Practical Issues in the Diagnosis of Myelodysplastic/ Myeloproliferative Overlap Syndromes

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The World Health Organization (WHO) classification of hematopoietic neoplasms, published in 2001, included the new category of “Myelodysplastic/ Myeloproliferative Diseases,” intended to accommodate those myeloid neoplasms that simultaneously showed features of chronic myeloproliferative disorders and myelodysplastic syndromes. Typically, myelodysplastic syndromes (MDS) are clonal neoplastic disorders characterized by accelerated apoptosis of the abnormal clone, resulting in a pattern of ineffective hematopoiesis, usually manifest by increased bone marrow cellularity in the presence of decreased peripheral blood counts (cytopenias) and absence of organomegaly. MDS also generally harbor some degree of morphologic dysplasia within one or more hematopoietic lineages. Conversely, the chronic myeloproliferative disorders (MPD) are myeloid neoplasms generally characterized by evidence of effective hematopoiesis, generally in the form of increased peripheral blood counts (“cytoses”), organomegaly, or both. Typically, MPD do not show prominent morphologic dysplasia of hematopoietic elements.

The WHO category of MDS/MPD overlap syndromes includes diseases that either have a proliferative component (increased peripheral blood counts, organomegaly) combined with significant morphologic dysplasia, or diseases that may vary in their degree of proliferative characteristics and morphologic dysplasia. The WHO classification lists four disease categories under the heading of MDS/MPD: 1) Chronic myelomonocytic leukemia (CMML), 2) Juvenile myelomonocytic leukemia (JMML), 3) Atypical chronic myeloid leukemia (aCML), and 4) Myelodysplastic/ myeloproliferative diseases, unclassifiable. There are many pitfalls that practicing hematopathologists may face in the diagnosis of MDS/MPD overlap syndromes. This session will focus on the following points:

1) Guidelines for morphologic recognition of the distinct categories of MDS/MPD outlined by the WHO, including recognition of clinical and morphologic heterogeneity in CMML
2) A discussion of the “unclassifiable” category, including the provisional entity “Refractory anemia with ringed sideroblasts associated with marked thrombocytosis.”
3) The distinction between CMML and acute myelomonocytic leukemia, which may be deceptively difficult
4) An illustration of mimickers of morphologic dysplasia in bone marrow that could lead to overdiagnosis of MDS/MPD.

A Brief Overview of Distinct Categories of MDS/MPD

Chronic Myelomonocytic Leukemia (CMML)

CMML was included initially in the French-American-British cooperative group (FAB) classification of myelodysplastic syndromes. As defined by the FAB, CMML shared many features in common with refractory anemia with excess blasts (RAEB), including peripheral cytopenias, usually in the setting of hypercellular marrow, marked multilineage dysplasia, and an increased blast percentage in the bone marrow (but not sufficient to diagnose frank acute leukemia). CMML differed, however, in a proliferative component of monocytosis, with the peripheral blood monocyte count by definition exceeding 1 x 10⁹/L. There remained, however, chronic myeloid neoplasms with a more proliferative phenotype (increased peripheral granulocyte counts, organomegaly, absence of significant morphologic dysplasia), but also marked by absolute peripheral monocytosis, and absence of the Philadelphia chromosome or BCR-ABL fusion. Some proposed that disorders fitting the latter description be termed “CMML,
myeloproliferative,” as opposed to the original FAB category, for which the term “CMML, myelodysplastic” was subsequently proposed. Subsequent clinicopathologic studies, however, failed to elucidate significant differences in clinical outcome between these two categories, and therefore they are consolidated under the single heading of CMML in the WHO classification.

Although CMML as defined by WHO is a diverse disorder that likely does not represent a single clinicopathologic disease entity, the CMML variant listed in the WHO classification as “CMML with eosinophilia” may be associated with the cytogenetic translocation t(5;12)(q31;p12), resulting in an abnormal fusion gene, $TEL/PDGFβR$, or other genetic abnormalities involving the $PDGFβR$ locus. It is important to recognize this unusual but distinct category, since such patients may respond extremely well to therapy with imatinib or other targeted tyrosine kinase inhibitors. In fact, such patients may show excellent response to only a fraction of the imatinib dose given to Philadelphia-positive chronic myelogenous leukemia patients.

