Bone Marrow at Initial Diagnosis: Clinical Associations and Approach to Diagnosis

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1. What is the relevance of morphological examination of bone marrow and the role of a surgical pathologist in the era of molecular diagnostics?

Despite the emergence of many ancillary tests, morphological examination of the bone marrow remains the mainstay of diagnostic work-up for almost all neoplastic hematologic conditions and many non-neoplastic conditions as well, because:

- Availability: Bone marrow aspiration for pathological diagnosis dates back to 1903 [1], and the trephine biopsy taken from the posterior superior iliac crest has been an integral part of the diagnostic workup since the mid-1960s [2]. Morphological examination of bone marrow is now an established technique, requiring relatively simple equipment and reagents which are almost universally available.
- Objective and comprehensive morphological information: Morphology of individual cells and overall histology can be assessed, using the aspirate smear, clot section, and

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core biopsy. Objective, quantitative, and qualitative findings from each preparation can be recorded independently. Laboratory technologists can perform quantitative assessment such as bone marrow differential counts, providing an extra layer of unbiased observation.

- Correct classification of most neoplastic/clonal conditions according to the latest WHO guidelines still requires accurate morphologic identification and enumeration of abnormal cell types in the marrow [3]. Well-stained preparations which allow detailed cytological and histological examination and precise qualitative and quantitative assessment of each marrow component are critical for accurate diagnosis and to assess prognosis.
- Specimen triage decisions: The repertoire of ancillary studies has steadily grown in the past two decades. It is the responsibility of the pathologist to properly triage low volume specimens for competing demands of various ancillary techniques in a timely manner, someties based on limited clinical information and laboratory results. Thus, familiarity with requirements of tissue handling, amount of tissue required, and the relative advantages and disadvantages of different tests in the clinical context are needed.
- New Challenges: While combinations of clinical presentation with peripheral blood and laboratory findings are associated with a limited set of bone marrow morphological findings, newer therapies can produce unanticipated morphological changes and create new diagnostic pitfalls and challenges. Novel applications of older drugs (arsenic, thalidomide analogues), designer molecules targeting subcellular organelles (bortezomib) or precise molecular defects in individual hematological malignancies, as well as the various modalities of immunotherapy improve survival but can cause unexpected morphological changes in the bone marrow and can induce therapy-related secondary pathology.

2. What are the indications of bone marrow examination?

As the primary function of the bone marrow is production and maturation of cellular components of the blood, and to a lesser extent of lymphoid tissues, it is not surprising that most bone marrow biopsies are performed to evaluate quantitative or qualitative abnormalities of the blood. Indications for bone marrow examination and the key variables which may provide etiological clues are listed below.

- Abnormalities of the complete blood count and/or peripheral smear:
  - Evaluation of cytopenias – cells involved, duration
  - Evaluation of cytosis – cells involved, duration
  - Evaluation of circulating immature/abnormal cells – leukoerythroblastosis, acute leukemia, other tumor cells
- Evaluation of a monoclonal paraprotein, lytic bone lesions, and suspected amyloidosis
- Follow-up after therapy for a marrow-based malignancy
- Staging of lymphomas and non-hematolymphoid malignancies
- Work-up of a fever of unknown origin/infection
- Evaluation of a storage disease
- Evaluation of suspected hemophagocytic syndrome

Additional clues to possible etiologies in common indications are:

- Cytopenias: Copper deficiency can cause pancytopenia [4] and a myelopathy which may develop with zinc excess, after gastric bypass, and with total parenteral nutrition [5] (Fig. 20.1a). Evaluation of cytopenias in patients with a diagnosis of a systemic autoimmune disease may be particularly challenging, and bone marrow examination may reveal a specific cause such as MDS in about 20% of cases [6]. In one study, evaluation provided no new information and dysplasia often was reactive [7]. Patients may develop autoimmune myelofibrosis which must be distinguished from primary myelofibrosis [8]. Isolated immune-mediated thrombocytopenia does not produce consistent morphologic changes in the marrow [9], and bone marrow examination should be undertaken only when an alternate or additional pathological process is suspected. Detailed lists of possible etiologies of pancytopenia and suggestions for additional testing for definitive diagnosis are available [10, 11].
- Immature cells in blood: Presence of circulating blasts suggests a primary hematopoietic neoplasm, and circulating abnormal lymphoid cells suggest lymphoma. A leukoerythroblastic reaction can occur not only in hematopoietic neoplasms (primary myelofibrosis, etc.) but also in marrow involvement by metastases from non-hematolymphoid neoplasms [12] and some benign conditions [13–15].
- Staging bone marrow: In areas with high incidence of HIV/AIDS, a significant proportion of previously unsuspected lymphomas may be diagnosed on bone marrow examination [16]. The diagnostic yield for non-hematological neoplasms in unselected bone marrow specimens was found to be about 1% among the more than 10,000 bone marrows analyzed retrospectively [17]. Frequent clinical indications in these cases were microangiopathic hemolytic anemia, leukoerythroblastosis, or unexplained anemia [18]. About 50% of these metastases came from cancers of the lung, GI tract, and breast. Metastases are often associated with marrow fibrosis [12], which may be the reason why malignant cells cannot be

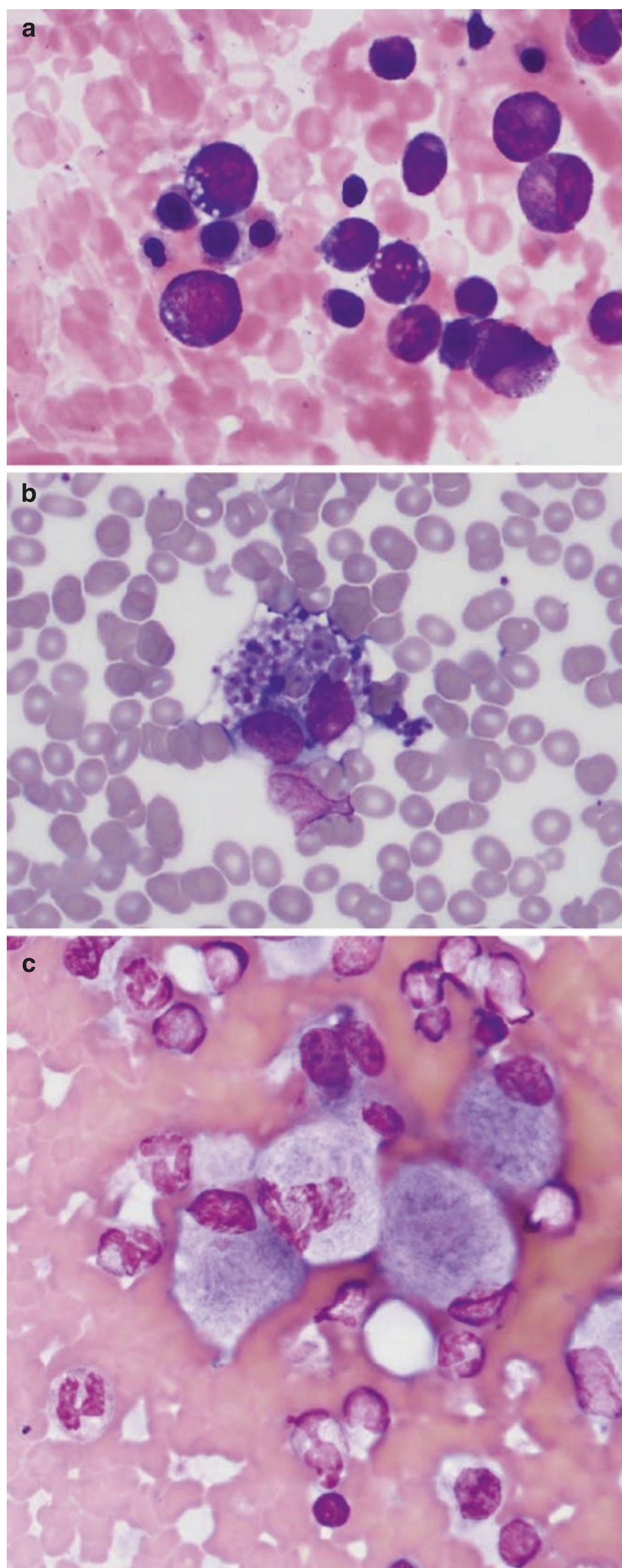

Fig. 20.1 Unusual bone marrow abnormalities in non-neoplastic bone marrow disorders (aspirate smears, Wright stain, 1000×). (**a**) Vacuolated red and white cell precursors in copper deficiency. (**b**) Hemophagocytic histiocytes in hemophagocytic lymphohistiocytosis. (**c**) Histiocytes with abundant "tissue paper" cytoplasm in Gaucher disease

identified in the aspirate smears from more than 25% of these cases.

