FLOW CYTOMETRY UPDATES IN LYMPHOPROLIFERATIVE DISORDERS

CANCER RESEARCH CENTER-IBSAL
UNIVERSITY & UNIVERSITY HOSPITAL,
SALAMANCA (SPAIN)

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DISCLOSURES

The EuroFlow Scientific Consortium I am co-chair of receives royalties from patents I am co-inventor from Cytognos SL and Becton/Dickinson Biosciences. All co-inventors of these patents and their institutions, including myself have declined receiving any compensation or royalty.

FLOW CYTOMETRY DIAGNOSTICS IN LYMPHOPROLIFERATIVE DISORDERS

1. Making the diagnosis
   Normal ↔ reactive/regenerating ↔ malignant

2. Classification of hematopoietic malignancies
   - relation with prognosis
   - relevance of risk-group definition in treatment protocols

3. Disease staging
   (e.g. in case of secondary CNS Lymphoma)

4. Identification of therapeutic targets
   (e.g. for antibody therapy)

5. Evaluation of treatment effectiveness
   Detection of minimal residual disease (MRD)

The EuroFlow comprehensive approach

Clinical question

Immunophenotyping

Mantle cell lymphoma

For sure (probability = 100%)
Not for sure (probability <100%)

Comprehensive network of panels aiming at the diagnosis and characterization of the major WHO entities

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Clinical question

Screening tube

Diagnostic panel

MRD

Immunophenotyping

Mantle cell lymphoma

For sure (probability = 100%)
Not for sure (probability <100%)

Comprehensive network of panels aiming at the diagnosis and characterization of the major WHO entities
**Monoclonal component**

- Non-IgM, Bone lesions

**BM plasmacytosis**

**ALO**

**TL S**

**PCS T**

**T**

**first tube of PCD**

**SS**

**T**

**Sustained monocytosis**

**Unexplained Eosinophilia**

**High suspicion of acute leukemia**

- e.g. blast cells observed

**Unexplained cytopenia**

- Atypical lymphocytes

- Splenomegaly

- Lymphocytosis

- LN enlargement

**High monoclonal component non-IgM**

**Suspicion of lymphoma**

**localization in “small cell number” samples e.g. CS F, vitreous**

**Pac Blue**

**Pac Orange**

**FITC PE**

**PerCP Cy5.5**

**PE Cy7**

**APC APC H7**

**CD20**

**CD45**

**CD4**

**CD56**

**Kappa**

**CD5**

**CD19**

**TCR JG**

**CD3 CD38**

**LST – Lymphocytosis screening tube**

**Unable to identify all the sample major populations:**

- Non-hematopoietic cells

- T lymphocytes (T-cell subpopulations)

- B lymphocytes (B-cell light chain restriction)

- NK cells

- Plasma cells

**B-NHL panel backbone**

**GATING B-CELLS IN THE LST TUBE**

**GATING IN THE LST TUBE:**

- 35 different cell populations x mean of 3 gates (105 gates)

**GATING IN THE LST TUBE:**

- 35 different cell populations x mean of 3 gates (105 gates)
GATING IN THE LST TUBE:
35 different cell populations x mean of 3 gates (105 gates)

Automated gating
How does it work?

Identifying the pathways that link individual events in an (N)-dimensional space

A software tool similar to Compass based on a Reference Database

Clustering phase
Groups of events
Classification phase
Cell populations

Responsible scientists: Rafael Flaxa, Juan Hernandez, Quentin Lecrevisse
**Classification phase**

Groups of events to be reclassified into cell populations

Output for the cluster: Events classified as cell populations

**Reference Database**

**COMPASS**

**Automated gating**

**LYMPHOCYTE SCREENING TUBE: DIAGNOSTIC SCREENING OF B-CLPD**

**LYMPHOCYTE SCREENING TUBE: DIAGNOSTIC SCREENING OF B-CLPD**

**LYMPHOCYTE SCREENING TUBE (LST): Automated gating and identification of tumor B-cells**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>N. of Cases (%</th>
<th>% blasts identified</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCLPD</td>
<td>113/113 (100%)</td>
<td>100%</td>
</tr>
<tr>
<td>OTHER</td>
<td>0/27 (0%)</td>
<td>0%</td>
</tr>
</tbody>
</table>

