Mod Pathol 37 (2024) 100397

MODERN PATHOLOGY

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Journal homepage: https://modernpathology.org/

Review Article

Fifth Edition of the World Health Classification of Tumors of the Hematopoietic and Lymphoid Tissue: Myeloid Neoplasms

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ARTICLE INFO

Article history:
Received 17 August 2023
Revised 14 November 2023
Accepted 21 November 2023
Available online 1 December 2023

Keywords:
acute myeloid leukemia
clonal cytopenia of undetermined significance
hematopathology
myelodysplastic neoplasm
myeloproliferative neoplasm
myeloid
World Health Organization

ABSTRACT

In this manuscript, we review myeloid neoplasms in the fifth edition of the World Health Organization classification of hematolymphoid tumors (WHO-HEM5), focusing on changes from the revised fourth edition (WHO-HEM4R). Disease types and subtypes have expanded compared with WHO-HEM4R, mainly because of the expansion in genomic knowledge of these diseases. The revised classification is based on a multidisciplinary approach including input from a large body of pathologists, clinicians, and geneticists. The revised classification follows a hierarchical structure allowing usage of family (class)-level definitions where the defining diagnostic criteria are partially met or a complete investigational workup has not been possible.

Overall, the WHO-HEM5 revisions to the classification of myeloid neoplasms include major updates and revisions with increased emphasis on genetic and molecular drivers of disease.

The most notable changes have been applied to the sections of acute myeloid leukemia and myelodysplastic neoplasms (previously referred to as myelodysplastic syndrome) with incorporation of novel, disease-defining genetic changes. In this review we focus on highlighting the updates in the classification of myeloid neoplasms, providing a comparison with WHO-HEM4R, and offering guidance on how the new classification can be applied to the diagnosis of myeloid neoplasms in routine practice.

Clonal Hematopoiesis and Clonal Cytopenia of Uncertain Significance

For the first time, the World Health Organization classification of hematolymphoid tumors (WHO-HEM5)[1] recognizes clonal hematopoiesis (CH) as a potential precursor of myeloid malignancies. CH is an age-related condition, defined by the detection of somatic variants (either somatic mutations or acquired chromosomal mosaicism) in hematopoietic stem and progenitor cells with the potential to expand over time under selective pressure. Clonal hematopoiesis of indeterminate potential (CHIP) is defined as somatic mutations in genes recurrently implicated in myeloid malignancies with a minimum variant allelic fraction (VAF) of 2% (4% for X-linked gene mutations in males) in peripheral blood (PB)

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https://doi.org/10.1016/j.modpat.2023.100397

Table 1
Recurrent driver mutations implicated in clonal hematopoiesis of indeterminate potential (CHIP) and clonal cytopenia of uncertain significance (CCUS)

Epigenetic modifiers
ASXL1
ASXL2[a]
DNMT3A
DNMT3B[a]
EZH2
IDH1
IDH2
SETD2[a]
SUZ12[a]
TET2
Spliceosome
SF3A1
SF3B1
SRSF2
U2AF1
U2AF2
ZRSR2
Cell Signaling
BRAF
CALR
CBL
CSF1R
CSF3R
EGFR[a]
FLT3[a]
GNAS
JAK2
JAK3
KIT
KRAS
MAP2K1[a]
MPL
NRAS
NOTCH1
NOTCH2[a]
NOTCH3[a]
PDGRFA[a]
PIK3CA[a]
PTPN11
RET[a]
SH2B3[a]
STAT3
Cohesin complex
PDS5B[a]
SMC1A
SMC3
STAG1[a]
STAG2
Ubiquitination
CBLC[a]
FBXW7[a]
UBA1[a]
Immune/complement
CD79B[a]
MYD88
PIGA
TLR2[a]
DNA repair and tumor suppressor
CHEK2[a]
FANCL[a]
RAD21
BCOR
BCORL1
PHF6
PPM1D
PTEN
TP53
Nuclear function/export/transcription
CEBPA
ETV6
GATA1[a]
GATA2
GATA3[a]
MEF2B[a]
NPM1[a,b]
RB1[a]
RUNX1
XPO1[a]
Miscellaneous
ATM[a]
BCL11B[a]
BRCC3
CNOT3[*]
CREBBP
CTCF
CTNNB1[a]
CUX1
EED[a]
EP300[a]
GNB1
KMT2A
KDM6A
LUC7L2[a]
NT5C2[a]
PIM1[a]
PRPF40B
RIT1[a]
RPL10[a]
SETBP1
SF1
SRCAP
WHSC1[a]
WT1

Adapted from Mangaonkar AA, *Am J Hematol.* (2023).[2]

[a] Genes not specifically included in WHO-HEM5 but implicated in other studies.

[b] *NPM1* mutations are considered an AML-defining alteration in WHO-HEM5; however, the significance of very low level (low VAF) *NPM1* mutation remains uncertain.

or bone marrow (BM) of individuals without unexplained cytopenias, hematologic neoplasms, or other clonal disorders. The most frequent mutations involve *DNMT3A*, *TET2* and *ASXL1* but many additional genes can be affected (Table 1).[2] Clonal cytopenia of undetermined significance (CCUS) is defined as persistent (>4 months) unexplained cytopenia(s) (hemoglobin <13 g/dL in men and <12 g/dL in women; absolute neutrophil count $<1.8 \times 10^9$; and platelet count $<150 \times 10^9$) and demonstration of somatic pathogenic variants (minimum VAF 2%; typically at higher VAF: $\geq$10%-20%) or clonal chromosomal abnormalities in myeloid cells in the absence of myelodysplastic neoplasm (MDS) diagnostic criteria ($\leq$10% dysplastic cells of any hematopoietic cell lineage in BM and absence of excess blasts, and absence of acute myeloid leukemia (AML)-defining cytogenetic abnormalities) or other myeloid neoplasms. Distinguishing CHIP and CCUS is based on the presence of cytopenia(s), whereas distinguishing between CCUS and MDS is based on the presence of morphologic dysplasia. Notably, WHO-HEM5 does not recognize MDS-defining genetic abnormalities in isolation as a diagnostic criterion for MDS without morphologic dysplasia (Fig. 1).

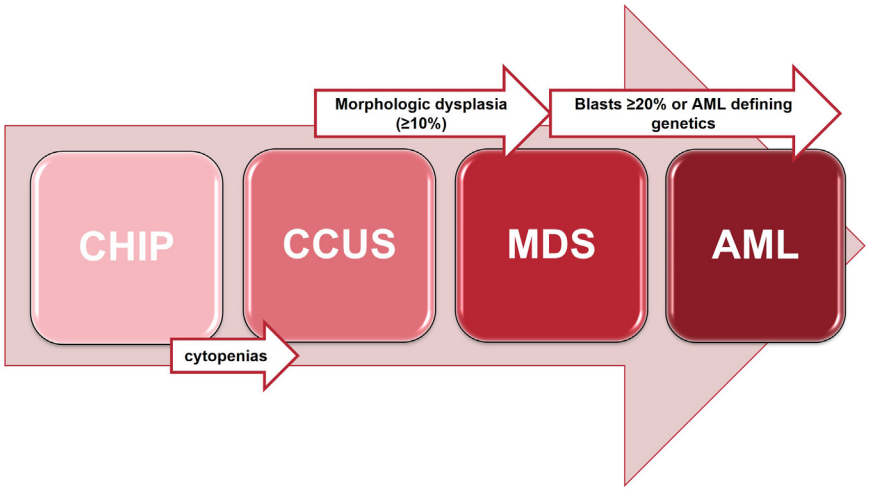

Figure 1.
The continuous spectrum of myeloid neoplasia. The distinction between clonal hematopoiesis of indeterminate potential and CCUS relies on the presence of cytopenia(s), whereas the distinction between CCUS and MDS relies on the presence of morphologic dysplasia (≥10% in one or more lineages). The boundary between MDS and AML is separated by the presence of AML-defining genetic abnormalities or increased (≥20%) blasts. AML, acute myeloid leukemia; CCUS, clonal cytopenia of undermined significance; MDS, myelodysplastic neoplasm.

The risk of progression from CH and CCUS to a frank myeloid neoplasm is variable, with CCUS bearing a higher risk for progression than CH (0.5%-1% per year). The risk of progression is influenced by mutation characteristics (number of mutations, VAF) as well as intrinsic and extrinsic factors, including aging, inflammation, smoking, cytotoxic therapy and/or radiation, and/or hematopoietic stem cell transplantation.[2,3] A CH risk score (CHRS)[1,4-6] is a recently proposed prognostic model incorporating several of these factors (Table 2) in an attempt to define CHIP/CCUS groups with low, intermediate, or high risk for progression to myeloid neoplasm. Using this model, the 10-year cumulative incidence of a myeloid neoplasm was 52.2% ± 4.96%, 7.83% ± 0.807%, and 0.669% ± 0.0827% in the high-, intermediate-, and low-risk groups, respectively.

Myeloproliferative Neoplasms

The main entities among the myeloproliferative neoplasms (MPNs) remain unchanged in WHO-HEM5, although there are subtle changes in terminology from WHO-HEM4R. MPNs include chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET), primary myelofibrosis (PMF), chronic neutrophilic leukemia (CNL), chronic eosinophilic leukemia (CEL), juvenile myelomonocytic leukemia (JMML), and MPN, not otherwise specified. MPNs are characterized by an abnormal clonal expansion/proliferation of one or more terminally differentiated myeloid lineage cells in the PB and BM. With disease progression and accumulation of genomic aberrations, terminally differentiated cells are replaced by immature cells leading to a "blastic" phase of disease.

Specific Types of MPNs

Chronic Myeloid Leukemia

CML is a genetically defined MPN and is uniformly *BCR::ABL1* positive. The qualifier "*BCR::ABL1* positive" has been omitted from the name, as the presence of *BCR::ABL1* is a requirement for diagnosis. An important difference from WHO-HEM4R is omission

Table 2
Clonal hematopoiesis risk score

	Assigned score				
Prognostic variable	0.5	1	1.5	2	2.5
Single *DNMT3A* mutation	Present	Absent			
High-risk mutation(s) • Splicing factor genes (*SRSF2*, *SF3B1*, *ZRSR2*) • AML-like genes (*IDH1*, *IDH2*, *FLT3*, and *RUNX1*) • *JAK2* • *TP53*		Absent			Present
Mutation number		1		≥2	
Variant allele fraction		<0.2 (20%)		≥0.2 (20%)	
Red cell distribution width-coefficient of variation (%)		<15			≥15
Mean corpuscular volume (fL)		<100			≥100
Cytopenia		CHIP (no cytopenia)	CCUS (+cytopenia)		
Age (y)		<65	≥65		

Adapted from Weeks LD et al., *NEJM Evidence*. (2023).[5]
Clonal hematopoiesis risk score (CHRS) categories: low: CHRS ≤ 9.5; intermediate: CHRS 10-12; and high: CHRS ≥ 12.5.
Of note, *U2AF1* was omitted from the model because of an erroneous duplication on chromosome 21 in the hg38 reference genome.
CHIP, clonal hematopoiesis of indeterminate potential; CCUS, clonal cytopenia of undetermined significance.

Table 3
High-risk features for progression in chronic phase chronic myeloid leukemia at diagnosis and during therapy with TKI

High-risk features of chronic myeloid leukemia-chronic phase, at diagnosis
High ELTS score[a]
10%-19% Myeloid blasts[b] in the PB and/or BM
≥20% Basophils in the PB
Additional chromosomal aberrations in Philadelphia (Ph) chromosome-positive (Ph+) cells (3q26.2 rearrangements, monosomy 7, isochromosome 17q, and/or complex karyotype). Or trisomy 8, 11q23 rearrangements, trisomy 19, trisomy 21, additional Ph+ in Ph+ cells (evidence of association with disease progression less clear)
Clusters of small megakaryocytes associated with significant BM fibrosis (MF2-3)
High-risk features chronic myeloid leukemia-chronic phase during treatment with TKI
Failure to achieve a complete hematologic response to the first TKI
Development of hematologic, cytogenetic, or molecular indications of resistance to 2 sequential TKIs
Development of new additional chromosomal abnormalities, and/or occurrence of compound[a] mutations in the *BCR::ABL1* fusion gene during TKI therapy

BM, bone marrow; ELTS, European Treatment and Outcome Study (EUTOS) long-term survival; PB, peripheral blood; TKI, tyrosine kinase inhibitor.
[a] $0.0025 \times (\text{age}/10)^3 + 0.0615 \times \text{spleen size [cm]} + 0.1052 \times \text{PB blasts} + 0.4104 \times (\text{platelet count}/1000)^{-0.5}$ (high risk: >2.2185); ≥2 mutations in the same *BCR::ABL1* molecule.
[b] Presence of bona fide lymphoblasts in the PB or BM (even if <10%) is indicative of blast-phase disease.

of the "accelerated phase" of CML. This change was adopted partly due to the impact of tyrosine kinase inhibitors (TKI) on disease course resulting in a reduction in the proportion of patients developing progression. However, WHO-HEM5 recognizes that certain features in the chronic phase of CML (CML-CP) signify a higher risk for disease progression and resistance to TKI. High-risk features in CML-CP are summarized in Table 3. Figure 2 depicts an example of one such case. The definition of blast phase of CML (CML-BP) mostly remains unchanged, however, the presence of bona fide lymphoblasts with aberrant immunophenotype by flow cytometry in the PB or BM (even if <10%) is sufficient for a diagnosis of CML-BP. The precise cutoff for lymphoblasts as a criterion for diagnosis of CML-BP remains unclear and requires additional studies. The significance of submicroscopic aberrant lymphoid

