Flow Cytometric Evaluation of Plasma Cell Dyscrasias

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General Considerations

• The flow cytometric diagnosis of plasma cell and lymphoproliferative disorders relies on two broad approaches:
  
  • Demonstration of clonality
  
  • Demonstration of immunophenotypic aberrancies (i.e. differential antigen expression from a normal counterpart)

• In mature B-cell LPDs, demonstration of clonality is accomplished through surface immunoglobulin light chain analysis
  
  • Often represents the primary method of flow cytometric diagnosis
General Considerations

- In plasma cell myeloma (PCM) and related disorders, the primary approach to the flow cytometric diagnosis relies on the identification of an aberrant immunophenotype
  - In the presence or absence of distinct cytoplasmic light chain restriction
  - Particularly in the MRD analysis setting
- CD38 is brightly expressed on normal PCs and, in conjunction with CD138, used as a lineage-defining antigen
  - Other maturing bone marrow elements also express CD38
Normal vs. Abnormal PCs

• The most common IP of normal PCs may be described as CD38 bright (+), CD138 bright (+), CD19 (+), CD20 (-), CD27 bright (+), CD28 (-), CD56 (-), CD81 (+), CD117 (-), CD200 predominantly (-), and polytypic cytoplasmic immunoglobulin.

• The typical IP of PCM shows various combinations of aberrancies, including CD38 (+) (often dimmer than normal PCs), CD138 bright (+), CD19 (-), CD20 (-) (may be positive in a subset of PCM), CD27 (-), CD28 (+), CD56 (+), CD81 (-), CD117 (-) (may be positive in a subset of PCM), CD200 (+), and monoclonal light chain expression.
  • Usually defined as a cytoplasmic kappa:lambda ratio >5 or <0.5.
# Frequency of IP Aberrancies in PCM

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Frequency of expression</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19 (-)</td>
<td>~ 95%</td>
</tr>
<tr>
<td>CD20 (+)</td>
<td>15-20%</td>
</tr>
<tr>
<td>CD27 (-)/ dim (+)</td>
<td>45-65%</td>
</tr>
<tr>
<td>CD28 (+)</td>
<td>15-45%</td>
</tr>
<tr>
<td>CD45 (-)</td>
<td>45-80%</td>
</tr>
<tr>
<td>CD56 (+)</td>
<td>75%</td>
</tr>
<tr>
<td>CD81 (-)</td>
<td>45-55%</td>
</tr>
<tr>
<td>CD117 (+)</td>
<td>35%</td>
</tr>
<tr>
<td>CD200 (+)</td>
<td>75-80%</td>
</tr>
</tbody>
</table>
Normal PCs

- Side scatter
- Forward scatter
- Isotype ctrl. FITC
- Isotype ctrl. PE
- CD38 APC
- CD20 PerCP
- CD19 PE
- CD10 FITC
- CD45 PerCP
- CD56 PE
- CD117 PerCP
- CD200 PE
- Surface lambda
- Surface kappa
- I.C. lambda
- I.C. kappa
Aberrant PCs in PCM
Normal and Aberrant PCs
Case #1
36-year-old M with massive splenomegaly

- Several month history of abdominal pain
  - Also, night sweats, weight loss, increasing fatigue
  - Splenomegaly (25 cm) on CT abdomen/pelvis
  - Diffuse marrow uptake on PET-CT
  - Pancytopenia

- BM biopsy performed for further work-up:
  - Hypercellular core (95%) with 60% plasma cells
  - No JAK2 mutation detected
  - Normal male karyotype
- Yellow (1.1% plasma cells): 0.56% intracellular kappa (+) (blue) and 0.48% intracellular lambda (+) (cyan); ic kappa:lambda = 1.2:1
- Violet (0.75% immature B cells / hematogones)
Bone marrow flow cytometry

- Two aberrant populations of plasma cells:
  - Yellow and blue (0.56%): CD38 bright (+), CD19 (-), CD56 (+), CD45 (+), CD200 (-), intracellular kappa restricted
  - Yellow and cyan (0.48%): CD38 bright (+), CD19 (-), CD56 (+), CD45 (+), CD200 (-), intracellular lambda restricted

