Fifth edition WHO classification: myeloid neoplasms

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ABSTRACT

The fifth edition of the WHO classification of haematolymphoid tumours (WHO-HEM5) introduces significant advancements in the understanding and diagnosis of myeloid neoplasms, emphasising molecular and genetic insights. This review highlights key updates from the revised fourth edition (WHO-HEM4R), particularly the integration of genetic criteria for disease classification. Many entities are now defined by specific genetic abnormalities, enhancing diagnostic precision and prognostic assessment. Notably, the elimination of the 20% blast threshold for acute myeloid leukaemia (AML) with specific defining genetic alterations reflects a shift towards genomic-driven diagnostics. Additional updates include the refined subclassification of myelodysplastic neoplasms (MDS) and MDS/myeloproliferative neoplasms, as well as the recognition of novel entities such as clonal haematopoiesis and MDS with biallelic TP53 inactivation, further expanding the spectrum of myeloid neoplasms. WHO-HEM5 illustrates the diagnostic utility of morphology, flow cytometry, immunohistochemistry and next-generation sequencing in resource-rich settings. However, its implementation in low-income and middle-income countries (LMICs) remains challenging due to limited access to advanced diagnostic tools. This review explores strategies to optimise diagnosis in resource-constrained environments, where morphology and immunophenotyping remain fundamental. By integrating molecular diagnostics with traditional methods, WHO-HEM5 aims to refine classification and facilitate risk stratification in the era of personalised medicine, providing haematopathologists and clinicians with an essential framework to navigate the complexities of myeloid neoplasms. The emphasis on advancing haematopathology practices worldwide, including in LMICs, demonstrates the ongoing commitment to improving global outcomes in haematological malignancies.

This review provides a concise overview of myeloid neoplasms as outlined in the fifth edition of the WHO classification of haematolymphoid tumours (WHO-HEM5), highlighting changes from the revised fourth edition (WHO-HEM4R). Table 1 summarises the introduced modifications. Haematolymphoid neoplasms are recognised based on morphology, cyto/histochemistry and various ancillary techniques such as flow cytometry, immunohistochemistry (IHC), cytogenetics and molecular diagnostics. Diverse genetic subtypes may correlate with clinical, morphological and immunophenotypic features. The current classification includes many entities primarily defined by genetic abnormalities. Beyond diagnosis, these biomarkers hold prognostic and therapeutic implications.

Most diagnostic laboratories within a cancer centre setting possess a histopathology laboratory supported by immunophenotyping (IHC and flow cytometry). Cytogenetics and molecular diagnostics are available in limited centres across developing countries. In the absence of these techniques, applying WHO-HEM5 guidelines becomes challenging. In the era of theranostics, the diagnosis, treatment and monitoring of chronic myeloid leukaemia (CML) rely on molecular techniques and targeted therapies. However, in many low-income and middle-income countries (LMICs), CML diagnosis continues to be primarily based on clinical examination and peripheral blood (PB) smear morphology. Patients often require referral to neighbouring states or countries for comprehensive evaluation.

While this review addresses recent advancements, it also emphasises haematopathology practices in resource-constrained settings. Morphology, combined with immunophenotyping, remains fundamental for diagnosing these neoplasms. A 'not further classifiable' (NFC) category can be used when diagnoses rely solely on morphology and immunophenotyping. High-quality Romanowsky stains, H&E sections and skilled haematopathologists are essential for haematolymphoid neoplasm diagnosis, particularly in LMIC settings.

CLONAL HAEMATOPOIESIS

The hypothesis of clonal haematopoiesis (CH) emerged in the 1960s following the seminal observation of skewed X chromosome inactivation patterns in females. This pattern was more pronounced in blood cells and increased with age.[1] Large-scale exome sequencing studies of unrelated disorders (psychiatric disorders, cardiovascular disease and non-haematological cancers) by three independent groups identified mutations in several genes.[2–4] Collectively termed CH, these alterations increased in frequency with age (rarely present in individuals under 40) and were associated with increased risks of blood cancers, cardiovascular diseases and all-cause mortality.

A distinction is necessary between CH, age-related clonal haematopoiesis (ARCH), CH of indeterminate potential (CHIP) and clonal cytopenia of undetermined significance (CCUS). CHIP and CCUS have been formally defined by WHO-HEM5.[5] ARCH refers to CH associated with ageing in individuals without a diagnosed blood disorder or cytopenia. Specific genetic criteria

Table 1 WHO classification of myeloid proliferations and neoplasms: a comparison of the revised fourth edition with the fifth edition

WHO-HEM4R	WHO-HEM5
Myeloid precursor lesions/clonal haematopoiesis	
Not included	Clonal haematopoiesis
Not included	Clonal cytopenias of undetermined significance
Myeloproliferative neoplasms (MPN)	
Chronic myeloid leukaemia, *BCR-ABL1*-positive	Chronic myeloid leukaemia
Chronic phase	Chronic phase
Accelerated phase	Deleted
Blast phase	Blast phase
Chronic eosinophilic leukaemia, not otherwise specified (NOS)	Chronic eosinophilic leukaemia
Juvenile myelomonocytic leukaemia (previously considered myelodysplastic neoplasm (MDS/MPN)	Juvenile myelomonocytic leukaemia
MPN, unclassifiable	MPN, NOS
MDS	
MDS, with defining genetic abnormalities	
MDS with isolated del(5q)	MDS with low blasts and 5q deletion
MDS with ring sideroblasts (single lineage dysplasia and multilineage dysplasia)	MDS with low blasts and *SF3B1* mutation
	MDS with biallelic *TP53* inactivation
MDS, morphologically defined	
MDS with single/multilineage dysplasia	MDS with low blasts
	MDS, hypoplastic
MDS with excess blasts	MDS with increased blasts
MDS of childhood	
Refractory cytopenia of childhood	Childhood MDS with low blasts
	cMDS-LB, hypocellular
	cMDS-LB, NOS
Childhood myelodysplastic syndrome with increased blasts	Childhood MDS with increased blasts
MDS/MPN	
Chronic myelomonocytic leukaemia (CMML)	CMML
	Myelodysplastic CMML
	Myeloproliferative CMML
CMML0	Deleted
CMML1	CMML1
CMML2	CMML2
Atypical chronic myeloid leukaemia, BCR-ABL1-negative	MDS/MPN with neutrophilia
MDS/MPN with ring sideroblasts and thrombocytosis	MDS/MPN with *SF3B1* mutation and thrombocytosis
MDS/MPN, unclassifiable	MDS/MPN, NOS
Acute myeloid leukaemia (AML) AML with defining genetic abnormalities	
Acute promyelocytic leukaemia with *PML::RARA*	Acute promyelocytic leukaemia with *PML::RARA* fusion
AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*	AML with *RUNX1::RUNX1T1* fusion
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	AML with *CBFB::MYH11* fusion
AML with t(6;9)(p23;q34.1); *DEK-NUP214*	AML with *DEK::NUP214* fusion
AML (megakaryoblastic) with t(1;22)(p13.3;q13.1); *RBM15-MKL1*	AML with *RBM15::MRTFA* fusion
AML with *BCR-ABL1*	AML with *BCR::ABL1* fusion
AML with t(9;11)(p21.3;q23.3); KMT2A-MLLT3	AML with *KMT2A* rearrangement
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2*, *MECOM*	AML with *MECOM* rearrangement
	AML with *NUP98* rearrangement
AML with mutated *NPM1*	AML with *NPM1* mutation
AML with biallelic mutation of *CEBPA*	AML with *CEBPA* mutation
AML with mutated *RUNX1*	Deleted
AML with myelodysplasia-related changes	AML, myelodysplasia-related
	AML with other defined genetic alterations
AML, defined by differentiation	
Acute monoblastic and monocytic leukaemia	Acute monocytic leukaemia
Acute panmyelosis with myelofibrosis	Deleted
Myeloid neoplasms, secondary Myeloid neoplasms and proliferations associated with antecedent or predisposing conditions	
Therapy-related myeloid neoplasms	Myeloid neoplasm post cytotoxic therapy
Myeloid neoplasms associated with germline predisposition	Additional subsets recognised

The table includes new entities, deleted entities and entities with subtle changes in the terminology. For brevity, entities that have had no changes are not listed.

are not required for ARCH diagnosis. CHIP is a CH subtype defined by somatic mutations in genes implicated in myeloid malignancies at a variant allele fraction (VAF) of ≥2% (≥4% for X-chromosome gene mutations in males) in individuals without a diagnosed haematological disorder or unexplained cytopenia. CCUS is diagnosed when CHIP is detected in a patient with unexplained cytopenia. While the list of genes implicated in CHIP is extensive, mutations in *DNMT3A, TET2, ASXL1, JAK2, TP53, SF3B1, PPM1D* and *SRSF2* genes are relatively common.[5] Among these, mutations in *DNMT3A, TET2* and *ASXL1*, collectively termed 'DTA gene mutations,' account for over 70% of CHIP cases.[6]

What are the implications for CHIP and CCUS for pathologists?

