Review Article

Histopathology in the diagnosis of high-risk myelodysplastic syndromes

Hidekazu Kayano

Myelodysplastic syndromes (MDS) are clonal diseases characterized by cytopenia and dysplasia in the peripheral blood, and risk of transition to acute myeloid leukemia (AML) in the bone marrow.   In the current revision of the World Health Organization (WHO) classification for hematopoietic tissues, MDS are divided into low-, intermediate-, and high- risk groups according to their frequency of leukemic transformation and other biological indicators.   Accuracy in histological evaluation plus blast counting on bone marrow biopsy is essential for the differentiation of high-risk MDS from AML.   In this review, the value of histopathology in the diagnosis of high-risk MDS is discussed.

Keywords: Histopathology, Diagnosis, Myelodysplastic syndromes

BASIC CONCEPTS OF MYELODYSPLASTIC SYNDROMES

Myelodysplastic syndromes (MDS) refer to the clonal proliferation of hematopoietic stem cells that are characterized by cytopenia and dysplasia of the peripheral blood, ineffective hematopoiesis in the bone marrow, recurrent genetic abnormalities, and risk of transition to acute myeloid leukemia (AML).[1,2]   Classically, MDS develop in older adults presenting with macrocytic–normocytic anemia of unknown causes with a gradual increase in myeloblasts over several years, followed by the development of AML (Figure 1). Cytopenia, including anemia, in MDS is refractory to treatment and previously referred to as preleukemia because its pathological condition changes with time.   The leading phenotype of AML is myeloid, and contrary to its rarity, cases of monocytic or megakaryoblastic acute leukemias have also been reported.[2]

The term "MDS" encompasses different pathological conditions presenting hematopoietic disorders that are categorized into three major groups: low-, intermediate-, or high-risk, based on the frequency of transition to AML, prognosis prediction, and treatment selection.   Although low-risk MDS progress relatively slowly, they require differentiation from cytopenia and/or dysplasia because of other causes (Table 1). Regarding the high-risk group, differentiation from AML is imperative and accurate blast counting of the peripheral blood and bone marrow is an issue directly associated with the diagnosis and treatment selection, with a significant

impact on patient prognosis.

NEW METHODS OF PERCEIVING MDS

Tumors of hematopoietic and lymphoid tissues are diagnosed based on the World Health Organization (WHO) classification.   The third edition was published in 2001, incorporating genetic information, which was then followed by the fourth edition in 2008.   Upon further accumulation of data on gene mutations, a revision of the fourth edition of the WHO classification was announced in 2016 and released in a printed form in 2017 (the WHO 2016).[3]   The WHO 2016 attempted to unify and organize the disease nomenclature and counting methods for cytopenia and blast cell percentages (Table 2).[1]   Disease names, including expressions suggestive of the clinical course (e.g., refractory cytopenia) are overall avoided, and MDS is used as the unified disease name with adjective phrases, such as those suggesting the number of dysplastic lines (single or multilineage dysplasia), the presence of ring sideroblasts (RS), and the degree of blast increase (excess blasts or none).   However, refractory cytopenia of childhood, a provisional entity in the WHO 2016, remains unchanged.   Low-risk MDS comprise MDS with single lineage dysplasia (SLD), MDS-RS-SLD, and MDS with isolated del(5q); intermediate-risk MDS comprise MDS with multilineage dysplasia (MLD) and MDS-RS-MLD; and high-risk MDS comprise MDS with excess blasts (EB). Those that do not fall into any category are classified as MDS unclassifiable (MDS-U), but it is challenging to stratify
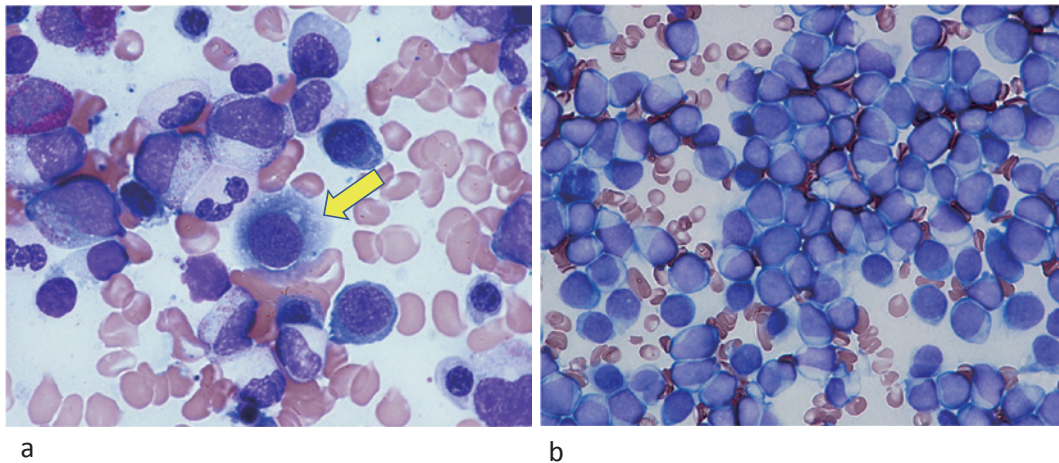
Fig. 1. An example of MDS evolving to AML
Bone marrow aspirates taken at diagnosis (***a***) and two years later (***b***) : (***a***) At diagnosis, small megakaryocytes (arrow) with hypolobated nuclei and cytoplasmic hypo-granulation of neutrophils were observed. (***b***) Leukemic transformation.

