Belgian consensus recommendations for flow cytometric immunophenotyping. The Belgian Association for Cytometry / Belgische Vereniging voor Cytometrie / Association Belge de Cytometrie.


Van Bockstaele DR, Deneys V, Philippé J, Bernier M, Kestens L, Chatelain B, De Waele M, Demanet C
Ten years after
Guidelines for an integrated diagnostic approach of chronic lymphoproliferative diseases in the routine laboratory of haematology in Belgium

Acta Clin Belg 2009: ?

BVAC/ABCA +/or experts in molecular analyses
Why guidelines?

Opportunity to meet colleagues active in the field of laboratory hematology and to discuss on how to apply theory in practice

Update of the guidelines of 1999 published by BVC/ABC (BVAC/ABCA)
Ten years after…in 2009

The field of laboratory hematology has dramatically changed since 1999, with especially more emphasis on molecular analyses (and cytogenetics).

Introduction of molecular analyses in the list of reimbursed biomedical tests.

Introduction of the new ISO 15189 certification with emphasis on integration of results (cytomorphologic, immunophenotypic, molecular, cytogenetic, anatomopathologic data) with the final goal of improving the quality of the results generated by medical labs.
Results of all debates…

A paper to be published in Acta Clinica Belgica, entitled “Guidelines for an integrated diagnostic approach of chronic lymphoproliferative diseases in the routine laboratory of haematology in Belgium.”

minimal workout of chronic lymphoproliferative disorders in a routine laboratory of haematology
conclusions drawn in dialogue with the clinician and experts in cytogenetics and histopathology
integration of cytomorphological, immunophenotypical and molecular data
these guidelines are not intended to be used as universal ‘diagnostic pathways’, but should be useful in developing local diagnostic pathways
starting point was essentially based upon clinical and/or haematological indications …. 
Clinical and/or Haematological indications

peripheral blood lymphocytosis
cytopenia (anaemia, leukopenia, thrombocytopenia or pancytopenia)
lymphadenopathy or extranodal masses
monoclonal gammopathy or unexplained plasmocytosis of bone marrow
(hepato)splenomegaly
skin rash
fever of unkown origin
Peripheral blood lymphocytosis

Fresh blood smear
- Reactive lymphocytes → nothing / serology
- If still doubts → screening panel for immunophenotyping (CD3-CD4-CD8-CD19), gating based upon CD45/SSC.

B-cell lymphocytosis > 500/µL → clonality?
- Three main goups: CD5+/CD10-
  CD5-/CD10+
  CD5-/CD10-
- STORE DNA!
- Prepare 10 blood smears → FISH
  (In case of rare events → purify by MACS or FACS)
CD5+/CD10- B-cell lymphocytosis

CLL?
  • To be recognized by its typical morphology
CD5+/CD10- B-cell lymphocytosis

**CLL**

- Typical surface markers: CD23+ / CD79bw / FMC7- / weak expression of Ig / CD20w
- If count < 5000/µL → MLUS
- ZAP-70 and CD38: prognostic markers, but NOT routine
- General remark: if clonality is doubtful by FC → molecular analysis focused on multiple targets (BIOMED-2)
- Patients < 65y → mutational analysis may be considered
- FISH: 17p- (and 11q-) ~ worse prognosis
- Determination of therapeutic targets: CD20 (rituximab), CD52 (alemtuzumab), CD23 (lumiliximab)
CD5+/CD10- B-cell lymphocytosis

Mantle cell lymphoma
  • Morphology
**CD5+/CD10- B-cell lymphocytosis**

**MCL**

- Surface markers: CD23- / FMC7+ / CD20+ / Ig expression is strong / $\lambda > \kappa$
- FISH is the preferred method for detection of $t(11;14)(q13;q32)$
- If FISH+ → PCR (if also+ to be used for follow-up)
CD5-/CD10+ B-cell lymphocytosis

Demonstrate monoclonality by FC
Leukemic phase of follicular lymphoma
Leukemic phase of DLBCL
Leukemic phase of Burkitt lymphoma

! HISTOPATHOLOGY !
But cytomorphology, even more than FC may be helpful in finding a DLBCL
CD5-/CD10+ B-cell lymphocytosis

FL
- \( t(14;18)(q32;q21) + \) in about 80% (FISH)
- If + → PCR, and if also + → follow-up

DLBCL
- \( t(14;18)(q32;q21) + \) in about 30% (FISH)
- Occasionally C-MYC rearrangement (FISH)

BL
- C-MYC rearrangement in 100% (FISH)
CD5-/CD10- B-cell lymphocytosis

The most frequent mature B cell leukaemias in this group are

- Hairy Cell Leukemia
- Marginal Zone Lymphoma (MALT, SLVL, nodal MZL)
- Lymphoplasmacytic Lymphoma
CD5-/CD10- B-cell lymphocytosis

HCL

- IF: CD20++ / CD22++ / CD11c+ / CD25+ / CD103 / FMC7+ / CD23-
- No specific cytogenetic abnormalities
CD5-/CD10- B-cell lymphocytosis