**Diagnostic Criteria for CMML (from WHO, 2001):**

1. Persistent peripheral blood monocytosis $>1 \times 10^9/L$
2. No Philadelphia chromosome or $BCR/ABL$ fusion gene
3. Less than 20% blasts in the blood or bone marrow
4. Dysplasia in one or more myeloid lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML may still be made if the other requirements are present and:
   - an acquired, clonal cytogenetic abnormality is present in the marrow cells, or
   - the monocytosis has been persistent for at least 3 months and
   - all other causes of monocytosis have been excluded

**Juvenile Myelomonocytic Leukemia (JMML)**

JMML is a rare but distinct disease entity that generally affects young children, with three-fourths of cases occurring in patients under 3 years of age. The pathogenesis of most cases of JMML is linked directly to abnormalities in the molecular pathway responsible for conducting the stimulatory signal of granulocyte-monocyte colony stimulating factor (GM-CSF), resulting in marked hypersensitivity to GM-CSF *in vitro*, and abnormal myelomonocytic proliferations *in vivo*.

There is an increased prevalence of JMML in children with neurofibromatosis type 1, and in children with Noonan syndrome (a complex syndrome of mild facial dysmorphism and other anatomic malformations including cardiac anomalies). This increased prevalence can be linked to abnormalities in the NF1 gene in neurofibromatosis, and the PTPN11 gene in Noonan syndrome. NF1 is an important regulator of the Ras pathway of signal transduction, which regulates cellular response to GM-CSF. PTPN11 is the gene encoding SHP-2, a tyrosine phosphatase required for Ras-dependent functions. Mutations of PTPN11 have been shown to induce hypersensitivity of hematopoietic precursors to GM-CSF stimulation.

The diagnostic criteria for JMML are listed below. The morphologic features are not necessarily specific, and *in vitro* assays for GM-CSF may be difficult to obtain in routine clinical practice. Therefore, the correlation of morphologic findings with the clinical history, as well as documentation of elevated hemoglobin F levels by hemoglobin electrophoresis or other method, are often crucial to diagnosis. Although it is not formally classified as a form of acute leukemia, JMML usually follows an aggressive clinical course despite therapy.

**Diagnostic criteria for JMML (from WHO, 2001):**

1. Peripheral blood monocytosis $>1 \times 10^9/L$
2. Blood and marrow blasts $<20\%$
1. Peripheral blood monocytosis >1 x 10⁹/L
3. No Ph chromosome or BCR/ABL fusion gene
PLUS 2 or more of the following:
Hemoglobin F increased for age
Immature granulocytes in peripheral blood
WBC > 10x10⁹/L
Clonal chromosomal abnormality (may be monosomy 7)
GM-CSF hypersensitivity of myeloid progenitors in vitro

Atypical Chronic Myeloid Leukemia (aCML)

Despite the name, aCML likely bears no relationship to true Philadelphia-chromosome-positive CML. In fact, in the drafting of the current WHO classification, alternative names for this entity were debated, but the consensus was reached that aCML was acceptable provided a clear distinction was made from true CML.

ACML is a very rare disorder, but likely represents a distinct disease entity. It is marked by increased peripheral granulocyte counts, splenomegaly, and distinct and marked morphologic dysplasia in circulating neutrophils in the form of hypolobation (pseudo-Pelger-Huet changes). ACML is more clinically aggressive than true CML, with median survival quoted in the literature as less than 20 months. While most reported cases of aCML are associated with clonal cytogenetic abnormalities, no single consistent abnormality has been reported, although three reported cases associated with t(4;11)(q12;q11), and the detection of Ras mutations in a subset of aCML cases suggests potential common pathobiology to other types of MDS/MPD.

Myelodysplastic/ Myeloproliferative Disease, Unclassifiable

Invariably, any classification scheme will have to accommodate disorders not readily classifiable into other distinct categories. Such is the case in the WHO classification of MDS/MPD overlap syndromes. One subtype under the “unclassifiable” moniker does, however, deserve mention since it may represent a distinct clinical entity.