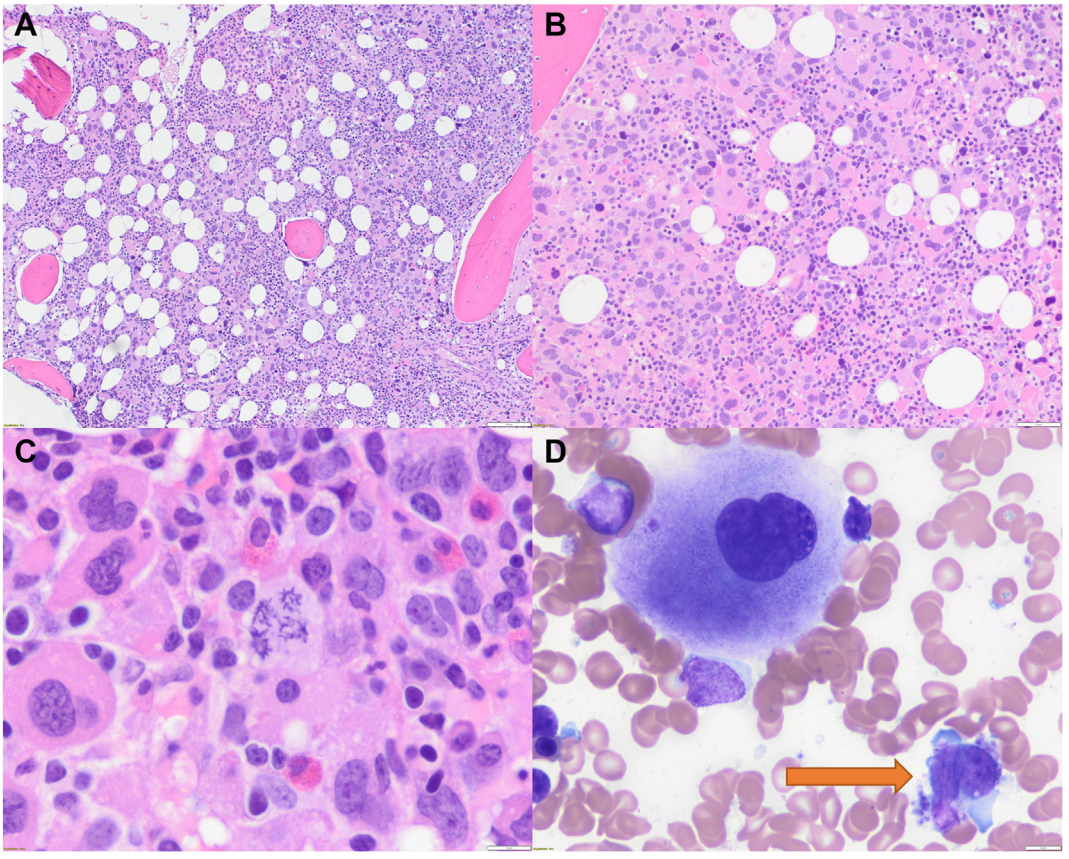

Figure 2.
Chronic myeloid leukemia (CML), with clustering megakaryocytic hyperplasia. Among features recognized in fifth edition of WHO Classification[1] as high risk for progression in chronic phase CML is the presence of clusters of small megakaryocytes associated with increased bone marrow fibrosis. This bone marrow is hypercellular ~90% (A; hematoxylin and eosin; ×100) and shows marked megakaryocytic hyperplasia with substantial clustering (B; hematoxylin and eosin; ×200). The megakaryocytes are small and hypolobated and admixed with eosinophils and eosinophilic precursors (C; hematoxylin and eosin; ×100). Bone marrow aspirate smear shows a micromegakaryocyte (arrow) compared with a relatively normal-sized megakaryocyte. (D; Giemsa; ×1000).

Table 4
WHO-HEM5 diagnostic criteria for polycythemia vera[a]

Major criteria
1. ↑ Hb concentration (>16.5 g/dL in ♂; >16.0 g/dL in ♀) or ↑ hematocrit (>49% in ♂; >48% in ♀)
2. BM biopsy showing age-adjusted hypercellularity with trilineage growth (panmyelosis), including prominent erythroid, granulocytic, and megakaryocytic proliferation with pleomorphic, mature megakaryocytes
3. Presence of *JAK2* p.V617F or *JAK2* exon 12 mutation
Minor criterion
1. Subnormal serum erythropoietin level
Diagnostic criteria for postpolycythemia vera (PV) myelofibrosis
Required criteria
1. Documentation of a previous diagnosis of WHO-HEM5-defined PV
2. Bone marrow fibrosis of grade 2-3 on a 0-3 scale
Additional criteria (2 are required)
1. Anemia or sustained loss of requirement of either phlebotomy or cytoreductive treatment for erythrocytosis
2. Leukoerythroblastosis
3. Increasing splenomegaly (increasing palpable splenomegaly of >5 cm from baseline (distance from the left costal margin) or newly palpable splenomegaly)
4. Development at least 2 of the following constitutional symptoms: >10% weight loss in 6 mo, night sweats, unexplained fever (>37.5°C)

Measurement of Cr-labeled red cells has become uncommon in routine clinical practice, it has been omitted as a diagnostic criterion.
[a] Diagnosis requires either all 3 major criteria or the first 2 major criteria + the minor criterion.

populations detected by more sensitive methods such as flow cytometry, without morphologically appreciable increase in blasts, is unclear and does not qualify for CML-BP.

Polycythemia Vera

The criteria for diagnosis of PV remain the same (Table 4), but the minor criterion of measurement of Cr-labeled red cells (red cell mass) has been eliminated as this test is uncommonly employed in routine clinical practice. Risk factors for leukemic and postpolycythemic myelofibrosis (Table 4), thrombotic episodes and outcomes in patients with PV include age (≥60 years); history of thrombotic events; white blood cell (WBC) ≥ 15 × 10^9/L; persistent leukocytosis; platelets >550 × 10^9/L; palpable splenomegaly; BM reticulin fibrosis (grade ≥ 1 at diagnosis); abnormal karyotype (deletion 20q, +9, +8, double abnormalities and complex karyotypes [≥3 abnormalities involving chromosomes 1, 5, 7, and 9]); *JAK2* p.V617F allelic burden (>50%); a progressive increase of *JAK2* p.V617F VAF while undergoing cytoreductive therapy; *JAK2* p.V617F homozygosity; and presence of additional mutations involving *ASXL1*, *SRSF2*, *IDH1*, *IDH2*, *RUNX1*, and/or *TP53*.

Table 5
WHO-HEM5 diagnostic criteria for essential thrombocythemia[a]

Major criteria
1. Platelet count ≥450 × 10^9/ L
2. BM biopsy showing proliferation mainly of the megakaryocytic lineage, ↑enlarged, mature megakaryocytes with hyperlobulated nuclei; no significant increase or left shift in neutrophil granulopoiesis or erythropoiesis; very rarely a minor (grade 1) increase in reticulin fibers
3. WHO criteria for CML (*BCR::ABL1*), PV , PMF , or other myeloid neoplasms are not met
4. *JAK2*, *CALR*, or *MPL* mutation
Minor criteria
1. Presence of a clonal marker or
2. Exclusion of reactive thrombocytosis
Diagnostic criteria for postessential thrombocythemia (ET) myelofibrosis
Required criteria
1. Documentation of a previous diagnosis of WHO-HEM5-defined ET
2. Bone marrow fibrosis of grade 2-3 on a 0-3 scale
Additional criteria (2 are required)
1. Anemia and a >2 g/dL decrease from baseline hemoglobin concentration
2. Leukoerythroblastosis
3. Increasing splenomegaly (increasing palpable splenomegaly of >5 cm from baseline (distance from the left costal margin) or newly palpable splenomegaly)
4. Elevated lactate dehydrogenase level (above the reference range)
5. Development of at least 2 of the following constitutional symptoms: >10% weight loss in 6 mo, night sweats, unexplained fever (>37.5°C)

[a] Diagnosis requires that either all major criteria or the first 3 major criteria + the minor criterion are met.

Essential Thrombocythemia

The criteria for ET diagnosis remain unchanged (Table 5). Risk factors for leukemic and post-ET myelofibrosis (Table 5), thrombotic episodes, and outcomes in patients with ET include: age (≥60 years); male sex; history of thrombotic events; WBC ≥ 11 × 10^9/L; platelets >1000 × 10^9/L; BM reticulin fibrosis; abnormal karyotype; presence of *JAK2* p.V617F mutation; *JAK2* p.V617F allele burden (>50%); presence of additional mutations involving *SRSF2*, *SF3B1*, *U2AF1*, *ASXL1*, *RUNX1*, and/or *TP53*; and therapy resistance, specifically hydroxycarbamide.

Primary Myelofibrosis

Similar to WHO-HEM4R, in WHO-HEM5 PMF is subclassified into prefibrotic and fibrotic stages, and distinguishing prefibrotic PMF from ET and PV is critical for patient management. There are a few changes to the diagnostic criteria of prefibrotic PMF (Table 6). Leukoerythroblastosis and splenomegaly (on clinical examination or by imaging) have been added as minor criteria. Megakaryocytic activation (M-ACT) identified in core biopsy specimens with the triad of emperipolesis, clustering (3 or more), and perimegakaryocytic fibrosis is a good predictor of fibrotic evolution in patients with prefibrotic PMF.[7] In addition to driver mutations (*JAK2*, *MPL*, or *CALR*), additional mutations can be found, including *TET2*, *ASXL1*, *DNMT3A*, *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *EZH2*, *IDH1*, *IDH2*, *CBL*, *KRAS*, *NRAS*, *STAG2*, and *TP53*. del(13)(q12-22) and der(6)t(1;6)(q21-23;p21.3) are frequent in PMF. [7,8] Various risk stratification systems exist for PMF that incorporate patient age, constitutional symptoms, PB counts, blast counts in PB, grade of fibrosis, karyotype, and mutations. Additional mutations are acquired as the disease progresses from prefibrotic to fibrotic stages. Mutations in *ASXL1* and *EZH2* are more frequent in the fibrotic stage of disease, and *TP53* mutation is associated with leukemic transformation.

Apart from specific genetic abnormalities, BM morphology plays a critical role in diagnosing and managing patients with PV, ET, and PMF (Fig. 3). BM cellularity and myeloid:erythroid ratio are valuable features for establishing the diagnosis. Estimation of myeloid immature or precursor cells using CD34 or CD117 immunostains is helpful in the phasing and prognostication of disease. Megakaryocytes display characteristic morphologic features in these entities and can be highlighted using immunohistochemistry (IHC) with markers such as CD61 or CD42b. Morphologic features, with or without the aid of immunostains, are also helpful in identifying evolving dysplastic features during disease progression. In general, a reticulin stain should be performed in all MPN cases, and a trichrome stain should be added in cases with increased reticulin or when fibrosis is suspected.

Accelerated Phase

The concept of "accelerated phase" (with 10%-19% blasts) is maintained in the 3 major disease types of non-CML MPNs: PV, ET, and PMF. A subset of patients with PMF develops blast phase (BP)

Table 6
WHO-HEM5 diagnostic criteria for primary myelofibrosis

Prefibrotic stage	Fibrotic stage
Major criteria 1. Megakaryocytic proliferation and atypia, without reticulin fibrosis grade >1, ↑ BM cellularity, granulocytic proliferation, and (often) decreased erythropoiesis 2. WHO criteria for CML (*BCR::ABL1*), PV, ET, MDS, or other myeloid neoplasms are not met 3. *JAK2, CALR, or MPL* mutation OR • Presence of another clonal marker OR • Absence of minor reactive myelofibrosis	Major criteria 1. Megakaryocytic proliferation and atypia, + reticulin/collagen fibrosis grade 2-3[a] 2. WHO criteria for CML (*BCR::ABL1*), PV, ET, MDS, or other myeloid neoplasms are not met 3. *JAK2, CALR, or MPL* mutation OR • Presence of another clonal marker OR • Absence of reactive myelofibrosis[b]
Minor criteria	
Presence of at least one of the following, confirmed in 2 consecutive determinations: 1. Anemia not attributed to a comorbid condition 2. Leukocytosis $\geq 11 \times 10^9$/ L 3. Splenomegaly detected clinically and/or by imaging 4. ↑ LDH 5. Leukoerythroblastosis	

CML, chronic myeloid leukemia; LDH, lactate dehydrogenase; PMF, primary myelofibrosis.
Diagnosis requires that all 3 major criteria +at least 1 minor criterion.
[a] Reticulin/collagen fibrosis grade 2-3.
[b] Reactive reticulin fibrosis secondary to infection, other malignancies, autoimmune disorders, or other chronic inflammatory conditions must be excluded, as they would not fulfill criteria for PMF-related myelofibrosis.

(>20% blasts), which is less frequent among patients of PV and ET. Of note, the designation of "blast phase" disease for leukemic transformation of chronic MPNs is preferred over "secondary AML" in WHO-HEM5.

Chronic Neutrophilia Leukemia

There are no significant changes in the criteria for CNL (Table 7). Mutations in *CSF3R* occur in >60% of cases, and co-occurring mutations in other genes are frequent; a median of 4 variants of known significance is observed, including genes frequently mutated in other myeloid neoplasms[9] Coexisting *ASXL1* mutation is associated with a poorer prognosis.[9,10] The development of dysplastic features may signal impending transformation to blast-phase disease; however, a distinct "accelerated phase" is not recognized in this disease subtype.