SLVL

- IF: cfr HCL, but CD20 and CD22 less bright
- Allelic loss of cr 7q31-32 (40%)
CD5-/CD10- B-cell lymphocytosis

| MALT lymphoma | t(11;18); *API2-MALT1* (50 in lung, 20 in stomach) t(14;18); *IGH-MALT1* (20) t(3;14); *IGH-FOXP1* (50 in thyroid, 20 in ocular adnexa) t(1;14); *BCL10-IGH* (5) |
CD5-/CD10- B-cell lymphocytosis

LPL

- IF: CD11c+ / CD25+ / CD103-
- No specific cr or oncogenic abnormalities
Clinical and/or Haematological indications

*peripheral blood lymphocytosis*

cytopenia (anaemia, leukopenia, thrombocytopenia or pancytopenia)
lymphadenopathy or extranodal masses
monoclonal gammopathy or unexplained plasmocytosis of bone marrow
(hepato)splenomegaly
skin rash
fever of unknown origin
Plasma cell leukemia

Uncommon
Morphology is variable

IF: CD38++ / CD138+ / CD45- / CD19- / CD56+
FISH: **del(17p)** (involving the p53 gene), **del (13p)** (involving the Rb1 gene) and **t(4;14)**, all ~ worse prognosis
and **t(11;14)** ~ good prognosis
T cell lymphocytosis

Clinical information!
Morphology: highly variable
IF: aberrant markers are suggestive, but never the proof of malignancy
-aberrant expression strength
## T cell lymphocytosis

<table>
<thead>
<tr>
<th>CD4+/CD8- T-cell LPD</th>
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<tbody>
<tr>
<td>Sézary syndrome/ cutaneous T-cell lymphoma</td>
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<tr>
<td>Adult T-cell lymphoma/leukaemia</td>
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<tr>
<td>Anaplastic large cell lymphoma (may be CD8+)</td>
<td>t(2;5) ; NPM-ALK (75) t(1;2) ; TPM3-ALK (17)</td>
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<tr>
<td>T-Prolymphocytic leukaemia (may be CD8+)</td>
<td>Inv(14), t(14;14) ; TCRA/D-TCL1A (70) t(X;14) ; TCRA/D-TCL1B (13)</td>
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<td>CD4-/CD8+ T-cell LPD</td>
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<tr>
<td>T-cell large granular lymphocytic leukaemia</td>
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</table>
NK cell lymphocytosis (CD3-)

Chronic lymphoproliferative disorders of NK cells
- Indolent
  - CD57+ / CD56+ / EBV-

Aggressive NK-cell leukemia
- Aggressive
  - CD57- / CD56+ / EBV+
  - Associated with cytopenias
Polyclonal B cell lymphocytosis

Serum IgM ↑

HLA DR7

i(3q)

multiple Bcl-2/IgH gene rearrangement.
Cytopenia(s)

Multiple causes
Typically with HCL (‘dry tap’)
Association with hemophagocytic syndrome
Lymphadenopathy and extranodal masses

Example: Ghent University Hospital

- Fresh biopsies (in isotonic saline):
  - operating theatre → histopathology
  - histopathology → lab of cytogenetics
  - → lab of flow cytometry
  - → lab of cytomorphology
  - → lab of molecular analyses
Lymphadenopathy and extranodal masses

Actions in the hematology lab:

- Imprint + staining: morphology
- Single cell suspension (50-70 µM filter) + viability assay
- Immunophenotyping on fresh material
- Molec. anal.: Ig & TCR gene rearr
  - FISH first, eventually PCR
- Consult the histopathologist!
<table>
<thead>
<tr>
<th>Type of lymphoproliferative disorder (LPD)</th>
<th>Balanced translocation and related gene rearrangement (frequency in %)</th>
<th>Other chromosomal aberrancies (frequency in %)</th>
<th>Technique of choice</th>
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<tbody>
<tr>
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<td>Chronic lymphocytic leukaemia</td>
<td>t(11;14) ; IgH-CCND1 (exceptional)</td>
<td>Del 13q (55)</td>
<td>FISH/PCR</td>
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<td></td>
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<td>Del 11q (18)</td>
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<td>Del 17p (7)</td>
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<td>Tris 12 (16)</td>
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<tr>
<td>Mantle cell lymphoma</td>
<td>t(11;14) ; IGH-CCND1 (100)</td>
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<tr>
<td>Follicular lymphoma</td>
<td>t(14;18) ; BCL2-IGH (90)</td>
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<td>FISH/PCR</td>
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<td>Diffuse large B-cell lymphoma</td>
<td>t(14 ;18) ; BCL2-IGH (15)</td>
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<td>t(3 ;14) ; IGH-BCL6 (40)</td>
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<td>t(8;14) ; IGH-MYC (5-10)</td>
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<td>Burkitt lymphoma</td>
<td>t(8 ;14) ; IGH-MYC (80)</td>
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<td>t(2 ;8) ; MYC-IGK (15)</td>
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**OVERVIEW**
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<td>t(4 ;14) ; <em>IGH-MMSET</em> and *IGH-</td>
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<td>t(14 ;18) ; (multiple) <em>IGH-BCL2</em></td>
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<td>(100)</td>
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Collaborators

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