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Preface

This book presents a comprehensive and practical approach to the diagnosis of diseases that primarily affect the blood and bone marrow. These diseases encompass both benign and neoplastic disorders that affect cells of hematopoietic lineages (leukocytes, red cells and their precursors, platelets, and megakaryocytes) and present with abnormalities primarily manifesting in the blood. The diagnostic approach to malignant hematologic diseases has recently been updated in the revised *World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues* published in 2017 and this book uses the current classification and nomenclature.

The diagnosis and classification of abnormalities of hematopoiesis are increasingly complex and constantly changing, in part due to the rapid advance in molecular genetic testing and accelerating development of new therapies that requires us to constantly refine our ability to effectively diagnose and classify these diseases. Next-generation sequencing represents a powerful tool that is rapidly entering routine clinical practice. While sequencing results help us understand disease biologies and are critical in diagnosing certain neoplasms, they add to the large amounts of information that must be digested and incorporated for individual cases. This data includes clinical features, bone marrow and blood morphology, flow cytometry immunophenotyping, and cytogenetics, as well as molecular genetic information. This comprehensive information set must be integrated by the diagnostician for each case in arriving at a specific and correct diagnosis and classification, which should ideally inform clinical management. Moreover, the diagnostician must weigh each piece of data to resolve contradictory information that often emerges. The purpose of this book is to guide the diagnostician as he/she navigates this process, providing detailed approaches to hematologic abnormalities and their differential diagnosis.

The book is uniquely organized by the specific clinical scenarios in which blood and bone marrow diseases present, rather than by each individual disease subtype. This allows the book to be used in real life situations, in which the diagnostician is confronted by abnormal blood and/or bone marrow testing (e.g. thrombocytosis or pancytopenia) that has a broad differential diagnosis. Each chapter has been organized to maximize its practical utility, including introductory information about hematopoietic cell types in normal and abnormal states, as well as clinical features, morphology, immunophenotype, genetics, and differential diagnosis of each specific disease. All chapters are extensively illustrated with images that underscore the salient features of

the diseases discussed in the text and with tables that present diagnostic criteria for neoplasms and differential diagnoses. Most chapters additionally contain algorithms that present a framework for the diagnostic approach to the specific clinical scenarios.

We are deeply indebted to all the authors who have contributed to this book and who have shared their collective wealth of experience in these challenging topics. We learned a great deal from our author colleagues in preparing this book; we hope that our readers will likewise benefit from the expertise put forth by this outstanding group of hematopathologists.

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Contents

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1 Identifying Blood and Bone Marrow Abnormalities in the Laboratory

Aliyah R. Sohani

Overview

Automated analysis of peripheral blood samples for complete blood count (CBC) and white blood cell (WBC) differential evaluation was introduced in the early 1950s and since that time has developed into the primary approach by which blood samples are analyzed for cellular constituents and the main avenue through which hematologic abnormalities first come to clinical attention. Prior to that time, the CBC, one of the most commonly ordered laboratory tests, was performed using entirely manual methods. For example, WBC, red blood cell (RBC), and platelet counts were performed using dilution of whole blood samples followed by manual counting under the microscope using a hemocytometer counting chamber; hematocrit was determined by high-speed column centrifugation; and WBC differential analysis always required examination, classification, and enumeration of cells under the microscope. Inarguably, these methods proved very time-consuming in a high-volume laboratory. Furthermore, manual WBC differential analysis is susceptible to errors related to consistency of cell classification between different observers, cell distribution artifact that results in larger cell types (e.g., neutrophils, monocytes, and eosinophils) being spread along the edges of a blood smear, and the inherent statistical limitation that stems from enumerating a relatively small number of cells, typically in the range of 100–200 per sample.

Modern-day hematology instrumentation comprises multichannel analyzers that employ a combination of methods, including electrical impedance, light scatter, radiofrequency conductivity, and/or cytochemistry, to perform cell counts [1]. The technology has evolved to the point that automated approaches to CBC and WBC differential analysis have proven to be efficient and cost-effective, as well as accurate and reliable in detecting clinically significant abnormalities. However, automated cell counters come with their own sources of error, confounding variables, and artifacts that may impede in the timely detection of peripheral blood abnormalities or result in falsely abnormal test results. In addition, it is generally accepted that these instruments serve a dual purpose as both diagnostic tools and screening devices, the latter implying that certain numerical abnormalities or analyzer-specific operator alerts (also known as "instrument flags") should trigger microscopic blood smear review by a skilled laboratory technologist or pathologist, particularly at the time of their initial occur-

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rence. For these reasons, a general understanding of the methods underlying automated CBC and WBC differential analysis is critical to a sound diagnostic approach to disorders involving the blood and, by extension, originating from the bone marrow.

This chapter provides an overview of automated analysis of whole blood constituents, including RBCs, platelets, and WBCs, with a focus on interferences, sources of error, and findings for which microscopic review or confirmation should be considered. In addition, situations in which peripheral blood flow cytometric analysis may be warranted will be addressed. Finally, appropriate preparation and microscopic evaluation of peripheral blood and bone marrow aspirate smears and trephine biopsies will be discussed.

Analysis of Red Blood Cells and Associated Parameters

The standard CBC includes the following RBC parameters: RBC count, hemoglobin concentration (HGB) measured in grams/deciliter (g/dL), hematocrit (HCT), mean corpuscular volume (MCV) measured in femtoliters (fL), mean corpuscular hemoglobin (MCH) measured in picograms/cell, mean corpuscular hemoglobin concentration (MCHC) measured in g/dL, and red cell distribution width (RDW). Reference ranges vary by age and gender and are readily obtained by consulting reference hematology or laboratory medicine texts [1]. Typical reference ranges for adults are listed in Table 1.1. However, it is recommended that individual laboratories confirm standard reference ranges for the specific patient population(s) they serve, as reference ranges for certain parameters may be affected by ethnic and geographic variations.

Most automated cell counters measure HGB using a spectrophotometric method, following RBC lysis and conversion of the HGB molecule to a derivative that can be measured using light absorbance at a specific wavelength. Historically, this process has required the use of potassium cyanide to convert HGB to hemiglobincyanide; however, cyanide-free measurement methods now exist [1]. RBC count is determined in many instances via electrical impedance, whereby cells

Table 1.1 Red blood cell indices: calculation, normal ranges, and interpretation in patients with anemia

Red blood cell (RBC) parameter	Definition/calculation	Normal range[a]	Abnormal values and some common associations
Mean corpuscular volume (MCV) (fL)	Average size of RBCs =HCT/RBC × 10	80–100	**Decreased MCV and decreased MCH:** iron deficiency, lead poisoning, thalassemia
Mean corpuscular HGB (MCH) (pg)	Amount of HGB per RBC =HGB/RBC × 10	26–33	**Normal MCV and normal MCH:** sudden blood loss, chronic inflammation, kidney failure, pregnancy **High MCV:** vitamin B12 or folate deficiency, certain drugs, MDS, hemolysis
Mean corpuscular HGB concentration (MCHC) (g/dL)	Amount of HGB per cell relative to RBC size =HGB/HCT × 100	31–36	**Decreased:** lead poisoning, vitamin B6 deficiency, copper deficiency **Normal:** iron deficiency **Increased:** hereditary spherocytosis, sickle cell disease, and homozygous hemoglobin C disease
Red cell distribution width (RDW) (%)	Variability in RBC size = coefficient of variation or standard deviation of MCV × 100%	12–15	**Increased:** recent transfusion, iron deficiency, vitamin B12 or folate deficiency, RBC fragmentation or agglutination, sideroblastic anemia, MDS **Decreased:** Not clinically relevant

HCT hematocrit, *MDS* myelodysplastic syndrome, *HGB* hemoglobin
[a]May vary by patient population and between laboratories

passing through a narrow aperture across which an electrical current is maintained cause changes in resistance, which can be measured in terms of pulse amplitude (corresponding to cell volume and size) and number of pulses (corresponding to the number of cells in a solution of known volume). Other instruments utilize purely optical-based methods of light scatter (allowing for determination of cell size and other physical properties) to enumerate RBCs, but regardless of the exact method used, an RBC volume distribution curve, histogram, or scatter plot is typically generated (Figs. 1.1a and 1.2a).

HCT, MCV, and RDW can be derived from the RBC volume histogram, depending on the type of technology used by the instrument. For example, some impedance-based instruments directly measure the HCT by summing the heights of all individual RBC pulses as each RBC passes through the aperture, a process known as cumulative pulse height detection. Once the HGB, RBC count, and HCT are known, the remaining RBC parameters, including MCV, can be calculated using the following formulae:

- MCV = HCT/RBC × 10
- MCH = HGB/RBC × 10
- MCHC = HGB/HCT × 100

Alternatively, other instruments may directly measure the MCV using an optical-based method, allowing for calculation of the HCT and other RBC parameters based on the MCV, RBC count, and HGB, as shown above. The RDW is another parameter calculated from the RBC volume distribution curve as either the coefficient of variation (CV) or the standard deviation (SD) of red cell size, thereby reflecting the degree of anisocytosis in an RBC population. It is helpful in the evaluation of anemia, particularly in distinguishing common causes of microcytic anemia from one another, as it is typically markedly elevated in the setting of recent transfusion or iron deficiency but only slightly elevated in thalassemia trait or anemia of chronic inflammation (Table 1.1). Knowledge of which RBC parameters are directly measured vs. calculated by a given analyzer is important, since factors causing

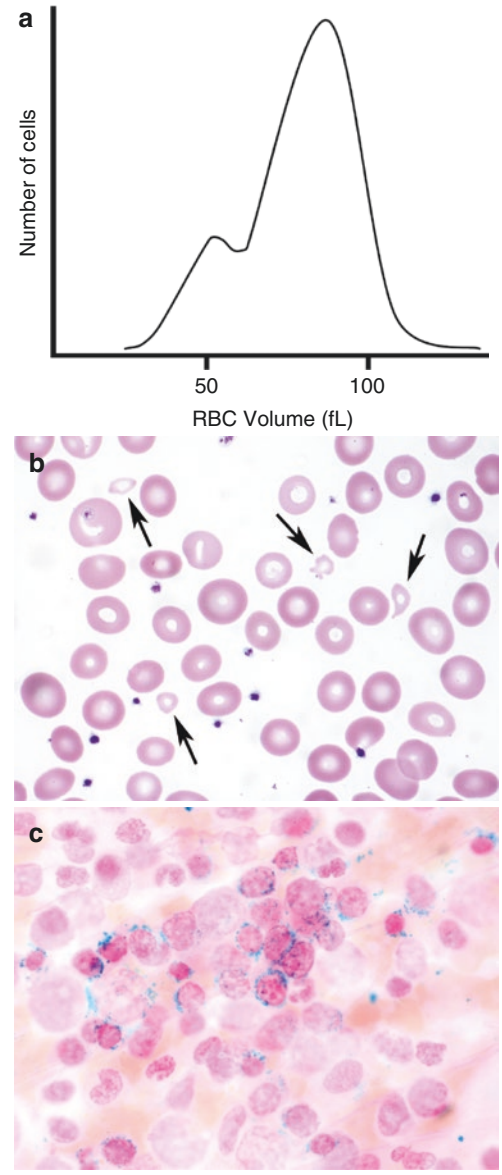

Fig. 1.1 Sideroblastic anemia with bimodal RBC volume distribution curve. (**a**) In this case of myelodysplastic syndrome with ring sideroblasts, the RBC volume distribution curve demonstrates a classic bimodal distribution with two distinct RBC populations, one that is microcytic and a second that is normocytic to macrocytic. (**b**) The corresponding peripheral blood smear demonstrates significant anisocytosis with a population of microcytic poikilocytes (arrows). This image also illustrates the ideal area of the blood smear for performing morphologic evaluation and platelet count estimates, where RBCs are evenly spaced and barely touching one other, although overlapping of two to three cells is acceptable. (**c**) Perls' iron stain of the corresponding bone marrow aspirate smear contains numerous ring sideroblasts, accounting for greater than 50% of erythroid precursors.

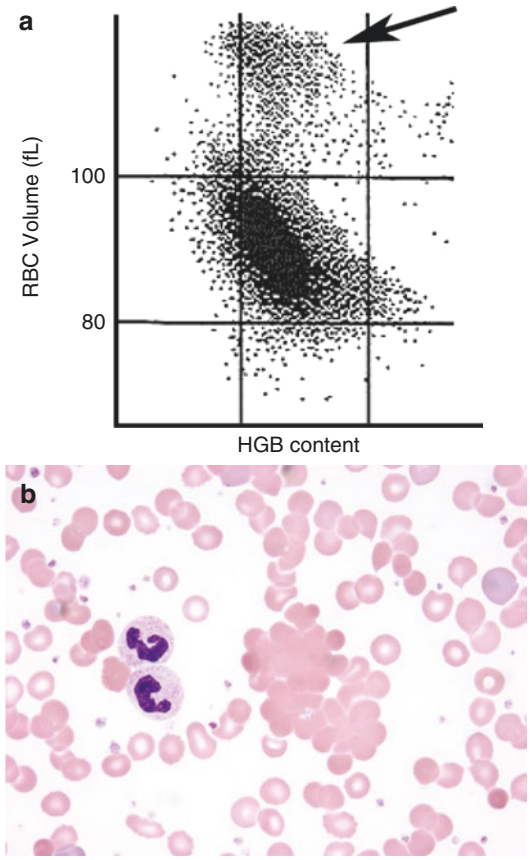

Fig. 1.2 RBC agglutination in cold agglutinin disease. (**a**) This scatter plot is taken from an optical hematology analyzer that measures RBC indices using light scatter following isovolumetric sphering of RBCs. Each dot represents a single RBC on a plot of volume vs. HGB content. In this example of cold agglutinin disease, RBC agglutinates appear as a separate cell population with very high volumes (arrow). (**b**) The corresponding blood smear shows prominent RBC agglutination

spuriously elevated or low counts can potentially affect the measured parameter, as well as indices deriving from it (Table 1.2).

The shape of the RBC volume histogram is usually Gaussian in nature, and changes in its symmetry can provide information about potential abnormalities present in a blood sample, prompting additional analysis and/or microscopic smear review. For example, a left-sided extension of the curve, or failure of the histogram to reach baseline on the left side between the platelet and RBC channels, may indicate the presence of a population of small RBCs, such as microspherocytes or RBC fragments, or may reflect the presence of large or giant platelets. A bimodal RBC volume distribution, in which two RBC populations of distinct sizes are present, may reflect prior transfusion or an underlying sideroblastic anemia (Fig. 1.1). A right-sided extension of the histogram may be seen in the setting of macrocytic anemia or reticulocytosis secondary to hemolysis. Such findings will often trigger an instrument flag indicating an abnormality in the RBC volume distribution, and laboratories should have mechanisms in place to review blood smears for underlying morphologic findings that may be clinically significant, such as schistocytes, microcytic spherocytosis, macrothrombocytes, or marked polychromasia. By contrast, low or high MCV values in the absence of RBC volume distribution abnormalities likely do not need to be confirmed by microscopic review. Another situation requiring additional investigation by the laboratory is that of RBC agglutination, which will result in extension of the RBC volume histogram to the extreme right, yielding an apparent population of very large RBCs and spuriously high MCV and MCHC instrument measurements (Table 1.2 and Fig. 1.2). In this circumstance, repeat analysis after sample warming should be undertaken; if the abnormalities do not correct after incubation at 37 °C, HCT may be determined manually via high-speed column centrifugation, but other RBC parameters are not considered reliable and should not be reported.

Measurement of reticulocytes, nonnucleated immature red cells recently produced by the bone marrow that contain high amounts of RNA, is a highly useful test in the diagnostic work-up of anemia by helping to distinguish causes related to impaired bone marrow production of erythrocyte precursors (associated with a low reticulocyte count) from those related to blood loss or hemolysis (associated with a high reticulocyte count). Traditionally, it is determined manually by incubating a peripheral blood smear with a supravital dye, either new methylene blue or brilliant cresyl blue, allowing for dark blue staining of the filamentous ribonucleoprotein complex contained within reticulocytes followed by their microscopic

Table 1.2 Causes of spurious results with automated hematology analyzers [1]

Parameter	Spurious increase	Spurious decrease
WBC count	• Cryoglobulins • Monoclonal proteins • Nucleated RBCs • Platelet clumps • Unlysed RBCs	• Clotted sample
RBC count[a]	• Cryoglobulins • Macrothrombocytes • Markedly high WBC count	• Clotted sample • In vitro hemolysis • Microcytic or fragmented RBCs • RBC agglutination
HGB[a]	• Cryoglobulins • Hyperbilirubinemia • In vitro hemolysis • Lipemia • Monoclonal proteins • Markedly high WBC count	• Clotted sample
HCT[a]	• Cryoglobulins • Hyperglycemia • Macrothrombocytes • Markedly high WBC count	• Clotted sample • In vitro hemolysis • Microcytic or fragmented RBCs • RBC agglutination
MCV[a]	• Hyperglycemia • Markedly high WBC count • Old sample (swollen RBCs) • RBC agglutination	• Cryoglobulins • In vitro hemolysis • Macrothrombocytes • Microcytic or fragmented RBCs
Platelets	• Cryoglobulins • In vitro hemolysis • Fragmented WBCs • Microcytic or fragmented RBCs • RBC inclusions	• Clotted sample • Macrothrombocytes • Platelet clumps or satellitosis

WBC white blood cell, *RBC* red blood cell, *HGB* hemoglobin, *HCT* hematocrit, *MCV* mean corpuscular volume
[a]Depending on the analyzer and the parameters that are directly measured vs. calculated, factors that affect these parameters may also affect other RBC parameters listed here, as well as the mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC)

identification and enumeration [1]. The reticulocyte count is subsequently reported as a percentage of total RBCs, based on a count of at least 1000 red cells, and typically falls in the range of approximately 1–2%. The absolute reticulocyte count is calculated by multiplying this percentage by the RBC count. More recently, automated cell counters have used optical methods employing fluorescent RNA-specific binding dyes to measure reticulocytes, precluding the need for more time-consuming microscopic evaluation and allowing for increased precision and accuracy. Regardless of whether the reticulocyte count is determined manually or via an automated method, it is important to note that the value reported by the laboratory is uncorrected for the degree of anemia and the longer circulating lifespan of reticulocytes in anemic states because of accelerated release from the bone marrow. Therefore, when evaluating an anemic patient, the reported reticulocyte count must be corrected for these two factors to yield a reticulocyte production index that can be used to help determine the cause underlying the anemia [2]. In order to calculate the reticulocyte production index from the reported reticulocyte count, first multiply the reported reticulocyte count by a correction factor derived from the patient's hematocrit divided by the expected normal hematocrit (typically 40% for women and 45% for men) to yield a corrected reticulocyte count. Then, adjust the corrected reticulocyte to account for the longer circulating lifespan of early reticulocytes. The exact adjustment depends on the degree of anemia, but a simple way is to divide the corrected reticulocyte

count by two (which presumes that in anemic states, reticulocytes circulate for about 48 hours, compared to about 24 hours in non-anemic states). For example, a male patient with a hematocrit of 15% and a reported reticulocyte count of 6% would have a corrected reticulocyte count of 2% (6%×15%/45%) and a reticulocyte production index of 1 (2%/2). In general, anemia due to impaired marrow production is associated with a reticulocyte production index of <2, while anemia related to blood loss or hemolysis has a reticulocyte production index of >2.

Automation of reticulocyte counts using optical methods has allowed for the development of additional, novel laboratory parameters that may have utility in various clinical circumstances. For example, the HGB content of reticulocytes (CHr or Ret-He) has been reported to be a useful screening test for early iron deficiency in both children and adults and has been identified as beneficial in monitoring the adequacy of iron therapy in iron deficiency anemia [3–8]. The immature reticulocyte fraction, indicating the proportion of reticulocytes with highest RNA content, has been heralded as potential laboratory indicator of bone marrow response to erythropoietin or exogenous iron therapy [9, 10]. More complex studies of RBC population dynamics enabled by optical hematology instrumentation have identified characteristics associated with certain anemic conditions and pre-anemic states, allowing for predictive modeling prior to the clinical development of anemia [11–13]; while such strategies have yet to be employed routinely, they illustrate the potential utility of systems-based approaches to diagnose and monitor hematologic diseases.

Analysis of Platelets and Associated Parameters

Although numerous methods exist for platelet enumeration, including manual, immunologic, and digital image analysis, most automated cell counters rely on impedance, optical light scatter, or a combination of the two. Impedance-based analysis is analogous to that described above for RBC analysis, where particles within a certain size range generate a platelet count, as well as volume distribution curve or histogram based on which possible impediments in count accuracy are flagged by the instrument. For example, failure of the platelet histogram to reach baseline at the low end or left side of the curve is an indicator of cytoplasmic fragments or electronic noise, while a similar pattern on the opposite side implies the presence of macrothrombocytes or microcytic RBCs. In these circumstances, microscopic smear review to estimate the platelet count (see the following section on Peripheral Blood Smear Evaluation) and compare it with that generated by the instrument is warranted. Optical counts rely on the light scatter properties of platelets and may incorporate a platelet-specific fluorescent dye, thereby limiting interference from cytoplasmic fragments or microcytic RBCs. Some platforms employ both an impedance and optical count, such that the latter is performed in instances when there is sufficient doubt about the accuracy of the impedance count. Immunologic counting of platelets using light scatter and platelet-specific antibodies, such as anti-CD41 or anti-CD61, is considered the reference method of platelet enumeration and demonstrates high accuracy, particularly in cases of severe thrombocytopenia [14–17].

An issue that interferes with all three of these methods is that of ethylenediaminetetraacetic (EDTA)-dependent agglutinins leading to platelet

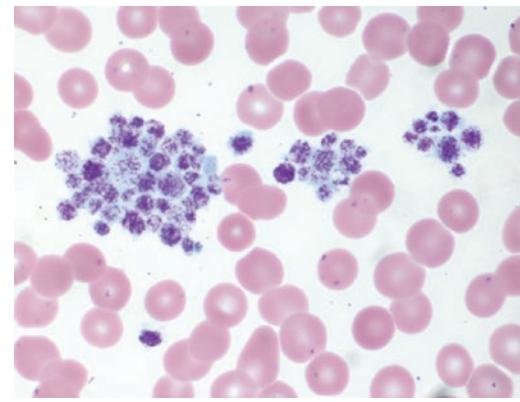

Fig. 1.3 Pseudothrombocytopenia with platelet clumping. In this patient with presumed immune thrombocytopenic purpura, review of the peripheral blood smear was performed because of the low platelet count; this revealed prominent platelet clumps, indicating that the instrument platelet count is falsely low. The platelet clumps are much larger than individual platelets that they are typically counted as WBCs by automated instruments

clumping or satellitism and underestimation of the platelet count, a phenomenon known as pseudothrombocytopenia (Table 1.2 and Fig. 1.3). Although automated instruments can flag for the presence of platelet clumps (see the following section on Analysis of White Blood Cells), an initial low platelet count below a certain threshold that approaches clinical significance should elicit smear review to exclude for the presence of platelet clumps. In circumstances of severe clumping, the instrument count is deemed unreliable, and an estimate based on smear review may also be limited in terms of accurately quantifying platelets, particularly if platelet transfusion is being considered. In such scenarios, remeasuring the platelet count on a sample collected in citrate- or heparin-anticoagulated plasma may be an option; however, in some individuals, platelet clumps persist despite collection using alternative anticoagulants. In such cases of multianticoagulant-dependent pseudothrombocytopenia, treatment of the sample with amikacin has been reported to dissociate platelet clumps, allowing for accurate platelet enumeration without affecting other CBC parameters [18].

Additional platelet-related parameters generated by certain automated cell counters include the mean platelet volume (MPV) and immature platelet fraction (IPF), both of which may help determine the underlying cause of thrombocytopenia (Table 1.3). The MPV, measured in fL, represents the average platelet size and is analogous to the MCV for RBCs, but unlike the MCV, the MPV normal range varies depending on the underlying technology used for measurement (impedance vs. optical) and is therefore instrument-specific. Under normal conditions, when the marrow and spleen are functioning appropriately, platelet size correlates inversely with number, such that the MPV will be elevated in conditions of destructive thrombocytopenia (e.g., immune thrombocytopenic purpura and microangiopathy). Other conditions associated with a high MPV include certain congenital thrombocytopenias, such as Bernard-Soulier syndrome, May-Hegglin anomaly, and gray platelet syndrome, some cases of myelodysplastic syndrome (MDS), and hyposplenism or asplenia (due to the tendency of the spleen to sequester larger platelets). In contrast, a low MPV may be seen in thrombocytopenia secondary to marrow hypoplasia or aplasia, splenomegaly, and congenital disorders, such as Wiskott-Aldrich syndrome. An abnormal MPV in the absence of bleeding or thrombocytopenia is unlikely to be clinically significant. It should also be noted that MPV may be affected by preanalytical variables related to the decreased stability of platelet shape immediately after collection and the propensity for platelets to swell in EDTA after prolonged exposure. Therefore, appropriate evaluation of a persistently abnormal MPV includes peripheral smear review to look for morphological clues,

Table 1.3 Mean platelet volume (MPV) and immature platelet fraction (IPF) in patients with thrombocytopenia

Parameter	Definition	Normal range	Abnormal values and possible associations
Mean platelet volume (MPV) (fL)	Average platelet size	Instrument-specific	**Increased:** destructive thrombocytopenia (such as ITP and TTP); congenital thrombocytopenias, such as Bernard-Soulier syndrome, May-Hegglin anomaly, and gray platelet syndrome; some cases of MDS; hyposplenism or asplenia **Decreased:** marrow hypoplasia or aplasia, splenomegaly, Wiskott-Aldrich syndrome
Immature platelet fraction (IPF) (%)	The fraction of reticulated platelets newly released by the bone marrow	1–7	**Increased:** peripheral platelet destruction (e.g., ITP, TTP, DIC); recovering marrow after chemotherapy and stem cell transplant **Decreased:** bone marrow failure; MDS; bone marrow suppression due to infection, chemotherapy, or other drugs

ITP immune thrombocytopenic purpura, *DIC* disseminated intravascular coagulation, *MDS* myelodysplastic syndrome, *TTP* thrombotic thrombocytopenic purpura

such as RBC fragments, abnormal leukocyte inclusions, hypogranular platelets, or dysplastic neutrophils, suggesting one of the above diagnoses. The MPV may also be used in conjunction with the IPF or the reticulated platelet count in distinguishing conditions associated with impaired platelet production from those related to accelerated destruction, as the latter typically show an elevated IPF [19, 20]. Analogous to reticulocytes, reticulated platelets represent young platelets recently released by the marrow with a higher RNA content that allows them to be identified optically on some platforms with the aid of a platelet-specific binding reagent.

Analysis of White Blood Cells and Nucleated Red Blood Cells

Most hematology analyzers provide automated WBC differential counts that include, at a minimum, the five main leukocyte subsets: neutrophils, lymphocytes, monocytes, eosinophils, and basophils. Depending on the platform, these are determined using a combination of technologies, including impedance (direct current and radiofrequency conductivity) and/or optical light scatter, in conjunction with various cell-specific lysing and staining reagents. Automated instruments provide both relative and absolute counts for WBC subsets; the latter are considered more clinically meaningful and should be included on CBC/differential reports issued by the laboratory [21]. Many newer analyzers also provide quantification of nucleated RBCs (in both relative [per 100 WBCs] and absolute units) with correction of total WBC count, since nucleated RBCs lead to spuriously high WBC counts when present in significant numbers (Table 1.2). Traditionally, the presence of any circulating nucleated RBCs has been considered to be an abnormal finding; however, the high sensitivity offered by modern automated cell counters has led to the recognition that small numbers of nucleated RBCs may be seen in healthy individuals without implying underlying pathology, particularly if other CBC parameters are normal [22]. Quantification of immature granulocytes (IGs) is another novel parameter offered by certain platforms; it represents the combined total number of circulating promyelocytes, myelocytes, and metamyelocytes. Studies have demonstrated good correlation with reference flow cytometry-based methods and suggest that elevated levels may be useful as an indicator of infection and other conditions related to bone marrow stress in both adult and pediatric populations [23–25]. At the very least, it likely represents a more objective and less time-consuming method of detecting a granulocytic "left shift" over traditional band neutrophil counts, which suffer from lack of reproducibility and limited sensitivity and specificity [26].

As with RBC and platelet analysis, cell counters act as screening devices for WBC abnormalities that fall out of expected parameters based on impedance, light scatter, and staining characteristics. Instrument flags are generated for the possible abnormalities that require peripheral blood smear review to confirm or exclude their presence. These include signal interference with the lymphocyte cluster by similarly sized cells or particles (e.g., nucleated RBC or platelet clump flags), interference at the interface of the lymphocyte and monocyte clusters (e.g., atypical lymphocytes or blast flags), interference at the interface of the monocyte and neutrophil clusters (e.g., blast flag), or altered positioning of the neutrophil cluster (e.g., left shift or immature granulocyte flags). Smears are typically reviewed by a medical technologist with training and expertise in peripheral blood smear morphology and cell classification. When abnormal cells such as blasts or atypical lymphocytes are identified, a traditional 100- or 200-cell manual differential count should be performed and resulted, supplanting the automated differential count. Smear review by the laboratory may also be considered when certain numerical thresholds are met or exceeded. Examples of the latter include absolute monocytosis or basophilia, moderate to severe pancytopenia that is new for a patient, or an absolute lymphocytosis in a child or adolescent to exclude the possibility of small lymphoblasts being erroneously categorized as lymphocytes by the analyzer. In addition, each laboratory should establish its own criteria for escalating a case further for pathologist review. Typically, this is performed in a subset of cases in which the findings are believed to represent a significant change from the patient's baseline that may be clinically significant (e.g., circulating blasts

or abnormal lymphoid cells suggesting the possibility of a previously undiagnosed or recurrent hematologic malignancy). However, these criteria vary depending on the complexity of the patient population served by a given laboratory.

While automated and manual differential analysis is useful in detecting abnormal circulating cell populations, immunophenotyping by flow cytometry is best used as an ancillary test to confirm CBC/differential findings and to determine the lineage of the abnormal cells [27]. For example, flow cytometry can help to establish a diagnosis of chronic lymphocytic leukemia in the setting of marked absolute lymphocytosis or can distinguish myeloid from lymphoid blasts in a case of previously undiagnosed acute leukemia. Flow cytometry is also helpful in situations requiring minimal residual disease evaluation, where neither automated cell counter-flagging nor morphological enumeration is sufficiently sensitive to detect small abnormal circulating populations. However, flow cytometry is unlikely to provide additional information over a manual differential count in cases of marked granulocytic hyperplasia with left shift, where the differential diagnosis includes a leukemoid reaction vs. chronic myeloid leukemia in chronic phase; in both instances, the small myeloid blast population is identifiable morphologically, and its neoplastic potential is not discernible by flow cytometry. Flow cytometry should also not be relied upon as a substitute for the morphologic blast count in the peripheral blood, since blasts may deviate from expected immunophenotypic characteristics (e.g., CD34 and CD117 negativity) [28].

Peripheral Blood Smear Evaluation

The number of samples requiring manual slide review greatly influences laboratory costs, productivity, and turnaround time. At the very least, manual smear review should be undertaken when instrument flags are triggered by abnormalities in the distribution of cells and/or particles within the RBC, PLT, and WBC analysis channels, as described previously (Table 1.4). In addition, some suggested numerical thresholds prompting smear review are also provided in Table 1.4, based in part

Table 1.4 Suggested criteria for peripheral blood smear review based on automated count and instrument flags

Numerical abnormalities	Threshold
Pancytopenia	WBC $<4 \times 10^9$/L and/or ANC $<1 \times 10^9$/L and/or HGB <7 g/dL and/or PLT $<100 \times 10^9$/L
Initial platelet count	$<50 \times 10^9$/L (to rule out pseudothrombocytopenia due to platelet clumping or satellitism)
Lymphocytes	$>5 \times 10^9$/L
Monocytes	$>1.5 \times 10^9$/L
Eosinophils	$>2 \times 10^9$/L
Basophils	$>0.5 \times 10^9$/L
Immature granulocytes[a]	>5%
Instrument flags	
Dimorphic red blood cell population	
RBC fragments or abnormal distribution	
Nucleated RBCs[b]	
Platelet clumping	
Giant platelets or abnormal platelet distribution	
Immature granulocytes[b]	
Variant lymphocytes	
Blasts	

ANC absolute neutrophil count, *HGB* hemoglobin, *PLT* platelet, *RBC* red blood cell, *WBC* white blood cell
[a]For analyzers that provide an automated immature granulocyte count
[b]May not be necessary for analyzers that can enumerate these cells, unless the count is flagged or exceeds a certain threshold

on consensus recommendations published by the International Society for Laboratory Hematology [29]. The latter may be modified by individual laboratories based on the automated platform in use, patient population served, clinician input, and following validation on patient samples.

The most commonly used stain for morphologic evaluation of the peripheral blood smear is the Wright or Wright-Giemsa stain. This staining protocol contains methanol, which fixes the cells to the glass slide, and both acidic and basic dyes that are variably taken up by cellular constituents at a pH of 6.4 maintained by a buffer, allowing for recognition of distinct cell and particle types at different stages of maturation. Best staining results are obtained from slides prepared within a few hours of sample collection [30]. Proper blood smear preparation and staining are essential to accurate assessment of cellular morphology and detection of abnormalities, particularly when evaluating neutrophil and platelet granularity (Fig. 1.4) [28]. Table 1.5 lists artifacts related to poorly prepared or stained smears and possible causes.

Peripheral smear examination should occur sequentially at low (×10 objective, ×100 total), intermediate (×40 high dry or ×50 oil immersion objective, ×400 or ×500 total), and high (×100 oil immersion objective, ×1000 total) magnifications. Low magnification allows for assessment of overall smear quality, including identification of artifacts (Table 1.5), as well as abnormalities in the distribution of RBCs and platelets such as rouleaux or clumps, while higher magnifications allow for assessment of individual cellular morphology and estimation of the platelet count in situations where the count provided by the instrument requires verification (see previous section on Analysis of Platelets and Associated Parameters) [30]. WBC abnormalities can be suspected at intermediate magnification but should be confirmed at high magnification. In addition, if a formal manual leukocyte differential is undertaken because abnormal WBCs are identified, this should generally be performed at high power using the ×100 objective (×1000 total magnification). The appropriate area for leukocyte evaluation and classification resides in the region of the smear where the RBCs are arranged as a monolayer, evenly spaced, and are close to one another but not quite touching (Fig. 1.1b); in a sample with a normal RBC count, this area contains about 200–250 RBCs per ×100 oil immersion field. This ensures that WBC evaluation is performed in an area of the smear where leukocyte morphology is optimal and devoid of artifacts seen in thick (dark, shrunken cells) or thin (fragmented cells) areas of the smear. To allow for systematic differential analysis and minimize slide distribution error (the phenomenon whereby larger cells, such as monocytes, granulocytes, and blasts, tend to localize at the edges of the smear), the smear should be examined from one edge to another in a sweeping up and down pattern without skipping areas, and consecutive WBCs should be counted [30]. Typically, 100 WBCs are counted, but a count of 200 cells is recommended if the WBC count is moderately elevated (>40 × 10^9/L) or in patients with a myeloid neoplasm [28]. Preparation of buffy coat smears from the peripheral blood [31] or digital image analysis (see below) can permit adequate cell enumeration in leukopenic samples. Nucleated red blood cells should be counted separately as a relative count (per 100 WBCs); however, if the instrument can report a valid numerical nucleated RBC count, then repeating the count manually is unnecessary.

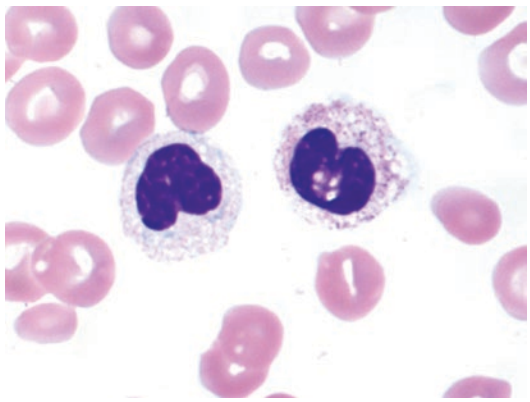

Fig. 1.4 Dysplastic neutrophils in a case of myelodysplastic syndrome. In this image, the cytoplasm of the neutrophil on the left is hypogranular compared to that on the right. Other fields demonstrated nuclear lobation abnormalities within neutrophils. RBCs are well-stained, indicating that the hypogranular cytoplasm is not simply an artifact of suboptimal, pale staining

The platelet count estimate, if needed, may be performed in the same region of the smear where leukocytes are evaluated, also under ×1000 total magnification. The average number of platelets,

Table 1.5 Peripheral blood smear artifacts and their causes [30]

Issue(s)	Possible cause(s)
Dark stain: • RBCs appear gray in color • WBCs too dark in color • Eosinophil granules appear gray instead of orange	• Stain or buffer too alkaline • Inadequate rinsing • Prolonged staining • Heparinized blood sample
Pale stain: • RBCs pale or red in color instead of pink • WBCs barely visible	• Stain or buffer too acidic • Over-rinsing • Inadequate buffering
Insufficient air-drying artifact: • RBCs contain refractile or punched-out central area	• Slide not allowed to dry sufficiently prior to staining • Equipment used to make slide is wet or contains condensation
Stain precipitate on slide	• Old or contaminated staining reagents • Equipment used to make slide is dirty or contains staining reagent residue
Smear too short; covers less than two-thirds of slide length	• Size of blood drop used to make smear too small • Elevated HCT in blood sample
Feathered edge not smoothed and rounded but irregular or bullet-shaped	• Spreader slide pushed too quickly
Smear contains irregularities, holes, or streaks	• Spreader slide chipped or rough-edged • Hesitation or uneven pressure in forward motion of spreader slide • Dirt on slide used for smear
Smear too narrow; does not span nearly the whole width of the slide	• Drop of blood not allowed to spread across width of spreader slide
Poor leukocyte distribution (monocytes and granulocytes mostly at edges of smear)	• Spreader slide pushed too slowly

RBC red blood cell, *WBC* white blood cell, *HCT* hematocrit

based on counting five to ten ×100 oil immersion fields, is multiplied by 20,000 to provide an estimate of the total number of platelets per microliter [30]. This result can then be compared to the instrument count, and if the instrument count is deemed inaccurate, the estimated platelet count may be reported as decreased, normal, or elevated per the laboratory's reference range. Caution should be exerted in reporting platelet count estimates numerically, particularly in cases of severe thrombocytopenia where platelet transfusion may be a consideration.

As with CBC and WBC differential analysis, peripheral smear preparation and evaluation have entered the age of automation. Many automated hematology platforms now offer the convenience and advantages of automated slide preparation and staining systems. These so-called auto slide maker stainers aspirate an aliquot of EDTA-anticoagulated whole blood and prepare a wedge-type blood smear similar to a manual smear prepared using the traditional push/pull method. In addition, instrument software systems, known as "middleware," allow for optimization of smear preparation using HCT information from the blood sample, thereby minimizing smearing artifacts resulting from abnormalities inherent to a sample (Table 1.5). Once dried, the smear is automatically advanced to the staining area of the instrument where stain and buffer are dispensed onto the slide per the staining protocol. In many instances, the slide maker stainer is physically connected to the main cell counter on a robotic track or line, allowing for smears to be prepared automatically on only those samples requiring microscopic review according to predetermined numerical or instrument flagging criteria and minimizing the need for operator intervention. These instruments are also flexible, allowing for staining of previously made blood smears or other sample types, such as body fluid cytospin and bone marrow aspirate smears, depending on the preferences and needs of the laboratory.

More recently, digital image analysis of prepared, stained blood smears via platforms such as CellaVision (CellaVision AB, Lund, Sweden) provides an opportunity for laboratories to ensure interobserver consistency in reporting practices and potentially to improve sensitivity in the detection of rare cell types [32]. These automated slide scanning devices help to locate and preclassify (via an artificial neural network) WBCs and nucleated RBCs and allow for evaluation of larger fields of view for characterization of RBC morphology and estimation of platelet counts. Images of pre-classified WBCs require confirmation by a trained morphologist (usually a medical technologist) prior to finalization of the manual differential count. The instrument software allows for side-by-side morphologic comparison of different groups of leukocytes present in the same smear, making it relatively easy to distinguish two or more populations with overlapping characteristics from one another (e.g., monocytes and variant lymphocytes). Laboratories can also create reference libraries of images illustrating typical or unusual findings, underscoring the potential educational utility of such platforms. Studies of this technology have demonstrated very good correlation with traditional 100-cell manual differentials, with superior accuracy depending on cell type and operator experience [33, 34]. Digital image analysis also allows for counting a larger number of cells in leukopenic samples, a frequent issue in posttreatment settings, improving sensitivity and turnaround times [34, 35].

Despite these advances in hematology automation, laboratory personnel and pathologists need to be cognizant of situations requiring procedural adjustments. For example, we have found in our laboratory that samples with extremely high WBC counts exceeding 400×10^9/L are poorly stained, making it difficult to evaluate cellular stage of maturation and cytoplasmic granularity and leading to a high rate of WBC misclassification on digital image analysis and traditional microscopic review. In such cases, our laboratory manually prepares the smear following dilution of the sample, allowing leukocytes to take up sufficient amounts of stain on the automated stainer and more accurate cell classification. Another example relates to the presence of large numbers of smudge cells, a characteristic feature of chronic lymphocytic leukemia, but also seen in other conditions associated with leukocytosis. Since certain cell types have greater fragility and propensity to rupture during the smearing process, basing a leukocyte differential on a sample with many smudge cells can lead to erroneous counts. Manual preparation of the blood smear following sample dilution with bovine serum albumin can minimize cell fragmentation and ensure evaluation of sufficient numbers of intact cells for a more accurate differential [36].

Bone Marrow Aspirate Evaluation

Unlike the situation described in the previous sections, preparation, staining, and evaluation of bone marrow aspirate are largely manual processes. Air-dried bone marrow aspirate smears may be prepared at the bedside or from the buffy coat layer of an EDTA-anticoagulated aspirate sample, in a manner similar to peripheral smears. Alternatively, marrow particles can be identified grossly and crushed between two slides (crush preparation), or touch imprints of marrow particles or of the core biopsy specimen can be performed; the latter is particularly helpful in the setting of bone marrow fibrosis eliciting a "dry tap" with collection of limited aspirate material. Smeared marrow particles (spicules) and fat droplets can be visualized on an unstained slide with the naked eye, providing assurance of bone marrow, rather than peripheral blood, sampling [1]. A standard Romanowsky-type staining method, such Wright-Giemsa or May-Grünwald-Giemsa, should be employed for routine morphologic analysis. As with peripheral smears, well-controlled staining protocols are essential for accurate morphological evaluation, particularly in cases where a diagnosis of myelodysplastic syndrome is suspected. A differential count of 500 nucleated cells (excluding macrophages and megakaryocytes) is recommended. This should be performed in areas of the smear adjacent to,

but not overlying, spicules, thereby limiting the effects of smear thickness and hemodilution. Additional stains that can be performed on bone marrow aspirate smears include cytochemical stains for enzyme constituents, such as myeloperoxidase (indicative of myeloid differentiation) and nonspecific esterases (indicative of monocytic differentiation) to help in determining blast lineage in cases of acute myeloid leukemia; however, some laboratories have replaced these tests with the use of flow cytometric immunophenotyping [28]. Perls' iron stain (Prussian blue reaction) to assess for storage iron in bone marrow macrophages and dendritic cells, sideroblasts (iron-containing erythroid precursors), and ring sideroblasts (erythroid precursors with five or more iron-containing granules encircling at least one-third of the nucleus) remains a standard part of morphologic assessment and particularly critical in evaluating cases of myelodysplastic syndrome with fewer than 5% blasts (Fig. 1.1c) [1, 28, 37].

Bone Marrow Trephine Biopsy Evaluation

Proper evaluation of the bone marrow biopsy mandates proper application of several preanalytic techniques: (1) obtaining an adequate core biopsy, which should be at least 1.5 cm in length and be taken at a right angle to the cortical bone surface so as to sample deeply into the hematopoietic marrow space, (2) adequate fixation, (3) proper decalcification, (4) adequate processing, and (5) careful histologic sectioning, generating intact 2–3 μm sections [38]. Neutral-buffered formalin is an adequate fixative for bone marrow, provided fixation time is sufficient and processing and sectioning are done properly, but many labs use fixatives containing metals, such as Zenker's, Bouin's, B5, aceto-zinc formalin, and B-plus, which improve nuclear detail [39, 40]; issues with toxicity and disposal of mercury have led to less frequent use of mercury-containing fixatives (Zenker's and B5). Decalcification agents include strong acids, buffered acids, and agents containing calcium chelators, often combined with acids. Decalcification time should be as short as possible, since acids have deleterious effects on the morphology and immunogenicity; agitation of the decalcification solution can help speed the decalcification process. Clot sections, representing either loose portions of the biopsy specimen that lack bone or clotted aspirated marrow, do not require decalcification. While clot specimens often are not optimal for evaluating marrow cellularity and may miss pathology associated with the bone trabeculae, they often provide superior immunostaining results and are also more amenable for molecular genetic testing than the decalcified bone marrow biopsy. Formalin-based fixation, paraffin processing, and decalcification all leach iron from the bone marrow, and thus iron stains on bone marrow biopsies (and to a lesser extent, clot sections) are less sensitive than iron stain on aspirate smears. While many labs perform a routine iron stain on the bone marrow biopsy, when assessment of storage iron or ring sideroblasts is essential for diagnosis, an iron stain on the bone marrow aspirate smear should always be performed [41].

In addition to at least one level stained with hematoxylin and eosin, many laboratories perform additional stains on the bone marrow sections, either routinely or as on an as-needed basis depending on the clinical suspicion and findings on routine histology. These stains are listed in Table 1.6. Because of the small size of the bone marrow core and tissue wastage between sectioning, when special stains and/or immunostains are ordered, additional blank slides may be cut up front for use if additional staining is needed. Of note, some immunostains on decalcified material may give different results from non-decalcified material. Thus, demonstration of staining of internal control cells in the bone is preferable to external non-decalcified control tissue in verifying a successful stain; alternatively, a decalcified bone marrow external control may be used. In some cases, different antigen retrieval or staining techniques may be needed for some immunostains (e.g., CD34) in bone marrow to ensure adequate sensitivity for detecting the cells of interest.

Table 1.6 Stains performed on bone marrow biopsies

Stain	Usage	Role
Hematoxylin and eosin	Routine: one or two levels	Evaluates routine histologic features; screens for the need for additional stains
Giemsa	Routine in many labs	Enhances the visualization of eosinophils, plasma cells, mast cells, and early erythroid forms
Iron	Routine in many labs	Evaluates storage iron but less sensitive than iron stain on the aspirate. Ring sideroblasts may sometimes be visualized in non-decalcified clot sections
Reticulin	Routine in many labs	Evaluates reticulin fibers in the marrow; essential for evaluating myeloproliferative neoplasms and also helpful in evaluating all myeloid neoplasms and some nonneoplastic conditions
Periodic acid Schiff	Routine in some labs	Enhances the visualization of megakaryocytes; detects fungal organisms
Trichrome	Typically only if Grade 2–3/3 fibrosis is seen on reticulin stain	Evaluates collagen fibrosis in the marrow; positivity usually indicates an advanced state of fibrosis
Other special stains	As needed, depending on clinical and histologic findings	Includes stains for microorganisms and Congo red
Immunohistochemistry	As needed, depending on clinical and histologic findings	Results may be different from non-decalcified tissue

References

1. Vajpayee N, Graham SS, Bem S. Basic examination of blood and bone marrow. In: McPherson RA, Pincus MR, editors. Henry's clinical diagnosis and management by laboratory methods. 23rd ed. St. Louis: Elsevier; 2017. p. 510–39.
2. Mathur SC, Hutchison RE, Mohi G. Hematopoiesis. In: McPherson RA, Pincus MR, editors. Henry's clinical diagnosis and management by laboratory methods. 23rd ed. St. Louis: Elsevier; 2017. p. 540–58.
3. Bakr AF, Sarette G. Measurement of reticulocyte hemoglobin content to diagnose iron deficiency in Saudi children. Eur J Pediatr. 2006;165(7):442–5.
4. Brugnara C, Laufer MR, Friedman AJ, Bridges K, Platt O. Reticulocyte hemoglobin content (CHr): early indicator of iron deficiency and response to therapy. Blood. 1994;83(10):3100–1.
5. Brugnara C, Zurakowski D, DiCanzio J, Boyd T, Platt O. Reticulocyte hemoglobin content to diagnose iron deficiency in children. JAMA. 1999;281(23):2225–30.
6. Ullrich C, Wu A, Armsby C, Rieber S, Wingerter S, Brugnara C, et al. Screening healthy infants for iron deficiency using reticulocyte hemoglobin content. JAMA. 2005;294(8):924–30.
7. Mast AE, Blinder MA, Dietzen DJ. Reticulocyte hemoglobin content. Am J Hematol. 2008;83(4):307–10.
8. Mast AE, Blinder MA, Lu Q, Flax S, Dietzen DJ. Clinical utility of the reticulocyte hemoglobin content in the diagnosis of iron deficiency. Blood. 2002;99(4):1489–91.
9. Geldard AR, Tobin DJ, Cuthbert A. Immature reticulocyte fraction as a useful parameter for blood transfusion assessment in anaemia. Br J Biomed Sci. 2009;66(2):98–101.
10. Chang CC, Kass L. Clinical significance of immature reticulocyte fraction determined by automated reticulocyte counting. Am J Clin Pathol. 1997;108(1):69–73.
11. Higgins JM. Red blood cell population dynamics. Clin Lab Med. 2015;35(1):43–57.
12. Higgins JM, Mahadevan L. Physiological and pathological population dynamics of circulating human red blood cells. Proc Natl Acad Sci U S A. 2010;107(47):20587–92.
13. Malka R, Delgado FF, Manalis SR, Higgins JM. In vivo volume and hemoglobin dynamics of human red blood cells. PLoS Comput Biol. 2014;10(10):e1003839.
14. Harrison P, Ault KA, Chapman S, Charie L, Davis B, Fujimoto K, et al. An interlaboratory study of a candidate reference method for platelet counting. Am J Clin Pathol. 2001;115(3):448–59.
15. Segal HC, Briggs C, Kunka S, Casbard A, Harrison P, Machin SJ, et al. Accuracy of platelet counting haematology analysers in severe thrombocytopenia and potential impact on platelet transfusion. Br J Haematol. 2005;128(4):520–5.
16. Kunz D, Kunz WS, Scott CS, Gressner AM. Automated CD61 immunoplatelet analysis of thrombocytopenic samples. Br J Haematol. 2001;112(3):584–92.
17. Hong KH, Kim MJ, Lee KW, Park KU, Kim HS, Song J. Platelet count evaluation using three automated haematology analysers compared with the immunoplatelet reference method, and estimation of possible inadequate platelet transfusion. Int J Lab Hematol. 2009;31(3):298–306.

18. Zhou X, Wu X, Deng W, Li J, Luo W. Amikacin can be added to blood to reduce the fall in platelet count. Am J Clin Pathol. 2011;136(4):646–52.
19. Briggs C, Kunka S, Hart D, Oguni S, Machin SJ. Assessment of an immature platelet fraction (IPF) in peripheral thrombocytopenia. Br J Haematol. 2004;126(1):93–9.
20. Kickler TS, Oguni S, Borowitz MJ. A clinical evaluation of high fluorescent platelet fraction percentage in thrombocytopenia. Am J Clin Pathol. 2006;125(2):282–7.
21. Etzell JE. For WBC differentials, report in absolute numbers. CAP Today [Internet]. March 2010. http://www.captodayonline.com/Archives/0310/0310d_for_wbc_differentials.html. Accessed 23 May 2017.
22. Hwang DH, Dorfman DM, Hwang DG, Senna P, Pozdnyakova O. Automated nucleated RBC measurement using the Sysmex XE-5000 hematology analyzer: frequency and clinical significance of the nucleated RBCs. Am J Clin Pathol. 2016;145(3):379–84.
23. Roehrl MH, Lantz D, Sylvester C, Wang JY. Age-dependent reference ranges for automated assessment of immature granulocytes and clinical significance in an outpatient setting. Arch Pathol Lab Med. 2011;135(4):471–7.
24. Fernandes B, Hamaguchi Y. Automated enumeration of immature granulocytes. Am J Clin Pathol. 2007;128(3):454–63.
25. Ansari-Lari MA, Kickler TS, Borowitz MJ. Immature granulocyte measurement using the Sysmex XE-2100. Relationship to infection and sepsis. Am J Clin Pathol. 2003;120(5):795–9.
26. Cornbleet PJ. Clinical utility of the band count. Clin Lab Med. 2002;22(1):101–36.
27. Craig FE. The utility of peripheral blood smear review for identifying specimens for flow cytometric immunophenotyping. Int J Lab Hematol. 2017;39(Suppl 1):41–6.
28. Vardiman JW, Brunning RD, Arber DA, Le Beau MM, Porwit A, Tefferi A, et al. Introduction and overview of the classification of the myeloid neoplasms. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press; 2008. p. 18–30.
29. Simson E, McFadden SL, Barnes PW, Machin SJ. The international consensus group for hematology review: consensus guidelines. International Society for Laboratory Hematology: Glenview; 2015. http://www.islh.org/web/consensus_rules.php. Accessed 17 Aug 2017.
30. Rodak BF, Carr JH. Clinical hematology atlas. 4th ed. St. Louis: Elsevier; 2013.
31. Brunning RD, Orazi A, Germing U, Le Beau MM, Porwit A, Baumann I, et al. Myelodysplastic syndromes/neoplasms, overview. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press; 2008. p. 88–93.
32. Sohani AR. Simple matters in complex times. Am J Hematol. 2017;92(3):230–1.
33. Briggs C, Longair I, Slavik M, Thwaite K, Mills R, Thavaraja V, et al. Can automated blood film analysis replace the manual differential? An evaluation of the CellaVision DM96 automated image analysis system. Int J Lab Hematol. 2009;31(1):48–60.
34. Park SH, Park CJ, Choi MO, Kim MJ, Cho YU, Jang S, et al. Automated digital cell morphology identification system (CellaVision DM96) is very useful for leukocyte differentials in specimens with qualitative or quantitative abnormalities. Int J Lab Hematol. 2013;35(5):517–27.
35. Rumke CL. Statistical reflections on finding atypical cells. Blood Cells. 1985;11(1):141–4.
36. Lunning MA, Zenger VE, Dreyfuss R, Stetler-Stevenson M, Rick ME, White TA, et al. Albumin enhanced morphometric image analysis in CLL. Cytometry B Clin Cytom. 2004;57(1):7–14.
37. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
38. Naresh KN, Lampert I, Hasserjian R, Lykidis D, Elderfield K, Horncastle D, et al. Optimal processing of bone marrow trephine biopsy: the Hammersmith Protocol. J Clin Pathol. 2006;59(9):903–11.
39. Mullink H, Henzen-Logmans SC, Tadema TM, Mol JJ, Meijer CJ. Influence of fixation and decalcification on the immunohistochemical staining of cell-specific markers in paraffin-embedded human bone biopsies. J Histochem Cytochem. 1985;33(11):1103–9.
40. Bonds LA, Barnes P, Foucar K, Sever CE. Acetic acid-zinc-formalin: a safe alternative to B-5 fixative. Am J Clin Pathol. 2005;124(2):205–11.
41. Stuart-Smith SE, Hughes DA, Bain BJ. Are routine iron stains on bone marrow trephine biopsy specimens necessary? J Clin Pathol. 2005;58(3):269–72.

Cytopenias: Reactive and Neoplastic

2

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Overview

Cytopenias are the most common peripheral blood count abnormality that come to medical attention and, in a subset of cases, stimulate performance of a bone marrow biopsy. Cytopenias encompass anemia, leukopenia (most often reduction of the absolute neutrophil count, but also including monocytopenia and lymphopenia), and thrombocytopenia. They may be isolated, involving only one cell line, or may involve two or all three cell lines (pancytopenia).

This chapter covers the spectrum of benign/reactive causes for cytopenias as well as the main neoplastic cause of cytopenia, myelodysplastic syndrome (MDS). The first part of the chapter presents the benign diseases associated predominantly with anemia, leukopenia, and thrombocytopenia and their differential diagnoses, followed by the second part which presents the clinical, pathologic, and genetic features of MDS and its broad differential diagnosis. Some specific scenarios that often cause cytopenia are covered in other chapters: congenital causes of cytopenia are mainly discussed in Chap. 3, aplastic anemia and paroxysmal nocturnal hemoglobinuria are discussed in Chap. 4, while lymphomas and plasma cell myeloma, diseases that often cause cytopenia, are discussed in Chaps. 11, 12, and 13.

Anemia

Erythropoiesis is a tightly regulated process characterized by continuous renewal of red blood cells by bone marrow erythroid precursors, which is in turn regulated by renal erythropoietin production in response to hypoxemia [1]. Anemia represents a reduction in one or more of the red blood cell indices and is generally defined as a hemoglobin (HGB) concentration of less than 13 g/dL in men, less than 12 g/dL in nonpregnant women, and less than 11 g/dL in pregnant women. The clinical presentation of anemia can vary greatly depending on the severity, rapidity of onset, and age of the patient, among other factors. Patients may be asymptomatic, but common symptoms include exercise intolerance, palpitations, dyspnea, headache, and fatigue. Ischemic symptoms, including angina, claudication, and heart failure, are usually an indication of severe, long-standing anemia. Anemias are

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divided into three main etiologic categories: (a) those that result from low red blood cell (RBC) production, (b) those that result from abnormal RBC maturation, and (c) those that result from increased RBC destruction. A comprehensive approach to anemia should involve detailed clinical and laboratory evaluation. The initial screening workup should include a complete blood count (CBC) to include RBC count, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width (RDW), and reticulocyte count, as well as serum iron profile and serum vitamin B12 and folate levels. A bone marrow examination may be required based on the results of the initial studies once iron deficiency, folate/B12 deficiency, and other common identifiable causes of anemia, such as alcohol, hemolysis, and drug-induced anemia, are excluded.

Anemias can be broadly divided into normocytic, macrocytic, and microcytic types based on the MCV. Microcytic anemias are defined by MCV below the lower level of normal range for age (80 fL in adults). Hypochromic (low MCHC) microcytic anemias are caused by abnormal hemoglobinization and/or maturation of RBC cytoplasm, which in turn result from defects in heme or globin chain synthesis or iron incorporation. Macrocytic anemia is characterized by anemia with an MCV of >99 fL in adults. Anemias can also be divided into cases associated with increased reticulocyte index and those associated with decreased reticulocyte production index (see Chap. 1 for discussion of measurement of reticulocytes).

Microcytic Anemias

Iron-Deficiency Anemia

Iron deficiency is the most common cause of microcytic anemia among children and adults. Deficient iron supplies result in decreased RBC production by the marrow and poor hemoglobinization of circulating RBCs. The two major causes of iron deficiency are reduced dietary supply of iron (more common in developing countries) and chronic blood loss (more common in developed countries). Iron influences the cell cycle of bone marrow normoblasts and a reduction in iron supplies results arrest of the normoblasts in G1/S phase, which further results in decreased proliferative response to erythropoietin [2]. Hepcidin, a protein secreted by the liver, plays a major role in the regulation of iron uptake from the intestine as well as release of storage iron.

The various stages of iron deficiency are further classified as iron store depletion (often due to inadequate iron intake, rapid growth, or normal menstruation), iron-deficient erythropoiesis (often due to excessive menstrual blood loss, pregnancy, malabsorption, inflammation, infection, or repetitive phlebotomy), and iron-deficiency anemia (usually reflecting chronic conditions such as chronic blood loss, hemolysis, hookworm infestation, severe malabsorption, celiac sprue, or enteritis). The earliest change in laboratory tests during the course of iron deficiency is a reduction of serum ferritin level, which is a surrogate for tissue iron stores. Following the depletion of iron stores, the serum iron level decreases and serum transferrin (the iron transport protein) increases, which in turn results in increased transferrin iron binding capacity (TIBC). Serum soluble transferrin receptor (sTfR) and sTfR/ferritin index are increased in iron-deficiency anemia. As such, evaluation for iron-deficiency anemia should include at least measurement of serum iron, TIBC, and serum ferritin level. Measurements of the red cell protoporphyrin level and the sTfR/ferritin index may also be helpful.

RBC morphology is affected during the later stages of iron deficiency: RBCs are initially microcytic and normochromic and eventually become microcytic and hypochromic. There is also usually marked anisocytosis (elevated RDW) and prominent elliptocytes. Reactive thrombocytosis can occur in association with iron-deficiency anemia (Fig. 2.1a). Bone marrow examination is rarely indicated for iron-deficiency anemia as the diagnosis can be established with fairly good reliability based on CBC and serum iron studies. However, bone marrow examination may be performed in order to assess marrow reticuloendothelial iron stores in ambiguous cases and in patients with additional comorbidities. The bone

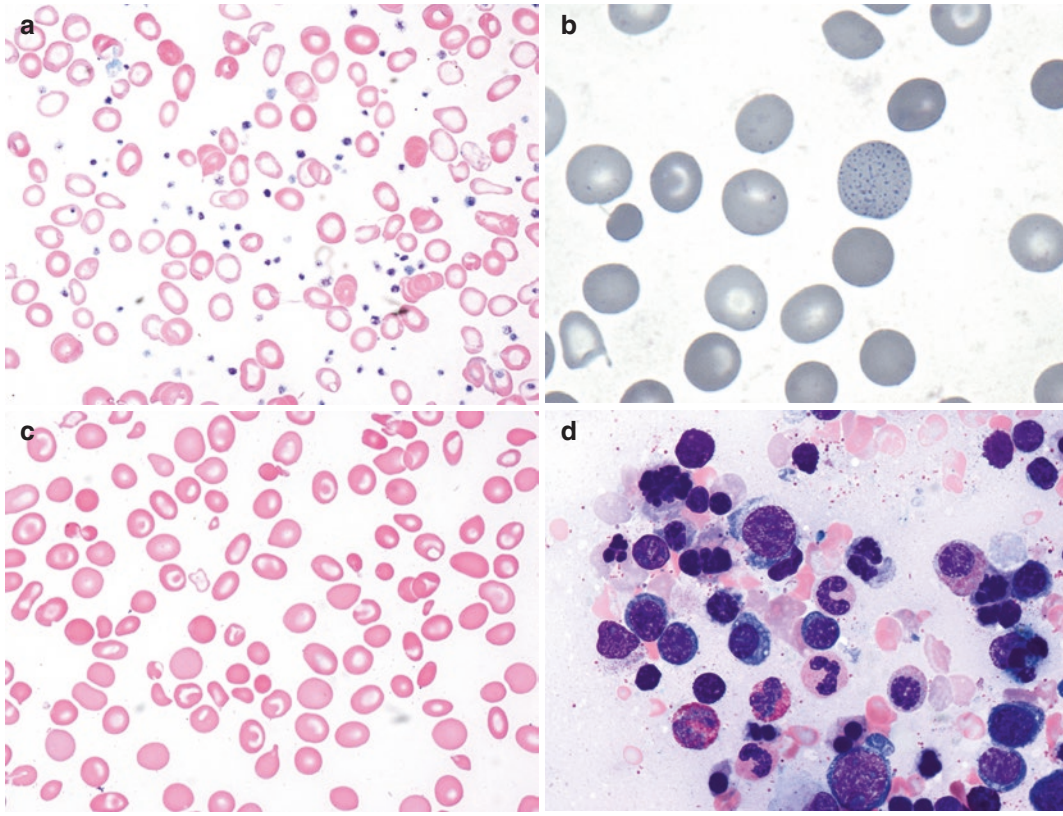


Fig. 2.1 Microcytic anemias. (**a**) Peripheral blood smear of iron-deficiency anemia. There is red cell microcytosis, anisopoikilocytosis, and hypochromia. Thromobocytosis, as seen in this case, occurs in a subset of cases. (**b**) Peripheral blood smear of β-thalassemia minor. Red cells show only mild anisocytosis and also exhibit basophilic stippling. (**c**) Peripheral blood smear of α-thalassemia major (hemoglobin H disease). There is anisopoikilocytosis with frequent target cells. (**d**) Bone marrow aspirate of α-thalassemia major. There is erythroid hyperplasia with striking reactive dyserythropoiesis ("stress erythropoiesis")

marrow aspirate smear is the optimal sample for evaluation of storage iron and sideroblastic iron incorporation, since iron is leached out during aqueous processing and decalcification of the paraffin-embedded bone marrow biopsy specimen. Approximately 40–60% of developing normoblasts have siderotic iron granules in their cytoplasm in healthy individuals with normal iron stores. Patients with iron deficiency usually have absent stainable iron on Prussian blue stain, with decreased sideroblastic iron incorporation.

The main differential diagnosis for iron-deficiency anemia is thalassemia. Thalassemias typically have moderate-to-severe microcytosis with varying degrees of anemia. A summarized comparison of the various causes of microcytic anemias is presented in Table 2.1. Serum iron studies and red blood cell morphology are usually sufficient to distinguish between these two entities. A more difficult distinction is between iron deficiency secondary to blood loss and iron deficiency associated with inflammation. Generally the anemia associated with inflammatory states is mild (HGB >10 g/dL) and RBC morphology is unaffected. However, with long-standing, chronic inflammatory illnesses, reduced iron supply and defective iron incorporation can lead to a moderately severe microcytic/hypochromic anemia. Serum iron studies are helpful in distinguishing true iron deficiency from anemia of chronic disease/inflammation and thalassemia (Table 2.1). Iron-deficiency anemia is characterized by low serum iron, high TIBC (percent saturation <10%), low ferritin, elevated sTfR/ferritin

Table 2.1 Microcytic anemias and their pertinent CBC and peripheral blood smear findings

Type of microcytic anemia	RBC count	MCV	MCHC	RDW	Reticulocyte production index	Peripheral blood smear findings
Iron-deficiency anemia	↓	↓	↓↓	↑↑	↓	Increased pencil cells (elliptocytes), blister cells (perkeratocytes), +/– target cells (codocytes)
Thalassemias	Normal or ↑	↓↓	Normal or ↓	Normal or ↑	↑	Increased codocytes and coarse basophilic stippling
Sideroblastic anemia	Normal or ↓	Normal or ↓	Normal or ↓	↑	↓	Dimorphic RBCs, increased hypochromic dacryocytes, coarse basophilic stippling, Pappenheimer bodies
Anemia of chronic disease/ chronic inflammation	↓	Normal or ↓	Normal or ↓	Normal	↓	

RBC red blood cell, *MCV* mean corpuscular volume, *MCHC* mean corpuscular hemoglobin concentration, *RDW* red blood cell distribution width

index, and absent marrow iron stores. While some patients with anemia of chronic disease/inflammation may have low serum iron and low transferrin saturation, marrow storage iron will still be present or even increased, serum ferritin will be normal or elevated, and sTfR/ferritin ratio will be not be increased. Additional rare conditions included in the differential diagnosis of microcytic anemia are conditions associated with mutations in genes regulating divalent metal transporter 1 (*DMT1*), ferroportin, ceruloplasmin, hepcidin, transferrin, and ferroxidase.

Thalassemias

Thalassemias are hereditary diseases that comprise the second leading etiology of microcytic anemias in pediatric and adult populations, with nearly 200 million patients affected worldwide. They are disorders associated with defective synthesis of α- or β-globin subunits of HbA ($\alpha_2\beta_2$). β-Thalassemia is most common in individuals of African and Mediterranean decent, whereas α-thalassemia and hemoglobin E disease are common in individuals from Southeast Asia. A diagnosis of thalassemia may be suspected in a patient with microcytic anemia based on various factors, including family history and ethnic background. The workup and diagnosis of thalassemia should include detailed clinical history, serum iron studies, and quantification of hemoglobins.

Thalassemia is further divided into thalassemia minor, thalassemia intermedia, and thalassemia major, depending on the severity of disease secondary to the anemia and other signs and symptoms. Thalassemia minor is the most common form of thalassemia and results from heterozygous mutations in either the α-globin genes (*HBA*) or β-globin gene (*HBB*). These patients are either asymptomatic or have very mild anemia. α-Thalassemia trait (deletion of 2 *HBA* genes), patients with heterozygous *HBB* mutations, and those with partial heterozygous deletions of both *HBA* and *HBB*, including HGB Lepore and hereditary persistence of fetal hemoglobin (HPFH), also show microcytosis and hypochromia out of proportion to the degree of their anemia. Patients with α-thalassemia (deletion of a single gene), hemoglobin Constant Spring [(αCS), a non-deletion form of α-thalassemia], and certain subtypes of β-thalassemia have little or no disease manifestations and are considered to be silent carriers. Patients with thalassemia intermedia include those with milder forms of homozygous β-thalassemia, combined α- and β-thalassemia defects, β-thalassemia with high levels of hemoglobin F, or $(\delta\beta)^0$-thalassemia. These patients typically present with severe microcytic hypochromic anemia as well as signs of exuberant compensatory erythropoiesis, including organomegaly and secondary bone changes. β-Thalassemia major (also known as Cooley anemia) is a life-threatening ane-

mia that most often results from homozygous deletions of the β-globin gene, although rare cases result from double-heterozygous mutations and other abnormal hemoglobins, such as hemoglobin C, E, and Lepore. It frequently manifests during the first 2 years of life. These patients are transfusion dependent and as a result develop severe transfusion-related sequelae as well as marked skeletal changes due to compensatory marrow hyperplasia and growth retardation. Patients with β-thalassemia major have elevated serum iron, saturated TIBC, and marked tissue iron overload. Other less common thalassemias include hemoglobin H disease (double heterozygotes for α^0 and α^+ or αCS) and hydrops fetalis (homozygous α^0). Hemoglobin H comprises 4 β-chains and is highly unstable, leading to a hemolytic component.

Patients with thalassemia minor have hypochromic, microcytic RBCs (MCV less than 75 fL) with normal or borderline low HGB levels. In contrast to iron-deficiency anemia, the RBC count is usually elevated. The peripheral blood smear often shows increased codocytes (target cells) as a result of excess membrane accumulation. Course basophilic stippling of erythrocytes is often seen in β-thalassemia (Fig. 2.1b). There is little anisocytosis and therefore the RDW is not increased. Patients with thalassemia intermedia and major have more profound anemia with marked microcytosis and hypochromia (Fig. 2.1c). The RDW is also increased, with red cell anisopoikilocytosis on the peripheral blood smear. The reticulocyte index is lower than that expected for the degree of anemia.

The diagnosis and classification of thalassemia ultimately rely on quantitative measurement of various cellular hemoglobins including hemoglobin A, A2, F, and H. Measurement of other rare abnormal hemoglobins such as S, C, D, E, Lepore, and Constant Spring is recommended in populations with high frequency of thalassemia. Qualitative measures of hemoglobin H include the use of supravital stain brilliant cresyl blue followed by quantitation by gel electrophoresis. Various methods of hemoglobin measurement are currently available for use including high-pressure liquid chromatography (HPLC), capillary electrophoresis, isoelectric focusing, and cellulose acetate membrane electrophoresis. [3]. In patients with microcytic hypochromic anemia and normal HbA2 and iron levels, secondary screening methods for thalassemia may be useful. These include assessment for HbH inclusions (insoluble β-globin tetramers) using oxidative dyes such as brilliant cresyl blue. Insoluble α-globin chain inclusions are highlighted with methyl violet and resemble Heinz bodies.

A bone marrow examination is usually not required in the workup of thalassemia. If performed, the bone marrow is generally hypercellular for age and shows erythroid hyperplasia. Dysplastic changes in the erythroid series can be prominent, reflecting so-called stress erythropoiesis (Fig. 2.1d).

The genetics of thalassemia is heterogeneous, resulting from approximately 200 known gene mutations/deletions involving the *HBA* or *HBB* genes on chromosomes 16 and 11, respectively. DNA-based molecular diagnosis offers means for definitive diagnosis and classification of thalassemia. Loss of the *HBA* gene, commonly through deletions and less commonly through non-deletional mutations causing impaired function and early protein termination, is responsible for α-thalassemia. Deletions and non-deletional mutations may coexist, resulting in different phenotypic manifestations. The majority of β-thalassemias result from point mutations in *HBB*. Many PCR-based methods, including allele-specific oligonucleotide hybridization, reverse dot blot analysis, amplification refractory mutation-PCR, gap-PCR, and direct sequencing as well as multigene next-generation sequencing panels, are available to detect these mutations [3].

The main differential diagnosis for thalassemia is iron-deficiency anemia. Unlike patients with thalassemia, those with severe iron-deficiency anemia usually do not show bone marrow erythroid hyperplasia. Patients with thalassemia often have increased serum bilirubin and LDH levels (indicative of increased cell turnover), which are not increased in patients with iron-deficiency anemia. Unlike patients with iron-deficiency anemia, those with thalassemia often show evidence of iron overload, with high serum iron levels and markedly elevated TIBC saturation, markedly increased serum ferritin, and increased stainable bone marrow iron. In addition, the degree of microcytosis in patients with thalassemia, particu-

larly thalassemia minor, is out of proportion to the level of anemia whereas in iron deficiency, microcytosis appears only after the HGB levels drop to below 10–11 g/dL. RDW is another helpful index in distinguishing these two entities: iron-deficiency anemia is associated with elevated RDW whereas thalassemia patients usually have normal or only mildly elevated RDW.

Sideroblastic Anemias

Sideroblastic anemias have an overall incidence of 1–3 per 100,000 population and are characterized by bone marrow ring sideroblasts as shown on Prussian blue stain [4]. They can be congenital or acquired and are primarily linked to alterations of the heme biosynthesis and iron-sulfur biogenesis pathways, as well as defects in the translation of mitochondrial proteins. The anemia is due to ineffective erythropoiesis, which results in systemic iron overload and its associated sequelae.

Congenital sideroblastic anemias are divided into syndromic and non-syndromic types, the former being defined by the presence of non-hematologic abnormalities in the affected individuals. The clinical, laboratory, and genetic features of genetically defined congenital sideroblastic anemias are summarized in Table 2.2 [5]. The genetic defect is unknown in approximately 40% of cases. The anemia of patients with congenital sideroblastic anemia is characteristically microcytic and hypochromic, with decreased MCV and MCHC. RBCs show prominent anisopoikilocytosis. Notably, the peripheral blood smear may show a dimorphic RBC population comprised of microcytic, hypochromic red cells and normocytic red cells. This phenomenon may be seen in patients with the autosomal form of disease as well as affected males and female carriers of the X-linked form of disease. MCV may also be elevated, particularly in the mitochondrial forms of the disease.

Table 2.2 Laboratory features of congenital sideroblastic anemias

Disease	MCV	Genetic defect	Other features
Nonsyndromic			
X-linked (XLSA)	↓	*ALAS2* mutations	Mild anemia or asymptomatic; MCV may be normal or increased in women with skewed X-chromosome inactivation in hematopoietic tissue
SLC25A38 deficiency	↓	*SLC25A38* mutations	Severe, transfusion-dependent anemia, presents early in life
Glutaredoxin 5 deficiency	↓	*GLRX5* mutations	Very rare; typically transfusion-dependent anemia
Erythropoietic protoporphyria (EPP)		*FECH* mutation	Mild anemia presenting in childhood; photosensitivity
Syndromic			
XLSA with ataxia	↓	*ABCB7* mutations	Mild anemia presenting in childhood, neurologic defects; mild increase in RDW and few circulating siderocytes may be seen in female carriers
CSA, B-cell immunodeficiency, periodic fevers, and developmental delay (SIFD)	↓	Unknown	Severe anemia from infancy
Pearson marrow-pancreas syndrome	↑	Mitochondrial DNA deletions, duplications, and rearrangements	Presents in infancy; marked vacuolization of erythroid and myeloid progenitors in bone marrow; abnormalities in mitochondrial ultrastructure in skeletal muscle
Myopathy, lactic acidosis, and sideroblastic anemia (MLASA)	Normal or ↑	*PUS1* and *YARS2* mutations	Presents in childhood with anemia and muscle weakness; vacuolization of bone marrow progenitors and abnormalities in mitochondrial ultrastructure in skeletal muscle
Thiamine-responsive megaloblastic anemia (TRMA) syndrome	↑	*SLC19A2* biallelic mutations	Variable age of onset; cellular thiamine deficiency

MCV mean corpuscular hemoglobin, *RDW* red blood cell distribution width, *XLSA* X-linked sideroblastic anemia, *CSA* congenital sideroblastic anemia

Chronic alcohol ingestion, copper deficiency, and hypothermia are the most common causes of acquired metabolic sideroblastic anemias. Many drugs can cause sideroblastic anemia, including various antibiotics, hormones, and chemotherapeutic agents. Some myelodysplastic syndromes are also associated with acquired ring sideroblasts and these are discussed later in this chapter.

Normocytic Anemias

Anemia of Chronic Disease/Anemia of Chronic Inflammation

Patients with chronic inflammatory diseases (including infections and collagen vascular diseases), renal insufficiency, and hypothyroidism can be affected by anemia of chronic disease/inflammation. The major cause for this condition is an imbalance between serum erythropoietin levels and bone marrow response to erythropoietin. Accordingly, the reticulocyte index and red cell synthesis are reduced in these patients. Bone marrow response to renal erythropoietin is a tightly regulated process that directly correlates with an individual's degree of anemia and relies on adequate iron supplies. Appropriate release of iron from the reticuloendothelial system in turn relies on hepcidin. Inflammatory cytokines, such as IL6, result in overexpression of hepcidin and increased levels of circulating iron, which in turn lead to reduced levels of erythropoietin and decreased erythropoiesis in the bone marrow. The severity of anemia generally correlates with the severity of the primary illness.

A comprehensive workup for anemia in patients with chronic diseases should include CBC, reticulocyte count, and serum iron studies (serum iron, TIBC, ferritin, and sTfR). Red blood cell morphology can range from normocytic and normochromic to microcytic and hypochromic depending on the degree of anemia, albeit the degree of microcytosis and hypochromia seldom reaches that of iron-deficiency anemia. Table 2.3 summarizes the pertinent laboratory studies in patients with anemia of chronic disease/inflammation compared to those with iron deficiency.

A bone marrow examination is rarely indicated in these patients as the diagnosis can be established by careful history and comprehensive laboratory studies. Bone marrow iron stores may be increased.

The differential diagnosis includes iron-deficiency anemia and other anemias associated with low red blood cell production.

Acquired Pure Red Cell Aplasia

Pure red cell aplasia is characterized by isolated anemia as a result of failure of erythropoiesis. The diagnosis relies upon the presence of anemia (which may be normocytic or macrocytic), low reticulocyte production index, and absent or markedly hypoplastic bone marrow erythropoietic precursors. Platelet and leukocyte counts are usually normal and serum iron studies usually show increased iron saturation and ferritin levels. In contrast, aplastic anemia applies to patients with pancytopenia and markedly reduced bone marrow cellularity with reduction of all three hematopoietic lineages (see Chap. 4). The pathogenesis is due to antibody and cellular mediated immune inhibition of erythropoiesis. It is most frequently encountered in association with autoimmune diseases (such as rheumatoid arthritis,

Table 2.3 Comparison of serum iron profile in patients with iron-deficiency anemia and anemia of chronic disease

Condition	Serum iron	TIBC	Serum ferritin (μg/L)	Serum sTfR/ ferritin	Marrow iron stores
Iron-deficiency anemia	↓	↑↑	↓(<30)	>2.5	↓
Anemia of chronic disease due to chronic renal failure	Normal[a]	Normal[a]	Normal	Variable	Normal
Anemia of acute or chronic inflammatory diseases	↓	↓	↑(>100)	<2.5	↑
Anemia of chronic disease due to hypothyroidism or pituitary dysfunction	Normal[a]	Normal[a]	Normal	Variable	Normal

TIBC total iron binding capacity, *sTfR* soluble transferrin receptor

[a]Serum iron and TIBC may be abnormal in patients receiving chronic hemodialysis where the iron stores are depleted and patients with transfusion-dependent chronic renal failure may show signs of iron overload

systemic lupus erythematosus, and myasthenia gravis), certain neoplasms (particularly thymoma [6] and lymphomas, including chronic lymphocytic leukemia/small lymphocytic lymphoma and large granular lymphocytic leukemia [7]), certain drugs, and viral infection. Many cases are idiopathic, with no identified association.

The infection most strongly associated with pure red cell aplasia is parvovirus B19, which preferentially infects erythroid precursors and arrests red cell production. Although the majority of patients have self-limited and even asymptomatic infections, persistent infection due to ineffective host antibody response (usually in immunocompromised patients) may lead to severe anemia or pancytopenia [8, 9]. Severe anemia, termed "transient aplastic crisis" also characterizes parvovirus B19 infection in patients with hemolytic anemias. Due to the increased cell turnover associated with hemolytic anemias, even a transient arrest in erythropoiesis rapidly leads to severe anemia. Transient aplastic crisis is most frequently associated with sickle cell disease, but also can occur in patients with hereditary spherocytosis, thalassemia, and other hemolytic anemias [10].

Several medications have been linked to pure red cell aplasia, including diphenylhydantoin, sulfa and sulfonamide drugs, azathioprine, allopurinol, isoniazid, procainamide, ticlopidine, ribavirin, and penicillamine [11]. Rarely, pure red cell aplasia may occur during pregnancy and in such cases, the anemia can be severe, with transfusion dependency.

Bone marrow examination demonstrates complete absence or markedly reduced (<5%) erythroid precursors (Fig. 2.2a, b). Myelopoiesis and

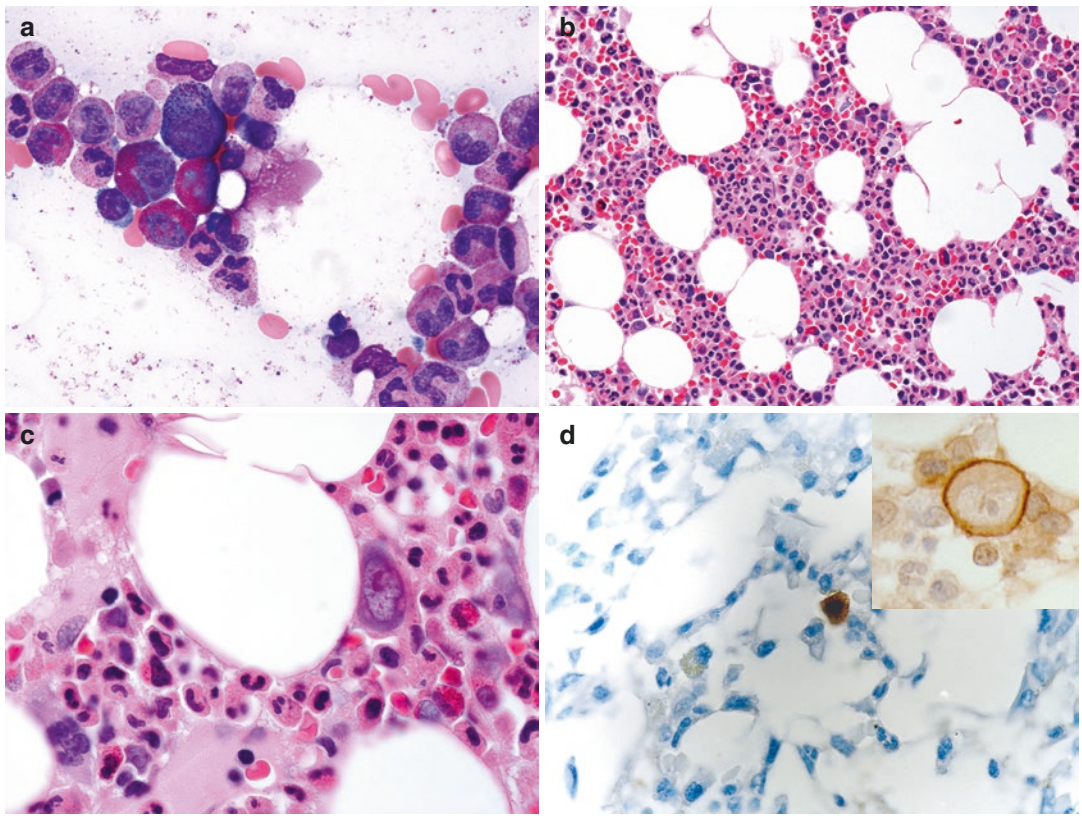


Fig. 2.2 Pure red cell aplasia. Bone marrow aspirate (**a**) and biopsy (**b**) in pure red cell aplasia secondary to thymoma. There is an almost complete absence of erythroid elements, with preserved myelopoiesis; in this case, megakaryocytes are also decreased. In pure red cell aplasia due to parvovirus infection (**c**), there is also a marked paucity of erythroid elements in the bone marrow biopsy, with occasional markedly enlarged primitive erythroblasts (giant pronormoblasts). The virally infected erythroblasts can be demonstrated by immunostaining for parvovirus (**d**) and the giant pronormoblasts are highlighted by glycophorin immunostain (**d**, inset)

megakaryopoiesis are normal. In parvovirus B19 infection, markedly enlarged primitive erythroid precursors ("giant pronormoblasts") can often be seen in both biopsy and aspirate specimens; some cells may contain visible viral inclusions. Infected erythroid cells in the marrow are highlighted by immunostaining for parvovirus B19 (Fig. 2.2c, d).

The differential diagnosis includes myelodysplastic syndromes with pure red cell aplasia [12]. Bone marrow examination for assessment of morphologic dysplasia and cytogenetics can aid in making this distinction. Idiopathic pure red cell aplasia is a diagnosis of exclusion and requires a comprehensive workup, including imaging studies to exclude thymoma and lymphoproliferative disorders, peripheral blood flow cytometry for exclusion of lymphoid leukemias, and bone marrow examination to exclude MDS. Parvovirus B19 infection should always be investigated in these patients by serology, PCR, and/or immunostaining of bone marrow for parvovirus.

Anemia Associated with Bone Marrow Infiltration (Myelophthisic Anemia)

Myelophthisic anemia is caused by bone marrow infiltration by abnormal cells or extracellular material, usually metastatic tumor [13] or fibrosis, but also including granulomatous inflammation, storage diseases, bone marrow necrosis [14], or rarely noncellular deposits such as oxalosis [15]. A myelophthisic picture is frequently encountered in myeloproliferative neoplasms with extensive bone marrow fibrosis, particularly advanced primary myelofibrosis. Anemia is often accompanied by a leukoerythroblastic picture in the peripheral blood smear (defined as increased dacryocytes erythrocytes, left-shifted leukocytosis, and circulating nucleated red blood cells). Very severe bone marrow infiltrative processes can be associated with pancytopenia. Table 2.4 provides a summary of conditions commonly associated with myelophthisic anemia.

The anemia ranges from mild to severe. Circulating nucleated red blood cells and increased dacryocytes are the hallmarks of this condition (Fig. 2.3). Bone marrow examination, particularly a biopsy, is indicated if the peripheral blood smear shows evidence to suggest a myelophthisic process. Severe marrow infiltration and fibrosis frequently lead to an inaspirable marrow, but aggregates of tumor cells may be identifiable even in hemodilute aspirate smears or on touch preparations prepared from the bone marrow core.

The main differential diagnosis is between an advanced, fibrotic myeloproliferative neoplasm and a marrow infiltrative process; this distinction relies on bone marrow examination. Patients with advanced myeloproliferative neoplasms and a leukoerythroblastic picture almost invariably have splenomegaly, a feature that is uncommon in most other conditions associated with myelophthisic anemia. Sepsis, acute severe hypoxia, sudden cardiac arrest, thalassemia major, congestive heart

Table 2.4 Causes of myelophthisic anemia

Metastatic solid tumor
Myeloproliferative neoplasms with myelofibrosis
Lymphoma involving bone marrow
Hairy cell leukemia with marrow fibrosis
Pulmonary hypertension with marrow fibrosis
Granulomatous inflammation
Non-cellular deposits
• Oxalosis
Storage diseases
• Gaucher disease
• Niemann-Pick disease
Hemophagocytic syndrome
Bone marrow necrosis
• Sickle cell crisis
• Infectious/sepsis
• All-trans retinoic acid therapy of acute promyelocytic leukemia
Disseminated intravascular coagulation

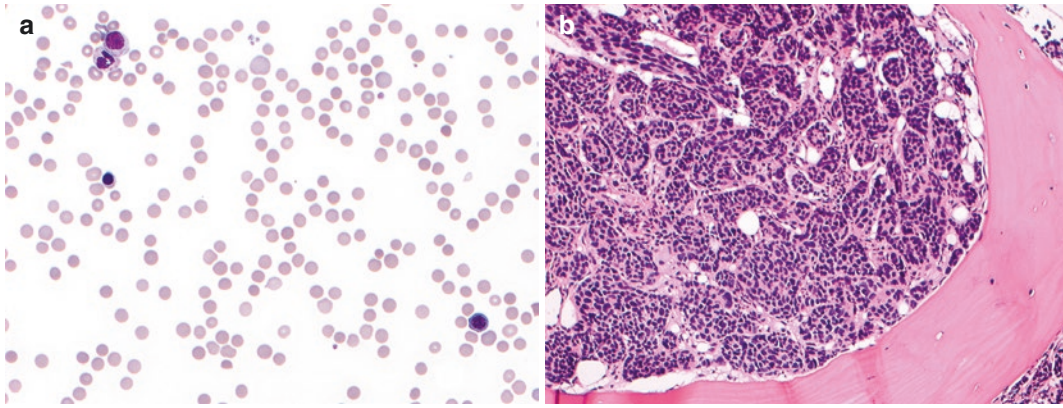


Fig. 2.3 Myelophthisic anemia in patient with metastatic breast cancer. (**a**) The peripheral blood smear shows anemia with circulating nucleated RBCs and occasional dacryocytes. A circulating myelocyte is seen in the upper left corner of the image. (**b**) The bone marrow biopsy shows extensive involvement by metastatic adenocarcinoma

failure, and severe hemolytic anemia may also present with leukoerythroblastosis, potentially mimicking a myelophthisic process [16].

Hemolytic Anemias

Hemolytic anemias encompass a broad spectrum of disorders associated with an increased rate of red blood cell destruction. These conditions are further categorized based on the etiology of hemolysis which is broadly divided into four major groups: defects inherent to the RBC (intrinsic RBC defects), immune-meditated factors, mechanical factors, and infections. Intrinsic hemolytic anemias include disorders of HGB (hemoglobinopathies), the red cell membrane, or red cell enzymes. The majority are inherited, but paroxysmal nocturnal hemoglobinuria (discussed separately in Chap. 4) is an exception. Table 2.5 provides a comprehensive list of intrinsic hemolytic anemias with pertinent clinical, laboratory, and genetic features of the most common forms [17]. Patients present with symptoms of anemia and hemolysis, including jaundice and dark urine. Diagnostic evaluation of intrinsic hemolytic anemias includes evaluation of RBC morphology, RBC membrane protein analysis, HGB electrophoresis, RBC enzyme measurement, and potentially genetic testing. Bone marrow examination is rarely indicated.

Hereditary Spherocytosis

Hereditary spherocytosis [18] is an intrinsic hemolytic anemia characterized by an increase in osmotically fragile, spherical shaped red blood cells on the peripheral blood smear. It is the most common form of inherited hemolytic anemia in northern European Caucasians, but affects all racial groups. Inheritance is autosomal dominant in the majority of cases. Hemolysis results from membrane fragility due to defects in membrane proteins, including spectrin, ankyrin, band 3, and protein 4.2, which lead to decreased surface area relative to intracellular volume causing the characteristic spherical shape. Damaged RBCs are ultimately destroyed in the spleen.

Patients present clinically with varying degrees of hemolysis leading to anemia, jaundice, increased reticulocyte index, gallstones, and splenomegaly. A positive family history of hemolysis is helpful in suspecting this diagnosis. Most patients have mild-to-moderate normocytic anemia with HGB in the range of 9–12 g/dL. Review of the peripheral blood smear demonstrates a variable number of spherocytes (RBCs lacking central pallor). The MCHC is increased in half of patients, but the MCV is usually normal. The reticulocyte index is markedly increased, often disproportionate to the degree of anemia. Serum lactate dehydrogenase, urinary and fecal urobilinogen, and indirect bilirubin are increased and serum haptoglobin is decreased.

The diagnosis is established by demonstrating increased osmotic fragility with hypotonic concentrations of sodium chloride. Flow cytometric assessment using eosin 5′-maleimide (EMA), a

Table 2.5 Inherited hemolytic anemias

Disorder	Gene/inheritance	RBC morphology	Diagnosis
RBC membrane disorders			
Hereditary spherocytosis (HS)	*ANK1* (AD/AR) *SPTB* (AD) *SPTA1* (AR) *SLC4A1* (AD) *EPB42* (AR)	Spherocytes	RBC morphology Osmotic fragility test
Hereditary elliptocytosis	*SPTA1* (AD) *SPTB* (AD) *EPB41* (AD)	Elliptocytes >25%	Coombs negative
Hereditary pyropoikilocytosis	*EPB41* (AR) *SPTA1* (AR) *SPTB* (AR)	Bizarre RBC fragments	Demonstration of eosin-5'-maleimide-labeled RBCs by flow cytometry
Dehydrated hereditary stomatocytosis	*PIEZO1* (AD) *KCNN4* (AD)	Macrocytosis; stomatocytes, target cells, schistocytes, spiculated cells	RBC morphology
Overhydrated hereditary stomatocytosis	*RHAG* (AD)		RBC morphology
Southeast Asian ovalocytosis	*RHAG* (AD)	↑↑Ovalocytes with transverse bars or single longitudinal slit	RBC morphology
RBC enzyme disorders			
G6PD deficiency	*G6PD* (XLR)	Usually normal ; rarely bite cells	Enzyme measurement
Pyruvate kinase deficiency	*PKLR* (AR)	Usually normal; occasionally contracted echinocytes	Enzyme measurement
Enolase deficiency	*ENO1* (AD)	Normal	Enzyme measurement
Adenylate kinase deficiency	*AK1* (AR)	Normal	Enzyme measurement
Glucose phosphate isomerase deficiency	*GPI* (AR)	Normal	Enzyme measurement
Pyrimidine 5' nucleotidase (UMPH1) deficiency	*NT5C3A* (AR)	Prominent stippling	Enzyme measurement
Gamma-glutamylcysteine synthetase deficiency	*GCLC* (AR)	Normal	Enzyme measurement
Glutathione peroxidase deficiency	*GPX1* (AR)	Normal	Enzyme measurement
Glutathione reductase deficiency	*GSR* (AR)	Normal	Enzyme measurement
Glutathione synthetase deficiency	*GSS* (AR)	Normal	Enzyme measurement
Hexokinase deficiency	*HK1* (AR)	Normal	Enzyme measurement
Bisphosphoglycerate mutase deficiency	*BPGM* (AR)	Normal	Enzyme measurement
Phosphoglycerate kinase 1 deficiency	*PGK1* (XLR)	Normal	Enzyme measurement
Triosephosphate isomerase deficiency	*TPI1* (AR)	Normal	Enzyme measurement
Hemoglobinopathies			
β-Thalassemia	*HBB* (AD/AR)	Target cells	HGB electrophoresis, isoelectric focusing, and high-performance liquid chromatography
α-Thalassemia	*HBA1* (AR) *HBA2* (AR)		
Sickle cell disease	*HBB* (AR)	Sickle cells	

AD autosomal dominant, *AR* autosomal recessive, *XLR* X-linked recessive, *HGB* hemoglobin, *G6PD* glucose-6-phosphate dehydrogenase, *RBC* red blood cell
Modified from Kim et al. [17]

fluorescent dye that binds to the transmembrane proteins band 3, Rh protein, Rh glycoprotein, and CD47, may be useful for diagnosis: patients with hereditary spherocytosis show decreased fluorescence compared to healthy controls. Decreased fluorescence may also be seen in patients with hereditary elliptocytosis and hereditary pyropoikilocytosis, a subset of red cell enzymopathies (see below) and other abnormalities of band 3, including congenital dyserythropoietic anemia type II.

Hereditary Elliptocytosis and Hereditary Pyropoikilocytosis

Hereditary elliptocytosis (HE) is a relatively common disorder characterized by the presence of increased elliptocytes on the peripheral blood smear [18, 19]. HE has a worldwide distribution, but it has the highest prevalence (up to 2% of population) in regions where malaria is endemic, such as West Africa. It is inherited in an autosomal dominant fashion. Clinical presentation is heterogeneous, ranging from asymptomatic carriers to severe, life-threatening hemolytic anemia. The disorder is caused by mutations in *SPTA1* (65%), *SPTB* (30%), and *EPB41* (5%), which encode for the cytoskeletal proteins α-spectrin, β-spectrin, and protein 4.1R, respectively. Decreased membrane stability as the result of defective interactions of the RBC cytoskeletal proteins, including spectrin, actin, and 4.1R junctional complex, causes weakened lateral interactions of the spectrin-based membrane skeleton. The severity of the anemia is related to the extent of decreased membrane stability. Splenectomy alleviates the severity of the anemia by increasing the life span of damaged RBCs.

The reticulocyte production index is increased and other hemolysis-associated laboratory findings (increased LDH, decreased haptoglobin, and increased indirect bilirubin) are present. The osmotic fragility test shows abnormal results in severe forms of HE. Other diagnostic tests include quantification of RBC membrane proteins by proteomic-based analyses. Ektacytometry, a method used for characterizing erythrocyte deformability, may also be useful establishing the diagnosis. Molecular testing for assessment of mutations is rarely needed, but may be indicated in cases where laboratory results are ambiguous.

Review of the peripheral blood smear shows the characteristic cigar-shaped elliptocytes ranging from few to nearly all RBCs. Other misshapen RBCs may also be seen. The degree of hemolysis does not correlate with the number of elliptocytes.

Hereditary pyropoikilocytosis (HPP) is a severe form of HE due to homozygous or compound heterozygous mutations of *SPTA1*, *SPTB*, or *EPB41*, leading to very severe disruption of spectrin self-association. Patients present in infancy or early childhood with a severe hemolytic anemia. RBCs show increased thermal sensitivity and marked poikilocytosis, mimicking the peripheral blood features of patients with severe thermal burns [20].

The differential diagnosis of HE includes other disorders with increased elliptocytes on the blood smear such as megaloblastic anemia, iron-deficiency anemia and thalassemia, and myeloid malignancies such as MDS and primary myelofibrosis. Elliptocytes generally comprise less than 25% of RBCs in these conditions. Pseudo-elliptocytosis is an artifactual phenomenon that results from PB smear preparation. In contrast to the randomly distributed long axes of true elliptocytes, the long axes of pseudo-elliptocytes are uniformly parallel [21].

Red Blood Cell Enzymopathies

Red cell enzyme disorders comprise a broad range of RBC enzyme deficiencies that lead to varying degrees of hemolytic anemia. G6PD deficiency is the most common RBC enzymopathy, with a worldwide prevalence of approximately 400 million people [17]. It is particularly common in areas where malaria is endemic. Neonatal jaundice is the most serious complication of G6PD deficiency. It has an X-linked recessive mode of inheritance. The *G6PD* mutations lead to decreased enzyme stability and defective ability to effectively synthesize glutathione, which leads to RBC membrane damage and a shortened RBC life span. The decreased enzyme activity also leads to increased stress-induced destruction of RBCs. Measurement of G6PD enzyme activity levels is commonly used as a screening tool for G6PD deficiency when a clini-

cal suspicion is raised. *G6PD* mutations are highly heterogeneous, but most are single-nucleotide substitutions leading to missense mutations [17]. In the more common forms, hemolysis occurs only during periods of stress induced by exogenous factors, typically infection or administration of oxidative drugs [22], and in some patients with ingestion of fava beans (Favism). Patients with the less common, severe variants of G6PD experience chronic hemolysis. Varying degrees of anemia and reticulocytosis are the main laboratory features of patients with G6PD deficiency.

Review of the peripheral blood smear demonstrates increased spherocytes and schistocytes during severe episodes of hemolytic anemia. Heinz bodies (inclusions within red blood cells composed of denatured HGB) are seen only during episodes of hemolysis. Other laboratory features of hemolysis, including reticulocytosis (sometimes accompanied by increased MCV), increased indirect bilirubin, decreased haptoglobin, and increased serum LDH, are present during episodes of active hemolysis. Diagnosis depends on the demonstration of decreased G6PD enzyme activity using any of a number of commerically available qualitative and quantitative assays [23]. Of note, enzyme measurement assays may show false-negative results in patients who have been recently transfused with normal red blood cells.

Hemoglobinopathies

Hemoglobinopathies are the most common inherited disorder of red blood cells. Approximately 5% of the general population are carriers of hemoglobinopathy genes. Among this heterogeneous group of disorders, sickle cell disease and thalassemias comprise the vast majority of cases. Thalassemias are discussed in the section on microcytic anemia above. Sickle cell anemia is highly prevalent in sub-Saharan and equatorial Africa, closely mimicking the worldwide distribution of malaria falciparum, followed by the Middle East, India, and the Mediterranean regions. Sickle HGB is the defective product resulting from a glutamic acid-to-valine substitution at the sixth amino acid of the *HBB* gene. Sickle cell disease is an autosomal recessive disorder resulting from homozygous *HBB* p.E6V mutation or from compound heterozygosity for sickle HGB and β-thalassemia or other abnormal β-globin variants. The sickle HGB molecule is insoluble upon deoxygenation and RBCs containing deoxy HbS polymer are abnormally rigid. This results in RBC membrane changes and abnormal interaction of sickle HGB with endothelial cells and leukocytes, nitric oxide depletion, release of inflammatory cytokines, and activation of the coagulation cascade. The constellation of these processes leads to hemolysis and an increased risk for vaso-occlusive episodes and multiorgan damage.

The peripheral blood smear shows "sickled" or crescent-shaped RBCs, because hemoglobin SS is prone to crystallization in RBCs in the presence of low oxygen tension (Fig. 2.4a). These abnormal RBCs adhere to one another and tend to sludge in capillaries, resulting in decreased blood flow and further reduced oxygen tension, thus creating a "vicious cycle" of sickling.

Solubility testing and assessing sickling of RBCs in the presence of sodium metabisulfite are good screening tests for sickle cell anemia, but they are not very specific. Definitive diagnosis of sickle cell anemia can be established using high-performance liquid chromatography (HPLC) and isoelectric focusing to identify hemoglobin S [24]

Bone marrow biopsy is rarely indicated in sickle cell anemia. If performed, there is usually erythroid hyperplasia, sometimes with dyserythropoiesis reflecting "stress erythropoiesis," similar to that seen in thalassemia and other hemolytic anemias (Fig. 2.4b). Bone marrow necrosis is a complication of sickle cell disease and necrotic bone marrow fragments may embolize to the lung, causing acute chest syndrome.

Autoimmune Hemolytic Anemia

Autoimmune hemolytic anemia (AHA) is caused by the interaction of autoantibodies with polysaccharide or protein antigens on the RBC membrane, leading to shortened RBC survival. They are divided into primary (idiopathic) and secondary forms. Conditions associated with secondary AHA include autoimmune diseases, infections (particularly infectious mononucleosis and *Mycoplasma pneumoniae*), drugs, and exposure

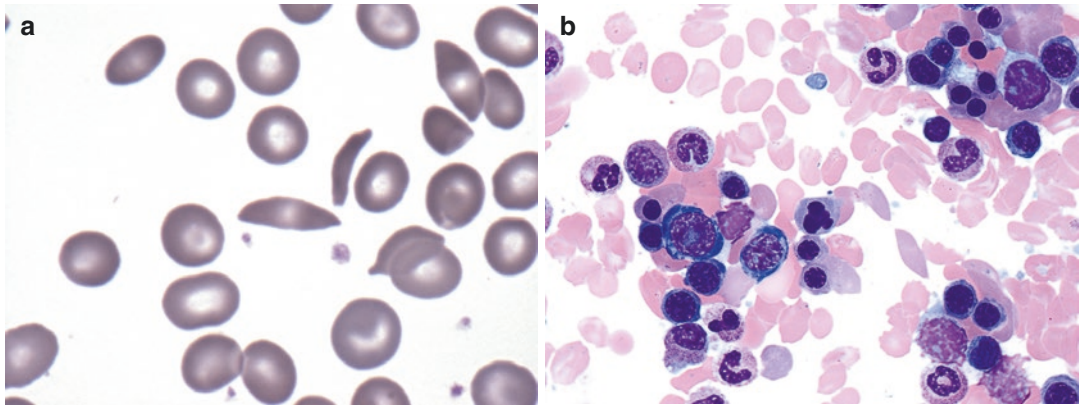


Fig. 2.4 Sickle cell anemia. (**a**) The peripheral blood smear shows sickled erythrocytes, which are elongated, curved, and densely stained. (**b**) The bone marrow aspirate smear shows marked erythroid hyperplasia, with erythroid nuclear irregularities reflecting stress erythropoiesis. Sickled erythrocytes are also seen

to foreign antigens that result in antibodies cross-reacting with self-RBC antigens. AHA can also occur as a paraneoplastic phenomenon, over half of which are associated with lymphoid neoplasms, particularly chronic lymphocytic leukemia (CLL). Autoantibodies associated with hemolysis are generally classified into "warm autoantibodies" which are more common, react most robustly at 37 °C, and are primarily IgG class and rarely IgA, and "cold autoantibodies," which react best at temperatures below 25 °C and are primarily IgM. These antibodies induce hemolysis by agglutinating RBCs and activating complement. Most warm autoantibodies are directed against Rh protein, while cold agglutinins are commonly directed against I antigen of RBCs.

Patients with AHA may be asymptomatic or present with signs of severe hemolysis. Splenomegaly is common. Episodic, acute hemolysis induced by cold temperatures may be seen in a subset of patients with cold agglutinins and RBC agglutination in cold temperatures may lead to vaso-occlusive events, including cyanosis involving the fingers, toes, nose, and ears or very rarely ulceration and necrosis of skin. Laboratory testing demonstrates anemia with an increased reticulocyte count. Other cytopenias are less common, but occasionally AHA can be associated with immune-mediated thrombocytopenia, also referred to as Evans syndrome. The diagnosis of AHA can be established by demonstrating the presence of immunoglobulin and/or complement bound to the patient's RBCs. The Coombs or direct antiglobulin test (DAT) is used as a screening method. This assay contains antibodies against human immunoglobulin and complement components (principally C3). If DAT is positive and agglutination is observed, additional testing is done with antisera that react selectively with IgG or C3 [25].

The peripheral blood smear of patients with AHA shows polychromasia indicative of reticulocytosis. Partial phagocytosis of antibody-coated RBC membranes results in the formation of spherocytes in patients with warm AHA (Fig. 2.5a, b). Spherocytes are less common in patients with cold agglutinin antibodies; rather, the PB smear may exhibit prominent RBC agglutination. Bone marrow examination is rarely indicated, but if performed, the bone marrow biopsy usually shows hypercellularity with erythroid hyperplasia and left shift. Mild stress-induced dyserythropoiesis may be observed (Fig. 2.5c, d).

The differential diagnosis includes hereditary spherocytosis, which is also characterized by increased spherocytes on the PB smear and splenomegaly, and other hemolytic anemias. The DAT test in patients with hereditary spherocytosis is negative. Drug-induced hemolytic anemia should be considered if there is a history of exposure to hemolysis-inducing drugs. Spider bite (particular the brown recluse spider) can lead to a severe

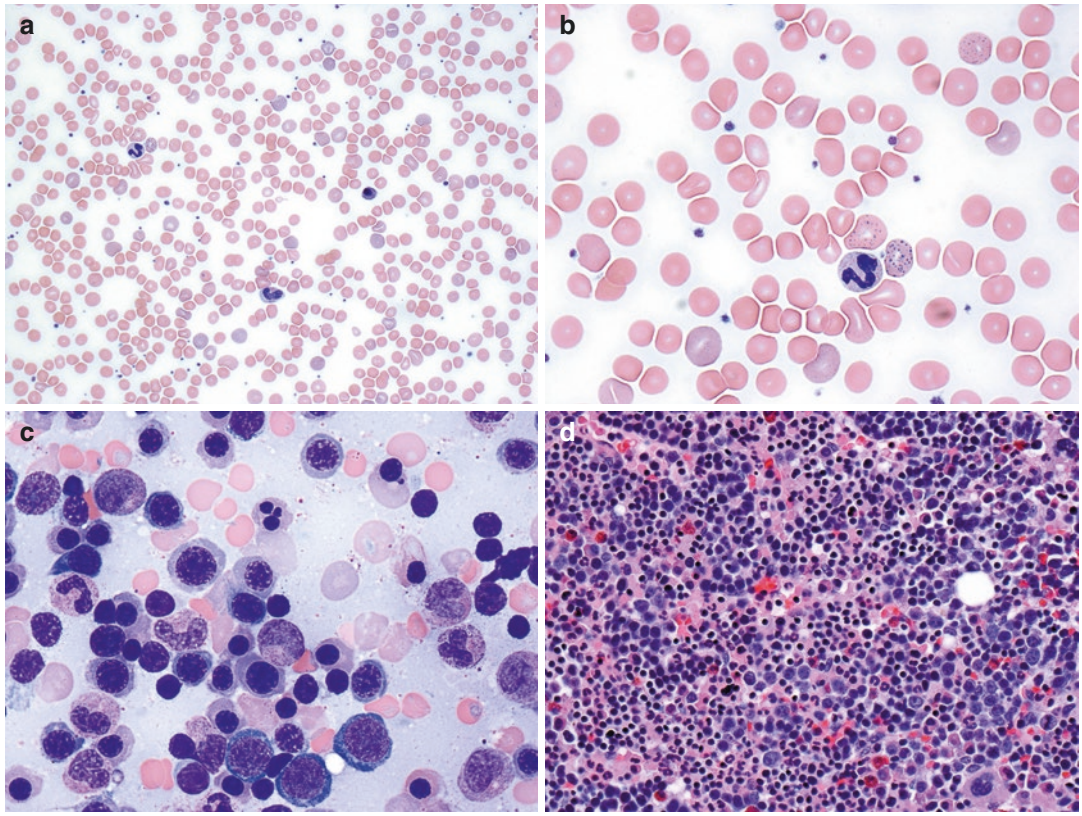


Fig. 2.5 Autoimmune hemolytic anemia. (**a**, **b**). In the peripheral blood smear, RBCs show anisocytosis, with frequent microspherocytes (small RBCs lacking central pallor). There is prominent polychromasia and many RBCs show basophilic stippling. A nucleated red blood cell is present in panel (**a**). (**c**) Marked erythroid hyperplasia and left shift are seen in the bone marrow aspirate smear. Nuclear irregularities reflecting stress erythropoiesis are present. (**d**) The bone marrow biopsy is profoundly hypercellular with marked erythroid hyperplasia and left shift

hemolytic anemia with positive DAT (IgG or complement) and PB spherocytosis. Alloimmune hemolytic anemia is a condition in which a donor's antibodies destroy RBCs and it may be suspected in patients with recent history of transfusion. Hematopoietic stem cell transplant recipients may develop donor-derived autoantibodies against RBC antigens produced by the grafted bone marrow. Solid organ transplant recipients can also develop alloantibody-mediated or lymphocyte-derived autoantibody-mediated hemolysis.

Hemophagocytic Syndrome

Hemophagocytosis is the occurrence of intact hemopoietic cells (particularly erythrocytes, but also nucleated hematopoietic elements of any lineage and platelets) within the cytoplasm of phagocytic histiocytes. Hemophagocytic lymphohistiocytosis (HLH), also known as hemophagocytic syndrome, is an inherited or acquired histiocyte activation syndrome characterized by hemophagocytosis. *Primary (familial) HLH* is a rare, autosomal recessive disease that usually manifests in infancy or early childhood [26]. A large subset of familial HLH is due to mutation in the *PRF1* gene encoding perforin [27]. Defects in other genes, including *UNC13D* and *STX11* [28], cause HLH in smaller subsets of patients. These mutations result in impaired cytotoxic T-cell and NK-cell function that leads to inappropriate activation of macrophages and uncontrolled macrophage phagocytosis of hema-

topoietic elements. Primary HLH may also occur in patients with inherited immunodeficiency syndromes such as Chediak-Higashi and X-linked lymphoproliferative syndrome [29]. *Secondary HLH* is more common than primary HLH and can be induced by an infection, tumor, or chronic inflammatory process [30, 31]. Infections are the inciting factor in over 50% of HLH cases, and include herpesviruses (especially EBV), HIV, other viruses, and occasionally bacterial, fungal, and parasitic infections (particularly leishmania, malaria, and toxoplasma in immunosuppressed patients). Compared to uncomplicated infectious mononucleosis, EBV viral titers are typically very high in HLH associated with EBV infection [32]. Of note, infections can precipitate the first episode of hemophagocytosis in patients with familial HLH, and thus identifying an underlying infection does not exclude a genetic/familial etiology; moreover, some forms of familial HLH may not manifest until adult life [33, 34]. The most common lymphomas associated with HLH are adult T-cell leukemia/lymphoma, aggressive NK-cell leukemia, hepatosplenic lymphoma, subcutaneous panniculitis-like T-cell lymphoma, diffuse large B-cell lymphoma (especially intravascular large B-cell lymphoma and EBV+ large B-cell lymphomas), and classical Hodgkin lymphoma.

HLH patients typically present with fever, splenomegaly, and anemia, usually with other cytopenias. The criteria required to make the diagnosis are shown in Table 2.6. Other abnormal clinical and laboratory findings that can be clues to the diagnosis of HLH (but are not considered diagnostic criteria) are meningeal symptoms, lymphadenopathy, edema, skin rash, elevated liver function tests, hypoproteinemia, and hyponatremia [35]. Patients are often critically ill at presentation and rapid diagnosis is important to ensure prompt institution of therapy, since HLH is an aggressive disease with a high mortality. Bone marrow biopsy and aspirate are often performed to evaluate a clinical suspicion of HLH, since hemophagocytosis is a key diagnostic feature of the disease and is usually readily identified in the marrow in affected patients. However, hemophagocytosis may be a nonspecific finding and has been identified in up to 50% of patients who undergo bone marrow examination as part of a workup for fever and also after blood transfusions [35, 36]. Hemophagocytic histiocytes are less frequent in these settings than in cases fulfilling diagnostic criteria for HLH. Conversely, a diagnosis of HLH can be made even if hemophagocytosis is not seen in the bone marrow, provided that sufficient other diagnostic criteria (see Table 2.6) are fulfilled.

The hemophagocytic histiocytes identified in the bone marrow are large and contain one or often multiple ingested hematopoietic cells, including erythrocytes, nucleated erythroid and myeloid elements, and sometimes platelets. They may be seen on either the aspirate or the biopsy specimens (Fig. 2.6). Phagocytic histiocytes, which are commonly seen in lymphoid tissues with high cell turnover, contain pyknotic cellular debris, which is different from the intact cells found within the

Table 2.6 Diagnostic criteria for hemophagocytic lymphohistiocytosis (Adapted from [35])

The diagnosis of hemophagocytic lymphohistiocytosis (HLH) can be established if either criterion A or criterion B is fulfilled
Criterion A. A molecular diagnosis consistent with HLH (primary HLH)
Criterion B. At least five of the following eight diagnostic criteria are fulfilled:
1. Fever
2. Splenomegaly
3. Cytopenias (affecting at least two of the three lineages in the peripheral blood)
a. HGB <9 g/dL (in infants <4 weeks of age, HGB <10 g/dL)
b. Platelets $<100 \times 10^9$/L
c. Neutrophils $<1.0 \times 10^9$/L
4. Hypertriglyceridemia and/or hypofibrogenemia
a. Fasting triglycerides ≥265 mg/dL
b. Fibrinogen ≤1.5 g/L
5. Hemophagocytosis in bone marrow, spleen or lymph nodes
6. Low or absent NK cell activity (according to local laboratory reference)
7. Serum ferritin ≥500 μg/L
8. Soluble CD25 (i.e., soluble IL-2 receptor) ≥2400 U/mL

IL-2 interleukin 2, *NK* natural killer

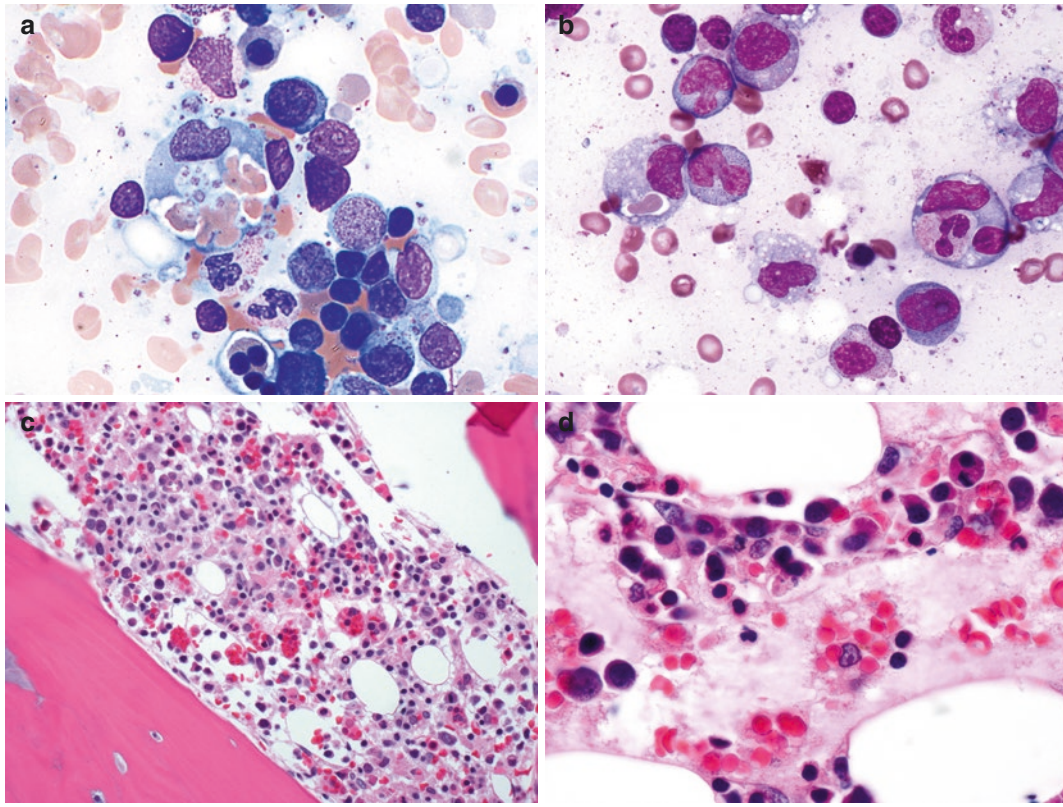


Fig. 2.6 Hemophagocytic syndrome. In the bone marrow aspirate smear, large histiocytes are present containing intact intracellular RBCs, platelets, granulocytic elements, and lymphocytes (**a**, **b**). Hemophagocytic histiocytes can also be appreciated in the bone marrow biopsy, in which numerous intact erythrocytes are present within the cytoplasm of histiocytes (**c**, **d**)

cytoplasm of true hemophagocytic histiocytes. Evaluation of a bone marrow specimen procured to evaluate for possible HLH requires careful review, because hemophagocytic cells are often rare (usually only 1 in 500 cells in the bone marrow aspirate). Repeat bone marrow sampling may be indicated if HLH is strongly suspected but cannot be diagnosed on the initial bone marrow sample or based on clinical criteria alone [35]. Immunostaining for CD163 can help facilitate the identification of the hemophagocytic histiocytes in the bone marrow biopsy specimen [37]. Of note, the amount of hemophagocytosis identified in the bone marrow does not appear to correlate with disease probability, whereas highly elevated ferritin, fever, and cytopenias show better correlation with a diagnosis of HLH [38].

Aside from the presence of hemophagocytic histiocytes, the bone marrow appearance of HLH is variable. The marrow can range from hypocellular to hypercellular and the myeloid-to-erythroid ratio is variable. Because HLH commonly occurs secondary to infection or a neoplastic process, the bone marrow may be concurrently involved by the inciting process, such as an EBV+ lymphoma. The identification of unexplained hemophagocytosis in a bone marrow sample in which some clinical features of HLH are present should precipitate a search for possible infectious, neoplastic, or inflammatory causes. Familial HLH should also be considered, with genetic testing for mutations in HLH-associated genes indicated if suspected.

Macrocytic Anemia

Megaloblastic Anemia

Megaloblastic anemias manifest as macrocytosis and are caused by impaired DNA synthesis

due to a variety of etiologies. They are characterized by the presence of megaloblastic changes in erythroids, granulocytes, and megakaryocytes. Megaloblastic hematopoietic cells are much larger than their normal counterparts, and have a relative abundance of cytoplasm and RNA compared with their nuclear DNA content, thus showing asynchronous nuclear-cytoplasmic maturation. The most common causes of megaloblastic anemia are folate and cobalamin (vitamin B12) deficiency. A number of medications are known to interfere with DNA synthesis and cause megaloblastic anemia. These include antifolate drugs, purine and pyrimidine analogues, ribonucleotide reductase inhibitors (hydroxyurea, cytarabine), anticonvulsants, and other medications (including omeprazole/lansoprazole, metformin, colchicine, neomycin, and arsenic) [39]. Patients present with symptoms of chronic anemia. Jaundice may be present in a subset of patients and the anemia may be associated with mild leukopenia and/or thrombocytopenia. Patients with folic acid deficiency have low levels of serum folate and lack of neurologic signs, while patients with cobalamin deficiency often show characteristic neurologic abnormalities.

The peripheral smear shows macrocytic RBCs and often hypersegmented neutrophils (Fig. 2.7a). Bone marrow examination is generally not indicated, but may be performed if the anemia is accompanied by other cytopenias. The bone marrow is usually markedly hypercellular with prominent erythroid hyperplasia including numerous early erythroid forms that appear megaloblastic, showing premature hemoglobinization of cytoplasm (Fig. 2.7b, d). The differential diagnosis of megaloblastic anemia includes MDS, as megaloblastic anemias may have marked dyserythropoiesis. However, the knowledge of serum vitamin B12 and red cell folate levels, the absence of dysplasia in the other hematopoietic lineages, and the absence of a cytogenetic or molecular marker of clonal hematopoiesis aid in making this distinction. Giant band forms and metamyelocytes in the bone marrow can be a clue to the diagnosis, as they are rarely seen in MDS (Fig. 2.7b). However, hypersegmented neutrophils are also seen in MDS as a manifestation of granulocytic dysplasia.

Neutropenia

Neutropenia is the most common form of leukopenia and is defined as an absolute neutrophil count of less than two standard deviations below the population's normal mean, typically <1.5–1.8 $\times 10^9$/L in Caucasian adults and children, depending on the individual laboratory's reference range patient population. The number of neutrophils in blood varies depending on age, race and ethnicity, physical activity, and other environmental factors. Hispanic individuals have slightly higher baseline neutrophil counts, while people of African and Middle Eastern descent have slightly lower baseline neutrophil counts. Neutropenia is associated with increased predisposition to infections, directly proportional to the degree of neutropenia. Neutropenia may be encountered in isolation or accompanied by other cytopenias and it is further subcategorized based on its etiology, as (1) conditions that lead to decreased granulopoiesis; (2) ineffective granulopoiesis; (3) increased utilization or increased destruction of neutrophils; (4) increased margination of neutrophils; or (5) a combination of these scenarios.

Typically, patients with acute-onset severe neutropenia present with fever, pharyngitis, and other forms of mucocutaneous infection. Assessment should include detailed clinical history, including medication intake, careful physical examination, and CBC with differential. Bone marrow examination may be considered if the etiology of neutropenia remains unknown. Chronic neutropenia is often discovered incidentally. Particular attention should be given to the presence of other cytopenias or cytoses in these patients, which could suggest a myeloid neoplasm. Evaluation of serum immunoglobulin levels is recommended for patients with chronic neutropenia to evaluate for congenital immunological disorders and immunodeficiency syndromes. Morphologic assessment of blood and bone marrow can aid in identifying a specific cause of neutropenia such as MDS or AML, discussed later in this chapter.

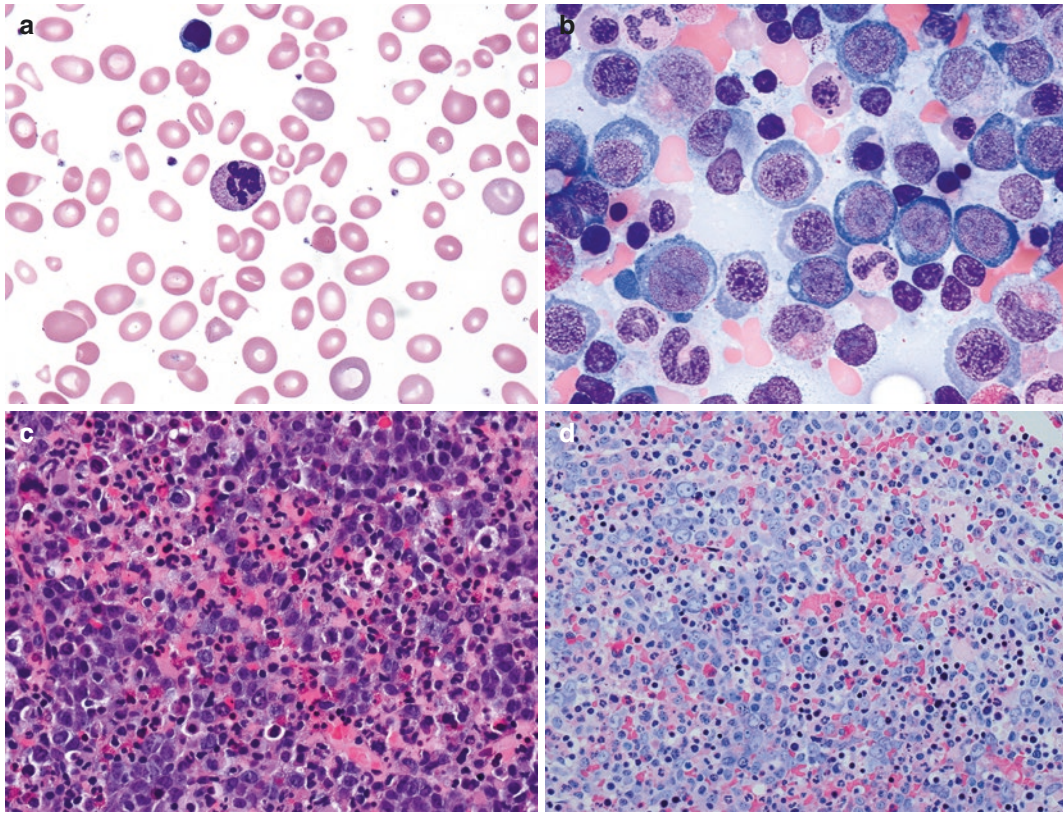


Fig. 2.7 Megaloblastic anemia due to vitamin B12 deficiency. (**a**) The peripheral smear shows macrocytosis with marked anisopoikilocytosis. The neutrophil shows nuclear hypersegmentation (seven distinct nuclear lobes). (**b**) The bone marrow aspirate smear shows striking erythroid hyperplasia and left shift. Some maturing erythroid elements show advanced cytoplasmic hemoglobinization, despite retained nuclear immaturity (lower right). A giant band form is present (lower left). (**c**) The bone marrow biopsy specimen is markedly hypercellular with left-shifted erythroid maturation; prominent clusters of early erythroids may raise the differential diagnosis of pure erythroid leukemia. (**d**) Giemsa stain of the bone marrow biopsy shows numerous early erythroids with basophilic cytoplasm. Dyserythropoiesis, indicated by nuclear budding and irregularities of late erythroid forms, is common in megaloblastic anemia

Neutropenia includes congenital and acquired forms. Congenital neutropenias comprise a heterogeneous group of genetically inherited disorders that are associated with increased cell turnover of granulocytes and ineffective granulopoiesis. Acquired neutropenias are more common. The most frequent causes in developed countries are iatrogenic due to exposure to cytotoxic and/or immunosuppressive therapeutic agents used for the treatment of malignancy or autoimmune diseases. Many of these agents result in impaired production of neutrophils and their precursors and are associated with a striking maturation arrest of granulocytes in the bone marrow and profound agranulocytosis in the blood (Fig. 2.8a, b). A summarized list of medications commonly associated with neutropenia is provided in Table 2.7. Several acute and chronic infections are associated with acquired neutropenia. The mechanisms of infection-related neutropenia are broad. Some viral agents (EBV, HBV, HCV, and HIV) cause neutropenia by directly infecting hematopoietic precursor cells. The mechanisms for other infections are varied, and include splenic sequestration, direct infection of the bone marrow, and endothelial cell infection causing altered endothelial-neutrophil interactions [40].

Neutropenia is often related to chronic autoimmune diseases. Thus, clinical history intake, physical examination, and laboratory workup including

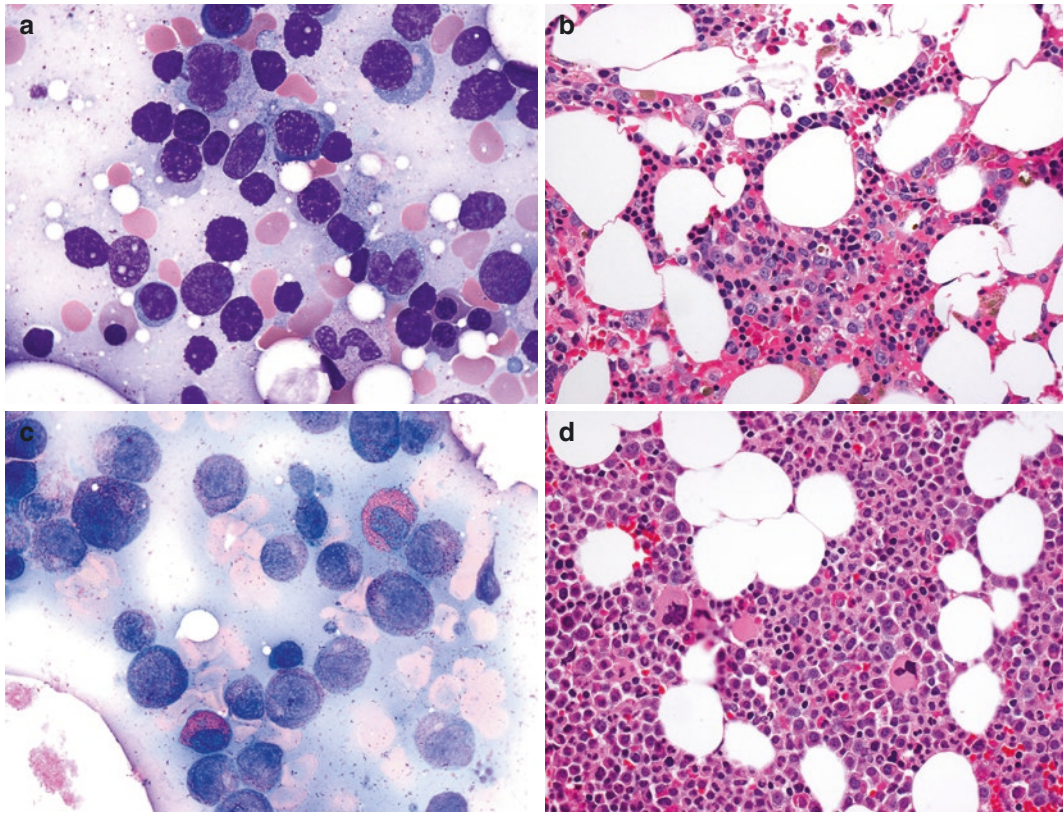


Fig. 2.8 Acquired neutropenia. (**a**, **b**) Secondary neutropenia due to antibiotic therapy. The bone marrow aspirate (**a**) shows granulocytic hypoplasia, with a predominance of erythroid elements. The bone marrow biopsy (**b**) shows predominantly maturing erythroid elements, with scattered left-shifted myeloid forms and a marked paucity of mature neutrophils. (**c**, **d**) Chronic idiopathic neutropenia. The marrow aspirate (**c**) shows granulocytic left shift with maturation arrest at the myelocyte stage. The marrow biopsy shows myeloid hyperplasia with marked left shift. Erythroid elements and megakaryocytes appear unremarkable

assessment of antinuclear antibodies (ANA) and rheumatoid factor titers and other serologic tests for autoimmune diseases may be useful. Examination of the peripheral blood smear may be helpful in identifying large granular lymphocytes, which are often increased in autoimmune diseases associated with neutropenia.

Congenital Neutropenias

The main forms of inherited neutropenia, including severe congenital neutropenia, Kostmann syndrome and Shwachman-Diamond syndrome, are discussed separately in Chap. 3. Chédiak-Higashi syndrome is characterized by partial oculocutaneous albinism and characteristic giant intracytoplasmic granules in neutrophils, monocytes, and lymphocytes. Mild neutropenia and recurrent infections are constant features. The condition is inherited in an autosomal recessive fashion and due to recurrent mutations in *LYST* leading to dysregulated lysosomal trafficking [41]. Griscelli syndrome is inherited in autosomal recessive fashion and is characterized by pigmentary dilution and varying degrees of cellular immunodeficiency. The syndrome is subdivided into three different types; neutropenia is associated with type 2 only. The neutropenia is mild, but it is often associated with pancytopenia. The syndrome is linked to *RAB27A* mutations leading to defects in guanosine triphosphatase (GTPase) and defective phagocytosis [42]. WHIM (warts, hypogamma-

Table 2.7 Medications associated with acquired neutropenia

Thiopurines (6-mercaptopurine and 6-thioguanine)
Fludarabine phosphate
Thalidomide
Pomalidomide
Tyrosine kinase inhibitors [210, 211]
Ruxolitinib [212]
Ibrutinib [213, 214]
Rituximab [215]
Ofatumumab [216]
Obinutuzumab [217]
Alemtuzumab [218]
Brentuximab Vedotin [219]
Cytotoxic chemotherapy
Antibiotics
Anticonvulsants
Anti-inflammatory agents
Diuretics
Cardiovascular drugs
Sedatives
Antidepressants
Antithyroid agents

globulinemia, infections, and myelokathexis) syndrome is a rare autosomal dominant disorder characterized by severe neutropenia and lymphopenia. Myelokathexis is believed to be due to retention of neutrophils in the bone marrow, leading to neutrophil hypersegmentation and eventual intramedullary destruction. WHIM syndrome is due to germline mutations in *CXCR4*, the gene encoding CXCL12, leading to dysregulation of the trafficking of hematopoietic cells in the blood and tissues. The bone marrow is usually hypercellular and shows marked myeloid hyperplasia, with mature neutrophils showing nuclear hypersegmentation, condensed pyknotic nuclei, and cytoplasmic vacuoles. The neutropenia is responsive to G-CSF, but these patients are at increased risk of developing myeloid neoplasms [43]. Many other forms of inherited neutropenia have also been reported in which the underlying genetic defect is yet to be determined. Neutropenia may also be a feature of a variety of congenital syndromes, discussed separately in Chap. 3.

Cyclic neutropenia is characterized by cyclic episodes of severe neutropenia that typically occur every 21 days and last 3–6 days. It is inherited in an autosomal dominant fashion, although sporadic cases may also occur [44]. This condition is due to recurrent mutations in *ELANE,* the gene encoding for neutrophil elastase [45]. Patients may present with fever, malaise, failure to thrive, oral ulcers, and cervical lymphadenopathy. The diagnosis of cyclic neutropenia must be established by demonstrating neutropenia on serial differential white blood cell counts, at least two or three times per week for a minimum of 6 weeks. Genetic confirmation is rarely needed. The prognosis is favorable and the severity of symptoms seems to diminish following puberty. Unlike patients with severe congenital neutropenias, children with cyclic neutropenia are not predisposed to developing myeloid malignancies.

Acquired Neutropenias

Chronic Idiopathic Neutropenia

Chronic idiopathic neutropenia (CIN) is an isolated form of neutropenia with a marked female predominance that typically presents between the ages of 18 and 35 years. Antineutrophil antibodies and autoantibodies, including antinuclear or antimitochondrial antibodies, are absent. The pathogenesis is attributed to increased apoptosis of neutrophils and neutrophilic precursors through mechanisms induced by Fas-ligand or interferon gamma [46]. The frequency and degree of symptoms and infections are directly proportional to the degree of neutropenia. The bone marrow may show mild granulocytic hypoplasia with left-shifted maturation and without significant dysplasia (Fig. 2.8c, d). Lack of significant morphologic dysplasia in granulocytes on the blood smear and bone marrow, normal bone marrow karyotype, and lack of MDS-type mutations aid in differentiating CIN from MDS, which would be very rare in the age group in which CIN typically presents.

Pure White Cell Aplasia

This is a rare acquired form of severe isolated neutropenia with nearly absent granulocytic precursors in the bone marrow which can be caused as a result of exposure to certain drugs (including

ibuprofen and chlorpropamide), infections, and inflammatory conditions (Fig. 2.9). The differential diagnosis includes aplastic anemia and erythroid-predominant MDS. Hairy cell leukemia and large granular lymphocytosis may occasionally be associated with pure white cell aplasia [40].

Nutritional Deficiencies

Neutropenia is often present in megaloblastic anemias due to vitamin B12 or folate deficiency and in such cases it is accompanied by macrocytosis. Copper deficiency in undernourished children and patients on total parenteral nutrition can cause neutropenia. The differential diagnosis for both conditions includes MDS, as varying degrees of dyserythropoiesis can be seen in both megaloblastic anemia and copper deficiency and the latter can also show multilineage dysplasia [47].

Immune-Mediated Neutropenia

Autoimmune neutropenia may be encountered in both children and adults. It is mediated by antineutrophil antibodies that cause decreased neutrophil survival. The diagnosis can be established by demonstrating the presence of one or more antineutrophil antibodies in the serum. The HGB, platelet, lymphocyte and monocyte counts are nearly always normal. The neutropenia is responsive to G-CSF and the overall prognosis and likelihood of spontaneous recovery are better in children than adults [48, 49]. Systemic autoimmune diseases may be associated with immune-mediated neutropenias, including systemic lupus erythematosus, rheumatoid arthritis (particularly when complicated by Felty syndrome, which is the presence of neutropenia and splenomegaly in the setting of rheumatoid arthritis), and Sjögren syndrome [50]. The neutropenia associated with autoimmune diseases is typically responsive to G-CSF.

Bone marrow granulocytic precursors and mature neutrophils in the blood have unremarkable morphology, but there is often marked left-shifted maturation in the bone marrow (Fig. 2.10). The main differential diagnosis is chronic idiopathic neutropenia. As well as Felty syndrome, conditions associated with neutropenia and splenomegaly include sarcoidosis, lymphoma, tuberculosis, malaria, visceral leishmaniasis, and Gaucher disease. The neutropenia is often accompanied by thrombocytopenia and anemia in these other diseases.

Alloimmune neonatal neutropenia is a rare condition affecting approximately 1 in 2000 live births and is due to maternal-fetal neutrophil antigen incompatibility. Maternal antibodies are transferred into the fetus's bloodstream via the placenta

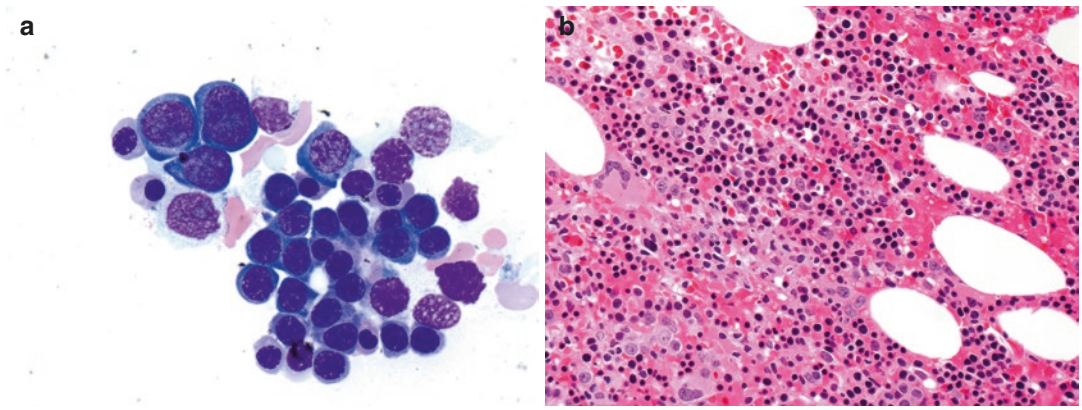
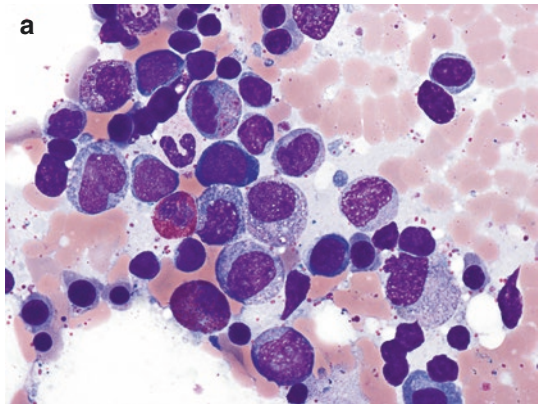


Fig. 2.9 Pure white cell aplasia. (**a**) Bone marrow aspirate smear shows exclusively erythroid elements, with a complete absence of granulocytes. (**b**) The bone marrow biopsy similarly shows intact maturing erythropoiesis and megakaryocytes, but absence of neutrophils. This 80-year-old man presented with profound neutropenia following a viral illness; neutrophils spontaneously recovered after 2 weeks



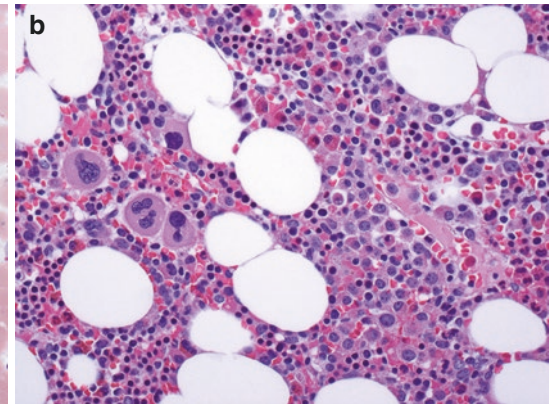


Fig. 2.10 Autoimmune neutropenia. (**a**) The bone marrow biopsy shows preserved granulopoiesis, but with marked left shift and increased myelocytes. The bone marrow biopsy shows normal megakaryocytes and erythroid elements as well as frequent eosinophils. Granulocytes are left shifted

and attack paternally inherited antigens expressed on the surface of the fetus's neutrophils. The most commonly involved alleles are those of human neutrophil antigen (HNA) and Fcgamma receptor IIIb. The degree of neutropenia is variable, but may be marked and can be associated with severe infections. The lymphocyte and monocyte counts are usually normal. The neutropenia may last up to 6 months, despite the fact that the causative maternal antibodies are usually cleared within the first 6 weeks of life. The main differential diagnosis is neonatal sepsis [51].

Lymphopenia

Lymphopenia is defined as an absolute lymphocyte count (ALC) of less than 1.5×10^9/L in adults or less than 2×10^9/L in children. Lymphopenias can be further subdivided into B-cell lymphopenia, T-cell lymphopenia, NK-cell lymphopenia, or various combinations thereof. Detailed clinical history and physical examination to assess for the presence of hepatosplenomegaly, lymphadenopathy, and chronic mucocutaneous infections are essential. The etiologies of lymphopenia are broad and can be generally divided into inherited and acquired causes. Inherited causes include severe combined immunodeficiencies, common variable immunodeficiency (CVID), ataxia telangiectasia, Wiscott-Aldrich sydnrome, and other known or unknown genetic defects. Acquired lymphopenia is most commonly due to infections, iatrogenic causes (chemotherapy, radiation therapy, or immunosuppression, including steroids), autoimmune disease, or aplastic anemia [52].

Monocytopenia

Monocytopenia is defined as an absolute monocyte count of less than 0.2×10^9/L. Monocytopenia in the context of aplastic anemia can be profound and is usually associated with other cytopenias, particularly neutropenia [53]. Although patients with hairy cell leukemia (HCL) often present with pancytopenia, the presence of monocytopenia is nearly ubiquitous and is considered an important clue in the diagnosis of HCL. The features and diagnosis of HCL are discussed in Chap. 12. Chronic glucocorticoid exposure can lead to monocytopenia through induction of apoptosis of hematopoietic cells including monocytes and macrophages [54, 55].

Profound monocytopenia can be observed in patients with germline mutations of *GATA2* that cause a spectrum of bone marrow failure disorders including MonoMAC syndrome (monocytopenia with mycobacterial infections); dendritic cell, monocyte, and B and NK lymphoid deficiency (DCML deficiency); primary lymphedema and MDS (Emberger syndrome); and familial MDS/acute myeloid leukemia (MDS/AML) [56]. These entities are discussed in Chap. 3.

Thrombocytopenia

Thrombocytopenia is one of the most common causes for hematologic consultation. It is defined by a platelet count of less than 150×10^9/L and may be caused by increased destruction, increased utilization, abnormal distribution, or reduced production of platelets. The major consequence of thrombocytopenia is increased risk of bleeding. Bone marrow examination may be indicated for assessment of megakaryocyte number and morphology in patients with unexplained thrombocytopenia. Table 2.8 provides a summary of conditions associated with thrombocytopenia according to their main mechanism of action.

Table 2.8 Conditions associated with thrombocytopenia

Impaired platelet production
Inherited platelet disorders
Nutritional deficiencies
Alcohol-induced thrombocytopenia
Hemophagocytosis
Immune thrombocytopenia (ITP)
Drug-induced thrombocytopenia
Pregnancy-related thrombocytopenia
Paroxysmal nocturnal hemoglobinuria
Aplastic anemia
Myeloid neoplasms
Myelophthisic processes (metastatic tumor, etc.)
***Abnormal* platelet distribution**
Hypersplenism
Hypothermia
Massive blood transfusions
Volume overload
Increased platelet destruction
Immune thrombocytopenia (autoimmune, alloimmune)
Thrombotic microangiopathies
Disseminated intravascular coagulopathy
Kasabach-Merritt syndrome
Drug-induced immune thrombocytopenia
Pregnancy-related thrombocytopenia
Artificial surfaces (hemodialysis, etc.)
Type 2B von Willebrand disease
Cyclic thrombocytopenia
Pure megakaryocytic aplasia

Spurious Thrombocytopenia

Spurious thrombocytopenia (pseudothrombocytopenia) comprises up to 30% of cases of isolated thrombocytopenia. The most common causes are ex vivo agglutination of platelets, incorrect platelet counting due to an increased number of giant platelets, and incorrect preparation of blood samples. Ex vivo antibody-induced platelet agglutination can be induced by antiplatelet antibodies or by activation of the platelets during blood collection. It is most notable in the presence of EDTA anticoagulant and can be prevented by using non-EDTA anticoagulants during blood collection. Another mechanism is platelet satellitism, in which naturally occurring antibodies directed against glycoprotein IIb/IIIa react with the leukocyte Fcγ receptor III and attach the platelets to neutrophils (or rarely monocytes), forming a rosette around the periphery of the white cell. Antiplatelet antibodies that cross-react with antiphospholipid and anticardiolipin antibodies have also been implicated in spurious thrombocytopenia: these antibodies have the ability to bind to negatively charged phospholipids or antigens modified by EDTA on the surface of platelet during blood collection. Glycoprotein IIb/IIIa agonists used in the treatment of acute coronary syndrome have also been implicated [57].

Inherited Platelet Disorders

Inherited platelet disorders are a heterogeneous group of diseases caused by a variety of genetic defects affecting platelet production, function,

or both. A subset of these disorders is associated with predisposition to developing myeloid malignancy. These conditions may present as isolated thrombocytopenia or as part of a syndrome. The individual disorders are discussed separately in Chap. 3. Patients typically present with mucocutaneous bleeding or menorrhagia. Careful clinical history intake, including family history of bleeding tendency, and detailed physical examination to assess for any associated physical anomalies are essential in establishing a suspicion of an inherited platelet disorder and distinguishing these from acquired causes of thrombocytopenia. Laboratory studies demonstrate impaired platelet function with light transmission aggregometry in a subset of these disorders, but platelet function studies may be unremarkable in others. Flow cytometric evaluation can be used to evaluate platelet surface glycoprotein expression. Numerous associated gene mutations have been identified, some of which are associated with characteristic peripheral blood and/or bone marrow morphology; these are discussed in Chap. 3.

Immune-Mediated Thrombocytopenia

Immune-mediated thrombocytopenia is divided into primary and secondary forms. The primary form, idiopathic thrombocytopenic purpura (ITP), is the most common cause of isolated thrombocytopenia. It is characterized by immune-mediated platelet destruction and compromised platelet production. ITP can affect patients of all ages, although it is more common in childhood. An association with preceding viral infections or vaccinations has been shown in childhood ITP. The prognosis is good and the thrombocytopenia is typically self-limited in children, while adult ITP has a more protracted course and rarely resolves spontaneously. The antiplatelet antibodies are predominantly IgG antibodies that are directed against glycoproteins IIb/IIIa in about 80% of patients and against glycoproteins Ib-IX–V complex and other platelet glycoproteins such as GP IV and GPIa-IIa in smaller subsets of patients [58]. IgM and IgA pathogenic antiplatelet antibodies are exceedingly rare.

Examination of the peripheral blood smear in ITP shows isolated thrombocytopenia with increased platelet anisocytosis, including abnormally large as well as small platelets. Cases of combined ITP and autoimmune hemolytic anemia (Evans syndrome) may also occur. Bone marrow examination in patients with ITP shows an increased number of megakaryocytes, which may include many small and immature forms potentially mimicking the megakaryocytes seen in MDS; however, micromegakaryocytes that characterize MDS cases with dysmegakaryopoiesis are rare in ITP (Fig. 2.11) [59]. Patients with ITP treated with thrombopoietin analogues (such as romiplostim or eltrombopag) may show increased reticulin fibrosis, with or without associated leukoerythroblastosis. Examination of the bone marrow in such cases can show increased fibrosis above baseline, which appears to resolve upon discontinuation of the drug [60, 61]. Alterations in megakaryocyte morphology resembling those seen in myeloproliferative neoplasms may be observed in such cases (Fig. 2.12).

Secondary immune-mediated thrombocytopenia is due to a variety of causes, including drugs, systemic diseases, and chronic infections. Among these, drug-induced thrombocytopenia is the leading cause of secondary immune-mediated thrombocytopenia [57]. The mechanism of thrombocytopenia depends on the implicated drug, but suppression of thrombopoiesis in the marrow and immune-mediated destruction of platelets are the most common. Thrombocytopenia may range from mild to severe. Heparin-induced thrombocytopenia (HIT) is one of the most severe forms of drug-induced thrombocytopenia. It is an immune-mediated condition caused by antibodies against the neoepitope in platelet factor 4 (PF4) that is exposed when PF4 binds heparin. This results in activation of cellular FcgRIIA on platelets and monocytes which leads to activation of the coagulation cascade and potentially life-threatening venous and arterial thrombosis. The diagnosis is established by demonstrating thrombocytopenia and/or thrombotic events in

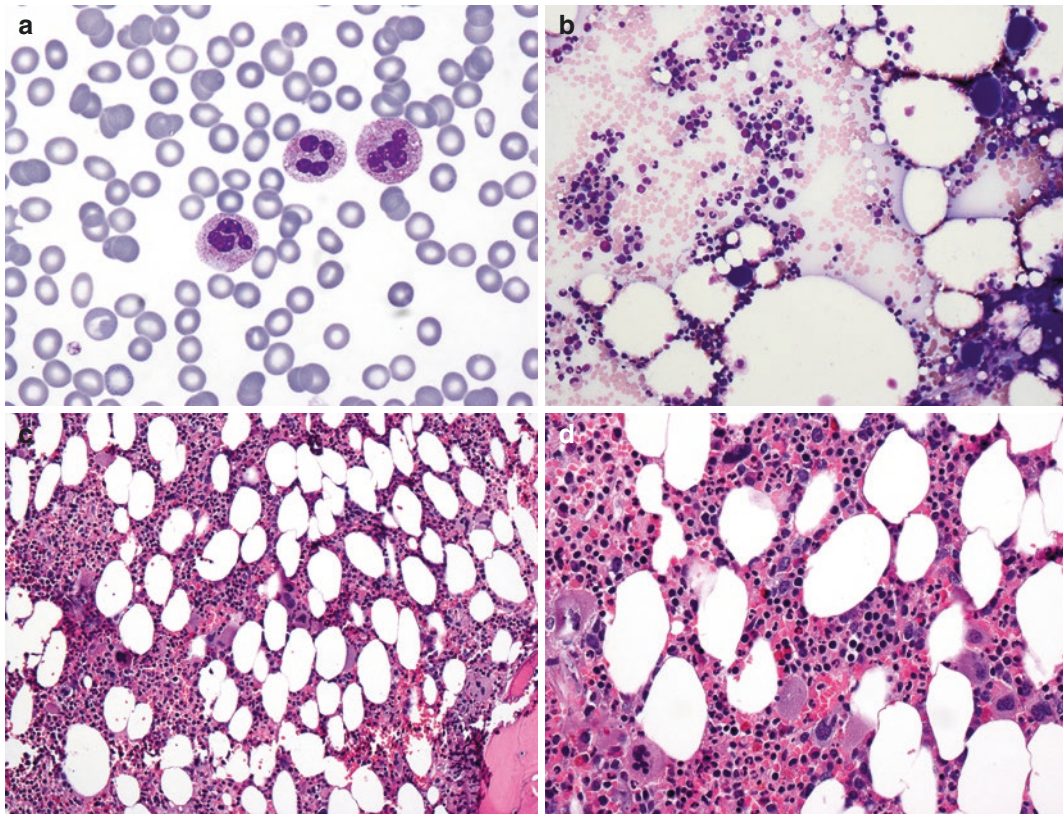


Fig. 2.11 Idiopathic (immune) thrombocytopenia. (**a**) The peripheral blood smear shows normally granulated neutrophils and unremarkable erythrocytes, with marked thrombocytopenia. (**b**) The bone marrow aspirate smear shows normal trilineage hematopoiesis, including several megakaryocytes identified in this low-power field. (**c**, **d**) The bone marrow biopsy is hypercellular, with increased megakaryocytes. Some megakaryocytes show simplified nuclear contours and are small in size, consistent with early or regenerating megakaryocytes. Micromegakaryocytes typical of MDS are absent

temporal association with heparin exposure in the absence of other causes for thrombocytopenia. Thrombocytopenia may be absolute or may manifest as a relative reduction in an individual's platelet count. The diagnosis is established by demonstrating the presence of anti-PF4/heparin antibodies [62].

Pure megakaryocyte aplasia is a rare cause of isolated severe thrombocytopenia. In a subset of patients it may progress to aplastic anemia or MDS. The exact pathogenesis is not fully understood, but it is thought to be due to immune-mediated suppression of megakaryopoiesis. Pure megakaryocyte aplasia may be associated with systemic autoimmune diseases or infections (especially hepatitis C) or it may be idiopathic. Anti-thrombopoietin (TPO) and anti-TPO receptor antibodies have both been implicated. Morphologic examination of the bone marrow shows markedly reduced to absent megakaryocytes and may show mild dyserythropoiesis. Patients may respond to immunosuppressive therapy [63].

Myelodysplastic Syndromes

The myelodysplastic syndromes (MDS) are clonal hematopoietic neoplasms characterized by progressive peripheral cytopenias and dysplastic morphology of hematopoietic cells. MDS encompasses a broad range of disease subtypes: some are characterized by genetic instability and rapidly wors-

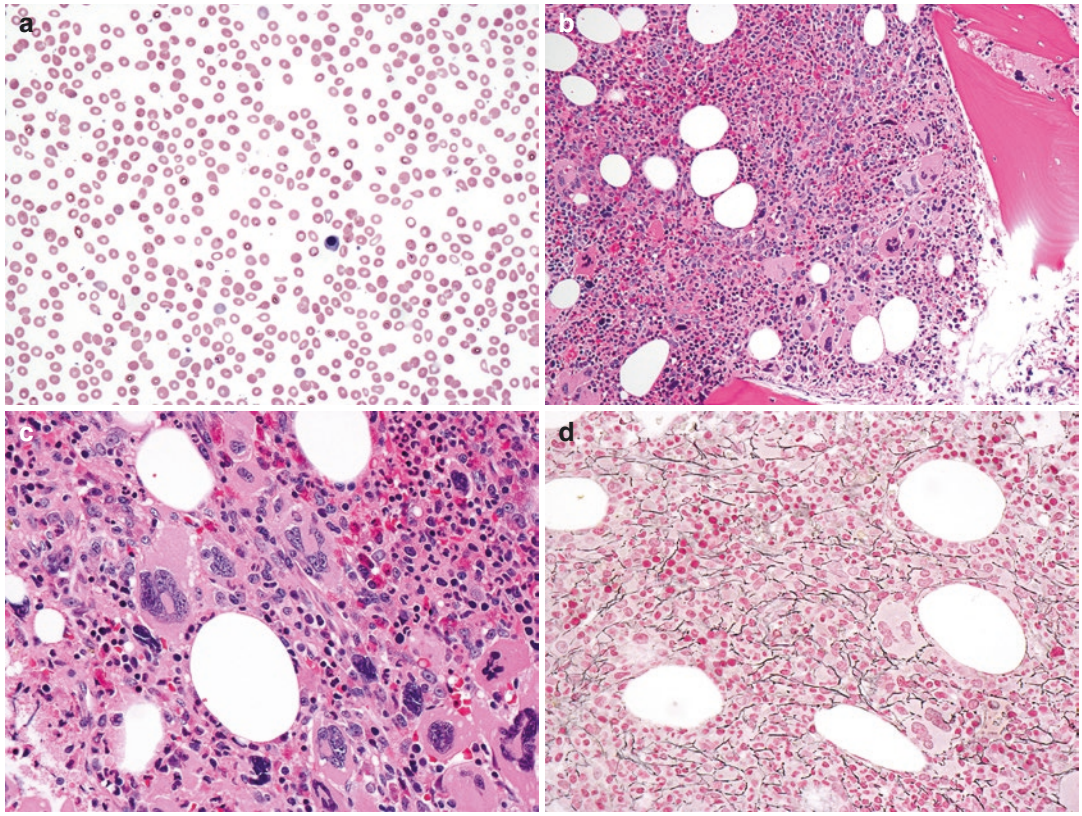


Fig. 2.12 Bone marrow fibrosis and atypical megakaryocyte proliferation associated with thrombopoietin analogue therapy for idiopathic thrombocytopenia. (**a**) The peripheral blood smear shows thrombocytopenia, with nucleated red blood cells and several dacryocytes. (**b**, **c**) The bone marrow biopsy is markedly hypercellular, with a proliferation of clustered megakaryocytes showing complex, hyperchromatic nuclei reminiscent of myeloproliferative neoplasms. (**d**) There is mild reticulin fibrosis (grade 1 of 3). These changes resolved following cessation of therapy

ening clinical disease over time, often culminating in acute myeloid leukemia (AML), while others manifest as relatively stable disease. The three main challenges in approaching MDS from a diagnostic standpoint are (1) establishing a definitive diagnosis of MDS versus another neoplastic or nonneoplastic cause of cytopenia; (2) correctly assigning an established MDS case to a disease subtype according to the 2016 WHO Classification; and (3) obtaining sufficient morphologic, clinical, and genetic information to provide additional prognostic guidance, mainly through the Revised International Prognostic Scoring System (IPSS-R) [64]. Testing for mutations that have known prognostic impact on MDS is an evolving area that continues to accumulate data.

To evaluate a potential case of MDS, the minimal requisite studies are a CBC with WBC differential; examination of the peripheral smear, a Wright-Giemsa-stained bone marrow aspirate smear, a bone marrow biopsy section, and an iron stain on a bone marrow aspirate smear to evaluate for ring sideroblasts; and a full bone marrow karyotype. Flow cytometry is not essential in all cases, but is helpful in characterizing the blast phenotype if increased and also can provide information to support an MDS diagnosis (see "Immunophenotype" section below). Importantly, abnormalities disclosed by the above tests are not always specific for MDS. In fact, no specific feature can be used in isolation to unequivocally diagnose MDS. Rather, clinicopathologic correlation

and integration of information from the multiple diagnostic modalities are essential for an accurate and reliable diagnosis. If the diagnosis is ambiguous and cytopenias persist, a repeat marrow sample may be taken after 6 months, which may allow confirmation of the diagnosis [65].

Clinical Features

MDS is primarily a disease of older adults: its incidence increases exponentially with age, with a median age estimated at approximately 76 years [66–72]. MDS is rare in children and young adults, with an annual incidence of <0.2/100,000 compared to 25/100,000 for individuals over 70 years of age [68–70, 73–75]. The overall incidence of MDS may be underestimated due to various factors that influence its reporting in national databases. MDS is generally more common in males, with a male-to-female ratio approaching 2:1.

MDS typically presents with symptoms and signs related to single or multiple peripheral blood cytopenias, most frequently those reflecting anemia (weakness, pallor, fatigue) or thrombocytopenia (petechiae, bleeding), but patients can also present with recurrent infections due to neutropenia. Occasional patients are recognized when asymptomatic cytopenias are noted during a routine CBC or when telltale abnormalities are noted in a peripheral blood or bone marrow sample obtained for other reasons. Although between 10 and 40% of MDS cases ultimately progress to AML and most of those patients die of complications of leukemia, overall the greatest morbidity and mortality in MDS are due to complications of cytopenias.

By definition, all MDS patients have some cytopenia at diagnosis that is sustained and progressive without therapeutic intervention. The 2016 WHO MDS guidelines suggest thresholds of cytopenias at a HGB level <10 g/dL, absolute neutrophil count $<1.8 \times 10^9$/L, and platelet count $<100 \times 10^9$/L, but it is recognized that a diagnosis of MDS may be made in patients with a milder cytopenia (below the reference range for the individual laboratory), provided that the cytopenia is persistent and unexplained and if other diagnostic criteria are present. Patients typically present with anemia that is often macrocytic, less commonly normocytic, and only rarely microcytic [76]. There is frequently an increased RDW. The anemia is often accompanied by neutropenia and/or thrombocytopenia [77] and patients may infrequently present with isolated neutropenia or thrombocytopenia, without anemia [78]. Although reticulocyte production is typically low in MDS, the reticulocyte count may be spuriously elevated in some patients owing to basophilic stippling in circulating RBCs, potentially suggesting an erroneous diagnosis of hemolytic anemia [79]. A small subset of MDS patients experience a component of hemolysis that contributes to the anemia.

