CSP04 Topics in Hematopathology: Flow Cytometry and Molecular Genetics as Tools for Understanding, Diagnosing, and Treating Hematolymphoid Malignancy

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CSP04 Topics in Hematopathology: Flow Cytometry and Molecular Genetics as Tools for Understanding, Diagnosing, and Treating Hematolymphoid Malignancy

The aim of this session is to introduce to the audience emerging areas of scientific knowledge in the pathogenesis and identification of hematologic malignancies and illustrate how this knowledge is being used to shape and improve patient care. Along these lines, the speakers will present topics in T-cell lymphoma pathogenesis and treatment, advanced flow cytometry in the diagnosis of myeloid neoplasms and plasma cell proliferative disorders, and the pathogenesis and diagnosis of extramedullary blastic myeloid neoplasm (myeloid sarcomas). Each of the speakers will be discussing from their own published work as well as the published literature, but the presentations will emphasize the practical, day-to-day implications of the findings for practicing pathologists and laboratorians. As such, these studies will not only be informative for the individual topic, but will also provide a tangible context for how advances in scientific knowledge are effecting our daily clinical practice. Therefore the topics should be of keen interest to a broad audience. Each presenter will deliver the content in a 45 minute lecture followed by a 10 minute question and answer session with the audience.

- Recognize how identifying disease pathogenesis provides opportunity for disease specific treatments.
- Identify the role of ancillary studies such as immunohistochemistry, flow cytometry, and molecular pathology in disease diagnosis and prognostication.
- Recognize how scientific discovery has direct impact on patient care.

FACULTY:

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Practicing Pathologists
Hematopathology
New Techniques
3.0 CME/CMLE Credits

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Myeloid Sarcoma

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Washington University School of Medicine
St Louis, Missouri

Disclosure Information

I have no relevant conflicts of interest.

Myeloid sarcoma

The World Health Organization defines myeloid sarcoma (synonyms: granulocytic sarcoma, chloroma) as "a tumor mass consisting of myeloid blasts with or without maturation occurring at an anatomical site other than the bone marrow."

Myeloid sarcoma

- Also referred to as granulocytic/monoblastic sarcomas or extramedullary myeloid tumors
- Originally termed chloromas because of the greenish color (Gr. Χλωρός = "green") imparted on gross examination due to the production of myeloperoxidase

Sauter (2008)

Myeloid sarcoma

- Wide age distribution; more common in the pediatric age group with an incidence of up to 30% in some studies of pediatric AML versus 2-5% in adults
- Most commonly presents concurrently with a new diagnosis of AML or as evidence of disease recurrence, a significant subset of myeloid sarcomas (27% in one large study) will present as de novo disease
- Studies that define outcomes and treatment response are limited and there are no established guidelines for clinical decision-making.
- Therapies typically include local radiation, systemic chemotherapy, immunotherapy and donor lymphocyte infusions.
- Outcomes are usually poor, although may be slightly better than outcomes of primary or relapsed AML without extramedullary involvement

Myeloid sarcoma

- Reported at nearly every anatomic site, but are most frequently encountered in the skin and soft tissues, lymph nodes, and gastrointestinal tract
- Clinical symptoms are largely dependent on the site of involvement

Photos: University of Virginia Medical School
Myeloid sarcoma

- May occur in the presence or absence of bone marrow disease
- Its presence is sufficient to establish a clinical diagnosis of AML
- Bone marrow involvement will become detectable in nearly all patients who originally presented with isolated myeloid sarcoma, with a mean interval of 10 months
- Isolated myeloid sarcoma also appears to be more common at relapse in patients who have undergone allogeneic stem cell transplantation, occurring in 8-20% of transplanted patients (graft-vs-leukemia surveillance or the biology of high risk AML treated with transplantation?)

Myeloid sarcoma

- Due to the aggressive nature of the disease, necrosis may be present, as well as areas with numerous mitotic figures and tingible-body macrophages.
- The pattern of infiltration is highly dependent on the tissue involved

![Image: Large intestine (Pileri, 2007)]

Myeloid sarcoma

- Leukemic blasts will diffusely infiltrate tissue within extranodal sites.
- Can appear to be cohesive when there are areas with dense fibroconnective tissue or a prominent stromal reaction, thus mimicking metastatic carcinoma.
- Infiltrates within lymph nodes may obliterate the entire nodal architecture or be confined to the paracortex or sinuses with occasional residual germinal centers.

![Image: Lymph node paracortex (Pileri, 2007)]
Myeloid sarcoma

- Though myeloblasts, or blast equivalents such as promonocytes, are the predominant cell population, varying degrees of myeloid maturation may be present within the leukemic infiltrate.
- Transformation of a myeloproliferative neoplasm can demonstrate evidence of more than one lineage.

Myeloid sarcoma

- Previously subdivided by morphologic features into granulocytic sarcomas and monoblastic sarcomas.
- Granulocytic sarcomas were further divided based on the extent of maturation into blastic, immature or differentiated variants.
Myeloid sarcoma

- Myelomonocytic forms, similar to acute myelomonocytic leukemia, are also common and myeloid sarcomas with erythroid and megakaryoblastic differentiation have also rarely been reported.
- Cases of extramedullary acute promyelocytic leukemia are rare and the majority occur at relapse with a preference for central nervous system involvement.