- Suspected infections: The diagnostic yield for identification of infection is higher among immunosuppressed patients such as renal transplant recipients (about 10% compared to 1–2% in unselected bone marrows) [19, 20]. In contrast, bone marrow examination in liver transplant recipients did not detect specific infection or granulomata [21]. In HIV-AIDS patients, special stain for fungi on bone marrow biopsies appears to be as sensitive as blood and/or bone marrow culture, but only 30% bone marrows from patients with positive mycobacterial cultures demonstrate acid-fast organisms [22]. In immunocompetent patients with a fever of unknown origin, bone marrow examination is much more likely to show an underlying hematologic malignancy compared to an infectious etiology [23], and using a simple scoring system can increase the likelihood of a diagnostic bone marrow examination in such cases [24].
- Hemophagocytic syndrome: or hemophagocytic lymphohistiocytosis (HLH), often presents as fever of unknown origin (Fig. 20.1b). Hereditary abnormalities of cytotoxic molecules are responsible in a minority of cases, which generally present in children, while HLH in the vast majority of patients is triggered by infections or as paraneoplastic effect of a variety of malignancies, including lymphomas [25]. Rare nucleated red cells can be found inside macrophages in many bone marrow aspirates, without evidence of HLH, reducing the specificity of this finding in the diagnosis of HLH [26]. Conversely, the absence of microscopically demonstrable erythrophagocytosis in the marrow does not rule out the diagnosis [27]. Using "bone marrow index" incorporating laboratory values which reflect the functional status of the bone marrow is reported to be an independent predictor of HLH [28].

3. Are there specific indications in certain patient groups?

- Children and infants (Table 20.1): The indications in infants are evaluation of cytopenias and suspicion of storage disorders [29] (Fig. 20.1c). In one study, biopsies in infants constituted about 10% of pediatric bone marrow biopsies and yielded a satisfactory sample in over 95% biopsies, all of which were performed by pathologists. The commonest diagnoses were acute leukemia and storage disorders. In older children, cytopenias affecting more than one line account for over half the bone marrow examinations performed [30]. In these children, simultaneous occurrence of anemia and thrombocytopenia is the commonest finding, often accompanied by circulating blasts.
- In resource-limited settings, the indications are primarily pancytopenia, anemia, and suspected leukemia

Table 20.1 Clinical indications for bone marrow evaluation in children [49]

Peripheral blood numerical abnormalities	Cytopenias (isolated or pancytopenia)
	With or without dysplastic features
	With or without circulating blasts
	Leukocytosis
	Blasts
	Neutrophilia and/or monocytosis with or without blasts
Systemic findings	Fever
	Lymphadenopathy and/or hepatosplenomegaly
	Bone pain
	Lytic bone lesions
	Masses suspicious for malignancy in anatomic sites that are difficult to sample; biopsy of bone marrow metastases may serve as the diagnostic sample
	Clinical manifestations of osteopetrosis

according to a study from Sudan [31]. The diagnostic yield of such bone marrows was over 75%, with only a small minority of cases being normal. In a study of over 1100 bone marrows obtained over a 4-year period in Iran [20], about 10% of specimens were unsatisfactory. Sixty percent of the technically satisfactory specimens yielded a definite diagnosis. The likelihood of a definitive diagnosis varied according to the clinical indication for the bone marrow, being highest in suspected leukemia (54%), followed by plasma cell myeloma (30%), myeloproliferative neoplasms (25%), and lymphoma (16%). On the other hand, bone marrow examination rarely provided definite diagnosis in suspected storage disorders or infection (≤2%).

4. What are the contraindications for a bone marrow biopsy?

- Absolute contraindications: In adults, there are no absolute contraindications.
- Relative contraindications, particularly in children, are [32]:
  - A hemorrhagic disorder – correction of coagulation factor deficiency is advisable, but severe thrombocytopenia is not a contraindication if sufficiently prolonged pressure is applied post-biopsy. In obese patients, correction of severe thrombocytopenia is advisable.
  - Hereditary or acquired bone disorders such as osteogenesis imperfecta or osteomyelitis.
  - Skin infection or recent radiation to the biopsy site.

5. What is the optimal procedure for obtaining and processing bone marrow samples?

Guidelines for adult patients and pediatric patients differ to some extent:

Adults Instructional videos demonstrating the technique for obtaining the bone marrow sample are available (e.g., https://www.youtube.com/watch?v=EYd7OnCt7ug from the University of Oslo, https://www.youtube.com/watch?v=3hzVvCl8UkM by Dr. Alejandro Calvo), and various monographs and textbooks provide protocols for processing specimens for examination [33] or for harvesting stem cells [34].

- If needed, specimen quality can be improved through a systematic quality improvement initiative involving pathologists and relevant clinicians/ physician extenders [35]. The International Council for Standardization in Hematology has provided guidelines for a universal protocol for procurement and the contents of the pathology report [36].
- If aspirate smears are inadequate, touch imprints from marrow core biopsies are quite helpful. When correctly prepared, such “touch preps” have the advantage of transferring sufficient cells to the slide from fibrotic or otherwise inaspirable marrows and providing some architectural details in addition to good cytomorphology [37].
- A trephine core biopsy of the marrow provides information that is complimentary to the cytological preparations mentioned above. The biopsy can be done with a Jamshidi needle or a powered drill, which has been introduced relatively recently. The diagnoses from aspirate smears and core biopsies can be discordant in 20–30% cases [38]. The Hammersmith protocol, in which biopsy cores are fixed in acetic acid-zinc-formalin fixative and decalcified in 10% formic acid-5% formaldehyde, before processing for paraffin embedding, is widely adopted, as it allows sectioning at 1–2 micron thickness and renders excellent cytological and architectural details [39]. However, 10% buffered formalin can be used if other fixatives are not readily available.
- The precise site and order of the core biopsy with respect to the bone marrow aspiration are important determinants of the “aspiration artifact” in the core biopsy (Fig. 20.2a), which has the potential for limiting the usefulness of the core biopsy [40]. If the aspirate is performed first, care must be taken to biopsy from an area away from the site of the aspirate. Unilateral biopsy appears to be adequate for staging of non-Hodgkin lymphomas, provided the core is of sufficient length (≥26 mm according to one study) [16].

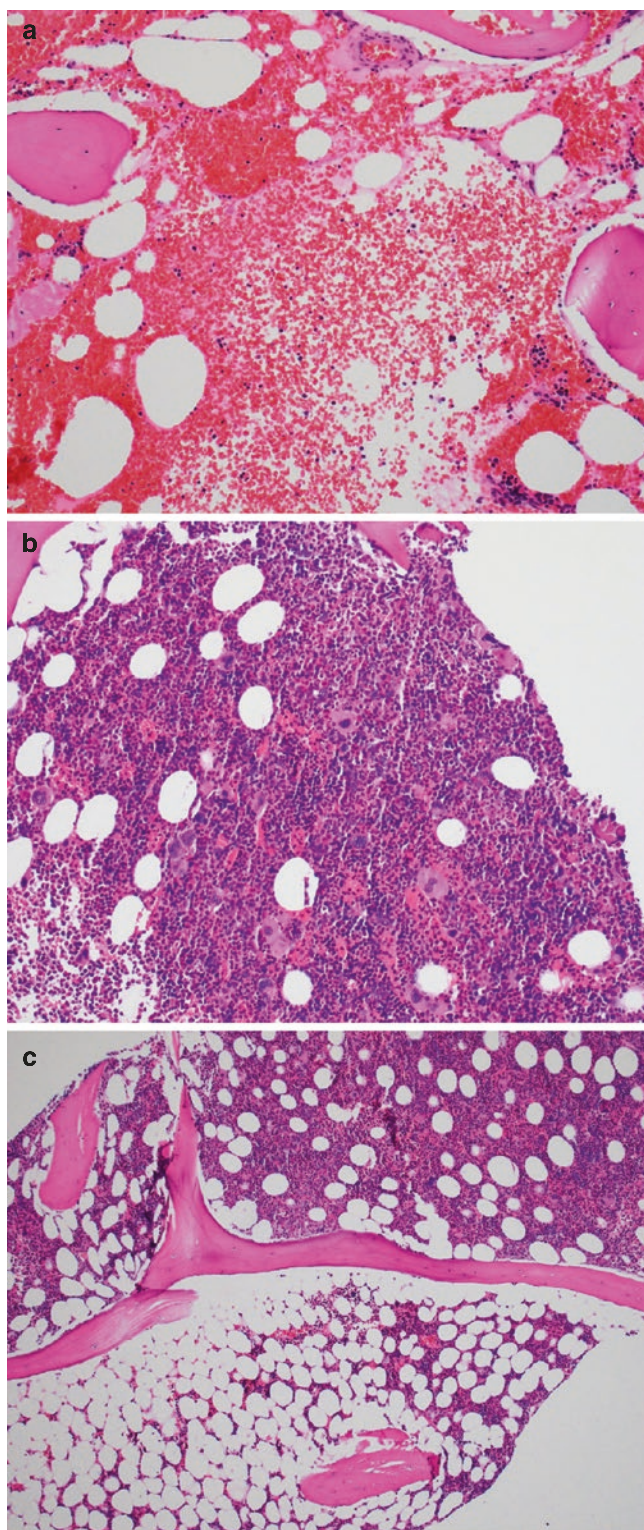

Fig. 20.2 Core biopsy, initial assessment (core biopsies, hematoxylin and eosin stain, 100×). (**a**) Aspiration artifact. The marrow contains hemorrhage and a paucity of hematopoietic cells as aspirate smear was obtained first and in the same location where the core biopsy was done. (**b**) Hypercellular marrow from an adult. (**c**) Variably cellular marrow from an adult

- While the unfixed cells obtained from an aspirate are essential for critical ancillary studies such as flow cytometry and cytogenetics, much of the same information can now be obtained from paraffin-embedded core biopsies. If decalcification and/or zinc-containing fixative preclude any type of testing, clot preparations prepared from residual aspirate material can be used. Most molecular tests for DNA and RNA as well as many fluorescent in situ hybridization (FISH) assays and immunohistochemistry can be performed on formalin-fixed, paraffin-embedded (FFPE) tissue. Alternatively, immunostaining protocols can be modified to work on decalcified and fixed bone marrow cores [41]. In resource-limited regions of the world, bone marrow aspiration by itself, when performed in patients with appropriate clinical presentations, can provide definitive diagnosis in a high proportion of cases [31].