**PERCENT TUMOR B-CELLS BY MANUAL vs AUTOMATED GATING (n=113)**
**The EuroFlow comprehensive approach**

Comprehensive network of panels aiming at the diagnosis and characterization of the major WHO entities

**Flow Cytometric Analysis of CSF Samples from B-NHL**

Stabilized CSF samples (Transfix)

- Add PBS (4/mL)
- Centrifuge-wash (2x) and concentrate (150µL)
- Cell staining of 1/3 sample (50µL)
- Measure in the flow cytometer

Restain (if negative) vs panel adapted to B-NHL phenotype

**Flow Cytometry vs Cytology for Detection of CSF in Aggressive B-NHL (n=123)**

Flow Cytometry vs Cytology

<table>
<thead>
<tr>
<th>Flow Cytometry</th>
<th>Cytology</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive CSF</td>
<td>27/123 (22%)</td>
<td>7/123 (6%)</td>
</tr>
</tbody>
</table>

Flow Cytometry

<table>
<thead>
<tr>
<th>Cytology</th>
<th>Flow Cytometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>95/123 (77%)</td>
</tr>
<tr>
<td>+</td>
<td>123 (1%)*</td>
</tr>
<tr>
<td>Susp</td>
<td>3/123 (2%)</td>
</tr>
</tbody>
</table>

*The presence of neoplastic cells was ruled out by further immunocytochemical analyses.*

**Diagnosis of Leptomeningeal Disease in NHL: Cytology vs Flow Cytometry**

<table>
<thead>
<tr>
<th></th>
<th>Schmitt*</th>
<th>Beermer</th>
<th>Am</th>
<th>Stachi</th>
<th>Bencser</th>
<th>Matsui</th>
<th>Matsui</th>
<th>Matsui</th>
<th>Matsui</th>
</tr>
</thead>
<tbody>
<tr>
<td>No cases</td>
<td>27</td>
<td>70</td>
<td>94</td>
<td>174</td>
<td>113</td>
<td>326</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No samples</td>
<td>37</td>
<td>62</td>
<td>30</td>
<td>125</td>
<td>91</td>
<td>246</td>
<td></td>
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<tr>
<td>N.S.L.B.</td>
<td>33</td>
<td>40</td>
<td>3</td>
<td>10</td>
<td>8</td>
<td>186</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Cytology</td>
<td>19%</td>
<td>29%*</td>
<td>7</td>
<td>16%</td>
<td>4%</td>
<td>7%</td>
<td>5%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>% Flow</td>
<td>30%</td>
<td>28%*</td>
<td>8%</td>
<td>26%</td>
<td>10%</td>
<td>22%</td>
<td>18%</td>
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</tr>
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</table>

* 30/37 FCM+.
* 4% False-positive results by cytology.

**Absolute and Relative Numbers of Neoplastic B-Cells in Infiltrated CSF Samples by FCM**

*Cut-off: <20% and <1 neoplastic B-cell/L

* p<0.001
It is recommended that (standardized) flow cytometry immunophenotyping is incorporated to routine diagnosis of leptomeningeal disease in high-risk DLBCL in combination with clinical presentation, imaging techniques and conventional cytology.