1. Implications for measurable residual disease (MRD) testing: DTA mutations persist following chemotherapy, even when patients achieve morphological or molecular remission. These DTA mutations disappear after successful bone marrow (BM) transplantation. It is important to note that persistent DTA mutations do not equate to evidence of MRD.[7 8]
2. Not all CHIP is the same: Individuals with CCUS exhibit higher VAF and a greater number of mutations compared with those with CHIP. Mutations in *SF3B1, SRSF2, ZRSR2, JAK2, TP53, RUNX1, FLT3, IDH1* or *IDH2* are considered high risk for developing a myeloid neoplasm. A CH Risk Score has been recently proposed, incorporating the presence of high-risk mutations along with other factors such as a single *DNMT3A* mutation, mutation count, VAF, red cell indices (red cell distribution width and mean corpuscular volume), cytopenia and age.[9] Furthermore, certain cancer chemotherapies like platinum-based chemotherapy, topoisomerase II inhibitors and radiation may accelerate CHIP in genes involved in DNA damage response pathways (*TP53, PPM1D, CHEK2*). These individuals face an increased risk of developing therapy-related myeloid neoplasms.[10]

MYELOPROLIFERATIVE NEOPLASMS

Myeloproliferative neoplasms (MPNs) are stem cell neoplasms of the myeloid lineage that fully differentiate to the most mature stages of normal haematopoiesis. These neoplasms present with increased proliferation of one or more myeloid lineages in the BM, resulting in splenomegaly and elevated counts of the affected lineage in the PB. They typically manifest with increased counts of erythrocytes, platelets, neutrophils or eosinophils.

The diagnostic criteria for CML in the chronic phase remain unchanged from WHO-HEM4R. However, gene expression studies have demonstrated a biphasic, rather than triphasic, pattern of expression.[11] Furthermore, the prognostic impact of the accelerated phase has diminished in the era of tyrosine kinase inhibitors, leading to its exclusion.[12 13] The blast phase definition has been modified to include the presence of bona fide lymphoblasts in the PB or BM (even if <10%), as these portend rapid progression to the blast phase.[14 15] CML diagnosis according to WHO-HEM5 requires no major additional technologies beyond morphology, routine cytogenetics for confirming *BCR::ABL1* and additional cytogenetic abnormalities, along with standard molecular techniques. Nevertheless, flow cytometry immunophenotyping is essential for early detection of lymphoblasts at diagnosis in a small subset of patients. The increasing use of next-generation sequencing (NGS) enhances sensitivity in detecting *ABL1* kinase domain mutations and mutations in other genes such as *ASXL1, RUNX1* and *IKZF1*. Genomics is expected to further facilitate early detection of advanced-phase CML.

Chronic neutrophilic leukaemia (CNL) is a rare entity with unchanged diagnostic criteria. However, differentiating CNL from neutrophilic leukemoid reactions associated with myeloma or monoclonal gammopathy of uncertain significance is crucial. A definitive diagnosis requires demonstrating the activating mutation *CSF3R T618I*, confirmable by simple allele-specific PCR in resource-limited settings. Identifying other *CSF3R* mutations necessitates NGS-based investigations. If the local infrastructure does not support mutation studies, persistent neutrophilia (≥3 months), splenomegaly and no identifiable cause of reactive neutrophilia including the absence of any evidence of a plasma cell neoplasm can be considered supportive of the diagnosis of CNL.

Chronic eosinophilic leukaemia (CEL) has replaced the previous term, CEL, not otherwise specified (NOS). The diagnostic criterion for hypereosinophilia duration has been shortened from 6 months to 4 weeks. BM exhibits abnormal features with hypercellularity, myeloid predominance, increased numbers of abnormal eosinophilic precursors and eosinophils (characterised by vacuoles, abnormal nuclear lobation and granulation), and abnormal megakaryocyte morphology with MPN-like, myelodysplastic neoplasms (MDS)-like or mixed MPN and MDS-like features.[16 17] Dysplastic features can manifest in both erythroid and neutrophilic series. Abnormal BM morphology in the context of hypereosinophilia strongly indicates CEL when other myeloid neoplasms are excluded. Increased numbers of mast cells and elevated serum tryptase levels raise the possibility of myeloid neoplasms other than CEL and systemic mastocytosis. Differentiating CEL from idiopathic hypereosinophilia can be challenging. Flow cytometry, cytogenetics and FISH are essential to rule out myeloid/lymphoid neoplasms associated with eosinophilia. Establishing clonality requires NGS, while RNA sequencing, though less widely available, aids in excluding rare translocations causing hypereosinophilia.

The diagnostic criteria for polycythaemia vera (PV) remain unchanged. Morphology and conventional molecular techniques, such as allele-specific PCR with fragment length analysis and real-time PCR, are sufficient for identifying the *JAK2* V617F mutation. Detecting rare *JAK2* exon 12 mutations requires NGS or fragment length analysis. NGS is also essential for measuring allele burden to assess prognosis. Similarly, there are no changes to the diagnostic criteria for essential thrombocythaemia (ET). Morphology and conventional molecular techniques, such as allele-specific PCR with fragment length analysis for *JAK2* V617F, *MPL* and *CALR* mutations, are required. The diagnostic criteria for both prefibrotic and fibrotic stages of primary myelofibrosis (PMF) remain unchanged, and the same conventional molecular techniques used for ET along with morphology suffice for diagnosis. In rare cases lacking *JAK2* V617F, *MPL* and *CALR* mutations, NGS using a myeloid gene panel is necessary to identify a clonal marker and differentiate from reactive causes of fibrosis. The concept of the accelerated phase (defined by 10%–19% blasts) persists in the three major non-CML MPN subtypes: PV, ET and PMF. A minority of PMF patients progress to the blast phase (>20% blasts), which is exceedingly rare in PV and ET. WHO-HEM5 outlines risk factors for leukaemic and myelofibrotic transformation, thrombotic episodes and outcomes in PV patients.

Morphological assessment of BM biopsies is crucial for diagnosing and managing patients with PV, ET and PMF. Due to haemodilution and a lack of BM particles caused by fibrosis in the aspirate, BM biopsies are often more informative.[18] BM

cellularity and the myeloid-to-erythroid ratio are valuable diagnostic parameters. Evaluating immature myeloid precursor cells using CD34 or CD117 IHC aids in assessing disease phase and progression. Megakaryocytes exhibit characteristic morphological features in these entities, which can be better highlighted using IHC (CD61 or CD42b).[19 20] Morphological assessment, with or without IHC, helps identify evolving dysplastic features during disease progression. Reticulin stain is essential in all cases, with trichrome stain added when reticulin is increased or collagen fibrosis is suspected.

Juvenile myelomonocytic leukaemia (JMML) was classified as an MPN/MDS in WHO-HEM4R and is now categorised as an MPN in WHO-HEM5; diagnostic criteria remain unchanged. Diagnosis requires a combination of clinical features, morphology and cytogenetic abnormalities like deletion 7q or others. In the absence of cytogenetic abnormalities, investigating mutations in *CBL, PTPN11, NF1, KRAS* and *NRAS* using a myeloid gene panel by NGS is necessary. About one-quarter of JMMLs are associated with germline genetic predisposition syndromes or RASopathies that include germline mutations in *NF1* with or without manifestations of neurofibromatosis type 1, Noonan syndrome and germline CBL syndrome.[21–23] Other clinical manifestations of these germline genetic syndromes can be a clue for appropriate workup and diagnosis.

The term, MPN, NOS is used to classify disorders presenting similarly to MPNs but failing to meet criteria for specific MPNs. These rare disorders must be differentiated from myeloid/lymphoid neoplasms and MDS/MPN. Diagnosis requires a comprehensive evaluation, including clinical features, morphology, cytogenetics and NGS-based testing to document mutations in genes like *TET2, ASXL1, SRSF2, RUNX1* and *DNMT3A*.

MYELODYSPLASTIC NEOPLASMS

In WHO-HEM5, the term 'myelodysplastic neoplasm' replaces 'myelodysplastic syndrome', emphasising the disease's neoplastic nature; however, the abbreviation 'MDS' persists. Cytopenia in at least one haematopoietic lineage is essential for an MDS diagnosis, defined as haemoglobin <13 g/dL in males and <12 g/dL in females, absolute neutrophil count $<1.8\times10^9$/L, or platelets $<150\times10^9$/L.[24] Cases of MDS with low blasts and 5q deletion can be associated with thrombocytosis. An MDS diagnosis may still be established in patients with milder anaemia if definitive morphological dysplastic features and cytogenetic abnormalities are present.[24] Dysplasia in one or more haematopoietic lineages (myeloid, erythroid or megakaryocytic) is characteristic of MDS, with a dysplasia threshold set at 10% for all lineages (figure 1). While blast counts may increase, they should remain below 20% in both BM and PB.

MDS entities are broadly categorised into three groups (table 2): (a) those defined by genetic abnormalities; (b) those defined morphologically and (c) those occurring in childhood. The morphologically defined MDS group is further classified based on BM and PB blast percentages, BM cellularity and marrow fibrosis. Childhood MDS is similarly categorised based on BM and PB blast percentages (table 2). Blast percentage cut-off values remain unchanged from WHO-HEM4R. The MDS group defined by genetic abnormalities encompasses MDS with low blasts and 5q deletion (MDS-5q), MDS with low blasts and *SF3B1* mutation (MDS-*SF3B1*) and MDS with biallelic *TP53* inactivation (MDS-bi*TP53*).

