Catlab Informa

Bulletin No.141

INITIAL EVALUATION OF PATIENTS WITH LYMPHOCYTOSIS

Lymphopoiesis:

The normal lymphoid system is made up of the primary or central lymphoid organs and the secondary or peripheral lymphoid organs. In adults, the bone marrow (BM) and the thymus function as primary organs; in these, B and T lymphocytes originate respectively from the pluripotent hematopoietic stem cell, subsequently maturing without requiring the presence of antigens. The immune responses are initiated in the secondary organs and are made up of the lymph nodes, the spleen, Waldeyer's ring and the lymphoid accumulations dispersed in the mucosa of the digestive tract (Peyer's patches), the respiratory tract and the genitourinary tract. Here, the lymphocytes find the appropriate environment to be able to interact with antigens and with antigen-presenting cells (APC).
B cells (B from bursa of Fabricius) are responsible for producing antibodies in response to antigenic stimuli. T cells (T from thymus) are responsible for the cellular immune response.
Mature B cells comprise 10–15% of peripheral blood lymphocytes, 25–50% of lymphocytes in the lymph node and spleen, and 10% of those in the bone marrow. These cells are characterized by the presence of surface Ig that serves as receptors for antigen recognition. “Naive” B cells are small lymphocytes that circulate in peripheral blood and also occupy primary follicles and the follicular mantle zones of lymph nodes. Upon encountering an antigen that specifically matches their surface receptors, “naive” B cells transform, proliferate and mature into plasma or memory cells. This maturation into short-lived plasma cells occurs directly after antigen binding outside the germinal center and is independent of T cells; this is the primary IgM antibody response. Other antigen-exposed B cells migrate to the center of the primary follicle, where they proliferate and transform into centroblasts and modulate the expression of various molecules. In the germinal center, somatic hypermutation of the genes of the variable regions of the heavy and light chains of Ig ocurs, as well as the switching of the heavy chain class from IgM to IgG or IgA. Centroblasts mature into centrocytes that express surface immunoglobulins (SIg) different from their precursors as a consequence of the mutations produced. Centrocytes with a higher affinity for the antigen that is exposed by the follicular dendritic cells, and thanks to the interaction with these and with T cells, they are rescued from apoptosis and differentiate into long-lived or memory plasma cells. Post-germinal memory B cells are located in the marginal zone of the lymphoid follicle, but also migrate to the peripheral blood and are located in the white pulp of the spleen and in the mucosa-associated lymphoid tissue (MALT).
T cells are responsible for cellular immunity, i.e. cytotoxicity, delayed hypersensitivity, graft rejection and graft-versus-host disease. Mature T cells account for 70-80% of normal peripheral blood cells, 90% of those in the thoracic duct and 30-40% of those in the lymph nodes and spleen. The differentiation and maturation of T cell precursors originating in the bone marrow (prothymocytes) occurs in different areas of the thymus under the influence of the epithelial microenvironment. Antigen-specific mature T cells are produced in the thymic cortex. T cells that recognise self-peptides are eliminated by apoptosis. Mature T cells pass into the peripheral blood and subsequently settle in the paracortical zone of the lymph node and form a perivascular cuff around the splenic arterioles.
T cells, and to a lesser extent B cells, maintain a constant recirculation between the blood and the tissues, which allows for permanent immunological surveillance.
In contrast to B cells, which recognize antigens in their primitive conformation, for T cell recognition to occur, the antigens must be processed into small peptides and presented together with the major histocompatibility complex (HLA) molecules. This antigen processing function is carried out by the APCs. Exposure to the foreign antigen determines the proliferation and subsequent differentiation of T cells into the various effector cell subtypes, in a complex and balanced process in which the cells of the phagocytic mononuclear system intervene and in which a large quantity of cytokines are released. Antigen recognition occurs through the T