Table 1. Non-MDS with cytopenia and/or >10% dysplasia (adopted from Castello A, 1992)

· Drug/toxins including recent(<6months) chemotherapy and alcohol abuse
· metabolic deficiencies; Vit B12, folate, copper, etc.
· "Stress erythropoiesis" due to hemoglobinopathy or hemolytic anemias
· Infections such as HIV and hepatitis C
· Autoimmune diseases
· Neoplasms other than MDS; Hairy cell leukemia, myeloma, large granular lymphocytosis, etc.

because the risk for the transition of MDS-U to AML varies by case. To date, in the classification of all MDS, how the conventional morphological classification of MDS and several newly found molecular abnormalities are integrated and reflected remains currently unknown.

Based on the WHO classification system, AML and MDS are diagnosed hierarchically.[1,2] First, AML and MDS with specific chromosome/gene abnormalities or those related to treatment are preferentially diagnosed irrespective of the blast percentage. Then, AML is diagnosed if blasts account for ≥20% of bone marrow cells. *De novo* AML cases are classified into the disease form of AML with myelodysplasia-related change if marked dysplasia (>50%) is noted for two or more hematopoietic lines, there is a history of MDS or MDS/MPN, or MDS-related chromosomal abnormalities are found.

High-risk MDS comprise two types of MDS-EB that are subclassified into MDS-EB1 if the blast percentage is ≥2% in the peripheral blood or ≥5% in the bone marrow and the blast percentage does not exceed 5% in the peripheral blood or 10% in the bone marrow, or into MDS-EB2 if the blast percentage does not exceed 10% in the peripheral blood or 20% in the bone marrow, or Auer bodies are found. Typically, blast percentage increase is indicative of malignancy of MDS independent of the chromosome analysis, cytopenia, and gene abnormalities. Thus, accurate blast counting is imperative for diagnosis.

HISTOPATHOLOGICAL DIAGNOSIS OF THE BONE MARROW INVOLVED WITH MDS

The diagnosis of MDS is based on morphological evidence of dysplasia upon visual examination of bone marrow aspirate and biopsy.[1-5] For diagnosis, evaluation of blood cell dysplasia should be performed cautiously because abnormalities in the blood cell morphology are non-specific to MDS (Table 3).[4-6] Findings with relatively high-diagnostic utility are micromegakaryocytes, mature neutrophils with hypo-segmented nuclei, neutrophils with cytoplasmic hypo-granulation, and ring sideroblasts (Figure 2). Dyserythropoiesis is less useful for diagnosis than dysplasia of other lines.[7,8] Information obtained from additional studies such as karyotype, flow cytometry or molecular genetics is usually complementary and may help refine the diagnosis.[5,9-11]

Bone marrow biopsy is useful for diagnosis of MDS, and facilitates the evaluation of bone marrow cellularity and interstitial changes such as fibrosis.[4] For example, it may also be applied to hypoplastic MDS for treatment selection such as prioritizing immunotherapy over chemotherapy.[5] The histological evaluation of bone marrow is also useful to eliminate reactive pathology and improves the accuracy of diagnosis.[4] Furthermore, improved accuracy of diagnosis of MDS and collection of new findings is anticipated using bone marrow immunohistochemistry and molecular genetics analysis methods with formalin-fixed, paraffin-embedded (FFPE) specimens.

Histopathological specimens of the bone marrow are primarily categorized into aspirated preparations and needle or trephine biopsies. As these each have advantages, both types should be prepared whenever possible. The former group comprises smear specimens and clot sections using FFPE specimens prepared from bone marrow aspirate. Bone marrow needle or trephine biopsies are used for the evaluation of cellularity, which is evaluated as area percentages of nucleated cells and adipose tissue, determination of predominant hematopoietic lines in the tissue, and assessment of tissue

Table 2. New terminology for MDS in the WHO 2016

WHO 2008	→	WHO 2016

WHO 2008	WHO 2016
· Refractory cytopenia with unilineage dysplasia (RCUD)	· MDS with single lineage dysplasia (MDS-SLD)
· Refractory cytopenia with multilineage dysplasia (RCMD)	· MDS with multilineage dysplasia (MDS-MLD)
· Refractory anemia with ring sideroblasts (RARS)	· MDS with ring sideroblasts
· Refractory cytopenia with multilineage dysplasia and ring sideroblasts (RCMD-RS)	- MDS-RS with single lineage dysplasia (MDS-RS-SLD) -MDS-RS with multilineage dysplasia (MDS-RS-MLD)
· MDS with isolated del (5q)	· MDS with isolated del (5q)
· MDS, unclassifiable (MDS, U)	· MDS, unclassifiable (MDS,U)
· Refractory anemia with excess blasts (RAEB)	· MDS with excess blasts (MDS-EB)
· Refractory cytopenia of childhood (RCC) *provisional*	· Refractory cytopenia of childhood (RCC) *provisional*

Table 3. Dysplastic changes in non-MDS (adopted from Orazi A. 2007)

