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Chapter 2

Cytopathology of MDS/MPN and AML by H&E Staining

Tatsuaki Tsuruyama

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Abstract

Bone marrow (BM) clots are routinely sampled in aspiration tests, and their sections are prepared for histological observation by hematoxylin and eosin (H&E) staining. However, H&E-stained sections are considered less informative than those stained by the May-Grünwald Giemsa (M-G) stain; thus, diagnosis using H&E-stained clot samples is challenging for pathologists. In fact, the diagnostic evaluation is limited to the observation of cellular morphology and the myeloid-erythroid cell ratio. Pathologists leave cellular observation to laboratory hematologists, who generally use M-G staining. In this chapter, the utility of bone marrow clot specimens for diagnosis by H&E staining is reviewed. Specifically, the review provides a descriptive and illustrative explanation of the diagnosis of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myelodysplastic syndrome/myelocytic proliferative neoplasm (MDS/MPN) and demonstrates the possibility of diagnosis on the basis of the characteristic features of blast cells. Clot specimens appear to be useful for the diagnosis of hematopoietic dysplasia by pathologists, and this approach can provide more informative findings for hematologists.

Keywords: bone marrow clots, hematoxylin and eosin, acute myeloid leukemia

1. Introduction

Bone marrow (BM) clots and their specimens can be evaluated histopathologically by hematoxylin and eosin (H&E) staining [1, 2]. However, this method is considered to yield limited information regarding cellular morphology, myeloid-erythroid cell ratio, size and morphology of megakaryocytes [3], increase in multiple myeloma [4], involvement of lymphoma, and metastatic tumor such as neuroblastoma [5–7]. In particular, BM clot sections comprising sinusoidal blood are inadequate for morphological interpretation [2]. However, the cellblock technique ensures the capture of cells required for diagnosis, and laboratories routinely apply this sample preparation technique for enhancing the usability of the BM clot specimen [2, 8].

To assist in morphologic diagnosis, immunohistochemistry (IHC) is available for assessment and immunophenotyping. For instance, by IHC, the marker glycophorin can assess the myeloid-erythroid cell ratio, and spectrin can efficiently detect erythroid cellular lineage [9]. P53 can also be used for differential diagnosis between refractory anemia and aplastic anemia [10]. An international Working Party for the Standardization of Bone Marrow IHC was formed by the International Council for Standardization in Hematology to prepare a set of guidelines for the standardization of handling BM specimens and reports [11]. While the histological examination of BM introduces new aspects of blood disease, the diagnosis of hematopoietic disorders using H&E-stained BM clot sections remains a challenge for nonhematopathologists. In fact, hematopoietic disorders have been diagnosed using May-Grünwald Giemsa (M-G) staining by hematologists and pathologists, and the findings obtained from H&E-stained BM clot sections are used as a reference for those obtained from M-G–stained samples.

This review discusses the information obtained from H&E-stained BM clot sections and its advantages and limitations over M-G–stained BM clot sections. The techniques are discussed with respect to examination of erythrocytes and myelocytes and the diagnosis of acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), and myelodysplastic syndrome/myelocytic proliferative neoplasm (MDS/MPN).

2. Overview of various blast cells

2.1. Erythroblasts

In H&E-stained samples, it is difficult to distinguish between erythroblasts, myeloblasts, and megakaryoblasts. Erythroblasts have a large nucleus that occupies the whole cell. In H&E-stained sections, the nucleus is observed to be pale, basophilic, and homogenous, with a fine appearance. Myeloblast lineages have more eosinophilic and rough cytoplasm than the erythroblasts, with distinct nucleoli (**Figure 1A** and **B**). Megakaryoblasts have transparent, lobulated nucleoli, with a higher nuclear-cytoplasmic ratio than that in megakaryocytes. The cytoplasmic border is frequently observed, and cell membranous projections are occasionally observed in some of the megakaryoblasts (**Figure 1C**).

2.2. Immature myelocytic lineage

In general, the morphological characteristics of myelocytic developmental stages have not been sufficiently described using H&E-stained clot samples. This is probably because of the difficulty in recognizing the cytoplasmic granules in the H&E-stained sections. However, H&E-stained sections provide incomplete morphological information regarding the developmental stages. In comparison with erythroblasts, myelocytic lineages are larger and have eosinophilic cytoplasm. Pro-myelocytes are smaller than myeloblasts and have a halo surrounding the nucleus, which is sometimes observed in M-G–stained samples (**Figure 2A**). Myelocytes and meta-myelocytes are easily identifiable owing to their segmented nuclei and smaller size than that of myelocytes (**Figure 2B** and **C**). Eosinophilic and basophilic lineages are identifiable because of the presence of distinct granules (**Figure 2D**).