cell antigen receptor, in connection with the HLA system; specifically, helper T cells (CD4+) recognize HLA class 2 determinants, and cytotoxic-suppressor T cells (CD8+) recognize class 1 determinants. A small number of T lymphocytes persist, after exposure to the antigen, as memory cells capable of developing a faster and more effective response if the organism is exposed again to the same antigen.
In addition to B and T cells, 15% of circulating lymphocytes are composed of so-called natural *killer* (NK) *cells*. NK cells have the morphology of large granular lymphocytes. These cells possess membrane receptors for the Fc fragment of IgG and exhibit direct cytolytic activity, either mediated by the Fc region of antibodies or independent of them (natural cytolytic activity, not mediated by the HLA system, dependent on activating *killer receptors* and inhibitory *killer receptors* [KIR]). NK cells play an important role in the surveillance and destruction of cells that spontaneously undergo malignant transformation and in the defense against viral infections.

Lymphocytosis:
In adults, lymphocytosis is considered to be an absolute lymphocyte count greater than $4x10^9$/L and in children up to 10 years of age, greater than $7x10^9$/L.
To assess lymphocytosis, it is necessary to take into account the patient's age, clinical history, time of onset and evolution of lymphocytosis, physical examination (lymph node areas, liver and spleen), laboratory parameters and morphological findings of lymphocytes.
The causes can be divided into two large groups: reactive lymphocytosis and clonal lymphocytosis or lymphoproliferative syndromes (LPS).

Initial study:
- **History:** Ask the patient about the reason for the consultation; personal and family history; use of drugs. Age and time of onset are important to determine the cause of lymphocytosis. Ask about B symptoms (weight loss, fever and/or night sweats). Assessment by systems.
- **Physical examination:** Oral mucosa, skin lesions, lymph node areas (cervical, axillary, inguinal), liver and spleen.
- **Analysis:** including complete blood count, ESR, kidney and liver function. Proteinogram, LDH and Beta-2 microglobulin in case of suspected PFS.
- **Blood smear morphology:** Indicated in persistent lymphocytosis, progressive increase or suspected LPS. As we will detail later, CATLAB uses criteria for the review of smears in routine samples and we will also describe the morphological alterations that are usually found in both reactive and pathological lymphocytosis.
- **Infectious serology:** Taking into account the patient's age and clinical symptoms, consider requesting serologies for the different infections that present in lymphocytosis described in the following section.
- **Immunophenotype of lymphoid population in peripheral blood:** Useful for the detection of clonal or pathological lymphocytes. If they are not detected, this does not rule out a LPS since some types of lymphoma do not usually have expression in peripheral blood, such as Hodgkin's lymphoma, diffuse large B-cell lymphoma, etc.
- **Imaging tests:** Chest X-ray and abdominal ultrasound to rule out splenomegaly. In the case of a LPS, to study the extent of the disease, a CT or PET-CT scan should be considered in clinical hematology depending on the type.

Other studies, which will be requested in Hematology in case of suspected LPS, to confirm the diagnosis and study its extension are: Bone marrow aspiration/biopsy, Lymph node biopsy, Cytogenetics and Molecular Biology.

Causes of lymphocytosis:

1) Reactive Lymphocytosis:

- Subacute/chronic bacterial infections: Brucellosis, tuberculosis, syphilis, cat scratch disease
- Mononucleosis syndromes (**Fig. 1**): Epstein-Barr virus (80-90% of cases), Cytomegalovirus (especially important in pregnant women and immunosuppressed patients), *Toxoplasma gondii*, primary HIV infection, human herpes virus type 6, rubella, varicella-zoster virus, hepatitis virus. The clinical profile is characterized by fever, odynophagia (suppurative in 30% of cases), cervical adenomegaly, asthenia and splenomegaly mainly. In addition to lymphocytosis, the laboratory analysis also shows elevated liver enzymes.
- Other infections: whooping cough, Rickettsiosis
- Chronic lymphocytosis: autoimmune diseases, solid tumors, thymoma, chronic inflammation, smoking, sarcoidosis, hyposplenia (functional or anatomical), Persistent polyclonal B-cell lymphocytosis (PPBL) a lymphocytosis generally associated with female smokers with the presence of binucleated lymphocytes in peripheral blood (**Fig. 2**).
- Acute Lymphocytosis: stress lymphocytosis (AMI, heart failure, septic shock, major surgery, trauma, status epilepticus, sickle cell crisis, hypersensitivity reactions), drugs (hydantoins, penicillins, phenylbutazone, para-aminosalicylic acid, phenothiazines).

