Review Article
Interpretation of Maturing Trilineage Hematopoiesis

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Abstract

Background: Normal adult bone marrow occupies the medullary spaces of large bones. The main function of bone marrow is the production of blood cells (hematopoiesis). Bone marrow is composed of a matrix requisite for hematopoiesis as well as erythriod precursors, myeloid precursors with monocyte macrophage system, megakaryocytes, lymphocytes, plasma cells, blood vessels and stroma. Interpretation of bone marrow maturing trilineage hematopoiesis includes the assessment of the maturation sequence and morphologic features for each lineage.

Aim: This review presents detailed guidelines for interpretation of bone marrow maturing trilineage hematopoiesis according to the cell line and the pathologic condition.

Keywords: Bone marrow, Trilineage hematopoiesis, Erythropoiesis, Granulopoiesis, Megakaryopoiesis.

Guidelines for Interpretation of Maturing Trilineage Hematopoiesis

Interpretation of bone marrow (BM) hematopoiesis includes the assessment of the maturation sequence and morphologic features for each lineage according to the cell line and the pathologic condition using the guidelines below.

Fat: Cell Ratio

BM cellularity is the volume ratio of hematopoiesis and fat. BM cellularity is age dependent; at birth and childhood it is 100% cellular, but with age, hematopoiesis diminishes and the amount of fat increases. Normal cellularity in an adult varies between 30:70 and 70:30 with increasing age. Beyond the seventh decade the bone marrow fat is expected to increase by 10% each passing decade.[1-5]

The fat: cell ratio is best evaluated using biopsy sections. Total fat replacement is called aplasia, where cellularity less than 30% in a young adult is considered hypocellular and more than 70% is considered hypercellular. Hypocellularity occurs following chemotherapy treatments and in cases with serous fat atrophy, hypoproliferative conditions and fibrosis, whereas myeloproliferative neoplasms and reactive conditions are associated with hypercellularity. In cases with unexplained aplasia or hypocellularity without peripheral blood cytopenia, it is recommended to rule out a sampling error by another contralateral biopsy. It is important to know that the subcortical hypocellular region becomes more prominent with advancing age and should be excluded from BM cellularity determinations.

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Myeloid: Erythroid Ratio

The myeloid:erythroid (M:E) ratio is calculated from a count of 100 myeloid and nucleated erythroid precursors. Normally the M:E ratio varies from 2:1 to 4:1.[1-6] An increase in ratio occurs in infection states, chronic myelogenous leukemia and erythroid hypoplasia. Decreased ratio means a depression of leucopoiesis or erythroid hyperplasia depending on BM total cellularity. The M:E ratio is best evaluated in aspirate smears and should be correlated with that in the core biopsy, or clot sections to rule out any bias introduced by sinusoidal blood or particle depleted smears.

Granulopoiesis

BM contains progenitor cells called stem cells which have the pluripotent capacity to conduct both re-newal and differentiation. These cells give rise to erythroid, granulocytic, megakaryocytic cell lines and monocytes. Table (1) lists cells of granulocytic and monocytic derivation alongside with their physiologic range as a percentage of the total BM cell count.[7, 8] Granulocytic precursors and mature forms can be readily identified in BM aspirate and biopsy. Small mononuclear macrophages and monocytes, however, cannot always be identified with certainty in routine stains of the aspirate and core. This is possible by using cytochemical studies for nonspecific esterase, or lysozyme and KP1 (CD68) immunostains.[9, 10]

In cases with normal BM maturation and normal M:E ratio, most observers do not consider it necessary to report a differential of granulocytic cell types. However, in pathologic states, it is of considerable diagnostic and prognostic value to enumerate abnormal cell types in percentile figures (e.g., myeloblasts in acute myeloid leukemia or myelodysplastic syndromes). The latest World Health Organization classification of tumors of hematopoietic and lymphoid tissues[11] recommended a manual count of 500 nucleated BM cells on cellular smears, preferably around the spicules, as a prerequisite for classification of myeloid neoplasms.