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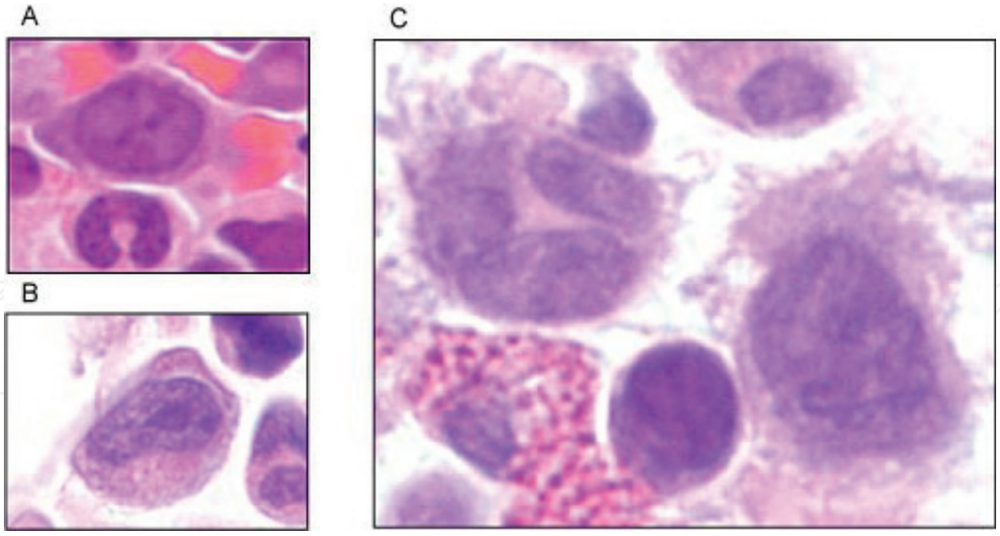

Figure 1. Morphology of erythroblasts and myeloblasts. (A) an erythroblast showing a transparent nucleus with condensed nucleoli (600×). (B) a myeloblast showing a transparent nucleus with one prominent eosinophilic nucleolus. The cytoplasm is eosinophilic, rough, and heterogeneously condensed (600×). (C) Two megakaryoblasts show transparent and lobulated nuclei with prominent eosinophilic nucleoli. The cytoplasm is eosinophilic, rough, and heterogeneously condensed. The edges are irregular, with a number of villi (600×).

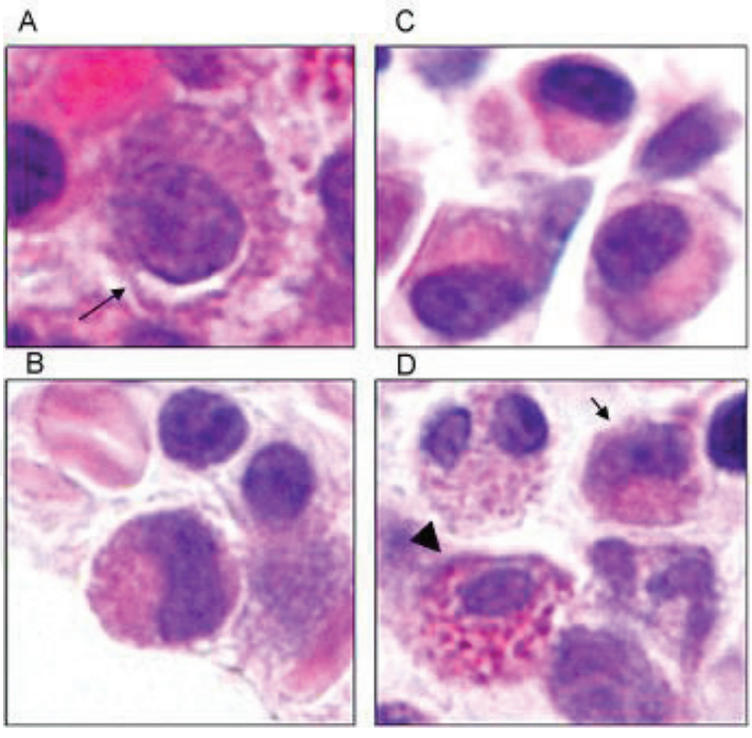

Figure 2. Morphology of myelocyte lineages detected by H&E staining. Immature neutrophil lineages (A–C) and immature eosinophil lineages (D). (A) A pro-myelocyte showing a nucleus surrounded by halo (represented by an arrow). Nucleoli are not prominent relative to myeloblasts. The cytoplasm is eosinophilic, rough, and heterogeneously condensed (600×). (B) A myelocyte showing a regular oval nucleus with a contour and without indentation. The cytoplasm is eosinophilic, rough, and heterogeneously condensed (600×). (C) A meta-myelocyte showing a U-shaped or bent nucleus. The cytoplasm is eosinophilic, rough, and heterogeneously condensed. No nucleoli are visible (600×). (D) Variable immature eosinophil lineages. The arrow indicates a pro-myelocyte, and the arrowhead indicates a myelocyte. A mature eosinophil can be seen in the upper-left corner (600×).