Megakaryoblastic AML (lymph node) CD41 (bottom)
Hiraga Y (2001)

Myeloid sarcoma


Myeloid sarcoma

- “Aleukemic” myeloid sarcomas can morphologically be easily confused with other hematologic malignancies:
  - Aggressive B-cell lymphomas
  - Non-hematolymphoid tumors
Myeloid sarcoma

Immunohistochemistry

- **CD43** and lysozyme
  - most sensitive markers as they are expressed in nearly 100% of cases in most studies
  - neither is specific
- **Myeloperoxidase** and **CD68** antigen are also frequently expressed; the KP-1 clone of anti-CD68 has higher sensitivity but lower specificity than PG-M1
- **CD34** immunoreactivity is not a consistent finding
  - present in 40/92 cases
  - frequently negative in cases with monocytic differentiation

- **Immunohistochemistry for CD45** is variable; in one study only 14 of 24 cases demonstrated positivity for CD45.
- **Other commonly used myeloid markers** include CD33 and CD117 (C-kit).
Myeloid sarcoma
Immunohistochemistry – Monocytic Differentiation

- Frequently negative for CD34 and/or CD117.
- Although myeloperoxidase is classically negative in acute monocytic leukemia, immunoreactivity for anti-myeloperoxidase is not sufficient for the exclusion of monocytic differentiation.
- CD68, CD43, CD33, and lysozyme are expressed by neoplastic monocytes within the bone marrow and in extramedullary sites.
- Immunohistochemistry for the hemoglobin scavenger receptor CD163 can also be positive in some cases with monocytic differentiation.

- CD56 (N-CAM) expression has been associated with monocytic differentiation, extramedullary disease, or t(8;21); however, this has not been a consistent finding.
- CD4 is also commonly expressed by monocytic leukemias, however, CD4 expression lacks specificity for myeloid sarcomas.
- As expected, myelomonocytic myeloid sarcomas will demonstrate a mixed pattern of immunoreactivity for the above-mentioned myeloid and monocytic markers.

Our group has also recently shown the usefulness of immunohistochemistry for CD14 and Kruppel-like factor 4 (KLF4) in monocytic leukemias.


Myeloid sarcoma
Immunohistochemistry

- Erythroid differentiation: glycophorin A, hemoglobin, or CD71 (transferrin receptor), although many of these markers may only show variable positivity.
- Occasional erythroid precursors can also show expression of CD117.
- CD71 expression from formalin fixed paraffin embedded (FFPE) tissue may be the most useful as its expression decreases during erythroid maturation, allowing for easier interpretation.
- Megakaryocytic differentiation: CD61 (platelet glycoprotein IIIa), CD41 (platelet glycoprotein IIb), CD42b (glycoprotein Ib, alpha polypeptide), linker for activation of T cells (LAT), vWF (factor VIII-related antigen) or CD31.
- CD31 is the least specific within this group, as it is expressed by endothelial cells, plasma cells and a subset of granulocytic elements.

Cutaneous myeloid sarcoma

- In the skin, lesions most commonly present as multiple papules, plaques, or nodules.
- Most common region of involvement is the torso, although the head and neck regions and extremities are also involved in many cases.

Photos: MY Hurley, MD, Saint Louis University

Retrospective study of 83 patients presenting with CMS over a 19 year period at 2 tertiary care institutions in the midwest United States.

- We emphasized the demographics, clinical presentation, and pathologic workup of these patients, and their response to therapy.

Hurley MY, et al, submitted
Materials and methods

- A search of the electronic databases of the Department of Dermatology, Section of Dermatopathology, Saint Louis University, and the Section of Anatomic and Molecular Pathology, Department of Pathology and Immunology, Washington University, was performed.
- Patient inclusion criteria for our search were less than 90 years of age and also a diagnosis of CMS from January 1st 1990 to June 1st 2009.
- Typical patient demographic data was collected (date of birth, gender, and race) along with dermatologic clinical CMS presentation regarding lesion size, character, and anatomical site.
- We also collected data concerning other anatomical involvement including liver, lymph node, and central nervous system, etc. both during clinical treatment and at autopsy when available.
- Patient survival status was also recorded.

Hurley MY, et al, submitted

Materials and methods

- This study also described bone marrow in comparison with cutaneous involvement of myeloid blast cells.
- Whenever available, we also included karyotype and fluorescence in-situ hybridization results.

Hurley MY, et al, submitted

Results

- CMS appears to affect genders randomly (p>0.05) but there is a male predominance (1.51:1).
- The disease appears to affect primarily Caucasians.

Hurley MY, et al, submitted
Results

- The mean age is 52 y with 4 patients being less than 1 year old
- The upper extremities are involved more frequently than would be predicted by body surface area (P<0.05)
- The most common region involved is the torso
- CMS most commonly presents as multiple papules, nodules, or plaques

Hurley MY, et al, submitted

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Results

- 19 pts had involvement of sites outside the bone marrow and skin
- CNS and lymph node are the most commonly involved extramedullary anatomic sites in patients with CMS
- Most (15/19) were from cases with monocytic differentiation
- Lower % than that reported by Kaddu et al (1999, 26 pts)

Hurley MY, et al, submitted

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Results

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Percent</th>
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<tr>
<td>AML before CMS</td>
<td>35</td>
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<tr>
<td>AML with CMS</td>
<td>21</td>
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<td>Total</td>
<td>61</td>
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</table>

- AML most commonly preceded the diagnosis of CMS (57%) or presented concurrently (34%) with CMS
- Most patients with a diagnosis of CMS were eventually diagnosed with AML (86%)