	Dysgranulopoiesis	Dyserythropoiesis	Dysmegakaryopoiesis
Paraneoplastic	○		○
B12/folate deficiency	○	○	
Infections	○	○ (HIV, B19)	○ (HIV)
Chemotherapy	○	○	○
Congenital	○ (Fanconi anemia)	○ (dysplastic anemia)	○ (Down syndrome)
Autoimmune		○	○
Transplantation		○	○
Aplastic anemia/PNH		○	

a   b   c
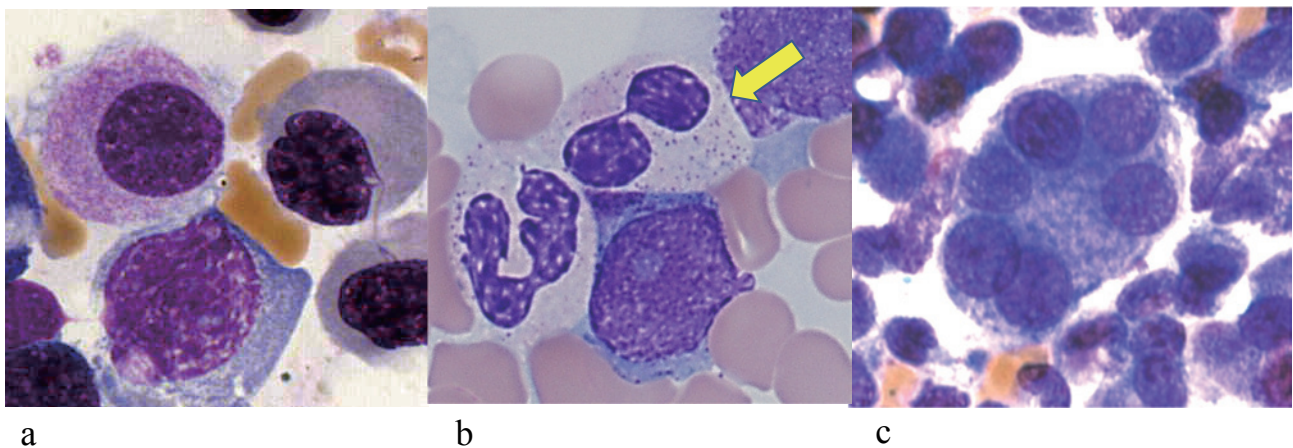
Fig. 2. Cytomorphology of MDS (bone marrow aspirate)
(***a***) Micromegakaryocyte with mature cytoplasm and round-shaped nucleus, and erythroblasts exhibiting nuclear bridging. (***b***) Mature neutrophil with bi-lobulated nucleus (arrow) (***c***) Megakaryocyte with round-shaped, separated nuclei

Table 4. Checklist for diagnosis of MDS on bone marrow biopsy (adopted from Orazi A. 2007)

□ Cellularity, lineage prevalence, architectural disturbance
□ Fibrosis and other stromal changes
□ Dysmegakaryopoiesis
□ Immunohistochemistry: CD34, P53, CD61,CD71, etc

construction (Table 4). Furthermore, bone marrow fibrosis, other interstitial changes, and trabecular changes are evaluated. For a comprehensive evaluation of the morphology of individual cells, bone marrow sections are of limited use compared with bone marrow aspirate films. As bone marrow needle biopsies require a decalcification step during the specimen preparation, these can be difficult to stain with iron staining and are sometimes limited in immunohistochemistry studies because of the possible loss of antigenicity; however, many antibodies have recently become available.

Desirable pathological specimens of the bone marrow typically meet the following criteria:

· Decalcification with EDTA is useful for immunohistochemical studies.

· Thin sections are preferred irrespective of clot sections or needle biopsies. As hematopoietic tissue comprises small cells compared with other tissues, determining the cell line with thick sections is challenging.

· In addition to basic H-E staining, the standard staining set to be performed includes Berlin blue staining, reticulum staining, trichrome staining, Giemsa staining, and Naphthol AS-D chloroacetate esterase (NASDA or Leder) staining. Berlin blue staining shows iron deposition. Reticulin staining and trichrome staining are used to observe interstitial features such as fibrosis and osteosclerosis. Double staining with high-quality LEDER staining and Giemsa staining readily distinguish cell lines. Furthermore, the PAS reaction has an advantage because megakaryocytes are readily visible; PAS-positive erythroblasts are characteristic of erythroleukemia.

## HISTOPATHOLOGY IN THE DIAGNOSIS OF HIGH-RISK MDS

When high-quality bone marrow specimens are obtained, the following points should be evaluated with bone marrow clot sections and/or needle biopsy specimens, in addition to dysplasia of blood cells and blast cell percentages with smear specimens.[4]

Bone marrow cellularity

In the bone marrow, adipose tissue increases with age and the proportion of area occupied by hematopoietic cells decreases. As a guide, 100 − age (%) has been traditionally used to calculate the approximate bone marrow cellularity. At the age of seventy or older, hypocellularity is defined as lower than 20% of hematopoietic tissue to the adjacent adipose tissues. In MDS, the bone marrow cellularity is often higher than the age-expected level, reflecting cytopenia and ineffective hematopoiesis; however, hypoplasia is observed in approximately 10% of cases. Even in these cases, care should be taken for blast cell counting because cases have to be classified as high-risk MDS if the proportion of blasts in the hematopoietic cells in the bone marrow is between 5 and 20%.