There are no significant changes to the diagnostic criteria for MDS-5q. In WHO-HEM5, a VAF of >5% for an *SF3B1* variant is essential for diagnosing MDS-*SF3B1*. When molecular analysis is unavailable, the presence of ≥15% ring sideroblasts (among erythroid precursors) serves as a surrogate, particularly relevant in resource-constrained settings. Within MDS-*SF3B1*, the term 'MDS with low blasts and ring sideroblasts' remains acceptable for cases with wild-type *SF3B1* or unknown mutation status and ≥15% ring sideroblasts. Morphologically, dysplastic features predominantly affect erythroid cells. Patients with MDS-*SF3B1* generally have better outcomes compared with other MDS types.[25–27] MDS-bi*TP53* is characterised by two or more *TP53* mutations or one *TP53* mutation and evidence of concurrent *TP53* copy loss or copy-neutral loss of heterozygosity (LOH). It may involve multiple *TP53* mutations or *TP53* mutations with allelic deletion. Diagnosis typically requires combining mutation analysis with copy number assessment (karyotype, FISH or array comparative genomic hybridisation). *TP53* mutations are usually of the missense type. A somatic *TP53* mutation with a VAF >49% strongly indicates an accompanying copy loss on the trans allele or copy-neutral LOH. Morphologically, BM exhibits marked dysplasia, often with higher blast counts and altered p53 expression by IHC. Overall, patients with MDS-bi*TP53* have higher risks of leukaemic transformation and poorer survival.[28–32] Inframe missense mutations (mostly occurring in the DNA binding domain) of *TP53* are associated with intense p53 positivity by IHC, and on the other hand, cases with truncations, frameshifts, splice site mutations and deep deletions of *TP53* are associated with loss of p53 expression ('null pattern').[33] Contrary to the mutant *TP53*, wild-type *TP53* is associated with weak/variable expression in a proportion of cells. The VAF of the mutant *TP53* also contributes to the p53 expression pattern.

WHO-HEM5 introduces the entity of MDS-hypoplastic (hMDS) for cases characterised by significantly reduced age-adjusted BM cellularity, as determined by trephine/core biopsy, and not attributable to non-neoplastic marrow failure

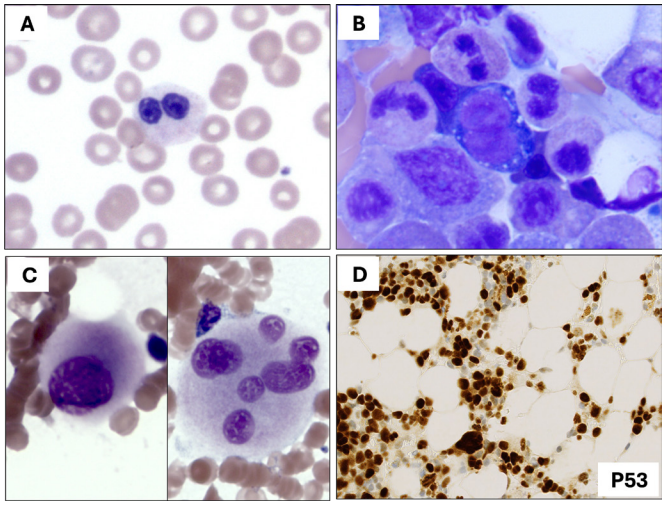

Figure 1 Evaluation of MDS by morphology and immunohistochemistry. (A) Dysplastic neutrophil in the peripheral blood, with hyposegmented nucleus. (B) Dysplastic erythroid precursor in the bone marrow aspirate, with binucleation and cytoplasmic vacuoles. (C) Dysplastic megakaryocytes in the bone marrow aspirate, with hypolobated nucleus or separated nuclear lobes. (D) P53 immunohistochemistry shows diffusely positive cells in the bone marrow biopsy. MDS, myelodysplastic neoplasms.

Table 2 Classification of myelodysplastic neoplasms in WHO-HEM5 and criteria for diagnosis

Entity	Blasts	Cytogenetics	Mutations
MDS with defining genetic abnormalities			
MDS-5q	<5% BM and <2% PB	Isolated 5q deletion or with one other abnormality other than monosomy 7 or 7q deletion	Any except multihit *TP53*
MDS-*SF3B1*	<5% BM and <2% PB	Absence of 5q deletion, monosomy 7 or complex karyotype	*SF3B1*
MDS-bi*TP53*	<20% BM and PB	Complex karyotype	Multi-hit *TP53* alterations (two or more *TP53* mutations, or one mutation with evidence of *TP53* copy-number loss or copy-neutral LOH)
MDS, morphologically defined			
MDS with low blasts	<5% BM and <2% PB	Any	Any except multihit *TP53* or *SF3B1*
MDS, hypoplastic	<5% BM and <2% PB	Any	
MDS with increased blasts 1 (MDS-IB1)	≥5% and <10% BM and/or ≥2% and <5% PB	Any	Any except multihit *TP53*
MDS with increased blasts 2 (MDS-IB2)	≥10% and <20% BM and/or ≥5% and <20% PB or Auer rods	Any	
MDS with increased blasts and fibrosis	≥5% and <20% BM and/or ≥2% and <20% PB	Any	
MDS of childhood			
Childhood MDS with low blasts	<5% BM and <2% PB	Any; Monosomy 7 common*.	Any; somatic *SETBP1*; germline *GATA2*, *SAMD9* or *SAMD9L**
Childhood MDS with increased blasts	≥5% and <20% BM and/or ≥2% and <20% PB	Any; 7q deletion, complex karyotype; exclusion of trisomy 21	Any; *PTPN11*

*A cautious approach is required as patients with germline mutations in *SAMD9* or *SAMD9L* can have adaptive genetic rescue via monosomy 7, which results in CH rather than MDS.[102] Furthermore, in patients with childhood MDS, screening for germline mutations is essential.
BM, bone marrow; CH, clonal haematopoiesis; LOH, loss of heterozygosity; MDS, myelodysplastic neoplasms; PB, peripheral blood.

conditions. By definition, BM cellularity is <30% in patients <70 years of age and <20% in patients ≥70 years.[34 35] The primary differential diagnosis is aplastic anaemia. MDS with increased blasts and fibrosis (MDS-F) has been introduced as a subtype of MDS with increased blasts (MDS-IB). MDS-F accounts for approximately 15% of MDS-IB cases, characterised by increased reticulin fibrosis in BM (WHO grade 2 or 3).[29 36]

Morphological dysplastic features, as described in table 3, can be appreciated in PB (particularly dysgranulopoiesis), BM aspirate smears, touch preparations or trephine/core biopsies. Beyond enumerating blasts based on CD34 and/or CD117, flow cytometry identifies abnormal maturational patterns in granulocytes (using a combination of CD11b, CD13 and CD16), monocytes (using CD13, CD14, CD64, HLA-DR, etc) and erythroid precursors (using CD36, CD71 and CD235a) based on aberrant antigen expression in addition to the abnormal blasts and altered immature progenitor cells.[37] IHC on BM core/trephine is crucial for evaluation of different lineages, especially in cases with marrow fibrosis or suboptimal aspirate quality. Dysplastic features in megakaryocytes (using CD61 or CD42b antibodies) and erythroid lineage cells (using antibodies to glycophorin or CD71) can be readily highlighted using IHC. Assessing blast percentage can be similarly aided by CD34 and/or CD117 IHC; clustering of CD34+cells in the trephine/core biopsy and associated morphological features of abnormal localisation of immature precursor are also helpful. CD34 expression by megakaryocytes provides another useful clue for dysplasia.[36 38–41]

MYELODYSPLASTIC/MYELOPROLIFERATIVE NEOPLASMS

MDS/MPN are a distinct category of haematological neoplasms characterised by overlapping MD and MP features. CH, arising from sequential somatic mutations in haematopoietic stem and progenitor cells, underlies the development of these neoplasms.[42] Although relatively rare, the true incidence of MDS/MPN may be higher due to diagnostic challenges associated with these overlapping syndromes.[43]

There are some important changes in chronic myelomonocytic leukaemia (CMML). WHO-HEM5 now allows

Table 3 Morphological dysplastic features

Lineage	Dysplastic changes—nuclear	Dysplastic changes—cytoplasmic
Dyserythropoiesis	Budding, internuclear bridging, multinuclearity, megaloblastic changes and karyorrhexis	Ring sideroblasts, vacuolisation and PAS positivity
Dysgranulopoiesis	Hyposegmentation (pseudo-Pelger-Huët) and hypersegmentation	Hypogranularity, pseudo-Chédiak-Higashi granules, small size and Auer rods
Dysmegakaryopoiesis	Hypolobation in megakaryocytes of all sizes and multinucleation (multiple widely separated nuclei)	Micromegakaryocytes

PAS, Periodic Acid-Schiff Stain.