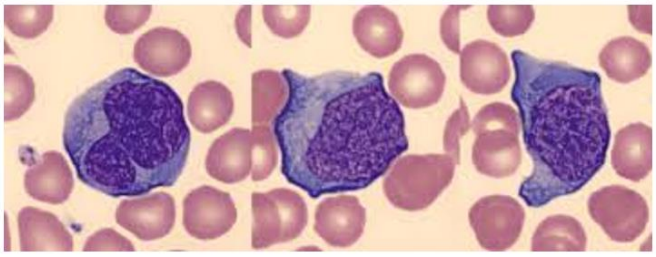

Fig. 1: Linfocitos activados o reactivos de la Mononucleosis infecciosa

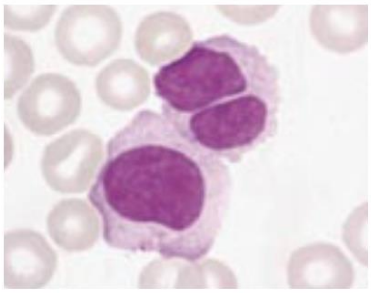

Fig. 2: Linfocito binucleado de la Linfocitosis B Policlonal Persistente

2) Clonal lymphocytosis or lymphoproliferative syndromes:

In this post we will not go into the latest WHO classification of lymphoid system neoplasms. Broadly speaking, they are divided into B-cell neoplasms, T-cell neoplasms and NK-cell neoplasms. Within each group, there are some lymphomas that have expression in peripheral blood (leukemic expression). Below, we show the most important ones with the typical morphology of the lymphocytes found in each of them:

- **From B cells:**

  . Chronic lymphatic leukemia (CLL):

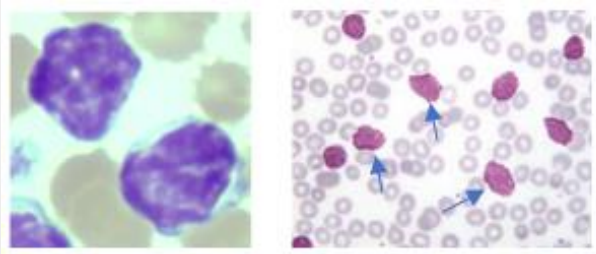

Fig. 3: Typical or classic CLL: small lymphocytes with condensed chromatin (turtle shell or lump appearance) and scarce cytoplasm. Proliferating lymphocytes are abnormally fragile, so they tear easily when extended, thus generating Gumprecht shadows (arrows).

. **Follicular lymphoma:**

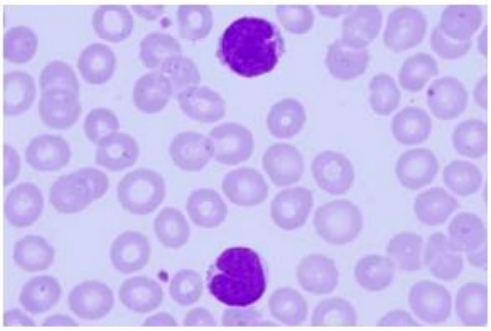

Fig. 4: Centrocytes with typical morphological features: small size, sparse cytoplasm and nuclear cleft.