3. H&E-stained AML

3.1. Identifying erythrocytic lineages and AML by H&E-stained BM clots

To determine the utility of H&E-stained BM clots for the diagnosis of megaloblastic leukemia, megaloblastoid cells in MDS, or erythroleukemia, the H&E staining properties of erythrocytic lineages were considered. Pro-erythroblasts are identifiable by their basophilic cytoplasm, transparent nucleus, and multiple deformed nucleoli. A basophilic erythroblast is smaller and has a relatively large nucleus, bearing thick or coarse chromatin (**Figure 3A**). The nucleus occupies a relatively small part of the cell compared to that in basophilic erythroblasts. Importantly, owing to two highly contrasting colors in H&E staining, two distinct types of erythroblasts can be observed: polychromatic and orthochromatic. The polychromatic erythroblast has a lacy nucleus and baso-eosinophilic cytoplasm, while the orthochromatic erythroblast has eosinophilic cytoplasm, in which the nucleus is small, pyknotic, homogeneous, and structure-less, and ultimately becoming blue-black (**Figure 3B**).

3.2. Identifying myeloblasts and AML by H&E-stained BM clots

In this study, the utility of H&E-stained BM clots in diagnosing classic AML subtypes was also examined. H&E-stained clots were prepared for M2, M3, M4, M5, M6, and M7 according to the classic French-American-British classification. Cases of M2, acute leukemia with maturation, as specified in WHO 2008 classification, were the most frequent AML cases

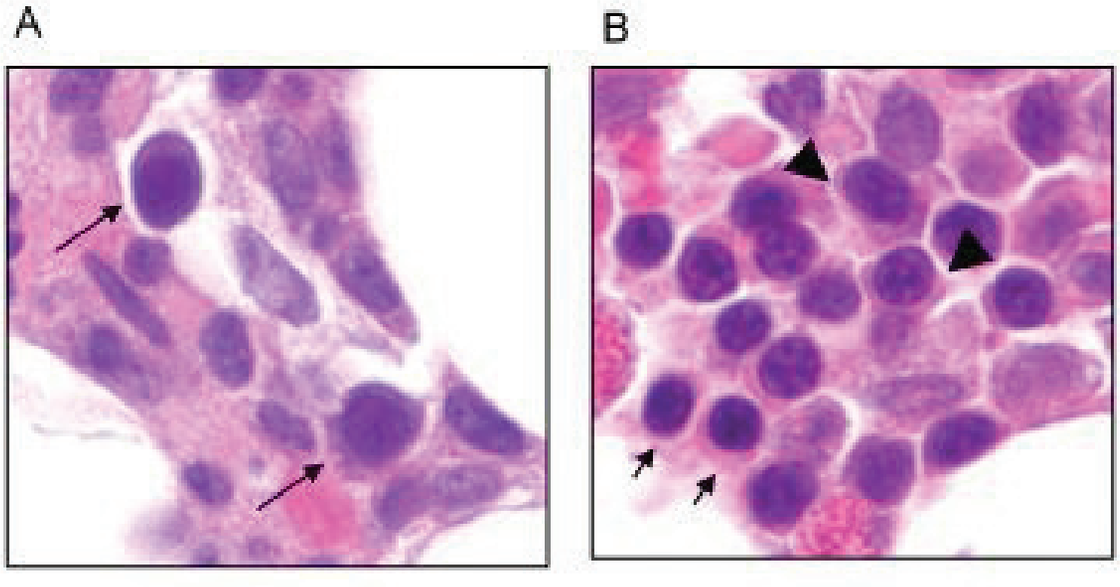

Figure 3. Erythroid lineages detected by H&E staining. (A) Two basophilic erythroblasts represent large nuclei (represented by arrows). Nucleoli are not prominent relative to pro-erythroblasts. The cytoplasm is basophilic, rough, and heterogeneously condensed (600×). (B) Three metachromatic erythroblasts represent lacy and oval nuclei (indicated by two arrowheads). The cytoplasm is baso-eosinophilic, rough, and heterogeneously condensed. Two orthochromatic erythroblasts represent condensed and oval nuclei (indicated by two arrowheads). The cytoplasm is eosinophilic and rough (600×).