Table 1. Types of myeloid precursors and their normal range in bone marrow

Cell Type	Range
Myeloblasts	*0%-1.5%*
Promyelocytes	*2.0%-4.1%*
Myelocytes	*8.2%-16%*
Metamyelocytes	*9.6%-24.6%*
Band forms	*9.5%-15.3%*
Segmented neutrophils	*6.0%- 12%*
Eosinophilic series(total)	*1.2%-5.3%*
Myelocytes	*0.2-1.3%*
Metamyelocytes	*0.4%-2.2%*
Band	*0.2-2.4%*
Segmented	*0%-1.3%*
Monocytes/macrophages	*0%-1%*
Basophils	*0%-1%*
Mast cells	*0%-1*

Mast cells are often concentrated in bone marrow particles, and as with the other myeloid elements, manifest reactive and neoplastic patterns of growth.[12, 13] In the core biopsy specimen, myeloid elements are randomly distributed among other hematopoietic and nonhematopoietic cells progressing away from bony trabeculae and toward the central medullary space. In contrast, abnormally located intertrablecular immature myeloid precursors (ALIP) [1, 14] may be observed in dysplastic states.

Routine microscopy remains the cornerstone of diagnosis of myeloid disorders enabling a choice of additional special procedures as needed. According to morphology, selective application of cytochemical stains such as myeloperoxidase, Sudan Black-B, periodic acid-Sciff (PAS) stain, terminal deoxynucleotidyl transferase (TdT), leucocyte alkaline phosphatase, α–naphthyl acetate esterase, α-naphthyl butyrate esterase and combined esterase can be applied with minimal cost and short turnaround time.[15, 16] Flow cytometry, cytogenetics, immuno-histochemistry and molecular studies[17-30] are also applied according to morphology. A flow cytometry panel for identification of immature elements (blasts) in different types of acute leukemias can be applied using the following markers: CD2, CD3, CD4, CD5, CD7, CD10, CD11b, CD13, CD14, CD15, CD19, CD20, CD33, CD34, CD68, CD79b, HLA-DR, myeloperoxidase and TdT. [17, 19, 23, 25]

Immunohistochemistry of the core biopsy specimen is of particular value in cases in which fresh cells for flow cytometry are not available or particle-depleted aspirate smears are obtained. The most valuable immunohistochemical stains for myelomonocytic lineage include myeloperoxidase, lysozyme, CD34, CD43, and CD68.[17, 31]

Lesions requiring conventional cytogenetics or molecular diagnostics, such as PCR, reverse transcriptase-PCR, FISH, and spectral karyotyping may be required in acute and chronic myeloid leukemias as well as myeloproliferative neoplasms and myelodysplastic syndrome.[27-29]

Erythropoiesis

The erythroid lineage matures from stem cell to proerythroblast, early normoblast, intermediate normoblast, late normoblast, and finally reticulocytes. It comprises 15-37% of nucleated BM cells.[1- 6] Erythropoiesis can be described as normoblastic, micronormoblastic, megaloblastic or megaloblastoid maturation. It is essential to correlate erythropoietic maturation with hemoglobin and hematocrit, erythrocyte indices, reticulocyte count, serum iron, total iron binding capacity, serum ferritin, vitamin B12, and folic acid levels.

With identification of any morphologic abnormalities such as giant pronormoblasts, and intranuclear inclusions in cases of parvovirus B19 infection, [32] multinuclearity observed in congenital dyserythropoietic anemia [33] and bizarre multinuclearity with megaloblastoid changes in cases with acute erythroid leukemia and myelodysplasia [34] are of great value.

Cytochemical stains play an important role in the identification of abnormal erythroid precursors in acute erythroid leukemia by displaying coarse blocks PAS-positivity.

Immunophenotyping using glycophorin and CD71 are used to identify erythroid elements.[17-19] Benign and malignant erythroid precursors may also be specifically identified in a core biopsy and clot sections by immunohistochemical

staining for adult hemoglobin.[5, 19, 24]

Megakaryopoiesis

Megakaryocytes are the least numerous hematopoietic cells accounting for less than 1% of all nucleated BM cells. [1- 6] They are considered the largest of the hematopoietic cells. Maturation of megakaryocytes from mononuclear agranular megakaryoblast with basophilic cytoplasm to large mature multilobated granular megakaryocyte is associated with an increase in cell size and cytoplasmic granularity. Mature megakaryocytes are PAS, α-naphthyl acetate esterase, FVIII related-antigen, FXIII, and von Willebrand factor positive.[35- 37]

BM biopsy sections are considered the best for evaluating the morphology, quantity and distribution of megakaryocytes. Assessment of megakaryopoiesis gives important clues to the diagnosis of the following: (1) Idiopathic thrombocytopenic purpura which is associated with megakaryocytic hyperplasia and many young forms but without clustering. (2) Myeloproliferative neoplasms which are associated with megakaryocytic hyperplasia with clustering of bizarre multilobated large megakaryocytes. (3) Myelodysplastic syndrome which is associated with hypolobated micromegakaryocyte. In cases of acute megakaryoblastic leukemia AML-M7 immunophenotyping using CD41 and CD61 can be applied to distinguish megakaryoblasts from myeloblasts. [5, 17, 19, 37]

Megakaryocytes should be differentiated form multinucleated cells in BM such as multinucleated plasma cells, Reed-Sternberg (RS) cells, metastatic tumor cells, histiocytes and osteoclasts.

