Flow cytometry in Lymphoproliferative Disorders
A case based approach

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Diagnosis of Lymphomas

Multidisciplinary Approach

• Adequate Clinical History and Examination

• Laboratory work up- CBC, ESR, LDH, B₂ microglobulin, etc.

• Radiological Evaluation- PET Scan, CT scan, etc.

• Molecular Studies

• Morphology – H & E

• Immunophenotyping- for diagnosis, subtyping, prognosis
Role of FCM in CLPD

• Diagnosis of CLPD – Reactive vs. Lymphoma
• Typing and Subclassification
  – B cell CLPD
  – T cell CLPD
  – NK cell CLPD
• Prognostic markers
  – eg. CD38 and ZAP-70 in CLL
B cell NHL

CD19 - Pan B cell Marker

CD5+
- CLL
  - CD23+
  - Dim CD20
  - Dim k/l
  - FMC7 neg
  - Cyclin D1-
- Mantle
  - CD23 +/-
  - Bright CD20
  - Bright k/l
  - FMC7 pos
  - Cyclin D1+
- PLL

CD5-
- CD10+
  - Follicular
  - DLBL
  - Burkitts
  - CD20+
  - k/l +
  - FMC7 +
- CD10-
  - HCL
  - HCL variant
  - SMZL
  - LPL

Additional Markers required - CD123, CD103, CD25, CD11c
## Antibody Panels

<table>
<thead>
<tr>
<th>NAME</th>
<th>FITC</th>
<th>PE</th>
<th>PerCP</th>
<th>PE-CY7</th>
<th>APC</th>
<th>APCH7</th>
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</thead>
<tbody>
<tr>
<td>B cell Tube 1</td>
<td>KAPPA</td>
<td>LAMBDA</td>
<td>CD5</td>
<td>CD19</td>
<td>CD23</td>
<td>CD20</td>
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<tr>
<td>B cell Tube 2</td>
<td>FMC7</td>
<td>CD22</td>
<td>CD38</td>
<td>CD19</td>
<td>CD10</td>
<td>CD45</td>
</tr>
<tr>
<td>T cell Tube 1</td>
<td>CD8</td>
<td>CD7</td>
<td>CD3</td>
<td>CD4</td>
<td>CD2</td>
<td>CD45</td>
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<tr>
<td><strong>Secondary Tubes as per case</strong></td>
<td></td>
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</tr>
<tr>
<td>Hairy cell tube</td>
<td>CD103</td>
<td>CD123</td>
<td>CD25</td>
<td>CD19</td>
<td>CD11c</td>
<td>CD20</td>
</tr>
<tr>
<td>Myeloma tube</td>
<td>CD20</td>
<td>CD138</td>
<td>CD38</td>
<td>CD19</td>
<td>CD56</td>
<td>CD45</td>
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</table>
Case 1
CLL
Case 2
Mantle Cell Lymphoma
Case 3
Follicular Lymphoma
Hematogones
Case 4
Case 4

CD10 pos B Cell NHL
Case 3

Burkitts / DLBL with high FSC

Case 4

Burkitts Lymphoma
CD 5 -ve CD 10-ve B Cell NHL
Additional Markers
Peripheral Smear
CD 5 -ve CD 10-ve B Cell NHL
CD 5 -ve CD 10-ve B Cell NHL c/w a SMZL
Case 8
Additional Markers
WHO Classification

- Mature B-cell Neoplasms
- Mature T- and NK- cell neoplasms
Problems in evaluating T cells

- T cells are functionally and phenotypically heterogeneous
- T cell malignancies are rare
- Phenotypic clonality assessment is not simple or routine
- T cells share many phenotypic features with NK cells
## Normal CD markers

<table>
<thead>
<tr>
<th>T Cells</th>
<th>NK Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CD3+</strong></td>
<td><strong>CD3-</strong></td>
</tr>
<tr>
<td><strong>TCRαβ+/TCRγδ+</strong></td>
<td><strong>TCRαβ-/TCRγδ-</strong></td>
</tr>
<tr>
<td>CD2+</td>
<td>CD2+</td>
</tr>
<tr>
<td>CD5+</td>
<td>CD5-</td>
</tr>
<tr>
<td>CD7+</td>
<td>CD7+</td>
</tr>
<tr>
<td>CD8+</td>
<td>CD8+</td>
</tr>
<tr>
<td><strong>CD16-</strong></td>
<td><strong>CD16+</strong></td>
</tr>
<tr>
<td><strong>CD56-</strong></td>
<td><strong>CD56+</strong></td>
</tr>
</tbody>
</table>
Normal Lymphocyte Subsets
CD4+ CD8+ Subset

A small subset of T cells (1-2%) are dual CD4+CD8+ with two different expression intensities (increases with age)

Case 1

Case 2
DIAGNOSIS OF A T CELL CLPD BY FLOW CYTOMETRY “REQUIRES”:

The presence of a discrete CD4+, CD8+, CD4+CD8+, or CD4-CD8- population based on FSC x SSC, CD45 x SSC or CD3 gating

AND

the presence within the gated population of a phenotypic abnormality defined as:-

-altered or absent expression of CD2, CD3, CD4, CD5, CD7, CD8, or CD26. (Altered CD5 expression should be accompanied by other abnormalities)