Hurley MY, et al, submitted
Bone marrow biopsy

- 70/83 (84%) patients had at least one bone marrow biopsy available for review.
- Lower percentage of M4/M5 cases than previously reported (Kaiserling et al [1994, 16 pts], Kaddu et al [1995, 26 pts])

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of cases</th>
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<tr>
<td>Acute myeloid leukemia (AML) with minimal differentiation (FAB: M0)</td>
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</tr>
<tr>
<td>AML without maturation (FAB: M1)</td>
<td>4</td>
</tr>
<tr>
<td>AML with maturation (FAB: M2)</td>
<td>6</td>
</tr>
<tr>
<td>Acute myelomonocytic leukemia (FAB: M4)</td>
<td>15</td>
</tr>
<tr>
<td>Acute monocytic/monoblastic leukemia (FAB: M5a/b)</td>
<td>13</td>
</tr>
<tr>
<td>Acute megakaryoblastic leukemia (FAB: M7)</td>
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<tr>
<td>AML, unclassifiable*</td>
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<tr>
<td>AML, myelodysplasia-related**</td>
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<tr>
<td>Biphenotypic acute leukemia (B-myeloid)</td>
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</tr>
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<td>Chronic myelogenous leukemia</td>
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<td>Chronic myelomonocytic leukemia***</td>
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<td>Myelodysplastic syndrome****</td>
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<td>Juvenile myelomonocytic leukemia</td>
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</tr>
<tr>
<td>No evidence of malignancy</td>
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</table>

* Not classified due to lack of enzyme cytochemical analysis of blasts
** Includes 2 cases of myelodysplastic syndrome which ultimately progressed to AML and 1 case presenting de novo with multilineage dysplasia
*** Includes 2 cases which progressed to acute myeloid leukemia
**** All cases presented with increased bone marrow blasts, 1 represented a therapy-related myelodysplastic syndrome

Hurley MY, et al, submitted

Cytogenetics and molecular genetics

- Cytogenetics abnormal in 23/28 (82%) of cases
  - 2 patients with loss of chromosome 7
  - 2 patients with loss of the long arm of chromosome 7
  - 7 patients with trisomy of chromosome 8
  - 5 patients with abnormalities of chromosome 11q23 involving the MLL locus
  - 7 patients had multiple nonspecific structural and/or numerical chromosomal abnormalities
  - 5 patients had normal conventional cytogenetic analysis

- Fluorescence in situ-hybridization confirmed the findings of MLL rearrangement in 4 patients and trisomy of chromosome 9 in 4 patients, and revealed low-level abnormal loss of AML1 locus in one patient with normal conventional cytogenetics.

Hurley MY, et al, submitted

Results

Skin biopsy - workup

- 19 cases with involved skin biopsies and a prior bone marrow diagnosis of acute myeloid leukemia were evaluated by a hematoxylin and eosin-stained section without additional studies
- An additional 19 patients, all with a prior history of bone marrow involvement by acute myeloid leukemia, had skin biopsies evaluated by a hematoxylin and eosin-stained section and a Leder stained section, in which the blasts were Leder negative
- The remaining 45 cases had immunohistochemistry analysis as part of their workup

Hurley MY, et al, submitted
Results

Skin biopsy

- Diffuse architecture - 18 cases
- Destruction of dermal microanatomy

Hurley MY, et al, submitted

Results

Skin biopsy

- Focal architecture - 38 cases
- Perivascular
- Periadnexal
- Interstitial

The microscopic features of skin disease are variable and do not correlate with clinical appearance of lesions.

Hurley MY, et al, submitted

Results

- The recent paper by Benet et al notes similar patterns of involvement of the skin: certain histologic patterns are associated with specific leukemia types, such as the association of acute myelomonocytic leukemia and acute monocytic leukemia with a granuloma annulare-type histology.
- Although Benet et al do not report survival data, the extent and pattern of involvement do not correlate with survival.
- The 1-year mortality status was could be determined for 59 (or 71.1%) of cases
- Of these 59 cases, 86.4% were deceased 1-year after the diagnosis date
- Of the cases who died during the observed period, the mean number of survival days after the diagnosis date was 227 ± 319 (range 14 to 1561).
- Survival does not appear to be impacted by more recent chemotherapeutic regimens

Hurley MY, et al, submitted
### Immunohistochemistry analysis of involved skin biopsies

<table>
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<tr>
<th>Antibody</th>
<th>Cases</th>
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</tr>
<tr>
<td>CD4</td>
<td>10</td>
<td>50%</td>
</tr>
<tr>
<td>CD43</td>
<td>12</td>
<td>100%</td>
</tr>
<tr>
<td>CD45</td>
<td>19</td>
<td>84%</td>
</tr>
<tr>
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<td>33%</td>
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<tr>
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<td>CD79a</td>
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<td>CD117</td>
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<td>Pan-cytokeratin</td>
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<tr>
<td>Myeloperoxidase</td>
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<td>Lysozyme</td>
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<tr>
<td>Chloroacetate esterase</td>
<td>19</td>
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Hurley MY, et al, submitted

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Hurley MY, et al, submitted

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### Lysozyme vs Myeloperoxidase

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<td>17</td>
<td>14</td>
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Lysozyme is very sensitive for the detection of myeloid disease but is relatively nonspecific for distinction of malignancies with monocytic differentiation (e.g. M4, M5, CML, JMML, CMML) from other leukemias without monocytic differentiation (e.g. M1, M2).