Children Guidelines provided by Abla et al. [32] stress that the procedure should be performed only by well-trained professionals. Need for conscious sedation or anesthesia and performing the procedure in an operating room must be carefully assessed. A platelet count is recommended in all children prior to undergoing the procedure and basic coagulation tests (PT and aPTT) in those with history of coagulation defects or anticoagulant therapy. Posterior superior iliac crest is preferred in most patients, but the anterior superior iliac crest is more accessible in obese patients. Irrespective of the biopsy site (anterior or posterior iliac crest), it appears that biopsies shorter than 1.5 cm in length are more likely to produce inadequate samples [42], which suggests that the generally accepted lowest threshold of 0.5 cm [43] may be too short.

6. What is the role of imaging studies in bone marrow examination?

While not as crucial as in other areas of pathology (i.e., bone and soft tissue), evaluation of imaging studies can be helpful in interpretation of bone marrow specimens. For example, the presence of lytic lesions can lead to a diagnosis of plasma cell myeloma if the marrow shows clonal plasma cells, and the presence of lymphadenopathy or splenomegaly may help in evaluating lymphoid infiltrates. Positron emission tomography (PET) scans may be valuable in suspected benign and malignant conditions affecting the bone marrow [44]. In some cases, a positive PET scan can obviate the need for a staging marrow in lymphoma [45–47].

7. What clinical information is needed to adequately evaluate a bone marrow specimen and what does the information imply for underlying disease?

Age of the patient: This is critical in narrowing the differential diagnoses in several ways (Table 20.2):

- Is the observed cellularity normal, high, or low (Fig. 20.2b, c)? The first step in examination of bone marrow is to determine if the observed cellularity is hypocellular, normocellular, or hypercellular. The simple formula to find the normal expected cellularity for a patient is 100 minus age of the patient. However, the calculated value is too high in children [48] and too low in very old patients. Age-specific cellularity considered together with the peripheral blood findings is useful to guide further evaluation.
- Which conditions are likely to involve the marrow at this age? The common conditions seen at different age groups in Western countries are shown in Table 20.2. The incidence of these conditions varies in different parts of the world, and awareness of the local epidemiology of hematological conditions is very useful to increase the efficiency of bone marrow examination.
- Is the presence and percentage of certain cells normal for the patient's age? The percentage of mature lymphocytes, including hematogones (normal B-precursor cells) (Fig. 20.3b), decreases from infancy to adulthood [49], but may aberrantly increase in a regenerating marrow as well as unrelated conditions such as copper deficiency [50]. Plasma cells and mature lymphocytes increase in older adults [51]. Furthermore, the presence of lymphoid aggregates (Fig. 20.3b) in older adults is not necessarily pathological (see also Chap. 26).

Family history of hematologic conditions A hemoglobinopathy or thalassemia can produce erythroid hyperplasia with mild dyserythropoiesis, while rare conditions like congenital dyserythropoietic anemia, hemophagocytic lymphohistiocytosis, and Fanconi anemia are causes of significant morphological alternations in pediatric marrows [10]. Some malignancies such as chronic lymphocytic leukemia and plasma cell myeloma have a familial predisposition.

Table 20.2 Common bone marrow findings in different age groups

Age group		Infants (0–1 yr)	Children (1–10 yrs)	Adolescent/young adults (10–25 yrs)	Adult (25–65 yrs)	Elderly (65+ yrs)
Normal marrow cellularity		Can be lower than calculated by formula	Can be lower than calculated by formula	As calculated by formula	As calculated by formula	Can be higher than calculated by formula
Pathology	More likely at this age	Iron deficiency, congenital hemolytic anemia, +21 related MPN[a]	AA, ALL, BL, congenital anemia, nutritional deficiency, storage disorders	BL, cHL, CML, DLBCL	BL, cHL, CML, DLBCL, ET	AML, CLL, FL, MDS, myeloma, metastasis
	Possible at this age	ALL, AML, BL, DLBCL, infant leukemia, mastocytosis, metastases	AML, DLBCL, FL, MCL, PTCL, mastocytosis, metastases, pediatric MDS	AA, AITL, EATL, MCL, MZL, PTCL	AA, AITL, AML, EATL, FL, PMF, PTCL, PV, myeloma	AA, ALL, CML, PMF, PTCL, PV
	Rare at this age	AA, MDS, MPN	CML, PMF, PV	AA, CLL, mastocytosis, metastases, myeloma, storage disorders	Congenital anemia, metastases, storage disorders	Storage disorders
	Not seen at this age	CLL, myeloma	CLL, myeloma			Congenital anemias
Non-hematopoietic cells often present		Hematogones++, mature lymphs++, mast cells	Hematogones++, mature lymphs+++, mast cells	Hematogones+, mature lymphs++	Few plasma cells, rarely lymphoid aggregates	Interstitial lymphoid aggregates, plasma cells
Non-hematopoietic cells usually absent/rare		Lymphoid aggregates, plasma cells	Lymphoid aggregates, plasma cells	Lymphoid aggregates, plasma cells	Hematogones +/−	Hematogones −/+

[a]Abbreviations (in alphabetical order): *AA* aplastic anemia, *AITL* angioimmunoblastic T-cell lymphoma, *ALL* acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *BL* Burkitt lymphoma, *cHL* classic Hodgkin lymphoma, *CLL* chronic lymphocytic leukemia, *CML* chronic myeloid leukemia, *DLBCL* diffuse large B-cell lymphoma, *EATL* enteropathy-associated T-cell lymphoma, *ET* essential thrombocythemia, *FL* follicular lymphoma, *MCL* mantle cell lymphoma, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm, *MZL* marginal zone lymphoma, *PMF* primary myelofibrosis, *PV* polycythemia vera, *PTCL* peripheral T-cell lymphoma, *NOS* not otherwise specified

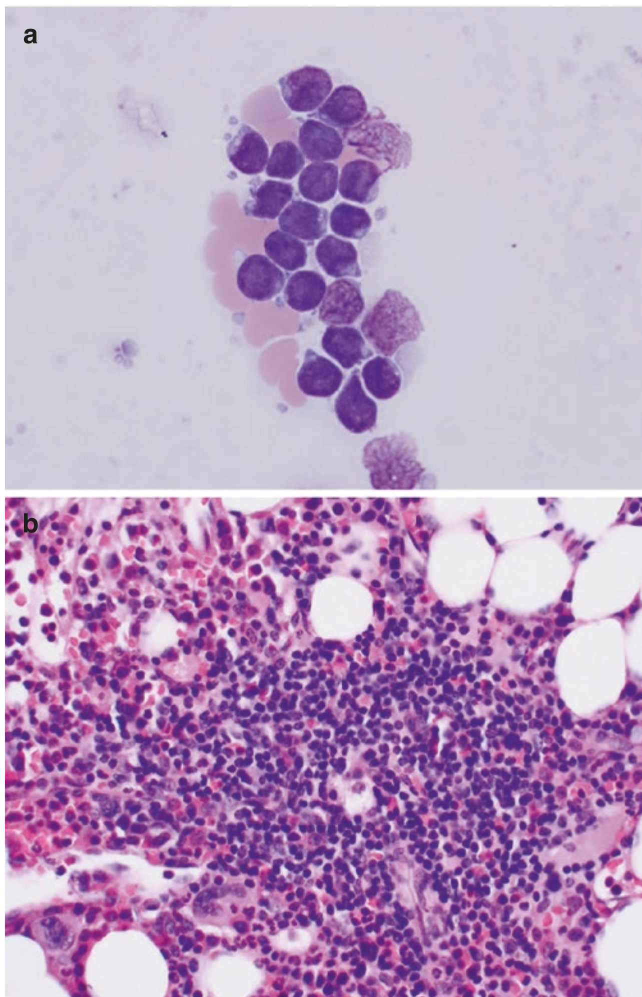

Fig. 20.3 Lymphoid cells in normal marrows. (**a**) Precursors of B lymphocytes (hematogones) in a marrow from a child undergoing evaluation for neuroblastoma; these cells with high nuclear/cytoplasmic ratios can be mistaken for blasts (aspirate smear, Wright stain, 1000×). (**b**) A well-circumscribed benign-appearing lymphoid aggregate. Lymphoid aggregates are seen with increased frequency in older patients (clot section, hematoxylin and eosin stain, 400×)

Prior malignancy and treatment Marrow recovery after chemotherapy and growth factor therapy can cause dyspoiesis and, in rare instances, increased blasts. History of prior cytotoxic drug or radiation therapy can lead to therapy-related myeloid neoplasms in a significant minority of patients [52]. Radiation field covering current biopsy site may lead to marrow suppression, fibrosis, and cellular atypia due to direct radiation effect. The effects of various immunotherapies, antibody based, stem cell based, or other, are not well documented but may mimic dysplasia or include left or right shift in maturation, hypoplasia, hyperplasia, and fibrosis.

Chronic diseases and medications Endocrinopathies including diabetes [53]and thyroid disease [54], chronic kidney disease [55], and chronic inflammatory conditions should be evaluated as there may be complex and unexpected interactive effects.

