**Immunological profile of lymphoproliferative disorders**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>CLL</th>
<th>Tricho</th>
<th>FL</th>
<th>MCL</th>
<th>MZL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kappa Lambda</td>
<td>+/++</td>
<td>+/++</td>
<td>+/++</td>
<td>+/++</td>
<td>+/++</td>
</tr>
<tr>
<td>CD19</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD10</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>CD20</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD22</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD23</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD61c</td>
<td>+</td>
<td>low</td>
<td>++</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD71</td>
<td>+/-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD71b</td>
<td>+/-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD103</td>
<td>+</td>
<td>low</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>CD43</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD79b</td>
<td>+/-</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>CD38</td>
<td>-/+</td>
<td>-/+</td>
<td>-</td>
<td>-/+</td>
<td>-/+</td>
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</tbody>
</table>

**Taking it further**

**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 method, the sensitivity is $10^4$ or $10^5$.

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**


**Focus on...**

- **Significance of circulatory lymphocyte immunophenotyping in hyperlymphocytosis diagnostics**
- **B lymphoproliferative disorders diagnostic procedure**

**Test conditions and results return time**

- Blood: 2 or 3 EDTA blood tubes
- 2 or 3 non-stained, non-fixed blood smears
- Result of lastest blood count.
- Specify “circulatory lymphocyte immunophenotype to screen for lymphoproliferative syndromes” on the order form
- If possible provide clinical information
- TAT: 5 working days

**Contacts**

**Malignant blood disease biological unit**

- Biomnis Ireland
  - Three Rock Road
  - Sandyford Industrial Estate
  - Sandyford
  - Dublin 18
  - Tel.: (01) 295 8545
  - Fax: (01) 295 5399
  - E-mail: sales@biomnis.ie
  - Web: www.biomnis.ie

**Immunological profile of lymphoproliferative disorders**

- **CLL:** Chronic lymphocytic leukaemia
- **FL:** Follicular lymphoma
- **MCL:** Mantle Cell Lymphoma
- **MZL:** Marginal zone lymphoma
- **Tricho:** hairy cell leukaemia

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**Taking it further**

**Supplementary tests**

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**References**

Hyperlymphocytosis is defined by a level of 4*10^9/L. It may consist of:
- Reaction hyperlymphocytosis.
- Tumoral hyperlymphocytosis, generally (over 90% of cases) T. It generally consists of Chronic Lymphocytic Leukaemia (CLL), followed by leukaemic phases of malignant lymphoma and tricholeukocytic leukaemia (rare).

### 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 reactive hyperlymphocytosis appear to be polymorphous generally with the presence of stimulated lymphocytes (plasmacytoid differentiation lymphocytes and/or hyperbasophilic +/- granular lymphocytes).
- Cases of tumoral hyperlymphocytosis are more or less monomorphic with an "atypical" cytology.
- In all cases of hyperlymphocytosis with a monomorphic and/or atypical cytology, 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 documented 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.

#### CD19 gating?

CD19 is the B lymphocyte marker. Other markers are also specific for B lymphocytes (although their expression may vary in a patholo- gical context).

- CD20, CD22.
- B lymphocytes are also characterised by the expression of immunoglobulins: IgG, IgM, IgA 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 monotypy.

<table>
<thead>
<tr>
<th>Membrane markers</th>
<th>Points</th>
<th>Interpretation of score:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface immunoglobulin (Kappa or Lambda) expression</td>
<td>Low</td>
<td>Moderate or high</td>
</tr>
<tr>
<td>CD5</td>
<td>-</td>
<td>Moderate/High</td>
</tr>
<tr>
<td>CD22</td>
<td>-/low</td>
<td>Moderate/High</td>
</tr>
<tr>
<td>CD23</td>
<td>+/-FM7</td>
<td>-</td>
</tr>
</tbody>
</table>

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

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