Topology in the marrow

In normal bone marrow, the three hematopoietic lines (erythroid, granulocytic or myeloid, and megakaryocytic series) are settled separately (Figure 3). In particular, the erythroblastic series form a cluster referred to as erythroblastic islands in the intertrabecular area, and megakaryocytes are scattered in the margin of the venous sinus. Immature granulocytic
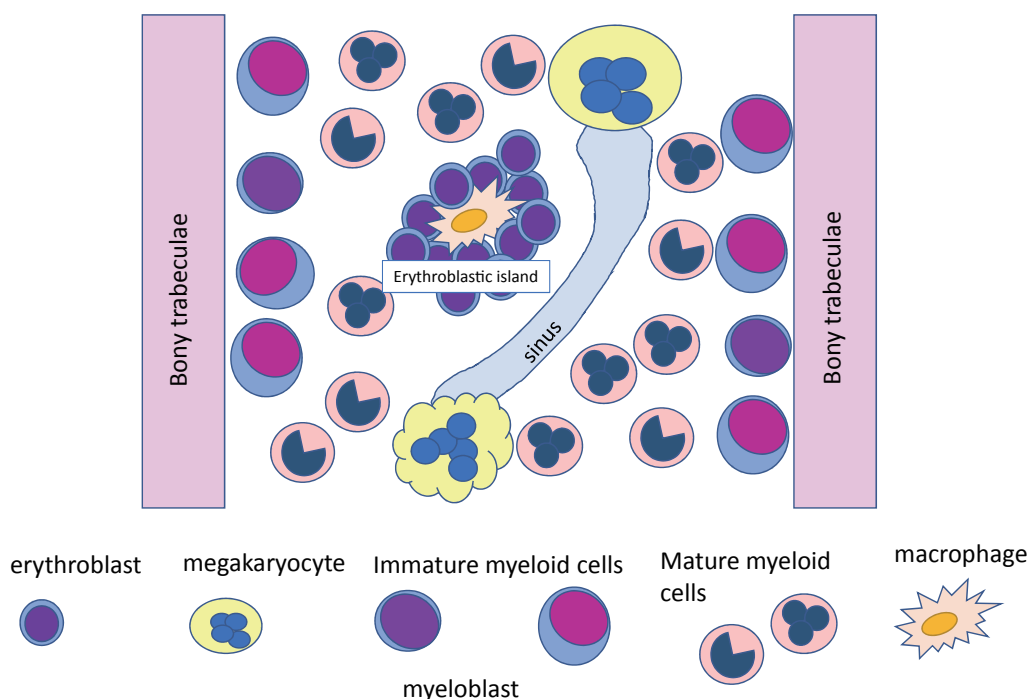

Fig. 3. Schematic illustration of normal bone marrow
Note paratrabecular granulopoiesis, intertrabecular erythroblastic island, and parasinusoidal megakaryocytes.

cells are found in the paratrabecular area in trabecular bones, suggesting migration to the intertrabecular area for maturation. Due to the distribution pattern of these three hematopoietic lines, certain patterns and gradations are observed when normal bone marrow tissue is observed under low magnification.

However, such topology is disturbed in MDS (Figure 4). In particular, immature myeloid cells migrate to the intertrabecular area and form clusters. On biopsy specimens, three or more aggregates of five or more immature granulocytic cells of myeloblasts or promyelocytes represent a pathological finding referred to as abnormal localization of immature precursors (ALIP). Although ALIP is comparatively easy to find in MDS-EB with increased blasts, ALIP can also be observed even in MDS where myelograms indicate a blast increase of <5%. The island formation of erythroblasts is often poor in MDS, and this is more common for high-risk MDS. As a result, the histology of normoplastic or hyperplastic bone marrow with MDS demonstrates a diffuse pattern with the configuration being lost; hypoplastic MDS share this lack of structure, and no noticeable differences are seen in cell density (Figure 5).
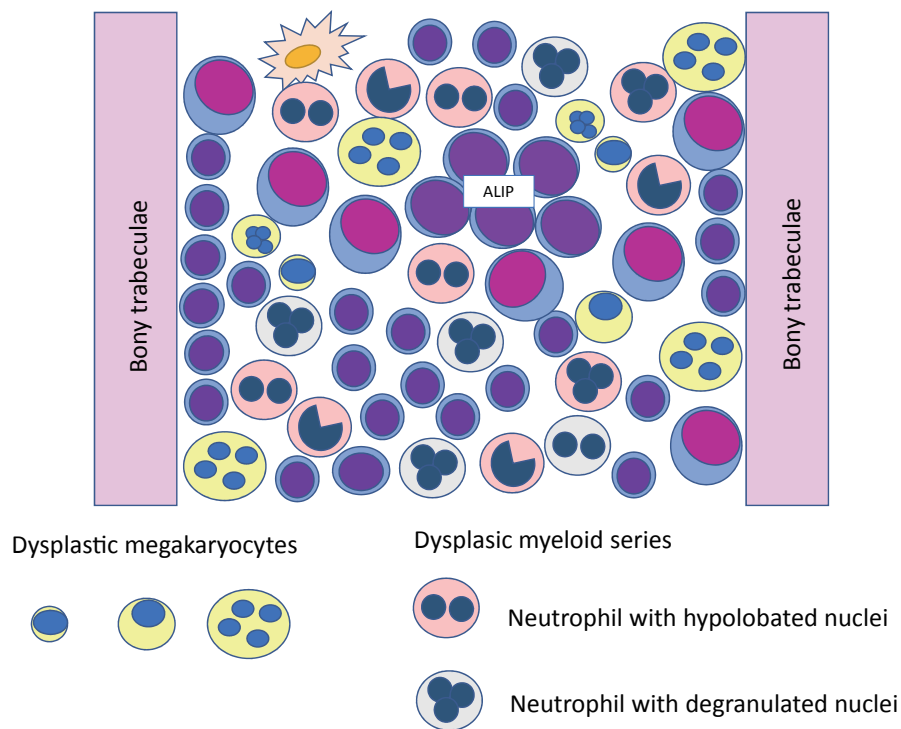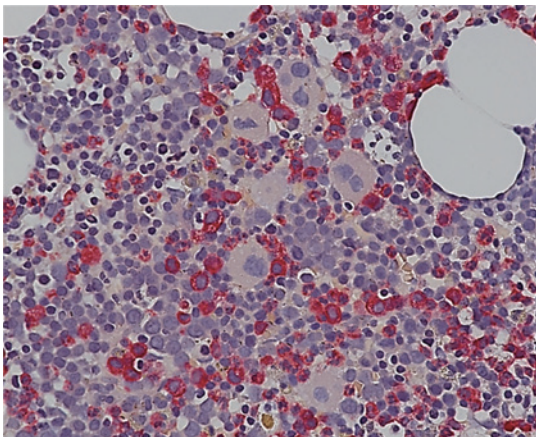