. Prolymphocytic Leukemia:

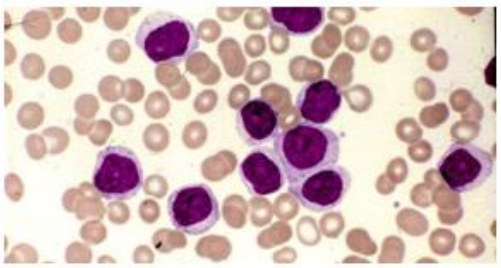

Fig. 5: Medium-sized prolymphocytes and semi-mature chromatin with a visible and prominent nucleolus of central location

. Mantle cell lymphoma:

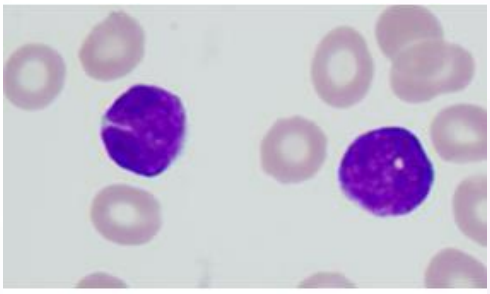

Fig. 6: The classical variant is polymorphous regarding the nuclear size and the irregularity of the nuclear contour. The cell size is more quite small and the nucleus, which occupies almost all of the cell, presents a chromatin of pointed aspect, with 1 or 2 small clefts.

. Splenic marginal zone lymphoma:

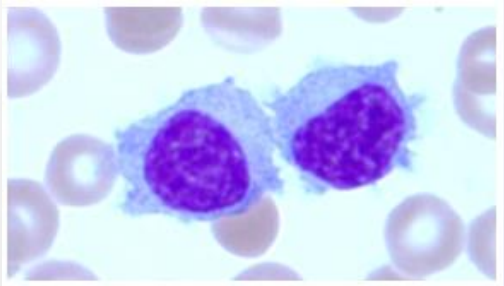

Fig. 7: Villous lymphocytes: larger than normal lymphocytes, round or oval nucleus with condensed chromatin, usually without visible nucleolus. The cytoplasm is basophilic with short villous extensions.

. Hairy cell leukemia:

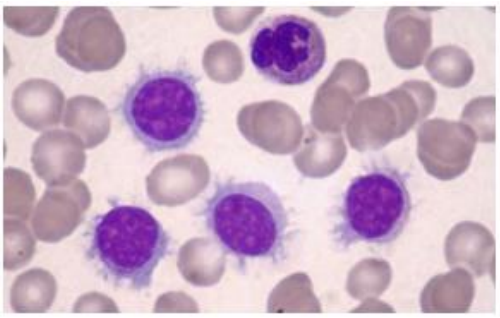

Fig. 8: Tricholeukocytes of medium or large size with nucleus with different configurations (round, ovoid or indented) of relatively eccentric position. The chromatin is lax and may show a small nucleolus. Broad cytoplasm, blue-gray in hue. The main feature is the numerous hair-like projections that give the cell its name.

. Lymphoplasmacytic lymphoma:

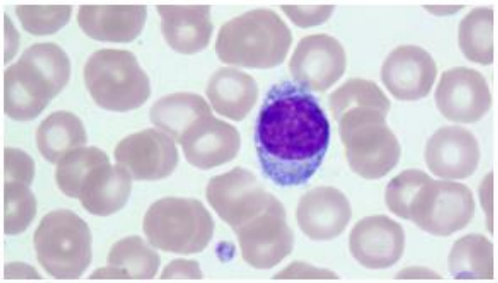

Fig. 9: Small/medium-sized lymphoplasmacyte, eccentric nucleus with condensed chromatin, with presence of Dutcher bodies (Ig nuclear inclusions), moderate, basophilic cytoplasm, occasionally with vacuoles. In addition, erythrocytes can be observed in Rouleaux formation.