encountered; in these cases, myeloblastic leukemic cells were observed. They have similar cytological features as those of normal myeloblasts: (i) eosinophilic, rough cytoplasm and (ii) a prominent nucleolus. **Figure 4A** shows leukemic marrow consisting of myeloblastic leukemic cells. M4, acute myelomonocytic leukemia (**Figure 4C**), and M5, acute monocytic leukemia (**Figure 4D**), have common features of monoblastic cells and are relatively easily identifiable due to a U-shaped nucleus with prominent dual nucleoli. Chromatin condensation was observed in the immature phenotype of monocytic leukemic cells in M4 and M5. The latter is a mix of monoblasts and monocytes and has pyknotic and condensed chromatin. In M6, acute erythroleukemia, atypical erythroblast-like leukemic cells were observed. The rough margin of the nucleus and basophil is a feature of these leukemic cells. In M7, acute megakaryocytic leukemia, the cells are transparent and lobulated without a distinct nucleus, which is observed in MDS. Furthermore, abnormal, deformed, and intensely stained nuclei are observed in MDS/MPN.

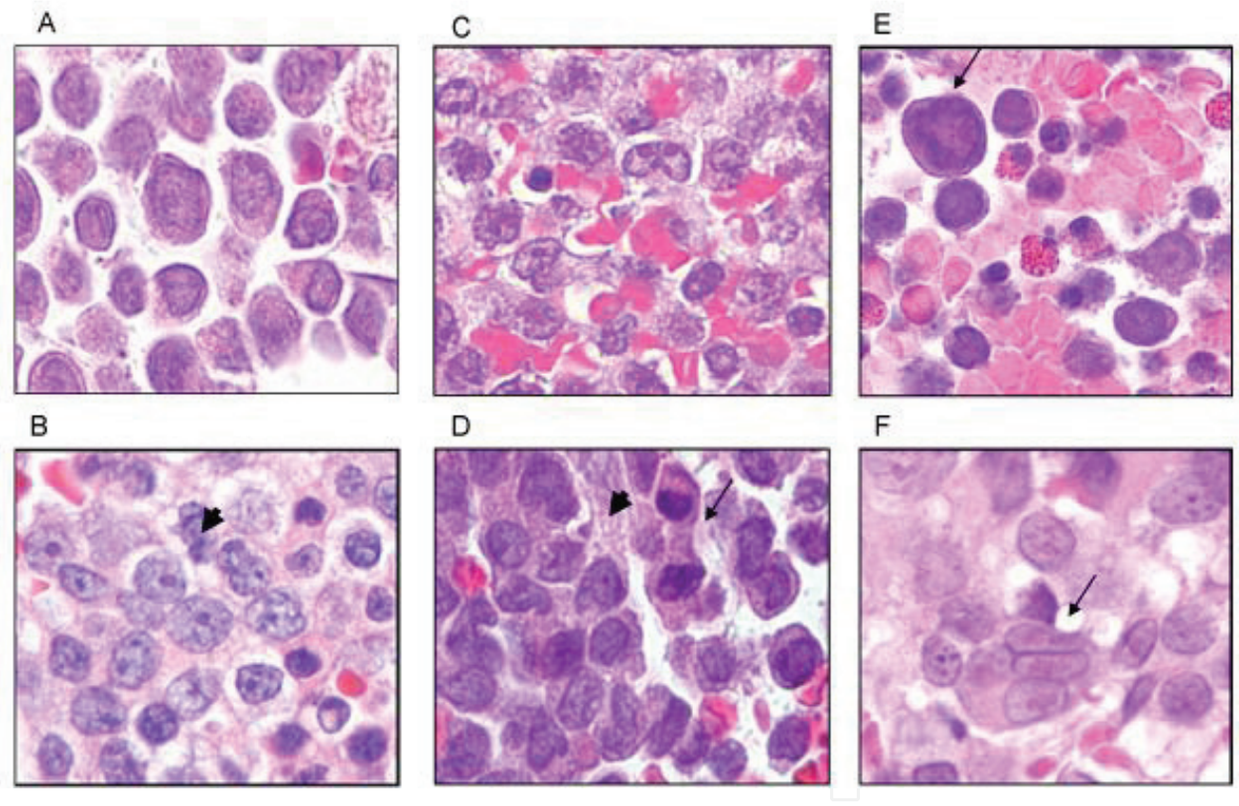

Figure 4. AML diagnosis using H&E staining. (A) Two M2 leukemic cells (AML with maturation) of various sizes mimic myeloblastic morphology and show irregular transparent nuclei with prominent eosinophilic nucleoli (600×). (B) Two M3 leukemic cells mimic pro-myelocytic morphology but present irregular transparent nuclei with prominent eosinophilic nucleoli (600×). (C) Two M4 acute myelomonocytic leukemic (AMMoL) cells, mimicking monocytic and myeloblastic morphology but presenting irregular transparent nuclei with prominent eosinophilic nucleoli (600×). (D) M5 acute monocytic leukemic cells are a mixture of two types of cells: Monoblasts and pro-monocytes. They mimic monoblastic morphology but present an irregular transparent nucleus with prominent eosinophilic nucleoli. Monoblasts have roughly circular and delicate lacy chromatin nuclei with one or two prominent nucleoli. Pro-monocytes have more convoluted nuclei, and the nucleoli are not prominent (600×). (E) M6 leukemic cells mimic erythroblastoid morphology but present irregular transparent nuclei with prominent eosinophilic nucleoli (600×). (F) M7 leukemic cells mimic myeloblastic morphology but present irregular transparent multilobed nuclei with prominent eosinophilic nucleoli (600×).