Fig. 4. Topographic distortion of hematopoiesis in MDS
In MDS, the precursors of the three cell lineages dispersed in all marrow regions in the bone marrow. ALIP; abnormal localization of immature precursors within the intertrabecular space.
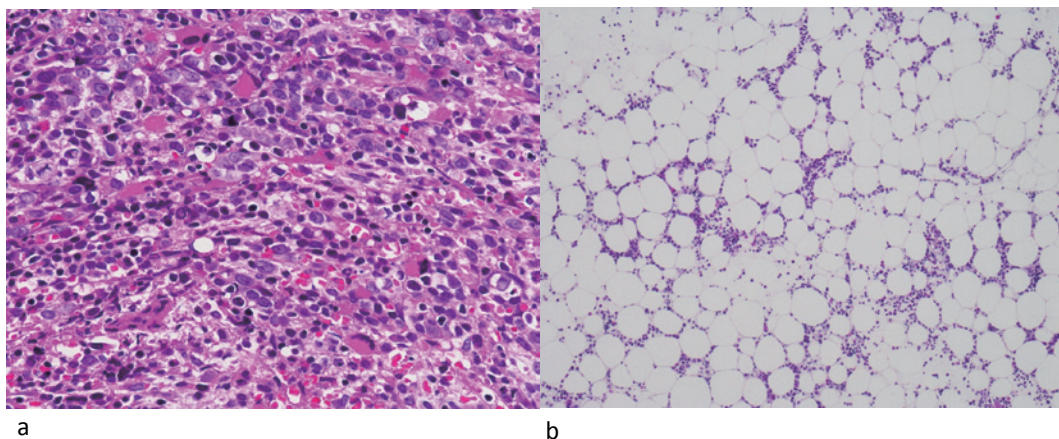
Fig. 5. Variations in the bone marrow cellularity due to MDS
(*a*) Hypercellular marrow intermingled with small megakaryocytes and dysplastic hematopoietic cells. (*b*) Hypocellular marrow showing diffuse distribution of hematopoietic components

Dysplasia

Morphological assessment of individual hematopoietic cells is not easy with tissue sections, but nuclear shape abnormalities of megakaryocytes are relatively easy to recognize (Figure 6). Separated circular nuclei in large megakaryocytes and non-segmented circular nuclei in small megakaryocytes are characteristic of MDS.[12] In MDS with chromosome 3q26 abnormality, megakaryocytic dysplasia is severe and the prognosis is similar to that of AML with the 3q26 abnormality.[2] Cell morphological abnormalities of erythroblasts are difficult to determine histologically and do not have a high impact on the diagnosis of MDS. Some bi-lobulated segmented nuclei and hypo-granulation of mature neutrophils can also be histologically observed. In MDS, iron deposition in bone marrow tissue is often increased. RS is an expression originally used to indicate five or more iron granules observed along the nucleus in smear preparations, but it can also be seen in tissue sections (Figure 7).
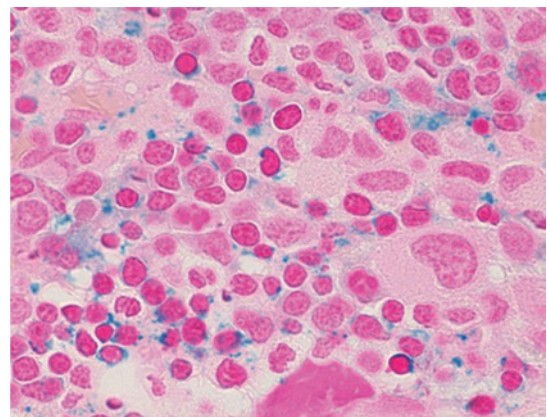
## IMMUNOHISTOCHEMISTRY USEFUL FOR DIAGNOSIS OF MDS

Although a substantial number of antibodies are available for immunohistochemical search of FFPE specimens, none are diagnostically specific to MDS. However, some antibodies provide useful information on the histopathological interpretation of bone marrow in MDS.

