Immunophenotypic characterisation of Chronic Lymphoproliferative Disorders in our healthcare catchment Area: EuroFlow screening results from Catlab

Lymphocytes are hematopoietic cells that belong to the immune system. Their function is to specifically and adaptively recognize and respond to various foreign agents while inducing tolerance to self-structures.

B lymphocytes acquire their immunological competence in the bone marrow. Their primary functions are producing antibodies against antigens, acting as antigen-presenting cells to facilitate the activation of the adaptive immune response, and eventually transforming into memory (long-lived) B cells following antigen activation, thus enabling a more rapid and effective response to future infection by the same pathogen.

T lymphocytes originate from a common lymphoid progenitor in the bone marrow that undergoes a maturation process and acquires immunocompetence in the thymus. Subsequently, they leave the thymus and move to peripheral blood and secondary lymphoid organs, transforming into functionally mature T-lymphocytes. There are two main populations of mature T-lymphocytes, CD4-expressing helper T-lymphocytes whose functions are to activate other immune cells, such as B-lymphocytes, cytotoxic T-lymphocytes and macrophages, by releasing cytokines, facilitate B-lymphocyte differentiation and are also crucial in the response to viral and bacterial infections, and the CD8 antigen-expressing cytotoxic T-population whose main function is to destroy virus-infected cells or tumour cells.

NK cells also originate in the bone marrow from haematopoietic stem cells, but unlike T cells, they do not require maturation in the thymus. They are part of the innate immune response.
In either case, the haematopoietic stem cell, located in the bone marrow, gives rise, among others, to lymphoblasts (more immature lymphoid cells) and from them, after successive maturation stages, mature lymphocytes originate. Neoplastic transformation of these cells can occur at any of these lymphocyte maturation stages and this gives rise to the different types of lymphoid neoplasms.

Chronic Lymphoproliferative Disorders (CLPD) are a heterogeneous group of hematologic malignancies characterized by the clonal expansion of mature-appearing lymphoid cells that resemble various stages of normal lymphocyte differentiation and that have a proliferative and/or survival advantage over their normal counterparts in different organs such as bone marrow, blood and lymph nodes, resulting in a progressive accumulation of clonal cells and their products in the different organs mentioned.

According to the World Health Organisation (WHO), from a phenotypic point of view and according to their origin, SLPC or Mature Cell Neoplasms are classified into SPLC-B and SLPC-T and NK. T and NK lymphocyte SLPC are considered together in the latest WHO classification (2022), because some types have similar clinical, morphological, immunophenotypic and evolutionary characteristics, independent of the expanded cell, and clearly different from mature B-lymphocyte neoplasms.

Immunophenotyping by multiparametric flow cytometry is very useful in the differential diagnosis between reactive lymphocytosis and chronic lymphoproliferative disorders. It is based on identifying a specific cell type according to the expression of individual antigenic markers of the cell using fluorochrome-conjugated antibodies.
Any type of sample from which a cell suspension can be obtained, such as bone marrow, whole blood, biopsy, lymph node, cerebrospinal fluid, etc., can be analysed.

- **Algorithm used in Catlab for the study of lymphocytes**:

When a CLPD is suspected, we follow the strategy recommended by the EuroFlow group (Figure 1). Using this diagnostic algorithm, when we want to know whether the sample to be studied has a population of mature B, T or NK cells with a normal/reactive or aberrant phenotype, we use the Lymphoid Screening Tube (LST).