Immunodeficiency and autoimmune/rheumatic diseases These conditions can have variable functional and structural effects on the marrow. Patients with HIV-AIDS may have fungal and mycobacterial infections, lymphomas [16, 22], and pathological changes indistinguishable from MDS [56]. The pathology of immunodeficiency-associated lymphoid neoplasms is covered in detail in Chaps. 10 and 11. Autoimmune conditions may present with unexplained cytopenias [6], “primary” bone marrow fibrosis [57–59], and sometimes as combination of pancytopenia and myelofibrosis [60, 61].

Significant physical examination findings Splenomegaly and/or hepatomegaly can be a key finding to differentiate between myelodysplasia with fibrosis and a myeloproliferative neoplasm as well as some lymphomas. Lymphadenopathy is important in proper assessment of marrow lymphocytosis. Skin lesions may suggest a mast cell or Langerhans cell neoplasm as well as T-cell lymphomas such as adult T-cell leukemia/lymphoma and peripheral T-cell lymphoma NOS.

8. Which laboratory test results are needed to adequately evaluate most bone marrow specimens?

- Complete blood count (CBC) and evaluation of a well-spread and stained peripheral blood smear: Evaluation of the presence and degree of either cytopenias or cytosis may be helpful in determining a diagnosis. The MCV may be beneficial in determining the cause for an anemia (macrocytosis is often associated with myelodysplastic syndromes).
- A well-stained blood smear is important because features such as neutrophil granulation are prone to artifacts and may be misinterpreted as dysplasia. The WHO recommends a 200-cell WBC differential, excluding any nucleated red blood cells. Identification of blasts, blast equivalents, and other immature cells is critical for any case in which acute leukemia, myelodysplastic syndrome, or myeloproliferative neoplasm is suspected.

9. Which additional laboratory tests are needed for specific indications listed above?

- Tests performed to *rule out* a neoplastic/clonal hematologic process: Ideally, clinicians should rule out reactive, nutritional, or toxic causes of the peripheral blood changes before performing the bone marrow aspiration; however, knowledge of vitamin B12, folate, serum copper, and heavy metal (lead, mercury, and zinc) levels is important to avoid overdiagnosis of MDS. Iron studies, tests for hemoglobinopathies and other congenital red cell abnormalities, tests for immune hemolytic anemia, and tests for paroxysmal nocturnal hemoglobinuria may be helpful in microcytic and normocytic anemia.
- Tests performed to *confirm/classify* suspected hematologic neoplasms: Cytogenetic and/or molecular tests for suspected myeloproliferative neoplasms are often performed on blood. A suspected plasma cell neoplasm is further investigated with serum and/or urine protein electrophoresis, immunofixation electrophoresis, and serum light chain evaluation. Serum tryptase may be helpful in the evaluation of mast cell disorders.
- Laboratory tests in systemic diseases with hematological manifestations: Suspected autoimmune, metabolic, or infectious diseases are investigated with appropriate laboratory tests prior to bone marrow biopsy to clearly formulate a rationale for the procedure and to guide additional testing on the bone marrow specimen.

10. What is the optimal specimen for cytological examination of the marrow?

- Wedge "pull" smear versus crush film smear [62, 63]: In the authors' experience, both "crush" smears made by placing marrow particles directly from the aspirating syringe on coverslips (Fig. 20.4a) and well-made "pull" smears made at the bedside (Fig. 20.4b) can provide consistently high-quality cytomorphology and uniform staining.
- Smears prepared from EDTA anticoagulated marrow are not inferior to those prepared directly from the aspirated marrow [64], but the WHO recommendation is to prepare smears from fresh marrow whenever possible, and smears prepared from anticoagulated marrow beyond 2 hours from collection are not suitable for determination of dysplastic changes.
- If the aspirate does not contain particles, the touch preparation (Fig. 20.4c) provides an alternative for cytological examination. Touch preps may provide diagnostic material while aspirate smears do not in focal involvement of the marrow by conditions such as metastatic carcinoma and plasma cell myeloma and diseases frequently

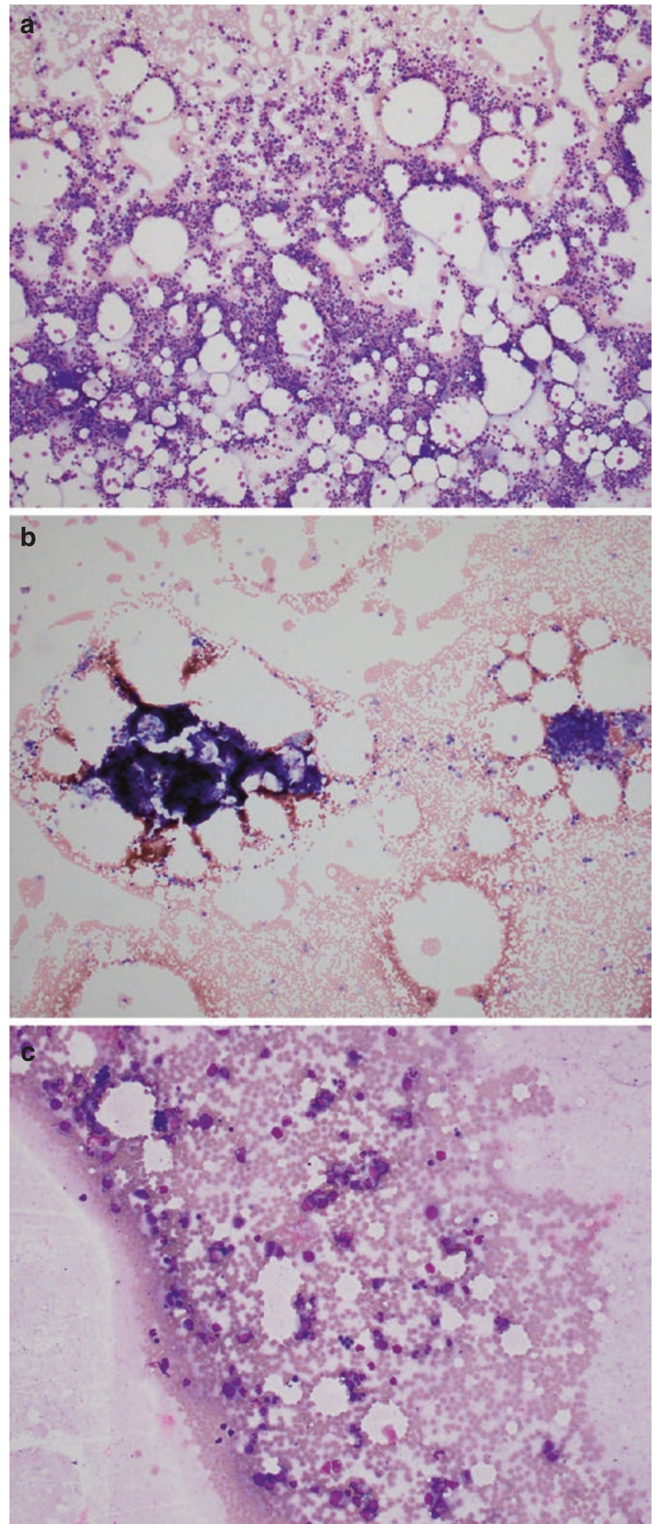

Fig. 20.4 Bone marrow aspirate preparations (aspirate smears, Wright stain, 100×). (**a**) Example of a crush preparation. (**b**) Example of a direct smear. (**c**) A touch preparation, made by touching the core biopsy on the glass slide. These can be helpful if the aspirate smear does not contain particles

associated with marrow fibrosis (various myeloproliferative neoplasms, MPNs, or MDS/PMN overlap).

11. How to judge the quality of aspirate smear?

- The presence of marrow particles (or spicules) in aspirate smears provides assurance that the findings are representative of the marrow. The proportion of various cell types expected in the marrow is best represented in the "tails" of smears following the marrow particles, where staining and cytologic details are optimal.
- Familiarity with the correct hues of basophilic, amphophilic, and eosinophilic staining at various stages of myeloid and erythroid precursors is essential to correctly assess dysplasia in these lineages. Avoid under-stained areas where blasts may be overestimated based on apparently fine chromatin.