Hurley MY, et al, submitted
Lysozyme  Myeloperoxidase  CD68

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MPO is frequently negative due to the high frequency of AML with monocytic differentiation

Hurley MY, et al. submitted

CD68 is reportedly similar to lysozyme in sensitivity, and is similarly nonspecific for distinction of malignancies with monocytic differentiation (e.g. M4, M5 CMML) from other leukemias without monocytic differentiation (e.g. M1, M2).

Hurley MY, et al. submitted

Immunohistochemistry of CMS

Analysis using CD14, CD33, KLF4, CD163

Case with monocytic differentiation and a bone marrow diagnosis of acute myelomonocytic leukemia (FAB-M4) showing positivity for lysozyme (A), CD14 (E) and KLF-4 (nuclear) (G), and negativity for the remaining markers (B, myeloperoxidase; C, CD163; D, CD117; F, CD34; H, CD33)

### Immunohistochemistry of CMS

**Analysis using CD14, CD33, KLF4, CD163**

Case with non-monocytic differentiation and a bone marrow diagnosis of AML with maturation (FAB-M2) showing positivity for lysozyme (A), myeloperoxidase (B), CD117 (D) and CD33 (H), and negativity for the remaining markers (C, CD163; E, CD14; F, CD34; G, KLF-4).


---

### Monocytic markers in monocyctic related AML cases

<table>
<thead>
<tr>
<th>Marker</th>
<th>Sens.</th>
<th>Spec.</th>
<th>PPV</th>
<th>NPV</th>
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<td>CD117</td>
<td>63%</td>
<td>49%</td>
<td>82%</td>
<td>29%</td>
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<tr>
<td>CD33</td>
<td>60%</td>
<td>60%</td>
<td>62%</td>
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<td>KLF-4</td>
<td>60%</td>
<td>60%</td>
<td>62%</td>
<td>60%</td>
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<tr>
<td>CD14</td>
<td>60%</td>
<td>60%</td>
<td>62%</td>
<td>60%</td>
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</table>

Regardless of AML subtype, a panel of antibodies that included lysozyme, CD117 and CD33, identified all cases, including those with monocytic differentiation.

Lysozyme was expressed in 52 (91%), CD33 in 34 (60%), myeloperoxidase in 31 (54%), CD34 in 22 (39%) and CD117 in 20 cases (38%).


---

In AML cases with monocytic differentiation (M4 and M5), CD14 and KLF-4 were significantly more commonly expressed than in AML cases of granulocytic lineage (M0, M1 and M2) (79% vs. 20%, p<0.001 and 50% vs. 13%, p<0.05, respectively).

Regardless of AML subtype, a panel of antibodies that included lysozyme, CD117 and CD33, identified all cases, including those with monocytic differentiation.

Lysozyme was expressed in 52 (91%), CD33 in 34 (60%), myeloperoxidase in 31 (54%), CD34 in 22 (39%) and CD117 in 20 cases (38%).

Flow cytometry vs IHC

- For CD33, CD34, CD117 and CD14 there was agreement between the bone marrow and skin antigen expression in 66%, 60%, 72% and 41% of the cases, respectively.
- In cases in which there was disagreement, the antigen expression was more commonly present in the bone marrow than in the skin for CD33 and CD117 (83 vs. 17% and 71 vs. 29%, respectively).
- Conversely, CD34 and CD14 were more commonly expressed in the skin than in the bone marrow (57 vs. 43% and 94 vs. 6%, respectively).


Differential diagnosis

- Commonly influenced by the clinical history, including the patient’s age and history of a possible antecedent or concurrent myeloid neoplasm.
- Ideally: fresh material will be sent for flow cytometric, cytogenetic and molecular studies.
- Clinical suspicion is very important in establishing the correct diagnosis; in one study of 26 cases of myeloid sarcoma, all 14 cases in which there was no antecedent myeloid neoplasm were initially misdiagnosed!

Skin biopsy

CD4 and CD56 expression

- CD56 expressed in 5/6 tested cases
- Coexpressed with CD4 in 3 cases: dx also includes blastic plasmacytoid dendritic cell neoplasm (blastic NK-cell lymphoma, agranular CD4+CD56+ hematodermic neoplasm)
- All 3 cases also expressed CD68 and lysozyme and had a history of acute myelomonocytic leukemia (2 cases) or relapsed acute myeloid leukemia (1 case)

Hurley MY, et al., submitted
Differential diagnosis
Blastic plasmacytoid dendritic cell neoplasm

Photo: JA Kozel, MD, Saint Louis University

Blastic plasmacytoid dendritic cell neoplasm

Photo: JA Kozel, MD, Saint Louis University

Blastic plasmacytoid dendritic cell neoplasm

Photo: JA Kozel, MD, Saint Louis University
**Blastic plasmacytoid dendritic cell neoplasm**

- Typically evolve to leukemic stage; may present with predominantly cutaneous disease
- Workup should include myeloperoxidase, lysozyme, CD33 (negative); CD43, CD123 (positive)
- Caveats: weak CD123 expression in some AMLs; CD43 is nonspecific
- BPDCN are occasionally CD68+ (dot-like peri-Golgi pattern)
- TCL1 more frequently positive in BPDCN vs. AML
- CMML may be associated with PDC proliferations

**Histiocytic sarcoma**

Histiocytic sarcoma

- Have overlapping histologic, clinical, and immunophenotypic features with AML
- CD163, CD68, lysozyme positive like AML
- CD13, CD33 negative

Granuloma annulare

“Generalized” – ddx includes AML
“Classic” – ddx doesn’t include AML

Photos: N Burkamper, MD, Saint Louis University

Photos: MY Hurley, MD, Saint Louis University
Differential diagnosis

Melanoma

S100 antigen, Melan-A (MART-1), HMB-45 and a pan-keratin marker, such as cytokeratin AE1/AE3, are usually sufficient for this distinction. However, rare AMLs have been reported to show scattered dot-like cytokeratin positivity.