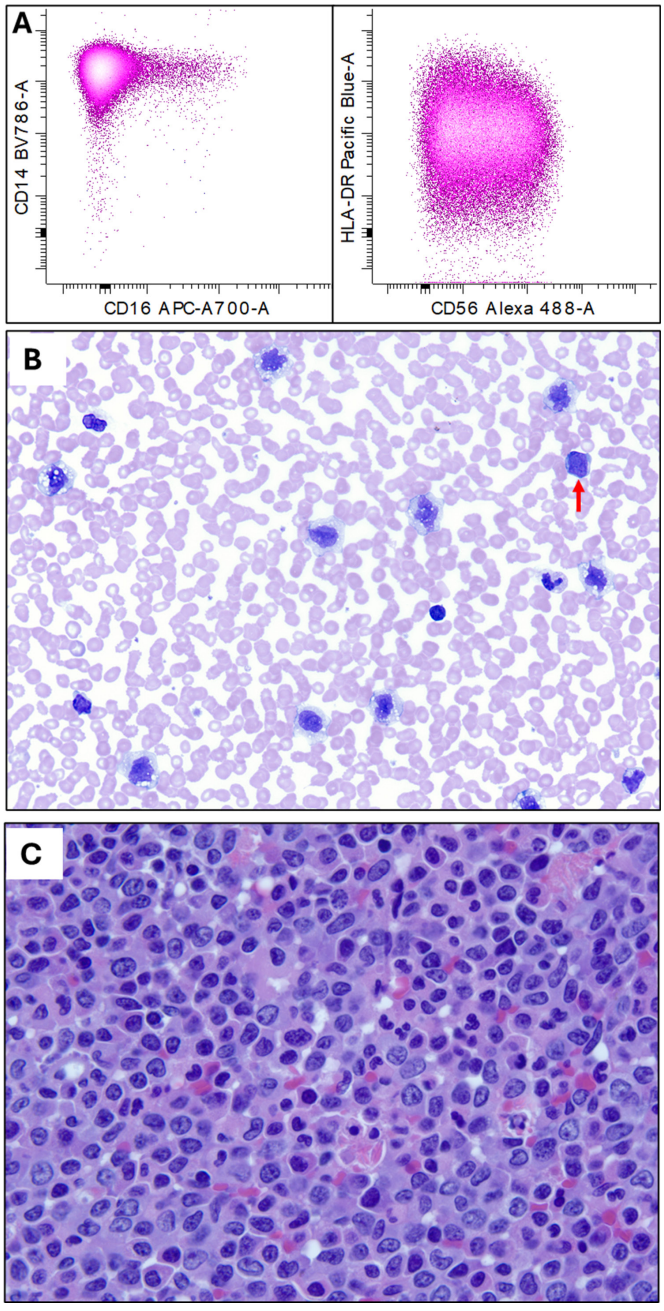

Figure 2 Flow cytometric and morphological features of chronic myelomonocytic leukaemia (CMML). (A) Flow cytometry: In peripheral blood, monocytes are proportionally increased at ~45% of the white blood cells and are largely (>95%) classical monocytes which lack expression of CD16. A subset of monocytes express CD56. (B) Peripheral blood smear from a patient with CMML shows absolute monocytosis and a rare blast (arrow). (C) H&E-stained section of bone marrow biopsy shows a diffuse infiltrate of monocytic cells.

for the inclusion of patients with absolute monocyte count $\geq 0.5\times10^9$/L, with monocytes comprising ≥10% of the white cell count (WCC) (Oligo monocytic (O-CMML)). This expansion requires the presence of morphological dysplasia in the BM and acquired clonal cytogenetic or molecular abnormalities; abnormal flow cytometry-based PB monocyte partitioning (classical monocytes fraction (CD14+CD16−)/M01>94%) using CD14 and CD16 is a supportive finding[44 45] (figure 2). CMML is categorised into MD (MD-CMML) and MP (MP-CMML) subtypes, distinguished by a WCC threshold of $\geq 13\times10^9$/L for MP-CMML. MP-CMML is associated with a higher prevalence of *RAS* pathway gene and *JAK2* mutations, distinct gene expression and methylation profiles, and a poorer prognosis. The previously defined CMML-0 category in WHO-HEM4R has been removed. Absolute monocyte counts, morphology and flow cytometry-based PB monocyte partitioning after exclusion of common MPN can help in the diagnosis of CMML in the absence of advanced molecular techniques.

MDS/MPN with neutrophilia (MDS/MPN-N), previously termed atypical CML is characterised by hepatosplenomegaly, anaemia, thrombocytopaenia and leucocytosis ($\geq 13\times10^9$/L) with neutrophilia.[46] The presence of ≥10% left-shifted myeloid cells alongside neutrophilic dysplasia differentiates it from CNL.[47] While the absolute monocyte count might be higher, a monocyte percentage <10% excludes CMML.[48] BM is hypercellular with granulocytic predominance and dysplasia in ≥10% of cells, with or without dysplastic features in megakaryocytic and erythroid lineages.[49] Criteria for acute leukaemia (blasts ≥20%), other MPNs, myeloid/lymphoid neoplasms with eosinophilia, and defining gene rearrangements, CMML or MDS/MPN-SF3B1-T should not be met.[50]

The absence of MPN-associated driver mutations, such as *JAK2, CALR* and *MPL*, coupled with the presence of *SETBP1* and *ETNK1* mutations, supports the diagnosis of MDS/MPN-N.[48] Clonal architectural reconstruction suggests that *ETNK1* mutations represent early events in MDS/MPN-N, with *SETBP1, SRSF2, RAS* and *CBL* mutations occurring secondarily.[42] Mutations commonly associated with CH (*TET2, ASXL1* and *DNMT3A*) are prevalent. Although *CSF3R* mutations can rarely be encountered in MDS/MPN-N, a prompt morphological review to exclude CNL is essential.[5 51] The involvement of multiple genes underscores the heterogeneous genetic basis of MDS/MPN-N compared with CML.[52] While CBL mutations demonstrate a trend towards favourable overall survival (OS),[53] mutations in *NRAS, GATA2, ASXL1, SETBP1, RUNX1, TP53* or *DNMT3A* are associated with inferior OS.[54] Karyotypic abnormalities, detected in 35%–40% of cases,[55] are linked to disease progression.[52] Age >65 years, female sex, haemoglobin level <10 g/dL, WCC $>50\times10^9$/L and thrombocytopaenia are established adverse prognostic factors in MDS/MPN-N.[56] Approximately 40% of MDS/MPN-N patients progress to acute myeloid leukaemia (AML).[47] Stem cell transplantation improves outcomes for these patients.[57]

MDS/MPN with *SF3B1* mutation and thrombocytosis (MDS/MPN-SF3B1-T), previously termed MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), is defined by the presence of an *SF3B1* mutation. While the term MDS/MPN-RS-T remains acceptable for cases with ≥15% ring sideroblasts and wild-type *SF3B1* or unknown mutation status, the emphasis on the SF3B1 mutation underscores the underlying pathophysiology. MDS/MPN-*SF3B1*-T predominantly affects females. Approximately 40% of patients present with splenomegaly and persistent thrombocytosis exceeding 450×10^9/L is characteristic.[58] Anaemia and low blast counts (<1% PB and <5% BM) are common.[5] BM is hypercellular with increased erythropoiesis and dyserythropoiesis, characterised by ring sideroblasts.[59 60] Iron stains highlight these cells, with at least five siderotic granules covering a third of the nuclear circumference.[61] While *SF3B1* mutations are the primary driver, concomitant activating mutations in *JAK2, MPL* or *CALR* signalling pathway genes occur in approximately 57% of cases[62 63] (figure 3). Low-level *JAK2* mutations often represent a small clone responsible for thrombocytosis and an ET-like phenotype.[64] In the absence of

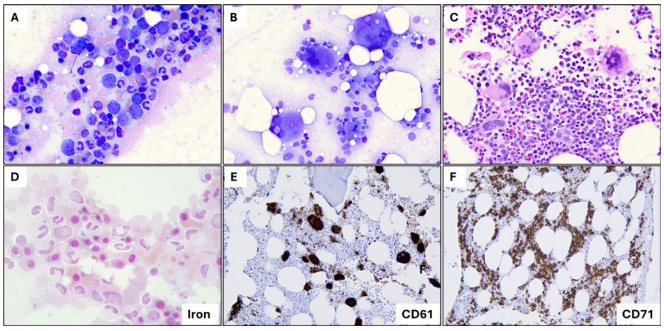

Figure 3 Myelodysplastic/myeloproliferative neoplasm with *SF3B1* and thrombocytosis. Next-generation sequencing of this sample revealed *SF3B1* p.K700E and *JAK2* p.V617F mutations. (A) Bone marrow aspirate reveals erythroid hyperplasia with dysplastic features including megaloblastic changes and binucleation. (B) Bone marrow aspirate reveals increased large, hyperlobulated megakaryocytes. (C) Bone marrow biopsy is hypercellular and shows increased large, hyperlobulated megakaryocytes. (D) Iron stain shows scattered ring sideroblasts (nucleated erythroid precursors with perinuclear iron granules). (E) CD61 immunohistochemistry highlights increased large, hyperlobulated megakaryocytes with loose clusters. (F) CD71 immunohistochemistry highlights erythroid hyperplasia.

molecular testing, sustained thrombocytosis for at least 3 months can serve as a surrogate for these mutations.[5] It is essential to exclude therapy-related myeloid neoplasms, MDS with isolated del(5q), myeloid neoplasms with double-hit *TP53* alteration, myeloid neoplasms with t(3;3)(q21.3;q26.2) and disease-defining gene fusions like *BCR::ABL1*.[5] MDS/MPN-*SF3B1*-T generally carries a favourable prognosis compared with other MDS/MPN subtypes. However, factors like abnormal karyotype, *ASXL1* and/or *SETBP1* mutations, and haemoglobin levels below 10 g/dL confer adverse risk.[42] Unique molecular characteristics beyond the *SF3B1* mutation suggest potential therapeutic avenues.[65]

ACUTE MYELOID LEUKAEMIA

WHO-HEM5 categorises AML into two primary groups: AML with defining genetic abnormalities and AML defined by differentiation. Changes can be summarised as (table 1):