12. What information is obtained from cytological examination of the marrow?

The aspirate smear (and/or the touch prep) is used for:

- Assessing proportion of erythroid and myeloid elements: A 500-cell differential of all nucleated cells is recommended, particularly when accurate and reproducible blast counts are critical for diagnosis or prognosis; however, in many cases, a 300-cell count suffices [61]. A differential count may be skipped if the total number of cells on the aspirate smear and touch preparation is limited as such counts are error-prone. The differential count includes myeloid (all three types of granulocytic and monocytic) and erythroid precursors as well as lymphocytes (mature and immature) and plasma cells (Fig. 20.5a). The megakaryocytes, mast cells, stromal cells, histiocytes, and any abnormal, non-hematopoietic cells, if present, are not included (Fig. 20.5b). The myeloid to erythroid ratio is calculated based on the differential count and should be normally 2–3:1. Ideally, the differential should be counted from several different slides.
- Assessing maturation of each line: Morphologic criteria for blasts and blast equivalents must be defined. Typical cells at various stages of myeloid and erythroid maturation are depicted in many standard texts, but it is important to recognize that maturation is a continuous process and each cell type spans a range of morphology. The laboratory should establish normal adult ranges for each cell type (Table 20.3). Widely accepted normal ranges for pediatric bone marrow are not available.
- Evaluating dysplasia: Despite the advances in molecular diagnostic methods, identification of cellular dysplasia is

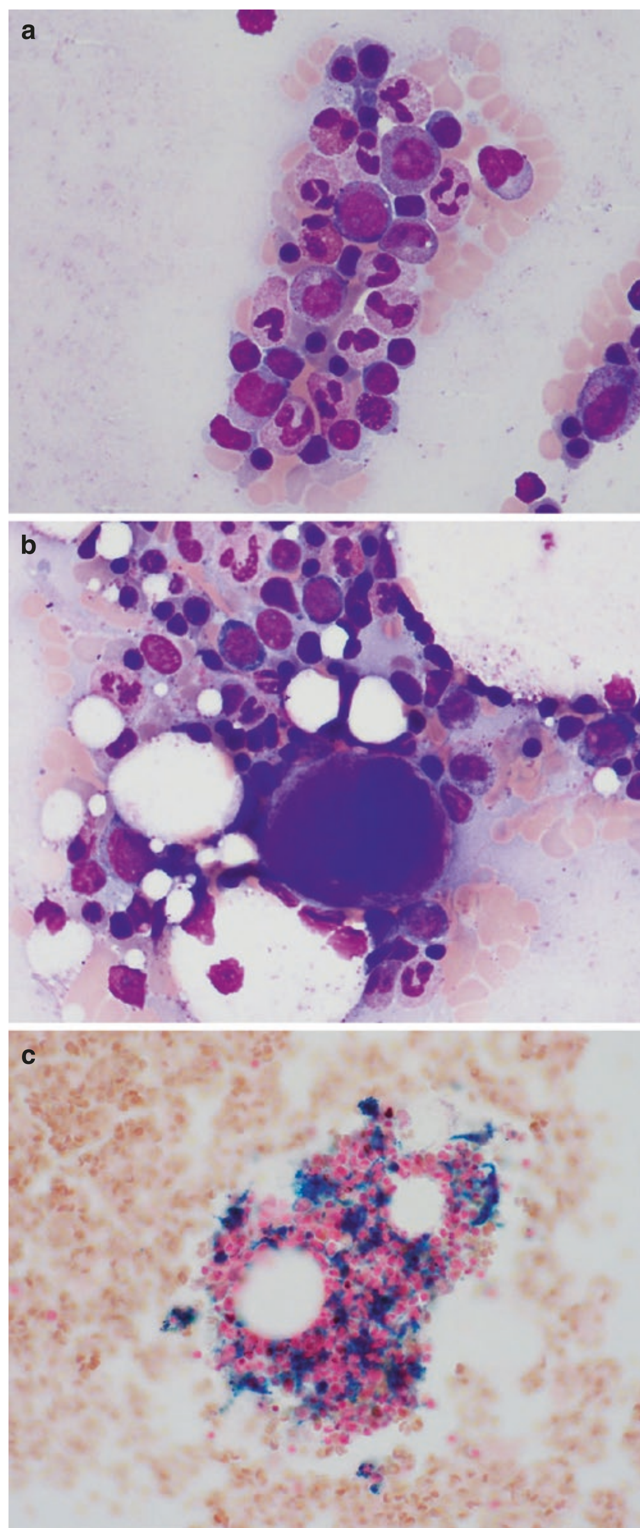

Fig. 20.5 Evaluation of bone marrow cells. (**a**) Normal myeloid and erythroid precursors. The myeloid to erythroid ratio should be approximately 2–3:1, and full maturation of both cell lines should be present (aspirate smear, Wright stain, 1000×). (**b**) Megakaryocytes are scattered on the smears (aspirate smear, Wright stain, 100×). (**c**) A Prussian blue for iron can be done on the aspirate smears or the clot sections (clot section Prussian blue stain, 40×)

Table 20.3 Reference ranges for bone marrow differential count in adults

Cell type	Reference range	Cell type	Reference range	Cell type	Reference range
Segs	(7–25)	Lymphocytes	(3–20)	Plasma cells	(0–3.5)
Bands	(6–36)	Atypical lymphocytes		RBC precursors	(10–30)
Metas	(9–25)	Lymphoblasts		Pronormoblasts	(0–3)
Myelos	(8–15)	Eosinophils	(0–4)	M:E ratio	
Promyelos	(1–6)	Basophils	(0–1)	Iron	(Scale 0–4+)
Myeloblasts	(0–3.5)	Monocytes	(0–2)		

a central pillar for diagnosis and categorization of myelodysplastic syndromes and some types of acute leukemia. The morphological findings of dysplasia are presented in Chap. 24. In addition to vitamin B12 and folate deficiency, copper deficiency as a cause of such changes should be kept in mind [65, 66].

- Storage and sideroblast iron: An iron stain should be performed on all initial adult bone marrow specimens. Aspirate smears containing particles are best suited for sensitive detection as well as quantification of storage iron [67]. We find a semi-quantitative, 4-point scale useful to grade iron stores; absent (grade 0 of 4) and trace of 4 stainable iron indicated reduced iron stores, grade 1–3 of 4 staining indicates normal stores, and grade 4 of 4 staining indicates increased stores. The amount of stainable iron in patients with normal iron stores varies widely, and only the two ends of the scale denote pathological finding.
  - Iron staining of clot section or core biopsy may be used if the aspirate does not contain particles and storage iron is not detected on the smear (Fig. 20.5c). The core biopsy is less sensitive because of tissue thickness and/or decalcification [68]. Iron-stained aspirate smears or touch preparations are required to assess ring sideroblasts which is important for classifying subtypes of myelodysplastic syndrome. Iron stains are less helpful in patients treated for a variety of hematologic malignancies as these patients often develop iron overload due to repeated transfusions.

13. What is the role of the core biopsy?

An H&E stained section (3–4uM) of a core biopsy, which is 1.5 cm in length and contains at least 10 marrow spaces, is optimal for examining the histological "architecture" of the marrow. This includes:

- Bone marrow cellularity: Cellularity is estimated in areas without significant aspiration or crush artifact. To estimate cellularity, it is useful to mentally estimate what percentage of the marrow space would be occupied if all the cells were together and all the fat was together. Highly fibrotic marrow may have very low proportion of fat, but also may contain few hematopoietic cells. A rare abnormality is gelatinous transformation or serous atrophy in which there is focal hypocellularity, accumulation of mucopolysaccharides, and normal fat replaced by a light pink granular material [69]. It occurs in a variety of clinical situations including anorexia nervosa, acute fever, HIV-AIDS, alcoholism, lymphoid and other malignancies, and chronic heart failure [70]. A similar change, called "marrow injury effect" or fibrinous necrosis (Fig. 20.6a) is often observed

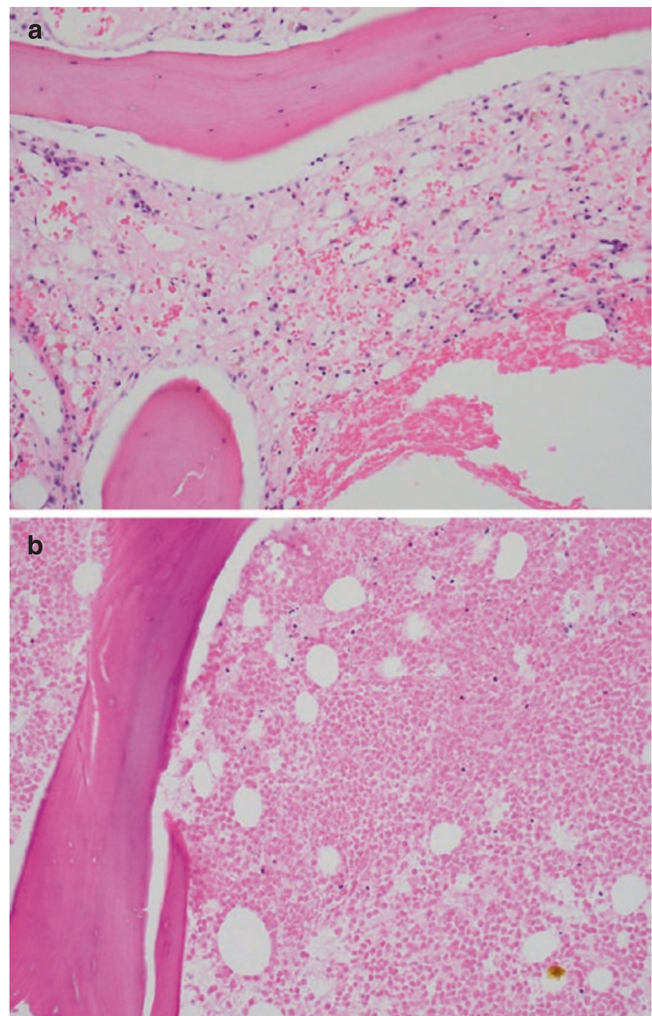

Fig. 20.6 Stromal abnormalities (core biopsies, hematoxylin and eosin stain, 100×). (**a**) Fibrinous necrosis. Stromal damage, hypocellularity, and focal hemorrhage due to recent chemotherapy. (**b**) Necrosis. Hypocellularity and necrotic tumor are seen in this example of marrow necrosis

after recent chemotherapy for a hematopoietic malignancy. It is more important to describe these abnormalities, rather than emphasizing a percentage cellularity. If the cellularity varies by more than 20%, the range should be mentioned and an average cellularity should be given. Subcortical marrow spaces can have lower cellularity than the rest of the marrow, and these should be avoided in calculating the average cellularity if possible.