Differential diagnosis

Extramedullary hematopoiesis

Extramedullary hematopoiesis can clinically present as a mass-forming lesion. Multilineage proliferation, or only erythroid if unilineage, and consists predominantly of mature terminally differentiated cells. It is important to report the finding of EMH, especially when there are atypical cytomorphicic features and aggregates of immature cells, as this finding may precede the eventual development of an acute leukemia.

Differential diagnosis

- **B-NHL**: CD20+, CD79a+ (rare AML cases with t(8;21) are weakly CD9a/ Pax5+)
- **T-NHL**: are CD43+/ CD45+ like AML; some AMLs are CD4+/ CD7+; rare AMLs have clonal TCRs
- **ALCL**: are occasionally CD13/ CD33+; AMLs are rarely CD30+
- Use myeloperoxidase, lysozyme, CD68, ALK to distinguish
Differential diagnosis

Pediatric cases

- Dx frequently includes Ewing sarcoma, PNET, medulloblastoma, other SRBCTs
- Workup includes CD99, MPO, lysozyme, CD43
- Beware: TdT (+) AMLs; CD99(+) AMLs - coexpressed in ~20% of AMLs!

Differential diagnosis

Recommended IHC

- CD43, lysozyme – positive in nearly all AMLs
- CD33, CD34, CD117
  - Identifies myeloid origin
  - Excludes NHLs, etc.
- Add other IHC as noted if warranted by clinical history, site

Molecular findings

- Trisomy 8, monosomy 7, MLL rearrangements most common
- Clinical Associations
  - AML1-ETO (RUNX1-RUNX1T1) translocations
    - t(8;21)(q22;q22) orbital region in children
  - inv(16)(p13.1q22)/t(16;16) (p13.1;q22) gastrointestinal tract or breast in adults
  - Trisomy 8: Skin involvement?
Molecular findings

- Rare cases of BCR-ABL1 positive myeloid sarcoma have been reported in which there is no systemic disease.
- Myeloid and lymphoid neoplasms with FGFR1 abnormalities/8p11 syndrome can have lymph node involvement by myeloid sarcoma, although involvement by T-lymphoblastic leukemia/lymphoma is more common in this disorder. Bilineal (T/Myeloid) extramedullary disease is also common in this syndrome with the myeloid component frequently in a perivascular distribution.

Vega (2008)

Molecular findings

- Myeloid sarcomas associated with FIP1L1-PDGFRA have also been reported.
- Increased local and peripheral eosinophils.
- Sensitive to treatment with tyrosine kinase inhibitors, such as imatinib.

Vedy (2010)

Molecular findings

- Small studies investigating the prevalence of Fms-like tyrosine kinase-3 (FLT3) and nucleophosmin (NPM1) mutations in myeloid sarcomas have been reported.
- FLT3-ITD (internal tandem duplication) in 3/20 cases with no D835 mutations. Lack of stability of the FLT3 mutation as cases with discrepancies either between the bone marrow and extramedullary site or the primary and recurrent specimens.
- NPM1 mutations have been found in 15% of myeloid sarcomas.
- The incidence of other recurring AML-associated mutations such as WT1, N/K-Ras, CEBPA, IDH1, IDH2 or DNMT3a have not been reported for myeloid sarcoma.
Molecular findings

• High-resolution genomic studies are limited for myeloid sarcoma.
• Deeb et al., array CGH of 7 cases: most common were
  – Gains involving chromosome 8 or 21q21.1-q21.3
  – Loss of 5q31.2-q31.3 C
  – Concordant findings between bone marrow and extramedullary disease.

Conclusions

Areas of Uncertainty

• What are the molecular events that allow some AMLs to have a predilection for extramedullary sites?
• What is the molecular relationship of the extramedullary tumor to the bone marrow disease?
• Does myeloid sarcoma represent clonal evolution of the original leukemia?
• Why do some AMLs originally manifest as extramedullary disease?

Conclusions

Future Research

• What is the incidence of the known genetic lesions associated with AML (i.e. IDH1, IDH2, DNMT3a, FLT3, NPM) in a large series of myeloid sarcomas and are these mutations stable when comparing the medullary and extramedullary tumors?
• High-resolution genomic studies (including whole genome sequencing) of a series of myeloid sarcomas with paired normal DNA and bone marrow disease to determine the molecular alterations associated with myeloid sarcoma.
Conclusions
Recommendations to the pathologist

• When possible, flow cytometric analysis of the specimen should be performed, since the challenges associated with diagnosis of myeloid sarcoma are compounded by the limitations of immunohistochemical analysis for AMLs with monocytic differentiation.

• A concurrent bone marrow biopsy is recommended to compare the immunophenotypic and morphologic features and to determine the extent of disease.

• Prudent use of immunohistochemistry is important to minimize the risk of misdiagnosis: e.g. CD7, CD4/ CD56

Conclusions
Recommendations to the pathologist

• Cytogenetic abnormalities are identified in a large percentage of tested cases. Identification of an AML-associated abnormality is helpful in arriving at the correct diagnosis.

• Correlation with past medical history is particularly important, since many patients have a concurrent or antecedent AML, simplifying the diagnosis and analysis of extramedullary disease.