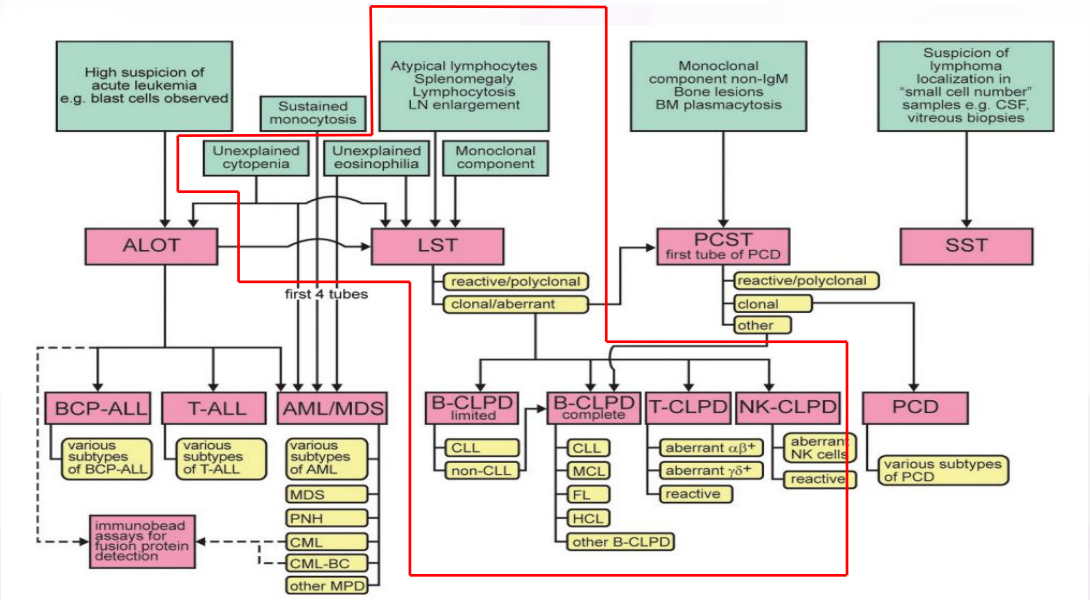

Figure 1. *Flowchart diagram of the EuroFlow strategy for immunophenotypic characterization of hematological malignancies.*
Abbreviations: ALOT, acute leukemia orientation tube; AML, acute myeloid leukemia; BC, blast crisis; BCP, B-cell precursor; BM, bone marrow; CLL, chronic lymphocytic leukemia; CLPD, chronic lymphoproliferative disorders; CML, chronic myeloid leukemia; CSF, cerebrospinal fluid; FL, follicular lymphoma; HCL, hairy cell leukemia; LN, lymph node; LST, lymphoid screening tube; MCL, mantle cell lymphoma; MDS, myelodysplastic syndrome; MPD, myeloproliferative disorders; PCD, plasma cell disorders; PCST, plasma cell screening tube; PNH, paroxysmal nocturnal hemoglobinuria; SST, small sample tube.

The combination of antibodies and fluorochromes of LST is shown in Table 1.

LST TUBE - LIMPHOCYTE SCREENING							
V450	V500	FITC	PE	PERCP-CY5.5	PE-CY7	APC	APC-H7
CD20 /CD4	CD45	CD8/ SmIgλ	CD56/ SmIgK	CD5	CD19/TCRγδ	CD3	CD38

Table 1: *Lymphocyte screening panel designed and validated by the Euroflow Consortium. Fluorochromes in the top row and labelled antibodies in the bottom row.*

This combination of eight fluorochromes/channels is detected with the three lasers of the Becton Dickinson cytometers (FACSCanto II™ and FACSLyric™) and allows us to confront 12 antibodies simultaneously (B, T and NK line antibodies) using the same exclusion antibodies in the same channel.

With the lymphocyte phenotypic pattern obtained from the analysis of the results of this tube using Infinicyt™ software, the study will be extended with one or another more specific panel in order to filter out the atypical population in question or, if no population with an aberrant phenotype suggestive of SLPC-B, T or NK is detected, as shown in the part marked in red in the diagram in figure 1, the study will no longer be continued.

- **Results of Euroflow Lymphocyte Screening in Catlab**:

In our laboratory, 927 LSTs were performed in 2023, of which 27% (249) were CLPD-B, 2% CLPD-T/NK (20), and 3% (30) were non-contributory results (these being considered samples with a lymphocyte count below the limit of quantification) and 6% (57) were follow-up or staging studies.