. Burkitt lymphoma:

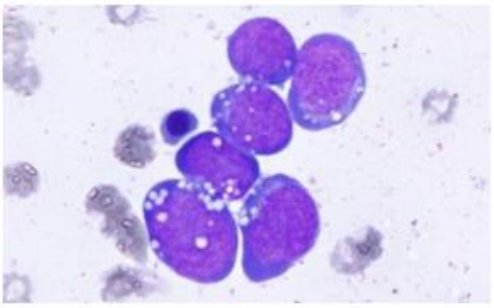

Fig. 10: Cells with a blastic, monomorphic appearance, of medium size, lax chromatin, one or more visible nucleoli and very basophilic cytoplasm with abundant vacuoles.

- **From T cells:**

. **Sézary Syndrome:** Leukemic Expression of Mycosis Fungoides (Cutaneous T-cell Lymphoma)

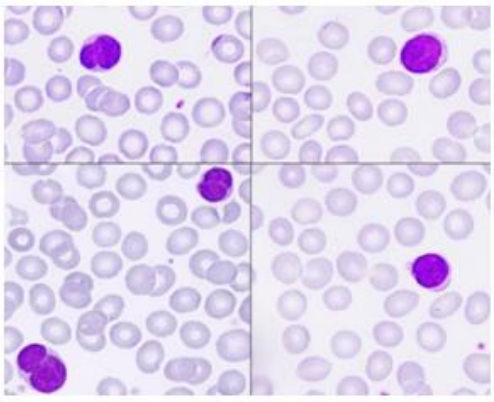

Fig. 11: Atypical lymphoid cells of small size, mature appearance with irregular "cerebriform" nucleus

. **Adult T-cell leukemia/lymphoma: associated** with the HTLV-1 virus

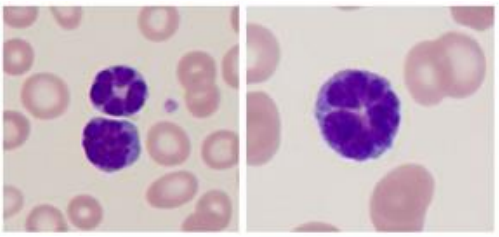

Fig. 12: Medium-sized lymphocytes with an irregular flower-shaped nucleus.

. **Large granular T-cell leukemia:**

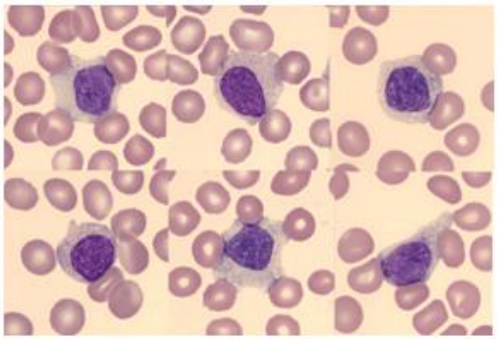

Fig. 13: Lymphoid cells of large size, round and dense nucleus, and large and clear cytoplasm in which the presence of azurophilic granules stands out.

- **From NK cells:**
  Aggressive NK cell leukemia:

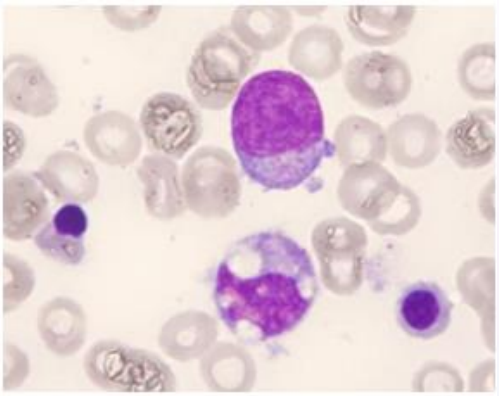

Fig. 14: Large lymphoid cells with abundant cytoplasmic granules.

Analysis of lymphocytosis in CATLAB:

The CATLAB central laboratory (Viladecavalls) analyses an average of 2,200 blood tests daily from 43 primary care centres and 3 secondary level hospitals in the Valles Occidental area, covering a population of 1,050,000 inhabitants.