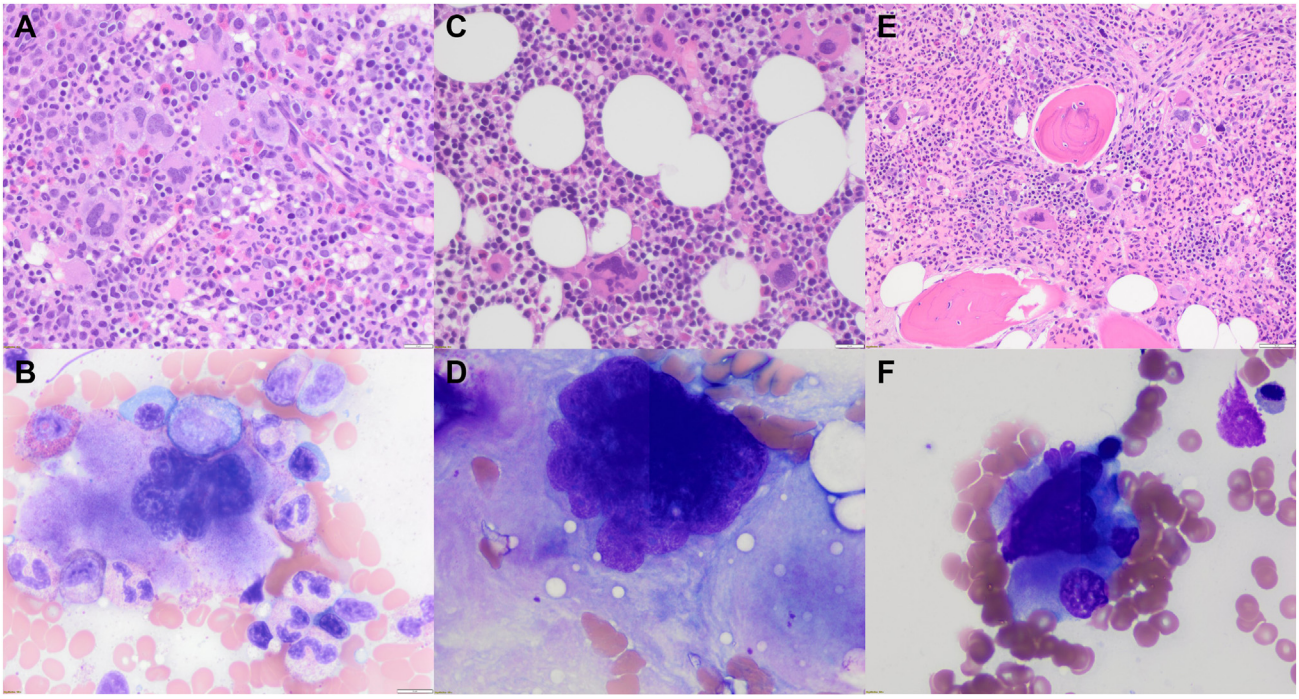

Figure 3.
Polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). The bone marrow biopsy is involved by PV and shows panmyelosis with megakaryocytic hyperplasia, forming loose clusters with pleomorphic morphology and cloud-like nuclei (A: hematoxylin and eosin; ×200; B: Giemsa; ×1000). The bone marrow biopsy is involved by ET and shows a relatively preserved myeloid:erythroid (M:E) ratio. A subset of megakaryocytes is large and hyperlobulated but with abundant cytoplasm (C: hematoxylin and eosin; ×200; D: Giemsa; ×1000). The BM involved by PMF shows cellular streaming (indicative of fibrosis), increased ratio, and increased megakaryocytes with clustering and pleomorphic morphology, some with prominent nuclear hyperchromasia, and increased nuclear to cytoplasmic ratio (E: hematoxylin and eosin; ×200; F: Giemsa; ×1000).

Table 7
WHO-HEM5 diagnostic criteria for chronic neutrophilic leukemia

WBC $\geq 25 \times 10^9$/ L • Neutrophils + bands ≥80% of WBC • Promyelocytes, myelocytes, metamyelocytes <10% WBC • Monocytes <10% WBC and $<1 \times 10^9$/ L • No dysgranulopoiesis • Myeloblasts rare (<2%) in blood
Hypercellular bone marrow • Neutrophils/granulocytes increased in % and number • Neutrophil maturation is normal • Myeloblasts <5%
Exclusion of reactive neutrophilia and other WHO-defined MPN and MDS/MPN
No *BCR::ABL1*, *PDGFRA*-r, *PDGFRB*-r, *FGFR1*-r, and *PCM1::JAK2* or other TKI fusions
CSF3R p.T618I or other activating *CSF3R* mutation OR Persistent neutrophilia (≥3 mo), splenomegaly, and no identifiable cause of reactive neutrophilia, including absence of a plasma cell neoplasm or, if a plasma cell neoplasm is present, demonstration of clonality of myeloid cells by cytogenetic or molecular studies

Chronic Eosinophilic Leukemia

CEL is characterized by a clonal proliferation of eosinophils and their precursors that result in persistent eosinophilia in PB and BM and which may involve other sites. The qualifier "not otherwise specified" has been omitted from the name. There are other changes to the diagnostic criteria (Table 8): There is no single defining genetic abnormality, and clonality can be inferred by clonal cytogenetic abnormalities and/or somatic mutations in genes associated with myeloid neoplasms. Dysplastic morphologic features are necessary to exclude reactive eosinophilia occurring in the background of CH. Patients with *STAT5B* p.N642H mutation or *SF3B1* as a sole abnormality have better overall survival than patients with other additional mutations.[11] Abnormal karyotype, atypical megakaryocytes, thrombocytopenia, and BM fibrosis are associated with poor outcomes.[12,13]

Juvenile Myelomonocytic Leukemia

This neoplasm was considered a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) in WHO-HEM4R but is now considered an RAS pathway activation-driven MPN of early childhood.[14,15] Dysplastic features are not prominent. Young children (median age at presentation: 2 years) present with granulocytosis and monocytosis with frequent organ infiltration by neoplastic cells. Activating mutations in the RAS pathway are generally mutually exclusive of one another and define subgroups with distinct clinical and hematologic presentations. Subtypes include *PTPN11*-mutated JMML; *NRAS*-mutated JMML; *KRAS*-mutated JMML; JMML in neurofibromatosis type 1 (*NF1*); JMML in children with *CBL* syndrome; and JMML-like disorders in children with Noonan syndrome (NS). The WHO-HEM5 diagnostic criteria for JMML are summarized in Table 9.

Nearly 30% of JMML patients evolve to blast-phase disease without identified predictive factors. In general, patients with poorer outcomes include those >2 years of age with low platelet counts ($<33 \times 10^9$/L); elevated fetal hemoglobin levels; overexpression of *LIN28B* (a regulator of hemoglobin F expression); mutations in *PTPN11* or *NF1;* additional mutations in *SETBP1, ASXL1, EZH2*, and other genes; and/or a DNA-hypermethylated profile[16–18]; however, a distinct "accelerated phase" is not recognized in this disease subtype. Spontaneous remission is reported in JMML patients with germline *CBL* mutation and in those with NS.

Table 8
WHO-HEM5 diagnostic criteria for chronic eosinophilic leukemia

PB eosinophilia $>1.5 \times 10^9$/L on at least 2 occasions over an interval of at least 4 weeks
Evidence of clonality in myeloid lineage
Abnormal bone marrow morphology (dysplasia)
WHO criteria for other myeloid or lymphoid neoplasms not met

Diagnosis requires all criteria.

Table 9
WHO-HEM5 diagnostic criteria for juvenile myelomonocytic leukemia

Clinical, hematologic, and laboratory criteria (all criteria are required) 1. Peripheral blood monocyte count $\geq 1 \times 10^9$/L 2. Blast and promonocytes in peripheral blood and bone marrow <20% 3. Clinical evidence of organ infiltration, most commonly splenomegaly 4. Lack of *BCR::ABL1* fusion 5. Lack of *KMT2A* rearrangement
Genetic criteria (1 criterion is sufficient) 1. Mutation in a component or a regulator of the canonical RAS pathway: a) Clonal somatic mutation in *PTPN11*, *KRAS*, or *NRAS* [a] b) Clonal somatic or germline *NF1* mutation and loss of heterozygosity or compound heterozygosity of *NF1* c) Clonal somatic or germline CBL mutation and loss of heterozygosity of *CBL*[b] 2. Noncanonical clonal RAS pathway pathogenic variant [c] or fusions causing activation of genes upstream of the RAS pathway, such as *ALK, PDGFRB*, and *ROS1*.
Other criteria (2 or more are required)[d] 1. Circulating myeloid (promyelocytes, myelocytes, metamyelocytes) and erythroid precursors. 2. Increased hemoglobin F for age. 3. Thrombocytopenia with hypercellular marrow often with megakaryocytic hypoplasia. Dysplastic features may or may not be evident. 4. Hypersensitivity of myeloid progenitors to GM-CSF (clonogenic assays or by measuring STAT5 phosphorylation in the absence or with low dose of exogenous GM-CSF).

GM-CSF, granulocyte-macrophage colony-stimulating factor; JMML, juvenile myelomonocytic leukemia.

[a] Germline mutation in PTPN11, KRAS, NRAS (Noonan syndrome) may produce JMML-like transient myeloproliferative disorder.

[b] Occasional cases have heterozygous splice-site mutations.

[c] Such as *RRAS*, *RRAS2*.

[d] Cases that do not meet any of the genetic criteria or if genetic testing is not available, must meet the following criteria in addition to the clinical, hematological, and laboratory criteria.

Myelodysplastic Neoplasms

Myelodysplastic neoplasms are a heterogenous group of clonal stem cell neoplasms characterized by progressive cytopenias, ineffective hematopoiesis, and morphologic dysplasia of hematopoietic precursors. Affected patients have an increased risk of developing AMLs. For the first time, WHO-HEM5 replaces the term myelodysplastic syndrome (MDS) with *myelodysplastic neoplasm* to indicate their neoplastic nature, retaining the traditional MDS abbreviation. [1]

The diagnostic criteria for MDS require the presence of cytopenias (hemoglobin <13 g/dL in men and <12 g/dL in women; absolute neutrophil count $<1.8 \times 10^9$; and platelets $<150 \times 10^9$) and morphologic dysplasia in at least 10% of cells in one or more lineages. In the presence of definitive features of MDS, such as typical genetic aberrations, milder degrees of cytopenia (below the reference range for the laboratory but not meeting above-indicated thresholds) can be considered. In addition, the reference range should be adjusted according to local population-related variables such as ethnicity and

altitude. Thrombocytosis (platelets $\geq 450 \times 10^9$) is permitted in the setting of MDS with low blasts and 5q deletion.[19]

Essential Diagnostic Criteria

Diagnostic evaluation for MDS should use a multimodal approach that includes microscopic examination of PB and BM smears and trephine biopsy; cytogenetic studies; molecular analysis, specifically for mutations involving *SF3B1* and *TP53*; and in some cases, flow cytometry immunophenotypic analysis. As in WHO-HEM4R, dysplasia in 10% or more of cells in a single lineage is essential to distinguish MDS from CCUS, regardless of the presence of *SF3B1* or *TP53* mutations, and to distinguish MDS from other entities such as MDS/MPN.[20,21]

The requirement of 20% blasts in either BM or PB for the diagnosis of AML was retained from WHO-HEM4R to avoid overtreatment of patients. This approach was taken to acknowledge the variability and subjectivity of evaluating blast percentages in challenging settings, such as poor sample quality, a background of cells showing severe dysplasia, and blasts of monocytic lineage. The percentage of BM and PB blasts should remain less than 20% and the presence of AML-defining genetic abnormalities excludes a diagnosis of MDS, particularly in cases with >2% PB blasts and >5% BM blasts. AML-defining genetic abnormalities include *PML::RARA, RUNX1::RUNX1T1, CBFB::MYH11, RBM15::MRTFA*, and *DEK::NUP214*; rearrangements involving *KMT2A*, *MECOM,* and *NUP98*; and *NPM1* mutation. The presence of bZIP *CEBPA* mutation with <20% blasts is not AML defining.

MDS is now broadly divided into 2 families: (1) MDS with defining genetic abnormalities and (2) MDS defined morphologically (Table 10). MDS with defining genetic abnormalities includes the following entities: MDS with biallelic *TP53* inactivation, MDS with low blasts and *SF3B1* mutation (MDS-*SF3B1*), and MDS with low blasts and del(5q). The major updates include the integration of additional genomic aberrations affecting *TP53* and *SF3B1.* MDS defined morphologically includes MDS with low blasts, hypoplastic MDS, MDS with increased blasts-1, MDS with increased blasts-2, and MDS with fibrosis. Subclassification based on the number of dysplastic lineages is no longer necessary and is considered optional.[20] With the formal recognition of CCUS in WHO-HEM5, and modification of the PB blast percentage to 2% for low blasts, MDS, unclassifiable, as defined in WHO-HEM4R, is no longer recognized (Table 9).

MDS with Defining Genetic Abnormalities

MDS with Biallelic TP53 Inactivation

The establishment of this entity is based on findings by Bernard et al[22] who showed that patients with MDS with >1 *TP53* aberration (mutation, deletion, or copy-neutral loss of heterozygosity [CN-LOH]), had a significantly poorer outcome compared with those with a single-hit (monoallelic) aberration; the latter group had outcomes similar to patients with MDS having wild-type *TP53*.[22-26] As the absolute determination of biallelic inactivation is not possible, for practical purposes, the WHO-HEM5 recognizes the following criteria as presumptive evidence for biallelic inactivation: presence of >1 *TP53* mutation, or a single *TP53* mutation with VAF of ≥50%, or a single *TP53* mutation associated with either deletion or CN-LOH of chromosome 17p/*TP53*. There is no minimum VAF for *TP53* mutation or blast percentage required. The presence of a complex karyotype is not equivalent to biallelic inactivation in the absence of other requirements listed. Blasts ≥20% is required for the diagnosis of AML, as biallelic *TP53* inactivation is not considered an AML-defining genetic abnormality. Assessment of p53 protein overexpression or complete loss (null mutations) by immunohistochemical staining can serve as a surrogate marker for mutation status, and the percentage of cells with overexpression (or complete lack of expression) frequently correlates with VAF and clonal burden of *TP53* mutation.[27-29] IHC is useful as a surrogate for rapid assessment of *TP53* mutation status and is particularly useful in low-resource settings; however, it is important to note that IHC is agnostic of the allelic state of *TP53* and cannot be used to distinguish monoallelic alterations from biallelic alterations (Fig. 4).