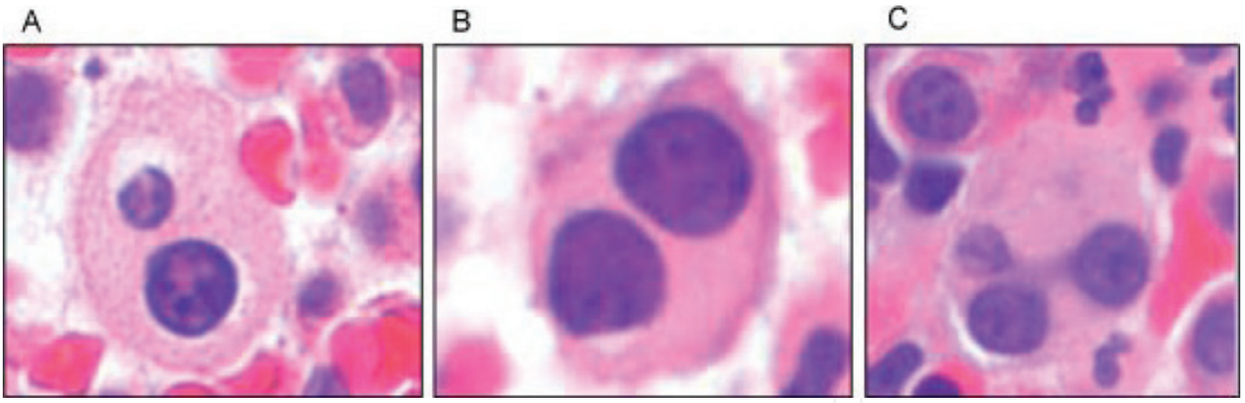

Figure 5. Dysplastic megakaryocytes detected by H&E staining. Megakaryocytes have round, dispersed nuclei (A, B, C). Two or three dispersed nuclei are observed (600×).