Careful physical exam is important in the initial evaluation of a patient presenting with possible MDS. Splenomegaly is uncommon and its presence may suggest an alternative cause for the cytopenias. Some patients experience cutaneous manifestations, particularly Sweet's syndrome (neutrophilic dermatosis) [80]. Myeloid sarcomas, which are extramedullary infiltrates of myeloblasts, can cause masses in lymph nodes, skin, or other extranodal sites in MDS patients. If a mass-forming collection of myeloblasts is confirmed histologically, this is considered to represent transformation to AML, even if the bone marrow myeloblast count remains below 20%.

Obtaining a detailed clinical and family history is also critical in approaching a new diagnosis of MDS. A variety of inherited conditions predispose to the development of MDS, including Down syndrome, Fanconi anemia, severe congenital neutropenia (Kostmann syndrome), Shwachman-Diamond syndrome, dyskeratosis congenita, amegakaryocytic thrombocytopenia, and Bloom syndrome [81]. These disorders are discussed in detail in Chap. 3. Cytotoxic agents that may damage the bone marrow, including ionizing radiation, alkylating agents, and other chemotherapeutic agents, as well as benzene, petrochemicals, agricultural chemicals, and smoking, have been linked to an increased incidence of MDS and AML. MDS cases developing after exposure to cytotoxic chemotherapy and/or radiotherapy have unique genetic features and aggressive clinical behavior and are classified in separate group of therapy-related myeloid neoplasms in the 2016 WHO Classification. Thus,

eliciting a history of such agents is critical when approaching any new MDS diagnosis.

Morphology

Despite peripheral cytopenias, the marrow is typically hypercellular for age [82–84]. Less commonly, it is normocellular and in about 10–15% of cases it is hypocellular. Hypocellularity is more frequent in pediatric MDS, following prior aplastic anemia, and in therapy-related MDS [85–87]. The marrow biopsy often exhibits disorganized hematopoiesis, whereby erythroid and myeloid elements are intimately admixed rather than forming discrete clusters in the marrow space. Although not pathognomonic for MDS, dysplastic morphology is a critical feature in establishing the diagnosis. Dysplastic findings in MDS are listed in Table 2.9 and are illustrated in Figs. 2.13–2.16. Some features are best seen in the peripheral smear, such as pseudo-Pelger-Huët anomaly and hypogranular cytoplasm in neutrophils, while others are more apparent in the bone marrow. Bone marrow blasts are increased in some MDS cases and may circulate in the peripheral blood, but are always <20% of the bone marrow and peripheral blood nucleated cells. Auer rods are uncommonly seen in MDS and, if present, indicate high-grade disease (MDS with excess blasts) [88]. Although experienced observers can generally agree on the presence of significant dysplasia [89], there is some subjectivity to interpreting and quantifying the degree of dysplasia [90]. The 2016 WHO Classification recommends that at least 10% of cells in a lineage demonstrate dysplastic features to be considered significant [91]. However, dysplastic features involving >10% of a hematopoietic lineage may be encountered in normal individuals and are even more frequently seen in patients with reactive cytopenias, such as those discussed earlier in this chapter [89, 92–94]. Thus, even if significant morphologic dysplastic changes are identified in a cytopenic patient, possible underlying secondary causes of cytopenia and hematopoietic dysplasia must be carefully excluded prior to rendering a diagnosis of MDS. These are discussed in the Differential Diagnosis section below.

It is critical to perform an accurate blast count on both the peripheral smear and the bone marrow in every MDS case. Optimally, at least 200 cells in the blood smear and at least 500 cells in the aspirate smear should be counted to ensure a precise blast percentage. The blast threshold for separating MDS from AML is always 20% in the bone marrow and/or blood. A rule in the previous 2008 WHO Classification that excluded erythroid precursors from the blast percentage calculation when erythroid precursors exceeded 50% of marrow cells to diagnose acute erythroid leukemia has been eliminated. Thus, the bone marrow myeloblast percentage is now always derived from all nucleated cells in all MDS and AML cases, irrespective of the percentage of erythroid cells [95]. An iron stain should be performed to evaluate for ring sideroblasts on a bone marrow aspirate smear rather than the biopsy specimen, because decalcification leaches iron from the biopsy sample. In some instances, ring sideroblasts may be identifiable in a non-decalcified paraffin-embedded particle clot section stained for iron (Fig. 2.16d). Reticulin staining demonstrates increased reticulin fibrosis in a subset of MDS cases and can be helpful in establishing the diagnosis, as significant reticulin fibrosis is uncommon in reactive conditions that cause cytopenia.

The 2016 WHO Classification recognizes several entities within MDS. The defining features of these entities are shown in Table 2.10 and are summarized below. Algorithms for classifying MDS cases without excess blasts, including MDS in children, are shown in Fig. 2.17. Although there is no formal grouping of the MDS entities in the WHO Classification, for the purposes of discussion below these are divided into *low-grade MDS* (with <5% bone marrow and <2% blood blasts), *high-grade MDS, therapy-related MDS,* and *MDS in children.* The special situations of hypoplastic MDS, MDS with erythroid predominance, and MDS with fibrosis are also discussed below.

Low-Grade MDS

Myelodysplastic syndrome with single-lineage dysplasia (MDS-SLD) encompasses those MDS cases lacking excess blasts or a significant number of ring sideroblasts, with morphologic dysplasia only manifesting in one hematopoietic lineage.

Table 2.9 Dysplastic morphologic features observed in MDS

Cell lineage	Peripheral blood	Bone marrow
Red blood cells and erythroids	• Macrocytes • Basophilic stippling	• Megaloblastoid change • Nuclear budding or other irregularities • Multinucleation • Vacuolated early erythroblasts • Internuclear bridging • Pyknotic nuclei • Ring sideroblasts
Granulocytes	• Pseudo-Pelger-Huët anomaly • Non-segmented nuclei • Abnormal chromatin clumping • Hypogranulation • Uneven granulation • Nuclear hypersegmentation	• Same abnormalities as seen in blood • Abnormal localization of immature precursors in clusters away from bone trabeculae
Platelets and megakaryocytes	• Large, vacuolated, or hypogranular platelets	• Small mononuclear forms (micromegakaryocytes) • Large forms with multiple small, rounded nuclei • Forms with hypolobated nuclei • Forms with hyperchromic nuclei and scant cytoplasm

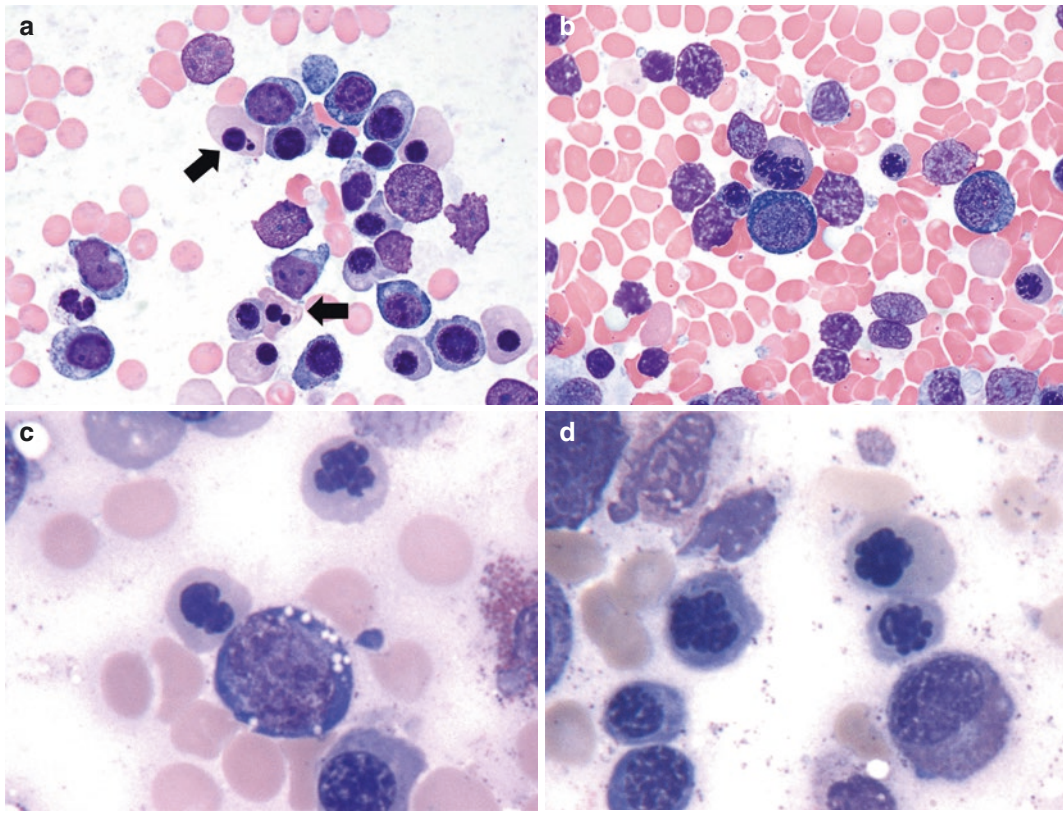


Fig. 2.13 Morphologic dysplasia seen in the erythroid lineage in MDS (bone marrow aspirate smears). (**a**) Nuclear budding of late forms (arrows). (**b**) Binucleation. (**c**) Vacuolation of early forms and nuclear irregularities in late forms. (**d**) Marked nuclear irregularities in late forms

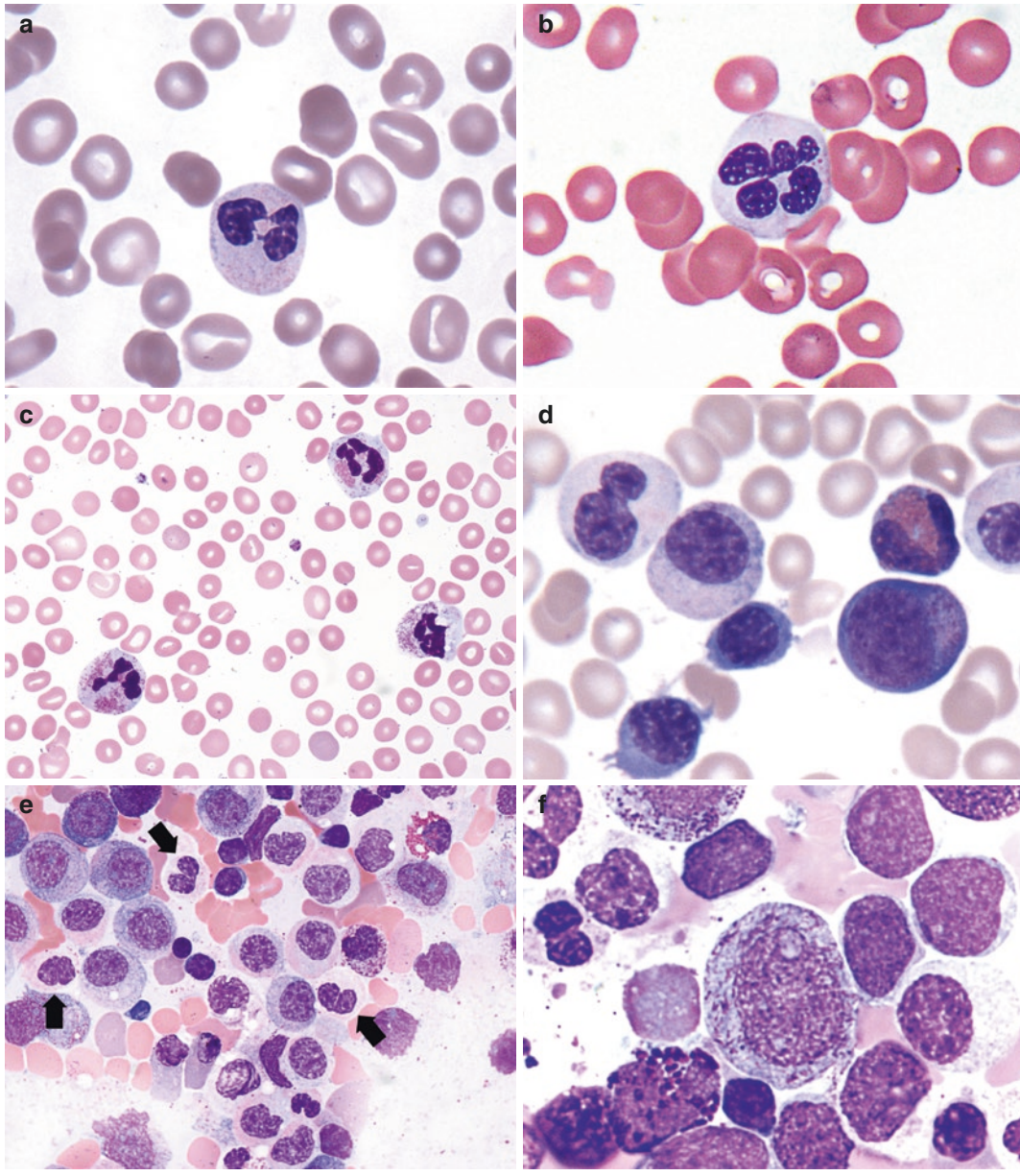


Fig. 2.14 Morphologic dysplasia in the granulocytic lineage in MDS. (**a**) Pseudo-Pelger-Huët neutrophil, with bilobed nucleus and hypogranular cytoplasm in the peripheral blood. (**b**) Dysplastic neutrophil with markedly hypogranular cytoplasm in peripheral blood. (**c**) Dysplastic neutrophils showing uneven cytoplasmic granulation and vacuolation in peripheral blood. (**d**) Dysplastic hypogranular myeloid forms in the bone marrow aspirate. (**e**) Bone marrow aspirate showing numerous dysplastic myeloid forms, including hypogranular myelocytes with abnormally clumped nuclear chromatin (center) as well as three pseudo-Pelger-Huët cells (arrows). (**f**) Abnormal promyelocyte (center) showing abnormally distributed and abnormally shaped (elongated) primary granules

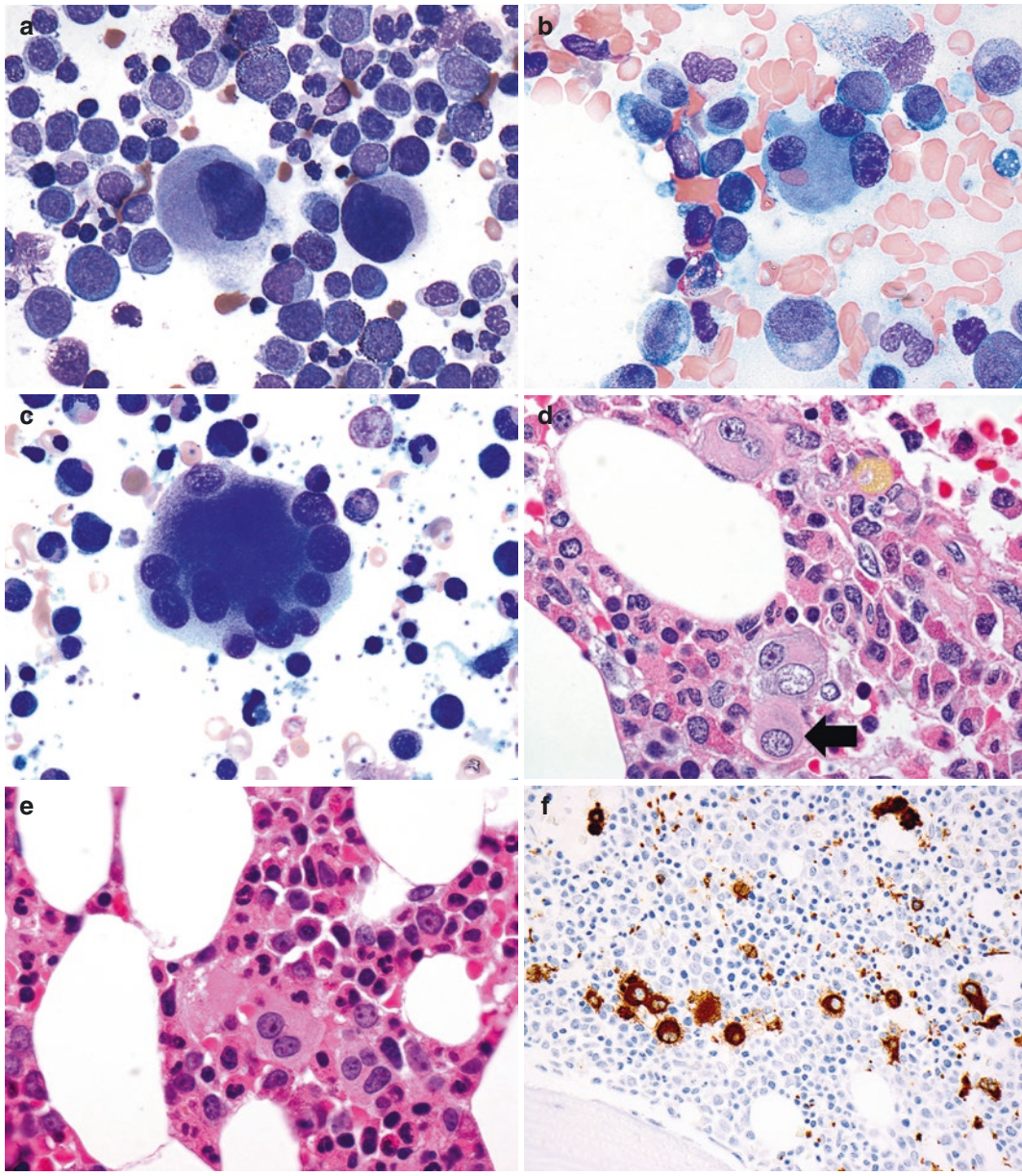


Fig. 2.15 Morphologic dysplasia in the megakaryocytic lineage in MDS. (**a**) Small, dysplastic megakaryocytes in the aspirate smear. (**b**) An abnormal form with three separated nuclei in the aspirate smear. (**c**) A large, dysplastic megakaryocyte with multiple separated nuclear lobes in the aspirate smear; in contrast to the large megakaryocytes seen in essential thrombocythemia, the nuclei are widely separated with rounded contours. (**d**) Several dysplastic forms in the biopsy, including a micromegakaryocyte (arrow). (**e**) A dysplastic binucleate megakaryocyte in the biopsy. (**f**) CD61 immunostain highlighting numerous small, non-lobated megakaryocytes and micromegakaryocytes in the biopsy

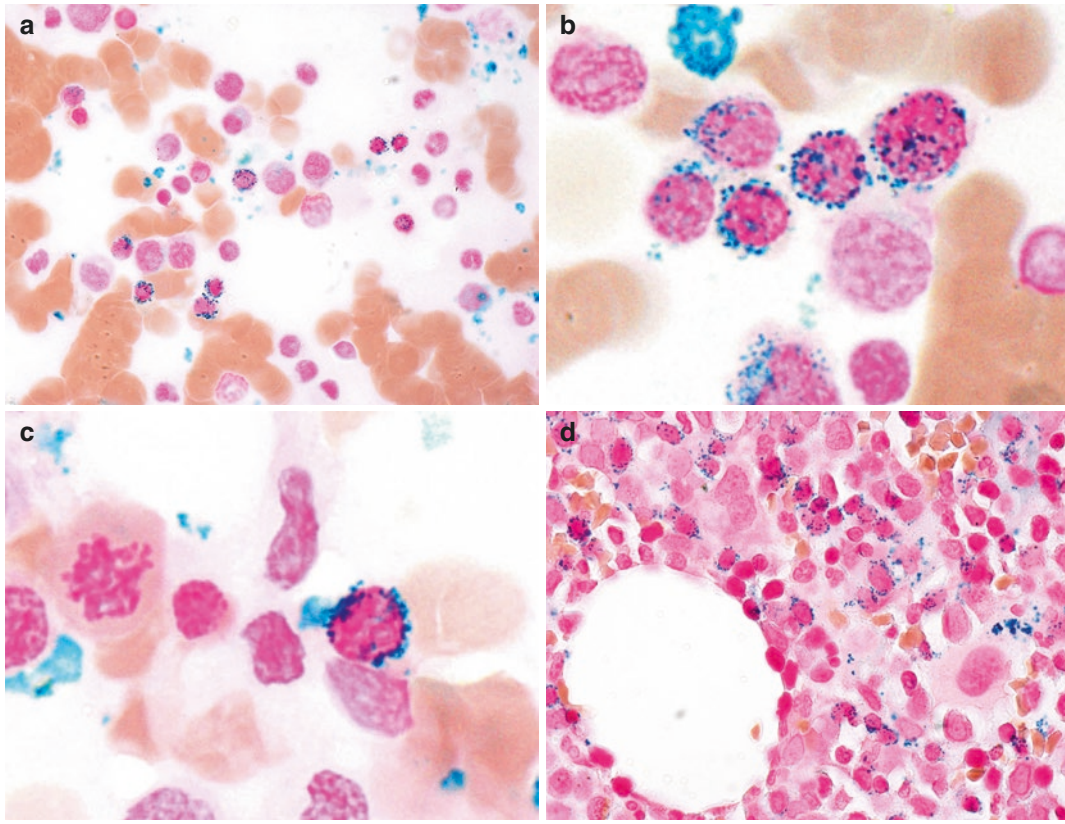


Fig. 2.16 Ring sideroblasts. (**a**) Low-power view of iron stain on bone marrow aspirate shows multiple ring sideroblasts. (**b**, **c**) Ring sideroblasts are nucleated erythroid elements in which multiple (at least five) siderotic granules are closely opposed to the nuclear membrane and partly or fully encircle the nucleus (**c**). (**d**) Multiple ring sideroblasts seen on an iron stain of this bone marrow clot specimen

Interestingly, the lineage manifesting dysplasia often does not coincide with the cytopenic lineage or lineages in the blood [96, 97]. Most cases of MDS-SLD present with anemia and isolated erythroid lineage dysplasia. Although bicytopenia is allowed in MDS-SLD, cases with single-lineage dysplasia and pancytopenia are placed in the MDS, unclassified (MDS-U) category, as they appear to have more aggressive behavior [96, 98].

Myelodysplastic syndrome with ring sideroblasts (MDS-RS) encompasses low-grade MDS cases in which ring sideroblasts are identified on an iron stain [99]. The presence of ring sideroblasts in all myeloid neoplasms is closely associated with mutation in the spliceosome gene *SF3B1*, which is in turn associated with a favorable prognosis in MDS (see "Cytogenetics and Molecular Genetics" section below) [100]. MDS-RS includes cases with an *SF3B1* mutation and as few as 5% ring sideroblasts, or with at least 15% ring sideroblasts irrespective of the *SF3B1* mutation status. The MDS-RS category is subdivided into cases with single-lineage dysplasia (MDS-RS-SLD) and multilineage dysplasia (MDS-RS-MLD). By definition, the erythroid lineage is dysplastic in all MDS-RS cases, since ring sideroblasts constitute a form of erythroid dysplasia; thus any additional dysplastic lineages mandate classification as MDS-RS-MLD, which has an inferior prognosis to MDS-RS-SLD [101–103].

Myelodysplastic syndrome with multilineage dysplasia (MDS-MLD) is the most common MDS subtype and encompasses cases lacking excess blasts, but with multilineage (two or three affected lineages) dysplasia and any number of cytopenias [104]. The prognosis of MDS-MLD is inferior to

Table 2.10 Diagnostic features of myelodysplastic syndrome (MDS) subtypes according to the 2016 World Health Organization

Entity name	Peripheral blood findings	Bone marrow findings	Cytogenetics and molecular genetics
MDS with single-lineage dysplasia (MDS-SLD)	1 or 2 cytopenias[a] <1% blasts; no Auer rods	<5% blasts; no Auer rods ≥10% dysplastic cells in 1 hematopoietic lineage <15% ring sideroblasts	Normal or any abnormalities, except isolated del(5q)[b] No *SF3B1* mutation if 5–15% ring sideroblasts are present.
MDS with multilineage dysplasia (MDS-MLD)	1–3 cytopenias[a] <1% blasts; no Auer rods	<5% blasts; no Auer rods ≥10% dysplastic cells in each of 2 or 3 hematopoietic lineages <15% ring sideroblasts	Normal or any abnormalities, except isolated del(5q)[b] No *SF3B1* mutation if 5–15% ring sideroblasts are present
MDS with ring sideroblasts and single-lineage dysplasia (MDS-RS-SLD)	1 or 2 cytopenias[a] <1% blasts; no Auer rods	<5% blasts; no Auer rods ≥10% dysplastic cells in 1 hematopoietic lineage ≥5% ring sideroblasts	Normal or any abnormalities, except isolated del(5q)[b] *SF3B1* mutation required if 5–15% ring sideroblasts are present
MDS with ring sideroblasts and multilineage dysplasia (MDS-RS-MLD)	1–3 cytopenias[a] <1% blasts, no Auer rods	<5% blasts; no Auer rods ≥10% dysplastic cells in each of 2 or 3 hematopoietic lineages ≥5% ring sideroblasts	Normal or any abnormalities, except isolated del(5q)[b] *SF3B1* mutation required if 5–15% ring sideroblasts are present
MDS with excess blasts-1 (MDS-EB-1)	1–3 cytopenias[a] 2–4% blasts, no Auer rods	5–9% blasts; no Auer rods Any amount of dysplasia Ring sideroblasts may or may not be present	Normal or any abnormalities, except *PML-RARA*, inv(16)/t(16;16), or t(8;21)
MDS with excess blasts-2 (MDS-EB-2)	1–3 cytopenias[a] 5–19% blasts, or the presence of Auer rods	10–19% blasts, or the presence of Auer rods Any amount of dysplasia Ring sideroblasts may or may not be present	Normal or any abnormalities, except *PML-RARA*, inv(16)/t(16;16), or t(8;21)
MDS with isolated del(5q)	1–2 cytopenias[a]; platelet count may be elevated <1% blasts No Auer rods	<5% blasts; no Auer rods ≥10% dysplastic cells in at least 1 lineage (almost always megakaryocytic) Ring sideroblasts may or may not be present	Del(5q) only, or with 1 additional clonal abnormality excluding del(7q), -7
Refractory cytopenia of childhood (RCC) (provisional)	1–3 cytopenias[a] <2% blasts	<5% blasts; no Auer rods ≥10% dysplastic cells in at least 1 lineage (usually 2 or 3 lineages) No ring sideroblasts Marrow hypocellularity in most cases	Normal or any abnormalities, except isolated del(5q)[b]
MDS, unclassifiable (MDS-U)			
with 1% peripheral blood blasts	1–3 cytopenias[a] 1% circulating blasts demonstrated on 2 separate occasions; no Auer rods	<5% blasts; no Auer rods ≥10% dysplastic cells in at least 1 hematopoietic lineage	Normal or any abnormalities
with single-lineage dysplasia and pancytopenia	Pancytopenia[a] <1% blasts; no Auer rods	<5% blasts; no Auer rods ≥10% dysplastic cells in 1 hematopoietic lineage Ring sideroblasts may or may not be present	Normal or any abnormalities
with defining cytogenetic abnormalities	1–3 cytopenias[a] <1% blasts; no Auer rods	<5% blasts; no Auer rods No significant (<10%) dysplasia in any lineage <15% ring sideroblasts	An MDS-defining clonal cytogenetic abnormality (see Table 2.13)

[a]Cytopenia defined as HGB, absolute neutrophil count, and/or platelet count less than the normal reference range for each laboratory. Pancytopenia defined as HGB <10 g/dL, absolute neutrophil count $<1.8 \times 10^9$/L, and platelets $<100 \times 10^9$/L

[b]Isolated del(5q) is defined here as the presence of a del(5q) cytogenetic abnormality with or without one additional abnormality, except for -7 or del(7q)

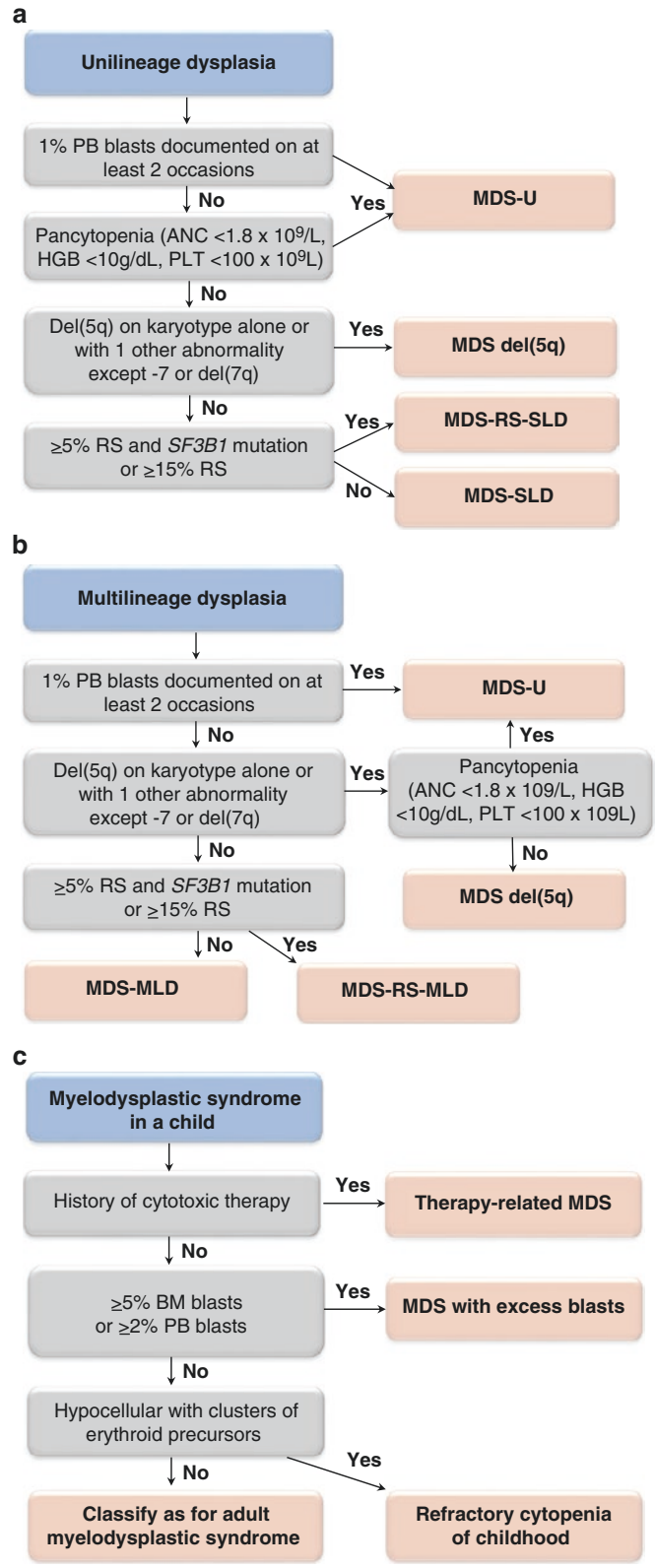


Fig. 2.17 A suggested algorithm for the differential diagnosis of MDS cases with unilineage dysplasia (non-therapy-related, without excess blasts) (**a**), multilineage dysplasia (non-therapy-related, without excess blasts) (**b**), and those occurring in children (**c**). *MDS-U* myelodysplastic syndrome, unclassifiable, *MDS* del(5q) myelodysplastic syndrome with isolated del(5q), *MDS-RS-SLD* myelodysplastic syndrome with ring sideroblasts and single lineage dysplasia, *MDS-SLD* myelodysplastic syndrome with single lineage dysplasia, *PB* peripheral blood, *ANC* absolute neutrophil count, *HGB* hemoglobin, *PLT* platelet count, *RS* ring sideroblasts, *MDS-MLD* myelodysplastic syndrome with multilineage dysplasia, *MDS-RS-MLD* myelodysplastic syndrome with ring sideroblasts and multilineage dysplasia, *MDS-EB* myelodysplastic syndrome with excess blasts

that of MDS-SLD and MDS-RS-SLD [101, 102, 105–107]. Thus, careful attention should be paid in evaluating dysplasia in each lineage in order to distinguish between single and multilineage dysplasia cases.

Myelodysplastic syndrome with isolated del(5q) is a discrete subset of MDS with a favorable prognosis [101, 102]. Patients typically present with macrocytic anemia, normal granulopoiesis and neutrophil count, and normal or increased platelets associated with increased and markedly dysplastic, mononuclear megakaryocytes in the marrow. The myeloid:erythroid ratio is usually elevated, with relative erythroid hypoplasia (Fig. 2.18).

Finally, the entity *Myelodysplastic syndrome, unclassifiable (MDS-U)* serves as a repository for those rare cases that fall into three specific categories [108]: (1) cases that resemble MDS-SLD, MDS-MLD, MDS with isolated del(5q), or MDS-RS, but with exactly 1% blasts in peripheral blood documented on two separate occasions; (2) cases that resemble MDS-SLD or MDS-RS-SLD, but with pancytopenia (HGB level <10 g/dL, absolute neutrophil count $<1.8 \times 10^9$/L, and platelet count $<100 \times 10^9$/L); and (3) cases with cytopenia and cytogenetic abnormalities typical of MDS (see Table 2.11), but lacking sufficient (<10%) dysplasia in any lineage. The prognosis of MDS-U is uncertain, but the subcategory with 1% peripheral blood blasts appears to have an inferior prognosis to low-grade MDS cases lacking circulating blasts and has an outcome similar to MDS with excess blasts [109].

High-Grade MDS

Myelodysplastic syndrome with excess blasts (MDS-EB) encompasses cases that have increased blasts in the bone marrow and/or blood (see Table 2.10 for specific blast thresholds), or Auer rods (Fig. 2.19) [88, 110]. MDS-EB can exhibit

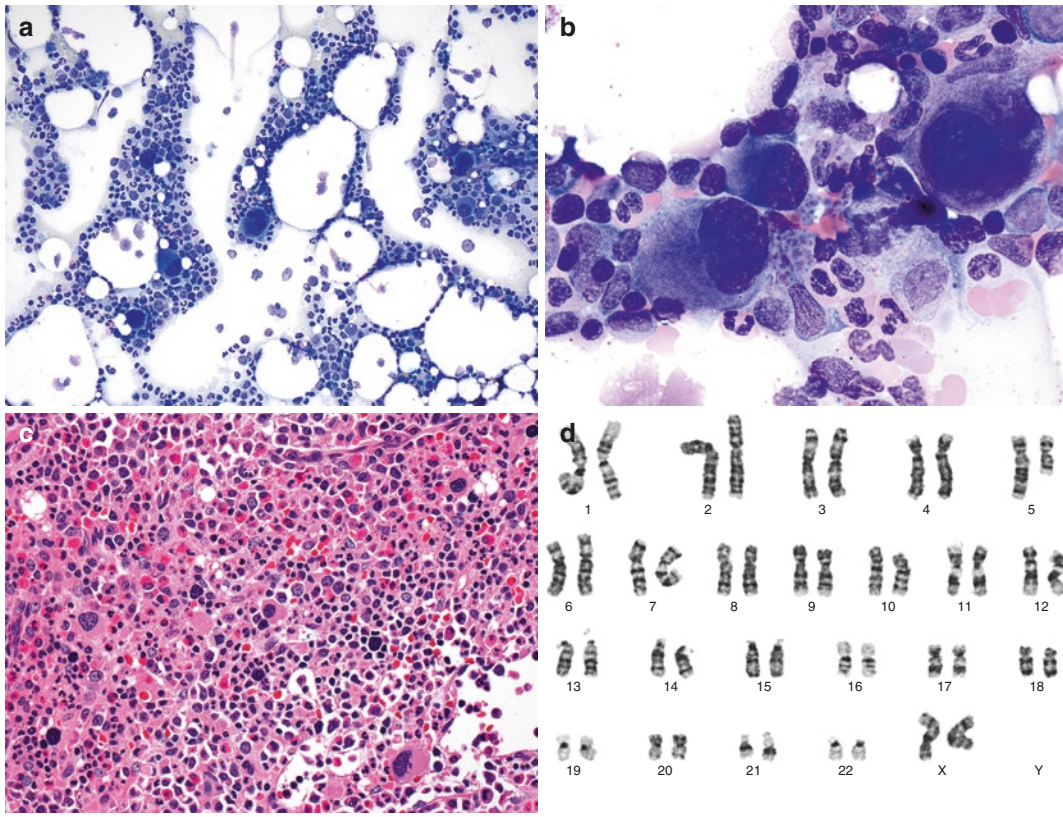


Fig. 2.18 MDS with isolated del(5q). (**a**) The bone marrow aspirate smear shows erythroid hypoplasia with multiple abnormal megakaryocytes with non-lobated, rounded nuclei. (**b**) Three dysplastic megakaryocytes seen in the aspirate smear. (**c**) The bone marrow biopsy specimen also shows relative erythroid hypoplasia and increased megakaryocytes which have round nuclei. (**d**) The bone marrow karyotype shows del(5q) as the only cytogenetic abnormality

Table 2.11 Cytogenetic abnormalities considered MDS defining according to the 2016 WHO classification

Unbalanced cytogenetic abnormalities
−7 or del(7q)
del(5q) or t(5q)
i(17q) or t(17p)
−13 or del(13q)
del(11q)
del(12p) or t(12p)
del(9q)
idic(X)(q13)
Balanced cytogenetic abnormalities
t(11;16)(q23;p13.3)
t(3;21)(q26.2;q22.1)
t(1;3)(p36.3;q21.2)
t(2;11)(p21;q23)
inv(3)(q21q26.2)/ t(3;3)(q21.3;q26.2)
t(6;9)(p23;q34)

any number of cytopenias, any degree of morphologic dysplasia, and variable cytogenetic findings. CD34 immunostaining of the biopsy section may help reveal increased blasts in cases with a hemodilute, nonrepresentative, or poorly-stained aspirate smear [111]. MDS-EB is divided into two strata (MDS-EB1 and MDS-EB2) based on specific cutoffs of bone marrow and blood blasts and presence or absence of Auer rods [105, 106, 112]. Importantly, the presence of excess blasts (or Auer rods) in the bone marrow or blood supersedes an isolated del(5q) cytogenetic abnormality or ring sideroblasts for the purposes of classifying MDS.

MDS in Children

MDS is rare in children and the distribution of disease subtypes differs from that of MDS in adults [113]. Most cases of MDS in children

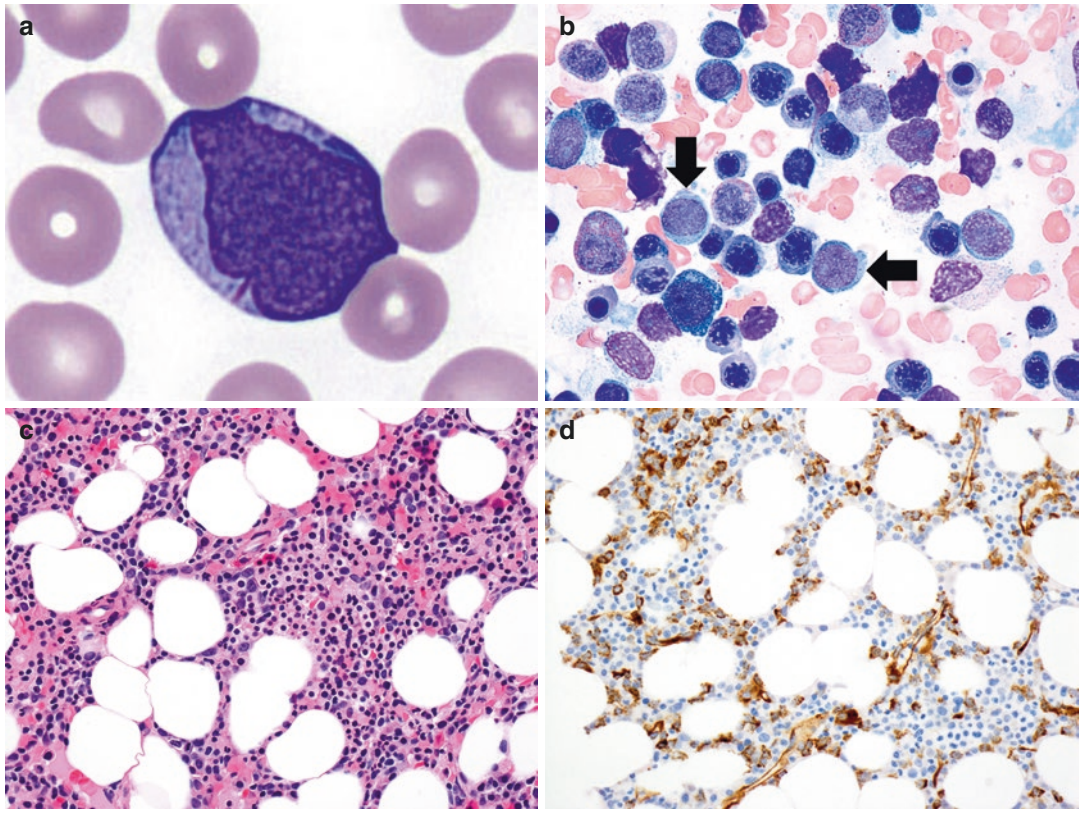


Fig. 2.19 MDS with excess blasts (MDS-EB). (**a**) This circulating blast in a patient with MDS contains an Auer rod, which mandates classification as MDS-EB-2, irrespective of the blast percentage. (**b**) Bone marrow aspirate smear from a patient with MDS-EB-1, showing two blasts (arrows). The blasts in MDS-EB are often small and may be missed in thick parts of the smear. (**c**) In this bone marrow biopsy from a patient with MDS-EB-2, blasts are difficult to enumerate on hematoxylin and eosin stain and do not appear to be increased. (**d**) CD34 immunostain on the same case shows frequent CD34-positive blasts comprising about 15% of the marrow cellularity and present in small clusters

manifest with multilineage dysplasia. MDS-RS is rare in children; if ring sideroblasts are observed in a pediatric patient, Pearson syndrome (discussed earlier in this chapter) must be considered [114]. MDS with isolated del(5q) does not occur in children. The main pediatric MDS entity is *refractory cytopenia of childhood (RCC)*, which comprises about half of all pediatric MDS cases. This disease is similar to MDS-MLD, but usually manifests with a hypocellular marrow with islands of erythroids in the biopsy and significant dyserythropoiesis, often with dysplasia in other lineages as well. Other MDS cases occurring in children are classified like adult MDS. Because of the frequent hypocellularity in pediatric MDS, distinction from aplastic anemia may be challenging. Although application of the diagnostic criteria of RCC (see Table 2.10) usually allows accurate distinction [85], in some cases extended observation may be required to distinguish RCC cases from aplastic anemia. Given this diagnostic difficulty, RCC is still considered as a provisional MDS entity in the 2016 WHO Classification. MDS in children frequently occurs in the background of a congenital germline predisposition syndrome (discussed in detail in Chap. 3), acquired bone marrow failure state (discussed in Chap. 4), or prior exposure to cytotoxic chemotherapy and/or radiotherapy.

Therapy-Related MDS

In the 2016 WHO Classification, *therapy-related MDS* is grouped together with therapy-related AML, as these diseases tend to have similarly poor prognosis irrespective of the blast count [115]. Therapy-related MDS occurs principally after exposure to agents that damage DNA (alkylating agents, platinum derivatives, nitrosoureas) or after exposure to ionizing radiation [116, 117]. The mechanism by which these agents cause disease is not fully understood. While it has generally been assumed that DNA damage initiated by these agents causes mutations in MDS-related oncogenes, more recent data suggest that cytotoxic effects on the marrow microenvironment may foster the expansion of preexisting clones bearing *TP53* or other mutations [118]. The onset of clinical disease may begin as early as 6 months after initiation of therapy with the causative agent, but more characteristically the latency is 2 or more years and peaks at 5–6 years. Patients may present with features of any MDS subtype. Regardless of the presentation, the prognosis is generally poor, with more rapid worsening of cytopenias and progression to AML and shorter survival compared to de novo MDS, mainly due to the high incidence of adverse cytogenetic features and *TP53* mutations in therapy-related MDS [119].

Hypoplastic MDS

Most cases of MDS have hypercellular marrow despite the presence of cytopenias, reflecting the ineffective hematopoiesis that is characteristic of the disease. However, 10–15% of MDS in adults (and a much higher percentage in children, as discussed above) has reduced marrow cellularity for age [120, 121]. Hypoplastic MDS is not a specific entity in the 2016 WHO Classification, and such cases should be classified as for other MDS. Rather, the term serves to identify a group of MDS cases that must be carefully distinguished from other hypoplastic marrow states. Because aplastic anemia can manifest mildly dysplastic morphology and even transient clonal cytogenetic abnormalities similar to MDS, distinction between hypocellular MDS and aplastic anemia may be difficult (see Chap. 4 and "Differential Diagnosis" section below) [87, 122]. Interestingly, hypoplastic MDS is more likely than more cellular MDS cases to respond favorably to immunosuppression, suggesting a possible biologic overlap with aplastic anemia [123–125]. A careful blast count on the aspirate smear must be performed to distinguish hypoplastic MDS-EB from hypoplastic AML. CD34 staining of the biopsy section is helpful in this regard [87] (Fig. 2.20).

Erythroid-Predominant MDS

Cases of MDS with erythroid predominance (≥50% erythroid elements) comprise about 15% of all MDS and are more frequently therapy related than MDS with <50% erythroids [126] (Fig. 2.21). Erythroid predominance does not define a specific MDS subtype in the 2016 WHO Classification.

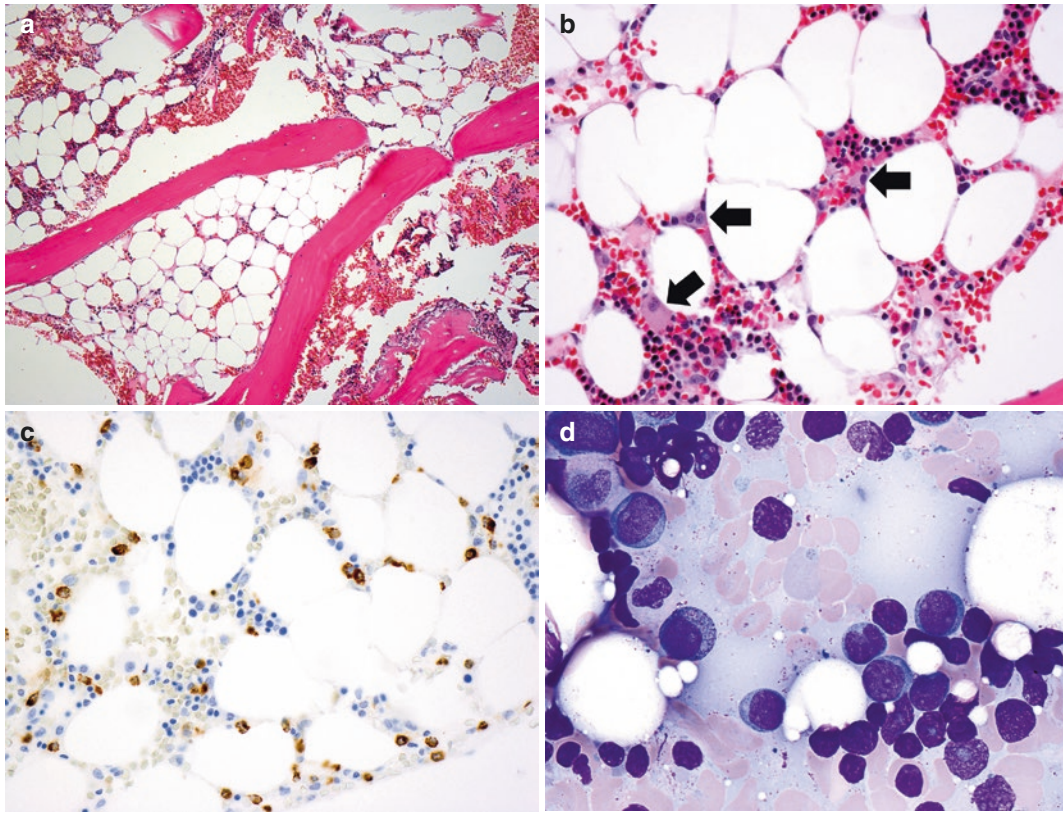


Fig. 2.20 Hypoplastic MDS, classified as MDS-EB-2. (**a**) The marrow biopsy is markedly hypocellular. (**b**) On high magnification, there are clusters of erythroid elements as well as scattered dysplastic megakaryocytes (arrows). (**c**) A CD34 immunostain highlights frequent myeloblasts, comprising 10% of the cellularity. (**d**) Although the aspirate smear is paucicellular, blasts are readily identified

These cases are most frequently classified as MDS-MLD, MDS-SLD, or MDS-RS [126]. In the 2016 WHO Classification, most erythroid-predominant cases previously classified as acute erythroid leukemia, erythroid/myeloid subtype in the 2008 WHO Classification are now classified as MDS-EB. This change was made because the method of counting blasts as a proportion of non-erythroid cells in acute erythroid leukemia did not necessarily predict clinically distinctive disease [127, 128]. Some studies have suggested that erythroid-predominant MDS cases with increased pronormoblasts have a more aggressive course [129]. However, enumeration of pronormoblasts in MDS is not part of the WHO Classification, in part because pronormoblast numbers and erythroid left shift may be labile and influenced by metabolic deficiencies and effects of exogenous growth factors.

MDS with Fibrosis

Moderate and occasionally marked reticulin fibrosis can occur in MDS [130, 131] (Fig. 2.22). Like hypoplastic MDS and erythroid-predominant MDS, MDS with fibrosis is not a distinct disease category in the 2016 WHO Classification and these cases are classified based on the same criteria used in other MDS cases. Significant reticulin fibrosis (grade 2 or 3 in the WHO myelofibrosis grading scheme) confers an inferior prognosis to MDS that is independent of other risk factors [132]. Many MDS cases with fibrosis have increased blasts and are classified as MDS-EB. In such cases, the fibrosis may interfere with the acquisition of an aspirate (so-called "dry tap"). Thus, accurate quantitation of blasts in fibrotic MDS often requires careful analysis of the core biopsy to estimate the blast count. Touch imprints

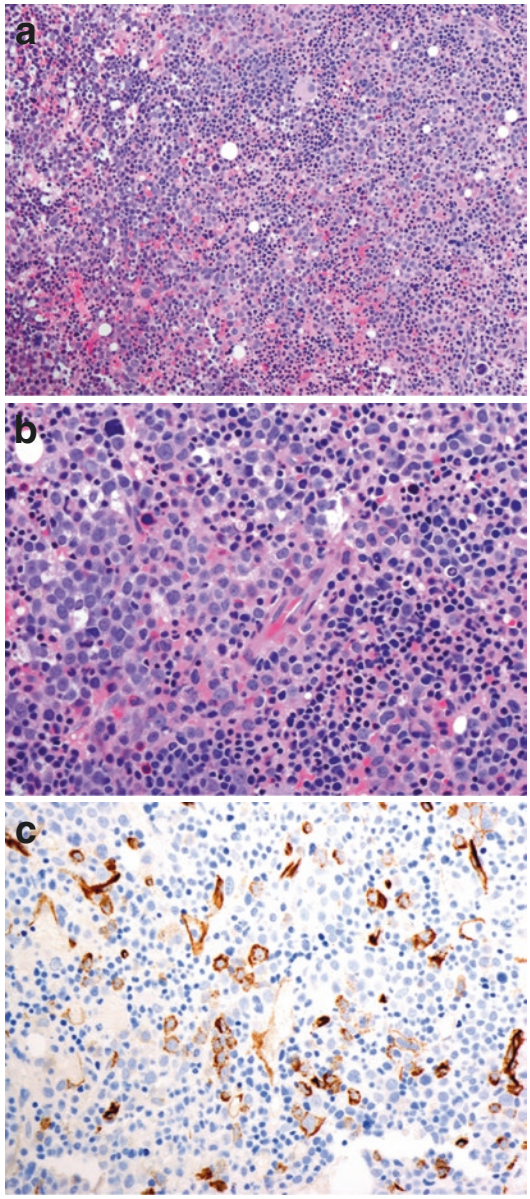


Fig. 2.21 MDS with erythroid predominance, classified as MDS-EB-1, on bone marrow biopsy. (**a**) The marrow is markedly hypercellular, with a prominent proliferation of erythroid elements. (**b**) Erythroid maturation is left shifted, but exhibits maturation, unlike pure erythroid leukemia, in which maturing erythroid elements are infrequent. Several late erythroid forms (lower right corner) exhibit dysplastic nuclear irregularities. (**c**) A CD34 immunostain highlights increased myeloblasts. Although relatively low in number, the percentage of blasts in the aspirate smear (not shown) comprised 25% of the non-erythroid elements and thus this case would have been diagnosed as acute erythroid leukemia (erythroid/myeloid subtype) in the 2008 WHO Classification. However, based on a 7% blast percentage of all cells in the aspirate smear, this case is classified as MDS-EB-1 in the 2016 WHO Classification

from the core biopsy should always be prepared in a "dry tap" situation. Immunohistochemical staining with CD34 to highlight possible increased blasts in the bone marrow biopsy is essential in fibrotic cases with a compromised aspirate smear. CD61 immunostaining is also helpful to reveal micromegakaryocytes that are commonly seen in MDS with fibrosis. MDS with fibrosis may mimic primary myelofibrosis, other chronic myeloproliferative neoplasms with fibrosis, and acute megakaryoblastic leukemia. These differential diagnoses are discussed below.

Immunophenotype

MDS hematopoietic cells exhibit quantitative and qualitative abnormalities in antigen expression and maturation patterns that can be evaluated by multiparameter flow cytometry immunophenotyping. These include abnormalities in the quantity and phenotype of blasts as well as the maturation phenotypic patterns and light scatter qualities of maturing granulocytic elements, and the maturation phenotypic patterns of erythroid cells and monocytes. A list of the main flow cytometry abnormalities observed in the most frequently assessed hematopoietic lineages (blasts, maturing myeloids, and monocytes) is shown in Table 2.12 and examples of normal and aberrant phenotypes of blasts and maturing hematopoietic cells are shown in Figs. 2.23, 2.24, and 2.25. These abnormalities correlate with the types and degrees of morphologic dysplasia and cytogenetic abnormalities [133], and predict prognosis independent of other known risk factors [134–136]. Although these phenotypic abnormalities have been validated in numerous studies, how flow cytometry should be optimally applied to diagnose MDS remains controversial. Some of the abnormalities in myeloid and monocytic maturation patterns that are typical of MDS may also be seen in reactive conditions such as HIV infection, bone marrow regeneration, and autoimmune conditions [137]. Assessing aberrant antigen expression patterns has proven difficult to implement in a clinical laboratory setting [138, 139]. In contrast, phenotypic aberrations

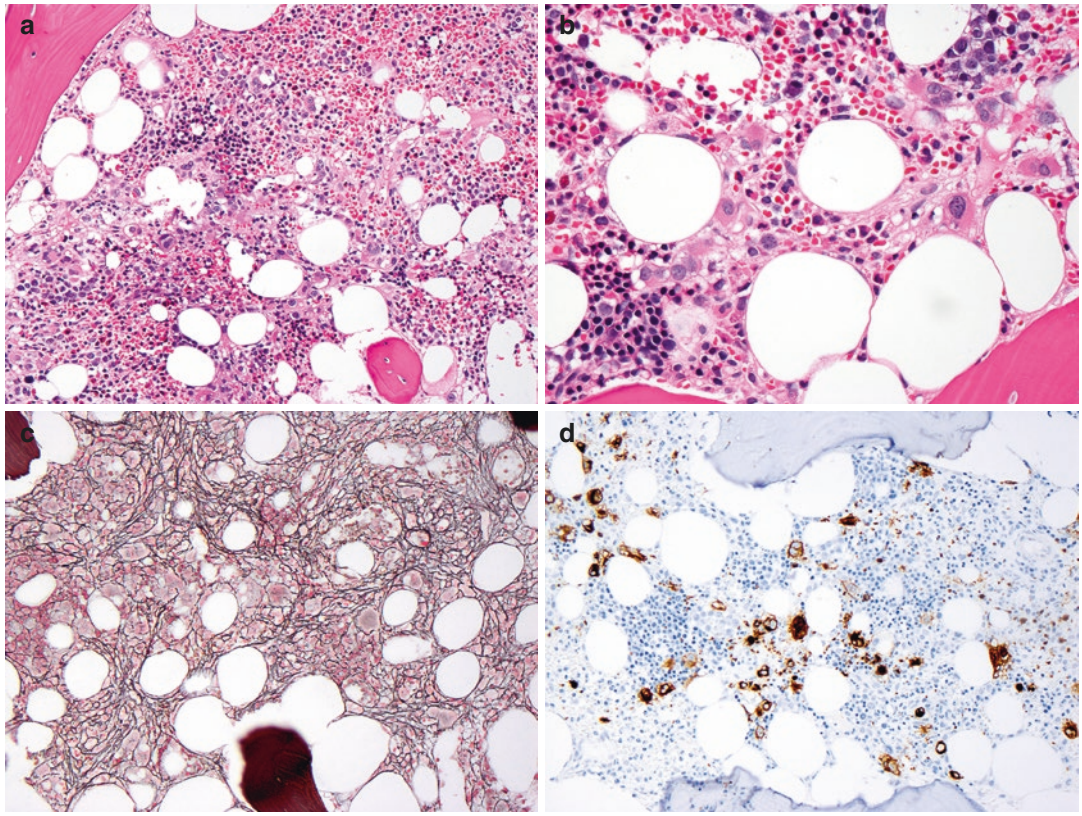


Fig. 2.22 MDS with fibrosis, classified as MDS-MLD, on bone marrow biopsy. (**a**) The marrow is markedly hypercellular, with streaming of cells in some areas suggesting fibrosis. (**b**) High magnification shows intact maturation of erythroid and myeloid elements and several small, dysplastic megakaryocytes. (**c**) There is moderate reticulin fibrosis (WHO MF grade 2) on a reticulin silver stain. (**d**) A CD61 immunostain highlights numerous micromegakaryocytes

observed in the blast compartment, such as a paucity of hematogones, altered expression of antigens such as CD45, CD34, CD117, CD33, CD13, or CD38, or aberrant expression of lymphoid antigens such as CD2, CD5, CD7, or CD56, appear to be more specific for MDS [140].

Implementation of flow cytometry evaluation of MDS in individual laboratories can be challenging, as it requires sufficient case volume and experience to validate. Ideally, published recommended panels should be used and multiple simultaneous abnormalities should be seen in the hematopoietic cells to suggest a diagnosis of MDS [141, 142]. A recent guideline from the European LeukemiaNet Working Group for Flow Cytometry in MDS recommended that either a limited "Ogata score" panel or a more comprehensive Euroflow panel be followed, that at least three aberrant findings in at least two cell compartments should be observed, and that flow cytometry findings should always be integrated into the diagnostic report together with the morphologic, cytogenetic, and any molecular genetic findings. Used appropriately, flow cytometry immunophenotyping in suspected MDS provides helpful information that can be used to support a diagnosis of MDS suspected on morphology and clinical features (particularly if the morphology is ambiguous and the karyotype is normal) [143–145]. Conversely, normal flow cytometry findings should stimulate careful investigations for non-MDS causes of cytopenia. However, according to current guidelines, including the 2016 WHO Classification, flow cytometry findings alone should not be used to make a diagnosis of MDS if other criteria are lacking, nor should a

Table 2.12 Abnormalities identified by flow cytometry in myelodysplastic syndrome (adapted from [142, 242])

Cell population	Abnormalities seen in MDS
CD34+ blasts	• Increased numbers (>2% or ≥3%) • Decreased CD19+CD10+ hematogones • Altered CD45 and side scatter • Increased CD13, CD34, CD117, CD123 expression levels • Decreased expression of CD38 or HLA-DR • Markedly increased cells with CD4, CD15, CD64, or CD65 expression • Aberrant expression of CD2,[a] CD5, CD7,[a] or CD56
Maturing myeloids	• Decreased side scatter relative to lymphocytes • Abnormal patterns of CD11b, CD13, and CD16 expression • Lack of CD10 expression on neutrophils[b]
Monocytes	• Decreased CD45 and/or side scatter • Decreased expression of HLA-DR, CD13, CD14, CD36 • Increased expression or CD15 or CD16 • Aberrant expression of CD56[c] (bright) and CD2

[a]CD2 and CD7 can be expressed on some normal blasts, but usually in the CD38 bright cells and CD13/CD33dim cells
[b]Lack of CD10 on granulocytes may also be seen in autoimmune neutropenia or hemophagocytic syndrome
[c]CD56 expression on monocytes is commonly seen in reactive or regenerative monocytes. In MDS monocytes, when expressed it is usually bright and seen in at least 25% of the monocytes

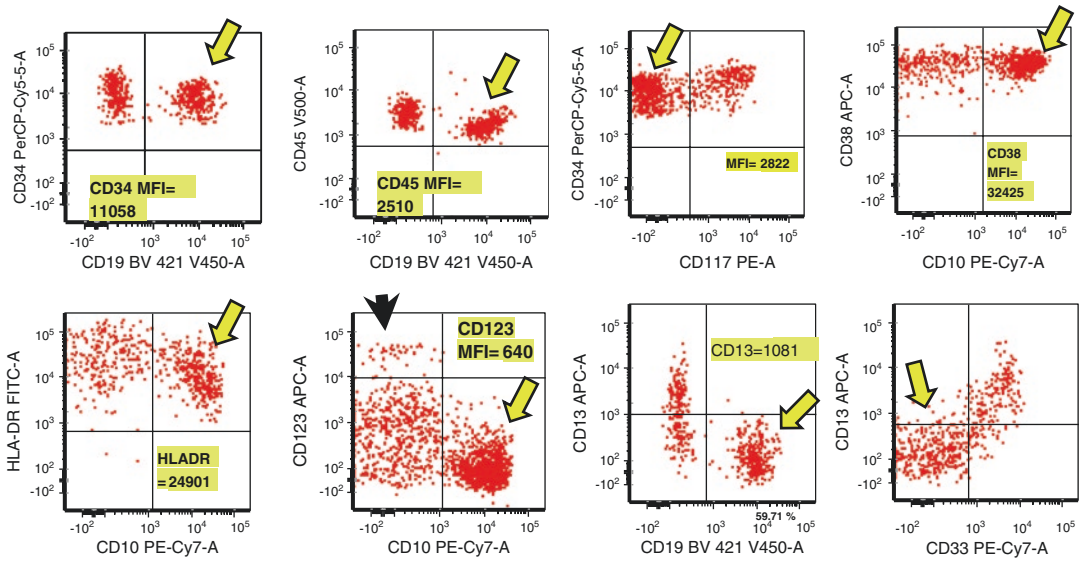


Fig. 2.23 The immunophenotype of CD34+ cells in a normal bone marrow. CD34+ cells are composed of myeloid precursors that are CD117+, CD13+, CD33+, CD123+, HLADR+ as well as various numbers of stage I hematogones (normal precursor B cells, shown by arrows) that are CD19+CD10+. Often, some plasmacytoid dendritic cell precursors are identified by CD123bright+HLADR+ expression (arrowhead). The normal expression levels of CD34, CD45, CD117, CD38, HLADR, CD123, and CD13 can be determined by visual inspection as well as measured by median florescence intensity (MFI) (measured here on myeloid precursors after excluding hematogones)

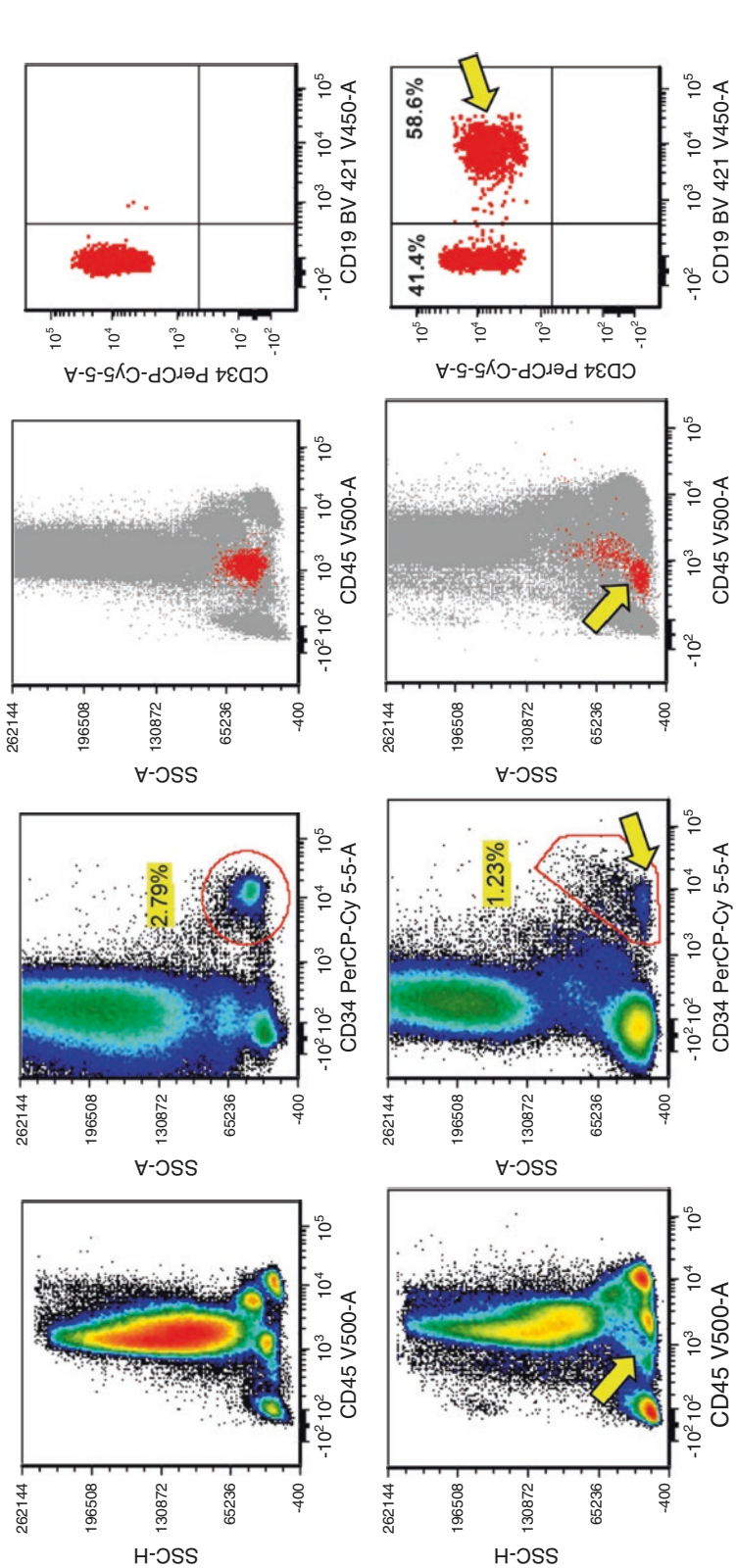


Fig. 2.24 Flow cytometry screening test, the MDS-Ogata score. Using three antibodies (CD45, CD34, CD19 or CD10) and four parameters (side scatter of granulocytes; percentage of CD34+ cells; location of CD34+ cells on CD45/SSC, and percentage of stage I hematogones as defined by CD34+CD19+ or CD34+CD10+), this simple flow cytometry panel provides an initial screen for bone marrows of patents with suspected MDS. Two or more abnormalities suggest the possibility of MDS. Upper panel: MDS case showing decreased side scatter; increased CD34+ cells (>2%); CD34+ cells with decreased CD45 on CD45/SSC, and lack of stage I hematogones, a total Ogata score of 4. Lower panel: Bone marrow from a patient who had prolonged bone marrow suppression after chemotherapy, but recovered subsequently and did not have MDS. The bone marrow shows decreased side scatter of granulocytes, but a normal percentage and location of CD34+ cells; and a large number of stage I hematogones (arrows)

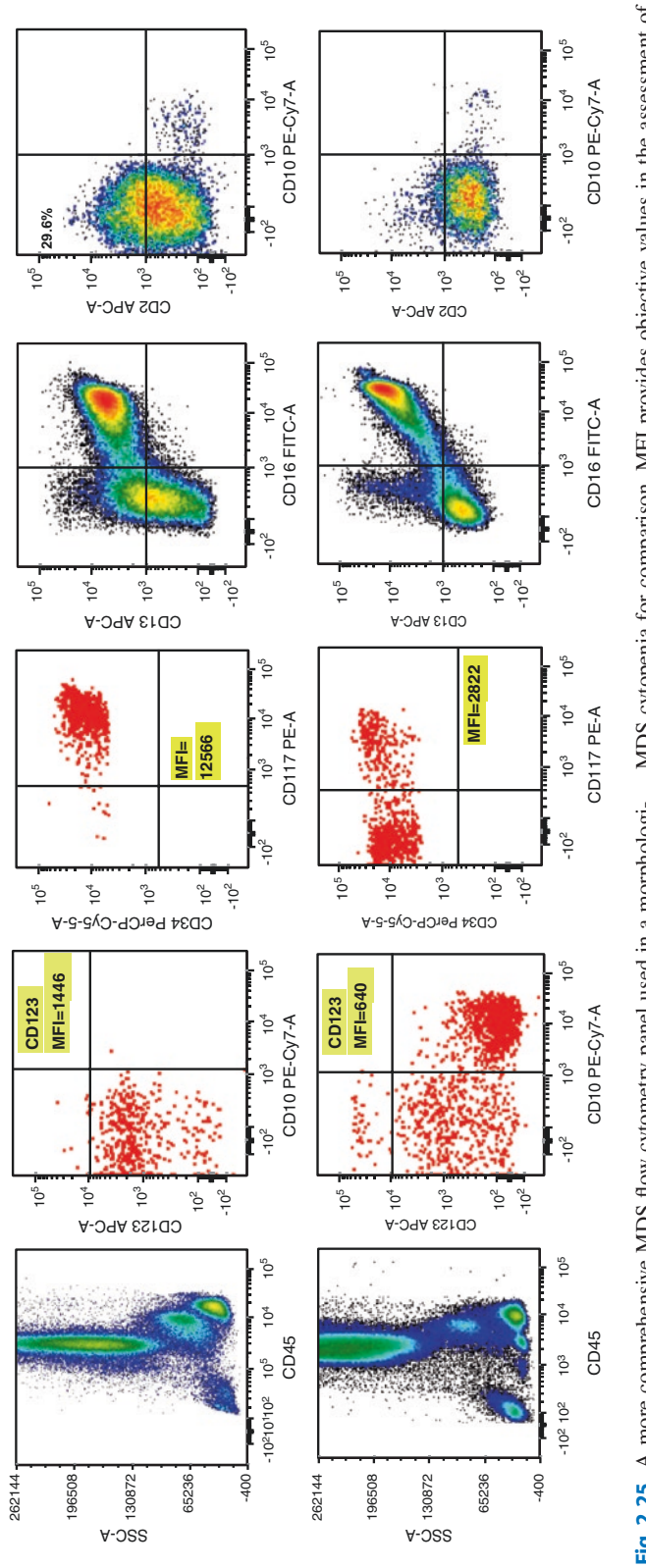


Fig. 2.25 A more comprehensive MDS flow cytometry panel used in a morphologically equivocal case. Upper panel: MDS case. The CD34+ myeloblasts (red) exhibit increased CD123 and CD117 expression, abnormal granulocyte maturation pattern on CD16 versus CD13, and aberrant CD2 expression on monocytes. Lower panel: Non-MDS cytopenia for comparison. MFI provides objective values in the assessment of the levels of antigen expression

negative flow cytometry result be used to unequivocally exclude the possibility of MDS.

Provided that a good bone marrow aspirate and peripheral smear are obtained and reviewed, immunohistochemistry is often not required to diagnose and classify MDS. However, in fibrotic bone marrow, cells may be crushed and difficult to identify and there may be no aspirate smear due to a "dry tap." In such cases, staining for lineage-specific markers (CD71, glycophorin, E-cadherin, or HGB for erythroids; MPO, lysozyme, or CD33 for granulocytic elements; and CD61, CD42b, or factor VIII for megakaryocytes) and CD34 can help identify cell types and quantify blasts. Myeloblasts in the vast majority of MDS cases are CD34 positive and this stain is more specific than CD117, which also stains some promyelocytes, early erythroid elements, and mast cells (Fig. 2.26a, b). Immunostaining for CD34 also helps highlight clusters of blasts away from bone trabeculae, which are associated with an adverse prognosis in MDS. Identification of very small dysplastic megakaryocytes (micromegakaryocytes), the presence of which strongly supports a diagnosis of MDS, is facilitated by immunostaining with a megakaryocyte marker (Fig. 2.26c). Finally, strong immunostaining for p53 in >1% of the bone marrow cells correlates with the presence of a *TP53* mutation, which is associated with a very poor prognosis in MDS (Fig. 2.26d) [146].

Cytogenetics and Molecular Genetics

Conventional karyotyping should be performed in all MDS cases at diagnosis, as cytogenetic abnormalities are seen in 50–60% of patients and the specific findings can help in establishing clonality and determining prognosis. Cytogenetic abnormalities, especially those involving chromosomes 5 and 7, are even more frequent in therapy-related MDS than in de novo disease: over 90% of therapy-related MDS cases show an abnormal karyotype [147]. FISH analysis for common abnormalities in MDS may be helpful if the karyotype fails or is insufficient (less than 20 metaphases) [100], but probably does not add information if 20 normal metaphases are obtained [148, 149]. The typical recurring clonal cytogenetic abnormalities seen in MDS are shown in Table 2.11 [91, 150–152]. Most of the abnormalities are losses or gains of large segments of chromosomes, the most frequent involving chromosomes 5, 7, and 8. Deletions or losses of chromosomal material may also result from unbalanced translocations in MDS, while balanced translocations are infrequent. According to the 2016 WHO Classification, the presence of any of the MDS-defining cytogenetic abnormalities listed in Table 2.11 is sufficient to confirm a diagnosis of MDS in a cytopenic patient, even if significant dysplasia is lacking. However, certain cytogenetic abnormalities [+8, del(20q), and -Y] are specifically excluded from this list, as they can be seen in normal aged individuals or in patients with non-MDS causes of cytopenia, such as aplastic anemia or immune thrombocytopenia [153–155]. Importantly, the cytogenetic abnormalities inv(16)/t(16;16), t(15;17), and t(8;21) are AML defining, even if bone marrow and blood blasts are <20%, and the presence of any of these abnormalities mandates a diagnosis of AML rather than MDS.

The only cytogenetic abnormality which defines a specific MDS subtype in the 2016 WHO Classification is an isolated del(5q) abnormality, reflecting its strong association with a particular disease phenotype and biology, responsiveness to a specific therapy (lenalidomide), and favorable prognosis [156]. It has recently been shown that MDS with del(5q) has a favorable prognosis if one, but not two or more, additional cytogenetic abnormalities is present [157, 158]. Thus, in the 2016 WHO Classification, one other cytogenetic abnormality (aside from monosomy 7 or deletion of 7q) is allowed in MDS with isolated del(5q).

In addition to isolated del(5q), other chromosomal abnormalities have important clinicopathologic associations in MDS. Cases with t(3;3)(q21.3;q26.2) and inv(3)(q21.3q26.2) tend to have pronounced megakaryocytic dysplasia, often an increased platelet count, and a poor prognosis similar to that of an aggressive AML [159, 160]. However, the t(3;3)/inv(3) abnormality is not considered AML defining, and cases with <20% blasts should be classified in the appropriate MDS category. MDS cases with chromosome 17p abnormalities typically demonstrate prominent pseudo-Pelger-Huët anomaly or nuclear monolobation in granulocytes [161];

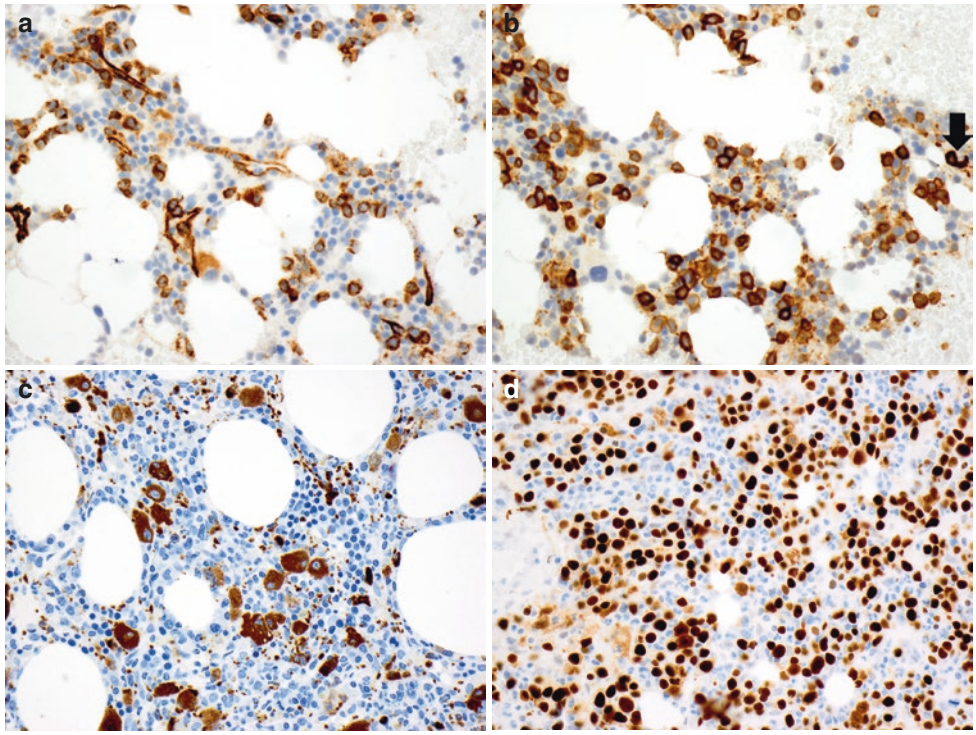


Fig. 2.26 Useful immunostains in evaluating a bone marrow biopsy involved by MDS. CD34 (**a**) is a relatively specific stain for blasts in the bone marrow: staining is limited to blasts and endothelial cells. CD34 staining of blasts is often weaker than that of endothelial cells and can show a granular staining pattern on blasts. CD117 performed on the same case (**b**) stains not only myeloblasts, but also early erythroblasts and promyelocytes. Myeloblasts typically stain most strongly than erythroblasts for CD117, while mast cells (arrow) stain very strongly and have a small, central nucleus. (**c**) CD61 immunostain helps identify small, dysplastic megakaryocytes and micromegakaryocytes, which are often underestimated on routine histologic stains. (**d**) p53 immunostain on a case of MDS-EB-2 shows strong staining of numerous marrow cells (mostly erythroid elements). This staining pattern correlates strongly with the presence of a *TP53* mutation

some have monocytosis or leukocytosis and are classified as a myelodysplastic/myeloproliferative neoplasm (MDS/MPN) rather than MDS. A common molecular genetic abnormality in some of these cases is deletion or inactivation of one copy of the *TP53* gene, but patients with specifically isochromosome 17q typically lack *TP53* mutations [162]. Beyond the abovementioned chromosomal abnormalities, many other specific karyotype abnormalities are significantly associated with patient outcome. The revised IPSS-R sheme divides recurrent cytogenetic abnormalities in MDS into five discrete risk groups, ranging from very good to very poor; normal karyotype, which is seen in 40–50% of MDS cases, is in the good prognostic group [64].

More recently, the significance of mutations in MDS has become increasingly apparent due to rapid advances in sequencing technologies. Between 80 and 90% of MDS patients have detectable aberrations in a typical panel of 50–100 genes evaluated by next-generation sequencing. A listing of the most commonly mutated genes in MDS is shown in Table 2.13. The commonly mutated genes in MDS affect the *spliceosome* (in aggregate present in about 50% of cases) [163] or epigenetic regulation of gene expression via mutations in genes controlling *DNA methylation* or *histone modification*. Other commonly mutated genes are those encoding hematopoietic *transcription factors*, *signaling proteins* (such as tyrosine kinases), the *tumor suppressor* p53, and the *cohesin complex*, which controls the cohesion of sister chromatids as well as DNA repair and transcriptional regulation. The mutational landscape of MDS is complex and dynamic in that multiple mutations usually are pres-

Table 2.13 Common recurrent gene mutations in myelodysplastic syndromes

Pathway and specific genes	Frequency in MDS	Comments
Splicing factors		
SF3B1	20–35%	Strongly associated with ring sideroblasts
SRSF2	10–15%	
U2AF1	5–10%	
ZRSR2	5–10%	
Epigenetic regulators		
ASXL1	15–20%	Frequently mutated in CHIP and aplastic anemia
EZH2	5–10%	
TET2	20–30%	Frequently mutated in CHIP
DNMT3A	10–15%	Frequently mutated in CHIP and aplastic anemia
IDH1/2	5%	
Transcription factors/tumor suppressors		
RUNX1	10–15%	
TP53	5–10%	
BCOR/BCORL	5%	Frequently mutated in aplastic anemia
ETV6	2-5%	
Growth factor/signaling		
NRAS/KRAS	5–10%	
CBL	5%	
JAK2	2–5%	More frequently mutated in MDS with fibrosis
Cohesin complex		
STAG2	4–7%	

CHIP clonal hematopoiesis of indeterminate potential

ent and show nonrandom but still poorly understood associations with one another. Further complicating interpretation, mutations may be present in only a subclone of the tumor cells and may change over time, with or without therapy [164].

Importantly, mutations identical to those seen in MDS can occur in apparently healthy individuals in the condition known as "clonal hematopoiesis of indeterminate potential" (CHIP, discussed later in this chapter). Thus, the presence of MDS-associated mutations does not necessarily prove the presence of MDS [76]. These CHIP mutations can have a relatively high mutant allele fraction similar to that seen in MDS patients. Thus, at the current time, the identification of MDS-type mutations alone, even in a patient with cytopenia, cannot be used to establish a diagnosis of MDS in the absence of other diagnostic criteria. Further study is needed to determine if particular mutation combinations, allele burden level, or the clinical context (such as patient age and degree of cytopenia) may eventually help establish criteria for a mutation-based diagnosis of MDS [76].

Beyond their role in helping establish the presence of clonal process, certain mutations correlate with specific MDS subtypes and/or clinical behavior. A spliceosome gene, *SF3B1*, is one of the most common genes mutated in MDS and has strong association with MDS-RS (as discsussed above) and with a favorable prognosis in MDS [103, 165–167]. *TP53* mutation is often associated with a complex karyotype and therapy-related disease and has a very poor prognostic impact on the disease course. Other mutations associated with an unfavorable prognosis in MDS include *ASXL1, CBL, DNMT3A, ETV6, EZH2*, *NRAS, RUNX1,* and *SRSF2* [168].

Differential Diagnosis

One of the most challenging aspects of MDS diagnosis is its differential diagnosis with the numerous other conditions that cause cytopenia, which have been discussed extensively earlier in this chapter. Dysplasia can be seen in many non-neoplastic conditions and can occur as a paraneoplastic phenomenon in some lymphomas and it is

also a feature of many non-MDS myeloid neoplasms. Navigating this difficult differential diagnosis requires consideration of all available diagnostic information and keeping an open mind when approaching a putative MDS diagnosis.

Secondary Causes of Cytopenia

In patients presenting with macrocytic anemia, megaloblastic anemia frequently manifests dysplastic features in the erythroid lineage that closely mimic MDS, as well as a markedly hypercellular marrow with prominent erythroid hyperplasia [83, 84]. In megaloblastic anemia, overt megaloblastic changes usually predominate over other dysplastic features, while the megaloblastoid changes in MDS are milder than those seen in megaloblastic anemia. Megaloblastic changes in the myeloid series seen in megaloblastic anemia (giant band forms and metamyelocytes) are helpful, as they are usually absent from MDS. Neutrophil hypersegmentation can be a manifestation of MDS dysplasia, but it is accompanied by hypogranular cytoplasm as opposed to the normally granulated cytoplasm of hypersegmented neutrophils in megaloblastic anemia. Whenever the differential diagnosis includes megaloblastic anemia, serum vitamin B12 and folate levels as well as gamma-levulinic acid should be measured prior to rendering a diagnosis of MDS.

Many chemotherapy agents induce dysplastic and megaloblastoid morphology in erythroid cells and also frequently cause cytopenias that are sometimes prolonged. The most striking megaloblastic changes are seen with folate antagonists (e.g., methotrexate) and antimetabolites such as hydroxyurea and 5-fluorouracil [169–171]. Distinguishing prolonged chemotherapy effects from an early therapy-related MDS may be difficult; identification of MDS-associated immunophenotypic abnormalities detected by flow cytometry can be helpful in this situation [172]. Care must be taken in interpreting the presence of mutations, since clonal mutated hematopoiesis may be observed in patients following chemotherapy. Although this condition appears to confer increased risk for subsequently developing MDS, it is not currently considered in isolation diagnostic of MDS. Cytogenetics may be helpful if an MDS-associated clonal abnormality such as -7 or del(5q) is demonstrated.

Copper and selenium deficiency and zinc toxicity [173] can cause pancytopenia and dysplastic marrow morphology (most often vacuolization of erythroids and granulocyte precursors), closely mimicking MDS [47, 174]. Copper deficiency can result from prolonged parenteral hyperalimentation, post-gastrectomy or malnourished states, or copper chelation therapy [175, 176]. Given the ability of copper deficiency to mimic MDS, it is prudent to evaluate copper levels in any patient with unexplained cytopenias. Arsenic trioxide (used to treat acute promyelocytic leukemia) causes striking dysplastic morphology in marrow, particularly in erythroid progenitors, mimicking the erythroid dysplasia of MDS [83, 177].

After an acute marrow injury (such as induced by chemotherapy, toxin, infection, or occasionally an unknown cause), there may be transiently increased blasts in blood or bone marrow accompanying hematopoietic recovery. Dysplastic changes may also be seen, especially in the erythroid and occasionally megakaryocytic lineages. Identification of left-shifted myeloid forms and toxic granulation in the blood can be helpful clues to appropriate marrow recovery rather than high-grade MDS with circulating blasts. Circulating granulocytic forms in MDS are often hypogranular, but only seldom show toxic granulation.

Viral and other infections can be associated with cytopenias and marrow changes potentially mimicking MDS. Bone marrow examination of cytopenic HIV patients often reveals dysplastic morphologic features, particularly in the erythroid and megakaryocytic lineages (Fig. 2.27) [83, 84]. However, the cytogenetic abnormalities of MDS are lacking in cytopenic HIV-infected patients and the cytopenias usually resolve following successful treatment of the infection. EBV, herpes, and cytomegalovirus infections may also present with hypercellular marrow and dysplastic marrow morphology [178]. Parvovirus B19 infection can mimic the small subset of MDS presenting with pure red cell aplasia; thus both MDS and parvovirus infection must be considered in the differential diagnosis of this condition [12, 179]. Morphologic abnormalities in

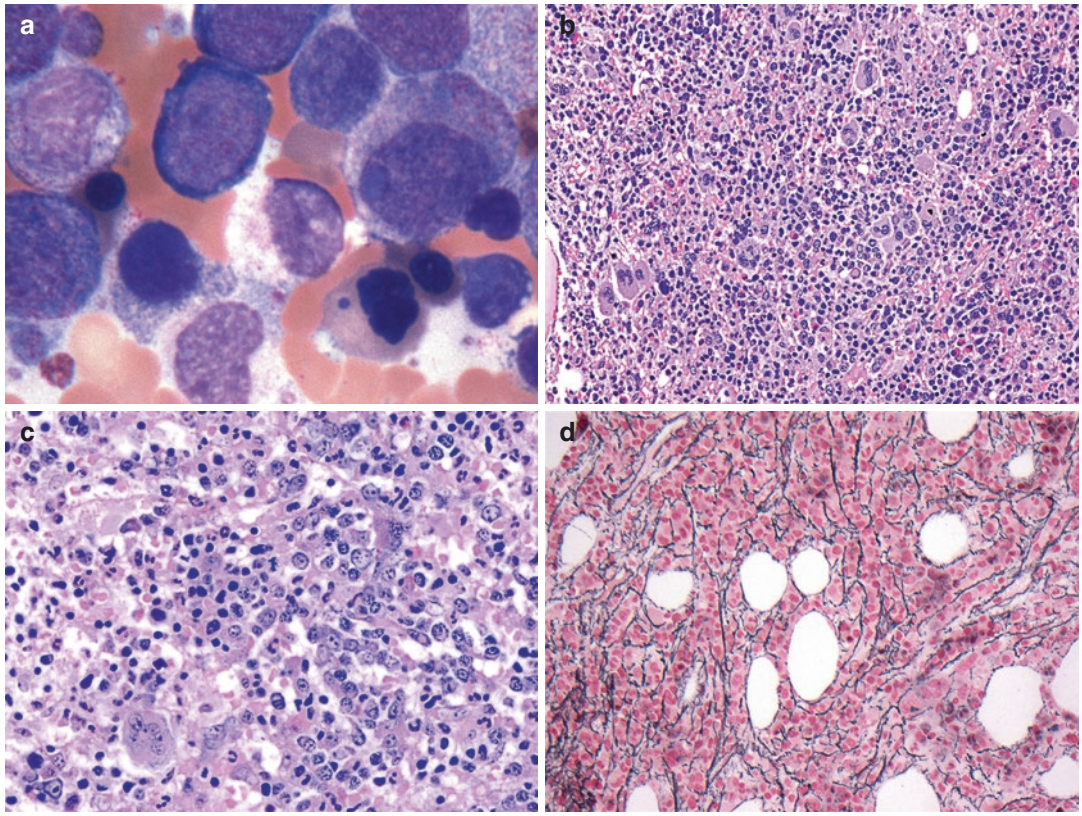


Fig. 2.27 Bone marrow findings in HIV infection, which can mimic MDS. (**a**) The bone marrow aspirate smear shows dysplastic vacuolation and nuclear irregularities in the erythroids. (**b**) The bone marrow biopsy is hypercellular with clustered megakaryocytes, some showing features reminiscent of those seen in MDS. (**c**) The marrow exhibits architectural disorganization (failure of erythroids to form well-defined islands) and increased plasma cells. (**d**) There is often increased reticulin fibrosis

parvovirus infection are usually restricted to the erythroid lineage and such cases demonstrate viral inclusions and positive parvovirus serology and immunohistochemistry.

A significant subset of MDS patients demonstrate subpopulations of hematopoietic cells with loss of glycosylphosphatidylinositol (GPI)-anchored proteins detected by flow cytometry, features shared with paroxysmal nocturnal hemoglobinuria (PNH). It is hypothesized that abnormal hematopoietic clones with the PNH anomaly arise through selective pressure on stem cells in the MDS bone marrow [180]. In most MDS cases, the PNH clone is small in size (<5% of cells), whereas the clone is typically >10% of cells in bona fide PNH [181]. However, about 5% of patients with overt PNH progress to MDS [182]. Thus, significant morphologic dysplasia or a clonal cytogenetic abnormality in the setting of a prior PNH diagnosis should raise concern for evolution to MDS.

Cytopenias, Mutations, and Dysplasia of Undetermined Significance

Patients who have persistent (>6 months), significant, and unexplained cytopenia, but lack MDS-defining dysplasia, excess blasts, or an MDS-defining cytogenetic abnormality, are designated as having "Idiopathic Cytopenia of Undetermined Significance" (ICUS) [183, 184]. It is recommended that these patients be followed with periodic blood counts and a repeat bone marrow examination should be performed if the cytopenias worsen significantly and remain unexplained. Over time, some ICUS patients progress to MDS or another myeloid neoplasm, such as AML. Flow cytometry aberrations and the presence of MDS-type mutations (see below) are

helpful in predicting the likelihood of ICUS progression to MDS or AML [185]. Cytopenia is a sine qua non of MDS, and therefore patients who undergo bone marrow examination for any reason that shows significant dysplasia in one or more lineages, but lack any cytopenia (over at least a 6 month period) cannot be diagnosed with MDS. This condition has been termed "Idiopathic Dysplasia of Undetermined Significance" (IDUS) [186]. In such situations, it is important to consider MDS/MPN overlap diseases that may manifest dysplasia but lack significant cytopenia. Plasma cell myeloma and some bone marrow lymphomas can produce reactive dysplastic changes in marrow hematopoietic elements. In addition, secondary dysplastic changes are caused by a variety of nonneoplastic conditions, including infections, autoimmune disease, and drugs, toxins, and metabolic deficiencies, as discussed above. IDUS patients may subsequently develop neoplasms, which are often neoplasms other than MDS, such as a myeloproliferative neoplasm (MPN), AML, or an MDS/MPN overlap.

Although certain cytogenetic abnormalities are considered sufficient to diagnose MDS in a cytopenic patient (see Table 2.11), the same is not true for mutations. As mentioned previously, MDS-type mutations as well as gene copy number abnormalities have been found in the blood of normal individuals who do not have MDS [187]. Hematopoietic stem cells progressively accumulate mutations as an individual ages (estimated at approximately 1.3 somatic mutations per stem cell per decade) [188]. Some of these mutations can affect key genes that confer growth advantage to the stem cell and allow it to outcompete its unmutated neighbors and expand within the bone marrow, eventually contributing enough to leukocyte production such that the mutation can be detected by sequencing a peripheral blood or bone marrow sample, a condition termed CHIP (defined previously) [76]. The presence of CHIP in the setting of unexplained cytopenia and lack of diagnostic findings of MDS on bone marrow examination has been termed "Clonal Cytopenia of Undetermined Significance" (CCUS). The incidence of CHIP is estimated at approximately 5–10% in individuals over 65 or 70 and approaches 20% in individuals over 90, while is rare in those under 40 [187, 189, 190]. Individuals with CHIP are at an increased risk of developing a subsequent hematologic malignancy compared to age-matched controls without CHIP, but this is only approximately 0.5–1% per year. It is currently unclear how individuals found to have CHIP should be managed, but at the current time, they are not considered to have a neoplasm and do not warrant therapy. Of note, the incidence of CHIP in elderly individuals is much higher than the incidence of MDS, thus the identification of MDS-type mutations in hematopoietic cells has low predictive value in this patient population, even among patients with unexplained cytopenia [191].

Some recent studies have suggested that certain specific mutation patterns in individuals with CCUS appear to predict higher likelihood of developing MDS. These include the presence of mutations with a mutant allele fraction of at least 10%, and those that involve either a spliceosome gene alone or one of the epigenetic regulating genes *TET2*, *DNMT3A,* or *ASXL1* in combination with at least one other mutation. The presence of *NPM1, CBL, NRAS*, and *IDH2* mutations, which are uncommon in CHIP, also appears to predict progression of CCUS to MDS or AML [192, 193].

Lymphomas mimicking MDS

While most lymphoid leukemias, such as chronic lymphocytic leukemia, present with overt lymphocytosis, some are associated with a cytopenic presentation. These may have lymphomatous infiltrates in the marrow that occur in a subtle interstitial or intrasinusoidal pattern that can be missed on casual inspection of the routine histology. These include hairy cell leukemia, large granular lymphocytic (LGL) leukemia, hepatosplenic T-cell lymphoma, and intravascular large B-cell lymphoma. Reactive dysplastic changes have been reported to occur in the maturing hematopoietic cells in LGL leukemia and conversely reactive lymphoid aggregates may be present in MDS. LGL leukemia patients present predominantly with neutropenia, whereas an anemic presentation is more common in MDS. Flow cytometry identifies an expanded population of immunophenotypically aberrant CD8+, CD57+ T cells and PCR study of blood and/or marrow reveals a clonal TCR rearrangement in LGL leukemia [194]. Application of T-cell markers (CD3, CD4, CD8), as well as CD57 and cytotoxic markers, can be helpful in identify-

ing the often subtle interstitial and intrasinusoidal LGL infiltrates in the bone marrow. Notably, MDS and LGL leukemia may rarely occur together and *STAT3* mutations that characterize LGL leukemia as well as clonal T-cell populations have been identified in MDS patients [195, 196]. Thus, in some cases it may be difficult to distinguish LGL leukemia from MDS with an expanded clonal T-cell population. Close clinical follow-up is advised in such cases, as the "true" disease often becomes apparent over time.

Hairy cell leukemia and plasma cell myeloma have both been associated with morphologic dysplastic changes in the bone marrow and may infiltrate the marrow biopsy in a subtle interstitial pattern obscured by reactive hematopoietic elements; these non-MDS neoplasms can be disclosed by applying appropriate immunostains.

Congenital Conditions

MDS may mimic the congenital dyserythropoietic anemias (CDAs) by exhibiting internuclear bridging, megaloblastoid hematopoiesis, and multinucleation of erythroid progenitors as manifestations of erythroid lineage dysplasia [197]. This differential diagnosis is critical, because the treatment and prognosis of MDS and CDAs differ. CDA types I and II typically present in childhood or adolescence, but even in infancy and childhood, MDS is more common and must be carefully ruled out before CDA is diagnosed. In the CDAs, dysplastic morphology and cytopenias are limited to the erythroid lineage. If neutropenia, thrombocytopenia, or dysplasia in the granulocytic or megakaryocytic lineages is present, a diagnosis of MDS should be strongly considered. Cytogenetics may be helpful, as well as clinical follow-up, because progressive disease over time is more suggestive of MDS.

Other Causes of Ring Sideroblasts

By far the most frequent cause of ring sideroblasts in bone marrow is MDS, but other diverse conditions are also associated with ring sideroblasts. Active alcohol abuse inhibits heme synthesis, resulting in the accumulation of mitochondrial iron as ring sideroblasts. Alcoholism may also cause megaloblastoid hematopoiesis due to dietary deficiency, as well as vacuolization of erythroid precursors, both findings seen in MDS [198]. Other reversible causes of ring sideroblasts include antituberculosis drugs (especially isoniazid) [199], copper deficiency or zinc toxicity (see below) [200], and penicillamine therapy [201]. Ring sideroblasts have also been reported to occur as a transient phenomenon following cytotoxic chemotherapy [202]. Congenital causes of sideroblastic anemia are discussed earlier in this chapter. In contrast to the typically macrocytic anemia of MDS, the anemia in most congenital sideroblastic anemias is microcytic and hypochromic and may respond to exogenous pyridoxine. Pearson syndrome is a mitochondrial cytopathy characterized by refractory sideroblastic anemia, vacuolated marrow erythroid precursors, and exocrine pancreatic dysfunction, which has an onset of symptoms in infancy. These congenital diseases are rare and in most cases the cytopenia and morphologic abnormalities are restricted to the erythroid lineage.

Fibrotic Bone Marrow

Mild (WHO grade 1) reticulin fibrosis in marrow is common in MDS, but significant marrow fibrosis in MDS (grade 2 or grade 3) may lead to confusion with primary myelofibrosis (PMF) or another MPN. This distinction has important clinical relevance, as the two diseases have different clinical courses and are treated differently. Splenomegaly is unusual in MDS and the presence of any extramedullary hematopoiesis tends to favor PMF over MDS. A leukoerythroblastic peripheral blood smear is more suggestive of PMF, although this finding can also be seen in MDS with fibrosis. Megakaryocyte morphology is the most helpful feature in this distinction: PMF typically has clusters of abnormal megakaryocytes in the bone marrow biopsy that are enlarged and hyperchromic with bulbous nuclei whereas in MDS the megakaryocytes are usually small with hypolobated nuclei and rounded rather than scalloped or highly lobulated nuclear contours. Mature collagen fibrosis, indicated by blue staining in marrow stroma on a trichrome stain, and osteosclerosis can be seen in advanced stages of PMF, but these findings are uncommon in MDS. Although many of the cytogenetic abnormalities in PMF overlap with those of MDS, mutation analysis can be helpful: *JAK2, MPL,* or *CALR* mutations are found in 80–90% of PMF

patients, while these mutations are rare in MDS, even in those cases with fibrosis [89, 203–205]. Advanced phases of polycythemia vera and chronic myeloid leukemia may also have marrow fibrosis, but clinical history, CBC findings, and genetic studies (*JAK2* and *BCR-ABL1* analyses) usually can reliably separate these entities from MDS.

Acute megakaryoblastic leukemia is frequently characterized by marrow fibrosis and a proliferation of dysplastic megakaryocytes, in addition to megakaryoblasts. In adults, it frequently evolves from MDS or has dysplastic background hematopoiesis, and thus may closely mimic MDS with fibrosis. Distinction of the two entities is based on the blast percentage. Megakaryoblasts (but not micromegakaryocytes, which are larger) are regarded as blast equivalents. Acute panmyelosis with myelofibrosis, a very rare AML subtype, shows increased marrow fibrosis, but also has at least 20% bone marrow or blood blasts. Since the marrow aspirate is often hemodilute, review of touch preparations to accurately enumerate the blasts is important in these cases.

Hypoplastic Bone Marrow

The distinction between hypoplastic MDS and aplastic anemia is often difficult. Aplastic anemia may display mild dysplastic morphology in hematopoietic progenitors [122]. Clonal proliferations of cells with loss of GPI-anchored proteins characteristic of PNH may be seen in both MDS and aplastic anemia. Recently, somatic mutations have been detected in approximately one-third of aplastic anemia patients, including genes that are frequently mutated in MDS, such as *BCOR, BCORL*, *DNMT3A*, and *ASXL1* [206]. In MDS, the dysplastic features tend to be more prominent than in aplastic anemia and the cytopenias are disproportionate to the degree of hypocellularity. In particular, the presence of frequent dysplastic micromegakaryocytes strongly favors MDS. Identification of micromegakaryocytes in the bone marrow biopsy section is facilitated by use of immunostains such as CD61. In addition, any persistent clonal cytogenetic abnormalities favor MDS. CD34 immunostaining on the bone marrow is useful at distinguishing hypoplastic MDS from aplastic anemia, since CD34 cells are not increased in aplastic anemia, whereas they are increased in the subset of hypoplastic MDS with excess blasts [87].

Other Myeloid Neoplasms Mimicking MDS

Most patients with MDS and neutropenia are unable to mount a significant leukocytotic response to an infection. Nevertheless, some MDS patients with a superimposed infectious or inflammatory process may develop a leukemoid reaction, mimicking a MDS/MPN overlap disease such as chronic myelomonocytic leukemia (CMML, if monocytosis is also present) or atypical CML, *BCR-ABL1* negative. The presence of persistent leukocytosis ($\geq 13 \times 10^9$/L) or absolute monocytosis ($\geq 1 \times 10^9$/L) at diagnosis excludes MDS and requires classification as MDS/MPN or MPN. However, some MDS patients subsequently develop monocytosis resembling CMML, which is considered to represent a type of disease progression that must be distinguished from a superimposed transient reactive condition [207]. The presence of a known infectious or inflammatory stimulus in an MDS patient with new leukocytosis should raise suspicion for a leukemoid reaction. Treatment of the underlying process results in resolution of a leukemoid reaction and reversion to peripheral blood counts typical of the underlying MDS.

Three cytogenetically-defined subtypes of AML can present with <20% bone marrow and peripheral blood blasts, potentially mimicking MDS with excess blasts. Cytogenetics performed on bone marrow sample is essential in the evaluation of all MDS in order to evaluate for *PML-RARA*, inv(16)/t(16;16), and t(8;21). Clues to suspecting one of these low-blast count AMLs include frequent promyelocytes with Auer rods in AML with *PML-RARA*, abnormal eosinophils with mixed eosinophilic-basophilic granules and monocytic blast morphologic in AML with inv(16)/t(16;16), and blasts with "salmon-colored" cytoplasm, fine granules, and Auer rods in AML with t(8;21). These AML entities tend to occur in a much younger age population compared to MDS [208]. Whether or not myeloblasts are increased, it is critical to distinguish MDS cases with erythroid predomi-

nance, in which erythroid elements are increased and dysplastic but exhibit complete maturation (Fig. 2.28), from pure erythroid leukemia, in which erythroid maturation is arrested and there is a proliferation of primitive erythroblasts. Pure erythroid leukemia is a highly aggressive AML subtype with very short survival and nearly universal highly complex karyotypes [209].

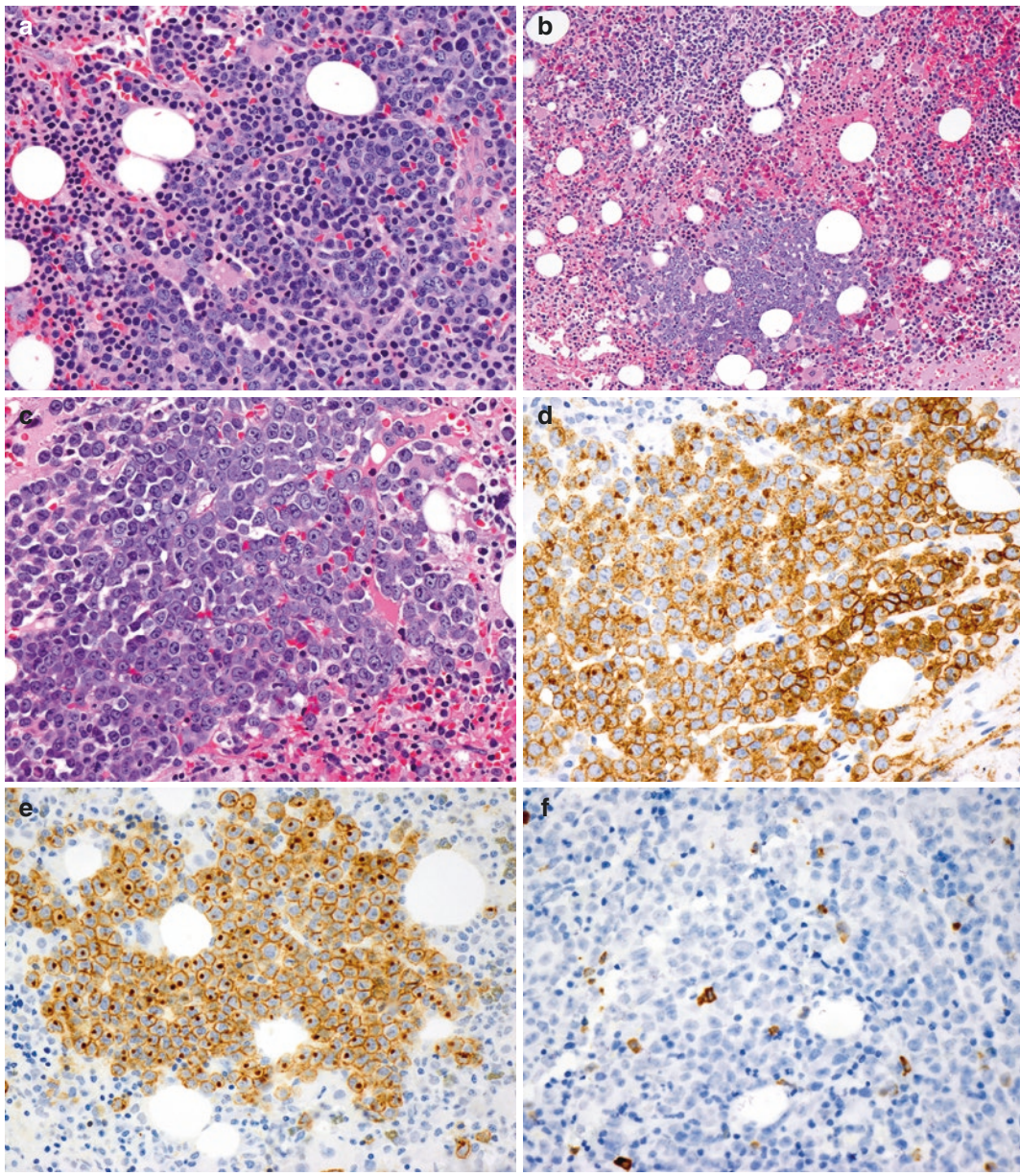


Fig. 2.28 MDS with multilineage dysplasia and erythroid predominance, with a striking left-shifted erythroid proliferation mimicking pure erythroid leukemia. (**a**) In most areas of the marrow biopsy, erythroids exhibit maturation, with frequent dysplastic nuclear irregularities. (**b**) Clusters of primitive cells (lower middle portion of image) are present multifocally. (**c**) On high magnification, the cohesive nature of the clusters and elongated nucleoli suggest early erythroid forms. Immunostains for E-cadherin (**d**) and glycophorin (**e**) are positive in the primitive cells, confirming erythroid lineage, while myeloperoxidase immunostain (**f**) and CD34 (not shown) are negative. Focal clusters of erythroblasts do not warrant a diagnosis of pure erythroid leukemia. Classification of erythroid-rich MDS cases depends on the percentage of myeloblasts, which were not increased in this case

References

1. Hillman RS, Ault KA, Leporrier M, Rinder HM. Normal erythropoiesis. In: Hematology in clinical practice, vol. 5e. New York: McGraw-Hill Medical; 2016.
2. Hillman RS, Ault KA, Leporrier M, Rinder HM. Iron-deficiency anemia. In: Hematology in clinical practice, vol. 5e. New York: McGraw-Hill Medical; 2016.
3. Barrett AN, Saminathan R, Choolani M. Thalassaemia screening and confirmation of carriers in parents. Best Pract Res Clin Obstet Gynaecol. 2017;39:27–40.
4. Hillman RS, Ault KA, Leporrier M, Rinder HM. The dysplastic and sideroblastic anemias. In: Hematology in clinical practice, vol. 5e. New York: McGraw-Hill Medical; 2016.
5. Bottomley SS, Fleming MD. Sideroblastic anemia: diagnosis and management. Hematol Oncol Clin North Am. 2014;28(4):653–70, v
6. Holbro A, Jauch A, Lardinois D, Tzankov A, Dirnhofer S, Hess C. High prevalence of infections and autoimmunity in patients with thymoma. Hum Immunol. 2012;73(3):287–90.
7. Go RS, Lust JA, Phyliky RL. Aplastic anemia and pure red cell aplasia associated with large granular lymphocyte leukemia. Semin Hematol. 2003;40(3):196–200.
8. Young NS, Brown KE. Parvovirus B19. N Engl J Med. 2004;350(6):586–97.
9. Wolfromm A, Rodriguez C, Michel M, Habibi A, Audard V, Benayoun E, et al. Spectrum of adult Parvovirus B19 infection according to the underlying predisposing condition and proposals for clinical practice. Br J Haematol. 2015;170(2):192–9.
10. Brown KE. Haematological consequences of parvovirus B19 infection. Bailliere's Best Pract Res Clin Haematol. 2000;13(2):245–59.
11. Young NS. Pure Red Cell Aplasia. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, et al., editors. Williams hematology. New York: McGraw-Hill Education; 2015. p. 9e.
12. Wang SA, Yue G, Hutchinson L, Landry ML, Hasserjian RP, Hao S, et al. Myelodysplastic syndrome with pure red cell aplasia shows characteristic clinicopathological features and clonal T-cell expansion. Br J Haematol. 2007;138(2):271–5.
13. Makoni SN, Laber DA. Clinical spectrum of myelophthisis in cancer patients. Am J Hematol. 2004;76(1):92–3.
14. Paydas S, Ergin M, Baslamisli F, Yavuz S, Zorludemir S, Sahin B, et al. Bone marrow necrosis: clinicopathologic analysis of 20 cases and review of the literature. Am J Hematol. 2002;70(4):300–5.
15. Halil O, Farringdon K. Oxalosis: an unusual cause of leucoerythroblastic anaemia. Br J Haematol. 2003;122(1):2.
16. Reddy VVB, Prchal JT. Anemia Associated with Marrow Infiltration. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, et al., editors. Williams hematology. New York: McGraw-Hill Education; 2015. p. 9e.
17. Kim Y, Park J, Kim M. Diagnostic approaches for inherited hemolytic anemia in the genetic era. Blood Res. 2017;52(2):84–94.
18. Narla J, Mohandas N. Red cell membrane disorders. Int J Lab Hematol. 2017;39(Suppl 1):47–52.
19. Gallagher PG. Hereditary elliptocytosis: spectrin and protein 4.1R. Semin Hematol. 2004;41(2):142–64.
20. Zarkowsky HS, Mohandas N, Speaker CB, Shohet SB. A congenital haemolytic anaemia with thermal sensitivity of the erythrocyte membrane. Br J Haematol. 1975;29(4):537–43.
21. Coetzer TL. Erythrocyte membrane disorders. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, et al., editors. Williams hematology. New York: McGraw-Hill Education; 2015. p. 9e.
22. van Solinge WW, van Wijk R. Erythrocyte enzyme disorders. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, et al., editors. Williams hematology. New York: McGraw-Hill Education; 2015. p. 9e.
23. LaRue N, Kahn M, Murray M, Leader BT, Bansil P, McGray S, et al. Comparison of quantitative and qualitative tests for glucose-6-phosphate dehydrogenase deficiency. Am J Trop Med Hyg. 2014;91(4):854–61.
24. Mario N, Baudin B, Aussel C, Giboudeau J. Capillary isoelectric focusing and high-performance cation-exchange chromatography compared for qualitative and quantitative analysis of hemoglobin variants. Clin Chem. 1997;43(11):2137–42.
25. Packman CH. Hemolytic anemia resulting from immune injury. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, et al., editors. Williams hematology. New York: McGraw-Hill Education; 2015. p. 9e.
26. Janka GE. Familial hemophagocytic lymphohistiocytosis. Eur J Pediatr. 1983;140(3):221–30.
27. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA, et al. Perforin gene defects in familial hemophagocytic lymphohistiocytosis. Science. 1999;286(5446):1957–9.
28. Zur Stadt U, Beutel K, Kolberg S, Schneppenheim R, Kabisch H, Janka G, et al. Mutation spectrum in children with primary hemophagocytic lymphohistiocytosis: molecular and functional analyses of PRF1, UNC13D, STX11, and RAB27A. Hum Mutat. 2006;27(1):62–8.
29. Rouphael NG, Talati NJ, Vaughan C, Cunningham K, Moreira R, Gould C. Infections associated with haemophagocytic syndrome. Lancet Infect Dis. 2007;7(12):814–22.
30. Dhote R, Simon J, Papo T, Detournay B, Sailler L, Andre MH, et al. Reactive hemophagocytic syndrome in adult systemic disease: report of twenty-six cases and literature review. Arthritis Rheum. 2003;49(5):633–9.
31. Verbsky JW, Grossman WJ. Hemophagocytic lymphohistiocytosis: diagnosis, pathophysiol-

ogy, treatment, and future perspectives. Ann Med. 2006;38(1):20–31.
32. Kelesidis T, Humphries R, Terashita D, Eshaghian S, Territo MC, Said J, et al. Epstein-Barr virus-associated hemophagocytic lymphohistiocytosis in Los Angeles County. J Med Virol. 2012;84(5):777–85.
33. Henter JI, Ehrnst A, Andersson J, Elinder G. Familial hemophagocytic lymphohistiocytosis and viral infections. Acta Paediatr. 1993;82(4):369–72.
34. Clementi R, Emmi L, Maccario R, Liotta F, Moretta L, Danesino C, et al. Adult onset and atypical presentation of hemophagocytic lymphohistiocytosis in siblings carrying PRF1 mutations. Blood. 2002;100(6):2266–7.
35. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer. 2007;48(2):124–31.
36. Risdall RJ, McKenna RW, Nesbit ME, Krivit W, Balfour HH Jr, Simmons RL, et al. Virus-associated hemophagocytic syndrome: a benign histiocytic proliferation distinct from malignant histiocytosis. Cancer. 1979;44(3):993–1002.
37. Filipovich AH. Hemophagocytic lymphohistiocytosis and related disorders. Curr Opin Allergy Clin Immunol. 2006;6(6):410–5.
38. Ho C, Yao X, Tian L, Li FY, Podoltsev N, Xu ML. Marrow assessment for hemophagocytic lymphohistiocytosis demonstrates poor correlation with disease probability. Am J Clin Pathol. 2014;141(1):62–71.
39. Green R. Folate, Cobalamin, and Megaloblastic Anemias. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, et al., editors. Williams hematology. New York: McGraw-Hill Education; 2015. p. 9e.
40. Dale DC, Welte K. Neutropenia and neutrophilia. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, et al., editors. Williams hematology. New York: McGraw-Hill Education; 2015. p. 9e.
41. Barbosa MD, Nguyen QA, Tchernev VT, Ashley JA, Detter JC, Blaydes SM, et al. Identification of the homologous beige and Chediak-Higashi syndrome genes. Nature. 1996;382(6588):262–5.
42. Griscelli C, Durandy A, Guy-Grand D, Daguillard F, Herzog C, Prunieras M. A syndrome associating partial albinism and immunodeficiency. Am J Med. 1978;65(4):691–702.
43. Hernandez PA, Gorlin RJ, Lukens JN, Taniuchi S, Bohinjec J, Francois F, et al. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. Nat Genet. 2003;34(1):70–4.
44. Dale DC, Link DC. The many causes of severe congenital neutropenia. N Engl J Med. 2009;360(1):3–5.
45. Horwitz M, Benson KF, Person RE, Aprikyan AG, Dale DC. Mutations in ELA2, encoding neutrophil elastase, define a 21-day biological clock in cyclic haematopoiesis. Nat Genet. 1999;23(4):433–6.
46. Papadaki HA, Palmblad J, Eliopoulos GD. Nonimmune chronic idiopathic neutropenia of adult: an overview. Eur J Haematol. 2001;67(1):35–44.
47. Gregg XT, Reddy V, Prchal JT. Copper deficiency masquerading as myelodysplastic syndrome. Blood. 2002;100(4):1493–5.
48. Dale DC. How I diagnose and treat neutropenia. Curr Opin Hematol. 2016;23(1):1–4.
49. Maheshwari A, Christensen RD, Calhoun DA. Immune-mediated neutropenia in the neonate. Acta Paediatr Suppl. 2002;91(438):98–103.
50. Starkebaum G. Chronic neutropenia associated with autoimmune disease. Semin Hematol. 2002;39(2):121–7.
51. Dale DC. How I manage children with neutropenia. Br J Haematol. 2017;178(3):351–63.
52. Vasu S, Caligiuri MA. Lymphocytosis and Lymphocytopenia. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, et al., editors. Williams hematology. New York: McGraw-Hill Education; 2015. p. 9e.
53. Twomey JJ, Douglass CC, Sharkey O Jr. The monocytopenia of aplastic anemia. Blood. 1973;41(2):187–95.
54. Viegas LR, Hoijman E, Beato M, Pecci A. Mechanisms involved in tissue-specific apoptosis regulated by glucocorticoids. J Steroid Biochem Mol Biol. 2008;109(3–5):273–8.
55. Fauci AS, Dale DC. The effect of in vivo hydrocortisone on subpopulations of human lymphocytes. J Clin Invest. 1974;53(1):240–6.
56. Ganapathi KA, Townsley DM, Hsu AP, Arthur DC, Zerbe CS, Cuellar-Rodriguez J, et al. GATA2 deficiency-associated bone marrow disorder differs from idiopathic aplastic anemia. Blood. 2015;125(1):56–70.
57. Diz-Küçükkaya R, López JA. Thrombocytopenia. In: Kaushansky K, Lichtman MA, Prchal JT, Levi MM, Press OW, Burns LJ, et al., editors. Williams hematology, vol. 9e. New York: McGraw-Hill Education; 2015.
58. He R, Reid DM, Jones CE, Shulman NR. Spectrum of Ig classes, specificities, and titers of serum antiglycoproteins in chronic idiopathic thrombocytopenic purpura. Blood. 1994;83(4):1024–32.
59. Dameshek W, Miller EB. The megakaryocytes in idiopathic thrombocytopenic purpura, a form of hypersplenism. Blood. 1946;1:27–50.
60. Kuter DJ, Mufti GJ, Bain BJ, Hasserjian RP, Davis W, Rutstein M. Evaluation of bone marrow reticulin formation in chronic immune thrombocytopenia patients treated with romiplostim. Blood. 2009;114(18):3748–56.
61. Brynes RK, Orazi A, Theodore D, Burgess P, Bailey CK, Thein MM, et al. Evaluation of bone marrow reticulin in patients with chronic immune thrombocytopenia treated with eltrombopag: Data from the EXTEND study. Am J Hematol. 2015;90(7):598–601.
62. Arepally GM. Heparin-induced thrombocytopenia. Blood. 2017;129(21):2864–72.
63. Bulchandani D, Nachnani J, Belt R, Hinton S. Acquired pure megakaryocytic aplasia: report of a single case treated with mycophenolate mofetil. Am J Hematol. 2007;82(7):650–1.

64. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Sole F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454–65.
65. Vardiman JW, Thiele J, Arber DA, Brunning RD, Borowitz MJ, Porwit A, et al. The 2008 revision of the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia: rationale and important changes. Blood. 2009;114(5):937–51.
66. Garand R, Goasguen J, Brizard A, Buisine J, Charpentier A, Claisse JF, et al. Indolent course as a relatively frequent presentation in T-prolymphocytic leukaemia. Groupe Francais d'Hematologie Cellulaire. Br J Haematol. 1998;103(2):488–94.
67. Forty-five Years of Cancer Incidence in Connecticut, 1935–79. Washington, DC: US Government Printing Office; 1986.
68. Cartwright RA, McNally RJQ, Rowland DJ, Thomas J. The descriptive epidemiology of leukaemia and related conditions in parts of the United Kingdom, 1984–1993. London: Leukemia Research Fund; 1997.
69. Germing U, Strupp C, Kundgen A, Bowen D, Aul C, Haas R, et al. No increase in age-specific incidence of myelodysplastic syndromes. Haematologica. 2004;89(8):905–10.
70. Schoch C, Schnittger S, Kern W, Dugas M, Hiddemann W, Haferlach T. Acute myeloid leukemia with recurring chromosome abnormalities as defined by the WHO-classification: incidence of subgroups, additional genetic abnormalities, FAB subtypes and age distribution in an unselected series of 1,897 patients with acute myeloid leukemia. Haematologica. 2003;88(3):351–2.
71. Smith MT, Linet MS, Morgan GJ. Causative agents in the etiology of myelodysplastic syndromes and the acute myeloid leukemias. In: Bennett JM, editor. The myelodysplastic syndromes, pathobiology and clinical management. New York: Marcel Dekker; 2002. p. 29–63.
72. Ma X, Does M, Raza A, Mayne ST. Myelodysplastic syndromes: incidence and survival in the United States. Cancer. 2007;109(8):1536–42.
73. Hasle H, Wadsworth LD, Massing BG, McBride M, Schultz KR. A population-based study of childhood myelodysplastic syndrome in British Columbia, Canada. Br J Haematol. 1999;106(4):1027–32.
74. Passmore SJ, Chessells JM, Kempski H, Hann IM, Brownbill PA, Stiller CA. Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia in the UK: a population-based study of incidence and survival. Br J Haematol. 2003;121(5):758–67.
75. Rollison DE, Howlader N, Smith MT, Strom SS, Merritt WD, Ries LA, et al. Epidemiology of myelodysplastic syndromes and chronic myeloproliferative disorders in the United States, 2001-2004, using data from the NAACCR and SEER programs. Blood. 2008;112(1):45–52.
76. Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9–16.
77. Gyan E, Dreyfus F, Fenaux P. Refractory thrombocytopenia and neutropenia: a diagnostic challenge. Mediterr J Hematol Infect Dis. 2015;7(1):e2015018.
78. Marinier DE, Mesa H, Rawal A, Gupta P. Refractory cytopenias with unilineage dysplasia: a retrospective analysis of refractory neutropenia and refractory thrombocytopenia. Leuk Lymphoma. 2010;51(10):1923–6.
79. Hertenstein B, Kurrle E, Redenbacher M, Arnold R, Heimpel H. Pseudoreticulocytosis in a patient with myelodysplasia. Ann Hematol. 1993;67(3):127–8.
80. Cohen PR, Talpaz M, Kurzrock R. Malignancy-associated Sweet's syndrome: review of the world literature. J Clin Oncol. 1988;6(12):1887–97.
81. Mast K, Taub J, Mosse CA, Matthew P, Hitzler J, Alonzo T, et al. Morphology of myelooid leukemia of down syndrome. Mod Pathol [Abstr]. 2013;26(Suppl 2):345A.
82. Brunning RD, McKenna RW. Atlas of tumor pathology: tumors of the bone marrow. Washington, DC: Armed Forces Institute of Pathology; 1994.
83. Foucar K. Bone marrow pathology. 2nd ed. Chicago: ASCP; 2001.
84. Naeim F. Atlas of bone marrow and blood pathology. Philadelphia: W.B. Saunders; 2001.
85. Baumann I, Fuhrer M, Behrendt S, Campr V, Csomor J, Furlan I, et al. Morphological differentiation of severe aplastic anaemia from hypocellular refractory cytopenia of childhood: reproducibility of histopathological diagnostic criteria. Histopathology. 2012;61(1):10–7.
86. de Planque MM, Kluin-Nelemans HC, van Krieken HJ, Kluin PM, Brand A, Beverstock GC, et al. Evolution of acquired severe aplastic anaemia to myelodysplasia and subsequent leukaemia in adults. Br J Haematol. 1988;70(1):55–62.
87. Orazi A, Albitar M, Heerema NA, Haskins S, Neiman RS. Hypoplastic myelodysplastic syndromes can be distinguished from acquired aplastic anemia by CD34 and PCNA immunostaining of bone marrow biopsy specimens. Am J Clin Pathol. 1997;107(3):268–74.
88. Willis MS, McKenna RW, Peterson LC, Coad JE, Kroft SH. Low blast count myeloid disorders with Auer rods: a clinicopathologic analysis of 9 cases. Am J Clin Pathol. 2005;124(2):191–8.
89. Della Porta MG, Travaglino E, Boveri E, Ponzoni M, Malcovati L, Papaemmanuil E, et al. Minimal morphological criteria for defining bone marrow dysplasia: a basis for clinical implementation of WHO classification of myelodysplastic syndromes. Leukemia. 2014;29(1):66–75.
90. Senent L, Arenillas L, Luno E, Ruiz JC, Sanz G, Florensa L. Reproducibility of the World Health Organization 2008 criteria for myelodysplastic syndromes. Haematologica. 2013;98(4):568–75.
91. Brunning RD, Orazi A, Germing U, Le Beau MM, Porwit A, Baumann I, et al. Myelodysplastic syndromes/neoplasms, overview. In: Swerdlow SH,

Campo E, Harris NL, Jaffe ES, Piler SA, Stein H, et al., editors. Who classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer; 2008. p. 88–93.
92. Bain BJ. The bone marrow aspirate of healthy subjects. Br J Haematol. 1996;94(1):206–9.
93. Parmentier S, Schetelig J, Lorenz K, Kramer M, Ireland R, Schuler U, et al. Assessment of dysplastic hematopoiesis: lessons from healthy bone marrow donors. Haematologica. 2012;97(5):723–30.
94. Steensma DP. Dysplasia has a differential diagnosis: distinguishing genuine myelodysplastic syndromes (MDS) from mimics, imitators, copycats and impostors. Curr Hematol Malig Rep. 2012;7(4):310–20.
95. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
96. Germing U, Strupp C, Giagounidis A, Haas R, Gattermann N, Starke C, et al. Evaluation of dysplasia through detailed cytomorphology in 3156 patients from the Dusseldorf Registry on myelodysplastic syndromes. Leuk Res. 2012;36(6):727–34.
97. Maassen A, Strupp C, Giagounidis A, Kuendgen A, Nachtkamp K, Hildebrandt B, et al. Validation and proposals for a refinement of the WHO 2008 classification of myelodysplastic syndromes without excess of blasts. Leuk Res. 2013;37(1):64–70.
98. Verburgh E, Achten R, Louw VJ, Brusselmans C, Delforge M, Boogaerts M, et al. A new disease categorization of low-grade myelodysplastic syndromes based on the expression of cytopenia and dysplasia in one versus more than one lineage improves on the WHO classification. Leukemia. 2007;21(4):668–77.
99. Hasserjian R, Gattermann N, Bennett JM, Brunning RD, Thiele J. Refractory anaemia with ring sideroblasts. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri S, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer; 2008. p. 96–7.
100. Malcovati L, Hellstrom-Lindberg E, Bowen D, Ades L, Cermak J, Del Canizo C, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood. 2013;122(17):2943–64.
101. Germing U, Strupp C, Kuendgen A, Isa S, Knipp S, Hildebrandt B, et al. Prospective validation of the WHO proposals for the classification of myelodysplastic syndromes. Haematologica. 2006;91(12):1596–604.
102. Malcovati L, Porta MG, Pascutto C, Invernizzi R, Boni M, Travaglino E, et al. Prognostic factors and life expectancy in myelodysplastic syndromes classified according to WHO criteria: a basis for clinical decision making. J Clin Oncol. 2005;23(30):7594–603.
103. Malcovati L, Karimi M, Papaemmanuil E, Ambaglio I, Jadersten M, Jansson M, et al. SF3B1 mutation identifies a distinct subset of myelodysplastic syndrome with ring sideroblasts. Blood. 2015;126(2):233–41.
104. Brunning RD, Hasserjian RP, Porwit A, Bennett JM, Orazi A, Thiele J, et al. Refractory cytopenia with unilineage dysplasia. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer; 2008. p. 94–5.
105. Cermak J, Michalova K, Brezinova J, Zemanova Z. A prognostic impact of separation of refractory cytopenia with multilineage dysplasia and 5q- syndrome from refractory anemia in primary myelodysplastic syndrome. Leuk Res. 2003;27(3):221–9.
106. Howe RB, Porwit-MacDonald A, Wanat R, Tehranchi R, Hellstrom-Lindberg E. The WHO classification of MDS does make a difference. Blood. 2004;103(9):3265–70.
107. Lee JH, Shin YR, Lee JS, Kim WK, Chi HS, Park CJ, et al. Application of different prognostic scoring systems and comparison of the FAB and WHO classifications in Korean patients with myelodysplastic syndrome. Leukemia. 2003;17(2):305–13.
108. Orazi A, Brunning RD, Baumann I, Hasserjian R. Myelodysplastic syndrome, unclassifiable. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri S, Stein H, et al., editors. Who classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer; 2008. p. 103.
109. Knipp S, Strupp C, Gattermann N, Hildebrandt B, Schapira M, Giagounidis A, et al. Presence of peripheral blasts in refractory anemia and refractory cytopenia with multilineage dysplasia predicts an unfavourable outcome. Leuk Res. 2008;32(1):33–7.
110. Amin HM, Yang Y, Shen Y, Estey EH, Giles FJ, Pierce SA, et al. Having a higher blast percentage in circulation than bone marrow: clinical implications in myelodysplastic syndrome and acute lymphoid and myeloid leukemias. Leukemia. 2005;19(9):1567–72.
111. Valent P, Orazi A, Busche G, Schmitt-Graff A, George TI, Sotlar K, et al. Standards and impact of hematopathology in myelodysplastic syndromes (MDS). Oncotarget. 2010;1(7):483–96.
112. Orazi A, Brunning RD, Hasserjian R, Germing U, Thiele J. Refractory anaemia with excess blasts. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri S, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer; 2008. p. 100–1.
113. Hasle H, Niemeyer CM, Chessells JM, Baumann I, Bennett JM, Kerndrup G, et al. A pediatric approach to the WHO classification of myelodysplastic and myeloproliferative diseases. Leukemia. 2003;17(2):277–82.
114. Chan GC, Head DR, Wang WC. Refractory anemia with ringed sideroblasts in children: two diseases with a similar phenotype? J Pediatr Hematol Oncol. 1999;21(5):418–23.

115. Vardiman JW, Arber DA, Brunning RD, Larson RA, Matutes I, Baumann I, et al. Therapy-related myeloid neoplasms. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri S, Stein H, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer; 2008. p. 127–9.
116. Dann EJ, Rowe JM. Biology and therapy of secondary leukaemias. Best Pract Res Clin Haematol. 2001;14(1):119–37.
117. Smith SM, Le Beau MM, Huo D, Karrison T, Sobecks RM, Anastasi J, et al. Clinical-cytogenetic associations in 306 patients with therapy-related myelodysplasia and myeloid leukemia: the University of Chicago series. Blood. 2003;102(1):43–52.
118. Wong TN, Ramsingh G, Young AL, Miller CA, Touma W, Welch JS, et al. Role of TP53 mutations in the origin and evolution of therapy-related acute myeloid leukaemia. Nature. 2015;518(7540):552–5.
119. Ok CY, Hasserjian RP, Fox PS, Stingo F, Zuo Z, Young KH, et al. Application of the international prognostic scoring system-revised in therapy-related myelodysplastic syndromes and oligoblastic acute myeloid leukemia. Leukemia. 2013;28(1):185–9.
120. Marisavljevic D, Cemerikic V, Rolovic Z, Boskovic D, Colovic M. Hypocellular myelodysplastic syndromes: clinical and biological significance. Med Oncol. 2005;22(2):169–75.
121. Tuzuner N, Cox C, Rowe JM, Watrous D, Bennett JM. Hypocellular myelodysplastic syndromes (MDS): new proposals. Br J Haematol. 1995;91(3):612–7.
122. Mikhailova N, Sessarego M, Fugazza G, Caimo A, De Filippi S, van Lint MT, et al. Cytogenetic abnormalities in patients with severe aplastic anemia. Haematologica. 1996;81(5):418–22.
123. Biesma DH, van den Tweel JG, Verdonck LF. Immunosuppressive therapy for hypoplastic myelodysplastic syndrome. Cancer. 1997;79(8):1548–51.
124. Lim ZY, Killick S, Germing U, Cavenagh J, Culligan D, Bacigalupo A, et al. Low IPSS score and bone marrow hypocellularity in MDS patients predict hematological responses to antithymocyte globulin. Leukemia. 2007;21(7):1436–41.
125. Sloand EM, CO W, Greenberg P, Young N, Barrett J. Factors affecting response and survival in patients with myelodysplasia treated with immunosuppressive therapy. J Clin Oncol. 2008;26(15):2505–11.
126. Wang SA, Tang G, Fadare O, Hao S, Raza A, Woda BA, et al. Erythroid-predominant myelodysplastic syndromes: enumeration of blasts from nonerythroid rather than total marrow cells provides superior risk stratification. Mod Pathol. 2008;21(11):1394–402.
127. Bacher U, Haferlach C, Alpermann T, Kern W, Schnittger S, Haferlach T. Comparison of genetic and clinical aspects in patients with acute myeloid leukemia and myelodysplastic syndromes all with more than 50% of bone marrow erythropoietic cells. Haematologica. 2011;96(9):1284–92.
128. Hasserjian RP, Zuo Z, Garcia C, Tang G, Kasyan A, Luthra R, et al. Acute erythroid leukemia: a reassessment using criteria refined in the 2008 WHO classification. Blood. 2009;115(10):1985–92.
129. Mazzella FM, Kowal-Vern A, Shrit MA, Wibowo AL, Rector JT, Cotelingam JD, et al. Acute erythroleukemia: evaluation of 48 cases with reference to classification, cell proliferation, cytogenetics, and prognosis. Am J Clin Pathol. 1998;110(5):590–8.
130. Imbert M, Nguyen D, Sultan C. Myelodysplastic syndromes (MDS) and acute myeloid leukemias (AML) with myelofibrosis. Leuk Res. 1992;16(1):51–4.
131. Lambertenghi-Deliliers G, Orazi A, Luksch R, Annaloro C, Soligo D. Myelodysplastic syndrome with increased marrow fibrosis: a distinct clinicopathological entity. Br J Haematol. 1991;78(2):161–6.
132. Fu B, Jaso JM, Sargent RL, Goswami M, Verstovsek S, Medeiros LJ, et al. Bone marrow fibrosis in patients with primary myelodysplastic syndromes has prognostic value using current therapies and new risk stratification systems. Mod Pathol. 2013;27(5):681–9.
133. Kussick SJ, Fromm JR, Rossini A, Li Y, Chang A, Norwood TH, et al. Four-color flow cytometry shows strong concordance with bone marrow morphology and cytogenetics in the evaluation for myelodysplasia. Am J Clin Pathol. 2005;124(2):170–81.
134. Kern W, Haferlach C, Schnittger S, Haferlach T. Clinical utility of multiparameter flow cytometry in the diagnosis of 1013 patients with suspected myelodysplastic syndrome: correlation to cytomorphology, cytogenetics, and clinical data. Cancer. 2010;116(19):4549–63.
135. van de Loosdrecht AA, Ireland R, Kern W, Della Porta MG, Alhan C, Balleisen JS, et al. Rationale for the clinical application of flow cytometry in patients with myelodysplastic syndromes: position paper of an International Consortium and the European LeukemiaNet Working Group. Leuk Lymphoma. 2012;54(3):472–5.
136. Wells DA, Benesch M, Loken MR, Vallejo C, Myerson D, Leisenring WM, et al. Myeloid and monocytic dyspoiesis as determined by flow cytometric scoring in myelodysplastic syndrome correlates with the IPSS and with outcome after hematopoietic stem cell transplantation. Blood. 2003;102(1):394–403.
137. Stachurski D, Smith BR, Pozdnyakova O, Andersen M, Xiao Z, Raza A, et al. Flow cytometric analysis of myelomonocytic cells by a pattern recognition approach is sensitive and specific in diagnosing myelodysplastic syndrome and related marrow diseases: emphasis on a global evaluation and recognition of diagnostic pitfalls. Leuk Res. 2008;32(2):215–24.
138. Bellos F, Alpermann T, Gouberman E, Haferlach C, Schnittger S, Haferlach T, et al. Evaluation of flow cytometric assessment of myeloid nuclear differentiation antigen expression as a diagnostic marker for myelodysplastic syndromes in a series of 269 patients. Cytometry B Clin Cytom. 2012;82(5):295–304.

139. McClintock-Treep SA, Briggs RC, Shults KE, Flye-Blakemore LA, Mosse CA, Jagasia MH, et al. Quantitative assessment of myeloid nuclear differentiation antigen distinguishes myelodysplastic syndrome from normal bone marrow. Am J Clin Pathol. 2011;135(3):380–5.
140. Ogata K, Kishikawa Y, Satoh C, Tamura H, Dan K, Hayashi A. Diagnostic application of flow cytometric characteristics of CD34+ cells in low-grade myelodysplastic syndromes. Blood. 2006;108(3):1037–44.
141. van de Loosdrecht AA, Alhan C, Bene MC, Della Porta MG, Drager AM, Feuillard J, et al. Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes. Haematologica. 2009;94(8):1124–34.
142. Westers TM, Ireland R, Kern W, Alhan C, Balleisen JS, Bettelheim P, et al. Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European LeukemiaNet Working Group. Leukemia. 2012;26(7):1730–41.
143. Kern W, Haferlach C, Schnittger S, Alpermann T, Haferlach T. Serial assessment of suspected myelodysplastic syndromes: significance of flow cytometric findings validated by cytomorphology, cytogenetics, and molecular genetics. Haematologica. 2013;98(2):201–7.
144. Ogata K, Della Porta MG, Malcovati L, Picone C, Yokose N, Matsuda A, et al. Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study. Haematologica. 2009;94(8):1066–74.
145. Truong F, Smith BR, Stachurski D, Cerny J, Medeiros LJ, Woda BA, et al. The utility of flow cytometric immunophenotyping in cytopenic patients with a non-diagnostic bone marrow: a prospective study. Leuk Res. 2009;33(8):1039–46.
146. Cleven AH, Nardi V, Ok CY, Goswami M, Dal Cin P, Zheng Z, et al. High p53 protein expression in therapy-related myeloid neoplasms is associated with adverse karyotype and poor outcome. Mod Pathol. 2015;28(4):552–63.
147. Zhou Y, Tang G, Medeiros LJ, McDonnell TJ, Keating MJ, Wierda WG, et al. Therapy-related myeloid neoplasms following fludarabine, cyclophosphamide, and rituximab (FCR) treatment in patients with chronic lymphocytic leukemia/small lymphocytic lymphoma. Mod Pathol. 2012;25(2):237–45.
148. Seegmiller AC, Wasserman A, Kim AS, Kressin MK, Marx ER, Zutter MM, et al. Limited utility of fluorescence in situ hybridization for common abnormalities of myelodysplastic syndrome at first presentation and follow-up of myeloid neoplasms. Leuk Lymphoma. 2013;55(3):601–5.
149. Yang W, Stotler B, Sevilla DW, Emmons FN, Murty VV, Alobeid B, et al. FISH analysis in addition to G-band karyotyping: utility in evaluation of myelodysplastic syndromes? Leuk Res. 2010;34(4):420–5.
150. Vallespi T, Imbert M, Mecucci C, Preudhomme C, Fenaux P. Diagnosis, classification, and cytogenetics of myelodysplastic syndromes. Haematologica. 1998;83(3):258–75.
151. Olney HJ, Le Beau MM. The cytogenetics and molecular biology of myelodysplastic syndromes. In: Bennett JM, editor. The myelodysplastic syndromes, pathobiology and clinical management. New York: Marcel Dekker; 2002. p. 89–119.
152. Raimondi SC. Cytogenetics in MDS. In: Lopes LFHH, editor. Myelodysplastic and myeloproliferative disorders in children. Sao Paulo: Le Mar; 2003. p. 119–61.
153. Gupta V, Brooker C, Tooze JA, Yi QL, Sage D, Turner D, et al. Clinical relevance of cytogenetic abnormalities at diagnosis of acquired aplastic anaemia in adults. Br J Haematol. 2006;134(1):95–9.
154. Jacobs KB, Yeager M, Zhou W, Wacholder S, Wang Z, Rodriguez-Santiago B, et al. Detectable clonal mosaicism and its relationship to aging and cancer. Nat Genet. 2012;44(6):651–8.
155. Soupir CP, Vergilio JA, Kelly E, Dal Cin P, Kuter D, Hasserjian RP. Identification of del(20q) in a subset of patients diagnosed with idiopathic thrombocytopenic purpura. Br J Haematol. 2009;144(5):800–2.
156. Woll PS, Kjallquist U, Chowdhury O, Doolittle H, Wedge DC, Thongjuea S, et al. Myelodysplastic syndromes are propagated by rare and distinct human cancer stem cells in vivo. Cancer Cell. 2014;25(6):794–808.
157. Mallo M, Cervera J, Schanz J, Such E, Garcia-Manero G, Luno E, et al. Impact of adjunct cytogenetic abnormalities for prognostic stratification in patients with myelodysplastic syndrome and deletion 5q. Leukemia. 2010;25(1):110–20.
158. Schanz J, Tuchler H, Sole F, Mallo M, Luno E, Cervera J, et al. New comprehensive cytogenetic scoring system for primary myelodysplastic syndromes (MDS) and oligoblastic acute myeloid leukemia after MDS derived from an international database merge. J Clin Oncol. 2012;30(8):820–9.
159. Morishita K, Parganas E, William CL, Whittaker MH, Drabkin H, Oval J, et al. Activation of EVI1 gene expression in human acute myelogenous leukemias by translocations spanning 300-400 kilobases on chromosome band 3q26. Proc Natl Acad Sci U S A. 1992;89(9):3937–41.
160. Rogers HJ, Vardiman JW, Anastasi J, Raca G, Savage NM, Cherry AM, et al. Complex or monosomal karyotype and not blast percentage is associated with poor survival in acute myeloid leukemia and myelodysplastic syndrome patients with inv(3)(q21q26.2)/t(3;3)(q21;q26.2): a Bone Marrow Pathology Group study. Haematologica. 2014;99(5):821–9.
161. Padua RA, Guinn BA, Al-Sabah AI, Smith M, Taylor C, Pettersson T, et al. RAS, FMS and p53 mutations and poor clinical outcome in myelodysplasias: a 10-year follow-up. Leukemia. 1998;12(6):887–92.
162. Kanagal-Shamanna R, Bueso-Ramos CE, Barkoh B, Lu G, Wang S, Garcia-Manero G, et al. Myeloid

neoplasms with isolated isochromosome 17q represent a clinicopathologic entity associated with myelodysplastic/myeloproliferative features, a high risk of leukemic transformation, and wild-type TP53. Cancer. 2012;118(11):2879–88.
163. Yoshida K, Sanada M, Shiraishi Y, Nowak D, Nagata Y, Yamamoto R, et al. Frequent pathway mutations of splicing machinery in myelodysplasia. Nature. 2011;478(7367):64–9.
164. Walter MJ, Shen D, Ding L, Shao J, Koboldt DC, Chen K, et al. Clonal architecture of secondary acute myeloid leukemia. N Engl J Med. 2012;366(12):1090–8.
165. Cazzola M, Rossi M, Malcovati L. Biologic and clinical significance of somatic mutations of SF3B1 in myeloid and lymphoid neoplasms. Blood. 2012;121(2):260–9.
166. Gerstung M, Pellagatti A, Malcovati L, Giagounidis A, Porta MG, Jadersten M, et al. Combining gene mutation with gene expression data improves outcome prediction in myelodysplastic syndromes. Nat Commun. 2015;6:5901.
167. Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D, et al. Somatic SF3B1 mutation in myelodysplasia with ring sideroblasts. N Engl J Med. 2011;365(15):1384–95.
168. Bejar R, Stevenson KE, Caughey B, Lindsley RC, Mar BG, Stojanov P, et al. Somatic mutations predict poor outcome in patients with myelodysplastic syndrome after hematopoietic stem-cell transplantation. J Clin Oncol. 2014;32(25):2691–8.
169. Davies SM, Robison LL, Buckley JD, Radloff GA, Ross JA, Perentesis JP. Glutathione S-transferase polymorphisms in children with myeloid leukemia: a Children's Cancer Group study. Cancer Epidemiol Biomarkers Prev. 2000;9(6):563–6.
170. Kishi S, Yang W, Boureau B, Morand S, Das S, Chen P, et al. Effects of prednisone and genetic polymorphisms on etoposide disposition in children with acute lymphoblastic leukemia. Blood. 2004;103(1):67–72.
171. Pui CH, Relling MV, Evans WE. Role of pharmacogenomics and pharmacodynamics in the treatment of acute lymphoblastic leukaemia. Best Pract Res Clin Haematol. 2002;15(4):741–56.
172. Tang G, Jorgensen LJ, Zhou Y, Hu Y, Kersh M, Garcia-Manero G, et al. Multi-color CD34(+) progenitor-focused flow cytometric assay in evaluation of myelodysplastic syndromes in patients with post cancer therapy cytopenia. Leuk Res. 2012g;36(8):974–81.
173. Ramadurai J, Shapiro C, Kozloff M, Telfer M. Zinc abuse and sideroblastic anemia. Am J Hematol. 1993;42(2):227–8.
174. Prodan CI, Holland NR, Wisdom PJ, Burstein SA, Bottomley SS. CNS demyelination associated with copper deficiency and hyperzincemia. Neurology. 2002;59(9):1453–6.
175. Condamine L, Hermine O, Alvin P, Levine M, Rey C, Courtecuisse V. Acquired sideroblastic anaemia during treatment of Wilson's disease with triethylene tetramine dihydrochloride. Br J Haematol. 1993;83(1):166–8.
176. Dunlap WM, James GW 3rd, Hume DM. Anemia and neutropenia caused by copper deficiency. Ann Intern Med. 1974;80(4):470–6.
177. Rezuke WN, Anderson C, Pastuszak WT, Conway SR, Firshein SI. Arsenic intoxication presenting as a myelodysplastic syndrome: a case report. Am J Hematol. 1991;36(4):291–3.
178. Kirby MA, Weitzman S, Freedman MH. Juvenile chronic myelogenous leukemia: differentiation from infantile cytomegalovirus infection. Am J Pediatr Hematol Oncol. 1990;12(3):292–6.
179. Hasle H, Kerndrup G, Jacobsen BB, Heegaard ED, Hornsleth A, Lillevang ST. Chronic parvovirus infection mimicking myelodysplastic syndrome in a child with subclinical immunodeficiency. Am J Pediatr Hematol Oncol. 1994;16(4):329–33.
180. Bagby GC, Meyers G. Bone marrow failure as a risk factor for clonal evolution: prospects for leukemia prevention. Hematology Am Soc Hematol Educ Program. 2007;2007:40–6.
181. Wang SA, Pozdnyakova O, Jorgensen JL, Medeiros LJ, Stachurski D, Anderson M, et al. Detection of paroxysmal nocturnal hemoglobinuria clones in patients with myelodysplastic syndromes and related bone marrow diseases, with emphasis on diagnostic pitfalls and caveats. Haematologica. 2009;94(1):29–37.
182. Socie G, Mary JY, de Gramont A, Rio B, Leporrier M, Rose C, et al. Paroxysmal nocturnal haemoglobinuria: long-term follow-up and prognostic factors. French Society of Haematology. Lancet. 1996;348(9027):573–7.
183. Valent P, Bain BJ, Bennett JM, Wimazal F, Sperr WR, Mufti G, et al. Idiopathic cytopenia of undetermined significance (ICUS) and idiopathic dysplasia of uncertain significance (IDUS), and their distinction from low risk MDS. Leuk Res. 2012;36(1):1–5.
184. Valent P, Horny HP, Bennett JM, Fonatsch C, Germing U, Greenberg P, et al. Definitions and standards in the diagnosis and treatment of the myelodysplastic syndromes: Consensus statements and report from a working conference. Leuk Res. 2007;31(6):727–36.
185. Ando K, Tanaka Y, Hashimoto Y, Ohyashiki JH, Sugimori N, Nakao S, et al. PNH-phenotype cells in patients with idiopathic cytopenia of undetermined significance (ICUS) with megakaryocytic hypoplasia and thrombocytopenia. Br J Haematol. 2010;150(6):705–7.
186. Valent P, Jager E, Mitterbauer-Hohendanner G, Mullauer L, Schwarzinger I, Sperr WR, et al. Idiopathic bone marrow dysplasia of unknown significance (IDUS): definition, pathogenesis, follow up, and prognosis. Am J Cancer Res. 2011;1(4):531–41.

187. Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488–98.
188. Welch JS, Ley TJ, Link DC, Miller CA, Larson DE, Koboldt DC, et al. The origin and evolution of mutations in acute myeloid leukemia. Cell. 2012;150(2):264–78.
189. Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med. 2014;20(12):1472–8.
190. Genovese G, Kahler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371(26):2477–87.
191. Kwok B, Hall JM, Witte JS, Xu Y, Reddy P, Lin K, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood. 2015;126(21):2355–61.
192. Malcovati L, Galli A, Travaglino E, Ambaglio I, Rizzo E, Molteni E, et al. Clinical significance of somatic mutation in unexplained blood cytopenia. Blood. 2017;129(25):3371–8.
193. Cargo CA, Rowbotham N, Evans PA, Barrans SL, Bowen DT, Crouch S, et al. Targeted sequencing identifies patients with preclinical MDS at high risk of disease progression. Blood. 2015;126(21):2362–5.
194. Ohgami RS, Ohgami JK, Pereira IT, Gitana G, Zehnder JL, Arber DA. Refining the diagnosis of T-cell large granular lymphocytic leukemia by combining distinct patterns of antigen expression with T-cell clonality studies. Leukemia. 2011;25(9):1439–43.
195. Huh YO, Medeiros LJ, Ravandi F, Konoplev S, Jorgensen JL, Miranda RN. T-cell large granular lymphocyte leukemia associated with myelodysplastic syndrome: a clinicopathologic study of nine cases. Am J Clin Pathol. 2009;131(3):347–56.
196. Jerez A, Clemente MJ, Makishima H, Rajala H, Gomez-Segui I, Olson T, et al. STAT3 mutations indicate the presence of subclinical T-cell clones in a subset of aplastic anemia and myelodysplastic syndrome patients. Blood. 2013;122(14):2453–9.
197. Marks PW, Mitus AJ. Congenital dyserythropoietic anemias. Am J Hematol. 1996;51(1):55–63.
198. Hines JD, Cowan DH. Studies on the pathogenesis of alcohol-induced sideroblastic bone-marrow abnormalities. N Engl J Med. 1970;283(9):441–6.
199. Sharp RA, Lowe JG, Johnston RN. Anti-tuberculous drugs and sideroblastic anaemia. Br J Clin Pract. 1990;44(12):706–7.
200. Beck EA, Ziegler G, Schmid R, Ludin H. Reversible sideroblastic anemia caused by chloramphenicol. Acta Haematol. 1967;38(1):1–10.
201. Kandola L, Swannell AJ, Hunter A. Acquired sideroblastic anaemia associated with penicillamine therapy for rheumatoid arthritis. Ann Rheum Dis. 1995;54(6):529–30.
202. Ok CY, Medeiros LJ, Hu Y, Bueso-Ramos CE, Wang SA. Transient/reversible ring sideroblasts in bone marrow of patients post cytotoxic therapies for primary malignancies. Leuk Res. 2011;35(12):1605–10.
203. Levine RL, Wadleigh M, Cools J, Ebert BL, Wernig G, Huntly BJ, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. Cancer Cell. 2005;7(4):387–97.
204. Nelson ME, Steensma DP. JAK2 V617F in myeloid disorders: what do we know now, and where are we headed? Leuk Lymphoma. 2006;47(2):177–94.
205. Ohyashiki K, Aota Y, Akahane D, Gotoh A, Miyazawa K, Kimura Y, et al. The JAK2 V617F tyrosine kinase mutation in myelodysplastic syndromes (MDS) developing myelofibrosis indicates the myeloproliferative nature in a subset of MDS patients. Leukemia. 2005;19(12):2359–60.
206. Yoshizato T, Dumitriu B, Hosokawa K, Makishima H, Yoshida K, Townsley D, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med. 2015;373(1):35–47.
207. Wang SA, Galili N, Cerny J, Sechman E, Chen SS, Loew J, et al. Chronic myelomonocytic leukemia evolving from preexisting myelodysplasia shares many features with de novo disease. Am J Clin Pathol. 2006;126(5):789–97.
208. Head DR. Revised classification of acute myeloid leukemia. Leukemia. 1996;10(11):1826–31.
209. Liu W, Hasserjian RP, Hu Y, Zhang L, Miranda RN, Medeiros LJ, et al. Pure erythroid leukemia: a reassessment of the entity using the 2008 World Health Organization classification. Mod Pathol. 2010;24(3):375–83.
210. Kantarjian HM, Giles F, Gattermann N, Bhalla K, Alimena G, Palandri F, et al. Nilotinib (formerly AMN107), a highly selective BCR-ABL tyrosine kinase inhibitor, is effective in patients with Philadelphia chromosome-positive chronic myelogenous leukemia in chronic phase following imatinib resistance and intolerance. Blood. 2007;110(10):3540–6.
211. Talpaz M, Shah NP, Kantarjian H, Donato N, Nicoll J, Paquette R, et al. Dasatinib in imatinib-resistant Philadelphia chromosome-positive leukemias. N Engl J Med. 2006;354(24):2531–41.
212. Marti-Carvajal AJ, Anand V, Sola I. Janus kinase-1 and Janus kinase-2 inhibitors for treating myelofibrosis. Cochrane Database Syst Rev. 2015;10(4):CD010298.
213. Burger JA, Keating MJ, Wierda WG, Hartmann E, Hoellenriegel J, Rosin NY, et al. Safety and activity of ibrutinib plus rituximab for patients with high-risk chronic lymphocytic leukaemia: a single-arm, phase 2 study. Lancet Oncol. 2014;15(10):1090–9.
214. O'Brien S, Furman RR, Coutre SE, Sharman JP, Burger JA, Blum KA, et al. Ibrutinib as initial therapy for elderly patients with chronic lympho-

cytic leukaemia or small lymphocytic lymphoma: an open-label, multicentre, phase 1b/2 trial. Lancet Oncol. 2014;15(1):48–58.
215. Cattaneo C, Spedini P, Casari S, Re A, Tucci A, Borlenghi E, et al. Delayed-onset peripheral blood cytopenia after rituximab: frequency and risk factor assessment in a consecutive series of 77 treatments. Leuk Lymphoma. 2006;47(6):1013–7.
216. Peyrade F, Bologna S, Delwail V, Emile JF, Pascal L, Ferme C, et al. Combination of ofatumumab and reduced-dose CHOP for diffuse large B-cell lymphomas in patients aged 80 years or older: an open-label, multicentre, single-arm, phase 2 trial from the LYSA group. Lancet Haematol. 2017;4(1):e46–55.
217. Grigg A, Dyer MJ, Diaz MG, Dreyling M, Rule S, Lei G, et al. Safety and efficacy of obinutuzumab with CHOP or bendamustine in previously untreated follicular lymphoma. Haematologica. 2017;102(4):765–72.
218. Keating MJ, Flinn I, Jain V, Binet JL, Hillmen P, Byrd J, et al. Therapeutic role of alemtuzumab (Campath-1H) in patients who have failed fludarabine: results of a large international study. Blood. 2002;99(10):3554–61.
219. Scott LJ. Brentuximab vedotin: a review in CD30-positive Hodgkin lymphoma. Drugs. 2017;77(4):435–45.
220. Brito-Zeron P, Soria N, Munoz S, Bove A, Akasbi M, Belenguer R, et al. Prevalence and clinical relevance of autoimmune neutropenia in patients with primary Sjogren's syndrome. Semin Arthritis Rheum. 2009;38(5):389–95.
221. Grunebaum E, Cohen A, Roifman CM. Recent advances in understanding and managing adenosine deaminase and purine nucleoside phosphorylase deficiencies. Curr Opin Allergy Clin Immunol. 2013;13(6):630–8.
222. Cassani B, Mirolo M, Cattaneo F, Benninghoff U, Hershfield M, Carlucci F, et al. Altered intracellular and extracellular signaling leads to impaired T-cell functions in ADA-SCID patients. Blood. 2008;111(8):4209–19.
223. Lagresle-Peyrou C, Six EM, Picard C, Rieux-Laucat F, Michel V, Ditadi A, et al. Human adenylate kinase 2 deficiency causes a profound hematopoietic defect associated with sensorineural deafness. Nat Genet. 2009;41(1):106–11.
224. Pannicke U, Honig M, Hess I, Friesen C, Holzmann K, Rump EM, et al. Reticular dysgenesis (aleukocytosis) is caused by mutations in the gene encoding mitochondrial adenylate kinase 2. Nat Genet. 2009;41(1):101–5.
225. Rochman Y, Spolski R, Leonard WJ. New insights into the regulation of T cells by gamma(c) family cytokines. Nat Rev Immunol. 2009;9(7):480–90.
226. Macchi P, Villa A, Giliani S, Sacco MG, Frattini A, Porta F, et al. Mutations of Jak-3 gene in patients with autosomal severe combined immune deficiency (SCID). Nature. 1995;377(6544):65–8.
227. Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency over 4 decades. Blood. 2012;119(7):1650–7.
228. Salzer U, Warnatz K, Peter HH. Common variable immunodeficiency: an update. Arthritis Res Ther. 2012;14(5):223.
229. Chen K, Coonrod EM, Kumanovics A, Franks ZF, Durtschi JD, Margraf RL, et al. Germline mutations in NFKB2 implicate the noncanonical NF-kappaB pathway in the pathogenesis of common variable immunodeficiency. Am J Hum Genet. 2013;93(5):812–24.
230. Lucas CL, Kuehn HS, Zhao F, Niemela JE, Deenick EK, Palendira U, et al. Dominant-activating germline mutations in the gene encoding the PI(3)K catalytic subunit p110delta result in T cell senescence and human immunodeficiency. Nat Immunol. 2014;15(1):88–97.
231. Angulo I, Vadas O, Garcon F, Banham-Hall E, Plagnol V, Leahy TR, et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. Science. 2013;342(6160):866–71.
232. Nowak-Wegrzyn A, Crawford TO, Winkelstein JA, Carson KA, Lederman HM. Immunodeficiency and infections in ataxia-telangiectasia. J Pediatr. 2004;144(4):505–11.
233. Ancliff PJ, Blundell MP, Cory GO, Calle Y, Worth A, Kempski H, et al. Two novel activating mutations in the Wiskott-Aldrich syndrome protein result in congenital neutropenia. Blood. 2006;108(7):2182–9.
234. Kainulainen L, Lassila O, Ruuskanen O. Cartilage-hair hypoplasia: follow-up of immunodeficiency in two patients. J Clin Immunol. 2014;34(2):256–9.
235. Etzioni A, Benderly A, Rosenthal E, Shehadah V, Auslander L, Lahat N, et al. Defective humoral and cellular immune functions associated with veno-occlusive disease of the liver. J Pediatr. 1987;110(4):549–54.
236. Grever MR, Abdel-Wahab O, Andritsos LA, Banerji V, Barrientos J, Blachly JS, et al. Consensus guidelines for the diagnosis and management of patients with classic hairy cell leukemia. Blood. 2017;129(5):553–60.
237. Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP, et al. BRAF mutations in hairy-cell leukemia. N Engl J Med. 2011;364(24):2305–15.
238. Hsu AP, Johnson KD, Falcone EL, Sanalkumar R, Sanchez L, Hickstein DD, et al. GATA2 haploinsufficiency caused by mutations in a conserved intronic

element leads to MonoMAC syndrome. Blood. 2013;121(19):3830–7. S1–7
239. Dickinson RE, Griffin H, Bigley V, Reynard LN, Hussain R, Haniffa M, et al. Exome sequencing identifies GATA-2 mutation as the cause of dendritic cell, monocyte, B and NK lymphoid deficiency. Blood. 2011;118(10):2656–8.
240. Hsu AP, Sampaio EP, Khan J, Calvo KR, Lemieux JE, Patel SY, et al. Mutations in GATA2 are associated with the autosomal dominant and sporadic monocytopenia and mycobacterial infection (MonoMAC) syndrome. Blood. 2011;118(10):2653–5.
241. Vinh DC, Patel SY, Uzel G, Anderson VL, Freeman AF, Olivier KN, et al. Autosomal dominant and sporadic monocytopenia with susceptibility to mycobacteria, fungi, papillomaviruses, and myelodysplasia. Blood. 2010;115(8):1519–29.
242. Porwit A, van de Loosdrecht AA, Bettelheim P, Brodersen LE, Burbury K, Cremers E, et al. Revisiting guidelines for integration of flow cytometry results in the WHO classification of myelodysplastic syndromes-proposal from the International/European LeukemiaNet Working Group for Flow Cytometry in MDS. Leukemia. 2014;28(9):1793–8.