Refractory anemia with ringed sideroblasts and thrombocytopenia is currently listed as a provisional entity under the broader heading of Myelodysplastic/ Myeloproliferative Disease, Unclassifiable in the WHO classification. Patients with this disorder otherwise fulfill criteria for refractory anemia with ringed sideroblasts (RARS), including normocytic or macrocytic anemia, dimorphic circulating red cells due to hypochromic microcytes admixed with normocytic or macrocytic red cells, erythroid expansion in the bone marrow with mild dyserythropoiesis, and greater than 15% ringed sideroblasts on examination of bone marrow aspirate smears. In contrast to classic RARS however, this disorder is characterized by marked and persistent thrombocytosis, and patients may present initially with clinical suspicion for essential thrombocythemia. (This disorder is in fact cross-referenced in the WHO classification under the heading of Essential Thrombocythemia.) A recent study noted that a substantial majority of cases of RARS with thrombocytosis harbor the JAK2 V617F mutation that has come to characterize a molecularly distinct subset of chronic myeloproliferative disorders (usually classifiable clinically as polycythemia vera, essential thrombocythemia, or chronic idiopathic myelofibrosis). This adds RARS with thrombocytosis to the list of JAK2 mutation-associated myeloid neoplasms, and suggests that it may represent a morphologically distinct variant of such disorders.

Diagnostic Criteria for aCML (from WHO, 2001):
1. Peripheral blood leukocytosis due to increased numbers of mature and immature neutrophils
1. Peripheral blood leukocytosis due to increased numbers of mature and immature neutrophils
2. Prominent dysgranulopoiesis
3. No Ph chromosome or BCR/ABL fusion gene
4. No or minimal absolute basophilia; basophils <2% of WBCs
5. No or minimal absolute monocytosis; monocytes <10% of WBCs
6. Hypercellular bone marrow with granulocytic proliferation and dysplasia, with or without dysplasia in the erythroid and megakaryocytic lineages
7. Fewer than 20% blasts in the bone marrow

**Pitfalls in the Differential Diagnosis of MDS/MPD**

**Acute Myeloid Leukemia**

True acute myeloid leukemia (AML) with monocytic differentiation may present with a deceptively mature appearance on examination of the peripheral blood. Patients may be referred for peripheral absolute monocytosis, organomegaly, and suspicion for CMML. However, subsequent bone marrow examination may show changes that fully meet criteria for acute leukemia.

One of the issues complicating the distinction between CMML and AML is the fact that morphologic criteria for a diagnosis of AML relies upon the quantification of true myeloblasts, as well as morphologically immature-appearing monocytes, termed promonocytes, which are counted as myeloblast equivalents for the purpose of acute leukemia diagnosis. Unfortunately, the morphologic criteria for the distinction of promonocytes from mature monocytes are not universally codified, and the interobserver reproducibility of the distinction between these two cell types is low. Therefore, the distinction between CMML and AML often relies on the subjective distinction between monocytes and promonocytes.

**Numerous Non-Neoplastic Conditions May Mimic True Myelodysplasia.**

Morphologic dysplasia (dyserythropoiesis, dysgranulopoiesis, dysmegakaryopoiesis) is a hallmark of myelodysplastic syndromes, and of many MDS/MPD overlap syndromes. However, many transient or non-neoplastic conditions can manifest with morphologic features indistinguishable from MDS.

*Dyserythropoiesis.* Dyserythropoiesis is relatively common in a variety of reactive conditions. Nutritional deficiencies (e.g. megaloblastic anemia, copper deficiency), toxic exposure (e.g. arsenic, lead, alcohol, etc), congenital red cell or metabolic abnormalities (e.g. congenital dyserythropoietic anemia, congenital sideroblastic anemia), infectious disease (particularly HIV infection) and treatment with certain chemotherapeutic agents can yield striking morphologic changes in red cell precursors. Often the degree of dysplasia in such conditions overlaps more or less completely with the morphologic changes that may be encountered in some forms of MDS.

The presence of ringed sideroblasts on Prussion blue staining is not pathognomonic for myelodysplasia, nor for that matter any single clinical disorder. Ringed sideroblasts are simply a form of dyserythropoiesis marked by the abnormal uptake of iron into the mitochondria of nucleated red cell precursors. Since mitochondria are situated adjacent to the cell nucleus, the ringed sideroblast is the light microscopic manifestation of this abnormal iron trafficking. Ringed sideroblasts, therefore, are an abnormality common to several different disorders including true myelodysplastic syndromes, acquired metabolic/ toxic forms of sideroblastic anemia, congenital abnormalities of porphyrin metabolism, and copper deficiency.

