Immunological profile of lympho-proliferative disorders

	CLL	Tricho	FL	MCL	MZL
Kappa Lambda	+	+/++	+++	++	+/++
CD19	++	+	+	+	+
CD10	-	-/+	+/++	-	-
CD20	+ low	+++	+	+	+
CD22	-	++	+	+	+++/+
CD23	++	-	+	-	-/+
FMC7	-	+++	+/-	++	++/-
CD5	++	+/-	-	++	-
CD11c	+ low	+	-	-	+/-
CD25	+ low	++	-	-	+/-
CD103	-	+++	-	-	-
CD43	++	-	-	+++/+	+/-
CD79b	+/-	-	++	++	+
CD38	-/+	-/+	-/+	-/+	-
CD43	+		-	+	

CLL: Chromic lymphocytic leukaemia; **FL:** Follicular lymphoma; **MCL:** Mantle Cell Lymphoma; **MZL:** Marginal zone lymphoma; **Tricho:** Tricholeukocytic leukaemia

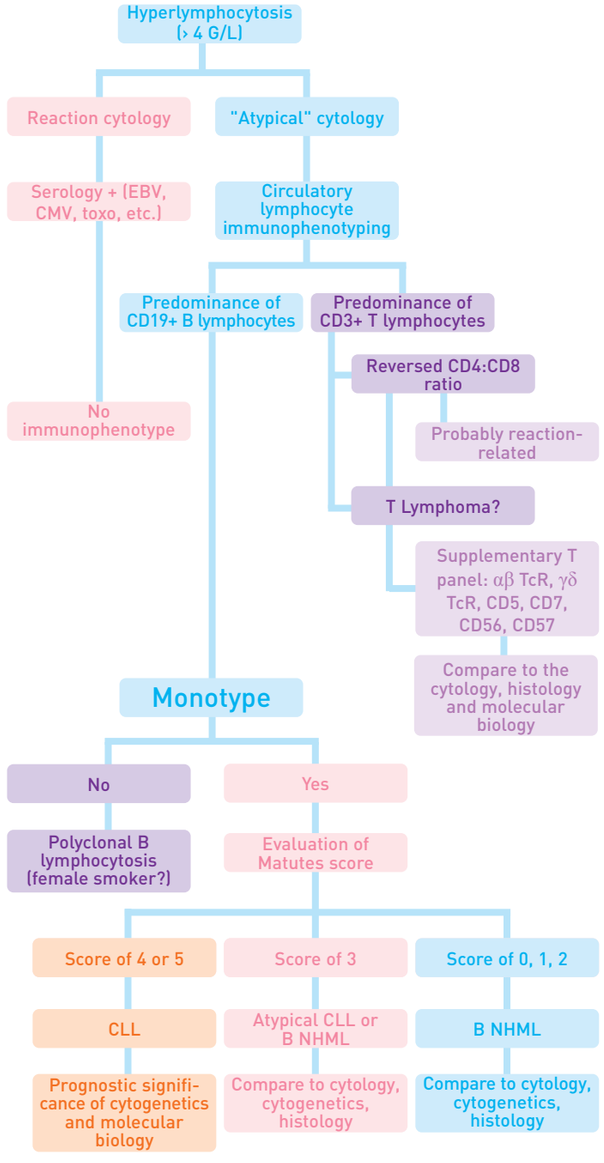
Taking it further
Supplementary tests

Study of residual disease in cases of CLL: it is possible to evaluate the response to treatment by quantifying the circulatory residual clone by means of flow cytometry. Depending on the teams, the sensitivity is 10-3 or 10-4.
A supplementary **cytogenetic study** is required to specify the diagnosis and assess the prognosis, both for CLL and MNHL (Malignant Non-Hodgkin's Lymphoma).
Molecular biology also makes it possible in cases of CLL to determine the mutational status of the genes of immunoglobulins of high prognostic value (bad if not mutated).

References

- The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. Matutes E, *et all*. Leukemia. 1994 Oct;8(10):1640-5.
- "WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues", Fourth Edition, Swerdlow, S.H., Campo, *et all*. WHO IARC Press 2008.
- International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia. Rawstron AC, *et all*. Leukemia. 2007 May;21(5):956-64. Epub 2007 Mar 15.

B lymphoproliferative disorders diagnostic procedure



Test conditions and results return time

- Blood/bone marrow: 1 EDTA tube if transport < 48h
  1 Heparin tube if transport > 48h
- 2 or 3 non-stained, non-fixed blood/bone marrow smears
- Result of last blood count.
- Specify "circulatory lymphocyte immunophenotype to screen for lymphoproliferative syndrome" on the order form.
- Always provide clinical information. Please use the specific B8-INTGB request form which can be downloaded from our website www.biomnis.com/international.

- **TAT:** 48 to 72 hours.

Contacts

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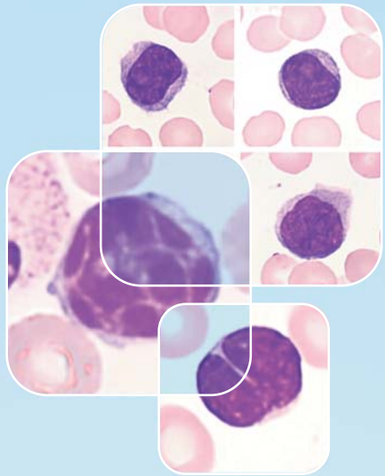
Focus on...



Significance of circulatory lymphocyte immunophenotyping in hyperlymphocytosis diagnostics

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Definition

Hyperlymphocytosis is defined by a **circulatory lymphocyte level of > 4G/L.**
It may consist of:

- Reaction hyperlymphocytosis.
- Tumoral hyperlymphocytosis, generally (over 90% of cases) type B. It generally consists of Chronic Lymphocytic Leukaemia (CLL), followed by leukaemic phases of malignant lymphoma and tricholeukocytic leukaemia (rarer).