3 Inherited Bone Marrow Failure Syndromes, Myeloid Neoplasms with Germline Predisposition and Myeloid Proliferations Associated with Down Syndrome

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Overview

The inherited bone marrow failure syndromes (IBMFS) are a group of genetic disorders associated with inadequate production of one or more blood cell lineages (Table 3.1). These disorders are rare and heterogeneous, but are clinically important due to cytopenias and propensity to progress to severe aplastic anemia, myelodysplastic syndromes, acute leukemia, and other malignancies. Broadly, IBMF syndromes can be divided into multilineage cytopenias (Fanconi anemia, dyskeratosis congenita, Shwachman-Diamond syndrome, congenital amegakaryocytic thrombocytopenia), unilineage cytopenia (Diamond-Blackfan anemia, severe congenital neutropenia, thrombocytopenia with absent radii), and congenital dyserythropoietic anemia. The incidence of IBMFS may be difficult to assess due to the absence of large-scale epidemiologic studies. However, the current literature and large-center studies suggest an annual incidence of 65 per million live births [1].

The gene mutations responsible for these conditions often impact the development and/or function of other tissues, resulting in birth defects or clinical disease in organs other than the bone marrow (BM). Sometimes, the birth defects are observed prior to the diagnosis of the marrow failure. These disorders may be present at birth (congenital) or may manifest at a later stage of life. Although inherited in the majority of patients, a de novo gene mutation acquired during early embryogenesis may also occur. Appropriate diagnosis and classification of these disorders have multiple implications in genetic counseling, management/treatment, and clinical surveillance. These implications are shown in Table 3.2.

IBMFS may be inherited as autosomal dominant, autosomal recessive, or X linked. As mentioned above, sporadic cases without a family history may occur as well. The defect may involve various cell functions, such as cell cycle control, DNA repair, ribosomal assembly and function, telomere maintenance, intracellular signaling, and protein folding (Fig. 3.1). Recent molecular genetic advances have identified genes commonly involved in these disorders, advancing our understanding of their pathophysiology. Inherited or de novo germline mutations in cer-

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Table 3.1 Common inherited bone marrow failure syndrome summary

Disorder	Inheritance	Associated malignancy	Affected cellular pathway
Fanconi anemia	AR (except *FANCB* X-linked)	MDS, AML, SCC, Wilms tumor, brain tumors	DNA repair
Dyskeratosis congenita	XL, AD, AR	MDS, AML, SCC, other solid tumors	Telomere maintenance
Shwachman-Diamond syndrome	AR	MDS, AML, possibly ALL	Ribosomal assembly
Diamond-Blackfan anemia	AD (except *GATA1* related, X-linked)	MDS, AML, osteosarcoma, colon carcinoma, possibly others	Ribosomal biogenesis
Congenital dyserythropoietic anemia (CDA)	AR (types I and II), AD (types III and IV)	Possible myeloma with type III	Unfolded protein response, cytokinesis, erythroid transcription factor
Severe congenital neutropenia *ELANE* mutation	AD	MDS/AML	Unfolded protein response
Congenital amegakaryocytic thrombocytopenia	AR	MDS, possible ALL	Megakaryopoiesis
Thrombocytopenia with absent radii	AR	AML, possibly ALL	Possible mRNA processing
Familial platelet disorder with associated myeloid malignancy (*RUNX1*)	AD	MDS, AML	Possibly hematopoietic stem cell differentiation
GATA2 deficiency	AD	MDS, AML	Hematopoietic stem cell differentiation

AR autosomal recessive, *AD* autosomal dominant, *XL,* X-linked, *DNA* deoxyribose nucleic acid, *mRNA* messenger ribose nucleic acid, *AML* acute myeloid leukemia, *ALL* acute lymphoblastic leukemia, *MDS* myelodysplastic syndrome, *SCC* squamous cell carcinoma

Table 3.2 Clinical implications and significance of a diagnosis of inherited bone marrow failure syndromes

	Clinical implications and significance
1	Genetic counseling for the patient and their family
2	Many cases may have morphologic BM features similar to acquired aplastic anemia (AAA) but will not respond to classic AAA treatment, such as immunosuppressant therapy
3	Some of these disorders are characterized by a predisposition for malignancies, such as myelodysplastic syndrome (MDS), acute leukemia, and solid tumors. Therefore, long-term follow-up is required
4	Disorders associated with ineffective erythropoiesis and BM erythroid hyperplasia, such as congenital dyserythropoietic anemia, may be associated with iron overload and its related complications
5	If family members are considered as donors for hematopoietic stem cell transplant, genetic screening needs to be performed to ensure that the donor is not a carrier or subclinically affected
6	For patients receiving hematopoietic stem cell transplant or myeloablative therapy for acute leukemia, reduced or alternative regimens may be instituted as the BM may be unusually sensitive to these regimens

tain genes can significantly increase the incidence of myeloid neoplasms (MDS and AML) in affected patients and families. A major change to the 2016 revision of the WHO Classification is the addition of the section of "myeloid neoplasms with germline predisposition." This category includes cases of MDS, myelodysplastic/myeloproliferative neoplasm (MDS/MPN), and acute leukemias that occur on the background of a predisposing germline mutation. The presence of the specific underlying genetic defect or predisposition syndrome should raise awareness of the need to screen family members for these aberrations. In the first part of this chapter, we review the epidemiology, clinical presentation, laboratory tests, and complications of these disorders. In the sec-

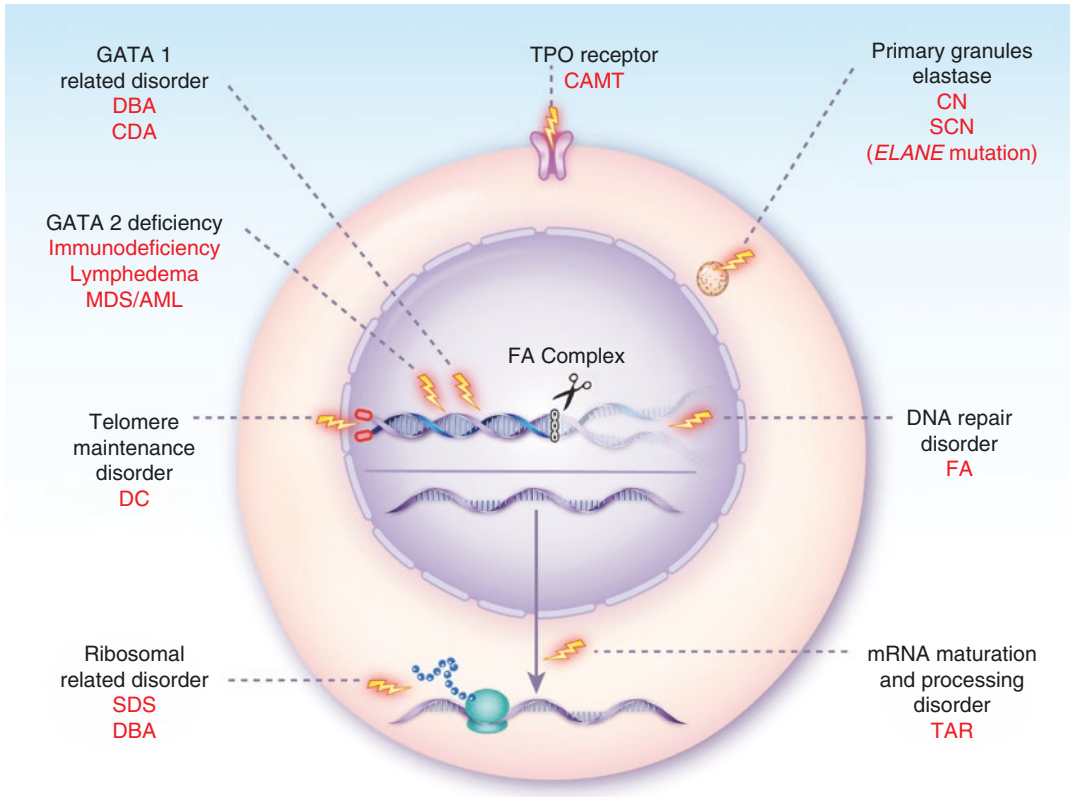

Fig. 3.1 Schematic representation of common inherited bone marrow failure syndromes and the associated genetic abnormalities. *DBA* Diamond-Blackfan anemia, *CDA* Congenital Dyserythropoietic Anemia, *DC* Dyskeratosis congenita, *SDS* Shwachman-Diamond syndrome, *TAR* Thrombocytopenia with absent radii, *FA* Fanconi anemia, *CN* Cyclic neutropenia, *SCN* Severe congenital neutropenia, *CAMT* Congenital amegakaryocytic thrombocytopenia

ond part, we discuss the recent recognized WHO category "myeloid neoplasms with germline predisposition." This will be followed by myeloid proliferations associated with Down syndrome.

Inherited Bone Marrow Failure Syndromes

Fanconi Anemia

Fanconi anemia (FA) is a chromosomal instability disorder with variable clinical presentations, which include congenital anomalies, progressive pancytopenia, and cancer susceptibility. FA was named after the Swiss pediatrician Guido Fanconi, who described the syndrome in 1927 [2]. The incidence of FA is difficult to ascertain. The Fanconi Anemia Research Fund, Inc., estimates that about 30 persons with FA are born in the United States each year; this indicates that the prevalence of FA is about 2.5 per million and the carrier frequency is approximately 1 in 180. A high prevalence of FA is found in individuals of Ashkenazi Jewish descent, who have an approximate heterozygous frequency of 1 in 77 [3, 4].

Clinical Features

The diagnosis of FA should be considered in patients with congenital anomalies, aplastic anemia, or a family history of bone marrow failure or cancer susceptibility. The median age of diagnosis is 6.5 years, but ranges from birth to well into adulthood. Approximately 40% of patients have no

reported physical findings. The male-to-female ratio is 1.2:1. Aplastic anemia or malignancy may be the presenting diagnoses of the underlying FA in the absence of physical anomalies or prior family history. The manifestations of FA can vary between affected members of the same family, suggesting that additional genetic, epigenetic, or environmental factors likely influence the disease characteristics and clinical course [5]. Short stature and cutaneous manifestations, such as generalized hyperpigmentation, café au lait spots, and hypopigmentation, are seen in approximately 40% of patients [6]. Thumb abnormalities are also common and are seen in approximately 35% of patients. These include absent or hypoplastic, bifid, duplicated, rudimentary, attached by a thread, triphalangeal, long, and low-set thumbs. Renal abnormalities, such as horseshoe, ectopic, pelvic, or hypoplastic kidneys, are reported in 20%.

Progressive bone marrow failure is one of the hallmarks of FA, although the hematologic findings vary. Thrombocytopenia and macrocytosis of red blood cells often precede anemia and neutropenia. The majority of FA patients (53%) already have pancytopenia at the time of diagnosis [2]. In a 2003 report from the International FA Registry that included 754 patients, 80% had BM failure at the time of enrollment; the cumulative incidence was 90% by age 40 years [7]. Elevated hemoglobin F levels and elevated expression of "i" antigen on red cells usually coincide with the macrocytosis and are likely manifestations of stress hematopoiesis. Serum alpha-fetoprotein levels are consistently elevated in FA patients irrespective of the presence of liver abnormalities. Erythropoietin levels are usually elevated in anemic FA patients.

The risk of cancer is about 500–700 times higher than in the normal population. By the age of 40 years, the cumulative incidence of solid tumors is estimated to be 26–30% [8]. The median age at development of cancer is 16 years, contrasting with 68 years for the same types of cancer in the general population. The cumulative incidence of myelodysplastic syndrome (MDS) in FA patients is approximately 30% and that of acute leukemia is about 10% by the age of 40 to 50 years. The leukemias are usually acute myeloid leukemia (AML), although patients with acute lymphoblastic leukemia (ALL) and chronic myelomonocytic leukemia (CMML) have been described. HSCT is the only curative therapy for the hematologic manifestations of FA.

Patients with FA are unusually sensitive to the toxicity of the usual chemotherapy and radiation regimens used in preparation for HSCT, particularly organ toxicity and graft-versus-host disease [5]. In addition, these regimens confer an increased risk of subsequent malignancies, primarily squamous cell carcinomas of the head and neck. For FA patients who survive the hematologic complications, follow-up surveillance for solid malignant tumors becomes increasingly important. The most common solid tumors in FA patients are head and neck carcinoma, hepatocellular carcinoma, and gynecological malignancies. The risk increases with age.

Morphology

Bone marrow specimens taken in infancy and early childhood may be normocellular with megaloblastic features; but by mid to late childhood, the BM often shows hypoplasia or aplasia. The BM at the aplastic stage shows reduced or completely depleted trilineage hematopoiesis, findings that are indistinguishable from those idiopathic/acquired AA. Rare patients present first with hypoplastic MDS or AML, without a prior documented aplastic phase. The differential diagnosis between hypoplastic MDS and AA in FA patients can follow the recommendations listed in Table 3.4 of Chap. 4.

Immunophenotype

While a paroxysmal nocturnal hemoglobinuria (PNH) clone is detected in 40–50% of idiopathic AA, PNH clones are not present in patients with Fanconi anemia with BM failure. In FA patients with MDS, the CD34+ myeloblasts show a number of abnormalities, including a decrease/absence of hematogones as well as other abnormalities, similar to those found in the blasts of MDS, or other myeloid neoplasms (see Chap. 2).

Cytogenetics and Molecular Genetics

The diagnosis of FA is based on the demonstration of increased chromosomal breakage in the

presence of DNA cross-linking agents, such as mitomycin C (MMC) or diepoxybutane (DEB). DEB is preferred in some centers, since it is associated with less variability in chromosomal breakage among normal controls. The chromosomal breakage test is usually performed on metaphase spreads of peripheral blood lymphocytes cultured with MMC or DEB. FA heterozygous carriers cannot be reliably detected with chromosomal breakage tests [5]. Some patients have somatic mosaicism for the FA genetic defect, and these patients may require testing skin fibroblasts for chromosomal breakage.

Pathogenetic variants in at least 20 different DNA repair genes of the FA/BRCA pathway have been shown to cause FA [9]. The majority of FA is inherited in an autosomal recessive manner. There is only one gene to date (*FANCB*), which is inherited in an X-linked manner. Somatic mosaicism can occur in some patients due to hematopoietic reversion, in which a point mutation or an intragenic recombination in a hematopoietic stem cell leads to correction of one FA allele and a consequent recovery of a normal or subnormal protein activity and the cellular phenotype. *FANCA* is the most common mutation, affecting approximately 60% of FA patients. The FA/BRCA DNA repair pathway repairs DNA interstrand cross-links (ICLs), which are covalent links between strands of DNA that interfere with DNA replication and transcription during cell mitosis. ICLs are produced by endogenous chemicals, such as reactive aldehydes, and environmental exposures, including natural psoralens and DNA cross-linking drugs, such as cisplatin. During mitosis, double-strand DNA gradually separates, creating a replication fork. When an ICL is encountered, polymerization is arrested and FANCM is recruited to the unwound DNA near the arrest site. This step is followed by the assembly of the FA core complex, which is comprised of FANC A, -B, -C, -E, -F, -G, and -L in addition to other proteins, including FAAP20, FAAP24, and MHF [10]. Once assembled, the core complex interacts with FANCL to catalyze the monoubiquitination of FANCD2 and FANCI. Monoubiquitination of FANCD2 and FANCI is clearly a critical step in ICL repair [9].

Patients with the *FANCD1/BRCA2* subtype constitute 3–4% of all FA patients and manifest an especially high rate of early-onset AML and specific solid tumors (brain tumors and Wilms tumor) compared with other FA subtypes.

Clonal cytogenetic abnormalities are found in 34–48% of FA patients. Fluctuations in karyotype are frequently observed, including the disappearance of clones, appearance of new clones, and clonal evolution. The most frequent chromosomal abnormalities in MDS occurring in FA patients are 1q+, 3q+, −7/7q, 11q−20q−, 6, 13, and 21q− [2].

Differential Diagnosis

A diagnosis of Fanconi anemia is established by a clinical suspicion with characteristic physical features and confirmed by chromosomal breakage testing that is quite sensitive for FA. However, about 25% of FA may not have the typical physical findings and additionally chromosomal breakage testing is not entirely specific for FA. Furthermore, FA tests can be falsely negative in patients who develop hematopoietic reversion and somatic mosaicism [11]. Several differential diagnoses should be considered.

Acquired aplastic anemia occurs frequently in children aged 5–15 years, similar to the age at which FA is often diagnosed. As mentioned above, FA patients may not have the typical physical findings and bone marrow failure may be the first manifestation of FA [12]. Moreover, the BM findings are indistinguishable between acquired AA and BM aplasia developed in FA. The presence of a PNH clone would essentially rule out FA and favor a diagnosis of acquired AA. Acquired AA typically has a more rapid onset and progression of cytopenias, lack of congenital anomalies and endocrine features of FA, a normal chromosomal breakage test, and lack of pathogenic germline mutations in FA genes. Unlike FA patients, AA patients usually respond to immunosuppressive therapy.

Other *inherited bone marrow failure syndromes* that present with BM failure include dyskeratosis congenita, reticular dysgenesis, Shwachman-Diamond syndrome, congenital amegakaryocytic thrombocytopenia, and Diamond-Blackfan anemia. These inherited BM

failure syndromes have characteristic physical findings and molecular genetic alterations specific for the disease (see Table 3.1) and a negative chromosomal breakage test.

Rare *non-FA chromosomal breakage syndromes* such as Nijmegen breakage syndrome, Bloom syndrome, and ataxia telangiectasia often demonstrate increased sensitivity to ionizing radiation and variable sensitivity to DNA cross-linking agents that often cause an abnormal result with chromosomal breakage testing. However, these patients show different physical anomalies and usually do not present with BM failure. Nevertheless, FA gene sequencing is recommended for all patients with a positive chromosomal breakage testing result.

Dyskeratosis Congenita

Dyskeratosis congenita (DC) is an inherited multi-organ disorder of the mucocutaneous and hematopoietic systems associated with a wide variety of other somatic abnormalities. It is now known that DC patients have a cellular defect in telomere maintenance. Originally, it was considered a dermatologic disease and was termed *Zinsser-Cole-Engman syndrome*. DC shows significant genetic and phenotypic heterogeneity. The traditional diagnostic ectodermal triad consists of reticular skin pigmentation of the upper body, mucosal leukoplakia, and nail dystrophy. The skin and nail findings usually become apparent during the first 10 years of life, but the leukoplakia is observed later. These manifestations tend to progress as patients get older.

About 550 cases have been reported in the literature. The incidence of DC in childhood is about four cases per million per year. The male:female ratio is 3.2:1 reflecting the X-linked recessive mode of inheritance in some patients [6].

Clinical Features

The severity of the clinical phenotype can vary widely in affected members of the same family. Most of the somatic abnormalities are not present early in life but rather develop progressively with age. The skin pigmentation is seen in >90% of patients and is typically reticular and mottled in appearance; it may be localized or widespread. Nail dystrophy is also frequent (>80% of patients) and varies in severity from minimal nail irregularities to progressive atrophy and even complete nail loss. Mucosal leukoplakia occurs frequently as well (>70% of patients) and typically involves the tongue, buccal mucosa, and oropharynx, with the tongue being the most frequently affected site. The median age of onset of the classical triad of abnormal skin pigmentation, nail dystrophy, and leukoplakia is between 6 and 8 years (range 0.5–26 years) [2, 5].

Cytopenias of one or more lineages are seen in approximately 85% of patients at presentation. Thrombocytopenia and anemia are often the first signs of bone marrow failure. Of note, cytopenias in early childhood are not necessarily due to bone marrow aplasia, but rather may be immune mediated or caused by organ dysfunction and/or bleeding. Some patients present early in life with isolated immune-mediated cytopenia involving single lineage, only to develop pancytopenia in later childhood or adolescence. Overall, the median age of onset of pancytopenia is 8 years, with 50% developing pancytopenia before the age of 10 and 95% by the age of 40. BM failure or its associated complications accounted for the majority of deaths in DC patients.

Approximately 20% of patients develop pulmonary disease with pulmonary fibrosis and vascular abnormalities and pulmonary disease account for nearly 10% of deaths in DC patients; pulmonary complications may also occur post-HSCT. Gastrointestinal findings, such as esophageal strictures, hepatomegaly, or cirrhosis, are seen in 10% of cases. Immune abnormalities are common, particularly in children with severe and early-onset disease. These include low or high immunoglobulin levels, reduced numbers of B or T cells, and reduced lymphocyte stimulation by phytohemagglutinin. Cognitive deficiencies, including learning disorders, are common in classic DC patients. Eye abnormalities are observed in approximately 50% of cases and include excessive tearing (epiphora) secondary to nasolacrimal duct obstruction. Other ophthal-

mologic manifestations include conjunctivitis, blepharitis, loss of eyelashes, strabismus, cataracts, and optic atrophy.

Among patients in the DC registry, malignancies were noted in 13 out of 148 patients (8.8%). These malignancies developed in older patients, generally after the second decade of life. A recent report from the National Cancer Institute cohort identified similar cancer risks in FA and DC, with cumulative incidences of 20–30% for solid tumors by age 50 years and 10% for AML by age 40 years. The majority of tumors in DC are head and neck squamous cell carcinoma [8].

The only curative treatment for BM failure, MDS, and AML in DC remains HSCT. Androgens improve BM function in approximately 50% of patients. A small number of patients may respond to G-CSF administration, with significant increase in neutrophil counts.

Morphology

The bone marrow findings may be dictated by the cause of the cytopenias (either as a result of immune dysregulation, organ dysfunction, or bleeding). The bone marrow cellularity may be normal or even increased early in the disease. At the advanced stages, the BM is typically hypocellular with depletion of all three lineages. Rarely hypoplastic MDS or AML may be diagnosed at the first BM examination. Periodic bone marrow examination is important for surveillance in the management of DC patients.

Immunophenotype

Telomere length analysis using multicolor flow cytometric fluorescent in situ hybridization (Flow-FISH) is a useful diagnostic screen for DC. DC patients exhibit very short telomeres (<1st percentile of age-matched controls) in multiple lymphocyte subsets. Like other inherited BM failure syndromes [13, 14], a PNH clone is not detected in dyskeratosis congenita because these inherited BM failure syndromes do not select for the outgrowth of PNH clones.

Cytogenetics and Molecular Genetics

DC is caused by abnormalities in the telomere maintenance system or its protective cap, also known as shelterin. Telomerase is an enzyme that adds DNA sequences to the ends of chromosomes (the telomeres) to prevent loss of terminal repeats (TTAGG) during DNA replication. Telomerase enzyme contains two core components, a reverse transcriptase, also known as TERT (telomere reverse transcriptase) and a ribose nucleic acid (RNA) molecule, also known as TERC (telomerase RNA component), which acts as a template for the synthesis of telomere repeats. Telomeres are important for the prevention of chromosomal fusions and rearrangements, which may explain why spontaneous unbalanced chromosomal rearrangements occur in fibroblasts and hematopoietic cells from DC patients. While telomerase activity is high early in development, in later life it is detectable only in a subset of tissues with dividing cells, including the progenitor cells of the hematopoietic system, basal layer of the epidermis, intestinal cells, and hair follicles. These tissues mirror those most severely affected in DC patients. Telomere loss has also been implicated in contributing to the process of aging and DC could be considered to represent a form of premature aging of tissues with a high replicative requirement [5].

There are four distinct genetic forms of DC: X-linked, autosomal dominant, autosomal recessive, and sporadic. The X-linked is the most common form and is almost always due to single-point mutation in the *DKC1* (Xq28) gene encoding the protein dyskerin. Autosomal dominant forms may be caused by mutations in the *TERT*, *TERT*, or *TINF2* genes. The latter encodes a shelterin component known as TIN2 and may be associated with a severe form of DC and also with Revesz syndrome, which manifests as exudative retinopathy in association with DC. Autosomal recessive DC is usually inferred when neither parent has any signs of DC but two or more children have the disease or when the parents of an affected child are consanguineous. Some families originally thought to have autosomal recessive DC were found to actually have autosomal dominant inheritance with variable penetrance and expressivity. The most common recessive forms of DC are due to mutations in *CTC1* and *RTEL*. Rare cases of autosomal recessive DC are due to

biallelic mutations in *TERT, TERC, NOP10, NHP2*, or *WRAP53*. Biallelic *TERT* mutations can result in a severe form of DC called *Hoyeraal-Hreidarsson syndrome,* which is characterized by hematologic and dermatologic manifestations of DC in addition to cerebellar hypoplasia. DC may also arise sporadically, with an absence of family history. Most cases due to *TINF2* mutations are sporadic, due to the fact that most patients with this severe form of DC do not reproduce.

Clonal BM cytogenetic abnormality is not a feature of most of the DC cases. However, similar to Fanconi anemia patients, clonal cytogenetic abnormalities may be observed in some patients with DC, which can be either stable or fluctuating over many years [6]. The presence of abnormalities on BM karyotype should not be used as presumptive evidence for an MDS diagnosis or to drive the decision to perform HSCT.

Differential Diagnosis

A diagnosis of DC can be established in any of the following conditions: (1) Patients have classic mucocutaneous triad, including dystrophic nails, lacy reticular pigmentation, and oral leukoplakia. (2) One of the mucocutaneous findings, plus bone marrow failure, plus two other multisystemic features known to occur in DC that include epiphora, developmental delay or mental retardation, pulmonary disease, periodontal disease, esophageal stricture, premature hair greying or loss, hyperhidrosis, or development of malignant lesions. (3) Four or more features of the Hoyeraal-Hreidarsson syndrome. (4) AA, MDS, or pulmonary fibrosis in the setting of a known genetic alteration affecting telomerase function. (5) Two or more features of DC and laboratory evidence of short telomeres in several lymphocyte subsets. The differential diagnoses, similar to Fanconi anemia, include *acquired BM failure*, and other *inherited BM failure syndromes*. In *sporadic MDS or AML,* the neoplastic cells may have very short telomeres on diagnostic testing, but these short telomeres are only in the hematopoietic cells, not lymphocytes or skin fibroblasts. Unlike DC, sporadic MDS/AML is not associated with mucocutaneous abnormalities of DC or pathogenic mutations affecting telomerase components.

Shwachman-Diamond Syndrome

Shwachman-Diamond syndrome (SDS) is a rare autosomal recessive multisystem disorder characterized by exocrine pancreatic insufficiency, BM dysfunction, and skeletal abnormalities, first described in the 1960s. Most recent data suggest an equal distribution between genders as expected from an autosomal recessive disorder. Based on data from the Canadian Registry, SDS is the third most common inherited BM failure syndrome with an incidence of 8.5 cases per million live births [1].

Clinical Features

SDS is characterized clinically by the combination of exocrine pancreatic insufficiency and BM failure. Exocrine pancreatic insufficiency typically presents in infancy with failure to thrive accompanied by loose, foul-smelling stools as indicators of steatorrhea. Fecal fat measurements may be helpful, but a normal test does not rule out the diagnosis of SDS, since exocrine pancreatic function may improve in a subset of patients with age. Fecal elastase may be decreased, but the sensitivity of this test as a screen for SDS remains to be ascertained. Imaging studies and pathologic examination of the pancreas in patients with SDS show fatty replacement of the pancreatic acini, with sparing of the ducts and islets. Deficiencies in fat-soluble vitamins (vitamins A, D, E, and K) may be seen. Exocrine pancreatic insufficiency can be tested by serum trypsinogen, which is generally depressed in SDS patients at any age, although it may improve with time. Isoamylase levels remain low after the age of 3 and are useful in this older age group.

Neutropenia (absolute neutrophil count $<1.5 \times 10^9$/L) is the most common hematologic finding in SDS and has been reported in 80–100% of patients, with approximately 50% of patients manifesting severe neutropenia (absolute neutrophil count $<0.5 \times 10^9$/L) [2]. Neutropenia may be either intermittent or persistent. In some SDS patients, neutrophils demonstrate abnormalities in chemotaxis and migration [15]. Patients are predisposed to infections, particularly bacterial and fungal. Abnormalities of T or B cells have also been

reported and may contribute to infectious complications. Macrocytic anemia and/or thrombocytopenia may also be seen and a subset of SDS patients develop aplastic anemia. SDS is characterized by a high propensity to develop MDS and leukemia, particularly AML. The overall incidence of combined MDS and AML in patients with SDS ranges from 8 to 33%. Due to the high incidence of developing MDS or AML, regular blood counts should be measured every 3–6 months to monitor the cytopenias.

Skeletal imaging studies may reveal a wide variety of abnormalities, which are often asymptomatic, although metaphyseal dysostosis may require treatment [5]. Hepatic abnormalities, including elevated liver enzymes and occasionally hepatomegaly, can be observed. Many patients with SDS suffer from neurocognitive deficits and psychosocial dysfunction. Dental abnormalities are also common. SDS patients may have some endocrine abnormalities, such as diabetes mellitus and growth hormone deficiency, and commonly have short stature.

Two international consensus reports have provided adequate guidelines for the diagnosis of SDS. One report was published prior to the identification of the *SBDS* gene [16] while a more recent report has included the use of genetic confirmation in the diagnostic criteria [15]. Both reports have used clinical and laboratory findings of BM failure and exocrine pancreatic insufficiency. At present, the only curative option for severe BM failure in SDS is HSCT, which may be indicated in patients with severe or symptomatic cytopenia, MDS with excess blasts, or acute leukemia [1].

Morphology

The current guidelines suggest performing annual BM biopsies for patients treated with G-CSF and biopsies every 1–3 years for patients with stable blood counts who are not receiving G-CSF [2]. The BM is usually hypocellular, but cases showing normal or even increased cellularity have also been observed, particularly early in the course of disease. The BM usually shows granulocytic hypoplasia with left-shifted granulopoiesis. Mild dysplastic changes, particularly in the granulocytic lineage, are commonly observed [17]. However, prominent multilineage dysplasia is unusual, and if identified it may indicate an evolution to MDS.

Immunophenotype

Similar to other inherited BM failure syndrome, a PNH clone is not detected in Shwachman-Diamond syndrome [13, 14]. Immunohistochemistry shows a reduced or diminished SBDS protein expression in all hematopoietic cell lineages and correlates with *SBDS* mutations [18]. SBDS expression is normal in patients with acquired AA and other inherited BM failure syndromes.

Cytogenetics and Molecular Genetics

SDS is inherited as autosomal recessive. It is caused by mutation in the *SBDS* gene on chromosome 7q11. There is an adjoining pseudogene *(SBDSP)* with 97% homology in its coding regions to *SBDS*. The *SBDS* gene encodes a 250-amino-acid protein product which is a member of a highly conserved protein family. The common *SBDS* mutations are composed of sequences that are homologous to *SBDSP*. Hence, these mutations are believed to result from recombination events whereby segments of *SBDSP* become incorporated into wild-type *SBDS* and interfere with its function, a process also known as gene conversion [1]. SBDS protein plays a major role in ribosomal assembly. The nascent large (60S) ribosomal subunit undergoes an ordered series of final maturation steps in the cytoplasm before it becomes competent to enter translation. Once mature, large (60S) and small (40S) ribosomal subunits combine to form an active translating ribosomal unit (80S). The large ribosomal units (S60) travel to the cytoplasm with an attached anti-association factor known as eukaryotic initiation factor 6 or eIf6. This factor must be removed in order to allow for the proper association of both ribosomal units. Under normal circumstances, SBDS directly interacts with the GTPase elongation factor-like 1 (EFL1). The interaction promotes eIF6 removal from the 60S subunit by a mechanism that requires guanosine triphosphate (GTP) binding and hydrolysis by EFL1. When SBDS protein is dysfunctional, ribosomal

assembly is impaired resulting in major cellular dysfunction [19].

BM karyotype is usually normal and the acquisition of clonal abnormalities is often associated with development of MDS or AML. Chromosomal abnormalities, in particular monosomy 7, are associated with poor outcomes and may be an indication for HSCT; however, i(7q) has questionable prognostic significance in SDS patients.

Differential Diagnosis

A clinical diagnosis of SDS is based on the findings of bone marrow failure and exocrine pancreatic dysfunction that are not attributable to other known causes. According to the consensus criteria guideline [15], cytopenia can be of any lineages, either neutropenia, anemia, or thrombocytopenia, but they have to be shown at least two occasions over a 2-month period. Pancreatic dysfunction is based on reduced levels of pancreatic enzymes. The detection of biallelic (homozygous or compound heterozygous) *SBDS* mutations confirms a clinical diagnosis of SDS and may allow an early diagnosis of SDS before it becomes clinically evident. However, a negative result does not exclude a diagnosis of SDS.

The differential diagnosis includes other inherited and acquired causes of marrow failure as well as other causes of exocrine pancreatic insufficiency, such as *cystic fibrosis, Pearson syndrome, and cartilage hair hypoplasia.* Pearson syndrome is caused by mitochondrial DNA abnormalities, due to either large deletions or rearrangements. Patients with Pearson syndrome present with pancytopenia, pancreatic insufficiency, lactic acidosis, and failure to thrive. Unlike SDS, the BM Pearson syndrome often shows a normal cellularity, numerous ring sideroblasts, and cytoplasmic vacuoles in myeloid precursors; the pancreas shows fibrosis rather than lipomatosis; and genetic testing reveals mitochondrial gene defects rather than *SBDS* mutations. Patients with cystic fibrosis, in addition to pancreatic insufficiency, often have lung disease, biliary cirrhosis, and infertility and do not have bone marrow failure.

Diamond-Blackfan Anemia

Diamond-Blackfan anemia (DBA) is a rare inherited BM failure syndrome of early infancy, affecting seven per million live births [20]. The disorder manifests as severe and isolated anemia due to pure red cell aplasia in a normocellular marrow. There are associated physical abnormalities which may be noted at the time of diagnosis or may become recognizable only after thorough physical examination. There is an increased incidence of cancers, specifically MDS, AML, colon cancer, osteosarcoma, and gynecologic cancers [6]. Recent studies have identified the genes involved in this entity as mostly related to ribosome biogenesis and function [21].

Clinical Features

Patients develop severe isolated anemia at a median age of 3 months, with ≥98% identified within the first year of life [6]; a very small subset of patients may present later in life (up to 64 years of age), making the diagnosis very challenging [20]. Asymptomatic carriers have also reported, reflecting incomplete penetrance [22]. There is no predilection for gender.

Patients with DBA have reticulocytopenia and increased level of hemoglobin F as well as erythrocyte adenosine deaminase (eADA). White blood cells and platelets are within normal limits. One-quarter of the patients have a physical birth defect, the most common being short stature [6]. The next most common abnormality is noted in the thumb, which may be triphalangeal, bifid, subluxed, or with thenar flattening, but with a normal radius. Additional defects include asymmetric scapula, fusion of cervical vertebrae, webbed neck, genitourinary, and heart defects [9]. Less than 5% of patients also have cleft lip and/or cleft palate [6]. Presentations of physical abnormalities are variable and a subset of patients may not have any associated congenital abnormalities [23].

Anemia responds to corticosteroid therapy in up to 80% of patients. HSCT is the only cure for DBA. Patients with DBA are at a fivefold increased risk of developing cancer compared to the general population, with the highest risk observed for

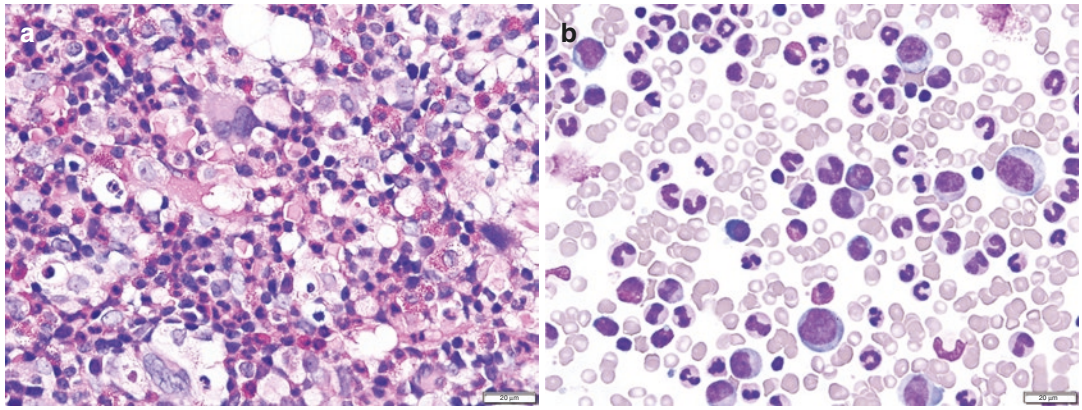

Fig. 3.2 Bone marrow of Diamond-Blackfan anemia. (**a**) Core biopsy shows normocellular marrow with almost complete absence of erythroid precursors. (**b**) Aspirate smear shows isolated marked erythroid hypoplasia/aplasia, with no dysplastic features in other cell lines

MDS, followed by colon carcinoma, osteosarcoma, AML, and genitourinary cancers [24].

Morphology

Bone marrow examination shows a normocellular marrow with isolated erythroid hypoplasia/aplasia and no significant dysplastic features; occasional very early erythroid precursors may be seen. Myeloid and megakaryocytic lineages are usually normal (Fig. 3.2), although some cases with mild dyspoietic features are reported [25].

Immunophenotype

Flow cytometry study reveals both quantitative and qualitative defects in erythroid progenitors that contribute to the defective erythropoiesis in DBA [26]. However, these tests are limited to laboratory research and are not available clinically.

Cytogenetics and Molecular Genetics

Inheritance of DBA is primarily an autosomal dominant pattern. It is caused by heterozygous mutations in genes of ribosomal proteins (for both 40S and 60S) [6]. Mutations of genes for the 40S subunit are reported in *RPS19*, *RPS10, RPS26, RPS24, RPS17, RPS29, RPS28, RPS27*, and *RPS20*, while mutations in genes for the 60S subunit are reported in *RPL11, RPL5, RPL35A, RPL26, RPL15, RPL31*, and *RPL27*. Rarely, patients with clinical presentation as DBA have had mutations reported in *GATA1* and *YSR2* genes and have an X-linked inheritance pattern [22]. *RPS19* mutation is the most common genetic cause of DBA and accounts for 25% of cases [9].

The complex process of ribosomal biogenesis and assembly begins with transcription of ribosomal RNA (rRNA) in the nucleus, along with simultaneous assembly of accessory factors (~200) and ribosomal proteins (~80). Pre-40S and pre-60S subunits are subsequently transported to the cytoplasm for complete assembly and function [9]. The mutations may lead to accumulation of free unassembled ribosomal proteins, which in turn leads to release and stabilization of p53, causing cell cycle arrest or apoptosis [6]. It is further hypothesized that due to the lack of a nucleus in the mature erythrocytes, there is a need for higher rates of ribosomal synthesis to sustain the life cycle of the cell; thus mutations affecting ribosomal biogenesis specifically affect the cells of the erythroid lineage. Recent studies suggest that cytosolic and mitochondrial accumulation of heme in DBA cells is toxic and may activate the apoptotic process [27].

There are no clonal cytogenetic abnormalities reported [25]. The presence of a persistent clonal cytogenetic abnormality should raise the possibility of disease progression to MDS.

Differential Diagnosis

DBA should be suspected in infants presenting with severe anemia and reticulocytopenia during the first year of life, particularly if it is accompanied by congenital malformations. The latter are present in about half of the patients. The diagnostic

criteria according to the international consensus group study [28] include (1) age <1 year; (2) macrocytic anemia with no other significant cytopenias; (3) reticulocytopenia; and (4) normal BM marrow cellularity with a paucity of erythroid precursors. In patients who do not meet all of these criteria, if there is a gene mutation associated with DBA and a positive family history, or elevated eADA activity, congenital anomalies associated with DBA, elevated hemoglobin F, and no evidence of another inherited bone marrow failure syndrome, a probable diagnosis of DBA can be made.

The major differential diagnosis of DBA is *transient erythroblastopenia of childhood* (TEC), which usually manifests in early childhood (typically around 2 years of age) and is transient with spontaneous recovery after one or two transfusions [6]. On the other hand, some DBA patients may not have the classical presenting features of DBA, but whenever a suspicion of an inherited BM failure syndrome is raised, genomic analysis should be performed targeting the genes associated with this disorder.

Patients with c*ongenital dyserythropoietic anemia* (CDA) also present with anemia and reticulocytopenia. However, unlike DBA, the bone marrow of CDA shows erythroid hyperplasia with marked dyserythropoiesis and multinucleated erythroblasts.

Congenital Dyserythropoietic Anemias

Congenital dyserythropoietic anemias (CDAs) are a group of rare hereditary disorders characterized by congenital anemia and ineffective erythropoiesis with distinct morphologic features of marrow late erythroblasts, with an accompanied hemolytic component. CDA has four major subtypes (CDA I–IV) that were originally defined based on morphology; more recently molecular techniques have unveiled the genes commonly mutated in the major CDA subtypes.

The overall prevalence of CDAs shows a wide range among families in Europe, with as low as 0.04 cases per million in North Europe and as high as 2.49 per million in Mediterranean countries such as Italy. Single cases are also reported from the United States, India, Japan, and China. CDA I is approximately three times less frequent than CDA II [29].

Clinical Features

Congenital dyserythropoietic anemia should be suspected based on the presence of symptoms and signs of increased red cell turnover, such as mild jaundice and low or absent haptoglobin, as in hemolytic anemias, with a low level of reticulocytosis that does not correspond to the degree of anemia. CDA I patients show variable degrees of macrocytic anemia, hepatosplenomegaly, skeletal abnormalities of mostly digits (syndactyly in hands or feet, absence of nails, or supernumerary toes), and progressive iron overload. CDA II may present as normocytic anemia, combined with jaundice (90% of cases), splenomegaly (70% of cases), and hepatomegaly (45% of cases).

Cholelithiasis, splenomegaly, and iron overload are the predominant complications of CDA I and CDA II. Secondary hemochromatosis is the most important long-term complication, which is mediated by the downregulation of the hepatic hormone hepcidin, leading to increased iron absorption and systemic iron overload. Hence it is crucial to recognize early iron overload and intervene with iron chelation therapy.

Aplastic crisis in CDA patients may occur as a result of viral infections. High levels of unconjugated hyperbilirubinemia are due to increased production associated with ineffective erythropoiesis: 62% of CDA II patients have neonatal jaundice and 84% of adult CDA II patients show splenomegaly. Rare CDA patients with refractory leg ulcers have been described; the etiology seems to be multifactorial, probably reflecting tissue hypoxia due to either anemia or thrombosis [29].

Cytogenetic and Molecular Genetics

CDA I is an autosomal recessive disease due to the mutation in *CDAN1* gene, which is located on chromosome 15 and encodes a 134-kDa ubiquitous protein (codanin-1). It is speculated that CDA I pathogenesis involves disruption of intrinsic connection between cell cycle dynamics and terminal erythroid differentiation. In up to 20% of cases, no *CDAN1* mutation can be identified. Homozygous mutations in the *C15ORF41* gene have been reported in some pedigrees [30].

CDA II is the most common form of CDA and has an autosomal recessive inheritance. *SEC23B* gene, located on chromosome 20, is mutated in CDA II. This gene encodes COPII

(coat protein), a protein involved in the secretory pathway of eukaryotic cells, mediating anterograde transport of correctly folded cargo from the endoplasmic reticulum towards the Golgi apparatus. The multinucleation of erythroid precursors could be secondary to the aberrant glycosylation of specific proteins required for cell division. Although disease inheritance is typically autosomal recessive, in 13% of cases only one *SEC23B* mutation is found, suggesting the presence of a second yet unidentified mutational event. Compound heterozygosity for missense and nonsense mutations tends to produce more severe clinical presentations.

CDA III is very rare, and has been reported in one American family and one large Swedish kindred. Both families showed autosomal dominant inheritance and the same mutation, P916R, in the *KIF23* gene located on chromosome 15q21. *KIF23* encodes a kinesin-superfamily protein which is a component for proper formation of central spindle and midbody, essential for cytokinesis. Defective cytokinesis leads to the formation of large multinucleated erythroblasts of CDA III. Other sporadic cases of CDA III may show possible autosomal recessive inheritance. These patients may exhibit mongoloid facies, mental retardation, extramedullary hematopoiesis, and some lymphomas (rarely myeloma).

CDA IV has been reported in four patients. All four patients show the same autosomal dominant mutation (E325K) in heterozygous state in the *KLF1* gene located at chromosome 19p13.2. This *KLF1* (erythroid Kruppel-like factor) gene encodes an essentially erythroid-specific transcriptional activator in erythropoiesis which plays a critical role in the switch between the fetal and adult hemoglobin (HGB) expression and is required for cell cycle progression in terminal erythroid differentiation. Figure 3.3 depicts the relation between the cell cycle and common CDAs (I–IV).

CDA variants other than I–IV have been described, each of which have relatively few patients, suggesting that these CDA subtypes are extremely heterogeneous and probably represent multiple unrelated genetic disorders. An X-linked recessive CDA variant due to mutation in the gene *GATA1* located on chromosome Xp11.23 has been recently identified [29, 31].

Morphology

Morphological analysis is the first step in the diagnosis of all subtypes of CDA, to be followed by confirmatory tests. The PB shows distinct anisopoikilocytosis, with ovalocytes, microspherocytes, teardrop cells, or irregularly contracted cells and nucleated red cells [32]. The degree and type of anemia (normocytic or macrocytic) along with bone marrow morphologic findings can provide a tentative diagnosis of the common types of CDAs (Table 3.3).

CDA I patients have macrocytic anemia. The BM shows incompletely divided erythroid cells with thin chromatin bridges between pairs of erythroblasts or between two nuclei in the same cell (Fig. 3.4a). Electron microscopic studies demonstrate that the heterochromatin is denser than normal and forms sharply delineated clumps with small translucent vacuoles, giving rise to the description of a "Swiss cheese" appearance in which the cytoplasm may penetrate through widened pores of the nuclear envelope.

CDA II patients have normocytic anemia with normal or slightly increased mean corpuscular

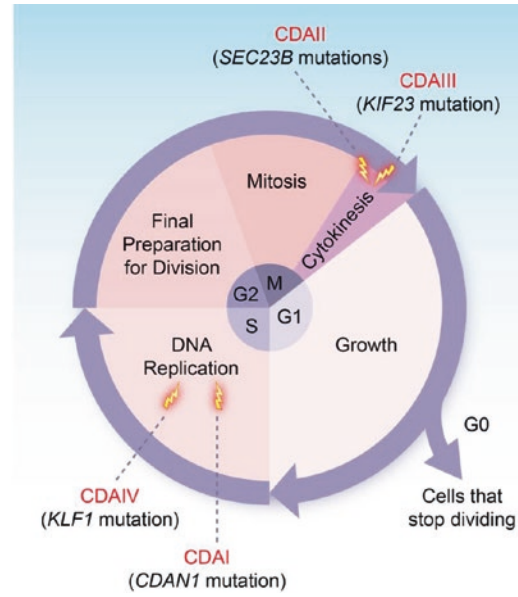

Fig. 3.3 Schematic representation of common congenital dyserythropoietic anemias (CDA) and their genetic abnormalities in relation to a cell cycle

Table 3.3 Features of various types of congenital dyserythropoietic anemias (CDA)

	Gene/inheritance	BM morphology	Associated features
CDA type I	*CDAN1, C15ORF41*/AR	Erythroblasts with internuclear bridges	Skeletal abnormalities
CDA type II	*SEC23B*/AR	Predominantly binucleated erythroblasts, rare multinucleated erythroblasts	May exist, variable
CDA type III, sporadic	Unknown	Giant multinucleated Normoblasts	Variable
CDA type III, familial	*KIF23*/AD	Giant multinucleated Normoblasts	Plasma cell neoplasms Angioid streaks
CDA type IV	*KLF1/AD*	Erythroblasts with internuclear bridging	Mild to moderate splenomegaly
CDA variants	*KLF1*, *GATA1*/AD, XLR	CDA I like, CDA II like Others	Central nervous system anomalies

CDA Congenital dyserythropoietic anemias, *AD* autosomal dominant, *AR* autosomal recessive, *XLR* X-linked recessive

volume with occasionally bilobed mature erythroblasts (Fig. 3.4b). The bone marrow is hypercellular with erythroid hyperplasia and a large number of binucleated (10–35%) and rarely multinucleated late polychromatophilic erythroblasts. Electron microscopy shows vesicles containing proteins of the endoplasmic reticulum beneath the plasma membrane.

CDA III patients have normocytic anemia with normal or slightly low reticulocytes. The peripheral smear shows macrocytes, poikilocytes, and occasionally giant red blood cells. The bone marrow is characterized by erythroid hyperplasia and large, pathognomonic multinucleated erythroblasts (Fig. 3.4c). Electron microscopy shows clefts in heterochromatin, autophagic vacuoles, and iron-laden macrophages with myelin figures in the cytoplasm [29].

CDA IV patients have normocytic anemia, generally severe, with a normal or slightly increased reticulocyte count. Hemoglobin F is elevated (>30%). The peripheral smear shows anisopoikilocytosis, schistocytes polychromasia, and nucleated red blood cells. The bone marrow shows erythroid hyperplasia, basophilic stippling of polychromatophilic erythroblasts and erythrocytes, and internuclear bridging. Electron microscopy shows immature erythroid progenitors with atypical cytoplasmic inclusions, enlarged nuclear pores, invagination of nuclear membrane, and marked heterochromatin [33]. Based on morphologic findings of the bone marrow, further genetic tests may be performed to establish the diagnosis (see below).

Immunophenotype

Flow cytometric analysis of erythrocytes and reticulocytes has shown utility in the differential diagnosis of CDA II from hereditary spherocytosis [34]. Compared to hereditary spherocytosis, CDA II shows a significantly higher red cell volume and HGB concentration and an increased distribution width for cellular HGB content in reticulocytes.

Differential Diagnosis

CDA may be confused with *thalassemias* and other *hemolytic anemia*. CDA is characterized by marked PB anisocytosis with a variegated picture, inadequate reticulocyte response, and presence of bizarre morphological abnormalities in erythroid precursors on BM examination. CDA II may bear a resemblance to *hereditary spherocytosis* (HS), which is a result of alterations in ankyrin, band 3 (the anion exchanger), alpha-spectrin, beta-spectrin, Rh-associated glycoprotein, and band 4.2. HS patients often have a positive family history, an elevated mean corpuscular hemoglobin concentration (MCHC usually >36 g/dL), the presence of spherocytes on the peripheral blood smear, and an abnormal osmotic fragility test. Furthermore, in some cases of CDA, due to iron overload, *hemochromatosis* may be a presenting feature of underlying congenital dyserythropoietic anemia.

Severe Congenital Neutropenia

Severe congenital neutropenia (SCN) is a heterogeneous group of disorders characterized by

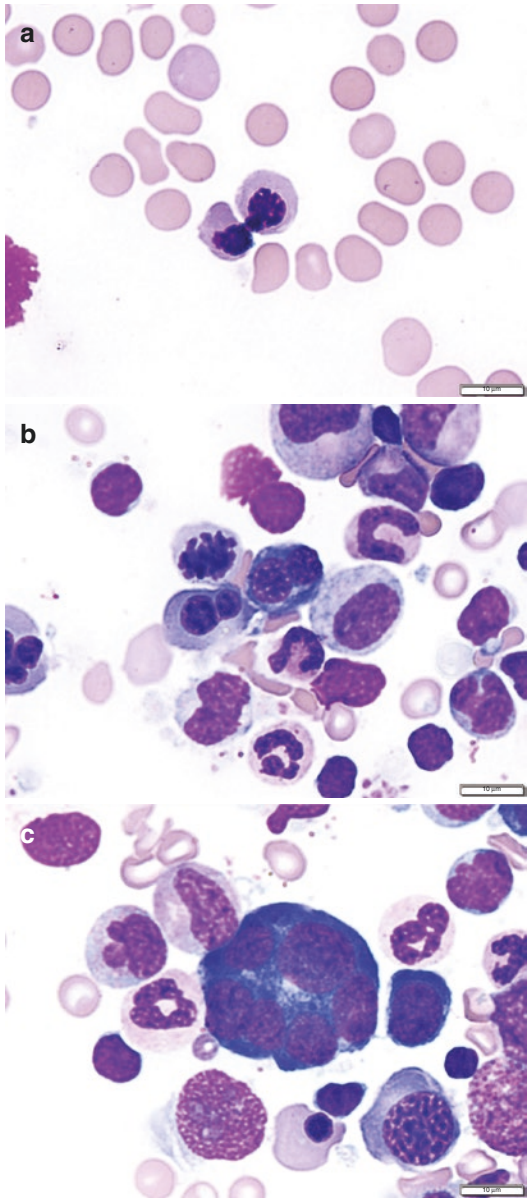

Fig. 3.4 Dyserythropoietic erythroblasts of congenital dyserythropoietic anemias (CDAs). (**a**) CDA I erythroblast showing internuclear bridge. (**b**) CDA II erythroblast showing binucleated forms. (**c**) CDA III erythroblast showing markedly enlarged multinucleated form

severe neutropenia presenting in the neonatal period or early infancy. HGB and platelet count are usually normal. There are no distinctive physical features or congenital anomalies associated with most cases [35]. The incidence of SCN is estimated to be approximately 1–2 cases per million and both genders are affected equally. It has been reported in a broad range of ethnic groups [2]. Inherited or sporadic point mutations in the *ELANE* gene, encoding neutrophil elastase, have been detected in 50–80% of patients with SCN [36]. The autosomal recessive form of SCN is also known as Kostmann syndrome.

Clinical Features

Patients present with recurrent bacterial infections such as skin and subcutaneous infections, abscesses, and pneumonia. Omphalitis is a typical neonatal presentation of SCN. HGB and platelet count are often within normal limits. In the majority of the patients, there are no other distinguishing physical features or congenital anomalies are seen that would suggest an inherited disorder [35]. Affected patients usually have isolated severe neutropenia (absolute neutrophil count $<0.5 \times 10^9$/L). There may also be a relative monocytosis with a monocyte percentage in the 30–50% range.

SCN is managed through administration of granulocyte colony-stimulating factor (G-CSF) to induce increased neutrophil count and prevent infections. HSCT is the only curative treatment. Patients with SCN are at increased risk of developing MDS and AML, with a cumulative incidence of MDS/AML of 21% by the age of 10 years.

Morphology

The neutropenia is associated with decreased production of myelocytes and granulocytic cells beyond the myelocyte stage. Bone marrow examination shows normal or slightly decreased cellularity with maturation arrest of the myeloid cells at the promyelocyte/myelocyte stage (Fig. 3.5). Myelocytes and promyelocytes may show atypical nuclei and cytoplasmic vacuolization. These morphologic findings do not correlate with the underlying mutations [37].

Immunophenotype

There are no specific flow cytometry finding in SCN. Flow cytometry and immunohistochemistry may be helpful to rule out neutropenia due to other causes, such as a proliferation of large granular lymphocytes or NK cells. Flow cytometry assay that measures neutrophil-associated immunoglobulin coating on neutrophil surface, especially the IgM form, helps to rule out autoimmune neutropenia and if positive would exclude a diag-

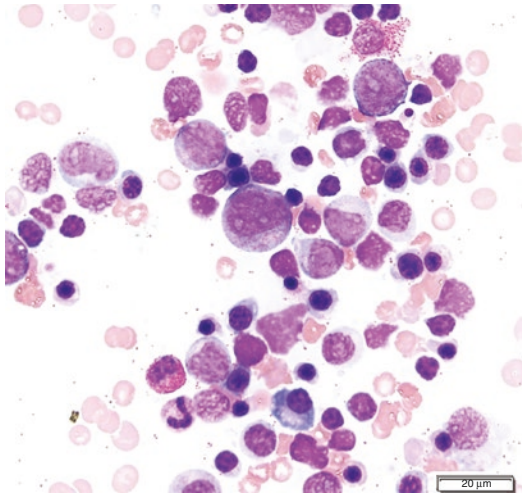

Fig. 3.5 Bone marrow aspirate of severe congenital neutropenia showing maturation arrest of myeloid cells at the myelocyte stage with only rare segmented neutrophils, while other cell lines are not affected

nosis of SCN [41]. This assay has been used clinically in some laboratories. The caveat is that neutrophil-associated immunoglobulin is commonly detected in patients with autoimmune diseases, solid-organ tumors, hematologic disorders, and lymphoma, albeit at a low level.

Cytogenetics and Molecular Genetics

Heterozygous mutations in *ELANE* gene are found in 50–75% of patients with SCN, inherited in an autosomal dominant or sporadic fashion. *ELANE* encodes the enzyme neutrophil elastase (NE), a serine protease component of the primary azurophilic granules of neutrophils. These mutations exhibit variable effects on NE enzymatic activity and its intracellular localization. Mutations in *ELANE* may also cause protein unfolding within the endoplasmic reticulum triggering unfolded protein response resulting in apoptosis. The mutational spectrum encompasses not only the region encoding the mature enzyme but also the prodomains and promoter region [38]. The autosomal recessive form, Kostmann syndrome, is caused by biallelic mutations in the *HAX1* gene. HAX1 is a mitochondrial protein, deficiency of which results in reduction of the inner mitochondrial membrane potential leading to pro-apoptotic state with engagement of BAX, release of cytochrome c, and activation of the apoptotic caspase cascade. An X-linked form of SCN is caused by mutations affecting the autoinhibitory domain of the WAS protein resulting in increased actin polymerization. Mutations in *G6PC3*, a gene involved in the glucose-6-phosphate pathway, have also been associated with congenital neutropenia; these patients manifested developmental anomalies of the cardiac and genitourinary systems. Rare patients with neutropenia and monocytosis have shown heterozygous dominant loss-of-function mutations in the transcriptional repressor GFI1 [6].

During the course of disease progression to MDS or AML, approximately 80% of patients acquire somatic mutations in the gene encoding the G-CSF receptor, *CSF3R*, specifically in the marrow myeloid cells, leading to truncation of G-CSFR C-terminus. These truncating mutations result in the deletion of the terminal negative autoregulatory region of the receptor, causing an increase in myeloid proliferation and resistance to apoptosis, but with impaired granulocytic differentiation. These findings suggest that *CSF3R* mutations contribute to leukemogenesis in SCN patients [36, 39]. The *CSF3R* mutant clones are highly dynamic and may disappear and reappear during continuous G-CSF therapy [40].

Differential Diagnosis

The most common causes of neutropenia, even in infants, are due to *infections, medications/drugs, nutritional deficiency, and autoimmune neutropenia*. Neutropenia can occur during bacterial, viral, parasitic, or rickettsial infections. Bacterial infection usually causes leukocytosis; however, typhoid fever, Shigella enteritis, brucellosis, tularemia, and tuberculosis are often associated with neutropenia. Viral infections commonly associated with neutropenia include human immunodeficiency virus (HIV), infectious mononucleosis, cytomegalovirus, hepatitis A, and viral exanthematous diseases. On the other hand, infections can trigger macrophage activation syndrome/hemophagocytic lymphohistiocytosis (HLH), which would lead to neutropenia, and often pancytopenia. The drugs frequently associated with severe neutropenia include clozapine, thionamides, and sulfasala-

zine. Neutropenia and/or agranulocytosis caused by drugs may be due to immune-mediated destruction of neutrophils by drug-dependent or drug-induced antibodies, or direct toxic effects on BM granulocytic precursors. Neutropenia may be secondary to nutritional deficiency including vitamin B12, folate, or copper deficiencies. *Isoimmune neonatal neutropenia* is secondary to transplacental passage of IgG antibodies that is directed against neutrophil-specific antigens inherited from the father. Most infants have only mild neutropenia with a mean duration of 7 weeks, but some infants may present with moderate-to-severe neutropenia. The infants are otherwise healthy. *Autoimmune neutropenia or chronic benign neutropenia* occurs primarily in infants and young children and is caused by the presence of antineutrophil antibodies that mediate neutrophil destruction either by splenic sequestration or by complement-mediated neutrophil lysis. The mean age at diagnosis is 6–12 months, with a range of 3–30 months. The mean duration of neutropenia is approximately 20 months and spontaneous recovery occurs by age 5. Many patients remain free of infections and maintain a normal lifestyle with no or minimal medical intervention. Antineutrophil antibodies are detected in almost all patients.

Chronic idiopathic neutropenia is used to describe chronic neutropenia for which there is no obvious cause. Chronic idiopathic neutropenia tends to occur in late childhood or adulthood and does not undergo spontaneous remission. There is a female preponderance. Patients often have no serious infections or splenomegaly, but some patients may have anemia. Antineutrophil antibodies are detected in about 20–30% of patients, indicating that in some cases neutropenia may be due to antibody-mediated neutrophil destruction [42]. Alternatively, there may be overproduction of inflammatory cytokines (such as Fas-ligand and interferon γ) by activated T lymphocytes within the bone marrow microenvironment, leading to inhibition of myelopoiesis [43]. *Pure white cell aplasia* is most often associated with thymoma, particularly medullary type. It is also reported in Goodpasture syndrome. The BM of pure white cell aplasia shows preserved erythropoiesis and megakaryocytopoiesis, but markedly decreased or absent granulopoiesis. Neutropenia also can occur in association with a number of *immune deficiency disorders*, such as hypergammaglobulinemia or hypogammaglobulinemia.

Cyclic neutropenia is a rare, autosomal dominantly inherited disorder. It is characterized by neutropenia that recurs in a median of 21 days of cycle (range 14–35 days). Most of the patients present with neutropenia in the first year of life. The neutrophil count waxes and wanes contrasting with the persistent neutropenia seen in SCN. Therefore, it is recommended to check white blood cell count and differentials 2–3 times a week for 6 weeks in order to understand the pattern of neutropenia. Subsequent genetic testing helps in the identification of the specific causative gene mutation. Compared to SCN, an even higher frequency of *ELANE* mutations (90–100%) is found in cyclic neutropenia. The mutations, however [44], occur predominantly in exon 4 or 5 or at the junction of exon 4 and intron 4. The cycling of neutropenia seems to decrease with age and can disappear by age 30 years in some patients [45]. Unlike SCN, cyclic neutropenia is not associated with the risk of AML transformation.

There is an acquired adult-onset form of cyclic neutropenia. However, it is autoimmune rather than genetic in nature and is often associated with a T-cell large granular lymphocyte proliferation. It has been suggested that T-cell immune response to granulocyte proteases may contribute to the phenomenon [46].

Shwachman-Diamond syndrome can present with isolated neutropenia. As discussed above, in addition to neutropenia, patients with SDS also show metaphyseal dysplasia and pancreatic insufficiency.

Myelokathexis is a rare congenital neutropenia resulting from impaired release of granulocytes from the bone marrow [47]. It is characterized by the accelerated apoptosis of granulocytes and the depressed expression of bcl-x of granulocyte precursor cells [48]. The bone marrow is often hypercellular, with an increased number of mature and hypersegmented neutrophils. The myelokathexis morphology is characterized by cytoplasmic vacuolization, nuclear hypersegmentation, and nuclear lobes separated by long

strands of chromatin. Patients usually show good response to G-CSF treatment [49].

WHIM syndrome is a rare disease characterized by warts (human papillomavirus infection), hypogammaglobulinemia, infections, and myelokathexis due to abnormal apoptosis and cell migratory function. Mutations in the chemokine receptor *CXCR4* [50] are identified in WHIM syndromes, resulting in impaired intracellular trafficking, leading to increased responsiveness to chemokine ligand and retention of neutrophils in bone marrow. The CXCR4 antagonist plerixafor is a potential therapy for myelokathexis and WHIM syndrome [51].

Chediak-Higashi syndrome is a rare autosomal recessive lysosomal disorder characterized by frequent infections, oculocutaneous albinism, bleeding diathesis, and progressive neurologic deterioration. About 80–85% patients eventually enter the "accelerated phase" of the disease, due to a lymphoproliferative infiltration of the bone marrow and reticuloendothelial system. The accelerated phase is characterized by fever, hepatosplenomegaly and lymphadenopathy, and pancytopenia and bleeding, features of hemophagocytic lymphohistiocytosis. Pathologic mutations in the lysosomal trafficking regulator (*LYST/CHS1*) gene localized at 1q42.1-2 are responsible for this defect [52, 53].

Table 3.4 lists various congenital disorders associated with neutropenia.

Congenital Amegakaryocytic Thrombocytopenia

Congenital amegakaryocytic thrombocytopenia (CAMT) is a rare disorder characterized by thrombocytopenia present at birth or in the neonatal period, associated with megakaryocytic hypoplasia/aplasia in the BM. Thus far, there are more than 100 cases reported. However, it is likely an underestimation as this disorder may go unrecognized. There is no reported male or female predilection.

Clinical Features

There are no characteristic congenital physical abnormalities associated with CAMT, except for a rare form associated with fusion of the radius and ulna (radial-ulnar synostosis). Approximately 70% of patients with CAMT have severe thrombocytopenia in the neonatal period and petechiae at birth and may develop intracranial or intestinal mucus membrane bleeding. Blood count commonly shows severe thrombocytopenia in the neonatal period, which may be as low as 16×10^9/L. The major treatment for bleeding is platelet transfusion. The disease progresses to bone marrow aplasia within the first few years of life (median age 3.7 years). Occasional patients may present with aplastic anemia, MDS, or AML without a preceding documented history of thrombocytopenia. HSCT remains the only curative therapy for CAMT [6, 35].

Table 3.4 Molecular genetics, mode of inheritance, and symptoms of congenital neutropenia

Disease category/entity	Gene/locus	Mode of inheritance	Associated sign/symptoms
Severe congenital neutropenia			
• Kostmann syndrome	*HAX1*	Autosomal recessive	+/− neurologic defects, +/− splenomegaly
• Sporadic and familial congenital neutropenia	*ELANE* (serine protease)	Autosomal dominant, or sporadic	Isolated neutropenia
	GFI1 (transcription factor)	Autosomal dominant	Monocytosis and defects in lymphocyte quantity and function
	WAS (cytoskeleton function)	X-linked recessive	Monocytopenia and T-cell activation
	G6PC3 (glucose metabolism)	Autosomal recessive	Cardiac and urogenital defects, thrombocytopenia
	CSF3R (G-CSF receptor)	Autosomal dominant	Severe myeloid hypoplasia in the BM G-CSF resistance Increased risk for AML

Table 3.4 (continued)

Disease category/entity	Gene/locus	Mode of inheritance	Associated sign/symptoms
Congenital immunodeficiency syndromes			
• X-linked agammaglobulinemia	*BTK*	X-linked	Defective B-cell development; recurrent bacterial infections
• Common variable immunodeficiency		Unknown	Hypogammaglobulinemia, sinopulmonary infections, autoimmune hemolytic anemia, and thrombocytopenia
• Wiskott-Aldrich syndrome	*WAS*	X-linked	Autoimmune-mediated neutropenia
• Dubowitz syndrome	*LIG4*	Autosomal recessive	Growth deficiency, cognitive defects, microcephaly, eczema, dysmorphic facial features
• Cartilage-hair hypoplasia	? Gene at 9p13 encoding for endoribonuclease	Autosomal recessive	Lymphopenia, short-limb dwarfism, metaphyseal chondrodysplasia, sparse thin hair, T-cell defects
• Griscelli syndrome, type 2	*RAB27A*	Autosomal recessive	Thrombocytopenia, partial albinism, lymphohistiocytosis
• Hyper IgM syndrome	*CD40LG*	Autosomal recessive X-linked	Recurrent bacterial infections, frequent autoimmune hemolytic anemia, and thrombocytopenia
• Selective IgA deficiency		Unknown	Autoimmune anemia and thrombocytopenia
• WHIM syndrome	*CXCR4*	X-linked	Warts, hypogammaglobulinemia, infection, myelokathexis, lymphopenia
• Reticular dysgenesis	*AK2*	Autosomal recessive	Neutropenia, thrombocytopenia
• Shwachman-Diamond syndrome	*SBDS*	Autosomal recessive	Short stature, pancreatic exocrine deficiency, and marrow failure Propensity for developing AML/MDS (up to 20%) and ALL
• Diamond-Blackfan syndrome (hereditary hypoplastic anemia)	19q13.2 and 8p23		Short stature, head and neck defects, maldevelopment of eyes, heart, visceral, organs and limbs, bifid thumb
• Chédiak-Higashi syndrome	*LYST*	Autosomal recessive	Partial oculocutaneous albinism, giant granules in neutrophils, monocytes, and lymphocytes, recurrent infections
• Cohen syndrome	*COH1*	Autosomal recessive	Mental retardation, postnatal microcephaly, facial dysmorphism, pigmentary retinopathy, myopia
• Cyclic neutropenia	*ELANE*	Autosomal dominant	Recurring episodes of severe neutropenia, usually every 21 days

WHIM warts, hypogammaglobulinemia, infections, and myelopathies

Morphology

Platelets show normal size and granularity. Bone marrow cellularity early in the course of disease may be normal with decreased or absent megakaryocytes; however, the cellularity decreases as the disease progresses. If not diagnosed early, many patients may present with pancytopenia and bone marrow aplasia.

Immunophenotype

Flow cytometric measurement of MPL (CD110) on platelets can help to distinguish between thrombocytopenia due to decreased megakaryocytes with a high plasma thrombopoietin (TPO) level or thrombocytopenia due to platelet destruction with a normal TPO level, but does not differentiate it from patients with a primary *MPL* defect. However, MPL (CD110) measured on CD34 + CD38lo/− progenitors is relatively independent from TPO plasma levels and can help in identifying conditions with impaired expression of MPL. Like other inherited BM failure syndromes, PNH test is recommended for patients at the first presentation of BM failure, in order to rule out PNH as well as to help in the differential diagnosis from idiopathic AA, which frequently has PNH clones.

Cytogenetics and Molecular Genetics

CAMT is an autosomal recessive disorder caused by homozygous or compound heterozygous mutations in *MPL* gene encoding the thrombopoietin (TPO) receptor. Nonsense mutations and deletions introduce premature stop codons that result in complete loss of the *MPL* function; other mutations (missense and splice-site mutations), though not completely defined, maintain some residual function of the TPO receptor function. There is evidence for genotype/phenotype correlation in patients with complete loss of the *MPL* function as they have permanently low platelet counts (CAMT I patients), while those with residual *MPL* activity demonstrate a transient amelioration of thrombocytopenia within the first year of life (CAMT II patients) [54]. The rare form of CAMT associated with radial-ulnar synostosis shows mutations in *HOXA11* gene that regulate megakaryocytic differentiation [35].

Chromosomal abnormalities are found in patients who develop bone marrow aplasia, MDS, and AML. The most common karyotype abnormalities are trisomy 8 and monosomy 7.

Differential Diagnosis

A diagnosis of CAMT is based on clinical and laboratory findings that are characterized as severe thrombocytopenia at birth due to ineffective megakaryocytopoiesis and no characteristic physical anomalies. The finding of germline *MPL* mutations helps to confirm the diagnosis, but it is not required because there are reports of patients with the characteristic clinical and laboratory findings of CAMT who do not have a *MPL* gene mutation [55].

Other causes must be ruled out, including prenatal causes such as vertical transmission of toxoplasma, rubella, cytomegalovirus, herpes, and other infections such as syphilis, varicella, parvovirus B19 (TORCH) infections, and neonatal allogeneic immune thrombocytopenia. The differential diagnoses also include *other inherited and acquired BM failure*.

Thrombocytopenia with Absent Radii

Thrombocytopenia with absent radii (TAR) is a rare genetic disorder presenting in newborns as thrombocytopenia and with characteristic absence of bilateral radii, but with presence of both thumbs. There is no predilection for gender.

Clinical Features

TAR is often diagnosed in utero or shortly after birth. The affected newborns present with thrombocytopenia and the characteristic physical finding of bilateral absent radii (unilateral in about 2% of cases). The thumbs are present, but may appear abnormal. Patients may have other birth defects such as hypoplastic ulnae, hypoplastic humeri, phocomelia, abnormal shoulders, bowed legs, hip dysplasia, abnormal knees, abnormal facies, renal malformations, and other findings. Gastroenteritis and cow's milk intolerance are frequent [6]. There is typically no anemia or leukopenia.

Approximately 60% of affected babies develop thrombocytopenia in the first few weeks of life. The thrombocytopenia is often severe with platelet counts less than 50×10^9/L [35]. There is bleeding tendency typically seen in the first years of life that can cause fatal hemorrhages. Platelet counts tend to increase over time, reaching normal values by adult life, although there may be fluctuation in the platelet count both in childhood and adult life. The bleeding tendency also improves with age. Early management of TAR is aimed at addressing the

major symptom, bleeding. Platelet transfusions are given as needed and usually are no longer required beyond the first year of life, when the platelet count improves. Early deaths are from bleeding, with a plateau of survival of 80% by age 1–2 years. HSCT is rarely required [6]. Leukemoid reaction has been reported and is usually transient. The development of aplastic anemia has not been observed, but rare cases of AML and ALL have been reported [2].

Morphology

The peripheral smear shows thrombocytopenia with normal platelet morphology. Other cell lines are unaffected. Bone marrow examination is often not required. Bone marrow megakaryocytes may be decreased, absent, or immature appearing, small sized, and with basophilic, vacuolated cytoplasm. Erythropoiesis and granulopoiesis are often normal (Fig. 3.6).

Immunophenotype

Flow cytometry assay for platelet activation markers such as CD62p, CD63, PAC-1, mepacrine, and glycoprotein CD41, CD42b, and CD61 [56] shows combined defect of platelet production and function in thrombocytopenia with absent radii syndrome [57].

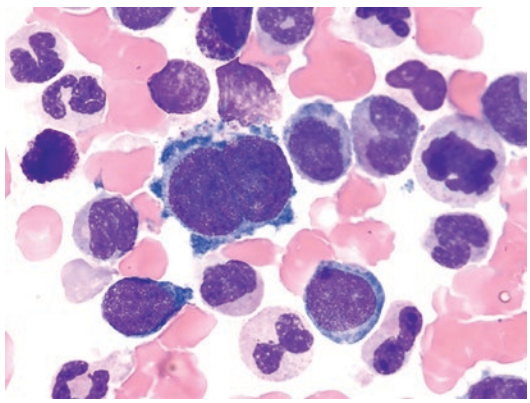

Fig. 3.6 Bone marrow aspirate of thrombocytopenia with absent radii (TAR). Megakaryocytes are often decreased, absent, or immature, with small, basophilic (center), and vacuolated cells. Erythropoiesis and granulopoiesis are often normal (courtesy of Julia Geyer, MD, Cornell Medical Center)

Cytogenetics and Molecular Genetics

TAR is inherited in an autosomal recessive fashion. Recently, a combination of comparative genomic hybridization and next-generation sequencing revealed a 200 kb microdeletion of chromosome 1q21.1 in one allele and two single-nucleotide polymorphisms (SNP) in *RBM8A* gene in the non-deleted allele. The microdeletion of 1q21.1 either is passed down from one parent (75% of cases) or occurs de novo (25% of cases). The Y14 subunit of the exon–junction complex encoded by *RBM8A* is less expressed on platelets of individuals with TAR, suggesting that the two noncoding SNPs are hypomorphic mutations affecting regulatory regions of the *RBM8A* gene [54]. Genetic testing for the 200 kb minimally deleted chromosomal band on chromosome 1q21.1 and *RBM8A* variant may aid in diagnosis [35].

Differential Diagnosis

It is important to distinguish TAR from the other inherited BM failure syndromes such as *Fanconi anemia,* since the clinical course and risks are very different; FA should be ruled out by testing chromosomal breakage. Malformations on physical exam, including hypopigmented spots, abnormality of thumbs, microcephaly, café au lait spots, and urogenital abnormalities and short stature, are seen in 60–70% of patients with FA.

Congenital amegakaryocytic thrombocytopenia can result in severe neonatal thrombocytopenia. The BM shows near absence of megakaryocytes. Unlike TAR, CAMT usually does not have characteristic congenital physical abnormalities. Pancytopenia develops in later childhood. This disorder is caused by homozygous or compound heterozygous mutations in *MPL* gene encoding the thrombopoietin (TPO) receptor.

Congenital platelet disorders including Wiskott-Aldrich syndrome, May-Hegglin anomaly, Bernard-Soulier syndrome, and Alport syndrome often show characteristic alterations in platelet size and morphology. Wiskott-Aldrich syndrome is caused by *WAS* gene mutations, mostly X-linked. WAS should be suspected in male patient presenting with bleeding disorder with congenital or early-onset thrombocytopenia associated with small platelet size (MPV 3–5 fL,

normal 7–10 fL). May-Hegglin anomaly is an autosomal dominant trait characterized by giant platelets (MPV 30–80 fL), often with moderate thrombocytopenia, and leukocyte inclusion bodies (Dohle-like bodies). Bernard-Soulier syndrome is an autosomal recessive disorder of platelet function presenting with mild thrombocytopenia, circulating "giant" platelets, and bleeding. In contrast to these disorders, the platelet size in TAR is normal.

GATA1 Mutation

Mammalian genomes encode six structurally related members of the GATA family, which act as tissue-specific master transcriptional regulators. GATA1 and GATA2 play critical roles in hematopoiesis and the mutations/deficiency in their respective genes produces several hematologic disorders. *GATA1* is an X-linked gene that is required for the development of erythroid cells, megakaryocytes, mast cells, eosinophils, and basophils. GATA2 expression is essential for maintenance of the pool of hematopoietic stem cells (HSCs), whereas GATA1 drives HSCs towards erythroid/megakaryocytic differentiation, leading to a loss of self-renewal capacity. During the differentiation of HSCs, through a phenomenon known as "the GATA switch," GATA2 activates GATA1, and then GATA1, in turn, represses GATA2 while displacing it from chromatin at various sites throughout the genome. The HSC compartment is therefore extremely sensitive to levels of GATA2{Crispino, 2017 #19}.

Inherited GATA1 mutations affect mostly erythroid and megakaryocytic maturation and may result in X-linked CDA, DBA, X-linked macrothrombocytopenia, X-linked thrombocytopenia with B-thalassemia, and occasional cases of inherited macrocytic anemia with mild neutropenia. Acquired GATA1 mutation has been associated with transient abnormal myelopoiesis in patients with trisomy 21. Recent studies suggest that GATA1 biology may play a role in some cases of primary myelofibrosis{Crispino, 2017 #19}.

Myeloid Neoplasms with Germline Predisposition

"Myeloid neoplasms with germline predisposition" is a new addition in the 2016 revision of the WHO classification. Such familial cases are further divided into three categories: (1) myeloid neoplasms with germline predisposition without a preexisting disorder or organ dysfunction; (2) myeloid neoplasms with germline predisposition and preexisting platelet disorders; and (3) myeloid neoplasms with germline predisposition and other organ dysfunction. The entities under each category are summarized in Table 3.5. These syndromes are very rare [58, 59]; however, they are likely under-recognized and underreported, especially in cases without physical anomalies and cases that do not yet have well-defined molecular genetic abnormalities.

Myeloid Neoplasms with Germline Predisposition Without a Preexisting Disorder or Organ Dysfunction

Myeloid Neoplasms with *CEBPA* Mutation

This is an autosomal dominant disorder caused by a single copy of germline mutation in the granulocytic differentiation factor gene *CEBPA* on chromosome 19q13 [60, 61]. The germline mutation is usually found in the N-terminal end of the gene and a somatic mutation at the C-terminal end of the other allele acquired at the time of progression to AML. The penetrance is close to 100%. Of note, approximately 10% of AML cases with biallelic *CEBPA* mutations represent a somatic mutation occurring on a background of a germline mutation. Thus, patients presenting with AML with biallelic *CEBPA* mutation are candidates for germline *CEBPA* mutation screening [62].

Patients present with AML at a young age and often do not have preceding hematologic abnormalities or an antecedent MDS phase. This disorder is not associated with non-hematologic manifestations. However, these patients usually have a family history of AML. The clinicopatho-

Table 3.5 Classification of myeloid neoplasms with germline predisposition

Myeloid neoplasms with germline predisposition without a preexisting disorder or organ dysfunction
• AML with germline *CEBPA* mutation
• Myeloid neoplasms with germline *DDX41* mutation
Myeloid neoplasms with germline predisposition and preexisting platelet disorders
• Myeloid neoplasms with germline *RUNX1* mutation
• Myeloid neoplasms with germline *ANKRD26* mutation
• Myeloid neoplasms with germline *ETV6* mutation
Myeloid neoplasms with germline predisposition and other organ dysfunction
• Myeloid neoplasms with germline *GATA2* mutation
• Myeloid neoplasms associated with BM failure syndromes
• Myeloid neoplasms associated with telomere biology disorders
• JMML associated with neurofibromatosis, Noonan syndrome, or Noonan syndrome-like disorders
• Myeloid neoplasms associated with Down syndrome

logical features are similar to sporadic biallelic *CEBPA*-mutated AML. Morphologically, mostly cases are AML with or without maturation, sometimes with Auer rods. Immunophenotypically, there is frequent aberrant CD7 expression on blasts. The karyotype is usually normal. Overall, AML with germline *CEBPA* mutation has a favorable prognosis [61]. However, the somatically acquired *CEBPA* may be unstable and novel independent leukemic clones may develop at disease recurrence [63].

Myeloid Neoplasms with Germline *DDX41* Mutation

DDX41 (DEAD/H-box helicase gene), located on chromosome 5q35.3, encodes an RNA helicase protein with a function in RNA splicing. DDX41 has been shown to contribute to multiple pathways and processes including mRNA splicing, innate immunity, and rRNA processing. Thus, mutations in *DDX41* potentially impact the initiation, maintenance, or progression of tumors [64]. *DDX41* mutations have been found in about 1.5% of myeloid neoplasms [66]. Similar to *CEBPA*, many patients with *DDX41* mutations have biallelic mutations. However, the frequency of one mutation being germline is about 50% of cases. The penetrance appears to be high. An autosomal dominant familial MDS/AML syndrome due to germline mutations in *DDX41* [65] has recently been recognized.

Patients with germline *DDX41* mutations have a late-onset presentation, with a median age of 62 years [40–85], similar to de novo MDS/AML with *DDX41* mutations. The neoplasms reported are mainly MDS, including MDS with multilineage dysplasia, MDS with excess blasts, and MDS with isolated del(5q), followed by AML. Chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), and lymphomas have also been reported [65]. Three families were found to be predisposed to immune disorders such as systemic lupus, eczema, vasculitis, and asthma. Aside from the most frequent acquired mutation of R525H on the second *DDX41* allele, several recurrent cooperating mutations in other genes have been identified, including *ASXL1, DNMT3A, JAK2, LUC7L2, SETBP1,* and *TP53,* especially during clonal evolution. Due to the lack of a clear premalignant phenotype and a typically late age of onset, the family history is easily overlooked by patients as well as treating physicians.

Patients with germline *DDX41* mutation who develop MDS/AML usually present with leukopenia or macrocytic anemia. The bone marrow is often hypocellular with marked dyserythropoiesis and increased blasts; a subset of cases presents with pure erythroid leukemia (PEL). Seventy percent of cases have a normal karyotype. The prognosis is generally poor, but early data suggest that patients may respond to lenalidomide.

Myeloid Neoplasms with Germline Predisposition and Preexisting Platelet Disorders

Myeloid Neoplasms with Germline *RUNX1* Mutation

RUNX1-associated MDS/AML is an autosomal dominant disorder caused by germline mutation in *RUNX1* on chromosome 21 [67]. RUNX1 is a transcription factor that plays a key role in megakaryocyte maturation, differentiation, and pro-platelet formation. *RUNX1* mutations are distributed throughout the gene. Numerous mutations have been identified; each mutation is usually unique to a given kindred.

The clinical presentation is variable, even within the same family. Patients classically present with lifelong mild-to-moderate thrombocytopenia (platelets $>50 \times 10^9$/L), which may be misdiagnosed as chronic immune thrombocytopenia (ITP). Platelets are morphologically normal, but often exhibit impaired function. Affected family members have varying risks of progression to myeloid neoplasms (range 11–100%, median 44%), with a median age onset of 33 years [68]. Progression may be associated with a second *RUNX1* mutation or acquisition of trisomy 21, but these are not required [62]. Other additional acquired abnormalities, including *CBL* and *ASXL1* mutations and loss of *NF1*, are reported. MDS and AML are the most common hematologic neoplasms. CMML, T-lymphoblastic leukemia, and rarely B-cell neoplasms including hairy cell leukemia are reported. Prognosis is uncertain due to limited long-term outcome data.

The bone marrow tends to be hypocellular for age. Abnormal megakaryopoiesis is described in both carriers and in patients with MDS/AML, characterized by micromegakaryocytes, bare megakaryocytic nuclei, and asynchronous nuclear cytoplasmic maturation (Fig. 3.7). Erythropoiesis and granulopoiesis are often unremarkable or only exhibit mild dyspoietic features. However, when MDS/AML progression occurs, patients often develop bi- or pancytopenia rather than mild thrombocytopenia, and the bone marrow and peripheral blood show multilineage dysplasia and often increased blasts (Fig. 3.8).

Myeloid Neoplasms with Germline *ANKRD26* Mutation

Mutations in ankyrin repeat domain 26 gene (*ANKRD26*) located on chromosome band 10p12.1 are inherited in autosomal dominant fashion; the mutations often occur in the 5′-untranslated region of *ANKRD26*, leading to impaired pro-platelet formation by megakaryocytes and abnormal megakaryocyte maturation. This disorder constitutes approximately 10% of inherited thrombocytopenias [69] and is characterized by normal platelet size and no or mild bleeding tendency. Patients usually have moderate thrombocytopenia, but some may have severe thrombocytopenia with a platelet count $<20 \times 10^9$/L [70]. HGB level and leukocyte count are usually normal, but some patients may have a high white blood cell count and/or high HGB level. Most patients have glycoprotein Ia and α-granule deficiency, but platelet aggregation studies are often normal. One large study has shown that approximately 8% of patients developed myeloid malignancies, including AML, MDS, and CML [71].

The bone marrow in patients without leukemia shows an increased number of dysplastic megakaryocytes [70, 71]. The megakaryocytes are small with monolobated or bilobed nuclei and some may have abnormal eosinophilic cytoplasm (Fig. 3.9). Serum TPO levels are elevated.

Myeloid Neoplasms with Germline *ETV6* Mutation

Familial *ETV6* mutations are characterized by autosomal dominantly inherited thrombocytopenia and an increased risk for hematological malignancies [72, 73]. *ETV6* is a transcriptional repressor involved in embryonic development and hematopoietic regulation, particularly the late phases of megakaryopoiesis. Germline

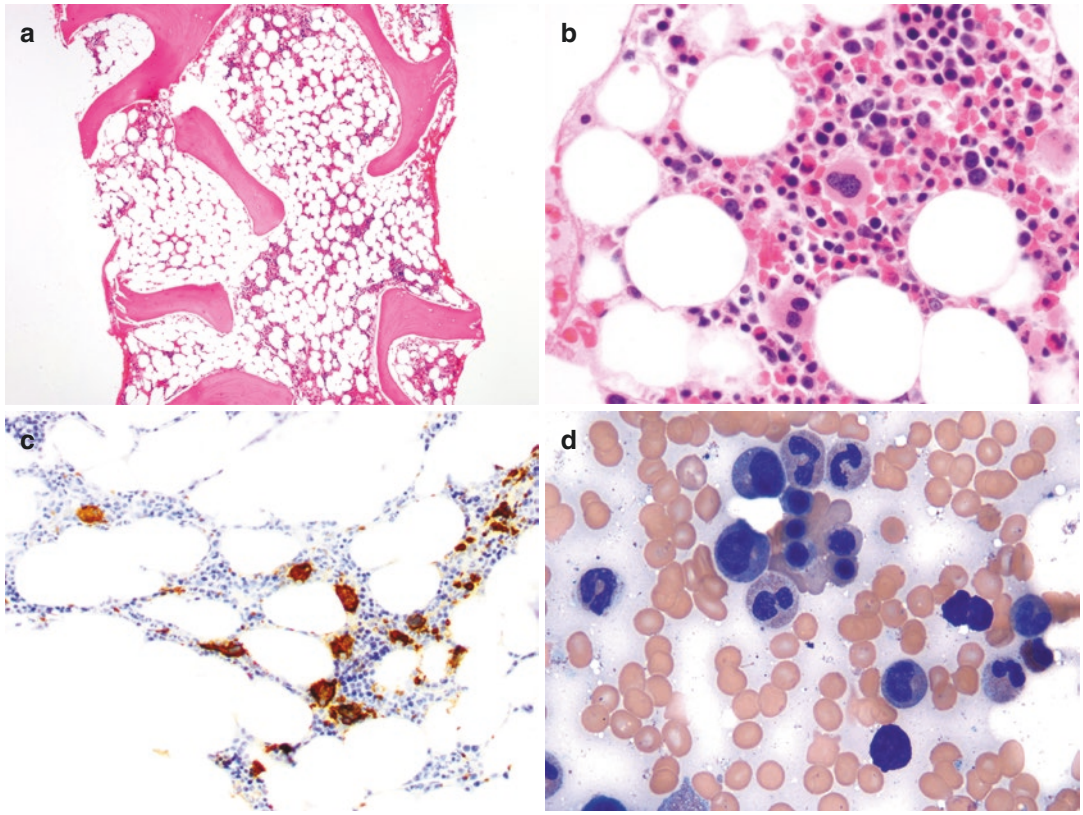

Fig. 3.7 Bone marrow biopsy from an asymptomatic 23-year-old patient with germline *RUNX1* mutation and mild thrombocytopenia. The bone marrow shows a hypocellularity for age (**a**); megakaryocytes are small and hypolobated (**b**); small dysplastic megakaryocytes are highlighted by CD42b immunohistochemistry stain (**c**); Bone marrow aspirate smear shows no significant dyserythropoiesis or dysganulopoiesis (**d**) (courtesy of Julia Geyer, MD, Cornell Medical Center)

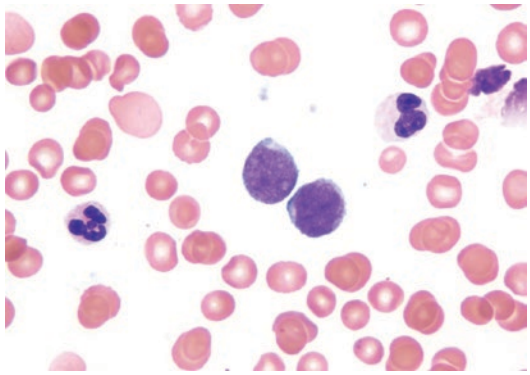

Fig. 3.8 Peripheral blood smear from a patient with germline *RUNX1* mutation who developed acute myeloid leukemia. There are circulating blasts, and dysgranulopoiesis (courtesy of Rashimi Kanagal, MD, MDACC)

ETV6 mutations account for 3–5% of cases of inherited thrombocytopenia [69]. Thrombocytopenia is often associated with mild-to-moderate bleeding tendency, occasionally presenting in infancy. Platelets are normal in size. Red cells may show macrocytosis. Germline *ETV6* mutation has been associated with a high frequency of hematologic malignancy, occurring in 25–40% of individuals, with the majority of cases being B-lymphoblastic leukemia (B-ALL), followed by MDS/AML, mixed-phenotype leukemia, CMML, and plasma cell myeloma. The B-ALL is often hyperdiploid [74]. Non-hematological neoplasms have also been reported in these families, including colorectal,

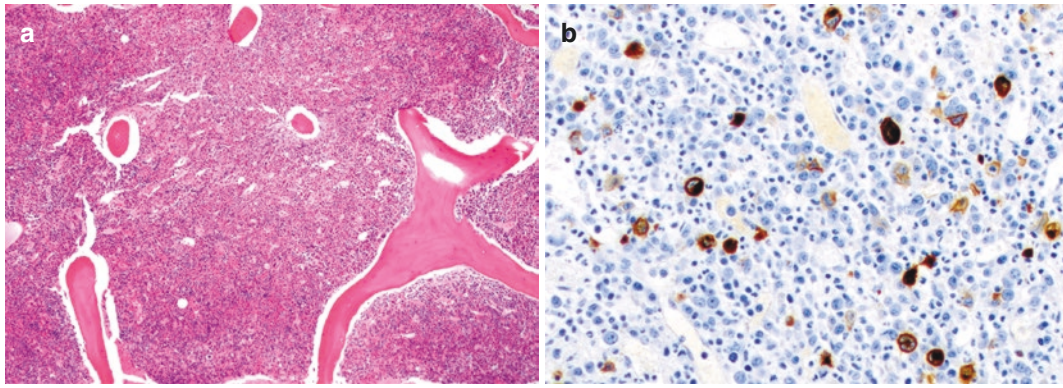

Fig. 3.9 Germline *ANKRD26* mutation in a patient with moderate thrombocytopenia. The bone marrow cellularity is high (**a**) showing increased number of megakaryocytes. Megakaryocytes are small and hypolobated, as being highlighted by CD42 stain (courtesy of Julia Geyer, MD, Cornell medical center). This case illustrates the difficulty in the diagnosis of myeloid neoplasm (MDS) progression in patients with germline predisposition and preexisting platelet disorders

kidney, and skin cancers. Of note, somatic mutation in *ETV6* is also detected in a wide variety of hematologic neoplasms [69].

The bone marrow from patients without leukemia often shows small, hypolobulated megakaryocytes and mild dyserythropoiesis.

Myeloid Neoplasms with Germline Predisposition Associated with Other Organ Dysfunction

Myeloid Neoplasms with Germline *GATA2* Mutation

GATA2 expression is essential for maintenance of the pool of hematopoietic stem cells (HSCs). GATA2 was first identified as an endothelial transcription factor and its haploinsufficiency causes abnormal vascular development. It has been shown that GATA2 is also required for the development of the semicircular canals and the perilymphatic space of the vestibular system [75]. GATA2 deficiency syndrome is an autosomal dominant disorder caused by heterozygous germline mutations, and in some cases large deletions, resulting in haploinsufficiency of *GATA2*. Germline mutations appear to arise spontaneously, but are then transmitted with autosomal dominant inheritance [75]. Mutations are identified in both coding and noncoding regions and there is no significant association between the specific mutation and clinical manifestations.

Earlier reports have described four clinical syndromes in association with GATA2 deficiency: (1) monocytopenia and atypical mycobacterial infection (MonoMAC syndrome); (2) dendritic cells, monocyte, B, and NK lymphoid deficiency; (3) familial MDS/AML; and (4) Emberger syndrome (primary lymphedema, warts, and a predisposition to MDS). More studies have also reported phenotypes of severe congenital neutropenia, aplastic anemia, chronic myelomonocytic leukemia, severe EBV infection, and EBV-associated neoplasms. Patients with GATA2 deficiency may develop non-hematological and noninfectious manifestations, such as deafness, pulmonary alveolar proteinosis, thrombosis, hypothyroidism, miscarriage, and autoimmune disorders.

Hematologic manifestations include peripheral cytopenias, particularly B- and NK-cell lymphopenia, monocytopenia (80% of cases), decreased CD4 count, and neutropenia (50% of cases). Some patients may present with MDS/AML without prior significant hematologic manifestations. MDS is extremely common in GATA2 deficiency, occurring in over 70% of patients at a median age of 29 years. The dysplasia in MDS manifests predominantly in megakaryocytes, which commonly show widely separated nuclear lobes. AML and CMML develop in approximately 15% of patients with GATA2 deficiency. Progression to MDS/AML is commonly associated with acquisition of secondary mutations in the gene encoding chromatin-

binding protein *ASXL1* [76, 77]. A recent European study has shown that inherited *GATA2* gene mutations account for 7% of all primary pediatric MDS; the vast majority of adolescents with MDS and monosomy 7 carry an underlying GATA2 deficiency [78]. However, many of them are mistaken as sporadic disease, since a family history of myeloid leukemia or GATA2-related symptoms is often obscure. Of note, the presence of these mutations does not appear to influence survival.

The bone marrow cellularity is often decreased and may show features of aplastic anemia (Fig. 3.10). Prominent dysmegakaryopoiesis is present, including micromegakaryocytes and megakaryocytes with widely separated nuclear lobes. Dysplastic features are often seen in erythroid and granulocytic cell lines as well. Increased reticulin fibrosis is common. Despite the absence of monocytes in the peripheral blood, bone marrow histiocytes and macrophages are often prominent [79].

Flow cytometry (Fig. 3.11) shows reduced BM NK cells and B cells, monocytopenia, and immunophenotypical alterations of CD34+ myeloblasts. The latter is similar to what is often observed in MDS myeloblasts. Granulopoiesis shows an abnormal maturation pattern. Plasma cells often show aberrant CD56 expression and loss of CD19 [79]. An atypical LGL population or LGL expansion is also common. These features may help identify the presence of *GATA2* mutation among patients who present with what appear to be "idiopathic" aplastic anemia.

Clonal cytogenetic abnormalities are identified in around 60% of patients, with monosomy 7 and trisomy 8 being the most frequent abnormalities.

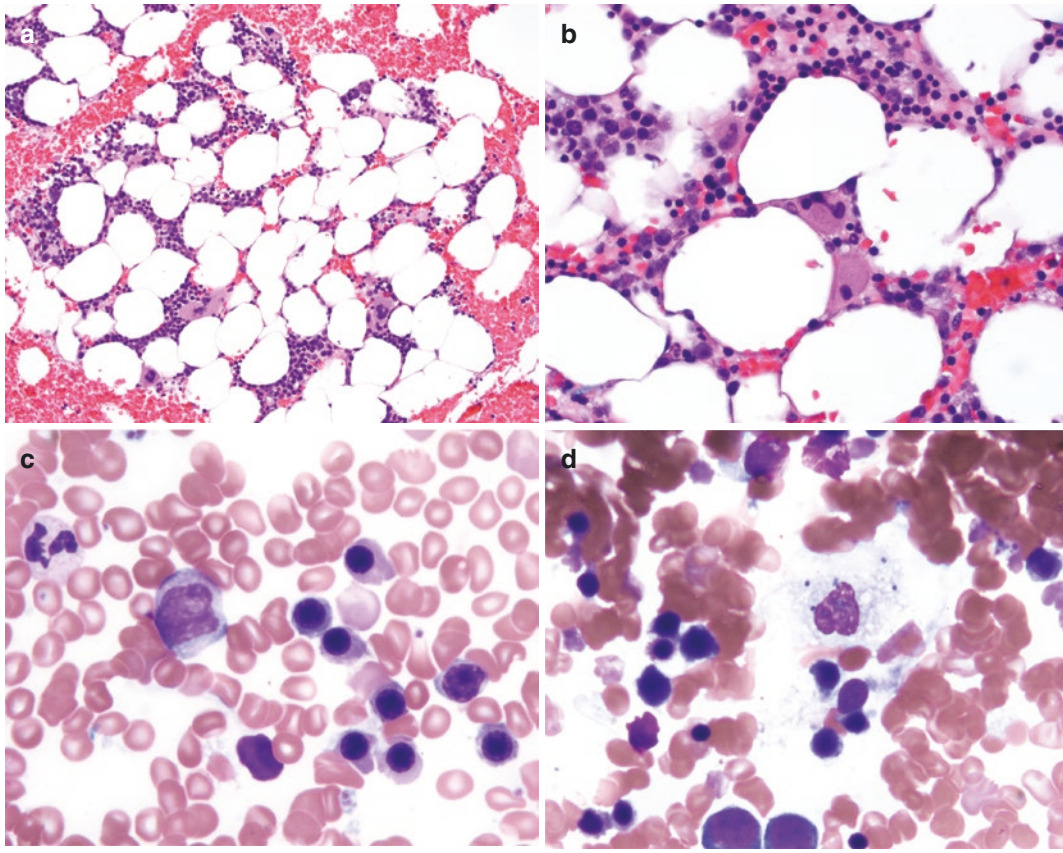

Fig. 3.10 Germline *GATA2* mutation in a 20-year-old patient with no significant cytopenia but a history of recurrent viral and bacterial infection. The bone marrow cellularity is significantly low, resembling aplastic anemia; (**a**) atypical megakaryocytes are present with widely separated nuclear lobes (**b**). There is erythroid predominance with markedly reduced myeloid lineage cells. But there is no dyserythropoiesis and no dysgranulopoiesis (**c**). Despite monocytopenia, histiocytes and macrophages are often present, in some cases, they are even increased (**d**)

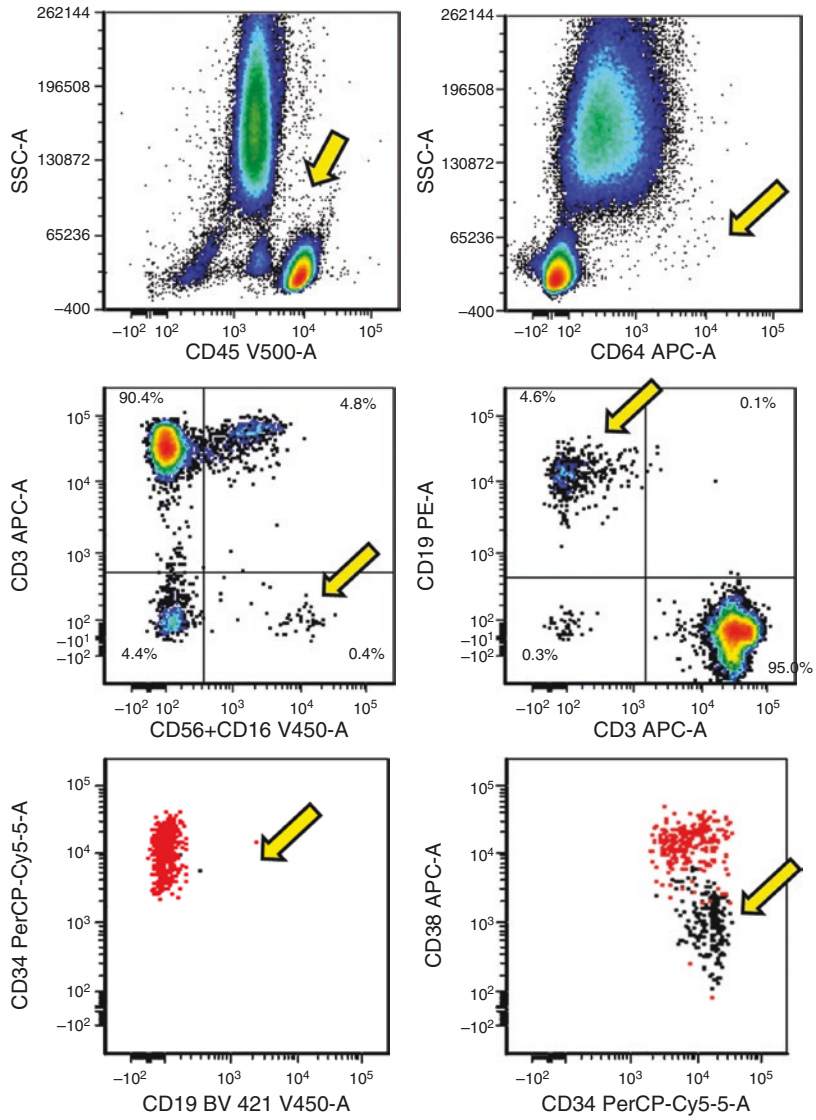

Fig. 3.11 Germline *GATA2* mutation in a 20-year-old patient with no significant cytopenia but a history of recurrent viral and bacterial infection. Upper: Peripheral blood and bone marrow both show nearly absence of monocytes (arrows) as demonstrated on CD45/SSC as well as by CD64. Middle: NK cells are markedly reduced, only 0.4% of total lymphocytes. B cells are also low, 4.6% of total lymphocytes. Lower: Of the bone marrow CD34+ cells, hematogones are absent; and a subset of myeloblasts exhibit aberrantly low CD38 expression

Myeloid Neoplasms Associated with Bone Marrow Failure Syndromes and Telomere Biology Disorders

This group includes the entities that have been extensively discussed in the first part of this chapter, including Fanconi anemia, severe congenital neutropenia, Shwachman-Diamond syndrome, Diamond-Blackfan anemia, dyskeratosis congenita, and related short telomere syndromes. Patients with these congenital syndromes need to be closely monitored due to the increased risk of myeloid neoplasms. It is noteworthy that the phenotypes of these disorders are highly variable; in some cases, patients may not be diagnosed until adulthood. MDS and AML are the most common hematological neoplasms, with some cases of lymphoblastic leukemia being reported [80].

Differential Diagnosis of Myeloid Neoplasms with Germline Predisposition

Myeloid neoplasms with germline predisposition are relatively obscure and remain underdiagnosed by pathologists and clinicians. In patients with myeloid neoplasms with germline predisposition and preexisting platelet disorders, the preexisting platelet disorders are frequently misdiagnosed as *chronic immune thrombocytopenia* (ITP) clinically. ITP is an acquired form of thrombocytopenia due to autoantibody-mediated destruction of platelets. Bone marrow examination is usually not required for diagnosis and is only performed for patients with other unexplained cytopenias (anemia, leukopenia), abnormalities on the peripheral blood smear, or clinically refractory disease. When performed, the BM in TIP shows a normal cellularity with increased, normal-appearing megakaryocytes. In contrast, the thrombocytopenia in myeloid neoplasm with germline predisposition is often associated with dysplastic megakaryocytes and a hypocellular BM.

The biggest challenge is diagnosing *MDS progression in patients with germline mutations*. The morphological distinction between the baseline abnormal marrow morphology and MDS can be difficult, due to the lack of an extensive description of the bone marrow findings in disease carriers. For example, congenital thrombocytopenia with germline *GATA2* and *ETV6* mutations shows abnormal megakaryopoiesis in the BM, similar to MDS dysmegakaryopoiesis. Although the criteria for MDS progression in this setting have not yet developed, a number of features have been suggested and should raise the concern for progression to MDS [81]: (1) development of anemia and/or neutropenia, in addition to the thrombocytopenia, with the exclusion of any nonneoplastic causes; (2) dysplasia in erythroid and/or myeloid precursors in the BM addition to dysmegakaryopoiesis; (3) an acquired clonal cytogenetic or molecular genetic abnormality; and (4) older patient age.

The same germline mutations can also occur as somatic acquired mutations in patients with de novo *MDS/AML*. Therefore, finding a pathogenic mutation is not sufficient for diagnosis; it is necessary to confirm that the abnormality is germline, preferably with skin biopsy testing. It is noteworthy that over half of patients with hereditary myeloid neoplasms have mutations that are as of yet unknown.

In summary, in practice, if a case is classic for a particular syndrome with typical physical anomaly or has a very-early-onset isolated cytopenia (either thrombocytopenia or anemia or severe neutropenia) typical of a particular syndrome, the test panel can be more selective for targeted abnormalities to confirm the diagnosis. For example, if flow FISH for telomere length is significantly shortened in a patient with mucocutaneous abnormalities, a diagnosis of dyskeratosis congenita can be easily established. However, many patients with inherited BM failure may not have associated classic presentation or some simply no physical abnormalities, and furthermore, there is substantial phenotypic overlapping of these congenital BM disorders. There is also significant overlapping with acquired BM failure. Pediatric patients with cytopenias may need a full workup that includes flow FISH for telomere length, DEB chromosomal breakage testing, hepatitis serological studies, PNH flow cytometry, parvovirus and other virus testing, autoimmune or autoantibody workup, etc. If unrevealing, a large gene panel may be tested to screen for underlying germline or somatic mutations.

Myeloid Proliferations Associated with Down Syndrome

Individuals with Down syndrome (DS) have a 10–100-fold increased risk of leukemia compared with non-DS individuals [82, 83]. There is an approximately 150-fold increase of AML in DS children <5 years of age and more than 50% of these neoplasms are acute megakaryoblastic leukemia (AMKL). In contrast, AMKL comprises only 3–6% of AML in non-DS children [84]. Transient abnormal myelopoiesis (TAM) affects 10% of DS neonates and 20–30% of these infants eventually develop AML in 1–4 years [85]. TAM may be morphologically and immunophenotypically indistinguishable from AMKL in DS. Due to the unique features of these

disorders, they are recognized as myeloid proliferations associated with DS.

It is noteworthy that there is overall increased risk of all types of leukemia in DS, including both lymphoblastic leukemia and other types of AML. The WHO recommends that these cases should not be automatically classified as myeloid leukemia associated with Down syndrome if they meet the diagnostic criteria for leukemia not associated with Down syndrome. Similar to any other types of acute leukemia, morphological, immunophenotypic, cytogenetic, and molecular proper characterization of leukemia must be performed to ensure proper diagnosis and right treatment.

Clinical Features

Transient abnormal myelopoiesis (TAM) associated with DS occurs in approximately 10% DS infants, and does not usually affect babies with mosaic trisomy 21. It typically occurs at 3–7 days of life. The clinical presentations of TAM are highly variable [85–87], ranging from asymptomatic (about 25%) to life threatening (25%). Patients often present with leukocytosis with circulating blasts and thrombocytopenia. Hepatosplenomegaly occurs in about 50–60% of patients. The severe forms show hydrops fetalis, hyperleukocytosis (>100 × 10^9/L), liver failure, cardiopulmonary failure, and pericardial and pleural effusions. Patients also have a wide range of physical and laboratory abnormalities at the time of diagnosis. In most patients, the process undergoes spontaneous remission within the first 3 months of life. Patients with life-threatening disease may have fatal clinical complications. Approximately 20–30% of the patients with TAM go on to develop AML within the first 4 years of life [88].

Acute myeloid leukemia associated with DS: About one-third to two-thirds of AML associated with DS is often preceded by a myelodysplastic syndrome (MDS)-like phase [89]. There are no biological differences nor are prognostic or therapeutic implications between MDS and overt AML; therefore, "myeloid leukemia associated with Down syndrome" includes both MDS and AML. It is noteworthy that some myeloid leukemia-DS cases have no documented history of TAM [86].

The MDS phase manifests as progressive thrombocytopenia followed by anemia. Neutropenia is not common. Some patients develop hepatomegaly and liver failure due to fibrosis and disease progression. AMKL develops during the first 4 years of life, with a median age onset of 2 years.

Unlike non-DS patients with AMKL who generally have a poor prognosis, DS patients with AML show excellent clinical outcome when treated with reduced-intensity chemotherapy protocols [90]. Myeloid leukemia in older children with DS with *GATA1* mutation has an inferior prognosis and the treatment outcome is comparable to *GATA1*+ AML in non-DS patients.

Morphology

There are the striking similarities between TAM and AML in DS children. Peripheral blood from both TAM and AML often shows circulating blasts with basophilic cytoplasm and cytoplasmic blebbing suggestive of blasts with megakaryoblastic differentiation (Fig. 3.12). TAM often presents with moderate leukocytosis with a WBC of 28–40 × 10^9/L). Twenty to thirty percent of patients present with hyperleukocytosis (WBC >100 × 10^9/L). HGB and platelet count are only mildly decreased or normal. Some patients may have peripheral blood basophilia. The bone marrow blast infiltrate (Fig. 3.13) in TAM may be less impressive than PB, with a lower number of blasts. Dyserythropoiesis and dysmegakaryopoiesis are often present in the bone marrow (Fig. 3.13). In patients with trisomy 21 mosaicism, AML with megakaryocytic differentiation may be the first presentation that leads to a diagnosis of Down syndrome (Fig. 3.14).

In patients with myeloid leukemia, the MDS-like phase often shows no increase in blasts with features similar to MDS-refractory cytopenia of childhood. Erythroid and megakaryocytic dyspla-

sia is often more pronounced than that of cases with an initial presentation of AML. In the bone marrow aspirate, the blasts often have round to slightly irregular nuclear contours and a moderate amount of basophilic cytoplasm; cytoplasmic blebs may be present. The blasts may contain basophilic granules that are MPO negative. Erythroid precursors often show megaloblastic changes and dysplastic features. Dysgranulopoiesis may be present. Fibrosis is frequently present.

Immunophenotype

The immunophenotype of leukemic blasts in TAM and AMKL is very similar [87, 91, 92]. In most of the cases, the blasts are positive for CD34, CD117, myeloid antigen CD13, and CD33, CD11b, megakaryocytic marker CD42, CD36, CD41, CD61, and frequent aberrant expression of CD7, CD56, and CD4, and they are negative for MPO, HLADR, CD15, CD14, and glycophorin A. Of note, this immunophenotype is different from AMKL in

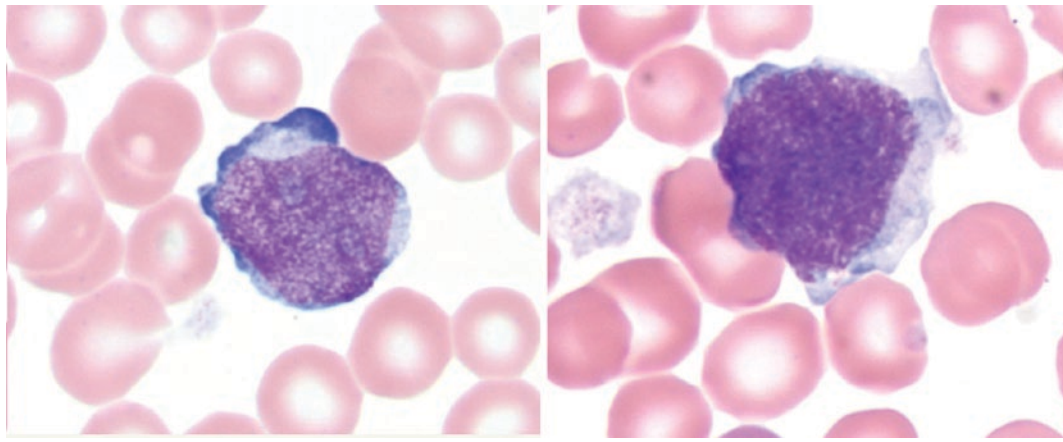

Fig. 3.12 Peripheral blood smear of transient abnormal myelopoiesis (TAM) in a neonate with Down syndrome. Circulating blasts are detected in the first week of life. The blasts are large with basophilic cytoplasm and cytoplasmic blebbing (courtesy of Julia Geyer, MD, Cornell Medical Center)

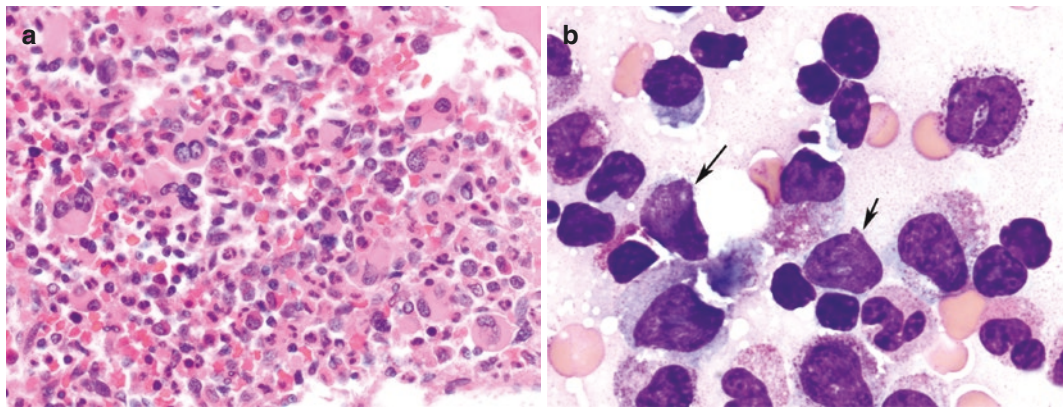

Fig. 3.13 Bone marrow of transient abnormal myelopoiesis (TAM) in a neonate with Down syndrome. Bone marrow biopsy (**a**) is hypercellular showing a proliferation of abnormal megakaryocytes. The megakaryocytes have widely separated nuclear lobes; some are small. There is retained granulopoiesis. On bone marrow aspirate smear (**b**), there are a few blasts (arrows), less impressive than the peripheral blood (Fig. 3.12). Many granulocytes and precursors are seen, with abundant cytoplasm and azurophilic and some basophilic granules (courtesy of Julia Geyer, MD, Cornell Medical Center)

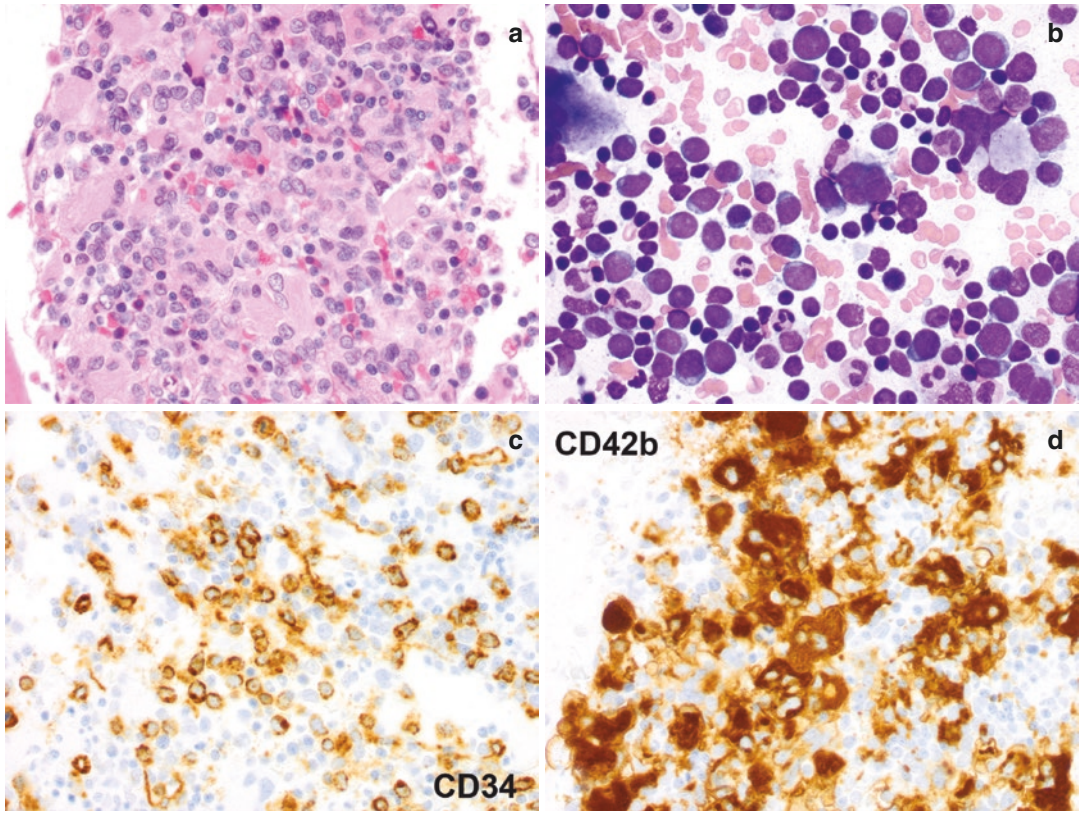

Fig. 3.14 Acute myeloid leukemia in a patient with constitutional trisomy 21 mosaicism. A 1-year-old child presented with acute leukemia. Bone marrow biopsy (**a**) is hypercellular showing a proliferation of abnormal megakaryocytes. The megakaryocytes are large with widely separated nuclear lobes. On bone marrow aspirate smear (**b**), there are many blasts, medium to large with very scant cytoplasm. The blasts are CD34+ (**c**) by immunohistochemistry. CD42b highlights large megakaryocytes, and a few monolobated forms/blasts (**d**). The patient was found to be mosaic for trisomy 21 (courtesy of Julia Geyer, MD, Cornell Medical Center)

adults that are often negative for CD117, CD13, and CD33. In AML associated with DS, about half of the cases are reported to be CD34 negative. The blasts may not express all megakaryocytic markers or only weak or partially express some of the markers (Fig. 3.15), especially with CD41. CD56 is less likely to be positive in AMKL comparing to TAM blasts. Immunohistochemical stains with CD41, CD42b, and CD61 may be particularly useful for identifying cells of megakaryocytic differentiation (Fig. 3.14), especially in cases with significant fibrosis.

It is noteworthy that in a subset of cases, the leukemia blasts do not show apparent megakaryoblastic differentiation. Megakaryocytic differentiation is not required for the diagnosis.

Cytogenetics and Molecular Genetics

GATA1 mutations are common in both TAM and AML, occurring in nearly 100% of cases [93, 94]. *GATA1* mutations are overall detected in about 3–5% of DS neonates by Sanger sequencing. Recently, targeted next-generation sequencing (NGS) has shown frequent (about 20%) low abundance of *GATA1* mutant clones in DS neonates; many of these neonates had no clinical features of TAM [95], but *GATA1* mutation might confer a 5–10% risk of subsequently developing AML.

Additional cytogenetic changes apart from the constitutional trisomy 21 can be seen in both TAM and AML, but are more frequent in AML (70–80% versus 20–30%) [96, 97]. Trisomy 8 is a common

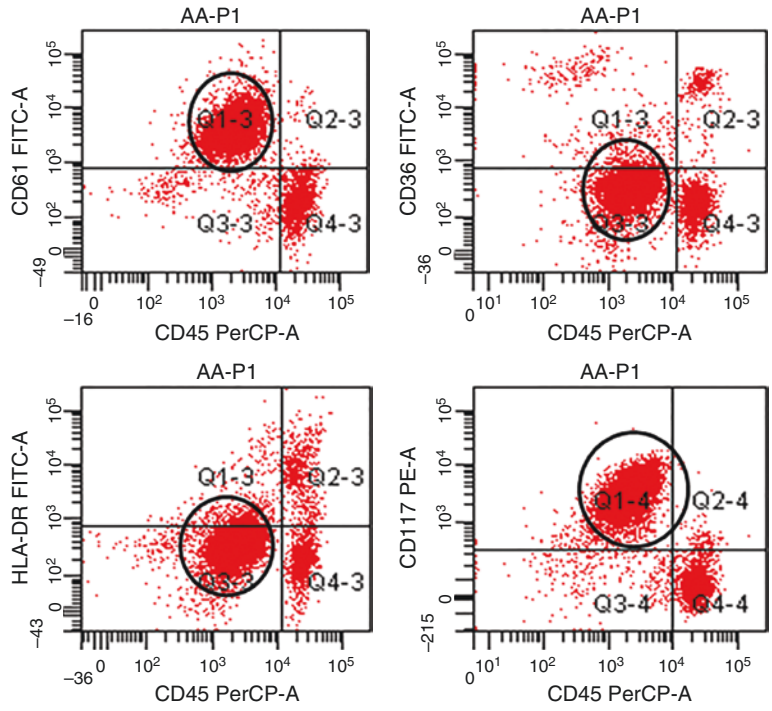

Fig. 3.15 Acute megakaryoblastic leukemia in a patient with Down syndrome. A 2-year-old child presented with acute leukemia. Flow cytometry shows that the blasts are CD61+, but CD36 negative, negative for HLADR, and positive for CD117 (courtesy of Silvia T. Bunting, MD, Children Health Care of Atlanta)

cytogenetic abnormality in AMKL syndrome, occurring in 13–44% of patients but monosomy 7 is very rare. Whole-genome sequencing data has shown that DS-related myeloid proliferations reflect a sequential acquisition of new mutations and clonal selection [98]. *GATA1* mutation appears to initiate the TAM phase and this proliferation may be further driven by mutations in cohesin *CTCF, EZH2,* or other epigenetic regulators, and *RAS,* leading to AMKL.

Differential Diagnosis

The most reliable criteria in distinguishing *TAM* versus *AMKL* lie in the clinical course. TAM occurs in the first week of life and AML has a median age of onset of 2 years. A significant fraction of patients with AML has a MDS phase with no increased blasts. Secondary chromosomal abnormalities, such as +8, are observed in a higher frequency in AMKL than TAM. In general, TAM should resolve within several weeks to 3–4 months: the study from the Children's Oncology Group reported a median of 36 days (2–126 days) to achieve spontaneous remission in TAM [85]. It is noteworthy that about 20–30% of TAM patients eventually develop AML; therefore patients with TAM will need close follow-up for subsequent MDS/AML.

TAM in non-DS individuals is rare. In reported cases [99, 100], the leukemic blasts carried cytogenetic abnormalities involving chromosome 21, mostly +21, but these abnormalities were not in the non-hematopoietic cells. One recent case report documented *GATA1* mutation in leukemic blasts, suggesting a molecular pattern of leukemogenesis similar to DS-associated TAM [101–103]. Examined skin fibroblasts may be needed to confirm no constitutional +21 abnormalities in these patients.

Acute megakaryoblastic leukemia with t(1;22)(p13;q13); RBM15-MKL1 is extremely rare generally showing megakaryocyte differentiation

and most commonly occurs in infants without DS, and is classified under AML with recurrent cytogenetic abnormalities. Acute megakaryoblastic leukemia (AMKL), NOS is defined as an AML with ≥20% blasts of which ≥50% are of megakaryocyte lineage in individuals without DS and often occur in adult patients.

References

1. Dror Y, Freedman MH. Inherited forms of bone marrow failure. In: Hoffman RB, Benz Jr EJ, Silberstein LE, Heslop H, Weitz J, Anastasi J, editors. Hematology: basic principles and practice. 6th ed. Philadelphia: Elsevier Health Sciences; 2013.
2. Bessler M, Mason PJ, Link DC, Wilson DB. Inherited bone marrow failure syndromes. In: Orkin SH, Nathan DG, Ginsburg D, Look AT, Fisher DE, Lux IV S, editors. Nathan and Oski's hematology and oncology of infancy and childhood. 7th ed. Philadelphia: Saunders; 2015.
3. Rosenberg PS, Tamary H, Alter BP. How high are carrier frequencies of rare recessive syndromes? Contemporary estimates for Fanconi Anemia in the United States and Israel. Am J Med Genet A. 2011;155(8):1877–83.
4. Fares F, Badarneh K, Abosaleh M, Harari-Shaham A, Diukman R, David M. Carrier frequency of autosomal-recessive disorders in the Ashkenazi Jewish population: should the rationale for mutation choice for screening be reevaluated? Prenat Diagn. 2008;28(3):236–41.
5. Shimamura A, Alter BP. Inherited aplastic anemia syndromes. In: Greer JP, Arber DA, Glader B, List AF, Means Jr RT, Frixos P, Rodgers GM, editors. Wintrobe's clinical hematology, 13th ed. Philadelphia: Wolters Kluwer Health/Lippincott Williams & Wilkins; 2014.
6. Shimamura A, Alter BP. Pathophysiology and management of inherited bone marrow failure syndromes. Blood Rev. 2010;24(3):101–22.
7. Kutler DI, Singh B, Satagopan J, et al. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood. 2003;101(4):1249–56.
8. Alter BP, Giri N, Savage SA, et al. Malignancies and survival patterns in the National Cancer Institute inherited bone marrow failure syndromes cohort study. Br J Haematol. 2010;150(2):179–88.
9. Wegman-Ostrosky T, Savage SA. The genomics of inherited bone marrow failure: from mechanism to the clinic. Br J Haematol. 2017;177(4):526–42.
10. Clauson C, Scharer OD, Niedernhofer L. Advances in understanding the complex mechanisms of DNA interstrand cross-link repair. Cold Spring Harb Perspect Biol. 2013;5(10):a012732.
11. Waisfisz Q, Morgan NV, Savino M, et al. Spontaneous functional correction of homozygous fanconi anaemia alleles reveals novel mechanistic basis for reverse mosaicism. Nat Genet. 1999;22(4):379–83.
12. Pinto FO, Leblanc T, Chamousset D, et al. Diagnosis of Fanconi anemia in patients with bone marrow failure. Haematologica. 2009;94(4):487–95.
13. DeZern AE, Symons HJ, Resar LS, Borowitz MJ, Armanios MY, Brodsky RA. Detection of paroxysmal nocturnal hemoglobinuria clones to exclude inherited bone marrow failure syndromes. Eur J Haematol. 2014;92(6):467–70.
14. Keller P, Debaun MR, Rothbaum RJ, Bessler M. Bone marrow failure in Shwachman-Diamond syndrome does not select for clonal haematopoiesis of the paroxysmal nocturnal haemoglobinuria phenotype. Br J Haematol. 2002;119(3):830–2.
15. Dror Y, Donadieu J, Koglmeier J, et al. Draft consensus guidelines for diagnosis and treatment of Shwachman-Diamond syndrome. Ann N Y Acad Sci. 2011;1242:40–55.
16. Rothbaum R, Perrault J, Vlachos A, et al. Shwachman-Diamond syndrome: report from an international conference. J Pediatr. 2002;141(2):266–70.
17. Myers KC, Bolyard AA, Otto B, et al. Variable clinical presentation of Shwachman-Diamond syndrome: update from the North American Shwachman-Diamond Syndrome Registry. J Pediatr. 2014;164(4):866–70.
18. Wong TE, Calicchio ML, Fleming MD, Shimamura A, Harris MH. SBDS protein expression patterns in the bone marrow. Pediatr Blood Cancer. 2010;55(3):546–9.
19. Weis F, Giudice E, Churcher M, et al. Mechanism of eIF6 release from the nascent 60S ribosomal subunit. Nat Struct Mol Biol. 2015;22(11):914–9.
20. Flores Ballester E, Gil-Fernandez JJ, Vazquez Blanco M, et al. Adult-onset Diamond-Blackfan anemia with a novel mutation in the exon 5 of RPL11: too late and too rare. Clin Case Rep. 2015;3(6):392–5.
21. Khincha P, Mirabello L, Ellis SR, Brodie S, Chandrasekharappa SC, Donovan FX, et al. Novel and known ribosomal causes of Diamond-Blackfan anaemia identified through comprehensive genomic characterization. J Med Genet. 2017;54(6):417–25.
22. Dietz AC, Mehta PA, Vlachos A, et al. Current knowledge and priorities for future research in late effects after hematopoietic cell transplantation for inherited bone marrow failure syndromes: consensus statement from the second pediatric blood and marrow transplant consortium international conference on late effects after pediatric hematopoietic cell transplantation. Biol Blood Marrow Transplant. 2017;23(5):726–35.
23. Steinberg-Shemer O, Keel S, Dgany O, et al. Diamond blackfan anemia: a nonclassical patient with diagnosis assisted by genomic analysis. J Pediatr Hematol Oncol. 2016;38(7):e260–2.

24. Vlachos A, Rosenberg PS, Atsidaftos E, Alter BP, Lipton JM. Incidence of neoplasia in Diamond Blackfan anemia: a report from the Diamond Blackfan Anemia Registry. Blood. 2012;119(16):3815–9.
25. Steele JM, Sung L, Klaassen R, et al. Disease progression in recently diagnosed patients with inherited marrow failure syndromes: a Canadian Inherited Marrow Failure Registry (CIMFR) report. Pediatr Blood Cancer. 2006;47(7):918–25.
26. Iskander D, Psaila B, Gerrard G, et al. Elucidation of the EP defect in Diamond-Blackfan anemia by characterization and prospective isolation of human EPs. Blood. 2015;125(16):2553–7.
27. Elghetany MT. Diamond-Blackfan anemia: death by heme toxicity? Eur J Haematol. 2016;96(4):333–4.
28. Vlachos A, Ball S, Dahl N, et al. Diagnosing and treating Diamond Blackfan anaemia: results of an international clinical consensus conference. Br J Haematol. 2008;142(6):859–76.
29. Gambale A, Iolascon A, Andolfo I, Russo R. Diagnosis and management of congenital dyserythropoietic anemias. Expert Rev Hematol. 2016;9(3):283–96.
30. Babbs C, Roberts NA, Sanchez-Pulido L, et al. Homozygous mutations in a predicted endonuclease are a novel cause of congenital dyserythropoietic anemia type I. Haematologica. 2013;98(9):1383–7.
31. Iolascon A, Heimpel H, Wahlin A, Tamary H. Congenital dyserythropoietic anemias: molecular insights and diagnostic approach. Blood. 2013;122(13):2162–6.
32. Heimpel H, Kellermann K, Neuschwander N, Hogel J, Schwarz K. The morphological diagnosis of congenital dyserythropoietic anemia: results of a quantitative analysis of peripheral blood and bone marrow cells. Haematologica. 2010;95(6):1034–6.
33. Iolascon A, Esposito MR, Russo R. Clinical aspects and pathogenesis of congenital dyserythropoietic anemias: from morphology to molecular approach. Haematologica. 2012;97(12):1786–94.
34. Danise P, Amendola G, Nobili B, et al. Flow-cytometric analysis of erythrocytes and reticulocytes in congenital dyserythropoietic anaemia type II (CDA II): value in differential diagnosis with hereditary spherocytosis. Clin Lab Haematol. 2001;23(1):7–13.
35. Khincha PP, Savage SA. Neonatal manifestations of inherited bone marrow failure syndromes. Semin Fetal Neonatal Med. 2016;21(1):57–65.
36. Qiu Y, Zhang Y, Hu N, Dong F. A truncated granulocyte colony-stimulating factor receptor (G-CSFR) inhibits apoptosis induced by neutrophil elastase G185R mutant: implication for understanding CSF3R gene mutations in severe congenital neutropenia. J Biol Chem. 2017;292(8):3496–505.
37. Zeidler C, Germeshausen M, Klein C, Welte K. Clinical implications of ELA2-, HAX1-, and G-CSF-receptor (CSF3R) mutations in severe congenital neutropenia. Br J Haematol. 2009;144(4):459–67.
38. Bellanne-Chantelot C, Clauin S, Leblanc T, et al. Mutations in the ELA2 gene correlate with more severe expression of neutropenia: a study of 81 patients from the French Neutropenia Register. Blood. 2004;103(11):4119–25.
39. Skokowa J, Dale DC, Touw IP, Zeidler C, Welte K. Severe congenital neutropenias. Nat Rev Dis Primers. 2017;3:17032.
40. Klimiankou M, Mellor-Heineke S, Zeidler C, Welte K, Skokowa J. Role of CSF3R mutations in the pathomechanism of congenital neutropenia and secondary acute myeloid leukemia. Ann N Y Acad Sci. 2016;1370(1):119–25.
41. Hwang K, Park CJ, Huh HJ, Han SH, Jang S, Chi HS. Flow cytometric detection of neutrophil-associated immunoglobulin in patients with or without neutropenia and establishment of the reference interval. Ann Clin Lab Sci. 2011;41(2):144–9.
42. Logue GL, Shastri KA, Laughlin M, Shimm DS, Ziolkowski LM, Iglehart JL. Idiopathic neutropenia: antineutrophil antibodies and clinical correlations. Am J Med. 1991;90(2):211–6.
43. Papadaki HA, Eliopoulos AG, Kosteas T, et al. Impaired granulocytopoiesis in patients with chronic idiopathic neutropenia is associated with increased apoptosis of bone marrow myeloid progenitor cells. Blood. 2003;101(7):2591–600.
44. Dale DC, Person RE, Bolyard AA, et al. Mutations in the gene encoding neutrophil elastase in congenital and cyclic neutropenia. Blood. 2000;96(7):2317–22.
45. Haurie C, Dale DC, Mackey MC. Cyclical neutropenia and other periodic hematological disorders: a review of mechanisms and mathematical models. Blood. 1998;92(8):2629–40.
46. Armistead PM, Wieder E, Akande O, et al. Cyclic neutropenia associated with T cell immunity to granulocyte proteases and a double de novo mutation in GFI1, a transcriptional regulator of ELANE. Br J Haematol. 2010;150(6):716–9.
47. Hord JD, Whitlock JA, Gay JC, Lukens JN. Clinical features of myelokathexis and treatment with hematopoietic cytokines: a case report of two patients and review of the literature. J Pediatr Hematol Oncol. 1997;19(5):443–8.
48. Aprikyan AA, Liles WC, Park JR, Jonas M, Chi EY, Dale DC. Myelokathexis, a congenital disorder of severe neutropenia characterized by accelerated apoptosis and defective expression of bcl-x in neutrophil precursors. Blood. 2000;95(1):320–7.
49. Wetzler M, Talpaz M, Kellagher MJ, Gutterman JU, Kurzrock R. Myelokathexis: normalization of neutrophil counts and morphology by GM-CSF. JAMA. 1992;267(16):2179–80.
50. Al Ustwani O, Kurzrock R, Wetzler M. Genetics on a WHIM. Br J Haematol. 2014;164(1):15–23.
51. Dale DC, Bolyard AA, Kelley ML, et al. The CXCR4 antagonist plerixafor is a potential therapy for myelokathexis, WHIM syndrome. Blood. 2011;118(18):4963–6.

52. Nagle DL, Karim MA, Woolf EA, et al. Identification and mutation analysis of the complete gene for Chediak-Higashi syndrome. Nat Genet. 1996;14(3):307–11.
53. Sanchez-Guiu I, Anton AI, Garcia-Barbera N, et al. Chediak-Higashi syndrome: description of two novel homozygous missense mutations causing divergent clinical phenotype. Eur J Haematol. 2014;92(1):49–58.
54. Balduini CL, Savoia A. Genetics of familial forms of thrombocytopenia. Hum Genet. 2012;131(12):1821–32.
55. Ballmaier M, Germeshausen M. Advances in the understanding of congenital amegakaryocytic thrombocytopenia. Br J Haematol. 2009;146(1):3–16.
56. Schmitz G, Rothe G, Ruf A, et al. European Working Group on Clinical Cell Analysis: Consensus protocol for the flow cytometric characterisation of platelet function. Thromb Haemost. 1998;79(5):885–96.
57. Fiedler J, Strauss G, Wannack M, et al. Two patterns of thrombopoietin signaling suggest no coupling between platelet production and thrombopoietin reactivity in thrombocytopenia-absent radii syndrome. Haematologica. 2012;97(1):73–81.
58. Bannon SA, DiNardo CD. Hereditary predispositions to myelodysplastic syndrome. Int J Mol Sci. 2016;17(6):838.
59. Duployez N, Lejeune S, Renneville A, Preudhomme C. Myelodysplastic syndromes and acute leukemia with genetic predispositions: a new challenge for hematologists. Expert Rev Hematol. 2016;9(12):1189–202.
60. Renneville A, Mialou V, Philippe N, et al. Another pedigree with familial acute myeloid leukemia and germline CEBPA mutation. Leukemia. 2009;23(4):804–6.
61. Smith ML, Cavenagh JD, Lister TA, Fitzgibbon J. Mutation of CEBPA in familial acute myeloid leukemia. N Engl J Med. 2004;351(23):2403–7.
62. Babushok DV, Bessler M. Genetic predisposition syndromes: when should they be considered in the work-up of MDS? Best Pract Res Clin Haematol. 2015;28(1):55–68.
63. Tawana K, Wang J, Renneville A, et al. Disease evolution and outcomes in familial AML with germline CEBPA mutations. Blood. 2015;126(10):1214–23.
64. Cheah JJC, Hahn CN, Hiwase DK, Scott HS, Brown AL. Myeloid neoplasms with germline DDX41 mutation. Int J Hematol. 2017;106(2):163–74.
65. Polprasert C, Schulze I, Sekeres MA, et al. Inherited and somatic defects in DDX41 in myeloid neoplasms. Cancer Cell. 2015;27(5):658–70.
66. Lewinsohn M, Brown AL, Weinel LM, et al. Novel germ line DDX41 mutations define families with a lower age of MDS/AML onset and lymphoid malignancies. Blood. 2016;127(8):1017–23.
67. Owen C, Barnett M, Fitzgibbon J. Familial myelodysplasia and acute myeloid leukaemia—a review. Br J Haematol. 2008;140(2):123–32.
68. West AH, Godley LA, Churpek JE. Familial myelodysplastic syndrome/acute leukemia syndromes: a review and utility for translational investigations. Ann N Y Acad Sci. 2014;1310:111–8.
69. Melazzini F, Palombo F, Balduini A, et al. Clinical and pathogenetic features of ETV6 related thrombocytopenia with predisposition to acute lymphoblastic leukemia. Haematologica. 2016;101(11):1333–42.
70. Noris P, Perrotta S, Seri M, et al. Mutations in ANKRD26 are responsible for a frequent form of inherited thrombocytopenia: analysis of 78 patients from 21 families. Blood. 2011;117(24):6673–80.
71. Noris P, Favier R, Alessi MC, et al. ANKRD26-related thrombocytopenia and myeloid malignancies. Blood. 2013;122(11):1987–9.
72. Noetzli L, Lo RW, Lee-Sherick AB, et al. Germline mutations in ETV6 are associated with thrombocytopenia, red cell macrocytosis and predisposition to lymphoblastic leukemia. Nat Genet. 2015;47(5):535–8.
73. Zhang MY, Churpek JE, Keel SB, et al. Germline ETV6 mutations in familial thrombocytopenia and hematologic malignancy. Nat Genet. 2015;47(2):180–5.
74. Moriyama T, Metzger ML, Wu G, et al. Germline genetic variation in ETV6 and risk of childhood acute lymphoblastic leukaemia: a systematic genetic study. Lancet Oncol. 2015;16(16):1659–66.
75. Spinner MA, Sanchez LA, Hsu AP, et al. GATA2 deficiency: a protean disorder of hematopoiesis, lymphatics, and immunity. Blood. 2014;123(6):809–21.
76. Crispino JD, Horwitz MS. GATA factor mutations in hematologic disease. Blood. 2017;129(15):2103–10.
77. Micol JB, Abdel-Wahab O. Collaborating constitutive and somatic genetic events in myeloid malignancies: ASXL1 mutations in patients with germline GATA2 mutations. Haematologica. 2014;99(2):201–3.
78. Wlodarski MW, Hirabayashi S, Pastor V, et al. Prevalence, clinical characteristics, and prognosis of GATA2-related myelodysplastic syndromes in children and adolescents. Blood. 2016;127(11):1387–97. quiz 518
79. Calvo KR, Vinh DC, Maric I, et al. Myelodysplasia in autosomal dominant and sporadic monocytopenia immunodeficiency syndrome: diagnostic features and clinical implications. Haematologica. 2011;96(8):1221–5.
80. Malkin D, Nichols KE, Zelley K, Schiffman JD. Predisposition to pediatric and hematologic cancers: a moving target. Am Soc Clin Oncol Educ Book. 2014;34:e44–55.
81. Tsang HC, Bussel JB, Mathew S, et al. Bone marrow morphology and disease progression in congenital thrombocytopenia: a detailed clinicopathologic and genetic study of eight cases. Mod Pathol. 2017;30(4):486–98.
82. Fong CT, Brodeur GM. Down's syndrome and leukemia: epidemiology, genetics, cytogenetics and mechanisms of leukemogenesis. Cancer Genet Cytogenet. 1987;28(1):55–76. Epub 1987/09/01.
83. Hasle H, Clemmensen IH, Mikkelsen M. Risks of leukaemia and solid tumours in individuals with Down's syndrome. Lancet. 2000;355(9199):165–9. Epub 2000/02/16.

84. Lange BJ, Kobrinsky N, Barnard DR, et al. Distinctive demography, biology, and outcome of acute myeloid leukemia and myelodysplastic syndrome in children with Down syndrome: Children's Cancer Group Studies 2861 and 2891. Blood. 1998;91(2):608–15. Epub 1998/01/15.
85. Gamis AS, Alonzo TA, Gerbing RB, et al. Natural history of transient myeloproliferative disorder clinically diagnosed in Down syndrome neonates: a report from the Children's Oncology Group Study A2971. Blood. 2011;118(26):6752–9; quiz 996
86. Klusmann JH, Creutzig U, Zimmermann M, et al. Treatment and prognostic impact of transient leukemia in neonates with Down syndrome. Blood. 2008;111(6):2991–8.
87. Massey GV, Zipursky A, Chang MN, et al. A prospective study of the natural history of transient leukemia (TL) in neonates with Down syndrome (DS): Children's Oncology Group (COG) study POG-9481. Blood. 2006;107(12):4606–13.
88. Roberts I, Izraeli S. Haematopoietic development and leukaemia in Down syndrome. Br J Haematol. 2014;167(5):587–99.
89. Lange B. The management of neoplastic disorders of haematopoiesis in children with Down's syndrome. Br J Haematol. 2000;110(3):512–24.
90. Creutzig U, Reinhardt D, Diekamp S, Dworzak M, Stary J, Zimmermann M. AML patients with Down syndrome have a high cure rate with AML-BFM therapy with reduced dose intensity. Leukemia. 2005;19(8):1355–60. Epub 2005/05/28.
91. Bombery M, Vergilio JA. Transient abnormal myelopoiesis in neonates: GATA get the diagnosis. Arch Pathol Lab Med. 2014;138(10):1302–6.
92. Khan I, Malinge S, Crispino J. Myeloid leukemia in Down syndrome. Crit Rev Oncog. 2011;16(1–2):25–36. Epub 2011/12/14.
93. Groet J, McElwaine S, Spinelli M, et al. Acquired mutations in GATA1 in neonates with Down's syndrome with transient myeloid disorder. Lancet. 2003;361(9369):1617–20. Epub 2003/05/16.
94. Hitzler JK, Cheung J, Li Y, Scherer SW, Zipursky A. GATA1 mutations in transient leukemia and acute megakaryoblastic leukemia of Down syndrome. Blood. 2003;101(11):4301–4. Epub 2003/02/15.
95. Roberts I, Alford K, Hall G, et al. GATA1-mutant clones are frequent and often unsuspected in babies with Down syndrome: identification of a population at risk of leukemia. Blood. 2013;122(24):3908–17. Epub 2013/09/12.
96. Forestier E, Izraeli S, Beverloo B, et al. Cytogenetic features of acute lymphoblastic and myeloid leukemias in pediatric patients with Down syndrome: an iBFM-SG study. Blood. 2008;111(3):1575–83. Epub 2007/11/01.
97. Picos Cardenas VJ, Meza Espinoza JP, Garibaldi Covarrubias RF, Barajas Torres RL, Gonzalez Garcia JR. Transient myeloproliferative disorder progression and acquired chromosomal abnormalities in children with Down syndrome. Pediatr Blood Cancer. 2012;59(5):962–3. Epub 2012/07/20.
98. Yoshida K, Toki T, Okuno Y, et al. The landscape of somatic mutations in Down syndrome-related myeloid disorders. Nat Genet. 2013;45(11):1293–9. Epub 2013/09/24.
99. Apollonsky N, Shende A, Ouansafi I, Brody J, Atlas M, Aygun B. Transient myeloproliferative disorder in neonates with and without Down syndrome: a tale of 2 syndromes. J Pediatr Hematol Oncol. 2008;30(11):860–4. Epub 2008/11/08.
100. Wang SA, Hasserjian RP. Acute erythroleukemias, acute megakaryoblastic leukemias, and reactive mimics: a guide to a number of perplexing entities. Am J Clin Pathol. 2015;144(1):44–60.
101. Carpenter E, Valverde-Garduno V, Sternberg A, et al. GATA1 mutation and trisomy 21 are required only in haematopoietic cells for development of transient myeloproliferative disorder. Br J Haematol. 2005;128(4):548–51. Epub 2005/02/03.
102. Cushing T, Clericuzio CL, Wilson CS, et al. Risk for leukemia in infants without Down syndrome who have transient myeloproliferative disorder. J Pediatr. 2006;148(5):687–9. Epub 2006/06/02.
103. Tsai MH, Hou JW, Yang CP, et al. Transient myeloproliferative disorder and GATA1 mutation in neonates with and without Down syndrome. Indian J Pediatr. 2011;78(7):826–32. Epub 2011/02/03.

4 Cytopenias: Acquired Bone Marrow Failure

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Overview

Acquired bone marrow failure can attribute to a number of mechanisms, such as the loss of pluripotent hematopoietic stem cells; bone marrow replacement by metastatic carcinoma, lymphoma, leukemia or fibrosis; maturation arrest as a result of nutritional/metabolic deficiency or clonal hematopoietic stem neoplasm such as myelodysplastic syndrome (MDS). BM failure due to an infiltrative process and MDS are discussed under Chapter 2, and BM failure associated with a congenital genetic predisposition under Chapter 3. This chapter will focus on Aplastic anemia and Paroxysmal nocturnal hemoglobinuria (PNH).

Aplastic Anemia

Aplastic anemia (AA) is characterized by diminished or absent hematopoietic precursors in the bone marrow (BM), most often due to injury to the hematopoietic stem cells. AA can be congenital but is more frequently acquired. Acquired AA preferentially affects young adults (20–25 years) and individuals over the age of 55–60 years [1]. The incidence of acquired AA is estimated at two per million annually, and occurs at higher rates in countries with increased rates of viral hepatitis [2]. Table 4.1 lists the causative agents that have been reported associated with acquired AA. However,

Table 4.1 Major causes of acquired aplastic anemia

Idiopathic (70% of all aplastic anemia)
Cytotoxic drugs and radiation
• Drugs
– Antibiotics: Chloramphenicol, sulfonamides
– Anticonvulsants: Felbamate, carbamazepine, phenytoin, valproic acid, phenytoin, nifedipine
– Anti-inflammatory: Phenylbutazone, indomethacin
• Chemicals: Industrial chemicals such as benzene, and pesticides
• Radiation
Viral infections
• Parvovirus B19
• Hepatitis A, B, G
• Human immunodeficiency virus infection (HIV)
• Epstein-Barr virus (EBV), cytomegalovirus (CMV)
Immune disorders including autoimmune diseases
• Eosinophilic fasciitis
• Systemic lupus erythematosus
• Graft-versus-host disease (GvHD), including transfusion-related GvHD
Miscellaneous
• Paroxysmal nocturnal hemoglobinuria (PNH)
• Thymoma, thymic carcinoma
• Pregnancy

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despite numerous, diverse possible causes, from chemicals and drugs to viral, collagen vascular diseases and pregnancy, in about 70% of patients, the cause cannot be clearly determined and the AA is considered to be idiopathic [3]. It has been suggested that immune-mediated destruction/suppression may be the underlying cause in many of the patients with idiopathic AA. It has been postulated that damage induced by chemicals, drugs, viruses, or antigens leads to lymphocyte activation resulting in destruction of BM hematopoietic cells [4]. Studies have shown that autoreactive T-lymphocytes from the BM of patients with AA can inhibit hematopoiesis when co-cultured with normal marrows [5, 6]. This inhibition may be mediated by the release of marrow-suppressing cytokines, such as interferon gamma (IFN-γ), tumor necrosis factor (TNF), and interleukin-2 [1], ultimately leading to apoptosis of BM hematopoietic stem cells. IFN-γ may also lead to increased expressions of the Fas and Fas receptor [7]. Telomeric attrition resulting in critically shortened telomeres, prompting cellular senescence or crisis, has also been considered as one of the underlying causes of AA. Inherited heterozygous mutations in the genes that repair or protect telomere may limit marrow stem cell self-renewal and predispose some patients to marrow failure [8, 9].

In recent years, it has been increasingly recognized that acquired AA, at least in a large fraction of patients, is linked to clonal hematopoiesis [10] (see "Molecular Genetics" section below).

Clinical Features

The clinical presentation of acquired AA is variable. Disease onset is often insidious, and the initial symptoms are usually related to anemia or bleeding, and infections. Anemia is usually normocytic but occasionally may be macrocytic, and is associated with absolute reticulocytopenia. Infections are typically bacterial, including sepsis, pneumonia, and urinary tract infection. Invasive fungal infection is a common cause of death, especially in subjects with prolonged and severe neutropenia [11]. Patients usually do not have splenomegaly or lymphoadenopathy.

Serologic testing for hepatitis and other viral pathogens, such as Epstein-Barr virus (EBV), cytomegalovirus (CMV), and human immunodeficiency virus (HIV), and an evaluation for autoimmune diseases may be helpful. However, the association between onset of AA and exposure to the offending agent varies greatly, and in many patients, a cause is never identified.

The clinical outcome and management decision of acquired AA depend in part upon the severity of AA, which is based on the degrees of the cytopenia(s). Significant cytopenias are defined as HGB < 10 g/L, absolute neutrophil count (ANC) < 1.5×10^9/L, and platelets <50×10^9/L [12]. To define AA, there must be at least two of the three cytopenias reaching the above levels [12, 13]. Moderate (non-severe) AA is defined as no severe pancytopenia but shows at least two cytopenia(s) below the above mentioned ranges but with an ANC ≥0.5×10^9/L. Severe and very severe AA are defined as the presence of severe pancytopenia, of which an ANC count of $0.2–0.5 \times 10^9$/L is considered as "severe" and ANC <0.2 as "very severe." The definitions of AA severity are shown in Table 4.2.

Morphology

Bone marrow (BM) biopsy is required to assess BM cellularity. Non-severe (moderate) AA usually has a cellularity lower than age-appropriate cellularity, often <50% in children and <30% in adults, while severe and very severe AA have a cellularity <25%. Typical BM biopsy (Fig. 4.1) shows a profound hypocellularity with decreased trilineage hematopoiesis. Erythroid islands are generally small if present. Megakaryocytes may be difficult to visualize. In addition, there is a relative increase in small lymphocytes, plasma cells, stromal cells, and mast cells. In some patients with severe and very severe AA, the BM cellularity may be higher than 30%, but comprises predominantly of inflammatory cells rather than hematopoietic cells. The BM aspirate is often hypocellular, containing acellular spicules.

In AA, the residual hematopoietic cells are often morphologically normal, but some mild dyserythropoiesis with megaloblastoid maturation may be observed. Megakaryocytes in AA are within the normal limits morphologically, but the low num-

Table 4.2 Aplastic anemia (AA) grading: moderate, severe, and very severe

	Moderate (or non-severe) AA	Severe AA	Very severe AA
Cytopenia(s) defined as • HGB <10 g/L • ANC <1.5 × 10^9/L • Platelets <50 × 10^9/L	Two of the three cytopenias, no severe pancytopenia	Severe pancytopenia	Severe pancytopenia
– ANC (×10^9/L)	≥0.5	0.2–0.5	<0.2
– Platelets(×10^9/L)	Usually 80–100	Usually <20	
Reticulocytes (mL^{-1})	Usually 40–60,000	<40,000	
Bone marrow cellularity	Usually <30–50% in children and <30% in adults	<25% or, <50% but hematopoietic cells constitute <30% of cellularity	
Median survival and outcome	60–70 months, spontaneous recovery may occur	Most patients <12 months if untreated. Spontaneous recovery is unlikely	
Treatment	No consensus recommendations	Immunosuppressive therapy Hematopoietic cell transplantation	

HGB hemoglobin, *ANC* absolute neutrophil count

ber of megakaryocytes may make the evaluation of megakaryocytic dysplasia difficult; applying immunohistochemistry stains to help visualize megakaryocytes (such as CD61 and/or CD42b) may aid in revealing if the megakaryocytes appear normal or dysplastic. Morphological assessment for dysgranulopoiesis can be performed on a peripheral smear in addition to the BM aspirate.

Immunophenotype

Flow cytometric studies show that the bone marrow of AA patients has a decreased CD34+ cell count. Within the CD34+ cell compartment, there is retained differentiation to immature precursor B cells (hematogones) (Fig. 4.2). The CD34+ cells have a normal immunophenotype (Fig. 4.2). The myelomonocytic maturation analyzed by flow cytometry is often normal in AA.

Blood cells deficient in glycosyl phosphatidylinositol-anchored membrane proteins (GPI proteins)-paroxysmal nocturnal hemoglobinuria (PNH) cells have been reported in 30–60% AA patients, and the presence of a PNH clone can be used to support a diagnosis of AA [1, 14]. The mechanism of PNH appearance in AA likely involves an escape of PNH cells from the immune attack associated with acquired AA [15]. Interestingly, PNH virtually never arises from inherited forms of AA [16]. Thus, in children and young adults, the presence of a small PNH clone in the setting of a hypocellular BM virtually excludes a diagnosis of an inherited BM failure disorder.

The PNH clones are frequently below 1%, and in the majority, <10% of the respective cell lineage cells analyzed. Since most of these clones are small, they do not lead to clinical manifestations of hemolysis or thrombosis. The presence of a minor population of PNH-type cells in AA correlates with a positive immunosuppressive therapy response and a favorable prognosis [17]. The detection of a small PNH clone relies on a high-sensitivity flow cytometric analytic method (please see the details under PNH disease). FLAER (fluorochrome-conjugated version of a non-lysing mutated form of proaerolysin) [18] assay is the recommended test in the detection of small PNH clones (Fig. 4.3). Analysis should be performed on granulocytes and monocytes, which are generally more sensitive than red blood cells in detecting a small PNH clone. To define PNH+ cells, GPI-deficient cells should be found in ≥2 different cell lineages at a frequency of >0.01% of all cells [19, 20].