- Appropriate distribution, proportion, and maturation of hematopoietic elements: Myeloid cells proliferate in paratrabecular areas (Fig. 20.7), and more mature myeloid elements are present in the interstitium. Erythroid cells are present as small colonies of cells at various stages of maturation in the interstitial areas. Megakaryocytes should be evenly distributed, away from bone. Adequacy of megakaryocytes is best assessed on the core biopsy as well as megakaryocyte dysplasia and clustering. The M:E ratio is assessed independently on the core biopsy and compared to that calculated on the aspirate smear. Left shift in maturation and collections of five or more immature precursors (myeloblasts and promyelocytes, proerythroblasts, monoblasts, and promonocytes) can be appreciated in the core biopsy, while it is generally not possible to verify minimal increase in immature cell types which are normally present in the marrow. An immunohistochemical stain for CD34 may be helpful in enumerating blasts.
- Marrow sinuses and vessels: The sinuses are inconspicuous unless they are dilated and/or filled with hematopoietic cells (as in primary myelofibrosis) (Fig. 20.8a) or abnormal infiltrating cells (as certain types of B- or T-cell lymphomas do). The normal marrow microvasculature is barely noticeable unless the vascularity is increased or the individual vessels have pathological changes. Small arteries are seen occasionally and when present are useful to assess amyloid deposition.
- Marrow fibrosis: A reticulin stain may be routinely or selectively performed depending on the institution but is essential in myeloproliferative neoplasms for accurate grading of the disease (Fig. 20.8b). It also can highlight early mastocytosis or minimal involvement by lymphoma. Fibrosis is rarely seen in conditions such as CLL/SLL, plasma cell neoplasms, and MDS. When present in these conditions, it may have prognostic significance [71]. A trichrome stain is required in selected cases of primary or secondary myelofibrosis for accurate grading.
- Infiltrative lesions: These may arise from neoplastic or inflammatory processes. The former may be hematolymphoid malignancies or other solid tumors metastasizing to the marrow (see also Chap. 27). Benign lymphoid aggre-

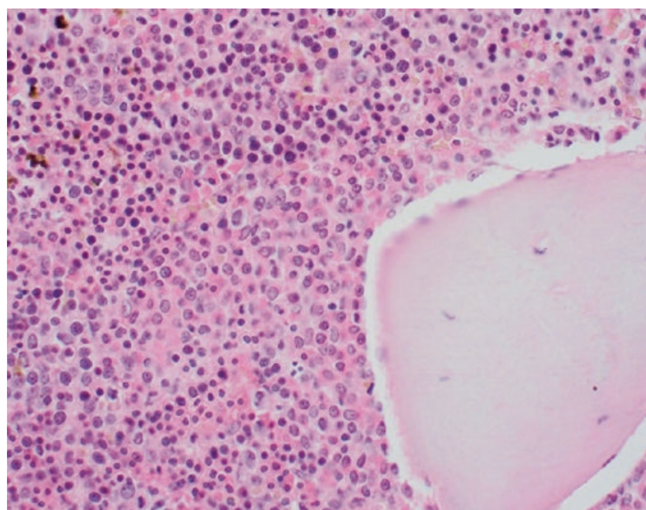

Fig. 20.7 Myeloid maturation (core biopsy, hematoxylin and eosin stain, 400×). Early myeloid precursors are seen in a paratrabecular location in this normal bone marrow

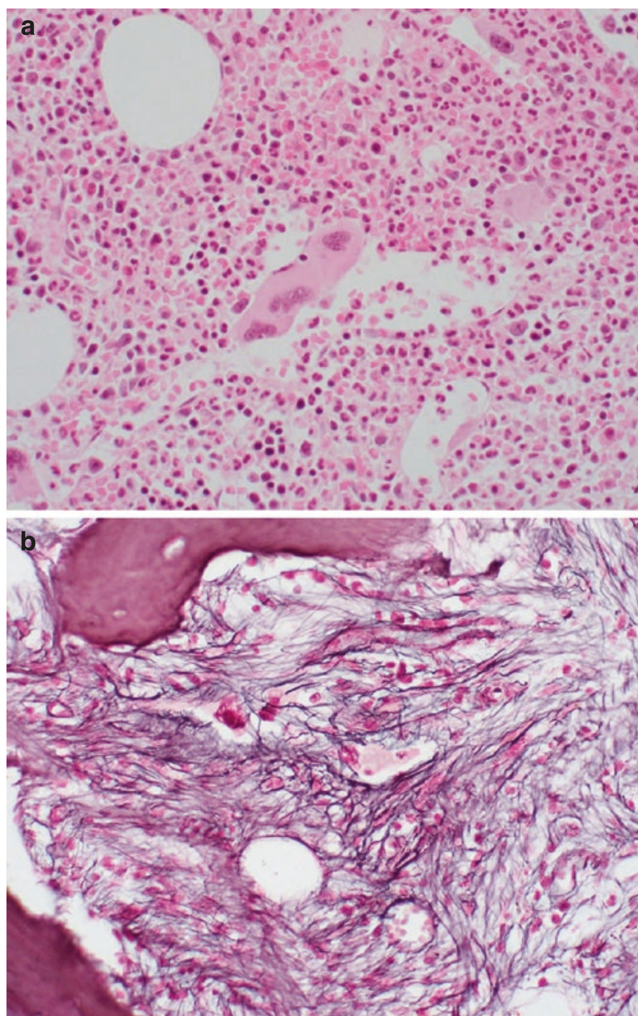

Fig. 20.8 Marrow sinusoids and fibrosis (core biopsies, hematoxylin and eosin stain, reticulin stain, 200×). (**a**) Dilated marrow sinusoids with intrasinusoidal hematopoiesis are present from a patient with primary myelofibrosis. (**b**) Increased reticulin fibrosis is present. Reticulin stains are essential in the evaluation of myeloproliferative disorders

gates may be seen with aging or a systemic chronic inflammatory condition. Other infiltrative/focal lesions most often are due to plasma cell neoplasms, systemic mastocytosis, Langerhans cell histiocytosis, and rarely histiocytic sarcomas. Granulomas are also seen in marrows and may be due to a variety of underlying causes [72].

- Necrosis: Diffuse necrosis of the bone marrow is seen in less than 1% bone marrows (Fig. 10.6b) and is almost always associated with metastatic or hematopoietic malignancy [73]. In some instances, extensive necrosis can make it difficult to determine the nature of the underlying malignancy.
- Changes in bone: While primary bone pathology is an infrequent finding in the bony trabeculae, evidence of hyperparathyroidism or Paget disease of bone may be present. Diffuse necrosis of bone marrow may be associated with karyolysis of osteocytes in the bony trabeculae and should be distinguished from true avascular necrosis of bone.
- Presence of unexpected "second" pathological process or disease: Particularly in older individuals, one can find evidence of a clinically unsuspected hematolymphoid process [74]. When both processes are relatively common, it is difficult to determine if they have any clonal or causal relationship or it is a chance occurrence [75]. (See Chap. 30 for more details).

14. What additional studies should be considered in the evaluation of a bone marrow?

Ancillary studies vary based on the indication for performing the bone marrow aspiration. The menu can be optimized by creating standard protocols [76, 77]. Some studies can be performed on routinely processed tissue, but some require additional specimen and decision to do these tests must be made at the time of collection. These studies include:

- Flow cytometry: A specimen should be collected in nearly every case. Two ml to 5 ml of marrow in a heparinized syringe is optimal. It is recommended that the first pull should be used for minimal residual disease (MRD) detection, especially for plasma cell myeloma [78]. In resource-poor settings, actual analysis can be safely delayed in almost all cases for 24–72 hours if the indication is not definitive.
- Karyotyping and FISH: Conventional cytogenetic analysis requires 2 ml or more of fresh (unfixed) heparinized marrow collected under aseptic conditions. FISH tests are often performed as a panel. FISH for *PML/RARA* translocation needs a rapid turnaround time if acute promyelocytic leukemia is suspected. These concerns must be communicated promptly to the performing laboratory so the test can be set up appropriately.
- Molecular tests: Most DNA-based tests can be performed on paraffin-embedded tissue, but decalcification can hamper some tests. However, DNA, and even more so RNA, promptly purified from fresh bone marrow collected in EDTA is more intact and provides more reliable results. Heparin may interfere in some PCR reactions. See Chap. 2 for additional precautions in collecting specimen for molecular tests.
- Bone marrow culture: When an infectious process is suspected, marrow should be submitted for culture, which can be more sensitive than morphological identification of mycobacteria (but not fungi) and blood culture [22].
- Additional unstained aspirate smears should be saved for performing cytochemical stains, including iron stain, as well as for FISH studies, particularly those involving numeric abnormalities.
- Ancillary tests which are performed on FFPE bone marrow biopsy and clot section include the following (see Chaps. 1 and 2 for more information about these tests):
  – Immunohistochemistry
  – Histochemical stains (Congo red, PAS, trichrome, etc.)
  – Paraffin FISH
  – Molecular tests (PCR-based amplification and sequencing, next-generation sequencing, RNA-based assays for gene expression)

15. Which findings are of immediate importance and should be reported to a clinician?

The following findings in the aspirate smear and/or touch preparation should be reported immediately to the clinician:

- Increased blasts (>20%) suggestive of acute leukemia, particularly if the morphology is suggestive of acute promyelocytic leukemia (see Chap. 21). Determining lymphoid versus myeloid leukemia may be difficult in the absence of Auer rods.
- When the definitive diagnosis from a set of limited differential diagnoses based on clinical or radiographic findings is obtained by morphological examination, the preliminary diagnosis should be conveyed to the clinicians; for example, metastasis versus plasma cell neoplasm causing lytic bone lesions.
- Presence of fungal or other organisms in the bone marrow – Histoplasma and candida may be detected in routinely stained aspirate smears. Prompt notification may help timely treatment and isolation of the patient.