What is the diagnostic approach?

- The cytological study is and should remain the cornerstone of the diagnostic approach in cases of hyperlymphocytosis in the laboratory.
- In cytological terms, cases of reaction hyperlymphocytosis appear to be polymorphous generally with the presence of stimulated lymphocytes (plasmocytic differentiation lymphocytes and/or hyperbasophilic +/- granular lymphocytes).
- Cases of tumoral hyperlymphocytosis are more or less monomorphous with an "atypical" cytology. For example, in cases of CLL, this consists of small lymphocytes with clustered chromatin accompanied by Gumprecht shadows.
- With respect to CLL, the latest guidelines **exclude bone marrow differential counts in the diagnostic evaluation.**

When should circulatory lymphocyte immunophenotyping be prescribed?


CLL cell

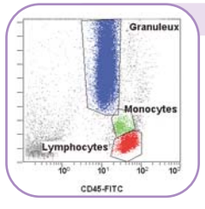
- In all cases of hyperlymphocytosis with a monomorphous and/or atypical cytology.
- In cases of atypical lymphoid elements without hyperlymphocytosis, the request for immunophenotyping is to be discussed in each individual case with the attending physician according to the clinical context.

When is it not necessary to prescribe immunophenotyping?

- When the diagnosis is already known apart from the evaluation of the residual disease.
- In cases of clearly identified mononucleosis (optimally documents with viral screening tests).

How should the results be interpreted?

In all immunophenotypes, a panel is used. A panel is a set of leukocytic markers or CD (Cluster of Differentiation). Each marker is more or less specific for a leukocytic type or subtype, for example CD3 is specific for T lymphocytes and CD19 is specific for B lymphocytes.

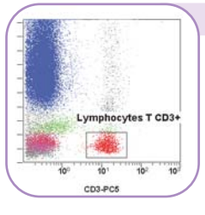


CD45 gating?

CD45 is the panleukocytic antigen enabling the effective separation of granulocytes, monocytes and lymphocytes. With this marker, it is possible to target lymphocytes precisely to establish the lympho gram, i.e. the evaluation of lymphocyte subpopulations, i.e.:

- T lymphocytes expressing CD3, N = 55 to 85%
- B lymphocytes expressing CD19, N = 10 to 20%
- NK lymphocytes expressing CD16/56, N = 10 to 20%
- NKT lymphocytes expressing both CD3 and CD16/56

The sum of T+B+NK must be approximately 100%.

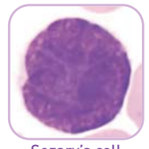


CD3 gating?

CD3 is the T lymphocyte marker. There are two types of T lymphocytes, CD4+ T lymphocytes, or T helpers, and CD8+ T lymphocytes, or cytotoxic T cells.

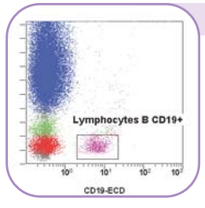
In healthy subjects, the CD4:CD8 ratio is greater than 1.

A reversal in the CD4:CD8 ratio indicates lymphocytosis reaction (current viral infection, mononucleosis) or a dysimmune state (e.g. HIV infection).


Sezary's cell

Finally, there are **γδ** T lymphocytes which are CD4-CD8, which represent a T lymphocyte sub-population of normally less than 5%.

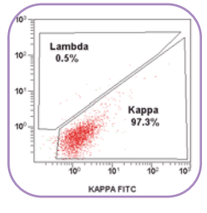
It may be useful in suspected cases of T lymphoma to study other T markers: CD2, CD5, CD7, CD56, CD57.



CD19 gating?

CD19 is the B lymphocyte marker. Other markers are also specific for B lymphocytes (although their expression may vary in a pathological context): CD20, CD22.

B lymphocytes are also characterised by the expression of immunoglobulins: IgG, IgM, IgA, IgD and their Kappa and Lambda light chains. For technical reasons, it is easier to study the expression of Kappa and Lambda light chains on the surface of B lymphocytes. In a polyclonal B population, approximately 2/3 Kappa B lymphocytes are found for 1/3 Lambda B lymphocytes. Monotypic population is observed when all the B lymphocytes are Kappa or Lambda.



Once monotypism has been defined, the immunological profile of this monotypic population is studied by determining the Matutes score.

What is the Matutes score?

The Matutes score corresponds to an immunological marker profile to confirm or exclude CLL in the presence of B monotypism.

Membrane markers	Points	
	1	0
Surface immunoglobulin (Kappa or Lambda) expression	Low	Moderate or high
CD5	+	-
CD22	-/low	Moderate/High
CD23	+	-
FMC7	-	+

Interpretation of score:

- **Score of 5/5 and 4/5: CLL.**
- **Score of 3/5: atypical CLL or B lymphoma.**
- **Scores of 0/5, 1/5 and 2/5: not CLL, but B lymphoma.**

There are variants of the Matutes score. Some laboratories replace, for the score calculation, CD22 by CD79b; others calculate a 6-point score, including CD79b in the score.

Why use other markers than those in the Matutes score?

Some markers have a prognostic value:
a positive CD38 and ZAP70 result is associated with a poor prognosis in the case of CLL.

Some markers indicate a given B NHML: CD10 for follicular lymphoma or CD43 for mantle cell lymphoma.

Some markers may have therapeutic significance. For example, CD20 and CD52 are therapeutic targets for Mabthera and Campath, respectively.


Tricholeukocytic

Some associations of markers are characteristic: cases of tricholeukocytic leukaemia are CD103+ CD11c+ CD25+.