CD34

In the bone marrow, CD34 is positive in myeloblasts and the vascular endothelium. The blast percentage is critical because it is directly related to the subtyping of MDS. Of note, the blast percentages can be difficult to evaluate with smear specimens or imprint specimens, especially when the bone marrow is hypoplastic or fibrotic. Thus, CD34 immunostaining is a useful alternative to blood cell counting with smear specimens, especially when there is myelofibrosis or the quality of the aspirates is unsatisfactory (Figure 8).

Fig. 6. Dysplastic megakaryocytes in MDS (Leder stain)
Note the dysplastic megakaryocytes with non-lobulated nuclei.

Fig. 7. Ring sideroblasts (RS) in MDS (Berlin blue stain)
Ring sideroblasts (RS) can be detected even in FFEP sections, although RS-counting may not be consistent between sections and aspirate smears.

a   b
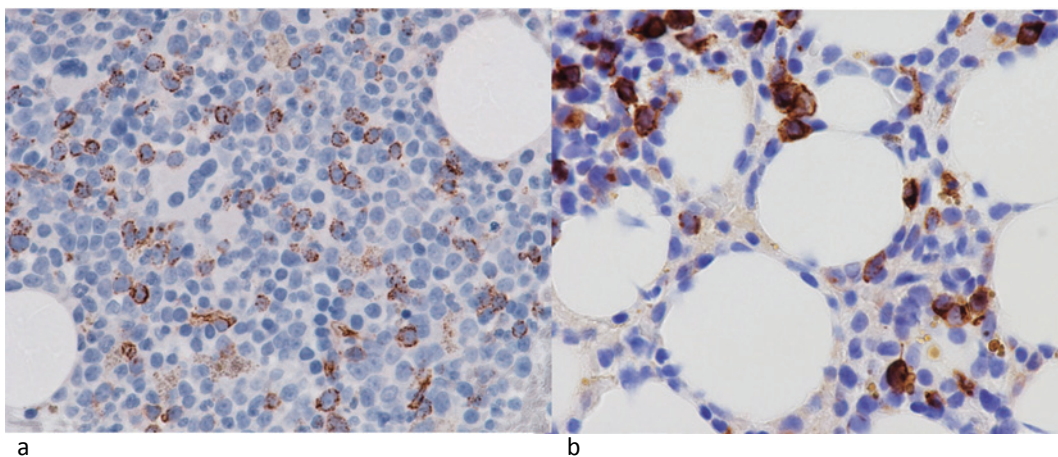
Fig. 8. CD34 immunostaining in MDS
In high-risk MDS, CD34-positive cells are frequently observed either in scattered (*a*) or clustered (*b*) patterns, the latter referred to as "abnormal multifocal accumulation (AMA) of CD34-positive cells"

Abnormal multifocal accumulation of CD34^{+} cells is a cluster of CD34^{+} blasts often detected in high-risk MDS, and is an expression of the ALIP immunophenotype that can only be confirmed by biopsy.[13,14] Hot spots, in which CD34^{+} blasts are abundantly found, are also diagnostically useful. In addition, immunostaining of CD34 is useful for assessing the microvessel density of the bone marrow and is expected to be applied to prognostic predictions.[15-17] However, it should be noted that CD34 is occasionally positive in megakaryocytes and erythroblasts. Markers applicable for detecting the myeloid–monocytic lineage comprise myeloperoxidase (MPO), CD68, and CD117 (c-Kit).

Megakaryocyte markers

In normal bone marrow tissue, megakaryocytes account for 1% of nucleated cells, approximately two cells per field are observed under high magnification, and small or micro megakaryocytes usually do not exceed 10% of the overall megakaryocytes but are not readily recognized with normal H-E sections. Some useful megakaryocyte markers are CD61, CD42b, and CD41, which are also useful for detecting small or micro megakaryocytes (Figure 9).[18] Typically, increases/decreases in megakaryocytes and abnormal distribution patterns (paratrabecular distribution) are also easier to evaluate.