It is noteworthy that classic PNH can be associated with marrow failure (the so-called aplastic anemia/PNH syndrome), and all PNH patients show evidence of underlying hematopoietic deficiency [21]. It has been suggested that some HLA-dependent immune mechanism plays an important role in the occurrence or selection of a

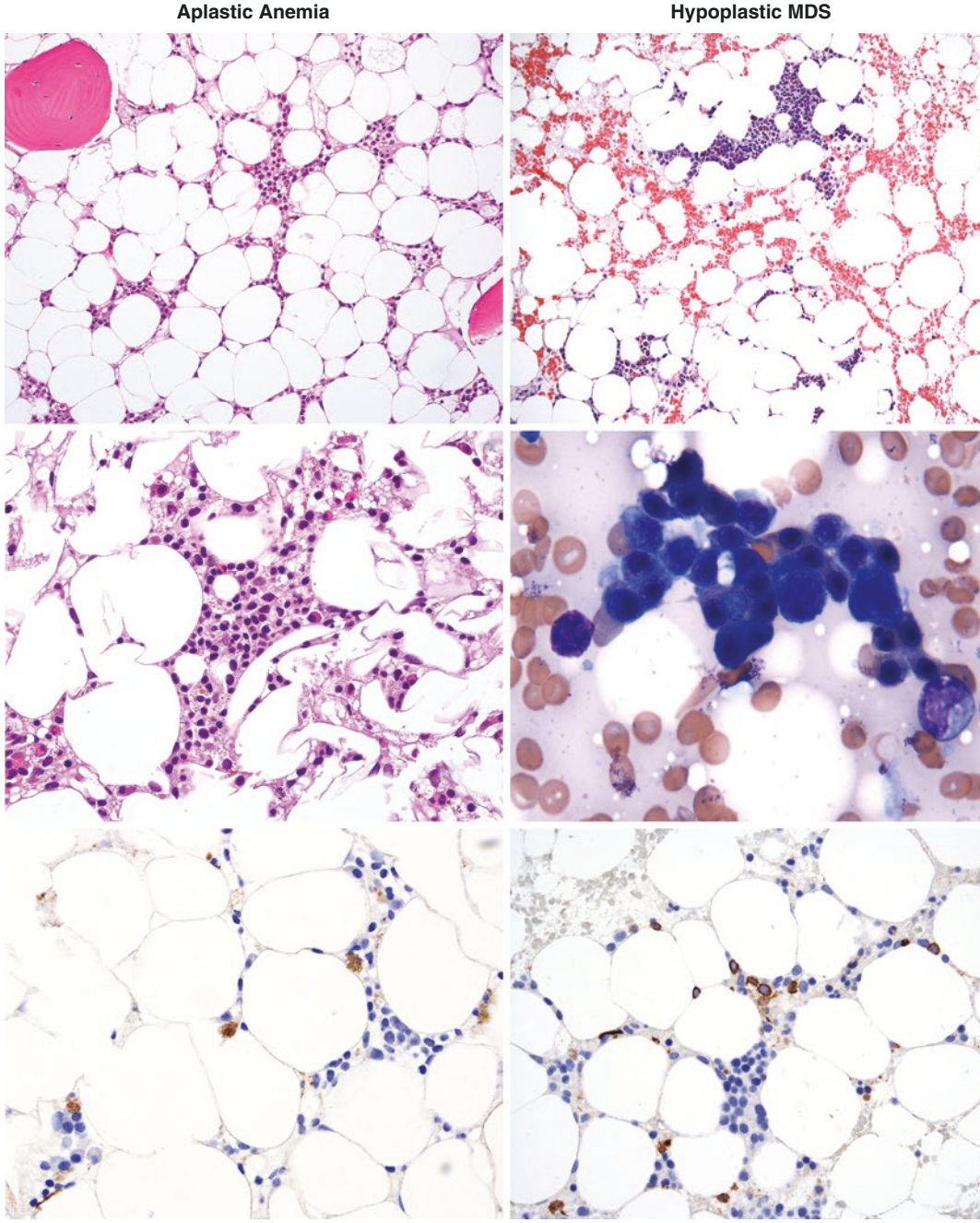


Fig. 4.1 Bone marrow findings of very severe aplastic anemia (AA) (*Left*) versus hypoplastic MDS (*Right*). In AA, the bone marrow trephine biopsy is profoundly hypocellular with trilineage hypoplasia (*Upper Left*). The small areas of preserved cellularity comprise mainly small lymphocytes, plasma cells, and stromal cells (*Middle Left*). CD34 immunohistochemistry stain shows decreased or absent CD34+ hematopoietic cells (*Lower Left*). Hypoplastic MDS also shows a hypocellularity, but with focal clusters of hematopoietic cells (*Upper Right*). The aspirate smear is hypocellular, but erythroid clusters with dyserythropoiesis are present (*Middle Right*). CD34 immunohistochemistry stain shows easily identifiable CD34+ blasts, focally clustering (*Lower Right*). These morphologic features differ from those of AA

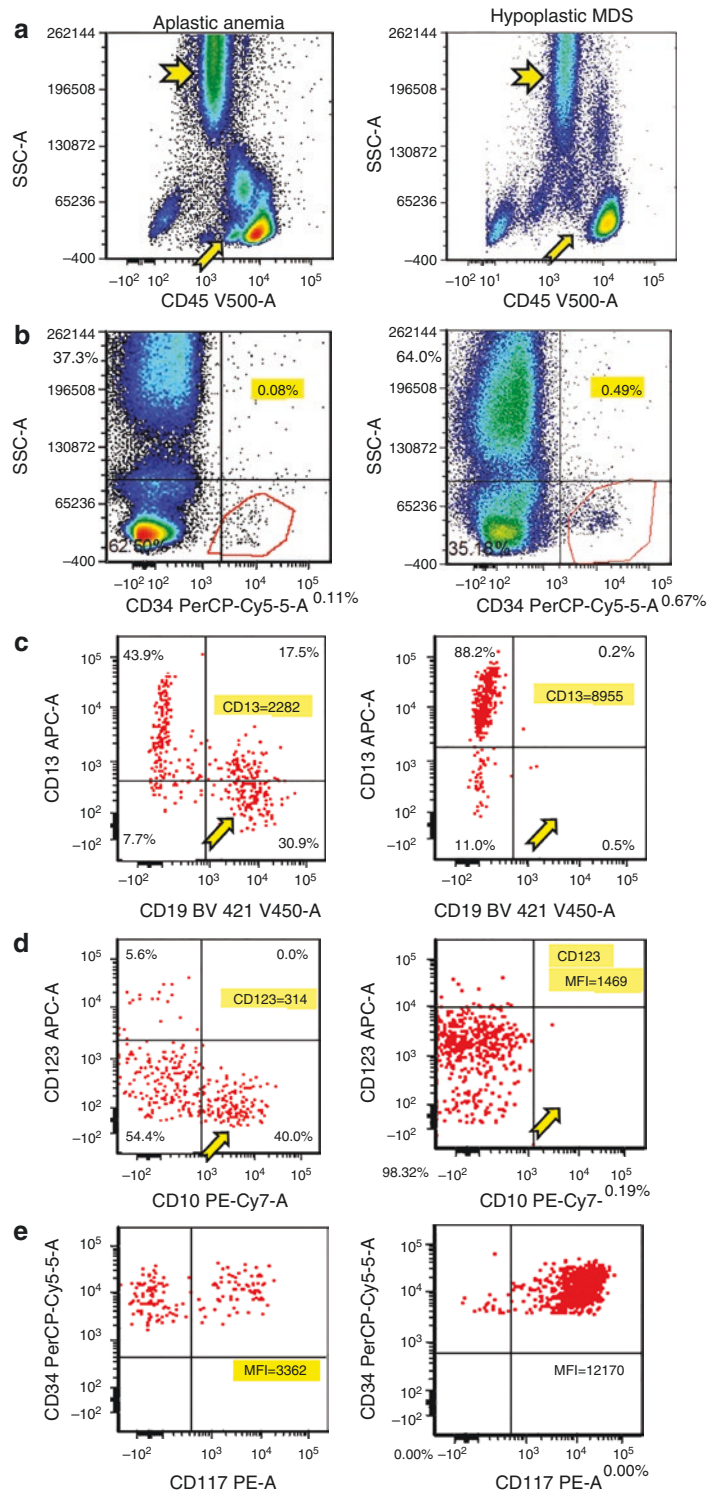


Fig. 4.2 Flow cytometry comparison of aplastic anemia (AA, left column) and hypoplastic myelodysplastic syndrome (MDS, right column). On CD45/SSC (**a**), compared to AA, MDS shows decreased side scatter of granulocytes (short arrows) and lack of hematogones (long arrows). (**b**) CD34+ cells are very low in number (0.08%) in AA versus a more distinct CD34+ population (0.49%) in hypoplastic MDS. (**c**)–(**e**) Within the CD34+ population (*all red dots*), AA shows retained stage I hematogones (arrows, about 40–50% of total CD34+ cells are CD19+ CD10+ cells) and normal expressions of CD13, CD123, and CD117 on myeloid precursors (not pointed by arrows). In contrast, hypoplastic MDS (*Right*) shows an absence of stage I hematogones and increased expression of CD13, CD123, and CD117 on myeloblasts. The mean florescence intensity (MFI) of each marker on immature myeloid cells is shown on the respective plot

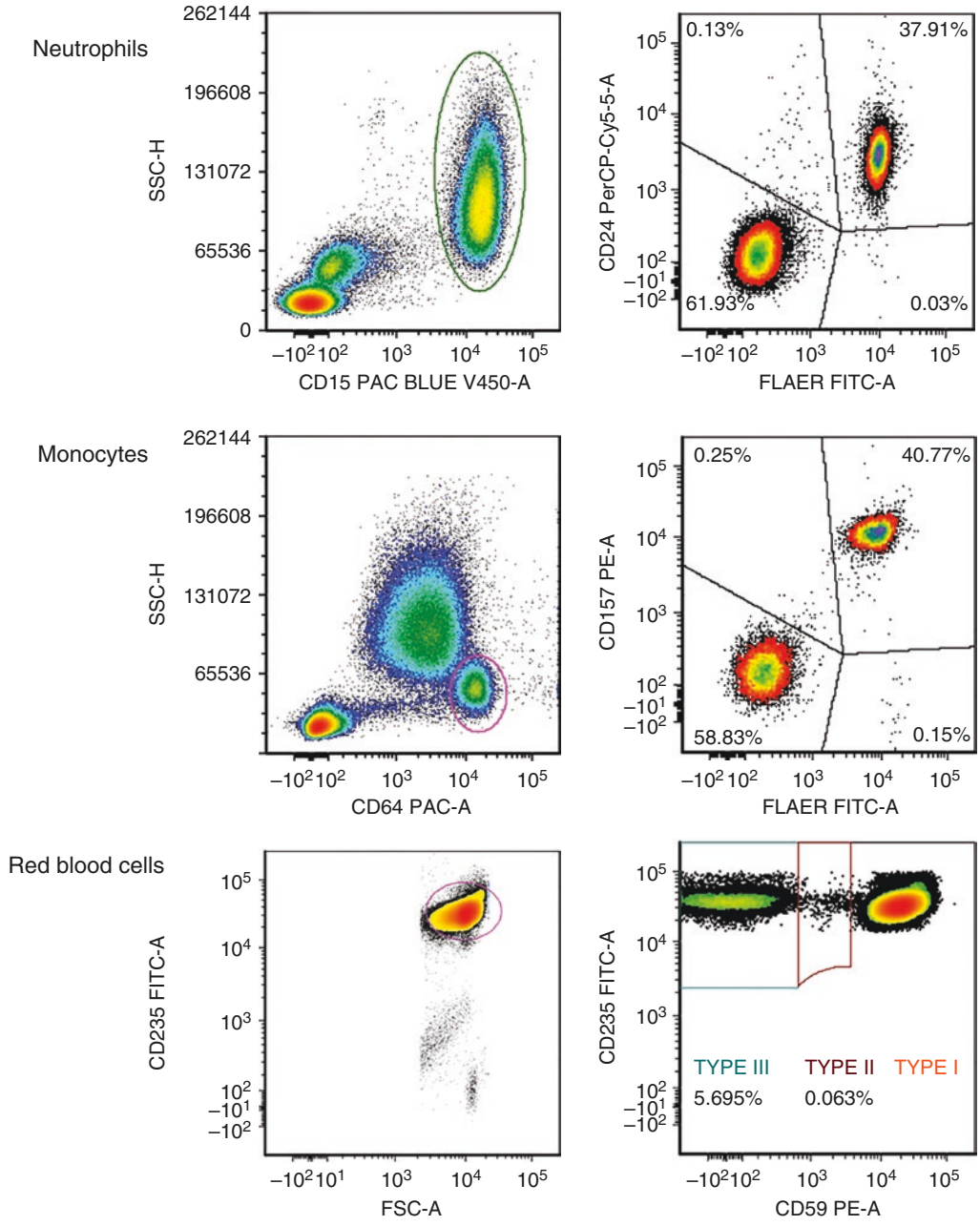


Fig. 4.3 Flow cytometry assay for paroxysmal nocturnal hemoglobinuria (PNH). A lineage marker is required to define (gate) the cell population for analysis. The left column shows CD15 for neutrophils, CD64 for monocytes, and CD235a (glycophorin) for red blood cell (RBC) gating. Leukocytes are better than RBCs for the detection of small PNH clones. In this case, 61.93% granulocytes, 58.83% of monocytes and 5.695% of RBCs completely lack GPI-linked proteins (PNH type III cells). In addition, 0.063% of RBCs show partial loss of GPI proteins (PNH type II cells). Type II PNH cells are not seen in the granulocytes and monocytes

PNH clone and GPI itself may be a target for cytotoxic T lymphocytes [22].

In AA patients treated with immunosuppressive therapy, the PNH clones may reduce in size (Fig. 4.4), persist, or expand.

Cytogenetics and Molecular Genetics

In recent years, with the advance of next-generation sequencing (NGS) technology, clonal hematopoiesis has been found in nearly half of AA patients [23]. Healthy individuals are now known to accumulate structural genomic rearrangements and somatic mutations as a part of normal aging mutations [24]; in AA, these preexisting age-related somatic mutations are expanded through autoimmune selection and hematopoietic stress. It has been shown that aging-related mutations in *DNMT3A, TET2*, and spliceosome factor genes are more frequent and occur at a higher mutant allele fraction in older AA patients than younger AA patients [23, 25]. Additionally, mutations in *ASXL1 and BCOR/BCORL1* [23, 26] are significantly overrepresented in AA compared to their relative prevalence in similarly aged individuals, outnumbering *DNMT3A and TET2*. Notably, in all age groups of AA patients, mutations in *PIGA* and loss of HLA class I alleles (such as copy number neutral loss of heterozygosity on chromosome 6p: 6p CN-LOH), two prototypical markers of immune escape in AA, are the most prevalent [27, 28]. It is likely that genetic mutations in hematopoietic stem cells confer a selective advantage in the AA BM environment.

The implications of age- and MDS-related somatic mutations in AA are complex and require a cautious interpretation. The detection of a PNH clone, or loss of HLA alleles through 6p CN-LOH or somatic inactivation, supports a diagnosis of AA. Patients with *PIGA, BCOR*, or *BCORL1* mutations are found to have an improved response to immunosuppressive therapy and therefore prognostically favorable [23]. *DNMT3A, ASXL1, TP53, RUNX1*, and *PRM1D* have been reported to be associated with a shorter overall survival [23], particularly in patients <60 years of age, whereas the significance of other age-related somatic mutations is less clear.

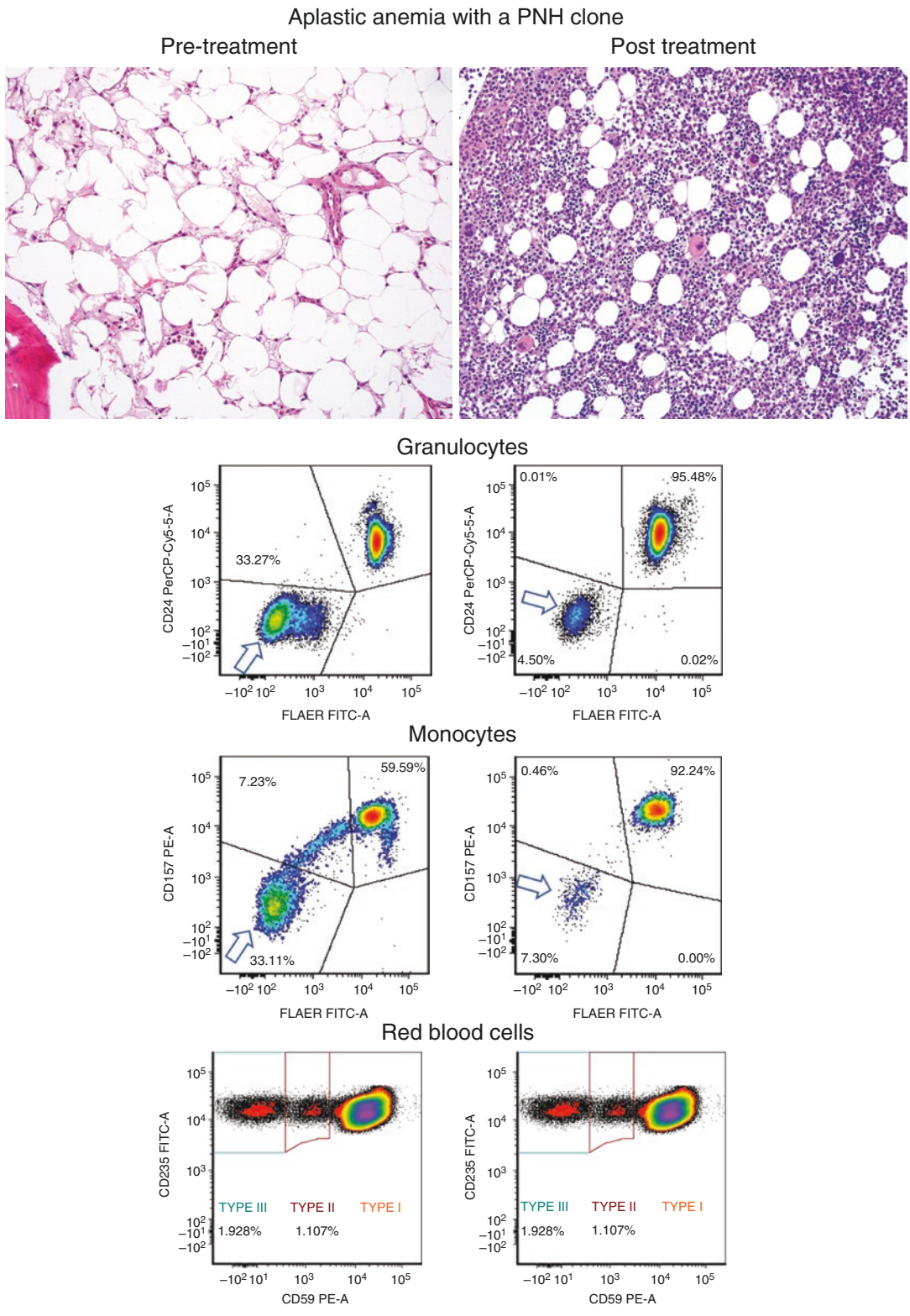
Bone marrow cytogenetic abnormalities can occur infrequently at the time of AA diagnosis, with an incidence of approximately 4–5% [29–32]. The most common abnormalities are del(6), del(5q), del(13), del(20q), -7, and +6, which may persist or disappear with hematopoietic recovery after treatment. Except for +6 which often shows no response to immunosuppressive therapy [31, 32], AA with other clonal chromosomal abnormalities and AA with a normal karyotype show a similar response to immunosuppressive therapies and have a comparable risk for MDS or AML progression. Therefore, the presence of clonal cytogenetic abnormalities should not be used as presumptive evidence of MDS in the absence of other features of MDS. Cytogenetic abnormality detected in AA at MDS evolution include -7, 11q23 abnormalities, and chromosomal 9 abnormalities [33, 34]. Unlike a cytogenetic abnormality present at diagnosis, the emergence of a new clonal karyotypic abnormality in a patient with AA generally heralds MDS progression.

Clonal evolution occurs in about 10–20% of AA patients; in other cases, the disease may be stable for many years without progression. The incidence of clonal evolution increases substantially among patients who are successfully treated by immunosuppressive therapy and hematopoietic growth factors [6, 33, 35–37]. AA can progress to PNH, MDS, or AML.

Approximately half of patients with AA have a detectable PNH clone (see "Immunophenotyic Studies" section above) that can expand during immunosuppressive therapy, and these patients may have increased vulnerability to complement-mediated hemolysis. Progression to clinically significant PNH has been described in 15–25% of the treated AA patients [16, 38, 39]. The evolution rate of AA to MDS is estimated at 5–15%.

Differential Diagnosis

The main differential diagnosis of AA is hypoplastic/hypocellular MDS; in some cases, it is virtually impossible to separate these entities


Aplastic anemia with a PNH clone
Pre-treatment
Post treatment
Granulocytes
CD24 PerCP-Cy5-5-A
FLAER FITC-A
33.27%
0.01%
95.48%
4.50%
0.02%
Monocytes
CD157 PE-A
FLAER FITC-A
7.23%
59.59%
33.11%
0.46%
92.24%
7.30%
0.00%
Red blood cells
CD235 FITC-A
CD59 PE-A
TYPE III
TYPE II
TYPE I
1.928%
1.107%

clinically and morphologically. The presence of mild dyserythropoiesis is common in AA, and should not be used to exclude an AA diagnosis. A number of subtle bone marrow findings may assist in the differential diagnosis of AA versus hypoplastic MDS (Table 4.3). Accurate evaluation of the blast percentage and presence of myeloid and megakaryocytic dysplasia in a severely hypoplastic marrow can be particularly challenging [38]. A CD34 stain by immunohistochemistry often reveals decreased CD34+ cells in AA [40] (Fig. 4.1) while they are normal or increased in hypocellular MDS (Fig. 4.1). P53 immunohistochemistry should not show strong-expressing cells in AA (<1%), but can be positive in a subset of MDS. Age-related mutations are very common in AA and therefore cannot be used to reliably differentiate AA from MDS.

Determining MDS evolution in AA patients who are treated with immunosuppressive therapy can be even more challenging. Notably, AA patients who are treated with immunosuppressive therapy may recover the BM cellularity, but frequently remain cytopenic; and when a complete clinical response is obtained, recurrence is common. MDS evolution in AA is characterized by a diffuse or patchy increase in bone marrow cellularity (27% of cases), while a continued hypocellularity is found in one-third of the patients with MDS evolution. Treated AA often displays mild-to-moderate dysplasia, especially in the erythroid lineage. Certain drugs that may be used in AA patients, such as antimetabolites, and superimposed viral infections can also produce BM dyspoietic changes that can complicate the evaluation for an evolving MDS.

A diagnosis of MDS evolution in AA can only be made by combining clinical, laboratory, BM morphology, cytogenetic, and immunophenotypic data. The following findings in this setting are considered as highly suggestive of evolution to MDS: (1) increased BM or peripheral blood blasts; (2) marked dysplasia in the granulocytic or megakaryocytic lineage; or (3) a newly emerging MDS-related clonal cytogenetic abnormality. As for mutations, in treated patients, clonal hematopoiesis can be detected by NGS in about 85% of patients with adult-onset AA and in over 60% of patients with pediatric-onset AA. It is debatable if the presence of unfavorable somatic mutations, such as *DNMT3A, ASXL1, TP53, RUNX1*, *and PRM1D*, in patients with persistent cytopenia but lack of other criteria of MDS is sufficient to diagnose a MDS evolution in AA patients.

Flow cytometry immunophenotyping can be particularly useful in this setting. While identification of a PNH clone is common in AA patients and supports an AA diagnosis, a PNH clone, often detected at a lower level (<1%), has been reported in about 10% of MDS cases without excess blasts. MDS cases with a PNH clone often show dysplasia limited to erythroid lineage cells; with little or no dysplasia in

Table 4.3 Markers recommended for PNH testing by flow cytometry

	Lineage-defining markers	GPI markers	Other GPI markers that may be used
Granulocytes	CD15	FLAER, CD24	CD16, CD66b
Monocytes	CD64	FLAER, CD157	CD14
Red blood cells	CD235a (glycophorin)	CD59	CD55

Fig. 4.4 Aplastic anemia with a paroxysmal nocturnal hemoglobinuria (PNH) clone, pre- and post treatment. A patient with severe aplastic anemia at the time of diagnosis (*Left side figures*) showed a profound marrow hypocellularity and a large PNH clone (arrows) in granulocytes (33.27%), monocytes (Type III = 33.11%, Type II = 7.23%), and RBCs (Type III = 1.928%, Type II = 1.107%). After immunosuppressive therapy (*Right side figures*), the bone marrow recovered trilineage hematopoiesis and a normal cellularity. The PNH clone decreased to 4.5% in granulocytes and 7.3% in monocytes, but the PNH clone relatively increased in RBCs, likely due to no longer requiring blood transfusions

myeloid and megakaryocytic lineages [41]. Flow cytometry immunophenotying of CD34+ cells is a very useful method to determine a neoplastic versus nonneoplastic process in this setting (Fig. 4.2). On flow cytometry study of bone marrow from AA patients, CD34+ blasts are often very low in number, do not form a discrete population, and show retained hematogones (normal immature B-cell precursors), and lack immunophenotypic aberrancies. In contrast, hypoplastic MDS or MDS evolving from AA often show a discrete myeloblast population, which demonstrates various immunophenotypic aberrancies [42]. The most common findings include loss of hematogones; altered expression level of CD13 (increased), CD38 (decreased), CD117 (increased), and/or CD123 (increased); or aberrant expression of lymphoid antigens. The identification of immunophenotypic aberrancies in blasts is highly suggestive of a diagnosis of MDS rather than AA.

Idiopathic AA must also be distinguished from transient myelosuppression caused by drug, toxin, or infection. In transient cytopenia(s) with a hypocellular marrow, interrogation of the clinical history is critical in identifying the causative agent; in some cases, the cytopenias resolve spontaneously without identifying an underlying cause, which may be an unrecognized viral infection. In order to exclude the possibility of a transient hypoplastic process due to an exogenous factor, it is recommended that an AA diagnosis should only be considered if cytopenias are persistent and the clinical and laboratory work-up is extensive and thorough but unrevealing.

Other conditions that can cause a profound hypocellular BM include anorexia nervosa or prolonged starvation, mycobacterial infection, and lymphomas. The differential diagnosis of AA with hereditary bone marrow failure syndromes and with pediatric MDS (refractory cytopenia of childhood) is discussed separately in Chap. 3.

Paroxysmal Nocturnal Hemoglobinuria (PNH)

Paroxysmal nocturnal hemoglobinuria (PNH) has long been classified as acquired hemolytic anemia, but it is now recognized as a stem cell disorder due to an acquired mutation in the *PIGA* gene that is located on the X chromosome. *PIGA* is involved in the synthesis of the glycosylphosphatidylinositol (GPI) anchor proteins that link dozens of cell-surface proteins to the plasma membrane on hematopoietic cells. GPI-link protein CD55 (decay-accelerating factor) prevents the formation of C3 convertases in the complement cascade, and CD59 inhibits assembly of the membrane attack complex of complement. Deficiency in these proteins on red cell surface is responsible for complement sensitivity of red cells in PNH. Classical PNH is characterized by complement-mediated intravascular hemolysis, as well as extravascular hemolysis. The latter is due to complement-mediated destruction of red blood cells by reticuloendothelial macrophages in the liver and spleen. PNH can arise de novo or in the setting of an underlying bone marrow disorder such as aplastic anemia (AA). The reported incidence of clinically significant PNH is 1–10 cases per million population [43].

In recent years, the clinical heterogeneity of PNH has been increasingly recognized. While classic PNH manifests complement-mediated hemolysis, in other patients, bone marrow failure dominates the clinical picture with modest or even no evidence of hemolysis. This clinical heterogeneity likely reflects the close relationship between PNH and immune-mediated bone marrow failure, and the fact that PNH is an acquired, clonal disease of the hematopoietic stem cells. Bone marrow failure complicates the management of PNH, because the intrinsically defective hematopoiesis contributes at varying degrees of anemia and other cytopenias; in addition, the extent to which the mutant stem cell clone expands in an individual patient determines the magnitude of the hemolytic component of the disease. The International PNH Interest Group (I-PIG) has proposed a working diagnostic classification that includes the following three categories: (1) classic PNH; (2) PNH in the context of another bone marrow disorder; and (3) subclinical PNH. The definitions, clinical and laboratory features, bone marrow findings, and PNH clone size of these three groups are summarized in Table 4.4.

Table 4.4 Comparison of aplastic anemia and hypoplastic MDS

	Aplastic anemia	Hypoplastic MDS
Bone marrow examination		
• Erythroid precursors	Lack, or very small erythroid clusters (often <10 cells/cluster), dyserythropoiesis either absent or mild	Patchy distribution of erythroid clusters, left shifted. Dyserythropoiesis is often seen
• Megakaryocytes	Often absent, or too few to assess morphology	Reduced, but more easily identifiable with dysplastic features
• Myeloid cells	Decreased, dysgranulopoiesis often absent	Decreased, more pronounced dysplasia
• Increased mast cells, lymphocytes, plasma cells	Often present	Often present
• Reticulin fibrosis	Absent	Can be present in 20% cases
Immunohistochemistry		
CD34	Decreased to absent (<1%)	Increased in some cases; if not increased, they are easily identified
TP53	Negative (lack of strongly positive cells)	Some cases may be positive (presence of strongly positive cells)
Flow cytometry immunophenotyping		
• CD34+ myeloblasts	Deceased; a normal immunophenotype	Increased CD13, CD34, CD117, CD123 expression levels; decreased CD38; aberrant lymphoid antigen expressions
• Hematogones	Often present	Often absent or markedly decreased
• PNH clones	40–50% cases	Much less frequent (10% very low grade MDS), if present, the clone size is very small
Cytogenetics	Often normal; some abnormalities may be seen, often involving small number of metaphases, could be transient	50% abnormal; however, markedly fatty marrows may lead to cytogenetic failures. FISH may be used in cases with insufficient metaphases
Molecular genetic studies	Mutations are very common, in about 50% of patients *PIGA, BCOR*, and *BCORL1* mutations are in favor of AA; *ASXL1,* and other age-related somatic mutations can be detected	*SF3B1, SRSF2, TET2, AXSL1, RUNX1, U2AF1, TP53, IDH1/2, EZH2*

Clinical Features

PNH is mostly a disease of adults, with the median age of onset in the 30s, and occurs equally in men and women [44, 45]. From the data from the international PNH registry [44], frequently reported symptoms include fatigue (80%), dyspnea (64%), hemoglobinuria (62%), abdominal pain (44%), and chest pain (33%).

Patients with classic PNH present with unexplained hemolytic anemia with jaundice, and red or pink urine. Laboratory workup shows anemia, increased lactate dehydrogenase (LDH) and bilirubin, decreased haptoglobin with a negative direct antiglobulin (Coombs) test, and an increased reticulocyte count, consistent with hemolysis. Thrombosis, mainly venous, occurs in about 5% of patients at the time of diagnosis, but the incidence of thrombotic events is as high as 40% over the entire course of disease. Thrombosis can involve the hepatic vein, inferior vena cava, and portal or splenic veins, and is the leading cause of death in patients with PNH. Patients with classic PNH have preserved overall bone marrow function and mainly present with anemia. However, many cases of PNH have some degree of bone marrow failure that either exacerbates the anemia or leads to pancytopenia. One study consisting of 220 PNH patients reported a 15% inci-

dence of pancytopenia at the 8 years of follow-up [45]. Some of these patients may belong to the group "PNH in the context of another bone marrow disorder," who show evidence of not only hemolysis but also another primary BM abnormality such as AA, MDS, or primary myelofibrosis (PMF) (Table 4.4).

"Subclinical PNH" is defined by the presence of a small population of PNH cells, without clinical or laboratory evidence of hemolysis, often in association with AA or MDS. It may be also observed in some patients with cytopenia(s) but not filling the criteria for AA or MDS. As discussed above, a PNH clone is identified in up to 60% of AA patients and in about 10% of MDS without excess blasts. Over time, the PNH clone size in AA may increase, and a subset of patients may develop classic PNH [46].

Morphology

In classic PNH where there is significant hemolysis, PB smears often show anisopoikilocytosis, increased polychromatic cells, and some nucleated RBC, features of intravascular and extravascular hemolysis. In patients who develop BM failure, PB often shows pancytopenia.

Bone marrow examination is not required for a diagnosis of PNH, but should be performed in patients with significant cytopenia(s) other than anemia. The BM in classic PNH (Fig. 4.5) is usually normocellular or slightly hypercellular with erythroid hyperplasia due to the intravascular and extravascular hemolysis. Mild dyserythropoiesis is common as a manifestation of "stress erythropoiesis." Stainable iron is often absent. Of patients with PNH in the context of another bone marrow disorder, the most commonly associated disease is AA, followed by MDS, and with rare cases of primary myelofibrosis [47] reported. Similarly, patients with subclinical PNH mostly have underlying AA and less frequently MDS. The diagnosis of these entities in patients with PNH clones should follow the standard diagnostic approach for AA and MDS.

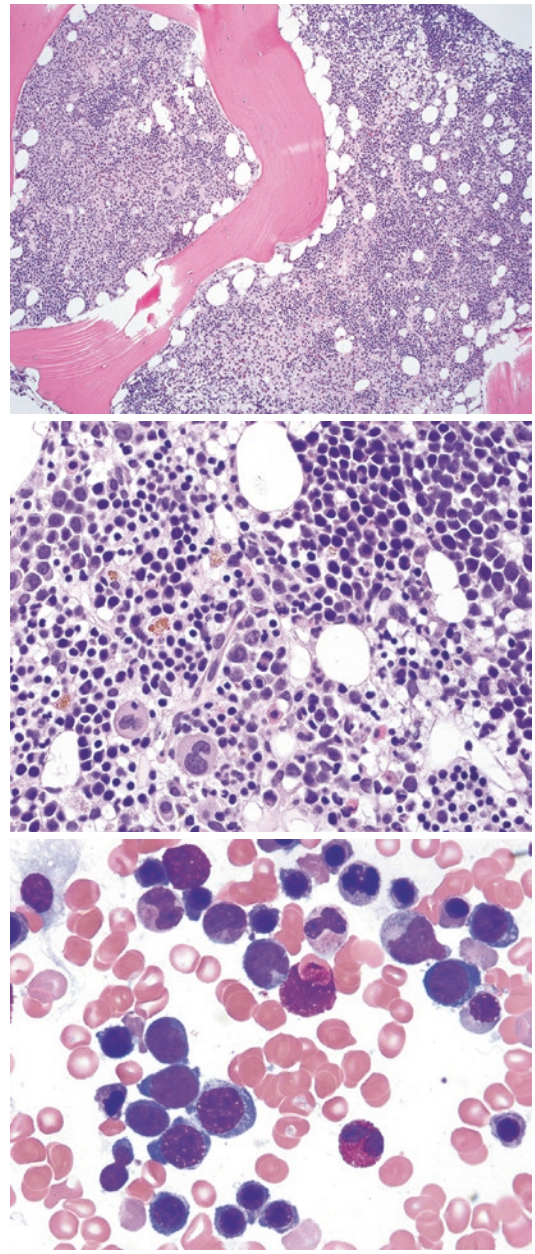


Fig. 4.5 Classic paroxysmal nocturnal hemoglobinuria (PNH). The bone marrow biopsy shows a marked hypercellularity (*Top Figure*) with normal megakaryocytes and erythroid hyperplasia (*Middle Figure*). The bone marrow aspirate smear shows erythroid predominance with dyspoietic changes ("stress erythropoiesis) secondary to hemolysis (*Bottom Figure*)

Immunophenotype

Flow cytometry to detect populations of GPI-deficient cells is the method of choice for diagnosis and monitoring PNH. A consensus guideline on screening criteria, reagents, methodology, and data analysis has been published by the International Clinical Cytometry Society (ICCS) [20]. The recommendation guidelines can be summarized as follows: (1) since PNH flow cytometry testing is to detect cells that are negative for GPI proteins, a lineage marker that is not GPI-anchored protein is required to define the population being analyzed. For example, CD15 is recommended to use for granulocytes, CD64 for monocytes, and CD235a (glycophorin) for red blood cells; (2) assessment of PNH populations in leukocytes is the best method to determine the size of a PNH clone. Neutrophils and monocytes should be assessed separately; (3) FLAER, a fluorochrome-conjugated inactive variant of aerolysin, which binds specifically to GPI anchors, is a better reagent than other individual antibodies for the detection of the PNH phenotype in neutrophils and monocytes. In addition to FLAER, assessing another GPI-linked protein is recommended to increase the reliability. The recommended markers are shown in Table 4.5. (4) Testing of red blood cells alone is not adequate for the evaluation of PNH patients, because hemolysis and transfusion may greatly underestimate the size of the PNH clone. However, if a large PNH clone is identified in leukocytes, RBC analysis is required to assess the severity of hemolysis by measuring cells lacking the expression of GPI-anchored proteins (PNH type III and type II cells, see below). (5) In order to detect a small PNH clone, the assay should reach 0.01% sensitivity. The flow cytometry test is illustrated in Fig. 4.3.

CD55 and CD59 are GPI proteins on RBCs. CD59 is often brighter than CD55, and is more closely associated with intravascular hemolysis. Three types of PNH cells have been described according to the abundance of GPI-anchored proteins on the cell surface. PNH type I cells have a normal level of GPI protein expressions; PNH type II cells show a low level (partial) of GPI protein expression and PNH type III cells show absence of GPI-linked proteins. PNH type II cells may be shown as a distinct population or a "transitional" population between PNH type I and PNH type III cells (Figs. 4.3 and 4.4). PNH type II cells may be due to a mutation in the *PIGA* gene [48] causing limited rather than completely abolished production of GPI-linked proteins. In general, the degree of hemolysis correlates with the size of the erythrocyte PNH clone; however, patients with a large percentage of type II RBCs

Table 4.5 Classification of paroxysmal nocturnal hemoglobinuria (PNH)

	Classic PNH	PNH in the setting of another bone marrow disorder	Subclinical PNH (PNH-sc)
Clinical intravascular hemolysis	Usually	Usually	No
Laboratory hemolysis (increased LDH, indirect bilirubin, low hepatoglobin)	Yes	Usually	No
Peripheral blood	Anemia	Anemia but frequent pancytopenia	Anemia but frequent pancytopenia
Bone marrow	Hypercellular with erythroid hyperplasia	Aplastic anemia or evidence of myelodysplastic syndrome or (less commonly) another myeloid neoplasm	Aplastic anemia or evidence of myelodysplastic syndrome
PNH clone size	Large (>10%)	Large (>10%)	Small (<10%)

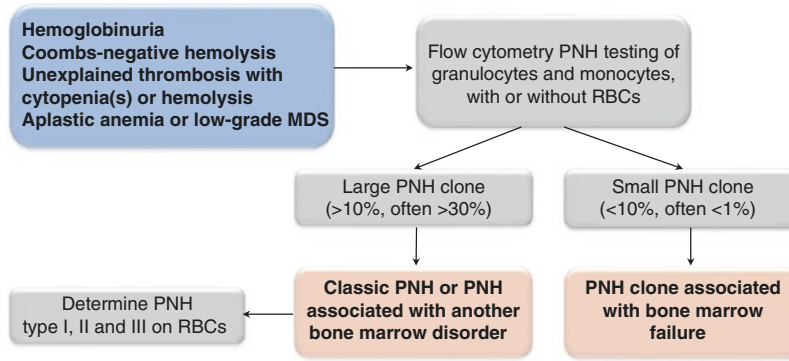


Fig. 4.6 Algorithm for paroxysmal nocturnal hemoglobinuria (PNH) testing. *RBC* red blood cell, *MDS* myelodysplastic syndrome

often have less hemolysis than patients with mostly or exclusively type III RBCs. Type II and type III PNH cells can be determined in leukocytes as well (Fig. 4.4). Figure 4.6 shows an algorithm of flow cytometry immunophenotyping studies of PNH phenotype cells.

Cytogenetics and Molecular Genetics

Acquired mutations in *PIGA* gene, which is located on the X chromosome, occur in early hematopoietic stem cells. These acquired *PIGA* mutations show a large spectrum of involved loci, spreading throughout the entire coding region of the *PIGA* gene, without any specific mutational "hot spot" [49, 50]. The majority are frame-shift mutations that create a premature stop codon, resulting in a truncated protein product that is unstable and rapidly degraded. Interestingly, rare circulating blood cells with *PIGA* mutations and a PNH immunophenotype have been detected in healthy blood donors [51]. These mutations are polyclonal and do not involve lymphocytes, indicating that the mutations occur in the hematopoietic cells. In contrast to patients with classic PNH and/or small PNH clones associated with AA/MDS, the *PIGA* mutations in healthy individuals appear to arise in hematopoietic precursor cells that lack the capacity for self-renewal, rather than in hematopoietic stem cells or earlier progenitor cells [52]. In PNH secondary to AA and MDS, the hematopoietic stem cell carrying a *PIGA* mutation can undergo clonal expansion due to a number of reasons, including immune escape, survival advantage, and clonal evolution.

Differential Diagnosis

Coombs-negative hemolytic anemia can mimic PNH, and may be caused by (1) hereditary red blood cell membrane or enzymatic defects, such as hereditary spherocytosis, sickle cell anemia, glucose-6-phosphate dehydrogenase deficiency, and pyruvate kinase deficiency; (2) drug- or toxin-induced hemolysis; (3) paroxysmal cold hemoglobinuria due to the presence of cold-reacting IgG antibodies; (4) microangiopathic hemolytic anemia; and (5) rarely autoimmune hemolysis, due to excessive hemolysis of antibody-coated cells that prevent their detection by the Coombs test. Unlike PNH, these conditions do not have PNH-type cells and are associated with characteristic red blood cell morphologies on the peripheral blood smear and other distinctive clinical features.

Thrombosis caused by an inherited or acquired hypercoagulable state can mimic the thrombosis associated with PNH. A variety of conditions can cause abdominal and cerebral venous thrombosis. Inherited conditions include factor V Leiden, prothrombin gene mutation, and protein S and C deficiency. Acquired conditions include myeloproliferative neoplasms, solid tumors associated with hypercoagulability, inflammatory diseases, compression of blood vessels, and antiphospholipid syndrome. Thrombosis in PNH is usually associated with evidence of intravascular hemolysis and often has a large population of PNH-type cells detected by flow cytometry.

Finally, as mentioned above, PNH and other bone marrow failure syndromes or myeloid neoplasms can coexist, and a diagnosis of one does not eliminate the possibility of the other.

Therefore, if there is evidence of hemolysis, it is important to follow the standard criteria for PNH screening in order not to miss the diagnosis of PNH whenever it may occur together with other disease processes.

References

1. Maciejewski JP, et al. Distinct clinical outcomes for cytogenetic abnormalities evolving from aplastic anemia. Blood. 2002;99(9):3129–35.
2. Nathan DG, Oski FA. Hematology of infancy and childhood. Philadelphia: WB Saunders; 2009.
3. Fuhrer M, et al. Immunosuppressive therapy for aplastic anemia in children: a more severe disease predicts better survival. Blood. 2005;106(6):2102–4.
4. Young NS, Maciejewski J. The pathophysiology of acquired aplastic anemia. N Engl J Med. 1997;336(19):1365–72.
5. Shimamura A, Guinan EA. Acquired aplastic anemia. In: Nathan D, Orkin SH, editors. Hematology of infancy and childhood. Philadelphia: WB Saunders; 2003. p. 256.
6. Young NS. Acquired aplastic anemia. Ann Intern Med. 2002;136(7):534–46.
7. Maciejewski JP, et al. Increased expression of Fas antigen on bone marrow CD34+ cells of patients with aplastic anaemia. Br J Haematol. 1995;91(1):245–52.
8. Ly H, et al. Functional characterization of telomerase RNA variants found in patients with hematologic disorders. Blood. 2005;105(6):2332–9.
9. Yamaguchi H, et al. Mutations in TERT, the gene for telomerase reverse transcriptase, in aplastic anemia. N Engl J Med. 2005;352(14):1413–24.
10. Stanley N, Olson TS, Babushok DV. Recent advances in understanding clonal haematopoiesis in aplastic anaemia. Br J Haematol. 2017;177:509.
11. Torres HA, et al. Infections in patients with aplastic anemia: experience at a tertiary care cancer center. Cancer. 2003;98(1):86–93.
12. Killick SB, et al. Guidelines for the diagnosis and management of adult aplastic anaemia. Br J Haematol. 2016;172(2):187–207.
13. Marsh JC, et al. Guidelines for the diagnosis and management of aplastic anaemia. Br J Haematol. 2009;147(1):43–70.
14. Nakao S, Sugimori C, Yamazaki H. Clinical significance of a small population of paroxysmal nocturnal hemoglobinuria-type cells in the management of bone marrow failure. Int J Hematol. 2006;84(2):118–22.
15. Young NS, Calado RT, Scheinberg P. Current concepts in the pathophysiology and treatment of aplastic anemia. Blood. 2006;108(8):2509–19.
16. de Planque MM, et al. Long-term follow-up of severe aplastic anaemia patients treated with antithymocyte globulin. Severe Aplastic Anaemia Working Party of the European Cooperative Group for Bone Marrow Transplantation (EBMT). Br J Haematol. 1989;73(1):121–6.
17. Sugimori C, et al. Minor population of CD55-CD59- blood cells predicts response to immunosuppressive therapy and prognosis in patients with aplastic anemia. Blood. 2006;107(4):1308–14.
18. Sutherland DR, et al. Diagnosing PNH with FLAER and multiparameter flow cytometry. Cytometry B Clin Cytom. 2007;72(3):167–77.
19. Morado M, et al. Diagnostic screening of paroxysmal nocturnal hemoglobinuria: prospective multicentric evaluation of the current medical indications. Cytometry B Clin Cytom. 2016;92:361.
20. Borowitz MJ, et al. Guidelines for the diagnosis and monitoring of paroxysmal nocturnal hemoglobinuria and related disorders by flow cytometry. Cytometry B Clin Cytom. 2010;78(4):211–30.
21. Shichishima T, Noji H. A new aspect of the molecular pathogenesis of paroxysmal nocturnal hemoglobinuria. Hematology (Amsterdam, Netherlands). 2002;7(4):211–27.
22. Young NS. Paroxysmal nocturnal hemoglobinuria: current issues in pathophysiology and treatment. Curr Hematol Rep. 2005;4(2):103–9.
23. Yoshizato T, et al. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med. 2015;373(1):35–47.
24. McKerrell T, et al. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. Cell Rep. 2015;10(8):1239–45.
25. Young NS, Ogawa S. Somatic mutations and clonal hematopoiesis in aplastic anemia. N Engl J Med. 2015;373(17):1675–6.
26. Kulasekararaj AG, et al. Somatic mutations identify a subgroup of aplastic anemia patients who progress to myelodysplastic syndrome. Blood. 2014;124(17):2698–704.
27. Inaguma Y, et al. Induction of HLA-B*40:02-restricted T cells possessing cytotoxic and suppressive functions against haematopoietic progenitor cells from a patient with severe aplastic anaemia. Br J Haematol. 2016;172(1):131–4.
28. Katagiri T, et al. Frequent loss of HLA alleles associated with copy number-neutral 6pLOH in acquired aplastic anemia. Blood. 2011;118(25):6601–9.
29. Ohga S, et al. Treatment responses of childhood aplastic anaemia with chromosomal aberrations at diagnosis. Br J Haematol. 2002;118(1):313–9.
30. Mikhailova N, et al. Cytogenetic abnormalities in patients with severe aplastic anemia. Haematologica. 1996;81(5):418–22.
31. Appelbaum FR, et al. Clonal cytogenetic abnormalities in patients with otherwise typical aplastic anemia. Exp Hematol. 1987;15(11):1134–9.
32. Moormeier JA, et al. Trisomy 6: a recurring cytogenetic abnormality associated with marrow hypoplasia. Blood. 1991;77(6):1397–8.
33. Fuhrer M, et al. Relapse and clonal disease in children with aplastic anemia (AA) after immunosuppressive therapy (IST): the SAA 94 experience. German/

Austrian Pediatric Aplastic Anemia Working Group. Klin Padiatr. 1998;210(4):173–9.
34. Kojima S, et al. Risk factors for evolution of acquired aplastic anemia into myelodysplastic syndrome and acute myeloid leukemia after immunosuppressive therapy in children. Blood. 2002;100(3):786–90.
35. Yamazaki E, et al. The evidence of clonal evolution with monosomy 7 in aplastic anemia following granulocyte colony-stimulating factor using the polymerase chain reaction. Blood Cells Mol Dis. 1997;23(2):213–8.
36. Doney K, et al. Primary treatment of acquired aplastic anemia: outcomes with bone marrow transplantation and immunosuppressive therapy. Seattle Bone Marrow Transplant Team. Ann Intern Med. 1997;126(2):107–15.
37. Socie G, et al. Malignant tumors occurring after treatment of aplastic anemia. European Bone Marrow Transplantation-Severe Aplastic Anaemia Working Party. N Engl J Med. 1993;329(16):1152–7.
38. Frickhofen N, et al. Antithymocyte globulin with or without cyclosporin A: 11-year follow-up of a randomized trial comparing treatments of aplastic anemia. Blood. 2003;101(4):1236–42.
39. Rosenfeld S, et al. Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome. JAMA. 2003;289(9):1130–5.
40. Orazi A, et al. Hypoplastic myelodysplastic syndromes can be distinguished from acquired aplastic anemia by CD34 and PCNA immunostaining of bone marrow biopsy specimens. Am J Clin Pathol. 1997;107(3):268–74.
41. Wang SA, et al. Detection of paroxysmal nocturnal hemoglobinuria clones in patients with myelodysplastic syndromes and related bone marrow diseases, with emphasis on diagnostic pitfalls and caveats. Haematologica. 2009;94(1):29–37.
42. Stetler-Stevenson M, et al. Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. Blood. 2001;98(4):979–87.
43. Gulbis B, et al. Epidemiology of rare anaemias in Europe. Adv Exp Med Biol. 2010;686:375–96.
44. Schrezenmeier H, et al. Baseline characteristics and disease burden in patients in the International Paroxysmal Nocturnal Hemoglobinuria Registry. Haematologica. 2014;99(5):922–9.
45. Socie G, et al. Paroxysmal nocturnal haemoglobinuria: long-term follow-up and prognostic factors. French Society of Haematology. Lancet. 1996;348(9027):573–7.
46. Moyo VM, et al. Natural history of paroxysmal nocturnal haemoglobinuria using modern diagnostic assays. Br J Haematol. 2004;126(1):133–8.
47. Parker C, et al. Diagnosis and management of paroxysmal nocturnal hemoglobinuria. Blood. 2005;106(12):3699–709.
48. Rollinson S, et al. Both paroxysmal nocturnal hemoglobinuria (PNH) type II cells and PNH type III cells can arise from different point mutations involving the same codon of the PIG-A gene. Blood. 1997;89(8):3069–71.
49. Ostendorf T, et al. Heterogeneous PIG-A mutations in different cell lineages in paroxysmal nocturnal hemoglobinuria. Blood. 1995;85(6):1640–6.
50. Nafa K, et al. The spectrum of somatic mutations in the PIG-A gene in paroxysmal nocturnal hemoglobinuria includes large deletions and small duplications. Blood Cells Mol Dis. 1998;24(3):370–84.
51. Araten DJ, et al. Clonal populations of hematopoietic cells with paroxysmal nocturnal hemoglobinuria genotype and phenotype are present in normal individuals. Proc Natl Acad Sci U S A. 1999;96(9):5209–14.
52. Hu R, et al. PIG-A mutations in normal hematopoiesis. Blood. 2005;105(10):3848–54.

Leukocytosis: Neutrophilia, Basophilia, and Blasts

5

Chi Young Ok and Robert P. Hasserjian

Overview

White blood cells (WBCs) are generally classified into two groups based on the density of granules: granulocytes (polymorphonuclear leukocytes) and agranulocytes (mononuclear leukocytes). The granulocytes contain azurophilic primary granules (lysosomes) and specific secondary granules and can be subdivided into neutrophils, eosinophils, and basophils depending on the shape of the nucleus and type of granules. The agranulocytes contain only azurophilic granules and include lymphocytes and monocytes. The specific granules have specific functions and bind neutral, basic, or acidic components of the dye mixture. All granulocytes differentiate from CD34+ hematopoietic stem cells in the bone marrow, which also serves as a site of maturation and a storage pool for granulocytes. In the bone marrow of healthy individuals, all stages of granulocytes (myeloblasts, promyelocytes, myelocytes, metamyelocytes, band forms, and mature granulocytes) are observed. Following egress from the bone marrow, granulocytes circulate in the blood vessels (the "circulating pool") or are adherent to vascular endothelium (the "marginated pool") and finally migrate into the tissue (the "tissue pool").

Neutrophils constitute approximately 60–70% of circulating WBCs. In healthy individuals, mostly mature neutrophils with occasional (3–5% of all WBCs) band forms are observed in peripheral blood. When there is a significant increase in the band forms or more immature neutrophilic precursors (metamyelocytes or myelocytes), the term "left shift" is often used. Neutrophils are 12–15 μm in diameter and have 2–5 nuclear lobes connected by fine threads of nuclear material. The half-life of neutrophils is 6–8 h in peripheral blood [1]. The azurophilic primary granules are large, dense vesicles that contain several antibacterial proteins and proteases including myeloperoxidase and lysozyme. The specific secondary granules are not as large as azurophilic granules and are faint pink. They play a role in antibacterial function and phagolysosomal killing.

Basophils have a similar size to neutrophils and constitute less than 1% of circulating WBCs. The nucleus has two irregular lobes, but the nucleus is usually obscured by the large specific basophilic granules. The half-life of basophils is estimated at a few days in circulation [2]. The specific granules stain metachromatically with basic dyes (e.g., toluidine blue or Alcian blue) due to the presence of heparin and other sulfated glucosaminoglycans. These constituents stain deeply basophilic on Wright-Giemsa stain of air-dried

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smear preparations, but are water soluble and thus are not visible in tissue sections that have passed through aqueous processing. The specific granules also contain histamine, hydrolase, platelet-activating factor, and phospholipase A.

In healthy adults, the normal total WBC count ranges from 4 to 11 × 10^9/L. Leukocytosis is defined as a value of WBC greater than 11 × 10^9/L in adults or a total WBC exceeding two standard deviations (SDs) above the mean. Depending on the specific cell type that is increased, leukocytosis can be subcategorized as granulocytosis (due to neutrophilia, eosinophilia, and/or basophilia), lymphocytosis, and monocytosis; occasionally, an absolute increase in more than one cell type is observed.

This chapter presents the neoplastic and non-neoplastic conditions that are mainly associated with neutrophilia and basophilia. Disorders of eosinophils, lymphocytes, and monocytes are addressed in separate chapters. The chapter also covers acute myeloid leukemias, acute leukemias of ambiguous lineage, and blastic plasmacytoid dendritic cell neoplasm and the differential diagnosis of increased blasts in the blood and bone marrow. The features of acute lymphoblastic leukemia are discussed in Chaps. 11 and 12, together with T-cell and B-cell lymphocytosis, respectively.

Reactive Neutrophilia and Basophilia

Neutrophilia is defined as an elevated absolute neutrophil count (ANC) that is >7.7 × 10^9/L in adults or that is greater than two SDs above the mean [3]. The ANC can be calculated as the WBC count multiplied by the percentage of mature granulocytes and band forms. Neutrophilia can be seen with or without circulating neutrophilic precursors. Based on its etiology, neutrophilia can often be divided into secondary (reactive) neutrophilia and primary neutrophilia (Table 5.1). Infrequently, spurious neutrophilia can be present due to artifacts such as platelet clumping (with platelet clumps erroneously counted as neutrophils by automated analyzers) or cryoglobulinemia (precipitated particles of which may be counted as neutrophils by automated analyzers when samples are at temperatures below 30 °C), so care should be taken [4, 5].

The causes of secondary neutrophilia are listed in (Table 5.1) [6–17]. Leukemoid reaction is a term used to describe reactive leukocytosis with a WBC count of >50 × 10^9/L, often with circulating neutrophilic precursors, without bone marrow involvement by a myeloid neoplasm. A leukoerythroblastic smear refers to the presence of circulating neutrophilic precursors as well as nucleated red cells and often teardrop erythrocytes. It is often associated with marrow fibrosis due to a reactive or neoplastic condition, but occasionally can be seen transiently in the same situations that cause secondary neutrophilia.

Constitutional primary neutrophilia is rare; some of the constitutional disorders have been associated with particular gene mutations. Primary constitutional neutrophilias include hereditary chronic neutrophilia (OMIM# 162830), leukocyte adhesion deficiency, type I (OMIM# 116920), and idiopathic Sweet syndrome (OMIM# 608068) [18–21].

Table 5.1 Causes of neutrophilia

Secondary neutrophilia	Primary neutrophilia	Spurious neutrophilia
• Smoking	• Constitutional	• Platelet clumping
• Infection	• Myeloid neoplasm associated	• Cryoglobulinemia
• Inflammation	• CML, *BCR-ABL1*+	
• Drugs	• CNL	
• Stress	• aCML and other MDS/MPN	
• Hemorrhage	• MPN (PV, ET, PMF)	
• Myelophthisis	• AML	
• Paraneoplastic syndromes		
• Asplenism		

CML chronic myeloid leukemia, *CNL* chronic neutrophilic leukemia, *aCML* atypical chronic myeloid leukemia, *BCR-ABL1* negative, *MDS/MPN* myelodysplastic/myeloproliferative neoplasm, *MPN* myeloproliferative neoplasm, *PV* polycythemia vera, *ET* essential thrombocythemia, *PMF* primary myelofibrosis, *AML* acute myeloid leukemia

Primary neutrophilias associated with myeloid neoplasms are discussed later in this chapter.

Basophilia is defined as an elevated circulating basophil count (>0.3 × 10^9/L in adults) [3]. Reactive basophilia can be associated with hypersensitivity reactions, infections, inflammation, drugs, or endocrine diseases. True reactive/secondary basophilias are rare and sustained basophilia is usually indicative of a neoplastic process.

Clinical Features

Smoking is one of the most common causes of mild neutrophilia. One single-institution study of 300 smokers demonstrated that both leukocytosis and neutrophilia are significantly associated with smoking [22]. In smoking-related reactive neutrophilia, the neutrophilia is mild, with a WBC count that is less than 20 × 10^9/L. The leukocytosis can persist up to 10 years even after smoking cessation [9].

Leukocytosis with left-shifted granulocytic elements is a common finding in pyogenic bacterial infection or occasionally viral infections (e.g., herpes viruses) [7]. Patients with acute infection usually have more severe leukocytosis (>25 × 10^9/L) compared to smokers. Elevation of neutrophils occurs within hours of the infection, suggesting release of mature neutrophils from the bone marrow storage and marginated pools. Neutrophilia can also be present in autoimmune diseases, including Kawasaki's disease, adult-onset Still's disease, Crohn's disease, and ulcerative colitis, and has been associated with disease flare-ups [6, 10]. Neutrophilic infiltrates in tissues can be seen in patients with psoriasis, rheumatoid arthritis, systemic lupus erythematosus, and anti-neutrophil cytoplasmic antibody (ANCA)-related vasculitides. It is well known that several drugs induce neutrophilia. Granulocyte-colony-stimulating factor (G-CSF) directly stimulates the production of neutrophils in the bone marrow and their release to circulation. G-CSF is often administered to patients being treated with chemotherapy for acute leukemia or solid tumors, and can cause neutrophilia with circulating immature granulocytes and even a transient increase in circulating blasts [11]. Flow cytometric immunophenotyping (by identifying an aberrant immunophenotype on the circulating blast cells) as well as cytogenetics and molecular genetic testing can be helpful to exclude persistent disease or relapse in leukemia patients with leukocytosis and circulating blasts on G-CSF therapy; the neutrophilia and circulating blasts usually resolve within 7–10 days following cessation of the G-CSF. Steroid or epinephrine administration can cause mild neutrophilia, as well as emotional or physical stress that increases the levels of adrenocortical steroids [12]. Neutrophilia usually occurs within minutes of the exercise or stress and appears to result from release of neutrophils from the marginated pool to the circulation [13]. Similarly, neutrophilia can be seen in the first 3 days in newborns or in women postpartum.

Myelophthisis refers to the replacement of hematopoietic cells and stroma in the bone marrow with abnormal cells, including metastatic tumors, granulomas, lipid-laden macrophages secondary to storage diseases, or fibrosis due to various causes, and may manifest as leukocytosis or leukoerythroblastosis [23]. In this condition, organomegaly due to extramedullary hematopoiesis is often present.

Paraneoplastic leukocytosis has long been recognized in cancer patients, particularly those with plasma cell myeloma or lung cancer [17, 24]. Although precise mechanisms are unknown, secretion of colony stimulating factors by tumor cells is a likely etiology for the leukocytosis [15, 17, 25]. Of note, neutrophils in paraneoplastic leukocytosis are generally deformable and are unlikely to cause leukostasis. Therefore, leukapheresis or cytoreductive therapies are not usually necessary in paraneoplastic leukocytosis.

The spleen contains a significant number of marginated neutrophils and thus asplenia (the absence of the spleen or its function) can induce moderate neutrophilia. Asplenia can be related to congenital diseases (e.g. Ivemark syndrome and sickle cell disease) or surgical splenectomy for hemoglobinopathies, storage disease, or trauma [26]. After acute hemorrhage, a significant increase in neutrophils is observed without increment in the ratio of band forms to mature neutrophils, suggesting release of neutrophils from the marginated splenic pool [27].

Morphology

Review of peripheral blood smear is an important first step to evaluate whether leukocytosis is reactive or may be part of neoplastic processes. In reactive neutrophilia, the peripheral blood usually shows mainly mature neutrophils and band forms without circulating blasts, significant granulocytic dysplasia, eosinophilia, or basophilia. There is often an increased proportion of maturing neutrophils (myelocytes and metamyelocytes) and band forms in the blood (Fig. 5.1a, b). In some cases, promyelocytes and/or blasts can circulate in the blood, potentially mimicking a myeloid neoplasm (see Differential Diagnosis section below). In neutrophilias associated with infection, neutrophils may show toxic granulation, vacuoles, or Döhle bodies (remnants of rough endoplasmic reticulum) in the cytoplasm. Similar changes are also often seen following G-CSF administration or following marrow recovery from chemotherapy (Fig. 5.1c). Aside from these changes, the neutrophils in most secondary neutrophilias are morphologically unremarkable, although hypogranulation may be observed in severe infections.

In severe, unexplained neutrophilia or leukoerythroblastosis, bone marrow examination including flow cytometry immunophenotyping, conventional karyotyping, and molecular testing to investigate for the various myeloid neoplasms

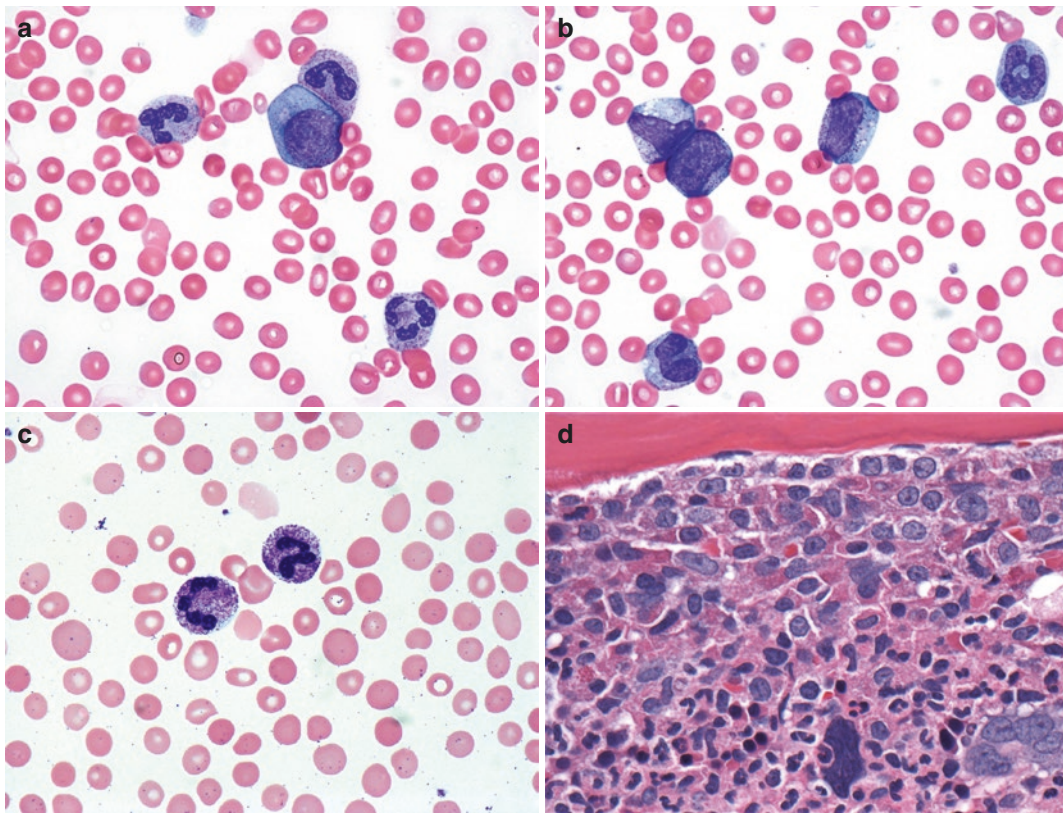

Fig. 5.1 Reactive leukocytosis. (**a**) In reactive leukocytosis due to an infection, increased immature granulocytic forms are often present in the blood. (**b**) Marrow stimulation by myeloid growth factors (G-CSF) can also result in circulating left-shifted granulocytic forms, including blasts and promyelocytes. (**c**) Toxic granulation, in which the neutrophil granules appear dark purple, is frequently seen in infection, G-CSF stimulation, and marrow recovery from chemotherapy or other insult. (**d**) In reactive neutrophilia, there is often hyperplasia and left shift of myeloid elements within the bone marrow, which may be markedly hypercellular; in such cases, normal marrow topography is preserved in that the most immature myeloid elements are adjacent to bone trabeculae while mature neutrophils are found further from the trabecular surface

that can cause neutrophilia (see below) may be indicated. The bone marrow in secondary neutrophilia can be hypercellular, particularly when it occurs after marrow injury with destruction of blood cells, but is more often normocellular for age. Normal maturation is present in all lineages of hematopoietic cells, without significant dysplasia. The topography of hematopoietic cells in the bone marrow is preserved: when increased granulocytic precursors are seen, they are located adjacent to the bone trabecular surface or are perivascular (Fig. 5.1d). Blasts in the normal bone marrow should be less than 5% of all cells, but they may increase transiently after administration of G-CSF.

Immunophenotype

Reviewing a peripheral smear is an important first step in approaching an unexplained neutrophilia or leukocytosis, because if no blasts or abnormal cells are seen on the smear, flow cytometry has limited utility and is usually not indicated. If review of the peripheral blood smear identifies circulating blasts (that are not explained by a known underlying disease or inciting factor) or abnormal cells, flow cytometry can help determine the immunophenotype of these cells and thereby narrow the differential diagnosis. It is important to note that in bone marrows heavily infiltrated by a nonmyeloid neoplasm (including acute lymphoblastic leukemia), the circulating blasts may be myeloblasts due to the myelophthisic process, and the neoplastic cells may not be in the circulation. Thus, the presence of a small number of circulating myeloblasts in a patient with neutrophilia or leukoerythroblastosis suspected of having a bone marrow malignancy should not be used to infer a diagnosis of acute myeloid leukemia (AML) or any myeloid neoplasm. If performed, flow cytometry does not demonstrate an aberrant blast immunophenotype in secondary neutrophilia.

Cytogenetics and Molecular Genetics

Evaluation for *BCR-ABL1* rearrangement by RT-PCR and/or FISH testing of peripheral blood is important to exclude CML in cases of unexplained neutrophilia and/or basophilia. Testing of a blood sample for the presence of mutations that are typically found in other myeloproliferative neoplasms (*JAK2*, *MPL*, *CALR*, and *CSF3R*) can be considered to evaluate an unexplained neutrophilia, but caution must be taken in interpreting a positive result in isolation, since mutations in hematopoietic cells can occur in aging individuals without a hematopoietic malignancy [28].

Differential Diagnosis

When leukocytosis is first noticed, artifacts such as platelet clumping or cryoglobulinemia first need to be considered and excluded. If neutrophilia is a real finding, a thorough review of medical history, family history, medication history, and physical examination is necessary to identify possible findings that suggest reactive leukocytosis. If family history is suggestive of a constitutional disease or syndrome, genetic consultation is also recommended.

The presence of circulating blasts and/or dysgranulopoiesis raises concern for a neoplastic process and, if persistent and unexplained, bone marrow examination may be indicated. Leukocytosis with eosinophilia and/or basophilia can be seen in myeloproliferative neoplasms, particularly CML, *BCR-ABL1+,* which shows characteristic bone marrow morphology and has a disease-defining *BCR-ABL1* rearrangement (see below). Conventional karyotype, FISH or RT-PCR, to evaluate for *BCR-ABL1* rearrangement can be sent from a blood specimen to help exclude the possibility of CML. Mutational analysis for *CSF3R* can be considered, because its presence (in the absence of *BCR-ABL1* rearrangement or severe neutrophil dysplasia) would suggest a diagnosis of chronic neutrophilic leukemia. Most other myeloproliferative neoplasms (MPN) that may present with neutrophilia (polycythemia vera, essential thrombocythemia, and primary myelofibrosis) have mutations in either *JAK2*, *MPL*, or *CALR* genes. Bone marrow examination is usually indicated to distinguish among these MPN, as they have characteristic bone marrow morphologies despite overlapping mutation profiles.

A suggested diagnostic algorithm for the workup of neutrophilias is shown in Fig. 5.7.

Neoplastic Causes of Neutrophilia

Chronic Myeloid Leukemia

Chronic myeloid leukemia, *BCR-ABL1*+ (CML), is a myeloproliferative neoplasm originating from an abnormal pluripotent bone marrow stem cell bearing the *BCR-ABL1* fusion gene in the Philadelphia (Ph) chromosome [29]. CML is divided into three phases of disease based on the bone marrow and peripheral blood blast counts and other clinical and pathologic features: chronic phase (CP), accelerated phase (AP), and blast phase (BP). The blast phase of CML fulfills features of an acute myeloid, lymphoid, or mixed lineage leukemia. Most patients present in CP, but occasional patients may be diagnosed first in the accelerated or even blastic disease phases.

Clinical Features

The median age at diagnosis is 65 years, but children as young as 3 years can be affected. A slight male predominance is observed. The annual incidence of disease is 1–2 per 100,000 population. Patients are often initially identified due to leukocytosis being found during routine blood work, because approximately half of the patients at diagnosis do not have symptoms. The other half complain of fatigue, malaise, weight loss, or night sweats. Splenomegaly and hepatomegaly are seen in about 50% and 20% of patients, respectively.

Morphology

In the chronic phase of CML (CML-CP), patients usually present with leukocytosis with left-shifted neutrophilic maturation, eosinophilia, and basophilia in the peripheral blood (Fig. 5.2a). The bone marrow demonstrates marked hypercellularity for age, usually approaching 100%, with extreme granulocytic proliferation at all maturational stages (Fig. 5.2b). There is usually a markedly elevated myeloid:erythroid ratio. Megakaryocytes are increased (>6/high-power field) in about half of the cases, and often form clusters. They are usually smaller than normal megakaryocytes and have hypolobated nuclei (so-called "dwarf megakaryocytes") (Fig. 5.2c, d). Histiocytes with foamy or striated cytoplasm (in the core biopsy or clot sections) or blue cytoplasm with striation (in the aspirate smears) are commonly seen (Fig. 5.2d). These cells are called pseudo-Gaucher histiocytes or sea-blue histiocytes and have excess cytoplasmic phospholipid from increased cellular turnover (Fig. 5.2e). Marrow eosinophils and basophils are increased in virtually all cases. Blasts comprise <5% of nucleated cells in the bone marrow in most cases at diagnosis. Variable degrees of reticulin fibrosis can be present. Significant dysplasia is not a feature of CML-CP, but can be a feature seen during disease progression to accelerated or blast phase (Fig. 5.2f).

In accelerated phase (CML-AP), (1) persistent or increased WBC count ($>10 \times 10^9$/L) uncontrolled by therapy, (2) persistent thrombocytosis ($>1000 \times 10^9$/L) uncontrolled by therapy, (3) persistent thrombocytopenia ($<100 \times 10^9$/L) unrelated to therapy, (4) persistent or increasing splenomegaly unresponsive to therapy, (5) ≥20% basophils in the peripheral blood, or (6) 10–19% blasts in the blood or bone marrow are present. In the revised 2016 WHO Classification, certain additional chromosomal abnormalities at diagnosis also can define CML-AP (see "Cytogenetics and Molecular Genetics" section below). The revised classification also introduced provisional CML-AP criteria based on responsiveness to treatment with tyrosine kinase inhibitors (TKI) [29].

In blastic phase (CML-BP), ≥20% blasts are present in the blood or bone marrow. A mass-forming blast proliferation that disrupts architecture in an extramedullary tissue (myeloid sarcoma) is also considered to define blast phase in CML.

Immunophenotype

Flow cytometry is required to determine the blast phenotype in the blastic phase of CML. In approximately three-quarters of CML-BP, the blasts are of myeloid lineage and the remaining cases are lymphoid (nearly always B-lymphoblastic, rarely T-lymphoblastic or NK-cell) lineage. The blast phase of CML may also occasionally demonstrate a mixed (B/myeloid) lineage. Of note, although ≥20% lymphoid blasts are required to make a diag-

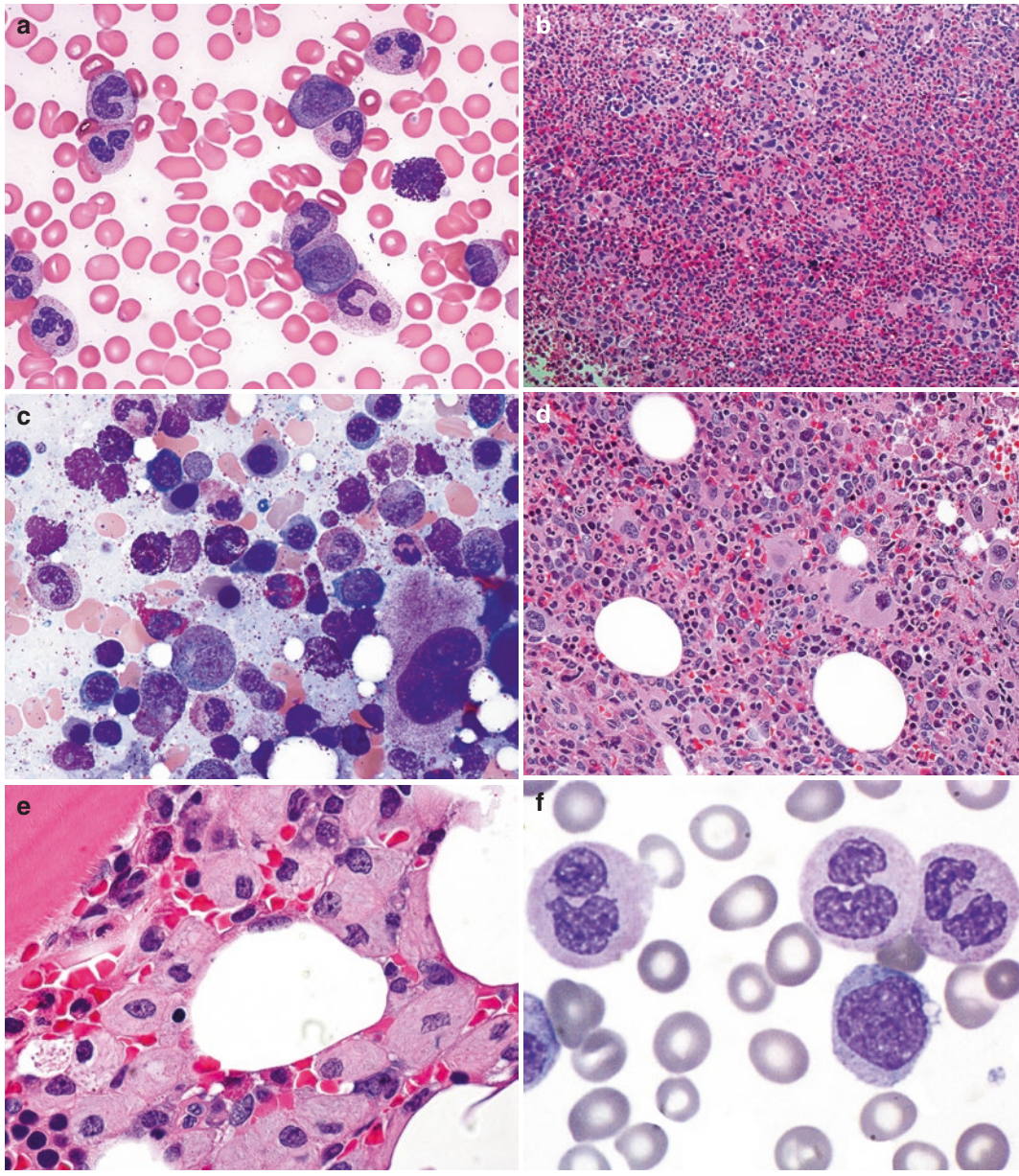

Fig. 5.2 Chronic myeloid leukemia, *BCR-ABL1*+. (**a**) The peripheral blood shows leukocytosis with left-shifted granulocytic forms, as well as increased basophils. (**b**) The marrow biopsy is markedly hypercellular at diagnosis, with a predominance of maturing granulocytic forms and often increased megakaryocytes and eosinophils. (**c**) In the bone marrow aspirate, the myeloid:erythroid ratio is elevated, basophils are increased, and small megakaryocytes with rounded nuclei are evident. (**d**) Small "dwarf" megakaryocytes form clusters amid numerous maturing myeloid elements. (**e**) Pseudo-Gaucher cells can be seen in the aspirate and the biopsy (illustrated) and have pale, striated cytoplasm. (**f**) Dysplastic granulocytic forms are usually not seen in the chronic phase of disease, but may be seen in accelerated phase (illustrated) or blast crisis

nosis of lymphoid BP in CML, the identification of even small numbers of lymphoid blasts is very worrisome for rapid progression to BP and such patients should be followed closely [30] (Fig. 5.3).

Cytogenetics and Molecular Genetics

The presence of the Ph chromosome, resulting from t(9;22)(q34.1;q11.2), is a hallmark of CML. The Ph chromosome contains the *BCR-*

ABL1 fusion gene encoding BCR-ABL1 oncoprotein that has constitutively activated tyrosine kinase activity. Breakpoints in the *BCR* gene at 22q11.2 are relatively consistent, whereas breakpoints in the *ABL1* gene at 9q34.1 are variable and can occur in exons 1–2 (minor breakpoint cluster region), 12–16 (major breakpoint cluster region), or 17–20 ("micro" breakpoint cluster region), generating p190, p210, or p230 fusion proteins, respectively. Almost all patients with CML harbor the p210 fusion protein. The p190 fusion protein is seen in approximately half of *BCR-ABL1*+ B-ALL and is only rarely seen in CML; this small subset of CML patients often present with monocytosis, mimicking chronic myelomonocytic leukemia. The p230 fusion protein is another very rare form of the BCR-ABL1 oncoprotein in CML. Patients most often present with neutrophilia and/or thrombocytosis, mimicking chronic neutrophilic leukemia (CNL) or essential thrombocythemia (ET).

The *BCR-ABL1* translocation can be detected in peripheral blood or bone marrow aspirate by cytogenetics, FISH, or RT-PCR (Fig. 5.4a). However, by conventional karyotype, the Ph chromosome is absent in approximately 5% of CML patients due to a variant translocation or a cytogenetically cryptic *BCR-ABL1* fusion; these variant *BCR-ABL1* rearrangements can be detected by FISH or qualitative RT-PCR. Importantly, false-negative results can occur with quantitative RT-PCR, which is usually performed in CML at diagnosis to establish a baseline level of *BCR-ABL1* transcript to compare subsequent levels on therapy. In many labs, quantitative RT-PCR only detects the major breakpoint-region transcripts and may miss the alternative

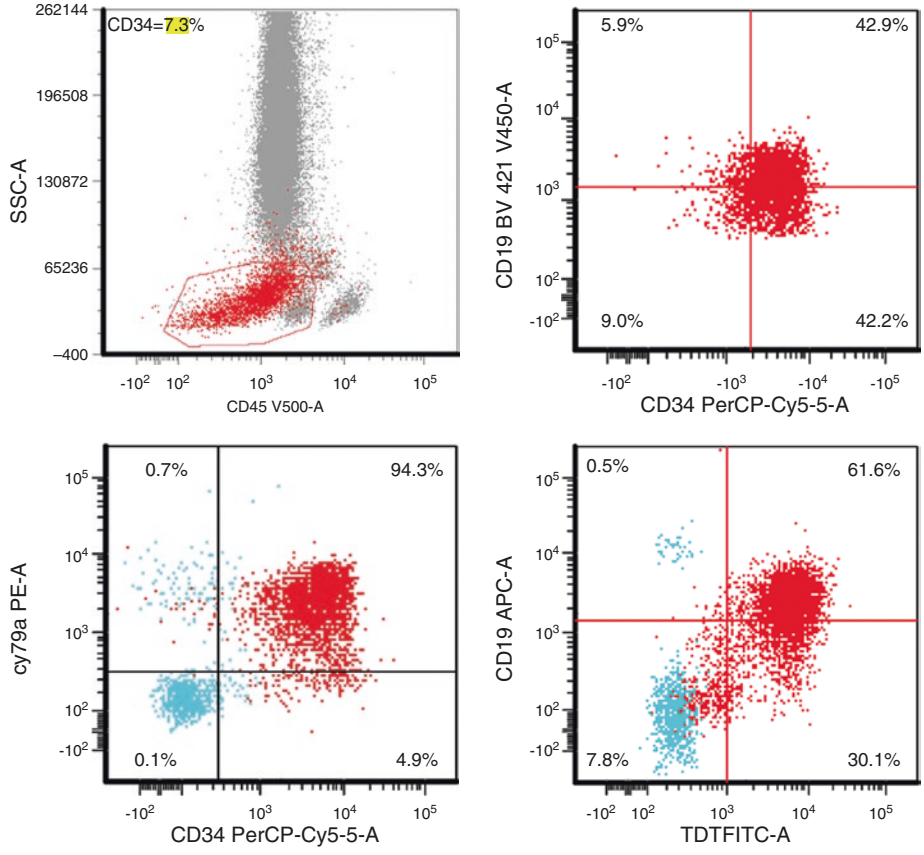

Fig. 5.3 Flow cytometry from a case of chronic myeloid leukemia with increased B-lymphoblasts. Although the B-lymphoblasts (CD19+, CD34+, cytoCD79a+, TdT+) only comprised 7.4% of all events, the patient rapidly progressed to B-lymphoid blast crisis

transcripts due to p190 or p230 BCR-ABL1 variants. Thus, if an unexpectedly false RT-PCR *BCR-ABL1* test is obtained in a case that is suggestive of CML, qualitative RT-PCR, and/or confirmatory FISH and conventional karyotyping should be performed. Although *BCR-ABL1* fusion detected by RT-PCR or FISH can confirm the presence of *BCR-ABL1* rearrangement, conventional karyotype of bone marrow aspirate (or blood if the marrow is inaspirable) is required at diagnosis and follow-up because it can demonstrate cytogenetic abnormalities other than the Ph chromosome. Some additional abnormalities (extra Ph chromosome, isochromosome 17q, +8, +19, abnormalities of 3q26.2, or complex karyotype), when present in the CML cells at initial diagnosis or in follow-up samples, are considered to define accelerated phase in the revised 2016 WHO Classification [29] (Fig. 5.4b).

Differential Diagnosis

In contrast to CML, chronic neutrophilic leukemia (CNL) demonstrates neutrophilia without increased neutrophilic precursors or basophilia and lacks *BCR-ABL1* rearrangement. Atypical CML, *BCR-ABL1* negative (which is a distinct myelodysplastic/myeloproliferative neoplasm that is completely unrelated to "true" CML despite its similar name), shares neutrophilia with circulating immature granulocytic precursors with CML, but demonstrates significant neutrophil dysplasia and lacks basophilia, which is present in most CML cases. CML presenting in accelerated phase may manifest dysgranulopoiesis, potentially mimicking atypical CML. *BCR-ABL1* genetic testing is helpful in confirming the diagnosis in such cases, since atypical CML by definition lacks *BCR-ABL1* rearrangement.

Cases of CML with prominent eosinophilia can mimic hypereosinophilic syndrome, chronic eosinophilic leukemia, or one of the genetically defined eosinophilic neoplasms. Again, genetic testing for *BCR-ABL1* rearrangement helps confirm CML and excludes primary eosinophilic neoplasms in such cases.

CML cases often have concomitant thrombocytosis and/or monocytosis, which may raise the differential diagnosis of another MPN (particularly essential thrombocythemia) or chronic myelomonocytic leukemia (CMML). In particular, the rare CML cases with the p190 BCR-ABL1 fusion protein often present with monocytosis with minimal leukocytosis and the rare cases bearing p230 fusion protein often present with neutrophilia and thrombocytosis. Evaluating for *BCR-ABL1* rearrangement by qualitative RT-PCR, FISH, and/or conventional karyotyping is critical in avoiding a misdiagnosis as another myeloid neoplasm, particularly given the highly specific and effective TKI therapies available to treat CML. Bone marrow morphology can be very helpful, because the markedly elevated myeloid:erythroid ratio and small,

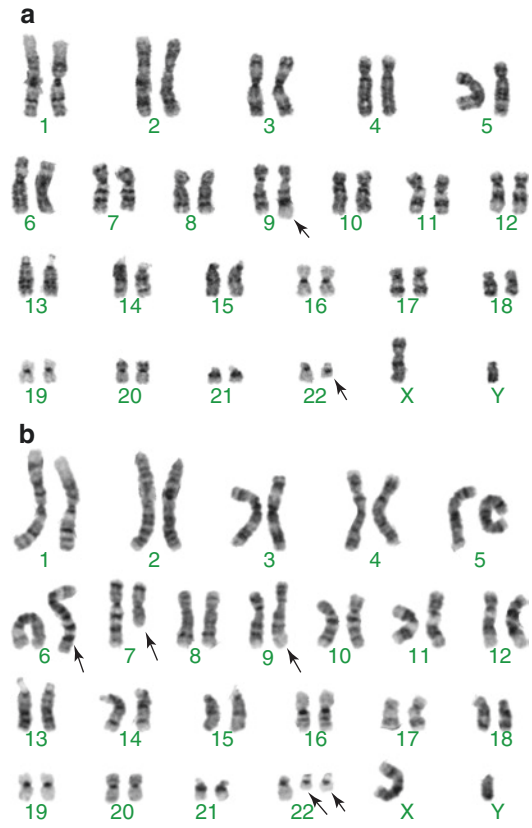

Fig. 5.4 (**a**) The t(9;22) associated with *BCR-ABL1* rearrangement is usually demonstrated by conventional bone marrow karyotype (illustrated), and can also be confirmed by interphase FISH or RT-PCR. (**b**) Certain karyotype findings can confirm a diagnosis of accelerated phase either at initial diagnosis or at progression, such as this karyotype showing an additional copy of the Philadelphia chromosome

hypolobated megakaryocytes of these variants are typical of classical CML, despite the clinical picture mimicking other myeloid neoplasms.

CML can occasionally present in blast crisis and in such cases it may be difficult to distinguish from a de novo AML or acute lymphoblastic leukemia (ALL). AML with *BCR-ABL1* rearrangement is a new provisional AML entity in the 2016 revised WHO Classification [29], and requires exclusion of any antecedent history of CML. Compared to AML with *BCR-ABL1*, CML in blast crisis more often has splenomegaly and basophilia [31]. Most cases of de novo B-ALL with *BCR-ABL1* express the p190 isoform and conversely CML-BC almost always expresses the p210 isoform. In cases without any documented history of CML presenting with a *BCR-ABL1*-positive acute leukemia and lacking stigmata of CML (such as prominent neutrophilia and basophilia accompanying the blasts in the blood), a diagnosis of a *BCR-ABL1*-positive AML or ALL is reasonable [32]. Deletions of the *IKZF1* and *CDKN2A* gene loci in *BCR-ABL1*-positive AML and deletions within *IGH* and loci in *BCR-ABL1*-positive B-ALL have recently been reported and may aid in distinguishing these entities from CML-BL [33]; however, this testing is not available in most labs and requires further validation.

Chronic Neutrophilic Leukemia

Chronic neutrophilic leukemia (CNL) is a rare myeloproliferative neoplasm characterized by increased blood neutrophils without a significant increase in circulating immature granulocytic forms, dysgranulopoiesis, or basophilia. The WHO Classification provides specific diagnostic criteria for CNL (Table 5.2) [29].

Clinical Features

The median age at CNL diagnosis is 66 years [34]. There is a slight male predominance. Most patients are incidentally found to have asymptomatic leukocytosis. When symptoms are present, fatigue, weight loss, night sweat, bone pain, and easy bruising can be seen. Splenomegaly is present in almost all cases at diagnosis.

Morphology

The peripheral blood shows leukocytosis (typically 40–50 × 10^9/L) predominantly composed

Table 5.2 WHO diagnostic criteria of chronic neutrophilic leukemia

Peripheral blood
• White blood count ≥25 × 10^9/L
• Segmented neutrophils plus band forms ≥80% of white blood cells
• Neutrophilic precursors (promyelocytes, myelocytes, and metamyelocytes) <10% of white blood cells
• Myeloblasts absent or rare
• Peripheral blood monocytes <1 × 10^9/L
• No dysgranulopoiesis
Bone marrow
• Hypercellularity
• Neutrophil granulocytes increased with normal maturation
• Myeloblasts <5% of nucleated cells
Other criteria
• Not meeting WHO criteria for CML, PV, ET, or PMF
• No rearrangement of *PDGFRA*, *PDGFRB*, *FGFR*, or *PCM1-JAK2*
• Presence of *CSF3R* p.T618I or other activating *CSF3R* mutation, *OR* if *CSF3R* is wild-type, persistent (> 3 months) neutrophilia, splenomegaly, and no identifiable cause of reactive neutrophilia including the absence of a plasma cell neoplasm; if there is a plasma cell neoplasm, clonality of myeloid cells by cytogenetics or molecular genetic studies should be demonstrated

Modified from [29]
MPN myeloproliferative neoplasm, *PV* polycythemia vera, *ET* essential thrombocythemia, *PMF* primary myelofibrosis

of mature neutrophils and band forms (comprising ≥80% of WBCs). Toxic granulation and Döhle bodies are often seen. By definition, neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes together) should comprise <10% of WBCs. Myeloblasts, eosinophils, and monocytes are not increased. Dysplasia in neutrophils is not a feature of CNL (Fig. 5.5a, b).

The bone marrow is hypercellular for age due to a neutrophilic proliferation. The maturation pattern is normal, with predominant neutrophilic cells ranging from myelocytes to mature neutrophil stages. Myeloblasts are not increased. Erythroid precursors are decreased, resulting in an elevated myeloid:erythroid ratio, which may approach 20:1. Megakaryocytes are usually normal in quantity or can be slightly increased and are morphologically unremarkable; they are widely distributed throughout the bone marrow space without forming clusters (Fig. 5.5c, d). Reticulin fibrosis, if present, is minimal.

Immunophenotype

There are no specific immunophenotypic features reported for chronic neutrophilic leukemia.

Cytogenetics and Molecular Genetics

The majority of patients have a normal karyotype, but -7, del(20q), +21, del(11q), and del(12p) abnormalities have been reported in some patients [34, 35]. Mutation in *CSF3R* is virtually a disease-defining molecular marker of CNL and is included as a diagnostic criterion in the WHO

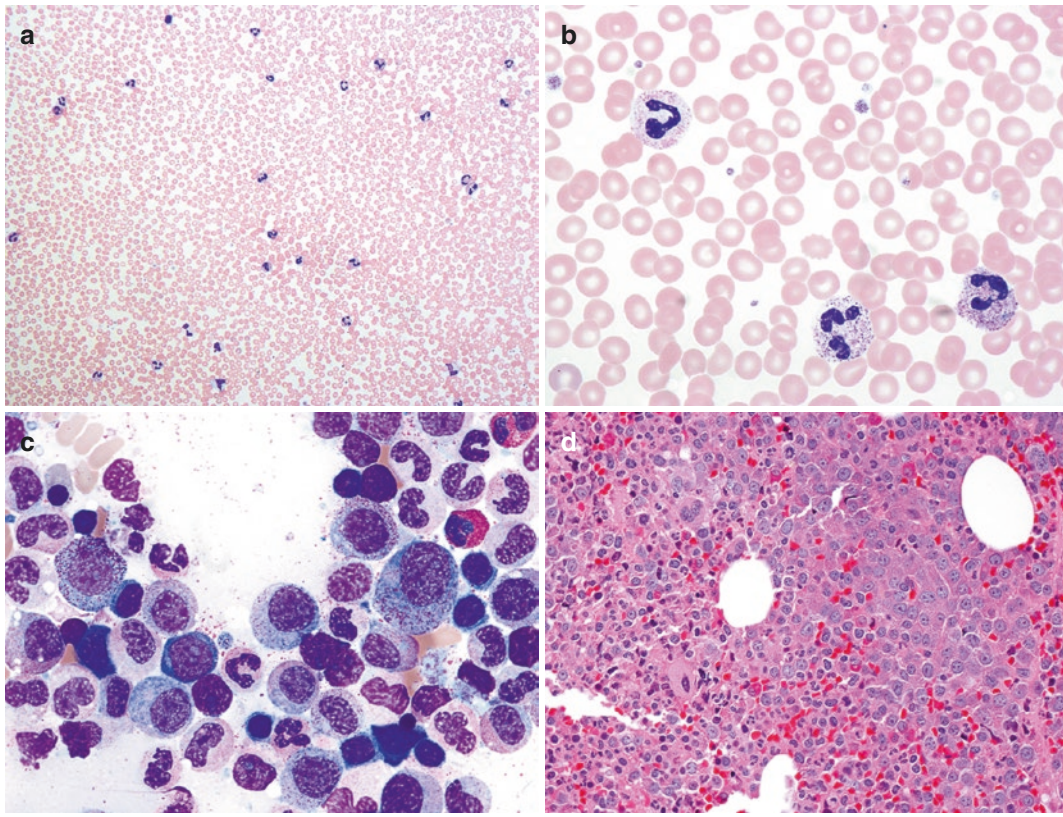

Fig. 5.5 Chronic neutrophilic leukemia. (**a**) The peripheral blood smear shows increased neutrophils, without a significant number of circulating immature granulocytic forms. (**b**) The neutrophils lack morphologic dysplasia, with normal nuclear lobation and normal granulation. (**c**) The bone marrow aspirate shows a myeloid predominance with complete maturation and without granulocytic dysplasia. (**d**) The bone marrow biopsy is markedly hypercellular with a predominance of myeloid forms. The histology may resemble chronic myeloid leukemia, *BCR-ABL1+;* however, megakaryocytes are usually less prominent in chronic neutrophilic leukemia

2016 Classification [29, 36]. A *CSF3R* mutation is not present in most other myeloid neoplasms with leukocytosis, such as AML, primary myelofibrosis, and CMML. Although the *CSF3R* mutation has been reported to occur in up to 45% of patients with atypical CML, *BCR-ABL1* negative (aCML), most studies have found a much lower incidence in this disease relative to CNL.

The *CSF3R* gene encodes the receptor for colony-stimulating factor 3 (CSF3), which is the main growth factor that stimulates neutrophil production [37]. The majority of *CSF3R* mutations occur at the proximal region of the receptor (i.e., p.T618I and p.T615A), mediating proliferative and survival signals via the JAK-STAT pathway. Less commonly, nonsense mutations occur with truncation of the cytoplasmic tail which activates the Src family-TNK2 kinase domain [36]. A mouse model transplanted with *CSF3R* p.T618I-expressing hematopoietic cells develops increased mature granulocytosis in blood, hypercellular bone marrow, and granulocytic infiltration in the liver and spleen, supporting that an activating *CSF3R* mutation is sufficient to develop a CNL-like myeloproliferative neoplasm [38].

Differential Diagnosis

Reactive paraneoplastic neutrophilia (often associated with monoclonal gammopathy and plasma cell neoplasms) can mimic CNL, but lacks *CSF3R* mutation or other evidence of neutrophil clonality. In fact many such cases met the prior 2008 WHO Classification criteria for CNL, but have been shown to have significantly superior survival compared to true CNL patients, suggesting that the neutrophilia in these patients is a reactive paraneoplastic phenomenon [39].

When neutrophilia is accompanied by significant dysplasia and frequent immature granulocytic cells in the blood, a diagnosis of aCML rather than CNL should be suspected [40]. In aCML, the peripheral blood demonstrates increased (>10%) neutrophilic precursors, without significant monocytosis or basophilia. Dysplasia in granulocytes, which is usually marked, is a part of the definition and dysplasia in other lineages is not uncommonly seen on bone marrow examination (Fig. 5.6); additional images and description of aCML are provided in Chap. 10. Molecular testing often shows S*ETBP1* or *ETNK1* mutations in atypical CML [41, 42], while *CSF3R* mutation occurs at a much lower frequency compared to CNL in most studies. However the presence of a *CSF3R* mutation does not exclude a diagnosis of atypical CML if other features are typical for that entity. Thus, rigorous application of the diagnostic criteria for CNL and atypical CML is required to accurately distinguish between these diseases. There may be mild absolute monocytosis in CNL, but if monocytes comprise ≥10% of the leukocytes, a diagnosis of CMML should be considered. Finally, it is essential to rigorously exclude the possibility of a *BCR-ABL1* rearrangement (including tests that

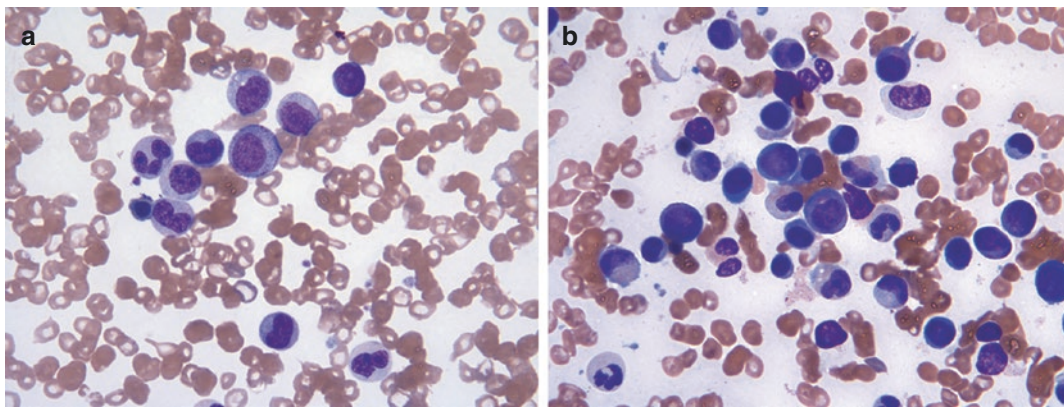

Fig. 5.6 Atypical chronic myeloid leukemia, *BCR-ABL1* negative. (**a**) The bone marrow aspirate shows a predominance of myeloid forms. (**b**) Marked dysgranulopoiesis is evident, with frequent bilobed and non-lobated neutrophils

evaluate for p190 and p230 isoforms) prior to rendering a diagnosis of CNL.

Other Myeloid Neoplasms Associated with Neutrophilia

Neutrophilia can be associated with other myeloid neoplasms, including primary myelofibrosis, polycythemia vera, essential thrombocythemia, or proliferative-type CMML. These entities are discussed in separate chapters. AML and ALL can present with leukocytosis due to increased blasts, but there is usually no significant increase in mature neutrophils; rather, there is often neutropenia. However, some patients with AML (particularly those with *FLT3* or *NPM1* mutations) may have significantly increased neutrophils in addition to circulating blasts [43, 44]. Therefore, careful examination of the smear for increased blasts should be done in all cases of unexplained neutrophilia.

An algorithm for the workup of neutrophilia is shown in Fig. 5.7.

Myeloid Neoplasms Associated with Basophilia

Basophilia can be seen in any myeloproliferative neoplasm, but it is most common and prominent in CML (see above). Basophilia is seen only rarely in myelodysplastic syndromes (MDS) and is associated with an adverse prognosis in such cases [45, 46]. Acute basophilic leukemia and AML with t(6;9)(p23;q34) are two types of acute leukemia that may be associated with basophilia (Fig. 5.8). Acute basophilic leukemia is very rare and only few case reports are available [47]. AML with t(6;9)(p23;q34);*DEK-NUP214* is also a very rare form of AML, constituting about 1% of all AML cases. The median age at diagnosis is 23 years with a broad age range of affected patients (2–66 years) [48]. Patients usually present with cytopenias, but basophilia is significantly more common compared to other AML subtypes [48]. The bone marrow typically shows multilineage dysplasia. Bone marrow basophils are increased about half of the cases.

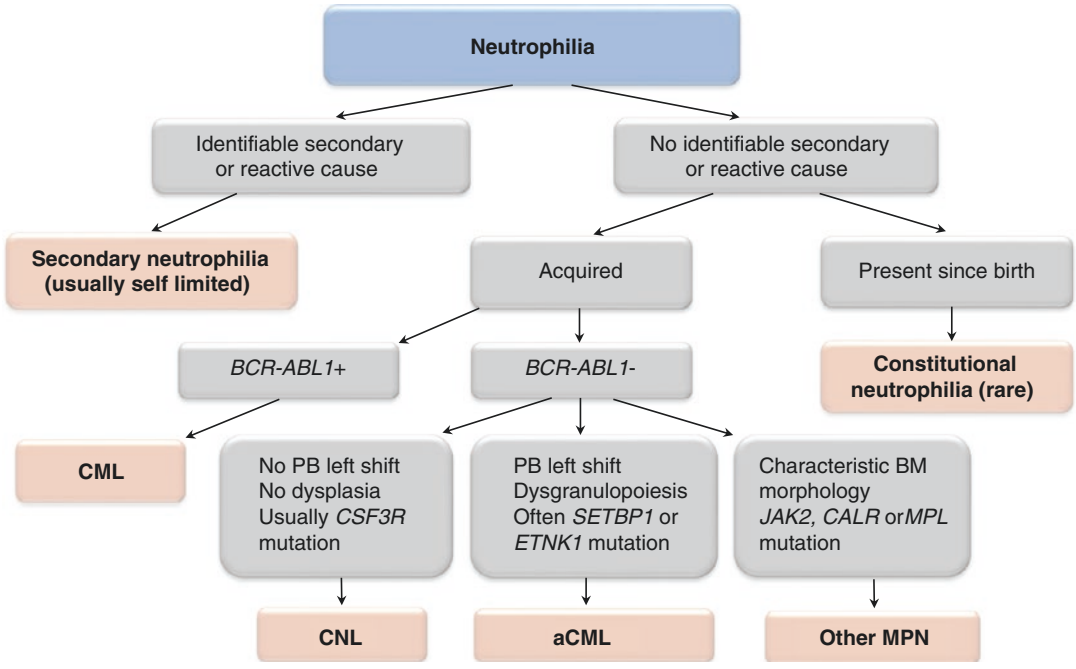

Fig. 5.7 Proposed algorithm for the workup of neutrophilia. *CML* chronic myeloid leukemia, *BCR-ABL1+*, *CNL* chronic neutrophilic leukemia, *aCML* atypical chronic myeloid leukemia, *BCR-ABL1-*, *MPN* myeloproliferative neoplasm, *BM* bone marrow

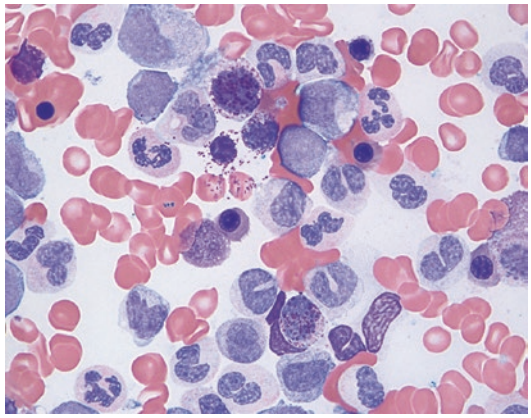

Fig. 5.8 Acute myeloid leukemia with t(6;9)(p23;q34); *DEK-NUP214*. Scattered blasts are present (comprising 22% of the aspirate smear cells) in a background of prominent dysgranulopoiesis and increased basophils

Acute Myeloid Leukemia

Acute myeloid leukemia (AML) is an aggressive myeloid neoplasm characterized by arrested maturation of hematopoiesis, leading to an accumulation of myeloblasts in bone marrow and/or blood. According to the 2016 WHO Classification, myeloblasts must comprise at least 20% of nucleated cells in bone marrow or blood in order to establish a diagnosis of AML, with rare exceptions discussed below. The 2016 WHO Classification recognizes several subtypes of AML, which have distinct clinical presentations, morphology, genetics, and biologic behavior. The defining features of these AML subtypes are shown in Table 5.3.

Clinical Features

The annual incidence of AML in the United States is approximately 4 per 100,000 per year. Its incidence increases with age, with a median age of 67–70 years and with a male:female ratio of 1.5:1. In children, AML is most common in infants under 1 year of age and is very rare between the ages of 4 and 18 years. The presenting symptoms of most AML patients reflect the effects of the disease on the quantity and function of mature hematopoietic cells. Symptoms related to cytopenias are common, such as bleeding, fatigue, or recurrent infections; almost all AML patients are anemic at presentation. The white blood count and percentage of blasts among circulating leukocytes are highly variable, ranging from marked leukopenia with no circulating blasts to marked leukocytosis in which most circulating cells are blasts. About 10% of patients have a circulating blast count $>100 \times 10^9$/L. AML patients with high levels of circulating blasts may present with symptoms related to leukostasis, such as respiratory distress and/or mental status changes. Tumor lysis syndrome is also a risk in AML with high blast count. Disseminated intravascular coagulation (DIC) can be caused by release of procoagulant factors upon lysis of blasts. DIC is a relatively common and serious complication of the AML subtype acute promyelocytic leukemia (APL) and a subset of APL patients succumb to DIC sequelae before treatment can be instituted. Thus, rapid diagnosis of APL with prompt institution of appropriate therapy (all-trans retinoic acid, ATRA) is critical. It is important to note that DIC can occur with other AML subtypes and is not specific for APL and conversely, APL patients may present without any evidence of DIC [49].

A small subset of AML patients present (or develop later in the course of disease) with blast infiltration of extramedullary tissues termed myeloid sarcoma. This can affect any anatomic site, but is most commonly seen in soft tissues, lymph nodes, skin, and mucosal sites such as the gums. Myeloid sarcomas are most frequently seen in patients with high levels of circulating blasts and with leukemias showing monocytic differentiation, but can occur with any AML subtype and can in some cases precede overt involvement of the blood or bone marrow.

Clinical history is essential in correctly diagnosing and classifying AML [50]. The WHO Classification defines therapy-related AML (t-AML) as any AML occurring in patients who have received cytotoxic chemotherapy and/or radiotherapy that exposed hematopoietic bone marrow. The chemotherapeutic agents include all alkylating agents, topoisomerase II inhibitors, antimetabolites such as thiopurines or purine/pyrimidine analogues, and antitubulin agents such as vincristine or paclitaxel. Although complex karyotype, *TP53* mutations, and background dys-

Table 5.3 Revised 2016 WHO classification acute myeloid leukemia (AML) subtypes [29]

WHO AML subtype	Defining morphologic features	Defining genetic features	Other features
AML with recurrent genetic abnormalities (AML-RGA)[a]			
AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*	Diagnosis may be made with <20% blasts if genetic finding is demonstrated	t(8;21)(q22;q22.1), with *RUNX1-RUNX1T1* rearrangement	Blasts are usually large and often granulated with frequent Auer rods. Dysgranulopoiesis is common
AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	Diagnosis may be made with <20% blasts if genetic finding is demonstrated	inv(16)(p13.1q22) or t(16;16)(p13.1;q22) with *CBFB-MYH11* rearrangement	Blasts usually have myelomonocytic features. Increased bone marrow (but not blood) eosinophils with abnormal mixed eosinophilic-basophilic granules
Acute promyelocytic leukemia with *PML-RARA*	Diagnosis may be made with <20% blasts if genetic finding is demonstrated	*PML-RARA* rearrangement, usually resulting from a t(15;17)(q24;q21.2). Variants may have *RARA* rearranged with other partners	Numerous promyelocytes with Auer rods; microgranular variant with bilobed nuclei
AML with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*	≥20% bone marrow or blood blasts	t(9;11)(p21.3;q23.3) with *KMT2A-MLLT3* rearrangement	Blasts usually have monocytic features
AML with t(6;9)(p23;q34.1); *DEK-NUP214*	≥20% bone marrow or blood blasts	t(6;9)(p23;q34.1) with *DEK-NUP214* rearrangement	Variable blast morphology; basophilia in about half of cases
AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*	≥20% bone marrow or blood blasts	inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2) juxtaposing *GATA2* and *MECOM* genes	Thrombocytosis may occur. Frequent background dysplasia, especially in megakaryocytes
AML with t(1;22)(p13;q13.1);*RBM15-MKL1*	≥20% bone marrow or blood blasts	t(1;22)(p13;q13.1) resulting in *RBM15-MKL1* rearrangement	Blasts have megakaryocytic features. Most often occurs in infants
AML with *BCR-ABL1* (provisional entity)	≥20% bone marrow or blood blasts	*BCR-ABL1* rearrangement, usually resulting from a t(9;22)(q34;q11.2)	De novo disease, with no antecedent or concurrent evidence of CML
AML with mutated *NPM1*	≥20% bone marrow or blood blasts	*NPM1* mutation; no defining cytogenetic features of AML-MRC	Blasts often have monocytic features and "cup-shaped" nuclear contour; de novo disease
AML with biallelic mutation of *CEBPA*	≥20% bone marrow or blood blasts	Biallelic (double) mutation of *CEBPA*; no defining cytogenetic features of AML-MRC	Can occur in the setting of germline single *CEBPA* mutation; de novo disease
AML with mutated *RUNX1* (provisional)	≥20% bone marrow or blood blasts	*RUNX1* mutation; no defining cytogenetic features of AML-MRC	Defined as a de novo disease

(continued)

Table 5.3 (continued)

WHO AML subtype	Defining morphologic features	Defining genetic features	Other features
AML with myelodysplasia-related changes (AML-MRC)			
Diagnosis may be made if any either the defining morphologic, genetic, or other features are present, in the absence of any prior cytotoxic therapy	≥20% bone marrow or blood blasts and at least 50% dysplastic cells in at least two hematopoietic lineages	≥20% bone marrow or blood blasts and complex karyotype (≥ 3 abnormalities), -7, del(7q), del(5q), t(5q), i(17q), t(17p), -13, del(13q), del(11q), del(12p), t(12p), idic(X)(q13), t(11;16)(q23.3;p13.3), t(3;21)(q26.2;q22.1), t(1;3)(p36.3;q21.2), t(2;11)(p21;q23.3), t(5;12)(q32;p13.2), t(5;7)(q32;q11.2), t(5;17)(q32;p13.2), t(5;10)(q32;q21), or t(3;5)(q25.3;q35.1)	≥20% bone marrow or blood blasts and documented history of prior MDS or MDS/MPN
Therapy-related AML (t-AML)			
Diagnosis requires defining morphologic and other features	≥20% bone marrow or blood blasts; cases with <20% may be diagnosed as therapy-related MDS, but are considered together with therapy-related AML	No defining features, but >90% have abnormal karyotype, most commonly -7, del(7q), del(5q), complex karyotype, or rearrangements of 11q23 (*KMT2A*). 50–80% have *TP53* mutations	Documented history of exposure to cytotoxic chemotherapy and/or radiation therapy exposing areas of hematopoietic marrow
AML, not otherwise specified (AML-NOS)[b]			
AML with minimal differentiation (M0)	≥20% blasts without expression of MPO or NSE, but with myeloid markers detected by flow cytometry	None	Blasts often small with scant cytoplasm
AML without maturation (M1)	≥20% blasts with expression of MPO (≥3%), and <10% maturing myeloid elements	None	
AML with maturation (M2)	≥20% blasts and >10% differentiated myeloid elements	None	
Acute myelomonocytic leukemia (M4)	≥20% blasts with expression of MPO (≥3%) and >20% monocytic cells (by morphology and/or NSE cytochemistry)	None	Blasts often have monocytic features and often include promonocytes; Auer rods may be present
Acute monoblastic/monocytic leukemia (M5)	≥20% blasts with ≥80% monocytic cells (by morphology and/or NSE cytochemistry)	None	Blasts have monocytic features and often include promonocytes
Acute erythroid leukemia, pure erythroid leukemia subtype (M6B)	>80% erythroid cells in the bone marrow, of which ≥30% are pronormoblasts, without increased myeloblasts	None; highly complex karyotype and *TP53* mutation are characteristic	Markedly atypical pronormoblasts with maturation arrest; very rare

Table 5.3 (continued)

WHO AML subtype	Defining morphologic features	Defining genetic features	Other features
Acute megakaryoblastic leukemia (M7)	≥20% blasts, with ≥50% expressing megakaryocytic markers (CD41, CD42, or CD61)	None	Large blasts which may show surface pseudopods
Acute basophilic leukemia	≥20% blasts with basophilic morphology and positivity for basophil markers (CD9, CD25, CD123, CD203c, CD11b)	None	Very rare
Acute panmyelosis with myelofibrosis	≥20% blasts in a diffusely fibrotic stroma with panmyelosis and markedly dysplastic megakaryocytes	None	Very rare

[a]AML-RGA has no history of prior cytotoxic therapy
[b]AML, NOS encompasses cases not meeting criteria for AML-MRC, AML-RGA, or t-AML

plasia are characteristic of t-AML, they can occur in de novo disease and conversely some cases of t-AML may have a normal karyotype and lack significant background dysplasia. Thus, obtaining history of any prior cytotoxic therapy exposure is mandatory in correctly diagnosing this AML subtype. Similarly, AML with myelodysplasia-related changes (AML-MRC) represents an AML that bears some relationship to myelodysplastic syndromes (MDS) and typically has a poor prognosis. The diagnosis of AML-MRC may be made in any case of AML following a prior documented diagnosis of MDS or MDS/MPN, underscoring the importance of accessing history of prior hematologic neoplasms in any newly diagnosed AML patient. Interestingly, patients with AML who have antecedent cytopenias, but no documented diagnosis of prior hematologic neoplasm, appear to have a similarly poor prognosis [51]; however, such cases are not classified as AML-MRC according to current criteria.

Finally, a history of Down syndrome impacts the classification of AML and related myeloid neoplasms. Down syndrome patients have a much higher frequency of AML than the general population, and AML affecting these patients is frequently of the megakaryoblastic type. Moreover, some individuals may experience a transient myeloproliferative disorder manifesting with leukocytosis and increased circulating blasts that mimics AML, but spontaneously resolves. Due to their unique features, AML and related myeloid neoplasms occurring in the setting of Down syndrome are considered separately in a category of "Myeloid proliferations associated with Down syndrome" in the 2016 WHO Classification. These proliferations are discussed further in Chap. 3. A detailed family and personal history should be taken from all patients with AML. The presence of a family history of cytopenias and/or myeloid neoplasms or a personal history suggesting lifelong cytopenia raise the possibility of AML arising in the setting of a germline predisposition syndrome. Germline predisposition conditions are discussed separately in Chap. 3.

Morphology

In the 2016 WHO Classification, a diagnosis of AML requires that myeloblasts comprise ≥20% of nucleated cells in bone marrow or blood. This cutoff is admittedly arbitrary: cases of AML with low blast counts (20–30%) may exhibit more indolent behavior than high-blast-count AML and conversely some myeloid neoplasms with blast counts of <20% may exhibit highly aggressive behavior akin to AML [52]. Nevertheless, it is critical to obtain an accurate blast count per-

formed on at least 500 nucleated bone marrow cells and at least 200 peripheral blood leukocytes in all putative AML cases, in order to achieve as precise a blast count as possible. The "gold standard" for the blast count in establishing an AML diagnosis is manual counting of the aspirate and blood smear. Neither flow cytometry nor biopsy blast estimate should be used in lieu of the aspirate blast count [53]. In instances of an unobtainable aspirate due to "dry tap," a blast count can be performed on an air-dried touch preparation. In some cases, there may be no aspirate smear or touch preparation, or these specimens may be nonrepresentative due to extensive hemodilution or uninterpretable due to cell degeneration or other artifacts. In such instances, a diagnosis of AML may be made based on the presence of ≥20% blasts in the biopsy specimen, if supported by the use of immunostains showing expression of blast markers such as CD34 and/or CD117 (see Immunphenotype section below). It is also important to note that the presence of ≥20% blasts in the blood establishes a diagnosis of AML, irrespective of the findings in the bone marrow.

The cytology of AML myeloblasts overlaps significantly with that of normal myeloblasts, and it is their inappropriately high number (not a particular cytologic feature) that identifies them as malignant. However, Auer rods, cytoplasmic crystalline structures containing components of myeloid primary granules, are restricted to malignant myeloblasts. Auer rods can be identified in about 50% of AML cases, but often are found in only a small subset of the blasts.

Exceptions to the requirement of at least 20% myeloblasts in blood or bone marrow for establishing an AML diagnosis are as follows:

1. In cases with *PML-RARA*, inv(16)/t(16;16), or t(8;21), a diagnosis of AML can be made irrespective of blast count (see Table 5.3).
2. In cases with monocytic differentiation, promonocytes (see below) are considered blast equivalents, and are included along with blasts in the blast count.
3. In cases of pure erythroid leukemia, undifferentiated erythroblasts (see below) are considered as blast equivalents, even though myeloblasts are not increased; however, these must comprise ≥30% of the marrow cells.
4. A mass-forming extramedullary accumulation of myeloblasts (myeloid sarcoma) is considered equivalent to a diagnosis of AML, even if blasts are not increased in the bone marrow or blood.

Blasts in AML have highly variable morphology, ranging from small forms only slightly larger than a mature lymphocyte to large, pleomorphic cells. The most constant feature that distinguishes myeloblasts from more mature hematopoietic cells is finely dispersed chromatin in smear preparations, in contrast to the more condensed, "chunky", or mottled chromatin pattern of mature cells. The cytologic features of various blast types in AML as they appear in smear preparations are as follows:

1. Myeloblasts range from small (approaching that of a small lymphocyte) to large in size and often have prominent central nucleoli, but nucleoli may be small and multiple or entirely absent. The nuclear contour can be rounded, finely irregular, indented, or convoluted. The cytoplasm is usually scant and pale and may contain a few granules. Auer rods are often present (Fig. 5.9a, b).
2. Monoblasts are usually large in size with oval, indented, or folded nuclei, prominent nucleoli, and moderately abundant grey cytoplasm that may have a few vacuoles and/or granules (Fig. 5.9c).
3. Promonocytes (considered as blast equivalents in AML cases with monocytic differentiation) are medium in size with delicately folded nuclei (often with visible "creasing" of the nucleus), fine chromatin, small or indistinct nucleoli, and abundant faintly granulated cytoplasm that often contains vacuoles (Fig. 5.9d).
4. Promyelocytes are medium to large cells with abundant cytoplasm. In the cells seen in the *classic variant* of APL, the cytoplasm contains numerous purple granules and often Auer rods, including cells with multiple Auer rods (Fig. 5.9e). In the *microgranular variant*

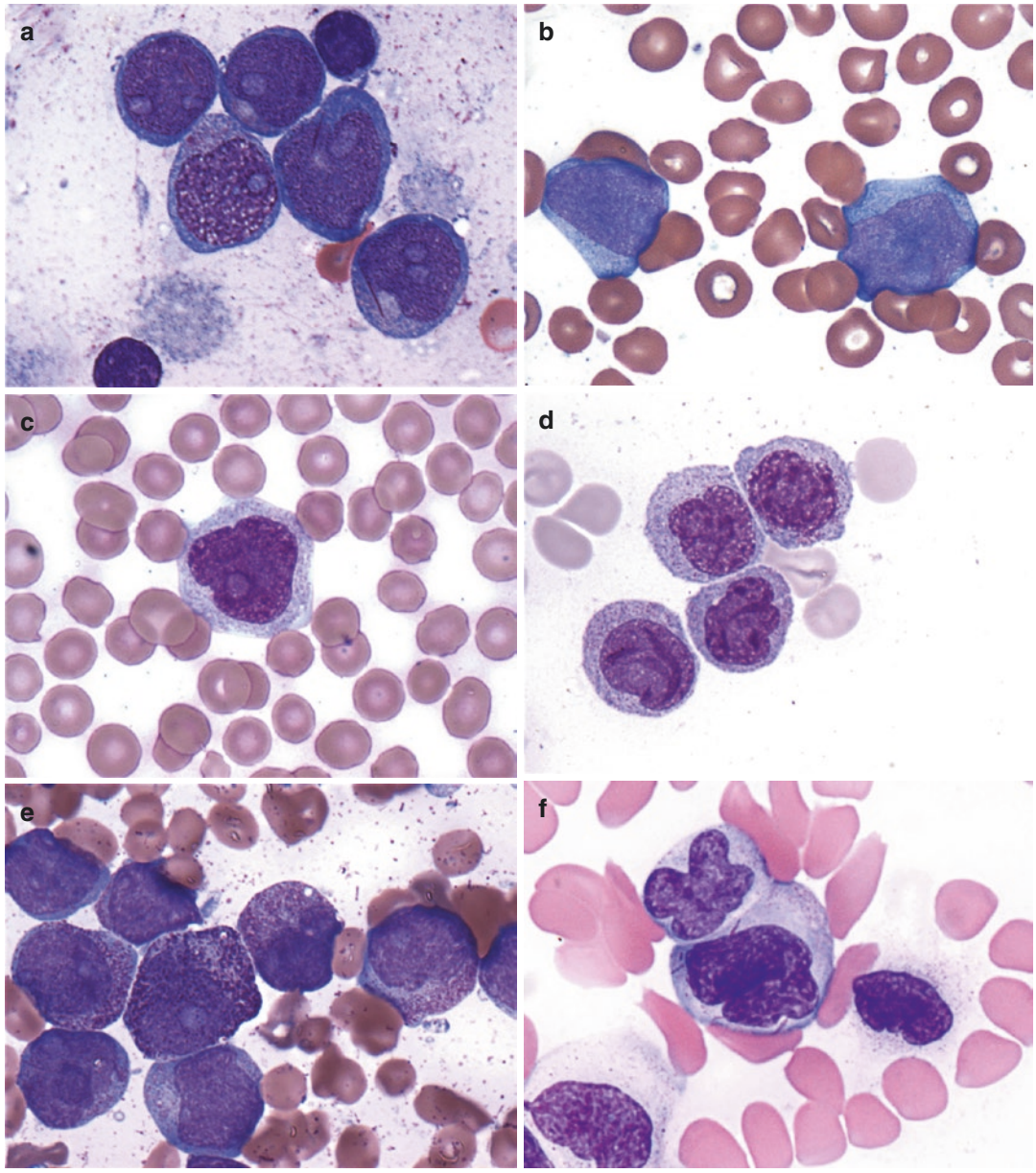

Fig. 5.9 Illustrations of different types of blasts and "blast equivalents" in peripheral blood and bone marrow aspirates. (**a**) Myeloblasts usually have prominent nucleoli and scant cytoplasm. Auer rods, as seen in this case, are proof of myeloid differentiation. (**b**) Some myeloblasts have more abundant cytoplasm and irregular nuclei; a few granules may be present in the cytoplasm. (**c**) Monoblasts tend to be larger than myeloblasts, with more irregular nuclei and more abundant cytoplasm that can appear grey or pale and basophilic, sometimes with sparse, small vacuoles. (**d**) Promonocytes, regarded together with monoblasts in establishing a diagnosis of acute leukemias with monocytic differentiation, have more abundant granular or vacuolated cytoplasm than monoblasts and have delicately folded, often highly lobulated nuclei. (**e**) Promyelocytes from a case of classic acute promyelocytic leukemia with *PML-RARA,* showing blasts with prominent cytoplasmic granulation. Auer rods are present. (**f**) Promyelocytes from a case of microgranular variant acute promyelocytic leukemia with *PML-RARA,* showing blasts with bilobed "butterfly-shaped" nuclei, set in an abundant cytoplasm. The cell in the center contains multiple Auer rods

of APL, the blasts have bilobed "dumbbell-" or "butterfly"-shaped nuclei that lack discernible granules, but often include some cells with Auer rods (Fig. 5.9f).

5. Malignant pronormoblasts in pure erythroid leukemia are medium to large in size with round nuclei, finely dispersed chromatin, often multiple nucleoli, and deeply basophilic cytoplasm that frequently contains vacuoles. The vacuoles are typically large and may be elongated [54]) (Fig. 5.10).
6. Megakaryoblasts are medium to large in size. In some cases, cytoplasmic pseudopod formation may be seen, but this is an inconsistent finding.

In evaluating the bone marrow of an AML patient, in addition to enumerating blasts, attention must be paid to the non-blast marrow elements to evaluate for dysplasia. Dysplasia in circulating mature granulocytic elements in the peripheral blood should also be evaluated in all cases. Significant morphologic dysplasia in maturing elements in AML has been associated with poor prognosis [55, 56], and defines the specific AML category in the 2016 WHO Classification, AML-MRC (Fig. 5.11). In contrast to the 10% threshold used to define a dysplastic lineage establishing a diagnosis of MDS, the threshold of dysplastic cells to define a lineage as dysplastic for the purposes of diagnosing AML-MRC is 50% and at least two lineages must be affected. Although all lineages are considered equally in terms of scoring dysplasia to diagnose AML-MRC, recent evidence suggests that dysmegakaryopoiesis (specifically micromegakaryocytes) appears to have the strongest adverse prognostic impact, while dyserythropoiesis may not have prognostic relevance [56, 57].

The main defining roles for morphology in AML in the 2016 WHO Classification are in establishing a diagnosis of AML-MRC and in separating the subgroups of AML-NOS. However, certain genetic subtypes of AML have distinctive morphologies that can provide clues to the underlying genetic abnormality. Indeed, morphologic evaluation of smears is usually possible within a few hours of procuring a bone marrow sample, whereas the typical turnaround time of cytogenetics and molecular genetic testing is 2–3 weeks. Moreover, some cytogenetic aberrations can be cryptic or manifest as unusual variants. Thus, a

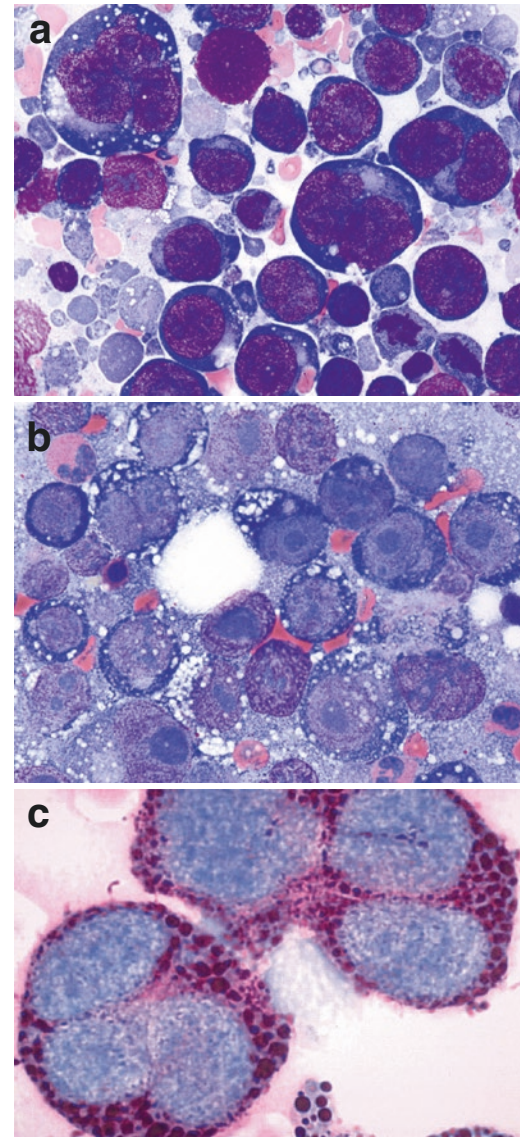

Fig. 5.10 Pure erythroid leukemia in the bone marrow aspirate smear. (**a**) The leukemic erythroblasts are often highly pleomorphic, with deeply basophilic cytoplasm. A perinuclear hof, similar to that seen in normal pronormoblasts, may be seen. (**b**) Prominently vacuolated cytoplasm is common. (**c**) The malignant pronormoblasts in pure erythroid leukemia contain large PAS+ cytoplasmic globules

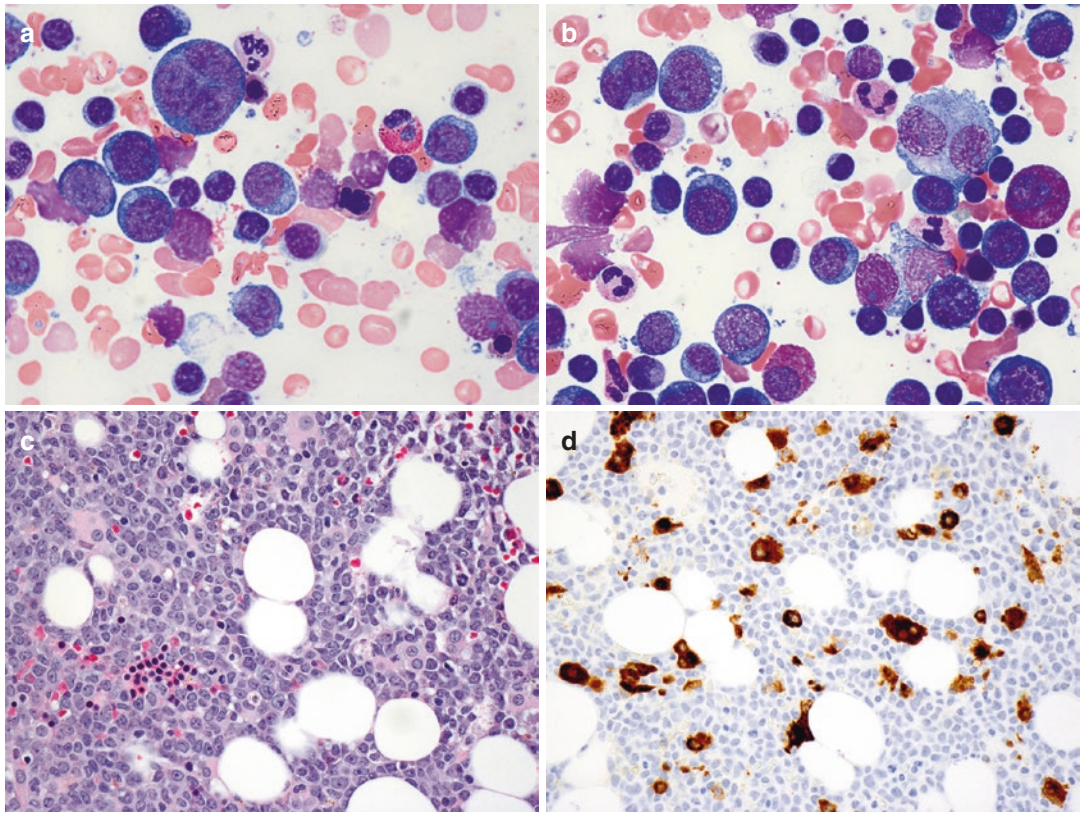

Fig. 5.11 (**a**) AML with myelodysplasia-related changes. (**a**) Blasts occur in a background of dysplastic maturing erythroid and myeloid elements in the bone marrow aspirate smear. (**b**) Hypogranulated neutrophils and a dysplastic megakaryocyte with two separated nuclei are seen. (**c**) In the bone marrow biopsy, many small megakaryocytes and micromegakaryocytes with non-lobated or bilobed nuclei are present. (**d**) As the micromegakaryocytes may be overlooked in the predominant population of blasts, CD61 immunostain can help to reveal their presence

strong morphologic suspicion of a particular genetic subtype can help guide confirmatory additional testing if the karyotype is negative. The main AML genetic subtypes that have distinctive (although not always specific) morphologies are APL with *PML-RARA*, AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1,* AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);*CBFB-MYH11,* AML with t(6;9)(p23;q34.1);*DEK-NUP214,* and AML with mutated *NPM1*. The morphologic features are listed in Table 5.3 and are illustrated in Figs. 5.12, 5.13, 5.14, and 5.15. Fig. 5.8 illustrates the features of AML with t(6;9). Many other AML subtypes tend to show certain morphologic features, such as monocytic blasts in AML with t(9;11) and markedly dysplastic megakaryocytes in AML with inv(3)/t(3;3), but these are seen across many different AML subtypes. Therapy-related AML tends to show significant background dysplasia, similar to AML-MRC.

Immunophenotype and Cytochemistry

The myeloid lineage of blasts may be established by the identification of Auer rods (see above), presence of myeloperoxidase (MPO) or nonspecific esterase (NSE, alpha-naphthyl butyrate or alpha-naphthyl acetate esterase) enzymatic activity in blasts by cytochemistry on air-dried aspirate smears, or expression of sufficient myeloid markers by flow cytometry. Flow cytometry should be performed in all newly diagnosed cases

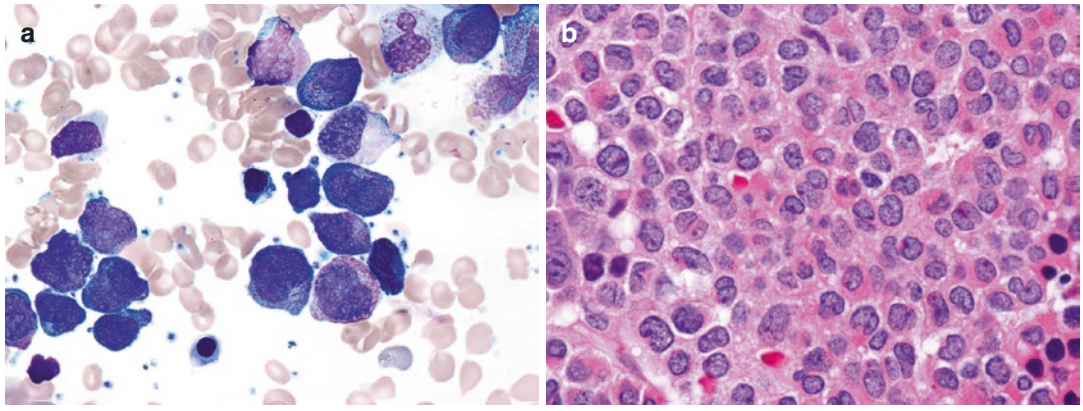

Fig. 5.12 Acute promyelocytic leukemia with t(15;17). (**a**) In the bone marrow aspirate of this case, many blasts have a microgranular appearance with bilobed nuclei, but one blast (upper center) has numerous promyelocytic granules. An Auer rod is present (center). (**b**) Neoplastic promyelocytes in the bone marrow biopsy also show bilobed or folded nuclei and abundant pink cytoplasm

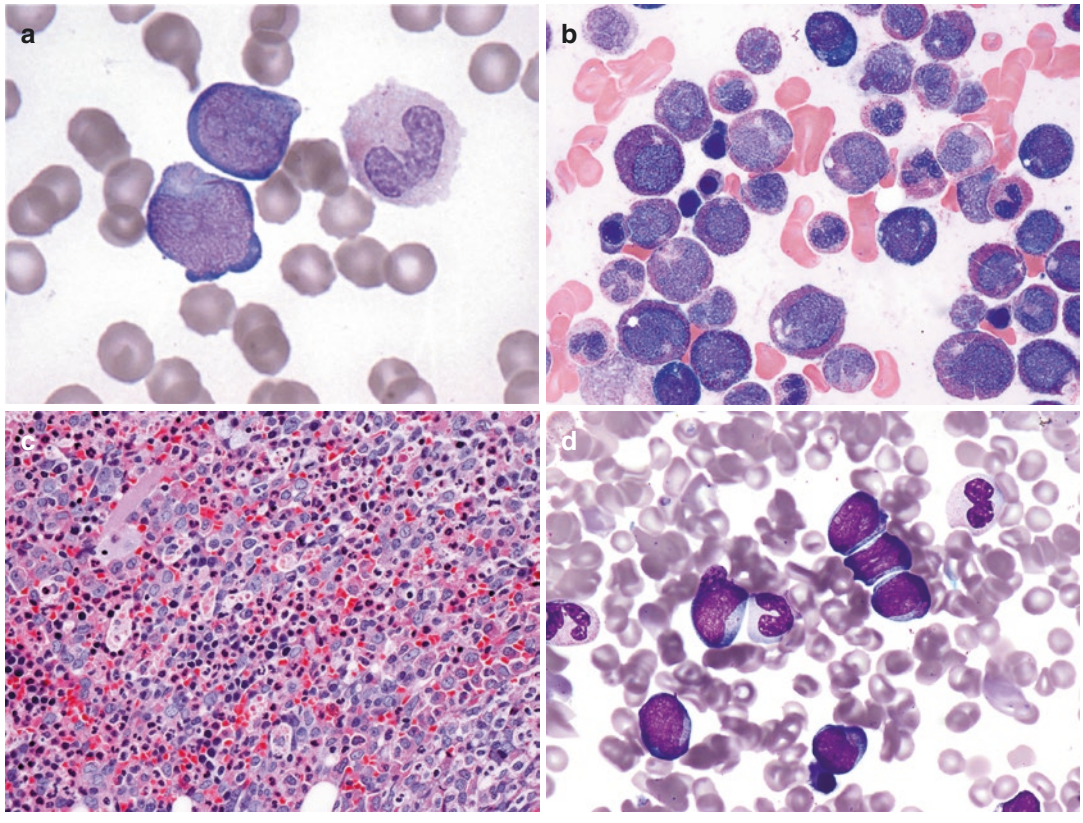

Fig. 5.13 AML with t(8;21)(q22;q22.1);*RUNX1-RUNX1T1*. (**a**) The blasts in this case are large with basophilic cytoplasm, one containing an Auer rod. (**b**) In another case, there is an extensive background of maturing hematopoiesis with only 10% blasts. Cytogenetics and FISH confirmed a t(8;21)(q22;q22.1) with *RUNX1-RUNX1T1* rearrangement, allowing a diagnosis of AML despite <20% blasts. (**c**) Bone marrow biopsy from same case as (**b**); large blasts are evident, but comprise <20% of the cellularity in a background of maturing myeloid and erythroid elements. (**d**) Peripheral blood smear showing circulating blasts and hypogranular neutrophils

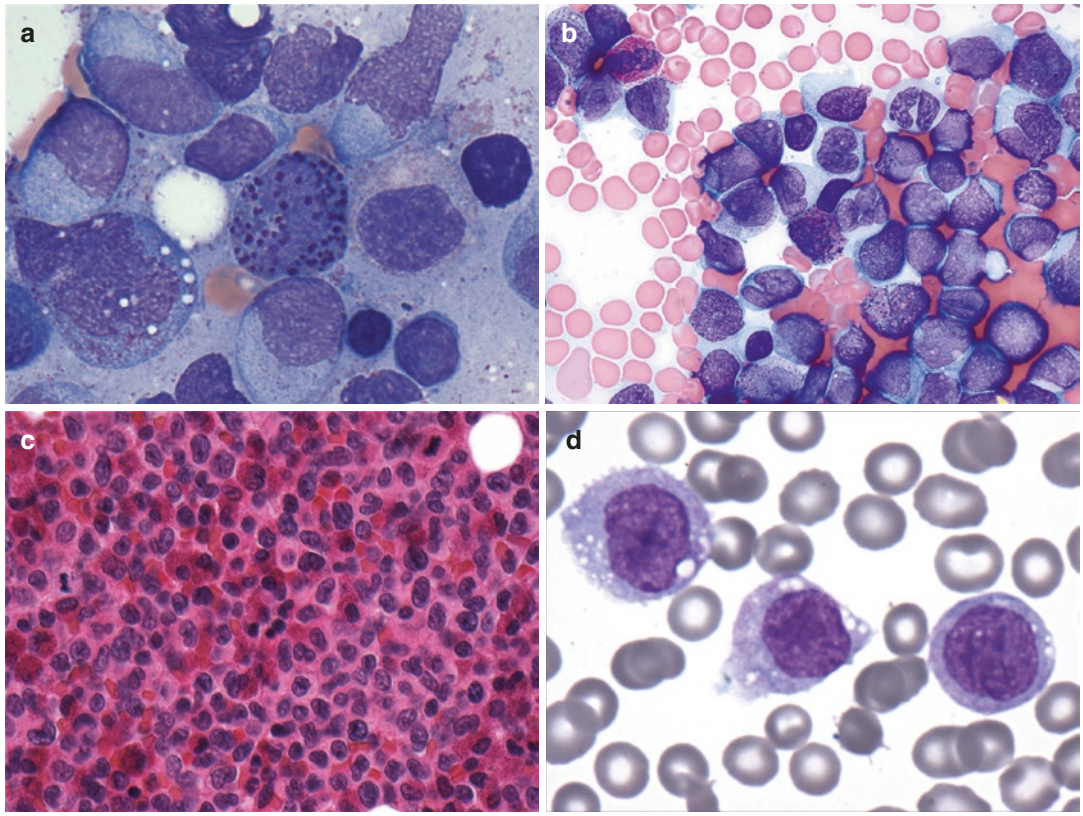

Fig. 5.14 AML with inv(16)(p13.1q22);*CBFB-MYH11*. (**a**) In the bone marrow aspirate, an abnormal eosinophil containing basophilic granules is present (center), surrounded by blasts showing monocytic features. (**b**) In another case, numerous blasts with folded nuclei are present amid abnormal eosinophils in the bone marrow aspirate. (**c**) In the biopsy section, a mixture of blasts with folded and lobated nuclei are mixed with numerous eosinophils. (**d**) The peripheral blood in this case shows many circulating promonocytes, but no eosinophilia

of acute leukemia and is considered current standard of care: it is more sensitive than cytochemistry, as cases of AML with limited maturation lack MPO staining by cytochemistry yet express surface antigens that reveal myeloid differentiation [50]. AML blasts should express at least two markers of granulocytic differentiation (CD13, CD15, CD33, CD117, and/or MPO) and/or monocytic differentiation (CD14, CD64, CD11c, and CD11b). The precursor cell marker CD34 is present on both AML and ALL, but a significant subset of AML cases (particularly those with monocytic, erythroid, or megakaryocytic differentiation) lack CD34 expression. Terminal deoxytransferase (TdT), an enzyme expressed in normal B- and T-cell precursors and in almost all ALL cases, is aberrantly expressed in a high proportion of AML (especially in AML with minimal differentiation) and is not useful in distinguishing AML from ALL.

In AML-NOS, the "gold standard" for determining myeloid versus myelomonocytic versus monocytic subtypes (see Table 5.3) used to be enzyme cytochemistry. However, in recent years, flow cytometry has become a much better tool to determine monocytic differentiation. Monocytic differentiation is determined by the expression of two or more monocytic markers (CD14, CD36, CD64, CD11c, or CD11b) and often CD4 (dimly) and HLA-DR by flow cytometry (Fig. 5.16). Monocytes, either mature or immature, usually show no CD34 expression. CD117 is absent or only expressed in a very small subset of monocytic blasts. Immature monocytes/monoblasts often demonstrate immunophenotypic differences from

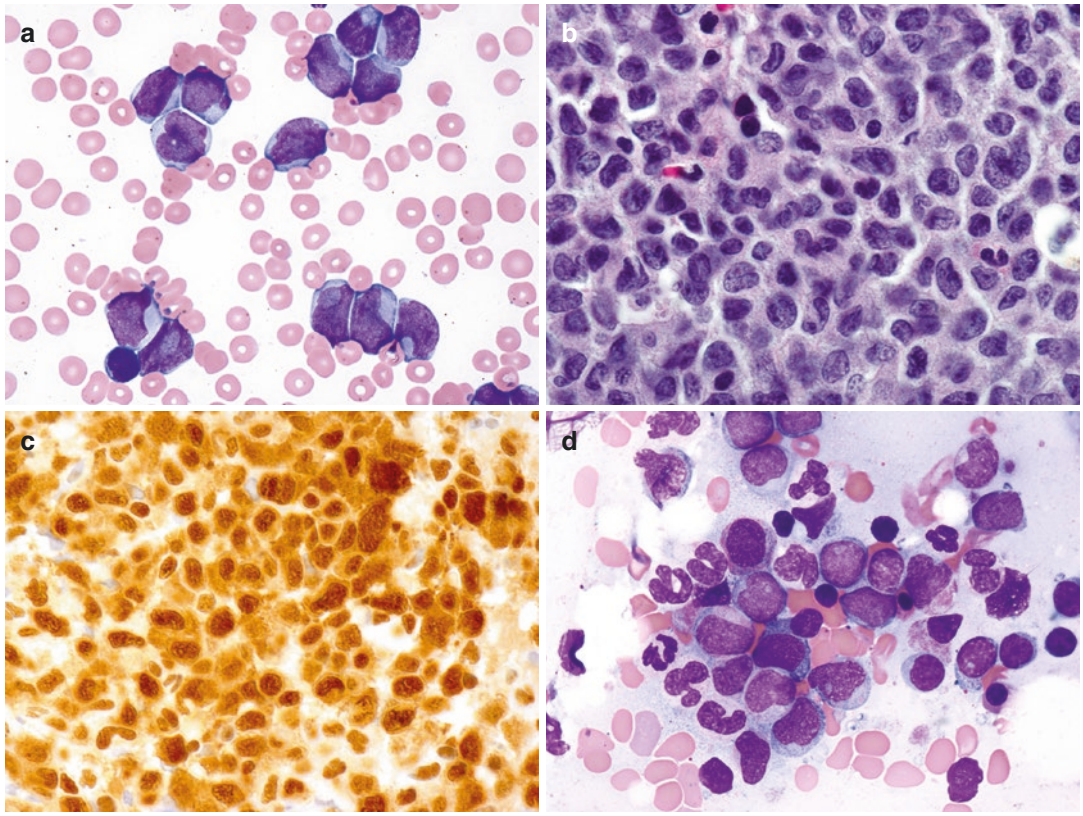

Fig. 5.15 AML with mutated *NPM1*. (**a**) The blasts in this peripheral blood smear have irregular nuclei with prominent cup-shaped nuclear indentations. (**b**) In the bone marrow biopsy, the blasts often show monocytic features, with folded and lobulated nuclei. (**c**) NPM1 immunohistochemistry discloses aberrant cytoplasmic staining for the NPM1 protein, as well as demonstrating its expected normal staining in the nucleus. (**d**) In the bone marrow aspirate, blasts have monocytic features with folded nuclei. Dysgranulopoiesis may be seen

mature monocytes, including markedly decreased CD13, CD14, and CD36 and increased CD15 expression. However, this is not a uniform finding: in some cases, monocytic blasts can show a completely mature immunophenotype resembling normal monocytes. The distinction between mature and immature monocytes/monoblasts can be difficult by immunophenotyping in many cases, and morphologic evaluation is always required to determine the blast count. AML of myelomonocytic type is composed of a population of myeloblasts with or without MPO expression and an often distinct population of monocytic blasts, which can be monoblasts or promonocytes, while AML of monocytic type is comprised almost exclusively of monocytic blasts. In acute megakaryoblastic leukemia, megakaryoblasts constitute 50% or more of the total blast population. Megakaryoblasts are CD117−, HLA-DR−, and CD13− and express one or more megakaryocytic markers (CD41, CD42b, and/or CD61); they may or may not express CD34 (Fig. 5.17). Especially in adults, there is often a smaller population of MPO-negative myeloblasts with an otherwise typical myeloid blast immunophenotype (CD34+, CD117+, CD13+, CD33+, HLA-DR+). The blast equivalents in pure erythroid leukemia are CD71bright+, CD36+, CD235a (glycophorin)+ and HLA-DR− abnormal pronormoblasts. They often show partial expression of CD117 and aberrant expression of CD33 and CD4, but are negative for CD34, CD13, MPO, and megakaryocytic markers. The blasts in acute basophilic leukemia express common myeloid antigens (CD13 and CD33) as well as CD9, CD11b, bright CD123, and CD25, and lack HLA-DR and MPO expression.

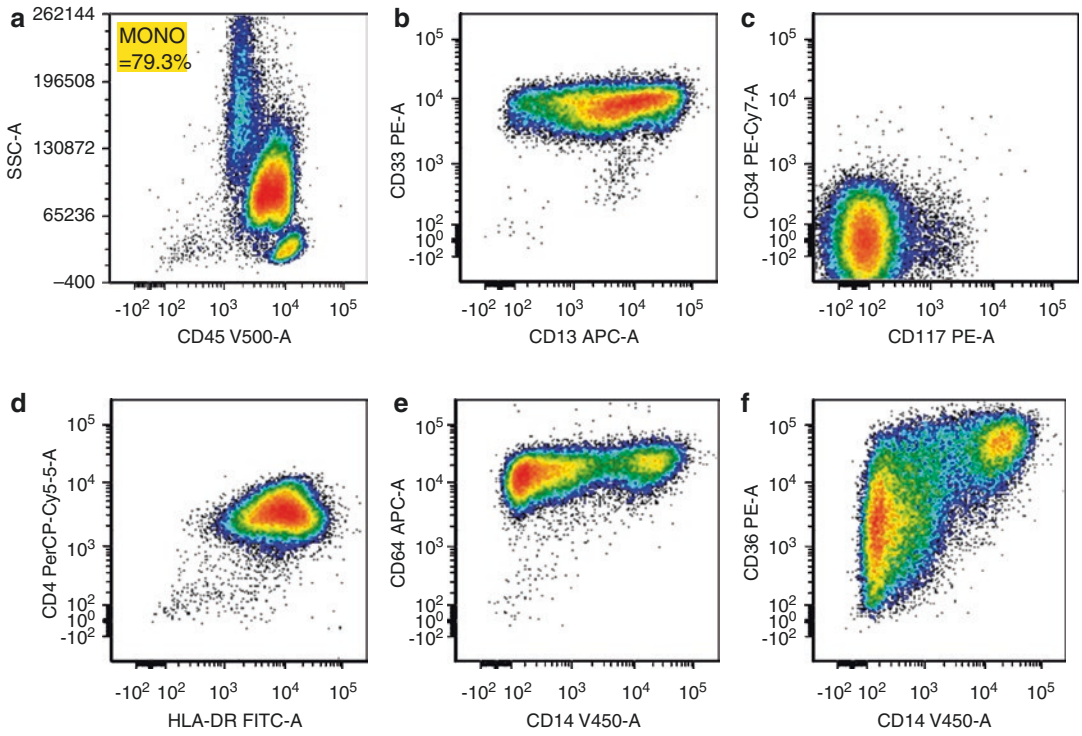

Fig. 5.16 Flow cytometry characteristics of acute monoblastic leukemia. (**a**) On CD45/SSC, the monocytic cells are markedly increased (79.3% of total cells), and granulocytes are markedly reduced; this is in contrast to chronic myelomonocytic leukemia that usually shows a retained large number of granulocytes. (**b**) The monoblasts, similar to mature monocytes, show bright CD33 and variable CD13 expression. (**c**) They are negative for CD34 and are mostly negative for CD117. (**d**) They express CD4 and HLA-DR. The monoblasts also show reduced CD14 (**e**) and CD36 (**f**) expression compared to mature monocytes

In cases where an aspirate cannot be obtained and minimal or no circulating blasts are present in the blood, immunohistochemistry may be performed on the bone marrow biopsy. By immunohistochemistry, AML blasts usually express lysozyme, CD117, and CD33, and many cases express MPO. CD34 can be helpful in confirming a primitive hematopoietic stage of differentiation. Although it does not distinguish between AML and ALL blasts, CD34 immunohistochemistry can also help establishing sufficient (at least 20%) blasts in the bone marrow biopsy to confirm a diagnosis of AML in cases where the aspirate is inadequate. However, it is noteworthy that CD34 can be negative in myeloblasts and is almost always negative in monocytic blasts. Monoblasts and promonocytes are both CD34− and CD117−, but are positive for CD68, lysozyme, CD11c, and often CD14 by immunohistochemistry. Similar to flow cytometry, TdT is frequently expressed in the blasts of AML by immunohistochemistry and its presence does not necessarily indicate an ALL or a mixed-lineage leukemia.

Immunohistochemistry has limited role in the subclassification of AML. Pure erythroid leukemia often requires the application of immunohistochemistry, because the large blasts may not be easily detected by flow cytometry and are usually negative for CD45. Pure erythroid leukemia blasts stain positively for E-cadherin and CD117 and are variably positive for other erythroid markers such as CD71, glycophorin, and hemoglobin (HGB) [58]. Of note, AML with t(8;21) may show expression of CD19 and/or PAX5, but lacks expression of other B-cell markers [59]. AML with mutated *NPM1* shows aberrant cytoplasmic staining of NPM1 protein in the cyto-

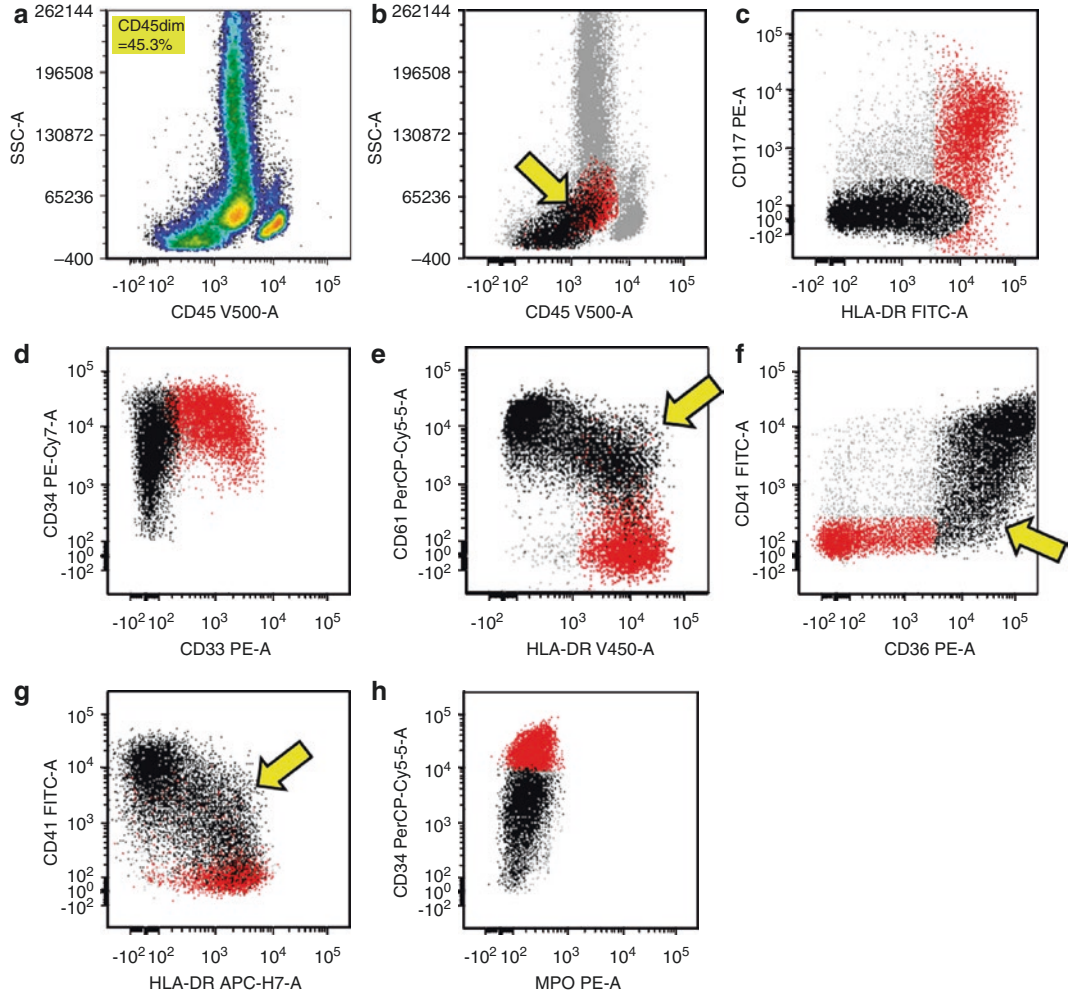

Fig. 5.17 Flow cytometry characteristics of acute megakaryoblastic leukemia. (**a**) On CD45/SSC, there are two populations of blasts with different levels of CD45 expression. (**b**) The black population is megakaryoblasts and the red is myeloblasts. The myeloblasts are CD117+ and HLA-DR+ (**c**), CD33+ (**d**), and are negative for CD61, CD41, and CD36 (**e**–**g**). The megakaryoblasts are CD117−, HLA-DR−, and CD33−, but are CD61+, CD41+, and CD36+ (**c**–**g**). Both populations are CD34+ and MPO− (**h**). The transition between myeloblasts to megakaryoblasts is indicated by arrows

plasm of the neoplastic blasts; normal NPM1 protein is confined to the nucleus in nondividing cells [60] (Fig. 5.15c). Application of CD61 or other megakaryocytic markers may aid in identifying micromegakaryocytes when evaluating for the possibility of AML-MRC (Fig. 5.11d).

Cytogenetics and Molecular Genetics

Recurring translocations that activate oncogenes are a hallmark of many types of AML and karyotype abnormalities are strongly correlated with clinical behavior in AML. Cytogenetic abnormalities are present in about 50% of AML cases at diagnosis. The cytogenetic findings provide critical diagnostic and prognostic information and a conventional karyotype should always be performed on bone marrow taken at the time of initial diagnosis [50]. Targeted FISH studies that interrogate for specific translocations may be helpful if the karyotype fails or is insufficient (less than 20 metaphases) [61] and are helpful in investigating for cryptic abnormalities such as some 11q23 rearrangements.

A listing of the cytogenetic aberrations that define AML subtypes in the 2016 WHO Classification is shown in Table 5.3. Importantly, three cytogenetic abnormalities—*PML-RARA,* inv(16)(p13.1q22) or t(16;16)(p13.1;q22), and t(8;21)(q22;q22.1)—are considered AML defining, irrespective of the blast count. Identification of *PML-RARA* rearrangement to confirm a diagnosis of APL is especially critical, since this unique AML subtype requires specific up-front therapy with ATRA. Institution of inappropriate therapy may precipitate fulminant DIC, which is a major cause of early death in APL patients. *PML-RARA* rearrangement should be rapidly investigated in all new acute leukemias where APL is a diagnostic consideration [50]. The *PML-RARA* rearrangement can be rapidly demonstrated (in <24 hours) by a direct harvest karyotype, targeted FISH, or RT-PCR. Immunofluorescent staining showing aberrant PML nuclear staining pattern can also confirm *PML-RARA*, but it is not widely available [62–64].

While the importance of karyotype abnormalities in defining AML subtypes with unique clinical and pathologist features has been established for decades, in more recent years the important impact of gene mutations on the biologic behavior of AML has become recognized. The mutation status of three genes (*NPM1*, *FLT3*, and *CEBPA*) has been used for over 10 years in combination with the cytogenetic risk group to guide treatment. In particular, AML patients with normal karyotype and *NPM1* or biallelic *CEBPA* mutations and lacking *FLT3*-ITD have a favorable prognosis and may not require treatment with stem cell transplantation [65]. For these reasons, as well as their other unique clinicopathologic features, AML with mutated *NPM1* and AML with biallelic *CEBPA* mutations represent specific mutationally defined subgroups in the 2016 WHO Classification. This revised classification additionally includes mutation in *RUNX1*, which confers an unfavorable prognosis, as a new provisional genetically-defined subgroup [29]. More recently, a large amount of data has been accumulating concerning the effect of other mutations on AML outcome. AML cases often have multiple mutations and the optimal way to combine karyotype with mutation data (particularly in the context of multiple mutations or several distinct subclones) to guide treatment decisions is still under active investigation.

According to the current standard of care, the complete genetic workup of a newly diagnosed AML case should always include a complete karyotype of bone marrow as well as assessment for *NPM1, FLT3*, and *CEBPA* mutations. Assessment for other mutations, including *KIT* [for cases of AML with t(8;21) or inv(16)/t(16;16)], *IDH1, IDH2, TET2, WT1, DNMT3A, TP53, RUNX1*, and *ASXL1*, has also been suggested by several recent guidelines [50, 66], but there is not yet universal agreement on the specific list of mutations which should be tested, nor is it clear how these results should impact patient management. It is hoped that ongoing studies will help provide guidance and achieve consensus on the optimal use of advanced mutation testing for AML management. The decision as to which mutations to test may also be driven by institution-specific clinical trials evaluating therapies targeting specific genes or the karyotypic features of the leukemia.

Differential Diagnosis

The main questions that must be answered in approaching a possible diagnosis of AML are the following:

1. Are abnormal cells identified in the blood or bone marrow blasts (or blast equivalents, as defined above)?
2. Are the blasts (plus blast equivalents) at least 20% of the blood or bone marrow leukocytes?
3. Are the blasts of myeloid phenotype?

A diagnosis of AML requires a "yes" answer to all three questions, with the exception of the three cytogenetically-defined AML entities (Table 5.3) that do not require the presence of at least 20% blasts.

In most patients first presenting with AML, the diagnosis is suspected based on abnormal cells seen in the blood. Since AML blasts have varied sizes and morphologies, they can be mimicked by many other cells. Patients may present with large numbers of circulating abnormal cells

that are initially thought to be blasts, but actually represent other neoplastic or even nonneoplastic cells. Small AML blasts can be confused with lymphocytes or hematogones (Fig. 5.18a, b), medium-sized blasts can be confused with reactive atypical lymphocytes, monocytes, or lymphoma cells (particularly blastoid mantle cell lymphoma and Burkitt lymphoma), while larger blasts can resemble megaloblastic erythroid precursors, large-cell lymphomas, or hypogranular dysplastic promyelocytes. Close attention to the fine chromatin typically found in myeloblasts versus the coarser chromatin in more mature cell types is critical in avoiding misdiagnosis in these scenarios. Flow cytometry immunophenotyping should be performed in all new AML diagnoses and can quickly clarify the diagnosis, as all of the above cell types are immunophenotypically distinct from AML blasts. One exception is mature monocytes, which can have an identical immunophenotype to the promonocytes in monocytic AML. In such cases, distinction relies on recognizing the typical immature nuclear cytology of promonocytes.

Distinguishing left-shifted neoplastic or nonneoplastic erythroid elements from myeloblasts is often difficult. Myeloblasts may have basophilic cytoplasm resembling pronormoblasts and conversely, in megaloblastic anemia, the proliferation of left-shifted, megaloblastic erythroid elements can be misdiagnosed as an acute leukemia (usually the pure erythroid leukemia

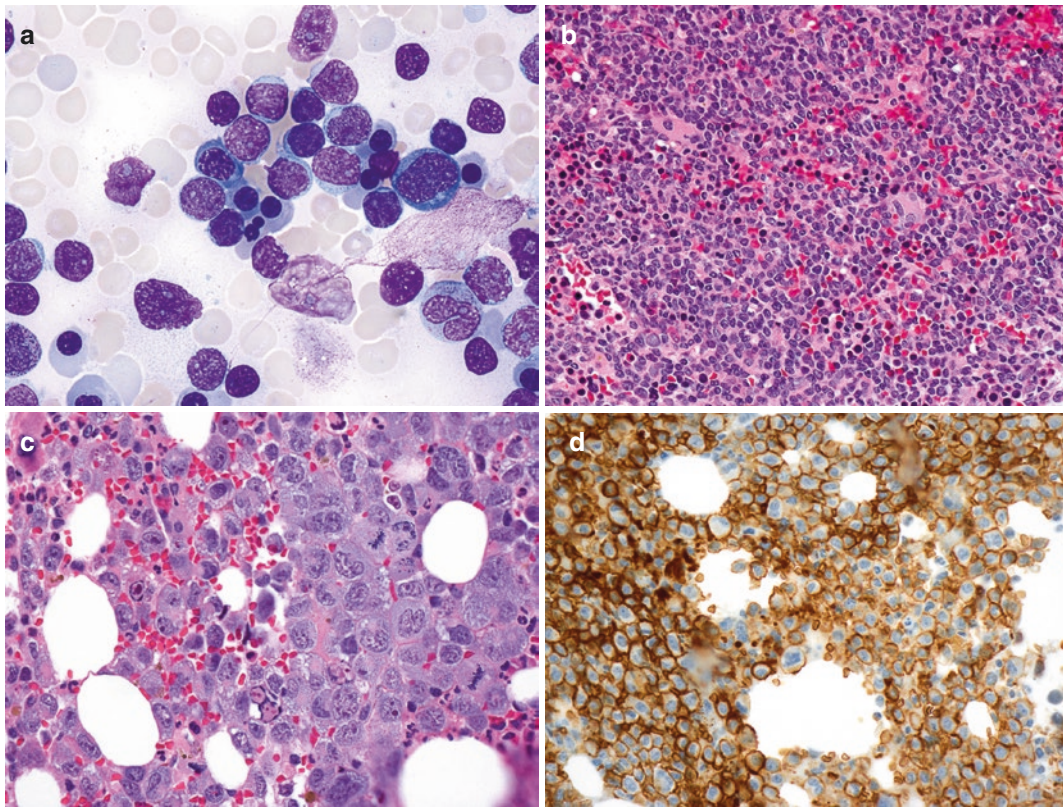

Fig. 5.18 Unusual AML morphologies that can cause diagnostic difficulties. Some cases of AML have small blasts, mimicking a lymphoblastic leukemia or even a mature lymphoma on both the bone marrow aspirate smear (**a**) and biopsy (**b**). In this case, immunophenotyping showed typical expression of AML markers CD34, CD117, and MPO. (**c**) Pure erythroid leukemia can mimic a high-grade lymphoma or metastatic solid tumor; because it is negative for CD45 and CD34, a hematopoietic neoplasm may not be initially considered. (**d**) In this case, glycophorin positivity confirmed the erythroid lineage of the malignant cells. The erythroblasts were also positive for E-cadherin and were variably positive for CD117 (not shown)

subtype) if these large cells are interpreted as blasts. Close attention to the WHO diagnostic criteria for pure erythroid leukemia (PEL) is essential to avoiding its overdiagnosis in situations of florid reactive erythroid proliferations [54]. The presence of a significant maturing erythroid component, hypersegmented neutrophils, and giant band forms are helpful clues to a megaloblastic anemia. In PEL, the BM biopsy almost always demonstrates diffuse sheets of immature erythroid cells; in some cases, there may be intrasinusoidal collections of malignant erythroblasts. The malignant PEL cells may resemble normal pronormoblasts, but they can be shown to contain large cytoplasmic PAS+ globules [54] (Fig. 5.10c). Ring sideroblasts also may be seen [67]. Use of immunohistochemistry for E-cadherin and other erythroid markers is helpful in confirming the diagnosis (Fig. 5.18c, d). PEL almost always has a highly abnormal complex karyotype [67] and frequently has *TP53* mutation (which can be suspected by demonstrating strong p53 immunopositivity in the neoplastic erythroid cells), unlike reactive erythroid proliferations. However, p53 overexpression (but not mutation) has been reported to occur in the bone marrow in cases of megaloblastic anemia [68].

