer. Different fluorochromes are excited by different wavelengths and emit light at different wavelengths too. The emitting light of each cell is captured and each cell is analysed based on cell size, granularity, viability and immunophenotype (cell markers/ antigens). The intensity of the emitted light reflects the level of expression of the antigen of interest.



All malignant processes are clonal, however, not all clonal populations are malignant – some infectious or inflammatory causes may be associated with oligoclonal expansions of lymphocytes. Also, premalignant disorders (Monoclonal B-cell lymphocytosis) or early stages of some leukaemias may exhibit low levels of clonal lymphocytes among a larger population of polyclonal lymphocytes – this will necessitate follow-up (clinical monitoring with repeat FBC+/- flow cytometry) to fulfil the diagnosis.

Flow cytometry service is provided by Pathcare Vermaak .

The turnaround time is about 48hrs and the specimen requirement is an EDTA sample.

Consultation with the haematologist on call is recommended.

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