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JA Kozel, MD

Saint Louis University School of Medicine
GK Ghahramani
Flow cytometry in acute and chronic myeloid neoplasms

Horatiu Olteanu, MD, PhD
Assistant Professor of Pathology
Director, Flow Cytometry Laboratory
Medical College of Wisconsin
Disclosure information

• I do not have any relevant financial relationships with any commercial interests.
Overview

• Flow cytometric immunophenotyping of myeloblasts

• Chronic myeloid neoplasms – MPN diagnosis
• Acute myeloid leukemias – diagnosis
• Acute myeloid leukemias – MRD analysis
Overview

• Flow cytometric immunophenotyping of myeloblasts
• Chronic myeloid neoplasms – MPN diagnosis
• Acute myeloid leukemias – diagnosis
• Acute myeloid leukemias – MRD analysis

• Rationale
• Technical aspects
Overview

• Flow cytometric immunophenotyping of myeloblasts

• Chronic myeloid neoplasms – MPN diagnosis
  • Acute myeloid leukemias – diagnosis
  • Acute myeloid leukemias – MRD analysis
FC in MPN diagnosis - Rationale

• Integrated diagnostic approach
  – Morphology
  – Immunophenotype
  – Cytogenetics
  – Molecular analysis
  – Clinical data
Technical aspects in FC of MPN

• Specificity of IP alterations in myeloblasts
• Heterogeneity of the CD45/SSC “blast gate”
Immune phenotype of normal blasts

- **CD7** (-)
- **CD11b** (-)
- **CD13** (v +)
- **CD14** (-)
- **CD15** (predom -)
- **CD16** (-)
- **CD33** (+)
- **CD34** (+)
- **CD36** (-)
- **CD38** (mod br +)
- **CD45** (mod +)
- **CD56** (-)
- **CD64** (-)
- **CD117** (+)
- **HLA-DR** (mod br +)
1345  A Dissection of the CD45/Side Scatter (SS) Blast Gate in Non-Acute Myeloid Disorders and Non-Neoplastic Bone Marrows

AM Harrington, H Olteanu, SH Kroft. Medical College of Wisconsin, Milwaukee, WI.

Mod Pathol (2010) 23;S1: 301A
CD45/SSC “Blast gate”

• Most common approach for defining blasts

• Imprecise, due to presence of other contaminating populations

• “Cluster analysis”: flexible, iterative analytic strategy; more robust
  (Kroft and Karandikar Flow Cytometry in Clinical Diagnosis, 2007)
Traditional gating vs. cluster analysis

• Traditional gating: based on relatively rigid quad marker application, dictated by presupposed antigen expression / light scatter characteristics of a population of interest

• Cluster analysis: detection of a “cluster” (population) residing in an abnormal location of the multidimensional flow space, based on global patterns of aberrant antigen expression
Granulocytes, monocytes, lymphocytes, erythroids, basophils, blasts, hematogones.

(Harrington, et al Mod Pathol, 2010)
Granulocytes, monocytes, lymphocytes, erythroids, basophils, blasts, hematogones.

(Harrington, et al Mod Pathol, 2010)
“Blast gate” composition

<table>
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<th>MDSs</th>
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<td>% Basos in BG</td>
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<td>11.5 (0-27)</td>
<td>35.4 (0-68.7)</td>
<td>3 (0-7.1)</td>
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<td>% Hgones in BG</td>
<td>10.5 (0-38.9)</td>
<td>1.3 (0-12.4)</td>
<td>2.2 (0-18.5)</td>
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<td>% Erythroids in BG</td>
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<td>9.8 (0-58.2)</td>
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(Harrington, et al Mod Pathol, 2010)
Granulocytes, monocytes, lymphocytes, basophils, blasts, hematogones.

The Specificity of Immunophenotypic Alterations in Blasts in Nonacute Myeloid Disorders

Alexandra Harrington, MD, Horatiu Olteanu, MD, PhD, and Steven Kroft, MD

Key Words: Blasts; Immunophenotype; Myelodysplastic syndromes; Myeloproliferative neoplasms; Flow cytometry
Percentage of IP changes in cytopenias/cytoses (CCs), MDSs, MPNs, and CMMLs.
Control samples (A, D, G, and J) and cytopenias/cytoses (B, E, H, and K)

Blast IP changes

Shared with reactive BMs

- CD13(bright +)
- CD33(variably +)
- CD34(slightly bright +)
- CD38(variably +)
- CD117(bright/dim/variably +)
- HLA-DR(bright +)

Neoplasia-specific

- CD7(partial +)
- CD11b(bright +)
- CD13(partial + or -)
- CD15(partial +)
- CD33(partial + or -)
- CD34(bright/variably +)
- CD36(partial +)
- CD38(partial +)
- CD45(dim +)
- CD56(partial +)
- CD117(partial +)
- HLA-DR(dim/variably +)

Neoplasia-specific IP alterations in MPNs

- Expression of CD7, CD36, CD56
- Variable expression of HLA-DR
- Loss of CD13
- Underexpression of CD45 and HLA-DR
- Partial loss of CD13, CD33, and CD117

Abnormal blast IP in non-CML MPNs

(Kussick and Wood Am J Clin Pathol, 2003)
Overview

- Flow cytometric immunophenotyping of myeloblasts
- Chronic myeloid neoplasms – MPN diagnosis
- Acute myeloid leukemias – diagnosis
- Acute myeloid leukemias – MRD analysis
Role of FC in diagnosing AML