- The 20% blast threshold, previously mandatory for all AML cases, is eliminated for those with defining genetic abnormalities. The list of genetic abnormalities defining specific AML subgroups has expanded from WHO-HEM4R to include novel variant translocations involving *RARA*, *KMT2A*, *MECOM* and *NUP98*, as well as other rearrangements and gene mutations like *NPM1* and *CEBPA*. The 20% blast cut-off remains for AML with *BCR::ABL1* fusion and AML with *CEBPA* mutations due to insufficient evidence to modify the diagnostic threshold.
- WHO-HEM5 removes the provisional entity of AML with mutated *RUNX1* from WHO-HEM4R.
- AML with myelodysplasia-related changes is now termed AML, myelodysplasia-related (AML-MR), requiring ≥20% blasts expressing a myeloid phenotype and harbouring specific MDS-associated genetic abnormalities, either de novo or evolving from a preexisting MDS or MDS/MPN. Morphological evidence of dysplasia is no longer a diagnostic criterion. Instead, the classification relies on a the presence of somatic mutations in one of the eight genes (*SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*, *ASXL1*, *EZH2*, *BCOR*, *STAG2*), and/or defining cytogenetic abnormalities including complex karyotype commonly mutated in secondary AML.[66]
- AML with other defined genetic alterations encompasses emerging or provisional AML subtypes with distinct genetic aberrations, including AML with *RUNX1T3(CBFA2T3)::GLIS2*, *KAT6A::CREBBP*, *FUS::ERG*, *MNX1::ETV6* or *NPM1::MLF1*. While these entities offer valuable insights, large-scale studies are necessary to establish their prognostic significance.
- AML cases lacking defining genetic abnormalities are classified based on differentiation through immunophenotyping. Acute erythroid leukaemia, previously termed pure erythroid leukaemia, is now recognised as a distinct entity within this group, with biallelic *TP53* mutations playing a pivotal role in pathogenesis.[67 68] The AML NOS category has been eliminated.

These changes reflect a deeper understanding of AML pathogenesis and aim to enhance classification accuracy and clinical utility.

Potential challenges for LMIC implementation and managing AMLs with limited resources

Diagnosis and classification of AML according to WHO-HEM5 require a comprehensive approach encompassing microscopic examination (including cytochemistry), immunophenotyping, cytogenetics and molecular studies. Resource-limited settings lacking specialised flow cytometry, cytogenetics and molecular facilities face significant challenges in performing detailed immunophenotyping and identifying cytogenetic and molecular abnormalities essential for definitive AML subclassification.[69–71] Accurately classifying AML subtypes defined by genetic aberrations and AML-MR, which rely on chromosome assays, or RNA and DNA NGS assays, is particularly challenging in these contexts. In such settings, treatment decisions may guide laboratory investigations.

Traditionally, acute leukaemia is defined by an increased blast count (≥20%) in PB or BM smears. While Auer rods are characteristic of AML, they are absent in many cases. Cytochemical MPO or NSE positivity (>3% blasts) further supports myeloid or monocytic AML. Morphology and cytochemistry can diagnose some AML with myeloid or monocytic differentiation, but immunophenotyping is essential for determining blast lineage (myeloid, monocytic, megakaryocytic, erythroid and basophilic) and diagnosing mixed-phenotypic leukaemia[72] (figures 4 and 5). Typical acute promyelocytic leukaemia (APL) cases can be diagnosed based on morphology and strong MPO positivity, but confirmation requires cytogenetic or molecular methods to document *PML::RARA* fusion. It should be noted the typical hypergranular APL characteristically lacks expression of CD34 and HLA-DR, which serves as a diagnostic clue along with high side scatter and forward scatter on flow cytometry. In resource-limited settings, morphology, cytochemistry and immunophenotyping are crucial for AML classification and treatment initiation. Genetic analysis, particularly for common fusions and mutations like *NPM1* and *CEBPA*, aids in risk stratification and treatment planning. Morphological and immunophenotypic findings can guide genetic testing. Examples include (a) AML with coexpression of lymphoid markers such as dim CD19 and CD56 suggest the presence of a *RUNX1::RUNX1T1* fusion; (b) AML with cup-like nuclei and absence of CD34 or HLA-DR expression predict the presence of *NPM1* mutations and *FLT3-ITD*. In a patient with blasts having cup-like nuclei, mutant NPM1 protein can be confirmed by IHC in BM biopsies and an accurate diagnosis

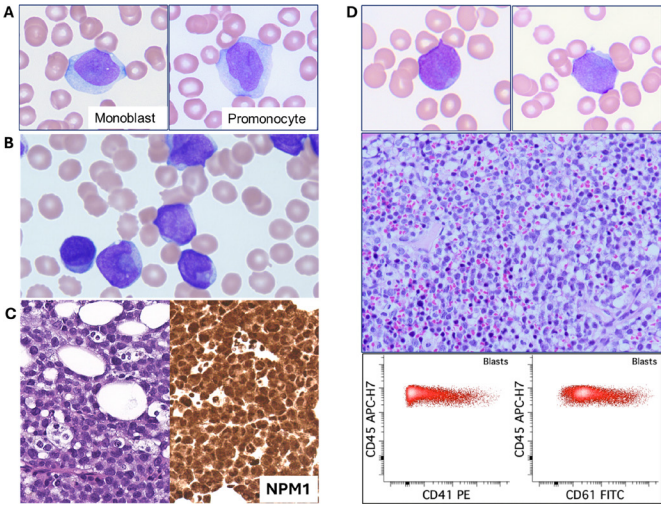

Figure 4 Examples of AML. (A) AML with NPM1 mutation. Blasts equivalents in the peripheral blood include monoblasts and promonocytes. (B) AML with NPM1 mutation. Blasts with cup-like nuclei in peripheral blood smear. (C) Bone marrow biopsy in a patient with AML with NPM1 mutation. Marrow shows sheets of blasts with strong cytoplasmic and nuclear NPM1 expression by immunohistochemistry. (D) AML with megakaryocytic differentiation. Bone marrow shows a diffuse infiltrate of large blastic cells. Flow cytometry reveals variable CD41 and CD61 expression on the blasts. AML, acute myeloid leukaemia.

can be achieved even in patients with <10% blasts (figure 4); (c) In paediatric acute megakaryoblastic leukaemia that is not associated with Down syndrome (DS), *CBFA2T3::GLIS2* fusion is the most frequent genetic aberration and is associated with RAM phenotype characterised by expression of CD56 (bright), CD33 (moderate to bright), CD34 (variable or moderate) and CD117 (moderate to bright) and dim to absent CD11b, CD13, CD36, CD38, CD45 and HLA-DR and (d) Paediatric myeloid leukaemia in children with DS are associated with *GATA1* mutations in addition to trisomy 21 and other genetic abnormalities, can have <20% blasts in the BM and express megakaryocytic markers.[73–82]

In situations where immunophenotypic or genetic information is unavailable, AML can be categorised as 'AML, NFC'. The newly defined genetic entities like AML-MR and those with other specified genetic alterations require advanced molecular techniques such as DNA and RNA sequencing. Notably, these entities, associated with poor prognosis, are rare and comprise a small fraction of AML cases. Accurate identification of these entities is crucial for appropriate risk stratification and improved OS in regions with insufficient resources, expertise and access to advanced therapies.

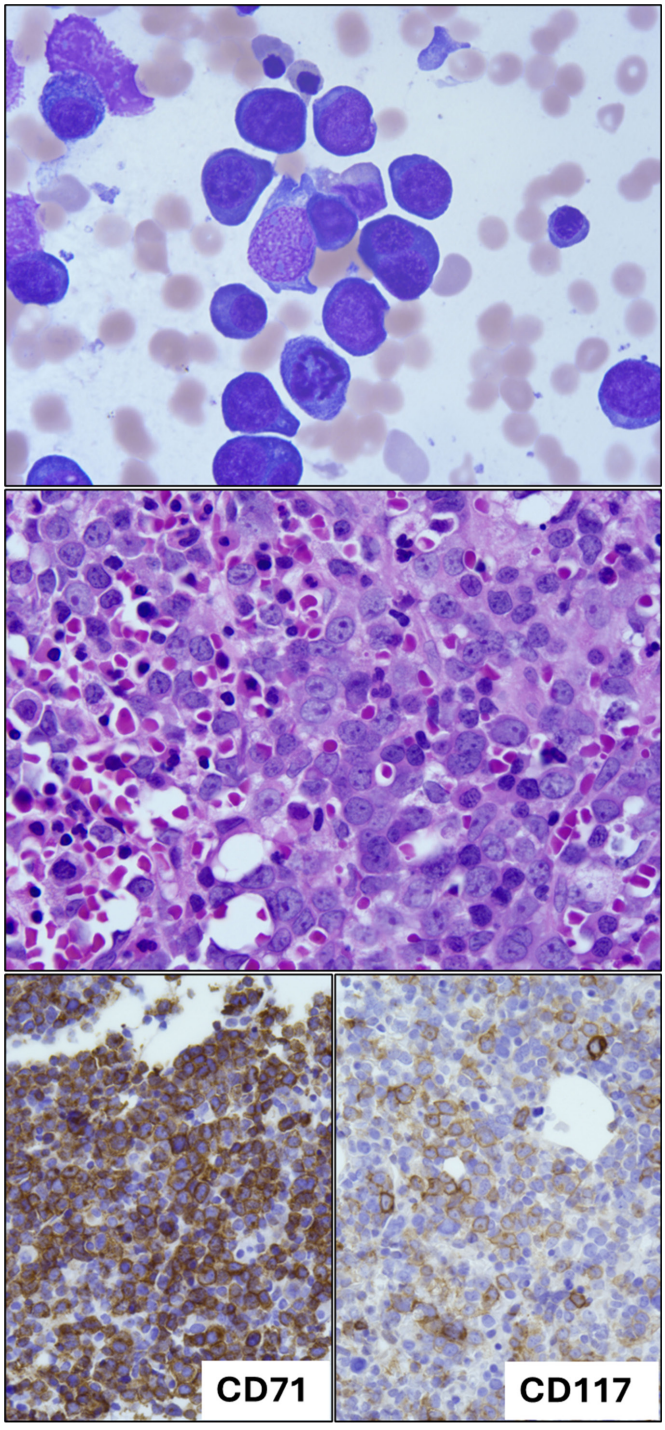

Figure 5 Acute erythroid leukaemia. Bone marrow aspirate smear and biopsy show ≥30% proerythroblasts, with expression of CD71 and CD117.