It is important to note that even if myeloblasts in the blood are confirmed by flow cytometry, their presence does not in isolation confirm a diagnosis of AML or even a neoplastic process. Blasts are normally absent from the blood, but may circulate in reactive conditions such as infections, following treatment with myeloid growth factors, or upon early marrow recovery post-chemotherapy; in such situations, they are almost always <5% of the white cells. Myeloid growth factors frequently cause the appearance of variable numbers of blasts in the blood and can rarely cause transient increases of blasts to above 20% in patients with MDS who already have excess blasts. In such situations, it is prudent to avoid diagnosing AML unless the blast percentage persists above 20% after the effect of growth factor has dissipated (typically 7–10 days after cessation of growth factor administration).

Regarding blast enumeration, if the myeloblast percentage is close to 20%, the differential diagnosis includes other myeloid neoplasms with elevated blasts. MDS with excess blasts, myeloproliferative neoplasms in accelerated phase, and advanced myelodysplastic/myeloproliferative neoplasm entities can have blast and blast equivalent percentages approaching 20% in the blood or marrow. While the 20% cutoff is admittedly arbitrary, it may dictate different therapeutic options; thus, as accurate a blast count should be derived as possible from the available material. As discussed above, this requires careful counting of the bone marrow aspirate or touch prep and sampling different areas of the slides, since there may be variability in the distribution of blasts in the aspirate smear. Review of the peripheral smear is mandatory, as some AML cases may have <20% blasts in the marrow, but ≥20% circulating blasts.

Unless Auer rods are present, immunophenotyping is essential to confirm myeloid phenotype of the blasts and to distinguish AML from other neoplasms that can mimic blasts. The immunophenotypic features of the various AML subtypes are discussed above. Within AML, the microgranular variant of APL can have some morphologic similarities to monocytic AML. Flow cytometry can aid in this distinction, because the blasts and promyelocytes of APL typically lack expression of HLA-DR, CD11b, and CD11c, unlike monocytic AMLs, which express most or all of these antigens [69] and are negative for CD34, which is expressed in most other AML subtypes (Fig. 5.19).

The distinction between AML and an acute leukemia of ambiguous lineage or a mixed phenotype acute leukemia (MPAL) is diagnostically challenging. Mixed-phenotype acute leukemia includes biphenotypic leukemias, where a single population of blasts co-expresses markers of two (or rarely three) lineages (B cell, T cell, and/or myeloid), and bi-lineage leukemias, in which there are two (or rarely three) distinct blast populations, each expressing markers of a different lineage. It is important to follow the strict definition of lineage-defining markers when making a diagnosis of MPAL (Table 5.4). Of note, AML with minimal differentiation (M0) shows no or

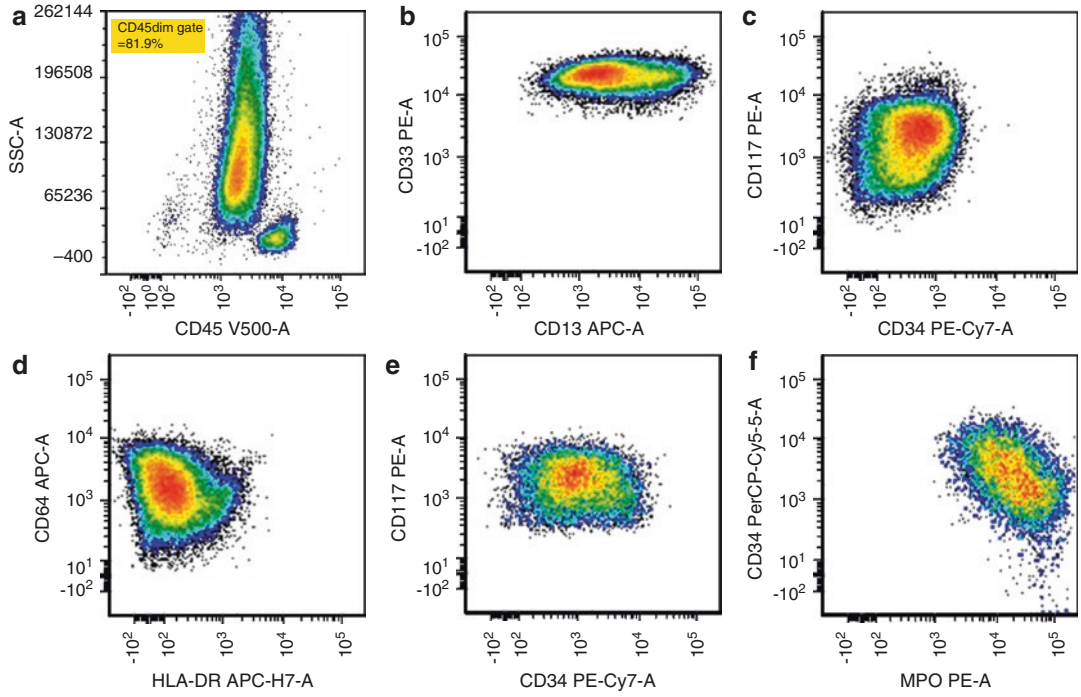

Fig. 5.19 Flow cytometry characteristics of acute promyelocytic leukemia (APL). (**a**) APL blasts have very high side scatter on CD45/SSC mimicking hypogranulated granulocytes. (**b**) They are CD33bright+ and CD13+. They are also CD117+ (**c**) and negative for CD34 and HLA-DR (**d**). Microgranular variant APL often can express dim or partial CD34 (**e**), but like the classical type of APL shows strong, uniform MPO expression (**f**)

minimal (<3%) MPO expression by cytochemistry, but a diagnosis can be made if blasts express two or more myeloid markers such as CD117 and CD13 and lack expression of B- or T-lineage-defining markers. On the other hand, cases with an immunophenotype typical for B- or T-lymphoblastic leukemia and with a few MPO+ cells demonstrated by either flow cytometry or cytochemistry should not be diagnosed as MPAL. Typical examples of mixed-phenotype acute leukemias are illustrated in Figs. 5.20 and 5.21. Acute undifferentiated leukemia shows no lineage-defining markers. The blasts are often CD34+ with CD7 expression, but lack MPO and CD117 and are negative for B- and T-lineage markers. Regarding the use of immunostains to diagnose MPAL cases, care must be taken with MPO immunostaining, which can be positive in some ALL cases. Thus, expression of MPO as the only myeloid marker by immunohistochemistry in isolation does not establish a myeloid lineage.

Blastic Plasmacytoid Dendritic Cell Neoplasm

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is an aggressive, primitive hematologic neoplasm derived from the precursors of plasmacytoid dendritic cells, which are type I interferon-producing dendritic cells. It most commonly simultaneously involves the skin and bone marrow and for these reasons had previously been called "primitive hematodermic neoplasm." The tumor can occur in any age group, but usually affects patients in the seventh decade of life or older. It has a male predominance. The skin is involved in nearly all cases, with patients presenting with often multiple cutaneous nodules or papules that are variable in size. The bone marrow is involved in up to 90% of cases and if the involvement is extensive, patients may manifest one or more cytopenias, with a leukemic phase often developing later in the course of disease [70]. Interestingly,

Table 5.4 Lineage-defining markers in diagnosing acute leukemias of ambiguous lineage

Myeloid lineage
• Myeloperoxidase (MPO) positive by cytochemistry (≥3% of blasts) or flow cytometry, or expression of at least two of the following monocytic markers: CD11c, CD14, CD64, lysozyme, nonspecific esterase
B lineage
• Bright surface CD19 plus at least one of the following markers: Cytoplasmic CD79a, surface or cytoplasmic CD22, CD10, *OR*
• Weak surface CD19 plus at least two of the following markers: Cytoplasmic CD79a, surface or cytoplasmic CD22, CD10
T lineage
• Cytoplasmic CD3 (in at least a subset of the blasts), expressed at the level of normal T cells

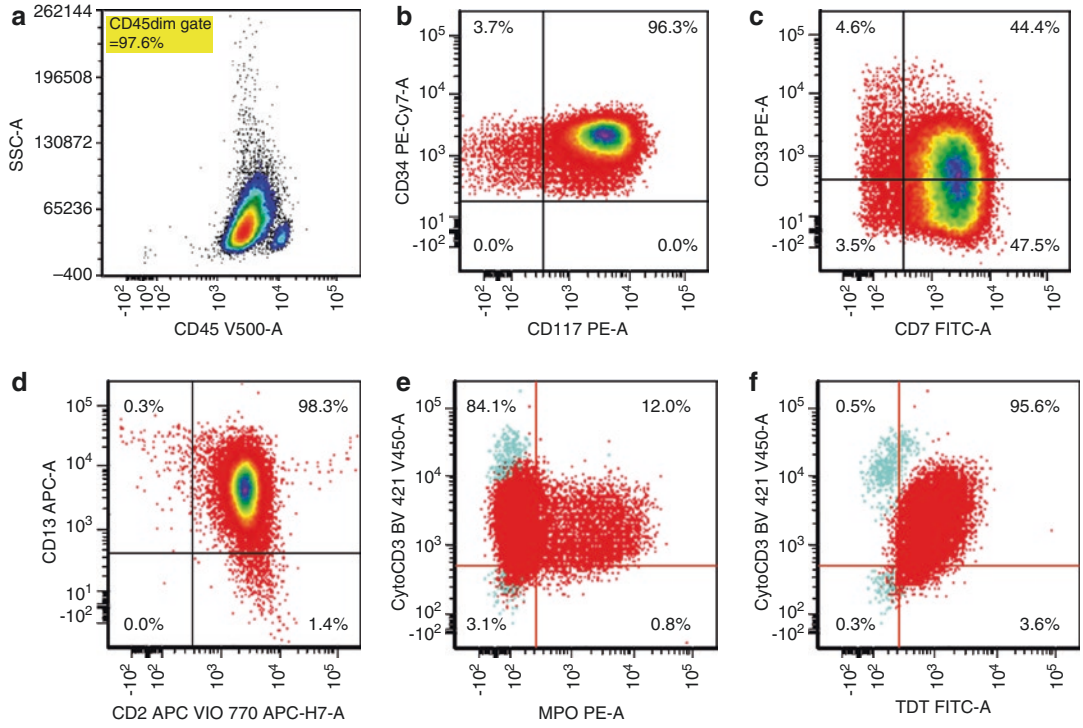

Fig. 5.20 Mixed-phenotype acute leukemia, T/myeloid subtype (biphenotypic). (**a**) A large population of CD45dim+ cells is seen on CD45/SSC. The blasts are uniformly positive for CD34 and CD117 (**b**), CD7 and partial/dimCD33 (**c**), bright CD13 and CD2 (**d**), cytoplasmic CD3 and MPO (subset) (**e**), and TDT (**f**). Turquoise color represents lymphocytes as internal control cells. The T-cell and myeloid markers are co-expressed on the same cell population, characteristics of biphenotypic acute leukemia

10–20% of BPDCN are associated with a concomitant myeloid neoplasm, most commonly MDS, CMML, or AML [71, 72]. The clinical course of BPDCN is aggressive, with most reported median survivals of less than 2 years.

In blood and BM smears, the neoplastic cells resemble blasts, with irregular nuclei, finely dispersed chromatin, and scant, agranular cytoplasm that may contain peripherally located small vacuoles; Auer rods are absent. In the bone marrow, the blastic infiltrate is usually diffuse, although the cells may be admixed with maturing hematopoietic elements (Fig. 5.22). As discussed above, other hematopoietic elements should be carefully examined for evidence of another concomitant hematopoietic neoplasm, which occurs in a subset of cases.

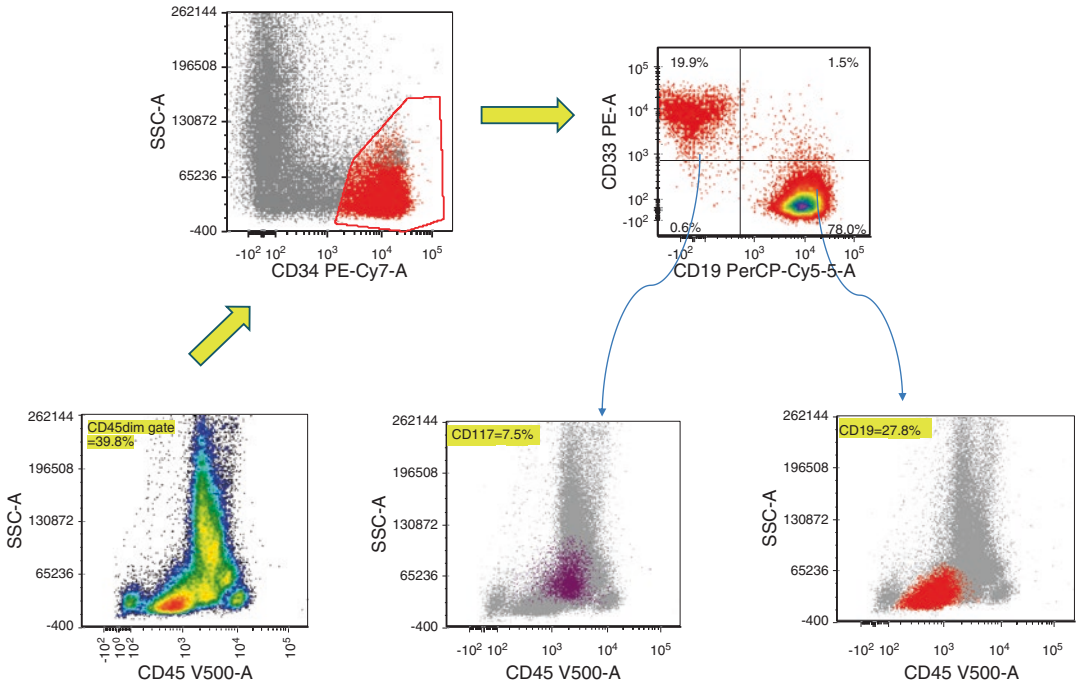

Fig. 5.21 Mixed-phenotype-lineage acute leukemia, B/myeloid (bi-lineal). A large population of CD34+ blasts is detected, containing two immunophenotypically completely different blast populations: one is CD33+/CD19− and another is CD33−/CD19+. They also show different levels of CD45 expression (lower right panels). Further characterization confirmed that the CD19+ population represents B lymphoblasts and the CD33+ population represents myeloblasts

By cytochemistry, the BPDCN cells are uniformly negative for NSE and MPO. The typical immunophenotype by immunohistochemistry is CD4+, CD56+, CD123+, and TCL1+ and most cases express all of these markers; however, about 10% of cases may fail to express CD4 or CD56 and negativity for one of these markers does not exclude the diagnosis if other features fit with BPDCN [71, 73, 74]. Other commonly expressed, but less specific, markers are CD68, CD33, and CD7. TdT, CD2, CD5, and CD117 are expressed in a subset of cases [74]. Specific markers defining B cell (CD19, CD20), T cell (CD3), or myeloid (MPO, lysozyme) lineages are negative. By flow cytometry, BPDCN express the same markers found by immunohistochemistry, with bright CD123 expression and dim CD45 expression. CD303 is also commonly expressed [75].

There is relatively little data available on the genetic profile of BPDCN. About half of cases that involve the bone marrow show an abnormal karyotype, with the most frequent abnormalities involving chromosomes 5q, 12p, and 13q. Interestingly, the gene expression profile of BPDCN is distinct from both AML and ALL [76], which validates its classification as a separate entity among the acute leukemias in the 2016 WHO Classification, rather than as a subtype of AML. However, the pattern of gene mutations, which includes *TET2* as well as *ASXL1*, *NRAS*, and *KRAS*, is more akin to myeloid neoplasms such as MDS rather than lymphoid neoplasms [74, 77, 78].

The main differential diagnosis is with AML. The blasts of AML with monocytic differentiati n can express CD4, CD56, and CD123 and BPDCN cells may resemble myeloblasts or monoblasts. Thus, demonstration of negativity for MPO, lysozyme, and NSE is important, as positivity for any of these markers would favor AML rather than BPDCN. One

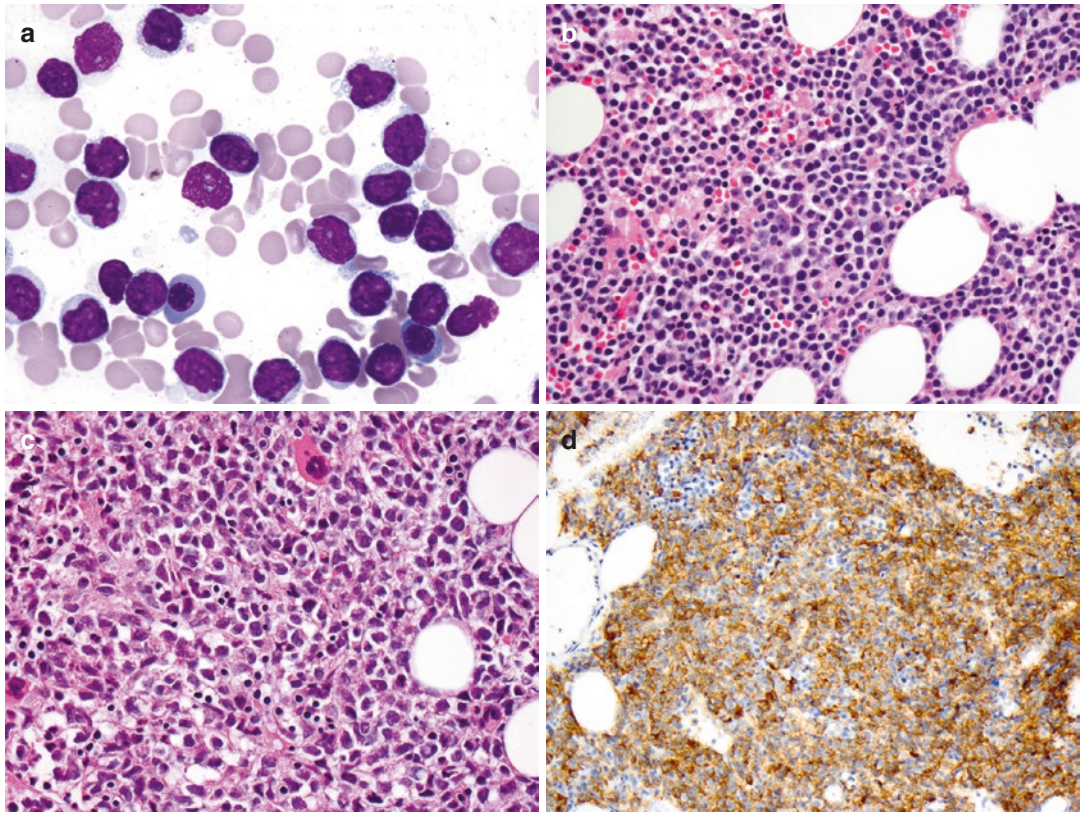

Fig. 5.22 Blastic plasmacytoid dendritic cell neoplasm (BPDCN) involving the bone marrow. (**a**) In the aspirate smear, the tumor cells in this case are small with moderately dispersed chromatin, one to two distinct nucleoli, irregular nuclei, and scant cytoplasm. Peripherally located vacuoles are present in some of the cells. (**b**) The bone marrow biopsy contains a heavy interstitial infiltrate of tumor cells with irregular nuclei. (**c**) Another example of BPDCN involving the bone marrow biopsy, showing more pronounced cellular pleomorphism. (**d**) An immunostain for CD123 is positive in the tumor cells

recent study suggested that a panel of immunohistochemical markers including MPO, CD56, CD123, TCL1, TdT, and myxovirus A (MxA) was most helpful in distinguishing between AML and BPDCN [79]. Mature nodules of plasmacytoid dendritic cells can occur in the bone marrow of patients with myeloid neoplasms, particularly CMML, and are not equivalent to a diagnosis of BPDCN. Unlike the tumor cells of BPDCN, the cells in mature plasmacytoid dendritic cell nodules have abundant, eccentric cytoplasm reminiscent of plasma cells and mature chromatin. They are also generally negative or only very weakly positive for CD56 and have a much lower Ki67 proliferation index compared to BPDCN [80].

References

1. Summers C, et al. Neutrophil kinetics in health and disease. Trends Immunol. 2010;31(8):318–24.
2. Stone KD, Prussin C, Metcalfe DD. IgE, mast cells, basophils, and eosinophils. J Allergy Clin Immunol. 2010;125(2 Suppl 2):S73–80.
3. George TI. Malignant or benign leukocytosis. Hematology Am Soc Hematol Educ Program. 2012;2012:475–84.
4. Savage RA. Pseudoleukocytosis due to EDTA-induced platelet clumping. Am J Clin Pathol. 1984;81(3):317–22.
5. Patel KJ, Hughes CG, Parapia LA. Pseudoleucocytosis and pseudothrombocytosis due to cryoglobulinaemia. J Clin Pathol. 1987;40(1):120–1.
6. Park HJ, et al. Delta neutrophil index as an early marker for differential diagnosis of adult-onset Still's disease and sepsis. Yonsei Med J. 2014;55(3):753–9.

7. Lawrence YR, et al. Extreme leukocytosis in the emergency department. QJM. 2007;100(4):217–23.
8. Juturi JV, Hopkins T, Farhangi M. Severe leukocytosis with neutrophilia (leukemoid reaction) in alcoholic steatohepatitis. Am J Gastroenterol. 1998;93(6):1013.
9. Kawada T. Smoking-induced leukocytosis can persist after cessation of smoking. Arch Med Res. 2004;35(3):246–50.
10. Biezeveld MH, et al. Sustained activation of neutrophils in the course of Kawasaki disease: an association with matrix metalloproteinases. Clin Exp Immunol. 2005;141(1):183–8.
11. Meyerson HJ, Farhi DC, Rosenthal NS. Transient increase in blasts mimicking acute leukemia and progressing myelodysplasia in patients receiving growth factor. Am J Clin Pathol. 1998;109(6):675–81.
12. Dale DC, et al. Comparison of agents producing a neutrophilic leukocytosis in man. Hydrocortisone, prednisone, endotoxin, and etiocholanolone. J Clin Invest. 1975;56(4):808–13.
13. Christensen RD, Hill HR. Exercise-induced changes in the blood concentration of leukocyte populations in teenage athletes. Am J Pediatr Hematol Oncol. 1987;9(2):140–2.
14. Marinella MA. Extreme leukemoid reaction associated with retroperitoneal hemorrhage. Arch Intern Med. 1998;158(3):300–1.
15. Ascensao JL, et al. Leukocytosis and large cell lung cancer. A frequent association. Cancer. 1987;60(4): 903–5.
16. Kayashima T, et al. Leukemoid reaction associated with diabetic ketoacidosis—with measurement of plasma levels of granulocyte colony-stimulating factor. Intern Med. 1993;32(11):869–71.
17. Kohmura K, et al. Granulocyte colony stimulating factor-producing multiple myeloma associated with neutrophilia. Leuk Lymphoma. 2004;45(7):1475–9.
18. Plo I, et al. An activating mutation in the CSF3R gene induces a hereditary chronic neutrophilia. J Exp Med. 2009;206(8):1701–7.
19. Cohen PR. Sweet's syndrome—a comprehensive review of an acute febrile neutrophilic dermatosis. Orphanet J Rare Dis. 2007;2:34.
20. Zhou Q, et al. Brief report: cryopyrin-associated periodic syndrome caused by a myeloid-restricted somatic NLRP3 mutation. Arthritis Rheumatol. 2015;67(9):2482–6.
21. Etzioni A. Genetic etiologies of leukocyte adhesion defects. Curr Opin Immunol. 2009;21(5):481–6.
22. Stemmelin GR, et al. Smoking as a cause for mild chronic neutrophilia. Blood. 2004;104:3796.
23. Ishii K, Young NS. Anemia of central origin. Semin Hematol. 2015;52(4):321–38.
24. Jardin F, et al. Intense paraneoplastic neutrophilic leukemoid reaction related to a G-CSF-secreting lung sarcoma. Am J Hematol. 2005;80(3):243–5.
25. Kusaba N, et al. Granulocyte-colony stimulating factor-producing myeloma with clinical manifestations mimicking chronic neutrophilic leukemia. Rinsho Ketsueki. 2004;45(3):228–32.
26. Loomba RS. Prevalence of isomerism from a European registry: live births, fetal deaths, and terminations of pregnancy. Congenit Anom (Kyoto). 2016;56(6):256–7.
27. Gaylor MS, Chervenick PA, Boggs DR. Neutrophil kinetics after acute hemorrhage. Proc Soc Exp Biol Med. 1969;131(4):1332–6.
28. Steensma DP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9–16.
29. Arber DA, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
30. El Rassi F, et al. Predicting early blast transformation in chronic-phase chronic myeloid leukemia: is immunophenotyping the missing link? Cancer. 2015;121(6):872–5.
31. Soupir CP, et al. Philadelphia chromosome-positive acute myeloid leukemia: a rare aggressive leukemia with clinicopathologic features distinct from chronic myeloid leukemia in myeloid blast crisis. Am J Clin Pathol. 2007;127(4):642–50.
32. Konoplev S, et al. Molecular characterization of de novo Philadelphia chromosome-positive acute myeloid leukemia. Leuk Lymphoma. 2013;54(1):138–44.
33. Nacheva EP, et al. Does BCR/ABL1 positive acute myeloid leukaemia exist? Br J Haematol. 2013;161(4):541–50.
34. Elliott MA, et al. WHO-defined chronic neutrophilic leukemia: a long-term analysis of 12 cases and a critical review of the literature. Leukemia. 2005;19(2):313–7.
35. Elliott MA, et al. ASXL1 mutations are frequent and prognostically detrimental in CSF3R-mutated chronic neutrophilic leukemia. Am J Hematol. 2015;90(7):653–6.
36. Maxson JE, et al. Oncogenic CSF3R mutations in chronic neutrophilic leukemia and atypical CML. N Engl J Med. 2013;368(19):1781–90.
37. Beekman R, Touw IP. G-CSF and its receptor in myeloid malignancy. Blood. 2010;115(25):5131–6.
38. Fleischman AG, et al. The CSF3R T618I mutation causes a lethal neutrophilic neoplasia in mice that is responsive to therapeutic JAK inhibition. Blood. 2013;122(22):3628–31.
39. Pardanani A, et al. CSF3R T618I is a highly prevalent and specific mutation in chronic neutrophilic leukemia. Leukemia. 2013;27(9):1870–3.
40. Wang SA, et al. Atypical chronic myeloid leukemia is clinically distinct from unclassifiable myelodysplastic/myeloproliferative neoplasms. Blood. 2014;123(17):2645–51.
41. Piazza R, et al. Recurrent SETBP1 mutations in atypical chronic myeloid leukemia. Nat Genet. 2013;45(1):18–24.
42. Gambacorti-Passerini CB, et al. Recurrent ETNK1 mutations in atypical chronic myeloid leukemia. Blood. 2015;125(3):499–503.
43. Thiede C, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. Blood. 2002;99(12):4326–35.

44. Dohner K, et al. Mutant nucleophosmin (NPM1) predicts favorable prognosis in younger adults with acute myeloid leukemia and normal cytogenetics: interaction with other gene mutations. Blood. 2005;106(12):3740–6.
45. Matsushima T, et al. Prevalence and clinical characteristics of myelodysplastic syndrome with bone marrow eosinophilia or basophilia. Blood. 2003;101(9):3386–90.
46. Wimazal F, et al. Evaluation of the prognostic significance of eosinophilia and basophilia in a larger cohort of patients with myelodysplastic syndromes. Cancer. 2010;116(10):2372–81.
47. Quelen C, et al. Identification of a transforming MYB-GATA1 fusion gene in acute basophilic leukemia: a new entity in male infants. Blood. 2011;117(21):5719–22.
48. Chi Y, et al. Acute myelogenous leukemia with t(6;9)(p23;q34) and marrow basophilia: an overview. Arch Pathol Lab Med. 2008;132(11):1835–7.
49. Lee HJ, et al. Comparison of laboratory characteristics between acute promyelocytic leukemia and other subtypes of acute myeloid leukemia with disseminated intravascular coagulation. Blood Res. 2013;48(4):250–3.
50. Arber DA, et al. Initial diagnostic workup of acute leukemia: guideline from the College of American Pathologists and the American Society of Hematology. Arch Pathol Lab Med. 2017;141(10):1342–93.
51. Huck A, et al. Prior cytopenia predicts worse clinical outcome in acute myeloid leukemia. Leuk Res. 2015;39:1034.
52. Hasserjian RP, et al. De novo acute myeloid leukemia with 20–29% blasts is less aggressive than acute myeloid leukemia with >/=30% blasts in older adults: a Bone Marrow Pathology Group study. Am J Hematol. 2014;89(11):E193–9.
53. Vardiman JW, et al. Introduction and overview of the classification of the myeloid neoplasm. In: Swerdlow S, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer (IARC); 2008. p. 18–30.
54. Wang SA, Hasserjian RP. Acute erythroleukemias, acute megakaryoblastic leukemias, and reactive mimics: a guide to a number of perplexing entities. Am J Clin Pathol. 2015;144(1):44–60.
55. Weinberg OK, et al. Clinical characterization of acute myeloid leukemia with myelodysplasia-related changes as defined by the 2008 WHO classification system. Blood. 2009;113(9):1906–8.
56. Weinberg OK, et al. Reproducibility and prognostic significance of morphologic dysplasia in de novo acute myeloid leukemia. Mod Pathol. 2015;28(7):965–76.
57. Weinberg OK, et al. NPM1 mutation but not RUNX1 mutation or multilineage dysplasia defines a prognostic subgroup within de novo acute myeloid leukemia lacking recurrent cytogenetic abnormalities in the revised 2016 WHO classification. Am J Hematol. 2017;92(7):E123–4.
58. Ohgami RS, et al. E-cadherin is a specific marker for erythroid differentiation and has utility, in combination with CD117 and CD34, for enumerating myeloblasts in hematopoietic neoplasms. Am J Clin Pathol. 2014;141(5):656–64.
59. Valbuena JR, et al. Expression of B cell-specific activator protein/PAX5 in acute myeloid leukemia with t(8;21)(q22;q22). Am J Clin Pathol. 2006;126(2):235–40.
60. Falini B, et al. Immunohistochemistry predicts nucleophosmin (NPM) mutations in acute myeloid leukemia. Blood. 2006;108(6):1999–2005.
61. Malcovati L, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood. 2013;122(17):2943–64.
62. Sanz MA, et al. Management of acute promyelocytic leukemia: recommendations from an expert panel on behalf of the European LeukemiaNet. Blood. 2009;113(9):1875–91.
63. Dimov ND, et al. Rapid and reliable confirmation of acute promyelocytic leukemia by immunofluorescence staining with an antipromyelocytic leukemia antibody: the M. D. Anderson Cancer Center experience of 349 patients. Cancer. 2010;116(2):369–76.
64. Falini B, et al. Immunocytochemical diagnosis of acute promyelocytic leukemia (M3) with the monoclonal antibody PG-M3 (anti-PML). Blood. 1997;90(10):4046–53.
65. Schlenk RF, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. N Engl J Med. 2008;358(18):1909–18.
66. Dohner H, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–47.
67. Liu W, et al. Pure erythroid leukemia: a reassessment of the entity using the 2008 World Health Organization classification. Mod Pathol. 2011;24(3):375–83.
68. Yadav MK, Manoli NM, Madhunapantula SV. Comparative assessment of vitamin-B12, folic acid and homocysteine levels in relation to p53 expression in megaloblastic anemia. PLoS One. 2016;11(10):e0164559.
69. Dong HY, et al. Flow cytometry rapidly identifies all acute promyelocytic leukemias with high specificity independent of underlying cytogenetic abnormalities. Am J Clin Pathol. 2011;135(1):76–84.
70. Feuillard J, et al. Clinical and biologic features of CD4(+)CD56(+) malignancies. Blood. 2002;99(5):1556–63.
71. Petrella T, et al. Blastic NK-cell lymphomas (agranular CD4+CD56+ hematodermic neoplasms): a review. Am J Clin Pathol. 2005;123(5):662–75.
72. Vitte F, et al. Specific skin lesions in chronic myelomonocytic leukemia: a spectrum of myelomonocytic and dendritic cell proliferations: a study of 42 cases. Am J Surg Pathol. 2012;36(9):1302–16.
73. Julia F, et al. Blastic plasmacytoid dendritic cell neoplasms: clinico-immunohistochemical correlations in a series of 91 patients. Am J Surg Pathol. 2014;38(5):673–80.
74. Alayed K, et al. TET2 mutations, myelodysplastic features, and a distinct immunoprofile characterize blastic plasmacytoid dendritic cell

neoplasm in the bone marrow. Am J Hematol. 2013;88(12):1055–61.
75. Garnache-Ottou F, et al. Extended diagnostic criteria for plasmacytoid dendritic cell leukaemia. Br J Haematol. 2009;145(5):624–36.
76. Sapienza MR, et al. Molecular profiling of blastic plasmacytoid dendritic cell neoplasm reveals a unique pattern and suggests selective sensitivity to NF-kB pathway inhibition. Leukemia. 2014;28(8):1606–16.
77. Menezes J, et al. Exome sequencing reveals novel and recurrent mutations with clinical impact in blastic plasmacytoid dendritic cell neoplasm. Leukemia. 2014;28(4):823–9.
78. Stenzinger A, et al. Targeted ultra-deep sequencing reveals recurrent and mutually exclusive mutations of cancer genes in blastic plasmacytoid dendritic cell neoplasm. Oncotarget. 2014;5(15):6404–13.
79. Sangle NA, et al. Optimized immunohistochemical panel to differentiate myeloid sarcoma from blastic plasmacytoid dendritic cell neoplasm. Mod Pathol. 2014;27(8):1137–43.
80. Facchetti F, et al. Neoplasms derived from plasmacytoid monocytes/interferon-producing cells: variability of CD56 and granzyme B expression. Am J Surg Pathol. 2003;27(11):1489–92; author reply 1492–3.

Leukocytosis: Eosinophilia

6

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Overview

A normal peripheral blood level of eosinophils ranges between 0.0 and 6.0% of leukocytes with an absolute count of 0.05–0.5 × 10^9/L. The normal range for eosinophils in bone marrow aspirates is 1–6%, but this percentage is less reproducible in clot or core biopsy specimens. Eosinophilia is the increase of eosinophils in the peripheral blood or tissues above what is considered the normal range. The peripheral blood threshold for eosinophilia of clinical significance is ≥1.5 × 10^9/L [1]. This threshold applies to both reactive and neoplastic processes alike and it has been used in the diagnosis of a variety of disorders. Above this threshold, eosinophilia is usually associated with skin rash or autonomic symptoms such as bronchoconstriction. A definition of bone marrow eosinophilia has been suggested when ≥20% of marrow cells are eosinophils, with or without peripheral blood eosinophilia [2, 3]. In extramedullary sites such as the gastrointestinal tract mucosa, thymus, spleen, or lymph nodes, scattered eosinophils are normally found; tissue eosinophilia is based on a level above what is expected, except for certain specific diseases, such as eosinophilic esophagitis, which have defined levels of tissue eosinophilia.

Eosinophils are normally controlled by cytokines [4]. The most potent physiologic growth factors and activators of eosinophils are produced by T lymphocytes, mast cells, and stromal cells, and include IL-5, GM-CSF, and IL-3 [4]. However, eosinophils in clonal myeloid disorders are derived from progenitors bearing chromosomal rearrangements of oncogenic tyrosine kinase receptors such as *PDGFRA*, *PDGFRB*, or *FGFR1* [5, 6] and in many circumstances the mechanisms of these clonal eosinophilias are not apparent. The granules of eosinophils contain active molecules such as eosinophil peroxidase, eosinophil cationic protein, major basic protein, and cytokines such as TFG-β [4]. Upon activation, eosinophils release their granules that when persistent may lead to fibrosis, thrombosis, and organ damage [4].

Primary eosinophilia, which encompasses chronic eosinophilic leukemia (CEL) and idiopathic hypereosinophilic syndrome (HES), is associated with organ damage due to the release of eosinophilic granules, which often causes organ fibrosis. In both reactive and neoplastic processes, eosinophilia can be associated with an increase or a stimulation of other cells such as mast cells, or sometimes secondary increase of bone marrow cellularity. When eosinophilia

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Table 6.1 Classification of hypereosinophilia (HE) [2]

Proposed terminology	Proposed abbreviation	Definition/pathogenesis
Hereditary (familial) HE	HE_{FA}	Unknown pathogenesis
		Familial clustering
		No evidence of hereditary immunodeficiency
		No evidence of reactive or neoplastic disorder associated with HE
HE of undetermined significance	HE_{US}	No underlying cause of HE
		No familial history
		No evidence of reactive or neoplastic disorder associated with HE
		No evidence of organ damage attributable to HE
Primary (clonal/neoplastic) HE	HE_{N}	Underlying stem cell, myeloid, or eosinophilic neoplasm (WHO criteria)
		Eosinophils are neoplastic cells
Secondary (reactive) HE	HE_{R}	Underlying condition or disease in which eosinophils are nonclonal cells
		Cytokine driven in most cases

is associated with other diseases such as classical Hodgkin lymphoma, Langerhans cell histiocytosis, or peripheral T-cell lymphoma, it can be a diagnostic hint for the underlying disease. In some cases, the significance of eosinophilia may not be apparent or may be obscured by other more ominous manifestations of disease in cases of acute leukemia, where eosinophils can be part of the neoplastic clone, or secondarily increased due to tumor-releasing cytokines. A current classification of hypereosinophilia is shown in Table 6.1.

The neoplastic eosinophilias discussed in this chapter are CEL, idiopathic HES, and the WHO category of myeloid/lymphoid neoplasms with eosinophilia and rearrangements of *PDGFRA, PDGFRB*, *FGFR1*, or *PCM1-JAK2*. The latter encompasses a rare group of neoplasms that results from gene fusions involving one of several tyrosine kinase genes: a variety of gene fusion partners have been found with *PDGFRA, PDGFRB*, and *FGFR1,* while the *JAK2*-rearranged category only includes rearrangement with *PCM1* [5–7]. Although most patients with these neoplasms present with eosinophilia, the presentations can be highly variable and not all have eosinophilia; early recognition of these neoplasms is important because patients with neoplasms associated with *PDGFRA* or *PDGFRB* fusions often respond to tyrosine kinase inhibitor therapy. The main differential diagnoses to address are myeloid neoplasms that are defined by other parameters such as recurrent cytogenetic or molecular abnormalities, but that on occasion associate with clonal eosinophilia. To better understand the concepts of neoplastic eosinophilias, it is important to first consider the mechanisms of secondary eosinophilia.

Secondary Hypereosinophilia

Secondary hypereosinophilia (HE) is a polyclonal, reactive expansion of eosinophils driven by a primary disease process, often due to cytokine overproduction. In secondary/reactive HE, the high eosinophil count can be a hint and an adjuvant to the diagnosis of parasites, drug effects, allergies, and autoimmune diseases. Eosinophilia in patients who recently visited developing countries may be due to parasitic infections, and a search for parasites in skin, respiratory secretions, or stool is warranted. Although in general the

eosinophilia disappears with treatment of the parasite, there are cases when the eosinophilia persists. There is a number of systemic, allergic, or autoimmune disorders that associate with eosinophilia, including allergy and hypersensitivity, drug reactions, collagen-vascular disease, pulmonary eosinophilic disease, allergic gastroenteritis, and adrenal insufficiency among others. It is important to note that reactive processes affecting the gastrointestinal tract, such as eosinophilic esophagitis, are characterized by tissue eosinophilia (with or without peripheral blood eosinophilia) that can lead to organ damage.

Several nonmyeloid neoplasms are associated with secondary eosinophilia resulting from secretion of cytokines such as IL-3, IL-5, and GM-CSF that promote eosinophilic differentiation. The best known lymphoid neoplasms that associate with secondary eosinophilia are lymphoblastic leukemia/lymphoma [8], peripheral T-cell lymphoma [9, 10], and classical Hodgkin lymphoma (Fig. 6.1) [11]. Hypereosinophilia can also be associated with solid tumors such as squamous cell carcinoma of the head and neck, renal cell carcinoma, small-cell carcinoma of the lung, transitional cell carcinoma, and breast cancer. Eosinophilia in this setting is considered as a paraneoplastic phenomenon, but in some cases may be a reaction to cancer treatment drugs.

Lymphocyte variant hypereosinophilic syndrome (L-HES) is an entity characterized by hypereosinophilia associated with clonal circulating CD3-CD4+ T cells. The CD3-CD4+ T cells are often CD7dim/negative, CD2bright+, and CD5bright+. The median percentage of CD3-CD4+ lymphocytes out of total lymphocytes is 17% (range 0.5–96%) (Fig. 6.2). These T cells have a Th2 profile and are able to produce eosinophilopoietic cytokines such as IL-4, IL-5, and/or IL-13 [12]. This T-cell secretion of cytokines leads to overproduction of eosinophils [13]. In this disease, the T lymphocytes are clonal in most cases, but the eosinophils are reactive. Recently, a gain-of-function *STAT3* mutation was found in a patient with L-HES, and by cell sorting the mutation was identified in the CD3-CD4+ T cells [14]. Patients with L-HES usually present with skin manifestations and rheumatologic symptoms, and less common with lymphadenopathy, gastrointestinal, and pulmonary symptoms. Although L-HES is considered an indolent disease, about 5–20% patients reportedly go on to develop T-cell lymphoma [15]. The bone marrow is in general unremarkable, except for increased eosinophils that may lead to a slightly increased cellularity. Eosinophils often do not show significant abnormalities. Trilineage hematopoiesis is retained and exhibits no significant dyspoiesis. There may be scattered spindle mast cells, but no aggregates, and no *KIT* mutations. The immunophenotype of the Th2 type T cells shows striking overlapping with the neoplastic T cells of angioimmunoblastic

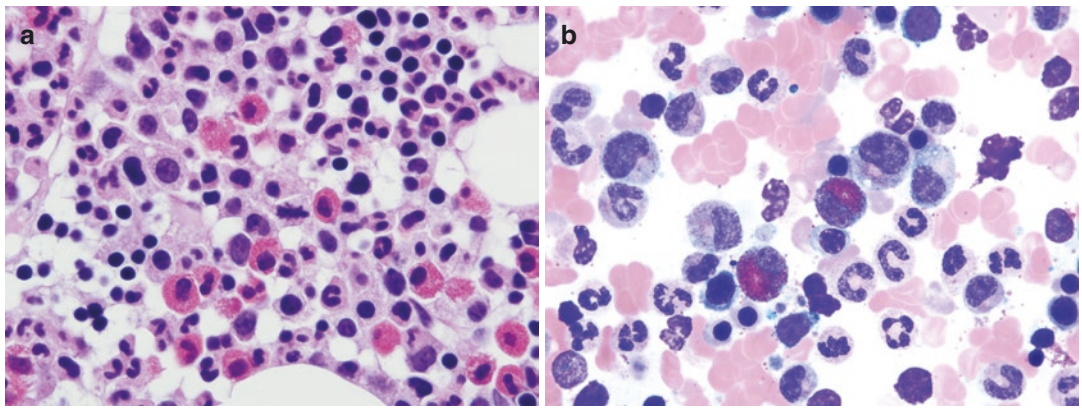

Fig. 6.1 Bone marrow of a patient with nodular sclerosis Hodgkin lymphoma shows mild hypercellularity and eosinophilia (**a**), but no involvement by Hodgkin lymphoma; the aspirate smear shows increased eosinophil precursors and also an increased myeloid-to-erythroid ratio (**b**)

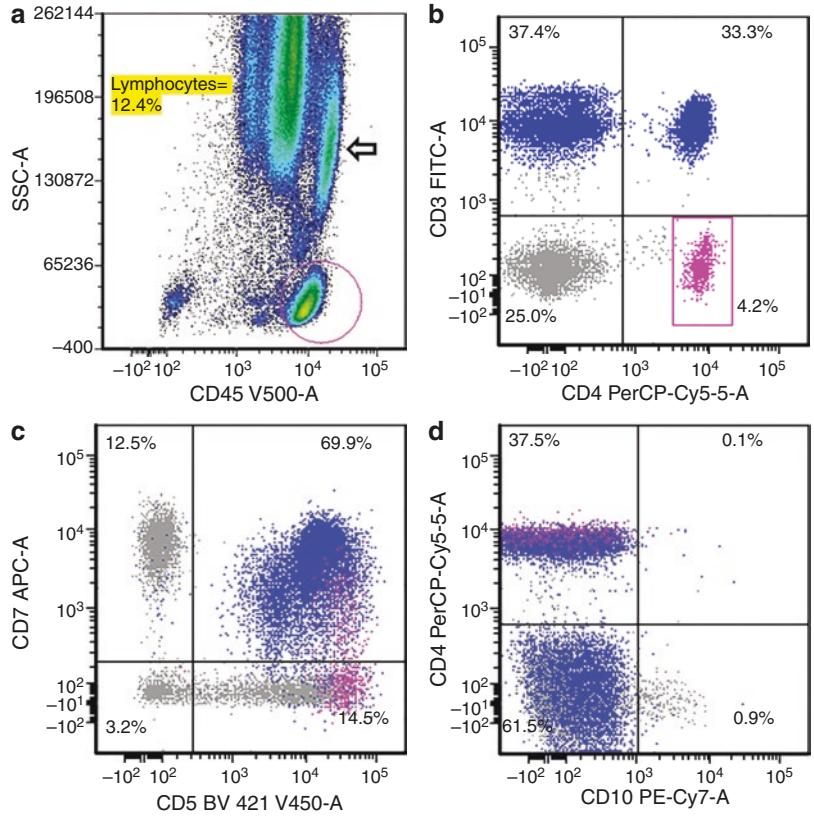

Fig. 6.2 Th2-type T cells detected by flow cytometry in a bone marrow of lymphocyte-variant hypereosinophilic syndrome. On CD45/SSC (**a**), the arrow points at markedly increased eosinophils that have bright CD45 expression. With CD3+ T cells highlighted in blue, the Th2-type T cells (pink, 4.2% of lymphocytes) are CD3 negative (**b**), showing a significant loss of CD7 but bright CD5 expression (**c**) and negativity for CD10 (**d**)

T-cell lymphoma (AITL). It is noteworthy that a significant number of AITL patients have a small number of circulating CD3-CD4+ T cells [16]. However, unlike AITL (Fig. 6.3), CD10 expression and T-follicular helper cell markers are not detected in L-HES, except in patients with L-HES transformed to AITL [17].

Chronic Eosinophilic Leukemia, Not Otherwise Specified (CEL, NOS) and Idiopathic Hypereosinophilic Syndrome (HES)

Chronic eosinophilic leukemia, not otherwise specified (CEL, NOS), is currently considered as a myeloproliferative neoplasm (MPN) that is associated with persistent eosinophilia in peripheral blood, bone marrow, or extramedullary tissues [18]. Organ damage may occur because of leukemic infiltration or because eosinophils release cytokines or enzymes. Since eosinophilia is not uncommon in most other MPNs (with the exception of CML), a thorough diagnostic workup is necessary to exclude all the other defined MPNs, including assessment of karyotype by conventional cytogenetics, fluorescence in situ hybridization (FISH) for specific translocations such as *BCR-ABL1*+ CML, and molecular genetic testing for mutations commonly detected in a variety of myeloid neoplasms (Table 6.2). Subsets of acute myeloid leukemia (AML) may also associate with eosinophilia; thus the blast count should be <20% in peripheral blood and bone marrow before CEL, NOS can be entertained as a diag-

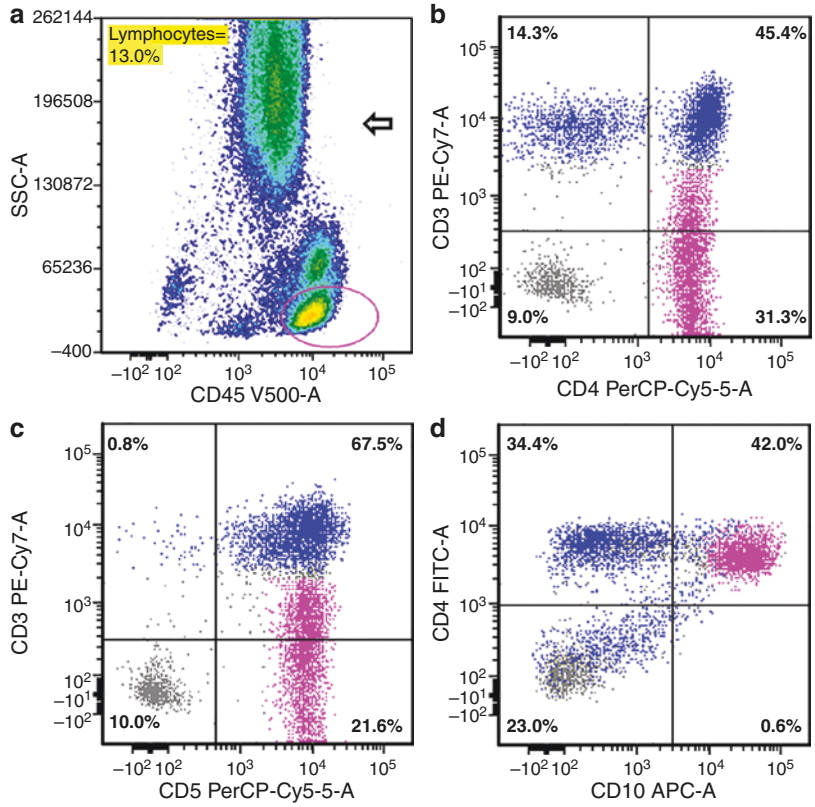

Fig. 6.3 A case of angioimmunoblastic T-cell lymphoma (AITL) in volving bone marrow. On CD45/SSC (**a**), the arrow shows no significant eosinophils in the bone marrow. With CD3+ T cells highlighted in blue, the lymphoma (pink) is CD4+ with significant CD3 loss (**b**), but retained CD5 expression (**c**), and aberrant CD10 expression (**d**). Aberrant expression of CD10 is often not observed in the Th2 cells in lymphocyte-variant HES unless it shows progression to AITL

nosis [18]. The criterion of neoplasia in CEL, NOS is fulfilled if clonality is demonstrated. One or two criteria that indicate clonality are accepted: (1) the presence of any clonal genetic abnormality after excluding those abnormalities that define other specific myeloid neoplasms, and (2) an increase of myeloblasts in peripheral blood or bone marrow (Table 6.2). The diagnosis of idiopathic hypereosinophilic syndrome (HES) is rendered when there is evidence of organ damage by eosinophils, but no evidence of clonality, and no cause is identified for eosinophilia that is at least 6 months in duration (Table 6.3) [19]. The distinction between CEL, NOS and idiopathic HES in cases with no genetic abnormality or increased blasts is difficult. Using NGS technology, somatic mutations associated with myeloid neoplasms have been detected in patients with eosinophilia, mostly involving genes that influence DNA methylation and histone modification. While such mutations can support a diagnosis of CEL, NOS, they have also been reported in some aging individuals lacking evidence of a myeloid neoplasm; thus, it is important to exclude all possible reactive causes of eosinophilia before considering a mutation as evidence of clonality to make a diagnosis of CEL, NOS [20, 21]. Morphology may play an important role in identifying the neoplastic nature of CEL, NOS and distinguishing it from idiopathic HES. Importantly, cases that resemble CEL, NOS or HES but have rearrangements of *PDGFRA, PDGRB, FGFR1,* or *PCM1-JAK2* [5, 6] are currently classified as separate entities, and not considered HES or CEL, NOS

Table 6.2 Diagnostic criteria for chronic eosinophilic leukemia, not otherwise specified

1. Eosinophilia (eosinophil count ≥1.5 × 10^9/L)
2. WHO criteria for *BCR-ABL1-positive* chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, primary myelofibrosis, chronic neutrophilic leukemia, chronic myelomonocytic leukemia, and *BCR-ABL1*-negative atypical chronic myeloid leukemia are not met
3. No rearrangement of *PDGFRA, PDGFRB*, or *FGFR1,* and no *PCM1-JAK2, ETV6-JAK2, or BCR-JAK2 fusion*
4. Blast cells constitute <20% of the cells in the peripheral blood and bone marrow, and inv(16)(p13.1q22), t(16;16)(p13.1;q22), t(8;21)(q22;q22.1), and other diagnostic features of acute myeloid leukemia are absent
5. There is a clonal cytogenetic or molecular genetic abnormality[a] or blast cells account for ≥2% of cells in the peripheral blood or ≥5% in the bone marrow

[a]Because some clonal molecular genetic abnormalities (e.g., mutations in *TET2, ASXLI,* and *DNMT3A*) can occur in a minority of elderly people in the absence of any apparent hematological abnormality, it is essential to exclude all possible causes of reactive eosinophilia before making this diagnosis solely on the basis of a molecular abnormality in an elderly person.

Table 6.3 Diagnostic criteria for idiopathic hypereosinophilic syndrome (HES)

1. Eosinophilia (eosinophil count ≥1.5 × 10^9/L) associated with tissue damage[a]
2. WHO criteria for *BCR-ABL1-positive* chronic myeloid leukemia, polycythemia vera, essential thrombocythemia, primary myelofibrosis, chronic neutrophilic leukemia, chronic myelomonocytic leukemia, and *BCR-ABL1*-negative atypical chronic myeloid leukemia are not met
3. No rearrangement of *PDGFRA, PDGFRB*, or *FGFR1,* and no *PCM1-JAK2, ETV6-JAK2, or BCR-JAK2 fusion*
4. Blast cells constitute <2% of the cells in the peripheral blood and <5% in bone marrow
5. There is no clonal cytogenetic or molecular genetic abnormality[b]
6. Rule out lymphocyte variant HES ([a]cytokine-producing, immunophenotypically aberrant T-cell population or Th2-type cells)

[a]In the absence of tissue damage, the preferred term is idiopathic hypereosinophilia
[b]Because some clonal molecular genetic abnormalities (e.g., mutations in *TET2, ASXLI,* and *DNMT3A*) can occur in a minority of elderly people in the absence of any apparent hematological abnormality, mutation results must be interpreted in the context of the overall clinical and bone marrow morphologic features

despite their common presentation with peripheral blood eosinophilia. Thus, CEL, NOS and HES are diagnoses of exclusion.

Clinical Features

CEL, NOS and HES are rare diseases [22]. Despite significant overlap between CEL, NOS and HES, recent studies [23] revealed some differences in clinical characteristics. There is a male predominance in both entities; however, the median age is in the fourth decade for idiopathic HES, and in the sixth decade for CEL, NOS. The median white blood cell count (WBC) is 25–30 × 10^9/L for CEL, NOS and 10–12 × 10^9/L for HES patients. HES patients more frequently present with symptoms associated with eosinophil activation, such as pulmonary, gastrointestinal, and rheumatologic manifestation, and humoral related symptoms such as angioedema, pruritus, and diarrhea [24]. Endomyocardial fibrosis and central nervous system (CNS) involvement are the most serious complications. Endomyocardial fibrosis may lead to restrictive cardiomyopathy, while fibrosis of the atrioventricular valves may lead to valvular regurgitation and thrombi [18]. Tissue damage is a result of eosinophilic infiltration and release of eosinophils' mediators. Spleen and liver infiltration occurs in 30–50% of cases [19]. On the other hand, CEL, NOS patients frequently present with constitutional symptoms, anemia, thrombocytopenia, or thrombocytosis (> 450 × 10^9/L), and symptoms related to those cytopenias, frequent organomegaly, elevated lactate dehydrogenase levels, and less frequently with allergy, urticaria/rash, edema, asthma, myalgia/arthralgia, or eosinophil-mediated organ injury.

Patients with CEL, NOS who have either a karyotypic abnormality or increased blasts have a median overall survival of 12–18 months. The outcome of patients with CEL, NOS based on a molecular genetic abnormality identified by NGS is uncertain, but one recent study found that the clinicopathological features and survival outcomes of these patients were intermediate between CEL, NOS with an abnormal karyotype and idiopathic HES with no mutations [23, 25]. Most CEL, NOS patients die of either AML progression or bone marrow failure. In contrast, the survival of HES patients is variable, and overall survival is 80% at 5 years [23, 26]. The most common causes of death of patients with HES are heart failure or brain infarct due to eosinophilic infiltration or release of eosinophil mediators. The heterogeneity of outcomes of patients with idiopathic HES is likely due to the difficulty or limitations in proving clonality; as a result, this entity likely includes true neoplastic disease as well as reactive/secondary HE whose cause cannot be identified.

Morphology

The peripheral blood (PB) in CEL, NOS/HES patients shows mature eosinophils, $\geq 1.5 \times 10^9$/L; less common are eosinophils at the myelocyte and promyelocyte stages of maturation. The absolute eosinophil count can be higher than 100×10^9/L and the percentage of eosinophils can be more than 70% [24]. On peripheral blood or bone marrow (BM) aspiration smears, it is common to observe abnormalities in the eosinophils such as large forms, decreased granularity, cytoplasmic vacuoles, and nuclear hypo- or hypersegmentation (Fig. 6.4). Although these features are also seen in reactive processes and HES [27], they are much more frequently observed in CEL, NOS. The normal range of eosinophils in bone marrow aspirate smears is 3–6% of all marrow cells, and elevations can range from 7 to 57% [24]. Criteria to define hypereosinophilia in the BM biopsy or other tissue specimens have been suggested [2], and include the following: (1) ≥20% of marrow cells are eosinophils, (2) an experienced pathologist deems there are too many eosinophils in the biopsied tissue, or (3) in the absence of obvious eosinophilia, immunohistochemistry for major basic protein (MBP) demonstrates extensive extracellular deposition of eosinophilic granules, i.e., evidence of eosinophilic degranulation [2].

Although not acknowledged in the current WHO classification, there are PB and BM morphological features that can assist in the separation of CEL, NOS from HES. The bone marrow of HES is usually normal or slightly hypercellular mainly due to

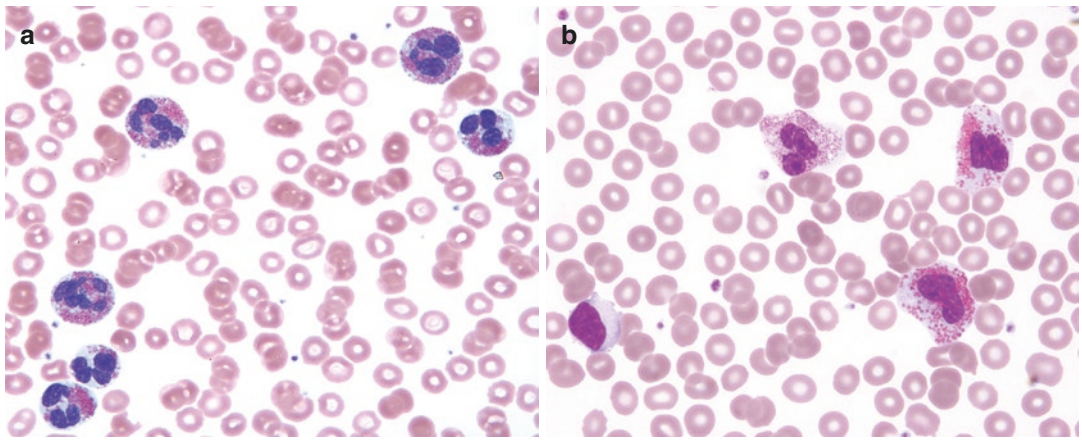

Fig. 6.4 Peripheral blood from a patient with chronic eosinophilic leukemia, not otherwise specified (CEL, NOS), displays increased eosinophils, some with hypogranulation or uneven granulation, nuclear hypersegmentation (**a**) and hyposegmentation (**b**)

increased eosinophils (Fig. 6.5). Eosinophil maturation is orderly, and most cells are mature eosinophils, often morphologically normal. Charcot-Leyden crystals may be seen. Megakaryocytes are morphologically unremarkable. Mild dyserythropoiesis may be seen after hydroxyurea treatment, but marked dyserythropoiesis is uncommon. There is often no dysgranulopoiesis. Myeloblasts are < 5%, by definition. Mild marrow fibrosis (up to MF grade 1 of 3) can be seen in some cases, while fibrosis is a common finding in extramedullary sites [28].

In contrast, CEL, NOS often shows marked hypercellularity, not only due to the increase of eosinophils, but also due to increased granulocytes. An increase of histiocytes, stromal cells, and vessels may also contribute to the overall appearance of increased cellularity. Blasts are usually less than 2% in the blood and less than 5% in the marrow, but can be elevated, which is one of the criteria to distinguish between CEL, NOS and idiopathic HES. Megakaryocytes can be increased, or decreased, and often exhibit abnormal features. Most of the megakaryocytes are not typical of those seen in other myeloproliferative neoplasms (large, hyperlobulated forms with clustering); instead, they more frequently show features of myelodysplasia (MDS)-like or are mixed MDS-like and MPN-like megakaryocytes (Fig. 6.6). Dysgranulopoiesis and or dyserythropoiesis may be present in some cases. Eosinophil abnormalities, including marked hypogranulation, abnormal uneven granulation, abnormal nuclear hypo- or hyperlobulation, and markedly increased immature forms, are frequently observed in CEL, NOS. Myelofibrosis grade 1 of 3 (MF-1) fibrosis can be seen in both CEL, NOS and HES, but MF-2 and MF-3 fibrosis are more common in CEL, NOS than in idiopathic HES. Mast cells may be increased in rare cases; however, they are often scattered and do not form large aggregates; the presence of aggregates of ≥15 mast cells is suggestive of systemic mastocytosis associated with eosinophilia.

Immunophenotype

The CD34+ myeloblasts, although not increased, often exhibit immunophenotypical aberrancy in CEL, NOS, but not in true reactive HE or HES. Frequent changes observed are decreased CD38 and HLA-DR expression and increased CD123, CD117, and CD13 expression. Immunophenotypical study for T cells and mast cells should be performed in every patient who undergoes workup for eosinophilia. Finding an aberrant T-cell population or Th2 type T cells helps to identify L-HES or HE driven by an underlying T-cell lymphoproliferative process, whereas finding a small number of mast cells with aberrant expression of CD25

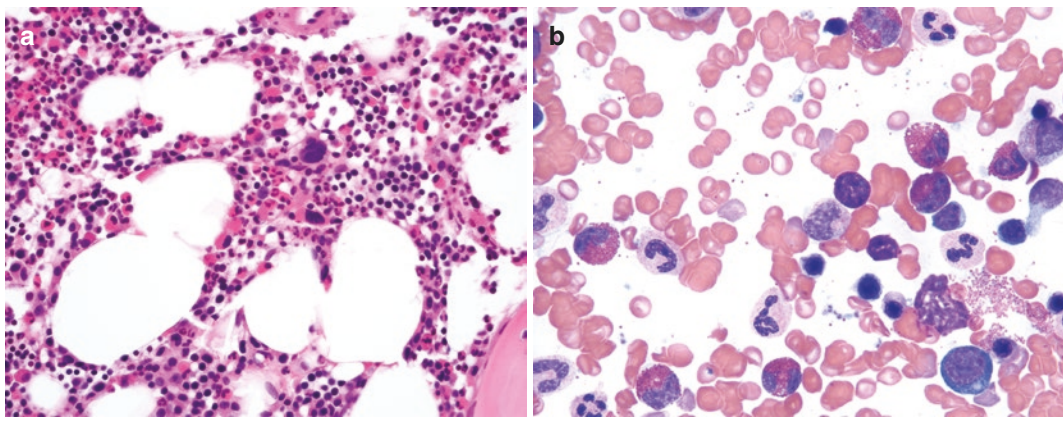

Fig. 6.5 A case of idiopathic hypereosinophilic syndrome (HES) with no clonal cytogenetic abnormality or mutations in a 45-year-old man with a long history of skin rash and upper respiratory symptoms. The bone marrow biopsy shows normal to slight hypercellularity with increased eosinophils and normal-appearing megkakaryocytes (**a**); bone marrow aspirate smear shows increased eosinophils and their precursors, which are morphologically unremarkable (**b**)

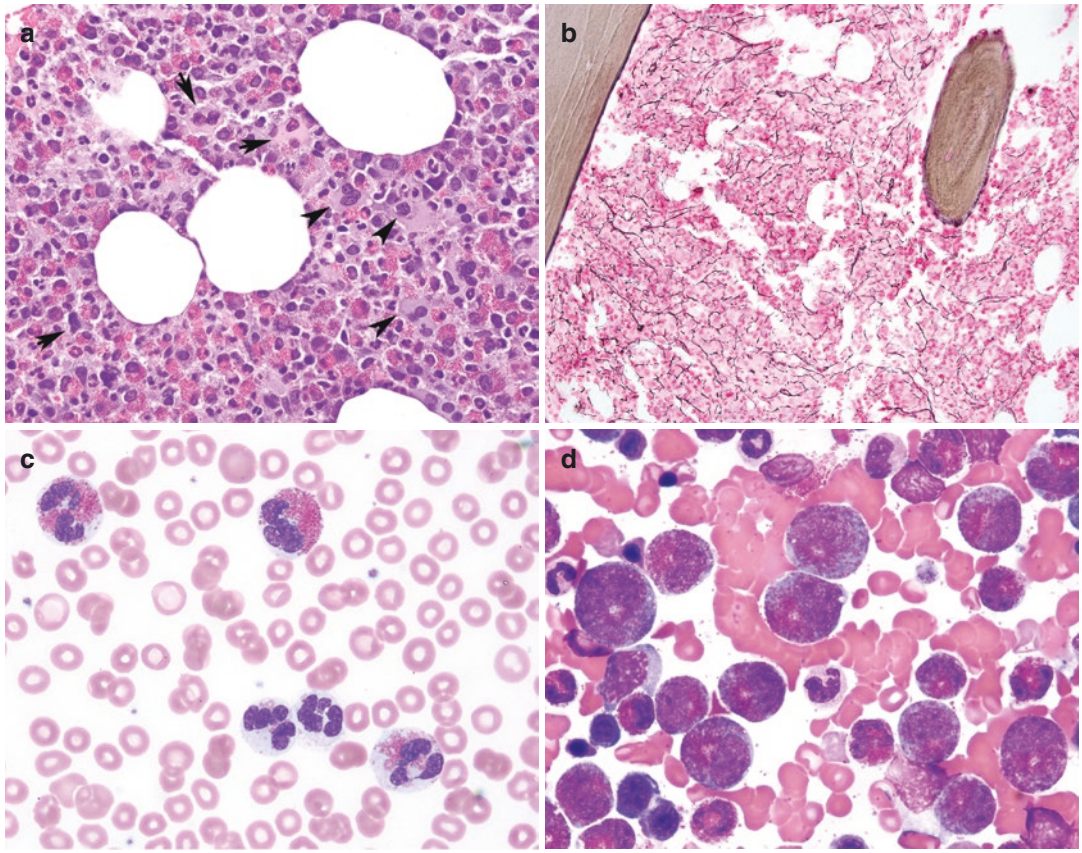

Fig. 6.6 Chronic eosinophilic leukemia, not otherwise specified (CEL, NOS). The hypercellular bone marrow contains frequent dysplastic megakaryocytes (**a**, arrows). Mild fibrosis is very common, but not specific (**b**, reticulin stain); the peripheral blood shows dysplastic eosinophils with reduced granules and abnormal nuclear hypersegmentation (**c**); the bone marrow aspirate smear shows many eosinophil precursors. These morphological findings highly suggest a neoplastic process even in the absence of clonal cytogenetic abnormality or increased blasts

may indicate a clonal process. However, the significance of the latter needs further study.

Cytogenetics and Molecular Genetics

There are no specific cytogenetic or molecular abnormalities that define CEL, NOS. When patients with hypereosinophilia are found to harbor certain specific or non-random cytogenetic abnormalities (see below), they are assigned to other disease categories.

The distinction between CEL, NOS and idiopathic HES remains challenging, even with the advent of NGS. The presence of a clonal karyotypic abnormality supports a diagnosis of CEL, NOS according to WHO criteria. However, as in the other MPNs, karyotypic abnormalities only occur in a subset of cases. Furthermore, there is no unique karyotypic abnormality in CEL, NOS; complex karyotype and trisomy 8 are the most common aberrancies. Mutations in genes frequently associated with MPNs, such as *JAK2, MPL, CALR, RAS,* and *KIT*, are very infrequent in CEL, NOS. A recent study applied NGS for cases of hypereosinophilia and identified mutations in 25–30% of cases that would otherwise be called idiopathic HES [23] (i.e., have a normal karyotype and no increase in blasts). The mutations were mostly in genes related to DNA methylation and histone modifications such as *ASXL1, TET2, EZH2,* and *DNMT3A* [23]. However, while these mutational data provide evidence of clonality, careful interpretation needs to consider the caveat

of prevalent clonal hematopoiesis of indeterminate potential ("CHIP") in the elderly population [20, 21]. Moreover, the detection of mutations by NGS usually relies on a testing panel of mutations, which may vary in the number of genes sequenced as well as the depth of sequencing that is variable among institutions.

Differential Diagnosis

A summary of the entities in the differential diagnosis of CEL, NOS and idiopathic HES is shown in Table 6.4. The list includes reactive and neoplastic processes that present with eosinophilia $\geq 1.5 \times 10^9$/L of at least 6 months in duration, or a shorter time if clonality can be demonstrated up front. An alternative to the 6-month observation period that has been proposed is the concept of persistent eosinophilia, which means that the eosinophilia has to be detected in two or more samples taken at least 4 weeks apart [2]. The optimal diagnostic approach requires a systematic evaluation of possible disorders in the differential diagnosis, and should include thorough clinical history, physical examination, laboratory data (LDH, tryptase, IgE, etc.), peripheral blood and bone marrow examination, flow cytometry immunophenotype, cytogenetics and its companion FISH (*BCR-ABL1, PDGFRA, PDGFRB, FGFR1, PCM1-JAK2*), and ideally molecular diagnostics testing that includes targeted assessment of translocations associated with myeloid neoplasm as well as evaluation for mutations of genes associated with myeloid neoplasms. A rationale for the assessment of variables and diagnostic hints are shown in Table 6.5.

Reactive eosinophilia can often be suspected from a detailed clinical history that discloses allergies, use of medications associated with eosinophilia, or travel to endemic areas of parasitic infections. When neoplastic processes are considered, biopsies of any involved tissue should be considered. If there is no localized or defined lesion, bone marrow aspirate and biopsy are recommended. Eosinophilia secondary to lymphomas such as Hodgkin lymphoma or non-Hodgkin lymphoma may be associated with marrow hypercellularity, whether or not the lymphoma involves the marrow (Fig. 6.7). A detailed clinical history, physical examination, and imaging studies may reveal lymphadenopathy, hepatomegaly, splenomegaly, or extranodal involvement in patients with lymphoma.

Eosinophilia associated with other myeloid neoplasms is suspected in bone marrow specimens that show hypercellularity, increased number of blasts, dysplastic features, or moderate-to-marked marrow fibrosis. Clonal eosinophilia has been documented in patients with chronic myeloid leukemia (CML), chronic myelomonocytic leukemia (CMML), MDS, MPN, and MDS/MPN overlap syndromes and in patients with systemic mastocytosis. CML, *BCR-ABL1*+ (Fig. 6.8) commonly has eosinophilia, while this is less common in the classic MPN with mutations in *JAK2*, *MPL*, and *CALR*. Eosinophilia in MDS is associated with a poor prognosis and an abnormal karyotype [29]. In the presence of $\geq 1 \times 10^9$/L monocytes in the peripheral blood, a diagnosis of CMML supersedes a diagnosis of CEL, NOS (Fig. 6.9). AML with inv(16) or t(16;16)(p13.1;q22)/*CBFB-MYH11* presents characteristically as acute myelomonocytic leukemia with bone marrow (but typically not peripheral blood) eosinophilia, including abnormal-appearing eosinophils with mixed eosinophilic and basophilic granules (M4eo). AML with t(8;21)(q22;q22)/*RUNX1-RUNX1T1* also can present with significantly increased eosinophils. In all of the above myeloid neoplasms, the eosinophils are also clonal (Fig. 6.10). Approximately 20–30% of patients with systemic mastocytosis present with eosinophilia (Fig. 6.11) [30, 31], and the presence of eosinophilia does not appear to confer prognostic significance [32]. However, systemic mastocytosis with lymphadenopathy and eosinophilia appears to be clinically aggressive. Finally, B-acute lymphoblastic leukemia (B-ALL) with t(5;14)(q31;q32);*IL3-IGH* is a rare B-ALL subtype associated with marked peripheral blood eosinophilia, often lacking circulating blasts and thus potentially mimicking HES or CEL, NOS. Unlike the AML entities associated with eosinophilia discussed above, in B-ALL with t(5;14) the eosinophils are not part of the leukemic clone.

Table 6.4 Differential diagnosis of clonal and reactive eosinophilia

Diagnosis	Example	Chromosomal abnormality	Molecular genetic abnormality/ abnormalities	Main compartment: (1) blood; (2) bone marrow; (3) tissue
HES		None	None[a]	1, 2, 3
CEL, NOS		Clonal but no unique abnormalities[b]	Not defined; most common are *EZH2, SETBP1, ASXL1, TET2*	1, 2, 3
AML	Inv(16)	inv(16)(p13.1q22) or t(16;16)(p13.1;q22)	*CBFB-MYH11; KIT* (subset)	1, 2
CML	Chronic phase	t(9;22)(q34;q11.2); *BCR-ABL1*	NA	1, 2
MPN	PMF	Up to 30%, no unique abnormalities	*JAK2, MPL,* or *CALR*	1, 2
CMML		About 30%, +8, -7 or del(7q)	*ASXL1, TET2, SRSF2, CBL, RAS*	1, 2
PDGFRA		*CHIC2* microdeletion (requires FISH)	*PDGFRA-FIP1L1*	1, 2, 3
PDGFRB		t(5;12)(q31 ~ q33;p12)	*PDGFRB-ETV6* or > 20 other partners	1, 2, 3
FGFR1		t(8;13)([11/2q12/1); t(8;22)(p11.2;q11.2), and 12 other rearrangements	*ZMYM2/FGFR1; BCR/FGFR1* or 12 other partners	1, 2, 3
Systemic mastocytosis		Normal or abnormal; no unique abnormalities	*KIT D816V*	1, 2
Drug	Penicillin	None	None	1
Parasite	Strongyloides	None	None	1, 3
Allergy	Pollen	None	None	1
Autoimmune	Lupus	None	None	1, 3
ALL		Various, according to the subtype	Various, according to the subtype	1, 2
PTCL	AITL	Various, according to the subtype	*TET2, DNMT3A*	1, 2, 3
HL		NA	NA	1, 2, 3
Solid tumor	Squamous cell carcinoma of head and neck	NA	NA	1, 3
Lymphocyte variant hypereosinophilic syndrome		None	*TRB* and/or *TRG* gene rearrangement *STAT3* mutation in sorted Th2 type T cells	1, 2, 3

Abbreviations: *CEL, NOS* chronic eosinophilic leukemia, not otherwise specified, *CML* chronic myeloid leukemia, *CMML* chronic myelomonocytic leukemia, *HES* hypereosinophilic syndrome, *HL* Hodgkin lymphoma, *PTCL* peripheral T-cell lymphoma.

[a]Be aware of clonal hematopoiesis of indeterminate potential (CHIP)

[b]Can be normal if morphologically abnormal with or without a somatic mutation

Both idiopathic HE and idiopathic HES have persistent hypereosinophilia ($\geq 1.5 \times 10^9$/L) and lack any clonal genetic abnormality or increased blasts. Idiopathic HE fulfills all HES criteria, but there is no organ damage or symptoms related to the eosinophilia. This latter definition does not consider the possibility that the longer the disease persists, the higher the likelihood of organ damage, and thus idiopathic HE and idiopathic HES may be part of the spectrum of the same disease.

Table 6.5 Rationale for a comprehensive diagnostic evaluation of patients with eosinophilia and possible associations

1. Clinical history	***Possible Associations***
Time of onset of disease	HES requires > 6 months of symptoms; recent onset of symptoms may suggest a reactive process
Exposure to medications	Hypersensitivity
Atopy	Allergy
Travel to underdeveloped countries	Parasites, infectious diseases
2. Physical examination	
Skin rash	Allergy
Hepatomegaly	MPN or lymphoma
Splenomegaly	MPN or lymphoma
Lymphadenopathy	Hodgkin or non-Hodgkin lymphoma
Lung abnormalities	HES with pulmonary eosinophilic disease
3. Complete blood count	
Anemia	MDS, MDS/MPN
Leukocytosis	MPN, MDS/MPN
Lymphocytosis	Non-Hodgkin lymphoma
Monocytosis	CMML
Thrombocytosis	MPN, MDS/MPN
Thrombocytopenia	MDS, MDS/MPN, MPN
≥20% blasts	AML or ALL
≥2% but < 20% blasts	CEL, NOS, MDS, MPN, MDS/MPN, AML, ALL.
4. Bone marrow examination	
Hypercellularity	MPN, MDS, MDS/MPN
Dysplastic features	MDS, MDS/MPN, or CEL, NOS
Abnormal lymphocytes	Lymphoma
Lymphoid aggregates	Lymphoma
≥20% blasts	AML or ALL
≥ 5% but <20% blasts	CEL, NOS, MDS, MPN, MDS/MPN, ALL, other myeloid neoplasms
Abnormal mast cells	Systemic mastocytosis
Hodgkin or RS cells	Hodgkin lymphoma
Abnormal Langerhans cells	Langerhans cell histiocytosis
5. Flow cytometry immunophenotype	
Clonal B cells	B-cell lymphoma
T-cell aberrancies	Lymphocyte-variant HES or T-cell lymphoma
Blast abnormalities	CEL, NOS, MDS, MPN, MDS/MPN, ALL, other myeloid neoplasms
6. Cytogenetic analysis: Conventional karyotype and FISH	
t(9;22)(q34;q11.2)	Chronic myeloid leukemia
PDGFRA-FIP1L1	*PDGFRA* rearrangement
t(5;12)(q31–35;p13)	*PDGFRB* rearrangement
t(8;13)(p11;q12)	*FGFR1* rearrangement
t(8;21)(q21;q21)	AML
inv(16)(p13.1q22) or t(16;16)(p13.1;q22)	AML
t(5;14)(q31;q32)	B-lymphoblastic leukemia

Table 6.5 (continued)

7. Molecular genetic testing[a]	
BCR-ABL1	CML
CBFB-MYH1	AML
JAK2 V617F, MPL, or CALR mutations	MPN: ET, PMF, or PV
TRB, TRG or IGH gene rearrangements	Non-Hodgkin lymphoma, ALL, or lymphocyte-variant HES

Abbreviations: *AML* acute myeloid leukemia, *aCML* atypical chronic myeloid leukemia, *CEL* chronic eosinophilic leukemia, *CML* chronic myeloid leukemia, *CMML* chronic myelomonocytic leukemia, *ET* essential thrombocythemia, *HES* hypereosinophilic syndrome, MDS/MPN myelodysplastic/myeloproliferative neoplasm, *MPN* myeloproliferative neoplasm, *PMF* primary myelofibrosis, *PV* polycythemia vera

[a]Includes common translocations, next-generation sequencing panel, specific mutations of myeloid disorders

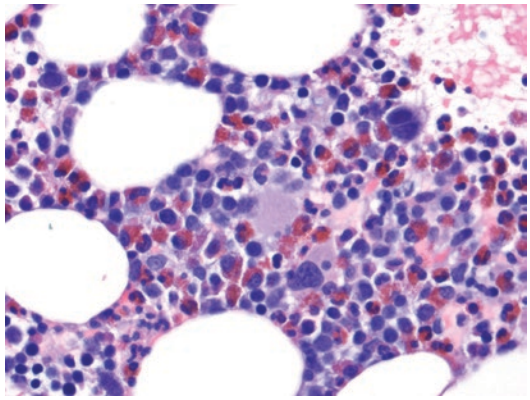

Fig. 6.7 Bone marrow clot specimen from a patient with peripheral T-cell lymphoma shows hypercellularity and increased eosinophils; however the marrow is not involved by lymphoma

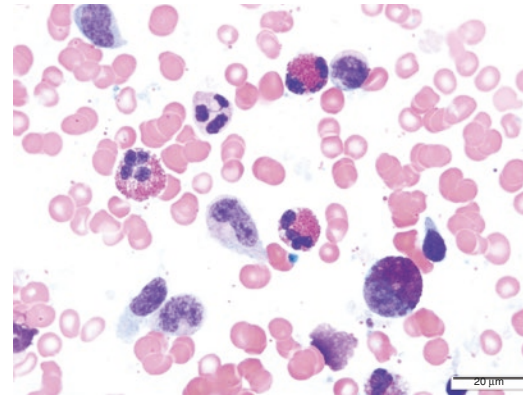

Fig. 6.9 Peripheral blood from a patient with hypereosinophilia displays increased eosinophils; however there is also an increase of monocytes and dysgranulopoiesis, which fits better with a diagnosis of chronic myelomonocytic leukemia

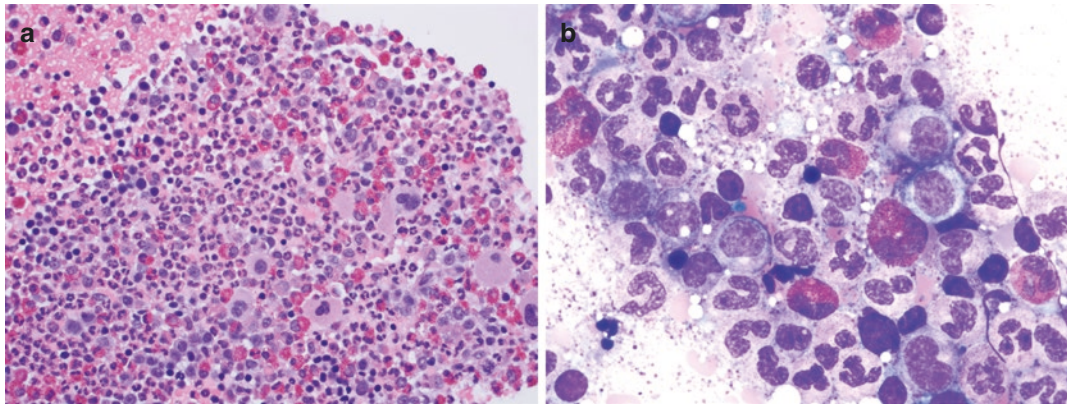

Fig. 6.8 Bone marrow section from a patient with *BCR-ABL1*+ chronic myeloid leukemia (CML) shows hypercellularity, a predominance of neutrophils with numerous eosinophils, and small, hypolobated megakaryocytes (**a**). The aspirate smear shows many eosinophils and neutrophils, which are morphologically unremarkable (**b**)

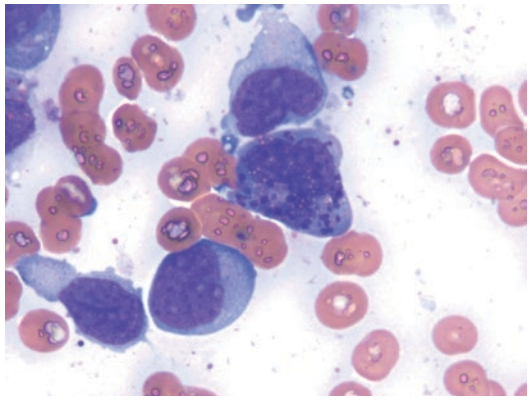

Fig. 6.10 Bone marrow aspirate of acute myeloid leukemia with inv(16)(p13.1q22) shows abnormal eosinophils with large, basophilic and eosinophilic granules

Myeloid/Lymphoid Neoplasms with Eosinophilia and Rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2*

Myeloid/Lymphoid Neoplasms with *PDGFRA* Rearrangement

Clinical Features

The most common disease within this category of neoplasms results from rearrangements of *PDGFRA*. The most common fusion gene partner for *PDGFRA* is *FIP1L1* [33]. It has been estimated that 10–20% of patients with unexplained eosinophilia in developed countries harbor *PDGFRA* rearrangements. In addition to eosinophilia (present in 70% of patients [34]), patients often have splenomegaly (about 60%), anemia, thrombocytopenia, and neutrophilia. The disease at presentation may resemble CEL/idiopathic HES, systemic mastocytosis with eosinophila, CML or atypical chronic myeloid leukemia (aCML), and *BCR-ABL1*-negative lymphoblastic leukemia (T as well as B); rare cases may present as AML [35, 36]. Patients presenting with AML or T-lymphoblastic lymphoma (T-LBL) often have a pre-phase or coexisting PB eosinophilia. After diagnosis, progression to acute lymphoblastic leukemia or AML may also occur. Symptoms related to the release of eosinophilic granules occur and commonly patients exhibit skin rash and erythema, and less frequently pulmonary, gastrointestinal tract, and/or cardiac manifestations. Endomyocardial fibrosis and restrictive cardiomyopathy may occur in patients who have this disease for a prolonged time interval.

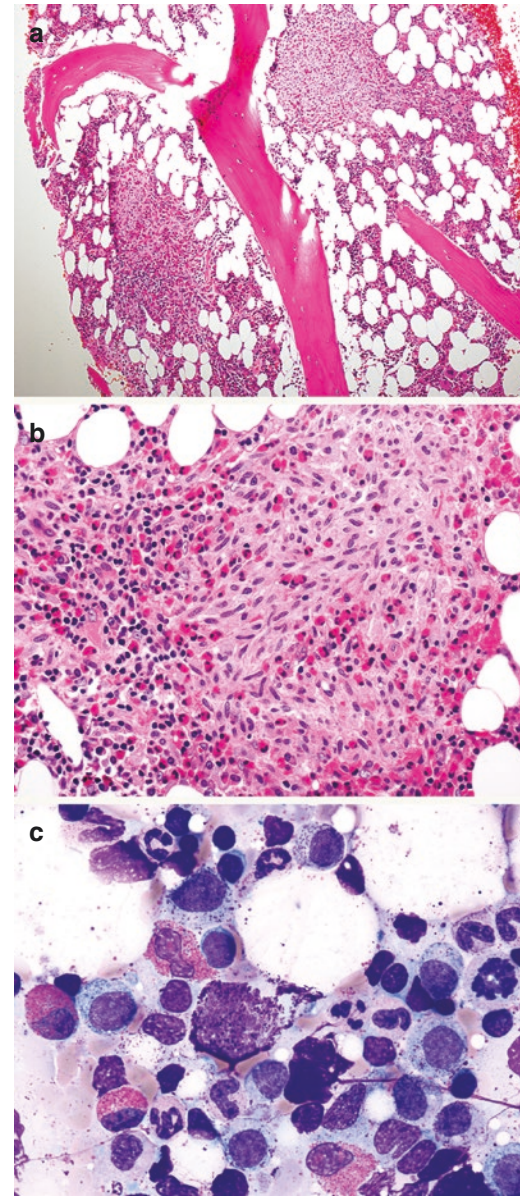

Fig. 6.11 Bone marrow from a patient with systemic mastocytosis and eosinophilia. The bone marrow biopsy shows multiple large mast cell aggregates (**a**). High magnification shows spindled mast cells admixed with numerous eosinophils (**b**). The bone marrow aspirate shows increased mast cells with reduced granules and normal-appearing eosinophils and their precursors (**c**)

Almost all patients with the *FIP1L1-PDGFRA* fusion are sensitive to tyrosine kinase inhibitors such as imatinib [37–39]. Primary or secondary resis-

tance to imatinib is unusual in these patients [40, 41]. Discontinuation of therapy can lead to relapse of disease in a subset of patients [42]. Resistance involves the T674I mutation within the ATP-binding domain of *PDGFRA*. Rearrangements of *PDGFRA* with other gene partners also respond to tyrosine kinase inhibitor. Additional chemotherapy may be needed when patients present as acute leukemia.

Morphology

The BM is usually hypercellular with increased eosinophils, including eosinophilic precursors (Fig. 6.12). Dysplastic features of granulocytes and or erythroids or an excess of blasts are uncommon. Megakaryocyte abnormalities may be present, resembling megakaryocytes in MDS or MPN or mixed MDS/MPN features or showing some larger megakaryocytes with separated nuclear lobes. Spindled mast cells are often observed, but they are usually scattered and do not form aggregates. Mast cells show frequent aberrant expression of CD25 (around 60% of cases). Myelofibrosis of at least grade 1 of 3 is very common, and is found in more than two-thirds of patients. Of note, the absolute and relative numbers of eosinophils in BM or peripheral blood are not useful to differentiate *PDGFRA*-rearranged from non-clonal eosinophilia. However, morphological abnormalities of eosinophils are frequently observed in cases with PDGFRA rearrangement. These abnormalities include hypogranulation, degranulation, or uneven granulation, cytoplasmic vacuolation, nuclear hyper- or hyposegmentation, large size and many immature forms, often more pronounced in PB than BM.

Immunophenotype

The immunophenotype of CD34+ blasts, as well as the presence of scattered (not aggregated) spin-

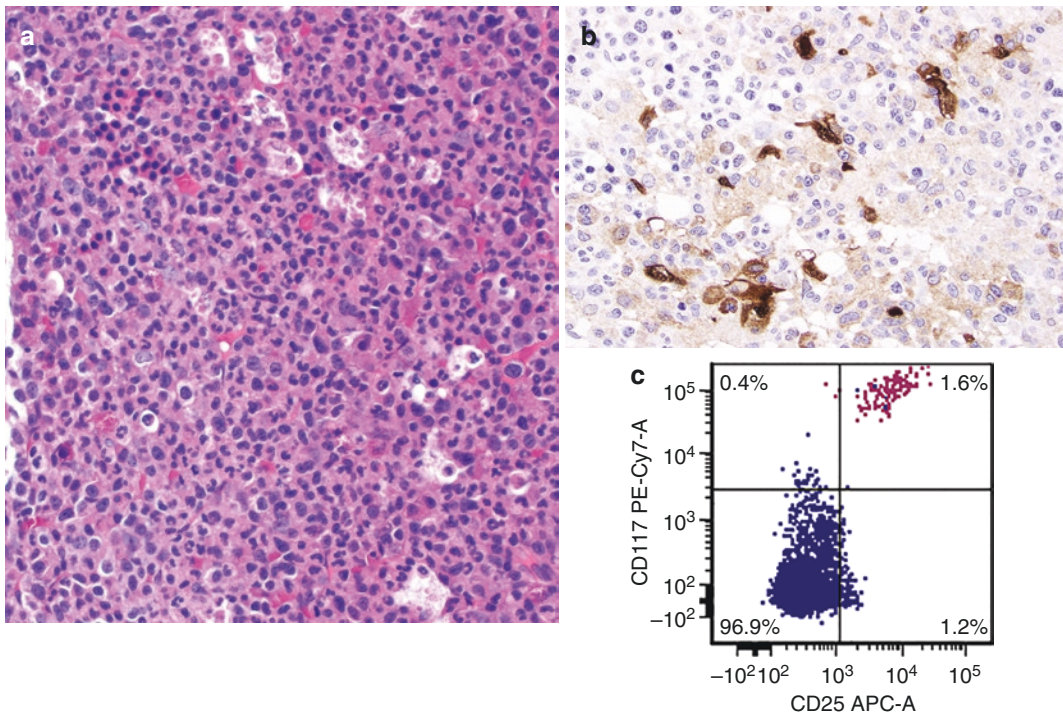

Fig. 6.12 Bone marrow clot specimen shows hypercellularity and eosinophilia in a patient with *FIP1L1-PDGFRA* gene fusion (**a**); spindled mast cells are highlighted by tryptase immunohistochemistry stain (**b**), but the mast cells are scattered and do not form large aggregates; they often show aberrant CD25 and CD117 co-expression as shown by flow cytometry study in this case (**c**)

dled CD25+ mast cells, can provide useful clues to raise the suspicion of a neoplastic process.

Cytogenetics and Molecular Genetics

The interstitial deletion of 800 kb that leads to *FIP1L1-PDGFRA* fusion is undetectable by conventional cytogenetics, a technique with a level of resolution estimated at only 10–15 Mb. Therefore, conventional cytogenetics often shows a diploid karyotype in these cases. Routine detection of the interstitial deletion in clinical practice is best achieved by interphase or metaphase FISH. Since the *CHIC2* gene is contained in the deleted region, the FISH test to detect *FIP1L1-PDGFRA* gene fusion is often referred to as "*CHIC2* deletion." The fusion can be detected in peripheral blood, BM smears, or involved tissues. It has been shown in human CD34+ cells that expression of FIP1L1-PDGFRA fusion protein induces cell proliferation and differentiation toward eosinophilic lineage in the absence of cytokines; these effects are mediated by the activation of NF-κB and STAT5 [43].

Myeloid/Lymphoid Neoplasms with *PDGFRB* Rearrangement

Clinical Features

Myeloid/lymphoid neoplasms with *PDGFRB* rearrangement are rare, with a reported incidence of 1.8% of all MPN [3, 44]. Most affected patients are adult men, with a median age in the fifth decade of life [44–46]. Patients usually present with anemia, leukocytosis, monocytosis, eosinophilia, and splenomegaly [44, 47]. Eosinophilia is common, but not invariably present in cases with *PDGFRB* fusion genes. These neoplasms at the time of presentation most often resemble chronic myelomonocytic leukemia (CMML), CEL, NOS, atypical CML, *BCR-ABL1* negative, or occasionally MDS. Presentation as AML is uncommon. T-lymphoblastic leukemia has been occasionally reported at presentation or progression, but cases of *PDGFRB* rearrangement presenting as B-ALL are best considered as *BCR-ABL1*-like B-ALL [48].

Morphology

A significant proportion of patients present as CMML with eosinophila or hypereosinophilia with monocytosis (Fig. 6.13). Some patients may show hematological features of atypical chronic myeloid leukemia (aCML) with increased eosinophils, or CEL, NOS or other MPN with increased eosinophils. If any of the above morphological entities show significant eosinophils in PB and or bone marrow, not necessarily $\geq 1.5 \times 10^9$/L, *PDGFRB* rearrangement should be worked up.

Immunophenotype

Similar to cases with *PDGFRA* rearrangements, immunophenotypic studies for CD34+ myeloblasts, T cells, and mast cells are highly suggested.

Cytogenetics and Molecular Genetics

The rearrangements of *PDGFRB* are usually not cryptic and thus conventional cytogenetics is the best approach to identify involvement of *PDGFRB* at chromosome 5q33. The breakpoints can be detected using FISH with break-apart probes to the *PDGFRB* locus; however, this approach does not identify the gene partner. More than 20 different partners of *PDGFRB* have been identified. These gene fusions lead to the creation of chimeric proteins that have enhanced tyrosine kinase activity. The most common translocation is t(5;12)(q33;p13)/*ETV6-PDGFRB*. As a consequence of the t(5;12), the extracellular ligand-binding domain of PDGFRB is replaced by the pointed domain of ETV6, resulting in enforced PDGFRB dimerization by the PNT domain. The chimeric protein ETV6-PDGFRB stimulates hematopoietic cell proliferation leading to a MPN and eosinophilic proliferation [43, 49]. These effects are mediated by STAT5, NF-κB, and ERK signaling activation.

Most hematolymphoid neoplasms associated with translocations of *PDGFRB* are sensitive to tyrosine kinase inhibitors. Patients with these neoplasms respond to imatinib therapy with excellent hematopoietic and molecular responses. Primary or secondary resistance to imatinib is uncommon [45, 50]. Additional therapy is needed when patients present with or develop acute leukemia.

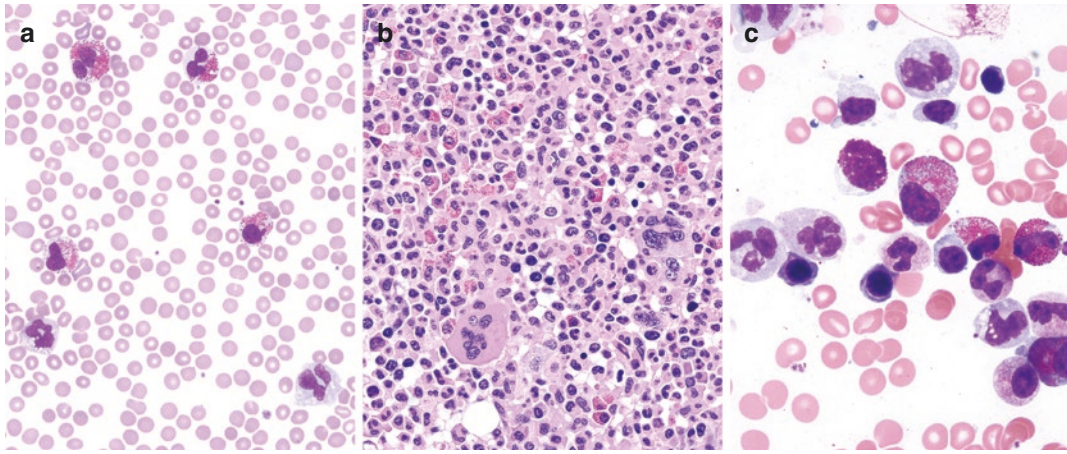

Fig. 6.13 Hypereosinophilia associated with monocytosis and *PDGFRB* gene fusion. Peripheral blood shows eosinophilia, and monocytosis (**a**); the bone marrow biopsy shows hypercellularity with eosinophilia and megakaryocytes are pleomorphic with separated nuclear lobes (**b**); the bone marrow aspirate shows increased and abnormal monocytes and eosinophilia (**c**)

Myeloid/Lymphoid Neoplasms with *FGFR1* Rearrangement

Clinical Features

Myeloid/lymphoid neoplasms associated with *FGFR1* rearrangement are rare and aggressive tumors. This group of diseases equally affects males and females with a reported male:female ratio of 1.2 to 1, and the median age is 44 years [7]. Patients usually present with systemic symptoms including fatigue, night sweats, weight loss, and fever. Lymphadenopathy and hepatosplenomegaly are common. Unlike myeloid/lymphoid neoplasms associated with *PDGFRA and PDGFRB*, *FGFR1*-rearranged neoplasms are usually not responsive to first-generation tyrosine kinase inhibitor (imatinib) therapy. Prognosis is poor and aggressive chemotherapy and often hematopoietic stem cell transplantation are often needed.

Morphology

The bone marrow is usually hypercellular with eosinophilia and other features of MPN, such as a hypercellularity (Fig. 6.14), increased megakaryocytes with clustering, myeloid hyperplasia, increased stromal cells and vessels, and fibrosis. These neoplasms frequently progress to AML or lymphoblastic leukemia. Most patients with neoplasms associated with t(8;13)(p11;q12) (see below) develop T-lymphoblastic lymphoma or a T/myeloid neoplasm involving nodal or extranodal sites. Most tumors at nodal or extranodal sites have a predominant T-lymphoblastic component admixed with scattered or perivascular myeloid blasts [51–53] (Fig. 6.15).

Immunophenotype

In addition to the study of T cells and mast cells recommended to all patients with hypereosinophilia, immunophenotypical studies should pay specific attention to the blast immunophenotype in such cases due to a frequent coexistence of lymphoblasts and myeloblasts. Especially in lymph node, it often shows a mixture of both types of blasts (Fig. 6.15).

Cytogenetics and Molecular Genetics

An association between the t(8;13) and the triad of T-lymphoblastic lymphoma/leukemia, eosinophilia, and myeloid malignancy was first reported in 1992 [54]. Since most neoplasms with rearrangements of *FGFR1* present with demonstrable translocations, conventional cytogenetics is the best technique to identify involvement of *FGFR1* at 8p11. The t(8;13) is the most common rearrangement observed in this disease (Fig. 6.16), in which the partner of *FGFR1* is *ZMYM2*. Patients with the *ZMYM2-FGFR1* rearrangement most often present

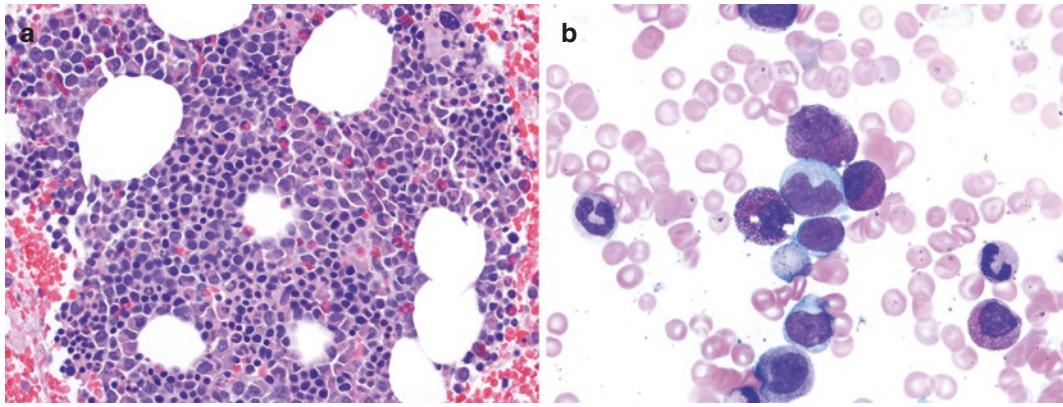

Fig. 6.14 Bone marrow clot specimen shows hypercellularity and eosinophilia, in a patient with myeloproliferative neoplasm with *FGFR1* rearrangement (**a**). The bone marrow aspirate smear shows myeloid cells admixed with small blasts, confirmed to be T lymphoblasts (**b**)

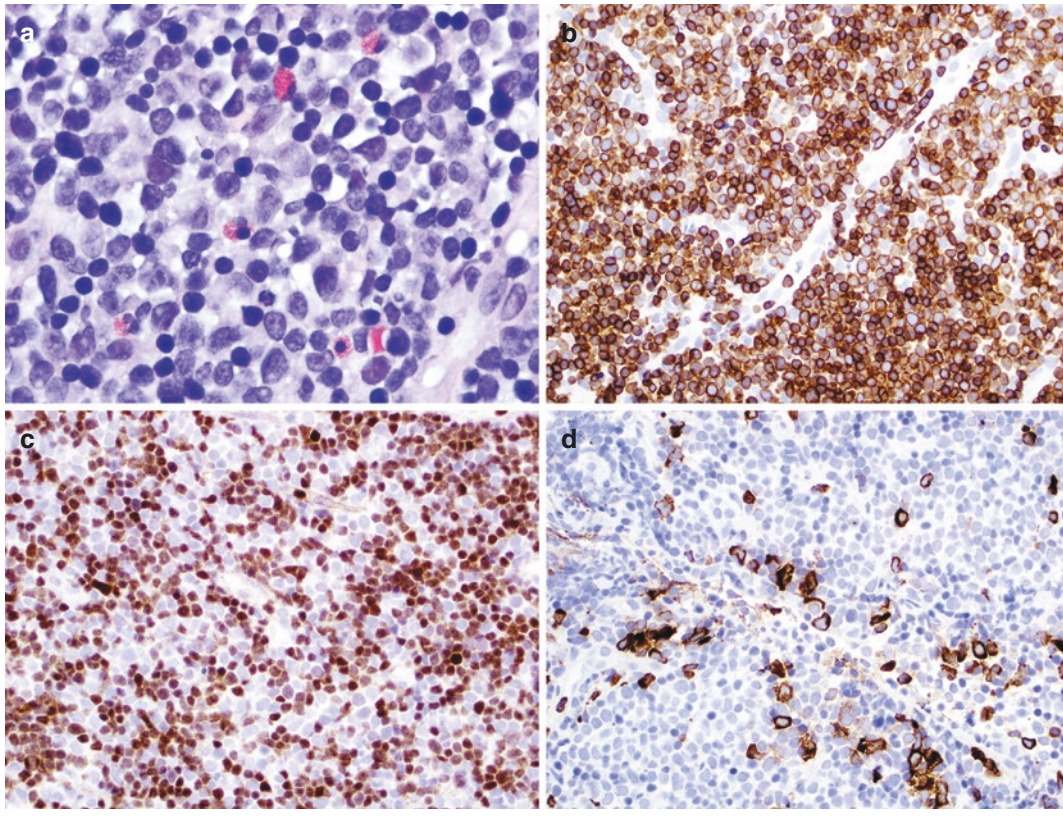

Fig. 6.15 Lymph node with blastic T/myeloid neoplasm with t(8;13)(p11;q12)(*ZMYM2-FGFR1*) rearrangement. The lymph node shows complete effacement of the architecture due to an infiltrate by intermediate-sized mononuclear cells (**a**). Immunohistochemistry shows that the tumor cells are CD3+ (**b**) and many are TdT+ (**c**). Scattered cells are positive for myeloperoxidase (**d**)

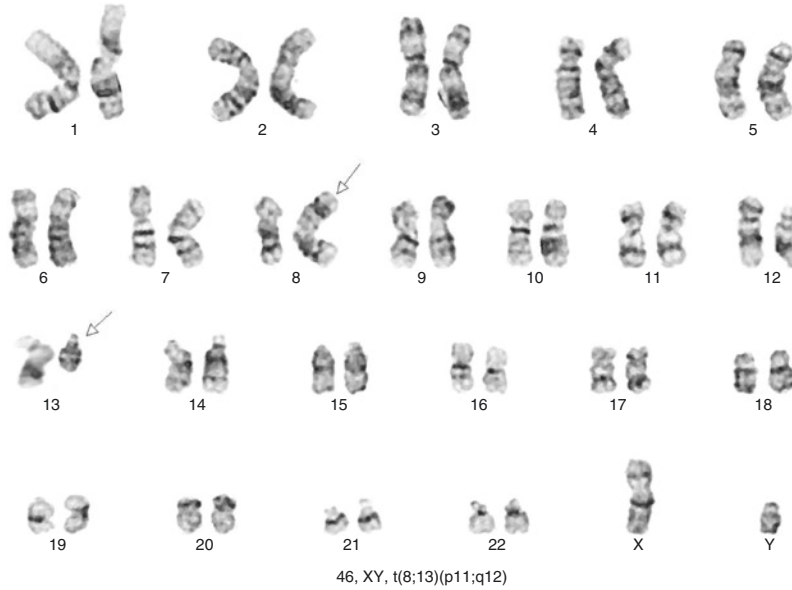

Fig. 6.16 Karyotype of bone marrow from a patient who presented with myeloproliferative neoplasm and eosinophilia demonstrates 46, XY, t(8;13)(p11;q12), consistent with rearrangement of *FGFR1* at 8p11

with an eosinophilic MPN and T-lymphoblastic lymphoma or blastic T/myeloid neoplasm.

The diagnosis of blastic T/myeloid neoplasm with *FGFR1* rearrangement can be confirmed by using FISH with break-apart probes for *FGFR1*. There are 14 other partners that can fuse with the *FGFR1*. However, manifestations may differ according to the gene partners. For example, myeloid/lymphoid neoplasms with t(8;22)(p11.2;q11.2)*; BCR-FGFR1* patients usually manifest with monocytosis and transformation is to B-lymphoblastic leukemia [55, 56].

Myeloid/Lymphoid Neoplasms with *PCM1-JAK2* Rearrangement

The identification of neoplasms with eosinophilia and specific tyrosine kinase rearrangements has led to increased recognition of hematolymphoid neoplasms presenting with eosinophilia, but lacking *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangements. This field will continue to evolve as new recurrent molecular genetic alterations are discovered. An example is eosinophilia associated with *PCM1-JAK2* [57], which has been incorporated in the group as myeloid/lymphoid neoplasm-associated eosinophilia and specific gene rearrangements as a new provisional entity in the 2016 WHO revision. By conventional karyotyping, a t(8;9) (p22;p24.1) is detected which results in a *PCM1-JAK2* rearrangement.

The t(8;9) (p22;p24.1) with *PCM1-JAK2* rearrangement should be distinguished from t(8;9) (p11;q33)/*CEP110-FGFR1*. FISH for *JAK2* and *FGFR1* molecular abnormalities should be performed to confirm the rearrangement suggested by the karyotyping. Some patients with *PCM1-JAK2* rearrangement present with marked eosinophilia, while other patients present with features of an MPN or MDS/MPN lacking prominent eosinophilia. This rare entity is characterized by a combination of eosinophilia with BM findings of left-shifted erythroid predominance, lymphoid aggregates, and often myelofibrosis (Fig. 6.17), potentially mimicking PMF. It may also rarely present as T-cell or B-cell lymphoblastic leukemia. While response to tyrosine kinase inhibitors

is poor, targeted therapy with JAK2 inhibitors offers potential benefit. Some patients have achieved excellent outcome after allogeneic stem cell transplant [6]. Variant *JAK2* rearrangements *BCR-JAK2* and *ETV6-JAK2* can also occur, but these have more heterogeneous presentations than *PCM1-JAK2*-rearranged neoplasms.

A diagnostic algorithm for the diagnosis of hypereosinophilia is illustrated in Fig. 6.18.

Differential diagnosis

The differential diagnoses for "Myeloid/lymphoid neoplasms with eosinophilia and rearrangements of *PDGFRA, PDGFRB*, or *FGFR1* or with *PCM1-JAK2*" include a wide range of disorders and entities. Since the clinical manifestations and morphological findings at time of presentations are highly variable, the scope of differential diagnoses needs to tailor to each individual case. For cases presenting with eosinophilia, the differential diagnosis include reactive/secondary eosinophilia, hypereosinophilia in the context of another well-defined myeloid neoplasm, chronic eosinophilic leukemia, not otherwise specified (CEL, NOS), and idiopathic hypereosinophilic syndromes (HES). For cases with no significant eosinophilia or with a presentation of acute leukemia, a diagnosis can only be achieved by the clinical suspicion coupled with the selection of appropriate cytogenetic and molecular genetic testing. If there is a karyotype indication of a rearrangement of *PDGFRA*,

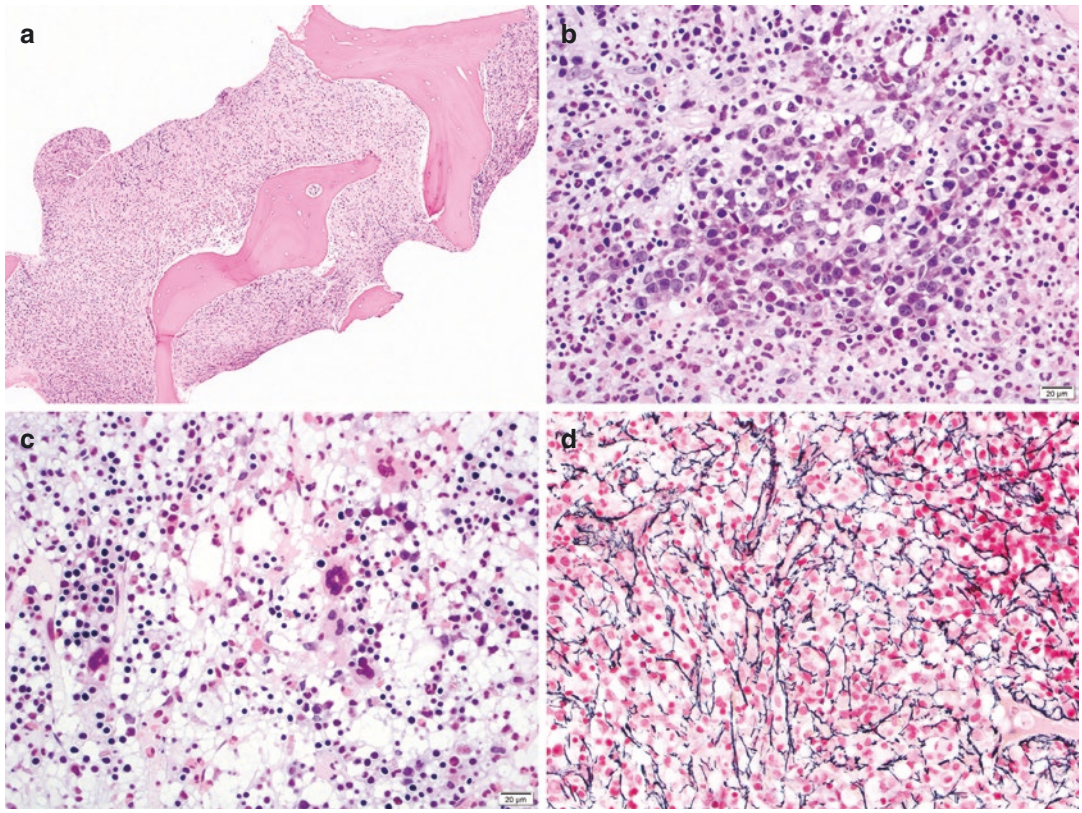

Fig. 6.17 A case of eosinophilia with t(8;9)(p22;p24.1); *PCM1-JAK2* rearrangement. Bone marrow biopsy at a low-power view shows diffuse fibrosis and bone remodeling changes (**a**). At higher power, there are large erythroid islands consisting of immature erythroblasts (**b**); megakaryocytes are not increased and do not cluster, but include some dysplastic forms. An eosinophil infiltrate is also seen (**c**). Reticulin stain confirms the presence of increased fibrosis (**d**)

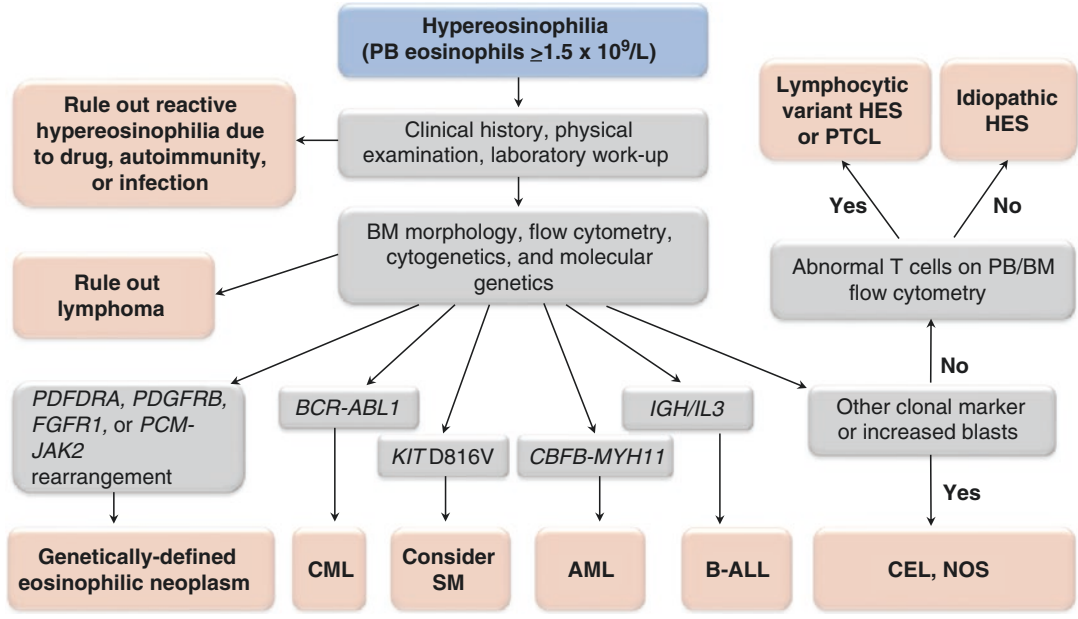

Fig. 6.18 An algorithm recommended in the workup of patients with hypereosinophilia. *CML* chronic myeloid leukemia, *BCR-ABL1+*, *SM* systemic mastocytosis, *AML* acute myeloid leukemia, *B-ALL* B-lymphoblastic leukemia, *CEL* chronic eosinophilic leukemia, *NOS* not otherwise specified, *HES* hypereosinophilic syndrome, *PTCL* peripheral T-cell lymphoma, *BM* bone marrow, *PB* peripheral blood

PDGFRB, or *FGFR1* or with *PCM1-JAK2*, FISH studies are definitely recommended to confirm or to exclude such rearrangements. Since *PDGFRA-FIP1L1* is cryptic, it is encouraged to perform *PDGFRA* FISH if there is any suspicion. For cases presenting with B-cell lymphoblastic leukemia/lymphoma, there is substantial molecular genetic overlapping with Ph-like/BCR-ABL1-like ALL. A number of rearrangements with specific partner genes in cases of B-ALL are best fit for Ph-like/BCR-ABL1-like ALL than "Myeloid and lymphoid neoplasms with eosinophilia". These include *EBF1-PDGFRB, SSBP2-PDGFRB, TNIP1-PDGFRB, ZEB2-PDGFRB* and *ATF7IP-PDGFRB*; *JAK2* with *SSBP2, PAX5, RFX3, USP25*, and *ZNF274*. Recently, a case of *B-ALL* with *FIP1L1-PDGFRA* was reported to have a Ph-like ALL signature, expanding the spectrum of these overlapping genetic entities.

These cases often do not have a chronic myeloproliferative neoplasm that may present pre-, concomitant or post-chemotherapy for B-ALL.

References

1. Gotlib J. World Health Organization-defined eosinophilic disorders: 2015 update on diagnosis, risk stratification, and management. Am J Hematol. 2015;90:1077–89.
2. Valent P, Klion AD, Horny HP, et al. Contemporary consensus proposal on criteria and classification of eosinophilic disorders and related syndromes. J Allergy Clin Immunol. 2012;130:607–12.e9.
3. Reiter A, Gotlib J. Myeloid neoplasms with eosinophilia. Blood. 2017;129:704–14.
4. Gleich GJ. Mechanisms of eosinophil-associated inflammation. J Allergy Clin Immunol. 2000;105:651–63.
5. Bain BJ, Horny H-P, Arber DA, Tefferi A, Hasserjian RP. Myeloid/lymphoid neoplasms with eosinophilia and rearrangements of PDGFRA, PDGFRB or FGFR1, or with PCM1-JAK2. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J. (eds.). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition). Lyon: IARC; 2017:72–9.
6. Vega F, Medeiros LJ, Bueso-Ramos CE, Arboleda P, Miranda RN. Hematolymphoid neoplasms associated with rearrangements of PDGFRA, PDGFRB, and FGFR1. Am J Clin Pathol. 2015;144:377–92.
7. Jackson CC, Medeiros LJ, Miranda RN. 8p11 myeloproliferative syndrome: a review. Hum Pathol. 2010;41:461–76.

8. Catovsky D, Bernasconi C, Verdonck PJ, et al. The association of eosinophilia with lymphoblastic leukaemia or lymphoma: a study of seven patients. Br J Haematol. 1980;45:523–34.
9. Kawasaki A, Mizushima Y, Matsui S, Hoshino K, Yano S, Kitagawa M. A case of T-cell lymphoma accompanying marked eosinophilia, chronic eosinophilic pneumonia and eosinophilic pleural effusion. A case report. Tumori. 1991;77:527–30.
10. Jin JJ, Butterfield JH, Weiler CR. Hematologic malignancies identified in patients with hypereosinophilia and hypereosinophilic syndromes. J Allergy Clin Immunol Pract. 2015;3:920–5.
11. Endo M, Usuki K, Kitazume K, Iwabe K, Okuyama Y, Urabe A. Hypereosinophilic syndrome in Hodgkin's disease with increased granulocyte-macrophage colony-stimulating factor. Ann Hematol. 1995;71:313–4.
12. Bank I, Amariglio N, Reshef A, et al. The hypereosinophilic syndrome associated with CD4+CD3- helper type 2 (Th2) lymphocytes. Leuk Lymphoma. 2001;42:123–33.
13. Simon HU, Plotz SG, Dummer R, Blaser K. Abnormal clones of T cells producing interleukin-5 in idiopathic eosinophilia. N Engl J Med. 1999;341:1112–20.
14. Walker S, Wang C, Walradt T, et al. Identification of a gain-of-function STAT3 mutation (p.Y640F) in lymphocytic variant hypereosinophilic syndrome. Blood. 2016;127:948–51.
15. Lefevre G, Copin MC, Staumont-Salle D, et al. The lymphoid variant of hypereosinophilic syndrome: study of 21 patients with CD3-CD4+ aberrant T-cell phenotype. Medicine (Baltimore). 2014;93:255–66.
16. Loghavi S, Wang SA, Medeiros LJ, et al. Immunophenotypic and diagnostic characterization of angioimmunoblastic T-cell lymphoma by advanced flow cytometric technology. Leuk Lymphoma. 2016;57(12):2804.
17. Lefevre G, Copin MC, Roumier C, et al. CD3-CD4+ lymphoid variant of hypereosinophilic syndrome: nodal and extranodal histopathological and immunophenotypic features of a peripheral indolent clonal T-cell lymphoproliferative disorder. Haematologica. 2015;100:1086–95.
18. Bain BJ, Horny, H-P, Hasserjian RP, Orazi A. Chronic eosinophilic leukemia, NOS. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J. (eds.). WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues (Revised 4th edition). Lyon: IARC; 2017:54–6.
19. Weller PF, Bubley GJ. The idiopathic hypereosinophilic syndrome. Blood. 1994;83:2759–79.
20. Cargo CA, Rowbotham N, Evans PA, et al. Targeted sequencing identifies patients with preclinical MDS at high risk of disease progression. Blood. 2015;126:2362–5.
21. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126:9–16.
22. Crane MM, Chang CM, Kobayashi MG, Weller PF. Incidence of myeloproliferative hypereosinophilic syndrome in the United States and an estimate of all hypereosinophilic syndrome incidence. J Allergy Clin Immunol. 2010;126:179–81.
23. Wang SA, Tam W, Tsai AG, et al. Targeted next-generation sequencing identifies a subset of idiopathic hypereosinophilic syndrome with features similar to chronic eosinophilic leukemia, not otherwise specified. Mod Pathol. 2016;29:854–64.
24. Ogbogu PU, Bochner BS, Butterfield JH, et al. Hypereosinophilic syndrome: a multicenter, retrospective analysis of clinical characteristics and response to therapy. J Allergy Clin Immunol. 2009;124:1319–25.e3.
25. Podjasek JC, Butterfield JH. Mortality in hypereosinophilic syndrome: 19 years of experience at Mayo Clinic with a review of the literature. Leuk Res. 2013;37:392–5.
26. Lefebvre C, Bletry O, Degoulet P, et al. Prognostic factors of hypereosinophilic syndrome. Study of 40 cases. Ann Med Interne (Paris). 1989;140:253–7.
27. Bain BJ. Eosinophilic leukaemias and the idiopathic hypereosinophilic syndrome. Br J Haematol. 1996;95:2–9.
28. Gotlib J, Cools J, Malone JM 3rd, Schrier SL, Gilliland DG, Coutre SE. The FIP1L1-PDGFRalpha fusion tyrosine kinase in hypereosinophilic syndrome and chronic eosinophilic leukemia: implications for diagnosis, classification, and management. Blood. 2004;103:2879–91.
29. Matsushima T, Handa H, Yokohama A, et al. Prevalence and clinical characteristics of myelodysplastic syndrome with bone marrow eosinophilia or basophilia. Blood. 2003;101:3386–90.
30. Miranda RN, Esparza AR, Sambandam S, Medeiros LJ. Systemic mast cell disease presenting with peripheral blood eosinophilia. Hum Pathol. 1994;25:727–30.
31. Pardanani A, Lim KH, Lasho TL, et al. Prognostically relevant breakdown of 123 patients with systemic mastocytosis associated with other myeloid malignancies. Blood. 2009;114:3769–72.
32. Pardanani A. Systemic mastocytosis in adults: 2015 update on diagnosis, risk stratification, and management. Am J Hematol. 2015;90:250–62.
33. Gotlib J, Cools J. Five years since the discovery of FIP1L1-PDGFRA: what we have learned about the fusion and other molecularly defined eosinophilias. Leukemia. 2008;22:1999–2010.
34. Vandenberghe P, Wlodarska I, Michaux L, et al. Clinical and molecular features of FIP1L1-PDFGRA (+) chronic eosinophilic leukemias. Leukemia. 2004;18:734–42.
35. Metzgeroth G, Walz C, Score J, et al. Recurrent finding of the FIP1L1-PDGFRA fusion gene in eosinophilia-associated acute myeloid leukemia and lymphoblastic T-cell lymphoma. Leukemia. 2007;21:1183–8.
36. Schwaab J, Jawhar M, Naumann N, et al. Diagnostic challenges in the work up of hypereosinophilia: pitfalls in bone marrow core biopsy interpretation. Ann Hematol. 2016;95:557–62.

37. Cools J, DeAngelo DJ, Gotlib J, et al. A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med. 2003;348:1201–14.
38. La Starza R, Specchia G, Cuneo A, et al. The hypereosinophilic syndrome: fluorescence in situ hybridization detects the del(4)(q12)-FIP1L1/PDGFRA but not genomic rearrangements of other tyrosine kinases. Haematologica. 2005;90:596–601.
39. Pardanani A, Ketterling RP, Li CY, et al. FIP1L1-PDGFRA in eosinophilic disorders: prevalence in routine clinical practice, long-term experience with imatinib therapy, and a critical review of the literature. Leuk Res. 2006;30:965–70.
40. Lierman E, Michaux L, Beullens E, et al. FIP1L1-PDGFRalpha D842V, a novel panresistant mutant, emerging after treatment of FIP1L1-PDGFRalpha T674I eosinophilic leukemia with single agent sorafenib. Leukemia. 2009;23:845–51.
41. SQ Q, Qin TJ, ZF X, et al. Long-term outcomes of imatinib in patients with FIP1L1/ PDGFRA associated chronic eosinophilic leukemia: experience of a single center in China. Oncotarget. 2016;7:33229–36.
42. Baccarani M, Cilloni D, Rondoni M, et al. The efficacy of imatinib mesylate in patients with FIP1L1-PDGFRalpha-positive hypereosinophilic syndrome. Results of a multicenter prospective study. Haematologica. 2007;92(9):1173.
43. Montano-Almendras CP, Essaghir A, Schoemans H, et al. ETV6-PDGFRB and FIP1L1-PDGFRA stimulate human hematopoietic progenitor cell proliferation and differentiation into eosinophils: the role of nuclear factor-kappaB. Haematologica. 2012;97:1064–72.
44. Arefi M, Garcia JL, Penarrubia MJ, et al. Incidence and clinical characteristics of myeloproliferative neoplasms displaying a PDGFRB rearrangement. Eur J Haematol. 2012;89:37–41.
45. David M, Cross NC, Burgstaller S, et al. Durable responses to imatinib in patients with PDGFRB fusion gene-positive and BCR-ABL-negative chronic myeloproliferative disorders. Blood. 2007;109:61–4.
46. Steer EJ, Cross NC. Myeloproliferative disorders with translocations of chromosome 5q31-35: role of the platelet-derived growth factor receptor Beta. Acta Haematol. 2002;107:113–22.
47. Lierman E, Cools J. TV6 and PDGFRB: a license to fuse. Haematologica. 2007;92:145–7.
48. Ondrejka SL, Jegalian AG, Kim AS, et al. PDGFRB-rearranged T-lymphoblastic leukemia/lymphoma occurring with myeloid neoplasms: the missing link supporting a stem cell origin. Haematologica. 2014;99:e148–51.
49. Cain JA, Xiang Z, O'Neal J, et al. Myeloproliferative disease induced by TEL-PDGFRB displays dynamic range sensitivity to Stat5 gene dosage. Blood. 2007;109:3906–14.
50. Apperley JF, Gardembas M, Melo JV, et al. Response to imatinib mesylate in patients with chronic myeloproliferative diseases with rearrangements of the platelet-derived growth factor receptor beta. N Engl J Med. 2002;347(7):481.
51. Vega F, Medeiros LJ, Davuluri R, Cromwell CC, Alkan S, Abruzzo LV. t(8;13)-positive bilineal lymphomas: report of 6 cases. Am J Surg Pathol. 2008;32:14–20.
52. Macdonald D, Aguiar RC, Mason PJ, Goldman JM, Cross NC. A new myeloproliferative disorder associated with chromosomal translocations involving 8p11: a review. Leukemia. 1995;9:1628–30.
53. Miranda RN, Medeiros LJ. Blastic T/Myeloid neoplasm associated with ZMYM2-FGFR1. In: Medeiros LJ, Miranda RN, (eds.). Diagnostic Pathology: Lymph Nodes and Extranodal Lymphomas. Second ed. Salt Lake City, UT: Elsevier; 2018:802–11.
54. Abruzzo LV, Jaffe ES, Cotelingam JD, Whang-Peng J, Del Duca V Jr, Medeiros LJ. T-cell lymphoblastic lymphoma with eosinophilia associated with subsequent myeloid malignancy. Am J Surg Pathol. 1992;16:236–45.
55. Wang W, Tang G, Kadia T, et al. Cytogenetic evolution associated with disease progression in hematopoietic neoplasms with t(8;22)(p11;q11)/BCR-FGFR1 rearrangement. J Natl Compr Cancer Netw. 2016;14:708–11.
56. Montenegro-Garreaud X, Miranda RN, Reynolds A, et al. Myeloproliferative neoplasms with t(8;22)(p11.2;q11.2)/BCR-FGFR1: a meta-analysis of 20 cases shows cytogenetic progression with B-lymphoid blast phase. Hum Pathol. 2017;65:147–56.
57. Reiter A, Walz C, Watmore A, et al. The t(8;9)(p22;p24) is a recurrent abnormality in chronic and acute leukemia that fuses PCM1 to JAK2. Cancer Res. 2005;65(7):2662.

Monocytosis

7

Julia Turbiner Geyer

Overview

Monocytes develop in bone marrow from myelomonocytic progenitor cells. Their number fluctuates in response to cytokines associated with systemic inflammation or infection. Thus, the presence of transient peripheral blood monocytosis is a very common and nonspecific finding that can be seen in a variety of autoimmune, infectious, and neoplastic conditions. However, sustained monocytosis, especially in conjunction with anemia or thrombocytopenia, can raise the possibility of a myeloid malignancy and often warrants a bone marrow biopsy. The differential diagnosis of persistent monocytosis includes chronic myelomonocytic leukemia (including the "oligomonocytic" form of disease) and acute myeloid leukemia with monocytic differentiation, as well as other less frequent myeloid neoplasms, such as juvenile myelomonocytic leukemia and monocytic progression of an underlying myeloproliferative neoplasm or myelodysplastic syndrome. Close attention to the peripheral blood and bone marrow morphology, flow cytometric findings, and cytogenetics is essential to navigate the differential diagnosis of monocytosis; more recently, molecular genetic testing has also been found to provide valuable information in this context. An algorithm for the suggested workup of monocytosis is presented at the end of the chapter (Fig. 7.30).

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Reactive Monocytosis

Monocytes develop in bone marrow and spleen from myelomonocytic progenitor cells under the influence of various cytokines, in particular the macrophage colony-stimulating factor (M-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) (see Fig. 7.1) [1]. Monocytic production in bone marrow is estimated to take 2–3 days. Mature monocytes are released into the circulation, where they have a half-life of 1–2 days [1]. Classical inflammatory monocytes egress with marked diurnal variations [2]. In addition, monocytes leave the bone marrow during both infection and sterile inflammation, such as myocardial infarction and atherosclerosis [3]. Monocytes then enter tissues and body fluids, where they develop a phenotype characteristic of the specific tissue of residence (see Table 7.1). Monocytes are also recruited from the blood to sites of infection or inflammation where they can either directly take part in the inflammatory response or differentiate into tissue macrophages [4].

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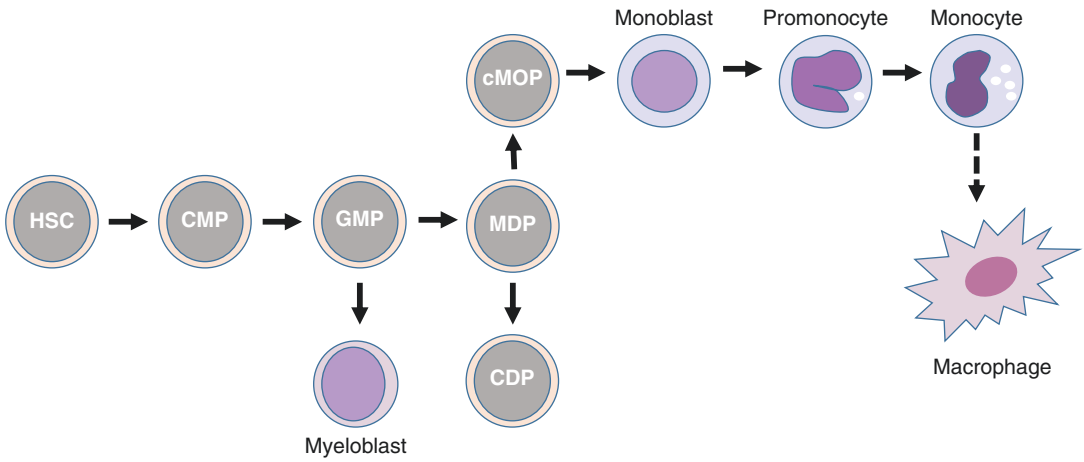

Fig. 7.1 Stages of normal monocytic differentiation in bone marrow. Monocytes originate from hematopoietic stem cells (HSCs) and develop through sequential differentiation stages: the common myeloid progenitor (CMP), the granulocyte-macrophage progenitor (GMP), the common macrophage and dendritic cell precursor (MDP), and finally the committed monocyte progenitor (cMoP). MDP also gives rise to common dendritic progenitors (CDP), whose differentiation is restricted to the dendritic cell lineage. The cMoP develops into the monoblast, which is the first morphologically recognizable monocytic cell type in bone marrow. Monoblasts mature into promonocytes and finally monocytes. After passage through peripheral blood, monocytes may develop into tissue macrophages

Table 7.1 Terminally differentiated monocytic cells and specialization of macrophages in particular microenvironments

Body site	Cell type
Liver	Kupffer cells
Brain	Microglia
Bone	Osteoclasts
Lung	Alveolar macrophages
Multiple tissues	Histiocytes

Clinical Features

Absolute monocytosis is defined as peripheral blood monocyte count of more than 0.8×10^9/L, or above each aboratory normal reference range for monocytes. For the purposes of defining chronic myelomonocytic leukemia (CMML), absolute monocytosis is a peripheral blood monocyte count of more than 1.0×10^9/L. The proportion of monocytes in peripheral blood may be increased in virtually all inflammatory and immune reactions [4], such as in pregnancy, autoimmune diseases, gastrointestinal disorders, cancer, or sarcoidosis (see Table 7.2). A large number of specific infections have been associated with monocytosis, including brucellosis, varicella zoster, bacterial endocarditis, tuberculosis, malaria, typhoid fever, syphilis, and trypanosomiasis [4]. Treatment with corticosteroids or colony-stimulating factors frequently induces monocytosis. In addition, patients with marked neutropenia may develop transient relative monocytosis. Thus, bone marrow biopsies performed following G-CSF administration in the course of chemotherapy treatment, especially in patients with acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), may show subtle to marked monocytosis that may include immature forms, raising the differential diagnosis of phenotypic shift of patient's known myeloid neoplasm or emergence of new myelomonocytic neoplasm [5, 6]. During early bone marrow reconstitution following chemotherapy or stem cell transplant, a transient blood and bone marrow monocytosis may develop even in the absence of exogenous growth factor therapy. However, as opposed to true monocytic malignancy, a reactive monocyte proliferation is fully reversible after discontinuation of the growth factor and upon complete bone marrow reconstitution [5–7]. Thus, clinical follow-up by repeat blood count examinations is often helpful in clarifying the

Table 7.2 Causes of secondary (reactive) monocytosis

Infection	Bacterial Viral Protozoal Mycobacterial
Autoimmune disease	Systemic lupus erythematosus Rheumatoid arthritis Polyarteritis nodosa Temporal arteritis Myositis Immune thrombocytopenia Immune hemolytic anemia
Gastrointestinal disorders	Alcoholic liver disease Inflammatory bowel disease Celiac sprue
Hematologic malignancy	Classical Hodgkin lymphoma Plasma cell myeloma B cell lymphoma T cell lymphoma
Drug induced	Glucocorticoids Exogenous cytokine administration
Non-hematopoietic malignancy	Colorectal cancer Breast carcinoma Soft tissue sarcoma
Others	Sarcoidosis Depression Splenectomy Marathon running Hemolytic anemia Thermal injury Myocardial infarction Cardiac bypass surgery Tetrachloroethane poisoning Parturition Wiskott-Aldrich syndrome Kawasaki disease Drug-induced myelosuppression

reactive nature of these transient monocytic proliferations.

Morphology

Monocytes are the largest (12–20 μm) circulating mononuclear cells. They are characterized by abundant blue-gray cytoplasm and a folded, lobated, indented, or oval nucleus (Fig. 7.2a, b). Nuclear chromatin is not as clumped as that of neutrophils or mature lymphocytes (Fig. 7.2c). Nucleoli are typically absent or inconspicuous. Monocytes frequently contain cytoplasmic vacuoles and often have very fine azurophilic granulation (Fig. 7.2d). While the terminology of "dysplasia" or "dyspoiesis" is used for granulocytic precursors, erythroid progenitors and megakaryocytes, it is not considered appropriate to apply to monocytic cells. Instead the term "abnormal" or "immature monocytes" is applied to monocytic cells with finer chromatin distribution, variably indented and folded nuclei, and more abundant cytoplasmic granules. These cells need to be distinguished from promonocytes, which are considered blast equivalents (see section on AML below).

Bone marrow monocytes are morphologically identical to circulating monocytes. They typically comprise ≤3% of bone marrow cellularity (Fig. 7.3). Promonocytes and monoblasts are not conspicuous in normal or reactive bone marrow samples. The monocyte composition of the bone marrow depends on age. Although

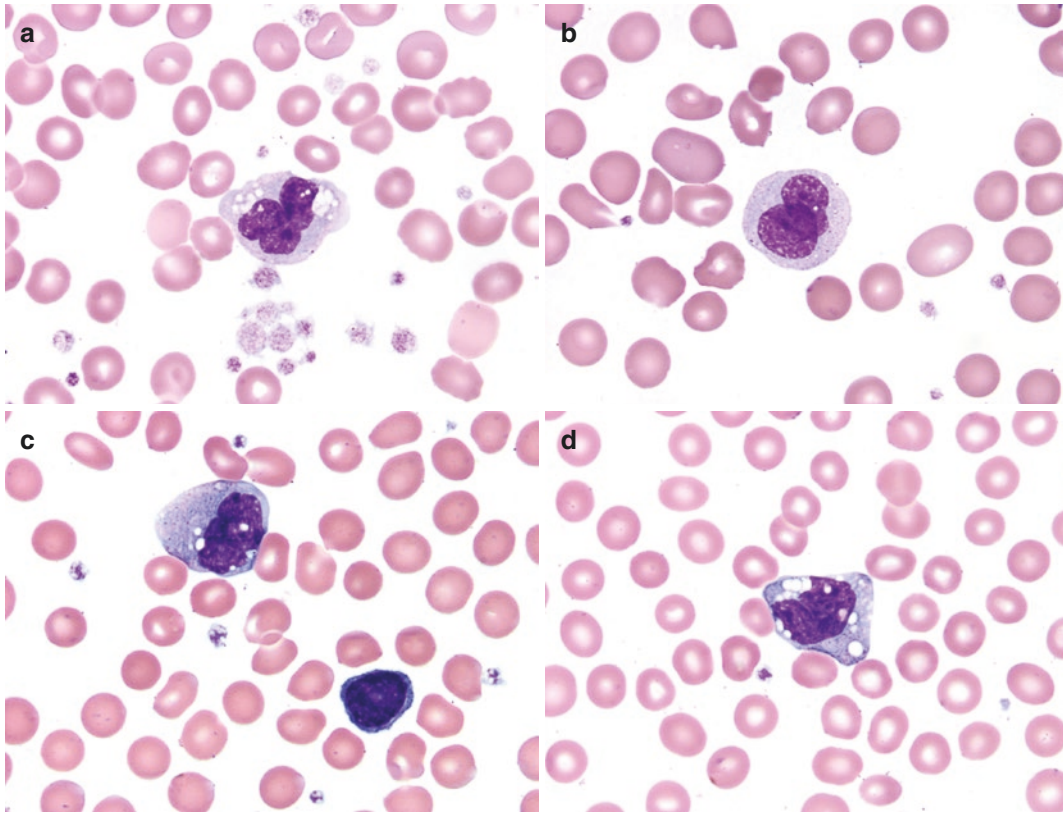

Fig. 7.2 (**a**, **b**) Mature monocyte morphology in peripheral blood. (**c**) Compared to a reactive lymphocyte (lower right), the mature monocyte is significantly larger, has more abundant and differently colored cytoplasm and an irregularly shaped nuclear contour with a more finely dispersed chromatin. (**d**) Mature monocytes contain fine to occasionally more visible, evenly distributed pink azurophilic granules, as well as sparse to numerous cytoplasmic vacuoles

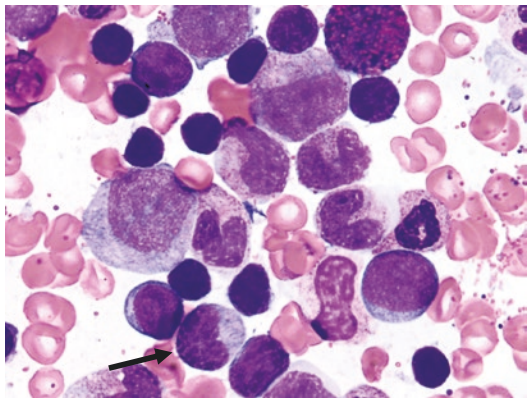

Fig. 7.3 Normal bone marrow aspirate composition including myeloid progenitors in various stages of maturation and rare monocytes (arrow)

young children have myeloid-predominant bone marrow, the percentage of monocytes is lower in children compared to adults (~1% of marrow cells in infants and ≤2% in children).

Immunophenotype and Cytochemistry

The content of cytoplasmic granules contributes to the strong cytoplasmic staining of the monocytic cells with alpha-naphthyl acetate esterase or alpha-naphthyl butyrate esterase used alone or with naphthol-ASD-chloroacetate esterase (cytochemical stains performed on bone marrow aspirate or peripheral blood).

Based on flow cytometry, monocytes have a predictable immunophenotypic profile, with expression of CD13, CD33, bright HLA-DR, bright CD11b, CD35, CD36, CD64, CD300e,

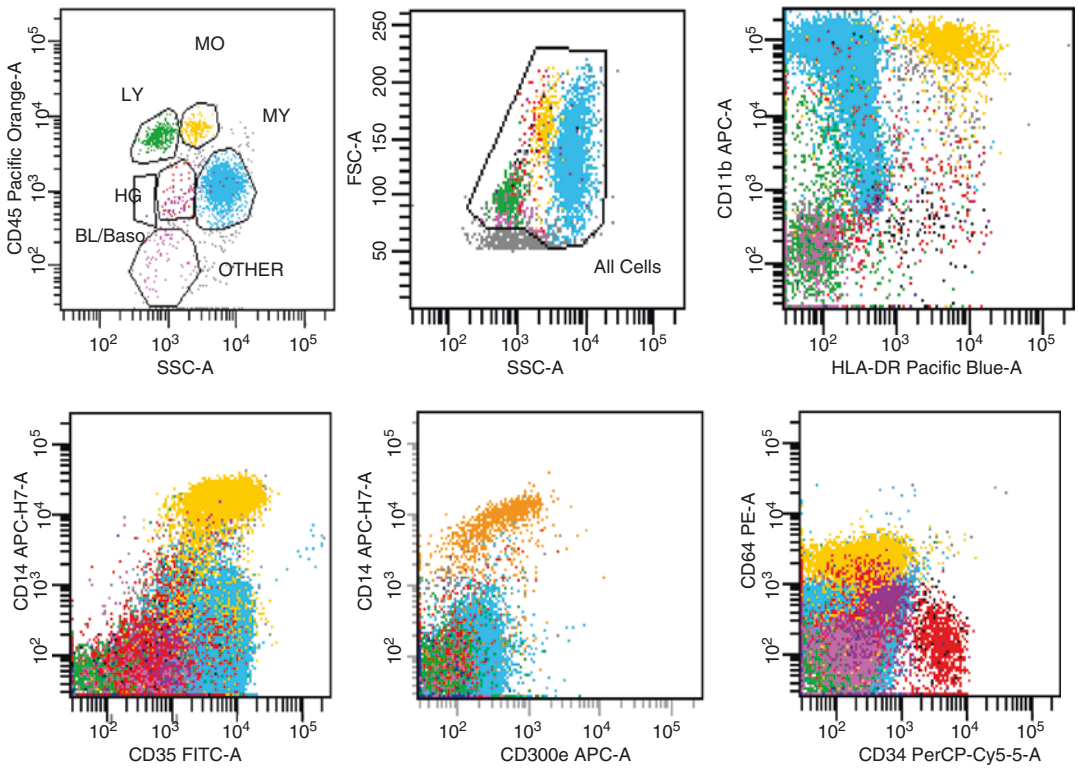

Fig. 7.4 Immunophenotypic profile of mature monocytes. Monocytes (in yellow) have moderate side scatter (SSC-A), high forward scatter (FSC-A), and bright CD45 expression. They also express bright HLA-DR, CD11b, CD14, CD35, CD300e, and CD64. By comparison, lymphocytes are colored green, neutrophils blue, immature myeloid cells red and erythroid precursors purple

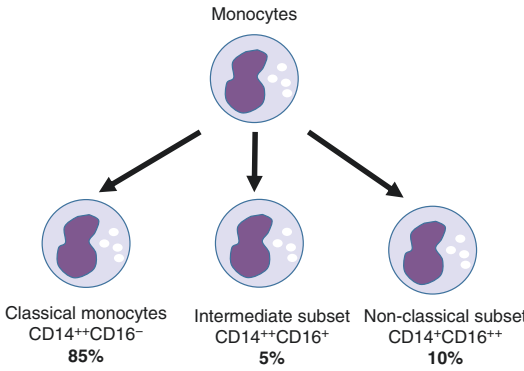

Fig. 7.5 Normal monocytes are grouped into three subsets based on the Nomenclature Committee of the International Union of Immunologic Societies [11]

and CD14 [8] (Fig. 7.4). At least three human monocyte subsets have been identified: $CD14^{++}$ $CD16^{-}$ (classical) is the most prevalent subset with phagocytosis properties; $CD14^{++}$ $CD16^{+}$ (intermediate) has a pro-inflammatory role and $CD14^{+}$ $CD16^{++}$ monocytes have patrolling and antiviral behavior [4, 9–11] (Fig. 7.5). Abnormal antigen expression is quite frequently present in reactive monocytes, including coexpression of CD2 and CD56 or underexpression of HLA-DR or CD13 [12, 13]. However, aberrant expression is usually limited to one marker. The presence of two or more abnormally expressed antigens is significantly more frequent in myeloid and monocytic neoplasms such as CMML compared to reactive monocytosis [13].

Immunohistochemistry can be performed on the bone marrow trephine to identify and quantify the monocytic cells. CD14 is the most sensitive and specific marker, followed by CD68 (KP1 or PGM-1 epitopes) and CD163 [8] (Fig. 7.6).

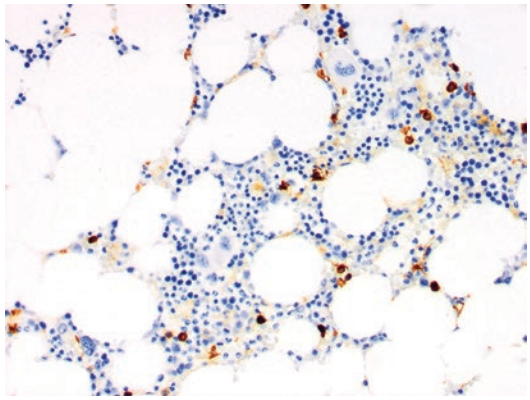

Fig. 7.6 Immunohistochemical stain for CD14 highlights monocyte distribution in normal bone marrow biopsy

Cytogenetics and Molecular Genetics

Cytogenetic or molecular genetic abnormalities are not features of secondary (reactive) monocytosis; confirmation of a normal karyotype and a lack of any myeloid-associated mutations (see below) can be supportive of a reactive rather than neoplastic etiology of a peripheral blood monocytosis. However, a subset of older patients may display clonal hematopoiesis with myeloid-type mutations in the absence of defining features of CMML or other myeloid neoplasm (so-called clonal hematopoiesis of indeterminate potential or CHIP). Thus, the presence of a single myeloid-type mutation, particularly if at low mutant allele fraction (<10%), may be an incidental finding and does not exclude a reactive process.

Differential Diagnosis

The main differential diagnosis with reactive monocytosis is a neoplastic monocytic proliferation, particularly chronic myelomonocytic leukemia or an acute myeloid leukemia with monocytic features (see discussion below). In deciding if a monocytosis is reactive or neoplastic, it is helpful to assess its duration and trajectory: reactive monocytosis is typically transient or intermittent, while monocytic neoplasms exhibit persistent (6 months or greater) and often increasing monocytosis. It is also important to determine any factors in the patient's history that may account for a reactive monocytosis, such as growth factor administration or an infection. There is significant morphologic and immunophenotypic overlap between reactive and neoplastic monocytosis; however, the presence of primitive monocytic forms (promonocytes or monoblasts) in the blood, unless rare, is worrisome for a hematologic malignancy. If monocytosis is persistent and remains unexplained, even after careful interrogation of the clinical record, a bone marrow examination is warranted to evaluate for the possibility of a monocytic neoplasm.

Chronic Myelomonocytic Leukemia (CMML)

CMML is a myeloid neoplasm with features of both myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN). The updated 2016 WHO Classification recognized the heterogeneity of this entity and distinguishes between "myelodysplastic" type of CMML (WBC of $<13 \times 10^9$/L and prominent dysplasia) and a "proliferative" type of CMML (WBC count of $\geq 13 \times 10^9$/L and frequent hepatosplenomegaly) [14]. In the majority of patients with the proliferative form of CMML, the WBC is increased due to monocytosis and, occasionally, neutrophilia. The current diagnostic criteria of CMML require a persistent, unexplained absolute peripheral blood monocytosis $\geq 1 \times 10^9$/L, as well as monocytes representing ≥10% of the blood leukocytes (Table 7.3).

Table 7.3 Updated WHO diagnostic criteria for chronic myelomonocytic leukemia

• Persistent monocytosis $\geq 1 \times 10^9$/L and ≥10% of the leukocytes
• No *BCR-ABL1, PDGFRA, PDGFRB, FGFR1*, *or PCM1-JAK2* gene rearrangements
• <20% blasts in blood or bone marrow
• Dysplasia in one or more of the lineages. If myelodysplasia is absent or minimal, the diagnosis of CMML can still be made if the other requirements are met and
– An acquired clonal cytogenetic or molecular abnormality is present—OR
– The monocytosis has been present for at least 3 months and all causes of reactive monocytosis have been excluded

Table 7.4 Updated WHO subclassification for chronic myelomonocytic leukemia

	Peripheral blood	Bone marrow
CMML-0	<2% blasts	<5% blasts
CMML-1	2–4% blasts	5–9% blasts
CMML-2	5–19% blasts or Auer rods	10–19% blasts or Auer rods
AML	≥20% blasts	≥20% blasts

The updated WHO Classification divides CMML into three categories, CMML-0, CMML-1, and CMML-2, based on the percentage of myeloid blasts (including promonocytes) in peripheral blood and bone marrow (Table 7.4). The finding of ≥20% blasts in the blood or the bone marrow indicates AML with monocytic differentiation rather than CMML. The value of subdividing CMML into three subgroups is based on the different survivals of these patients [15, 16].

The so-called oligomonocytic CMML refers to cases of myeloid neoplasms showing increased PB monocytes (>0.5 × 10^9/L) and relative monocytosis (>10%), but not at the level of absolute monocytosis required by the WHO Classification. Currently, these cases do not fit any well-defined WHO category and are usually classified as MDS or as myelodysplastic/myeloproliferative neoplasm, unclassifiable (MDS/MPN, U). Based on results of one study, oligomonocytic CMML patients have low-normal WBC values and typically present with anemia, macrocytosis, thrombocytopenia, and/or neutropenia [17]. Approximately one-third of patients with oligomonocytic CMML progress to overt CMML and a significant subset (25%) progress to AML.

Clinical Features

CMML mostly occurs in older adults (median age at diagnosis, 70 years). There is a moderate male predominance. In a subset of patients, CMML may be associated with immune-mediated processes and/or autoimmune disorders, such as systemic vasculitis, immune thrombocytopenia, psoriasis, and gout [18, 19]. Many patients may be asymptomatic and have abnormalities found on routine blood counts. Other patients present with complications resulting from cytopenias, skin lesions, or symptoms related to splenomegaly. Splenomegaly is present in up to 50% of patients with CMML and is often accompanied by hepatomegaly, lymphadenopathy, or nodular cutaneous leukemic infiltrates [20]. Patients with "proliferative"-type CMML tend to have more constitutional symptoms (fevers, unexplained weight loss, and night sweats), organomegaly, as well as pleural and pericardial effusions and ascites [21].

Morphology

Cases of CMML are characterized by abnormal (immature) monocytes circulating in the blood, which may display unusual nuclear lobation, dense but delicate nuclear chromatin, and abnormal cytoplasmic granules [22] (Fig. 7.7a–d); however, many or most of the circulating monocytes in CMML may be morphologically indistinguishable from reactive monocytes. Blasts and promonocytes may be seen, but represent <20% of the blood and bone marrow cells. There is often evidence of dysgranulopoiesis, especially in patients with dysplastic type of CMML [22].

The bone marrow is hypercellular in over 75% of cases, usually due to a granulocytic proliferation and with variable monocytic proliferation [23] (Fig. 7.8). Most cases have myeloid and megakaryocytic dysplasia and a subset demonstrates dyserythropoiesis [23]. Megakaryocytes are typically small and hypolobated, resembling those seen in MDS, and do not form clusters [23]; however, many cases may have mostly normal-appearing megakaryocytes. On close inspection of the biopsy section, monocytes with irregular and angulated nuclear contours, distinguishable from the granulocytic precursors that have oval or folded nuclei, can often be visualized. Up to 30% of the cases may have significant marrow fibrosis. Frequently, nodules of plasmacytoid dendritic cells may be seen in CMML [24] (Fig. 7.9). The plasmacytoid dendritic cells are mature cells closely related to monocytes, and are not considered blast equivalents [24]. In systemic mastocytosis associated with hematological neoplasm, CMML is one of the most common associations. The CMML cells and mast cells

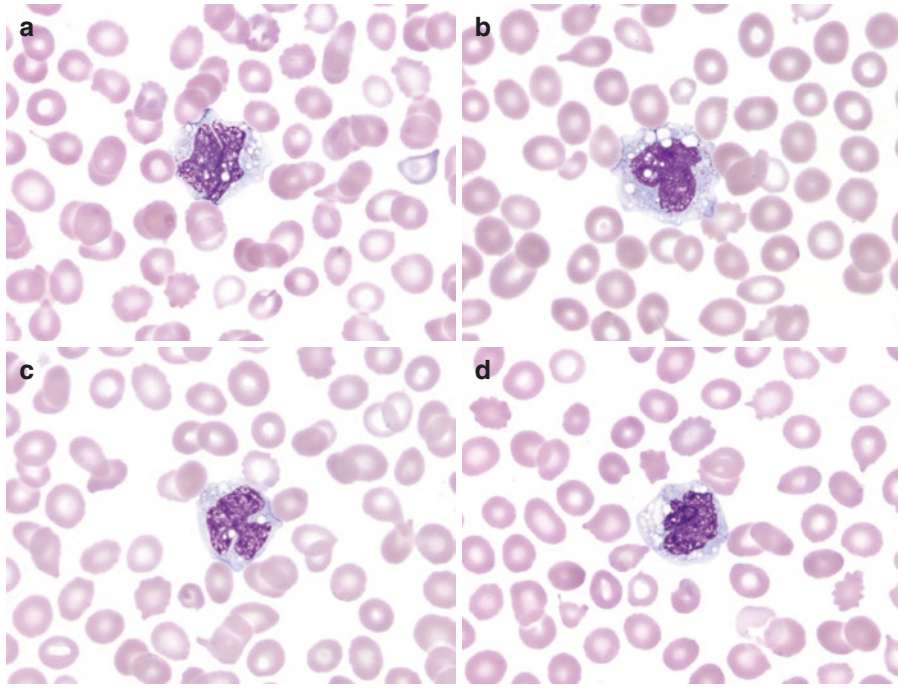

Fig. 7.7 Chronic myelomonocytic leukemia in blood. (**a**–**d**) Abnormal (immature) monocytes may have unusual nuclear lobation, dense to delicate nuclear chromatin, and abnormal cytoplasmic granules

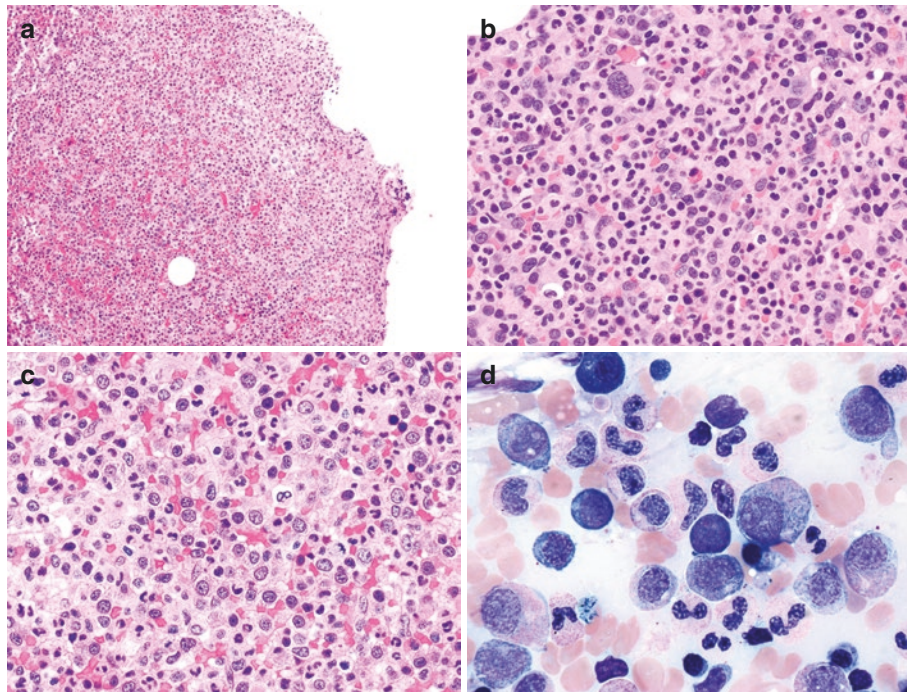

Fig. 7.8 Chronic myelomonocytic leukemia in bone marrow. (**a**) The bone marrow biopsy is hypercellular with marked myeloid predominance. (**b**) Higher magnification demonstrates maturing granulopoiesis and evidence of dysmegakaryopoiesis; monocytic cells are not conspicuous in this case. (**c**) Some cases have morphologic evidence of myeloid immaturity, suggestive of increase in blasts and higher grade disease. (**d**) Dysgranulopoiesis is frequently seen in the bone marrow aspirate

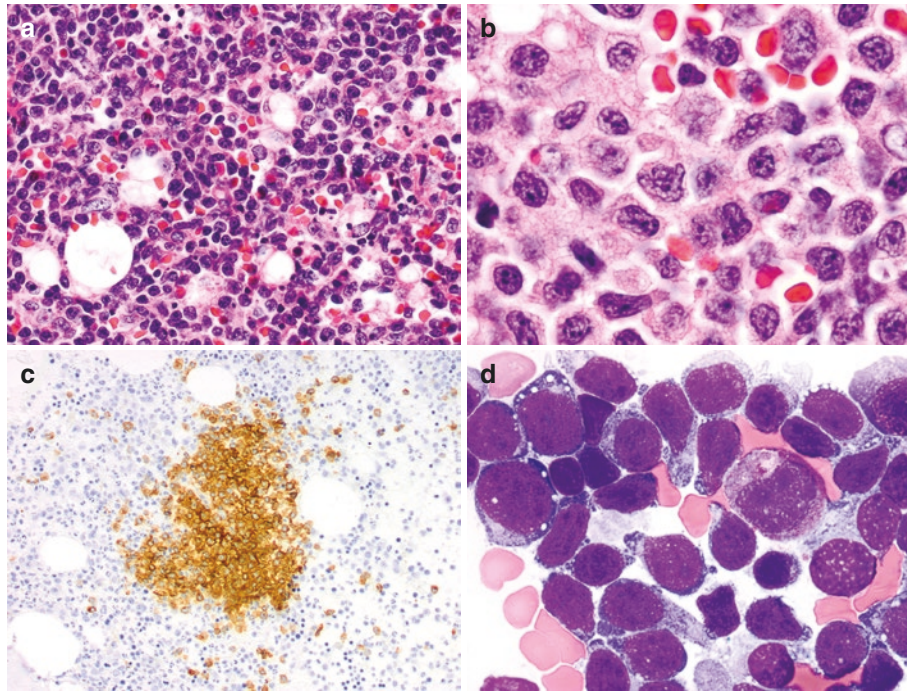

Fig. 7.9 Plasmacytoid dendritic cells in chronic myelomonocytic leukemia. (**a**) Nodule of plasmacytoid dendritic cells in bone marrow trephine biopsy. (**b**) Plasmacytoid dendritic cell morphology at high magnification. (**c**) Plasmacytoid dendritic cells strongly express CD123. (**d**) Aggregate of plasmacytoid dendritic cells in bone marrow aspirate

share the same cytogenetic abnormalities [25] as well as *KIT* mutations, indicating a same clonal origin.

In oligomonocytic CMML, the bone marrow biopsy also demonstrates hypercellularity. The myeloid-to-erythroid ratio may be decreased, normal, or increased. Bone marrow monocytes are increased in the majority of the patients with oligomonocytic CMML and dysplasia in at least one lineage is seen in more than half of the cases (Fig. 7.10). Ring sideroblasts may be present.

Immunophenotype and Cytochemistry

On the bone marrow aspirate, the monocytic proliferation can be confirmed by cytochemical staining with nonspecific esterase (Fig. 7.11).