- Allows distinction between AML and ALL
- Certain immunophenotypes correlate with the presence of recurrent cytogenetic or molecular abnormalities
- Establishes an immunophenotypic fingerprint for MRD analysis at follow-up
AML with minimal differentiation
AML with minimal differentiation
AML with t(8;21)(q22;q22)
AML with $t(8;21)(q22;q22)$
AML with $t(15;17)(q22;q12)$
AML with t(15;17)(q22;q12)
AML with “cup-like” inclusions
AML with “cup-like” inclusions

NPM1 mut(+)
AML with t(1;22)(p13;q13)
Overview

- Flow cytometric immunophenotyping of myeloblasts
- Chronic myeloid neoplasms – MPN diagnosis
- Acute myeloid leukemias – diagnosis
- Acute myeloid leukemias – MRD analysis
MRD detection in AML - Rationale

• Standard of care: induction chemotherapy + consolidation (chemotherapy and/or SCT) (Dohner, et al Blood, 2010)

• Patients with AML show heterogeneous response to therapy, ranging from cure to refractory disease

• CR is formally defined based on BM morphology (<5% blasts) – low sensitivity (Cheson, et al J Clin Oncol, 2003)
MRD detection in AML - Rationale

• Risk stratification includes cytogenetic and molecular abnormalities (2008 WHO classification)

• Other risk factors:
  – Drug efflux transporters
  – Pharmacologic resistance
  – BM environment

MRD detection in AML - Rationale

• Prognosis in individual patients cannot be estimated accurately
  – Very good risk patients may relapse
  – Very poor risk patients may be cured

• MRD analysis may potentially guide treatment
  – More chemotherapy for high risk patients
  – Less chemotherapy for low risk patients

• MRD analysis may offer a short-term surrogate endpoint for assessing new therapies
Toward Optimization of Postremission Therapy for Residual Disease–Positive Patients With Acute Myeloid Leukemia

Luca Maurillo, Francesco Buccisano, Maria Ilaria Del Principe, Giovanni Del Poeta, Alessandra Spagnoli, Paola Panetta, Emanuele Ammatuna, Benedetta Neri, Licia Ottaviani, Chiara Sarlo, Daniela Venditti, Micol Quaresima, Raffaella Cerretti, Manuela Rizzo, Paolo de Fabritiis, Francesco Lo Coco, William Arcese, Sergio Amadori, and Adriano Venditti
Minimal residual disease-directed therapy for childhood acute myeloid leukaemia: results of the AML02 multicentre trial

Jeffrey E Rubnitz, Hiroto Inaba, Gary Dahl, Raul C Ribeiro, W Paul Bowman, Jeffrey Taub, Stanley Pounds, Bassem I Razzouk, Norman J Lacayo, Xueyuan Cao, Soheil Meshinchi, Barbara Degar, Gladstone Airewele, Susana C Raimondi, Mihaela Onciu, Elaine Coustan-Smith, James R Downing, Wing Leung, Ching-Hon Pui, Dario Campana

Summary

Background We sought to improve outcome in patients with childhood acute myeloid leukaemia (AML) by applying risk-directed therapy that was based on genetic abnormalities of the leukaemic cells and measurements of minimal residual disease (MRD) done by flow cytometry during treatment.
• 232 patients
• MRD levels used to allocate gemtuzumab and to determine timing of 2nd induction
• MRD and cytogenetic abnormalities were combined to determine final risk stratification

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<th>Overall survival</th>
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<td>HR (95% CI)</td>
<td>p value</td>
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<td>0.483</td>
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<td>Induction 1 MRD ≥1%</td>
<td>2.41 (1.36–4.26)</td>
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<tr>
<td>CBF</td>
<td>0.32 (0.13–0.79)</td>
<td>0.013</td>
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<td>Age at diagnosis*</td>
<td>1.65 (0.92–2.95)</td>
<td>0.095</td>
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<tr>
<td>Other 11q23</td>
<td>2.05 (0.96–4.34)</td>
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<td>M7 without t(1;22)</td>
<td>2.17 (0.86–5.45)</td>
<td>0.099</td>
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<td>FLT3-ITD</td>
<td>1.64 (0.82–3.27)</td>
<td>0.163</td>
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</tbody>
</table>

Significance of MRD in AML

• “MRD detection methods... should be part of the routine management of AML patients to guide therapy.”

(Bene and Kaeda Haematologica, 2009)
(Grimwade, et al Curr Opin Oncol, 2010)
Techniques for MRD detection in AML

• PCR and FC

• Real-time quantitative RT-PCR (RQ-PCR) has clinical utility in directing treatment in APL
  (Grimwade, et al J Clin Oncol, 2009)

• FC is useful for MRD detection and quantification, and can provide prognostic information
  (Kern, et al Cancer, 2008)
  (Buccisano, et al Curr Opin Oncol, 2009)
MRD analysis by RQ-PCR

• Advantages:
  – Very sensitive \((10^{-6})\)
  – Quantitative

• Disadvantages:
  • Applicable to a limited number of molecular targets
    – PML-RARA
    – CBF leukemias
    – NPM1

(Perea, et al Leukemia, 2006)
(Schnittger, et al Blood, 2009)
MRD analysis by FC

• Advantages:
  – Sensitive \((10^{-4})\)
  – Rapid
  – Quantitative
  – Applicable in ≥95% patients

• Disadvantages:
  – Lacks standardization
  – Immunophenotypic stability of LAIP
Technical aspects in FC of AML

• MRD analysis
  – How to gate?
  – Beware of blast autofluorescence
  – What antibody combinations to use?
  – How many colors (4, 6, 8)?
  – What MRD cut-off values to use / how many events are required?
  – Immunophenotypic shifts
Leukemia-associated immunophenotype