MASTOCYTOSIS

Similar to WHO-HEM4R, mastocytosis is categorised into three primary types in WHO-HEM5: cutaneous mastocytosis, systemic mastocytosis (SM) and mast cell sarcoma.[83] WHO-HEM5 refines diagnostic criteria for SM, now including CD30 expression in mast cells and additional activating *KIT* mutations as minor criteria.[84–89] Baseline serum tryptase >20 ng/mL, adjusted in cases of known hereditary alpha-tryptasemia, is also considered a minor criterion for SM. BM mastocytosis, previously a subtype of indolent SM in WHO-HEM4R, is now listed as a separate subtype of SM in WHO-HEM5, defined by SM criteria, absence of skin lesions and B findings, basal serum tryptase <125 ng/mL and no dense mast cell infiltrates in extramedullary organs. Well-differentiated SM may be seen in any SM subtype and is characterised by round, granulated mast cells that are typically positive for CD30 and negative for CD2 and CD25, and lack *KIT* p.D816V mutation but carry other *KIT* mutations that do not cause resistance to imatinib.[90–93] WHO-HEM5 modifies B findings (disease burden) to include *KIT* D816V mutation with a variant allele frequency (VAF) of ≥10% as an indicator of high mast cell burden.

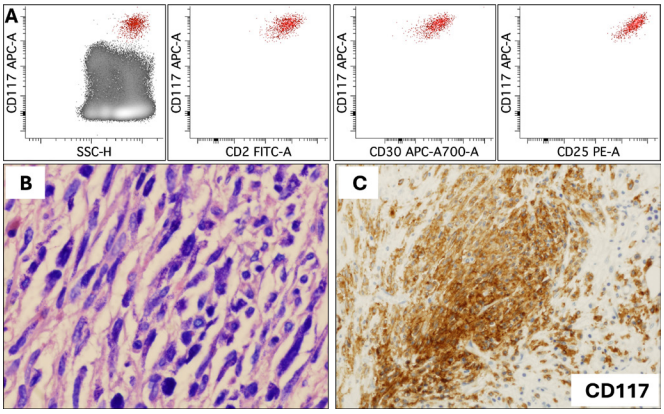

Figure 6 Systemic mastocytosis. Molecular studies detected *KIT* p.D816V mutation. (A) Flow cytometry: A CD117-bright mast cell population is identified in the bone marrow with expression of CD2, CD25 and CD30 (dim). (B) Giemsa-stained section of bone marrow biopsy shows mast cell aggregates with spindled morphology. (C) The mast cell aggregates are highlighted by CD117 immunohistochemistry.

CD30 is present in most cases of SM, including well-differentiated SM, which typically lacks CD25.[85 87 88] CD30, when combined with CD25, has been shown to improve the diagnostic accuracy of SM and is recognised as a potential target for antibody-based therapy.[85 86 88] Routine CD30 assessment via IHC or flow cytometry is essential for SM evaluation.

KIT p.D816V mutation is present in >90% of patients with SM and results in constitutively active *KIT* receptor tyrosine kinase, conferring resistance to imatinib. Multilineage involvement by *KIT* D816V mutation has been demonstrated in essentially all patients with aggressive SM and 20%–30% indolent SM.[94] Other rare activating *KIT* mutations have been detected in the extracellular, transmembrane and juxtamembrane domains and are sensitive to imatinib.[95 96] Additional somatic mutations, such as *TET2*, *SRSF2*, *ASXL1*, *EZH2*, *CBL*, *RUNX1*, *JAK2* and *RAS*, have been identified in the majority of advanced SM, but less frequently found in indolent and smouldering SM. Since *KIT* p.D816V mutation or other activating *KIT* mutations detected in PB, BM or other extracutaneous organ(s) serves as a minor criterion for SM, molecular studies including highly sensitive PCR assays to detect recurrent *KIT* p.D816V mutation and NGS panel to identify mutations in other domains of *KIT* gene and the broader genetic landscape of SM, are crucial complementary tools in diagnosing and subclassifying SM.[97 98]

Integrating morphology (BM mast cell infiltrate), laboratory (serum tryptase), immunophenotype (CD2, CD25, CD30) and molecular data (*KIT* mutations, other gene mutations) is essential for SM diagnosis, prognosis and treatment planning (figure 6). In resource-limited settings, morphology, laboratory testing and IHC should guide SM diagnosis based on WHO criteria.

Diagnostic challenges myeloid neoplams in LMICs

We recognise the challenges of implementing the latest WHO-HAEM5 genetic and molecular AML classification systems in LMICs. LMICs may not have skilled flow cytometry, cytogenetic and molecular facilities, limiting capacity to perform a detailed immunophenotyping analysis and identify cytogenetic and molecular abnormalities for definitive subclassification of myeloid neoplasms.[69 71] In a dated reference from 2012, 10% of NGS labs reside in LMICs, and specifically none in low-income countries.[70] In particular, classifying myeloid neoplasms with defining genetic abnormalities including gene mutations and fusions, which are best detected through RNA and DNA NGS assays will pose a significant challenge. In such a scenario, the available treatment options can guide the laboratory workup.

Significant improvements in pathology and laboratory medicine services in LMICs are needed.[99] Key barriers include developing a skilled workforce, providing education and training, establishing well-equipped laboratories and hospital infrastructure, ensuring international quality standards and accreditation, and sustaining reagent and equipment supply.[99] Recent advancements in digital pathology offer opportunities for disseminating virtual pathology education and accessing expertise.[100 101] By adopting these strategies, LMICs can address challenges, improve patient outcomes and enhance therapeutic strategies.

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REFERENCES

1 Ayachi S, Buscarlet M, Busque L. 60 Years of clonal hematopoiesis research: From X-chromosome inactivation studies to the identification of driver mutations. *Exp Hematol* 2020;83:2–11.
2 Xie M, Lu C, Wang J, *et al*. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med* 2014;20:1472–8.
3 Genovese G, Kähler AK, Handsaker RE, *et al*. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* 2014;371:2477–87.
4 Jaiswal S, Fontanillas P, Flannick J, *et al*. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* 2014;371:2488–98.
5 Khoury JD, Solary E, Abla O, *et al*. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Myeloid and Histiocytic/Dendritic Neoplasms. *Leukemia* 2022;36:1703–19.
6 Kusne Y, Xie Z, Patnaik MM. Clonal hematopoiesis: Molecular and clinical implications. *Leuk Res* 2022;113:106787.
7 Jongen-Lavrencic M, Grob T, Hanekamp D, *et al*. Molecular Minimal Residual Disease in Acute Myeloid Leukemia. *N Engl J Med* 2018;378:1189–99.
8 Patkar N, Kakirde C, Shaikh AF, *et al*. Clinical impact of panel-based error-corrected next generation sequencing versus flow cytometry to detect measurable residual disease (MRD) in acute myeloid leukemia (AML). *Leukemia* 2021;35:1392–404.
9 Weeks LD, Niroula A, Neuberg D, *et al*. Prediction of risk for myeloid malignancy in clonal hematopoiesis. *NEJM Evid* 2023;2.
10 Bolton KL, Ptashkin RN, Gao T, *et al*. Cancer therapy shapes the fitness landscape of clonal hematopoiesis. *Nat Genet* 2020;52:1219–26.
11 Yong ASM, Melo JV. The impact of gene profiling in chronic myeloid leukaemia. *Best Pract Res Clin Haematol* 2009;22:181–90.
12 Rea D, Etienne G, Nicolini F, *et al*. First-line imatinib mesylate in patients with newly diagnosed accelerated phase-chronic myeloid leukemia. *Leukemia* 2012;26:2254–9.
13 Lauseker M, Bachl K, Turkina A, *et al*. Prognosis of patients with chronic myeloid leukemia presenting in advanced phase is defined mainly by blast count, but also by age, chromosomal aberrations and hemoglobin. *Am J Hematol* 2019;94:1236–43.
14 Vijayasekharan K, Chatterjee G, Ramanathan S, *et al*. Sudden blast phase in pediatric chronic myeloid leukemia-chronic phase with abnormal lymphoid blasts detected by flow cytometry at diagnosis: Can it be considered a warning sign? *Cytometry B Clin Cytom* 2021;100:345–51.
15 El Rassi F, Bergsagel JD, Arellano M, *et al*. Predicting early blast transformation in chronic-phase chronic myeloid leukemia: is immunophenotyping the missing link? *Cancer* 2015;121:872–5.