When breaking down the CLPD-B into the various subtypes, the results obtained were:

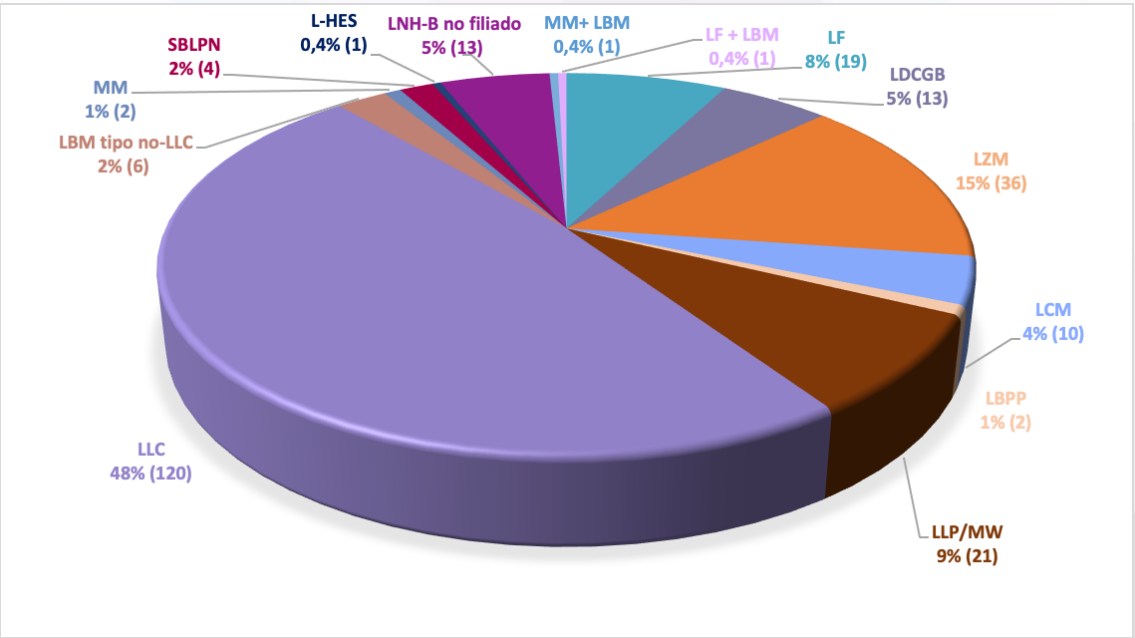

Figure 2: *Chronic B-lymphoproliferative syndromes diagnosed in CATLAB from LST.*

CLL: chronic lymphocytic leukaemia; MM: multiple myeloma; SBLPN: splenic B-cell lymphoma/leukaemia with prominent nucleolus; NHL-B-NHL: non-affiliated non-Hodgkin B-cell lymphoma; L-HES: lymphocytic variant hypereosinophilic syndrome; NHL-B-NHL: non-affiliated non-Hodgkin B-cell lymphoma; MZL: monoclonal B-lymphocytosis non-CLLL type; MF: multiple myeloma; HES: hypereosinophilic lymphocytic syndrome of lymphocytic variant: FL: follicular lymphoma; DLBCL: diffuse large B-cell lymphoma; MZL: marginal zone lymphoma; MCLL: mantle cell lymphoma; PPLL: persistent polyclonal B lymphocytosis; LLP/MW: lymphoplasmacytic lymphoma/Waldenström's macroglobulinaemia; ALCL: anaplastic large cell lymphoma.

It should be noted that the most common type detected was Chronic Lymphocytic Leukaemia, an entity that is often underdiagnosed because it is asymptomatic (WHO, 2017). In our centre, as it is a routine laboratory in which basic health studies are performed and where, from the haematology department, blood samples are sent to us for immunophenotypic study of those haemograms in which atypical morphologies suggestive of lymphoproliferative syndrome are detected, in most cases we do detect asymptomatic patients with no clinical symptoms.