Degree of evidence*: 1+
Recommendation*: A

*Scottish Intercollegiate Guidelines Network (SIGN)

Compatible with B-cell chronic lymphocytic leukemia

CONSTRUCTION OF EUROFLOW LEUKEMIA/LYMPHOMA IMMUNOPHENOTYPING ANTIBODY PANEL

LST + BCLPD classification panel

LST + BCLPD classification panel

Responsible scientist: Sebastian Bottcher
**Backbone markers:**
- Should identify all B cells
- Aberrant underexpression of CD19 and/or CD20 frequently observed
- sIgκ/CD37/sIgλ/CD19/CD22/CD20 tested in 69 B-NHL cases

**Conclusion:** CD37 & CD22 redundant, as CD20 PE-Cy7 plus CD19 PE-Cy7 were sufficient to identify all malignant B cells in all cases

**Characterization markers**

- CD10
- CD20
- CD22
- CD24
- CD27
- CD38
- CD39
- CD43
- CD63
- CD81
- CD95
- CD138
- Bcl-2
- HLA-DR
- IgM
- CD5
- CD23
- CD25
- CD79b
- CD103
- CD200
- sIg

**MCL vs CLL: PCA of total immunophenotype**

**MCL vs CLL: 1 X 1 Differential Diagnosis**
### Separation power of different types of BCLPD

<table>
<thead>
<tr>
<th></th>
<th>CLL</th>
<th>DLBCL CD10+</th>
<th>DLBCL CD10-</th>
<th>FL</th>
<th>HCL</th>
<th>LPL</th>
<th>MCL</th>
<th>MZL</th>
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<tbody>
<tr>
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<td>CLL</td>
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<tr>
<td>DLBCL CD10+</td>
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<tr>
<td>DLBCL CD10-</td>
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<td>FL</td>
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<td>HCL</td>
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<td>LPL</td>
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<tr>
<td>MCL</td>
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<tr>
<td>MZL</td>
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</tbody>
</table>

Responsible scientist: S. Böttcher

1 x 1 comparison

n = 150

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### Expert pathologist agreement with the consensus diagnosis

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>% of cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular, low grade</td>
<td>90</td>
</tr>
<tr>
<td>Follicular, high grade</td>
<td>12</td>
</tr>
<tr>
<td>Marginal zone B-cell, FL</td>
<td>54</td>
</tr>
<tr>
<td>Marginal zone B-cell, MALT</td>
<td>94</td>
</tr>
<tr>
<td>Marginal zone B-cell, DLBCL</td>
<td>93</td>
</tr>
<tr>
<td>Reactive lymphoid follicles</td>
<td>53</td>
</tr>
<tr>
<td>Reactive lymphoid follicles, FL</td>
<td>53</td>
</tr>
<tr>
<td>Reactive lymphoid follicles, MALT</td>
<td>53</td>
</tr>
</tbody>
</table>

The NHL Classification Project, Bodd 1997:89-3909-3918

Kindly provided by Raul Braylan

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### PCA of B-CLPD panel

Responsible scientist: S. Böttcher

Designed by: Q Lecrevisse

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### BCLPD classification panel: modular design

Responsible scientist: Sebastian Bottcher

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### BCLPD classification panel: modular design

Tubes 1 & 2 only: resolve 100% of CLL and 85% MCL cases
Summary:

EuroFlow LST tube and BCLPD Panel for diagnosis and classification of mature B-cell malignancies

- The EuroFlow BCLPD panel consists of a total of 5 tubes containing information about 30 markers, useful for the diagnostic screening and classification of the major BCLPD diagnostic WHO2008 subtypes.
- For an optimized efficiency the panel may be applied stepwise. Thus, with only the LST tube, around half CLL cases and a significant percentage of mantle cell lymphoma cases may be unequivocally identified.
- Tubes 1 & 2 are typically sufficient for the diagnosis of CLL and the differential diagnosis between CLL and MCL.
- Similarly, the combination of tubes 1 & 3 are sufficient for the diagnosis of MCL.
- Usage of different multivariate approaches is associated with variable levels of discrimination among the distinct diagnostic categories of BCLPD.
- Despite all the above the differential diagnosis between a few entities still remains a challenge.
EuroFlow consortium aims at innovation in flow cytometry (www.euroflow.org)

THE CIC/USAL-IBSAL TEAM

THANK YOU