MDS with Low Blasts and SF3B1 Mutation

This diagnosis is based on low blasts (<5% BM and <2% PB blasts) with *SF3B1* mutation (minimum VAF of 5%) in the absence of del(5q), monosomy 7, complex karyotype or biallelic *TP53*

Table 10
WHO-HEM5 classification of myelodysplastic neoplasms (MDS)

	Blasts	Cytogenetics	Mutation(s)
MDS with defining genetic abnormalities			
MDS with biallelic *TP53* inactivation (MDS-biTP53)	<20% BM and PB	Often complex	Two or more *TP53* mutations, or 1 mutation with evidence of *TP53* copy number loss or cnLOH
MDS with low blasts and *SF3B1* mutation* (MDS-SF3B1)	<5% BM and <2% PB	Absence of del(5q), monosomy 7, or complex karyotype	*SF3B1* (VAF ≥5%)
MDS with low blasts and 5q deletion (MDS-5q)	<5% BM and <2% PB	Isolated del(5q), or with 1 additional abnormality other than monosomy 7 or del(7q)	
MDS, morphologically defined			
MDS with low blasts (MDS-LB)	<5% BM and <2% PB		
MDS, hypoplastic (MDS-h)	<5% BM and <2% PB		
MDS with increased blasts (MDS-IB)			
MDS-IB1	5%-9% BM or 2-4% PB		
MDS-IB2	10%-19% BM or 5%-19% PB Auer rods		
MDS with fibrosis (MDS-f)	5%-19% BM; 2%-19% PB		

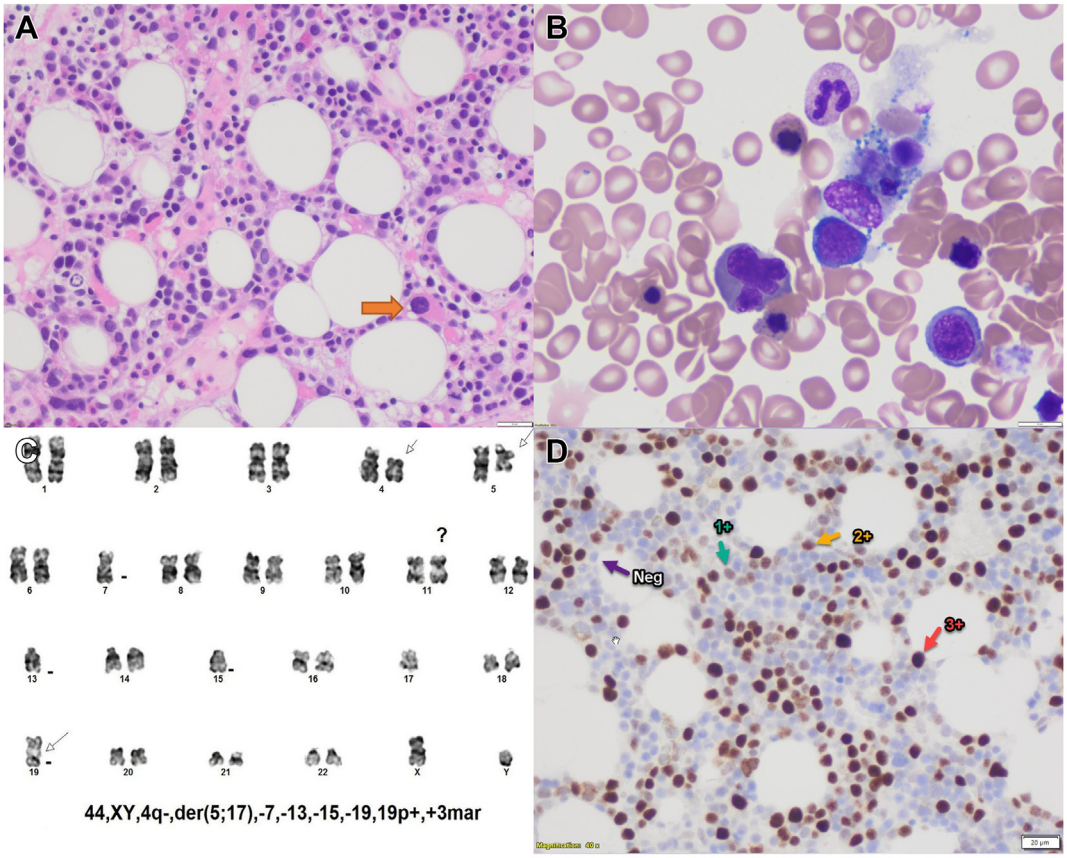

Figure 4.
Myelodysplastic neoplasm (MDS) with biallelic *TP53* inactivation. The bone marrow biopsy is hypercellular and shows a small dysplastic megakaryocyte (arrow) (A; hematoxylin and eosin; ×200). The bone marrow aspirate smear shows prominent erythroid dysplasia (B; Giemsa; ×1000). Conventional karyotyping showed a complex karyotype including loss of one copy of chromone 17 (*TP53* is located at 17p13). Next-generation sequencing detected a *TP53* p.R248Q mutation with a variant allelic fraction of 60%. Immunohistochemistry (IHC) for p53 shows aberrant overexpression (strong, 3+ nuclear staining) in a subset of nuclei (IHC with hematoxylin counterstain; ×400). Only 3+ staining nuclei should be taken into account when using IHC to assess for aberrant overexpression. Mild (1+) or moderate (2+) expression should not be overinterpreted as a mutant pattern of staining.

aberrations. MDS with low blasts and SF3B1 mutation (MDS-*SF3B1*) is a distinct entity with favorable overall survival.[30,31] *SF3B1*-mutated MDS is strongly associated with increased ring sideroblasts (RS), and patients show sustained hematologic responses to luspatercept treatment.[32,33] The presence of ≥15% ring sideroblasts can be alternatively used to designate cases as MDS-*SF3B1* when genetic testing is unavailable. If *SF3B1* is proven to be wild-type and ≥15% RS are present, a diagnosis of MDS with low blasts and RS can be rendered to cover mutations affecting other RNA-splicing genes. There is no minimum threshold for RS if the presence of an *SF3B1* mutation with VAF of ≥5% is documented. Distinguishing between single lineage and multilineage dysplasia is considered optional. Others have shown that the favorable outcome associated with *SF3B1* mutation may be specific to hotspot K700E mutations and that patients with other *SF3B1* mutations, such as K666 and H662, may have a worse outcome.[34,35]

MDS with Low Blasts and del(5q)

Diagnostic criteria are unchanged from WHO-HEM4R. These criteria include low blasts (<5% BM and <2% PB blasts) and del(5q) as a sole abnormality or with one additional cytogenetic abnormality other than monosomy 7, and absence of biallelic *TP53* aberration. Neither the presence of *SF3B1* mutation nor monoallelic *TP53* aberration overrides this diagnostic entity, as both can occur as secondary events. Patients generally present with anemia and thrombocytosis with characteristically increased small hypolobated and monolobated megakaryocytes. It is important to perform molecular testing for *TP53* mutation to identify those cases that might be resistant to lenalidomide therapy.[36-38]

MDS – Morphologically Defined

MDS with low blasts implies <5% BM and <2% PB blasts without biallelic *TP53* inactivation, *SF3B1* mutation, del(5q), or other AML-defining genetic abnormalities. Cases with low blasts and AML-defining genetic abnormalities should be approached conservatively with respect to diagnostic classification; a descriptive diagnosis may be more appropriate for such cases. Careful evaluation to exclude secondary causes of cytopenias such as nutritional deficiencies, thorough assessment of dysplastic changes, and ancillary workup for detection of clonal abnormalities are warranted. Most cases have a normal karyotype.[39] The most frequent somatic mutations affect *TET2*, *DNMT3A*, *U2AF1*, *RUNX1*, *ASXL1*, and *SRSF2*.[40] A specific subtype of hypoplastic MDS is recognized under this category when BM cellularity is <30% of normal cellularity [(100 − age) ± 10%] in patients <70 years, or <20% in patients ≥70 years.[41] There must be no clear etiology for BM hypocellularity such as aplastic anemia (AA), paroxysmal nocturnal hemoglobinuria (PNH), or germline predisposition syndromes.[41,42] It is often

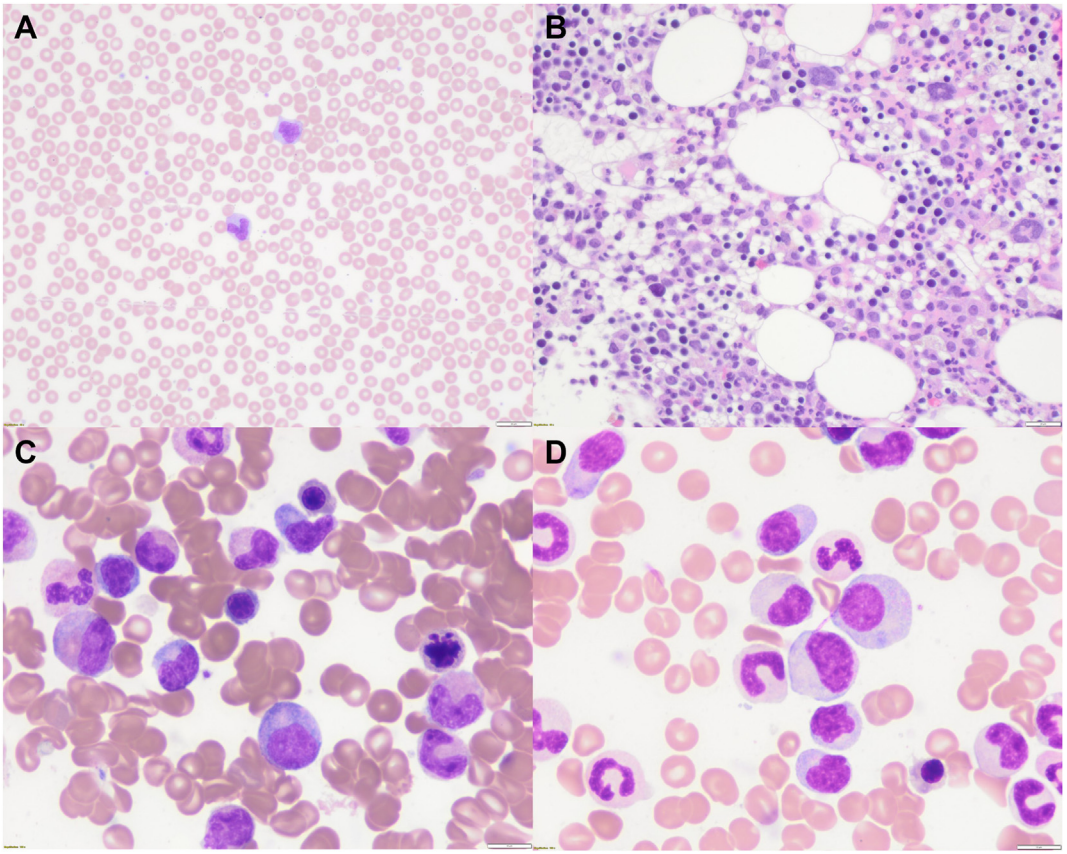

Figure 5.
Oligomonocytic chronic myelomonocytic leukemia (CMML). This patient presented with thrombocytopenia (platelets 84 × 10^9), macrocytosis (hemoglobin 13.4 g/dL, MCV 100 fl/L) with adequate white blood cell count and relative (17%) monocytosis (absolute monocyte count, 800 × 10^9). A couple of monocytes are shown on the peripheral blood smear (A, Giemsa; ×1000). The bone marrow core biopsy shows mildly atypical megakaryocytes with abnormal nuclear lobation (B; hematoxylin and eosin; ×200). The bone marrow aspirate smears show increased monocytes and monocytic precursors with erythroid and granulocytic dysplasia (C, D, Giemsa; ×1000). Next-generation sequencing of this sample showed mutations involving *ASXL1*, *SRSF2*, and *TET2*.

difficult to distinguish these cases from AA and PNH, and CD34 immunohistochemical stain for microclusters of blasts (3 or more blasts in the aggregate; although the total percentage of blasts must remain below 5% of total cellularity), CD61 to highlight micromegakaryocytes, and flow cytometry immunophenotypic studies can be useful. These patients show favorable outcomes and sustained responses to immunosuppressive therapies targeting AA, such as antithymocyte globulin and cyclosporine A.[43] These patients typically have fewer driver somatic mutations, specifically those involving splicing factors, *ASXL1* and *IDH1/2*. While we acknowledge that hypoplastic MDS (MDS-h) is a heterogenous group of diseases, it is plausible that in a subset of patients, cytopenias associated with MDS-h may be attributable to destruction of hematopoietic stem and progenitor cells by immune-mediated mechanisms such as abnormal oligoclonal populations of CD8-positive cytotoxic T cells with overproduction of proinflammatory cytokines such as interferon-gamma, interleukin 17, and tumor necrosis factor-alpha.[44-46]

MDS with increased blasts (MDS-IB) is further divided into MDS with increased blasts-1 (MDS-IB1; blasts: BM, 5%-9%; PB, 2%-4%), and MDS with increased blasts-2 (MDS-IB2; blasts, BM: 10%-19%; PB, 5%-19%; or the presence of Auer rods). These cases generally demonstrate florid morphologic dysplasia and increased blasts (aggregates of 3-5 cells shown by CD34 IHC), and are frequently associated with high-risk cytogenetic and molecular abnormalities such as monosomy 7; del(7q), complex karyotype; and mutations affecting *ASXL1*, *RUNX1*, *EZH2*, *NRAS, KRAS*, and *TP53*.[40,47,48] The presence of dysplastic changes in at least 1 lineage (10% or more dysplastic cells) is a required criterion. A separate category of MDS with increased blasts and fibrosis (MDS-F) has been recognized under MDS with increased blasts (5%-19% BM blasts, 2%-19% PB blasts) and reticulin fibrosis (grade 2 or 3). MDS-F is enriched for *TP53* mutation,[49] whereas monoallelic *TP53* mutation is acceptable in MDS-F. The diagnosis of MDS with biallelic *TP53* inactivation supersedes MDS-F, and cases with biallelic *TP53* inactivation should not be classified as MDS-F, even if marked fibrosis is present. Because of the aparticulate nature of BM aspirate smears associated with fibrosis, CD34 IHC performed on the biopsy specimen may be essential to highlight blasts.[50] These cases are generally associated with numerous small dysplastic megakaryocytes that are highlighted by CD61 immunohistochemical stain.