Erythroid markers

Markers applicable for the detection of the erythroid series include hemoglobins, glycophorins, glucose transporter (Glut-1), and others. CD71 (transferrin receptor-1) is the leading marker used for immunohistochemistry and flow cytometry (Figure 10). Unlike other erythroblast markers,[19] CD71 offers the advantage of not being expressed in mature erythrocytes, making it easier to assess the increase/decrease and distribution pattern of erythroblasts.[20,21] However, CD71 is expressed in several types of cells and is occasionally highly expressed even in malignant lymphoma that has invaded the bone marrow. E-cadherin, an epithelial cell adhesion factor that interacts with β-catenin, is positive in immature, large erythroblasts such as proerythroblasts and
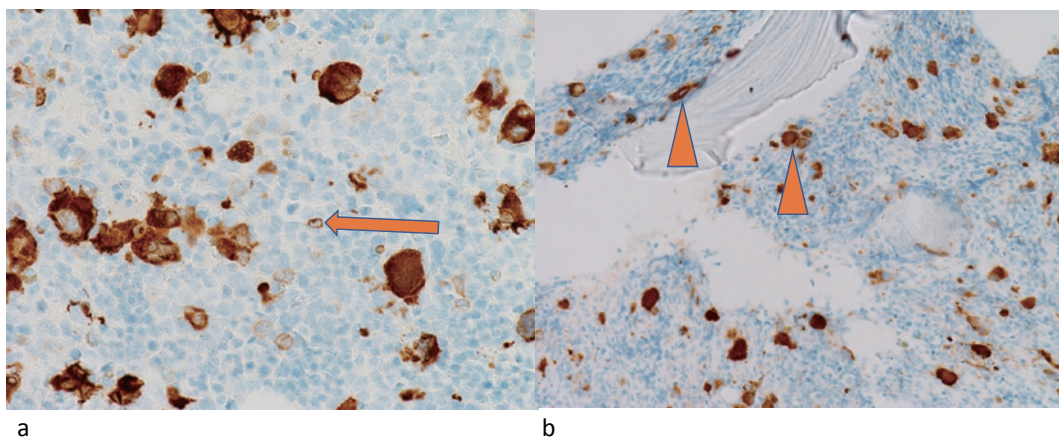
Fig. 9. CD61 immunostaining in MDS
Immunohistochemical detection of megakaryocytes highlights micromegakaryocytes (***a***, arrow) and paratrabecular distribution (***b***, arrow heads).
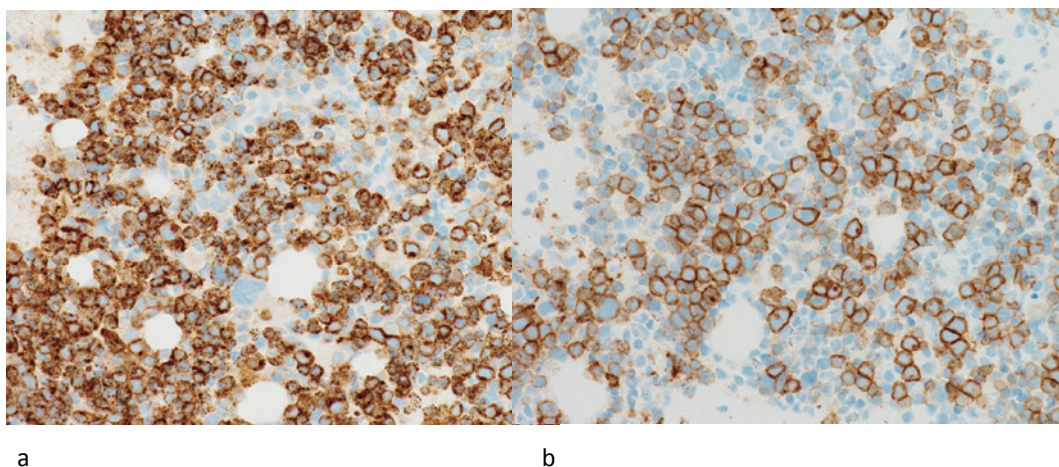
Fig. 10. Immunohistochemical detection of erythroblasts
CD71 (transferrin receptor-1, ***a***) is expressed by nucleated, mature and immature erythroblasts, whereas E-cadherin (***b***) is mainly expressed by immature erythroblasts.

basophilic erythroblasts.[22]

TP53 protein

TP53 is a classical tumor-suppressor gene. Previously, several studies on MDS have been reported, many of which have established its correlation with chromosomal abnormalities, blast percentages, myelofibrosis, and poor prognosis.[23-27] Positive outcomes of immunostaining are not necessarily attributable to gene mutations and should be interpreted judiciously. In particular, intranuclear positive images are often observed for proliferating erythroblasts, but the expression intensity varies from cell to cell (Figure 11).

DIFFERENTIAL DIAGNOSIS OF HIGH-RISK MDS

Hypoplastic MDS

In approximately 10% of MDS cases, the bone marrow cellularity is too low for the patient's age.[13] It is slightly more common in females and is commonly MDS-SLD. Of note, dysplastic erythroblasts can be found in aplastic anemia (AA), and there are some common features of low-risk MDS, such as the risk for developing MDS. In the bone marrow of AA, the decline of the three hematopoietic lineages, especially megakaryocytes, is pronounced, but megakaryocytes are frequently conserved in hypoplastic MDS. In hypoplastic MDS, small aggregates of blast cells can be observed in the fat septum, and CD34 immunostaining is helpful for differentiation from AML.

MDS with fibrosis (MDS-F)

Overall, 10%–15% patients with MDS have grade 2/3 or severer fibrosis in the bone marrow (Figure 12).[28,29] Cases related to increased myeloblasts are classified as MDS-EB. In MDS-F, dysplastic and pleomorphic megakaryocytes are often noticeable. In MDS, myelofibrosis is a sign of poor prognosis. In addition, fibrosis of the bone marrow may accompany several bone marrow tumors, including

therapy-related MDS, and can also be detected in infectious diseases and autoimmune diseases, thereby necessitating careful differentiation.

MDS with erythroid proliferation

Approximately 15% of patients with MDS exhibit erythroblast proliferation exceeding 50% of nucleated cells and confluent distribution (Figure 13). Although not listed as a classification item in the WHO classification, the accurate histopathological evaluation of erythroblast proliferation in the bone marrow is essential because of poor prognosis, indicated by chromosomal abnormalities, and treatment relevance in this group.[30,31] Cases with a myeloblast-to-non-erythroblastic cell percentage>20%, formerly classified as erythroleukemia in the WHO 2008, are currently classified as MDS-EB because the blast counting was revised in the WHO 2016. Although the uniqueness of MDS-E remains unclear, chromosomal abnormalities in the high-risk group and gene mutations, such as *TP53*, are considered to be associated with prognosis.