16. What is the optimal organization of the bone marrow report?

- Bone marrow reports often include morphological descriptions. Pertinent parts of a concurrent CBC and microscopic findings in the peripheral blood and bone marrow aspirate (and/or touch preparation and clot section and core biopsy), including results of special stains, should be given. The choice of a narrative description versus a tabular reporting of findings varies from institution to institution. The College of American Pathologists has proposed synoptic report templates for hematologic neoplasms [79].
- In addition to morphology, flow cytometric, immunohistochemical, cytogenetic, FISH, and molecular results which are available at the time of completing the report may influence the specificity of the report.
- Bone marrow diagnoses can be broadly categorized based on degree of diagnostic certainty:
  - Definitive diagnosis of an entity: For neoplastic conditions, diagnosis according to WHO classification should be the goal [80]. Other clinically important findings or presence of a second pathological process should be mentioned as secondary diagnoses. In a comment, mention the key ancillary findings which support the diagnosis, such as positive FISH for *BCR/ABL* in a case of CML.
  - In case of a new malignant diagnosis, when all ancillary studies are completed, it is recommended that a comprehensive addendum diagnosis is issued which correlates the diagnostic, therapeutic, and prognostic impact of the additional results.
  - In follow-up marrows after therapy, the presence or absence of residual disease should be mentioned prominently so clinicians can easily find this information.
  - A descriptive diagnosis is appropriate when:
  - There are no pathological findings: The normal state of the marrow is conveyed by a phrase such as "Normocellular marrow with adequate trilineage hematopoiesis" along with status of iron stores.
- The pathological findings are not specific: The distinction between neoplastic and non-neoplastic conditions cannot be made with certainty, either because there is insufficient evidence of a clonal process (blast count is not high, flow cytometry is not diagnostic, etc.) or the morphological findings such as dysplasia, megakaryocytic abnormalities, or reticulin fibrosis are not well developed, or nutritional deficiencies, toxins, or infections can cause identical morphological change. In these situations, the key morphological findings, both normal and abnormal, are listed to provide a snapshot of the likely functional status of the marrow. Particularly in cases of cytopenias, the distinction between inability to produce enough cells or their destruction in the marrow or in the periphery is helpful. In these cases, the comment should include the likely differential diagnoses, the ancillary studies, and/or clinical/radiographic findings which can narrow the differential or provide a definitive diagnosis.

17. What are the mimics and what is the clinical relevance of misinterpretation between the true diagnosis and mimics with reference to mistreatment and/or wrong prognosis?

Caution should be exercised in interpreting following morphological findings, because they can be deceptively similar in the conditions listed below in the absence of ancillary studies:

- Small (8–9 uM diameter) blasts with high N:C ratio – hematogones (Fig. 20.3a), B-ALL (Fig. 20.9a), minimally differentiated AML

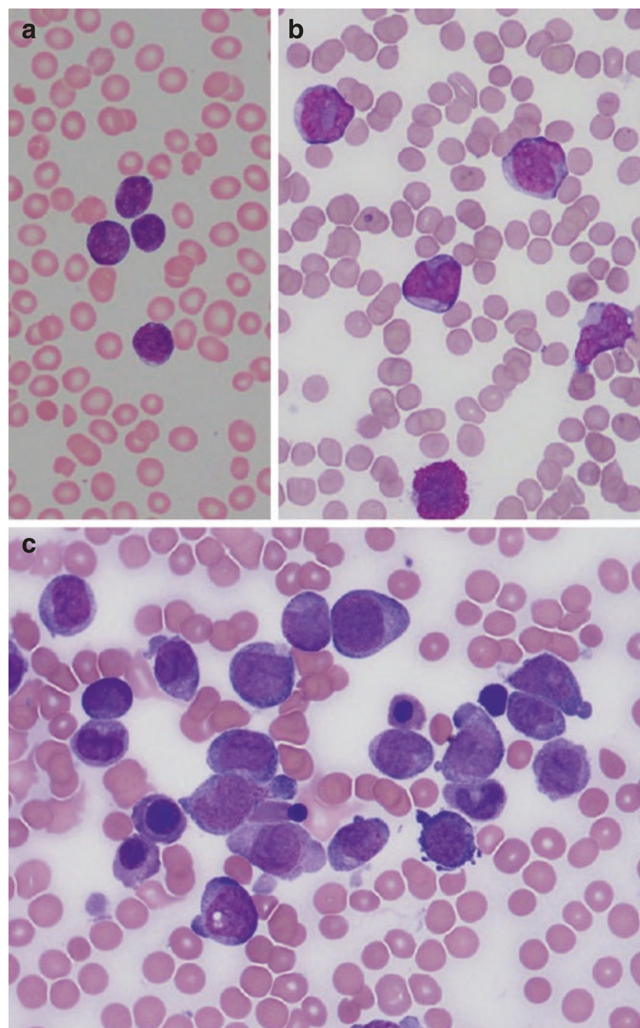

Fig. 20.9 Mimics (aspirate smears, Wright stain, 1000×). (**a**) Small blasts in B lymphoblastic leukemia should be differentiated from hematogones. Similarly, (**b**) hypogranular acute promyelocytic leukemia must not be mistaken for (**c**) Monocytic leukemia

- Large (12–18 uM diameter) immature cells with folded nuclei – hypogranular APL, monocytic acute leukemia (Fig. 20.9b,c)
- Blastoid cells – myeloblasts, plasmablasts, megaloblasts, metastases of small blue cell tumors (Fig. 20.10a), blastic plasmacytoid dendritic cell neoplasm, large cell lymphoma (Fig. 20.10b)
- Spindle cells, particularly in paratrabecular location – systemic mastocytosis (Fig. 20.11a), osteosclerotic myeloma, follicular lymphoma
- Giant cells – abnormal megakaryocytes, inflammatory giant cells (Fig. 20.11b), osteoclasts
- Abnormal granulocytes: toxic granules in infection and growth factor administration, hypolobate nuclei in benign Pelger-Huet anomaly or MDS

18. Which morphological findings in the peripheral blood/BM aspirate/biopsy are reliably diagnostic? Which ones suggest the diagnosis? Which is (are) unreliable for diagnosis? Which findings rule out the diagnosis?

Most morphological marrow findings must be interpreted in the context of clinical and other findings. Few are completely diagnostic or completely rule out a process.

- Pathognomonic findings: Auer rods (Fig. 20.12) are pathognomonic to distinguish abnormal myeloid from lymphoid blasts, but not acute leukemia from myelodysplasia. Amyloid identified by Congo red stain is consid-

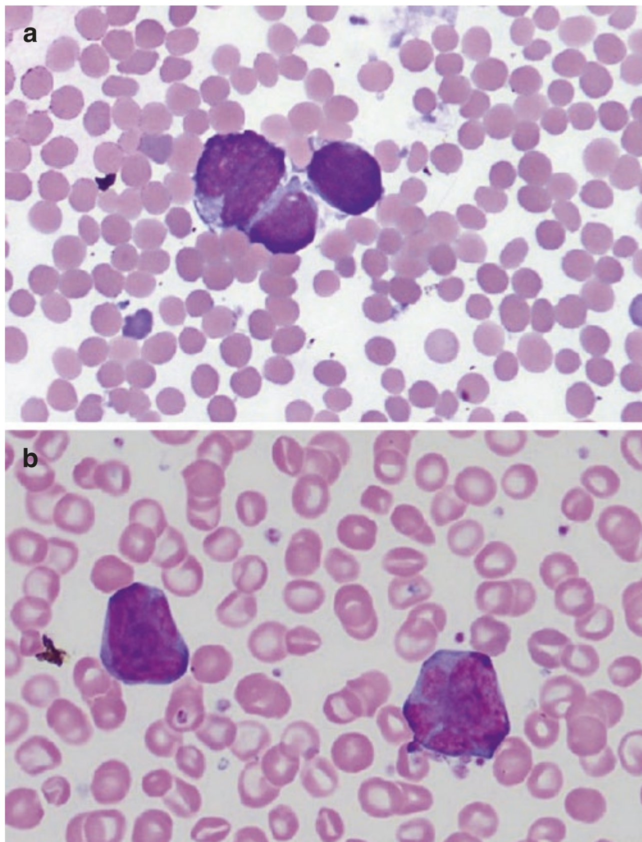

Fig. 20.10 Blastoid cells (aspirate smears, Wright stain, 1000×). (**a**) Metastatic small round blue cell tumor (neuroblastoma). These can be separate cells and resemble lymphoblasts. (**b**) Lymphoma cells. These cells may resemble blasts