-aberrant expression of CD10, CD158k.
T Cell CLPD

• True” evidence of clonality:
  – Vbeta analysis
  – KIR analysis

• Neoplasia can be supported by:
  – Quantitative abnormalities
  – abnormal uniform expression of antigens such as CD25, HLA-DR, CD45RA/CD45RO, CD16, and CD56
  – Expression of CD30
Abnormalities in antigen expression in T cell lymphoproliferative disorders

<table>
<thead>
<tr>
<th>Antigen</th>
<th>% Abnormal</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td>20%</td>
</tr>
<tr>
<td>CD3</td>
<td>50%</td>
</tr>
<tr>
<td>CD5</td>
<td>40%</td>
</tr>
<tr>
<td>CD7</td>
<td>60%</td>
</tr>
<tr>
<td>CD45</td>
<td>10%</td>
</tr>
<tr>
<td>Light scatter</td>
<td>40%</td>
</tr>
</tbody>
</table>

- Abnormalities in 90-95% of cases
- Two or more antigens affected in 60-80%

Mature T- and NK- cell neoplasms

- T-cell PLL
- T-cell LGL
- CLPD of NK cells
- Aggressive NK cell leukemia
- ATLL
- Extranodal NK/T cell Lymphoma, nasal type
- EATCL
- HSTCL
- MF/Sezary
- ALCI
- AITL
- Cutaneous T cell Lymphomas
<table>
<thead>
<tr>
<th></th>
<th>MF/SS</th>
<th>ATLL</th>
<th>T-PLL</th>
<th>HSTCL</th>
<th>T-LGL</th>
<th>AILD</th>
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</thead>
<tbody>
<tr>
<td>CD2</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>CD3</td>
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<td>++</td>
<td>/+++</td>
<td>/+++</td>
<td>/+++</td>
</tr>
<tr>
<td>CD4</td>
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<td>++</td>
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<td>-</td>
<td>-</td>
<td>++</td>
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<tr>
<td>CD5</td>
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<td>/+</td>
<td>/+</td>
<td>++</td>
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<td>CD7</td>
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<td>/+++</td>
<td>++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>CD8</td>
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<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>
<td>+</td>
</tr>
<tr>
<td>CD11b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>/+</td>
<td>/+</td>
<td>-</td>
</tr>
<tr>
<td>CD16</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>/+</td>
<td>/++</td>
<td>-</td>
</tr>
<tr>
<td>CD25</td>
<td>/+</td>
<td>++</td>
<td>/+</td>
<td>/+</td>
<td>/+</td>
<td>/+</td>
</tr>
<tr>
<td>CD26</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>CD30</td>
<td>-</td>
<td>/+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>/+</td>
</tr>
<tr>
<td>CD56</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>--/+</td>
<td>-</td>
</tr>
<tr>
<td>CD57</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>/+</td>
<td>/++</td>
<td>-</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>/+</td>
<td>/+++</td>
<td>/+</td>
<td>/+</td>
<td>/+</td>
<td>/+</td>
</tr>
<tr>
<td>TCR ab</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>--/+</td>
<td>++/-</td>
<td>+++</td>
</tr>
<tr>
<td>TCR gd</td>
<td>--/+</td>
<td>-</td>
<td>-</td>
<td>+++/-</td>
<td>--/+</td>
<td>-</td>
</tr>
<tr>
<td>EBV</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+++</td>
</tr>
</tbody>
</table>
Case 9

84/M skin lesions
PS – 12% Atypical lymphoid cells / Blasts
CD4 pos T cell NHL
Advised work up for MF/SS in view of skin lesions
Case 10

63/F, P.S. - 68% small atypical lymphoid cells
T cell CLPD
Case 11

44- Male

?CLPD
CD4+CD25+ T cell NHL
HTLV1 positive
ATLL
Case 12

• 55/M
• 4 months history of fatigue and abdominal pain
• Low grade fever of and on
• Spleen- 3cm
• CBC-
  Hb 7g%
  WBC - 24000/ul showing 67% lymphocytes
  Platelets-1.40 lacs
Peripheral Smear
Summary

Positive markers
CD2 / CD7 / CD8 / CD16 / CD45 / CD94

Negative Markers
TCRab / TCRgd / Cyto CD3 / tdt
CD19/ CD20 / Kappa/ Lambda
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<tr>
<td>TCR&lt;sub&gt;ab&lt;/sub&gt;+/TCR&lt;sub&gt;gd&lt;/sub&gt;+</td>
<td>TCR&lt;sub&gt;ab&lt;/sub&gt;-/TCR&lt;sub&gt;gd&lt;/sub&gt;-</td>
</tr>
<tr>
<td>CD2+</td>
<td><strong>CD2+</strong></td>
</tr>
<tr>
<td>CD5+</td>
<td>CD5-</td>
</tr>
<tr>
<td>CD7+</td>
<td><strong>CD7+</strong></td>
</tr>
<tr>
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- HSTCL
- MF/Sezary
- ALCI
- AITL
- Cutaneous T cell Lymphomas
Case 12-Diagnosis

Chronic Lympho Proliferative Disorder of NK Cells
Acknowledgements

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Dr Sumeet Gujral
Dr PG Subramanian
Dr Prashant Tembhare
Dr Ashok Kumar
Y Badrinath
Sitaram G

PD Hinduja Hospital

Dr Shanaz Khodaiji
Dr Preeti Mansukhani

Thank You