Myelodysplastic/Myeloproliferative Overlap Neoplasms

MDS/MPN-overlap neoplasms in WHO-HEM4R included 5 entities: chronic myelomonocytic leukemia (CMML); atypical chronic myeloid leukemia (aCML), *BCR::ABL1* negative; MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T); MDS/MPN-unclassifiable (MDS/MPN-U); and JMML. [51] In WHO-HEM5, JMML is eliminated from the overlap neoplasms category

Table 11
Differences in WHO-HEM5 and WHO-HEM4R classification schema for myelodysplastic/myeloproliferative neoplasms

Diagnostic entities			Comments
CMML			
	Revised Fourth Edition of WHO Classification (WHO 2016)	Fifth Edition of WHO Classification	
Nomenclature and stratification	CMML-0, CMML-1, and CMML-2 MD-CMML and MP-CMML	CMML-1 and CMML-2 MD-CMML and MP-CMML CMML-0 eliminated	1. No prognostic significance for CMML-0. 2. MDS-CMML and MPN-CMML shown to have clear biological and prognostic differences.
Clinical and morphology criteria			
Monocytes	AMC $\geq 1.0 \times 10^9$/L with monocytes $\geq$10% of WBC differential	AMC $\geq 0.5 \times 10(9)$/L with monocytes $\geq$10% of WBC differential. If AMC $\geq$0.5 and $<1 \times 10^9$ /L, then the presence of BM dysplasia $\geq$1 myeloid lineage and an acquired clonal cytogenetic or molecular genetic abnormality must be present. Flow cytometry–based expansion of the Monocyte compartment (M01>94%) has been added as a supportive criterion.	Monocyte flow can be associated with false positive and negative repartitioning results, but overall is associated with good sensitivity and specificity rates.
Peripheral blood and bone marrow promonocytes/blasts	<20%	<20%	
Bone marrow findings	Bone marrow dysplasia involving $\geq$1 myeloid lineage seen in $\geq$10% of cells.	Bone marrow dysplasia involving $\geq$1 myeloid lineage seen in $\geq$10% of cells.	
Clonality	Presence of an acquired somatic cytogenetic or molecular abnormality with no VAF specification	Presence of an acquired somatic cytogenetic or molecular abnormality with no VAF specification	
Molecular exclusionary criteria	*BCR::ABL1* or myeloid/lymphoid neoplasms associated with TK fusions. Presence of MPN driver mutations such as *JAK2, CALR, MPL*, in the presence of MPN-like bone marrow features, tends to favor a MPN diagnosis	*BCR::ABL1* or myeloid/lymphoid neoplasms associated with TK fusions	MPN with monocytosis can be distinguished from CMML by using the monocyte repartitioning flow cytometry. In addition, MPL and CALR mutations are very uncommon in CMML.
Atypical CML, *BCR::ABL1* negative			
Nomenclature and stratification	Atypical CML	MDS/MPN with neutrophilia	Recommend MDS/MPN with dysplastic neutrophilia
Clinical and morphology criteria	WBC $\geq 13 \times 10^9$/L with immature myeloid cells $\geq$10% of the WBC differential, prominent dysgranulopoiesis, and monocytes and eosinophils comprising <10% of the differential.	WBC $\geq 13 \times 10^9$/L with immature myeloid cells $\geq$10% of the WBC differential, prominent dysgranulopoiesis, and monocytes and eosinophils comprising <10% of the differential.	
Molecular exclusionary criteria	*BCR::ABL1*, TK fusions, and MPN-associated driver mutations like *JAK2, MPL,* and *CALR*	*CSF3R* (desirable) *BCR::ABL1*, TK fusions, and MPN-associated driver mutations like *JAK2, MPL* and *CALR*	*CSF3R* is a known driver mutation for CNL but can be seen in other myeloid neoplasms.
Supportive diagnosis	*SETBP1* and *ETNK1* mutations	*SETBP1* and *ETNK1* mutations	Would consider *ASXL1* mutations in this list, given how frequent they are and the fact that they are often ancestral mutations in this neoplasm.

(*continued on next page*)

Table 11 (*continued*)

Diagnostic entities			Comments
CMML			
	Revised Fourth Edition of WHO Classification (WHO 2016)	Fifth Edition of WHO Classification	
MDS/MPN-RS-T			
Nomenclature and stratification	MDS/MPN-RS-T	MDS/MPN-*SF3B1*-T (in patients meeting morphologic criteria with *SF3B1* mutations) Retained MDS/MPN-RS-T (for *SF3B1* wild-type cases with ≥15% BM RS)	RS% ≥5% most likely to suffice for classification of *SF3B1* wild-type cases.
Morphology	Anemia associated with erythroid lineage dysplasia with or without multilineage dysplasia, ≥15% ring sideroblasts, <1% blasts in PB and <5% blasts in the BM. Persistent thrombocytosis with platelet count ≥450 3 109/L	Anemia associated with dysplastic erythropoiesis and ≥15% ring sideroblasts, with or without dysplasia in the megakaryocytic and erythroid lineages.	
Molecular exclusionary criteria	*BCR::ABL1*, MPN and myeloid/lymphoid neoplasms with TK fusions. Presence of t(3;3)(q21.3;q26.2), inv(3)(q21.3q26.2), and del(5q).	*BCR::ABL1*, MPN and myeloid/lymphoid neoplasms with TK fusions. Presence of t(3;3)(q21.3;q26.2), inv(3)(q21.3q26.2), isolated del(5q) (ie, MDS-del5q), and bi-allelic *TP53* inactivation	
MDS/MPN-U			
Nomenclature and stratification	MDS/MPN-U	MDS/MPN-NOS	
Clinical and morphology criteria	WBC ≥13 × 10^9/L and/or the platelet count ≥450 × 10^9/L are needed	WBC ≥13 × 10^9/L and/or the platelet count ≥450 × 10^9/L are needed	
Molecular exclusionary criteria	*BCR::ABL1*, myeloid/lymphoid neoplasms with TK fusions, MPN and other MDS/MPN-overlap neoplasms. t(3;3)(q21.3;q26.2), inv(3)(q21.3q26.2), and del(5q).	*BCR::ABL1*, myeloid/lymphoid neoplasms with TK fusions, MPN and other MDS/MPN-overlap neoplasms. t(3;3)(q21.3;q26.2), inv(3)(q21.3q26.2), and del(5q).	

aCML, atypical chronic myeloid leukemia; AMC, absolute monocyte count; CMML, chronic myelomonocytic leukemia; JMML, juvenile myelomonocytic leukemia; MD, myelodysplastic; MDS/MPN-RS-T, MDS/MPN with ring sideroblasts and thrombocytosis; MDS/MPN-U, MDS/MPN-unclassifiable; MP, myeloproliferative; MPN, myeloproliferative neoplasm; VAF, variant allele fraction; WBC, white blood cell count.

Table 12
WHO-HEM5 classification of acute myeloid leukemia with recurrent genetic abnormalities

Fifth Edition of WHO Classification	Revised Fourth Edition of WHO Classification (WHO 2016)	Clinicopathologic features
Acute promyelocytic leukemia with *PML::RARA* fusion	Acute promyelocytic leukemia with PML-RARA	Frequently associated with risk of coagulopathy and DIC Hypergranular (classic) and microgranular (hypogranular) variant. Characteristic cells containing bundles of Auer rods (faggot cells) Microgranular APL is characterized by blasts with bilobed nuclei, paucity or absence of granules and many present with a very high WBC Hypergranular APL is characterized by negative expression of CD34 and HLA-DR, high side scatter and forward scatter Microgranular APL or the bcr3 transcript of the PML-*RARA* fusion frequently show expression of CD34 and CD2 by at least subset of cells CD2 expression in APL has been associated with *FLT3*-ITD mutation Rare APL cases have cryptic rearrangements, but can be detected by FISH or molecular methods A subset of cases (~5%) show variant translocations involving *RARA* with various fusion partners and are resistant to tretinoin
AML with *RUNX1::RUNX1T1* fusion	AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*	Blasts are large with ample basophilic cytoplasm, often containing numerous azurophilic granules and perinuclear clearing Few blasts containing large Chediak-Higashi–like granules or Auer rods with a single long with tapered ends are frequently found Blasts characteristically show high-intensity expression of CD34, CD13, and MPO and aberrant expression of the lymphoid markers CD19, cytoplasmic CD79a, and PAX5 Blasts may show maturation asynchrony (eg, coexpressing CD34 and CD15) Usually associate with good response to intensive consolidation therapy *KIT* mutations (in adults) and CD56 expression are associated with worse prognosis
AML with *CBFB::MYH11* fusion	AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	Blasts usually have morphologic and immunophenotypic features of myelomonocytic differentiation Marrow eosinophilia and the presence of abnormal eosinophils with large dark purple–violet granules is a feature of AML with *CBFF::MYH11* fusion Case with suspicious morphologic features that lack inv(16) or t(16;16) on conventional karyotyping should be assessed by FISH or molecular diagnostics The prognosis of AML with *CBFB::MYH11* is usually favorable, but lack of molecular remission post induction therapy is associated with relapse and poor survival
AML with *DEK::NUP214* fusion	AML with t(6;9)(p23;q34.1); *DEK-NUP214*	Blasts commonly have morphologic and cytochemical features similar to AML with maturation or with myelomonocytic differentiation Multilineage dysplasia is common Usually associated with a poor prognosis and short overall survival *FLT3*-ITD mutations are very common and does not impact overall survival, but are associated with faster relapse
AML with *RBM15::MRTFA* fusion	AML (megakaryoblastic) with t(1;22)(p13.3;q13.1); *RBM15-MKL1*	Mostly occurs in infants (first 6 mo) and young children (age $\leq$ 3 y) without trisomy 21 (Down syndrome), F>M Blasts are usually medium to large, with irregular nuclei containing several nucleoli, and agranular, basophilic cytoplasm and sometimes with cytoplasmic blebs Blasts show megakaryocytic differentiation and express one or more platelet glycoproteins: CD41, CD61, and/or CD42b The bone marrow usually shows reticulin and collagenous fibrosis leading to a falsely low blast count; thus, a trephine biopsy is required for full evaluation
AML with *BCR::ABL1* fusion	AML with *BCR-ABL1*	Mainly occurs in adults, M>F The morphologic features are nonspecific Aberrant expression of CD19, CD7, and TdT is common, however, cases meeting the criteria for a mixed phenotype should be diagnosed as MPAL with BCR-ABL1 Cases with features of CML prior to or at diagnosis or after therapy are excluded
AML with *KMT2A* rearrangement	AML with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*	Blasts have broad range of morphology, with most cases having monocytic, monoblastic, or myelomonocytic features Affects any age group, but more common in infants and young children More than 80 fusion partners have been described in *KMT2A* rearranged AML (most common *MLLT3*, *AFDN*, *ELL*, and *MLLT10*) Partial tandem duplication in *KMT2A* is common in adults with AML and should not be diagnosed as AML with KMT2A rearrangement Identification of the *KMT2A* fusion partner is desirable due to its prognostic impact
AML with *MECOM* rearrangement	AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2);GATA2, *MECOM*	Aggressive disease with short survival irrespective of the blast percentage Multilineage dysplasia is frequent, most commonly in megakaryocytes More than 30 partner genes, have been described, *GATA2* being the most common partner gene

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Table 12 (*continued*)

Fifth Edition of WHO Classification	Revised Fourth Edition of WHO Classification (WHO 2016)	Clinicopathologic features
AML with NUP98 rearrangement	Not previously included	Morphologic findings vary considerably, blasts in up to 34% of patients (>3 y) show megakaryoblastic differentiation Commonly associated with acute leukemias with erythroid differentiation in children *NUP98* rearrangements are often cryptic; FISH and molecular methods are recommended diagnostic platforms Should be suspected in AML with concurrent *FLT3* and *WT1* mutations Associated with poor prognosis, up to 50% of pediatric patients with refractory AML have NUP98 rearrangement
AML with *NPM1* mutation	AML with mutated *NPM1*	Most often show normal karyotype Identification of >10% blasts with cup-like nuclear morphology is highly specific for AML with *NPM1* mutation Cytoplasmic *NPM1* by immunohistochemistry can be used as a surrogate marker of *NPM1* mutation
AML with *CEBPA* mutation	AML with biallelic mutation of *CEBPA*	Dysgranulopoiesis and dysmegakaryopoiesis are common Association with a favorable prognosis

AML, acute myeloid leukemia; APL, acute promyelocytic leukemia; CML, chronic myeloid leukemia; MPAL, mixed-phenotype acute leukemia; MPO, myeloperoxidase; TdT, terminal deoxynucleotidyl transferase.

and placed in the category of MPNs, largely due to its proliferative genetic mechanisms related to constitutive activation of the RAS pathway and the high tumor burden of JMML causing organ infiltration, akin to what is seen in MPN.[52] Criteria for CMML in both WHO-HEM4R and WHO-HEM5 include sustained monocytosis as an absolute monocyte count (AMC) $\geq 1 \times 10^9$/L, with monocytes comprising ≥10% of the WBC differential count. WHO-HEM5 also allows for the inclusion of patients with AMC $\geq 0.5 \times 10^9$/L, with monocytes comprising ≥10% of the WBC (also known as oligomonocytic [O-CMML] (Fig. 5), as long as these cases meet supporting criteria (Table 11). These supporting criteria include the presence of morphologic dysplasia, acquired clonal cytogenetic or molecular abnormalities, or abnormal flow cytometry–based monocyte partitioning (classical monocytes fraction/M01>94%) [the first 2 supporting criteria must be present for O-CMML].[1] Both WHO-HEM4R and WHO-HEM5 underscore the need to recognize distinct myelodysplastic (MD-CMML) and myeloproliferative (MP-CMML) subtypes, the latter requiring a WBC $\geq 13 \times 10^9$L.[1,51] This stratification is based on clinical (MP-CMML with shorter survival), genetic (MP-CMML enriched in RAS pathway and *JAK2* mutations), transcriptomic (unique gene expression profiles for both entities), and epigenetic differences (increased monomethylation of histone(H)3lysine(K)4 in MP-CMML) between the 2 subtypes. These subtypes also convey strong clinical and prognostic value.[4,5,53,54] WHO-HEM4R had stratified CMML into 2 categories based on PB and BM blasts/promonocytes, with CMML-0 being defined as having <2% PB blasts and <5% BM blasts. Due to the limited prognostic value of this stratification,[55] in WHO-HEM5 CMML-0 is eliminated and stratifies CMML as CMML-1 (<10% BM blasts and <5% PB blasts) and CMML-2 (10%-19% BM blasts and 5%-19% PB blasts), respectively. Importantly, *BCR::ABL1*, myeloid/lymphoid neoplasms with tyrosine kinase fusions, and MPN are excluded from CMML. In particular, distinguishing between CMML and MPN with monocytosis remains challenging, given that 10% of CMML can have *JAK2* V617F mutations.[56] Emphasis on somatic mutations (*MPL* and *CALR* mutations are infrequent in CMML), BM morphology (especially megakaryocytes and megakaryocyte clustering), and monocyte partitioning assessed by flow cytometry can help distinguish CMML from MPN with monocytosis.[8,56-58] Of note, some degree of reticulin fibrosis can be seen in both entities, but plasmacytoid dendritic cell nodules are more likely in CMML.[59]