- Splenectomy:
  - 2,170 g spleen with multifocal myeloma deposits
  - Fibrocongestive splenomegaly with multiple old infarcts
BM aspirate

BM core biopsy
Diagnosis:

Biclonal Plasma Cell Myeloma
PCM Unusual Findings

• Immunophenotypic aberrancies are more important in discriminating normal from abnormal plasma cells, than intracytoplasmic light chain restriction

• PCM may be diagnosed in younger patients and may show organomegaly, due to plasmacytomas

• PCM may be preceded by neutrophilia or eosinophilia, mimicking a MPN
General Technical Issues

- PCs show low recovery by flow cytometry
- Neoplastic PCs have variable light scatter properties and CD45 expression
- PCs have increased propensity to form complexes with granulocytes
- PCs show high autofluorescence
Low PC Recovery by FC

• PCs are under-represented in FC analysis
  • Only 1-30% recovery, compared to morphology

• Potential contributing factors:
  • Patchy, heterogeneous distribution of PCM cells in the BM
  • Relative higher degree of hemodilution in the specimen processed for FC
  • Differential distribution of PCs in lipid-enriched particles in the morphology slides, compared to the lipid-depleted liquid BM aliquot analyzed by FC
  • Further loss during processing, staining, and acquisition on the flow cytometer, related to particular physical characteristics of PCM cells, which render them potentially more susceptible to mechanical damage
Low PC Recovery by FC

- Accurate quantification of BM PCs has prognostic value in the MRD setting
- There also is literature data on the prognostic value of flow cytometric enumeration of PCM cells at diagnosis
- The percentage of PCs used to establish a diagnosis of PCM is generated from morphology slides (gold standard)
Variable Light Scatter and CD45 Properties in PCs

- Traditional FC data analysis employs gating approaches using FSC/SSC and CD45/SSC 2D-plots.
- This strategy offers a predictable and often reproducible pattern of normal cell subsets that can be found in BM specimens.
- PCM cells tend to vary in size (FSC) and cytoplasmic complexity (SSC), and show variable CD45 expression, which renders these fluorescence parameters inadequate for gating.
- Current combinations of recommended markers: CD38, CD138, and CD45.
Variable Light Scatter and CD45 Properties in PCs
PCs Tend to Form Complexes with Granulocytes

- This phenomenon may be responsible for both unusual light scatter properties (high FSC and SSC on a subset of PCs), as well as for over-interpretation of CD45
High Autofluorescence in PCs

- May lead to erroneously ascribing positive expression of certain antigens to PCM cells
- Use of an isotype control, multivariate computational tools and visualization plots may circumvent this issue
Added Value of FC in the Diagnostic Work-Up of PCM

- In general, PCM can be diagnosed by a combination of clinical, morphologic, radiologic, and laboratory criteria, and the contribution of FC in the initial evaluation is limited.

- FCIP plays a more important role in the DD of PCM:
  - Unusual morphologic variants of PCM vs. LPL
  - PCM vs. prominent reactive plasmacytosis
  - Coexistent PCM and unrelated B-cell clone of similar light chain restriction vs. low-grade B-cell lymphoma with extensive plasmacytic differentiation
  - PCM vs. MGUS or SMM
PCM with Lymphoplasmacytoid Morphology
Florid Reactive Plasmacytosis
Lymphoplasmacytic Lymphoma/
Waldenstrom Macroglobulinemia
PCM and Clonally Unrelated B-Cell NHL with Similar Light Chain Restriction
PCM vs. MGUS or SMM

- Clonal PCs found in MGUS, SMM and PCM are immunophenotypically aberrant and can be readily distinguished from normal ones.

- The presence of a large (>95%) proportion of abnormal PCs/BM PCs has been associated with a higher risk of progression of MGUS and SMM to PCM.