As for the CLPD-T/NK:

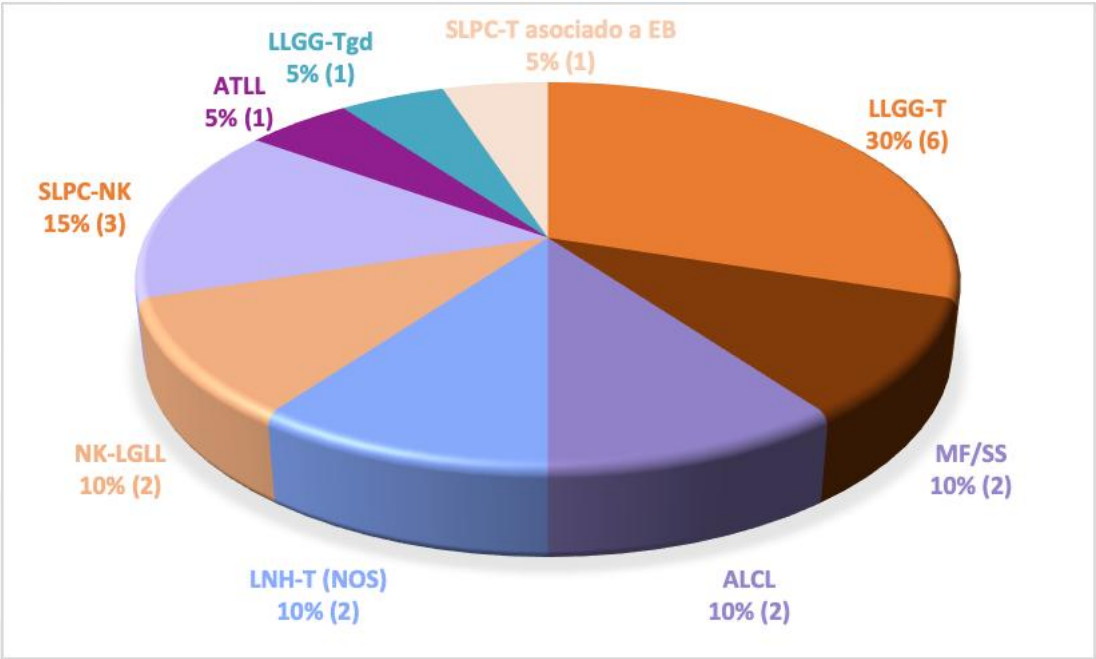

Figure 3: *Chronic T and NK lymphoproliferative syndromes diagnosed in CATLAB from LST. NHL-T (NOS): peripheral T-cell lymphoma (not otherwise specified); NK-LGLL: large granular NK-cell lymphocytic leukemia; ATLL: adult T-cell lymphoma; LLGG-Tgd: large granular gamma/delta T-cell lymphoma; CLPL-T associated with EB: chronic T-lymphoproliferative syndrome associated with Epstein-Barr infection; LLGG-T: large granular T-cell lymphoma; MF/SS: Mycosis fungoides/Sèzary syndrome.*

In the case of CLPD-T and NK, the most prevalent phenotype was Granular Large Lymphocyte Leukaemia.

In conclusion, atypical lymphocytes were detected in approximately one third of the 927 LSTs performed at Catlab in 2023, within a healthcare catchment area of roughly 1,050,000 inhabitants. Consistent with previous reports in the literature, CLPD-B phenotypes were markedly more prevalent than CLPD-T/NK phenotypes in the overall population, which is in agreement with our findings. In our study, the most frequent immunophenotypes among CLPD-B cases were CLL-B (120) and MZL (36), while among CLPD-T/NK cases were T-LGLL (6) followed by NK-LGLL (3).

We consider it very useful to use the Euroflow LST screening tube to rule out lymphoproliferative disorders and to assist our clinicians as well as to ensure patient safety.

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