Flow cytometry–based monocyte partitioning is a welcome addition to the WHO CMML classification criteria, as this assay has been validated clinically and helps distinguish reactive from clonal monocytosis and importantly helps segregate CMML from other

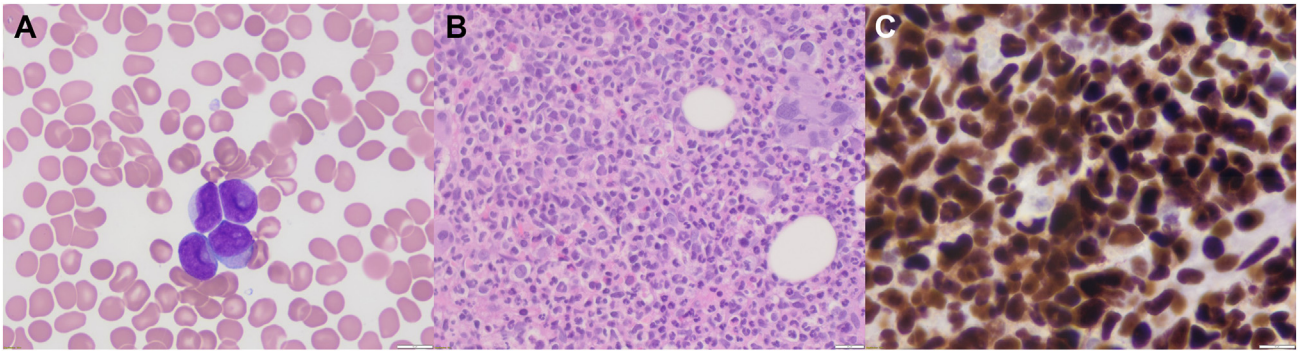

Figure 6.
Acute myeloid leukemia with *NPM1* mutation. Peripheral blood smear shows circulating blasts with "cup-like" nuclei (A, Giemsa; ×1000). The bone marrow biopsy is hypercellular and shows myelomonocytic hyperplasia with increased immature mononuclear cells and atypical (large/hyperlobated) megakaryocytes (B; hematoxylin and eosin; ×200). Immunohistochemistry for NPM1 shows an abnormal (mutant) pattern of staining, characterized by cytoplasmic staining of cells. Of note, wild-type NPM1 is ubiquitously expressed, but the expression is restricted to nuclei. The mutant pattern of staining is highly sensitive and specific for NPM1 mutation (immunohistochemistry with hematoxylin counterstain; ×400).

Table 13
Defining features of acute myeloid leukemia—myelodysplasia related

Defining cytogenetic abnormalities
Complex karyotype (≥3 abnormalities) -5/del(5q)
-7/del(7q)
del(11q)
del(12p)
-13/del(13q)
del(17p)
Isochromosome 17q
idic(X)(q13)
Defining somatic mutations
ASXL1, BCOR, EZH2, SF3B1, SRSF2, STAG2, U2AF1, or ZRSR2

clonal causes of monocytosis.[57] This test has been validated by the European Leukemia Net iMDS Flow Cytometry Group, demonstrating a sensitivity of 94% and specificity of 84% for CMML when performed on PB, and a sensitivity of 87% and specificity of 80% for CMML when performed on BM.[60] False-negative results can be seen in patients with CMML having concomitant systemic autoimmune and inflammatory diseases, largely due to an increase in the M02 fraction, which produces a characteristic "bullous profile" on the scatter curve. In CMML $CD14^{low}$, $CD16^{+}$ monocytes usually comprise <1.7% of all monocytes, providing an additional verification step for CMML diagnosis.[61] In addition, 6-sulfo-LacNac (slan), a carbohydrate modification of p-selectin glycoprotein ligand 1, has been identified as a reliable marker of nonclassical monocytes; although slan is not routinely used in most clinical laboratories.

Atypical CML, *BCR::ABL1* negative, is renamed in WHO-HEM5 as MDS/MPN with neutrophilia.[3] Morphologic criteria for diagnosis are unchanged from WHO-HEM4R, including WBC ≥ 13×10^9/L with immature myeloid cells ≥ 10% of the WBC differential count, prominent dysgranulopoiesis, and monocytes comprising <10%. WHO-HEM4R and WHO-HEM5 mandate the absence of *BCR::ABL1*, TK fusions, and MPN-associated driver mutations in MDS/MPN with neutrophilia; WHO-HEM5 also encourages exclusion of *CSF3R* mutations, now considered to be relatively specific for CNL[61,62]; and notes that somatic *SETBP1* and *ETNK1* mutations are supportive diagnostic criteria. In our experience the neutrophils in MDS/MPN with neutrophilia typically show prominent dysplasia and perhaps this feature should be emphasized more in future versions of the WHO classification. Given the high prevalence rate of ancestral *ASXL1* mutations, we strongly recommend adding somatic *ASXL1* mutations to the molecular signature for supportive diagnostic criteria [63] Although *CSF3R* mutation is a known driver for CNL, it can be seen in other myeloid neoplasms infrequently, such as MDS/MPN with neutrophilia, CMML, and MPNs.[63]

In WHO-HEM5, MDS/MPN-RS-T is redesignated as MDS/MPN-SF3B1-T in patients meeting morphologic criteria for MDS/MPN-RS-T with *SF3B1* mutations, with the term MDS/MPN-RS-T still retained for *SF3B1* wild-type cases with ≥15% BM RS. Sustained thrombocytosis for at least 3 months may substitute for *JAK2*, *MPL,* or CALR mutations and ≥15% RS may substitute for *SF3B1* mutation if the status is unknown or absent. In the absence of *SF3B1* mutation the diagnosis of MDS/MPN-RS-T may be rendered if ≥15% RS are present. We support and advocate for the creation of molecularly defined MDS/MPN-RS-T categories, and hope that additional concrete data will emerge in the future that will further elucidate the requirement of ≥15% BM RS in the *SF3B1* wild-type cases, given the lack of prognostic value of a precise RS percentage in MDS (especially ≥5%),[64] and the fact that patients with *SF3B1* mutant and wild-type MDS/MPN-RS-T have good outcomes, particularly in the absence of an abnormal karyotype.[63,65]

In WHO-HEM5, MDS/MPN-U is redesignated as MDS/MPN-not otherwise specified (NOS), retaining the core diagnostic features, underscoring the presence of dysplastic and proliferative features.[1] Both WHO-HEM4R and WHO-HEM5 endorse exclusion of *BCR::ABL1* and myeloid and lymphoid neoplasms with TK fusions, MPN, and other MDS/MPN entities. WHO-HEM5, however, does not acknowledge that the genetic signatures of most patients in this category resemble other overlap neoplasms, with a small subset having somatic *TP53* mutations and associated poor outcomes.[1,65] Further stratification of this category might help patients gain access to clinical trials and provide better prognostication.[66]

Acute Myeloid Leukemia

The WHO-HEM5 classification of AML has been updated and restructured to emphasize breakthroughs in our understanding. One of the most important changes is the separation of AML into 2 major groups, *AML with defining genetic abnormalities* and *AML defined by differentiation*, previously known as AML-NOS. The other major change in WHO-HEM5 is removing the 20% blast requirement for AML types with defining genetic abnormalities (except AML with *BCR::ABL1* fusion and AML with *CEBPA* mutation); although the diagnostic criteria reserve the diagnosis of

Table 14
Acute myeloid leukemia with other defined genetic alterations

Subtypes	Pathological features	Immunophenotype	Clinical features
AML with *CBFA2T3::GLIS2*, inv(16)(p13q24)	Usually associated with acute megakaryoblastic leukemia	>50% show the RAM phenotype; strong CD56 expression, lack of HLA-DR, and under-expression of CD45 and CD38	Children <5 y (mostly infants) Poor prognosis
AML with *KAT6A::CREBBP*, t(8;16)(p11.2;p13.3)	Myelomonocytic or monocytic Erythrophagocytosis	Positive: HLA-DR, CD15, MPO, CD13, CD33, sometimes CD56, CD14. Negative: CD34, CD117	Any age In children, peak in neonates
AML *with FUS::ERG*, t(16;21)(p11;q22)	Any, except erythroid differentiation Auer rods	Nonspecific	Young adults (median age of 30 y) Poor prognosis
AML with *MNX1::ETV6*, t(7;12)(q36;p13)	Usually minimal differentiation or without maturation	Positive: CD34, HLA-DR, often CD117, T cell markers CD7 and CD4	Children (one-third 0-2 y) Poor prognosis
AML with *NPM1::MLF1*, t(3;5)(q25;q35)	Nonspecific	Nonspecific	Poor prognosis

Table 15
WHO-HEM5 classification of acute myeloid leukemia, defined by differentiation

	Diagnostic criteria	Clinicopathologic features
AML with minimal differentiation	• <3% positivity for MPO (cytochemistry, flow cytometry or IHC) or SBB by cytochemistry • Expression of at least 2 myeloid-associated markers (MPO CD13, CD33, and CD117)	• Any age, M:F ration of 1.5:1 • Medium-sized blasts lacking cytoplasmic granules or Auer rods, occasionally resembling lymphoblasts • *BCL11B* rearrangement in ~30% of cases • *BCL11B* rearrangement is frequently associated with *FLT3* mutation (~85%, mostly as *FLT3*-ITD) • Frequent mutations: RUNX1 (~30%), *DNMT3A* (~20%), *IDH1/2*, *FLT3*-ITD (~20%)
AML without maturation	• ≥3% Positivity for MPO (cytochemistry, flow cytometry or IHC) and SBB and negative for NSE by cytochemistry • Maturing cells of the granulocytic lineage constitute <10% of the nucleated BM cells • Expression of 2 or more myeloid-associated antigens by flow cytometry, such as MPO, CD13, CD33, and CD117	• Medium to large-sized blasts with or without azurophilic granules and/or Auer rods • Frequent mutations: *DNMT3A* (~30%), *RUNX1* (~30%), *IDH2* (~25%), and *IDH1* (~20%)
AML with maturation	• ≥3% positivity for MPO or SBB by cytochemistry, flow cytometry or IHC • Maturing cells of the granulocytic lineage constitute ≥10% of the nucleated BM cells • Monocyte lineage cells constitute <20% of BM cells • Expression of 2 or more myeloid-associated antigens by flow cytometry, such as MPO, CD13, CD33, and CD117	• Medium to large-sized blasts with azurophilic granules and/or Auer rods • Frequent mutations: *RUNX1* (~30%), *IDH2* (~25%) and *DNMT3A* (~20%)
Acute basophilic leukemia	• Blasts and immature/mature basophils with metachromasia on toluidine blue staining • Blasts are negative for MPO, SBB, and NSE • No expression of strong CD117 equivalent to mast cells	• Medium to large-sized blasts with variable numbers of cytoplasmic coarse basophilic granules • Usually present with features of BM failure, or cutaneous involvement, hepatosplenomegaly, lytic bone lesions, and symptoms related to hyperhistaminemia • Recurrent translocation of t(X;6)(p11;q23) resulting in *MYB::GATA1* rearrangement has been reported in male infants • Cytogenetic and molecular characterization is needed to exclude potential differential diagnostic considerations such as AML with *DEK::NUP214*; AML with *BCR::ABL1*
Acute myelomonocytic leukemia	• ≥20% monocytes and their precursors • ≥20% maturing granulocytic cells • At least 3% of the blasts should show MPO positivity	• Medium to large-sized blasts with abundant, basophilic cytoplasm that is often vacuolated • Peripheral blood: increase in monocytes, which are often more mature than those in the bone marrow • Frequent mutations: *TET2, RUNX1*
Acute monocytic leukemia	• ≥80% monocytes and their precursors including monoblasts and promonocytes • <20% maturing granulocytic cells • Blasts and promonocytes expressing at least 2 monocytic markers including CD11c, CD14, CD36, and CD64 or NSE positivity on cytochemistry.	• Medium or large-sized blasts with abundant basophilic cytoplasm sometimes with fine azurophilic granules and cytoplasmic vacuoles • No specific molecular genetic profile is described
Acute erythroid leukemia	• ≥30% immature erythroid cells (undifferentiated or pronormoblastic) • No evidence of a significant myeloblastic component (a strict numerical cutoff is not defined at this time).	• More common in men, often late 60s • Erythroid predominance (usually ≥80% of marrow cellularity) • Usually medium to large-sized blasts with cytoplasmic blebs and vacuoles • PAS stain shows coarsely globular cytoplasmic staining • Dysmegakaryopoiesis is frequent • Erythroblasts are positive for CD36, CD71, and CD117 (often subset), negative for CD34 and MPO • CD235 (Glycophorin A) is usually positive in subset of CD117-negative blasts • Evidence of *TP53* mutation by molecular studies or p53 immunostaining supports diagnosis • Extremely poor prognosis, median survival of 2-4 mo
Acute megakaryoblastic leukemia	• Blasts express at least one or more of the platelet glycoproteins: CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIIa), or CD42b (glycoprotein Ib)	• More common in young children (4%-15% of childhood AML) and rare in adults (1%-2% of AML) • Mostly present with cytopenia, but some patients may have thrombocytosis • Some AMKL are associated with mediastinal germ cell tumors in young adult males • Down syndrome associated AMKL should be ruled out • Adult AMKL has an extremely poor prognosis

AML, acute myeloid leukemia; AMKL, acute megakaryoblastic leukemia; IHC, immunohistochemistry; MPO, myeloperoxidase; NSE, non-specific esterase; SBB, sudan black B.