16 Wang SA, Hasserjian RP, Tam W, *et al*. Bone marrow morphology is a strong discriminator between chronic eosinophilic leukemia, not otherwise specified and reactive idiopathic hypereosinophilic syndrome. *Haematologica* 2017;102:1352–60.
17 Morsia E, Reichard K, Pardanani A, *et al*. WHO defined chronic eosinophilic leukemia, not otherwise specified (CEL, NOS): A contemporary series from the Mayo Clinic. *Am J Hematol* 2020;95:E172–4.
18 Naresh KN. Classification of Philadelphia translocation-negative myeloproliferative neoplasms: its impact on data from clinical trials. *J Clin Oncol* 2009;27:e175–6.
19 Thiele J, Kvasnicka HM, Orazi A. Bone marrow histopathology in myeloproliferative disorders--current diagnostic approach. *Semin Hematol* 2005;42:184–95.
20 Thiele J, Kvasnicka HM, Beelen DW, *et al*. Megakaryopoiesis and myelofibrosis in chronic myeloid leukemia after allogeneic bone marrow transplantation: an immunohistochemical study of 127 patients. *Mod Pathol* 2001;14:129–38.
21 Side LE, Emanuel PD, Taylor B, *et al*. Mutations of the NF1 gene in children with juvenile myelomonocytic leukemia without clinical evidence of neurofibromatosis, type 1. *Blood* 1998;92:267–72.
22 Kratz CP, Niemeyer CM, Castleberry RP, *et al*. The mutational spectrum of PTPN11 in juvenile myelomonocytic leukemia and Noonan syndrome/myeloproliferative disease. *Blood* 2005;106:2183–5.
23 Hecht A, Meyer JA, Behnert A, *et al*. Molecular and phenotypic diversity of CBL-mutated juvenile myelomonocytic leukemia. *Haematologica* 2022;107:178–86.
24 Greenberg PL, Tuechler H, Schanz J, *et al*. Cytopenia levels for aiding establishment of the diagnosis of myelodysplastic syndromes. *Blood* 2016;128:2096–7.
25 Malcovati L, Karimi M, Papaemmanuil E, *et al*. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. *Blood* 2015;126:233–41.
26 Malcovati L, Stevenson K, Papaemmanuil E, *et al*. SF3B1-mutant MDS as a distinct disease subtype: a proposal from the International Working Group for the Prognosis of MDS. *Blood* 2020;136:157–70.
27 Duetz C, Westers TM, In 't Hout FEM, *et al*. Distinct bone marrow immunophenotypic features define the splicing factor 3B subunit 1 (SF3B1)-mutant myelodysplastic syndromes subtype. *Br J Haematol* 2021;193:798–803.
28 Tashakori M, Kadia T, Loghavi S, *et al*. TP53 copy number and protein expression inform mutation status across risk categories in acute myeloid leukemia. *Blood* 2022;140:58–72.
29 Loghavi S, Al-Ibraheemi A, Zuo Z, *et al*. TP53 overexpression is an independent adverse prognostic factor in de novo myelodysplastic syndromes with fibrosis. *Br J Haematol* 2015;171:91–9.
30 Bernard E, Nannya Y, Hasserjian RP, *et al*. Implications of TP53 allelic state for genome stability, clinical presentation and outcomes in myelodysplastic syndromes. *Nat Med* 2020;26:1549–56.
31 Zhang J, Walsh MF, Wu G, *et al*. Germline Mutations in Predisposition Genes in Pediatric Cancer. *N Engl J Med* 2015;373:2336–46.
32 Yoshizato T, Nannya Y, Atsuta Y, *et al*. Genetic abnormalities in myelodysplasia and secondary acute myeloid leukemia: impact on outcome of stem cell transplantation. *Blood* 2017;129:2347–58.
33 Sung YN, Kim D, Kim J. p53 immunostaining pattern is a useful surrogate marker for TP53 gene mutations. *Diagn Pathol* 2022;17:92.
34 Bono E, McLornan D, Travaglino E, *et al*. Clinical, histopathological and molecular characterization of hypoplastic myelodysplastic syndrome. *Leukemia* 2019;33:2495–505.
35 Marisavljevic D, Cemerikic V, Rolovic Z, *et al*. Hypocellular myelodysplastic syndromes: clinical and biological significance. *Med Oncol* 2005;22:169–75.
36 Della Porta MG, Malcovati L, Boveri E, *et al*. Clinical relevance of bone marrow fibrosis and CD34-positive cell clusters in primary myelodysplastic syndromes. *J Clin Oncol* 2009;27:754–62.
37 Chen X, Johansson U, Cherian S. Flow Cytometric Assessment of Myelodysplastic Syndromes/Neoplasms. *Clin Lab Med* 2023;43:521–47.
38 Porwit A, van de Loosdrecht AA, Bettelheim P, *et al*. Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes-proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. *Leukemia* 2014;28:1793–8.
39 Goasguen JE, Bennett JM, Bain BJ, *et al*. Proposal for refining the definition of dysgranulopoiesis in acute myeloid leukemia and myelodysplastic syndromes. *Leuk Res* 2014;38:447–53.
40 Tang G, Wang SA, Menon M, *et al*. High-level CD34 expression on megakaryocytes independently predicts an adverse outcome in patients with myelodysplastic syndromes. *Leuk Res* 2011;35:766–70.
41 Thiele J, Quitmann H, Wagner S, *et al*. Dysmegakaryopoiesis in myelodysplastic syndromes (MDS): an immunomorphometric study of bone marrow trephine biopsy specimens. *J Clin Pathol* 1991;44:300–5.
42 Palomo L, Meggendorfer M, Hutter S, *et al*. Molecular landscape and clonal architecture of adult myelodysplastic/myeloproliferative neoplasms. *Blood* 2020;136:1851–62.
43 Fontana D, Elli EM, Pagni F, *et al*. Myelodysplastic Syndromes/Myeloproliferative Overlap Neoplasms and Differential Diagnosis in the WHO and ICC 2022 Era: A Focused Review. *Cancers (Basel)* 2023;15:12.
44 Selimoglu-Buet D, Wagner-Ballon O, Saada V, *et al*. Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia. *Blood* 2015;125:3618–26.
45 Barge L, Gooch M, Hendle M, *et al*. Real world implementation of flow cytometric monocyte subset partitioning for distinguishing chronic myelomonocytic leukaemia from other causes of monocytosis. *Pathology (Phila)* 2023;55:827–34.
46 Kurzrock R, Bueso-Ramos CE, Kantarjian H, *et al*. BCR rearrangement-negative chronic myelogenous leukemia revisited. *J Clin Oncol* 2001;19:2915–26.
47 Wang SA, Hasserjian RP, Fox PS, *et al*. Atypical chronic myeloid leukemia is clinically distinct from unclassifiable myelodysplastic/myeloproliferative neoplasms. *Blood* 2014;123:2645–51.
48 Patnaik MM, Tefferi A. Atypical chronic myeloid leukemia and myelodysplastic/myeloproliferative neoplasm, not otherwise specified: 2023 update on diagnosis, risk stratification, and management. *Am J Hematol* 2023;98:681–9.
49 Arber DA, Orazi A, Hasserjian RP, *et al*. International Consensus Classification of Myeloid Neoplasms and Acute Leukemias: integrating morphologic, clinical, and genomic data. *Blood* 2022;140:1200–28.
50 Hernández JM, del Cañizo MC, Cuneo A, *et al*. Clinical, hematological and cytogenetic characteristics of atypical chronic myeloid leukemia. *Ann Oncol* 2000;11:441–4.
51 Meggendorfer M, Haferlach T, Alpermann T, *et al*. Specific molecular mutation patterns delineate chronic neutrophilic leukemia, atypical chronic myeloid leukemia, and chronic myelomonocytic leukemia. *Haematologica* 2014;99:e244–6.
52 Fontana D, Ramazzotti D, Aroldi A, *et al*. Integrated Genomic, Functional, and Prognostic Characterization of Atypical Chronic Myeloid Leukemia. *Hemasphere* 2020;4:e497.
53 Zhang H, Wilmot B, Bottomly D, *et al*. Genomic landscape of neutrophilic leukemias of ambiguous diagnosis. *Blood* 2019;134:867–79.
54 Piazza R, Valletta S, Winkelmann N, *et al*. Recurrent SETBP1 mutations in atypical chronic myeloid leukemia. *Nat Genet* 2013;45:18–24.
55 Montalban-Bravo G, Kanagal-Shamanna R, Sasaki K, *et al*. Clinicopathologic correlates and natural history of atypical chronic myeloid leukemia. *Cancer* 2021;127:3113–24.
56 Breccia M, Biondo F, Latagliata R, *et al*. Identification of risk factors in atypical chronic myeloid leukemia. *Haematologica* 2006;91:1566–8.
57 Koldehoff M, Beelen DW, Trenschel R, *et al*. Outcome of hematopoietic stem cell transplantation in patients with atypical chronic myeloid leukemia. *Bone Marrow Transplant* 2004;34:1047–50.
58 Broseus J, Florensa L, Zipperer E, *et al*. Clinical features and course of refractory anemia with ring sideroblasts associated with marked thrombocytosis. *Haematologica* 2012;97:1036–41.
59 Cazzola M, Malcovati L. Diagnosis and treatment of sideroblastic anemias: from defective heme synthesis to abnormal RNA splicing. *Hematology Am Soc Hematol Educ Program* 2015;2015:19–25.
60 Jeromin S, Haferlach T, Weissmann S, *et al*. Refractory anemia with ring sideroblasts and marked thrombocytosis cases harbor mutations in SF3B1 or other spliceosome genes accompanied by JAK2V617F and ASXL1 mutations. *Haematologica* 2015;100:e125–7.
61 Patnaik MM, Tefferi A. Refractory anemia with ring sideroblasts and RARS with thrombocytosis. *Am J Hematol* 2015;90:549–59.
62 Boissinot M, Garand R, Hamidou M, *et al*. The JAK2-V617F mutation and essential thrombocythemia features in a subset of patients with refractory anemia with ring sideroblasts (RARS). *Blood* 2006;108:1781–2.
63 Ceesay MM, Lea NC, Ingram W, *et al*. The JAK2 V617F mutation is rare in RARS but common in RARS-T. *Leukemia* 2006;20:2060–1.
64 Malcovati L, Della Porta MG, Pietra D, *et al*. Molecular and clinical features of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. *Blood* 2009;114:3538–45.
65 Montalban-Bravo G, Kanagal-Shamanna R, Darbaniyan F, *et al*. Clinical, genomic, and transcriptomic differences between myelodysplastic syndrome/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) and myelodysplastic syndrome with ring sideroblasts (MDS-RS). *Am J Hematol* 2021;96:E246–9.
66 Lindsley RC, Mar BG, Mazzola E, *et al*. Acute myeloid leukemia ontogeny is defined by distinct somatic mutations. *Blood* 2015;125:1367–76.
67 Fang H, Wang SA, Khoury JD, *et al*. Pure erythroid leukemia is characterized by biallelic *TP53* inactivation and abnormal p53 expression patterns in *de novo* and secondary cases. *Haematologica* 2022;107:2232–7.
68 Montalban-Bravo G, Benton CB, Wang SA, *et al*. More than 1 *TP53* abnormality is a dominant characteristic of pure erythroid leukemia. *Blood* 2017;129:2584–7.
69 Meillon-Garcia LA, Demichelis-Gómez R. Access to Therapy for Acute Myeloid Leukemia in the Developing World: Barriers and Solutions. *Curr Oncol Rep* 2020;22:125.
70 Kingsmore SF, Lantos JD, Dinwiddie DL, *et al*. Next-generation community genetics for low- and middle-income countries. *Genome Med* 2012;4:25.
71 Smith SM, Eadara A, Parkash V. Addressing quality and safety in anatomic pathology in low- and middle-income countries. *Front Med (Lausanne)* 2022;9:1060179.