- Similar prevalence of common antigen expression (CD19, CD56, CD20, CD45, CD117) in clonal PCs from MGUS and PCM, with perhaps the exception of CD200.
  - More frequently expressed in PCM (73%) compared to MGUS (54%).
Prognostic Utility of FC in PCM

• Qualitative prognostic markers
  • CD19, CD28, CD45, CD56, CD81, CD117, CD200

• Quantitative prognostic markers
  • Percentage of PCs (of total bone marrow cells)
  • Percentage of clonal PCs (of bone marrow PCs)
  • Number of circulating clonal PCs
  • Evaluation of PC DNA ploidy
Qualitative Prognostic Markers

- Expression of CD19 and CD28, and lack of CD117 was associated with worse PFS and OS
- Lack of CD45 or CD56 expression has been associated with less favorable outcome
- CD81 expression has been identified as an independent prognostic factor for worse PFS and OS
- Lack of CD200 expression was associated with a favorable outcome in most reports
Quantitative Prognostic Parameters

- The overall percentage of PCs (calculated as proportion of nucleated BM cells) and the relative percentage of myeloma cells (reported as the proportion of BM PCs) were identified as quantitative FC parameters with prognostic significance.

- A relative higher proportion of normal PCs (>5% of BM PCs) at diagnosis has been associated with a better PFS and OS.
Quantitative Prognostic Parameters

• The number of circulating PCs has also prognostic significance in PCM

• A subset of patients with SMM and high levels of circulating PCs (400) have an elevated risk of progression within the first 2-3 years following diagnosis

• Patients with newly diagnosed PCM may be stratified in a higher risk group (with worse OS), based on a cut-off of ≥400 clonal PCs

• Amongst patients with recurrent PCM, a higher number (≥100) of circulating clonal PCs may also predict for worse survival
Quantitative Prognostic Parameters

- FC can assess PC DNA content and proliferation

**G0/G1** – Quiescent phase/pre-DNA synthesis phase; cells in these phases have normal (diploid or 2N) DNA content.

**S-phase** – DNA synthesis phase; DNA content increases from 2N toward 4N.

**G2/M** – Post –DNA synthesis phase/mitosis phase; cells have doubled their DNA content to 4N and are ready to divide.
DNA index | Ploidy
---|---
<0.95 | Hypodiploid
0.95-1.05 | Diploid
1.06-1.74 | Hyperdiploid
>1.74 | Tetraploid
PC Proliferation Assessment

- Has several reportables with diagnostic and/or prognostic information:
  - PC clonality – diagnostic
  - Clonal PC DNA index (DNA ploidy) – diagnostic & prognostic
  - Clonal PC proliferation (S phase) – prognostic
  - Percent polyclonal PCs of total PCs – prognostic
DNA Ploidy Facilitates Detection of Clonal PCs

- Non-diploid PCs = abnormal
- Can establish clonality in a background of polytypic PCs
- May detect bi-clonal PC populations
- Increased level of detection of very small clonal population - analytic sensitivity $10^{-4} - 2 \times 10^{-5}$
Case #2
Kappa clone confirmed by hyperdiploidy

DNA index (kappa) : 1.15
- Hyperdiploidy
Case #3
Biclonal PCs confirmed by non-diploid DNA content

DNA index (kappa) : 1.23
- Hyperdiploidy

DNA index (lambda) : 0.93
- Hypodiploidy
Case #4

Dimmer CD38
Case #4 (LOD: 0.002%)

DNA index (kappa) : 1.47
- Hyperdiploidy
Hyperdiploidy is a Good Prognostic Factor

High S phase is a Poor Prognostic Factor

- S-phase $\geq 3.0$ – Worse prognosis
  S-phase $< 3.0$ – Good prognosis


- With the current version of the test, the best cutoff has been established at the S-phase of 2%
Mayo Stratification for Myeloma and Risk-adapted Therapy (mSMART)

mSMART 3.0: Classification of Active MM

<table>
<thead>
<tr>
<th>High-Risk</th>
<th>Standard-Risk$^a$</th>
</tr>
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<tbody>
<tr>
<td>- FISH$^{a,b}$</td>
<td></td>
</tr>
<tr>
<td>- Del 17p</td>
<td></td>
</tr>
<tr>
<td>- t(4;14)</td>
<td></td>
</tr>
<tr>
<td>- 1q gain</td>
<td></td>
</tr>
<tr>
<td>- t(14;16)</td>
<td></td>
</tr>
<tr>
<td>- t(14;20)</td>
<td></td>
</tr>
<tr>
<td>- RISS Stage 3</td>
<td></td>
</tr>
<tr>
<td>- High Plasma Cell S-phase$^c$</td>
<td></td>
</tr>
<tr>
<td>- GEP: High risk signature</td>
<td></td>
</tr>
</tbody>
</table>

All others including:
- Trisomies
- t(11;14)$^d$
- t(6;14)
MRD FC Analysis in PCM

- MRD analysis is likely to exert the most impact in evaluating the effectiveness of new therapeutic drugs and in potentially identifying subsets of patients at risk for progression and in need for more intensive treatment.