Lymphopoiesis

Lymphocytes are derived from the same stem cells that give rise to all hematopoietic elements and are therefore discussed here. They are normally present in BM and comprise 10-20% of all nucleated cells.[38, 39] The ratio of T-cells to B-cells is 3:1, and the CD4:CD8 ratio about 2:1. In

pediatric samples, the total lymphocytes count is higher, and comprises up to 40% of cells, which are sometimes designated as hematogones (benign B-lymphocytic precursors) that express CD34, CD10, CD19, and TdT.[40, 41]

Hematogones usually do not appear in peripheral blood, lack surface immunoglobulin, show a characteristic pattern of antigen expression in flow cytometry histograms (smear pattern) and do not reveal any cytogenetic abnormality.[41] They should be distinguished from immature lymphoblast in cases of acute lymphoblastic leukemia.

Beyond the third decade, benign nonparatrabecular lymphoid aggregates may be observed. Abnormal lymphocytes can be identified on both BM aspirate smears and biopsy sections. Cytologic features are best appreciated on aspirate smears, while BM biopsy sections are essential to determine the pattern. Paratrabecular, nonparatrabecular and intertrabecular monoclonal B-cell lymphoid aggregates are suggestive of lymphoproliferative B-cell neoplasms involving BM, which might not be identified on aspirate smears; such tumors are best identified using CD20 and other related specific immunostains.[42-44] T-cell lymphoproliferative neoplasms infiltrating BM can also be identified using pan T-cell markers (CD3 and CD5) with other related immunostains.[45, 46] In the cases of BM involvement by acute lymphoblastic leukemia and high-grade lymphoma (lymphoblastic lymphoma and Burkitt lymphoma), the correlation of morphology with immunophenotypic, cytochemical and cytogenetic data are of great value.

Hodgkin's disease in the BM is associated with fibrosis, accounting for the rarity of RS cells observed in aspirate preparations.[47] As with non-Hodgkin lymphomas, bilateral core biopsies are recommended for the staging of Hodgkin's disease. The true identity of mononuclear RS cells in the BM can be established with antibodies specific for CD15 and CD30 and other related immunostains.[48, 49]

Conclusion

Finally, interpretation of maturing trilineage hematopoiesis with other categories related to BM (not discussed in this review) such as stainable iron stores, plasma cell dyscrasias, granulomatous changes, metastatic disease, reticulin and collagen proliferation, stromal and vascular abnormalities, disorders of trabecular bone and lesions associated with abnormal macrophage activity, remains the corner stone in the BM reporting system and pathologic diagnosis.

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تقييم تكوّن ونضج سلالات الدم الثلاث

رائده إبراهيم العودات

الملخص

يشغل نخاع الدم في الشخص البالغ الفراغات النخاعية للعظام الكبيرة. الوظيفة الرئيسية لنخاع الدم هي انتاج خلايا الدم (تكوين الدم).

يتكون نخاع العظم من نسيج بمواصفات تضمن تكون الدم بالاضافة الى الخلايا المكوّنة لكريات الدم الحمراء، الخلايا المكوّنة لخلايا الدم البيضاء مع خلايا الوحيدات والماكروفاجات، الخلايا المكوّنة للصفائح الدموية، الليمفاويات، خلايا البلازما، الاوعية الدموية، والستروما.

تقييم تكوّن ونضج سلالات الدم الثلاث يتضمن تقييم تسلسل النضج والسمات المورفولوجية لكل سلالة.

تعرض هذه المراجعة التوجيهات المفصلة حول تقييم تكوّن ونضج سلالات الدم الثلاث بحسب خط الخلية والحالة المرضية.

الكلمات الدالة: نخاع العظم، تكوين سلالات الدم الثلاث، تكوين كريات الدم الحمراء، تكوين الخلايا المحبة، تكوين الصفائح الدموية.