- Sum of aberrant antigen expression on blasts
- Type of IP aberrancies:
  - Asynchronous antigen expression
  - Cross-lineage antigen expression
  - Antigen over- / underexpression
  - Abnormal forward / side scatter properties
Asynchronous antigen expression

CD34 and CD15 coexpression
Asynchronous antigen expression

CD34 and CD15 coexpression
Cross-lineage antigen expression

Partial CD7 and CD56 expression
Cross-lineage antigen expression

Partial CD2 expression
Cross-lineage antigen expression

Dim CD19 and partial CD56 expression
Antigen over- / underexpression

Bright CD34, CD33 and CD117
Dim HLA-DR
Antigen over- / underexpression

Partial CD34, CD117
Abnormal FSC / SSC

Increased side scatter
Abnormal FSC / SSC

Increased side scatter
Technical aspects: How to gate?

• CD45/side scatter ("blast gate")
• Back gating with CD34 and/or CD117 for refinement of "blast gate"
• "Cluster analysis" is more powerful, but potentially time consuming
Blasts ante portas
Technical aspects: Autofluorescence

• Blasts tend to have high autofluorescence
• Include appropriate negative isotype control antibody tube
CD10(+) vs. CD10(-)
CD10(+) vs. CD10(-)
Technical aspects: Antibodies

• LAIP found in 75-100% of cases; depends on:
  – Number of antibodies
  – Comparison with “normal”/reactive BM blasts
  – Individual laboratory thresholds for LAIP definition
• 5-color FC shows increased sensitivity vs. 4-color FC
• Suggested antibody panels are highly variable

(Campana and Coustan-Smith Cytometry, 1999)
### LAIP type and frequencies

<table>
<thead>
<tr>
<th>Report</th>
<th>No. of MFC Colors Used</th>
<th>LAPs (%)</th>
<th>Asynchronous Antigen Expression (%)</th>
<th>Lineage Infidelity (%)</th>
<th>Antigen Overexpression (%)</th>
<th>Aberrant Light-Scatter Properties (%)</th>
<th>Absence of Lineage-Specific Antigens (%)</th>
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Incidence, Sensitivity, and Specificity of Leukemia-Associated Phenotypes in Acute Myeloid Leukemia Using Specific Five-Color Multiparameter Flow Cytometry

Adhra Al-Mawali, MD,1,2 David Gillis, MBBS,1 Pravin Hissaria, MD,1 and Ian Lewis, MBBS, PhD2
Frequency of LAIPs in 51 AMLs

<table>
<thead>
<tr>
<th>LAIPs (n = 200)</th>
<th>No. of Cases</th>
<th>Range of Positive Cells in AML Bone Marrow (%)</th>
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<tr>
<td><strong>Lineage infidelity (n = 40)</strong></td>
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<td>CD34+/CD2+</td>
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<td>CD117+/CD2+</td>
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<td>CD117+/Glycophorin A+</td>
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<td><strong>Asynchronous antigen expression (n = 146)</strong></td>
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<td>CD33+/CD56+</td>
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<td><strong>Lack of lineage-specific antigen (n = 14)</strong></td>
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<td>CD33++/CD13−</td>
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<tr>
<td>CD33−/CD13++</td>
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<td>25-98</td>
</tr>
</tbody>
</table>

Sensitivity of aberrant LAIPs

Compared to normal BMs

Compared to reactive BMs

Technical aspects: How many events?

• Several studies have used an MRD cut-off of 0.03 – 0.1%
  (Kern, et al Haematologica, 2004)
  (Buccisano, et al Leukemia, 2006)

• For a 0.01% \((10^{-4})\) detection level, at least 200,000 events are required to detect 20 aberrant blasts

• In practice, up to 1,000,000 events may be necessary
AML - Diagnosis

20% blasts
AML – MRD at day 14

0.06% blasts
Technical aspects: IP stability

• Question: Is the aberrant blast IP relatively stable over time?
• Hematologic malignancies (ALL, PCM, T-LGLL, MF / SS) demonstrate temporal IP instability
• May occur in AML at follow-up

(San Miguel, et al Blood, 1997)
Issues to consider

• Revised definition for CR in AML
  – Similar to plasma cell myeloma
• What level of MRD correlates with cure?
• Role of additional therapy in MRD(+) cases
• MRD detection in peripheral blood / stem cells
• MRD analysis in CD34(-) AML
• Uniform analytic strategies
• FC vs. PCR comparison for MRD detection
Future developments

• Several single-institution, retrospective studies have been performed
• Standardize LAIP analysis to enable adequate multicenter clinical studies (initiated in Netherlands and Switzerland – HOVON/SAKK studies)
• Perform RQ-PCR in parallel with FC
• Prospective, randomized trials, to determine the clinical value of MRD status as predictive biomarker (underway in UK – AML 16 and 17 trials)
Conclusions

• There are technical issues associated with blasts IP in MPN
• Traditional CD45/SSC “blast gate” has a heterogeneous composition
• There is IP overlap between reactive and neoplastic blast aberrancies
• FC is useful and diagnosis and assessment of MRD in AML
Conclusions

• Currently, there are numerous technical limitations for MRD detection by FC
• MRD assessment may help in refining risk stratification of AML patients
• MRD assessment may apply to individual risk definition
• MRD assessment potentially constitutes a short-term surrogate endpoint for determining effectiveness of new therapies
References