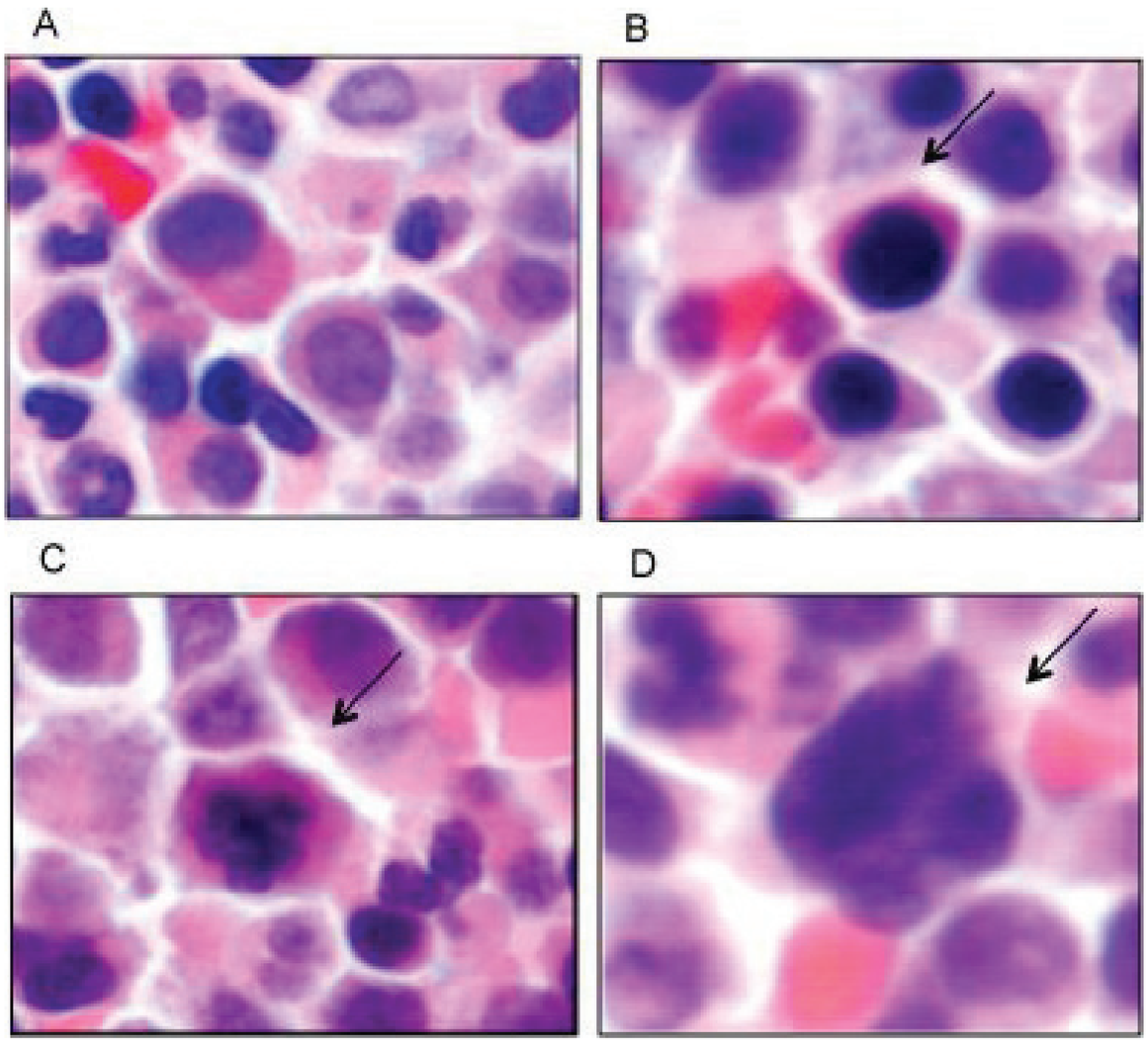

Figure 6. Dysplastic erythrocytes observed by H&E staining. (A, B) nuclear-cytoplasmic dissociation. Arrows represent the dysplastic erythroblasts that have baso-eosinophilic cytoplasm. (C, D) abnormal pro-erythroblasts. Arrows represent the abnormally hyperlobated nuclei.