With flow cytometry, immature monocytes have a similar immunophenotype to their normal counterparts, including expression of CD13, CD33, bright HLA-DR, bright CD11b, CD35, CD36, CD64, CD300e, and CD14 [8]. Several studies have found that flow cytometry monocyte quantification is very accurate and may improve on cytochemistry alone [26]. In addition, aberrant immunophenotype has been demonstrated in monocytes, granulocytes, and/or myeloid blasts in >95% of CMML cases [26]. CD34+ cells in bone marrow, although comprising only a small fraction of all cells, exhibit a large number of immunophenotypic alterations (Fig. 7.12a). The most common abnormalities are markedly increased CD13, CD117, and CD123, and decreased CD38 expression. The changes are similar to those seen in CD34 myeloblasts in cases of MDS, but appear to be more uniform as a group. Immature monocytes may have aberrant expression of CD56, CD2, and CD23 or abnormally dim expression of CD45, HLA-DR, CD11c, CD13, CD15, CD64, or CD36 [8, 26, 27] (Fig. 7.12b). A characteristic distribution of peripheral blood monocyte subsets due to an increase of "classical" CD14(+)/CD16(−) cells and a consequent relative reduction of CD14(+)/CD16(+) "intermediate" and CD14(low)/CD16(+) "nonclassical" monocytic cells has been recently

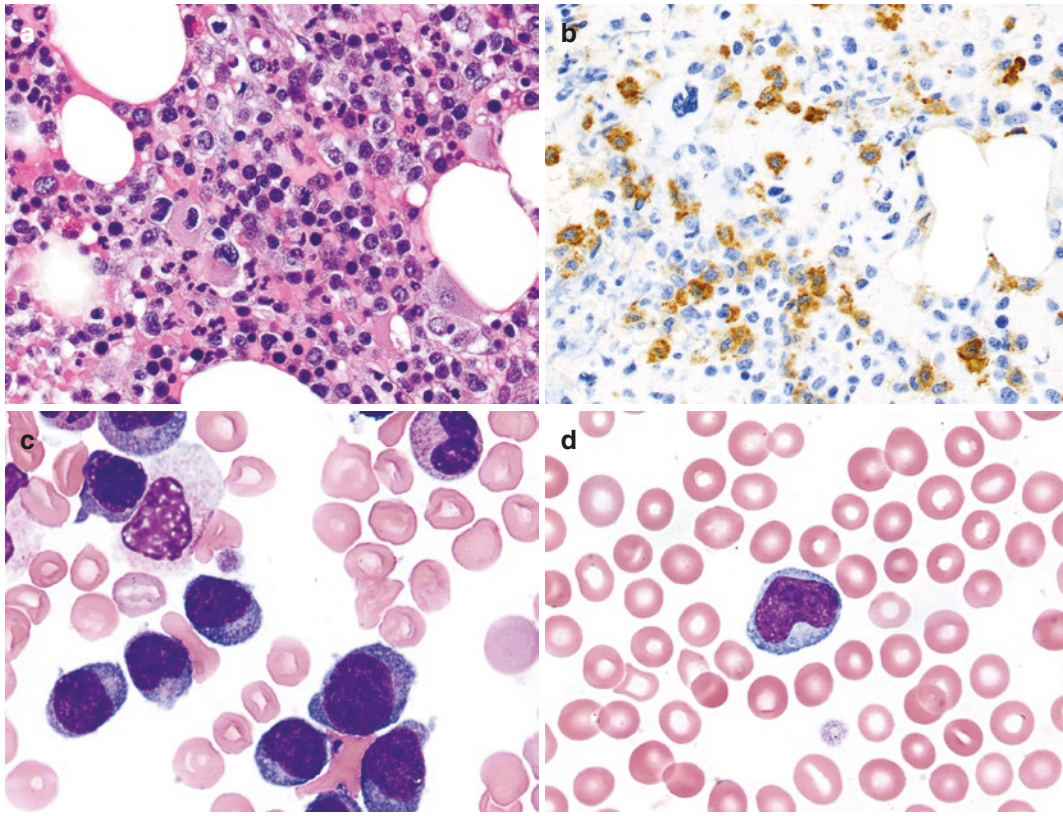

Fig. 7.10 Oligomonocytic chronic myeloid leukemia. (**a**) The bone marrow biopsy is hypercellular; monocytes are not morphologically conspicuous. Micromegakaryocytes and small hypolobated megakaryocytes are frequently seen. (**b**) CD14 is useful to highlight an increase in bone marrow monocytes. (**c**) The bone marrow aspirate demonstrates evidence of immature monocytosis. (**d**) The peripheral blood shows relative, but not absolute, monocytosis

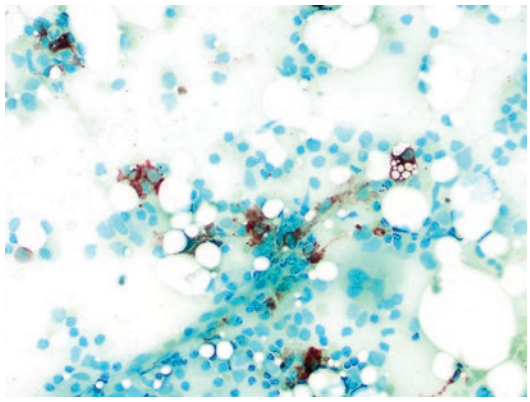

Fig. 7.11 NSE staining in the bone marrow aspirate of a patient with CMML shows a mild increase in monocytic cells

proposed as a possible diagnostic signature of CMML [28]. This flow cytometry pattern may help distinguish CMML from reactive forms of monocytosis [28]. CD56 expression with concomitant underexpression of a myeloid marker was suggested to be unique to CMML monocytes [13]. Granulocytes in CMML may also show abnormally decreased side scatter and aberrant phenotypic features, such as loss of CD10, disturbed CD11b/CD13/CD16 patterns, and ≥20% CD56 expression (25) (Fig. 7.12b).

Immunohistochemistry can be performed on the bone marrow trephine to identify and quantify the monocytic cells. CD14 is the most sensitive and specific marker, followed by CD68 (PGM-1) and CD163 [8] (Fig. 7.13). An increased number of CD34-positive blasts is associated with disease progression [15, 24] (Fig. 7.14). The most useful marker to highlight the nodules of mature plasmacytoid dendritic cells is CD123 (Fig. 7.9c). Plasmacytoid dendritic cells may also

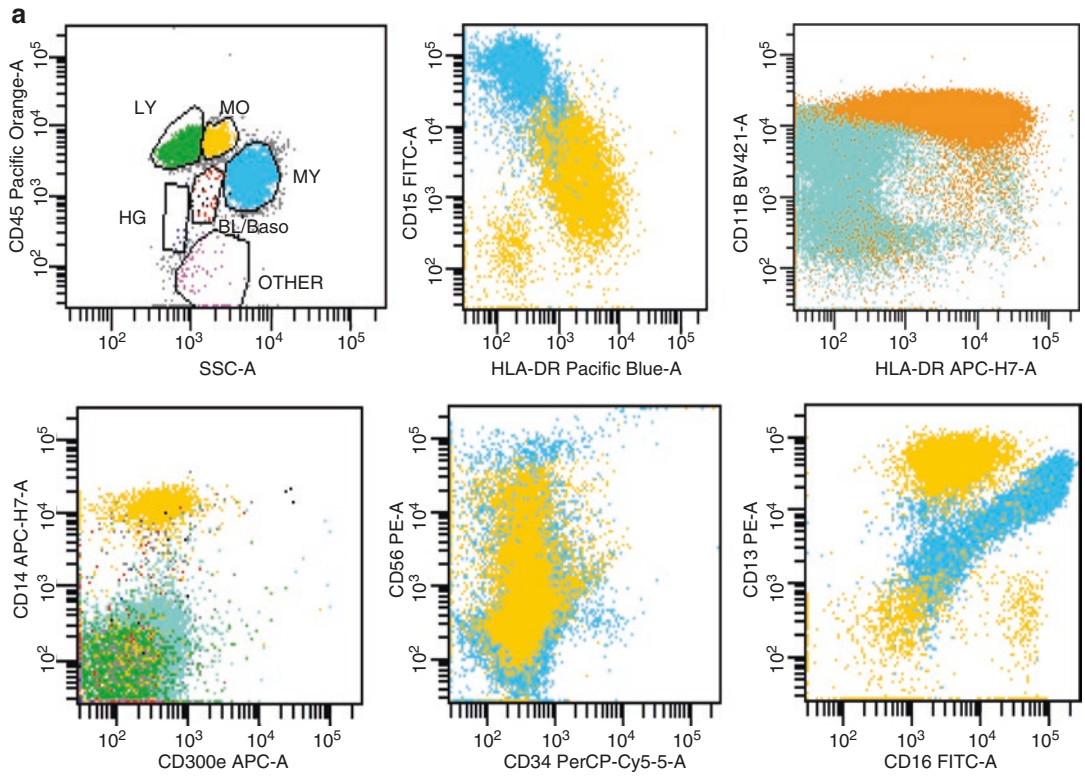

Fig. 7.12 Flow cytometry abnormalities in chronic myelomonocytic leukemia. (**a**) Immature monocytes. Monocytes (yellow) are increased and demonstrate partial expression of CD15 and HLA-DR, no expression of CD300e, and aberrant expression of CD56. The granulocytes (blue) show aberrant expression of CD56 and an abnormal pattern of maturation, with absence of CD13 on CD16-negative immature cells.

express CD14, CD43, CD68, CD45RA, CD33 (weak staining), CD4, and granzyme B. Of note, the blasts and promonocytes in CMML-1 and CMML-2 and even in AML transformed from CMML are often CD34 negative. Thus, CD34 evaluation by flow cytometry and/or immunohistochemistry is often unhelpful at detecting the increased blasts and promonocytes in such cases.

Cytogenetics and Molecular Genetics

Clonal cytogenetic abnormalities are found in 20–40% of patients with CMML. The most frequent recurring abnormalities include trisomy 8, monosomy 7, deletion of 7q, and structural abnormalities of 12p. On the other hand, certain gene mutations are enriched in CMML cases. As many as 40% of patients exhibit point mutations of *NRAS/KRAS* genes at diagnosis, or during the course of the disease, and the presence of mutations in both *TET2* and *SRSF2* has been found to be strongly associated with the diagnosis of CMML [29–32]. *TET2, SRSF2*, or *ASXL1* gene mutation is present in 90% of patients with CMML [31]. The presence of *NRAS, RUNX1, SETBP1*, or *ASXL1* mutations is capable of imparting a more aggressive course independently of the cytogenetic abnormalities [31–33]. The presence of *JAK2 V617F* mutation has been associated with myeloproliferative features and large megakaryocytes [34]. *NPM1* mutation has been described in a small percentage (3–5%) of CMML cases [35, 36]. In patients with monocytic proliferations associated with *NPM1* mutation, especially when at a high mutant allele frequency, careful review of the blast count is indicated to exclude the alternative diagnosis of AML with *NPM1* mutation [35]. Close follow-up of these patients is recommended, since presence of *NPM1*

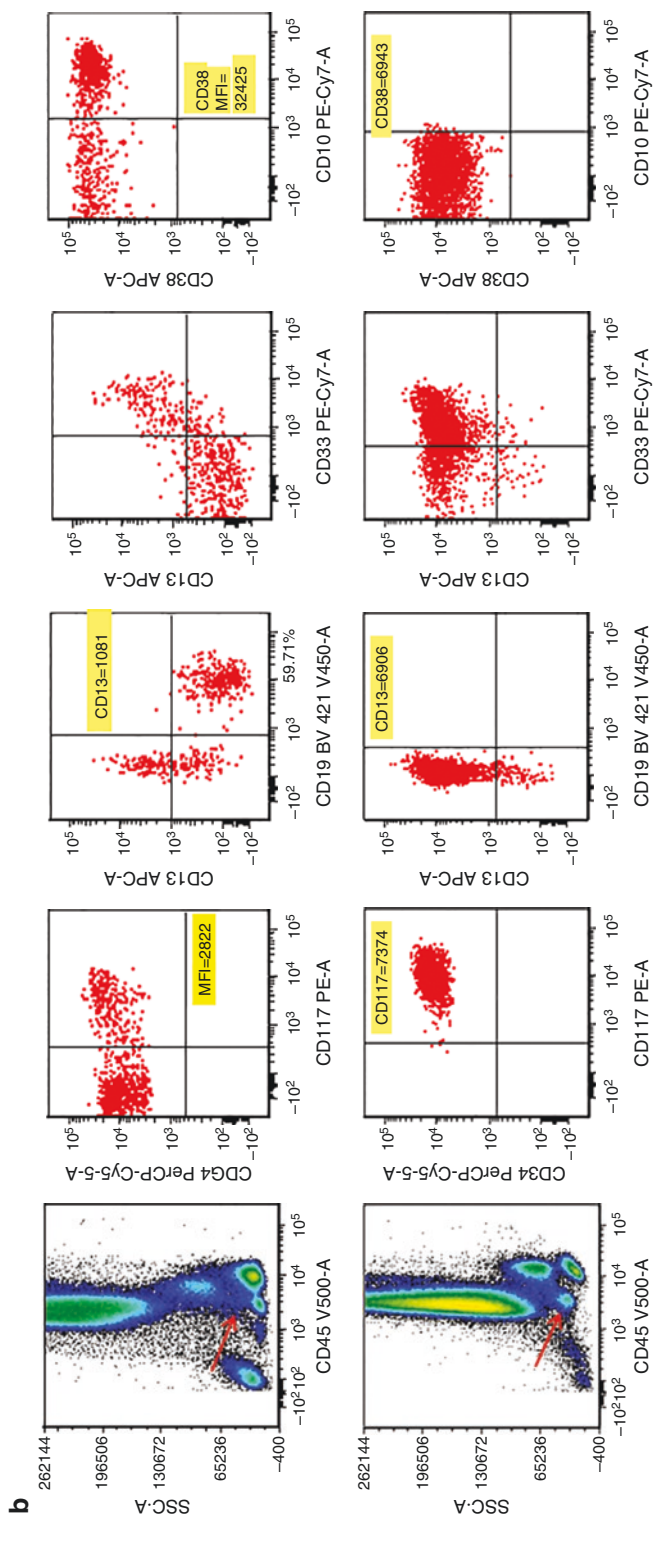

Fig. 7.12 (continued) (**b**) CD34+ cell abnormalities. Upper panel: bone marrow with reactive monocytosis; lower panel: chronic myelomonocytic leukemia. The CD34+ cells in CMML form a discrete cell population contrasting with a scattered pattern in normal bone marrow (arrows), absence of hematogones, increase in CD117 and CD13 expression, abnormal CD13/CD33 pattern, and decrease in CD38 expression

mutation or 11q23 rearrangement in CMML may herald rapid progression to acute leukemia [37].

Patients with oligomonocytic CMML frequently have mutations in *ASXL1*, *TET2*, and *SRSF2* genes. Approximately one-third of patients have concurrent *TET2* and *SRSF2* mutations.

Differential Diagnosis

The main differential diagnoses with CMML are with AML with monocytic differentiation, myelodysplastic syndrome, CML, and myeloproliferative neoplasms that have developed secondary monocytosis. The most important distinction is between CMML and AML, because these two diseases have different treatments and prognoses. Importantly, patients with overt monocytic or myelomonocytic AML may display a mature-appearing monocytosis in the blood, bone marrow,

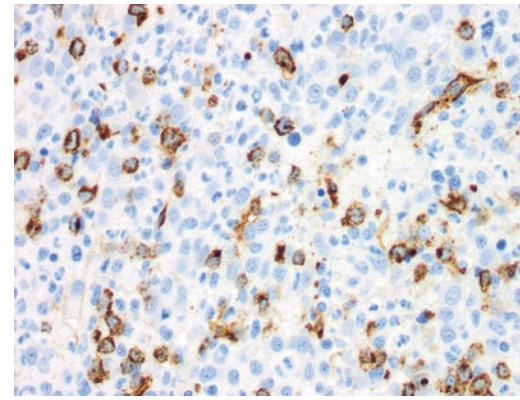

Fig. 7.14 Immunohistochemical staining for CD34 in chronic myelomonocytic leukemia. In this case, CD34 highlights approximately 10% blasts. Of note, the blasts in CMML are often monocytic and are negative for CD34 as well as CD117 [24]

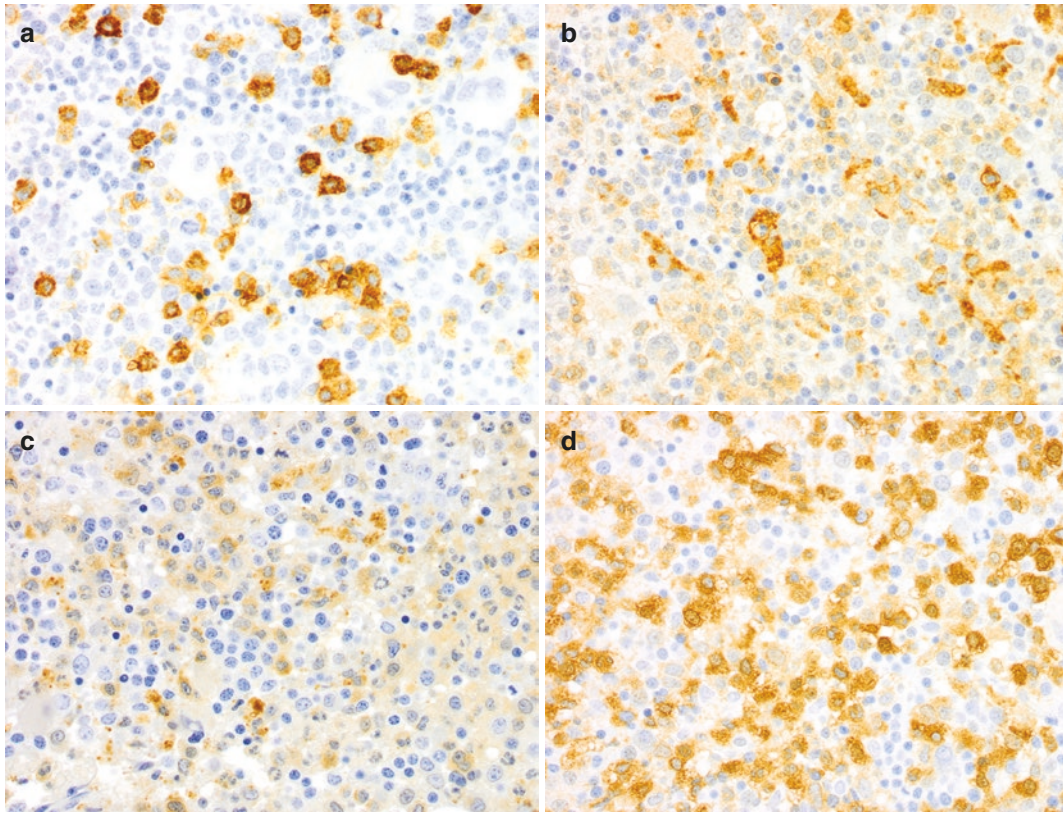

Fig. 7.13 Immunohistochemical profile of chronic myelomonocytic leukemia. (**a**) CD14 is the most sensitive and specific marker. (**b**) CD68 (KP-1) is a lysosomal membrane marker and stains numerous bone marrow cells, including monocytes and macrophages (strong staining) and neutrophils (weak staining). (**c**, **d**) Lysozyme (**c**) and myeloperoxidase (**d**) are both expressed in cells of myeloid lineage, predominantly the early precursors in addition to mature monocytes

or even in extramedullary sites such as the CSF, body fluids, or cutaneous tissue infiltrates. These mature monocytes, which presumably represent a mature circulating component of the clonal leukemic cells, may closely resemble reactive monocytes, thus mimicking a reactive monocytosis or CMML. For these reasons, even if a monocytosis is proven to be clonal, a bone marrow examination is essential prior to rendering a diagnosis of CMML: the presence of greater than 20% blasts and promonocytes in the bone marrow establishes a diagnosis of AML, even if there are few or no circulating blast equivalents in the blood. A diagnosis of CMML requires demonstration that there are <20% blast equivalents in the bone marrow. As discussed above, mature-appearing monocytosis with a *NPM1* mutation is particularly worrisome for a monocytic or myelomonocytic AML.

Once AML has been excluded, it is also important to exclude CML or a prior MDS or MPN prior to rendering a diagnosis of CMML. Chronic myelomonocytic leukemia should have monocytosis at the initial presentation and this diagnosis should not be made in patients already carrying a confirmed diagnosis of MDS or MPN. Classification of cases in which there is some evidence of a prior MDS (such as a history of progressive cytopenias) or MPN (such as megakaryocyte morphology closely resembling that of primary myelofibrosis and/or a concurrent *JAK2* mutation, which is uncommon in CMML), but no documented prior diagnosis, can be difficult. In such cases, a diagnosis of CMML may be rendered, but the possibility of progression of a prior MDS or MPN should be mentioned in the differential diagnosis. CML must always be excluded by testing for *BCR-ABL1* rearrangement by karyotype, FISH, and/or RT-PCR.

In monocytosis with concomitant eosinophilia, the differential diagnosis includes myeloid/lymphoid neoplasms with recurrent genetic abnormalities, especially *PDGFRB* rearrangement. In some of these cases, the peripheral blood eosinophils may not reach 1.5×10^9/L and the bone marrow eosinophil infiltrate may be <20%. Monocytes can exhibit abnormal features resembling CMML. The *PDGFRB* rearrangement is usually not cryptic and conventional chromosomal analysis usually reveals a rearrangement involving 5q32. However, some cases may show a normal karyotype either due to poor cell growth or cryptic *PDGFRB* rearrangement with unknown partners; therefore, *PDGFRB* FISH should be performed in cases of monocytosis with eosinophilia. If there are no recurrent genetic abnormalities and the monocytosis persists, these cases are classified as CMML, despite the presence of eosinophilia.

Situations of intermittent monocytosis, in which some but not all peripheral blood monocyte levels exceed 1×10^9/L and/or some but not all monocyte percentages exceed 10% of all leukocytosis over a 6-month time period, are also difficult to classify. The presence of increased bone marrow monocytes or a mutation pattern typical of CMML (with a combination of *TET2, SRSF2*, or *ASXL1* mutations) tends to favor a diagnosis of CMML in such cases.

Juvenile Myelomonocytic Leukemia

Juvenile myelomonocytic leukemia (JMML) is a rare, highly aggressive disorder of infancy and early childhood. The peripheral blood shows marked leukocytosis with absolute monocytosis (at least 1×10^9/L), as well as neutrophilia and presence of myeloid left-shift. Patients may have anemia and thrombocytopenia. Recent advances in molecular profiling of JMML patients have allowed the simplification and streamlining of the diagnosis of JMML. The current diagnostic criteria are summarized in Table 7.5.

Clinical Features

The majority of cases occur in children younger than 3 years of age. The incidence of JMML is markedly increased in patients with genetic syndromes arising from germline mutations in genes of the RAS/MAPK pathways, such as neurofibromatosis type 1 (NF1 mutation), Noonan syndrome (*PTPN11* mutation), and germline *CBL* mutation [38]. Approximately 10% of cases occur in children with neurofibromatosis type 1. Patients usually present with pallor and fever and may have a skin rash. There is leukocytosis (with a white blood cell count

Table 7.5 Updated WHO diagnostic criteria for juvenile myelomonocytic leukemia

I. Clinical and hematologic features (all four features mandatory)
• PB monocyte count ≥1 × 10^9/L
• Blast percentage in PB and BM <20%
• Splenomegaly
• Absence of Philadelphia chromosome (*BCR-ABL1* rearrangement)
II. Genetic studies (1 finding sufficient)
• Somatic mutation in *PTPN11*[a] or *KRAS*[a] or *NRAS*[a]
• Clinical diagnosis of NF1 or *NF1* mutation
• Germline *CBL* mutation and loss of heterozygosity of CBL[b]
III. For patients without genetic features, besides the clinical and hematologic features listed under I, the following criteria must be fulfilled:
• Monosomy 7 or any other chromosomal abnormality or at least two of the following criteria:
– Hemoglobin F increased for age
– Myeloid or erythroid precursors on PB smear
– GM-CSF hypersensitivity in colony assay
– Hyperphosphorylation of STAT5

[a]Germline mutations (indicating Noonan syndrome) need to be excluded
[b]Occasional cases with heterozygous splice-site mutations

typically between 25 and 35 × 10^9/L, but ranging from 10 to 100 × 10^9/L), absolute monocytosis (ranging from 1 to 60 × 10^9/L), and thrombocytopenia with or without anemia. However, the clinical features are relatively nonspecific and may closely resemble viral illness. There is usually hepatosplenomegaly and often lymphadenopathy. Café au lait spots are seen in patients with NF1.

Morphology

Morphologic evaluation of peripheral blood smear is crucial in suspecting this diagnosis. Peripheral blood examination shows immature monocytes, along with myelocytes, metamyelocytes, and nucleated red blood cells [39] (Fig. 7.15). Bone marrow morphology is relatively nonspecific [39]. It is hypercellular for age and typically shows myeloid predominance, although occasional cases are erythroid rich [40] (Fig. 7.16). Monocytes typically account for 5–10% of the cellularity. Blasts (including promonocytes) represent <20% of all cells. Significant dysplasia is not a feature of JMML. Some cases have marrow fibrosis [40].

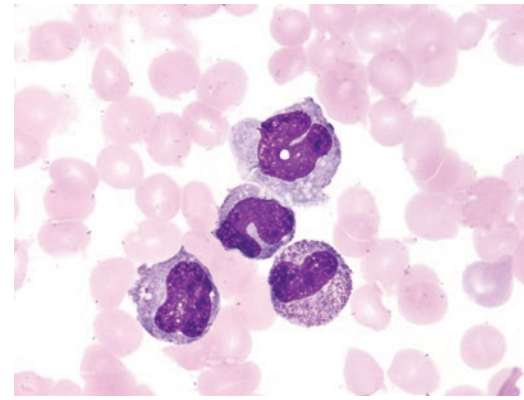

Fig. 7.15 Peripheral blood findings in a 9-month-old boy with juvenile myelomonocytic leukemia. CBC showed WBC of 10.9 × 10^9/L with 28% monocytes and significant myeloid left-shift. Molecular analysis showed evidence of a somatic *PTPN11* mutation

Immunophenotype

Aberrancies in antigen expression of myeloid progenitors are frequently observed in JMML, and the phenotypic abnormalities are similar to those found in adult MDS and CMML [42]. Although there are no specific or characteristic immunophenotypic abnormalities seen in JMML [40, 41], abnormal flow cytometry findings help to differentiate a neoplastic from reactive monocytosis.

Cytogenetics and Molecular Genetics

The majority of cases have a normal karyotype [41]. Monosomy 7 is present in ~25% of patients and ~10% of patients have evidence of other cyto-

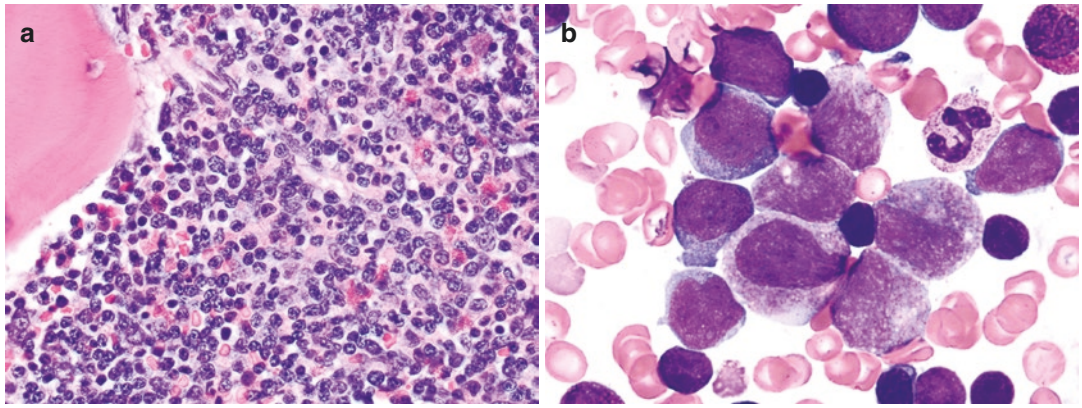

Fig. 7.16 Bone marrow findings in juvenile myelomonocytic leukemia. (**a**) The biopsy cellularity is markedly increased for age and myeloid maturation is left-shifted. Megakaryocytes are decreased in number and there are numerous hematogones. Monocytic cells are not conspicuous. (**b**) In the aspirate smear, the myeloid-to-erythroid ratio is increased due to increased granulopoiesis. Myeloid cells are left-shifted and there are scattered monocytes

genetic abnormalities [41]. Approximately 90% of patients carry either somatic or germline mutations of RAS pathway genes, including *PTPN11, KRAS, NRAS, CBL,* or *NF1* genes [43]; these genetic aberrations are largely mutually exclusive and activate the RAS/RAF/MAPK pathway.

Differential Diagnosis

In cases of suspected JMML where there is no clonal cytogenetic abnormality, all possible causes of infection and as well as autoimmune diseases should be excluded prior to rendering a diagnosis of JMML. Transient myeloproliferation in the setting of Down syndrome may mimic JMML. Finally, it is important to note that some children with Noonan syndrome, particularly those with heterozygous germline *PTPN11* mutation, may develop a transient myeloproliferative disorder in early infancy; in contrast to JMML, this can be transient with a benign course, although a subset of patients may subsequently acquire additional somatic genetic aberrations and develop overt JMML [44].

Acute Myeloid Leukemia with Monocytic Differentiation

This subtype of AML comprises acute myelomonocytic leukemia (AML-M4), acute monoblastic leukemia (AML-M5a), and acute monocytic leukemia (AML-M5b), as well as certain subtypes of AML with recurrent genetic abnormalities (see below). Acute myelomonocytic leukemia is characterized by a proliferation of both neutrophilic and granulocytic precursors with at least 20% bone marrow monocytic cells. It represents 5–10% of AML cases. Acute monoblastic leukemia is characterized by >80% monocytic cells with a predominance of monoblasts, while acute monocytic leukemia contains >80% monocytic cells with a predominance of promonocytes.

Up to a third of patients with CMML have disease progression to AML and most of these cases show monocytic differentiation, either myelomonocytic or monoblastic/monocytic variants [45]. According to the revised WHO Classification, these cases are classified as AML with myelodysplasia-related changes.

Clinical Features

Patients typically present with symptoms related to anemia or thrombocytopenia. Numerous mature-appearing monocytes may be present in the blood. Patients with AML following a diagnosis of CMML tend to have a significantly higher WBC and absolute monocyte count compared to de novo AML patients [45]. Acute monoblastic leukemia is more common in younger adults, while acute monocytic leukemia

is more frequent in older individuals. Patients with AML with monocytic features are particularly prone to present with extramedullary disease, including lymphadenopathy, organomegaly, pulmonary edema, skin and gingival infiltration, and central nervous system involvement.

Morphology

Monoblasts are large cells with abundant, moderately to intensely basophilic cytoplasm, which may demonstrate pseudopod formation, scattered fine azurophilic granules, and vacuoles. Nuclei are round with delicate lacy chromatin and one or more prominent nucleoli (Fig. 7.17a, b). Promonocytes are large cells with intermediate features between monoblasts and immature monocytes. They have less basophilic and sometimes more obviously granulated cytoplasm with occasional large azurophilic granules and vacuoles. The nucleus is irregular with a delicately convoluted configuration. A nucleolus may be present (Fig. 7.17c, d). However, the distinction between promonocytes and other abnormal marrow elements such as dysplastic myeloid precursors or immature monocytes is notoriously subjective and may be difficult (Fig. 7.18); moreover, cases of AML with monocytic differentiation may display a mature-appearing monocytosis in the blood and may have few or no circulating monoblasts or promonocytes, despite their presence in the bone marrow. The distinction between promonocytes and monoblasts can also be challenging; however it is not as critical, since both are considered blast equivalents.

Bone marrow examination typically shows increased cellularity due to extensive prolifera-

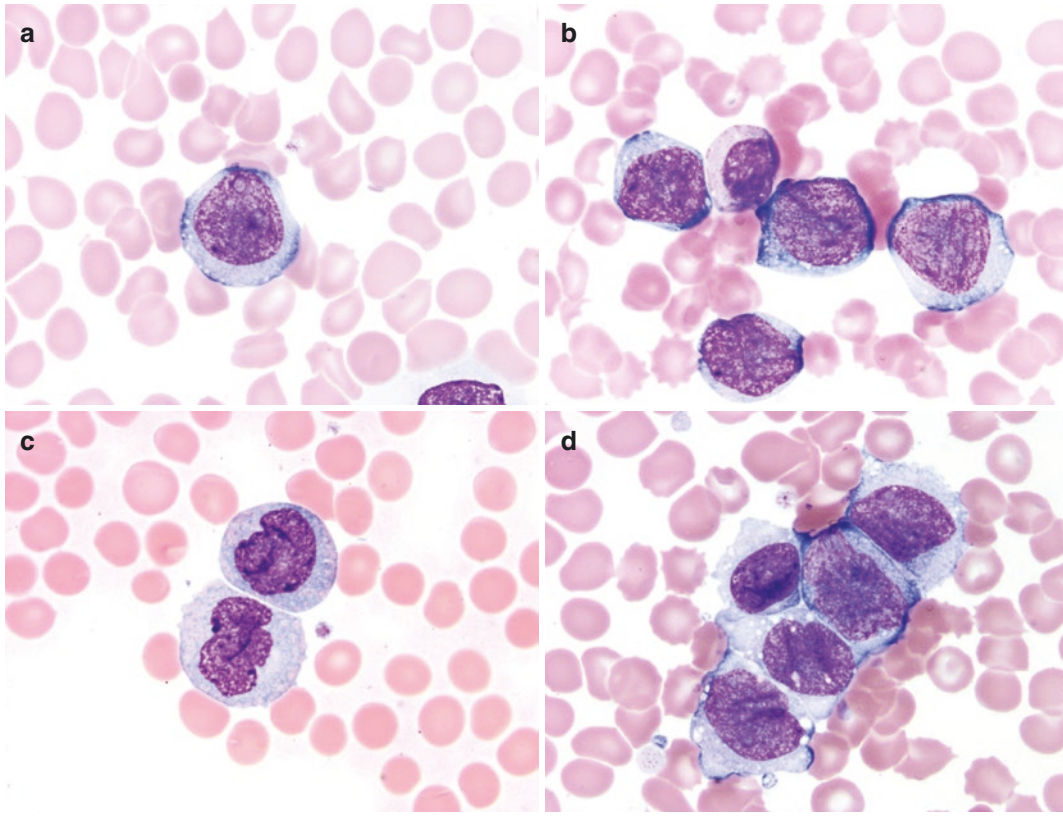

Fig. 7.17 Monoblasts and promonocytes in peripheral blood. (**a**, **b**) Monoblasts have abundant basophilic vacuolated cytoplasm and round nuclei with delicate lacy chromatin and one or more large prominent nucleoli. (**c**, **d**) Promonocytes have more abundant and more granulated cytoplasm with occasional large azurophilic granules and vacuoles. Compared to the monoblast, promonocyte nucleus is more irregular with a lobulated convoluted configuration. Small nucleoli are frequently present

tion of neoplastic immature monocytic cells (Figs. 7.19, 7.20, and 7.21). Normal trilineage hematopoiesis is markedly decreased or absent. AML cases that have progressed from a prior CMML have high bone marrow cellularity and increased bone marrow monocyte percentage. In one study the median bone marrow blast count in secondary AML cases following CMML was 40%, which was lower than in de novo acute myeloid leukemia [45] (Fig. 7.22a, b).

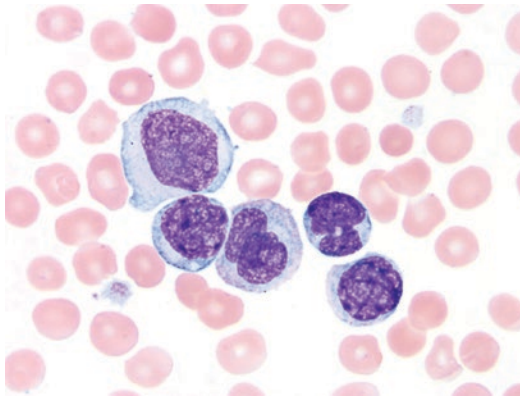

Fig. 7.18 Peripheral blood with marked immature monocytosis. There is a spectrum of morphology ranging from immature monocytes to promonocytes and blasts. The morphologic distinction between these elements may be subjective and somewhat arbitrary

Occasionally, cuplike nuclear invaginations are present in a proportion of myeloid blasts in cases of AML associated with *NPM1* and/or *FLT3* mutations [46, 47]. AML with cuplike blasts is typically defined as presence of ≥10% blasts with at least one prominent nuclear invagination that spans ≥25% of the greatest nuclear dimension (Fig. 7.23) [47]. The cutoff of ≥10% cuplike blasts appears to be highly specific for *NPM1* mutation-positive cases, although the sensitivity is low [48].

Immunophenotype and Cytochemistry

The monoblasts, promonocytes, and monocytes are usually positive with nonspecific esterase by cytochemistry, although absence of NSE does not exclude that cells are of monocytic derivation

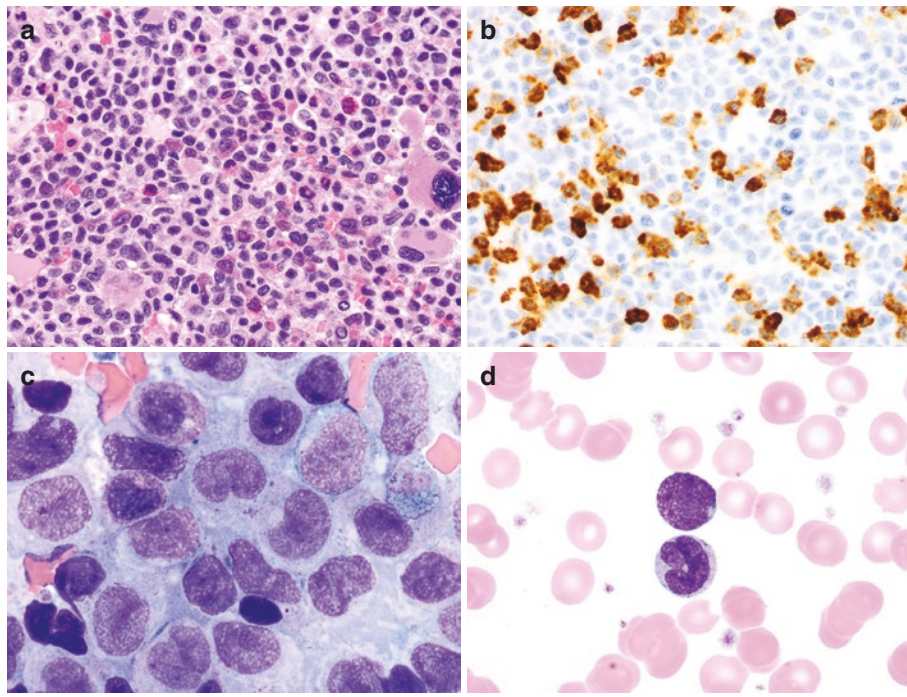

Fig. 7.19 Acute myelomonocytic leukemia. (**a**) The bone marrow biopsy is markedly hypercellular with myeloid predominance and an increase in blasts. Focally, maturing granulopoiesis is also present. (**b**) CD14 highlights an increased number of mature monocytes. (**c**) The bone marrow aspirate demonstrates an increase in blasts and monocytes. (**d**) The peripheral blood shows circulating blasts as well as mature monocytosis

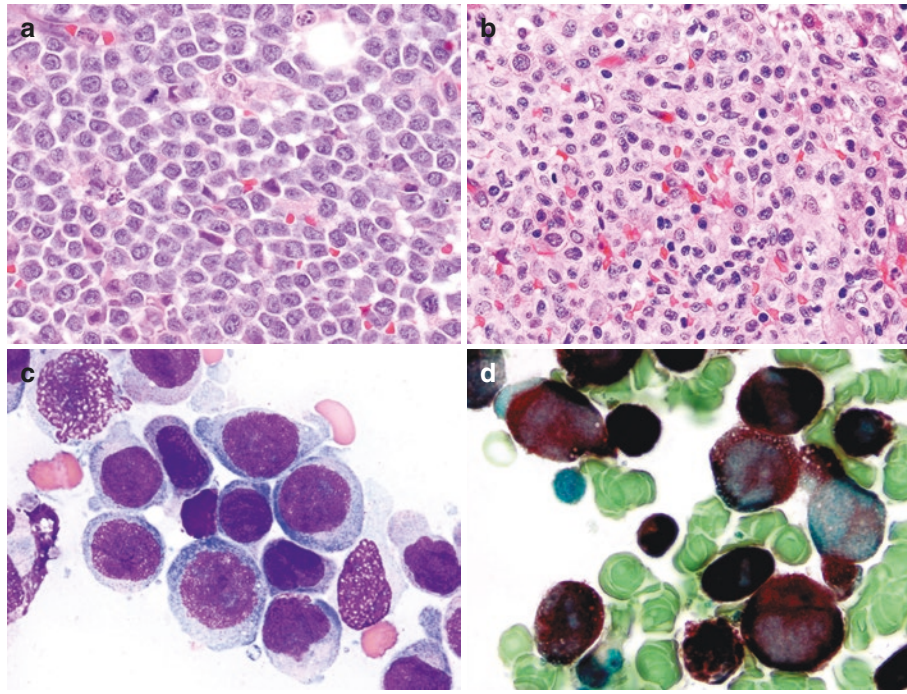

Fig. 7.20 Acute monoblastic leukemia. (**a**, **b**) Normal bone marrow elements are extensively replaced by sheets of blasts with monoblastic (**a**) or mixed monoblastic and monocytic morphology (**b**). (**c**) The bone marrow aspirate shows numerous monoblasts and occasional promonocytes. (**d**) NSE cytochemical stain is positive in the monoblasts

(Fig. 7.24a, b). Monoblasts are usually negative with myeloperoxidase cytochemistry, while promonocytes and monocytes can be weakly to moderately MPO positive (Fig. 7.24c, d). However it is possible to find cases where monoblasts are positive for both MPO and NSE.

Based on flow cytometry, myeloid antigens are variably expressed by the neoplastic cells and there is usually positivity for at least two markers of monocytic differentiation, such as CD14, CD4, CD11b, CD11c, CD64, and CD36, (Fig. 7.25a). Expression of NG2 by flow cytometry has been found to be a highly sensitive marker of *KMT2A (MLL)* gene rearrangement (see below) [49]. In acute myelomonocytic leukemia, there are often two populations of blasts; myeloblasts and monocytic blasts. The myeloid blasts typically express CD34 and CD117, while these markers are often negative in the monoblasts and promonocytes (Fig. 7.25b). Acute monoblastic and monocytic leukemia is composed almost exclusively of monocytic blasts (Fig. 7.26a). At the initial diagnosis, the CD34+ myeloblasts may be too low in numbers to characterize further. But if present in a discernible population, documentation of the immunophenotype is important: these CD34+ cells are more resistant to chemotherapy and can be detected in patients with minimal residual disease (MRD) post-induction chemotherapy (Fig. 7.26b). The monocytic blasts are negative for CD34 and are mostly negative for CD117 or only express CD117 in a small subset. Monocytic blasts share significant immunophenotypic overlapping with reactive or immature monocytes. They all express CD4, CD11b, CD11c, CD33, CD64, and CD36. While a significant decrease or loss of CD14 indicates immaturity, normal expression of CD14 does not guarantee maturity of monocytic cells. CD56 expression neither differentiates immature from mature nor abnormal from normal monocytes. Several changes that are often observed in immature monocytes include markedly decreased CD13, decreased or

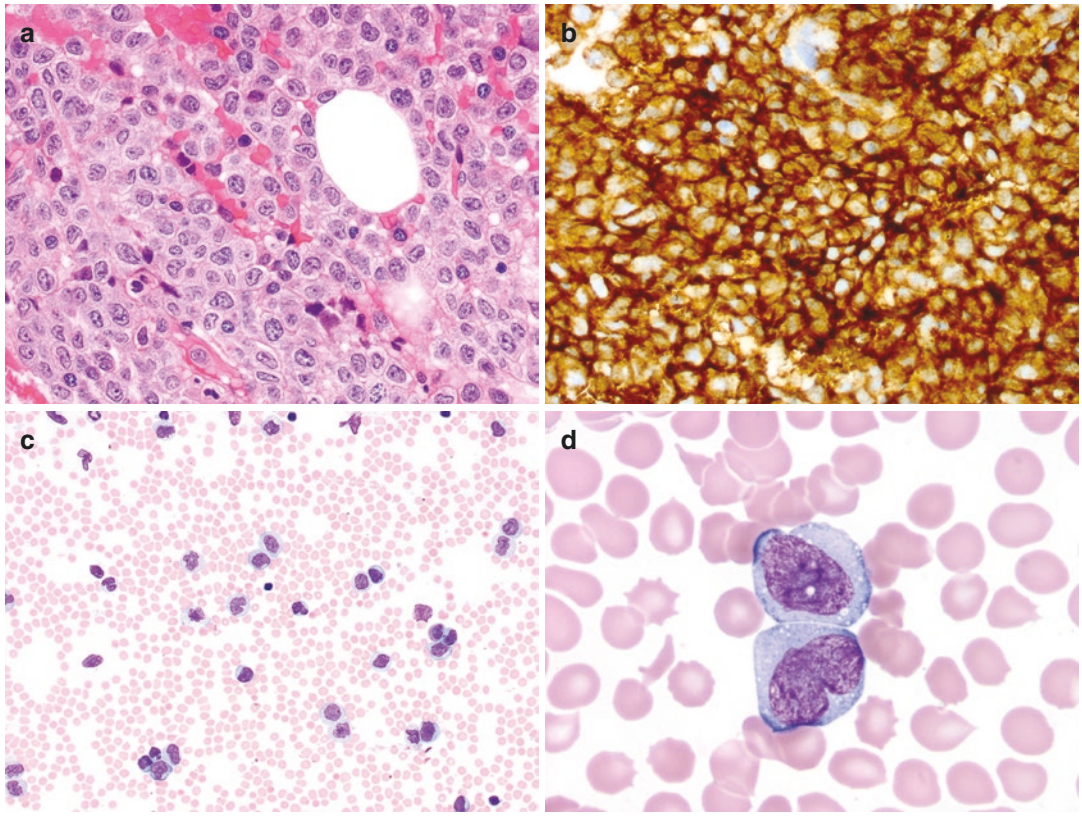

Fig. 7.21 Acute monocytic leukemia. (**a**) The bone marrow elements are replaced by immature cells with markedly irregular nuclear contours and high nuclear-to-cytoplasmic ratio (promonocytes). (**b**) Promonocytes are typically negative for the blast markers CD34 and CD117, limiting the utility of immunohistochemistry. Immunophenotypic aberrancies may be useful on a case-by-case basis: in this case, the neoplastic cells strongly overexpress CD56. (**c**) At presentation, the WBC was 76×10^9/L with 80% immature monocytic cells, which were composed of promonocytes (**d**)

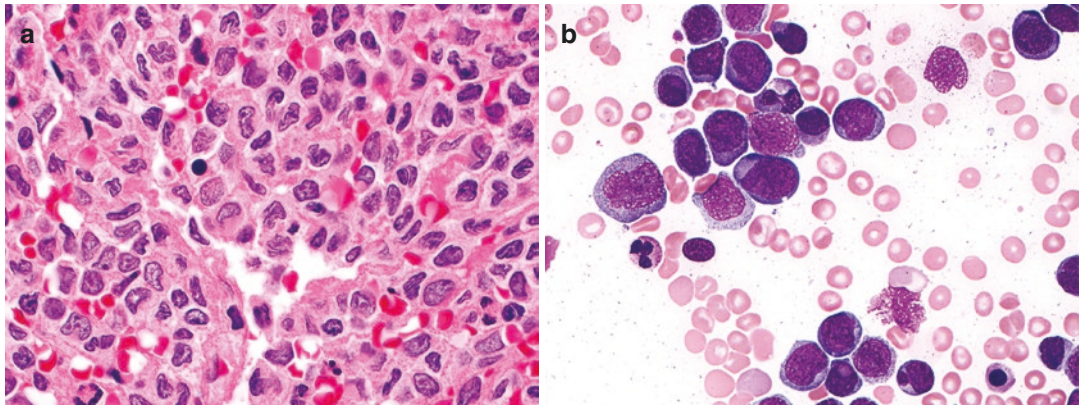

Fig. 7.22 AML following chronic myelomonocytic leukemia. (**a**) The bone marrow biopsy has high cellularity composed predominantly of immature monocytic cells with folded and angulated nuclei. (**b**) The bone marrow aspirate has an increased proportion of blasts, promonocytes, and immature monocytes (images courtesy of Dr. Elizabeth Courville, University of Minnesota)

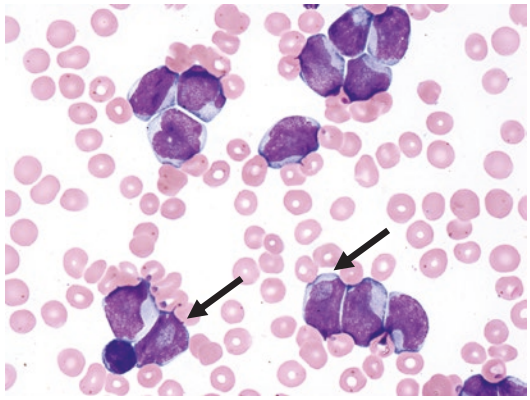

Fig. 7.23 AML with "cuplike" morphology in the peripheral blood (arrows). Such cases are strongly associated with monocytic differentiation and single *NPM1* or co-occurring mutations of *NPM1* and *FLT3*. This patient had concurrent *NPM1* and *FLT3* mutations (courtesy of Dr. Robert P Hasserjian, Massachusetts General Hospital)

absent CD14, and increased CD15 expression (Fig. 7.26a). In acute monocytic leukemia progressed from CMML, there is almost always a small population of abnormal CD34+ myeloblasts. These cells are also present in the CMML disease phase and can frequently be detected after chemotherapy. One multiparameter flow cytometry study found higher percentages of blasts and monocytic cells in AML with monocytic differentiation compared with the presence of more granulocytic cells in CMML [50].

Cytogenetics and Molecular Genetics

Certain recurrent genetic abnormalities are associated with a monocytic phenotype in AML [51]. AML with inv(16)(p13.1q22);*CBFB-MYH11* corresponds to acute myelomonocytic

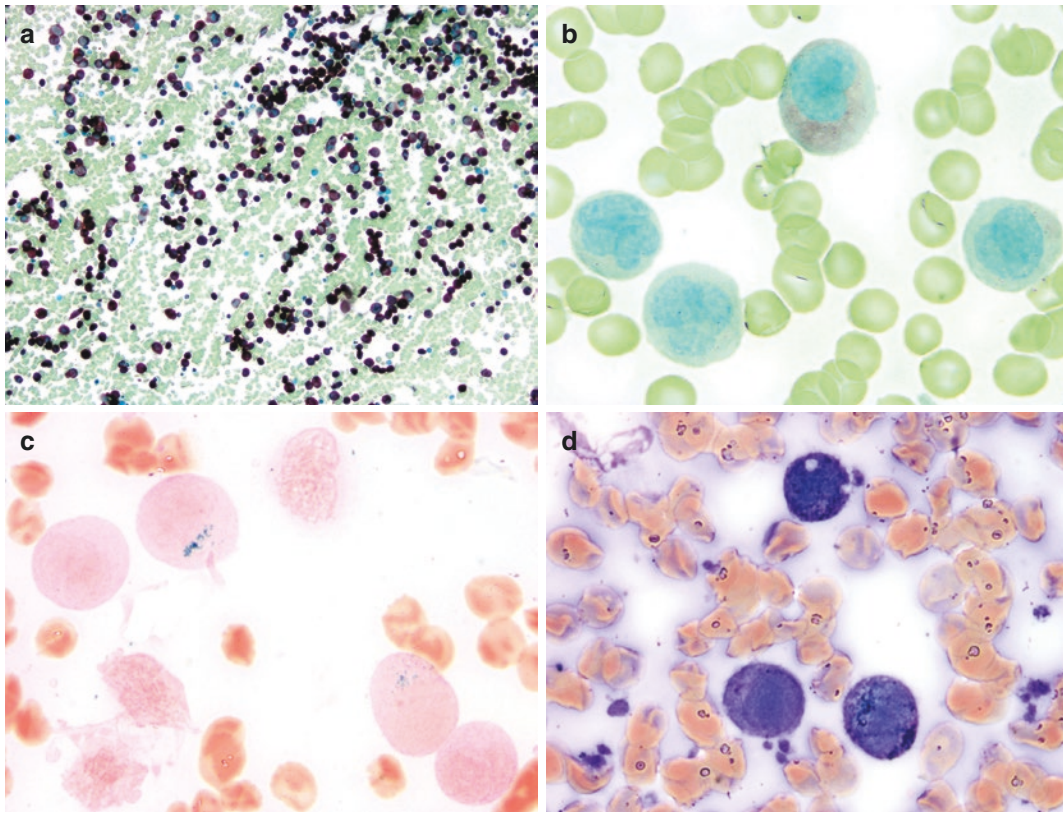

Fig. 7.24 Cytochemistry in acute monocytic/monoblastic leukemia. (**a**) NSE shows strong diffuse staining in the vast majority of the blasts in most cases. (**b**) However, absence of NSE staining of blasts does not exclude monocytic derivation. (**c**) Monoblasts are usually negative with myeloperoxidase cytochemical stain. (**d**) Promonocytes and monocytes are typically weakly positive by myeloperoxidase cytochemistry

leukemias with monocytic differentiation and an increased number of eosinophils with immature large purple granules (Fig. 7.27). AML with t(9;11)(p21.3;q23.3);*MLLT3-KMT2A,* as well as AML with other variant *KMT2A* rearrangements, usually presents as acute monoblastic or acute monocytic leukemia (Fig. 7.28). Translocation t(8;16)(p11.2;p13.3) may be seen in a subset of acute monocytic leukemia or acute myelomonocytic leukemia and typically is associated with hemophagocytosis by the neoplastic cells.

More recently, molecular profiling of de novo AML patients also revealed close genotype–phenotype associations. There appears to be a significant association between AML with monocytic differentiation and *NPM1, DNMT3A, TET2, and KRAS* mutations [52, 53]. *NPM1* mutation, in particular, appears very frequent in acute leukemia with monocytic differentiation

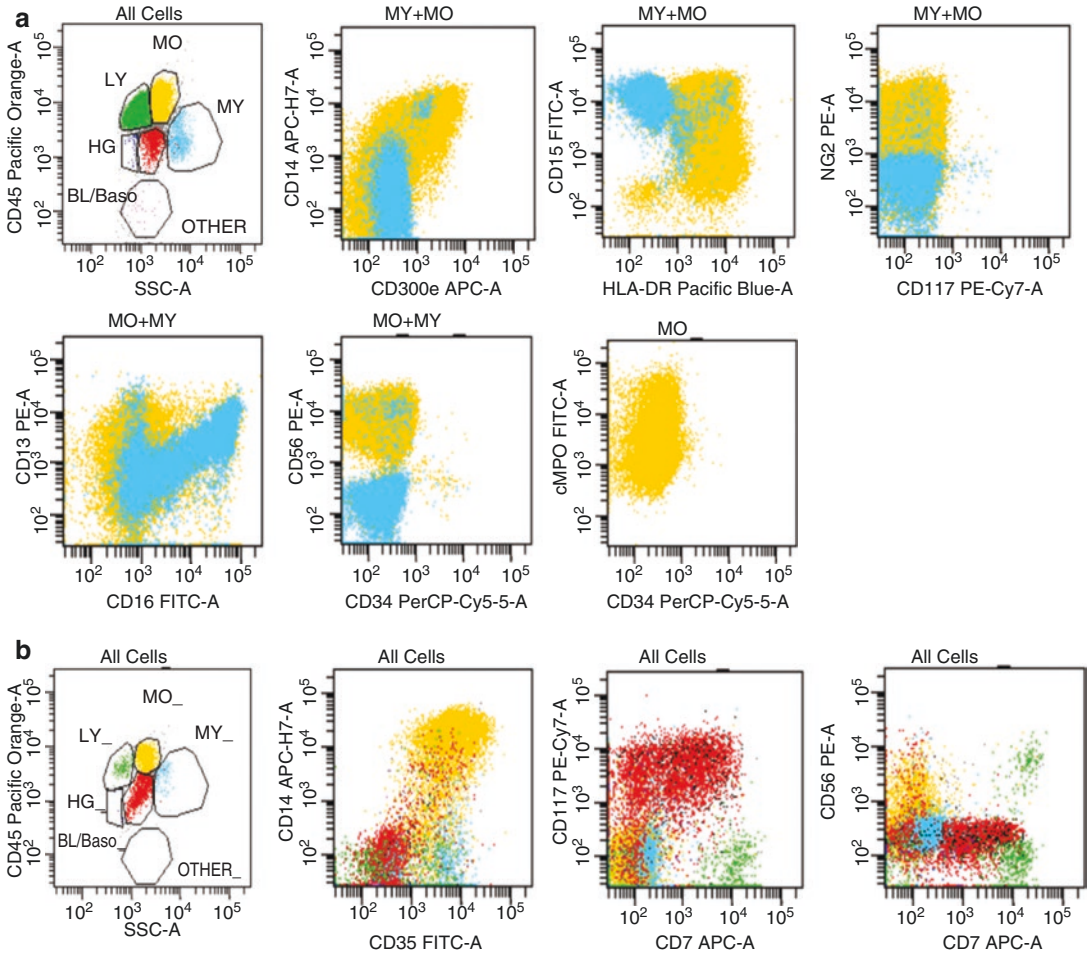

Fig. 7.25 Flow cytometry immunophenotype of AML with myelomonocytic differentiation. (**a**) There is a markedly increased number of monocytic cells, which have bright CD45 and moderate side scatter (yellow). They demonstrate variable expressions of mature monocytic markers CD14 and CD300e, which correlated with partial positivity for MPO. Neoplastic cells show partial expression of CD15, absent CD16, and aberrant uniform expression of CD56. The granulocytes are highlighted in blue. Overexpression of NG2 correlates with presence of *KMT2A (MLL)* rearrangement. (**b**) These leukemias frequently have several populations of blasts as illustrated here. Abnormal monocytic cells (yellow) have moderate side scatter and express bright CD45, CD14, and CD35. These cells show aberrant expression of CD56. A separate population of myeloid blasts (red) have dim CD45 and CD117 and aberrantly express CD7. These blasts are negative with monocytic markers or CD56

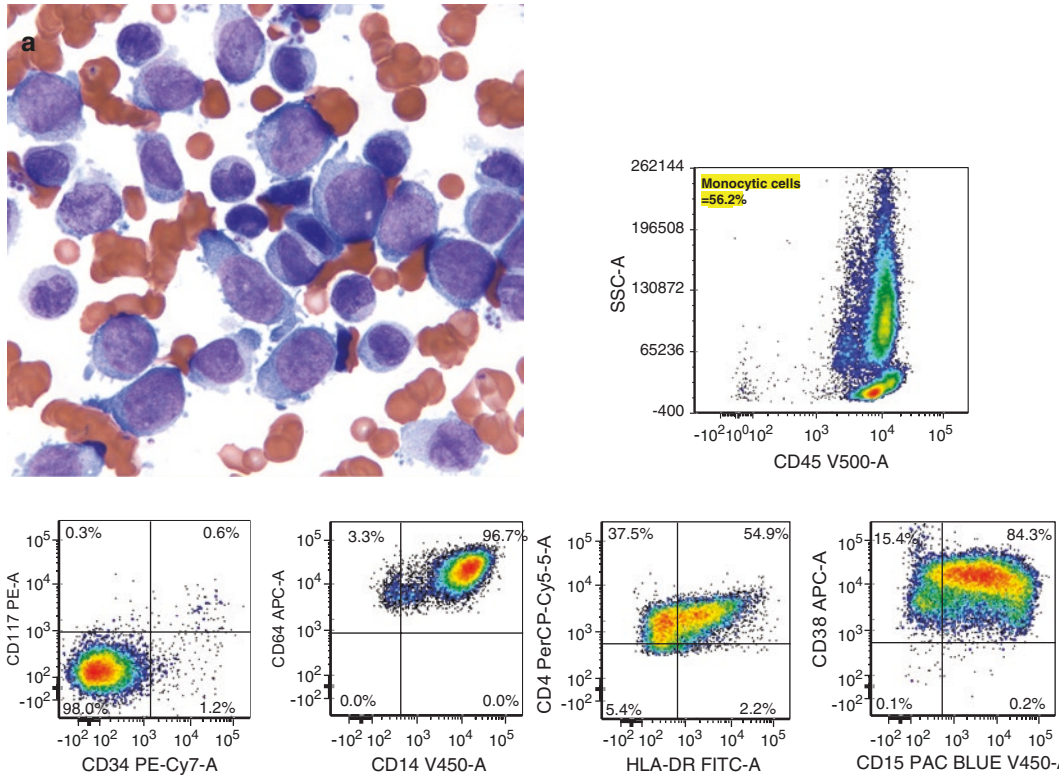

Fig. 7.26a Flow cytometry immunophenotype of AML with monocytic differentiation. (**a**) In acute monoblastic leukemia, the blasts are composed predominantly of monoblasts (upper left); on CD45/SSC, the monocytic cells are markedly increased 56.4%, granulocytes are markedly reduced to absent (upper right). The monocytes are negative for CD34 and CD117 (lower left) and uniformly positive for CD64 and CD14. While loss of CD14 indicates immaturity, expressing CD14 does not guarantee maturity of monocytes; the monocytes show markedly decreased HLA-DR and increased CD15 expression (lower right). Increased CD15 is often associated with immaturity of monocytes.

and has been extensively studied. In one large study, *ASXL1* mutations were significantly associated with a monoblastic differentiation and *DNMT3A* mutations with a monocytic phenotype within the *NPM1* mutated cohort of patients with AML [52]. AML with mutated *NPM1* usually has a normal karyotype.

Differential Diagnosis

The differential diagnosis from CMML has been discussed in the CMML section above. The other most important differential diagnosis with AML with monocytic differentiation is the microgranular variant of acute promyelocytic leukemia (APML) with *PML-RARA*. Morphologically, the blasts and promyelocytes of microgranular APML blasts have frequently bilobed or "butterfly-shaped" nuclei, compared to the more rounded and irregularly lobulated nuclei of true monoblasts and promonocytes (Fig. 7.17), or the "cup-shaped" blasts of AML with *NPM1* and/or *FLT3* mutations (Fig. 7.23). Frequent Auer rods tend to favor a diagnosis of APML, although myelomonocytic AML may also display Auer rods. Immunophenotype can be very helpful, as microgranular APML is negative for CD11b and CD11c and shows uniformly strong MPO expression by both cytochemistry and flow cytometry [54]. In ambiguous cases, a rapid assessment for *PML-RARA* rearrangement (by conventional karyotyping, FISH, RT-PCR, and/or immunofluorescent staining of nuclear PML protein) should be performed prior to instituting induction chemotherapy, since APML patients should be treated with all-trans retinoic acid (ATRA) prior to receiving any cytolytic chemotherapy.

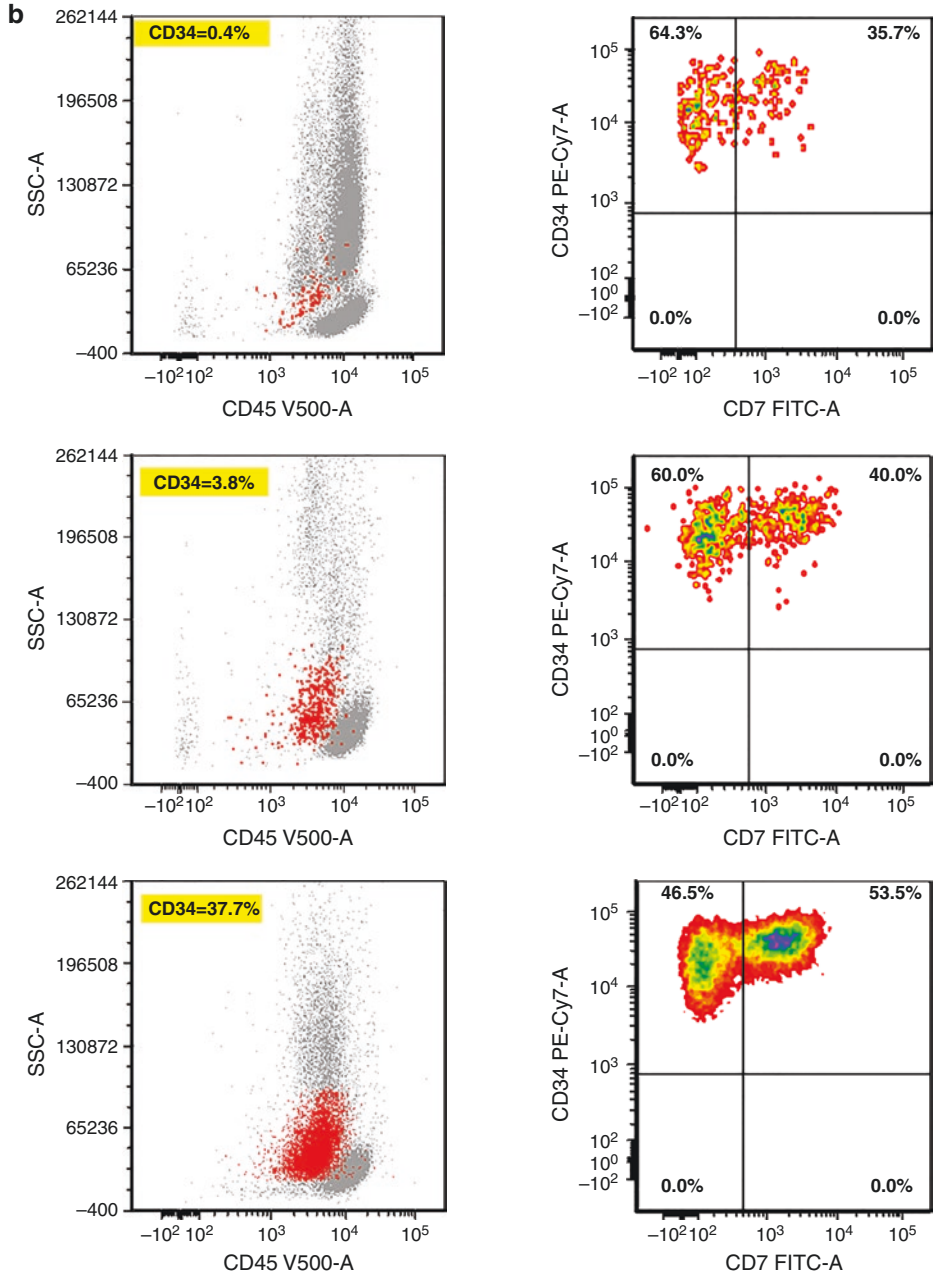

Fig. 7.26b The same case of acute monoblastic leukemia as shown in (**a**). At the time of diagnosis, the blasts were monoblastic; only 0.4% CD34+ myeloblasts were detected (upper left), showing increased CD45 with aberrant CD7 expression (upper right). Status post-induction (middle panel), in this hypocellular bone marrow sample, most of the monoblasts were eradicated; however, the same aberrant CD34+ myeloblasts were detected at 3.8%. The lower panel shows AML relapse, the blasts were exclusively of CD34+ myeloblasts showing the same aberrant CD7 expression (courtesy of Sa A Wang, MD Anderson Cancer Center)

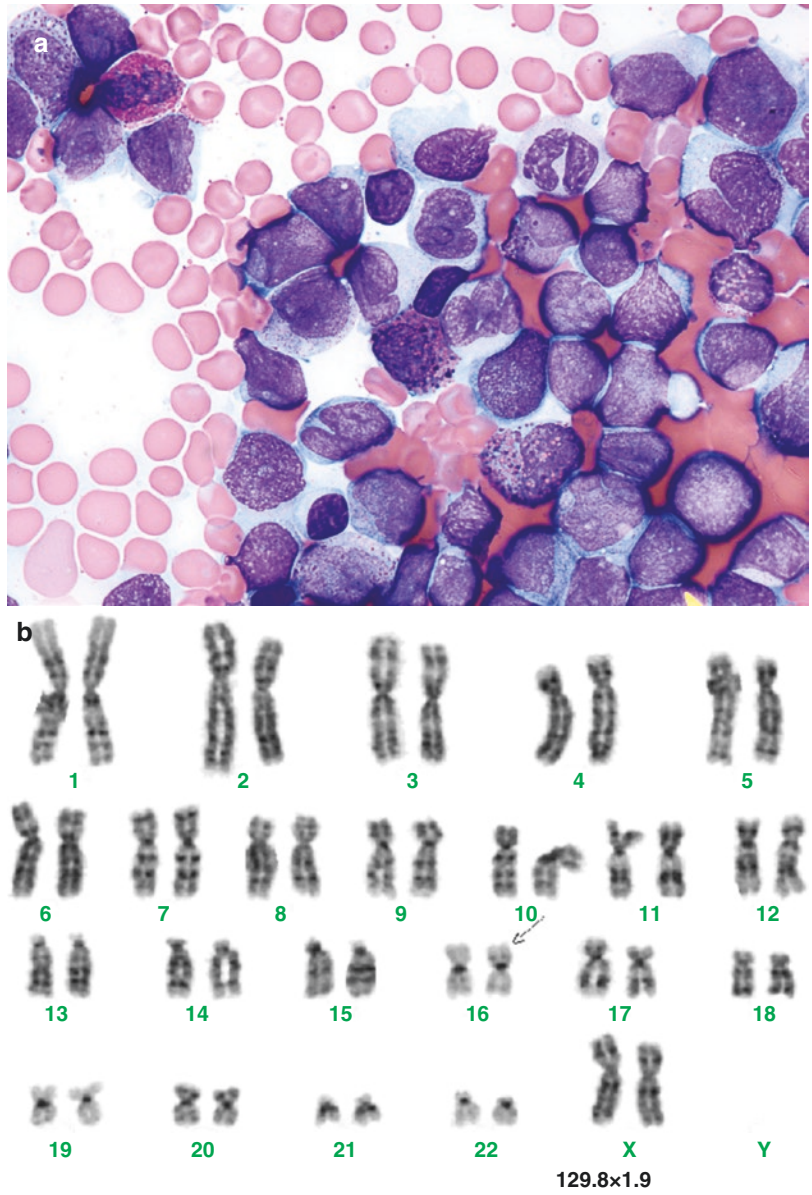

Fig. 7.27 AML with inv(16)(p13.1;q22). (**a**) The morphology has features of acute myelomonocytic leukemia, with a variable increase in bone marrow eosinophils. (**b**) Conventional cytogenetics shows a rearrangement on chromosome 16 (arrow), which may occasionally be overlooked. If morphologic features raise suspicion for AML with inv(16)(p13.1;q22), FISH analysis could be performed for confirmation

Myeloproliferative Neoplasms and Myelodysplastic Syndromes with Monocytic Proliferation

Primary myelofibrosis (PMF) is a myeloproliferative neoplasm characterized by megakaryocytic proliferation and atypia, accompanied by increased age-adjusted cellularity, increased granulopoiesis, and fibrosis [14, 51]. Primary myelofibrosis has the most aggressive clinical course among the JAK2-associated myeloproliferative neoplasms and the highest progression to bone marrow failure and/or AML. Some of these patients have an intervening acceler-

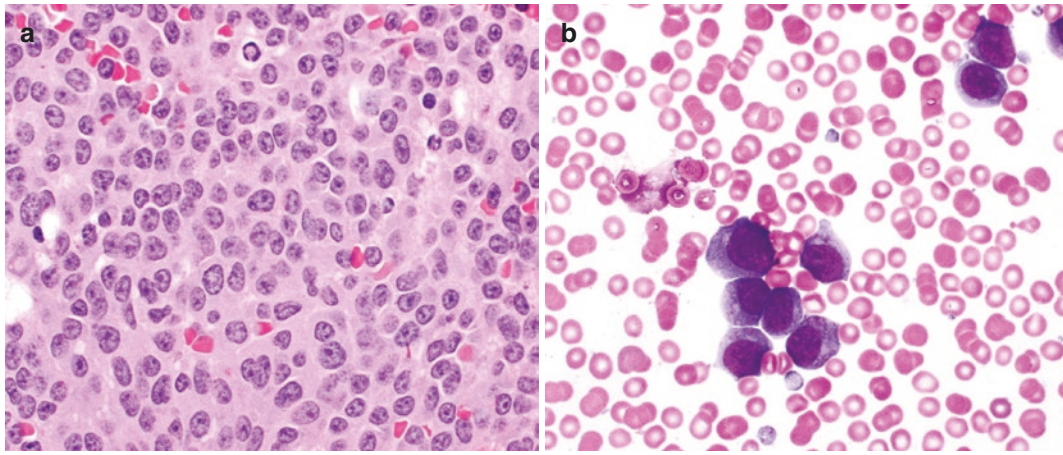

Fig. 7.28 AML with t(9;11)(p22;q23); *MLLT3-KMT2A*. (**a**) The bone marrow biopsy shows sheets of large monoblasts. (**b**) The peripheral blood has numerous blasts with features of monoblasts

ated phase and/or myelodysplastic syndrome-like progression, characterized by the presence of an increased number of blasts and often multilineage dysplasia including numerous micromegakaryocytes. A subset of these patients may also develop monocytosis, with CMML-like morphology developing during the course of disease [55]. Higher than expected prevalence of monocytosis has also been found in patients with polycythemia vera (PV); PV patients with monocytosis have a mutation profile and age distribution akin to those of patients with CMML [56]. Chronic myeloid leukemia patients frequently have absolute monocytosis at diagnosis and the presence of *BCR-ABL1* rearrangement excludes a diagnosis of CMML. While the presence of a more prominent granulocytic proliferation with eosinophilia and basophilia is an important clue to the diagnosis of CML rather than CMML, some CML patients may have a monocytosis that is more prominent than the granulocytic proliferation. This monocytic proliferation of CML is most commonly associated with the p190 isoform of the *BCR-ABL1* rearrangement.

Patients with MDS may also develop persistent absolute monocytosis after the initial diagnosis [57]. It is important to note that in cases of myeloproliferative neoplasms or MDS that develop a persistent monocytosis, the disease classification should not be changed to CMML, which requires that monocytosis be present at the time of initial diagnosis. Rather, the original diagnosis category should be retained, but the monocytosis noted as a type of progression.

Clinical Features

PMF patients typically have rapid disease progression and short survival following development of monocytosis [55]. In addition, the presence of absolute monocytosis at the time of initial diagnosis has been found to be associated with inferior survival in younger patients with primary myelofibrosis and in patients presenting with PV [56, 58, 59]. CMML-like progression in MDS is also associated with short survival and more frequent progression to AML [57].

Morphology

The clinical development of peripheral blood monocytosis in PMF is frequently associated with a marked bone marrow myelomonocytic proliferation and a high number of bone marrow neutrophils and monocytes [55]. This proliferation can be associated with the presence of increased number of hypolobated megakaryocytes (Fig. 7.29).

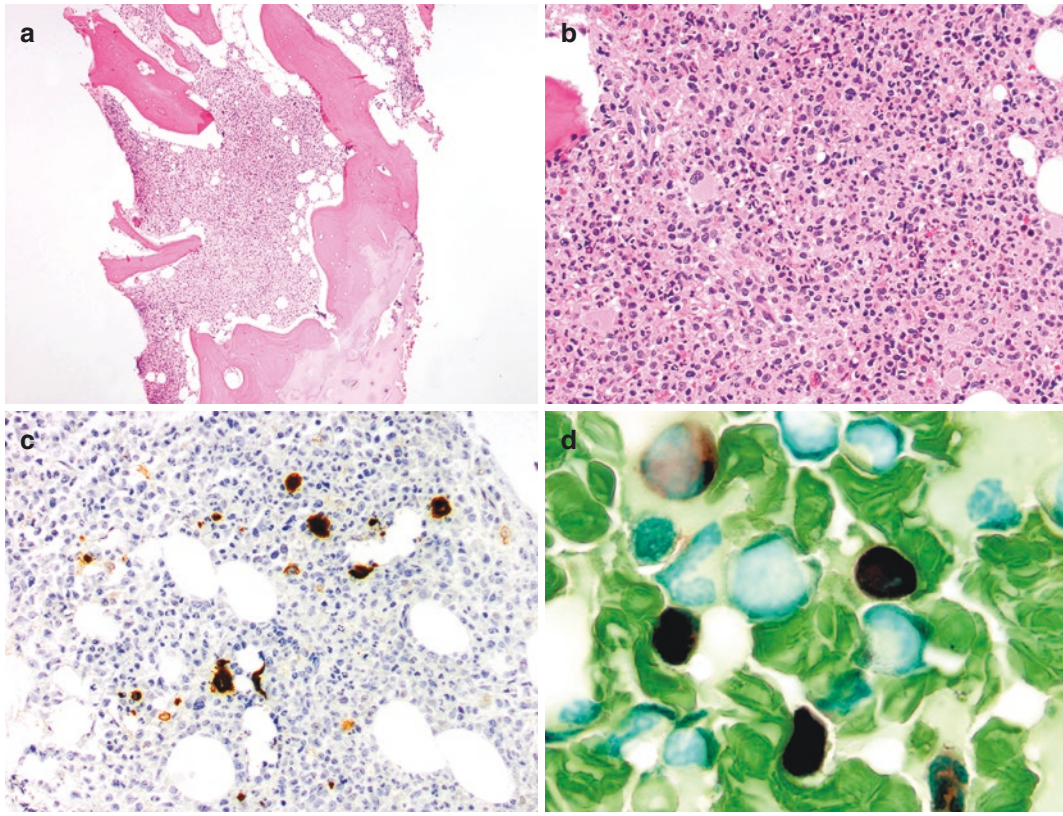

Fig. 7.29 Bone marrow biopsy in a patient with a 10-year history of marrow biopsy-confirmed primary myelofibrosis and persistent absolute monocytosis for 1 year. (**a**) The bone marrow biopsy shows evidence of osteosclerosis. Marrow cellularity is markedly increased. (**b**) A myelomonocytic proliferation with a high number of neutrophils and monocytes is noted. Megakaryocytes are predominantly small and hypolobated. (**c**) CD42b immunohistochemistry highlights the atypically small and dysplastic appearing megakaryocytes. (**d**) NSE cytochemistry on bone marrow touch preps shows an increased number of monocytic cells

Immunophenotype

Flow cytometry is not very informative in cases of advanced primary myelofibrosis, since the marrow aspirate samples are aspicular and hemodilute. With immunohistochemistry, the number of marrow monocytes is increased in a subset of the patients [55].

Cytogenetics and Molecular Genetics

There is no cytogenetic evolution or change in *JAK2* mutation status associated with the development of monocytosis in PMF [55], nor is there a known genetic correlate of CMML-like progression in MDS. PV patients with absolute monocytosis had significantly more frequent *SRSF2* and *TET2* mutations, compared to PV patients without monocytosis [56]. Interestingly, the p190 isoform of the *BCR-ABL1* rearrangement is associated with a predominantly monocytic proliferation mimicking CMML [60]. Thus, it is critical to perform *BCR-ABL1* testing that excludes a p190-type rearrangement in all cases of putative CMML. Such testing could be conventional bone marrow karyotype, FISH for BCR-ABL1 rearrangement, and/or qualitative *BCR-ABL1* RT-PCR that assesses for p190 rearrangement. However, many quantitative *BCR-ABL1* RT-PCR tests do not evaluate for the p190 isoforms, potentially leading to misdiagnosis of a CML with monocytosis as CMML if

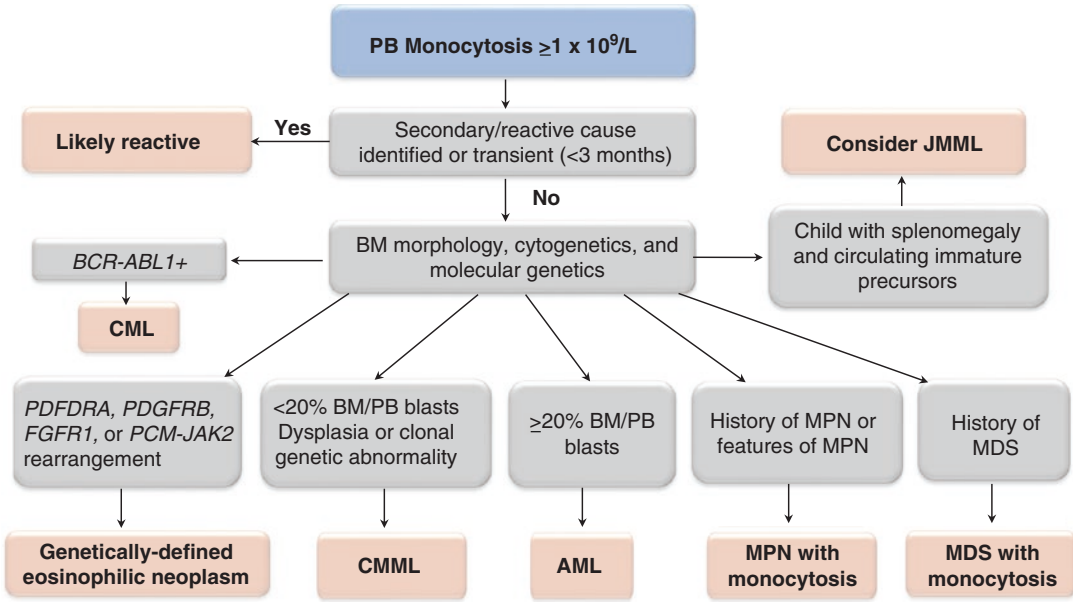

Fig. 7.30 Algorithm for suggested workup of peripheral blood monocytosis. *CML* chronic myeloid leukemia, *BCR-ABL1+*, *MDS* myelodysplastic syndrome, *MPN* myeloproliferative neoplasm, *CMML* chronic myelomonocytic leukemia, *JMML* juvenile myelomonocytic leukemia, *AML* acute myeloid leukemia, *BM* bone marrow, *PB* peripheral blood

this is the only up-front *BCR-ABL1* assessment that is performed (Fig. 7.30).

Differential Diagnosis

Monocytic progression of MDS and MPN must be distinguished from CMML; the latter is a disease characterized by monocytosis at the initial presentation, without a documented history of MDS or MPN. CML may present with monocytosis at the initial presentation. These cases almost always have a concomitant leukocytosis and can be distinguished from CMML by confirming the presence of *BCR-ABL1* rearrangement, as discussed above.

References

1. Italiani P, Boraschi D. From Monocytes to M1/M2 Macrophages: Phenotypical vs. Functional Differentiation. Front Immunol. 2014;5:514.
2. Nguyen KD, Fentress SJ, Qiu Y, Yun K, Cox JS, Chawla A. Circadian gene Bmal1 regulates diurnal oscillations of Ly6C(hi) inflammatory monocytes. Science (New York, NY). 2013;341(6153):1483–8.
3. Dutta P, Nahrendorf M. Regulation and consequences of monocytosis. Immunol Rev. 2014;262(1):167–78.
4. Shi C, Pamer EG. Monocyte recruitment during infection and inflammation. Nat Rev Immunol. 2011;11(11):762–74.
5. Reale MA, Yen Y, Strair RK, Flynn SD, Cooper DL. Pseudoleukemia after granulocyte colony-stimulating factor therapy. South Med J. 1995;88(4):462–4.
6. Liu CZ, Persad R, Inghirami G, Sen F, Amorosi E, Goldenberg A, et al. Transient atypical monocytosis mimic acute myelomonocytic leukemia in post-chemotherapy patients receiving G-CSF: report of two cases. Clin Lab Haematol. 2004;26(5):359–62.
7. Koenigsmann M, Hiddemann W, Buchner T. Reversible leukaemic regrowth under GM-CSF treatment after chemotherapy for AML. Leuk Res. 1991;15(1):37–41.
8. Dunphy CH. Comparative analysis of detecting monocytic cells and their aberrancy. Appl Immunohistochem Mol Morphol. 2011;19(4):336–40.
9. Ingersoll MA, Spanbroek R, Lottaz C, Gautier EL, Frankenberger M, Hoffmann R, et al. Comparison of gene expression profiles between human and mouse monocyte subsets. Blood. 2010;115(3):e10–9.
10. Wong KL, Yeap WH, Tai JJ, Ong SM, Dang TM, Wong SC. The three human monocyte subsets: implications for health and disease. Immunol Res. 2012;53(1–3):41–57.

11. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. Blood. 2010;116(16):e74–80.
12. Kampalath B, Cleveland RP, Chang CC, Kass L. Monocytes with altered phenotypes in posttrauma patients. Arch Pathol Lab Med. 2003;127(12):1580–5.
13. Xu Y, McKenna RW, Karandikar NJ, Pildain AJ, Kroft SH. Flow cytometric analysis of monocytes as a tool for distinguishing chronic myelomonocytic leukemia from reactive monocytosis. Am J Clin Pathol. 2005;124(5):799–806.
14. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
15. Orazi A, Germing U. The myelodysplastic/myeloproliferative neoplasms: myeloproliferative diseases with dysplastic features. Leukemia. 2008;22(7):1308–19.
16. Schuler E, Schroeder M, Neukirchen J, Strupp C, Xicoy B, Kundgen A, et al. Refined medullary blast and white blood cell count based classification of chronic myelomonocytic leukemias. Leuk Res. 2014;38(12):1413–9.
17. Geyer JT, Tam W, Liu YC, Chen Z, Wang SA, Bueso-Ramos C, Oak J, et al. Oligomonocytic chronic myelomonocytic leukemia (chronic myelomonocytic leukemia without absolute monocytosis) displays a similar clinicopathologic and mutational profile to classical chronic myelomonocytic leukemia. Mod Pathol. 2017;30(9):1213-22. doi: 10.1038/modpathol.2017.45. Epub 2017 May 26. PubMed PMID: 28548124.
18. Peker D, Padron E, Bennett JM, Zhang X, Horna P, Epling-Burnette PK, et al. A close association of autoimmune-mediated processes and autoimmune disorders with chronic myelomonocytic leukemia: observation from a single institution. Acta Haematol. 2015;133(2):249–56.
19. Grignano E, Mekinian A, Braun T, Liozon E, Hamidou M, Decaux O, et al. Autoimmune and inflammatory diseases associated with chronic myelomonocytic leukemia: a series of 26 cases and literature review. Leuk Res. 2016;47:136–41.
20. Tefferi A, Hoagland HC, Therneau TM, Pierre RV. Chronic myelomonocytic leukemia: natural history and prognostic determinants. Mayo Clin Proc. 1989;64(10):1246–54.
21. Germing U, Gattermann N, Minning H, Heyll A, Aul C. Problems in the classification of CMML—dysplastic versus proliferative type. Leuk Res. 1998;22(10):871–8.
22. Kouides PA, Bennett JM. Morphology and classification of the myelodysplastic syndromes and their pathologic variants. Semin Hematol. 1996;33(2):95–110.
23. Michaux JL, Martiat P. Chronic myelomonocytic leukaemia (CMML)—a myelodysplastic or myeloproliferative syndrome? Leuk Lymphoma. 1993;9(1–2):35–41.
24. Orazi A, Chiu R, O'Malley DP, Czader M, Allen SL, An C, et al. Chronic myelomonocytic leukemia: the role of bone marrow biopsy immunohistology. Mod Pathol. 2006;19(12):1536–45.
25. Wang SA, Hutchinson L, Tang G, Chen SS, Miron PM, Huh YO, et al. Systemic mastocytosis with associated clonal hematological non-mast cell lineage disease: clinical significance and comparison of chomosomal abnormalities in SM and AHNMD components. Am J Hematol. 2013;88(3):219–24.
26. Shen Q, Ouyang J, Tang G, Jabbour EJ, Garcia-Manero G, Routbort M, et al. Flow cytometry immunophenotypic findings in chronic myelomonocytic leukemia and its utility in monitoring treatment response. Eur J Haematol. 2015;95(2):168–76.
27. Sojitra P, Gandhi P, Fitting P, Kini AR, Alkan S, Velankar MM, et al. Chronic myelomonocytic leukemia monocytes uniformly display a population of monocytes with CD11c underexpression. Am J Clin Pathol. 2013;140(5):686–92.
28. Selimoglu-Buet D, Wagner-Ballon O, Saada V, Bardet V, Itzykson R, Bencheikh L, et al. Characteristic repartition of monocyte subsets as a diagnostic signature of chronic myelomonocytic leukemia. Blood. 2015;125(23):3618–26.
29. Kohlmann A, Grossmann V, Klein HU, Schindela S, Weiss T, Kazak B, et al. Next-generation sequencing technology reveals a characteristic pattern of molecular mutations in 72.8% of chronic myelomonocytic leukemia by detecting frequent alterations in TET2, CBL, RAS, and RUNX1. J Clin Oncol. 2010;28(24):3858–65.
30. Kosmider O, Gelsi-Boyer V, Ciudad M, Racoeur C, Jooste V, Vey N, et al. TET2 gene mutation is a frequent and adverse event in chronic myelomonocytic leukemia. Haematologica. 2009;94(12):1676–81.
31. Bacher U, Haferlach T, Schnittger S, Kreipe H, Kroger N. Recent advances in diagnosis, molecular pathology and therapy of chronic myelomonocytic leukaemia. Br J Haematol. 2011;153(2):149–67.
32. Ricci C, Fermo E, Corti S, Molteni M, Faricciotti A, Cortelezzi A, et al. RAS mutations contribute to evolution of chronic myelomonocytic leukemia to the proliferative variant. Clin Cancer Res. 2010;16(8):2246–56.
33. Itzykson R, Kosmider O, Renneville A, Gelsi-Boyer V, Meggendorfer M, Morabito M, et al. Prognostic score including gene mutations in chronic myelomonocytic leukemia. J Clin Oncol. 2013;31(19):2428–36.
34. Pich A, Riera L, Sismondi F, Godio L, Davico Bonino L, Marmont F, et al. JAK2V617F activating mutation is associated with the myeloproliferative type of chronic myelomonocytic leukaemia. J Clin Pathol. 2009;62(9):798–801.
35. Peng J, Zuo Z, Fu B, Oki Y, Tang G, Goswami M, et al. Chronic myelomonocytic leukemia with nucleophosmin (NPM1) mutation. Eur J Haematol. 2016;96(1):65–71.
36. Ernst T, Chase A, Zoi K, Waghorn K, Hidalgo-Curtis C, Score J, et al. Transcription factor mutations in myelodysplastic/myeloproliferative neoplasms. Haematologica. 2010;95(9):1473–80.

37. Schnittger S, Bacher U, Haferlach C, Alpermann T, Dicker F, Sundermann J, et al. Characterization of NPM1-mutated AML with a history of myelodysplastic syndromes or myeloproliferative neoplasms. Leukemia. 2011;25(4):615–21.
38. Niemeyer CM, Kang MW, Shin DH, Furlan I, Erlacher M, Bunin NJ, et al. Germline CBL mutations cause developmental abnormalities and predispose to juvenile myelomonocytic leukemia. Nat Genet. 2010;42(9):794–800.
39. Loh ML. Recent advances in the pathogenesis and treatment of juvenile myelomonocytic leukaemia. Br J Haematol. 2011;152(6):677–87.
40. Rosser A, Swallow G, Swann RA, Chapman C. Salmonella enteritidis necrotising fasciitis in a multiple myeloma patient receiving bortezomib. Int J Hematol. 2010;91(1):149–51.
41. Niemeyer CM, Kratz CP. Paediatric myelodysplastic syndromes and juvenile myelomonocytic leukaemia: molecular classification and treatment options. Br J Haematol. 2008;140(6):610–24.
42. Oliveira AF, Tansini A, Vidal DO, Lopes LF, Metze K, Lorand-Metze I. Characteristics of the phenotypic abnormalities of bone marrow cells in childhood myelodysplastic syndromes and juvenile myelomonocytic leukemia. Pediatr Blood Cancer. 2017;64(4):e26285.
43. Locatelli F, Niemeyer CM. How I treat juvenile myelomonocytic leukemia. Blood. 2015;125(7):1083–90.
44. Niemeyer CM. RAS diseases in children. Haematologica. 2014;99(11):1653–62.
45. Courville EL, Wu Y, Kourda J, Roth CG, Brockmann J, Muzikansky A, et al. Clinicopathologic analysis of acute myeloid leukemia arising from chronic myelomonocytic leukemia. Mod Pathol. 2013;26(6):751–61.
46. Chen W, Konoplev S, Medeiros LJ, Koeppen H, Leventaki V, Vadhan-Raj S, et al. Cuplike nuclei (prominent nuclear invaginations) in acute myeloid leukemia are highly associated with FLT3 internal tandem duplication and NPM1 mutation. Cancer. 2009;115(23):5481–9.
47. Kussick SJ, Stirewalt DL, Yi HS, Sheets KM, Pogosova-Agadjanyan E, Braswell S, et al. A distinctive nuclear morphology in acute myeloid leukemia is strongly associated with loss of HLA-DR expression and FLT3 internal tandem duplication. Leukemia. 2004;18(10):1591–8.
48. Bennett JM, Pryor J, Laughlin TS, Rothberg PG, Burack WR. Is the association of "cup-like" nuclei with mutation of the NPM1 gene in acute myeloid leukemia clinically useful? Am J Clin Pathol. 2010;134(4):648–52.
49. Wuchter C, Harbott J, Schoch C, Schnittger S, Borkhardt A, Karawajew L, et al. Detection of acute leukemia cells with mixed lineage leukemia (MLL) gene rearrangements by flow cytometry using monoclonal antibody 7.1. Leukemia. 2000;14(7):1232–8.
50. Kern W, Bacher U, Haferlach C, Schnittger S, Haferlach T. Acute monoblastic/monocytic leukemia and chronic myelomonocytic leukemia share common immunophenotypic features but differ in the extent of aberrantly expressed antigens and amount of granulocytic cells. Leuk Lymphoma. 2011;52(1):92–100.
51. Swerdlow S, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC; 2008.
52. Rose D, Haferlach T, Schnittger S, Perglerova K, Kern W, Haferlach C. Subtype-specific patterns of molecular mutations in acute myeloid leukemia. Leukemia. 2016;31:11.
53. Shen Y, Zhu YM, Fan X, Shi JY, Wang QR, Yan XJ, et al. Gene mutation patterns and their prognostic impact in a cohort of 1185 patients with acute myeloid leukemia. Blood. 2011;118(20):5593–603.
54. Dong HY, Kung JX, Bhardwaj V, McGill J. Flow cytometry rapidly identifies all acute promyelocytic leukemias with high specificity independent of underlying cytogenetic abnormalities. Am J Clin Pathol. 2011;135(1):76–84.
55. Boiocchi L, Espinal-Witter R, Geyer JT, Steinhilber J, Bonzheim I, Knowles DM, et al. Development of monocytosis in patients with primary myelofibrosis indicates an accelerated phase of the disease. Mod Pathol. 2013;26(2):204–12.
56. Barraco D, Cerquozzi S, Gangat N, Patnaik MM, Lasho T, Finke C, et al. Monocytosis in polycythemia vera: clinical and molecular correlates. Am J Hematol. 2017;92:640.
57. Wang SA, Galili N, Cerny J, Sechman E, Chen SS, Loew J, et al. Chronic myelomonocytic leukemia evolving from preexisting myelodysplasia shares many features with de novo disease. Am J Clin Pathol. 2006;126(5):789–97.
58. Elliott MA, Verstovsek S, Dingli D, Schwager SM, Mesa RA, Li CY, et al. Monocytosis is an adverse prognostic factor for survival in younger patients with primary myelofibrosis. Leuk Res. 2007;31(11):1503–9.
59. Beran M, Shen Y, Onida F, Wen S, Kantarjian H, Estey E. Prognostic significance of monocytosis in patients with myeloproliferative disorders. Leuk Lymphoma. 2006;47(3):417–23.
60. Melo JV. BCR-ABL gene variants. Baillieres Clin Haematol. 1997;10(2):203–22.

Thrombocytosis

8

Beenu Thakral and Sa A. Wang

Overview

Platelets are anucleate cells released from megakaryocytes under the influence of flow in capillary sinuses in the bone marrow. The normal blood platelet count in adults varies between 150 to 450 × 10^9/L. Thrombocytosis, defined as platelet count ≥ 450 × 10^9/L, is a common laboratory finding that is often detected incidentally during routine complete blood count (CBC) examination [1, 2]. Extreme thrombocytosis (platelet count ≥ 1000 × 10^9/L) is quite rare, as only 2–5.8% of patients with thrombocytosis have this degree of platelet count elevation [3, 4].

Secondary or reactive thrombocytosis is the most common cause in both adult and pediatric populations. A review of clinical history, prior platelet counts, and ultimate treatment of the underlying condition helps in the resolution of reactive thrombocytosis in the majority of the cases. If reactive causes of thrombocytosis are excluded, a bone marrow morphologic examination with ancillary testing (molecular genetic and cytogenetic analysis) helps in reaching a correct diagnosis of a clonal myeloid neoplasm causing primary thrombocytosis.

The various causes of thrombocytosis are illustrated in Table 8.1. In the majority of cases, the thrombocytosis is reactive and abates when the underlying condition is treated or resolves. Sometimes thrombocytosis can be due to primary BM causes. These primary thrombocytoses include myeloproliferative neoplasms such as chronic myeloid leukemia (CML), polycythemia vera (PV), primary myelofibrosis (PMF) or essential thrombocythemia (ET), myelodysplastic syndrome (MDS) such as MDS with del(5q) or inv(3)(q21q26.2)/t(3;3)(q21;q26.2) [1, 5], myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), or acute myeloid leukemia (AML). Thrombocytosis can also be familial.

Spurious thrombocytosis is rare and occurs when non-platelet structures in peripheral blood are counted as platelets by automated hematology analyzers. Examples of these include red cell microspherocytes or schistocytes (commonly seen in microangiopathic hemolytic anemia), cryoglobulin crystals, bacteria or fragments of leukemic cells that can mimic platelets when evaluated by hematology analyzers [6–8]. Examination of the peripheral blood smear is a simple method to confirm the presence of thrombocytosis and exclude spurious causes and should be an initial part of the laboratory evaluation of thrombocytosis. This should be followed by a review of the patient's history, particularly comorbid conditions, as well as other hematologic parameters and the platelet count history.

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Table 8.1 Causes of thrombocytosis[a]

(1) Primary/clonal thrombocytosis
• Myeloproliferative neoplasms (chronic myeloid leukemia, essential thrombocythemia, polycythemia vera, and primary myelofibrosis)
• Myelodysplastic syndrome (for example: MDS with isolated del5q)
• Myelodysplastic/myeloproliferative neoplasms (for example: MDS/MPN-RS-T and CMML)
• POEMS syndrome
• Familial thrombocytosis: thrombopoietin overproduction, *MPL* mutations
(2) Secondary/reactive thrombocytosis
• Infection/inflammation
• Tissue damage: collagen vascular disease or inflammatory bowel disease
• Iron deficiency anemia
• Hemolysis or acute hemorrhage
• Hyposplenism/postsplenectomy
• Malignancy
• Postoperative
• Drugs (gemcitabine, corticosteroids, adrenaline, TPO, following myelosuppressive chemotherapy)
• "Rebound"—correction of vitamin B_{12} or folate deficiency, post-ethanol abuse
(3) Spurious thrombocytosis
• Cryoglobulinemia
• Microspherocytes
• Schistocytes
• Bacteria
• Cytoplasmic fragments of neoplastic cells

[a]Modified from Harrison et al. [1]

The initial part of this chapter will focus on reactive thrombocytosis, followed by myeloid neoplasms that are most frequently associated with thrombocytosis, including ET and MDS/MPN-RS-T. Primary myelofibrosis, polycythemia, and CML are also frequently associated with thrombocytosis, but these entities will be discussed under other chapters. An algorithmic approach to thrombocytosis is illustrated in Fig. 8.1.

Secondary/Reactive Thrombocytosis

Secondary or reactive thrombocytosis is the most common etiology of thrombocytosis in both adult and pediatric populations, comprising 88–97% of thrombocytosis cases in adults [3, 4, 9] and nearly 100% in the pediatric population [10]. In adults, secondary thrombocytosis is typically acute and reactive, being attributed to tissue damage, chronic inflammation, or malignancy in over 75% of cases. In children, similar causes lead to reactive thrombocytosis, with the addition of hemolytic anemia (especially thalassemia) as a relatively common etiology [10].

Platelets are produced by megakaryocytes in the bone marrow (BM). Under normal physiological conditions, about 2×10^{11} platelets are produced per day, which have a normal life span of 7–10 days. Thrombopoiesis occurs in the setting of a complex cytokine milieu. The major regulator of platelet production is thrombopoietin (TPO), which is synthesized in the liver and kidney. TPO binds to its receptor MPL on megakaryocytes, activating the signal transduction pathways that regulate megakaryocyte proliferation, maturation, and platelet production. Under normal physiological conditions, TPO is mostly bound to MPL, with very low detectable free serum levels. The BM megakaryocytes play an important role in regulating serum TPO levels. Thrombopoietin levels are moderately elevated in secondary thrombocytosis, but due to a significant overlap with values of ET patients, serum TPO level cannot be used as a reliable

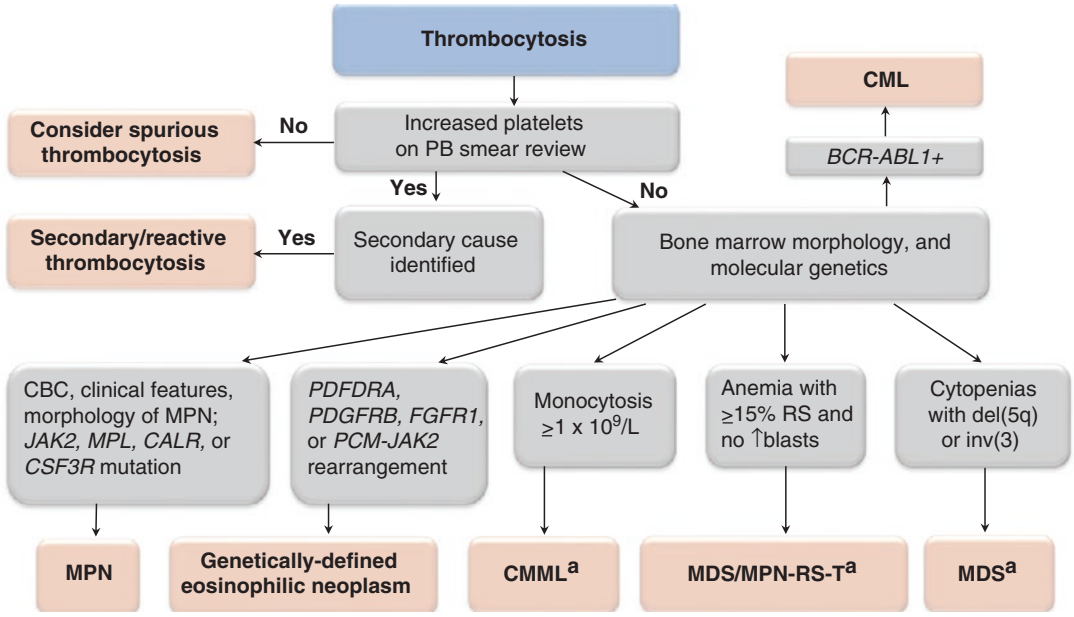

Fig. 8.1 An algorithm for diagnosis and differential diagnosis of thrombocytosis. *MDS*: myelodysplastic syndromes, *MPN*: myeloproliferative neoplasm, *MDS/MPN-U*: myelodysplastic/myeloproliferative–neoplasm unclassifiable, *RS*: ring sideroblasts, *MDS/MPN-RS-T*: myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis, *ET*: essential thrombocythemia, *PMF*: primary myelofibrosis

marker in distinguishing these two entities. Besides TPO, many other cytokines (IL-1, IL-4, IL-6, IL-11, and TNF) play an important role in the cause of thrombopoiesis, of which IL-6 plays a particularly important role. Synthesis of these cytokines is increased with inflammation. Specifically, IL-6 levels are usually elevated in patients with reactive thrombocytosis but not in primary thrombocytosis or in normal controls [11, 12]. However, utilizing cytokine levels as a diagnostic tool has been practically difficult, as the levels may not be uniformly high throughout the period of thrombocytosis and may return to normal even in the presence of persistent thrombocytosis. Clinical correlation, along with use of other surrogate markers of acute inflammation such as C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR), which are usually elevated in reactive thrombocytosis but not in primary thrombocytosis, can be useful in distinguishing between the two entities. In a study by Tefferi et al., 76% of patients with reactive thrombocytosis had elevated CRP (>1 mg/dL) as compared to only 10% of patients with clonal thrombocytosis [13]. Thus, although elevated acute phase reactants (IL-6 or CRP) are neither sensitive nor specific for the use as diagnostic markers, in the appropriate clinical testings, they can support a diagnosis of reactive thrombocytosis.

Iron deficiency anemia is another common cause of reactive thrombocytosis, and thus evaluation of serum ferritin and iron studies should be a part of initial evaluation of patients presenting with thrombocytosis. The pathophysiology of thrombocytosis in iron deficiency anemia, although not completely understood, does not appear to be due to elevated cytokines (IL-6, IL-11, and TPO). The levels of these cytokines are similar to iron-deficient patients without thrombocytosis, and there is no change observed upon iron repletion [14]. The only hematopoietic cytokine found to be elevated in patients with iron deficiency anemia (with or without thrombocytosis) is erythropoietin (EPO). The association of elevated EPO levels and thrombocytosis is also observed with administration of human recombinant erythropoietin (rh-EPO) in patients with chronic kidney disease. The latter can present with thrombocytosis of variable

degree and duration. It has been suggested that the homology between the erythropoietin receptor (EPO-R) and thrombopoietin receptor (MPL) contributes to the EPO-induced thrombocytosis phenomenon [15].

Approximately one-third of the platelets reside in the spleen, and relative thrombocytosis can be seen in up to 50% of patients with hyposplenism or postsplenectomy state [9]. Thrombocytosis is typically seen immediately postsplenectomy, and platelet count usually normalizes within months and only rarely after years.

Thrombocytosis can also be associated with malignancy as a paraneoplastic phenomenon and is observed in 40% of patients with lung and gastrointestinal cancers; 20% of patients with breast, endometrial, and ovarian cancers; and 10% of patients with lymphoma [16]. Patients with thrombocytosis are more likely to have advanced-stage disease and have a poorer prognosis than patients without thrombocytosis. In ovarian cancer, IL-6 has been shown to directly promote tumor growth. Paraneoplastic thrombocytosis typically does not require treatment other than the treatment for the underlying tumor.

Clinical Features

Reactive thrombocytosis is typically acute in onset and non-persistent, and patients are usually asymptomatic, as opposed to a MPN that is usually associated with persistent thrombocytosis and can be associated with hepatosplenomegaly, vasomotor symptoms, and thrombotic or bleeding complications [9, 17]. Thromboembolic complications are rare in reactive thrombocytosis compared to MPNs, unless provoked by underlying conditions such as malignancy or atherosclerosis.

Secondary thrombocytosis is usually a self-limited process that resolves with the resolution of the underlying disorder. The risk of thrombotic complications is low, even in patients with an extreme reactive thrombocytosis; it may occasionally occur due to the concomitant conditions such as trauma and coronary artery bypass grafting [18]. In one large series, thrombotic complications were seen in 1.6% of patients with secondary thrombocytosis [3]. The thrombotic events are venous thrombosis and are more prone to occur in a postoperative setting or in association with an underlying malignancy. In general, in reactive thrombocytosis, treatment of the underlying cause is most important, and usually no antiplatelet therapy is needed.

Patients with markedly elevated platelet counts (>1500 × 10^9/L), usually seen in the setting of MPN such as ET, have an increased risk of bleeding. The bleeding is in part due to acquired von Willebrand disease (vWD) that the platelet-vWF binding leads to removal of vWF from the circulation.

Morphology

The peripheral blood in secondary thrombocytosis shows increased platelets, comprising mainly of small granulated mature forms (Fig. 8.2). There is no dysgranulopoiesis and no basophilia. The peripheral blood smear in secondary thrombocytosis may show features of microcytic, hypochromic anemia that suggest iron deficiency anemia or neutrophils with toxic granulation suggesting an infectious or inflammatory etiology as the cause of thrombocytosis. Careful clinicopathologic correlation often identify the cause of secondary thrombocytosis, and a bone marrow biopsy is usually not required. However, if a BM evaluation is performed, the bone marrow biopsy often shows a normal cellularity (Fig. 8.2). Megakaryocytes are often not increased and are morphologically unremarkable, contrasting to clonal thrombocytosis that often shows abnormal megakaryocytes. Reticulin and trichrome special stains are usually normal in secondary thrombocytosis, with no significant fibrosis.

Immunophenotype

Immunophenotyping studies on bone marrow samples are often not necessary, unless there is a need to rule out lymphoma or acute leukemia that may be the underlying causes of thrombocytosis.

Cytogenetics and Molecular Genetics

Reactive thrombocytosis does not carry clonal cytogenetic abnormality in hematopoietic cells; however, transient cytogenetic abnormalities may be rarely detected in patients with acute bone marrow injury/insult.

JAK2V617F, *MPL*, and *CALR* mutations, present in about 85–90% of MPNs, are usually absent in reactive thrombocytosis. Finding a

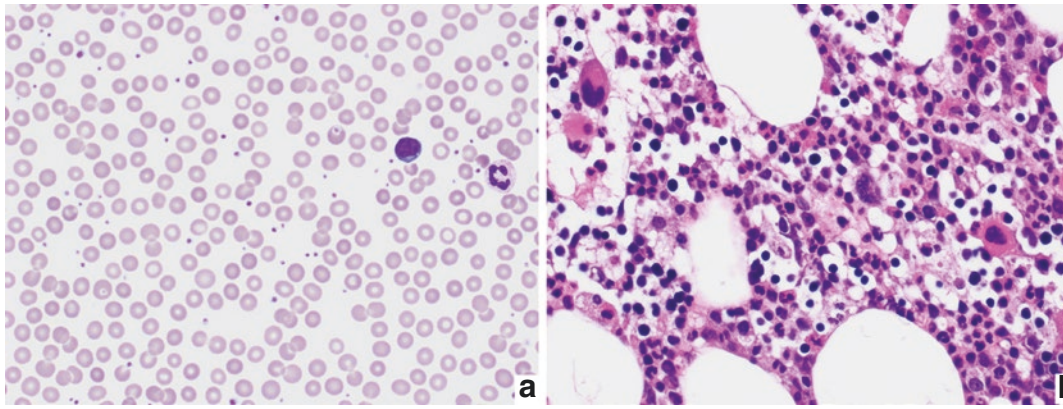

Fig. 8.2 Reactive/secondary thrombocytosis. Peripheral blood smear shows normocytic, normochromic anemia with reactive thrombocytosis in a patient with corticosteroid secreting adrenal adenoma (**a**). Bone marrow biopsy shows normocellular bone marrow with trilineage hematopoiesis; Megakaryocytes are not significantly increased in numbers and morphologically unremarkable (**b**)

Table 8.2 Clinicopathologic features helpful in distinguishing essential thrombocythemia from secondary/reactive thrombocytosis

Parameters	Essential thrombocythemia	Secondary thrombocytosis
Platelet increase	Persistent	Transient
History of thrombotic/bleeding episode/ vasomotor symptoms	Present	1–2%
Hepatosplenomegaly	Present	Absent
History of infections/inflammatory stimuli or non-myeloid malignancy	Absent	Present
Increased acute phase reactants (e.g., CRP)	Absent	Present
Megakaryocyte morphology in the bone marrow	Enlarged megakaryocytes including staghorn appearance and clustering present	Megakaryocytes with normal morphology can be increased and usually without clustering
Clonal molecular abnormality	Usually present (*JAK2V617F*, *MPLW515K/L*, or *CALR* mutations)	Absent

mutation (in *JAK2*, *MPLW515K/L*, or *CALR)* or a cytogenetic abnormality (such as *BCR-ABL1* rearrangement or del(5q)) can be helpful in excluding a reactive thrombocytosis in difficult cases. However, a low level of *JAK2*V617F mutation (0.03–1%) can be seen in healthy individuals [19, 20], and the significance of these findings in the absence of bone marrow morphological features of an MPN has not yet been determined. A low level of *JAK2V617F* allele burden may indicate the presence of a small mutated clone within an overall polyclonal normal hematopoiesis. A cutoff of allele burden ≥0.8% has been found significantly correlate with a concurrent or future diagnosis of MPN [21]. Sequential monitoring of the *JAK2V617F* mutation fraction is recommended in such cases.

Differential Diagnosis

A detailed history is essential to determine the duration of thrombocytosis and if it is isolated or associated with leukocytosis and/or anemia. Any history of a previous thrombotic or bleeding episode or an underlying illness or medication that might account for thrombocytosis needs to be documented. Laboratory assessment of acute phase reactants such as CRP, ESR, and serum ferritin can be useful. The major differential diagnosis is ET which will be discussed later in this chapter. Table 8.2 summarizes clinical and laboratory

parameters that can be helpful in distinguishing between secondary and primary thrombocytosis.

Essential Thrombocythemia (ET)

Essential thrombocythemia (ET) is a *BCR-ABL1*-negative myeloproliferative neoplasm that primarily manifests abnormal morphology in the megakaryocytic lineage and is characterized by sustained thrombocytosis of $\geq 450 \times 10^9$/L [1, 2].

Clinical Features

ET is a MPN with an incidence of 0.5–1.8 cases per 100,000 persons per year [22, 23]. The disease has a slight female predominance with a median age of about 60 years and is seen more commonly in Ashkenazi Jews. Clinically, patients are usually asymptomatic or may exhibit symptoms such as headaches, blurred vision, dizziness, and erythromelalgia (redness, burning, pain of distal ends of toes and fingers) due to thrombotic occlusion of the microvasculature. Catastrophic large vessel thrombosis includes stroke, myocardial infarction, deep venous thrombosis (including splanchnic vein thrombosis), and peripheral arterial thrombosis. Bleeding is less common than thrombosis, and patients at risk for bleeding usually have platelet counts $>1000 \times 10^9$/L due to an acquired vWF deficiency associated with decreased functional activity of vWF as measured by collagen-binding activity and ristocetin cofactor activity [24]. Hemorrhage can occur in mucocutaneous areas and most seriously in the gastrointestinal tract. Splenomegaly is not as prominent as that of the other MPNs, and it occurs in 15–20% of patients [25].

The prognosis of ET is favorable. The overall median survival from the time of initial diagnosis is approximately 20 years. Risk factors that predict a worse overall survival include age ≥60 years, a history of previous thrombosis, and a platelet count $\geq 1500 \times 10^9$/L. Other factors may include a high serum LDH level and WBC $>11 \times 10^9$/L [26]. The three most important complications that can occur during the follow-up in ET patients include [27, 28]: [1] thrombovascular complications, with a 15-year cumulative risk of thrombosis ranging from 10 to 25%, higher in patients with *JAK2* mutations than patients with *CALR* mutations; [2] progression to myelofibrosis occurring in about 5% of patients at 10 years and about 10% at 15 years, with a higher cumulative risk in type 1 *CALR*-mutated ET than in *JAK2*-mutated ET; and [3] rare leukemic transformation, occurring in about 1% of patients at 10 years and in about 3% at 15 years. Some studies suggest that *JAK2V617F*-mutated ET has a cumulative risk of transformation to a polycythemia vera phenotype in up to 29% of patients at 15 years, but it is not clear how many of these patients may have had a masked PV that were mistakenly diagnosed as ET [28].

Morphology

The peripheral blood smear in ET shows marked thrombocytosis with anisocytosis of platelets. Mean platelet volume (MPV) is generally lower than normal or reactive thrombocytosis, but mean platelet width is generally increased. There is usually no leukocytosis, no granulocytic left shift or dysplasia, and no absolute or relative basophilia [29–31]. Red blood cells are normocytic and normochromic, except in patients with significant hemorrhage and iron deficiency, in which case the red blood cells may be hypochromic and microcytic. Finding teardrop forms and a leukoerythroblastic picture should raise the possibility of PMF or other bone marrow fibrotic processes rather than ET.

A bone marrow biopsy is essential to diagnose ET and to distinguish it from early/prefibrotic PMF, PV, other MPN, and other myeloid neoplasms associated with thrombocytosis as well as reactive thrombocytosis. The bone marrow of ET is generally normocellular with normal erythropoiesis and granulopoiesis. The notable changes are in megakaryocytes that are abnormal but show features different from PV, PMF, and CML. In ET, megakaryocytes are generally large, with hyperlobulated nuclei or "stag-horn-shaped" nuclei, and have abundant cytoplasm, frequently containing other hematopoietic cells within the cytoplasm (emperipolesis). Megakaryocytes are scattered or focally

forming loose clusters. Bizarre nuclear forms with hypercondensed chromatin and tight clustering are typical of PMF, whereas small megakaryocytes with hypolobated nuclei are typical of CML and these features are not consistent with ET. Granulocytic proliferation is usually absent or, if present, only minimal, and there is no left shift or increase in blasts [24]. Reticulin fibrosis may be minimally increased (up to MF grade 1 of 3), but significant fibrosis at initial diagnosis should suggest another entity. Stainable iron is usually present though may be reduced, unlike polycythemia vera that storage iron is usually absent. In addition, the presence of ring sideroblasts (≥15%) in a patient with anemia in addition to the thrombocytosis would suggest a diagnosis of MDS/MPN with ring sideroblasts and thrombocytosis rather than ET.

Immunophenotype

Immunophenotyping studies on bone marrow samples are not necessary to diagnose ET. *CALR* mutations (see below) result in the expression of a novel epitope that can be detected by immunohistochemistry in fixed tissues using a mutation-specific monoclonal antibody (CAL2). CAL2 shows strong cytoplasmic staining of megakaryocytes as well as blasts, in both type 1 and type 2 *CALR*-mutated *MPNs*. Thus, the presence or absence of *CALR* mutations can now be easily tested in routine biopsy material [32].

Cytogenetics and Molecular Genetics

Cytogenetic abnormalities are rare in ET, in 5–10% of cases, and their presence would support a diagnosis of a clonal hematopoiesis. The abnormalities most commonly seen in ET include +8, +9, and del(13q) [33]. Of note, these cytogenetic changes are notspecific for ET and do not distinguish ET from PV or early PMF.

Mutations of *JAK2V617* are detected in 50–60% of ET, followed by *CALR* in 25–30% of cases and *MPL W515 L/K* in 3–5% of cases [34–38]. These three mutations are somatic, mutually exclusive and lead to constitutive aberrant activation of JAK/STAT pathways that stimulate megakaryocyte proliferation and platelet production. Mutation studies for *JAK2V617F, CALR*, and *MPL M515L/K* are particularly helpful in ruling out a reactive process, where they should be absent. In ET, the allele burden of the *JAK2V617* mutation is lower than that of PV, and its level does not appear to affect survival or leukemic transformation [36]. *CALR* mutations lead to an alteration of the C-terminal of the protein that results in loss of an endoplasmic reticulum retention motif and activates STAT signaling pathway. There are two types of mutations: a 52-bp deletion in exon 9 (type 1 mutation) and a 5-bp insertion (type 2 mutation), with type 1 mutations being more common than type 2 mutations. The mutation subtypes in *CALR* correlate with patient clinical phenotype and outcomes. In general, *CALR*-mutated ET affects relatively young individuals, especially males, and tends to have a higher platelet count (type 2 *CALR* more than type 1 *CALR*), lower hemoglobin (HGB), lower leukocyte count, and lower thrombotic risk as compared to *JAK2V617F* or *MPL M515L/K* mutated ET. Type 1 *CALR* mutations are associated with a significantly higher risk of myelofibrotic transformation in ET. Despite very high platelet counts, type 2 *CALR* mutations are associated with a low risk of thrombosis and an indolent clinical course. *MPL* mutations have been reported to be associated with an older age, a female gender, a lower HGB level, and a higher platelet count but no associations with survival or leukemic transformation [36, 39].

It is noteworthy that, in about 10% of ET cases, mutations in *JAK2V617F, CALR*, *or MPLW515* are not detected, and such cases are known as "triple-negative" cases [38, 40–42]. However, recent studies have shown that a few triple-negative ET patients may carry activating mutations of *MPL* outside exon 10 (*MPLS204P and MPLY591N* mutants), and functional studies have revealed that these are weak gain-of-function mutations that either increase MPL signaling or confer to a TPO hypersensitivity [40–42]. In addition, a few triple-negative cases of ET may include individuals with familial thrombocytosis, such as hereditary thrombocytosis and familial ET.

Besides these driver mutations, mutations in epigenetic regulatory genes can also be found in ET. Mutations in *SH2B3*, *SF3B1*, *U2AF1*, *TP53*,

IDH2, and *EZH2* have been reported to increase the risk of leukemic or fibrotic progression in ET [36]. While among all MPN patients, the presence of two or more somatic mutations represents a negative prognostic factor; the number of mutations in ET does not appear to provide additional prognostic information [35, 36].

Differential Diagnosis

The diagnoses of ET requires a sustained elevation in the platelet count (≥450 × 10^9/L); identifying BM morphologic findings consistent with the disease; excluding other MPN, MDS, or other myeloid neoplasms associated with elevated platelets; and demonstrating *JAK2*, *CALR*, or *MPL* mutations or a cytogenetic abnormality. In the absence of these molecular and cytogenetic abnormalities, a diagnosis of ET can be made with carefully ruling out reactive thrombocytosis and other myeloid neoplasms associated with thrombocytosis. Diagnostic criteria as per the WHO 2016 [1] are shown in Table 8.3.

The differential diagnoses include reactive conditions leading to elevated platelet counts, other MPNs with elevated platelet counts, and rarely AML, MDS, or MDS/MPNs associated with increased platelets.

Reactive/secondary thrombocytosis can be caused by infection, inflammatory diseases, blood loss and chronic iron deficiency, malignancy, trauma and surgery (especially splenectomy), and rebound following chemotherapy or replacement therapy for B_{12} or folate deficiency. Reactive conditions are more frequently associated with elevated acute phase reactants such as C-reactive protein. Reactive conditions should not be persistent or associated with splenomegaly, and they are not likely associated with a history of thrombotic episodes. Please refer to the earlier part of this chapter on secondary thrombocytosis for the discussion.

Hereditary thrombocytosis is caused by molecular alterations in the thrombopoietin gene (*THPO*) or in the gene for the thrombopoietin receptor (*MPL*). These mutations remove the inhibitory upstream open reading frame, leading to increased translation of the THPO mRNA and a high serum thrombopoietin. Mutation in *MPL*Ser505Asn is reported to associate with increased thrombotic risk, splenomegaly, and progression to bone marrow fibrosis [43]. Recent reports have described cases of hereditary thrombocytosis associated with noncanonical germ line mutations of *JAK2* with examples being *JAK2-V625F* and *JAK2-F556V* [40, 41]. In familial ET, *JAK2V617F* is always a somatically acquired event [38].

Patients with hereditary thrombocytosis present at a much younger age than ET patients, and there is often a familiar history of thrombocytosis. The bone marrow morphology may resemble ET or prefibrotic PMF. Depending on the age of the first BM examination, patients may show various degrees of BM fibrosis. Splenomegaly is more often seen in older patients.

Chronic myeloid leukemia (CML) can frequently present with thrombocytosis, but the prominent feature of CML is a myeloid proliferation. Peripheral blood shows leukocytosis with frequent myelocytes and basophilia, which is usually quite distinctive and not a feature of in ET. In addition, small "dwarf" megakaryocytes in the bone marrow are also distinctly different from the larger staghorn-like megakaryocytes in ET. CML is a genetically defined entity, and the detection of t(9;22) or *BCR-ABL1* will clarify any dilemma in this differential diagnosis. Some

Table 8.3 Diagnostic criteria for essential thrombocythemia (WHO 2016) [2] (A diagnosis requires all four major criteria or the first three major criteria and one minor criterion)

Major criteria
• Platelet count ≥ 450 × 10^9/L
• Bone marrow: normocellular bone marrow with a megakaryocytic proliferation of enlarged, mature megakaryocytes with hyperlobulated nuclei. No significant increase in left-shifted neutrophilic granulopoiesis or erythropoiesis and no or rarely minimal increase in reticulin fibrosis (up to grade 1)
• Not meeting the WHO criteria for *BCR-ABL1*-positive CML, PV, PMF, MDS, or other myeloid neoplasms
• Presence of *JAK2*, *CALR*, or *MPL* mutation
Minor criteria
• Presence of a clonal marker or absence of evidence of reactive thrombocytosis

patients with CML with markedly elevated platelets may have the p230 *BCR-ABL1* fusion transcript, and these cases may mimic ET.

MDS with isolated del(5q) can present with increased platelets, but patients almost always are anemic. The bone marrow megakaryocytes are small and hypolobated/monolobated, distinctively different from the large hyperlobulated megakaryocytes seen in ET. There is often erythroid hypoplasia, with dyserythropoiesis, features that are not typically seen in ET.

Acute myeloid leukemia (AML) can sometimes present with elevated platelets; this is particularly a feature of AML with inv(3)(q21;q26.2) or t(3;3)(q21;q26.2) or in some megakaryoblastic leukemias. In addition to the defining abnormal cytogenetic finding, these patients will have elevated blasts and usually show highly dysplastic megakaryocytes, including the classic micro-megakaryocytes that make them easily distinguishable from ET.

Other MPNs and MDS/MPNs with thrombocytosis that must be considered in the differential diagnosis include PV, PMF, MDS/MPN-RS-T, MPN-unclassifiable, and MDS/MPN-unclassifiable. These entities have clinicopathologic features different from ET that make the distinction possible. PV patients often have thrombocytosis at presentation; in cases of the so-called "masked" PV that HGB and hematocrit are only mildly elevated, the laboratory features closely mimicking ET [44, 45]. Moreover, ET cases with *JAK2* mutation often have higher HGB levels compared to ET cases with *CALR* or *MPL* mutations [28]. There are a number of features that help in the differential diagnosis between ET and PV: (1) PV has a high HGB/hematocrit ratio; (2) PV bone marrow shows a hypercellularity with panmyelosis; (3) megakaryocytes in PV are pleomorphic, with some normal-appearing ones admixed with large forms, contrasting with the more uniformly enlarged megakaryocytes in ET; (4) PV frequently has frequent biallelic *JAK2* mutations; and (5) PV is negative for *MPL515W* or *CALR* mutations. MDS/MPN-RS-T patients present with macrocytic anemia in addition to thrombocytosis, and BM reveals numerous ring sideroblasts and shows frequent *SF3B1* mutations. The most difficult differential diagnosis is between ET and the prefibrotic phase of PMF; features distinguishing these two entities are shown in Table 8.4 and illustrated in Fig. 8.3. There has been some controversy as to whether ET and prefibrotic PMF can be reliably distinguished

Table 8.4 Clinicopathological features of essential thrombocythemia (ET) versus prefibrotic primary myelofibrosis (PMF)

Features	ET	Prefibrotic PMF
Peripheral blood		
WBC	Usually normal, left-shifted granulocytes not seen	Variable, often increased with left-shifted granulocytes
Platelets	Increased ($\geq 450 \times 10^9$/L)	Often $\geq 450 \times 10^9$/L, may be normal or decreased
Anemia	No	May present
Bone marrow		
Cellularity	Usually normal	Increased
Megakaryocyte clustering, size, maturation, and nuclear shape	Mostly large, giant, mature, hyperlobulated, staghorn forms. Dispersed or loose clusters	Variable in size from small to large, immature to mature with hyperchromatic forms, some bizarre and bulbous forms. Tight clusters, endosteal translocation may be seen
M/E ratio	Often normal, no significant increase in granulo- and erythropoiesis	Increased, pronounced myeloid hyperplasia
Fibrosis	No	No or minimal to mild (MF0 and MF1)
Splenomegaly	No	No or minimal to mild enlarged
LDH	Normal	Normal or minimal to mildly elevated

ET essential thrombocythemia, *PMF* primary myelofibrosis

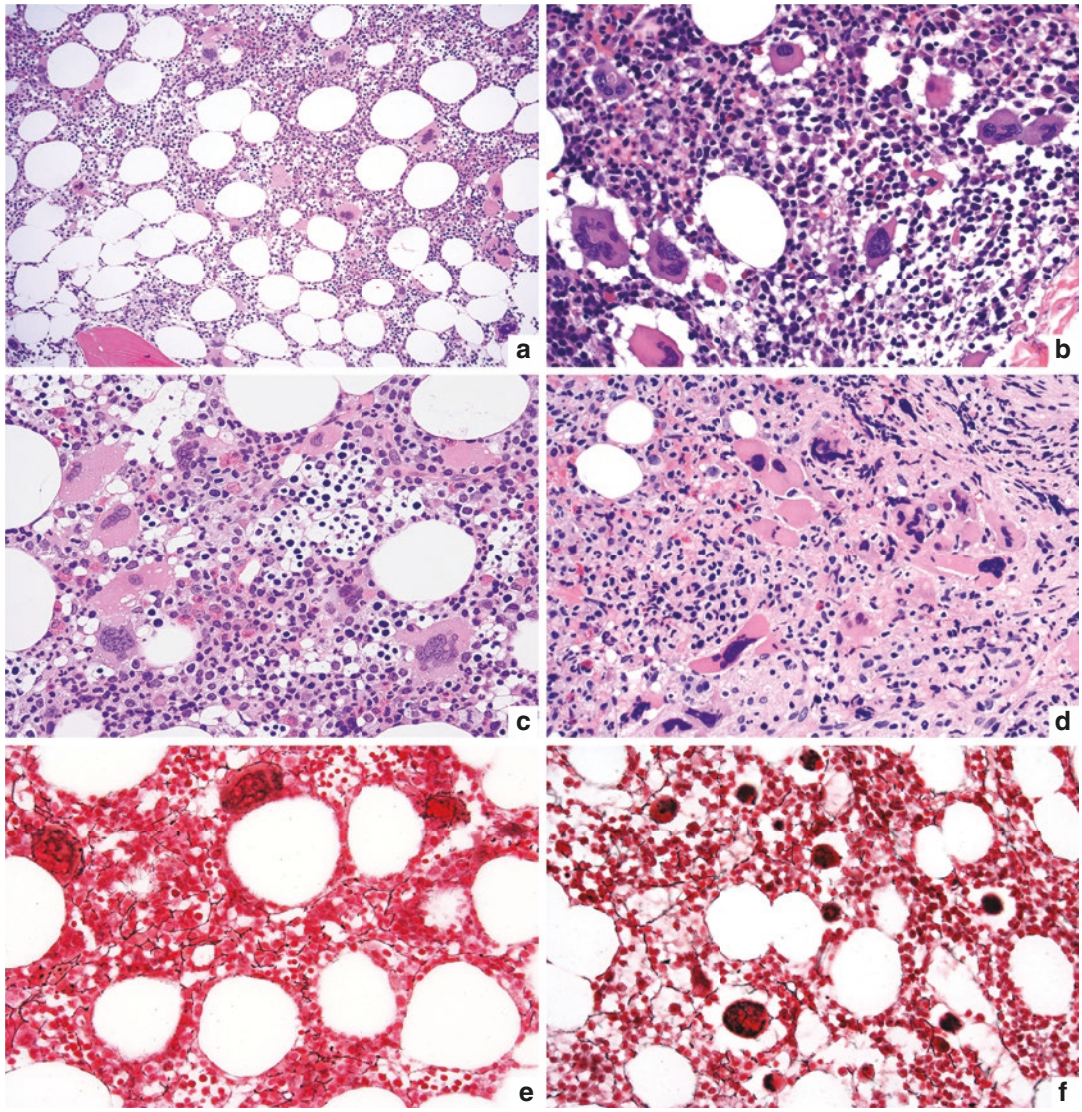

Fig. 8.3 Bone marrow of essential thrombocythemia (ET, **a**, **c**, **e**) versus pre-fibrotic primary myelofibrosis (PMF, **b**, **d**, **f**). The bone marrow of ET often shows an age-match cellularity (**a**); increased megakaryocytes with loose clustering, mostly large, mature, hyperlobulated, staghorn forms (**c**). No significant increase in reticulin fibrosis (**e**). Prefibrotic PMF BM shows a hypercellularity with increased bizarre appearing megakaryocytes, a few bulbous forms (**b**) and often hyperchromatic (more prominent in fibrotic stage, **d**). In the prefibrotic PMF, this case shows minimal to mild reticulin fibrosis (**f**)

from one another based on pathologic and clinical features. However, recent data suggest that by rigorously applying WHO morphologic criteria based on the bone marrow biopsy findings, these two entities can be reliably separated [29]. This distinction has prognostic relevance that prefibrotic PMF has a more aggressive course compared to ET [30].

Myelodysplastic/Myeloproliferative Neoplasm with Ring Sideroblasts and Thrombocytosis (MDS/MPN-RS-T)

Myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) was formerly referred to as

refractory anemia with ring sideroblasts and marked thrombocytosis (RARS-T). It was a provisional entity in the 2008 WHO classification of hematolymphoid neoplasms with overlapping features of myelodysplastic syndrome (MDS) and myeloproliferative neoplasm (MPN) (MDS/MPN). This group of neoplasms has been renamed as MDS/MPN-RS-T, based on recent clinical and molecular data and recognized as a formal subtype of MDS/MPN in the WHO 2016 revision [1, 2].

The question, if MDS/MPN-RS-T represents a distinct diagnostic category, a disease that evolved from MDS or MPN, or two distinct disorders occurring simultaneously, had been in debate in the past 10–15 years. Since *JAK2*V617F is closely correlated with MPN, the identification of *JAK2*V617F in approximately 50% of cases of MDS/MPN-RS-T suggests that this disease may be more close to MPN [46–50]. Until recently, studies of the mutational landscape show a high frequency of *SF3B1* mutation in MDS with RS and single-lineage dysplasia (about 80%) as well as MDS with RS with multilineage dysplasia (about 40%) [51–54]. An even higher frequency (80–90%) of *SF3B1* mutation has been found in MDS/MPN-RS-T. Furthermore, somatic mutations in epigenetic regulators characteristic of MDS [54, 55] such as *TET2, ASXL1, DNMT3A,* and *IDH2* are frequently found in MDS/MPN-RS-T, whereas other MPN-like mutations such as *CALR* and *MPL* are infrequent [56, 57]. The hybrid molecular findings as well as overlapping clinicopathologic features support the inclusion of MDS/MPN-RS-T as a formal distinct subtype of MDS/MPNs. Thus, the nomenclature has been changed to MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) in the 2016 WHO classification revision. The diagnosis of MDS/MPN-RS-T requires anemia with persistent thrombocytosis (platelet count ≥ 450 × 10^9/L), <1% blasts in the peripheral blood; dysplastic, ineffective erythropoiesis with ≥15% RS; and <5% myeloblasts in the bone marrow [1]. The current 2016 WHO criteria for diagnosing MDS/MPN-RS-T are shown in Table 8.5.

Clinical Features

MDS/MPN-RS-T is a rare entity with a slight female propensity, and the median age of presentation is 71–75 years, which is older than patients with ET [58, 59]. Symptoms are usually related to anemia that can be severe but can also be due to excessive thrombocytosis, with bleeding or thrombosis. Splenomegaly has been reported in about 40% of cases, and hepatomegaly can also occur [60]. The frequency of thrombotic events in MDS/MPN-RS-T is similar to that of ET (3.6 vs. 3.9/100 patient-years) [61, 53]. Older age (≥60 years) and a prior history of thrombosis are risk factors for thrombosis. However, unlike ET, the thrombotic events do not seem to have impact on overall survival [61]. MDS/MPN-RS-T is a relatively indolent disease with a median survival of 71 months [62].

Currently, there is no standard care for MDS/MPN-RS-T patients. Treatment includes management of anemia as well as thrombocytosis. Anemia management follows the same principle

Table 8.5 Features of myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) (WHO 2016) [2]

Diagnostic features of MDS/MPN-RS-T
• Anemia, often macrocytic, with <1% blasts in peripheral blood
• Persistent thrombocytosis with platelet count ≥ 450 × 10^9/L
• Bone marrow: erythroid lineage dysplasia with or without multilineage dysplasia, ≥15% ring sideroblasts[a], <5% blasts in bone marrow
• No *BCR-ABL1* fusion gene; no rearrangement of *PDGFRA, PDGFRB*, *FGFR1*, or *PCM1-JAK2*; and no isolated del(5q), t(3;3)(q21.3;q26.2), or inv(3)(q21.3q26.2)
• No preceding history of MPN, MDS (except for MDS-RS), or other types of MDS/MPN
• Presence of *SF3B1* mutation or, in the absence of *SF3B1* mutation, no history of recent cytotoxic or growth factor therapy that could explain the myelodysplastic/myeloproliferative features

[a]Requires ≥15% ring sideroblasts of the marrow erythroid precursors even in the presence of *SF3B1* mutation

of treatment of anemia in lower-risk forms of MDS, including MDS with RS [58], whereas, management of thrombocytosis generally follows the algorithms used for patients with ET [58, 63].

Morphology

A review of peripheral blood smears shows increased platelets with some anisocytosis including large and giant forms. The anemia is often macrocytic, and red cells often show a dimorphic picture with small and large forms (Fig. 8.4). Neutrophils usually lack of dysplastic features, and circulating blasts are absent (<1%).

Bone marrow examination (Fig. 8.5) shows increased erythroid precursors with or without significant dyserythropoiesis. Dyserythropoiesis, like MDS, can show asynchronous nuclear/cytoplasmic maturation, megaloblastoid features, binucleated erythroblasts, karyorrhexis, and nuclear budding. Iron stain shows RS that, by definition, must comprise ≥15% of erythroids, with a median value of 30–50%. The presence of ≥15% RS is required even if *SF3B1* mutation is detected, unlike MDS with RS in which if a *SF3B1* mutation is detected, 5% RS should be sufficient. The granulocytic lineage is often unremarkable or shows only mild dyspoietic features. Bone marrow blasts are <5%. The bone marrow biopsy is usually hypercellular, with decreased myeloid/erythroid ratio due to ineffective hematopoiesis. Megakaryocyte proliferation is invariably present in all cases, frequently with focal clustering. Initially, the proliferation of megakaryocytes was described similar to ET megakaryocytes. However, recent published studies show that the megakaryocytes in MDS/MPN-RS-T are more heterogeneous than what have been recognized previously [46, 57, 64, 65]. Megakaryocytes are often composed of predominantly large hypersegmented forms, but they typically show a significant degree of size variation, including some smaller hypolobated megakaryocytes. While megakaryocytes often resemble ET megakaryocytes, in some cases, they are akin to those in polycythemia vera or primary myelofibrosis. In a subset of cases, small hypolobated megakaryocytes may be prominent, similar to those seen in MDS. In some other cases, megakaryocytes show mixed features that can be difficult to characterize either as MPN-like or MDS-like. At initial diagnosis, various grades of reticulin fibrosis (ranging from MF1 to MF3) can be seen in some patients.

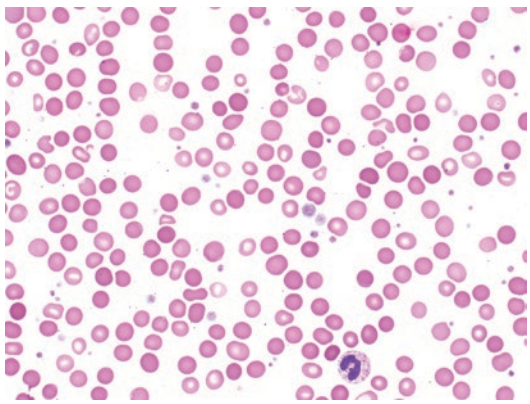

Fig. 8.4 Peripheral blood smears of a case of myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and marked thrombocytosis (MDS/MPN-RS-T) shows moderate anisocytosis of red blood cells with dimorphic morphology. Neutrophils are morphologically unremarkable. Platelets are increased showing anisocytosis including some large platelets

Immunophenotype

Immunophenotyping studies on bone marrow samples are not necessary for a diagnosis of MDS/MPN-RS-T.

Cytogenetics and Molecular Genetics

Overall, cytogenetic abnormalities are reported in 10–30% of cases [46, 64]. Among these, trisomy 8 appears to be the most common. However, there are no cytogenetic abnormalities that are specific for this entity. As recommended by the WHO classification, cases with isolated del(5q), t(3;3)(q21;q26.2), and inv(3)(q21q26.2) are excluded from MDS/MPN-RS-T.

*JAK2*V617F is a recurrent mutation in MDS/MPN-RS-T [46–50, 66] and is found in about 50% of cases. *JAK2*V617F has been reported to be associated with higher red cell, white cell, and platelet counts [47]. Homozygous mutation of *JAK2*V617F is common, and occasionally, mutation can occur in exon 12 of *JAK2* [67, 68]. *JAK2*V617F had been suggested to associate with a better prognosis in some studies, but it does not appear to be statistically significant in the multivariable analysis [69]. *MPL* W515L/K mutation has been identified in a

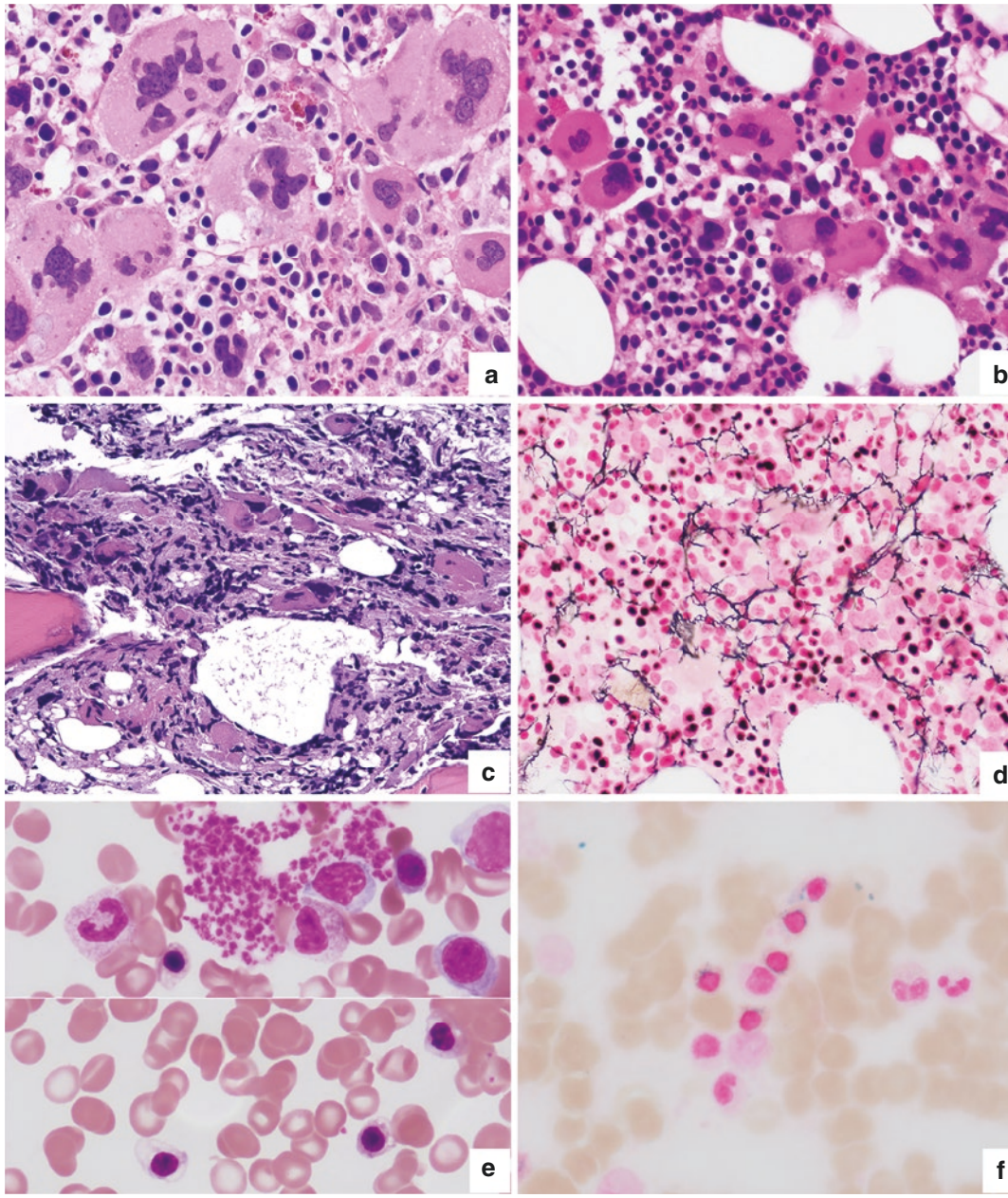

Fig. 8.5 Bone marrow morphologic features of myelodysplastic/myeloproliferative neoplasm with ring sideroblasts and marked thrombocytosis (MDS/MPN-RS-T) include a hypercellularity, erythroid hyperplasia, and proliferation of megakaryocytes. Megakaryocytes are often markedly increased in numbers, large, hypersegmented, and pleomorphic (**a**). In some cases, megakaryocytes are more variable in size, including a mixture of small, normal and large forms (**b**), In some MDS/MPN-RS-T, bone marrow may show significant myelofibrosis, and megakaryocytes can be hyperchromatic with bizarre nuclear segmentation/lobation, resembling megakaryocytes seen in primary myelofibrosis (**c**); a slight increase in reticulin fibrosis is very common (**d**). Bone marrow aspirate smears often show platelet clumps and mild megaloblastoid changes with mild dyserythropoiesis (**e**). Every case shows increased ring sideroblasts by Perl's iron stain (**f**)

low proportion of MDS/MPN-RS-T patients, up to 10% [55, 56, 68]. *MPL* mutation can coexist with *JAK2*V617F in some cases [68]. *CALR* mutations are extremely rare (0–5%) [55, 56, 70].

The frequency of *SF3B1* mutations in patients with MDS/MPN-RS-T patients is 85–90% [54, 55]. Mutations in other spliceosome genes, including *SRSF2*, *U2AF1*, and *ZRSR2,* are also detected in some MDS/MPN-RS-T cases. Approximately 50% of patients harbor both *JAK2V617F and SF3B1* mutations. Most of the *SF3B1* wild-type patients have *JAK2V617F* and *ASXL1* mutations. The co-occurrence of *SF3B1* and *JAK2*V617F mutations has been reported to associate with a better prognosis [71].

Mutations are also identified in epigenetic regulators including *TET2*, *ASXL1*, *DNMT3A*, *EZH2*, and *IDH2* and transcription factors *ETV6* and *RUNX1* [55]. *SETBP1* or *ASXL1* mutations are associated with a poorer prognosis.

Differential Diagnosis

In a patient with macrocytic anemia, thrombocytosis, and ring sideroblasts in the bone marrow, the differential diagnoses may include several conditions:

Benign/reactive causes of ring sideroblasts include alcohol ingestion, toxin exposure (e.g., benzene), drugs [72–74], mineral imbalance (e.g., copper deficiency or zinc excess), vitamin B12 deficiency [75], pregnancy [76], congenital disorders [77, 78], and in the clinical setting of cancer treatment with certain investigational drugs [79]. If there has been any recent cytotoxic or growth factor therapy, clinical and laboratory follow-up is essential to confirm that the PB and BM changes are not due to treatment. Cases of RS caused by drugs, toxins, and nutritional deficiency should be reversible once the underlying cause is addressed. Morphologically, these acquired reversible sideroblastic anemias often show erythroid hyperplasia with dyserythropoiesis in the bone marrow, but megakaryocytes are usually morphologically unremarkable with a normal topographic distribution.

Hereditary sideroblastic anemias include X-linked sideroblastic anemia (XLSA) secondary to mutations in delta-aminolevulinate synthase 2 gene (ALAS2) [77] or ATP-binding cassette subfamily B member 7 (ABCB7) [78]. The latter is often associated with nonprogressive spinocerebellar ataxia. Sideroblastic anemia is also seen as part of other syndromes as a result of mitochondrial cytopathy or other disturbances in cellular metabolism. One example is Pearson syndrome, which is characterized by sideroblastic anemia, neutropenia, and exocrine pancreatic dysfunction. Patients with thiamine-responsive megaloblastic anemia syndrome, a major defect in one of the thiamine transports, often present with megaloblastic anemia, diabetes mellitus, and deafness and respond to thiamine treatment. Congenital sideroblastic anemia often presents at an early age, is associated with other anomalies, and affects boys in XLSA. Of note, MDS with RS is exceedingly rare in children [80–86], and thus the finding of RS in children always should prompt for investigation of congenital/hereditary sideroblastic anemia. Similarly, MDS/MPN-RS-T is not a disease of childhood.

Myeloproliferative neoplasms (MPN) can develop anemia associated with secondary RS, either as a part of dyserythropoiesis or as a reversible finding secondary to treatment [87]. MDS/MPN-RS-T is a diagnosis of exclusion, and its diagnosis is most challenging in cases without a well-documented history and/or with the presence of significant myelofibrosis that makes morphologic assessment difficult. An *SF3B1* mutation with or without *JAK2*V617F mutation would favor a diagnosis of MDS/MPN-RS-T, whereas lack of *SF3B1* but positive *MPL* or *CALR* would argue against a diagnosis of MDS/MPN-RS-T. Nevertheless, in patients in whom an underlying or antecedent MPN cannot be identified or documented and who exhibit thrombocytosis, anemia, and RS at the initial presentation, a diagnosis of MDS/MPN-RS-T would be appropriate.

Other myelodysplastic/myeloproliferative neoplasms (MDS/MPN) are in the differential diagnosis with MDS/MPN-RS-T. If patients have persistent monocytosis or marked leukocytosis with left-shifted maturation and dysgranulopoiesis, the cases may be more appropriately classified as chronic myelomonocytic leukemia (CMML) or atypical chronic myeloid leukemia

(aCML), respectively. Of note, marked thrombocytosis is rare in these entities, although RS can occur in some CMML cases. By definition, if there are circulating blasts and/or ≥5% bone marrow blasts, the case is no longer considered MDS/MPN-RS-T and should be designated otherwise, such as MDS/MPN, unclassifiable.

Myelodysplastic syndromes (MDS) are also in the differential diagnosis. Approximately 5% of patients with isolated del(5q) harbor *JAK2*V617F mutation, and these patients often present with thrombocytosis [88, 89]. Of note, RS are infrequent in these cases. Although it is debatable whether MDS with isolated del(5q) and *JAK2*V617F mutation should be considered as a form of MDS/MPN, such cases are currently classified under MDS with isolated del(5q) due to their clinical features and response to lenalidomide [90]: the recommendation is to classify them as MDS with isolated del(5q) and to note the presence of *JAK2*V617F in the diagnosis. Some MDS cases with inv(3)(q21q26.2) may present with RS and thrombocytosis [91], and in such patients, the disease often has an aggressive clinical course even in the absence of increased blasts [92].

In addition, some cases of MDS may have borderline thrombocytosis at presentation, but the platelet counts may fluctuate or are only transiently increased. Also, RS may not be persistently detected. In the absence of pertinent clinical and laboratory data, these factors may lead to misdiagnosing a case of MDS as MDS/MPN-RS-T. Testing for *JAK2*V617F and *SF3B1* mutations will aid in the differential diagnosis of such cases.

References

1. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
2. Swerdlow SH, Campo E, Harris NL, Jaffe ES, The International Agency for Research on Cancer (IARC). WHO classification of tumours of haematopoietic and lymphoid tissue. 4th ed. Lyon: World Health Organiza-tion; 2008.
3. Griesshammer M, Bangerter M, Sauer T, Wennauer R, Bergmann L, Heimpel H. Aetiology and clinical significance of thrombocytosis: analysis of 732 patients with an elevated platelet count. J Intern Med. 1999;245(3):295–300.
4. Santhosh-Kumar CR, Yohannan MD, Higgy KE, al-Mashhadani SA. Thrombocytosis in adults: analysis of 777 patients. J Intern Med. 1991;229(6):493–5.
5. Thiele JKH, Orazi A, et al. Essential thrombocythemia. In: CE SSH, Harris NL, et al., editors. WHO classification of tumours of haematopoietic and lymphoid tissue. 4th ed. Lyon: IARC; 2008. p. 48–50.
6. Hutchinson CV, Stelfox P, Rees-Unwin KS. Needle-like cryoglobulin crystals presenting as spurious thrombocytosis. Br J Haematol. 2006;135(3):280.
7. Kakkar N. Spurious rise in the automated platelet count because of bacteria. J Clin Pathol. 2004;57(10):1096–7.
8. Kakkar N, Garg G. Spuriously elevated automated platelet count in severe burns--a report of two cases. Indian J Pathol Microbiol. 2004;47(3):408–10.
9. Sulai NH, Tefferi A. Why does my patient have thrombocytosis? Hematol Oncol Clin North Am. 2012;26(2):285–301, viii.
10. Yohannan MD, Higgy KE, al-Mashhadani SA, Santhosh-Kumar CR. Thrombocytosis. Etiologic analysis of 663 patients. Clin Pediatr (Phila). 1994;33(6):340–3.
11. Haznedaroglu IC, Ertenli I, Ozcebe OI, Kiraz S, Ozdemir O, Sayinalp NM, et al. Megakaryocyte-related interleukins in reactive thrombocytosis versus autonomous thrombocythemia. Acta Haematol. 1996;95(2):107–11.
12. Hsu HC, Tsai WH, Jiang ML, Ho CH, Hsu ML, Ho CK, et al. Circulating levels of thrombopoietic and inflammatory cytokines in patients with clonal and reactive thrombocytosis. J Lab Clin Med. 1999;134(4):392–7.
13. Tefferi A, Ho TC, Ahmann GJ, Katzmann JA, Greipp PR. Plasma interleukin-6 and C-reactive protein levels in reactive versus clonal thrombocytosis. Am J Med. 1994;97(4):374–8.
14. Akan H, Guven N, Aydogdu I, Arat M, Beksac M, Dalva K. Thrombopoietic cytokines in patients with iron deficiency anemia with or without thrombocytosis. Acta Haematol. 2000;103(3):152–6.
15. Balcik OS, Ozturk M, Dagli M, Kaftan O, Kosar A, Karaaslan Y, et al. Increased erythropoietin levels in reactive thrombocytosis. Haematologia (Budap). 2002;32(1):59–65.
16. Spivak JL. Polycythemia vera and other myeloproliferative neoplasms. In: Kasper D, Fauci A, Hauser S, Longo D, Jameson JL, Loscalzo J, editors. Harrison's principles of internal medicine. 19th ed. New York, USA: Mc-Graw-Hill Education; 2015. p. 898–904.
17. Harrison CN, Bareford D, Butt N, Campbell P, Conneally E, Drummond M, et al. Guideline for investigation and management of adults and children presenting with a thrombocytosis. Br J Haematol. 2010;149(3):352–75.

18. Buss DH, Stuart JJ, Lipscomb GE. The incidence of thrombotic and hemorrhagic disorders in association with extreme thrombocytosis: an analysis of 129 cases. Am J Hematol. 1985;20(4):365–72.
19. Sidon P, El Housni H, Dessars B, Heimann P. The JAK2V617F mutation is detectable at very low level in peripheral blood of healthy donors. Leukemia. 2006;20(9):1622.
20. Nielsen C, Birgens HS, Nordestgaard BG, Bojesen SE. Diagnostic value of JAK2 V617F somatic mutation for myeloproliferative cancer in 49 488 individuals from the general population. Br J Haematol. 2013;160(1):70–9.
21. Perricone M, Polverelli N, Martinelli G, Catani L, Ottaviani E, Zuffa E, et al. The relevance of a low JAK2V617F allele burden in clinical practice: a monocentric study. Oncotarget. 2017;8(23):37239.
22. Johansson P. Epidemiology of the myeloproliferative disorders polycythemia vera and essential thrombocythemia. Semin Thromb Hemost. 2006;32(3):171–3.
23. Deadmond MA, Smith-Gagen JA. Changing incidence of myeloproliferative neoplasms: trends and subgroup risk profiles in the USA, 1973–2011. J Cancer Res Clin Oncol. 2015;141(12):2131–8.
24. Harrison CN, Green AR. Essential thrombocythaemia. Best Pract Res Clin Haematol. 2006;19(3):439–53.
25. Passamonti F, Thiele J, Girodon F, Rumi E, Carobbio A, Gisslinger H, et al. A prognostic model to predict survival in 867 World Health Organization-defined essential thrombocythemia at diagnosis: a study by the International Working Group on Myelofibrosis Research and Treatment. Blood. 2012;120(6):1197–201.
26. Mudireddy M, Barraco D, Hanson CA, Pardanani A, Gangat N, Tefferi A. The prognostic relevance of serum lactate dehydrogenase and mild bone marrow reticulin fibrosis in essential thrombocythemia. Am J Hematol. 2017;92(5):454–9.
27. Passamonti F, Rumi E, Pungolino E, Malabarba L, Bertazzoni P, Valentini M, et al. Life expectancy and prognostic factors for survival in patients with polycythemia vera and essential thrombocythemia. Am J Med. 2004;117(10):755–61.
28. Rumi E, Pietra D, Ferretti V, Klampfl T, Harutyunyan AS, Milosevic JD, et al. JAK2 or CALR mutation status defines subtypes of essential thrombocythemia with substantially different clinical course and outcomes. Blood. 2014;123(10):1544–51.
29. Thiele J, Kvasnicka HM, Mullauer L, Buxhofer-Ausch V, Gisslinger B, Gisslinger H. Essential thrombocythemia versus early primary myelofibrosis: a multicenter study to validate the WHO classification. Blood. 2011;117(21):5710–8.
30. Barbui T, Thiele J, Passamonti F, Rumi E, Boveri E, Ruggeri M, et al. Survival and disease progression in essential thrombocythemia are significantly influenced by accurate morphologic diagnosis: an international study. J Clin Oncol. 2011;29(23):3179–84.
31. Gisslinger H, Jeryczynski G, Gisslinger B, Wolfler A, Burgstaller S, Buxhofer-Ausch V, et al. Clinical impact of bone marrow morphology for the diagnosis of essential thrombocythemia: comparison between the BCSH and the WHO criteria. Leukemia. 2016;30(5):1126–32.
32. Nomani L, Bodo J, Zhao X, Durkin L, Loghavi S, Hsi ED. CAL2 immunohistochemical staining accurately identifies CALR mutations in myeloproliferative neoplasms. Am J Clin Pathol. 2016;146(4):431–8.
33. Gangat N, Tefferi A, Thanarajasingam G, Patnaik M, Schwager S, Ketterling R, et al. Cytogenetic abnormalities in essential thrombocythemia: prevalence and prognostic significance. Eur J Haematol. 2009;83(1):17–21.
34. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. N Engl J Med. 2013;369(25):2379–90.
35. Milosevic JD, Kralovics R. Genetic and epigenetic alterations of myeloproliferative disorders. Int J Hematol. 2013;97(2):183–97.
36. Tefferi A, Barbui T. Polycythemia vera and essential thrombocythemia: 2017 update on diagnosis, risk-stratification, and management. Am J Hematol. 2017;92(1):94–108.
37. Xia D, Hasserjian RP. Molecular testing for JAK2, MPL, and CALR in myeloproliferative neoplasms. Am J Hematol. 2016;91(12):1277–80.
38. Rumi E, Cazzola M. How I treat essential thrombocythemia. Blood. 2016;128(20):2403–14.
39. Asp J, Andreasson B, Hansson U, Wasslavik C, Abelsson J, Johansson P, et al. Mutation status of essential thrombocythemia and primary myelofibrosis defines clinical outcome. Haematologica. 2016;101(4):e129–32.
40. Cabagnols X, Favale F, Pasquier F, Messaoudi K, Defour JP, Ianotto JC, et al. Presence of atypical thrombopoietin receptor (MPL) mutations in triple-negative essential thrombocythemia patients. Blood. 2016;127(3):333–42.
41. Milosevic Feenstra JD, Nivarthi H, Gisslinger H, Leroy E, Rumi E, Chachoua I, et al. Whole-exome sequencing identifies novel MPL and JAK2 mutations in triple-negative myeloproliferative neoplasms. Blood. 2016;127(3):325–32.
42. Harrison CN, Vannucchi AM. Closing the gap: genetic landscape of MPN. Blood. 2016;127(3):276–8.
43. Teofili L, Giona F, Torti L, Cenci T, Ricerca BM, Rumi C, et al. Hereditary thrombocytosis caused by MPLSer505Asn is associated with a high thrombotic risk, splenomegaly and progression to bone marrow fibrosis. Haematologica. 2010;95(1):65–70.
44. Barbui T, Thiele J, Carobbio A, Guglielmelli P, Rambaldi A, Vannucchi AM, et al. Discriminating between essential thrombocythemia and masked polycythemia vera in JAK2 mutated patients. Am J Hematol. 2014;89(6):588–90.
45. Barbui T, Thiele J, Gisslinger H, Finazzi G, Carobbio A, Rumi E, et al. Masked polycythemia vera (mPV): results of an international study. Am J Hematol. 2014;89(1):52–4.