- PCM has well-defined and nuanced response criteria and it is estimated that up to 33% of patients in CR, and 10% of those in near CR or VGPR may be considered potentially cured (i.e. relapse-free at 10-year follow-up).
MRD FC Analysis in PCM

• This data implies that a better definition for CR is necessary, since 40% of patients with PCM in CR will relapse, and 20% will succumb to their disease within 4 years of initial treatment.

• Accurate estimation of the depth of the response appears to correlate with better outcome in PCM, which supports the addition of MRD analysis to the definition of CR.
Prognostic influence of sequential MRD status by MFC before and after ASCT. (A) PFS and (B) OS (n = 157).

Test Design (EuroFlow Group)

• 10 color – 2 tubes with 6 anchor antigens:
  • Tube 1 CD38 CD138 CD45 CD19 CD56 CD27 CD81 CD117
  • Tube 2 CD38 CD138 CD45 CD19 CD56 CD27 kappa lambda

• CD38 is a multi-epitope antibody
• 10 million cells per tube, collect 5 million each tube.
• Infinicyt software for analysis
• Sensitivity of at least $10^{-5}$ if 2 million events are collected
Abnormal Plasma Cells

Polytropic PCs

Population Events Total %

Events 10000000 100.0000

Time/NonAggs 9467317 94.6732
Plasma Cells 47072 0.4707
Other Plasma Cells 0 0.0000
Poly PCs 46703 0.4670
Other Poly PCs 26160 0.2616
Poly Lambda 11247 0.2249
Poly Kappa 9296 0.1859
Abnormal PCs 369 0.0037
Other Abnormal PCs 190 0.0038
Abnormal Kappa 159 0.0032
Abnormal Lambda 20 0.0004
B CELLS 78728 0.7873
Other B CELLS 29884 0.2988
GAMMA+ 15331 0.3066
Cyto Lambda+ 8510 0.1702
Gomes 25003 0.2500
Mast Cells 0 0.0000

PC Aggs per all PCs 29.9860 %

Reportable Values:
9467317.0000
Total plasma cell events 47072.0000
% MRD (abnormal PC/nonaggs) 0.0039
% Poly Per all PCs 99.2161

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Multiple Myeloma MRD by Flow, BM

Results:

<table>
<thead>
<tr>
<th>Marker Name</th>
<th>Result</th>
<th>Unit</th>
<th>Result Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Minimal Residual Disease (MRD)</td>
<td>0.0039</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>% Normal Plasma Cells (of total PC)</td>
<td>99.2</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>Non-Aggregate Events</td>
<td>9467317</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Plasma Cell Events</td>
<td>47072</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly PC Events</td>
<td>46703</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abnormal PC Events</td>
<td>369</td>
<td></td>
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Final Diagnosis:

Bone marrow, flow cytometric immunophenotyping:

Monotypic kappa plasma cells identified (MRD= 0.0039%).

Viability: Sub-optimal, interpret with caution
Viable lymphocytes (7-AAD): 76%

Comment: This assay is intended for post-therapeutic assessment of patients with plasma cell neoplasms, when in complete remission. Correlation with clinical and other laboratory studies is recommended.

Plasma cell analysis was performed with antibodies to the following antigens: Myeloma MRD panel: CD138, CD27, CD38, CD56, CD45, CD19, CD117, CD81, kappa and lambda cytoplasmic immunoglobulin light chains.

Quality Assessment: Specimen received within validated guidelines.
Summary

• Routine flow cytometry in PCM requires careful consideration of a number of technical aspects related to plasma cell analysis

• Flow cytometry has applications in the differential diagnosis, prognosis, and treatment of PCM

• Minimal residual disease analysis by flow cytometry is a powerful predictor of outcome in myeloma, in the clinical trial setting
Thank you for participating!