Introduction to Cell Markers:
Chronic Lymphoproliferative disorders

Michelle Petrasich
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Objectives
- Be able to obtain results from dot plot statistics graphs
- Be able to identify common characteristic Immunophenotypes with the aid of a table
- Be able to identify a lymphocyte population by flow as small or large cell

Classifications
- 1832 - Thomas Hodgkin: first description of lymphoma
- 1966 - Rappaport
- 1973 - BNLI
- 1974 - Like/Lennert
- 1974 - Lukes/Collins
- 1976 - WHO
- 1982 - Working Formulation
- 1994 - REAL
- 2001 - WHO, updated 2008

Incidence of Lymphomas

WHO Classification

B cell Development
**T cell Development**

- Identifies lymphocytes subsets present: B, T, NK
- Lymphocytes: Normal/reactive?
- Lymphocytes: Malignant/clonal?

**B cell Clonality – Light chain restriction**

- B cells have surface immunoglobulin on membrane: heavy and light chains
- Single cell: either kappa or lambda (not both!)
- Clone: All B cells K or L: Light chain restriction

**Normal B population (2:1):**
2 Kappa cells : 1 lambda cell

**T cell clonality**

- PB : 2 CD4+: 1 CD8+ cell = ~ 2:1
- Skewing of the CD4:8 ratio is not enough
- Loss of lineage markers (pan T)
- Changes in staining intensity
- Clonality confirmed by molecular TCR gene rearrangement studies

**Common B cell Immunophenotypes**

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pan B</strong></td>
<td>Sig KAPPA/COD2 (FRR)</td>
</tr>
<tr>
<td><strong>Pan T</strong></td>
<td>Sig LAMBA/COD19 (FRR)</td>
</tr>
<tr>
<td><strong>B subets</strong></td>
<td>KAPPA (FRR)</td>
</tr>
<tr>
<td><strong>T subets</strong></td>
<td>LAMBA (FRR)</td>
</tr>
<tr>
<td><strong>Natural killer cells</strong></td>
<td>MK (FRR)</td>
</tr>
<tr>
<td><strong>B cell</strong></td>
<td>CD19 (FRR)</td>
</tr>
<tr>
<td><strong>T cell</strong></td>
<td>CD3 (FRR)</td>
</tr>
<tr>
<td><strong>Useful for hairy cells</strong></td>
<td>CD103 (FRR)</td>
</tr>
<tr>
<td></td>
<td>CD103 (FRR)</td>
</tr>
<tr>
<td></td>
<td>CD8 (FRR)</td>
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<tr>
<td></td>
<td>CD4 (FRR)</td>
</tr>
<tr>
<td></td>
<td>CD5 (FRR)</td>
</tr>
</tbody>
</table>

- Antigen not expressed: +, antigen expressed is less than 5% of patients; ++, antigen expressed in majority of patients;
- +, antigen expressed; --, weak expression; ×, strong expression.

**Chronic Panel for LPD**

- Chronic panel for LPD
- Pan B subsets
- Pan T subsets
- Natural killer cells
- B cell
- T cell
- Useful for hairy cells

T cell Leukaemias

Roth et al. Consensus Protocol for Immunophenotyping of Haematopoietic Malignancies. Working Group on Flow Cytometry and Image Analysis

<table>
<thead>
<tr>
<th>Antigen</th>
<th>T-PLL</th>
<th>hairy/MP</th>
<th>T-PLL</th>
<th>ATL</th>
<th>NK2.4+</th>
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<tbody>
<tr>
<td>CD3</td>
<td>0+</td>
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</tr>
</tbody>
</table>

- 0+ = antigen not expressed
- 0+ = antigen expressed in less than 50% of patients
- 1+ = antigen expressed in majority of patients

ATL, ALL T cell leukaemia. LGL, large granular lymphocyte leukaemia. MP, mycosis fungoides. T-PLL, T prolymphocytic leukemia

ATL (LGL) 50% of cases are CD8+. The remaining 20% are CD4+, or CD4+CD8+, or CD4-CD8-.

Patient A: Biopsy L neck node
Bilateral cervical lymphadenopathy, NHL? Urticarial angiodema

Patient B: Peripheral Blood
Persistent high lymphocyte count (14.65 x 10^9/L)

Patient C: Biopsy L axillary node
Enlarged lymph node, ? lymphoma
**Patient D: Peripheral blood**
Lymphocytosis, WCC : 38.97 x 10^9/L

**Patient E: Biopsy L cervical node**
Widespread lymphadenopathy from upper neck to groin

**Patient F: Bone Marrow**
Pancytopenia, ? Cause, ? MDS, increased monocytes

**Patient G: FNA R groin node**
Unexplained increase in LN size, no previous history of note

**Patient H: Peripheral blood**
On Clexane; known LPD; WCC = 881.37 x 10^9/L
Patient I: Peripheral blood
Mild fluctuating neutropenia, repeat immunophenotyping

Patient J: Biopsy L nasal passage
Increased awareness of blocked nasal passage - mass