ALIP-like findings

Although not specific, ALIP is a crucial finding for the diagnosis of MDS. The following factors should be histologically differentiated: erythroblastic islands (Figure 14), granulocyte proliferation foci because of G-CSF administration, and aggregated lymphocytes (Figure 15). It is imperative not to misidentify the proliferation of immature erythroid cells, such as megaloblastic anemia, as myeloblasts. The presence of a rod-like nucleolus is a diagnostic clue. The observation of aggregated lymphocytes in the bone marrow of MDS is not uncommon, and these primarily comprise CD20+ B lymphocytes and are accompanied by increased reticulin fibers and lower hemoglobin levels not related to age or prognosis.[32]

FURTHER PERSPECTIVES
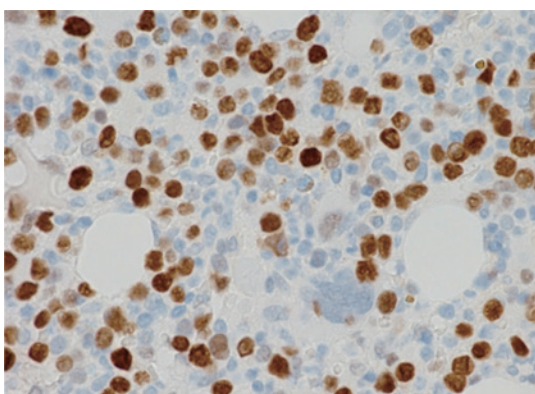
Available molecular data on MDS has markedly increased,

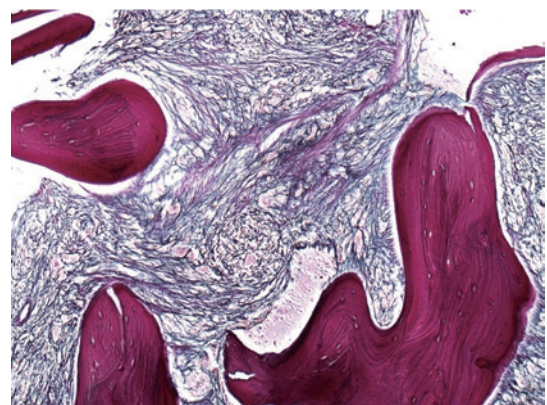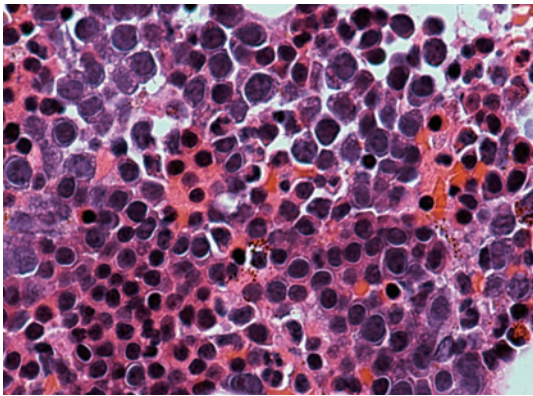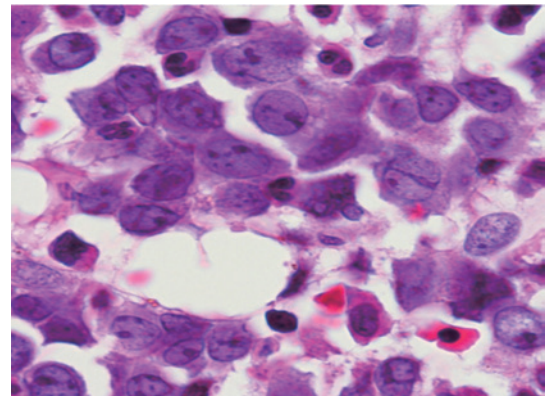**Fig. 11.** p53 immunostaining in the diagnosis of MDS

Fig. 12. MDS with fibrosis
Reticulin staining demonstrates black and fine reticulin fibers, as well as red and thick collagen fibers. Bone sclerosis is also observed corresponding to the 3/3 WHO grade.
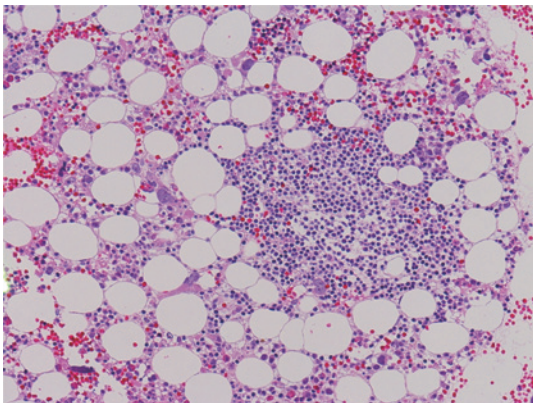
Fig. 13. MDS with erythroid predominance
Immature erythroids. Note the round nuclei with peri-
nuclear halo.

Fig. 14. Megaloblastic anemia
Immature erythroid precursors with rod-shaped
nucleoli.

Fig. 15. MDS with a lymphoid aggregate in the
marrow.

prompting future applications for the diagnosis of MDS and
stratification for treatment selection with FFPE specimens,
including the detection of genomic methylation, abnormali-
ties, and epigenetic abnormalities such as the presence of
microRNAs.[33]

CONFLICT OF INTEREST

The author declares no conflict of interest.

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