AML for cases with "increased" blasts, referring to cases with having ≥2% PB blasts and ≥5% BM blasts. A more conservative approach is recommended for cases with blasts below these thresholds. While promoting molecular classification principles, the latter change requires correlation between molecular genetic studies, morphologic findings, and clinical presentation. The

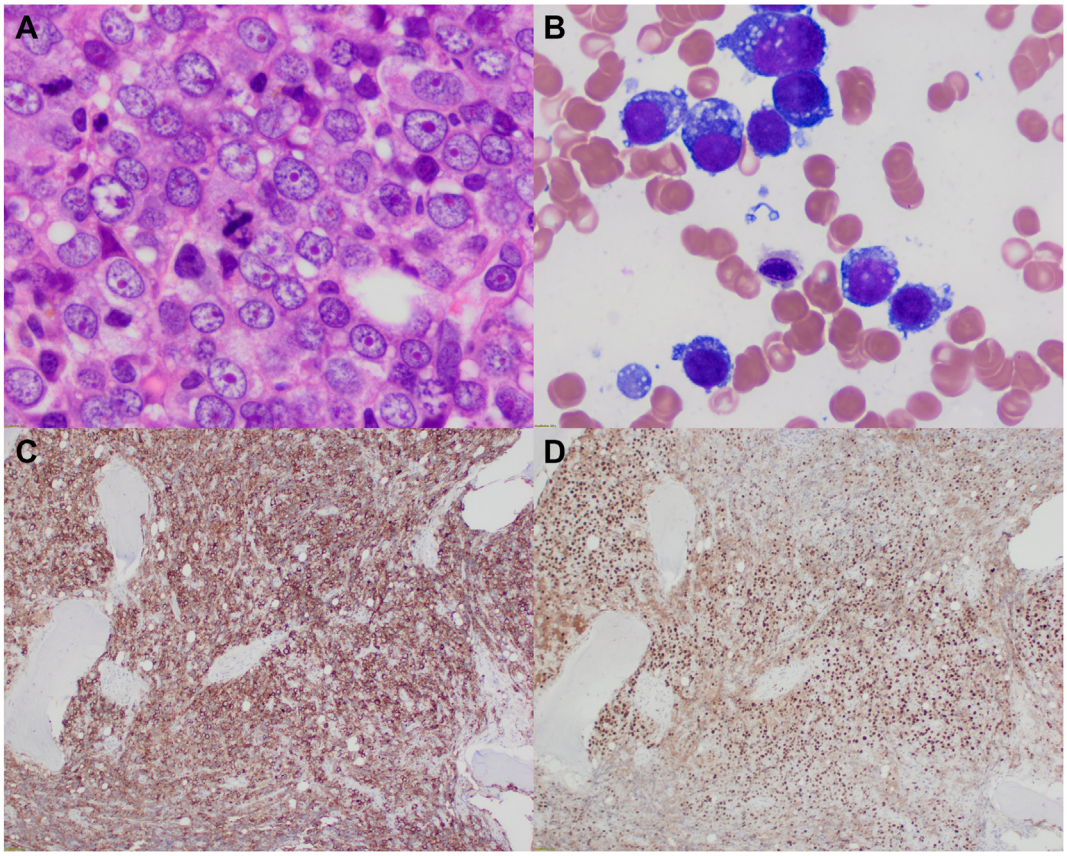

Figure 7.
Acute erythroid leukemia. The bone marrow biopsy shows sheets of immature mononuclear cells with characteristic features of erythroid differentiation (crisp nuclear membranes, vesicular nuclear chromatin, prominent nucleoli, some bound to nuclear membranes) (A; hematoxylin and eosin; ×1000). The bone marrow aspirate smear shows increased immature erythroid progenitors with frequent cytoplasmic vacuoles. A more mature dysplastic normoblast is seen in the center of the image (B; Giemsa; ×100). Immunohistochemistry for CD71 shows markedly increased erythroid cells; there is aberrant nuclear overexpression of p53 protein (C, D; immunohistochemistry with hematoxylin counterstain; ×400). This case had a complex karyotype including deletion 17(p) and a concurrent *TP53* A159V mutation signifying biallelic *TP53* inactivation.

WHO-HEM5 has not provided a cutoff blast percentage as a criterion for AML diagnosis, because evidence in the form of carefully conducted studies with respect to blast percentage currently does not exist. A 10% cutoff for blasts, although arbitrary, is a useful guideline in the context of AML with defining genetic abnormalities. The third major change in WHO-HEM5 is the introduction of a section of AML with *other defined* genetic alterations, which encompasses new and/or uncommon AML subtypes that may (or may not) become defined types in future editions of the classification.

AML With Defining Genetic Abnormalities

The diagnostic criteria for AML with *PML*::*RARA*, AML with *RUNX1*::*RUNX1T1*, and AML with *CBF*::*MYH11* largely remain unchanged from WHO-HEM4R. However, the importance of evaluating for measurable residual disease and detection of concurrent molecular alterations are further emphasized due to implications for patient management and treatment decisions in current practice. With the exception of eliminating the 20% blast requirement, the diagnostic criteria of AML with *DEK*::*NUP214* and AML with *RBM15*::*MRTFA* (formerly *RBM15*::*MKL1*) also remain mostly unchanged.[1] The blast cutoff of 20% is retained for AML with *BCR*::*ABL1* to avoid overlap with a *de novo* presentation of the myeloid blast phase of CML.

AML with *KMT2A* rearrangement is the new designation for AML with t(9;11)(p22;q23); *KMT2A-MLLT3*. This change underlines that more than 80 *KMT2A* fusion partners have been reported, with *MLLT3, AFDN, ELL*, and *MLLT10* being the most common. Identification of the *KMT2A* fusion partner is not required, but the partner can provide prognostic information and may impact disease monitoring. AML with monocytic differentiation and high WBC and blast counts is the most common presentation of AML with *KMT2A* rearrangement in adults. AML with *KMT2A*::*MLLT3* and *KMT2A*::*MLLT10* can present with megakaryoblastic differentiation or low blast counts, especially in children.

AML types with *MECOM* rearrangement [with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2)] and *NUP98* rearrangements are novel categories included in AML with defining genetic abnormalities in WHO-HEM5. Based on data showing that patients with <20% blasts with *KMT2A, MECOM*, and *NUP98* rearrangements have clinical and outcome features similar to those with higher blast counts, the requirement for 20% blasts has been eliminated.[67-69] Although not specific, *NUP98 rearrangment* can be suspected in younger patients, particularly when concurrent mutations in *FLT3/WT1* and/or *CEBPA* are present. *KMT2A-rearrangement* may be suspected in acute monoblastic leukemias with high WBC count in adults, and megakaryoblastic AML in pediatric patients. AML with *MECOM* rearrangement may show very small characteristic megakaryocytes. Rearrangements involving *KMT2A, MECOM,* and particularly *NUP98* may be cryptic

Table 16
Myeloid neoplasms associated with germline predisposition

Myeloid neoplasms with germline predisposition without a pre-existing platelet disorder or organ dysfunction
Germline *CEBPA* P/LP variant (*CEBPA*-associated familial AML)
Germline *DDX41* P/LP variant[a]
Germline *TP53* P/LP variant[a] (Li–Fraumeni syndrome)
Myeloid neoplasms with germline predisposition and pre-existing platelet disorder
Germline *RUNX1* P/LP variant[a] (FPD-MM)
Germline *ANKRD26* P/LP variant[a]
Germline *ETV6* P/LP variant[a]
Myeloid neoplasms with germline predisposition and potential organ dysfunction
Germline *GATA2* P/LP variant (GATA2-deficiency)
Bone marrow failure syndromes • Severe congenital neutropenia • Shwachman–Diamond syndrome • Fanconi anemia
Telomere biology disorders
RASopathies • Neurofibromatosis type 1 • CBL syndrome • Noonan syndrome or Noonan syndrome-like disorders[a]
Down syndrome[a]
Germline *SAMD9* P/LP variant (MIRAGE Syndrome)
Germline *SAMD9L* P/LP variant (SAMD9L-related Ataxia Pancytopenia Syndrome)
Biallelic germline *BLM* P/LP variant (Bloom syndrome)

FPD-MM, familial platelet disorder with associated myeloid malignancy; P/LP, pathogenic/likely pathogenic.
[a] Lymphoid neoplasms can also occur.

on conventional karyotyping, and in such cases, FISH break-apart probes, reverse-transcription PCR, and RNA sequencing are recommended, if readily available, when these AML subtypes are suspected.

AML with defining genetic abnormalities (Table 12) includes 2 novel AML types defined by mutations: AML with *NPM1* mutation and AML with *CEBPA* mutation. AML with *NPM1* mutation may have variable morphologic features; however, myelomonocytic or monocytic differentiation is common and multilineage dysplasia can be seen in 20% to 25% of cases and has no impact on clinical outcome.[70,71] Although blasts with cup-like nuclear morphology can be associated with both *NPM1* and *FLT3-ITD* mutations, identification of cup-like *nuclear invaginations* in more than 10% of blasts is highly specific for AML with *NPM1* mutation.[72] Approximately 80% of AML with *NPM1* mutation lack CD34 expression.[73] Several studies have shown that detection of cytoplasmic NPM1 by IHC can be used as a surrogate of *NPM1* mutation (Fig. 6).[73,74] The 20% blast threshold was eliminated from the diagnostic criteria in WHO-HEM5 based on results from several studies that have shown that patients with *NPM1*-mutated myeloid neoplasms with <20% blasts are associated with shorter survival compared with patients with wild-type AML and can benefit most from upfront intensive (AML-type) chemotherapy regimens.[75,76] Nevertheless, WHO-HEM5 calls for judicious clinicopathologic correlation for rare cases with a low blast count, particularly those with blasts <10% of BM cellularity and low *NPM1* mutant allelic burden before rendering a diagnosis of AML. The European LeukemiaNet recommends monitoring *NPM1* mutation transcript levels by sensitive molecular techniques for use as a marker of measurable residual disease to guide risk stratification and management decisions. [77]

The definition of AML with *CEBPA* mutation in WHO-HEM5 has been modified to include single mutations located in the basic leucine zipper (bZIP) region of the gene (smbZIP-CEBPA) in addition to biallelic (bi*CEBPA*) mutations based on recent studies showing a favorable prognosis for in-frame smbZIP-*CEBPA*, similar to bi*CEBPA* in children and adults up to 70 years old.[78-80] The current data do not support any change in the blast cutoff (>20%) for AML with CEBPA mutation; therefore, the 20% minimum blast count has been retained.[81]

The entity of AML, myelodysplasia related (AML-MR), formerly known as AML with myelodysplasia related change is defined as a myeloid neoplasm with ≥20% blasts harboring specific cytogenetic and/or molecular abnormalities associated with MDS (Table 13), arising de novo or in a patient with antecedent MDS or MDS/MPN. This AML subtype has undergone several revisions. The main changes include (1) elimination of morphologic changes as the sole diagnostic criterion; (2) balanced translocations are no longer considered defining AML-MR and modifications to cytogenetic criteria; and (3) addition of MR-defining mutations.[82,83] *TP53* mutation is frequently associated with AML-MR and a complex karyotype. Several studies have suggested that *TP53* mutation is an independent adverse prognostic factor in AML-MR and de novo AML[84,85] and data suggest that *TP53*-mutated myeloid neoplasms may represent a distinct group of diseases; however, further studies are needed to determine whether *TP53* status is by itself a class-defining genetic abnormality. Nevertheless, several studies suggest that for prognostic and therapeutic purposes MDS with increased blasts and *TP53* mutation[84,86] or MDS-bi*TP53* are highly aggressive and with very poor outcomes irrespective of the blasts count.[22,26]

Due to lack of sufficient specificity to define a distinct AML subtype, the provisional entity of AML with mutated *RUNX1* has been eliminated from WHO-HEM5. Notably, most of these cases will now be included in the AML-MR category, as *RUNX1* mutations frequently co-occur with other MR-defining mutations. [81,87,88]

AML with other defined genetic alterations is a newly proposed category that encompasses emerging or provisional AML subtypes with distinct genetic and associated clinicopathologic features that do not meet criteria for other distinct genetic subtypes (Table 14). This group also includes very rare cases of AML with *RARG* (12q13) fusions. These neoplasms typically present with acute promyelocytic leukemia-like features and resistance to all-trans retinoic acid–based therapy.

AML Defined by Differentiation

The category of AML defined by differentiation (Table 15), formerly known as AML-NOS in WHO-HEM4R, includes cases that lack defining genetic abnormalities. As new discoveries shed more light on this group of AML cases, it is predicted that many of these cases will be classified more specifically based on genetic alterations in the future. One example of these new discoveries is the association of *BCL11B* rearrangements with mixed phenotype acute leukemia T/Myeloid, early T-precursor acute lymphoblastic leukemia, acute leukemia of ambiguous lineage, and a subset of AML with minimal differentiation. This finding suggests a biologic and phenotypic continuum across these entities and will likely have implications for future editions of the WHO classification. Nonetheless, classification of AML cases based on differentiation in the absence of any defining genetic abnormalities provides practical and prognostic information for clinical purposes and continues to be important in low-resource settings. The differentiation markers and criteria of AML defined by differentiation have been updated (Table 15).