46. Wang SA, Hasserjian RP, Loew JM, Sechman EV, Jones D, Hao S, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis harbors JAK2 mutation and shows overlapping myeloproliferative and myelodysplastic features. Leukemia. 2006;20(9):1641–4.
47. Schmitt-Graeff AH, Teo SS, Olschewski M, Schaub F, Haxelmans S, Kirn A, et al. JAK2V617F mutation status identifies subtypes of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Haematologica. 2008;93(1):34–40.
48. Boissinot M, Garand R, Hamidou M, Hermouet S. The JAK2-V617F mutation and essential thrombocythemia features in a subset of patients with refractory anemia with ring sideroblasts (RARS). Blood. 2006;108(5):1781–2.
49. Szpurka H, Tiu R, Murugesan G, Aboudola S, Hsi ED, Theil KS, et al. Refractory anemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T), another myeloproliferative condition characterized by JAK2 V617F mutation. Blood. 2006;108(7):2173–81.
50. Ceesay MM, Lea NC, Ingram W, Westwood NB, Gaken J, Mohamedali A, et al. The JAK2 V617F mutation is rare in RARS but common in RARS-T. Leukemia. 2006;20(11):2060–1.
51. Patnaik MM, Lasho TL, Hodnefield JM, Knudson RA, Ketterling RP, Garcia-Manero G, et al. SF3B1 mutations are prevalent in myelodysplastic syndromes with ring sideroblasts but do not hold independent prognostic value. Blood. 2012;119(2):569–72.
52. Malcovati L, Papaemmanuil E, Bowen DT, Boultwood J, Della Porta MG, Pascutto C, et al. Clinical significance of SF3B1 mutations in myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms. Blood. 2011;118(24):6239–46.
53. Visconte V, Makishima H, Maciejewski JP, Tiu RV. Emerging roles of the spliceosomal machinery in myelodysplastic syndromes and other hematological disorders. Leukemia. 2012;26(12):2447–54.
54. Visconte V, Rogers HJ, Singh J, Barnard J, Bupathi M, Traina F, et al. SF3B1 haploinsufficiency leads to formation of ring sideroblasts in myelodysplastic syndromes. Blood. 2012;120(16):3173–86.
55. Jeromin S, Haferlach T, Weissmann S, Meggendorfer M, Eder C, Nadarajah N, et al. Refractory anemia with ring sideroblasts and marked thrombocytosis cases harbor mutations in SF3B1 or other spliceosome genes accompanied by JAK2V617F and ASXL1 mutations. Haematologica. 2015;100(4):e125–7.
56. Broseus J, Lippert E, Harutyunyan AS, Jeromin S, Zipperer E, Florensa L, et al. Low rate of calreticulin mutations in refractory anaemia with ring sideroblasts and marked thrombocytosis. Leukemia. 2014;28(6):1374–6.
57. Visconte V, Tabarroki A, Hasrouni E, Maciejewski JP, Hsi ED, Tiu RV, et al. Molecular and phenotypic heterogeneity of refractory anemia with ring sideroblasts associated with marked thrombocytosis. Leuk Lymphoma. 2016;57(1):212–5.
58. Patnaik MM, Tefferi A. Refractory anemia with ring sideroblasts and RARS with thrombocytosis. Am J Hematol. 2015;90(6):549–59.
59. Patnaik MM, Tefferi A. Refractory anemia with ring sideroblasts (RARS) and RARS with thrombocytosis (RARS-T): 2017 update on diagnosis, risk-stratification, and management. Am J Hematol. 2017;92(3):297–310.
60. Szpurka H, Jankowska AM, Makishima H, Bodo J, Bejanyan N, Hsi ED, et al. Spectrum of mutations in RARS-T patients includes TET2 and ASXL1 mutations. Leuk Res. 2010;34(8):969–73.
61. Patnaik MM, Lasho TL, Finke CM, Hanson CA, King RL, Ketterling RP, et al. Vascular events and risk factors for thrombosis in refractory anemia with ring sideroblasts and thrombocytosis. Leukemia. 2016;30(11):2273–5.
62. Shaw GR. Ringed sideroblasts with thrombocytosis: an uncommon mixed myelodysplastic/myeloproliferative disease of older adults. Br J Haematol. 2005;131(2):180–4.
63. Barbui T, Barosi G, Birgegard G, Cervantes F, Finazzi G, Griesshammer M, et al. Philadelphia-negative classical myeloproliferative neoplasms: critical concepts and management recommendations from European LeukemiaNet. J Clin Oncol. 2011;29(6):761–70.
64. Gurevich I, Luthra R, Konoplev SN, Yin CC, Medeiros LJ, Lin P. Refractory anemia with ring sideroblasts associated with marked thrombocytosis: a mixed group exhibiting a spectrum of morphologic findings. Am J Clin Pathol. 2011;135(3):398–403.
65. Mohamed M. Refractory anemia with ring sideroblasts associated with thrombocytosis (RARS-T). Blood. 2014;123(3):314.
66. Remacha AF, Nomdedeu JF, Puget G, Estivill C, Sarda MP, Canals C, et al. Occurrence of the JAK2 V617F mutation in the WHO provisional entity: myelodysplastic/myeloproliferative disease, unclassifiable-refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Haematologica. 2006;91(5):719–20.
67. Flach J, Dicker F, Schnittger S, Kohlmann A, Haferlach T, Haferlach C. Mutations of JAK2 and TET2, but not CBL are detectable in a high portion of patients with refractory anemia with ring sideroblasts and thrombocytosis. Haematologica. 2010;95(3):518–9.
68. Malcovati L, Della Porta MG, Pietra D, Boveri E, Pellagatti A, Galli A, et al. Molecular and clinical features of refractory anemia with ringed sideroblasts associated with marked thrombocytosis. Blood. 2009;114(17):3538–45.
69. Patnaik MM, Lasho TL, Finke CM, Hanson CA, King RL, Ketterling RP, et al. Predictors of survival in refractory anemia with ring sideroblasts and thrombocytosis (RARS-T) and the role of next-generation sequencing. Am J Hematol. 2016;91(5):492–8.
70. Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC, et al. Somatic CALR mutations in

myeloproliferative neoplasms with nonmutated JAK2. N Engl J Med. 2013;369(25):2391–405.
71. Broseus J, Alpermann T, Wulfert M, Florensa Brichs L, Jeromin S, Lippert E, et al. Age, JAK2(V617F) and SF3B1 mutations are the main predicting factors for survival in refractory anaemia with ring sideroblasts and marked thrombocytosis. Leukemia. 2013;27(9):1826–31.
72. Bernstein WB, Trotta RF, Rector JT, Tjaden JA, Barile AJ. Mechanisms for linezolid-induced anemia and thrombocytopenia. Ann Pharmacother. 2003;37(4):517–20.
73. Vial T, Grignon M, Daumont M, Guy C, Zenut M, Germain ML, et al. Sideroblastic anaemia during fusidic acid treatment. Eur J Haematol. 2004;72(5):358–60.
74. Rudek MA, Horne M, Figg WD, Dahut W, Dyer V, Pluda JM, et al. Reversible sideroblastic anemia associated with the tetracycline analogue COL-3. Am J Hematol. 2001;67(1):51–3.
75. Bharath V, Hsia CC. Vitamin B12 deficiency presenting as pancytopenia with ring sideroblasts. Blood. 2015;126(19):2255.
76. Natelson EA. Pregnancy-induced pancytopenia with cellular bone marrow: distinctive hematologic features. Am J Med Sci. 2006;332(4):205–7.
77. Cotter PD, Baumann M, Bishop DF. Enzymatic defect in "X-linked" sideroblastic anemia: molecular evidence for erythroid delta-aminolevulinate synthase deficiency. Proc Natl Acad Sci U S A. 1992;89(9):4028–32.
78. Guernsey DL, Jiang H, Campagna DR, Evans SC, Ferguson M, Kellogg MD, et al. Mutations in mitochondrial carrier family gene SLC25A38 cause nonsyndromic autosomal recessive congenital sideroblastic anemia. Nat Genet. 2009;41(6):651–3.
79. Ok CY, Medeiros LJ, Hu Y, Bueso-Ramos CE, Wang SA. Transient/reversible ring sideroblasts in bone marrow of patients post cytotoxic therapies for primary malignancies. Leuk Res. 2011;35(12):1605–10.
80. Chatterjee T, Mahapatra M, Dixit A, Naithani R, Tyagi S, Mishra P, et al. Primary myelodysplastic syndrome in children: clinical, hematological and histomorphological profile from a tertiary care centre in India. Hematology. 2005;10(6):495–9.
81. Hasle H, Kerndrup G, Jacobsen BB, Hasle H, Kerndrup G, Jacobsen BB. Childhood myelodysplastic syndrome in Denmark: incidence and predisposing conditions. Leukemia. 1995;9(9):1569–72.
82. Hasle H, Wadsworth LD, Massing BG, McBride M, Schultz KR, Hasle H, et al. A population-based study of childhood myelodysplastic syndrome in British Columbia, Canada. Br J Haematol. 1999;106(4):1027–32. [Research Support, Non-U.S. Gov't].
83. Luna-Fineman S, Shannon KM, Atwater SK, Davis J, Masterson M, Ortega J, et al. Myelodysplastic and myeloproliferative disorders of childhood: a study of 167 patients. Blood. 1999;93(2):459–66. [Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.]
84. Occhipinti E, Correa H, Yu L, Craver R, Occhipinti E, Correa H, et al. Comparison of two new classifications for pediatric myelodysplastic and myeloproliferative disorders. Pediatr Blood Cancer. 2005;44(3):240–4. [Comparative Study].
85. Passmore SJ, Hann IM, Stiller CA, Ramani P, Swansbury GJ, Gibbons B, et al. Pediatric myelodysplasia: a study of 68 children and a new prognostic scoring system. Blood. 1995;85(7):1742–50. [Research Support, Non-U.SGov't].
86. Sasaki H, Manabe A, Kojima S, Tsuchida M, Hayashi Y, Ikuta K, et al. Myelodysplastic syndrome in childhood: a retrospective study of 189 patients in Japan. Leukemia. 2001;15(11):1713–20.
87. Schmitt-Graeff A, Thiele J, Zuk I, Kvasnicka HM. Essential thrombocythemia with ringed sideroblasts: a heterogeneous spectrum of diseases, but not a distinct entity. Haematologica. 2002;87(4):392–9.
88. Ingram W, Lea NC, Cervera J, Germing U, Fenaux P, Cassinat B, et al. The JAK2 V617F mutation identifies a subgroup of MDS patients with isolated deletion 5q and a proliferative bone marrow. Leukemia. 2006;20(7):1319–21.
89. Patnaik MM, Lasho TL, Finke CM, Gangat N, Caramazza D, Holtan SG, et al. WHO-defined 'myelodysplastic syndrome with isolated del(5q)' in 88 consecutive patients: survival data, leukemic transformation rates and prevalence of JAK2, MPL and IDH mutations. Leukemia. 2010;24(7):1283–9.
90. Musto P, Simeon V, Guariglia R, Bianchino G, Grieco V, Nozza F, et al. Myelodysplastic disorders carrying both isolated del(5q) and JAK2(V617F) mutation: concise review, with focus on lenalidomide therapy. Onco Targets Ther. 2014;7:1043–50.
91. Jenkins RB, Tefferi A, Solberg LA Jr, Dewald GW. Acute leukemia with abnormal thrombopoiesis and inversions of chromosome 3. Cancer Genet Cytogenet. 1989;39(2):167–79.
92. Rogers HJ, Vardiman JW, Anastasi J, Raca G, Savage NM, Cherry AM, et al. Complex or monosomal karyotype and not blast percentage is associated with poor survival in acute myeloid leukemia and myelodysplastic syndrome patients with inv(3)(q21q26.2)/t(3;3)(q21;q26.2): a Bone Marrow Pathology Group study. Haematologica. 2014;99(5):821–9.

Erythrocytosis

9

Olga Pozdnyakova

Overview

Erythrocytosis (or polycythemia) is defined by an increased number of red blood cells (RBC) per unit volume of blood, an alteration that is reflected by increases in the hemoglobin (HGB) concentration and hematocrit (HCT). Because these RBC parameters are affected by plasma volume and the total RBC mass (RCM), erythrocytosis can be absolute (increased RCM, increased HGB and HCT, normal or increased plasma volume) or relative (normal RCM, increased HGB and HCT, decreased plasma volume) [1]. The most direct way to determine whether erythrocytosis is absolute or relative is to measure the RCM, which should exceed 125% of that predicted for sex and body mass [2]. However, RCM is rarely measured in practice for a variety of reasons, including the need to use radioisotopes and relatively low sensitivity and specificity. As a result most physicians utilize HGB and HCT as surrogates for RCM and make a provisional diagnosis of erythrocytosis when HGB and HCT levels exceed the upper limit of normal for age and gender (Table 9.1).

RBC production is a complex and multistep process that is primarily regulated by erythropoietin (Epo), a growth factor produced in specialized interstitial cells of the kidney that is essential for the growth and survival of erythroid progenitors [3]. The major physiologic regulator of Epo production is renal hypoxia, which in most instances results from reduced RCM (anemia) or hypoxemia (a decrease in RBC oxygenation), both of which diminish the oxygen carrying capacity of the blood. *EPO* gene expression is controlled by a family of transcription factors known as hypoxia-inducible factors (HIF) that play an essential role in cellular adaptation to reduced levels of oxygen [4, 5]. Once released from the kidney, Epo circulates to hematopoietic marrow where it binds and activates its receptor, EPOR, a tyrosine kinase normally expressed on erythropoietic progenitor cells. EPOR in turn phosphorylates and activates Janus kinase 2 (JAK2), a key regulator of signal transducer and activator of transcription 5 (STAT5) in erythroid progenitors, as well as phosphatidylinositol 3 (PI3) kinase. Signals transduced via JAK2/STAT5 and PI3 kinase lead to changes in gene expression that promote effective erythropoiesis by increasing proliferation and reducing apoptosis of red cell progenitors [6, 7].

There are many ways to approach an investigation into causes of excessive erythropoiesis leading to erythrocytosis. We will follow a practical approach that divides erythrocytosis into primary and secondary disorders. Primary erythrocytosis is associated with an intrinsic genetic

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defect, while secondary erythrocytosis occurs as a response to physiologic or pathophysiologic increase in Epo production due to various conditions and diseases. Although the causes of secondary erythrocytosis are much more numerous than the causes of primary erythrocytosis, the discovery of *JAK2* V617F mutation associated with polycythemia vera, the most common primary erythrocytosis, has greatly simplified our approach to evaluation of both groups.

Secondary Erythrocytosis

Secondary erythrocytosis results from an increase in RBC production due to elevated serum Epo levels, which is most often caused by tissue hypoxia and rarely by inherited defects in the renal oxygen-sensing pathway. Table 9.2 lists the numerous causes of secondary erythrocytosis, which include congenital (genetic defects in HGB leading to increased oxygen affinity or defects in HIF pathway) and acquired

Table 9.1 Normal hemoglobin (HGB) and hematocrit (HCT) values in children and adults [55]

Age	Males		Females	
	HGB, g/dL (mean ± SD)	HCT, % (mean ± SD)	HGB, g/dL (mean ± SD)	HCT, % (mean ± SD)
1–2 years	12 ± 0.8	36 ± 2	11.8 ± 0.9	36 ± 2
3–5 years	12.4 ± 0.8	37 ± 2	12.1 ± 0.8	37 ± 2
6–8 years	12.8 ± 0.8	38 ± 2	12.4 ± 0.8	38 ± 2
9–11 years	13.3 ± 0.8	39 ± 2	12.7 ± 0.8	39 ± 2
12–14 years	14.1 ± 1.0	42 ± 3	12.5 ± 0.9	40 ± 3
15–70 years	15.8 ± 0.8	46 ± 3	13.8 ± 0.9	40 ± 2

SD Standard deviation

Table 9.2 Causes of primary and secondary erythrocytosis

	Congenital	Acquired
Primary	*EPOR* mutation	Polycythemia vera
	LNK mutation	
Secondary	Increased HGB oxygen affinity	Physiologic increased erythropoietin production due to hypoxia
	• Structural mutations of globin	• Chronic lung disease
	• Methemoglobinemia	• Right-to-left cardiac shunt
	• Defects in of red cell 2,3-DPG	• Sleep apnea
	Oxygen-sensing pathway defects	• Massive obesity
	• *VHL* gene mutations	• High altitude
	• *PHD2* mutations	• Chronic CO poisoning
	• *HIF2A* mutations	• Smoking
		Erythropoietin-secreting tumors
		• Renal cell carcinoma
		• Pheochromocytoma
		• Cerebellar hemangioblastoma
		• Hepatocellular carcinoma
		• Meningioma
		• Parathyroid tumors
		• Uterine leiomyoma
		Renal lesions
		Post-renal transplant
		Exogenous
		• Epo administration
		• Androgen administration

DPG diphosphoglycerate, *Epo* erythropoietin, *CO* carbon monoxide

causes. Acquired causes can be further subdivided into conditions associated with either physiologic (hypoxia) or pathologic (tumor-associated or exogenous) increases in Epo production.

Increased HGB Oxygen Affinity States

Oxygen in blood is transported by HGB, a tetramer consisting of four heme moieties and four globin chains, two alpha-globins and two beta-globins. The binding and the release of oxygen by HGB can be expressed as a sigmoid-shaped oxygen association/dissociation curve. The sigmoid shape of the curve is explained by cooperativity: in the tense (deoxygenated) state, HGB has a lower affinity for oxygen than in the relaxed (oxygenated) state. Partial saturation of heme by oxygen increases the oxygen affinity of the remaining hemes, and at some point during the sequential addition of oxygen to the four hemes, a transition from the tense to the relaxed state occurs [1]. HGB oxygen affinity is expressed as P_{50}, the partial pressure of oxygen at which 50% HGB is saturated, and measures 27 mmHg at 37 °C and pH 7.4. HGB oxygen affinity is affected by blood pH, levels of 2,3-biphosphoglycerate (2,3-BPG), HGB structure, and temperature. A shift of the curve to the right indicates that a higher partial pressure of oxygen is required to saturate HGB, while a shift of the curve to the left indicates that a lower partial pressure of oxygen is required to saturate HGB. High oxygen affinity hemoglobinopathies and decreased levels of 2,3-BPG shift the curve to the left, indicating a decreased ability of HGB to release oxygen during flow through the microcirculation, leading to cellular hypoxia. Specialized interstitial cells in the inner cortex and outer medulla of the kidney appropriately respond to hypoxia by increasing Epo production and secondary RBC expansion. 2,3-BPG is a potent modulator of the affinity of the HGB for oxygen; indeed, increased RBC 2,3-BPG is an important normal adaptation to chronic hypoxia. It is synthesized in the RBC via a reaction that is catalyzed by the enzyme biphosphoglycerate mutase (BPGM).

Another condition leading to cellular hypoxia due to increased oxygen affinity of HGB is congenital methemoglobinemia. Heme iron in metHGB is in the ferric (Fe^{3+}) form, which is unable to bind oxygen; in addition, partial oxidation of heme iron diminishes the oxygen affinity of any heme moieties in the HGB tetramer that remain in the ferrous (Fe^{2+}) state [8]. Under physiologic conditions, heme iron in the Fe^{3+} state is reduced by enzymes such as cytochrome b5 reductase, and as a result, methemoglobin accounts for only 1% of the total HGB [9]. Congenital deficiency of cytochrome b5 reductase or cytochrome b5 or, less commonly, the presence of the HGB M variant, leads to increase in metHGB levels, cellular hypoxia, cyanosis, and secondary erythrocytosis. Patients with high-affinity HGB conditions typically present with isolated erythrocytosis and a benign clinical course. The absence of leukocytosis, thrombocytosis, or splenomegaly helps distinguish them from polycythemia vera (see below). Increased affinity HGB conditions are associated with decreased partial pressure of O2 at which 50% of HGB is saturated, which can be directly measured using a cooximeter, a preferred method, or calculated with the following formula: log PO2 (7.4) = log PO2 (observed) − [0.5 (7.4 − pH (observed))] [21]. Conditions of high-affinity HGB can be distinguished from each other by measuring serum Epo levels, 2,3-BPG activity, oxygen saturation, and the presence of structurally abnormal hemoglobins, among others. Table 9.3 summarizes the distinguishing features of congenital erythrocytosis associated with decreased P_{50} and increased oxygen affinity HGB states. Numerous point substitution mutations affecting alpha- and beta-globin genes have been described that lead to hemoglobins with increased oxygen affinity. These mutations impair the dissociation of oxygen through various mechanisms and include mutations that affect alpha-beta-globin interactions, the binding of 2,3-BPG, and the heme oxygen-binding pocket. HGB Chesapeake (HbA R92L) was the first high-affinity hemoglobinopathy to be described. The mutant alpha chain makes up to 20–25% of the total HGB [24]. Since then over 100 additional high-affinity HGB variants have been identified; the complete list of mutations can be found on the Globin Gene Server [25, 26]. Mutations in BPGM gene, an extremely rare congenital condition, are associated with decreased levels of 2,3-BPG, a right shift in the HGB dissociation curve, and chronic tissue hypoxia [27].

Table 9.3 Distinguishing diagnostic features of congenital erythrocytosis associated with increased oxygen affinity HGB and decreased P_{50}

	Serum Epo	PO_2 levels	O_2 saturation	2,3-BPG activity	Hemolysate affinity	Hemolysate absorption spectrum	HGB structure	Cytochrome b5R or b5 activity
High-affinity HGB variants	High	Normal	Normal	Normal	High	Normal	Abnormal	Normal
2,3-BPG mutase deficiency	Normal	Normal	Normal	Decreased	Normal	Normal	Normal	Normal
Methemoglobinemia	High	Normal	Low	Normal	High	Abnormal (increased COHb)	Normal (in the absence of hemoglobin M	Reduced

HGB hemoglobin, *Epo* erythropoietin, 2,3-*BPG* 2,3 biphosphoglycerate, *COHb* carboxyhemoglobin

Hypoxia-Inducible Factor (HIF) Pathway Defects

The HIF pathway is present in almost every cell of the body, regulating a cascade of downstream genes that allow adaptation to reduced levels of oxygen. HIFs are composed of two subunits and, while the HIFβ subunits are constitutively expressed, the HIFα subunits are activated only in response to hypoxia, promoting *EPO* gene expression by binding to a hypoxia-responsive element (HRE) on the *EPO* gene [10]. When tissues are adequately oxygenated, the HIFα subunits undergo proteasomal degradation due to the presence of an intracellular oxygen sensor that involves prolyl hydroxylase domain (PHD)-containing enzymes and the von Hippel-Lindau (VHL) tumor suppressor complex. Mutations that disable the HIF pathway result in stabilization of HIF-2α even when tissue oxygenation is adequate, producing increased *EPO* transcription and secondary erythrocytosis [11].

Patients with Chuvash erythrocytosis present with a variety of hematological and vascular abnormalities due to complications associated with erythrocytosis but no tumors. Some patients with HIF pathway genetic defects develop various tumors, such as paraganglioma, pheochromocytoma, and somatostatinoma [10].

Mutations that disable the HIF pathway include mutations in genes encoding VHL, PHD2, and HIF-2α [11]. The first congenital erythrocytosis associated with the oxygen-sensing pathway dysregulation was described in 81 families living in the Chuvash region in Russia [28]. Chuvash polycythemia develops due to the presence of a VHL gene mutation that produces a R200W substitution in the VHL protein. This in turn reduces the affinity of VHL for the hydroxylated HIFα subunits, allowing HIF to accumulate and drive increased expression of EPO . Recently it has been shown that, in addition to influencing Epo production, the VHL arg200trp mutation prevents EPOR degradation by reducing its affinity for suppressor of cytokine signaling leading to constitutive activation of Epo signaling pathway [10]. There is a growing list of other homozygous mutations causing HIF pathway defects, including Croatian VHL H191D and P138L, and other compound heterozygous germline mutations, such as VHL T124A and VHL L188 V [29–31].

Heterozygous loss-of-function mutations in the EGLN1 gene encoding prolyl hydroxylase domain protein 2 (PHD2), such as P317R, P371H, and H374R, affect the catalytic and substrate binding to PHD2, resulting in partial inhibition of HIFα subunit hydroxylation and degradation [10].

Gain-of-function mutations in the EPAS1 gene encoding HIF-2α alter HIF-2α hydroxylation and its subsequent binding to VHL, leading to an aberrant stabilization under normoxic conditions.

Physiologic Increase in Erythropoietin Production Due to Cellular Hypoxia

Secondary erythrocytosis can develop due to increased Epo levels as a physiologic compensa-

Table 9.4 Conditions and disorders associated with hypoxia and associated arterial blood gases measurements

Hypoxia mechanisms	Conditions/disorders	Arterial blood gases measurements			Additional tests
		PaO_2	P(A-a) O_2	PCO_2	
Hypoventilation	CNS depression (drug, lesions) Obesity (Pickwickian syndrome) Altered neural conduction (ALS, GBS, injury) Muscular weakness (myasthenia gravis, diaphragmatic paralysis, polymyositis, muscular dystrophy, severe hypothyroidism) Kyphoscoliosis	Low	Normal	High	Neuroimaging Chest imaging Toxicology screen Electromyography Nerve conduction studies CSF examination Disease-specific Ab serum levels Thyroid function tests Genetic studies
V/Q mismatch	Pulmonary vascular disease Pulmonary venous thromboembolism	Low	High	Normal	PFT V/Q scan Chest imaging Echocardiography
Right-to-left shunt	Physiologic (atelectasis, pneumonia, ARDS) Anatomic (intracardiac shunts, pulmonary AVM, hepatopulmonary syndrome)	Low	High	Normal	Echocardiography Cardiac catheterization Chest imaging Microbiological studies
Diffusion limitation	Interstitial lung disease Chronic obstructive lung disease	Low	High	Variable	PFT; V/Q scan; imaging
Reduced PiO_2	High altitude	Low	Normal	Normal	Travel history

V/Q ventilation-perfusion, *PiO_2* inspired oxygen tension, *CNS* central nervous system, *ALS* amyotrophic lateral sclerosis, *GBS* Guillain-Barre syndrome, *ARDS* acute respiratory distress syndrome, *AVM* arteriovenous malformation, *PFT* pulmonary function tests, *CSF* cerebrospinal fluid, *Ab* antibody

tion for low oxygen concentration in lungs and/or tissues due to chronic hypoxia. Hypoxia can be caused by hypoventilation, ventilation-perfusion (V/Q) mismatch, right-to-left shunt, diffusion impairment, or reduced inspired oxygen tension [22]. Table 9.4 lists the most common disorders and conditions associated with various defects that can lead to hypoxia, along with changes in arterial blood gas measurements.

The clinical workup of the hypoxemic conditions requires the integration of clinical history, multiple laboratory tests, pulmonary function tests, and imaging studies to elucidate the mechanism and cause of hypoxia. Blood and tissue oxygenation is measured by arterial oxygen saturation (SaO_2, noninvasive pulse oximetry), arterial oxygen tension (PaO_2, arterial blood gas), the alveolar to arterial oxygen gradient (A-a oxygen gradient, calculated based on the arterial blood gas and alveolar gas equation), arterial oxygen tension to the fraction of inspired oxygen ration (PaO_2/FiO_2, used in ventilated patients), the arterial to alveolar oxygen ratio (a-A oxygen ratio), and the oxygenation index (OI, used in neonates with persistent pulmonary hypertension). Table 9.4 lists laboratory testing to evaluate for hypoxic causes of polycythemia.

Inappropriate Epo Production Due to Neoplasms

Increased Epo production can also be seen as a paraneoplastic phenomenon in several tumors due to constitutive production. The most common tumors associated with Epo overproduction are hepatocellular carcinoma (seen in 23% of patients), renal cell carcinoma (seen in 1–5% of patients), cerebellar hemangioblastoma, uterine leiomyoma, pheochromocytoma, parathyroid adenoma, and meningioma [1]. All patients with erythrocytosis and inappropriately high Epo levels in the setting

of normal oxygen studies should undergo computer tomography or magnetic resonance imaging studies to exclude the presence of an occult neoplasm.

Inappropriate Erythropoietin Production Due to Renal Lesions

Increased Epo production through HIF pathway upregulation is seen in conditions associated with renal hypoxia or end-stage renal disease, such as renal artery stenosis, polycystic kidney disease, and hydronephrosis.

Erythrocytosis also occurs in up to 15% of patients following renal transplant and is defined as persistent (>6 months) increase in HGB and HCT in the absence of other CBC abnormalities [12]. Patients demonstrate an increase in serum Epo as well as other hematopoietic factors, such as insulin-like growth factor 1, insulin-like growth factor-binding proteins, and serum-soluble stem cell factor, which may either increase sensitivity to erythropoietin or enhance erythropoiesis directly [13, 14]. Almost all cases develop in patients who have retained native kidneys that appear to be the source of excess erythropoietin [15]. One proposed mechanism leading to increase in Epo production in the native or graft kidneys or direct stimulation of red cell precursors in the bone marrow is the activation of renin-angiotensin system, possibly through the deregulation of the HIF pathway [16, 17]. The differential diagnosis includes erythrocytosis associated with renovascular hypertension, renal cell carcinoma, and hepatocellular carcinoma (especially in patients with history of hepatitis B and C, for which the renal transplant population is at a higher risk). Post-renal transplant erythrocytosis usually occurs 8–24 months after transplantation, and the risk factors include male gender, a rejection-free course, preserved glomerular filtration rate, and history of polycystic kidney disease or glomerulonephritis [23].

TEMPI Syndrome

Erythrocytosis is a clinical manifestation of a recently described rare syndrome that is associated with telangiectasia, elevated Epo levels, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting (TEMPI) [18, 19]. Only 11 patients have been described to date. The *JAK2 V617F* is absent in all cases. The bone marrow biopsy findings are nonspecific without the characteristic changes present in polycythemia vera or other myeloproliferative neoplasms (see below). Although the pathogenesis of TEMPI syndrome is unknown, the remarkable response seen with bortezomib-based regimens suggests a central role of the plasma cell clone, and this disease should be considered in patients with concurrent erythrocytosis and a plasma cell neoplasm [18].

Iatrogenic Erythrocytosis

Drug-induced polycythemia can be seen with excess use of recombinant human erythropoietin or anabolic steroids. Abuse of both types of agents is seen in athletes and may be associated with increased thrombotic risk [20]. Sports federations have introduced mandatory monitoring of HGB, HCT, and reticulocytes to identify potential misuse of exogenous stimulants of erythropoiesis.

Polycythemia Vera

Primary erythrocytosis results from genetic defects in bone marrow precursors that drive increased red cell production. These genetic changes can be congenital or acquired. The most common cause of primary erythrocytosis is polycythemia vera (PV), a MPN that develops due to acquired gain-of-function mutations in the cytoplasmic tyrosine kinase *JAK2* that leads to hypersensitivity to cytokines and proliferation of all hematopoietic lineages.

Clinical Features

The incidence of PV is estimated at 1.9/100,000 per year with a slight male predominance and the average age of 60 at diagnosis [32–34]. Many patients are asymptomatic, and the diagnosis may be suspected by the findings of plethora and splenomegaly on examination or abnormalities on a routine blood count that, in addition to increased HGB and HCT, often include leukocytosis and/or

thrombocytosis. The main causes of morbidity and mortality are due to complications of blood hyperviscosity, which stems from increases in RCM and contributes to an increased risk of venous and arterial thrombosis [35]. These complications include hypertension, Raynaud phenomenon, claudication, peripheral gangrene, Budd-Chiari syndrome, myocardial infarction, and cerebrovascular accident. Hemorrhagic complications also occur less commonly. Other common symptoms include headache, dizziness, pruritus, or abdominal complaints related to splenomegaly, which is present in the majority of patients (Table 9.5).

The diagnosis of PV is based on WHO classification criteria that include clinical, laboratory, morphologic, and genetic findings (Table 9.6). The diagnosis requires the presence of the three major criteria: elevated HGB/HCT levels, characteristic bone marrow morphologic findings, and the presence of *JAK2* V617F or *JAK2* exon 12 mutations. In the absence of *JAK2* mutations (which occurs very rarely in PV), the diagnosis relies on the first two major criteria and subnormal serum Epo levels, the only minor criterion. Although relatively specific for PV, the sensitivity of subnormal Epo level is only 64–72%, and as a result, it is not required for a PV diagnosis if all three major criteria are met.

PV has three stages defined by clinical presentation and morphologic findings: pre-polycythemic/early phase, polycythemic phase, and post-polycythemic myelofibrosis phase (post-PV MF). Over the course of their disease, patients may progress

Table 9.5 Common clinical and laboratory findings in patients with polycythemia vera at diagnosis [32]

Clinical features	% of patients with finding
Skin plethora	67
Hypertension	46
Splenomegaly	36
Pruritus	36
Vasomotor symptoms	29
Arterial thrombosis	16
Venous thrombosis	7
Major hemorrhage	4
Laboratory features	**% of patients with finding**
WBC >10.5 × 10^9/L	49
Platelets >450 × 10^9/L	53
Increased lactate dehydrogenase	50
Presence of *JAK2* V617F mutation	98
Subnormal serum erythropoietin levels	81
Abnormal bone marrow karyotype	12

Table 9.6 2016 WHO criteria for polycythemia vera [56]

Major criteria
1. HGB >16.5 g/dL in men and >16.0 g/dL in women
Or
Hematocrit >49% in men and >48% in women
Or
Increased red cell mass (>25% above mean normal predicted value)
2. Bone marrow biopsy showing hypercellularity for age with trilineage growth (panmyelosis) including prominent erythroid, granulocytic and megakaryocytic proliferation with pleomorphic, mature megakaryocytes (different in size)
3. Presence of *JAK2 V617F* or *JAK2* exon 12 mutation
Minor criterion
Subnormal serum erythropoietin levels

Diagnosis of PV requires meeting either all three major criteria or the first two major criteria and the minor criterion

through all three stages or may remain in only one stage throughout their disease course. Early-stage PV (also known as "masked PV") may have HGB and HCT values that are just slightly elevated; such patients may be mistakenly diagnosed with essential thrombocythemia (ET), especially when presenting with very high platelet counts, and the correct diagnosis requires a bone marrow biopsy [36–38]. To better capture these patients, the WHO has lowered the HGB and HCT levels and included bone marrow biopsy evaluation as a requirement for PV workup. Most PV patients present in polycythemic phase that is associated with significantly increased RCM leading to markedly elevated HCT values, frequently to values above 60%, leukocytosis of greater than 10×10^9/L, and thrombocytosis of greater than 450×10^9/L.

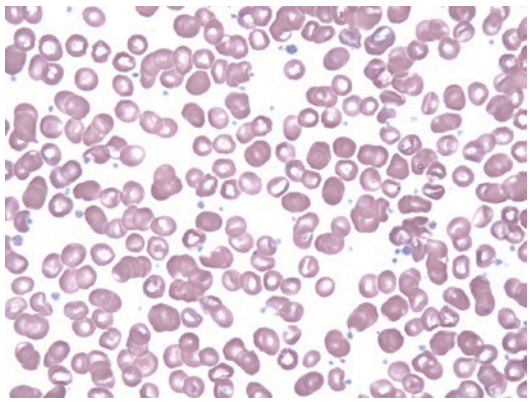

Fig. 9.1 Polycythemia vera, polycythemic phase. The peripheral blood smear shows hypochromic microcytic red blood cells due to iron deficiency. Platelets are increased. The CBC showed WBC of 4.7 × 109/L, Hg of 18.7 g/dL, MCV 78 fL, and platelets of 602 × 109/L

Morphology

Because the excessive RBC production depletes iron stores, the erythrocytes may be microcytic and hypochromic (Fig. 9.1). The post-PV MF stage is associated with leukoerythroblastic peripheral blood smear showing the presence of left-shifted myeloid cells, blasts, nucleated RBCs, and misshapen RBCs with numerous tear-drop forms (Fig. 9.2).

Bone marrow biopsy has become a requirement for PV workup and should include assessment of cellularity with focus on what lineage(s) is/are hyperplastic, megakaryocyte morphology, presence of megakaryocyte clusters, presence of fibrosis (reticulin and collagen) and/or osteosclerosis, and blast enumeration. This systematic evaluation helps establish the diagnosis, exclude other myeloproliferative neoplasms, and establish the disease stage and prognosis.

Both early/pre-polycythemic and polycythemic stages of PV show similar morphologic findings of age-adjusted hypercellularity, trilineage hyperplasia, and pleomorphic megakaryocytes that may occur in loose clusters [39] (Fig. 9.3). Some patients, especially those with markedly increased megakaryocytes, may present with substantial bone marrow fibrosis (Fig. 9.4).

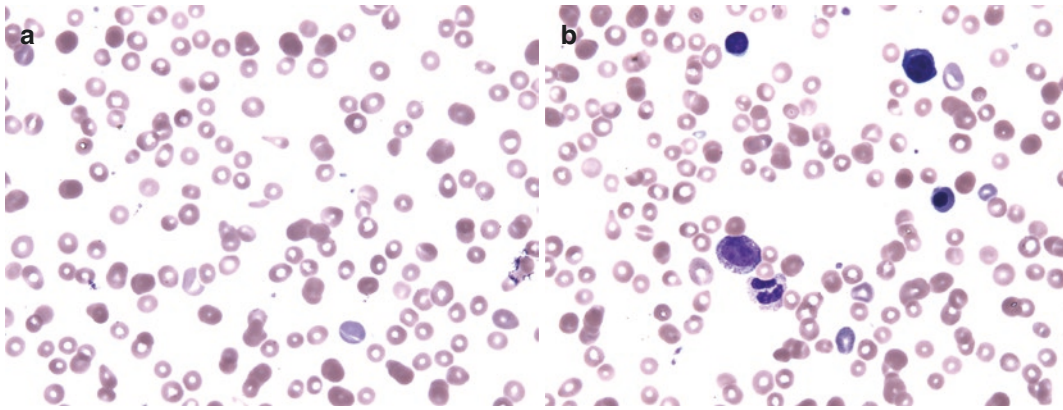

Fig. 9.2 Polycythemia vera, post-polycythemic myelofibrosis. The peripheral blood smear shows markedly decreased hypochromic RBCs with numerous misshapen forms, including tear-drop cells (**a**). Nucleated RBCs and myelocyte are seen (**b**). The CBC showed WBC of 3.09 × 10^9/L, RBC of 1.99 M/μL, HGB of 6.2 g/dL, and platelets of 57 × 10^9/L. Nucleated RBC counted 51.1 per 100 WBC. WBC differential included 29% neutrophils, 12% bands, 33% lymphocytes, 12% monocytes, 2% eosinophils, 4% metamyelocytes, and 8% myelocytes

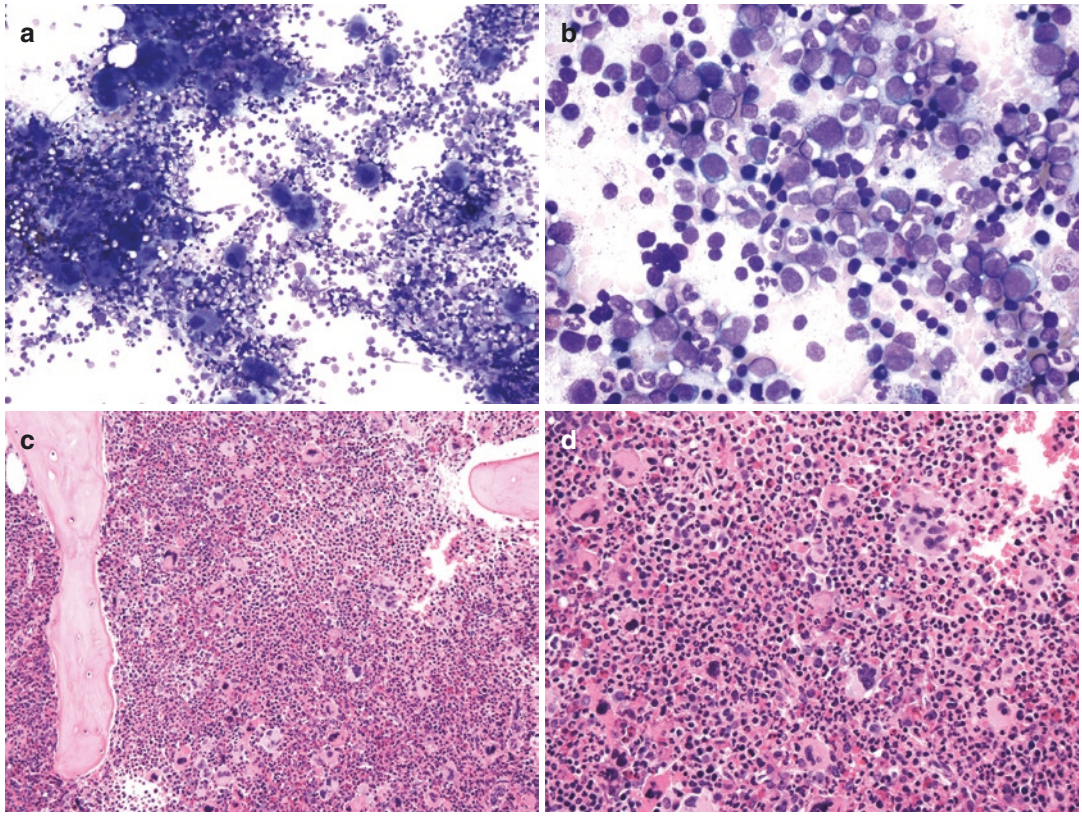

Fig. 9.3 Polycythemia vera, polycythemic phase. The aspirate smears are hypercellular showing trilineage proliferation (panmyelosis) with numerous polymorphous megakaryocytes (**a**). The image shows myeloid and erythroid hyperplasia with complete maturation and absence of dysplasia (**b**). The bone marrow biopsy is hypercellular for age (100% cellular) with trilineage proliferation (**c**). Megakaryocytes are increased, occur in loose clusters, and are pleomorphic. Complete maturation is seen in the myeloid and erythroid lineages (**d**)

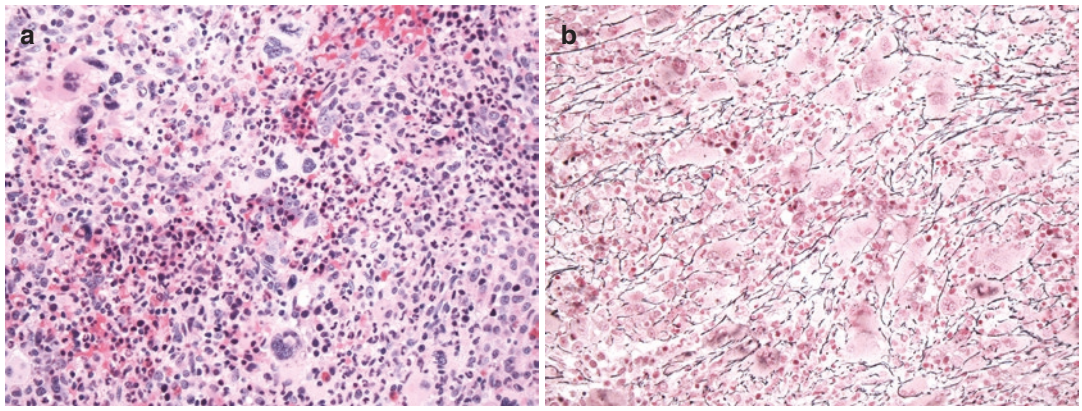

Fig. 9.4 Polycythemia vera, polycythemic phase. The biopsy shows a hypercellular for age marrow with panmyelosis. Although no obvious fibrosis is appreciated on H&E (**a**), the reticulin stain shows diffuse loose reticulin meshwork without intersections corresponding to WHO myelofibrosis grade 1 (**b**)

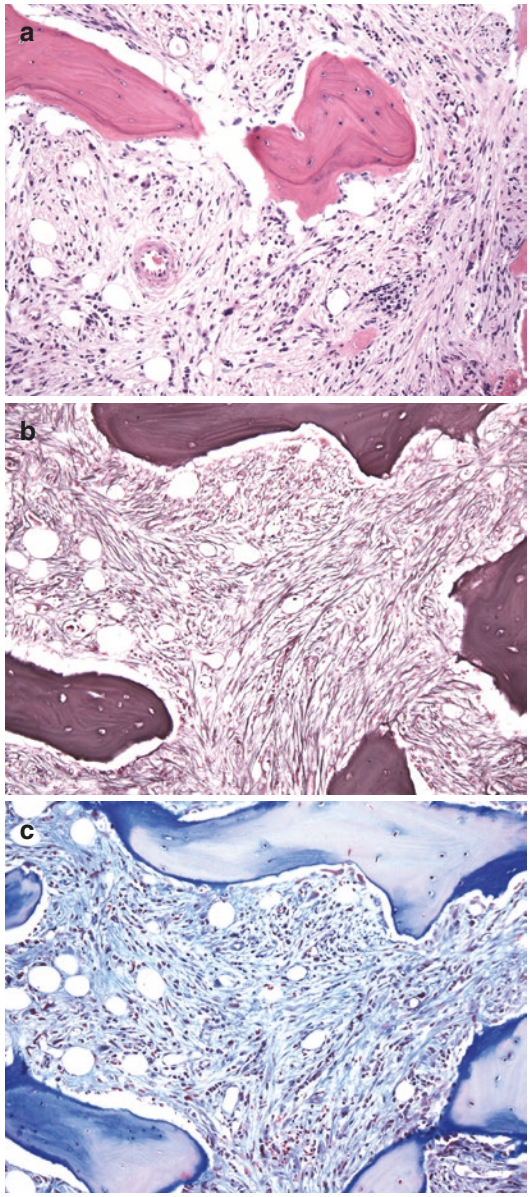

Fig. 9.5 Polycythemia vera, post-polycythemic myelofibrosis (post-PV-MF). The bone marrow biopsy shows hypocellular marrow with background fibrosis and osteosclerotic change (**a**). Dense intersecting reticulin fibers on reticulin stain (**b**) and extensive interstitial collagen deposition on trichrome stain (**c**) are consistent with WHO myelofibrosis grade 3

Although no significant differences in overall and leukemia-free survival are seen between the PV patients presenting with or without myelofibrosis, the latter group is significantly less prone to develop thrombosis [40]. Iron stores are nearly always absent. Approximately 12–21% of PV patients progress to post-PV MF [41]. Associated marrow findings include a variably cellular, often hypocellular, marrow with marked reticulin and/or collagen fibrosis, decreased erythropoiesis and granulopoiesis, persistence of megakaryocytes that often show dysplastic nuclear features, and, occasionally, osteosclerotic bone (Fig. 9.5). Increased blasts should raise the suspicion of an accelerated phase of disease (10–19% blood or bone marrow blasts) or evolution to acute myeloid leukemia (blast phase, with ≥20% blood or bone marrow blasts), which is seen in less than 10% of PV patients [42].

Immunophenotype and Cytochemistry

There is no role for immunophenotyping or cytochemistry in the diagnosis of polycythemia vera.

Cytogenetics and Molecular Genetics

The *JAK2 V617F* mutation is present in greater than 95% of PV patients and represents the third major criterion for PV diagnosis [43]. Its presence establishes the existence of clonal hematopoiesis but is not specific for PV, since the same mutation is present in other myeloproliferative neoplasms, particularly essential thrombocythemia and primary myelofibrosis. Overall, the variant allele burden and the frequency of *JAK2 V617F* homozygosity are significantly higher in PV [44, 45]. The presence of the *JAK2* V617F in several MPN may reflect the central role that JAK2 plays in the signaling from the Epo receptor (EPOR), thrombopoietin receptor (encoded by the *MPL* gene) [46], and granulocyte colony-stimulating factor (G-CSF) receptor. The *JAK2* V617F mutation induces constitutive signaling through these receptors via *STAT5*, *STAT3*, RAS-MAPK, and PI3-AKT pathways, leading to cellular proliferation independent of cytokines, including Epo, and hypersensitivity to cytokines [47–49].

The *JAK2* exon 12 mutation, a rare gain-of-function mutation seen in only 3% of PV patients, is specific for PV as it is not found in other MPN [50, 51]. Although not located in the pseudokinase domain, this mutation

acts very similarly to *JAK2* V617F. Other rare mutations seen in approximately 25% of the rare *JAK2* V617F-negative PV cases include somatic or congenital mutations in lymphocyte adaptor protein (*LNK*), which acts as a negative regulator of the *JAK-STAT* signaling pathway [52]. It is important to mention that although *JAK2* V617F mutation is considered to define clonality in MPN, including PV, recent studies have shown that *JAK2* mutation likely represents a late secondary molecular event [53, 54]. Although the initiating molecular change remains unknown, there is clear evidence that *TET2*, *ASXL1*, and *EZH2* mutations may precede *JAK2* V617F; on the other hand, mutations in these genes can also be associated with disease progression and transformation [49].

Differential Diagnosis

The approach to a patient with erythrocytosis should include clinical history, laboratory tests, and, in some cases, genetic testing to evaluate for secondary erythrocytosis (Fig. 9.6). The finding of decreased or subnormal serum Epo is quite specific for PV and helps establish the diagnosis in the setting of a *JAK2* mutation. However, Epo levels may be normal, especially in early stages of PV (pre-polycythemic or masked PV), requiring further investigation of erythrocytosis. The presence of accompanying leukocytosis and thrombocytosis, splenomegaly, and *JAK2* V617F or another clonal genetic abnormality helps exclude secondary causes of erythrocytosis (see Secondary Erythrocytosis section above) but does not differentiate between PV and other MPN, especially ET and early/pre-fibrotic primary myelofibrosis (PMF). Distinction between these three entities relies on a careful morphologic evaluation of a bone marrow biopsy, which has become a required major criterion in the revised 2016 WHO classification (Table 9.7).

The presence of lifelong erythrocytosis with early onset in the setting of low serum Epo levels should prompt an evaluation for an *EPOR* germline mutation, a rare primary cause of erythrocytosis. Unlike PV, patients with this rare autosomal dominant condition are usually asymptomatic, and the diagnosis can often be suspected on the basis of positive family history.

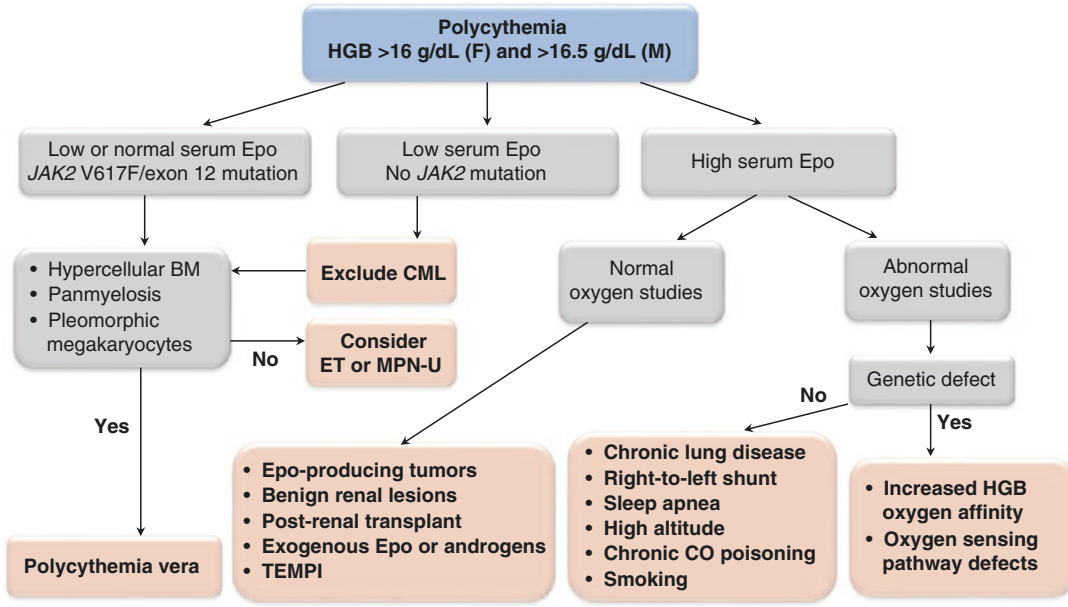

Fig. 9.6 Algorithm for the workup of polycythemia. *HGB* hemoglobin, *Epo* erythropoietin, *CML* chronic myeloid leukemia, *ET* essential thrombocythemia, *MPN-U* myeloproliferative neoplasm, unclassifiable, *TEMPI* telangiectasia, elevated erythropoietin levels, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting

Table 9.7 Distinguishing features between polycythemia vera, essential thrombocythemia, primary myelofibrosis

	Masked PV	PV	PMF	Early PMF	ET
Clinical features					
Symptoms	Asymptomatic	Increased BP pruritus	Splenomegaly	Asymptomatic	Asymptomatic
Laboratory features					
• HCT/HGB	Normal/ increased	Increased	Low	Normal/low	Normal
• Platelets	Increased	Increased	Low	Increased	Increased
• WBC	Increased	Increased	Increased/low/ normal	Increased	Normal
• Epo	Normal/ subnormal	Low	Normal	Normal	Normal
Mutations					
• *JAK2*	V617F or exon 12	V617F or exon 12	Often V617F	Often V617F	Often V617F
• *CALR*	None	None	Often mutated	Often mutated	Often mutated
• *MPL*	None	None	Occasionally mutated	Occasionally mutated	Occasionally mutated
Bone marrow morphology					
• Erythroid proliferation	Present	Present	Absent	Absent	Absent
• Myeloid proliferation	Present	Present	Present	Present	Absent
• Megakaryocytic proliferation	Present	Present	Present	Present	Present
• Megakaryocyte morphology	Pleomorphic	Pleomorphic	Bulbous	Bulbous	Large, staghorn
• Megakaryocyte clusters	Absent	Absent	Present, tight	Present, tight	Present, loose
• Fibrosis	MF0	MF0-1	MF2-3	MF0-1	MF0

BP blood pressure, *HCT* hematocrit, *HGB* hemoglobin, *MF* myelofibrosis grades 0–3, *Epo* erythropoietin, *WBC* white blood cells, *ET* essential thrombocythemia, *PMF* primary myelofibrosis

References

1. Patnaik MM, Tefferi A. The complete evaluation of erythrocytosis: congenital and acquired. Leukemia. 2009;23(5):834–44.
2. Keohane C, McMullin MF, Harrison C. The diagnosis and management of erythrocytosis. BMJ. 2013;347:f6667.
3. Blanchard KL, et al. Regulation of the erythropoietin gene. Stem Cells. 1993;11(Suppl 1):1–7.
4. Huang LE, et al. Regulation of hypoxia-inducible factor 1alpha is mediated by an O2-dependent degradation domain via the ubiquitin-proteasome pathway. Proc Natl Acad Sci U S A. 1998;95(14):7987–92.
5. Cockman ME, et al. Hypoxia inducible factor-alpha binding and ubiquitylation by the von Hippel-Lindau tumor suppressor protein. J Biol Chem. 2000;275(33):25733–41.
6. Witthuhn BA, et al. JAK2 associates with the erythropoietin receptor and is tyrosine phosphorylated and activated following stimulation with erythropoietin. Cell. 1993;74(2):227–36.
7. Constantinescu SN, Ghaffari S, Lodish HF. The erythropoietin receptor: structure, activation and intracellular signal transduction. Trends Endocrinol Metab. 1999;10(1):18–23.
8. Hong WJ, Gotlib J. Hereditary erythrocytosis, thrombocytosis and neutrophilia. Best Pract Res Clin Haematol. 2014;27(2):95–106.
9. Percy MJ, McFerran NV, Lappin TR. Disorders of oxidised haemoglobin. Blood Rev. 2005;19(2):61–8.
10. Franke K, Gassmann M, Wielockx B. Erythrocytosis: the HIF pathway in control. Blood. 2013;122(7):1122–8.
11. Prchal JT. Classification and molecular biology of polycythemias (erythrocytoses) and thrombocytosis. Hematol Oncol Clin North Am. 2003;17(5):1151–8, vi.
12. Einollahi B, et al. Erythrocytosis after renal transplantation: review of 101 cases. Transplant Proc. 2005;37(7):3101–2.

13. Kiykim AA, et al. Serum stem cell factor level in renal transplant recipients with posttransplant erythrocytosis. Artif Organs. 2009;33(12):1086–90.
14. Brox AG, et al. Erythrocytosis after renal transplantation represents an abnormality of insulin-like growth factor-I and its binding proteins. Transplantation. 1998;66(8):1053–8.
15. Dagher FJ, et al. Are the native kidneys responsible for erythrocytosis in renal allorecipients? Transplantation. 1979;28(6):496–8.
16. Richard DE, Berra E, Pouyssegur J. Nonhypoxic pathway mediates the induction of hypoxia-inducible factor 1alpha in vascular smooth muscle cells. J Biol Chem. 2000;275(35):26765–71.
17. Glicklich D, et al. Effects of erythropoietin, angiotensin II, and angiotensin-converting enzyme inhibitor on erythroid precursors in patients with posttransplantation erythrocytosis. Transplantation. 1999;68(1):62–6.
18. Rosado FG, et al. Bone marrow findings of the newly described TEMPI syndrome: when erythrocytosis and plasma cell dyscrasia coexist. Mod Pathol. 2015;28(3):367–72.
19. Sykes DB, Schroyens W, O'Connell C. The TEMPI syndrome: a novel multisystem disease. N Engl J Med. 2011;365(5):475–7.
20. Robinson N, et al. Erythropoietin and blood doping. Br J Sports Med. 2006;40(Suppl 1):i30–4.
21. Lichtman MA, Murphy MS, Adamson JW. Detection of mutant hemoglobins with altered affinity for oxygen. A simplified technique. Ann Intern Med. 1976;84(5):517–20.
22. Rodriguez-Roisin R, Roca J. Mechanisms of hypoxemia. Intensive Care Med. 2005;31(8):1017–9.
23. Razeghi E, et al. Risk factors of erythrocytosis post renal transplantation. Saudi J Kidney Dis Transpl. 2008;19(4):559–63.
24. Charache S, Weatherall DJ, Clegg JB. Polycythemia associated with a hemoglobinopathy. J Clin Invest. 1966;45(6):813–22.
25. http://globin.cse.psu.edu, The Globin Gene server.
26. Wajcman H, Galacteros F. Hemoglobins with high oxygen affinity leading to erythrocytosis. New variants and new concepts. Hemoglobin. 2005;29(2):91–106.
27. Rosa R, et al. The first case of a complete deficiency of diphosphoglycerate mutase in human erythrocytes. J Clin Invest. 1978;62(5):907–15.
28. Sergeyeva A, et al. Congenital polycythemia in Chuvashia. Blood. 1997;89(6):2148–54.
29. Lanikova L, et al. Novel homozygous VHL mutation in exon 2 is associated with congenital polycythemia but not with cancer. Blood. 2013;121(19):3918–24.
30. Lorenzo FR, et al. Novel compound VHL heterozygosity (VHL T124A/L188V) associated with congenital polycythaemia. Br J Haematol. 2013;162(6):851–3.
31. Tomasic NL, et al. The phenotype of polycythemia due to Croatian homozygous VHL (571C>G:H191D) mutation is different from that of Chuvash polycythemia (VHL 598C>T:R200W). Haematologica. 2013;98(4):560–7.
32. Tefferi A, et al. Survival and prognosis among 1545 patients with contemporary polycythemia vera: an international study. Leukemia. 2013;27(9):1874–81.
33. Berlin NI. Diagnosis and classification of the polycythemias. Semin Hematol. 1975;12(4):339–51.
34. Ania BJ, et al. Trends in the incidence of polycythemia vera among Olmsted County, Minnesota residents, 1935–1989. Am J Hematol. 1994;47(2):89–93.
35. Briere JB. Budd-Chiari syndrome and portal vein thrombosis associated with myeloproliferative disorders: diagnosis and management. Semin Thromb Hemost. 2006;32(3):208–18.
36. Thiele J, Kvasnicka HM, Diehl V. Initial (latent) polycythemia vera with thrombocytosis mimicking essential thrombocythemia. Acta Haematol. 2005;113(4):213–9.
37. Barbui T, et al. Discriminating between essential thrombocythemia and masked polycythemia vera in JAK2 mutated patients. Am J Hematol. 2014;89(6):588–90.
38. Barbui T, et al. Masked polycythemia vera (mPV): results of an international study. Am J Hematol. 2014;89(1):52–4.
39. Pozdnyakova O, et al. Impact of bone marrow pathology on the clinical management of Philadelphia chromosome-negative myeloproliferative neoplasms. Clin Lymphoma Myeloma Leuk. 2015;15(5):253–61.
40. Barbui T, et al. Initial bone marrow reticulin fibrosis in polycythemia vera exerts an impact on clinical outcome. Blood. 2012;119(10):2239–41.
41. Tefferi A, et al. Long-term survival and blast transformation in molecularly annotated essential thrombocythemia, polycythemia vera, and myelofibrosis. Blood. 2014;124(16):2507–13; quiz 2615.
42. Finazzi G, et al. Acute leukemia in polycythemia vera: an analysis of 1638 patients enrolled in a prospective observational study. Blood. 2005;105(7):2664–70.
43. Tefferi A, Spivak JL. Polycythemia vera: scientific advances and current practice. Semin Hematol. 2005;42(4):206–20.
44. Scott LM, et al. Progenitors homozygous for the V617F mutation occur in most patients with polycythemia vera, but not essential thrombocythemia. Blood. 2006;108(7):2435–7.
45. Godfrey AL, et al. JAK2V617F homozygosity arises commonly and recurrently in PV and ET, but PV is characterized by expansion of a dominant homozygous subclone. Blood. 2012;120(13):2704–7.
46. Emadali A, et al. Haploinsufficiency for NR3C1, the gene encoding the glucocorticoid receptor, in blastic plasmacytoid dendritic cell neoplasms. Blood. 2016;127(24):3040–53.
47. James C, et al. A unique clonal JAK2 mutation leading to constitutive signalling causes polycythaemia vera. Nature. 2005;434(7037):1144–8.
48. Wernig G, et al. Expression of Jak2V617F causes a polycythemia vera-like disease with associated myelofibrosis in a murine bone marrow transplant model. Blood. 2006;107(11):4274–81.

49. Vainchenker W, et al. New mutations and pathogenesis of myeloproliferative neoplasms. Blood. 2011;118(7):1723–35.
50. Scott LM, et al. Prevalance of JAK2 V617F and exon 12 mutations in polycythaemia vera. Br J Haematol. 2007;139(3):511–2.
51. Butcher CM, et al. Two novel JAK2 exon 12 mutations in JAK2V617F-negative polycythaemia vera patients. Leukemia. 2008;22(4):870–3.
52. Lasho TL, Pardanani A, Tefferi A. LNK mutations in JAK2 mutation-negative erythrocytosis. N Engl J Med. 2010;363(12):1189–90.
53. Kralovics R, et al. A gain-of-function mutation of JAK2 in myeloproliferative disorders. N Engl J Med. 2005;352(17):1779–90.
54. Rumi E, et al. JAK2 (V617F) as an acquired somatic mutation and a secondary genetic event associated with disease progression in familial myeloproliferative disorders. Cancer. 2006;107(9):2206–11.
55. Hollowell JG, et al. Hematological and iron-related analytes—reference data for persons aged 1 year and over: United States, 1988–94. Vital Health Stat 11. 2005;247:1–156.
56. Arber DA, et al. The 2016 revision to the World Health Organization (WHO) classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.

Mixed Cytoses and Cytopenias

10

Sanam Loghavi and Sa A. Wang

Overview

Leukocytosis is defined as leukocyte count ≥13 × 10^9/L; in this chapter, the focus is leukocytosis due to an increase of neutrophils and their precursors. Thrombocytosis is defined by a platelet count ≥450 × 10^9/L. Leukocytosis either in isolation or as the main abnormal feature of blood is discussed in Chap. 5, whereas thrombocytosis as the primary presenting abnormality is discussed in Chap. 8. This chapter covers conditions presenting with either leukocytosis or thrombocytosis and one or two cytopenias including reactive conditions that may present with this mixed picture in the blood as well as specific myeloid neoplasms that most commonly present with mixed cytosis and cytopenias: atypical chronic myeloid leukemia; myelodysplastic syndrome/myeloproliferative neoplasm (MDS/MPN)-unclassifiable; and primary myelofibrosis. A proposed algorithmic approach to the diagnosis of neutrophilic leukocytosis accompanied by anemia and/or thrombocytopenia is shown in Fig. 10.1. Myelodysplastic syndromes with isolated del(5q) and with inv(3) that can frequently present with thrombocytosis despite other cytopenias will also be discussed. The specific entity MDS/MPN with ring sideroblasts and thrombocytosis, also commonly presents with thrombocytosis and anemia, but this entity is discussed separately in Chap. 8.

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Reactive Cytosis with Associated Cytopenia(s)

Clinical Features

Reactive conditions presenting primarily with isolated leukocytosis or thrombocytosis are discussed separately in Chaps. 5 and 8, respectively. The causes of reactive/secondary leukocytosis and/or thrombocytosis are very broad, including infections, collagen vascular diseases, trauma, recent surgery, iron deficiency, hemolysis, splenectomy, paraneoplastic syndromes, drugs, smoking, growth factors, and corticosteroids. It is quite common to see concomitant anemia in these patients, due to the same underlying conditions that cause leukocytosis and/or thrombocytosis. Anemia can be a result of blood loss, hemolysis, iron deficiency, nutritional deficiency, or chronic diseases. Anemia is often microcytic if the underlying causes are iron deficiency and thalassemia, and reactive thrombocytosis frequently accompanies iron deficiency anemia. Blood loss, hypersplenism, and hemolysis often results in normocytic anemia. Macrocytic anemia can be seen in patients with vitamin B12 or folate deficiency, liver disease, excessive alcohol intake, hypothyroidism, or drugs/medications that can interfere with DNA synthesis.

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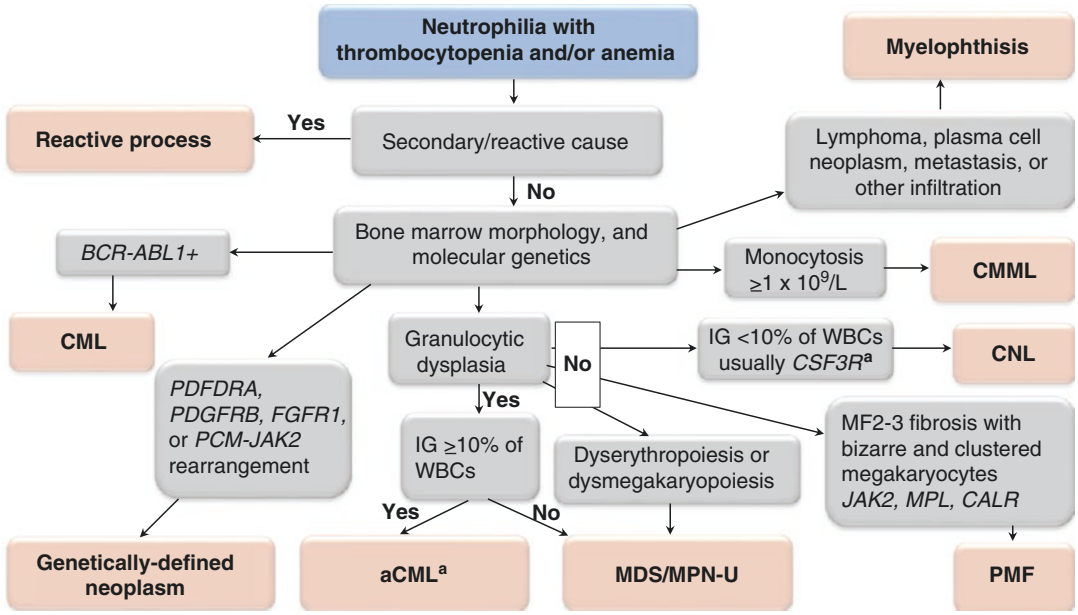

Fig. 10.1 Algorithm for the workup of neutrophilia with thromobcytopenia and/or anemia. [a]*CSF3R* mutations may be present in atypical CML but with much lower frequency compared with chronic neutrophilic leukemia (CNL), and presence of *SETBP1* and/or *ETNK1* mutations favor atypical CML. *IG*, immature granulocytes

On the other hand, leukocytosis with thrombocytopenia or thrombocytosis with leukopenia is less common in reactive conditions. Leukocytosis with thrombocytopenia can be seen in infections, including viral [1], bacterial, and mycobacterial infections, particularly in patients with sepsis [2]. Thrombocytopenia occurs in up to 20% of patients in the medical intensive care unit (ICU) and 35% of patients within the surgical ICU, and sepsis is a clear risk factor for thrombocytopenia. Thrombocytopenia may occur as a complication of heparin therapy also known as heparin-induced thrombocytopenia (HIT), and some patients may have undiagnosed or underlying chronic idiopathic thrombocytopenia purpura (ITP); both entities are discussed separately in Chap. 2. Leukocytosis with leukoerythroblastosis (the presence of left-shifted myeloid forms and nucleated red blood cells in the blood) and thrombocytopenia may be seen in an infiltrative process causing marrow fibrosis, such as metastatic tumor or primary myelofibrosis. Conversely, the combination of thrombocytosis and leukopenia has been reported in patients with celiac disease [3]. Thrombocytosis may be secondary to inflammatory mediators or, in some cases, secondary to iron deficiency anemia or functional hyposplenia, while leukopenia (which is less common than thrombocytosis in celiac disease patients) has been attributed to folate and/or copper deficiency. In addition, the combination of thrombocytosis and leukopenia may be seen in association with antipsychotic and antituberculosis drugs, rheumatoid arthritis, and patients with systemic lupus erythematosus or patients with non-Hodgkin lymphoma with or without bone marrow (BM) involvement [4].

Morphology

Bone marrow biopsy may be indicated in patients with persistently abnormal blood counts to rule out an underlying neoplastic condition. The morphologic findings are variable depending on the hematological manifestations and underlying cause. BM cellularity, myeloid/erythroid ratio, and megakaryocyte numbers are variable. Erythroid precursors are often increased in patients with anemia, but can be decreased or even absent in some cases, if associated with pure red cell aplasia (Fig. 10.2). Mild dyserythropoiesis is common,

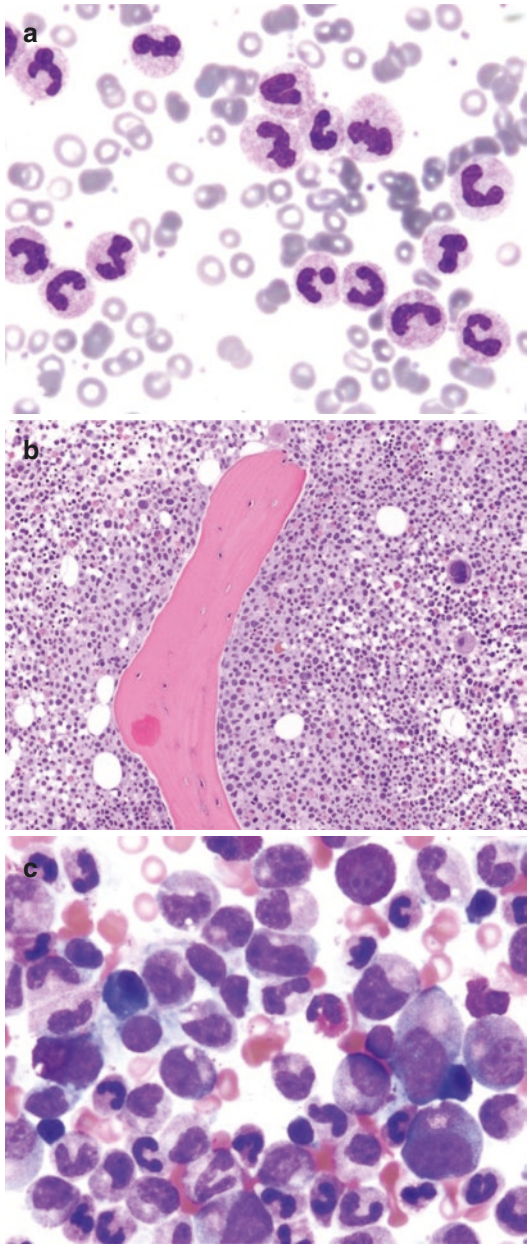

Fig. 10.2 Cytosis and cytopenia associated with paraneoplastic syndrome. A patient with widely metastatic ovarian carcinoma presented with marked leukocytosis (WBC 97.3 × 10^9/L), anemia (hemoglobin 7.9g/dL, MCV 93fL) and thrombocytosis (platelets 537 × 10^9/L). (**a**) Peripheral blood shows leukocytosis with mainly normal appearing neutrophils. (**b**) Bone marrow trephine biopsy shows a hyperceullar marrow with left shifted myeloid maturation with many early myeloid progenitors next to the bone trabeculae. (**c**) Bone marrow aspirate smear shows myeloid hyperplasia with left-shifted maturation. No dysplasia is observed. Erythrocytes are reduced. Cytogenetic studies showed a normal karyotype, and molecular studies showed wild-type *JAK2, MPL, CALR, CSF3R, KRAS, NRAS,* and *KIT*

reflecting stress-induced hematopoiesis, and may be quite marked in patients with active hemolysis. Myeloid lineage cells are often increased and can show left-shifted or arrested maturation. In patients treated with growth factor, myeloid precursors may be increased, but blasts usually comprise <5% of cells in BM and <2% of cells in blood. Small lymphocytes, lymphoid aggregates, and mature plasma cells are generally increased in autoimmune diseases and viral infections, such as human immunodeficiency virus (HIV) and hepatitis C infection.

The following features should be considered on morphologic examination of BM in patients with mixed cytoses and cytopenia: (1) Is there an infiltrative process by a lymphoid or plasma cell neoplasm or metastatic tumor? (2) Are megakaryocytes abnormal in morphology or distribution (abnormal clustering, location close to the bone trabecula)? (3) Is there significant dysplasia in any hematopoietic lineage? (4) Are blasts increased? (5) Is there significant reticulin fibrosis? (6) Are basophils and/or eosinophils increased? (7) Are abnormal mast cell clusters present?

Immunophenotype

Immunophenotyping can be particularly helpful in ruling out an infiltrative process or an intrinsic myeloid neoplasm. Flow cytometry study should include markers to assess B-cell clonality, T- and NK-cell subsets, and blasts. If the initial screening panel reveals anything unusual, then an extended panel of markers or immunohistochemistry studies may be performed for further investigation.

Cytogenetics and Molecular Genetics

Chromosomal analysis and mutation testing may help in identifying a clonal hematopoietic neoplasm; however, the interpretation of these abnormalities needs to be done with caution, considering the prevalence of somatic mutations (such as *DNMT3A*, *TET2*, and *ASXL1* [5–9]) in otherwise healthy aging population, known as clonal hematopoiesis of indeterminate potential (CHIP). Clonal chromosomal abnormalities

can be observed in patients who have received cytotoxic therapy, either transient or clinically "silent," which does not warrant a diagnosis of myeloid neoplasm. Therefore, like any disease, molecular genetic data need to be combined with other clinical, morphological, and immunophenotypical parameters.

Differential Diagnosis

The work-up of patients with cytosis and cytopenia, should exclude any possible underlying etiology that may cause secondary cytosis and cytopenia and assess for a potential hematopoietic neoplasm that may be responsible for the abnormal production of blood cells. Myeloid neoplasms with leukocytosis and/or thrombocytosis with cytopenia and their clinicopathological features are discussed below and represent the main differential diagnostic consideration in these patients.

Myeloid Neoplasms with Leukocytosis and/or Thrombocytosis with Cytopenia

Among myeloid neoplasms, the presence of a "hybrid" picture in the blood, with one elevated count and one or two cytopenias, is most suggestive of a disease in the MDS/MPN category. Within the category of MDS/MPN, chronic myelomonocytic leukemia (CMML) and juvenile myelomonocytic leukemia (JMML) present with a prominent monocytic proliferation and other cytopenias; these conditions are discussed in detail in Chap. 7. MDS/MPN with ring sideroblasts and thrombocytosis (MDS/MPN-RS-T) is discussed in Chap. 8. The remaining two MDS/MPN entities, atypical chronic myeloid leukemia (aCML) and MDS/MPN-unclassifiable (MDS/MPN-U), are discussed here. Primary myelofibrosis (PMF) is a pure myeloproliferative neoplasm (MPN); however, unlike polycythemia vera, essential thrombocythemia, chronic myeloid leukemia (CML), and chronic neutrophilic leukemia (CNL) which are characterized primarily by their respective cytoses, patients with PMF often present with hybrid cytoses and cytopenias. Many patients have mild leukocytosis and anemia, but others may have significant cytopenias that raise the question of MDS/MPN or MDS. Finally, MDS with del(5q) and inv(3) are discussed in this chapter because they frequently present with thrombocytosis with anemia and/or leukopenia.

Atypical Chronic Myeloid Leukemia, *BCR-ABL1* Negative

Atypical chronic myeloid leukemia (aCML) is a rare MDS/MPN characterized by the simultaneous presence of myelodysplastic and myeloproliferative features. The reported incidence is approximately 0.01–0.04 per 100,000 populations. Dysplastic neutrophilic leukocytosis with left-shifted neutrophilic precursors (≥10%) in the blood is the hallmark of this disease. There is no evidence of *BCR-ABL1* fusion or rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2* translocation, by definition [10].

Table 10.1 WHO 2016 diagnostic criteria for atypical chronic myeloid leukemiav, *BCR-ABL1* negative (aCML)

PB leukocytosis with neutrophils and neutrophilic precursors (promyelocytes, myelocytes, metamyelocytes) comprising ≥10% of WBCs
Dysgranulopoiesis, which may include abnormal chromatin clumping
No or minimal absolute basophilia; basophils typically <2% of WBCs
No or minimal absolute monocytosis; monocytes <10% of WBCs
Hypercellular BM with dysplastic granulocytic hyperplasia, with or without erythroid and megakaryocytic dysplasia
<20% blasts in the PB and BM
No evidence of *BCR-ABL1*, *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangement or *PCM1-JAK2*
Not meeting WHO criteria for PMF, PV, or ET and no previous history of these diseases
Presence of *SETBP1* and/or *ETNK1* mutations[a]
Absence of *CSF3R* mutation[a]

BM bone marrow, *ET* essential thrombocythemia, *PB* peripheral blood, *PMF* primary myelofibrosis, *PV* polycythemia vera, *WBC* white blood cell

[a]These features are not required for the diagnosis but are highly suggestive of aCML

The WHO 2017 diagnostic criteria for aCML are summarized in Table 10.1.

Clinical Features

Affected patients are typically elderly with a median age at presentation of 72 years [10–12]. There is a male predominance (2:1). Organomegaly (45%), anemia (median HGB 9.4 g/dL), thrombocytopenia (median platelet count 87 × 10^9/L), and leukocytosis (median WBC count, 40.8 × 10^9 /L) are common; circulating blasts are seen in 17% of patients. The presence of marked dysgranulopoiesis and circulating neutrophil precursors is helpful in distinguishing aCML from MDS/MPN-U and MPNs that can present with leukocytosis [11, 12]. Absolute monocytosis (≥1 × 10^9/L) due to a high white cell count is common, but the percentage of monocytes is always <10%. Elevated serum lactate dehydrogenase (LDH) is seen in approximately 85% of patients at presentation [12].

aCML is an aggressive disease, with a median overall survival of 14–30 months [10, 13–16]. Approximately 15–40% of cases progress to acute myeloid leukemia (AML), and the median transformation time is 18 months [10, 13, 14, 17]. Older age (>65 years) and leukocytosis >50 × 10^9/L have been reported to be associated with shorter overall survival, while organomegaly, monocytosis, BM blasts >5%, and transfusion dependency have been linked to increased rate of AML progression [14].

Morphology

The peripheral blood shows leukocytosis with increased neutrophils and neutrophilic precursors. By definition, neutrophil precursors including promyelocytes, myelocytes, and metamyelocytes comprise ≥10% of WBCs. Dysgranulopoiesis is present and is usually prominent (Fig. 10.3). The morphologic features defining dysgranulopoiesis in MDS, such as cytoplasmic hypogranulation, nuclear hypolobation, and pseudo-Pelger-Huët neutrophils, apply to aCML as well. In addition, dysgranulopoiesis may be present in a peculiar form that is uncommon in MDS (Fig. 10.4): the neutrophils appear to be hypersegmented with often normal granulation, but the nuclei

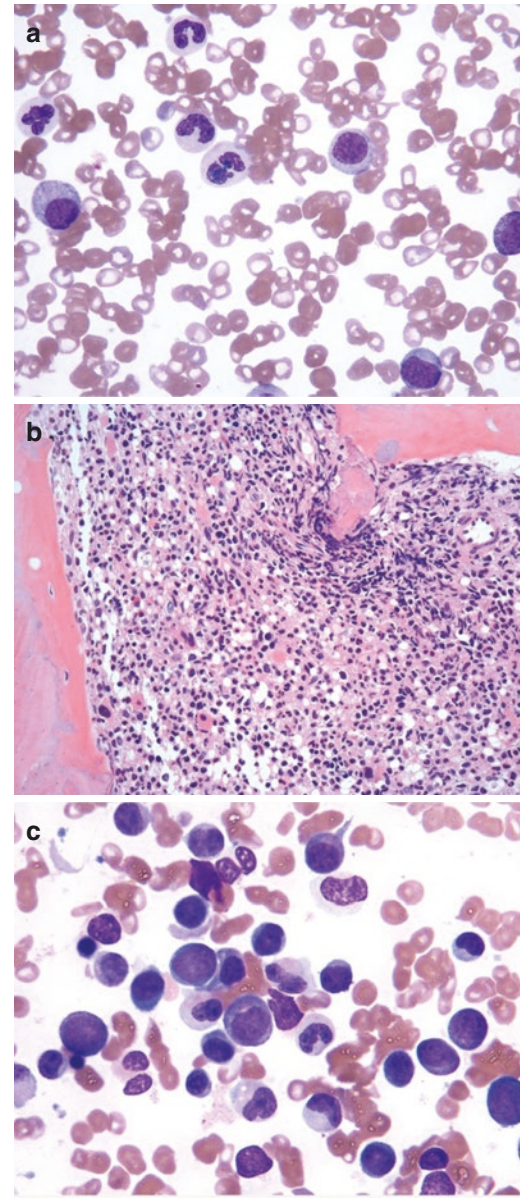

Fig. 10.3 Atypical chronic myeloid leukemia, *BCR-ABL1* negative. (**a**) Peripheral blood smear shows leukocytosis, with increased neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes). A blast is seen in the lower right corner. There is dysgranulopoiesis characterized by cytoplasmic hypogranulation and nuclear hypolobation, similar to the dysgranulopoiesis seen in myelodysplastic syndromes (MDS). (**b**) Bone marrow biopsy shows a hypercellular marrow with myeloid hyperplasia and many small dysplastic megakaryocytes. (**c**) Bone marrow aspirate smear shows myeloid hyperplasia with left-shift maturation and dysgranulopoiesis, with cytoplasmic hypogranulation, nuclear hypolobation

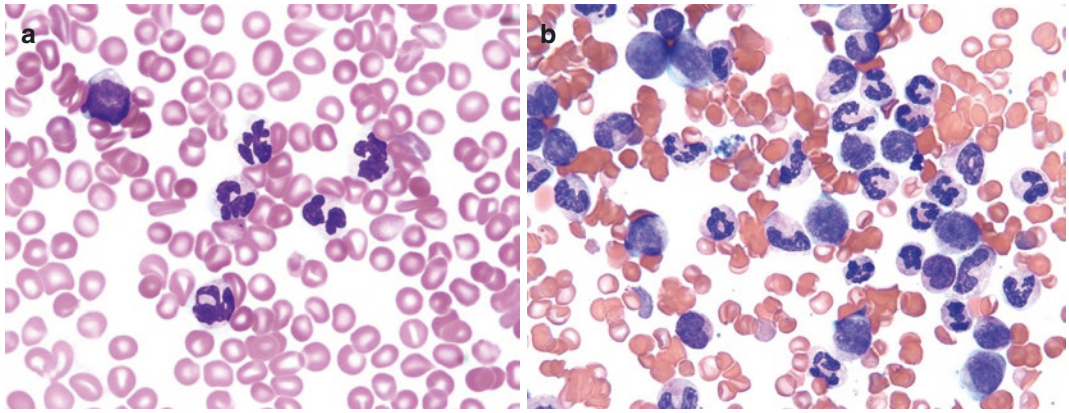

Fig. 10.4 Atypical chronic myeloid leukemia, *BCR-ABL1* negative with peculiar abnormal neutrophils. (**a**) Peripheral blood smear shows leukocytosis, with neutrophils showing abnormally twisted and branched nuclei that are hyperchromatic. This particular abnormality is not common in MDS dysgranulopoiesis and may be mistaken for neutrophil hypersegmentation. (**b**) Bone marrow aspirate smear shows myeloid hyperplasia, with similar abnormal neutrophils

are twisted and abnormally branched, unlike the neutrophil hypersegmentation seen in megaloblastic anemia. This form of dysgranulopoiesis is observed in about 20–30% aCML cases and should be recognized. There is generally no basophilia, but rare cases may show up to 5% peripheral blood basophils. Eosinophilia is also uncommon.

The BM is often hypercellular for age with a disproportionate increase in myeloid lineage cells: the median myeloid/erythroid ratio is 6. Severe granulocytic dysplasia characterized by increased pseudo-Pelger-Huët neutrophils, nuclear hyperchromasia, and abnormal nuclear segmentation and cytoplasmic granularity is invariably present and is helpful in distinguishing aCML from its mimics, particularly CNL. The peculiar form of dysgranulopoiesis with abnormal nuclear branching is also observed on BM aspirate smears. Erythroid precursors are often decreased, and dyserythropoiesis is present in approximately half of cases. Dysmegakaryopoiesis with increased small, hypolobated megakaryocytes, with or without megakaryocytic hyperplasia, is often seen. Blasts may be increased, but are less than 20% of all nucleated cells by definition. Various degrees of bone marrow reticulin fibrosis may be present at the time of initial diagnosis or may appear later in the course of disease.

Immunophenotype

No specific immunophenotypic features have been identified in aCML; however, myeloid blasts often exhibit an aberrant immunophenotype with alterations of CD34, CD38, CD117, CD123, and/or CD13 and/or aberrant expression of lymphoid markers by flow cytometric analysis [18]. Granulocytes often demonstrate hypogranulation (decreased side scatter) and abnormal maturation patterns (Fig. 10.5).

Cytogenetics and Molecular Genetics

An abnormal karyotype is identified in 30–40% of patients. No specific recurrent numerical or structural cytogenetic abnormalities have been identified in aCML. Trisomy 8 is the single most common cytogenetic abnormality (18%) [12]. del(20q) and abnormalities of chromosomes 12, 13, 14, 17, and 19 have also been reported [10]. Complex karyotype, monosomy 7, or del(7q) and i17(q) have been reported in approximately 8% of cases [12]. There is no evidence of *BCR-ABL1* or *PDGFRA*, *PDGFRB*, or *FGFR1* rearrangement, by definition [10]. Isolated cases with *BCR-JAK2* have been reported [12, 19–21].

High-throughput next-generation sequencing studies have identified recurrent somatic mutations which appear to be involved in the pathogenesis of aCML. These mutations span across various gene categories including those involved in epigenetic regulation [*SETBP1* (up to 33%); *ASXL1* (25%);

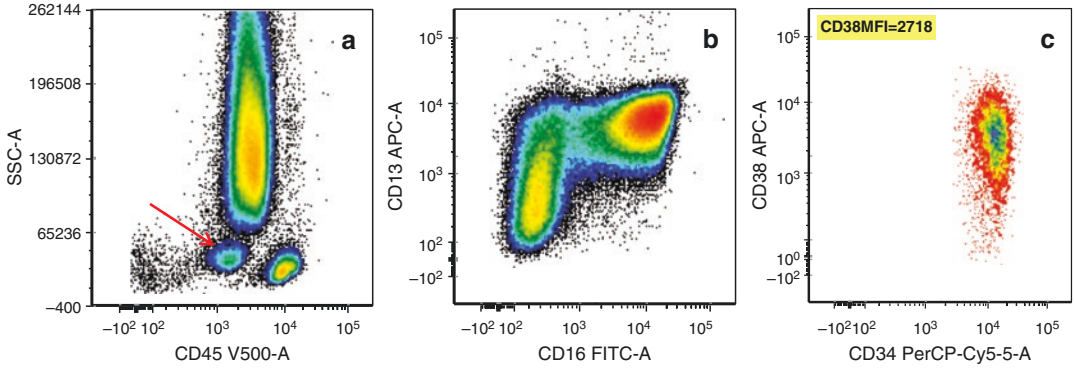

Fig. 10.5 Flow cytometric characterization of atypical chronic myeloid leukemia. (**a**) CD45 versus SCC: neutrophils and their precursors show markedly decreased SSC. There is a discrete blast population (*arrow*) showing decreased CD45 and low SSC; monocytes are nearly absent. (**b**) Neutrophils and their precursors show an abnormal CD13/CD16 maturation pattern (normal is a 'check mark' pattern). (**c**) CD34+ blasts show markedly decreased CD38 (normal CD38 APC MFI >20,000)

TET2 (25%); *EZH2* (up to 15%)] and cell signaling [*NRAS/KRAS* (up to 35%); *ETNK1* (9%); *JAK2* (7%); *CBL* (7%)] [11, 13, 22]. *CSF3R* mutations, frequently present in CNL, are notably less common in aCML (<10% of cases) [23, 24]. Other MPN-associated driver mutations (*CALR*, *MPL*) are also typically absent [25]. The exact mechanistic role of *SETBP1* in aCML is not well understood; there is some data suggesting that the stabilization of SET results in inhibition of the tumor suppressor protein phosphatase 2A (PP2A) which in turn leads to dysregulated cellular proliferation [13].

Unlike other myeloid neoplasms, there is no data to suggest a prognostic significance for specific cytogenetic alterations such as abnormalities of chromosome 7 or a complex karyotype [11]. Patients with *SETBP1*-mutated aCML have a shorter overall survival compared to those with *SETBP1*-wild-type disease [13].

Differential Diagnosis

The major differential diagnoses for aCML are other conditions presenting with marked leukocytosis.

Reactive/secondary leukocytosis with neutrophilia may be caused by smoking, infections, inflammation, drugs including growth factors, stress (physical or emotional), hemorrhage, myelophthisis, paraneoplastic syndromes, and asplenism [26–37]. Leukemoid reaction is a term used to describe reactive leukocytosis with WBC count >50,000/uL. Reactive leukocytosis with neutrophilia does not show dysplastic features; rather, neutrophils may show toxic granules, Döhle bodies, hypersegmentation, and/or increased band forms. A few neutrophil precursors, including promyelocytes, myelocytes, and metamyelocytes, may be present, but they rarely comprise >10% of WBCs. The BM findings of reactive/secondary leukocytosis include normal or increased cellularity with increased neutrophils and their precursors. Significant dysgranulopoiesis and dysmegakaryopoiesis are absent. The BM biopsy may reveal lymphoma, myeloma, or metastatic carcinoma that may be the underlying causes of leukocytosis.

Chronic myeloid leukemia (CML) presents with marked leukocytosis with increased neutrophil precursors in the peripheral blood. CML frequently shows basophilia and eosinophilia, which are usually not seen in aCML. Similar to aCML, the BM of patients with CML is hypercellular and shows increased neutrophils and neutrophilic precursors. However, there is no significant dysgranulopoiesis. There is often a prominent increase in myelocytes and neutrophils in CML (Fig. 10.6), which is not consistently observed in aCML. Megakaryocytes in CML are "dwarf" small and hypolobated forms, morphologically overlapping with the dysplastic megakaryocytes in aCML. All myeloid neoplasm presenting with leukocytosis should be checked for *BCR-ABL1* fusion, which defines CML and excludes aCML.

Chronic neutrophilic leukemia (CNL) is a myeloproliferative neoplasm with a median

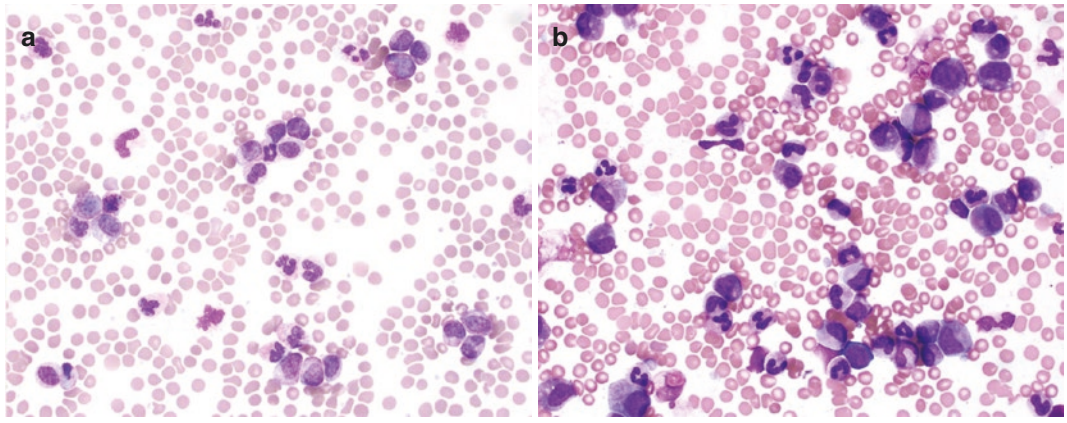

Fig. 10.6 Chronic myeloid leukemia (CML). (**a**) Peripheral blood shows leukocytosis, with increased neutrophil precursors (promyelocytes, myelocytes, metamyelocytes). Basophils are present. There is no significant dysgranulopoiesis. (**b**) Bone marrow aspirate smear shows myeloid hyperplasia with a bulging of neutrophils and myelocytes without dysgranulopoiesis

WBC of 40–50 × 10^9/L, similar to aCML. However, the leukocytes are predominantly composed of mature neutrophils and band forms (≥80% of WBCs). Neutrophil precursors (promyelocytes, myelocytes, and metamyelocytes) comprise <10% of WBCs (Fig. 10.7). Toxic granulation and Döhle bodies are often seen; however, significant dysgranulopoiesis is not present. The BM of patients with CNL is hypercellular due to neutrophilic proliferation with cells predominantly from metamyelocytes to mature neutrophil stages. Megakaryocytes are usually normal in quantity or can be slightly increased without significant clustering and may be atypical (large, hyperlobulated) or morphologically unremarkable. Mutation in *CSF3R* is virtually a disease-defining molecular marker of CNL and is included as a diagnostic criterion in the WHO update in 2017 [23, 25]. In contrast, *CSF3R* mutations are uncommon (<10%) in aCML, but can occur in a subset of cases and do not preclude the diagnosis if other features fit with a diagnosis of aCML.

Chronic myelomonocytic leukemia (CMML) is an MDS/MPN characterized by persistent monocytosis. In aCML cases with a very high WBC, the absolute monocyte count can easily surpass ≥1.0 × 10^9/L, underscoring the importance of the requirement of ≥10% monocytes for a diagnosis of CMML. Overall, if there is persistent monocytosis (both absolute number and percentage), a diagnosis of CMML supercedes a diagnosis of other MDS/MPNs, including aCML. It should be noted that in some patients with CMML, the PB at presentation may show features of aCML with borderline monocytosis (8–12%) that fluctuates with time. It is important to observe the monocyte count over a period of time in order to determine whether the degree of monocytosis increases and becomes a persistent feature, in which case a diagnosis of proliferative type CMML is warranted; otherwise, the case should be classified as aCML.

Myelodysplastic syndrome (MDS) cases may develop leukocytosis in the course of disease; and *myeloproliferative neoplasms (MPN)* may develop dysgranulopoietic leukocytosis. It is important to ensure that leukocytosis is not due to an underlying infection, growth factor treatment, or experimental drugs used to treat MDS patients. In some cases, leukocytosis may become a persistent unrelenting feature, meeting all criteria for aCML; however, the current recommendation is not to make such a diagnosis in patients with a previously established diagnosis of MDS or MPN.

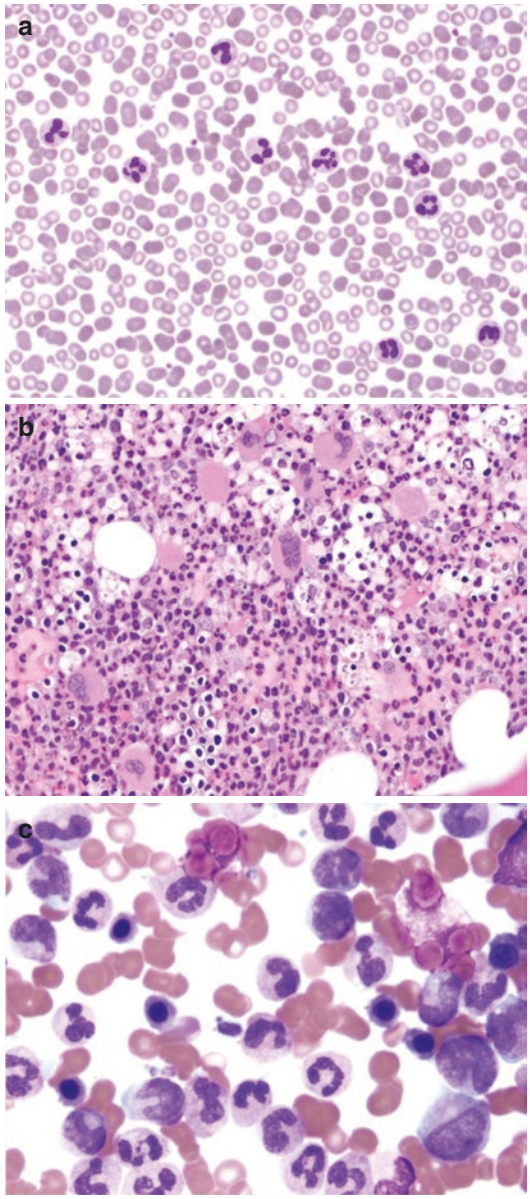

Fig. 10.7 Chronic neutrophilic leukemia (CNL). (**a**) Peripheral blood shows leukocytosis comprised mostly of neutrophils. Neutrophil precursors (promyelocytes, myelocytes, metamyelocytes) are not seen (by definition <10%). There is no dysgranulopoiesis. (**b**) Bone marrow is markedly hypercellular with increased megakaryocytes, large and multilobated, but not forming dense clusters. (**c**) Bone marrow aspirate smear shows myeloid hyperplasia with many neutrophils and notably absent dysgranulopoiesis

The differential diagnosis also includes MDS/MPN-unclassifiable, and PMF, which are discussed below.

Myelodysplastic Syndrome/ Myeloproliferative Neoplasm, Unclassifiable (MDS/MPN-U)

Myelodysplastic syndrome/myeloproliferative neoplasm-unclassifiable (MDS/MPN-U), is a rare myeloid stem cell neoplasm, overall accounting for less than 5% of all myeloid malignancies. It is characterized by overlapping myeloproliferative and myelodysplastic features at the time of initial diagnosis and no preceding history of MDS or MPN. By definition these cases do not fulfill the diagnostic criteria for other WHO-defined neoplasms in this category (CMML, JMML, aCML, or MDS/MPN-RS-T), and there is no evidence of *BCR-ABL1* or rearrangements of *PDGFRA*, *PDGFRB*, *FGFR1*, or *PCM1-JAK2*. Cases that fulfill criteria for MDS with isolated del(5q), according to the current classification, are excluded from this category [11, 38, 39]. MDS with inv(3)/t(3;3) also can have thrombocytosis, which is further discussed below.

Refractory anemia with ring sideroblasts and marked thrombocytosis (RARS-T) was placed within MDS/MPN-U as a provisional entity in the 2008 WHO classification. This MDS/MPN entity is now recognized in the 2017 WHO revision as a distinct form of MDS/MPN due to its unique clinicopathological and genetic features, with its revised name MDS/MPN-RS-T. This entity is discussed in Chap. 8.

Clinical Features

MDS/MPN-U affects primarily older patients with a median age of 71–73 years and a male-to-female ratio of 1.4–2:1 [12, 39, 40]. Patients typically present with anemia (median HGB 10.1 g/dL) with or without macrocytosis and leukocytosis ($\geq 13 \times 10^9$/L, median 19.4×10^9/L) or thrombocy-

tosis (≥450 × 10^9/L). There is no monocytosis and no significant eosinophilia or basophilia. Splenomegaly and monocytopenia are relatively common [12]. MDS/MPN-U is a diagnosis of exclusion, for cases not meeting other defined entities and patients who do not have a history of MDS or MPN. However the diagnosis does not exclude patients who may have a history of cytosis or cytopenia but have no documentation or confirmation of a diagnosis of MDS or MPN. If the first BM biopsy and hematological features are consistent with MDS/MPN-U, this diagnosis is appropriate.

The median overall survival of patients with MDS/MPN-U is reported to be between 12.4 and 21.8 months. Thrombocytopenia (<100 × 10^9/L) is the factor most prominently associated with shorter overall survival [12, 39]. Younger age (<60 years), thrombocytosis, lack of circulating blasts, and low BM blast count (<5%) are associated with favorable outcomes [39]. AML transformation is reported in about one quarter of patients and is associated with extremely poor prognosis.

Morphology

MDS/MPN-U is more heterogeneous than other better-defined MDS/MPN entities; therefore, the morphologic findings are more variable. Peripheral blood granulocytes and platelets may exhibit abnormal morphology. Some cases may show increased circulating granulocyte precursors, including promyelocytes, myelocytes, and metamyelocytes, but they are either <10% of WBC or not in conjunction with dysgranulopoiesis; otherwise, the case may be better classified as aCML. Circulating blasts may be present but are <20%. The BM is usually hypercellular and shows myeloid or megakaryocytic hyperplasia with morphologic dysplasia in one or more of the hematopoietic lineages. Megakaryocytes are often dysplastic, with small hypolobated forms or widely separated nuclear lobes (MDS-like); but in about 20–30% of cases, they may show a mixture of small hypolobated (MDS-like) and large hypersegmented megakaryocytes with loose clustering (MPN-like); and in 5–10% of cases, megakaryocytes may show only large hypersegmented MPN-like morphology. M:E ratio is more variable than aCML, overall increased, but some cases may show erythroid hyperplasia. MF2 and MF3 fibrosis is observed in about 20–30% of patients (Fig. 10.8).

Immunophenotype

Myeloid blasts often exhibit an aberrant immunophenotype with alterations of CD34, CD38, CD117, CD123, and/or CD13 +/− aberrant expression of lymphoid markers by flow cytometric analysis; however no specific immunophenotypic abnormalities have been defined. Neutrophils and their precursors, as well as monocytes, fre-

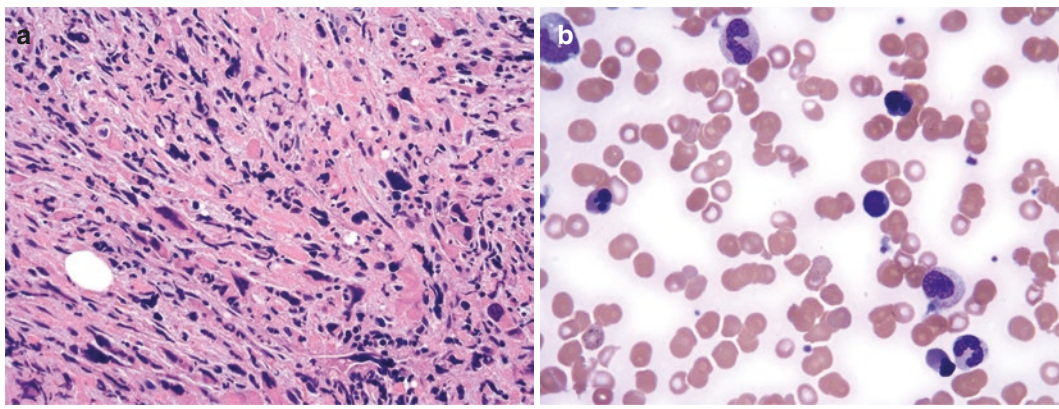

Fig. 10.8 Myelodysplastic/myeloproliferative neoplasm unclassifiable (MDS/MPN-U) with significant myelofibrosis. (**a**) Bone marrow biopsy shows marked myelofibrosis with many abnormal megakaryocytes. Megakaryocytes are variable in size including many small and hyperchromatic forms. (**b**) Bone marrow aspirate smear shows dyserythropoiesis and dysgranulopoiesis. This patient had no history of MDS or MPN prior to this bone marrow examination

quently show abnormal maturation patterns similar to what has been described in MDS.

Cytogenetics and Molecular Genetics

Approximately one third of MDS/MPN-U cases exhibit clonal numeric and structural chromosomal alterations; however no specific abnormalities have been identified. The most commonly detected alteration is trisomy 8, reported in approximately 15% of cases [12]. Other relatively more common abnormalities include monosomy 7, trisomy 9, or deletions of 7q, 13q, and 20q [41]. Single nucleotide polymorphism arrays (SNP-A) have identified large regions of uniparental disomy associated with gain-of-function mutations in known oncogenes or loss-of-function mutations in known tumor suppressors in ~38% of MDS/MPN-U [41–43].

JAK2 p.V617F is the most frequent genetic alteration in MDS/MPN-U with a reported frequency of ~20–25% [11, 12, 39]. This high frequency of *JAK2* p.V617F may be due to the inclusion of some cases with an undocumented history of MPN that have progressed to MDS/MPN at the time of initial diagnosis. Other genes involved in various cellular mechanisms including DNA methylation [*TET2* (30%), *IDH1/2* (5–10%), *DNMT3A* (4%)], transcription regulation [*RUNX1* (~14%), *CEBPA (*~4%)], histone modification [*EZH2* (10%)], and cell signaling pathways [*KRAS/NRAS* (~10%), *SETBP1* (~10%), *CBL* (~10%), and *FLT3* (~3%)] are also recurrently mutated [42, 44, 45]. *CSF3R* mutations are notably absent in MDS/MPN-U [12].

Differential Diagnosis

Reactive/secondary conditions causing leukocytosis or thrombocytosis associated with cytopenia as discussed in the beginning of the chapter need to be excluded before reaching a diagnosis of MDS/MPN-U. Reactive conditions often have an associated underlying cause, and blood count abnormalities should recover as the underlying condition resolves. The BM morphology in patients with reactive conditions is often unremarkable, whereas almost all cases of MDS/MPN-U show an abnormal BM.

Chronic myelomonocytic leukemia (CMML) supercedes a diagnosis of MDS/MPN-U. Persistent monocytosis, based not only on the absolute monocyte count but also on the monocyte percentage, is emphasized, since borderline or transient monocytosis may be seen in some cases of MDS/MPN-U.

Atypical chronic myeloid leukemia (aCML) shares overlapping features with at least a subset of MDS/MPN-U (Figs. 10.9 and 10.10), and in some cases, this separation is arbitrary. For example, if there is marked leukocytosis with marked dysgranulopoiesis but the PB has <10% neutrophil precursors (Fig. 10.9) or, conversely, if there is leukocytosis but the PB shows ≥10% neutrophil precursors and no dysgranulopoiesis despite the presence of dyspoietic features in other lineages (Fig. 10.10), diagnostic criteria for aCML are not fulfilled, and the features are more in keeping with MDS/MPN-U. One study [12] comparing aCML with MDS/MPN-U found that the presence of ≥10% neutrophil precursors without dysgranulopoiesis was an independent risk for an inferior prognosis in MDS/MPN-U.

Myelodysplastic syndromes with thrombocytosis or leukocytosis can be difficult to distinguish from MDS/MPN-U. Some MDS/MPN patients may have a preceding clinical prodrome suggesting MDS, for example, a history of anemia in the past 2–3 years, but never have an established diagnosis of MDS. Only if the patient presents with cytosis and cytopenia at the time of initial presentation is the case classified as MDS/MPN-U rather than MDS. Exceptions are MDS with isolated del(5q) or inv(3), which are known to be associated with thrombocytosis in some patients. However, marked persistent leukocytosis with no recent growth factor treatment is highly unusual for MDS, and such cases, despite the presence of isolated del(5q) or inv(3), are best classified as MDS/MPN-U rather than MDS. Thus, del(5q) and inv(3) cytogenetic abnormalities can be seen in MDS/MPN-U and do not exclude this diagnosis.

Myeloproliferative neoplasms (MPN) with cytopenia: Cytopenia(s) as well as dysplasia can develop in a patient with MPN and can represent signs of disease progression. On the other hand, if a diagnosis of MPN is not confirmed previously, the presence of significant cytopenia and dyspoie-

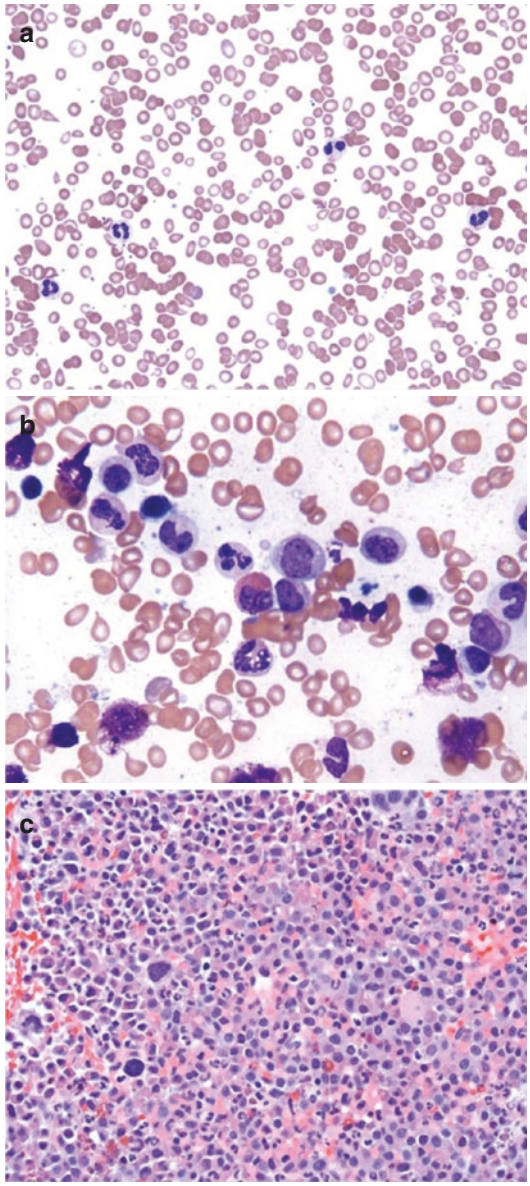

Fig. 10.9 Myelodysplastic /myeloproliferative neoplasm unclassifiable (MDS/MPN-U) or atypical chronic myeloid leukemia (aCML)? (**a**) Peripheral blood shows mild leukocytosis (WBC, 13.1 × 10^9/L), marked dysgranulopoiesis but lack of neutrophil precursors (promyelocytes, myelocytes, metamyelocytes). (**b**) Bone marrow aspirate smear shows myeloid hyperplasia, marked dysgranulopoiesis and dyserythropoiesis. (**c**) Bone marrow biopsy is hypercellular, with dysplastic megakaryocytes, and a markedly increased myeloid:erythroid ratio. This case, due to the absence of increased PB neutrophil precursors (<10%), is best classified as MDS/MPN-U

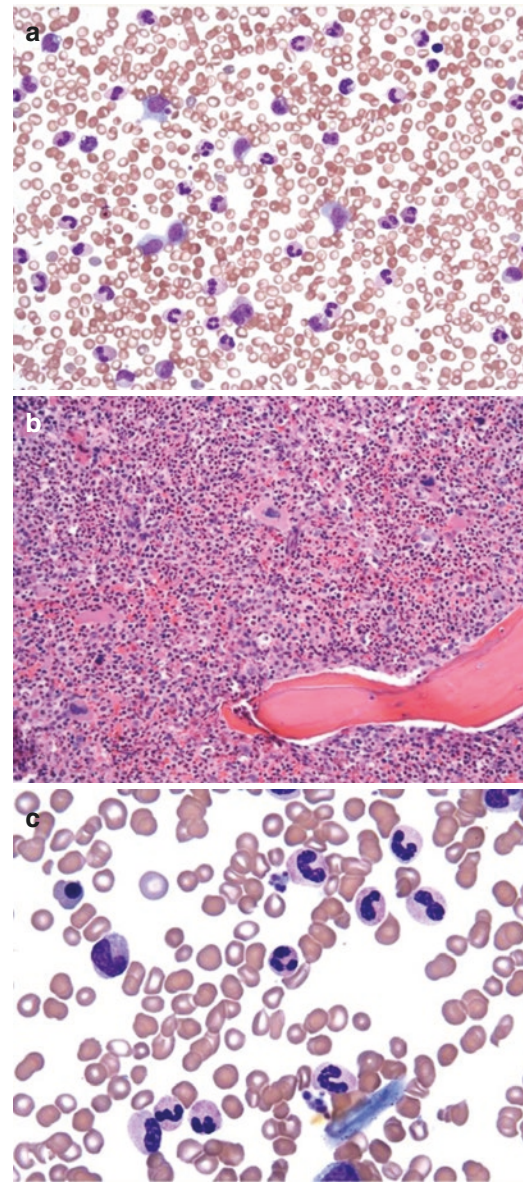

Fig. 10.10 Myelodysplastic /myeloproliferative neoplasm unclassifiable (MDS/MPN-U) or atypical chronic myeloid leukemia (aCML)? (**a**) peripheral blood shows marked leukocytosis with ≥10% neutrophil precursors (promyelocytes, myelocytes and metamyelocytes). (**b**) Bone marrow biopsy shows a hypercelluar (100%) marrow with dysplastic megakaryocytes, and markedly increased myeloid:erythroid ratio. However, there is no significant dysgranulopoiesis seen on bone marrow aspirate smear (**c**) or PB smear (**a**). This case, due to the absence of significant dysgranulopoiesis, is best classified as MDS/MPN-U

sis would favor a diagnosis of MDS/MPN-U over a diagnosis of "pure" MPN. *JAK2* p.V617F is detected in about 20–25% of MDS/MPN-U, indicating that some cases classified as MDS/MPN-U may indeed have developed from an undocumented MPN; thus, the detection of *JAK2* p.V617F does not help in the differential diagnosis between a "pure" MPN and MDS/MPN-U. Differentiating MDS/MPN-U from PMF is further discussed in this chapter in the PMF section below.

Primary Myelofibrosis

Primary myelofibrosis (PMF) is a clonal, stem cell-derived myeloproliferative neoplasm (MPN) characterized by the proliferation of megakaryocytic and granulocytic lineages and associated with reactive bone marrow fibrosis, osteosclerosis, angiogenesis, frequent extramedullary hematopoiesis, and altered chemokine production. PMF is further categorized into the initial pre-fibrotic (cellular) phase with a predominantly proliferative bone marrow morphology and absent or minimal reticulin fibrosis and the fibrotic or overt stage characterized by the presence of prominent reticulin and/or collagen fibrosis, osteosclerosis, leukoerythroblastosis, and hepatosplenomegaly. The diagnosis of PMF requires the integration of several clinical, laboratory, and pathologic findings. The WHO 2017 diagnostic criteria for PMF, both pre-fibrotic and fibrotic stages, are shown in Tables 10.2 and 10.3.

PMF, according to the degree of fibrosis, can be divided into pre-fibrotic (MF0 and MF1) and fibrotic phases (MF2 and MF3) [46], representing approximately 40% and 60% of PMF, respectively. PMF at pre-fibrotic phase often has no significant cytopenia or only mild to moderate anemia. In contrast, the combination of cytosis and cytopenia is common in patients in fibrotic phase, and for these reasons, PMF is discussed in this chapter.

Clinical Features

The overall incidence of PMF is approximately 0.5–1.5 per 100,000 population with a median age at diagnosis of ~65 years and a slight male predilec-

Table 10.2 WHO 2016 diagnostic criteria for early/pre-fibrotic primary myelofibrosis

Major criteria
Atypical megakaryocytic proliferation and <grade 2 reticulin fibrosis, with increased bone marrow cellularity for age and granulocytic proliferation
Presence of a *JAK2, MPL,* or *CALR* mutation
or presence of other clonal marker
or absence of evidence for reactive myelofibrosis if no clonal marker is demonstrated
Not meeting diagnostic criteria for other WHO-defined myeloproliferative neoplasms, myelodysplastic syndrome, or other myeloid neoplasms
Minor criteria
Requires at least one minor criterion confirmed in two consecutive occasions
Anemia not related to other comorbid conditions
Leukocytosis $\geq 11 \times 10^9$/L
Palpable splenomegaly
Increased serum LDH[a]

The diagnosis of PMF requires all three major criteria and at least one minor criterion

[a]Above the upper normal limit of institutional reference range

Table 10.3 WHO 2016 diagnostic criteria for overt/fibrotic stage primary myelofibrosis

Major criteria
Atypical megakaryocytic proliferation and ≥grade 2 reticulin and/or collagen fibrosis
Presence of a *JAK2*, *MPL*, or *CALR* mutation
or presence of other clonal marker
or absence of evidence for reactive myelofibrosis if no clonal marker is demonstrated
Not meeting diagnostic criteria for other WHO-defined myeloproliferative neoplasms, myelodysplastic syndrome, or other myeloid neoplasms
Minor criteria
Requires at least one minor criterion confirmed in two consecutive occasions
Anemia not related to other comorbid conditions
Leukocytosis $\geq 11 \times 10^9$/L
Palpable splenomegaly
Increased serum LDH[a]
Leukoerythroblastosis

The diagnosis of PMF requires all three major criteria and at least one minor criterion

[a]Above the upper normal limit of institutional reference range

tion [47]. The clinical manifestations of PMF are broad and include constitutional symptoms (fever, fatigue, night sweats, etc.), splenomegaly (up to 90%), splenic infarct, hepatomegaly (about 50%), portal hypertension, ascites, pleural effusion, bone pain, pruritus, thrombosis, and bleeding. The associated symptoms are thought to be the result of abnormal cytokine production by the clonal cells and the immune system of the host [48, 49].

Anemia is present in most patients with PMF, with ~30% having a HGB <10 g/dL. Causes of anemia include hemodilution, ineffective erythropoiesis, shortened RBC survival, large spleen, hemolysis, and/or folate and iron deficiency. Leukopenia ($<4 \times 10^9$/L) is observed in approximately 10–15% of patients, whereas leukocytosis ($\geq 13 \times 10^9$/L) may be observed in 30–40%. Either thrombocytosis or thrombocytopenia can be observed in 15–20% of patients, respectively.

Pre-fibrotic stage PMF [50, 46] shows slightly less male predominance, younger age, higher HGB, higher platelet count, lower white blood cell count, and smaller spleen index but higher incidence of splenic vein thrombosis. According to a large cohort study of 787 patients by the cooperative group AGIMM [46], patients diagnosed with pre-fibrotic PMF have a median OS of 14.7 years vs. 7.2 years in patients with fibrotic PMF.

Morphology

The PB shows leukoerythroblastosis and anisopoikilocytosis in patients with overt BM fibrosis (Fig. 10.11a). In pre-fibrotic PMF, the PB may not have significant findings (Fig. 10.11b). There is usually no dysgranulopoiesis. The majority of the patients with PMF are diagnosed at fibrotic stage. Atypical megakaryocytic hyperplasia and bone marrow fibrosis are the morphologic hallmarks of PMF and are required for establishing the diagnosis. The megakaryocytes of PMF are typically pleomorphic and include small, hypolobated, and hyperchromatic forms as well as large hyperchromatic forms with abnormally folded nuclei, high nuclear/cytoplasmic ratio, and nuclear hyperchromasia (Fig. 10.12a, b). "Naked" megakaryocytic nuclei are frequently appreciated on bone marrow aspirate smears. Granulocytic hyperplasia is present, and there is progressive depletion of erythroid precursors as the disease advances. Sometimes the BM is almost depleted of hematopoietic cells, with only small islands of hematopoietic precursors within the vascular sinuses. Mild dyserythropoiesis may be seen in PMF, particularly in the advanced stages of disease, or in patients treated with hydroxyurea. Lymphoid aggregates are often present. Osteosclerotic changes are variably seen, including scalloping of oste-

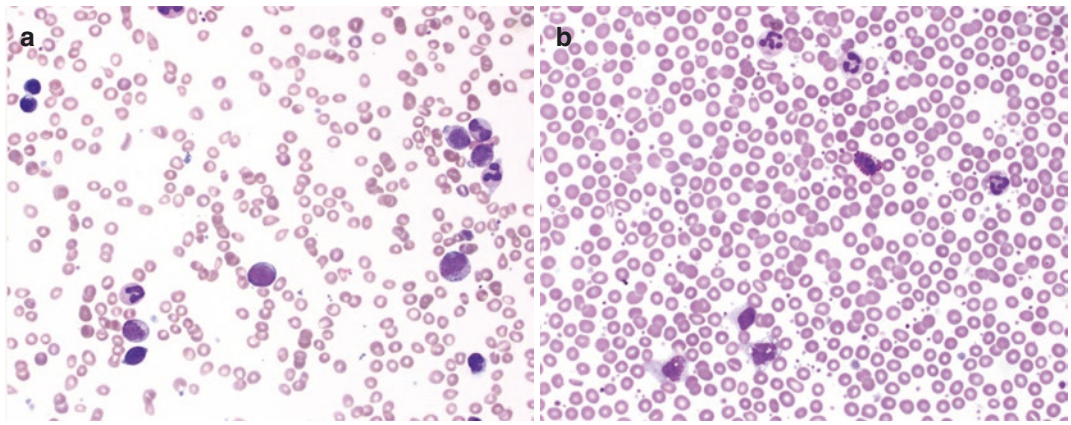

Fig. 10.11 Primary myelofibrosis (PMF), peripheral blood findings. (**a**) In PMF with significant fibrosis (MF2 and MF3), PB shows leukoerythroblastosis and anisopoikilocytosis of red blood cells. (**b**) In PMF with MF0 or MF1 (pre-fibrotic), the PB smear is often unremarkable. One basophil is in noted in this microscopic field

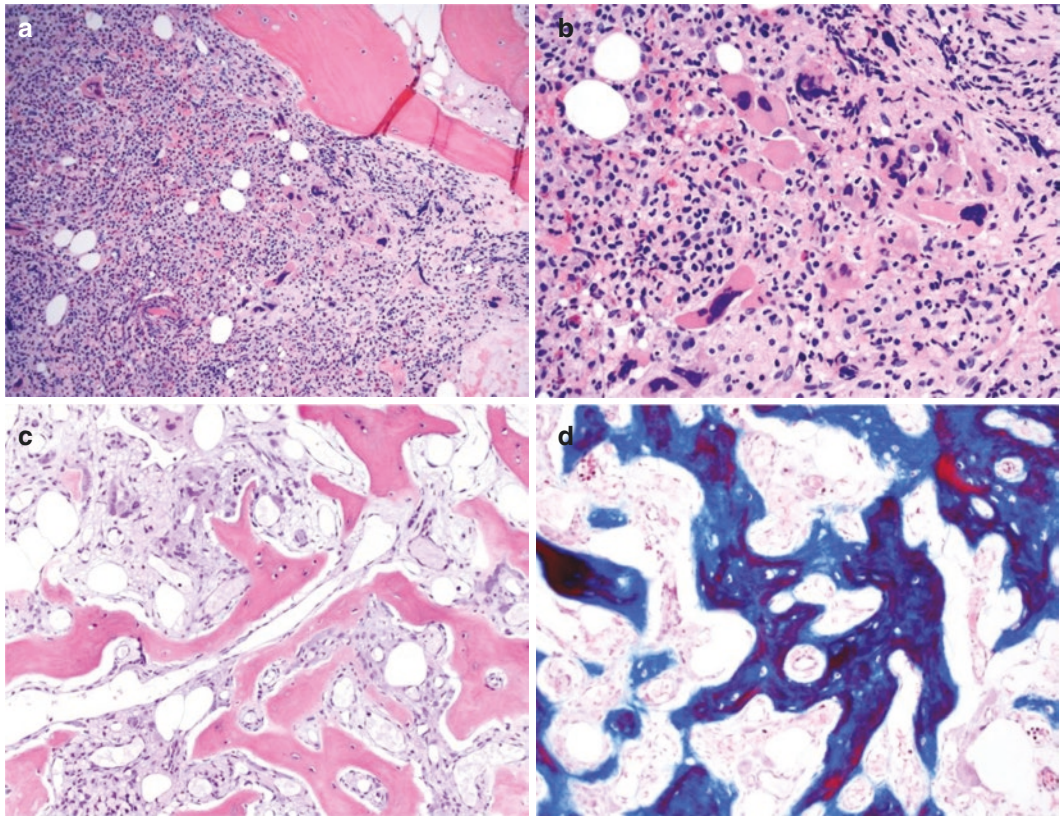

Fig. 10.12 Primary myelofibrosis (PMF), bone marrow biopsy findings. (**a**) In PMF with significant fibrosis (MF2 and MF3), BM cellularity can be variable, showing a megakaryocytic proliferation in a fibrotic background (**a**). Megakaryocytes are pleomorphic and include small, hypolobated and hyperchromatic forms as well as large hyperchromatic forms with abnormally folded nuclei, high nuclear:cytoplasmic ratio and nuclear hyperchromasia (**b**). In some cases, osteosclerosis may be marked (**c**). Megakaryocytes are increased despite the low overall cellularity. Masson-trichrome stain highlights periosteal collagenous fibrosis (**d**)

oid seams, mosaic changes, and increased thickness or volume of bone trabeculae with periosteal collagenous fibrosis (Fig. 10.12c, d).

Although the changes in pre-fibrotic PMF are less dramatic than fibrotic PMF, they are different from a reactive process and can be distinguished from other types of MPNs (Fig. 10.13). The BM is usually hypercellular for age with myeloid hyperplasia, which shows a predominance of metamyelocytes, bands, and segmented neutrophils. Megakaryocytes are abnormal, often hyperchromatic with bulbous, "cloud-like," or "balloon-shaped" nuclei and naked megakaryocytic nuclei, similar to those observed in fibrotic PMF. Megakaryocytes form dense clusters that are frequently adjacent to BM vascular sinuses and the bone trabeculae. Lymphoid aggregates are seen in about 20% of cases.

The degree of fibrosis correlates closely with disease progression. Reticulin stain should be performed in all cases, and trichrome should be performed in cases suspected to have MF2 or MF3 fibrosis. The criteria [51] by the European consensus on myelofibrosis are shown in Table 10.4. In some cases, fibrosis may be patchy and heterogeneous, and the recommendation is to score the highest fibrotic areas that involve at least 30% of BM. Grading is not only required at the time of diagnosis but also in the sequential follow-up BM biopsies in order to assess disease progression and response to treatment.

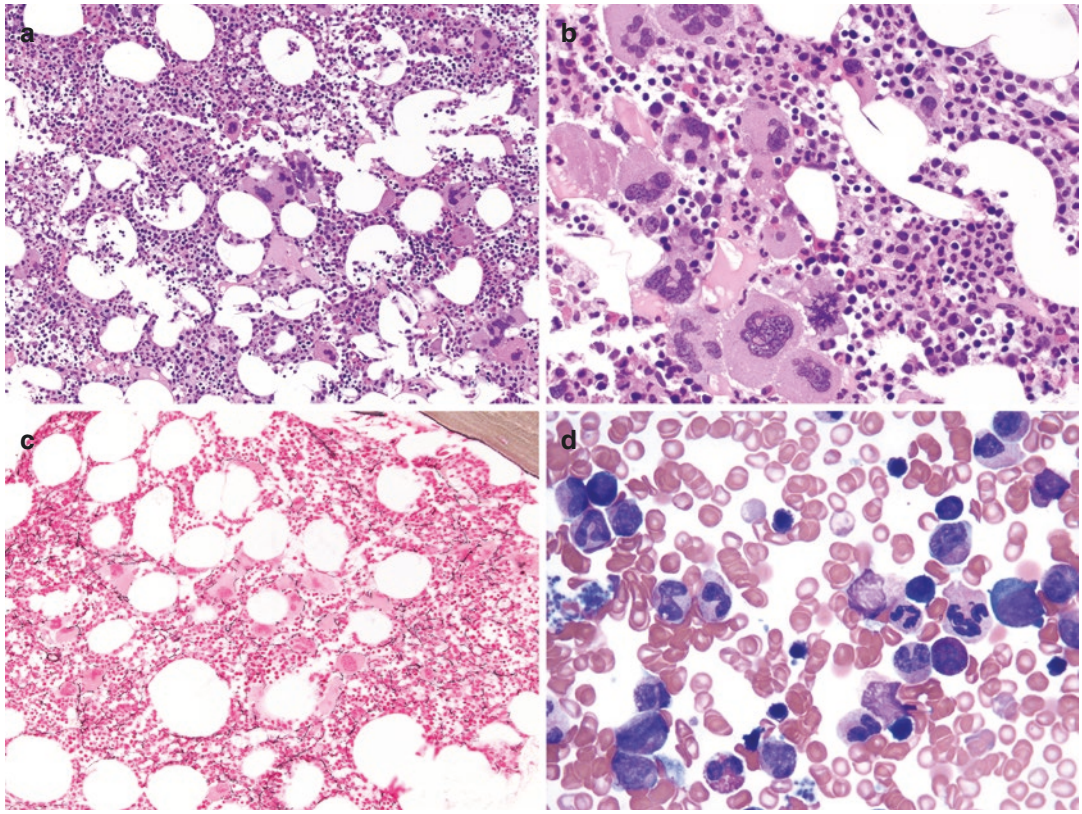

Fig. 10.13 Pre-fibrotic primary myelofibrosis (PMF), bone marrow findings. In pre-fibrotic PMF (MF0 and MF1), BM cellularity is usually high and there is a megakaryocytic proliferation without significant fibrosis on H&E stained sections. Focally, megakaryocytes form dense clusters (**a**). Megakaryocytes are pleomorphic with a predominance of large forms with mild nuclear hyperchromasia and a few small forms (**b**). Reticulin stain shows minimal to mild fibrosis(**c**). Due to the absence of significant fibrosis, bone marrow aspirate is often adequately cellular; here it shows myeloid hyperplasia, with no dyspoietic changes (**d**)

Table 10.4 Myelofibrosis grading in primary myelofibrosis

Grade	Description
MF0	Scattered linear reticulin with no intersections (crossovers) corresponding to normal bone marrow
MF1	Loose network of reticulin with many intersections, particularly in perivascular areas
MF2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of thick fibers mostly consistent with collagen, and/or focal osteosclerosis*
MF3	Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of thick fibers consistent with collagen, usually associated with osteosclerosis*

*If fibrosis is uneven, score the highest grade area that constitutes ≥30% assessable areas

Progression to AML occurs in ~20% of patients and is associated with very poor outcomes. Other causes of death include cardiovascular events and complications of cytopenia including bleeding and infection [48, 52].

Immunophenotype

Cases of PMF, particularly in the fibrotic stage of disease, exhibit immunophenotypic alterations both in the CD34+ myeloid blasts [decreased CD38; increased CD13, CD123, and CD117; altered CD45/SSC; aberrant expressions of lymphoid markers such as CD2, CD5, CD7, CD19, and CD56] and abnormalities in myelomonocytic cells [53, 18]. There is significant overlap between the immunophenotypic alterations seen in PMF and MDS [18].

Quantification of circulating CD34+ blasts has shown to correlate with myeloid metaplasia (extramedullary hematopoiesis) and prognosis. The presence of $\geq 0.1 \times 10^9$/L CD34+ cells correlates with shorter survival, leukemic transformation, and clinical progression. However, this significance is questionable when other confounding factors are co-analyzed.

Cytogenetics and Molecular Genetics

Approximately one third of PMF cases show numeric or structural chromosomal abnormalities. The most common alterations include del(20q), del(13q), trisomy 8, and trisomy 9 and alteration of chromosome 1 including duplication 1q. Other less frequent abnormalities are also seen including monosomy 7 or del(7q), del(5q), del(12p), +21, and der(6)t(1;6)(q21;p21.3). Isolated abnormalities of del(20q) or del(13q) are favorable cytogenetic findings, while other chromosomal abnormalities are unfavorable [54].

Somatic mutations in *JAK2* (65%), *CALR* (25%), and *MPL* (10%) are driver events in the pathogenesis of PMF. Approximately 10–15% of PMF cases are wild type for all three genes and are designated as "triple-negative" PMF. Some triple-negative PMF cases have been shown to have gain-of-function mutations (e.g., *MPL* p.S204P and *MPL* p.Y591N) by whole exon sequencing [55]. *CALR* mutations are associated with younger age, less profound anemia, a higher platelet count, lower DIPSS-plus scores (see below), and distinctive megakaryocytic morphologic features [56, 57]. Other recurrently mutated genes involve various cellular mechanisms including the spliceosome machinery [*SRSF2* (17%), *SF3B1* (7%)], pre-mRNA processing [*U2AF1* (16%)], DNA transcription [*ASXL1* (13%)], histone methylation [*EZH2* (7%)], DNA methylation [*DNMT3A* (7%)], epigenetic regulation [*IDH1/2* (4%)], and tumor suppression [*TP53* (4%)]. Mutation features known to be associated with inferior patient outcomes include nullizygosity for *JAK2* 46/1 haplotype, low *JAK2* p.V617F allelic fraction, and *ASXL1*, *EZH2*, *IDH*, and *SRSF2* mutations [48, 58–65]. Patients with mutant *CALR* and wild-type *ASXL1* have the longest overall survival (median 10.4 years), whereas patients with wild-type *CALR* and mutant *ASXL1* have the shortest overall survival (median 2.3 years) [66].

Differential Diagnosis

The BM of PMF is morphologically abnormal, and with the identification of *JAK2*, *CALR*, and *MPL* mutations, it is usually not difficult to differentiate from a reactive process. Autoimmune myelofibrosis is a rare inflammatory entity that can present with cytopenias and significantly increased BM reticulin fibrosis; it can occur in patients with or without an established diagnosis of a systemic autoimmune disease [67, 68]. Unlike PMF, the megakaryocytes in autoimmune myelofibrosis are morphologically unremarkable, and *JAK*, *MPL*, and *CALR* mutations are absent (Fig. 10.14).

Pre-fibrotic phase PMF versus essential thrombocythemia can pose a diagnostic challenge, but the distinction carries significant prognostic implications [69]. Careful evaluation of bone marrow morphology is required to make this distinction. The morphologic features of both entities are illustrated and compared in Chap. 8. In brief, unlike the large, mature-appearing, and hyperlobulated megakaryocytes of ET, the megakaryocytes of PMF frequently exhibit marked variation in size and shape and include hyperchromatic forms with aberrant maturation and abnormal nuclear lobation. In addition to the different megakaryocytic morphologies, prefibrotic PMF often shows an increased cellularity, granulocytic hyperplasia, and left-shifted granulocytic maturation in the peripheral blood. These features are unusual for ET and should raise suspicion for PMF [70].

Post-polycythemia vera and essential thrombocythemia myelofibrosis are essentially indistinguishable from fibrotic PMF [71], morphologically and prognostically. The original cellular composition (panmyelosis in PV and megakaryocytic hyperplasia in ET) may be focally retained in some post-PV/ET MF biopsies; however, they are not very reliable. The key

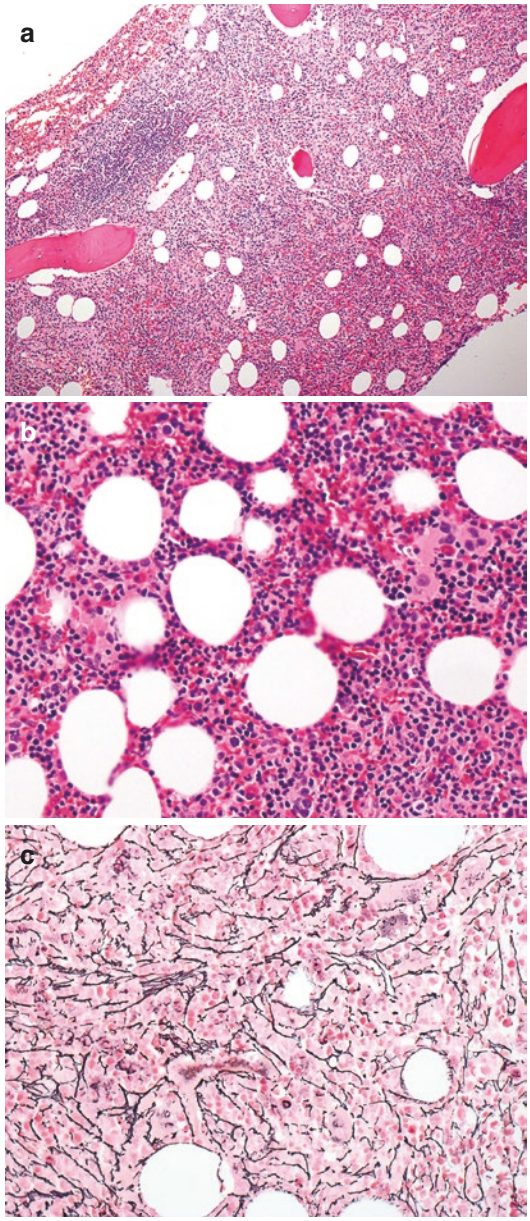

Fig. 10.14 Autoimmune myelofibrosis. (**a**) The bone marrow biopsy is markedly hypercellular and contains lymphoid aggregates. (**b**) On high magnification, there are increased interstitial small lymphocytes and relative erythroid predominance. Megakaryocytes are increased and they have normal morphology. (**c**) Reticulin staining shows grade moderate reticulin fibrosis

Table 10.5 Diagnostic criteria recommended by the International Working Group for Myelofibrosis Research and Treatment (IWG-MRT) for post-polycythemia vera or post-essential thrombocythemia myelofibrosis

Required criteria	
1	Documentation of a previous diagnosis of polycythemia vera (PV) or essential thrombocythemia (ET)
2	Bone marrow fibrosis ≥MF2
Additional criteria (two are required)	
1	Anemia (below the reference range appropriate for age, gender, and altitude) A. Or sustained loss of requirement of either phlebotomy or cytoreductive treatment for PV patients B. A 2 g/dL hemoglobin decrease from baseline for ET patients
2	A leukoerythroblastic peripheral blood picture
3	Increasing splenomegaly defined as either an increase in palpable splenomegaly of ≥5 cm or the appearance of newly palpable splenomegaly
4	Development of ≥1 of 3 constitutional symptoms: weight loss >10% of body weight in 6 months, night sweats, or unexplained fever >37.5 °C
5	Increased LDH above reference level (for ET patients only)

is to have a documentation of prior PV and ET. Of note, the finding of a fibrotic BM is not sufficient to diagnose post-PV and post-ET myelofibrosis, as patients need to meet at least 2 of 4 co-criteria, including anemia, leukoerythroblastosis, increased splenomegaly ≥5 cm, or newly developed constitutional symptoms (Table 10.5).

Myelodysplastic syndromes with fibrosis (MDS-F) versus PMF can be diagnostically challenging. Morphologically, some MDS-F cases closely resemble PMF with respect to BM morphology showing a proliferation of pleomorphic megakaryocytes with clustering in a fibrotic background (Fig. 10.15). Additionally, the aspirate smears are often suboptimal leading to suboptimal assessment for dysplasia. Conversely, PMF may exhibit some dyspoietic changes. The following features are helpful in this distinction [72, 73]: (1) MDS-F often presents with pancytopenia, and cytopenias are usually severe; in contrast, pancytopenia in fibrotic PMF is uncommon, which most often presents with normal or increased WBC, normal or increased platelet count, anemia, and a leukoerythroblastic blood picture. (2) *JAK2* mutation can be seen in about 15–20% of MDS-F; how-

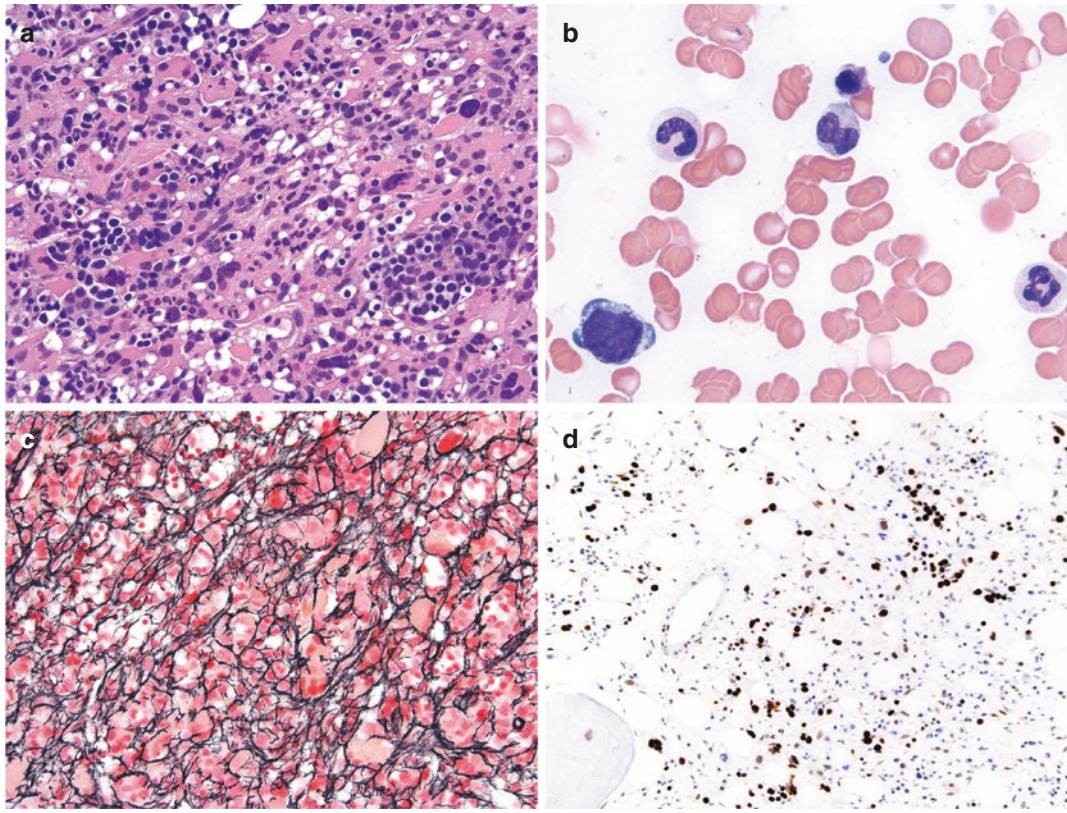

Fig. 10.15 Myelodysplastic syndrome with fibrosis (MDS-F). Approximately 10–15% of MDS cases may present with significant fibrosis. (**a**) BM cellularity is usually high, showing many dysplastic megakaryocytes, variable in size including many small forms. In contrast to primary myelofibrosis (PMF) that often shows an increased myeloid:erythroid (M:E) ratio, MDS-F often shows a decreased/reversed M:E ratio. (**b**) Bone marrow aspirate is often hypocellular due to dry tap, but shows dyspoietic features in granulocytes and erythrocytes. (**c**) Reticulin stain shows marked fibrosis. (**d**) A high fraction of MDS-F shows aberrant p53 expression (strong), which is not common in PMF

ever, *MPL* and *CALR* mutations are very uncommon. (3) While splenomegaly and markedly increased LDH are almost always seen in fibrotic PMF, they are uncommon in MDS-F. (4) The presence of ring sideroblasts or mutations in splicing genes such as *SF3B1* and the lack of *JAK2*, *MPL*, or *CALR* mutations support a diagnosis of MDS-F. In addition, a helpful clue in making this distinction is the frequent presence of *TP53* mutations and p53 overexpression in MDS with fibrosis, a finding that is relatively rare in PMF [73, 74].

Myelodysplastic syndrome/myeloproliferative neoplasm, unclassifiable (MDS/MPN-U) can present with fibrosis in about 20% of patients; furthermore, *JAK2* P.V617F mutations are present in about 20–25% of MDS/MPN-U patients. Megakaryocytes may show mixed small and large forms and may occur in dense clusters, resembling PMF. As discussed under the MDS/MPN-U section above, some MDS/MPN-U may have developed in a background of an undiagnosed MPN. The presence of significant dysplasia associated with significant cytopenia(s) in the BM or blood at the time of initial presentation is more in keeping with MDS/MPN-U. Mutations in *MPL* and *CALR* are more in favor of PMF, whereas *SETBP1* mutations are more common in MDS/MPN-U. The presence of either del(13)(q12–22) or der(6) t(1;6) (q21–23;p21.3) [75], although not diagnostic, is strongly suggestive of PMF.

Myelodysplastic syndromes with isolated del(5q) or with inv(3)/t(3;3). A subset of MDS may present with cytopenia and thrombocytosis, and they should be included in the differ-

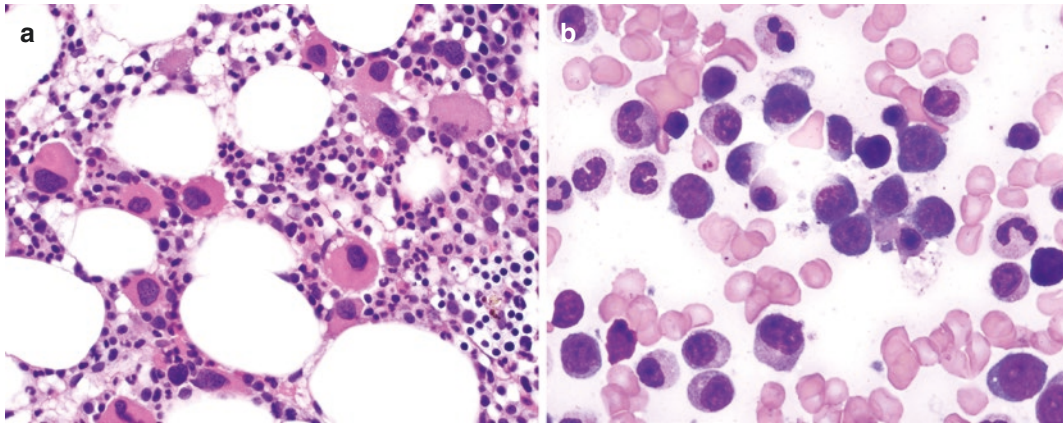

Fig. 10.16 Myelodysplastic syndrome with del(5q). About one third of MDS with del(5q) may present with thrombocytosis. Megakaryocytes are characteristically normal or slightly small in size and have monolobated or hypolobated nuclei. There is no clustering of megakaryocytes (**a**). Dysgranulopoiesis and dyserythropoiesis are uncommon (**b**)

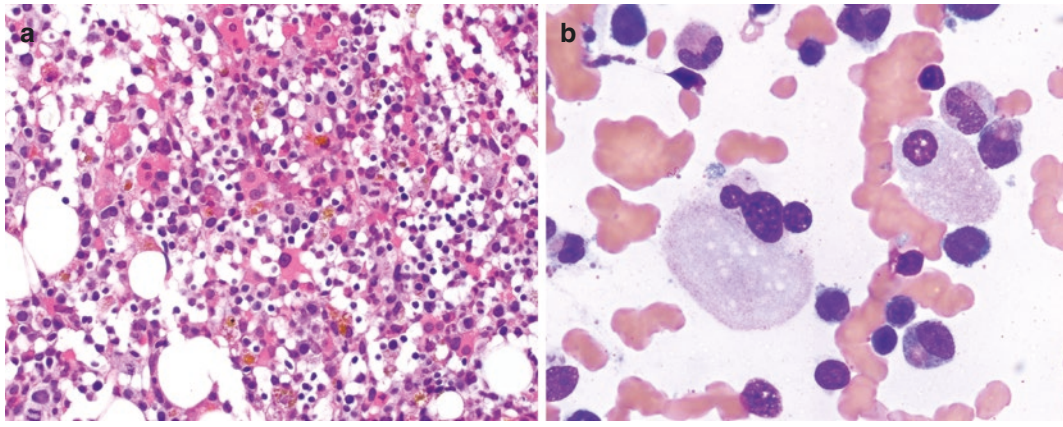

Fig. 10.17 Myelodysplastic syndrome with inv(3). About 5% of MDS with inv(3) may present with thrombocytosis. Dysmegakaryopoiesis is seen in virtually all cases, characterized by the presence of increased small, monolobated or bilobated megakaryocytes. (**a**) Bone marrow biopsy and (**b**) BM aspirate

ential diagnosis in patients presenting with hybrid cytopenia and cytosis.

MDS with isolated del(5q) is a unique MDS subtype characterized by anemia with deletion of the long arm of chromosome 5 as an isolated finding or with one additional karyotypic finding, excluding -7 or del(7q). Thrombocytosis is present in approximately one third of patients. Megakaryocytes are characteristically normal or slightly small in size and have monolobated or hypolobated nuclei. Dysgranulopoiesis and dyserythropoiesis are relatively uncommon (Fig. 10.16) [76, 77]. Blasts are less than 5% in the BM and <1% in the blood. *JAK2* P.V617F mutation has been reported in about 6% of MDS with isolated del(5q), and these patients appear to have particularly prominent thrombocytosis. However, the clinical implications of this finding are unclear, and a *JAK2* mutation should not be used to assign these cases to the MDS/MPN category [78]. The features of MDS with isolated del(5q) are discussed further in Chap. 2.

MDS with inv(3)/t(3;3) is a rare, aggressive myeloid neoplasm with high propensity for progression to AML [79]. A minor subset of patients (~5%)

present with thrombocytosis. Hepatosplenomegaly is seen in approximately 15–20% of patients. Most cases exhibit multilineage dysplasia and blasts are often increased. Dysmegakaryopoiesis is seen in virtually all cases, characterized by the presence of increased small, monolobated, or bilobated megakaryocytes (Fig. 10.17).

Importantly, while thrombocytosis is common in these subtypes of MDS, significant leukocytosis may favor a diagnosis of MDS/MPN over MDS since del(5q) and inv(3). It is also important to note that cases with anemia, thrombocytosis, and del(5q), but with one or more features precluding assignment to the MDS with isolated del(5q) category (such as increased PB or BM blasts, leukocytosis, abnormalities of chromosome 7, or two or more additional cytogenetic abnormalities), are best considered as MDS/MPN-U.

References

1. Al-Tawfiq JA, et al. Hematologic, hepatic, and renal function changes in hospitalized patients with Middle East respiratory syndrome coronavirus. Int J Lab Hematol. 2017;39(3):272–8.
2. Aird WC. The hematologic system as a marker of organ dysfunction in sepsis. Mayo Clin Proc. 2003;78(7):869–81.
3. Halfdanarson TR, Litzow MR, Murray JA. Hematologic manifestations of celiac disease. Blood. 2007;109(2):412–21.
4. Conlan MG, et al. Clinical significance of hematologic parameters in non-Hodgkin's lymphoma at diagnosis. Cancer. 1991;67(5):1389–95.
5. Steensma DP, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. Blood. 2015;126(1):9–16.
6. Xie M, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. Nat Med. 2014;20(12):1472–8.
7. Jaiswal S, et al. Age-related clonal hematopoiesis associated with adverse outcomes. N Engl J Med. 2014;371(26):2488–98.
8. Genovese G, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. N Engl J Med. 2014;371(26):2477–87.
9. Kwok B, et al. MDS-associated somatic mutations and clonal hematopoiesis are common in idiopathic cytopenias of undetermined significance. Blood. 2015;126(21):2355–61.
10. Vardiman JW, et al. Atypical chronic myeloid leukaemia, BCR-ABL1 negative. In: WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC; 2008. p. 80–1.
11. Talati C, Padron E. An exercise in extrapolation: clinical management of atypical CML, MDS/MPN-unclassifiable, and MDS/MPN-RS-T. Curr Hematol Malig Rep. 2016;11(6):425–33.
12. Wang SA, et al. Atypical chronic myeloid leukemia is clinically distinct from unclassifiable myelodysplastic/myeloproliferative neoplasms. Blood. 2014;123(17):2645–51.
13. Piazza R, et al. Recurrent SETBP1 mutations in atypical chronic myeloid leukemia. Nat Genet. 2013;45(1):18–24.
14. Breccia M, et al. Identification of risk factors in atypical chronic myeloid leukemia. Haematologica. 2006;91(11):1566–8.
15. Hernandez JM, et al. Clinical, hematological and cytogenetic characteristics of atypical chronic myeloid leukemia. Ann Oncol. 2000;11(4):441–4.
16. Kurzrock R, et al. BCR rearrangement-negative chronic myelogenous leukemia revisited. J Clin Oncol. 2001;19(11):2915–26.
17. Koldehoff M, et al. Outcome of hematopoietic stem cell transplantation in patients with atypical chronic myeloid leukemia. Bone Marrow Transplant. 2004;34(12):1047–50.
18. Ouyang J, et al. Flow cytometry immunophenotypic analysis of Philadelphia-negative myeloproliferative neoplasms: correlation with histopathologic features. Cytometry B Clin Cytom. 2015;88(4):236–43.
19. Xu Y, et al. A BCR-JAK2 fusion gene from ins(22;9)(q11;p13p24) in a patient with atypical chronic myeloid leukemia. Leuk Lymphoma. 2013;54(10):2322–4.
20. Bellesso M, et al. Atypical chronic myeloid leukemia with t(9;22)(p24,11.2), a BCR-JAK2 fusion gene. Rev Bras Hematol Hemoter. 2013;35(3):218–9.
21. Muta T, Osaki K, Yamano Y. Translocation t(9;22)(p23;q11) in atypical chronic myeloid leukemia (aCML) presenting osteolytic lesions. Int J Hematol. 2002;76(4):344–8.
22. Gambacorti-Passerini CB, et al. Recurrent ETNK1 mutations in atypical chronic myeloid leukemia. Blood. 2015;125(3):499–503.
23. Maxson JE, et al. Oncogenic CSF3R mutations in chronic neutrophilic leukemia and atypical CML. N Engl J Med. 2013;368(19):1781–90.
24. Geyer JT, Orazi A. Myeloproliferative neoplasms (BCR-ABL1 negative) and myelodysplastic/myeloproliferative neoplasms: current diagnostic principles and upcoming updates. Int J Lab Hematol. 2016;38(Suppl 1):12–9.
25. Arber DA, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.
26. Park HJ, et al. Delta neutrophil index as an early marker for differential diagnosis of adult-onset Still's disease and sepsis. Yonsei Med J. 2014;55(3):753–9.
27. Lawrence YR, et al. Extreme leukocytosis in the emergency department. QJM. 2007;100(4):217–23.
28. Juturi JV, Hopkins T, Farhangi M. Severe leukocytosis with neutrophilia (leukemoid reaction) in alcoholic steatohepatitis. Am J Gastroenterol. 1998;93(6):1013.

29. Kawada T. Smoking-induced leukocytosis can persist after cessation of smoking. Arch Med Res. 2004;35(3):246–50.
30. Biezeveld MH, et al. Sustained activation of neutrophils in the course of Kawasaki disease: an association with matrix metalloproteinases. Clin Exp Immunol. 2005;141(1):183–8.
31. Meyerson HJ, Farhi DC, Rosenthal NS. Transient increase in blasts mimicking acute leukemia and progressing myelodysplasia in patients receiving growth factor. Am J Clin Pathol. 1998;109(6):675–81.
32. Dale DC, et al. Comparison of agents producing a neutrophilic leukocytosis in man. Hydrocortisone, prednisone, endotoxin, and etiocholanolone. J Clin Invest. 1975;56(4):808–13.
33. Christensen RD, Hill HR. Exercise-induced changes in the blood concentration of leukocyte populations in teenage athletes. Am J Pediatr Hematol Oncol. 1987;9(2):140–2.
34. Marinella MA. Extreme leukemoid reaction associated with retroperitoneal hemorrhage. Arch Intern Med. 1998;158(3):300–1.
35. Ascensao JL, et al. Leukocytosis and large cell lung cancer. A frequent association. Cancer. 1987;60(4):903–5.
36. Kayashima T, et al. Leukemoid reaction associated with diabetic ketoacidosis: with measurement of plasma levels of granulocyte colony-stimulating factor. Intern Med. 1993;32(11):869–71.
37. Kohmura K, et al. Granulocyte colony stimulating factor-producing multiple myeloma associated with neutrophilia. Leuk Lymphoma. 2004;45(7):1475–9.
38. Vardiman JW, et al. Myelodysplastic/myeloproliferative neoplasm, unclassifiable. In: WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC; 2008. p. 85–6.
39. DiNardo CD, et al. Myelodysplastic/myeloproliferative neoplasms, unclassifiable (MDS/MPN, U): natural history and clinical outcome by treatment strategy. Leukemia. 2014;28(4):958–61.
40. Srour SA, et al. Incidence and patient survival of myeloproliferative neoplasms and myelodysplastic/myeloproliferative neoplasms in the United States, 2001–12. Br J Haematol. 2016;174(3):382–96.
41. Tiu RV, et al. Prognostic impact of SNP array karyotyping in myelodysplastic syndromes and related myeloid malignancies. Blood. 2011;117(17):4552–60.
42. Zoi K, Cross NC. Molecular pathogenesis of atypical CML, CMML and MDS/MPN-unclassifiable. Int J Hematol. 2015;101(3):229–42.
43. Dunbar AJ, et al. 250K single nucleotide polymorphism array karyotyping identifies acquired uniparental disomy and homozygous mutations, including novel missense substitutions of c-Cbl, in myeloid malignancies. Cancer Res. 2008;68(24):10349–57.
44. Clara JA, Sallman DA, Padron E. Clinical management of myelodysplastic syndrome/myeloproliferative neoplasm overlap syndromes. Cancer Biol Med. 2016;13(3):360–72.
45. Meggendorfer M, et al. SETBP1 mutations occur in 9% of MDS/MPN and in 4% of MPN cases and are strongly associated with atypical CML, monosomy 7, isochromosome i(17)(q10), ASXL1 and CBL mutations. Leukemia. 2013;27(9):1852–60.
46. Guglielmelli P, et al. Presentation and outcome of patients with 2016 WHO diagnosis of prefibrotic and overt primary myelofibrosis. Blood. 2017;129(24):3227–36.
47. Thiele J, et al. Primary myelofibrosis. In: WHO classification of tumours of haematopoietic and lymphoid Tissues. Lyon: IARC; 2008. p. 44–7.
48. Tefferi A. Primary myelofibrosis: 2017 update on diagnosis, risk-stratification, and management. Am J Hematol. 2016;91(12):1262–71.
49. Tefferi A. Pathogenesis of myelofibrosis with myeloid metaplasia. J Clin Oncol. 2005;23(33):8520–30.
50. Barosi G, et al. Evidence that prefibrotic myelofibrosis is aligned along a clinical and biological continuum featuring primary myelofibrosis. PLoS One. 2012;7(4):e35631.
51. Thiele J, et al. European consensus on grading bone marrow fibrosis and assessment of cellularity. Haematologica. 2005;90(8):1128–32.
52. Mesa RA, et al. Leukemic transformation in myelofibrosis with myeloid metaplasia: a single-institution experience with 91 cases. Blood. 2005;105(3):973–7.
53. Feng B, et al. Aberrant myeloid maturation identified by flow cytometry in primary myelofibrosis. Am J Clin Pathol. 2010;133(2):314–20.
54. Hussein K, Van Dyke DL, Tefferi A. Conventional cytogenetics in myelofibrosis: literature review and discussion. Eur J Haematol. 2009;82(5):329–38.
55. Milosevic Feenstra JD, et al. Whole-exome sequencing identifies novel MPL and JAK2 mutations in triple-negative myeloproliferative neoplasms. Blood. 2016;127(3):325–32.
56. Tefferi A, et al. CALR vs JAK2 vs MPL-mutated or triple-negative myelofibrosis: clinical, cytogenetic and molecular comparisons. Leukemia. 2014;28(7):1472–7.
57. Loghavi S, et al. Myeloproliferative neoplasms with calreticulin mutations exhibit distinctive morphologic features. Am J Clin Pathol. 2016;145(3):418–27.
58. Tefferi A, et al. JAK2 germline genetic variation affects disease susceptibility in primary myelofibrosis regardless of V617F mutational status: nullizygosity for the JAK2 46/1 haplotype is associated with inferior survival. Leukemia. 2010;24(1):105–9.
59. Tefferi A, et al. Low JAK2V617F allele burden in primary myelofibrosis, compared to either a higher allele burden or unmutated status, is associated with inferior overall and leukemia-free survival. Leukemia. 2008;22(4):756–61.
60. Guglielmelli P, et al. Identification of patients with poorer survival in primary myelofibrosis based on the burden of JAK2V617F mutated allele. Blood. 2009;114(8):1477–83.
61. Guglielmelli P, et al. JAK2V617F mutational status and allele burden have little influence on clinical phenotype and prognosis in patients with post-polycythemia vera and post-essential thrombocythemia myelofibrosis. Haematologica. 2009;94(1):144–6.

62. Tefferi A, et al. IDH mutations in primary myelofibrosis predict leukemic transformation and shortened survival: clinical evidence for leukemogenic collaboration with JAK2V617F. Leukemia. 2012;26(3):475–80.
63. Guglielmelli P, et al. EZH2 mutational status predicts poor survival in myelofibrosis. Blood. 2011;118(19):5227–34.
64. Lasho TL, et al. SRSF2 mutations in primary myelofibrosis: significant clustering with IDH mutations and independent association with inferior overall and leukemia-free survival. Blood. 2012;120(20):4168–71.
65. Vannucchi AM, et al. Mutations and prognosis in primary myelofibrosis. Leukemia. 2013;27(9):1861–9.
66. Tefferi A, et al. CALR and ASXL1 mutations-based molecular prognostication in primary myelofibrosis: an international study of 570 patients. Leukemia. 2014;28(7):1494–500.
67. Pullarkat V, et al. Primary autoimmune myelofibrosis: definition of a distinct clinicopathologic syndrome. Am J Hematol. 2003;72(1):8–12.
68. Vergara-Lluri ME, et al. Autoimmune myelofibrosis: an update on morphologic features in 29 cases and review of the literature. Hum Pathol. 2014;45(11):2183–91.
69. Barbui T, et al. Survival and disease progression in essential thrombocythemia are significantly influenced by accurate morphologic diagnosis: an international study. J Clin Oncol. 2011;29(23):3179–84.
70. Kvasnicka HM, Thiele J. Prodromal myeloproliferative neoplasms: the 2008 WHO classification. Am J Hematol. 2010;85(1):62–9.
71. Sangle N, et al. Myelofibrotic transformations of polycythemia vera and essential thrombocythemia are morphologically, biologically, and prognostically indistinguishable from primary myelofibrosis. Appl Immunohistochem Mol Morphol. 2014;22(9):663–8.
72. Fu B, et al. Bone marrow fibrosis in patients with primary myelodysplastic syndromes has prognostic value using current therapies and new risk stratification systems. Mod Pathol. 2014;27(5):681–9.
73. Fu B, et al. The clinical importance of moderate/severe bone marrow fibrosis in patients with therapy-related myelodysplastic syndromes. Ann Hematol. 2013;92(10):1335–43.
74. Loghavi S, et al. TP53 overexpression is an independent adverse prognostic factor in de novo myelodysplastic syndromes with fibrosis. Br J Haematol. 2015;171(1):91–9.
75. Dingli D, et al. Der(6)t(1;6)(q21-23;p21.3): a specific cytogenetic abnormality in myelofibrosis with myeloid metaplasia. Br J Haematol. 2005;130(2):229–32.
76. Hasserjian R, et al. Myelodysplastic syndrome with isolated del(5q). In: WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: IARC; 2008. p. 102.
77. Washington LT, et al. Myeloid disorders with deletion of 5q as the sole karyotypic abnormality: the clinical and pathologic spectrum. Leuk Lymphoma. 2002;43(4):761–5.
78. Ingram W, et al. The JAK2 V617F mutation identifies a subgroup of MDS patients with isolated deletion 5q and a proliferative bone marrow. Leukemia. 2006;20(7):1319–21.
79. Rogers HJ, et al. Complex or monosomal karyotype and not blast percentage is associated with poor survival in acute myeloid leukemia and myelodysplastic syndrome patients with inv(3)(q21q26.2)/t(3;3)(q21;q26.2): a Bone Marrow Pathology Group study. Haematologica. 2014;99(5):821–9.