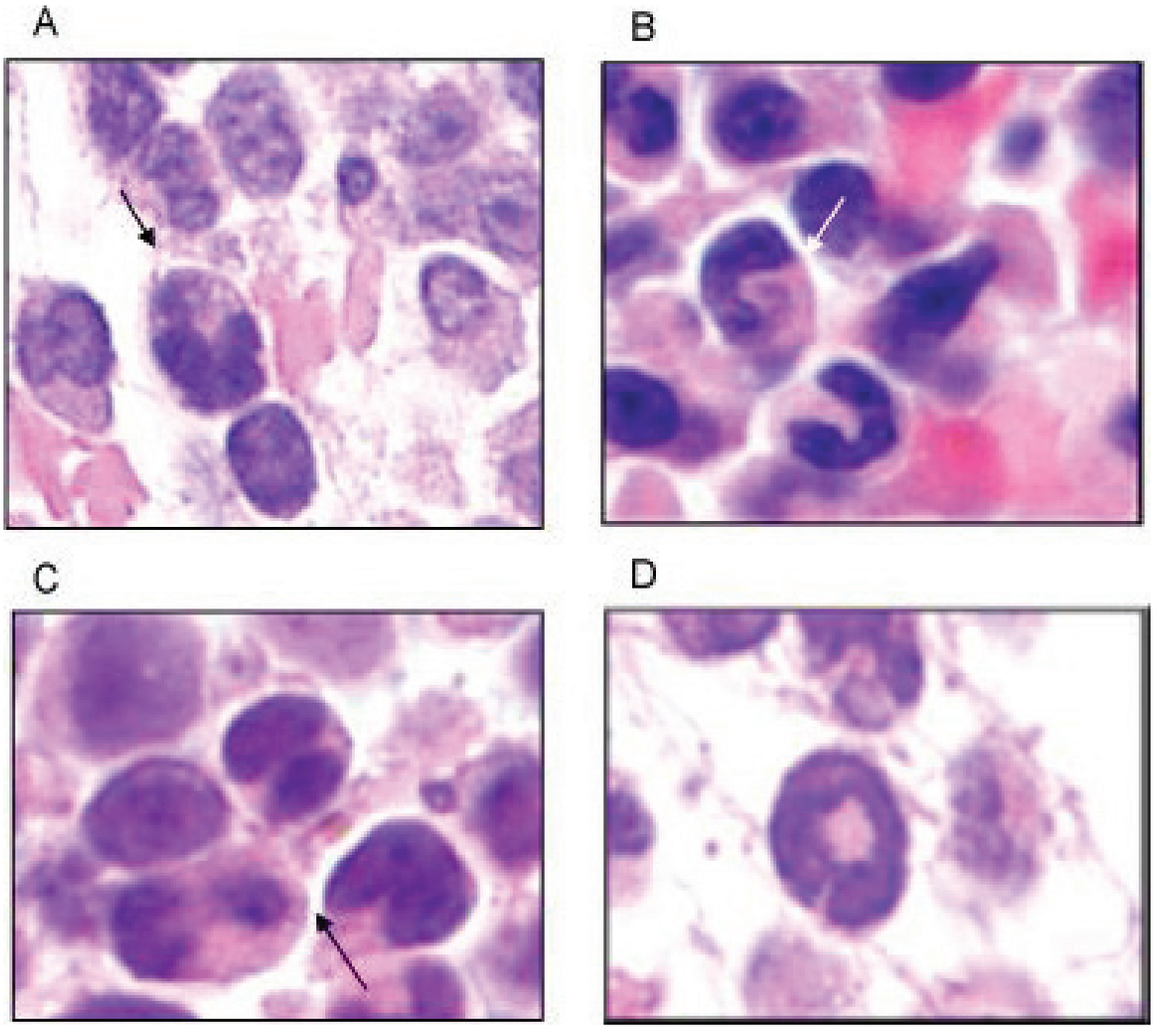

Figure 7. Dysplastic myelocytes observed by H&E staining. (A–D) dysplastic metamyelocytes maturing into neutrophils, which have blots in the cytoplasm (indicated by arrows).

3.3. Dysplastic megakaryocytes in refractory anemia with excess blasts and MDS/MPN

In H&E-stained clot specimens of refractory anemia with excess blasts and MDS, abnormal megakaryocytes, which include micromegakaryocytes and megakaryocytes with large mononuclear form, and dispersed round nucleoli were observed.

Morphological changes and increase in the number of megakaryocytes are informative for diagnosis. Micromononuclear megakaryocytes are detected in MDS, while large, over-mature megakaryocytes have been observed to increase in number in myeloproliferative neoplasms [12]. In anaplastic anemia, the megakaryocytes decrease definitively in number without any evident morphological abnormality [13]. By two-color contrast, megakaryocytic morphological abnormality is more distinct than that by M-G staining. Nuclear-cytoplasmic dissociation was more identifiable in H&E-stained specimens. As shown in **Figure 5**, polynucleated cells and cells with lobulated nuclei were identified in pro-erythroblasts of BM clot samples of MDS patients (**Figure 5A–C**).

In the erythroblast lineage, nuclear-cytoplasmic dissociation (**Figure 6A** and **B**) [2], evident as hemoglobin production in the cytoplasm without enucleation, is identifiable in H&E-stained specimens because of distinct two-color contrast.

Multinuclear abnormal pro-erythroblasts are also the hallmark of dysplasia (**Figure 6C** and **D**) [2]. Furthermore, basophilic heterogeneity in the cytoplasm is observed. Neutrophils exhibited a "blot" or localized heterogeneous staining in the cytoplasm, which is frequently seen in H&E-stained specimens (**Figure 7A–C**) [2]. In addition, a ring-shaped nucleus, which is observed in murine neutrophils, was observed in MDS and MDS/MPN (**Figure 7D**) [2].

4. H&E and M-G staining

Most pathologists are more familiar with H&E-stained samples than with M-G–stained samples in routine diagnostic work. As demonstrated in this study, H&E-stained specimens of BM, as well as M-G–stained samples, were available despite the difficulty in observation of granules in the cytoplasm in the myelocytic lineage. In this chapter, high-power magnification images are provided for understanding features of individual hematopoietic lineages for further clarifying morphological features of the subjects.

As demonstrated, H&E specimens allow the identification of myeloblasts. Therefore, the identification of "blasts" in H&E-stained sections should be clearly defined. In contrast to the M-G-stained sections, H&E-stained sections do show basophilic cytoplasm; rather, the transparency of the nucleus with one or more nucleoli is more identifiable as the feature of blasts in H&E staining. This phenomenon represents another feature of dysplasia with deformity in the shape with or without atypical morphological findings of the nucleus. In dysplasia, hyperlobulated or ring nuclei were observed, which is a diagnostic feature of dysmyelopoiesis. In addition, basophilic heterogeneity, exudation, or a "blot" in the erythroblast cytoplasm is identified in dysplasia. This basophilic exudation was clearer in H&E-stained specimens than in M-G-stained sections. In addition, H&E-stained clot samples are sufficiently useful for the observation of morphological changes and for the assessment of megakaryocytic features.