Acute erythroid leukemia (AEL) (Fig. 7) is characterized by neoplastic proliferation of erythroid precursors with features of

Table 17
WHO-HEM5 classification of systemic mastocytosis

Mastocytosis subtypes	Diagnostic criteria	Clinicopathologic features
Cutaneous mastocytosis (subtypes include: maculopapular cutaneous mastocytosis [Urticaria pigmentosa] which is further divided into monomorphic and polymorphic subtypes; Diffuse cutaneous mastocytosis; and cutaneous mastocytoyctona)	Essential • Skin lesions typical of mastocytosis • Absence of clinical signs of systemic mastocytosis; bone marrow evaluation (when indicated) not meeting the criteria for systemic mastocytosis; increased numbers of MCs in skin biopsies; MCs immunoreactive for CD117 and tryptase. Desirable • Expression of CD2, CD25 and/or CD30 • Detection of *KIT* mutation in skin lesions.	Monomorphic maculopapular cutaneous mastocytosis: more common in infants; generally, very rare. Generalized erythroderma, thickened skin, exaggerated folds and episodic blistering typically accompanied by severe systemic manifestations. Distinct skin lesions are not seen. Polymorphic maculopapular cutaneous mastocytosis: most common subtype in adults. May be present in childhood. Small, round, and pigmented maculopapular lesions (may be confluent) Diffuse cutaneous mastocytosis: most frequent type of mastocytosis in infants. Mastocytomas are typically solitary, but up to 3 lesions are accepted. Yellow to brown papules, nodules, or plaques, often with leathery texture. Less commonly, small and pigmented macules. Rubbing may cause wheals, blisters, and occasionally flushing and hypotension.
Systemic mastocytosis	Major criterion • Multifocal dense mast cell infiltrate (≥15 mast cells) in BM and/or other extracutaneous organ(s). Minor criteria • >25% of all mast cells have atypical morphology on BM smears or are spindle-shaped in dense and diffuse aggregates in BM biopsy or other extracutaneous organ(s) • Activating *KIT* mutation(s) at codon 816 or other *KIT* mutation with confirmed activating effect • Expression of CD2, CD25, and/or CD30 by mast cells • Baseline serum tryptase concentration >20 ng/mL in the absence of an associated myeloid neoplasm [a]	
Bone marrow mastocytosis	SM criteria fulfilled No skin lesions No B-finding(s) Basal serum tryptase <125 ng/mL No dense SM infiltrates in an extramedullary sites	Low mast cell burden in BM Compact mast cell infiltrates are common Morphologic features are typically similar to ISM and rarely like WDSM (if WDSM is observed consider testing for noncanonical *KIT* mutations)
Indolent systemic mastocytosis	SM criteria fulfilled Skin lesions are common ≤1 B-finding ISM without skin lesions: ≤1 B-finding and/or basal serum tryptase ≥125 ng/mL and/or dense SM infiltrates in an extramedullary site	Low mast cell burden in BM (usually <5%-10% of medullary space) Compact mast cell infiltrates is common, typically associated with eosinophils and small lymphocytes Usually >25% spindle-shaped mast cells Paratrabecular compact mast cell infiltrates often with prominent fibrosis and osteosclerotic changes Mast cell morphology is usually atypical but well-differentiated morphology may be seen. *KIT* p.D816V present in 90%-95% (if WDSM is observed consider testing for noncanonical *KIT* mutations)
Smoldering systemic mastocytosis	SM criteria fulfilled ≥2 B-findings No C-findings	High mast cell burden in the BM (>30% of medullary space) Diffuse-compact mast cell infiltration is most common Spindled mast cells are typically associated with dense fibrosis and osteosclerosis Mild dysplastic changes (not fulfilling WHO criteria for a myeloid neoplasm) are acceptable WD morphology is rare, but permitted. *KIT* p.D816V present in 90%-95% (if WDSM is observed consider testing for noncanonical *KIT* mutations) Tryptase level is typically high Organomegaly and skin lesions are common.
Aggressive systemic mastocytosis	SM criteria fulfilled ≥1 C-findings	Very high mast cell burden in BM (up to 80% of medullary space) Diffuse-compact mast cell infiltrates Spindle-shaped mast cells associated with dense fibrosis and osteosclerosis Increased mast cells on BM smears (>5% and <20%) indicated transformation (ASM-t) Mild dysplastic changes (not fulfilling WHO criteria for a myeloid neoplasm) are acceptable; WD morphology is permitted. *KIT* p.D816V nearly universally present (if WDSM is observed consider testing for noncanonical *KIT* mutations) Skin lesions are typically absent.

(*continued on next page*)

Table 17 (*continued*)

Mastocytosis subtypes	Diagnostic criteria	Clinicopathologic features
Systemic mastocytosis with an associated hematologic neoplasm[b]	Any subtype of SM and any type of WHO-defined myeloid neoplasm[b]	Immunostains to demonstrate neoplastic mast cells (tryptase, CD117) and show aberrant antigen expression (CD2, CD25, CD30) are recommended when SM-AHN is suspected WDSM morphology is permitted. KIT p.D816V is typically present; sensitive techniques may be required for detection. (if WDSM is observed consider testing for noncanonical *KIT* mutations) The associated myeloid neoplasm typically harbors other myeloid-malignancy related mutations *SRSF2, ASXL1, RUNX1* mutations are associated with poorer prognosis
Mast cell leukemia	SM criteria fulfilled ≥20% mast cells on BM smears ≥10% MCs in PB (classic) and <10% mast cells in PB (aleukemic)	Predominantly round and hypogranulated mast cell morphology. BM fibrosis is uncommon Well-differentiated morphology is permitted. *KIT* p.D816V 50%-70% (if WDSM is observed consider testing for noncanonical *KIT* mutations) Skin lesions are typically absent
Mast cell sarcoma	Essential: • Extramedullary infiltrative lesion of atypical mast cells • Criteria for SM are not fulfilled Desirable: • Expression of CD2, CD25, and/or CD30	

BM, bone marrow; ISM, indolent systemic mastocytosis; SM, systemic mastocytosis; SM-AHN, systemic mastocytosis with an associated hematologic neoplasm WDSM, well-differentiated systemic mastocytosis.
The diagnosis is SM requires at least 1 major and 1 minor or 3 minor criteria.
B findings: (1) High mast cell burden: infiltration grade (MC) in BM ≥ 30% in histology (IHC) and/or serum tryptase ≥ 200 ng/mL and/or *KIT* p.D816V VAF ≥ 10% in BM or PB leukocytes; (2) myeloproliferation and/or myelodysplasia: hypercellular BM with loss of fat cells and prominent myelopoiesis ± left shift and eosinophilia ± leukocytosis and eosinophilia and/or discrete signs of myelodysplasia (<10% neutrophils, erythrocytes, and megakaryocytes); (3) Organomegaly: Palpable (or documented by imaging) hepatomegaly without ascites or other signs of organ damage or/and palpable splenomegaly without hypersplenism and without weight loss or/and lymphadenopathy palpable or visceral lymph node enlargement found on imaging (>20 mm).
C findings: (1) Cytopenia(s) [ANC $<1 \times 10^9$/L; Hb <10 g/dL; Plt $< 1.0 \times 10^9$/L]; (2) ascites and elevated liver enzymes ± hepatomegaly or cirrhotic liver ± portal hypertension; (3) palpable splenomegaly with hypersplenism ± weight loss ± hypoalbuminemia; (4) malabsorption with hypoalbuminemia ± weight loss; and (5) large osteolysis (≥20 mm) ± pathological fracture ± bone pain.
[a] Serum tryptase level must be adjusted for patients with known alfa-1 antitrypsin deficiency.
[b] The associated hematologic neoplasm is almost always of myeloid origin; in our experience and based on other literature concurrent lymphoid neoplasms if present are often clonally unrelated and coincidental.

maturation arrest, usually representing ≥80% of BM elements, of which ≥30% are proerythroblasts (or pronormoblasts). These neoplasms commonly carry biallelic *TP53* alterations. Although de novo presentation may occur, most cases of AEL are associated with cytotoxic therapy or progression of a prior myeloid neoplasm, particularly MDS.[89-91] The WHO-HEM5 recommends specific designation of therapy-relatedness for AEL, when applicable. AEL supersedes the category of AML-MR if the criteria for AEL are met (in the presence of biallelic *TP53* inactivation, the erythroid and pronormoblast percentage requirements are not strictly enforced, and prominent erythroid differentiation is sufficient to meet diagnostic criteria for AEL). This approach is further supported by studies showing increased venetoclax resistance and BCL-XL dependency in cases of AML with marked erythroid or megakaryocytic differentiation. [92] However, for cases that overlap with AML-MR, such as those showing a complex karyotype, designation as AML-MR is preferred if ≥20% myeloblasts are present. The main differential diagnostic considerations for AEL include MDS-bi*TP53* and megaloblastic anemias. The distinction between AEL and MDS-bi*TP53* relies on maturation arrest in the erythroid lineage and increased immature erythroid precursors (typically ≥30%) in AEL. Megaloblastic anemia may have profound erythroid proliferation with increased left-shifted erythroid maturation and may show moderate overexpression of p53 by IHC staining and must be excluded in cases where there is no evidence of clonality.

Myeloid Sarcoma

Myeloid sarcoma (MS) is an extramedullary tumor composed of a neoplastic proliferation of myeloid or monocytic blasts, with or without maturation. De novo isolated MS is very rare.[93,94] MS typically occurs in association with concurrent or relapsed AML, transformed MDS, MDS/MPN, or MPN. The diagnostic criteria for MS have not changed compared with WHO-HEM4R. Diagnosis of MS is equivalent to AML and should prompt comprehensive evaluation including immunophenotypic and cytogenetic analysis for translocations, and molecular assessment gene mutations for accurate classification, prognostication, and treatment planning. MS and concurrent BM diseases share similar molecular alterations in ~70% of patients, suggesting derivation from a common hematopoietic stem or precursor cell. Some studies have shown that patients with molecularly discordant MS may have a poorer prognosis. [93,95,96]

Secondary Myeloid Neoplasms

WHO-HEM5 divides secondary myeloid neoplasms (sMN) into 2 broad categories: (1) sMN (AML, MDS, and MDS/MPN) that arise following exposure to cytotoxic therapy (DNA-damaging) therapy or large-field radiation therapy for an unrelated condition; and (2) sMN (AML, MDS, MPN, and MDS/MPN) that arise in

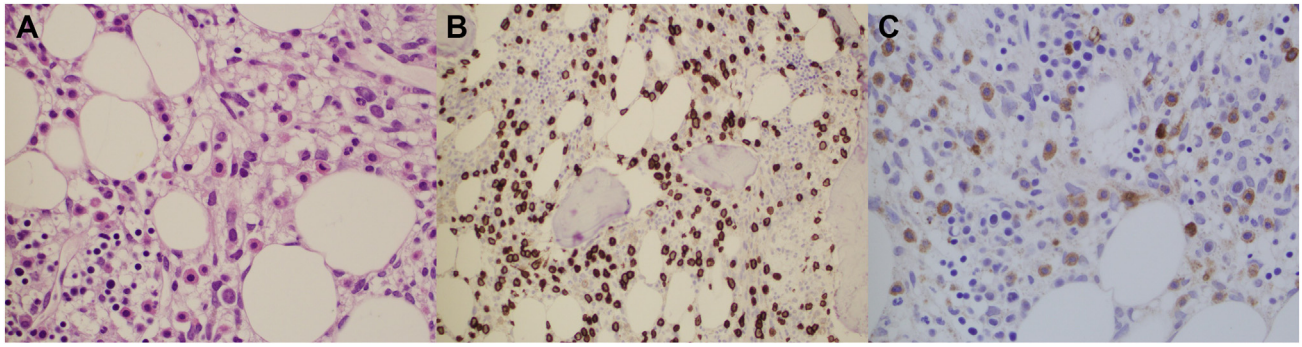

Figure 8.
Well-differentiated systemic mastocytosis. The bone marrow biopsy shows increased morphologically normal mast cells that are round and do not form clusters (A, hematoxylin and eosin; ×200). Increased mast cells are further highlighted by CD117 (B) and mast cell tryptase (C). (B, C: immunohistochemistry with hematoxylin counterstain; ×200).

a patient with germline predisposition to myeloid neoplasms[1] (Table 16). sMN excludes leukemic transformation evolving from antecedent MPN, which is retained in the MPN category as blast-phase MPN and AML transformation of MDS and MDS/MPN, which is classified as AML-MR. The nomenclature recommended by WHO-HEM5 is to add the relevant qualifiers of "postcytotoxic therapy" and "associated with germline [*gene*] variant" as disease attributes to the relevant myeloid disease types whose criteria are fulfilled as defined in WHO-HEM5, eg, "AML with *KMT2A rearrangement postcytotoxic* therapy" or "MDS with low blasts associated with germline *DDX41* variant" or "CMML postcytotoxic therapy."

Of note, CCUS is specifically excluded from the sMN as it does not fulfill the criteria for a WHO-HEM5–established MN. AML with a "de novo molecular signature" such as *NPM1* mutation and core-binding factor leukemias are still assigned to the category of sMN since the "*postcytotoxic therapy*" qualifier is based on clinical history. Exposure to PARP1 inhibitors [ie, olaparib (Lynparza), rucaparib (Rubraca), and niraparib (Zejula)] is added as a qualifying criterion for sMN, and methotrexate exposure is not considered as a qualifying criterion.

Mastocytosis Mastocytosis Encompasses a Group of Rare Neoplasms Characterized by a Neoplastic Proliferation of Mast Cells in the BM and/or Extramedullary Tissues

Debilitating comorbidities are common. WHO-HEM5 recognizes SM as a distinct class of myeloid neoplasms, which represents a change from WHO-HEM4R, which classified SM as a subtype of MPN. Similar to WHO-HEM4R, WHO-HEM5 recognizes 3 distinct subtypes of SM: systemic mastocytosis (SM), cutaneous mastocytosis, and mast cell sarcoma. Updated classification and diagnostic criteria, including essential and desirable criteria, are summarized in Table 17.[1] Mast cell lineage can be confirmed using IHC for CD117 and mast cell tryptase. CD117 is a sensitive but nonspecific marker of mast cells as it also can be positive in cells of myeloid and erythroid lineage cells.

The most common molecular driver mutation in mastocytosis is a point mutation in codon 816 of *KIT* (>90%) but other rare (<1%) activating *KIT* alterations also occur. The noncanonical mutations are particularly more common in indolent SM cases. Additional somatic mutations involving *TET2, SRSF2, ASXL1, RUNX1*, and *JAK2* may be seen in advanced disease. Associated myeloid neoplasms may be seen, most commonly CMML, in patients with mastocytosis.

The most significant changes in WHO-HEM5 compared with WHO-HEM4R include (1) inclusion of CD30 and the presence of any activating *KIT* mutation as minor criteria; (2) inclusion of elevated basal serum tryptase level (>20 ng/mL) as a minor criterion; (3) a novel subtype of "BM mastocytosis" is now recognized (Table 17); (4) definitions of B and C findings have been slightly modified, including inclusion of *KIT* p.D816V with VAF $\geq$ 10% as a B-finding; and (5) recognition of well-differentiated systemic mastocytosis (Fig. 8) as a distinct SM subtype, characterized by round and adequately granulated mast cells that aberrantly express CD30, are often negative for CD25 and CD2 and typically lack *KIT* codon 816 mutation.

Author Contribution

S.L., R.K.S., J.D.K., L.J.M., K.N.N., R.N., and M.M.P. wrote and edited the manuscript. S.L. provided the microscopic images. All authors have reviewed and approved the final draft of the manuscript.

Data Availability

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

Funding

The authors received no funding for this study.

Declaration of Competing Interest

None reported.

Ethics Approval and Consent to Participate

Not applicable.

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