There are a few additional common pitfalls that should be avoided in the interpretation of dyserythropoiesis. First, any circulating nucleated red blood cell may show dysmorphic nuclear features, whether its origin is in a reactive or neoplastic condition. The finding of “dysplastic”
circulating nucleated red cells therefore has no predictive value in the diagnosis of MDS. Second, the morphology of megaloblastic anemia may bear disturbing resemblance to acute leukemia to the inexperienced observer. The combination of giant pronormoblasts, markedly increased marrow cellularity, markedly elevated serum lactate dehydrogenase (LDH) and marked peripheral blood poikilocytosis (including teardrop forms, Howell-Jolly bodies, and dysmorphic nucleated red cells) can lead to erroneous diagnoses of leukemia or myelodysplasia. Finally, hypererythropoiesis due to high red blood cell turnover can cause significant dyserythropoiesis. Therefore, the diagnosis of myelodysplasia in the setting of a hyperproliferative anemia (as evidenced by a high absolute reticulocyte count) should be approached with extreme caution.

Dysgranulopoiesis. Several non-neoplastic conditions can yield striking dysgranulopoiesis. The congenital Pelger-Huet anomaly is a harmless (and therefore generally subclinical) autosomal dominant condition in which neutrophils are hyposegmented, with most showing one or two nuclear lobes despite a fully mature-appearing chromatin. The appearance of Pelger-Huet neutrophils can be indistinguishable from the “pseudo-Pelger-Huet” morphology associated with either myelodysplastic syndromes, MDS/MPD syndromes, or certain reactive conditions. If examination of the blood smear of an otherwise healthy individual reveals that virtually all of the neutrophils are hypolobated then the possibility of a true Pelger-Huet anomaly should be considered.

Pseudo-Pelger-Huet morphology can be seen in many reactive conditions, including therapy with certain drugs (e.g. colchicine, sulfonamides, and possibly other antimicrobial agents), mycoplasma infection, and human immunodeficiency virus infection. Pathologists should resist the temptation to assume MDS in the face of acquired P-H morphology, since the pseudo-PH changes associated with mycoplasma infection or other reactive conditions may be marked.

Neutrophil hypogranularity is a form of dysgranulopoiesis. However, artifacts of preparation or variability in staining methods can lead to the overdiagnosis of hypogranular neutrophils. Furthermore, since pathologists tend to examine abnormal blood smears in which neutrophils display some degree of toxic granulation, the normal pattern of neutrophil granulation may be misinterpreted as hypogranular. Remember that although primary myeloid granules are coarse and azurophilic, secondary (specific) neutrophil granules are tiny and barely discernable by light microscopy. Therefore, they show up as a pink hue in the neutrophil cytoplasm. On the other hand, true hypogranularity tends to appear as water-clear cytoplasm. When in doubt, preparation of a fresh, hand-stained peripheral blood smear (preferably made directly from a finger-stick) should be pursued to investigate the possibility of true hypogranulation of neutrophils.

Dysmegakaryopoiesis. Very few reactive conditions will yield the type of dysmegakaryopoiesis encountered in MDS. However, some observers overinterpret the natural variability of megakaryocyte cytology as evidence of dysmegakaryopoiesis. The dysmegakaryopoiesis associated with MDS manifests as megakaryocytes with separate, round hypolobated nuclei (as opposed to the single nuclei with multiple lobes seen in normal megakaryocytes). Most dysplastic megakaryocytes will contain one to three small round nuclei, and will be smaller than normal megakaryocytes. Such criteria for the diagnosis of dysmegakaryopoiesis should be applied fairly strictly, so as not to overinterpret dysplasia in megakaryocytes. Finally, be careful about diagnosing dysmegakaryopoiesis on paraffin-embedded bone marrow core biopsy sections (as opposed to bone marrow aspirate smears), since a section cut through one lobe of a normal megakaryocyte may look very similar to a section cut through a hypolobated, dysplastic megakaryocyte.
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