H&E-stained clot specimens are advantageous for diagnosing AML subtypes because they allow better identification of hematopoietic dysplastic cells than M-G–stained sections do. Pathologists can integrate findings from the three staining methods: H&E, M-G, and IHC: for the decisive diagnosis of AML subtypes. Routine histological observations using M-G or H&E staining will be more useful in combination with flow cytometry analysis for immunohistological assessment using anti-CD33 and anti-CD13, a set of antibodies used to accurately diagnose AML. Myelodysplasia is also identifiable by H&E staining. Using high magnification, such as 1000×, the blot and localized heterogeneous staining or budding of the nucleus is identifiable by H&E staining. In fact, this high-power magnification provides more cytological and histological findings for facilitating the diagnosis of MDS and MDS/MPN.

5. Discussion

5.1. Terminology: dysplastic, atypical, and blastic findings and interrelationship with hematological neoplasm

Dysplastic, atypical and abnormal, and blastic findings, together with hypercellularity, compose the core concepts of hematological neoplastic entities in BM pathology. It is possible to understand the interrelationship of the entities by organizing concepts on the basis of their morphological findings (**Figure 8**). Dysplasia refers to specified morphological features that are distinct from other abnormal or atypical morphologies. As an important example, dysplastic megakaryocytes have circular, dispersed nuclei, which differ from atypical and irregular megakaryocytes in MPN and MDS/MPN. Thus, the terms "abnormal" and "atypical" should be used to describe the morphological changes other than morphological changes in MDS. The cytological spectrum of atypical lymphocytes is extremely broad. For example, small- to large-sized cells with often regular or indented nuclei, moderately condensed chromatin, moderate-to-faint basophilic cytoplasm without azurophilic granules and sometimes with microvacuoles are atypical findings [14].

On the other hand, the term "blast" refers to "immature" cells, which have a transparent nucleus with a prominent clear nucleus. For example, the "centroblast" is a morphological concept that refers to lymphocytes that have a specified morphological feature, such as prominent nucleoli representing an immunologically activated state. As another example, "B-lymphoblasts" refer to the activated B-lymphocytes that undergo hypersomatic mutations and the class switch of immunoglobulin genes. In other words, blastic lymphocytes correspond to the lymphocytes with gene recombination. Myeloblasts and erythroblasts, however, do not necessarily correspond to specific genetic alterations but correspond to the activated state of the first stage of differentiation. Distinguishing these morphological features from hyperplastic marrow will elucidate the mutual relationships among AML, AML arising from MDS, MDS, MDS/MPN, and MPN. MDS, AML, and MPN are specific entities on the basis of dysplastic, blastic (>20% of nucleated cell in BM), and hypercellular findings, respectively.

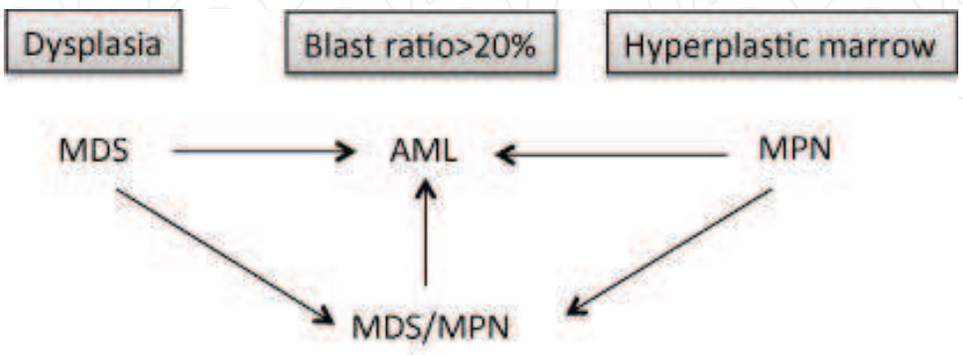

Figure 8. Interrelationships of hematopoietic neoplasms. Dysplastic, blastic findings, together with hypercellularity, compose the core concepts of hematological neoplastic entities.

AML arising from MDS and MDS/MPN are the hybrid entities of dysplasia and blastic findings and dysplastic and hypercellular findings, respectively. On the basis of these three morphological findings, the interrelationships among hematopoietic neoplasms can well be understood (**Figure 8**).

6. Conclusion

Diagnostic evaluation should include H&E-based assessment of morphological dysplastic findings. H&E-stained BM clot specimens have advantage in diagnosis of hematopoietic neoplasms, because they better allow identification of hematopoietic dysplastic and abnormal cells than M-G-stained sections by cases. Pathologists can integrate findings from the two staining methods, H&E, and M-G for decisive diagnosis of hematopoietic neoplasms.

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Author details

Tatsuaki Tsuruyama

Address all correspondence to: tsuruyam@kuhp.kyoto-u.ac.jp

Department of Pathology, Graduate School of Medicine, Kyoto University, Kyoto, Japan

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