72 Alaggio R, Amador C, Anagnostopoulos I, *et al*. The 5th edition of the World Health Organization Classification of Haematolymphoid Tumours: Lymphoid Neoplasms. *Leukemia* 2022;36:1720–48.
73 Yang D, Lee J, Mun Y, *et al*. Predictable prognostic factor of CD56 expression in patients with acute myeloid leukemia with t(8:21) after high dose cytarabine or allogeneic hematopoietic stem cell transplantation. *American J Hematol* 2007;82:1–5.
74 Shang L, Chen X, Liu Y, *et al*. The immunophenotypic characteristics and flow cytometric scoring system of acute myeloid leukemia with t(8;21) (q22;q22); RUNX1-RUNX1T1. *Int J Lab Hematol* 2019;41:23–31.
75 Falini B, Martelli MP, Bolli N. Immunohistochemistry predicts nucleophosmin (NPM) mutations in acute myeloid leukemia. *Blood* 2006;108:1999–2005.
76 Gruszka AM, Lavorgna S, Consalvo MI, *et al*. A monoclonal antibody against mutated nucleophosmin 1 for the molecular diagnosis of acute myeloid leukemias. *Blood* 2010;116:2096–102.
77 Kussick SJ, Stirewalt DL, Yi HS, *et al*. A distinctive nuclear morphology in acute myeloid leukemia is strongly associated with loss of HLA-DR expression and FLT3 internal tandem duplication. *Leukemia* 2004;18:1591–8.
78 Oertling E, Fuda F, Koduru P, *et al*. Distinct mutational and clinicopathologic profiles characterize acute myeloid leukemia with cup-like nuclei. *Leuk Res* 2023;124:106995.
79 Masetti R, Bertuccio SN, Pession A, *et al*. CBFA2T3-GLIS2-positive acute myeloid leukaemia. A peculiar paediatric entity. *Br J Haematol* 2019;184:337–47.
80 de Rooij JDE, Branstetter C, Ma J, *et al*. Pediatric non-Down syndrome acute megakaryoblastic leukemia is characterized by distinct genomic subsets with varying outcomes. *Nat Genet* 2017;49:451–6.
81 Labuhn M, Perkins K, Matzk S, *et al*. Mechanisms of Progression of Myeloid Preleukemia to Transformed Myeloid Leukemia in Children with Down Syndrome. *Cancer Cell* 2019;36:123–38.
82 Tunstall O, Bhatnagar N, James B, *et al*. Guidelines for the investigation and management of Transient Leukaemia of Down Syndrome. *Br J Haematol* 2018;182:200–11.
83 Valent P, Akin C, Gleixner KV, *et al*. Multidisciplinary Challenges in Mastocytosis and How to Address with Personalized Medicine Approaches. *Int J Mol Sci* 2019;20:12.
84 Valent P, Horny HP, Escribano L, *et al*. Diagnostic criteria and classification of mastocytosis: a consensus proposal. *Leuk Res* 2001;25:603–25.
85 Morgado JM, Perbellini O, Johnson RC, *et al*. CD30 expression by bone marrow mast cells from different diagnostic variants of systemic mastocytosis. *Histopathology* 2013;63:780–7.
86 Blatt K, Cerny-Reiterer S, Schwaab J, *et al*. Identification of the Ki-1 antigen (CD30) as a novel therapeutic target in systemic mastocytosis. *Blood* 2015;126:2832–41.
87 Sotlar K, Cerny-Reiterer S, Petat-Dutter K, *et al*. Aberrant expression of CD30 in neoplastic mast cells in high-grade mastocytosis. *Mod Pathol* 2011;24:585–95.
88 Valent P, Sotlar K, Horny HP. Aberrant expression of CD30 in aggressive systemic mastocytosis and mast cell leukemia: a differential diagnosis to consider in aggressive hematopoietic CD30-positive neoplasms. *Leuk Lymphoma* 2011;52:740–4.
89 Valent P, Akin C, Hartmann K, *et al*. Updated Diagnostic Criteria and Classification of Mast Cell Disorders: A Consensus Proposal. *Hemasphere* 2021;5:e646.
90 Akin C, Fumo G, Yavuz AS, *et al*. A novel form of mastocytosis associated with a transmembrane c-kit mutation and response to imatinib. *Blood* 2004;103:3222–5.
91 Álvarez-Twose I, Jara-Acevedo M, Morgado JM, *et al*. Clinical, immunophenotypic, and molecular characteristics of well-differentiated systemic mastocytosis. *J Allergy Clin Immunol* 2016;137:168–78.
92 Alvarez-Twose I, Matito A, Morgado JM, *et al*. Imatinib in systemic mastocytosis: a phase IV clinical trial in patients lacking exon 17 *KIT* mutations and review of the literature. *Oncotarget* 2017;8:68950–63.
93 Chen K, Hendrie PC, Naresh KN. Aleukemic mast cell leukemia, well-differentiated and chronic type. *Ann Hematol* 2023;102:1613–5.
94 Garcia-Montero AC, Jara-Acevedo M, Teodosio C, *et al*. KIT mutation in mast cells and other bone marrow hematopoietic cell lineages in systemic mast cell disorders: a prospective study of the Spanish Network on Mastocytosis (REMA) in a series of 113 patients. *Blood* 2006;108:2366–72.
95 Reiter A, George TI, Gotlib J. New developments in diagnosis, prognostication, and treatment of advanced systemic mastocytosis. *Blood* 2020;135:1365–76.
96 Arock M, Sotlar K, Akin C, *et al*. KIT mutation analysis in mast cell neoplasms: recommendations of the European Competence Network on Mastocytosis. *Leukemia* 2015;29:1223–32.
97 Kristensen T, Vestergaard H, Bindslev-Jensen C, *et al*. Sensitive *KIT* D816V mutation analysis of blood as a diagnostic test in mastocytosis. *American J Hematol* 2014;89:493–8.
98 Greiner G, Gurbisz M, Ratzinger F, *et al*. Digital PCR: A Sensitive and Precise Method for *KIT* D816V Quantification in Mastocytosis. *Clin Chem* 2018;64:547–55.
99 Wilson ML, Fleming KA, Kuti MA, *et al*. Access to pathology and laboratory medicine services: a crucial gap. *The Lancet* 2018;391:1927–38.
100 Hassell LA, Absar SF, Chauhan C, *et al*. Pathology Education Powered by Virtual and Digital Transformation: Now and the Future. *Arch Pathol Lab Med* 2023;147:474–91.
101 Seymour DJL, Graef KM, Iliyasu Y, *et al*. Pathology Training for Cancer Diagnosis in Africa. *Am J Clin Pathol* 2022;157:279–85.
102 Sahoo SS, Pastor VB, Goodings C, *et al*. Clinical evolution, genetic landscape and trajectories of clonal hematopoiesis in SAMD9/SAMD9L syndromes. *Nat Med* 2021;27:1806–17.