In our work routine, we have algorithms to perform smear reviews in patients with lymphocyte parameter alterations, taking into account the person's age, the absolute number of lymphocytes and the analyzer alerts. When a smear review is generated for a sample, a peripheral blood smear is performed using the SP50 extender/stainer and then analyzed by digital microscopy using the Cellavision® DI-60. Both analyzers work connected in a chain to the 5 Sysmex XN-9100 analyzers performing blood counts in the laboratory (**Fig. 15**).

Fig. 15: **Sysmex XN-9100 chain composed of, from right to left, 3 XN-10 analyzers; 2 XN-20 analyzers; a TS distributor; 1 SP-50 extender-stainer and a Cellavision ® DI-60**

The cellular images obtained by Cellavision® are initially reviewed by laboratory technicians (TEL) who perform the cell count and describe the main morphological alterations detected. The hematologist then reviews the report and decides whether to validate the result or perform a new review using optical microscopy. If a previously undiagnosed LPS is suspected, the study is expanded using flow cytometry and, if confirmed, a notice is sent to the requesting physician by email or phone call.

In 2023, we generated 238 immunophenotype studies to rule out T/B LPS, of which 154 (65%) were positive for some type of LPS. Of the positive ones, 140 (91%) had the morphological description that matched the LPS diagnosed by flow cytometry; and the most frequent LPS was chronic lymphocytic leukemia (CLL) with 56% of cases (**Fig. 16**).

In a future CATLAB-Informa of the cytometry department, the LPS diagnosed in the different biological samples from different origins will be described in greater detail.

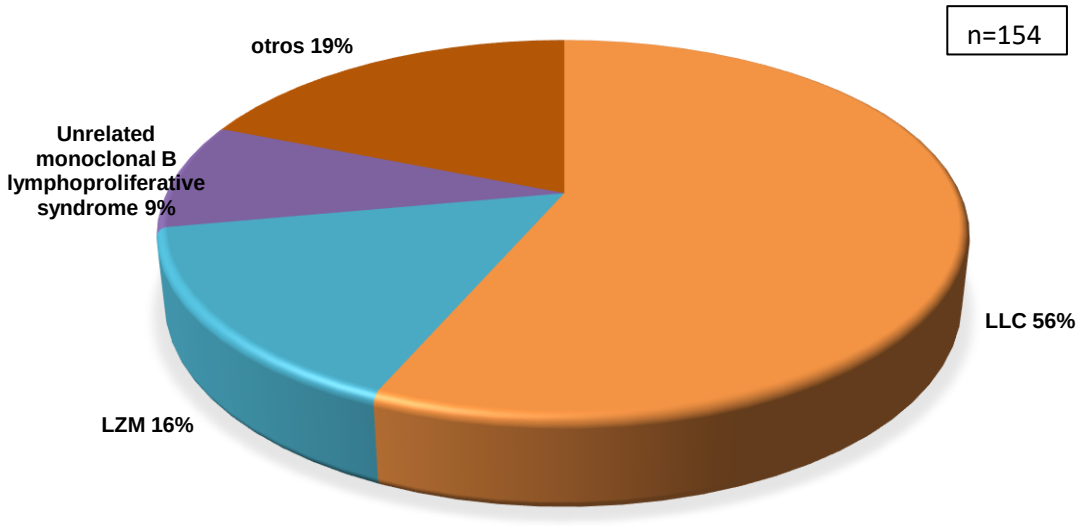

Fig. 16: Lymphoproliferative syndromes diagnosed in CATLAB expanded from smear review during 2023

DEPARTMENT OF HEMATOLOGY CATLAB

- **Jorge Medina** jmedina@catlab.cat
- **Teresa Villalba** tvillalba@catlab.cat
- **Miquel Diaz** mdiaz@catlab.cat

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