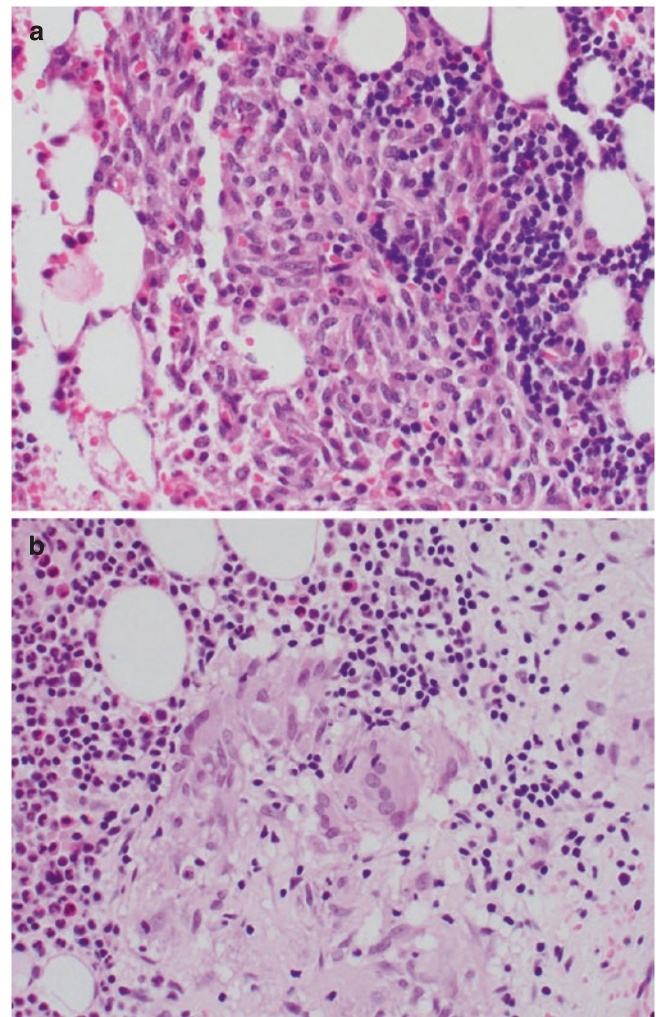

Fig. 20.11 Spindle cells and giant cells (core biopsy, hematoxylin and eosin stain, 400×). (**a**) Spindle cells in systemic mastocytosis shown here may be difficult to differentiate from fibroblasts. (**b**) Inflammatory giant cells need to be distinguished from megakaryocytes and osteoclasts

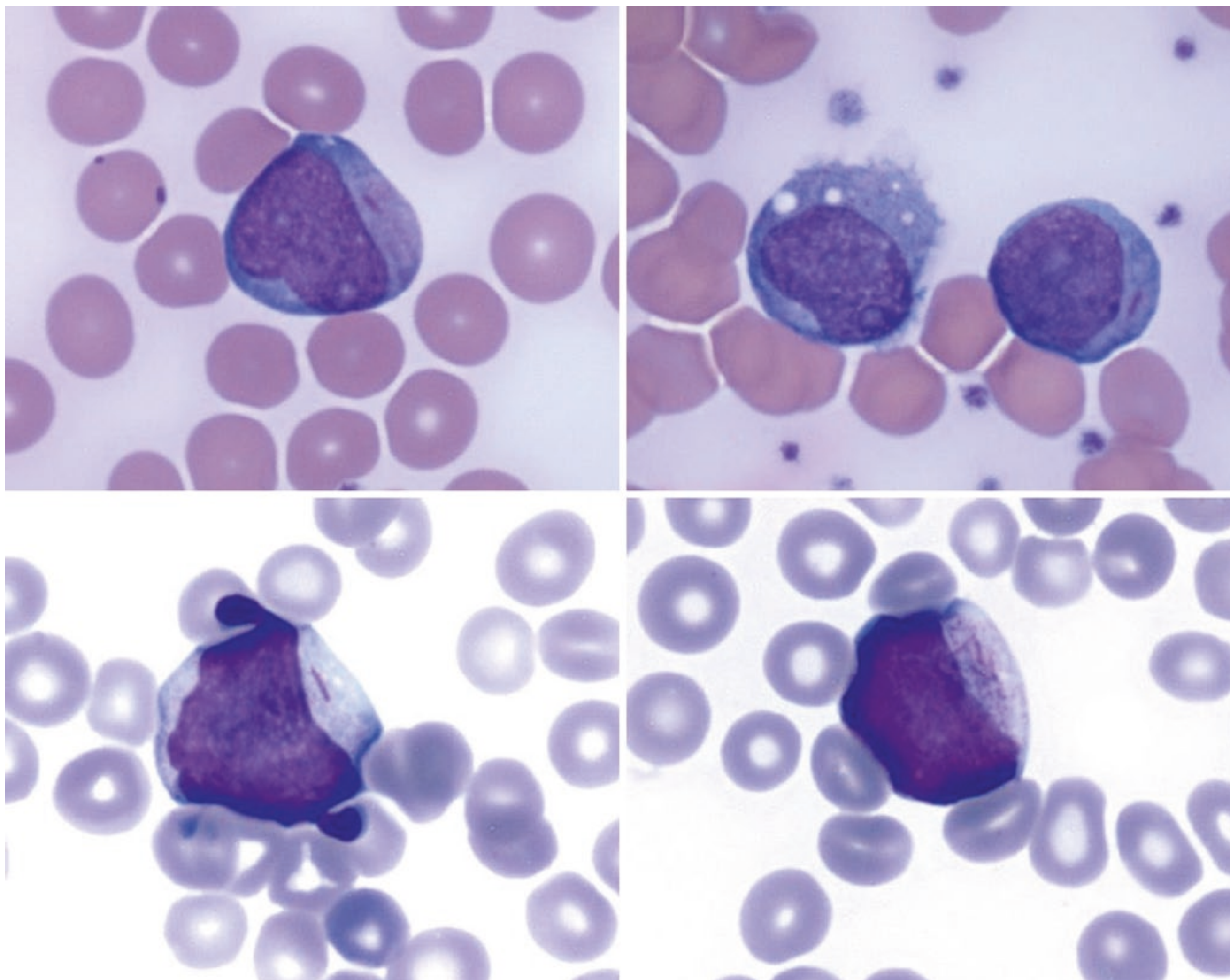

Fig. 20.12 Auer rods (Wright stain, 1000×). Auer rods are only seen in malignant myeloid blasts (upper panels) and abnormal promyelocytes (lower panels) and can be helpful in distinguishing lymphoid from myeloid blasts

ered specific; however; the determination of the underlying cause for the amyloid needs further study. Fungal and mycobacterial infections with appropriate special stains are considered sufficiently diagnostic even in the absence of additional proof by culture or molecular methods. A blast count above 20% of marrow cells based on morphological identification from a good quality aspirate smear is typically sufficient to diagnose acute leukemia, but exceptions occur such as infants with Down syndrome and patients receiving myeloid growth factor therapy.

- Suggestive of diagnosis: Dysplastic morphology is required for diagnosis in most MDS cases and is suggestive but is not sufficient for definitive diagnosis. Characteristic combinations of morphological findings are suggestive of the subtypes of MPNs, but diagnosis requires demonstration of specific mutations or ruling out reactive causes.
- Unreliable for diagnosis: See morphologic mimics above.
- Findings that rule out a diagnosis: Stainable storage iron rules out iron deficiency, but absence of stainable iron may be due to biological or technical reasons.

19. What should be the approach to provide maximum, but defensible information, from limited specimen or work-up?

- Following morphological findings tend to be unreliable in quantitatively limited specimens: proportion of various cells including M:E ratio and blast percentage in sparsely cellular aspirate smears, iron stores in aparticulate aspirate smears, marrow cellularity in subcortical marrow, and involvement by focal processes such as lymphoma, myeloma, metastases, etc., in small specimens (<5 mm long or <5 marrow spaces).

- Qualitatively suboptimal aspirate smears preclude accurate assessment of dysplastic changes and should not be used to count blasts. Often, some part of the specimen, either biopsy or clot section or aspirate or touch prep, has material of sufficient quality and quantity. Some of these parameters, such as blast count, can be measured by flow cytometry even when morphologically the specimen appears hopelessly inadequate with the caveat that blasts are often under-represented in flow cytometry specimens.
- The quality and quantity of the bone marrow core biopsy is less important in disorders like leukemia if there is a good aspirate smear compared to evaluation for infiltrative lesions like lymphoma or metastatic disease. Provided only the findings deemed reliable are reported, very few marrows are completely insufficient for diagnosis.

20. When is a diagnostic comment necessary and what should be discussed in the diagnostic comment?

A diagnostic comment is the rule rather an exception in bone marrow reports, because in many cases a definitive diagnosis of a well-defined entity is not possible. The report is part of an ongoing consultation between the hematopathologists and the clinicians. The content of the comment is dependent on whether the diagnosed entity is neoplastic or non-neoplastic and how definitive is the diagnosis (see the question about content of a bone marrow biopsy report).

21. When is it appropriate to seek external consultation for a bone marrow biopsy?

External consultation may be appropriate:

- If the clinical picture does not fit the pathological diagnosis. For example, the patient is clinically well and stable despite an apparent high-grade MDS.
- When there is a major discrepancy between the morphological diagnosis and the molecular/genetic results. For example, *BCR/ABL* translocation without any evidence of progressive leukocytosis, thrombocytosis, or acute leukemia.
- If certain pathological findings are not adequately explained by the overall diagnosis. Such findings may include reticulin fibrosis, histiocytic or lymphoid collections, deposition of acellular material of uncertain nature, etc.
- When the pathological findings are borderline or suggest a rare condition.
- In morphologically identical conditions with significant therapeutic differences.

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