T- and NK-Cell Lymphocytosis

11

Sa A. Wang

Overview

Peripheral blood (PB) lymphocytosis is one of the most common abnormalities encountered in clinical hematology laboratory. Lymphocytosis is an increase in the number and/or proportion of lymphocytes in the blood. The normal ranges of lymphocytes in adult patients are about 1.0–3.0 × 10^9/L and 20–40%. In adults, absolute lymphocytosis is defined as absolute lymphocyte count (ALC) greater than 4.0 × 10^9/L, while relative lymphocytosis is an increase in the percentage of lymphocytes >40%, but ALC is normal. In children, the reference ranges differ significantly with age and, in general, significantly higher than adults. Mayo Clinic laboratory reports a normal range of lymphocyte count of 2.0–11.0 × 10^9/L (26–36%) at birth, which increases over the first 6 months of life, and peaks at 6 months to 1 year 1.4–22 × 10^9/L (47–77%), then declines over time, and approaches to adult range between age 15 and 20 years. The reference ranges may vary depending on populations, individual laboratory, instruments, and methods.

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In a patient presenting with an increased lymphocyte count, a number of factors are important for the initial assessment. These include patient age, duration of lymphocytosis, clinical signs and symptoms, and accompanied cytopenia(s). While pediatric lymphocytosis is most commonly benign, lymphocytosis in adults requires a more diligent workup to exclude a neoplastic process. Reactive lymphocytosis is usually self-limiting and normalizes after cessation of antigenic stimulus. The most common and well-recognized reactive causes include viral infections [1, 2] (Epstein–Barr Virus, hepatitis, cytomegalovirus, others) and drug reactions. Transient stress lymphocytosis is an under recognized form of reactive lymphocytosis, which often resolves in a few hours to 1–2 days. It is observed in patients who have various medical conditions such as trauma, surgery, excessive exercise, sickle cell anemia, cardiac events, and intoxication [3, 4]. The potential causes of reactive lymphocytosis are listed in Table 11.1.

Peripheral blood smear review may provide useful information in the initial differential diagnoses [5–7]. Reactive lymphocytosis is predominantly composed of small- to medium-sized lymphocytes that are often pleomorphic with blue cytoplasm. Monomorphic lymphocytosis is much more concerning for a lymphoproliferative neoplasm; however, some reactive processes may be associated with monomorphic lymphocytosis. Neoplastic lymphocytosis often shows

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Table 11.1 Potential causes of reactive lymphocytosis

Underlying causes	Examples
Infection	
• Viral	Epstein–Barr virus; cytomegalovirus; adenovirus type 1/2; herpes virus-6; HIV-1 in early seroconversion; HTLV-I benign lymphocytosis; mumps, varicella, influenza, hepatitis, rubella, roseola, Coxsackie virus B2, poliovirus
• Bacteria	Acute infection: bordetella pertussis (whooping cough) Chronic infection: cat-scratch fever, brucellosis, secondary syphilis, tuberculosis
• Protozoa	Toxoplasmosis
• Parasites	Babesiosis
Autoimmune	Rheumatoid arthritis, vasculitis
Hypersensitivity	Drug-induced, such as anticonvulsants Serum sickness, Vaccination
Stress	Post trauma, splenectomy, cigarette smokers, psychiatric emergency, sickle cell disease
Endocrine	Hyperthyroidism, Addison's disease
Malignancy	Thymoma, myeloma

abnormal cellular features, such as cleaved or "buttock" cells, "flower cells," "cerebriform" cells, or cells with blastic chromatin and distinctive nucleoli. It can be challenging to recognize neoplastic lymphocytes in a background of normal lymphocytes.

Flow cytometry immunophenotyping (FCI) is widely available in clinical practice and has become an essential tool in the workup of patients with lymphocytosis. In patients with persistent lymphocytosis, atypical findings on peripheral blood smear review, or patients with constitutional or B symptoms, organomegaly, or associated cytopenia(s), FCI study is highly recommended. FCI can characterize the lineage of the lymphocytes, assess the clonality of B cells, and identify aberrancy on T cell/NK cells. In a positive case, FCI helps in the further classification of neoplastic lymphocytosis. In benign/reactive lymphocytosis, FCI not only helps in ruling out a neoplastic proliferation but also may provide useful information in identifying the underlying causes.

Both reactive and neoplastic processes will be discussed. This chapter will focus on lymphocytosis primarily involving T cells and NK cells. This chapter also includes T-cell lymphoblastic leukemia/lymphoma. The characteristic features of each entity and their major differential diagnoses will be discussed.

Reactive Lymphocytosis

Clinical Features

Depends on the underlying causes, patients may have different clinical presentations. In patients with viral infections, including EBV, CMV, influenza, adenovirus, and HIV, the common symptoms associated with lymphocytosis are fever, fatigue, sore throat, and general malaise. Some patients may have lymphadenopathy and/or splenomegaly. Lymphocytosis in patients with chronic infections, such as tuberculosis, brucellosis, or secondary syphilis, will have different presentations depending on the underlying diseases. Noninfectious causes include medications, trauma, vaccination, postsplenectomy, hypersensitivity reaction, smoking, and autoimmune diseases. In the study series of stress lymphocytosis in a hospital setting [3, 4], the common causes are trauma, predominantly motor vehicle accidents, followed by gynecologic and obstetric diagnoses, psychiatric emergencies, cardiac emergencies, thermal injury, seizure disorders, sickle cell disease, postoperative, acute drug toxicity, acute infection, and viral syndrome.

Morphology

In reactive lymphocytosis, lymphocytes are often heterogeneous in appearance. The PB smear often shows a diverse spectrum of lymphocytes

(Fig. 11.1), ranging from small lymphocytes with mature chromatin and inconspicuous nucleoli to large lymphocytes with abundant clear, light blue cytoplasm, or deeply basophilic cytoplasm, round to oval nuclei, and prominent nucleoli. The cytoplasm may appear to be indented by surrounding red blood cells (RBC). Circulating immunoblasts and plasma cells may also be present. Infectious mononucleosis from EBV infection is a classic example that often shows many reactive lymphocytes in blood. Large granular lymphocytes (LGLs) with azurophilic cytoplasmic granules may be significantly increased in patients with infection, autoimmune disease, drug/medications, or post-hematopoietic stem cell transplant. It can be difficult to distinguish these reactive processes from T-cell LGL leukemia or chronic lymphoproliferative neoplasm of NK cells, based on morphology alone. Small monotonous lymphocytes, although frequently associated with a neoplastic/clonal lymphoproliferative disorder, may be seen in *Bordetella pertussis* infection.

Immunophenotype

The normal reference ranges for lymphocyte subsets have to be established in individual laboratories, considering the factors of age, race, gender, as well as geographic location [8, 9]. The reference ranges can be further affected by nutritional status, stress, and underlying subclinical infections, overall immune status in any given "healthy" population [10–12]. In children, both the absolute and percentage of each lymphocyte subset not only significantly differ from that of adults but also vary among different age groups [13]. A recent study [9] based on the healthy population from South Florida reported the normal ranges of various lymphocyte subsets, and it is referenced in Table 11.2. However, the reference ranges can be very difficult to apply to patients with reactive lymphocytosis. Therefore, it is imperative to understand the normal immunophenotype of various lymphocyte subsets.

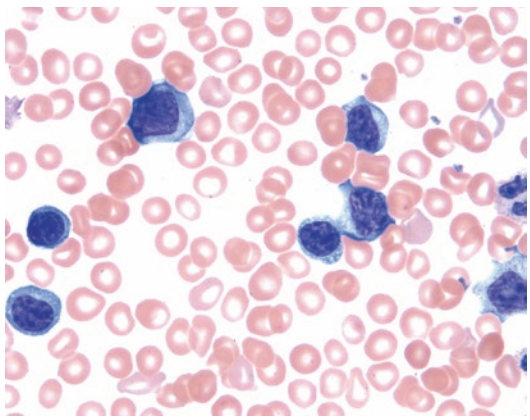

Fig. 11.1 Reactive lymphocytosis. Peripheral blood smear shows an array of lymphocytes, from small with mature chromatin to large lymphocytes with abundant light basophilic cytoplasm. Some cells contain azurophilic granules

The immunophenotype of B cells in PB and BM is discussed in Chap. 12. For T cells and NK cells, the CD4:CD8 ratio varies in the PB (Table 11.2), BM, spleen, and lymph nodes, which can be significantly skewed to either CD4 or CD8, due to the underlying medical conditions. Normal T cells express pan T-cell antigens CD2, CD3, CD5, and CD7. The majority of them are TCRαβ and a minor population is TCRγδ (Fig. 11.2). CD4+ T cells have brighter CD3 than CD8+ T cells. CD7-negative T cells are a part of normal T cells, more commonly negative in CD4 than in CD8 T cells [14, 15]. In reactive conditions, a number of variations can be seen that include increased CD2 and variably decreased CD7 on activated CD4+ T cells, decreased CD5 on CD8+ large granular lymphocytes (LGLs), increased CD3 and absent CD5 on gamma/delta T cells, variable expression of CD8 on a subset of CD4+ T cells or variable expression of CD4 on a subset of CD8+ T cells, and absent CD2 expression on a small subset of CD4+ or CD8+ T cells. In addition, the immunophenotypic aberrancies that are characteristic of T-cell lymphoma can also be seen in T cells in patients with no clinical or histologic evidence of T-cell lymphoma but are more often encountered in lymph nodes than blood and bone marrow [16, 17]. Normal T-LGL are CD3+, CD2+, CD5slightly dimmer+, CD7+, CD8+, CD57+, CD56subset+, CD16verydim+, and CD94dim+; CD5-negative T cells can be seen in normal, often more in TCRγδ T cells than TCRαβ T cells [18].

Table 11.2 Normal reference ranges of various lymphocyte subset markers in healthy adults and adolescents, modified based on a population study of South Florida [9]

	Adolescents (median, range)	95% confidence interval	Adults (median, range)	95% confidence interval
Age (years)	16 (12–18)	14.8–15.9	38 (21–67)	35.9–40.3
WBC (/μL)	6500 (2900–11100)	5948–7246	6700 (2600–11800)	6363–7069
Lymphocytes (% of total WBC)	35% (27–62)	34–39	32 (18–54)	31–34
Lymphocytes absolute count (/μL)	2250 (1263–4253)	2125–2635	2134 (1170–4698)	2021–2262
CD3+ T-cells (% of lymphocytes)	73 (49–83)	69–74	78 (65–88)	76–78
CD3+ T-cell absolute count (/μL)	1665 (939–2959)	1525–1880	1593 (983–3572)	1543–1730
CD3+CD4+ T cells (% lymphocytes)	40% (27–53)	37.5–41.8	47 (26–62)	45.7–48.5
CD3+CD4+ T-cell absolute count (/μL)	920 (467–1563)	838–1029	931 (491–2000)	942–1066
CD3+CD8+ T cells (% of lymphocytes)	27 (16–40)	25.1–29.1	27 (14–44)	26.4–28.9
CD3+CD8+ T-cell absolute count (/μL)	624 (259–1262)	565.7–739.7	522 (314–2087)	544.4–636.6
CD19+ B cells (% of lymphocytes)	17.2 (8–31)	15.6–18.8	11.6 (2–27)	11.1–12.8
CD19+ B-cell absolute count (/μL)	374 (169–1297)	344.6–479.7	234 (64–800)	231.8–280.8
CD16+CD56+ NK cells (% of lymphocytes)	8.5 (3–30)	8.3–13.2	9.3 (2–27)	9.0–10.8
CD16+CD56+ NK-cell absolute count (/μL)	198 (59–1178)	187.7–335.7	203 (27–693)	189.6–235.4
CD4:CD8 ratio	1.5 (0.7–2.6)	1.4–1.7	1.7 (0.6–4.4)	1.7–1.9

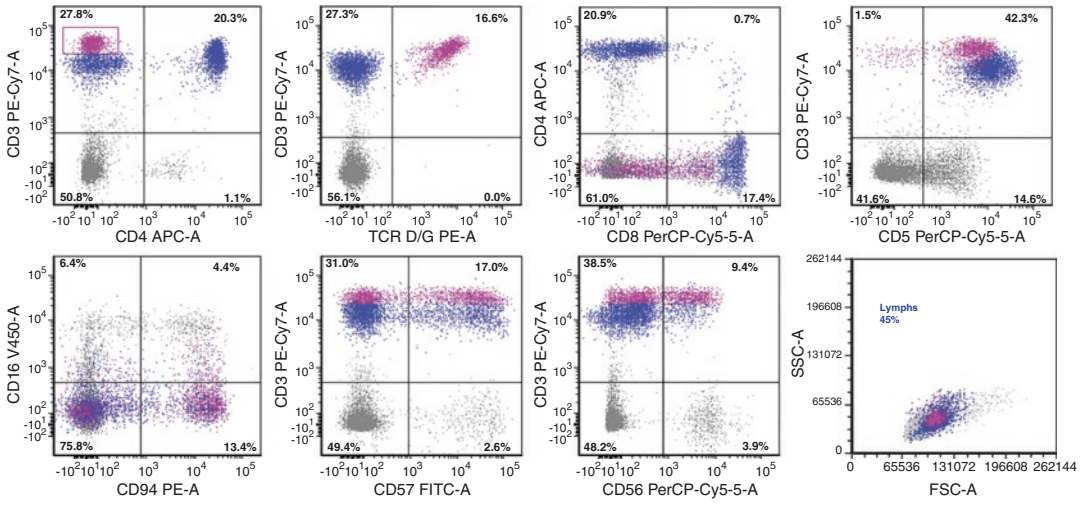

Fig. 11.2 Reactive γδ T-cells (CD3+ T cells are in *blue* and γδ T-cells in *pink*). Upper panel: normal γδ T-cells are CD3brighter+, CD8 subset+, CD4-negative, CD5-slightly dimmer+, CD16 small subset+, CD57subset+, CD56subset+, and CD94 subset+. They are small on forward scatter (*lower right plot*).

Reactive T-/NK-Cell Lymphocytosis

The majority of reactive lymphocytosis, in absolute numbers, is due to an expansion of T cells. Similar to B-cell lymphocytosis, peripheral blood smear review should serve as an initial screen tool. While reactive lymphocytosis is often polymorphic, some cases may show a marked expansion of large granular lymphocyte (LGL) cells. These LGL cells are small to medium, with moderate cytoplasm and fine cytoplasmic granules. Cells with LGL morphology are composed predominantly of T-cell LGL cells, NK cells, and some TCRγδ T cells. If of any concern, flow cytometry study should be performed.

Patients with autoimmune diseases tend to have an increased CD4/CD8 ratio, while those with acute viral infections often have a decreased CD4/CD8 ratio. However, CD4:CD8 ratio can vary significantly in any given condition, for example, may be high in early HIV infection, normalized after seroconversion, and gradually drops in patients with persistent infection. A marked increased CD4:CD8 ratio (10:1) or a markedly reversed ratio should prompt further investigation. In the following, reactive T-cell/NK-cell lymphocytosis will be discussed according to the expansion of different subsets of cells.

Increased T-Cell Large Granular Lymphocytes

LGL is a morphologically distinct lymphocyte subset that is larger than most circulating lymphocytes and has characteristic azurophilic granules with moderate cytoplasm (Fig. 11.3). In normal adults, LGL cells represent up to 10–15% of peripheral blood mononuclear cells (PBMCs) and 200–400/μL in absolute count. Cells with LGL morphology can be classified into two distinct lineages as either CD3+ CD8+ LGL or NK cells. Both cell types play important roles in the immune system antimicrobes. LGLs become activated through antigen recognition and undergo an significant expansion, followed by subsequent death through apoptosis upon antigen clearance. NK-cell lymphocytosis will be discussed separately in the following section.

T-cell LGLs are terminally differentiated effector memory T cells that are CD3+, CD45RA+, CD62L-neg, and CD57+. T-cell LGL expansions can be seen in a number of reactive conditions. The major clinical settings are infections particularly viral infections, autoimmune diseases, post splenectomy, an underlying malignancy, certain drugs/medications, and post-hematopoietic stem cell transplant (HSCT). Recently it has been recognized that in patients with chronic myeloid leukemia (CML) and Ph(+) acute lymphoblastic leukemia treated with dasatinib, a significant expansion of LGLs can be observed in about half of the patients. A LGL clonal expansion in dasatinib-treated patients is associated with early and long-lasting responses to tyrosine kinase inhibitor therapy [19, 20]. In patients who received allogeneic [21] and autologous HSCT [22], a LGL expansion that persists beyond the early transplantation period can be observed in nearly half of the patients [23]. The LGL expansions are usually polyclonal or oligoclonal and in some cases, clonal. The LGL proliferations in the post-HSCT setting are often nonprogressive and self-limiting, and it may be associated with thymoglobulin prophylaxis, reduced conditioning regimen, and acute and chronic graft-versus-host disease (GVHD).

Reactive LGL lymphocytosis is very common in patients with autoimmune diseases, especially in patients with rheumatoid arthritis; but unfortunately, T-cell LGL leukemia also commonly occurs in patients with an underlying autoimmune disease. Felty's syndrome, the triad of chronic arthritis, splenomegaly, and neutropenia, typically occurs in patients with severe, long-standing, seropositive rheumatoid arthritis and is often associated with other extraarticular manifestations. Some of these patients have been found to have a clonal proliferation of LGL meeting the criteria of T-cell LGL leukemia.

Due to significant overlap between LGL leukemia and LGL lymphocytosis, it is important to recognize the morphological features and understand the immunophenotype of normal and reactive T-cell LGL cells in order to identify real aberrancies. Normal T-cell LGL cells have small round or slightly elongated nuclei and moderate

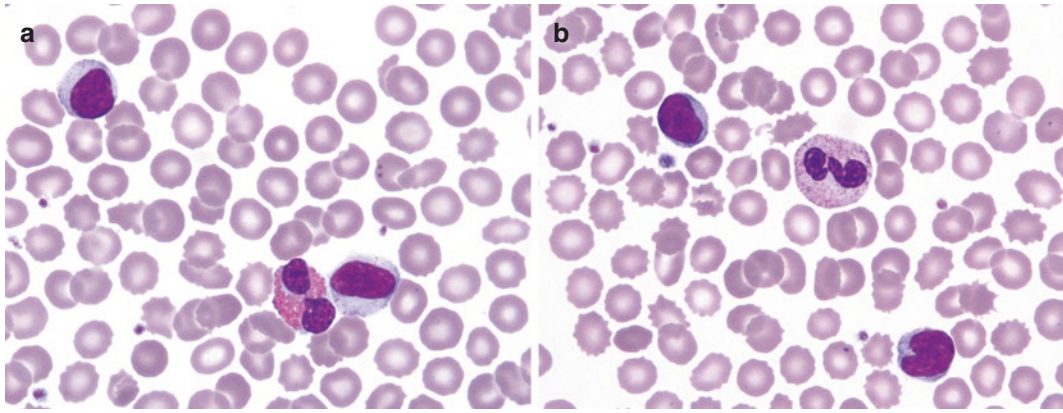

Fig. 11.3 Reactive large granular lymphocytosis. Lymphocytes are small to medium in size with moderate cytoplasm containing azurophilic granules (**a**). Some of the reactive LGL can show nuclear irregularity; (**b**) some may correspond to γδ T cells or NK cells

to abundant cytoplasm, with azurophilic granules (Fig. 11.3). Reactive LGL expansions are mainly seen in peripheral blood and do not show a BM infiltrate (see LGL leukemia for detailed illustration and discussion).

Immunophenotypically, normal/reactive T-cell LGL cells are CD2+, CD3+, CD8+, CD57+ (showing a spectrum of intensity), CD5 dimmer+, CD56 small subset+, CD16 very dim+/negative, CD94 dimmer+, and TCRαβ+ (Fig. 11.4). Occasionally, a subset of normal/reactive LGL cells can show dim/partial CD4 coexpression with CD8 and should not be interpreted as abnormal. In some cases, a small subset of LGL cells may show an aberrant immunophenotype in a background of reactive T cells; if the quantity of this abnormal LGL cells is low, it may represent T-cell clonopathy of unknown or undetermined significance (TCUS) [24, 25]. TCUS may represent a benign end of the spectrum of T-cell LGL proliferation with lower frequencies of symptoms and signs, rheumatoid serology, and treatment requirement.

PCR for TCR gene rearrangement may detect a clonal rearrangement in reactive, or oligoclonal LGL lymphocytosis, or a clonal LGL proliferation of unknown significance. Therefore, it should not be used as a sole evidence in the diagnosis of T-cell LGL leukemia, in the absence of morphological, clinical, and flow cytometry immunophenotypical support. Flow cytometry assessment of expression of Vβ repertoire is useful in the determination of T-cell clonality and quantification of the clonal cells [26, 27].

Increased Reactive TCRγδ T Cells

In healthy adults, γδ T cells constitute only a small proportion (1–5%) of the total T-lymphocyte pool in blood, and they are preferentially found in the spleen and epithelial-rich tissues, such as the skin, intestine, and reproductive organs [28, 29]. Although the precise function of TCRγδ T cells remains unclear, it is postulated that they play a sentinel role in both innate and antigen-specific mucosal immune responses [30, 31]. TCRγδ T cells can be significantly increased (>5% and $>0.2 \times 10^9$/L) in a number of conditions [18] including infection/inflammatory diseases, autoimmune diseases, an underlying malignancy, post-HSCT, and postsplenectomy. Increased blood TCRγδ T cells have been found in hospital workers who are in close contact with tuberculosis [32]. Of autoimmune diseases, increased TCRγδ T cells have been found in patients with rheumatoid arthritis, immune thrombocytopenic purpura (ITP), ankylosing spondylitis, Sjögren syndrome, celiac disease, Graves' disease, multiple sclerosis, pernicious anemia, and autoimmune-induced pancytopenia, primary sclerosing cholangitis, and autoimmune hepatitis [33].

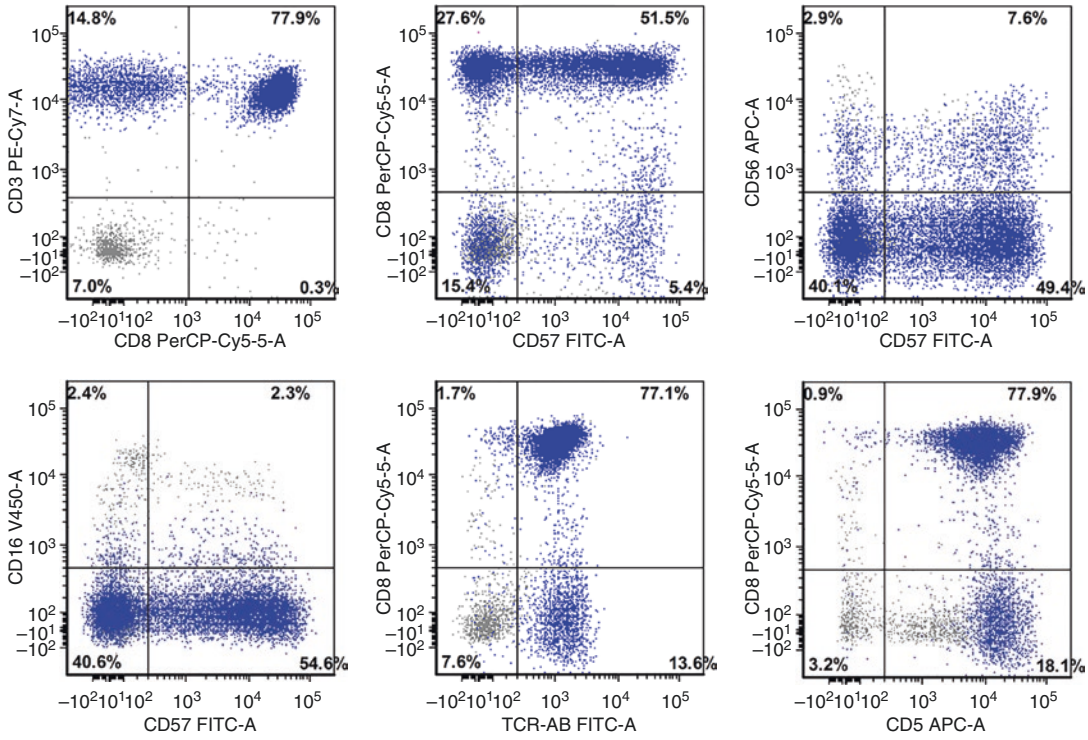

Fig. 11.4 Reactive T-cell large granular lymphocytosis (CD3+ T cells are in blue). A markedly reversed CD4:CD8 ratio (*upper left*); T-cell LGL cells show a variable CD57 expression (*upper middle*); a small subset of T-cell LGL cells express CD56 (*upper right*); reactive T-cell LGL cells have a very low expression or negative for CD16 (*lower left*); they are TCRαβ+ (*lower middle*) and show a slightly dimmer CD5 expression (*lower right*)

Additionally, increased blood TCRγδ T cells have been found in patients with Hodgkin lymphoma, diffuse large B-cell lymphoma, myelodysplastic syndromes, and solid tumor [18, 34].

Morphologically, mature γδ T lymphocytes are indistinguishable from either B or other (TCRαβ) T lymphocytes. Some may contain cytoplasmic granules, showing similar morphological features to T-cell LGL or NK cells (Fig. 11.3). Immunophenotypically, TCRγδ T cells are CD3bright+, CD2+, CD5+/−, CD7+, CD4−, and CD8small subset+ (Fig. 11.2). They frequently express cytotoxic antigens, such as CD16, CD56, CD57, and CD94, but the expressions are usually partial and heterogeneous, differing from a true neoplasm (see below γδ T-cell LGL leukemia and hepatosplenic γδ T-cell lymphoma) in the expression pattern and intensity. In reactive conditions, normal γδ T lymphocytes may show a partial loss of CD5 expression, or decreased CD7 expression [18, 34]. Failure to recognize normal γδ T cell immunophenotype could lead to an erroneous diagnosis of peripheral blood involvement by a γδ T-cell neoplasm.

Increased Natural Killer (NK) Cells

NK cells represent a small proportion of blood lymphocytes and do not express a specific receptor for antigens. NK cells are important in tumor surveillance and in the early control of microbial infections, including infections with viruses and intracellular parasites. A marked relative expansion of NK cells can be seen in various reactive conditions. It is well known that NK cells can be significantly increased in autoimmune diseases, infections, vaccination, malignancies, and therapeutic drugs. As mentioned previously, a marked expansion of large granular lymphocyte (LGL) cells has been reported in patients with *BCR-ABL1*+ chronic myeloid leukemia (CML) treated with dasatinib, a second-generation tyrosine

kinase inhibitor. Recently, it is shown [35] that NK cells are the dominant LGLs expanded in dasatinib-treated patients; and specifically, CMV-associated adaptive NK cells are responsible for the dasatinib-induced NK cell expansion. NK-LGL lymphocytosis during dasatinib treatment is associated with superior therapeutic responses [36, 37]. In patients who received allogeneic HSCT [38], NK cells usually take 1–2 months to normalize, and they are the first lymphocyte subset to return/reconstitute to a normal level after HSCT, whereas B- and T-cells' recovery often take months to years. NK cells have the ability to kill residual malignant cells in the very early phase of post-HSCT; and furthermore, NK cells are involved in the innate immune responses against antimicrobial threats as well as the development of adaptive immune responses [39]. A marked relative increase in NK cells is often observed in the early phase of post-HSCT.

Normal NK cells are surface CD3−, CD2+, CD5−, CD7+, CD8small subset+, CD94+, CD56+, and CD57subset+ (Fig. 11.5). In PB, five NK-cell subpopulations can be defined on the basis of their irrelative expressions of CD16 and CD56 [40]: (1) CD56bright CD16−, (2) CD56bright CD16dimmer, (3) CD56dimmer+ CD16−, (4) CD56dimmer CD16bright, and (5) CD56−CD16bright. In healthy individuals, populations (3) and (5) are in very small numbers. CD56−CD16bright NK cells often are dramatically expanded in HIV infection. The CD56dimmer CD16bright NK cells represent at least 90% of all PB NK cells. Up to 10% are CD56bright CD16 dimmer/negative NK cells. By immunohistochemistry, variable numbers of NK cells may show reactivity to CD3 staining, depending on the specificity of the anti-CD3 antibodies in recognizing cytoplasmic CD3 epsilon chain. NK cells express cytotoxic markers granzyme, TIA1, and perforin. NK-cell function is regulated by a series of receptors known as killer immunoglobulin-like receptors (KIRs) and their interaction with HLA ligands (Fig. 11.5). KIR antigen expression can be used to assess NK-cell clonality in cases where a NK-cell lymphoproliferative disorder is suspected.

Reactive lymphocytosis, both T-LGL and NK cells, should not show a bone marrow infiltration; please refer to the following discussion on T-cell LGL leukemia and NK-cell neoplasm for further discussion.

T- and NK-Cell Lymphoproliferative Disorders

T-Cell Large Granular Lymphocytic Leukemia

Large granular lymphocytic (LGL) leukemia is a clonal expansion of large granular lymphocytes (LGLs), either of cytotoxic T lymphocytes (CTLs) or natural killer (NK) cells, with a bone marrow infiltrate, frequent splenomegaly, and cytopenia(s), most commonly neutropenia followed by anemia. LGL leukemia is thought to arise from chronic antigen stimulation, which drives long-term cell survival through the activation of survival signaling pathways and suppression of pro-apoptotic signals. However, unlike their normal counterparts, leukemic LGL cells are resistant to subsequent Fas-mediated activation-induced cell death [41]. It is postulated that following antigen encounter, the dysregulation of several signaling pathways and cytokines such as interleukin-15, platelet-derived growth factor receptor, and signal transducer and activator of transcription 3 (STAT3) sustains a constitutively active clonal cell population [42]. The recent discoveries [43–45] of somatic mutations in *STAT3* and *STAT5B* in LGL leukemia further support the hypothesis.

NK-type LGL is renamed as chronic lymphoproliferative disorder of NK cells (CLPD-NK) in the 2008 World Health Organization (WHO) and has remained unchanged in the 2016 WHO revision [46]. The rationale is likely due to the difficulty in assessing clonality of NK cells, as well as the importance in distinguishing it from aggressive NK cell leukemia. In line with the change, T-cell LGL will be discussed here, and CLPD-NK will be discussed separately.

Clinical Features

T-LGL leukemia affects men and women with a similar proportion. Patients have a median age

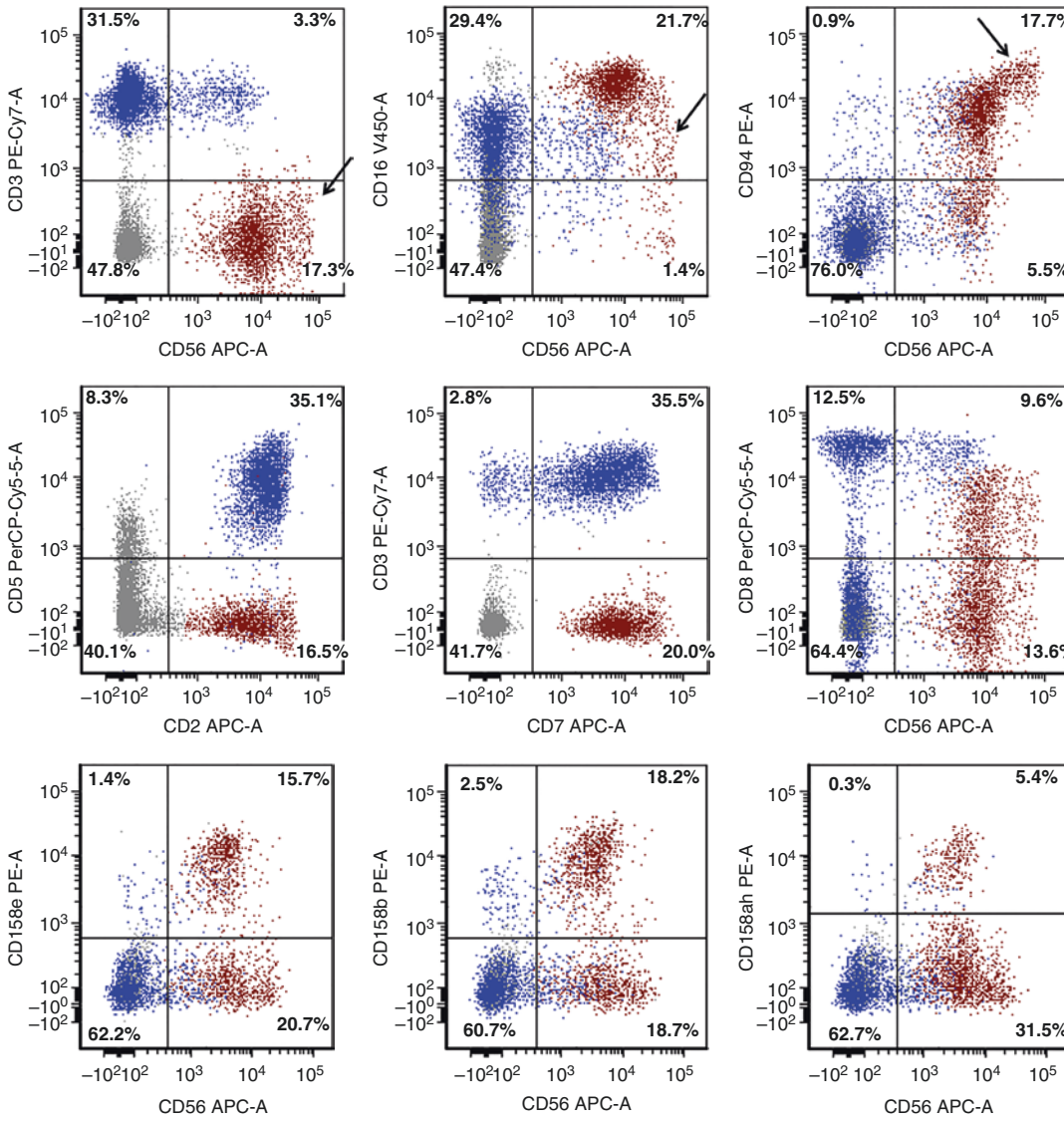

Fig. 11.5 Normal peripheral blood NK cells (CD3+ T cells are in *blue*, and NK cells are in *brown*). *Upper* and *middle* panels, normal NK cells are CD3−, CD56+, CD16+, CD94+, CD2+, CD5−, CD7+, CD8partial+, and CD57small subset (not shown). The majority of the NK cells are CD56dimmer+CD16bright+ with a small subset CD56brightCD94brightCD16dimmer+/negative (*arrows*). The latter corresponds to high effective NK cells. NK cells express various KIR antigens (CD158a, b, and h, *lower panel*) indicating polytypicality

of 60 years, and only 20–25% of patients are younger than 50 years. Most of the patients with T-cell LGL leukemia come to medical attention with cytopenia(s), frequently isolated neutropenia. Severe neutropenia may lead to recurrent infections, especially bacterial infection, which occurs in 15–56% of patients [47, 48]. Pure red cell aplasia (PRCA) has been reported in 8–19% of patients, and aplastic anemia has also been reported [25, 49]. Thrombocytopenia occurs in about 20% of patients, and immune thrombocytopenic purpura (ITP) is seen at an increased frequency in patients with T-cell LGL leukemia. Splenomegaly is observed in 25–50% of cases, whereas hepatomegaly and lymphadenopathy are very rare [47, 50]. Rheumatoid arthritis appears to be the most frequent autoim-

mune disease associated with T-cell LGL leukemia, reported in up to 36% of patients. Serologic abnormalities (rheumatoid factor, antinuclear antibody, and polyclonal hypergammaglobulinemia) are frequent, even in patients without clinical manifestation of an autoimmune disease [50, 51]. T-cell LGL leukemia is mostly of CD3+CD8+ immunophenotype and, occasionally, CD3+CD4+ with or without a dimCD8 coexpression. Like CD8+ T-cell LGL leukemia, CD4+ T-cell LGL leukemia or clonal lymphocytosis is also a consequence of chronic antigenic stimulation. However, CD4+ T-cell LGL appears to have clinicopathological features that are different from classic CD8+ T-LGL leukemia, with almost all patients lacking significant cytopenias or the association with an autoimmune disease. Instead, many patients with CD4+ T-LGL have an underlying primary malignancy [52–54].

Rare cases of T-cell LGL leukemia have also been reported in the post-HSCT setting [50, 55–57]. Patients with T-cell LGL leukemia arising in the post-HSCT setting generally have the typical manifestations of T-cell LGL leukemia such as cytopenia, recurrent infections, and splenomegaly. A T-cell LGL leukemia is also described in patients with a primary malignancy, such as B-cell lymphoma or monoclonal B-cell lymphocytosis [58, 59], monoclonal gammopathy of undetermined significance (MGUS), multiple myeloma [25, 60], solid tumor and myelodysplastic syndromes [61, 62]. However, in these settings, the concomitant conditions make it difficult to determine if cytopenia(s) are attributed to LGL leukemia; and it is uncertain if some of these reported cases are true LGL leukemia or an oligoclonal LGL proliferation due to underlying chronic antigenic stimulation.

Morphology

The number of T-cell LGL cells in T-cell LGL leukemia, according to the WHO guideline, is usually between 2 and 20 × 10^9/L [63]. From the French large cohort study consisting of 229 patients, lymphocytosis (>4 × 10^9/L) related to the LGL proliferation is observed in more than half of patients. However, in individual cases, absolute circulating LGL count varied widely, ranging from 0.5 × 10^9/L to 10 × 10^9/L [50]. The determination of the number of LGL cells, although not clearly stated in most of the studies, likely relied on flow cytometry immunophenotype rather than microscopic counting.

Normal or reactive LGL cells in peripheral blood often are small to medium in size, with abundant cytoplasm, and fine azurophilic granules (Fig. 11.3). In PB, cells with LGL morphology include T-cell LGL cells, NK cells, some cytotoxic CD3+CD8+ T cells, as well as some γδ T cells. While LGL cells in LGL leukemia can resemble normal/reactive LGL cells, in some cases, they may exhibit morphological abnormalities. These include reduced cytoplasmic granules, reduced amount of cytoplasm, smaller cell size, irregular nuclear contours, and condense chromatin (Fig. 11.6). Of note, aggressive NK-cell leukemia/lymphoma and some peripheral T-cell lymphoma of a cytotoxic immunophenotype may contain cytoplasmic granules, but they are often medium to large, with overt abnormal nuclear features (convoluted, hyperchromatic, smudgy chromatin), and should not be mistaken as LGL cells.

Immunophenotype

T-cell LGL leukemia consists of various immunophenotypic subtypes (Table 11.3). The most common immunophenotype is CD3+, CD4-, CD8+ and CD57+, TCRαβ+ (Fig. 11.7). Alterations frequently observed are increased expressions of CD57 (in over 75% of T-cell LGL cells, and bright and uniform) [64] and increased CD16. A decreased CD5 expression is also frequently observed. It is important to know that normal reactive T-LGL cells generally have a dimmer CD5 expression comparing to CD4+ T cells and other CD8+ T cells; however, in T-cell LGL leukemia, the decrease in CD5 expression usually is much pronounced. A complete lack of CD5 expression is not common in T-cell LGL leukemia but can be rarely seen. Other alterations include a complete loss of CD8 [65] and altered expression levels of CD2 and CD3. CD56 expression is generally completely negative in T-cell LGL leukemia, unlike normal/reactive T-LGL cells that often have a subset of cells positive for CD56. However, an uniform expression of CD56

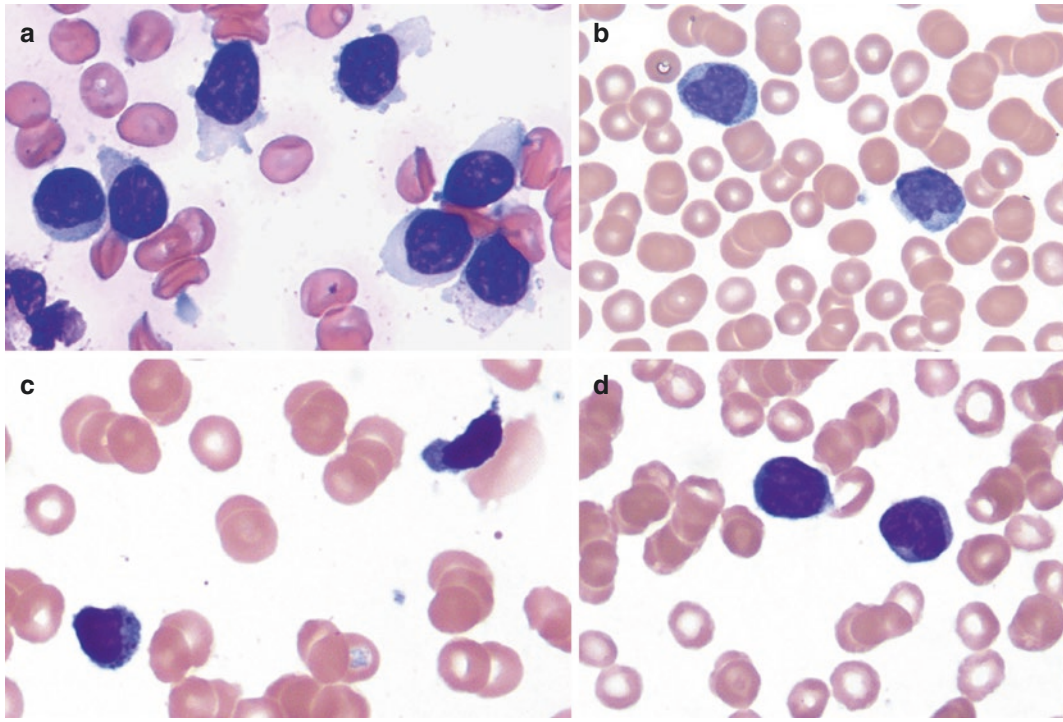

Fig. 11.6 Peripheral blood smears of T-cell large granular lymphocytic leukemia (LGL). (**a**) Markedly increased LGL that are small- to medium-sized cells with moderate cytoplasm containing azurophilic granules, typical LGL morphology; however, some features differing from normal LGL cells may be observed such as reduced cytoplasmic granules and irregular nuclear contours (**b**), small cell size (**c**), and reduced cytoplasm with irregular nuclei (**d**)

Table 11.3 Immunophenotype of normal T-cell large granular lymphocytes (LGL) cells, T-cell LGL leukemia and its immunophenotypical variants

	Frequency	Common Immunophenotype	Common alterations
Normal/reactive T-cell LGL		CD3+, TCRαβ+, CD8+, CD4−, CD57+, CD56 small subset, CD16−/very dim+, CD94dim+	CD5 dimmer than other CD8+ T cells; some may have a dim partial CD4 coexpression
LGL leukemia variants			
Common type	70%	CD3+, TCRαβ+, CD8+, CD4−, CD57+	↓CD8 on TCRαβ+ subtype ↑CD8 on TCRγδ+ subtype
TCRγδ variant	5–15%	CD3+, TCRγδ+, CD8−, CD4−, CD57+	Uniform or ↑CD57 expression; ↑CD16; ↑CD56 expression; Complete loss or markedly ↓CD2, CD5, CD7 expression
CD4+ variant	2–4%	CD3+, TCRαβ+, CD4+, CD8dimer/neg, CD56+, CD57+	

can be seen in some T-cell LGL leukemia. It has been suggested that CD56+ T-cell LGL leukemia is a clinically aggressive variant T-LGL leukemia. However, these reported CD56+ cases are all CD57-negative; and a significantly proportion of these cases show cytogenetic abnormalities containing i(7) or i(7q) [66]. It is possible that some of these cases represent hepatosplenic T-cell lymphoma, TCRαβ variant, with LGL morphology. Therefore, it is unclear if a CD56

coexpression has any clinical significance in a case with an immunophenotype, morphological characteristics, and clinical presentation typical of T-cell LGL leukemia.

TCR γδ variant T-cell LGL leukemia comprised ~10% (5–15%) of all T-cell LGL leukemia [67, 68]. The typical immunophenotype is CD3bright+, CD8−, CD4−, CD57+ (Fig. 11.8, upper panel), and TCRγδ+. CD56 is often negative but can be partial/subset+ or positive together with CD57. In some cases, the leukemic cells may be negative for both CD56 and CD57. CD5 is dimmer+ or partial+ and only occasionally completely negative. The critical differential

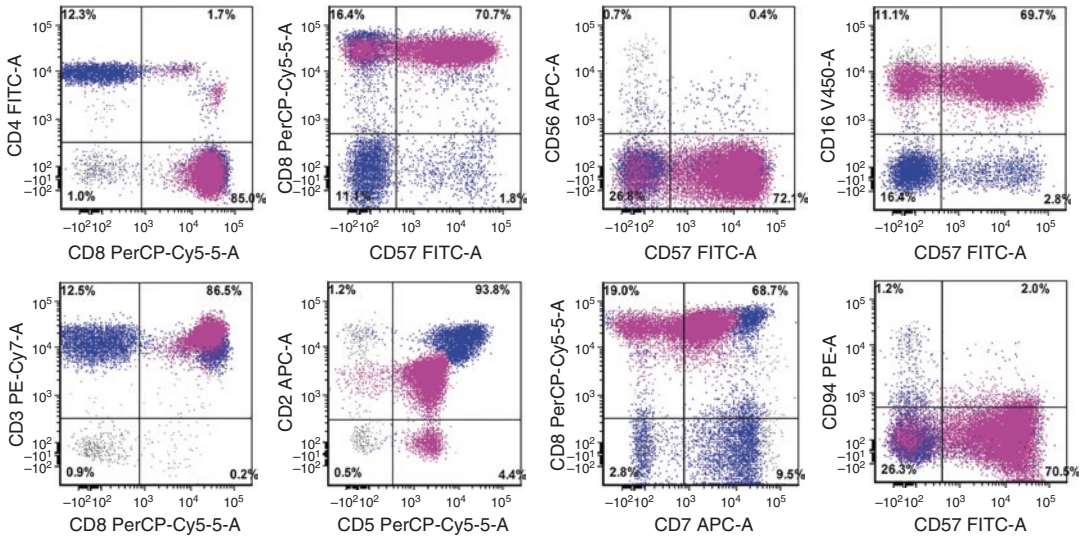

Fig. 11.7 A case of T-cell LGL leukemia with a common immunophenotype. The T-cell LGL leukemia is CD3+CD8+CD57mostly+TCRαβ+ (not shown). The LGL cells aberrantly express increased CD16 and CD3; decreased CD2, CD5, CD7; and a completely aberrant loss of CD56 and CD94

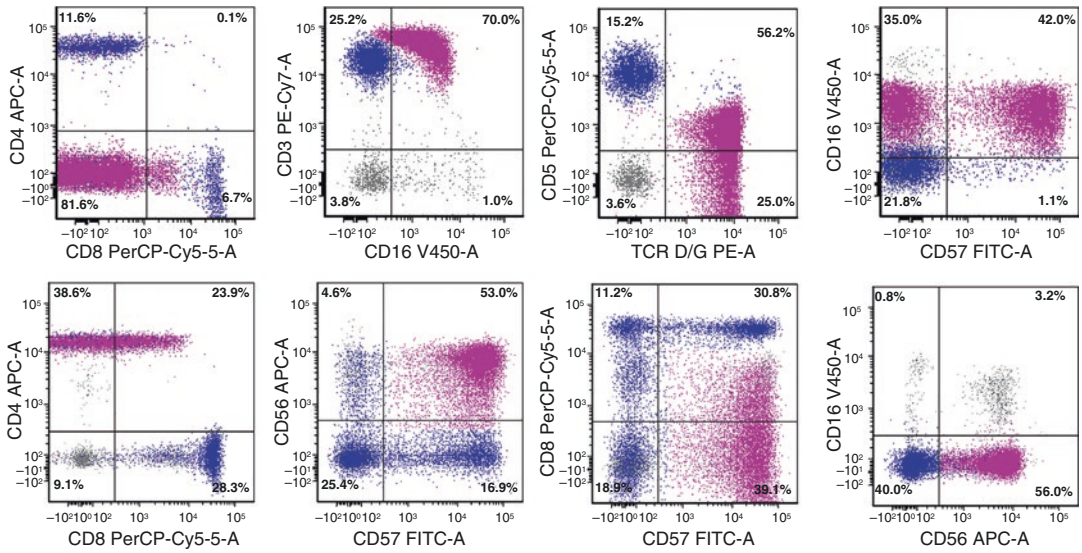

Fig. 11.8 Examples of T-cell LGL leukemia immunophenotypical variants. *Upper panel*: a case of TCRγδ T-cell LGL leukemia CD4−CD8− with bright CD3 and increased CD16, dim/partialCD5, TCRγδ, and subsetCD57+; *lower panel*: CD4+ variant T-cell LGL leukemia, the LGL cells are CD4+ with partialCD8+, CD56+, CD57 bright +, and CD16−

diagnosis is hepatosplenic γδ T-cell lymphoma or other γδ T-cell lymphomas, which are clinically aggressive and require different management. These γδ T-cell lymphomas often show a different immunophenotype from γδ LGL leukemia, with a strong uniform CD56 expression, absence of CD57, completely lack of CD5, and often have very bright CD16 and CD94 (Table 11.4).

Rare cases of T-cell LGL leukemia are composed of monoclonal CD4+ T cells with dim or no coexpression of CD8 (Fig. 11.8, lower panel), so-called CD4+ T-cell LGL leukemia [52–54]. The typical immunophenotype is CD3+, CD4+, CD56+, CD57+, and TCRαβ+, with a variable dim CD8 expression.

The presence of bone marrow infiltrate is strongly in favor of a T-cell LGL leukemia over a reactive LGL proliferation (Fig. 11.9). The latter often shows increased LGL in PB but lack of a BM infiltrate. The challenge is what would be considered as "a LGL infiltrate" since CD3+ T cells are invariably present in a normal bone marrow and may be significantly increased in reactive BM. A LGL infiltrate can be very difficult to appreciate on H&E stain; and therefore, immunohistochemistry stains (IHC) are necessary to identify and confirm the presence of the infiltrate. CD3, granzyme B, and TIA1 are considered to be the basic three IHC markers to identify a T-cell LGL infiltrate. Granzyme B stain is excellent to identify cytotoxic cells; however, in about 30% T-cell LGL leukemia, granzyme B may be aberrantly lost or partially lost; therefore, TIA1 stain is necessary. TIA1 stains neutrophils but with a different pattern from LGL cells, and the latter show darker, dot-like, and punctuate staining. CD57 is a good flow cytometry marker for T-cell LGLs but can be weak by IHC, often, only highlights a subset of T-LGL cells, underestimating the number of LGL cells. CD4 and CD8 IHC can be helpful to show the predominance of CD8+ T cells.

In the BM, the LGL infiltrate is often interstitial and with some cells in the sinuses. The intrasinusoidal infiltrate component can vary from cases to cases, from very minimal to prominent, but may not be present at all. The finding of a sinusoidal infiltrate by LGL cells is strongly favor a neoplastic process (Fig. 11.9) and may correlate

Table 11.4 Comparison of T-cell large granular lymphocytic leukemia (LGL) and hepatosplenic γδ T-cell lymphoma (HSTL)

	LGL	HSTL
Age	60(12–87)	34(16–58)
Male/female	1:1	5:1
CBC	Isolated neutropenia or pure red cell aplasia	Pancytopenia is common
Clinical presentations	Underlying autoimmune disease or autoantibodies, chronic infection	Fever, weight loss, night sweats, and fatigue. Hemophagocytosis is common. Association with immunosuppression
Organomegaly	Mild splenomegaly in 20–50%; hepatomegaly rare	Marked splenomegaly in 100%, hepatomegaly in 50%
Lymphadenopathy	Uncommon	Uncommon
Abnormal liver function	Uncommon	Common
Bone marrow infiltrate	Interstitial and or intrasinusoidal linear pattern; reactive lymphoid aggregates common	Intrasinusoidal/intravascular, with expansion of the sinuses
Immunophenotype	CD5dim+, CD56−/ or variably+, CD57+, CD16/CD94 dim	CD5 often negative, CD56 +, CD57−, CD16/CD94 frequently bright+
Cytotoxic granules	TIA1+, granzyme B may be lost in about 30–40% cases	TIA1+, granzyme B often negative
Cytogenetic/mutations	Mostly normal/*STAT3* in 30-40%, and rare *STAT5B*	i7q, +8/ *SETD2*, *STAT5B*, rare *STAT3*
Clinical course	Indolent	Aggressive

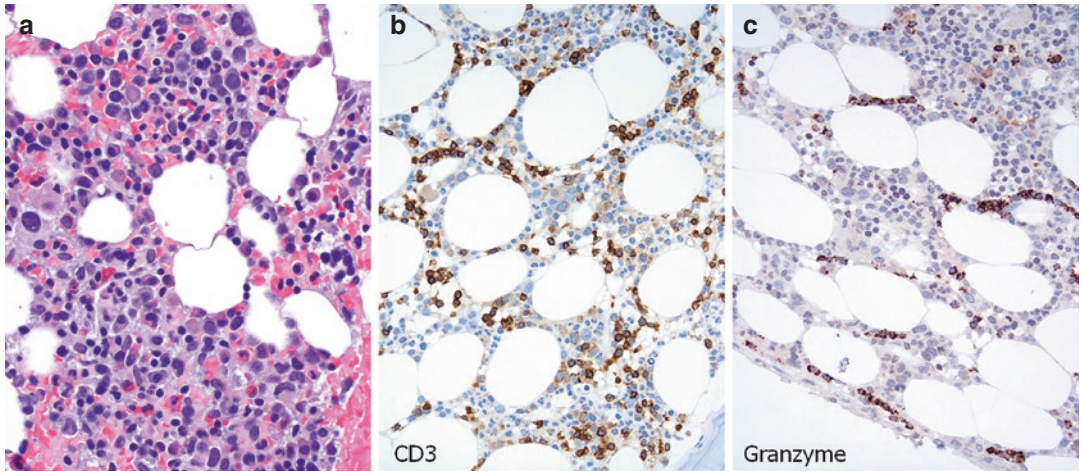

Fig. 11.9 Bone marrow biopsy confirms a diagnosis of T-cell LGL leukemia. (**a**) Bone marrow biopsy is unremarkable on routine H&E histology; a LGL infiltrate is difficult to see. (**b**) CD3 immunohistochemistry highlights a slight increase in T cells. (**c**) Granzyme B highlights the infiltrative T-cell LGL cells in a linear intrasinusoidal pattern

with clinical severe cytopenia [69]. However, the lack of an intrasinusoidal infiltrate cannot be used to rule out T-cell LGL leukemia. The sinusoidal infiltrate is linear and does not expand the sinuses. This feature is distinctively different from hepatosplenic T-cell lymphoma, which not only infiltrates the sinuses but also expands the sinuses (Table 11.4). In BM with a T-cell LGL leukemia infiltrate, there are often lymphoid aggregates. These lymphoid aggregates are not a part of LGL leukemic cells; but reactive lymphoid aggregates, which are likely due to frequent association with underlying autoimmune diseases. These lymphoid aggregates do not contain increased cytotoxic T cells.

Cytogenetics and Molecular Genetics

A minority of cases demonstrate numeric and/or structural chromosomal abnormalities involving chromosomes 7, 8, and 14 (i.e., inversion, trisomy, translocation); however, none of the changes is specific for T-cell LGL leukemia [70].

Identification of clonal TCR gene rearrangement by PCR helps for a diagnosis of T-cell LGL leukemia. However, due to the promiscuity of a positive TCR gene rearrangement rearrangement result in reactive, oligoclonal proliferation and a clonal T-cell population of unknown significance, the information should be interpreted in the context of other findings. Furthermore, clonal drift in TCR repertoire with a change in the dominant clone may be seen in approximately one-third of cases over the disease course, illustrating the heterogeneity of the disorder [71].

Point mutations in *STAT3* are found in around 30–40%, up to 70% [43–45] patients depending on the methods used, lower by capillary sequencing and higher by amplicon sequencing. Interestingly, around 20% *STAT3*-mutated LGL leukemia contain multiple lymphocyte subclones harboring different *STAT3* mutations [72], illustrating the complexity of the clonal landscape of LGL leukemia. *STAT3* mutations in LGL leukemia are gain-of-function mutations and are able to cause leukemic transformation in multiple settings. *STAT5B* [73] is found in a small subset of patients with LGL leukemia; the clinical course of patients with *STAT5B N642H* mutation is aggressive and fatal, clearly different from typical LGL leukemia with a relatively favorable outcome.

Differential Diagnosis

The major differential diagnoses are reactive (polyclonal) T-cell LGL lymphocytosis and clonal or oligoclonal T-cell LGL expansions in patients who may only have mild degrees of

cytopenia(s). The latter cases may represent a more benign end of the spectrum of clonal T-cell LGL expansions and have been given a name of "T-cell clonopathy of unknown or undetermined significance" (TCUS). The differential diagnosis between reactive T-cell LGL lymphocytosis vs. T-cell LGL leukemia becomes even more challenging in the post solid organ transplant or post HSCT or patients with aplastic anemia treated with immunosuppressant. In these settings, patients with reactive LGL lymphocytosis often have cytopenia(s) and various clinical symptoms related to underlying medical conditions, and, the expansion of LGL cells may last for a prolonged period of time [23]. In a substantial number of these cases, TCR PCR study [23] may be positive for a clonal rearrangement. In clinical practice, a definitive diagnosis is difficult and many of these patients are given a diagnosis of "LGL lymphocytosis vs. LGL leukemia," due to the uncertainty of the biological behavior of these LGL cells.

Recently, based on a largest series of LGL leukemia published by the French group [50], an algorithm in the diagnosis of LGL leukemia has been proposed [51]. Of note, the requirement of LGL cell count is only 0.5×10^9/L or more, much lower than what recommended by the WHO criteria [46] of $2–20 \times 10^9$/L. A BM examination is only recommended if clonal LGL cells are $<0.5 \times 10^9$/L. This algorithm can be used as a reference; however, as discussed above, LGL can be significantly increased in reactive conditions or TCUS where TCR gene rearrangement can be demonstrated by PCR in a reactive LGL lymphocytosis; and on the other hand, cytopenia(s) can attribute to other underlying medical conditions. To establish a diagnosis of LGL leukemia at the first time, the LGL proliferation has to be persistent, substantially increased (a requirement of 1-2 $\times 10^9$/L is more appropriate), demonstrate immunophenotypical aberrancies in addition to PCR TCR clonality, and associated with clinical symptoms. A neoplastic LGL process almost always shows an infiltrate in BM; whereas, a reactive process should not. Therefore, a BM biopsy with immunohistochemistry study is highly recommended to confirm a diagnosis of LGL leukemia. (Fig. 11.9).

Chronic Lymphoproliferative Disorder of NK Cells (CLPD-NK)

The WHO 2008 category of chronic lymphoproliferative disorder of NK cells (CLPD-NK) likely comprises a broad spectrum of NK-cell LGL expansions, ranging from reactive to monoclonal/neoplastic disorders. These disorders have been previously given a number of names, such as chronic NK-cell lymphocytosis, chronic NK-cell LGL or NK-cell LGL lymphocytosis, etc. This is largely due to the lack of a universal and specific marker for NK-cell clonality to reliably differentiate a reactive (polyclonal/oligoclonal) from (mono)clonal proliferation of NK cells.

CLPD-NK comprises approximately 5% of all patients with LGL leukemia. The pathogenesis is very similar to T-cell LGL leukemia, likely due to dysfunctional activation of survival pathways and the evasion of apoptosis with antigen stimulation. Constitutive activation of the Ras/MEK/ERK pathway has been found in many patients with CLPD-NK [74]. Activating mutations in *STAT3* [43] are detected in approximately 30% of these patients, similar to T-LGL leukemia.

Clinical Features

Patients with CLPD-NK have a very similar indolent clinical presentation as T-cell LGL leukemia. However, patients with CLPD-NK are less symptomatic and less likely associated with rheumatoid arthritis [50, 51, 75]. In contrast, autoimmune cytopenias, such as pure red cell aplasia, aplastic anemia, and mild thrombocytopenia, may be more frequently present in CLPD-NK, but the severity of neutropenia is often less than in that of T-cell LGL leukemia. Associations with vasculitis, including acute glomerulonephritis, urticarial vasculitis, and cutaneous polyarteritis nodosa, have been reported. Most patients have an indolent course, and without treatment, the number of circulating PB NK cells usually remains stable for a long period of time, and some cases have even been reported to show spontaneous regression [76].

Morphology

The cells of CLPD-NK are morphologically indistinguishable from T-cell large granular lymphocytes. They are generally small to median, having moderate cytoplasm with a few granules. Similar to T-cell LGL leukemia, they may show some morphological atypia, such as reduced granules, reduced cytoplasm, and irregularity of nuclear contours, differing from normal NK cells.

Immunophenotype

Normal NK cells are sCD3−, CD2+, CD5−, CD7+, CD8 subset+, CD16+, CD56+, CD57 small subset+, and CD94+. Normal NK cells are generally CD38 bright+ and HLADR-negative. Clonal CLPD-NK shows an aberrant immunophenotype in nearly all patients [77, 78]. In the majority of LGL-NK cell type or CLPD-NK (Fig. 11.10), the typical immunophenotype is sCD3−, CD2+, CD5−, CD7+, and CD8 either negative or uniformly+. Decreased or absent of CD56 expression is seen in about 50–60% cases and increased CD57 expression in about 60% cases. Other aberrancies frequently observed include decreased CD7; increased CD2, distinctly bright CD16 and CD94; or decreased or diminished CD16. Other changes include increased HLADR, granzyme B, and perforin and decreased CD38 and CD11b. Some NK-LGL can show an aberrant loss of granzyme B.

Killing inhibitory receptors (KIRs) of NK cells bind to major histocompatibility complex (MHC) class I molecules expressed on potential target cells to regulate effector cell activity. KIR antigens, CD158a, CD158b, and CD158e, can be assessed by flow cytometry to provide information of NK-cell clonality. Restricted expression of a single KIR isoform indicating a clonal NK cell expansion is reported in about 30–40% of clonal NK-CLPD cases. In the rest of the two-thirds of cases, they may show completely absent

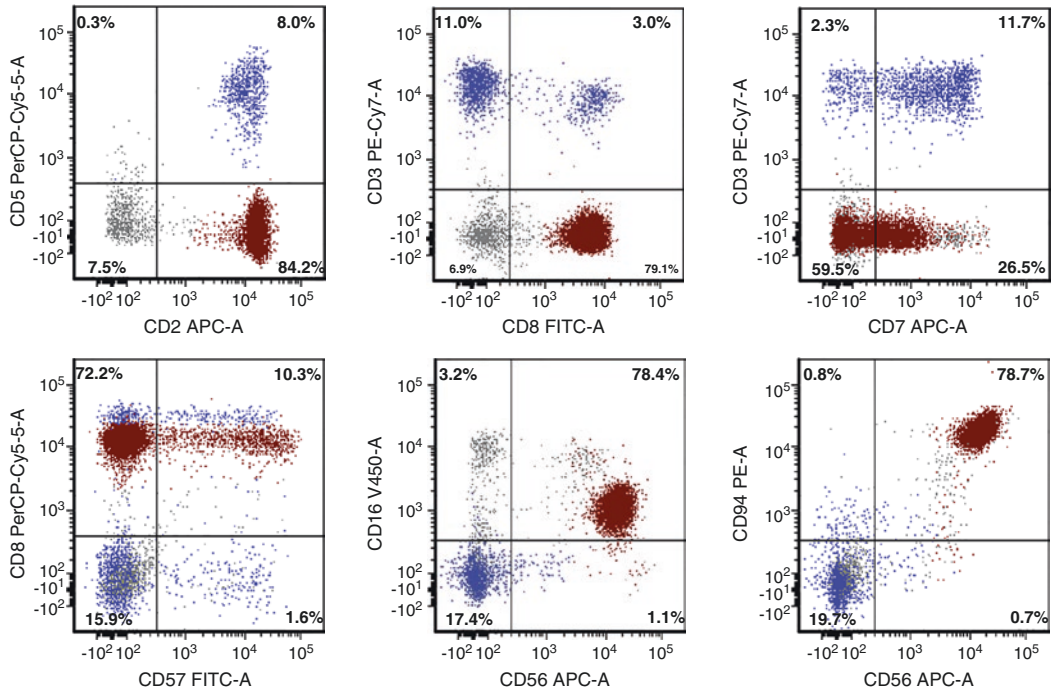

Fig. 11.10 Chronic lymphoproliferative disorder of NK cells (CLPD-NK) (T cells in *blue* and NK cells in *brown*). The NK cells are CD3−CD5−CD2+CD56+ but show aberrant uniform expressions of CD8, decreased CD7 and CD16, and increased CD94. A few normal NK cells are in the background (grey) for comparison.

of CD158a, CD158b, or CD158e expressions, indicating the presence of a clonal NK-cell proliferation with a KIR expression not detected by the antibodies used. It is reported [77] that in NK cell proliferations with the absence of KIR antigen expressions, a threshold of 7% of CD158a+CD158b+CD158e cells separates a clonal NK cell proliferation (≤7%) from non-clonal NK cell lymphocytosis (>7%). However, this value has to be validated in individual laboratories; and the assumption of clonality in the absence of KIR antigen expressions should be used in conjunction with other immunophenotypical aberrancies.

Essentially, with the assessment of KIR antigens by CD158a/b/e, all clonal CPLD-NK should demonstrate immunophenotypic aberrancies.

Cytogenetics and Molecular Genetics

In the majority of the CLPD-NK cases, karyotype is normal [79, 80]. Del(16q) has been reported in some patients [81].

A clonal NK cell proliferation does not show TCR gene rearrangements. The methods for NK-cell clonality assessment include the assessment of the pattern of inactivation of the X-chromosome (e.g., the human androgen receptor assay (HUMARA) [82], restricted (or aberrant) expression of a single isoform of KIR receptors [78, 80, 83], and, more recently, the presence of *STAT3* mutations (approximately 30–40% cases) [43]. However, these approaches have only proved to be helpful in a fraction of patients with chronic NK-cell expansions and some cases show inconclusive results.

Differential Diagnosis

The major differential diagnosis is reactive polyclonal NK cell lymphocytosis. This should follow the similar approach for differentiating T-cell LGL leukemia from reactive T-cell LGL lymphocytosis. Clonal CLPD-NK should have accompanied clinical symptom, an aberrant immunophenotype with an abnormal KIR expression pattern, as well as a BM infiltrate. In addition, patients with clonal CLPD-NK have a higher lymphocyte count with more than 3×10^9/L lymphocytes in almost all cases, as well as a higher absolute count of NK cells. NK cell lymphocytosis is persistent in clonal CLPD and may increase over time, contrasting to reactive NK-cell lymphocytosis that the lymphocyte count often decreases and returns to normal after a median follow-up of 24–36 months. Although some reactive/polyclonal NK cell cytosis may be persistent over a prolonged period, there are often not associated with clinical symptoms.

The other differential diagnosis is aggressive NK-cell leukemia, which is a highly aggressive hematological malignancy with a poor prognosis. The comparisons of their clinical, laboratory, morphological, and immunophenotypical features with CLPD-NK are shown in Table 11.5, following the discussion of aggressive NK-cell leukemia.

An algorithm in the workup of T-cell LGL leukemia and CLPD-NK is illustrated in Fig. 11.11.

Aggressive NK-Cell Leukemia

Aggressive NK-cell leukemia (ANKL) is a very rare and extremely aggressive neoplasm. It has been given a number of different names in the past, including "lymphoma of large granular lymphocytes," "leukemia of non-T lineage natural killer cells," "CD56 + NK lymphoma," "CD3-negative large granular lymphocyte leukemia," "aggressive nodal cytotoxic lymphoma of natural killer cell type," etc.

ANKL [84–86] has a higher prevalence among Asians. It is also encountered among indigenous populations in the Americas. Men and women are equally affected, and the disease usually manifests in the third or fourth decades of life. It has a strong association with EBV infection, which is considered as a part of disease definition. However, EBV-negative ANKL has been described [87]. Patients with EBV-negative disease tend to be older (median age 63 years), with a high incidence in non-Asian patients in western countries, but have a similar aggressive fulminant clinical course.

Table 11.5 Comparison of chronic lymphoproliferative disorder of NK cells (CLPD-NK) with aggressive NK-cell leukemia

	CLPD-NK	Aggressive NK-cell leukemia
Age	60 years	30–40 years
Male: female	3:1	1:1 to 2:1
Complete blood count (CBC)	Isolated cytopenia, anemia, thrombocytopenia, or neutropenia	Pancytopenia is common; some patients with a high WBC due to circulating tumor cells
Clinical presentations	Underlying autoimmune disease or autoantibodies common	Fever, constitutional symptoms, acutely ill. Hemophagocytosis is common
Organomegaly	Mild splenomegaly in 20–50%; hepatomegaly rare	Hepatosplenomegaly or isolated hepatomegaly or splenomegaly, seen in almost all patients
Lymphadenopathy	Uncommon	Common
Other presentations	Cytopenia related symptoms	CNS involvement, liver, gastrointestinal, and lung
LDH, liver transaminase	Normal	Elevated in the majority of cases
NK cell Cytology	Small, with round to slightly elongated nuclei, moderate cytoplasm with azurophilic granules	Often large, pleomorphic; some can be monotonous. Chromatin is often slightly immature. Some may have nucleoli. Cytoplasm has variable azurophilic granules, but some may not have visible granules
Bone marrow infiltrate	Interstitial and intrasinusoidal linear pattern	Interstitial, diffuse, rarely intrasinusoidal Hemophagocytosis is seen in almost one-third of patients
Immunophenotype	sCD3−, CD4−, CD5−, TCR− Often CD57+, CD56−/dim+ Often CD16+ Often CD7+	sCD3−, CD4−, CD5−, TCR− Often CD56 bright+ and CD57 negative CD16 dim+ or negative CD7dimmer+ or negative
EBER	Negative	Positive in >90% patients
Cytogenetic	Mostly normal	Abnormal in at least two-thirds, with frequent a complex karyotype
Clinical course	Indolent	Aggressive, fatal

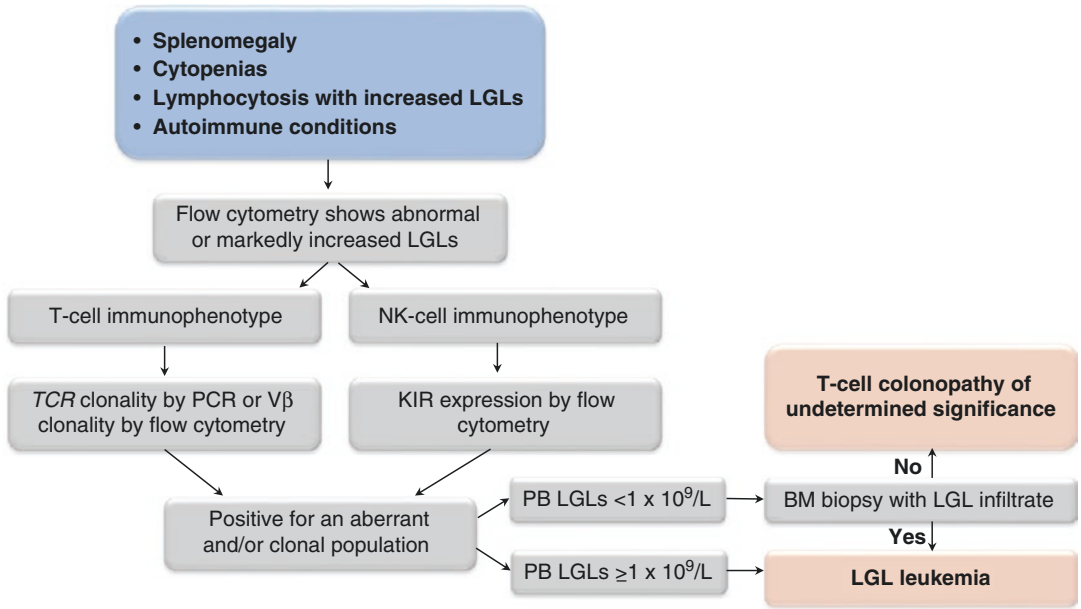

Fig. 11.11 A suggested algorithm in the workup of LGL proliferation. Modified according to Lamy T et al. Blood. 2011;117(10):2764–74 and Lamy T et al. Blood, 2017, 129(9):1082-1094

Clinical Features

Patients usually are extremely ill, with fever and other systemic symptoms, hepatosplenomegaly, pancytopenia, and abnormal liver function. Some patients have a high white cell count due to the presence of circulating tumor cells. There is frequent CNS involvement. Other systemic involvement includes lung and gastrointestinal tract. Skin involvement can be seen, but not as common as nasal NK-/T-cell lymphoma. Serum levels of LDH and Fas ligand (FasL) are often markedly increased. Increased levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) can be seen in about two-thirds of patients. Hemophagocytic syndrome is frequently present at the time of diagnosis or during the course of the disease, as a result of uncontrolled monocyte/macrophage activation in response to cytokines produced by the neoplastic NK cells. The disease course is fulminant; with multiorgan failure and disseminated intravascular coagulation, death usually occurs within a few weeks.

Morphology

Aggressive NK-cell leukemia cells could be quite variable in size and appearance. In some patients the leukemic cells are pleomorphic, larger with more open chromatin and distinct nucleoli. Some patients have blast-like morphology with monotonous, medium-sized lymphocytes that have fine chromatin and coarse azurophilic granules. However, in other patients, the cells have been described similar to LGL cells that the cytoplasm often contains variable numbers of azurophilic granules; and, in some cases, tumor cells may not have visible cytoplasmic granules (Fig. 11.12).

The BM involvement can be variable, from a massive infiltrate to a subtle involvement. The infiltrative pattern is often interstitial, followed by a diffuse, focal, and rarely intrasinusoidal pattern. Angiocentricity may be seen in some cases. Hemophagocytosis can be identified in a significant subset of patients, with increased mature histiocytes containing red blood cells or nucleated cells.

Immunophenotype

The tumor cells are negative for surface CD3 and CD5 and positive for CD2, CD56, and CD94. CD57 is generally negative (in over 90% cases). CD56 is generally bright and CD16 dimmer+ or lost. CD8 is often negative but can be uniformly positive in rare cases. A decrease or loss of CD7 expression is common. HLADR is often brightly positive. Other alterations include loss or decreased expression of CD11b and CD38. Granzyme B and TIA1 are generally positive [88], whereas perforin expression is decreased in approximately half of the patients. CD158a/h, CD158b, and CD158e are entirely lost in 70–75% of the patients, and a positive expression is only seen in about 20–25% cases. sCD3, CD4, CD5, TCRαβ, and TCRγδ are consistently absent.

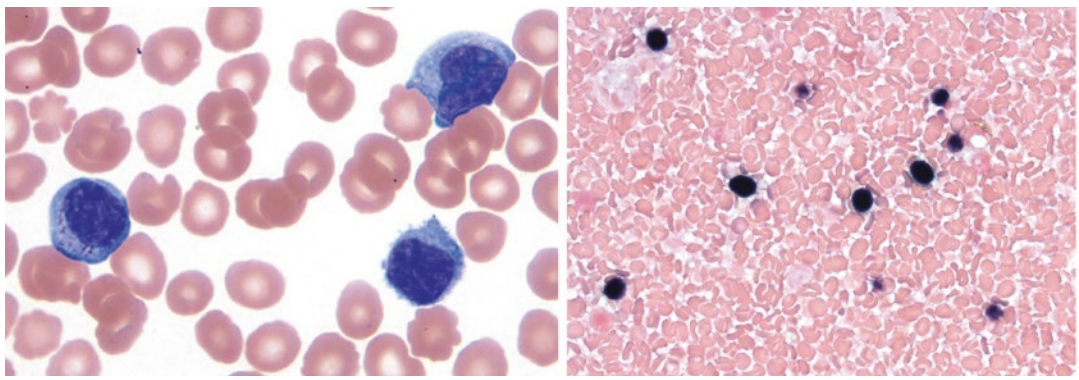

Fig. 11.12 Aggressive NK cell leukemia in the peripheral blood. (*Left*) The tumor cells have moderately abundant cytoplasm with azurophilic granules. However, these cells are much bigger than normal, reactive, or clonal large granular lymphocytes. (*Right*) They are EBER positive (performed on blood clot by in situ hybridization)

EBER in situ hybridization should be performed in all cases, and >90% cases are EBER+.

Cytogenetics and Molecular Genetics

Clonal cytogenetic abnormalities are detected in approximately two-thirds of cases, including a frequent complex karyotype but nothing specific. The most common abnormalities are in chromosome 13 and 11 (38.5%), and others include +8, –19, –21, +1, and +2. Del (6q) has been reported in nasal or nasal-type NK lymphoma but uncommon in aggressive NK-cell leukemia. *STAT3* and *STAT5B* mutations have been reported. TCR has a germ-line configuration.

Differential Diagnosis

One of the differential diagnoses is CLPD-NK, especially for EBER-negative aggressive NK-cell leukemia. The detailed clinicopathological features are illustrated in Table 11.5. The other most challenging diagnosis is nasal or nasal-type extranodal NK-/T-cell lymphoma (ENKTL) that is often also EBER+. Hemophagocytosis can occur in ENKTL as well. The immunophenotype (NK-cell type) of ENKTL is very similar to aggressive NK-cell leukemia. However, unlike aggressive NK-cell leukemia, patients with ENKTL often do not have a systemic leukemic presentation, and the CBC is often normal or near normal. Peripheral blood involvement is uncommon. BM involvement is essentially 100% in aggressive NK-cell leukemia but less than 50% in ENKTL cases. In contrast, patients with ENKTL almost always present with mass lesions, most commonly nasal lesions, followed by testicular and skin involvement. Additionally, liver function tests and coagulation studies that are often abnormal in aggressive NK-cell leukemia but are often unremarkable in patients with ENKTL.

Hepatosplenic T-Cell Lymphoma

Hepatosplenic T-cell lymphoma (HSTL) is an aggressive T-cell lymphoma, characterized by a primary extranodal involvement of medium-sized lymphoid cells, typically with a sinusoidal infiltration of the liver, spleen, and bone marrow. Patients often present with hepatosplenomegaly, peripheral blood cytopenia(s), and other related systemic symptoms [89]. Most cases are of γ/δ T-cell origin, and a small subset is of α/β T-phenotype.

Chronic antigen stimulation in the setting of immune deficiency or immune dysregulation might be important in the pathogenesis of HSTL [89, 90]. About 20% patients are immunosuppressed, such as solid organ transplant recipients [91] or patients with leukemia receiving chemotherapy. Some patients have a history of autoimmune disease, such as lupus and rheumatoid arthritis, or inflammatory bowel disease treated with tumor necrosis factor α inhibitors and immunomodulators [92], or viral infections.

The lymphoma is resistant to current chemotherapeutic regimens and has a rapidly progressive disease course. The diagnosis is usually established based on the combination of clinical findings, histologic features, and immunophenotyping result.

Clinical Features

Hepatosplenic T-cell lymphoma, TCRγ/δ subtype, occurs predominantly in adolescents and young adults, with a median age of 35 years (range, 15–65 years). Male to female ratio is about 9:1 [93]. Patients often present with fever, fatigue, weight loss, and abdominal discomfort due to hepatosplenomegaly and, sometimes, with jaundice because of liver involvement [91]. Lymphadenopathy is usually absent. Patients with HSTL of TCR α/β subtype show a similar clinical presentation, including frequent B symptoms, splenomegaly, hepatomegaly, lymphadenopathy, anemia, thrombocytopenia, leukopenia, leukocytosis, and bone marrow involvement; however, there is a female predominance and a bimodal age distribution, occurring in pediatric patients and patients older than 50 years of age [94]. HSTL of TCR α/β subtype may be more aggressive.

Peripheral blood often shows pancytopenia or bicytopenia(s) (anemia and thrombocytopenia). Cytopenia(s) may be a combination of hypersplenism, BM infiltrate, or abnormal cytokine release by tumor T cells with or without an underlying hemophagocytosis. Patients usually do not have peripheral lymphocytosis at initial presentation; however, a small population of circulating neoplastic lymphocytes may be seen at the presenta-

tion in approximately 50% of patients. Circulating tumor cells may increase as disease progresses. Other abnormal data include elevated serum liver transaminase and alkaline phosphatase. Patients often show laboratory data of coagulopathy, and some may have bleeding diathesis [91].

Morphology

Due to the presence of cytopenia(s) with no absolute lymphocytosis and abnormal liver function tests but no discrete liver mass lesion, a BM biopsy is often the first specimen to obtain rather than a PB sample, liver biopsy or splenectomy.

BM is almost always involved. The infiltrate is intrasinusoidal, and the infiltrate can be very subtle at the time of diagnosis. As the disease progresses, the neoplastic infiltrate can go beyond the sinuses, showing an interstitial or diffuse pattern. The cells in the intrasinusoidal spaces are often monomorphic and medium-sized, with slightly irregular nuclei, condensed chromatin, indistinct nucleoli, and moderate amount of clear cytoplasm (Fig. 11.13). In many cases, the infiltrate may be missed if immunohistochemistry (IHC) or flow cytometry study is not performed. For IHC markers, HSTL are positive for CD3, TIA1, granzyme M, and CD56; they are often negative for granzyme B and perforin. CD3 is often sufficient to highlight the intrasinusoidal infiltrative tumor cells. One of the important features of HSTL is that the lymphoma cells not only involve sinuses but also expand the sinuses (Fig. 11.14). This expansion of sinuses differs from LGL leukemia

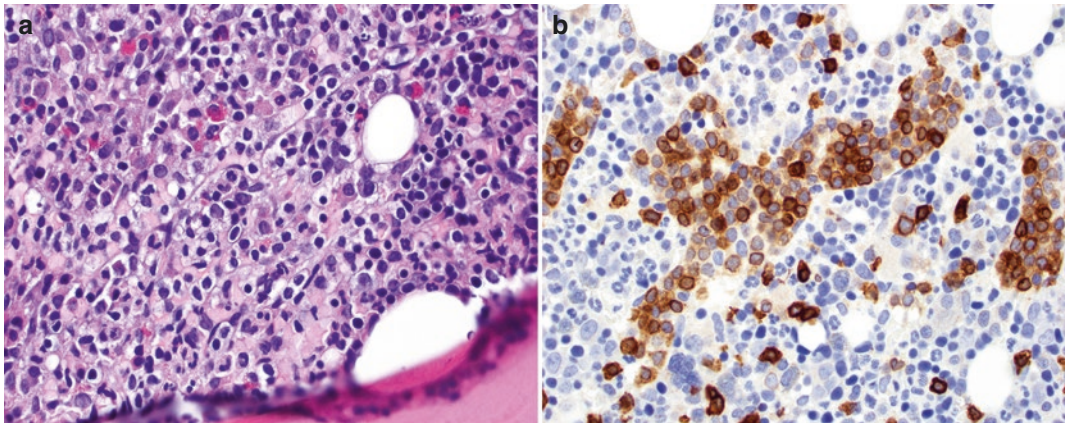

Fig. 11.14 Hepatosplenic T-cell lymphoma (HSTL). (**a**) Bone marrow biopsy shows a lymphoid infiltrate. (**b**) CD3 highlights the infiltrate with a sinusoidal expansile pattern, only very few tumor cells are scattered in the interstitial area

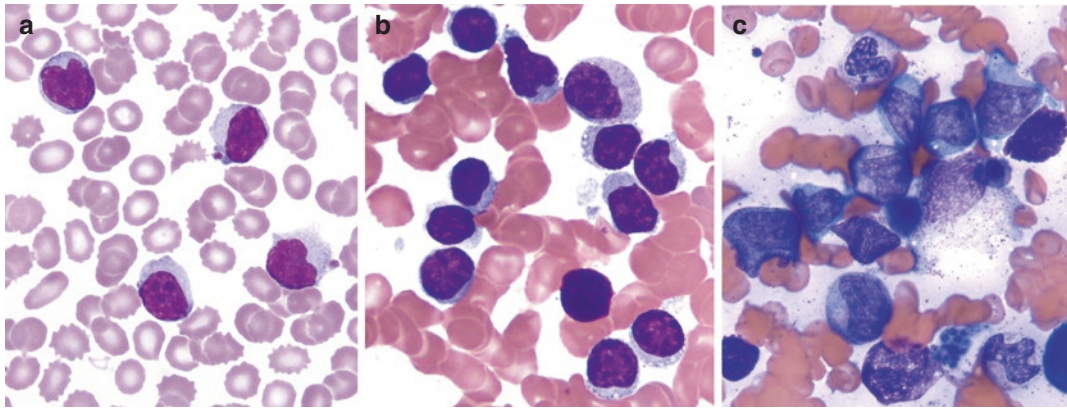

Fig. 11.13 Hepatosplenic T-cell lymphoma (HSTL). Peripheral blood (**a**) shows typical medium-sized cells with moderate cytoplasm with cytoplasmic granules. HSTL can be small to medium with an "innocent" appearance (**b**) or large with irregular nuclei (**c**). The cytoplasm either contains variable numbers of granules or agranular

with a sinusoidal involvement, which often shows a linear single layer of cells.

Bone marrow often shows a hypercellularity. Dyspoietic features involving 1–3 hematopoietic cell lineages can be frequently observed in patients with HSTL [95]. This is likely a result of cytokine effects or due to the perturbation of the BM microenvironment by lymphoma cells. Increased histiocytes may be seen in some cases, some with phagocytosis, particularly in patients with clinical and laboratory evidence of hemophagocytic syndrome. The lymphoma cells on BM aspirate smears can range from small to medium with "innocent" look to large lymphoma-like cells or blastic cells (Fig. 11.13). Lymphoma cells may contain variable numbers of azurophilic granules and, when abundant, may be mistaken as LGL cells.

When a liver biopsy is performed, HSTL often shows a sinusoidal pattern with dilatation of sinuses and sometimes with perisinusoidal fibrosis. The portal triad is often not involved. The infiltrative tumor cells are often very bland and may be difficult to appreciate on H&E slides. IHC is often necessary. If splenectomy is performed (which is not often done), spleen shows an expansion of the red pulp. The neoplastic cells infiltrate both splenic cords and sinuses. Similar to liver and BM, the cells in the sinusoidal distribution are predominantly medium in size and monotonous, with inconspicuous nucleoli.

Although patients often do not have PB lymphocytosis, the neoplastic cells are often present in peripheral blood in variable numbers (Fig. 11.13). The neoplastic cells on the PB smear are often intermediate to large cells with nuclear irregularity, condensed but dispersed chromatin, indistinct nucleoli, and basophilic cytoplasm, more abnormal than the small lymphocytes seen on BM and liver biopsy tissue.

Immunophenotype

In a series of 28 patients from a single institution [96] assessed by flow cytometry study, 74%

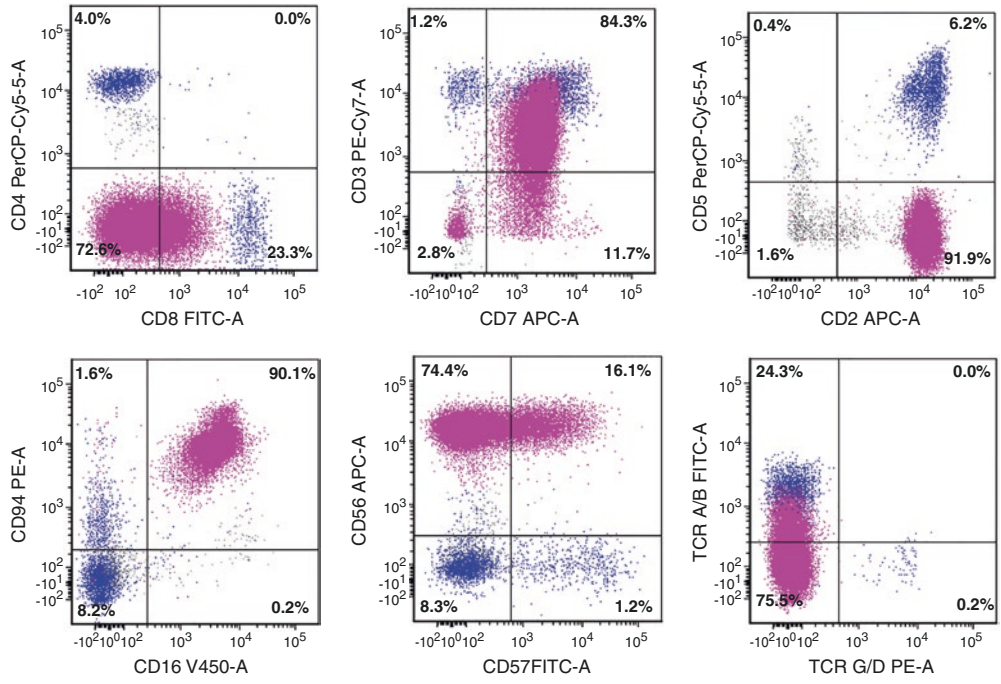

Fig. 11.15 A case of hepatosplenic T-cell lymphoma (HSTL), TCRαβ type. The HSTL cells are CD8partial/dim+, with decreased expressions of CD3 and CD7 and negative for CD5 and CD4 and with strong expressions of CD94, CD16, CD56, and only small subset CD57+. TCR αβ is partially positive and negative for TCRγδ

HSTL are γ/δ subtype, 19% α/β subtype (Fig. 11.15), and 7% are absent of TCR γ/δ and α/β. In γ/δ subtype, sCD3 is often bright+, double negative for CD4 and CD8. They are often positive for CD2, CD7, CD56, CD94, and CD16 but negative for CD5 and CD57. Occasionally, some cases may be CD8+. The TCR α/β subtype is generally CD2+, CD3+, CD4−, CD5−/+, CD7+/−, CD8+/−, TCR γ/δ−, TCR α/β +, and CD56+. HSTL is generally TIA1+, but perforin and granzyme B are generally negative or partially lost. CD57 expression is generally absent in TCR γ/δ+ HSTL but has been reported in TCR α/β HSTL. The latter is often in pediatric patients and often also expresses granzyme B and correlates with large cell morphology [94].

Flow cytometry is superior to immunohistochemistry for the detection of the surface TCR subtypes, because γ/δ TCR antibodies are often not reliably interpreted on decalcified, formalin-fixed, paraffin-embedded tissue. Of note, other markers by IHC are generally weaker than by flow cytometry method. EBER is in general negative but has been reported to be positive in a few cases. EBER+ cases tend to have pleomorphologic morphology or TCR α/β subtype [97].

Cytogenetics and Molecular Genetics

Isochromosome 7q is a recurrent cytogenetic abnormality, and a significantly number of patients with i7q also have trisomy 8 abnormality. FISH is more sensitive than conventional karyotyping in the detection of cytogenetic abnormalities. Sporadic case reports indicate the presence of i7q in almost all cases of HSTL [98, 99], but the case series showed [91, 94, 96] i7q in about 50–70% patients at the time of diagnosis. i(7q) may represent a primary chromosomal aberration, and trisomy 8 is a likely secondary event. Trisomy 8 has been reported to associate an inferior survival in patients with HSTL. Recently, whole genome sequencing has found a high frequency of *SETD2* (a tumor suppressor gene) mutations (71%) in patients with HSTL. *STAT5B* is around 30%, and *STAT3* is less common (9%). Other mutations reported include *INO80* (21%), *TET3* (15%), and *SMARCA2* (10%) [100].

Differential Diagnosis

The first time diagnosis of HSTL can be challenging, due to uncharacteristic clinical presentation and a subtle involvement of BM and PB. Some patients come in with a clinical suspicion of myelodysplastic syndrome, acute leukemia, infections or thrombotic thrombocytopenic purpura, or hemophagocytosis (HLH) with unidentified underlying causes. If HSTL is not considered in the differential diagnoses, it can be easily missed. As a result, more than 50% patients eventually undergo splenectomy and or liver biopsy to obtain materials for a diagnosis.

Due to the clinical presentation of cytopenia (s) and BM hypercellularity with dyspoietic changes in hematopoietic cells, as well as frequent presence of trisomy 8 abnormality, HSTL may be misdiagnosed as MDS. However, unlike HSTL, MDS patients often do not have marked splenomegaly, abnormal liver functions, or B symptoms. If patients present with HLH, a diagnosis should not stop at HLH but look for the possible cause of HLH, including T-cell lymphoma. PB blood often has a low level of lymphoma involvement, and a careful review of PB smear may identify abnormal tumor cells. BM is almost always involved; but the involvement can be very subtle and not visible on H&E sildes. Therefore, it is important that for any patients undergoing a BM or PB examination for a cause of cytopenia(s), or a suspicion for malignancy, flow cytometry study should be routinely performed and should include basic markers to assess B-cell clonality, T-cell immunophenotype (CD3, CD4, CD8, CD56), and immature marker CD34. In HSTL BM and often PB, a population of abnormal T cells is shown as brighter CD3+, CD4−, CD8−, and CD56 bright+, not corresponding to any of the normal subsets of T cells. The finding should prompt further workup for T-cell malignancies. CD3 immunohistochemistry should be performed on BM biopsy that will highlight the infiltrate.

Once an aberrant T-cell population is identified, the top differential diagnosis is T-cell large granular lymphocytic (LGL) leukemia, which is also a blood- and BM-based disease with frequent splenomegaly. This is especially problematic with TCRγ/δ variant LGL. The clinico-

pathological features between HSTL and LGL are compared in Table 11.4. LGL leukemia is an indolent T-cell neoplasm, whereas HSTL is clinically aggressive, requiring completely different treatment. A correct unambiguous diagnosis is critical for clinical management.

The other differential diagnosis is nonhepatosplenic γ/δ T-cell lymphomas. Nonhepatosplenic γ/δ T-cell lymphomas often occur in other sites, particularly mucosal (gastrointestinal tract) or cutaneous tissues. Dissemination to other extranodal sites is often seen, but splenomegaly, hepatomegaly, and BM intrasinusoidal involvement are infrequent. Thus, the sites of involvement and pattern of tissue infiltration are important features for the deferential diagnosis of HSTL, which are different from other T-/NK-cell lymphoma/leukemia. This is true for TCRα/β variant HSTL and peripheral T-cell lymphoma that clinical presentations and BM findings should provide useful clue for the differential diagnosis.

The differential diagnosis may also include aggressive NK cell leukemia (ANKL) due to a similar clinical presentation. Both occur in young adults and have a fulminant clinical course with hepatosplenomegaly, BM involvement, pancytopenia, coagulopathy, hemophagocytic syndrome, and multiorgan failure. ANKL may show an immunophenotypic profile overlapping with HSTL, including CD2+, CD5−, CD56+, CD4−/CD8−, and positivity for cytotoxic granules. ANKL by flow cytometry is negative for sCD3 and TCR expression, but by immunohistochemistry, CD3 may be positive due to the presence of CD3 ε chain of NK cells. It is important to know that ANKL infiltrate is typically interstitial, diffuse, or nodular, contrasting to HSTL of a predominant sinusoidal and intravascular pattern. Furthermore, ANKL is typically EBER+, whereas HSTL is generally EBER-negative or rarely in scattered bystander B cells.

Sézary Syndrome and Mycosis Fungoides

Sézary syndrome (SS) [101] is an aggressive type of cutaneous T-cell lymphoma characterized by an intensely pruritic erythroderma, with early systemic dissemination of clonal CD4+ T cells in blood, bone marrow, and lymph nodes. The tumor cells are considered to derive from skin-homing CD4+ T cells or central memory T cells with the capacity to traffic in and out of the skin in response to the appropriate chemokines. According to the consensus by the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC) [102, 103], SS requires to have erythroderma involving at least 80% of surface skin, a high number of circulating tumor cells ($\geq 1 \times 10^9$/L Sézary cells), and lymphadenopathy. The incidence of SS is about 0.8–0.9/million, predominantly occurring in older people (median age 60 years), with a slight male predominance (1.5–2:1), and more prevalent in African Americans. SS is predominantly de novo, and only a small number of cases are evolved from myelosis fungoides (MF). In either case, both MF and SS are staged by the same criteria [102]. In contrast to patch/plaque stage of MF, SS is much more symptomatic, has a lower potential for remission, and lower expected survival.

Clinical Features

Pruritic erythroderma and generalized lymphadenopathy often have developed over weeks to months. This can be preceded by a prodromal phase manifesting as pruritus or nonspecific dermatitis. Peripheral blood shows lymphocytosis with abnormal lymphocytes. Associated hypereosinophilia can be seen in some cases. For patients with a well-established history of MF, a diagnosis of SS must fulfill the criteria for SS, which means to be tumor stage IV (erythroderma ≥80% of surface area) and blood stage 2 (B2, $\geq 1 \times 10^9$/L Sézary cells). These cases should be designated as "SS preceded by MF."

The skin lesions may be associated with alopecia, ectropion, leonine facies, nail dystrophy, and plantar hyperkeratoses with extremely painful fissuring. Patients frequently have secondary bacterial infection or severe viral infection such as herpes and polyomavirus. This is a disseminated disease; however, visceral involvement is not very common. It may involve spleen, liver,

lung, central nervous system, and occasionally, other organs.

Patients also have been reported to have increased prevalence of secondary malignancies [104], especially lymphoma including Hodgkin and non-Hodgkin lymphoma, melanoma, and urinary tract cancer. This may be attributable to decreased normal CD4(+) T cells and impaired cellular and humoral immunity.

Morphology

Peripheral blood shows abnormal lymphocytes (Sézary cells) (Fig. 11.16). The cells generally have cerebriform nuclei, which can be variable in size from case to case. Small Sézary cells are described as <12 μm in diameter, and large Sézary cells are >14 μm in diameter. Small Sézary cells are not completely specific and can be reactive lymphocytes; in contrary, large Sézary cells are generally neoplastic tumor cells.

Of note, according to the consensus by the ISCL and EORTC, the number of tumor cells is based on morphological counting.

For skin biopsy, in ~2/3 patients with SS, skin biopsy shows diagnostic findings. The changes are similar to MF; however, epidermotropism can be variable that may be absent in some biopsy specimens. Tumor cells may be only seen in dermis and perivascular infiltrate. Tumor cell size can be variable but tends to be more monotonous than MF. In ~1/3 patients, skin biopsy may only show nonspecific changes without abnormal lymphocytes and may not be distinguishable from nonneoplastic erythroderma. In cases with inconclusive skin biopsy, the diagnosis of SS can be made based on a positive lymph node biopsy and or the presence of a large number of circulating lymphoma cells. For the latter, the blood tumor cells match the tumor infiltrate in the skin.

Lymph node shows partial or total effacement of normal architecture by Sézary cells. The tumor cells often show capsular invasion or extranodal invasion. Changes of dermatopathic lymphadenopathy are often present.

Bone marrow is often not involved or only minimal involvement. When the BM is involved, Sézary cell infiltrate is often sparse, predominantly a patchy or interstitial pattern.

Immunophenotype

The vast majority of SS cells are CD4+ T cells; CD8+ SS is exceedingly rare (<5%). These cells are TCR α/β(+) and TCRγ/δ (−). By flow cytometry immunophenotyping, the first notable change is a markedly increased CD4:CD8 ratio (≥10), which can be observed in about 90% patients. SS and MF cells often express CD3, CD2, and CD5; however, they may show altered levels of expressions of these antigens. The most common aberrancies are the loss of CD26 and CD7, occurring in approximately 80–90% cases [105] and 60–70% cases, respectively [106, 107]. It is important to remember that lack of CD7 expression can be seen in up to 40% CD4+ T cells and lack of CD26 in 30–40% of normal CD4+ T cells. These CD7- and

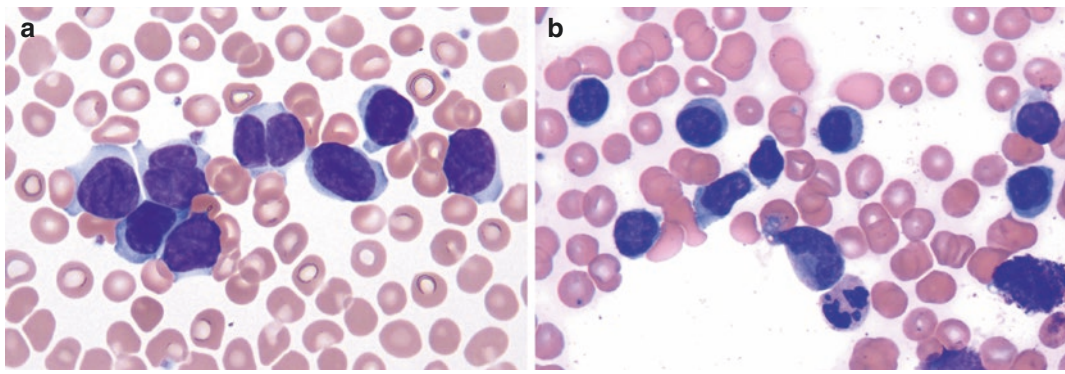

Fig. 11.16 Sézary syndrome. Typical large Sézary cells (**a**). In some cases, the cells may be small and lack of characteristic cerebriform cells (**b**)

CD26- cell numbers may be even higher in some reactive conditions. Therefore, it is important to assess the expression patterns instead of simply applying the cutoff numbers. In normal/reactive conditions, CD7-negative or CD26 negative CD4+ T cells are within a spectrum (Fig. 11.17) with the rest of CD4+ T cells, contrasting to MF/SS that often show a complete absence of expressions of these markers. Combining alterations in other T-cell markers, such as dimmer expressions of CD3, CD4, and negative CD7 with CD26 assessment will significantly increase the specificity of detection. Furthermore, SS/MF, though rare, may have an uniform expression of CD26, which is also considered as abnormal. It is noteworthy that the cutoff (30–40%) for CD26 negative cells is established on peripheral blood, not applicable to skin or lymph node samples. In tissues, normal/reactive CD4+ T cells can have significantly increased CD26-negative T cells.

In addition, lymphoma cells of MF or SS may express follicular center helper T-cell (TFH) markers CD10, Bcl-6, and PD-1 and occasionally CXCL-13 [108], which may cause confusion in distinguishing MF/SS from other TFH T-cell lymphomas with cutaneous manifestations. In fact, a TFH phenotype, defined as expressions of at least three of five markers (PD-1, CXCL-13, ICOS, Bcl-6, and CD10) in more than 10% of tumor cells, is very common in MF/SS [109]. PD1 expression is observed in most of SS, more frequently in SS than MF in skin biopsy. Nonetheless, the expression of TFH markers should not cause confusion in the differential diagnosis between MF/SS and T-cell lymphoma with a TFH immunophenotype.

Cytogenetics and Molecular Genetics

No specific chromosomal abnormalities have been identified in SS. Complex karyotypes are common,

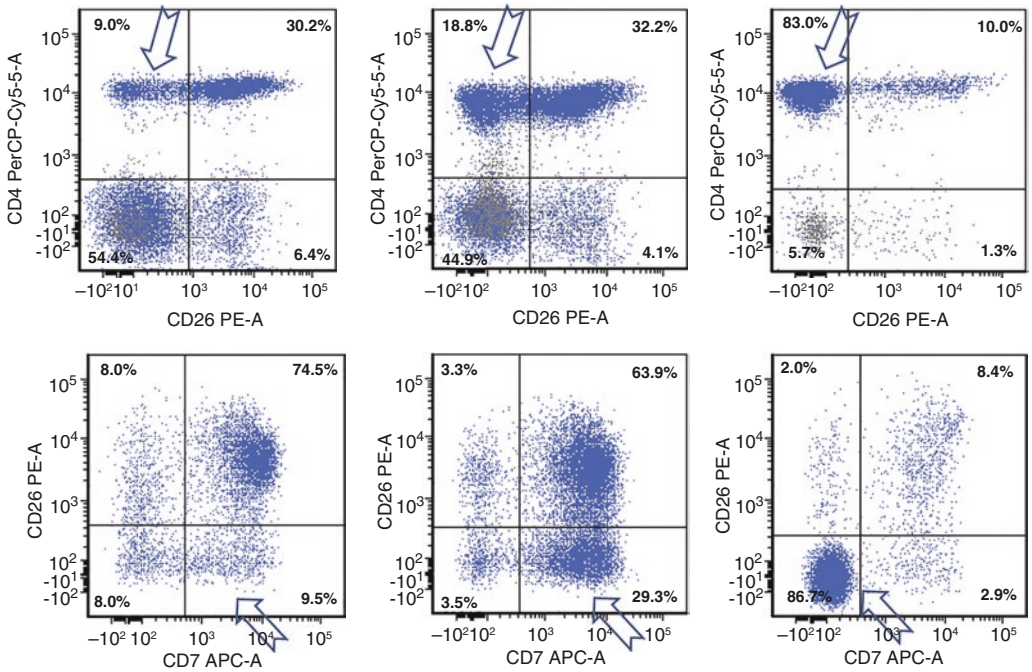

Fig. 11.17 CD26 and CD7 expression patterns in a normal blood (*Left*), negative lymph node (*Middle*) and a PB positive for MF/SS (*right*). (T cells in *blue*; *upper panel* showing the lymphocyte gate; *lower panel* showing CD7 and CD26 expressions on CD3+CD4+ T cells only). CD26-negative cells (*arrows*) comprise up to 35% of normal CD3+CD4+ T cells in peripheral blood (*left upper*); these cells can be seen in a higher percentage in reactive lymph node (*middle upper*) but both in a smear pattern; these CD4+CD26− cells show variable CD7 expression (*lower left* and *lower middle*). In contrast, blood of mycosis fungoides/Sézary syndrome shows a discrete CD26-negative cell population (*Upper Right*); these cells are also CD7 negative (*Lower Right*)

with a high frequency of unbalanced translocations and deletions of chromosome 1p, 6q, 10q, 17p, and 19. T-cell receptor genes are clonally rearranged. In recent years, exome and whole-genome sequencing [110–114] studies of MF/SS have reported frequent somatic mutations involving TCR/NFκB signaling pathway (*NFKB2, TNFAIP3, PLCG1, PRKCQ, and TNFAIP3*), Th2 differentiation (*ZEB1*), cell survival and fate (*PDGFR, ERK, JAK/STAT, MAPK*), epigenetic regulation (*DNMT3A, ASLX3, TET1-3*), homologous recombination (*RAD51C, BRCA2, POLD1*), and cell-cycle control (*TP53*). The discovery and understanding of these mutations will not only provide potential biomarkers for prognostication or monitoring of disease but also identify potential therapeutic targets.

Differential Diagnosis

Erythroderma can be caused by a number of erythrodermic inflammatory dermatoses (EIDs) not unique for MF/SS, such as psoriasis, atopic dermatitis, drug rash, pityriasis rubra pilaris, contact dermatitis, etc. The clinical differential diagnosis between MF/SS and various erythrodermic skin diseases can be quite challenging due to overlapping morphological features. However, compared to EID, SS shows more epidermotropism with intraepidermal atypical lymphocytes; and about 20% cases may show Pautrier microabscesses. The latter is not seen in EID. In SS, the cells in the dermis and perivascular locations are often cerebriform and some blastic. Immunophenotypically, SS shows more profound CD7 loss, significant PD1 expression, Mum1+, and high Ki67. They have less CD8+ T cells on the background [115]. The peripheral blood smears of patients with EID may show some cerebriform lymphocytes, but they are often small, with mature chromatin, so-called small Sézary cells, and the number, unlike SS, is low, not more than 1×10^9/L. Immunoph enotypically, they may show a decreased CD7 expression but often with no other aberrancies.

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell neoplasm associated with human T-lymphotropic virus, type I (HTLV-I). The acute variant (60% of cases) is characterized by a leukemic phase, often with a markedly elevated white blood cell count, skin rash, and generalized lymphadenopathy (see the following chapter on ATLL). Skin lesions often present as nodular and tumorous lesions, followed by erythematous plaques and macules. Erythroderma occurs in 3–5% of patients. Skin biopsy often shows an epidermal infiltrate with Pautries-like microabscesses. Dermal infiltrate is often perivascular. Peripheral blood shows medium-sized lymphocytes with condensed chromatin and bizarre hyperlobated nuclei, described as "clover leaf" or "flower cells." Immunophenotypically, like MF/SS, ATLL is often CD4+. Loss of CD7 as well as CD26 is very common; however, CD25 is often uniform and bright in nearly all cases of ATLL. FOXP3 is also positive in ATLL. Practically all patients with ATLL have serologic antibodies to HTLV-I.

T-prolymphocytic leukemia (T-PLL) is a T-cell leukemia, predominantly of CD4+ T cells and presenting with marked lymphocytosis, splenomegaly, and elevated LDH. Skin involvement can be observed in some patients; however, generalized erythema skin is exceedingly uncommon. Morphologically, T-PLL is characterized by small- to medium-sized lymphocytes with small but distinct nucleoli. However, some other morphological variants have been described, including cerebriform that can mimic small Sézary cells. Immunophenotypically, T-PLL are often bright CD7+ and CD26 uniformly+; and TCL1 is positive in 80% T-PLL (see details on T-PLL chapter).

Peripheral T-cell lymphoma, CD4+, is predominantly a lymph node-based disease. Peripheral blood is sometimes involved, but a leukemic presentation is uncommon; and skin is occasionally involved, but erythroderma is uncommon. Angioimmunoblastic T-cell lymphoma (AITL) can present with skin rash and have circulating lymphoma cells. However, the circulating tumor cells usually present in low numbers, often surface CD3 negative and CD4+, and some with an aberrant CD10 expression [116, 117].

T-Cell Prolymphocytic Leukemia

T-cell prolymphocytic leukemia (T-PLL) is an aggressive T-cell leukemia characterized by the proliferation of small- to medium-sized pro-

lymphocytes with a mature post-thymic T-cell phenotype involving the peripheral blood (PB), bone marrow (BM), lymph nodes, liver, spleen, and skin. T-PLL is an extremely rare disease, comprising approximately 2% of mature lymphocytic leukemias in adults. Approximately 90% of cases demonstrate chromosome abnormalities involving chromosome 14 with *TCL1* or homolog of *TCL1, MTCP1*. With the genetic information, it has been increasingly recognized that T-PLL is more heterogeneous than previously defined, showing variation in morphology, immunophenotype, and clinical behavior.

Clinical Features

T-PLL is a disease of elderly, with a median age of 65 years. There is a slight male predominance with a male to female ratio of 1.33 [118]. Patients with ataxia telangiectasia have a greatly increased incidence of T-PLL with a median age of onset of T-PLL about 30 years of age, and some cases may present in adolescence [119, 120].

Most patients with T-PLL present with an elevated white blood count (WBC). WBC is often markedly increased, >100 × 10^9/L; however, not uncommonly, some patients may present with a WBC around 20–30 × 10^9/L that often progressively increases in the course of disease. Hepatosplenomegaly (50–70%), generalized lymphadenopathy (50%), anemia and thrombocytopenia, skin infiltration (20–25%), and serous effusions (i.e., pleural) (15%) occur at various frequencies in patients with T-PLL [118]. Serum immunoglobulins are often normal. Serology for HTLV-I is negative.

Morphology

Typically, the tumor cells are small- to medium-sized lymphoid cells with moderately condensed chromatin and a visible nucleolus. The nucleus can be round or oval. The cytoplasm is usually moderately abundant and slightly basophilic without granules. Cytoplasmic blebs are common. In about 20–25% cases, the tumor cells are small with condensed chromatin and inconspicuous nucleoli, so-called small cell variant. In 5% T-PLL, the tumor cells have a very irregular nuclear outline resembling the cerebriform nucleus of Sézary cells seen in mycosis fungoides/Sézary syndrome (Fig. 11.18). The BM is almost always involved, showing an interstitial or a diffuse leukemic infiltrative pattern.

Skin and cutaneous involvement by T-PLL often shows no epidermotropism but a perivascular or more diffuse dermal infiltrate. Splenectomy is rarely performed, and if performed, the spleen shows a dense red pulp infiltration. T-PLL cells may invade the spleen capsule, blood vessels, and atrophied white pulp. In lymph nodes, the T-PLL infiltrate tends to predominate in the paracortical areas, sparing follicles, or efface the nodal architecture. High endothelial venules may be numerous, showing T-PLL cell trafficking.

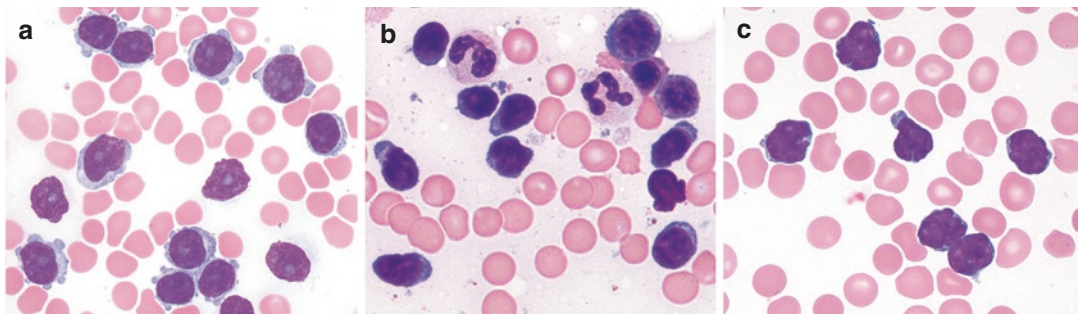

Fig. 11.18 T-cell prolymphocytic leukemia (T-PLL). (*Left*) Typical T-PLL cells in peripheral blood, small to medium with small but conspicuous nucleoli, cytoplasmic blebs. (*Middle*) "Small cell variant" T-PLL, cells have clumping chromatin, most cells with no visible nucleoli. (*Right*) Some cases, the tumor cells can have very irregular nuclear contours (cerebriform, Sézary-like)

Immunophenotype

T-PLL is predominantly CD4+, comprising about 60–70% of cases. Double expressions of CD4 and CD8 are reported in a quarter of the cases. Of CD4+CD8+ cases, some cases may show a partial/dim expression either of CD4 or CD8. About 5–10% cases are CD4−CD8+. Unlike other T-cell lymphoma/leukemia that shows frequent CD7 loss and CD26 negativity, the majority of T-PLL is positive for CD7 and CD26, often at an increased intensity [118, 121–123]. CD2 and CD5 are almost always positive, frequently showing an increased intensity as well. Surface CD3 and CD45 can be completely absent, in 5–10% cases (Fig. 11.19). CD52 is brightly positive in nearly all cases.

Immunohistochemistry study shows that about 80% cases are positive for TCL1. The overexpression of TCL1 often correlates with a *TCL1* rearrangement. However, TCL1 oncoprotein can be activated/upregulated through other mechanisms other than *TCL1* gene rearrangement, such as hypomethylation of the *TCL1* promoter region [124]. TCL1 immunohistochemistry is negative in T-PLL with t(X;14)(q28;q11) fusing *TCRA/D with MTCP.*

Cytogenetics and Molecular Genetics

Chromosomal abnormalities are common, observed in 60–70%, and the majority has a complex karyotype. Chromosome 14 abnormalities inv(14)(q11q32) and t(14;14)(q11;q32) involving *TCL1* rearrangement have been reported in up to 80% of the cases and have become the hallmarks of T-PLL [125–128]. Another abnormality, t(X;14)(q28;q11), involves a homolog of *TCL1, MTCP1* (mature T-cell proliferation 1 gene) [129]. Overall, if combining FISH and cGH, approximately 80–90% of cases demonstrate chromosome abnormalities involving chromosome 14.

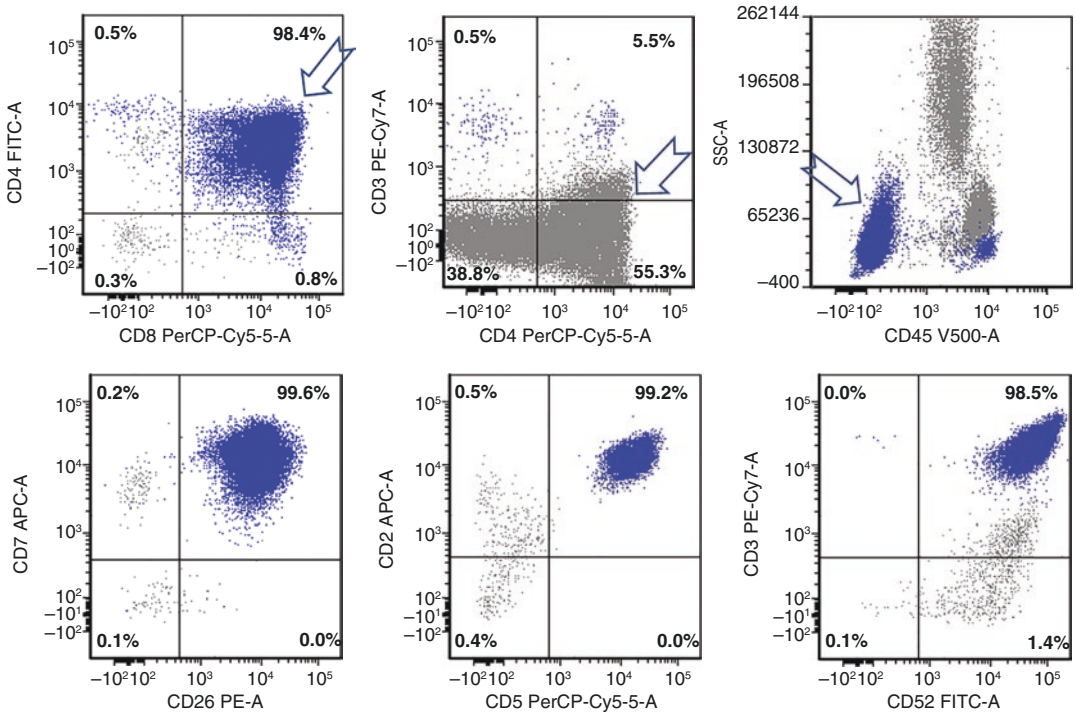

Fig. 11.19 Immunophenotypical variations of T-cell prolymphocytic leukemia (T-PLL) (CD3+ T cells are colored in *blue*). About a quarter of the T-PLL are CD4+CD8+ (*upper left*); some may be negative for surface CD3 (*upper middle*) or CD45 (*upper right*). T-PLL cells often have very bright CD7, CD26, CD2, and CD5, as well as CD52 (*Lower panel*).

Other recurrent chromosomal abnormalities include i(8)(q10), found in about 50% patients. It is suggested that [130] i(8)(q10) leads to increased expression of the *MYC* (8q24) and a deleterious loss of tumor suppressor genes on 8p such as *MTUS1*, synergistically contributing to the pathogenesis of T-PLL. Del(12)p13, abnormalities in chromosome 6 and chromosome 17, and deletion of *TP53* gene (17p13) are also commonly seen in T-PLL. In patients with ataxia telangiectasia with germ-line *ATM* mutation, mutations of the second *ATM* allele are detected in patients who developed T-PLL [131]. Biallelic mutations/deletions lead to premature truncation or alteration of the *ATM* gene product. Loss-of-function mutations and a complete absence of the normal copy of *ATM* resulting in somatic inactivation of this gene have also been observed in sporadic T-PLL [132].

T-cell receptor (TCR) genes are clonally rearranged. Activating mutations in the *JAK3* nonreceptor tyrosine kinases are detected in approximately one-third of cases [133, 134] and *STAT5B* mutations in about 20% cases. *JAK3 and STAT5B* mutations are mutually exclusive. Mutations in epigenetic regulators *EZH2, TET2, and BCOR* are also found in T-PLL [135]. Haploinsufficiency for the *CDKN1B* tumor suppressor gene is another common genetic lesion that occurs in approximately half of cases. The discovery of these mutations raises interest in possible therapeutic relevance of JAK inhibitors and epigenetic regulators in T-PLL.

Differential Diagnosis

As discussed under mycosis fungoides/Sézary syndrome, both T-PLL and MF are T-cell neoplasms that can involve the blood and skin. The morphology of the "cerebriform" (Sézary cell-like) variant seen in 5% of patients with T-PLL resembles the Sézary cells seen in MF. However, these two neoplasms can be easily distinguished by their immunophenotype. T-PLL is often brightly positive for CD7, CD26, and TCL1 (80% cases), whereas MF/SS are often negative for CD7 (60%), CD26 (80–90%), and TCL1.

Similar to T-PLL, chronic lymphocytic leukemia (CLL) shows lymphocytosis and splenomegaly. Historically, small cell variant T-PLL was given a name of T-cell CLL, due to its morphological resemblance to B-cell CLL. CLL can be easily differentiated from T-PLL by immunophenotyping; CLL is of B-cell lineage, CD19+, CD20+, CD5+, CD23+, and CD200+.

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell neoplasm associated with infection by the HTLV-I. The tumor cells are often medium to large, highly pleomorphic, with very irregular nuclear contours, "flower" like. ATLL shows frequent loss of CD7 and CD26, negative for TCL1, but positive for bright CD25. Serologic and PCR for HTLV-I are used to exclude this diagnosis (please refer to ATLL chapter).

Peripheral CD4+ T-cell lymphoma with a leukemic presentation can be difficult to distinguish from TCL1-negative T-PLL immunophenotypically. However, it is exceedingly rare for CD4+ PTCL to show bright and uniform expressions of CD7 and CD26.

Adult T-Cell Leukemia/Lymphoma

Adult T-cell leukemia/lymphoma (ATLL) is a peripheral T-cell neoplasm that is caused by the human retrovirus known as human T-cell leukemia virus type 1 (HTLV-I). HTLV-I is primarily transmitted by breastfeeding, although spread via blood transfusion, sharing of needles, and sexual intercourse also occurs. The incidence of ATLL varies in different geographic populations according to the prevalence of HTLV-I infection. Infection with HTLV-I is endemic in several islands in Southern Japan, the Caribbean basin Jamaica and Trinidad, Western Africa, Peru, Northeast Iran, and southeastern portion of the United States; most ATLL affects patients who live in or originate from these areas [136, 137].

ATLL is considered to derive from HTLV-I-infected memory T cells with stem cell-like properties. Exposure to the virus early in life increases the risk of eventual development of ATLL. A

short latency interval has been observed in patients who are treated with immunosuppressive agents. In the majority of the patients, the rate of ATLL development among HTLV-I infected individuals is relatively low, about 4–5% in the endemic areas [138]. The latency intervals are generally long, about several decades. The relatively low incidence among patients with HTLV-I infection and the long period of clinical latency indicate that infection with HTLV-I is not sufficient for malignant transformation [139]. Additional etiologic factors have not yet been identified.

Although it is considered as one of the highly aggressive T-cell non-Hodgkin lymphoma (NHL) variants, the disease course is variable and sometimes can be quite indolent. The clinical course of acute and lymphomatous ATLL is aggressive, and if no treatment, survival would be measured in months. In contrast, most cases of chronic or smoldering ATLL are relatively indolent that survival without treatment is measured in years.

Clinical Features

Clinically, there are several variants, including acute, lymphomatous, chronic, and smoldering forms. They differ in clinical presentations, treatment requirement, and prognosis [140, 141].

Acute variant is characterized by a leukemic presentation of ATLL. Patients frequently present with constitutional symptoms. Lymphadenopathy is seen in almost all patients. Organomegaly, either liver, spleen, or both, is observed in about 30% cases. A high white count due to circulating malignant cells is very common, and the white blood cell (WBC) count may be higher than 100×10^9/L. In some cases, there is an increase in eosinophils. Bone marrow (BM) involvement is only seen in about 5–35% cases. The number of circulating ATLL cells does not correlate with the degree of BM involvement, suggesting that circulating cells are recruited from other organs such as the skin.

Hypercalcemia with or without lytic bone lesions is very common, observed in approximately 40–50% patients at time of presentation and 30% patients who would develop hypercalcemia during the course of the disease. Approximately 25–50% patients will have skin lesions at diagnosis, which can be patches, plaques, papules, tumors, erythrodermic lesions, and purpuric lesions, similar to mycosis fungoides [142]. Less commonly, some patients may have pulmonary and CNS involvement.

The lymphomatous variant is characterized by prominent lymphadenopathy with no blood involvement. Patients frequently have elevated LDH and can have hypercalcemia. The prognosis is poor, with a survival similar to that of patients with the acute variant. The lymphomatous variant accounts for approximately 20% of all cases.

The chronic variant often shows exfoliative skin lesions, mild lymphadenopathy, and elevated lymphocyte count but tumor cells not numerous. Disease may be stable for months to years, and the median survival is 2–5 years. However, a subset of patients who have a low serum albumin, high LDH, or high blood urea nitrogen may have a poor prognosis similar to that of the acute and lymphomatous variants. The chronic variant comprises approximately 10% cases.

The smoldering variant is the least common subtype, accounting for less than 10% of cases. These patients are often asymptomatic except for skin and/or pulmonary lesions. They have normal blood lymphocyte counts with <5% circulating neoplastic cells and no hypercalcemia. Median survival without treatment is approximately 3 years [143].

Progression from chronic and smoldering disease to aggressive disease resembling the acute variant eventually occurs in up to 25% of cases. Patients with ATLL are immunosuppressed and at risk of developing opportunistic infections.

Morphology

In the PB, ATLL is mostly medium-sized cells, with polylobated nuclei, and described as "clover leaf" or "flower" cells [137, 144] (Fig. 11.20). These cells have condensed chromatin and invisible nucleoli and are highly pleomorphic. The cytoplasm is medium or scanty, basophilic cytoplasm, and agranular. In some cases, a few blasts or immunoblast-like cells with dispersed chromatin and prominent nucleolus may be pres-

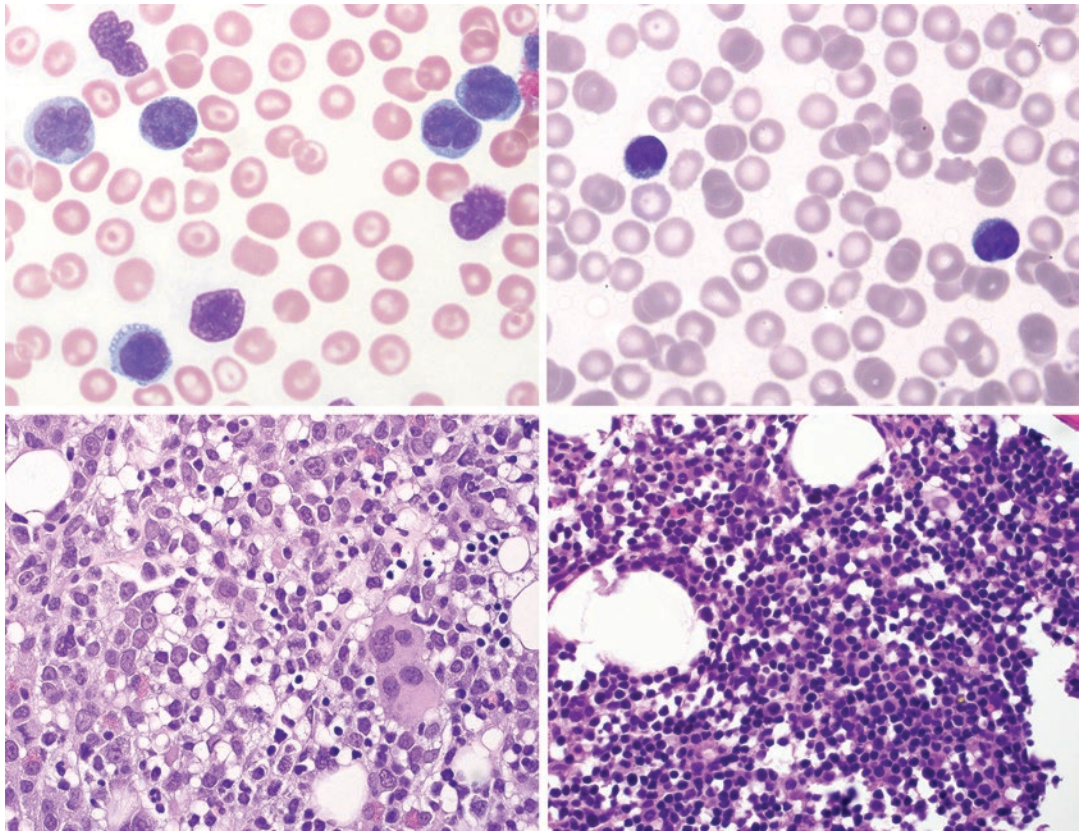

Fig. 11.20 Adult T-cell leukemia/lymphoma (ATLL). (*Upper Left*) In peripheral blood, ATLL is mostly medium-sized cells, with polylobated nuclei, and described as "clover leaf" or "flower" cells. (*Upper Right*) In some cases, the tumor cells can be very small. The bone marrow can be infiltrated by large cells (*Lowe Left*) or small cells. They can be interstitial or in aggregates (*Lower Right*)

ent in the blood. Patients may have variable anemia and thrombocytopenia and increased eosinophils. In some cases, the tumor cells can be very small.

Bone marrow infiltrates are usually patchy and subtle, even in patients with a leukemic presentation, ranging from sparse to moderate. Osteoclastic activity may be prominent, even in the absence of BM infiltration by neoplastic cells.

Lymph node involvement can be predominantly paracortical or show a leukemic pattern of infiltration, with preservation or dilation of lymph node sinuses that contain malignant cells. Tumor cells are highly variable, ranging from predominantly small to medium and large cell types or anaplastic and, rarely, resembling angioimmunoblastic T-cell lymphoma or Hodgkin lymphoma [145]. Overall, the lymph node histology of ATLL may be indistinguishable from those of other peripheral T-cell lymphomas.

Skin lesions are very common in ATLL, about 50% cases. Epidermal infiltration with Pautrier-like microabscesses is common. Dermal infiltration is mainly perivascular, but an extension to subcutaneous fat may be observed. The skin infiltrative pattern may be indistinguishable from that of mycosis fungoides and Sézary syndrome.

Immunophenotype

ATLL tumor cells often express pan-T-cell antigens CD2, CD3, and CD5 but usually lack CD7. CD26 is often negative [146]. While most cases are CD4+ and CD8−, a few are CD4−, CD8+, or double positive for CD4 and CD8. CD25 is

strongly expressed in nearly all cases. The large transformed cells may be positive for CD30, like in MF/SS. In addition, tumor cells frequently express the chemokine receptor CCR4 and FOXP3, features of regulatory T-cells.

A recent study [146] compared ATLL aggressive variant, indolent variant, and HTLV-I serology positive non-ATLL patients. HTLV-I-infected cells have a CD4+CCR4+ CD26− immunophenotype. Within this population, CD7− phenotype correlates with a diagnosis of ATLL; CCR7+ phenotype identifies the aggressive variant ATLL, and CCR7− CD127− phenotype identifies the indolent form of ATLL.

Cytogenetics and Molecular Genetics

ATLL exhibits a variety of cytogenetic abnormalities in almost all patients; however, no cytogenetic abnormalities are unique for ATLL [147, 148]. The most frequent aberrations include gains of chromosomes 14q, 7q, and 3p and losses of chromosomes 6q and 13q. Chromosomal imbalances, losses, and gains are more frequently observed in aggressive ATLL cases than in indolent ATLL cases. Clonal evolution has been observed during disease progression.

T-cell receptor genes are clonally rearranged, and neoplastic cells show monoclonal integration of HTLV-I. However, ATLL develops through a multistep carcinogenesis process involving approximately five or more genetic events [149]. Recent advance in molecular genetics has provided further understanding the landscape of genetic and epigenetic alterations in ATLL. ATLL shows alterations highly enriched in genes for TCR–NF-κB signaling such as *PLCG1, PRKCB, and CARD11* and gain-of-function mutations in *CCR4 and CCR7.* Mutations in *RHOA*, which encodes a GTP-binding protein, and *TET2*, an epigenetic regulator, as well as *MLL3,* have been identified in a subset of ATLL cases [157]. *EZH2* activation is induced by HTLV-I Tax and NF-κB.

Differential Diagnosis

"Flower cells" similar to those seen in ATLL can be seen in the peripheral blood of asymptomatic healthy carriers of HTLV-I. Such cases may be difficult to distinguish from smoldering ATLL. Proven clonal integration of HTLV-I the circulating cells would support a diagnosis of smoldering ATLL. TCR clonality study by PCR may help to determine a clonal process. Flow cytometry immunophenotyping shows loss of CD7 and CD26 expression, or other alterations in pan-T-cell antigens can help to determine a neoplastic process.

ATLL can be difficult to distinguish from MF/SS, as discussed in the MF/SS section above. Both of these disorders can have cutaneous manifestations, and circulating tumor cells. Morphologically, the skin infiltrate and the cytology of cells in PB may show significant similarity. They can also have a similar immunophenotype, as both may be CD4+, CD7−, and CD26−. CD25 expression can be observed in MF/SS but often variable and at a relatively low level contrasting bright and uniform CD25 expression in ATLL. The key distinguishing feature is the demonstration of HTLV1 infection in ATLL.

Anaplastic large cell lymphoma (ALCL) primarily involves the lymph nodes and skin but can have circulating malignant cells in blood and a BM involvement. Similar to ATLL, ALCL can be pleomorphic and often CD4+ and CD7−. However, ALCL often has strong, uniform expression of CD30 with a membrane and Golgi distribution and is usually positive for cytotoxic granule-associated protein. For small cell variant and lymphohistiocytic variant ALK1+ ALCL, CD30 can be weaker and partial. On the other hand, in ATLL, the large cells can be CD30 positive. ALK1 staining is essential to rule in or rule out small cell variant and lymphohistiocytic variant ALK1+ ALCL.

Angioimmunoblastic T-cell lymphoma (AITL) usually presents with generalized systemic symptoms, lymphadenopathy, hepatomegaly, bone marrow involvement, and pruritic skin rash that can mimic ATLL. However, in tissue biopsies, they often have prominent arborizing high endothelial venules and many reactive cells on the background. In AITL, the tumor cells have a follicular T-help immunophenotype, with a variable expression of PD-1, CXCL13, CD10, and BCL6. EBER-positive B cells are present in

a large subset of AITL. AITL can have circulating tumor cells but often at a low level. They are frequently surface CD3 negative and CD4+, with or without partial CD10 expression [117, 116].

ALK1+ Anaplastic Large Cell Lymphoma (ALK+ ALCL)

Clinical Features

ALK1+ ALCL comprises 3% of non-Hodgkin lymphomas in adults and 20–30% in children and adolescents. It is characterized by *ALK* rearrangement, frequently due to a t(2;5)(p23;q35) *ALK/NPM1* fusion and, less commonly, with other partner genes. ALK1+ ALCL usually involves lymph nodes and extranodal sites; involvement of bone marrow and peripheral blood is unusual in that it occurs in <10% of the cases.

Morphology

ALK+ ALCL exhibits a broad spectrum of morphology, including classic (70%), lymphohistiocytic (5–10%), small cell (5–10%), and sarcomatoid variants (<1%). The hallmark cells, which are typically large with eccentric horseshoe- or kidney-shaped nuclei, are seen in all variants and are helpful features in establishing the diagnosis.

Leukemic presentations of ALK1+ ALCL are most often associated with small variant ALCL [150–153]. In PB, the cells are small to medium in size, with condense chromatin, irregular nuclear contours, and scant to moderate cytoplasm with occasional cytoplasmic azurophilic granules (Fig. 11.21). Large tumor cells are often rare; if present, they generally have basophilic and vacuolated cytoplasm with moderately condensed chromatin and a single prominent nucleolus. In the BM,

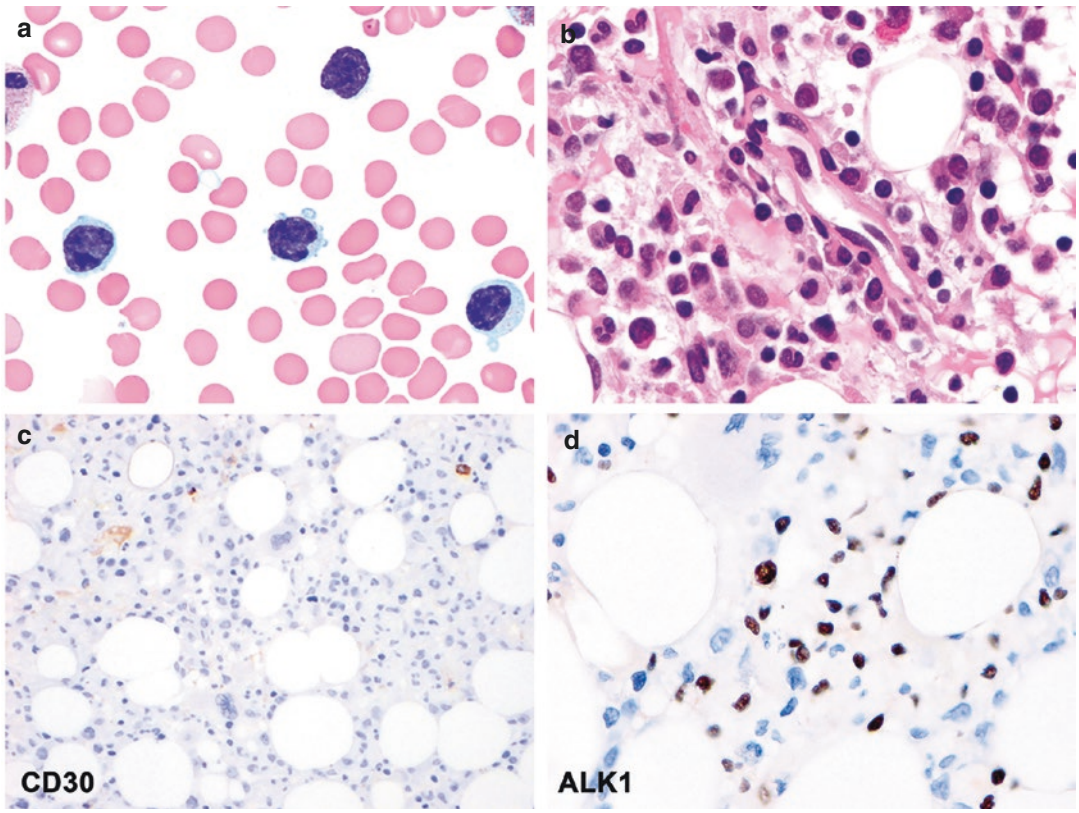

Fig. 11.21 Small cell variant ALK1+ anaplastic large cell lymphoma (ALCL) with a leukemic presentation. (*Upper Left*) Peripheral blood shows mature lymphocytes with condensed chromatin, irregular nuclear contours, scant to moderate cytoplasm, with sparse granules. (*Upper Right*) The infiltrate in bone marrow is very subtle on routine H&E histology. (*Lower Left*) CD30 stain is mostly negative; weaker/partial CD30 expression is common for small cell variant ALCL. (*Lower Right*) These cells are highlighted by ALK1 stain

the infiltrate is interstitial, which may be difficult to see due to small cell size and a subtle infiltrate.

Immunophenotype

CD30, a member of the tumor necrosis receptor family, is believed to be universally expressed in ALK1+ ALCL in an intense membranous and Golgi pattern. However, CD30 expression can be weaker and partial in small cell variant and lymphohistiocytic variant ALK1+ ALCL and is weak or negative in PB and BM lymphoma cells [154]. Moreover, ALK1+ ALCL often expresses at least one myelomonocytic marker, such as CD11b, CD13, or lysozyme [155, 150–153]. The lymphoma cells may lack surface CD3 expression, or even CD5 and CD2, with a null cell type.

Cytogenetics and Molecular Genetics

ALK+ ALCL is characterized by an *ALK* rearrangement, most frequently due to a t(2;5)(p23;q35) *ALK-NPM1* fusion and, less commonly, with other partner genes.

Differential Diagnosis

Because of its rarity, atypical clinical presentation, morphologic overlap with other T-cell lymphoma/leukemia, and weak/absent of CD30, as well as the expression of myelomonocytic markers, the leukemic phase of ALCL can be very difficult to diagnose, and the differential diagnoses include a long list of entities, such as T-lymphoblastic leukemia; T-cell prolymphocytic leukemia; T-cell large granular lymphocytic leukemia; peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS); Sézary syndrome; and adult T-cell leukemia/lymphoma (ATLL).

Different from T-PLL, PTCL, NOS and MF/SS, and T-LGL, ALK1+ ALCL is likely to occur in adolescent and young adults. The detection of myelomonocytic antigens in a mature T-cell neoplasm should prompt ALK1 immunohistochemistry study or FISH for *ALK* rearrangement. They differ from T-lymphoblastic leukemia in the maturity of the cells, bright expression of CD45, and lack of CD1a, TDT, and CD34 immature markers.

T-Lymphoblastic Leukemia/Lymphoma

Precursor T-lymphoblastic leukemia/lymphoma (T-LBL) is a neoplasm committed to the T-cell lineage. T-lymphoblastic lymphoma (T-LBL) is the appropriate term if T-LBL is confined to a mass lesion with no or minimal peripheral blood (PB) and bone marrow (BM) involvement. T-lymphoblastic leukemia (T-ALL) is used if there is an extensive PB and BM involvement. In general, or for protocol reasons, if there are >25% BM blasts, the case is qualified for leukemia (T-ALL), regardless of the presence or absence of a mass lesion. There is significant biological and clinical overlap between these neoplasms; therefore, T-LBL and T-ALL should be considered the same disease with different clinical presentations.

TALL/LBL was thought to arise from precursor T-precursor cells at varying stages of differentiation; however, like other acute leukemia, TALL/LBL is likely maintained by a small number of leukemia initiating cells, so-called leukemia stem cells [156]. In TALL/LBL, a small subpopulation of CD90 (Thy-1)+ and CD110+ (c-Mpl) cells has been shown to correlate with stem cell properties both in vitro and in transplantation experiments [157]. In recent years, a unique subset of TALL/LBL with limited early T-cell differentiation (also called early T-precursors) has been recognized. ETP TALL/LBL represents a subset of TALL/LBL that has not yet irreversibly committed to the T-cell lineage but rather retains myeloid/dendritic cell differentiation potential.

TALL/LBL occurs most frequently in late childhood, adolescence, and young adulthood, with a 2:1 male predominance; it comprises 15% of childhood and 25% of adult ALL. Treatment of TALL/T-LBL is generally more aggressive than that for precursor B-ALL and is the same for T-lymphoblastic lymphoma and leukemic presentations [158]. With aggressive chemotherapy regimens, TALL/LBL in children has an outcome similar to those of children with B-ALL. Among adults, TALL/LBL has a more favorable outcome than B-lineage ALL, likely attributing to a younger patient age and lack of distinctive adverse cytogenetic abnormalities in TALL/LBL.

Clinical Features

Patients are usually adolescent or young adult males who present with a bulky anterior mediastinal mass (50–75%) or lymphadenopathy involving cervical, supraclavicular, and axillary regions. The anterior mass may be complicated by pericardial and pleural effusions, superior vena cava syndrome, or tracheal obstruction. Some patients may have extranodal disease, such as skin, testicular, or bony lesions. Hepatosplenomegaly is not infrequent. Central nervous system (CNS) involvement can be seen, especially in patients with a leukemic presentation with a high white count and BM involvement. While many patients present with an acute course, some patients may have symptoms progressing slowly over weeks to months.

Morphology

On peripheral blood smears, lymphoblasts are often small to medium in size with scant cytoplasm. In some cases, TALL/LBL cells can be medium to large with moderate cytoplasm, mimicking AML blasts. Chromatin can range from disperse to condense, nuclei range from round and irregular to convoluted, and nucleoli range from invisible to prominent. TALL/LBL is indistinguishable from B-ALL/LBL. In some cases, the cells are hyperchromatic, with a few azurophilic cytoplasmic granules, resembling mature T-cell neoplasms.

On bone marrow trephine biopsy, lymphoblasts have scant cytoplasm, finely stippled chromatin and inconspicuous nucleoli. The infiltrate is often less extensive than B-ALL, often showing retained normal BM hematopoiesis.

In lymph node, the nodal architecture is generally effaced, with a diffuse involvement. In some cases, the infiltrate may predominantly involve paracortical areas and surrounds residual germinal centers. Occasionally, TALL/LBL can have a nodular growth pattern. The TALL/LBL cells are small to medium, with round, oval, or convoluted nuclei, indistinct nucleoli, and scant cytoplasm. Mitotic figures are often numerous, and some cases may have a starry sky pattern. In cases with increased eosinophils on the background, or with a myeloblast component, *FGFR1* rearrangement study should be performed to rule out "myeloid and lymphoid neoplasms with *FGFR1* abnormalities" [159].

Immunophenotype

The lymphoblasts in TALL/LBL are cytoplasmic CD3+ and CD7+. CD7 expression usually is uniform and brighter than normal mature T cells. TdT is positive in 70–80% cases by flow cytometry and positive in about 90% cases by immunohistochemistry. TdT IHC often stains a small subset of blasts that may not be picked up by flow cytometry [160]. TALL/LBL variably expresses CD1a, CD2, surface CD3, CD4, CD5, CD7, and CD8. CD10 can be seen in a subset of cases. Myeloid antigen CD13, CD33, or both can be seen in about 20–30% cases [161]. CD117 (cKIT) may be positive; such cases have been associated with activating mutations of *FLT3* [162]. Subset CD79a positivity has been observed in approximately 10% of cases of TALL/LBL [163]. Some cases may express partial CD19 in an otherwise typical TALL/T-LBL. These cases should not be called mixed-phenotype leukemia.

TALL/LBL has previously been stratified into four immunophenotypical subtypes according to the proposal by the European Group for the Immunological Characterization of Leukemias (EGIL). According to the stage of differentiation and maturation, TALL/LBL is divided into pro-T, pre-T, and cortical T- and medullary TALL/LBL [164]. The classification is difficult to apply in practice due to significant overlapping among these subgroups. Of note, many cases previously classified as pro-T or pre-T would now meet criteria for the entity early T-precursor ALL (ETP). The immunophenotype of ETP ALL [165] is shown in Table 11.6 and a case in Fig. 11.22. They are CD7+, negative for CD8 and CD1a, and positive for one or more of the myeloid/stem cell markers CD34, CD117, HLADR, CD13, CD33, CD11b, or CD65. Cytoplasmic CD3 is positive; however, it is common to see partial cCD3 expression, and the level of expression can be dim, not like other TALL/LBL that cCD3 expression, at least a subset of cells, is as bright as mature T cells. CD5 is either negative or dim, positive in <75% of the blast popula-

Table 11.6 The immunophenotype of early T-precursor lymphoblastic leukemia

Immunophenotypic markers	Description/comment
T-lineage markers	
• CD7	Usually bright and uniform
• Cytoplasmic CD3	Positive, but often weak/partial, and may not be as bright as mature T cells
Defining markers	
• CD1a and CD8 negative	Expression in <5% of blasts
• CD5 negative or weak+	Expression in <75% of blasts and 1 log lower than mature T cells in median fluorescence intensity
• Expression of at least one stem cell-associated or myeloid-associated antigen CD117, CD34, HLADR, CD13, CD33, CD11b, or CD65	Expression has to be in >25% of blasts
Other markers	CD2 often positive, CD4 may be positive, surface CD3 and CD10 often negative

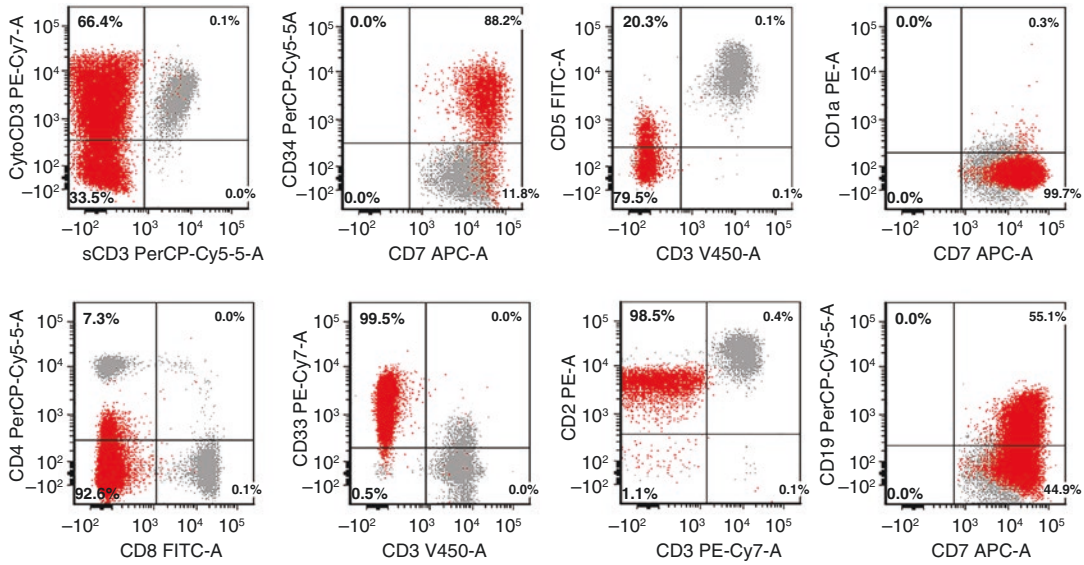

Fig. 11.22 A case of early T-precursor lymphoblastic leukemia (ETP-TALL) (blasts are colored in *red* and mature T cells in *gray*, quadrant % are calculated of the blast population only). Cytoplasmic CD3 is positive but often partial/weak; like other TALL, CD7 is often very bright. CD5 is negative, partial, or dim, expressed in <75% of blasts, and 1 log lower in mean fluorescence intensity comparing to mature T cells; CD1a and CD8 are negative; blasts express at least one myeloid/stem cell marker (in this case, CD34 and CD33). This case also expresses aberrant partial CD19

tion, and more than 1 log lower than the mature T cells in terms of mean florescence intensity (MFI). They typically also are positive for CD2 or may express some CD4, but these markers are not part of the definition. Myeloperoxidase is negative; if MPO+, a case would most likely meet the criteria for T/myeloid mixed-phenotype acute leukemia or AML. It has been suggested that leukemia with brighter or more uniform CD5 expression but otherwise meeting the criteria for ETP are called near-ETP ALL. ETP and near-ETP phenotype have been suggested to represent a high-risk group of TALL/LBL [165, 166]. The rates of minimal residual disease (MRD) at the end of induction therapy are higher than other TALL. However, recent studies show that with more effective therapy, the outcomes may not be significantly different [167, 168].

Cortical TALL/LBL often shows coexpression of CD4 and CD8, CD1a+ whereas medul-

lary TALL/LBL expresses either CD4 or CD8, may have surface CD3 expression; however, individual cases may not fit into the exact pattern. CD1a has been shown to correlate with a better prognosis in T-LBL/T-ALL. CD10 expresses more frequently in T-LBL than T-ALL. CD56 expression can be seen in some TALL/LBL.

Cytogenetics and Molecular Genetics

An abnormal karyotype is found in 50–70% of cases of TALL/LBL [169, 170]. The most common abnormalities are translocations involving *TCRα/δ* loci at 14q11.2, *TCRβ* at 7q35, and *TCRγ* at 7p14-15. The partner genes are variable and include *MYC* (8q24), *TAL1/SCL* (1p32), *LMO1* (11p15), *LMO2* (11p13), *HOX11* (10q24), *HOX11L2 (TLX3)* (5q35), and the cytoplasmic tyrosine kinase *LCK* (1p34). Deletion of *TAL1* locus at 1p32 is seen in about 25% cases. Deletions of 9p involving deletion of the p16 (ink4a) tumor suppressor gene (CDK4 inhibitor) are also seen in approximately 30% of T-ALL/LBL.

TALL/LBL almost always shows clonal rearrangements of the TCR gene; some may also show immunoglobulin heavy chain gene rearrangements [171]. *NOTCH1* gene, which encodes a protein critical for early T-cell development, is found mutated in approximately 50% cases [172]. *FBXW7* gene, a negative regulator of *NOTCH1* and *MYC*, is mutated in about 30% of cases [173]. *TP53* mutations are seen in 10–15% cases.

Gene expression profiling studies show that ETP ALL has an expression profile similar to that of normal early thymocyte precursors, whereas the other TALL/LBL has a signature of later thymocytes. ETP shows overexpression of myeloid or stem cell markers such as CD44, CD34, *KIT*, *GATA2,* and *CEBPA*, as well as immature T-ALL Lyl1. The mutational profile [174, 175] is also more similar to that of myeloid leukemia than to other T-cell leukemia showing frequent *FLT3, RAS, DNMT3A, IDH1,* and *IDH2* mutation and low frequencies of more typical ALL lesions such as *NOTCH1*-activating mutations or mutations in *CDKN1/2*.

Differential Diagnosis

Thymomas especially cortical lymphocytic thymoma (Type B2 thymoma) on fine-needle aspiration (FNA) specimen or core needle biopsy can be difficult to distinguish from T-LBL. Thymic epithelium can be scanty and difficult to see, and the background thymocytes can look immature (Fig. 11.23). Thymus hyperplasia can present as a mass lesion and frequently occurs in children and adolescents. Thymocytes are CD4+, CD8+, CD1a+, CD99+, and TDT+, mimicking T-LBL. Figure 11.23 shows a case of thymoma in comparison with a case of thymic cortical T-lymphoblastic leukemia. Flow cytometry demonstrates a maturation pattern in thymic lymphocytes of a thymoma that is not seen in T-LBL. There are three stages of maturation in thymocytes. The least mature cells have low-density CD45 with partial CD34 expression. These cells are either double negative for CD4 and CD8 or partial CD4; negative for CD8, with no surface CD3; and very few CD1a+ but with bright TDT and cytoplasmic CD3 expression (Fig. 11.24). The intermediate stage cells have higher intensity of CD45 and double positive for CD4 and CD8 and show heterogeneous surface CD3. They are negative for CD34. The mature cells (or medullary thymocytes) are mostly single positive either for CD4 or CD8 and positive for surface CD3. The heterogeneous expression of surface CD3, CD4, and CD8 also create a characteristic smearing pattern for these antigens. All three stages of thymocytes are CD7+, CD5+, and CD2+.

Some mature T-cell lymphoma/leukemias may lack surface CD3 expression; and some, especially T-prolymphocytic leukemia (T-PLL), can be CD45 negative. On the other hand, some TALL/LBL may not have immature markers such as CD34 and TDT, and some can show surface CD3 expression and single CD4 or CD8 expression. Mature T-cell neoplasms often have more condensed and mature chromatin and are more pleomorphic, whereas T-ALL/LBL often are medium-sized cells with immature chromatin and scant cytoplasm. CD45 is dimmer in T-ALL/LBL by flow cytometry, and they often show some immature markers such as CD10,

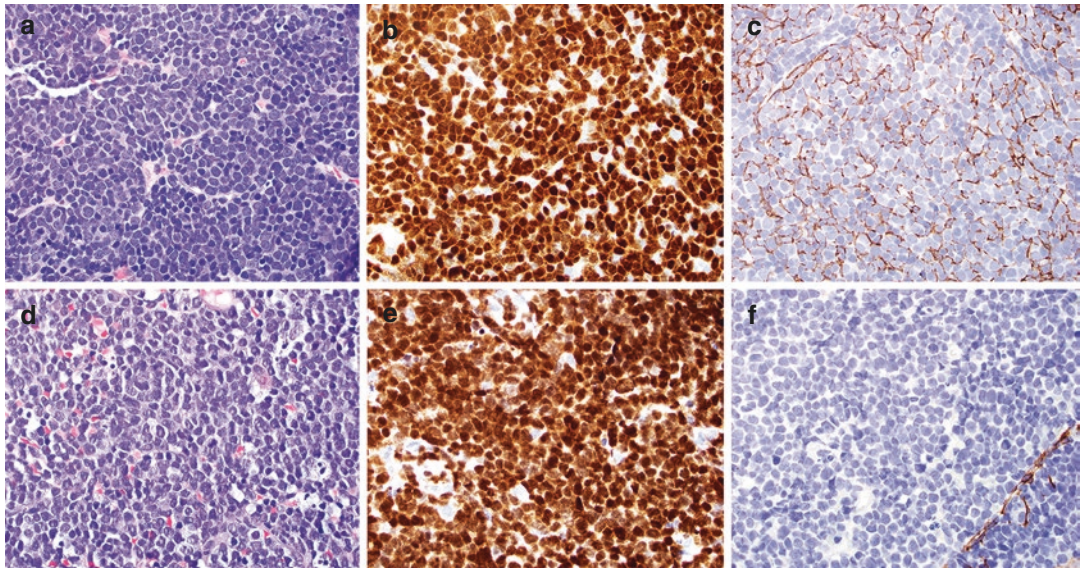

Fig. 11.23 A case of lymphocyte-rich thymoma (**a**–**c**) and a case of T-lymphoblastic lymphoma (T-LBL, **d**–**f**). In lymphocyte-rich thymoma (**a**), the background thymocytes can look immature and blastic, and they are strongly positive for TDT (**b**), similar to T-LBL (**d**, **e**) (also positive for CD3, CD1a). Cytokeratin (**c**) highlights epithelial cells in a lacy-like delicate pattern. In contrast, cytokeratin shows only rare normal residual thymic epithelial cells in T-LBL (**f**)

CD1a, CD34, TDT, and CD99, lack of surface CD3, and variable expressions of myeloid antigens CD13 and CD33. They don't show cytotoxic granules.

Acute leukemia of ambiguous lineage, including undifferentiated, mixed T/myeloid, or precursor NK-cell lymphoblastic leukemia/lymphoma can show substantial overlap with T-ALL/LBL, especially the ETP type. The presence of myeloid markers does not exclude a diagnosis of TALL/LBL nor does it indicate a mixed-phenotype acute leukemia, T/myeloid. To call a mixed T/myeloid leukemia, the leukemia cells either show very convincing MPO expression on cCD3+ cells (biphenotypic) or in a complete different subset of blasts (mixed lineages), or a subset of blasts are monocytic (CD64, CD11b, CD4, CD36, CD14). MPO assessment should not be solely based on immunohistochemistry due to its nonspecific nature. CD117 is often a good marker for myeloid but also can be expressed in TALL/LBL, especially in cases with *FLT3 ITD* mutations. In ETP ALL, cCD3 can be partial and weaker by flow cytometry, and immunohistochemistry can help to confirm the T-lineage differentiation of the blasts. In contrast, acute leukemia, undifferentiated, should not have cytoplasmic CD3 expression. On the other hand, if a case is typical of AML, very dim/weak cytoplasmic CD3 expression on myeloblasts is not sufficient to call a case myeloid/T-mixed-phenotype acute leukemia. In such instance, cytoplasmic CD3 should be as bright as mature T cells, at least in a subset of blasts. CD56 expression in TALL/LBL is not common but can be observed in about 5% cases. Precursor NK-cell lymphoblastic leukemia/lymphoma often expresses CD2, CD7, and CD56. NK-ALL/LBL often expresses CD94. cCD3 may be positive by flow cytometry if the antibody used is specific for the CD3ξ chain. TCR gene rearrangement is germ line.

Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is derived from the precursors of plasmacytoid dendritic cells, with a high frequency of cutaneous and bone marrow (BM) involvement and leukemic dissemination. Recent studies have shown that BPDCN originates from hematopoietic stem cells and share a clonal origin with chronic myelomonocytic leukemia [176]. BPDCN are CD4+, CD123bright+, and CD56+;

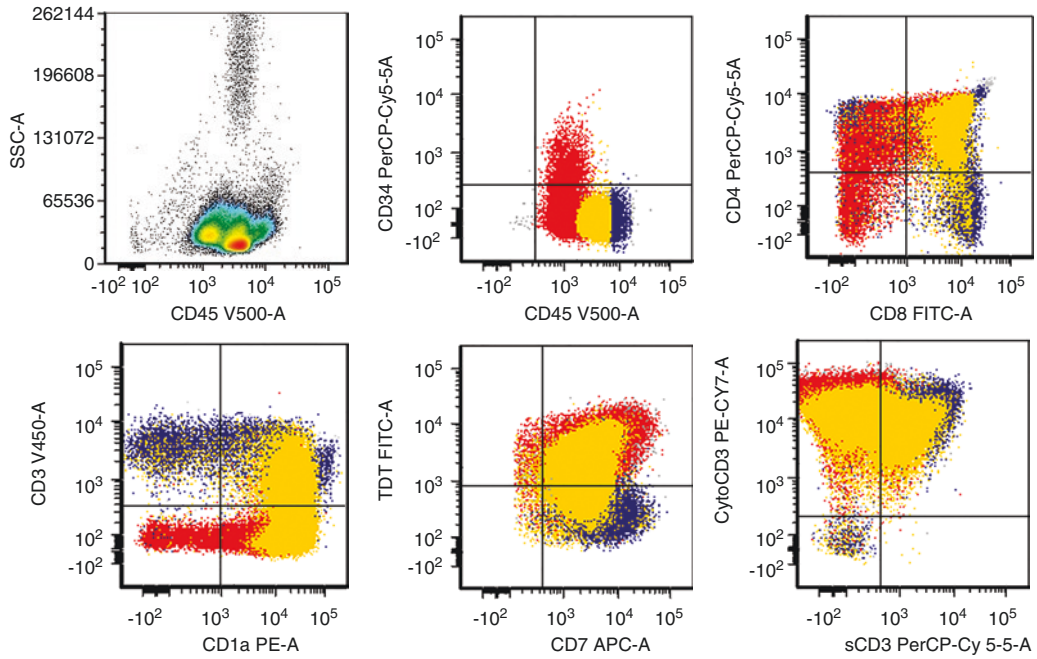

Fig. 11.24 Thymic lymphocytes in lymphocyte-rich thymoma demonstrate a normal thymocyte maturation pattern. Three populations of cells are seen on CD45/SSC-A plot (*upper left*). The population with the lowest CD45 expression represents more immature thymocytes (*colored in red*), which shows partial CD34 expression, either double negative for CD4 and CD8 or partial CD4, with no surface CD3, very few CD1a, but bright TDT and cytoplasmic CD3 expression. The majority of the cortical thymocytes (*colored in yellow*) are CD45 slightly dimmer, double positive for CD4 and CD8, bright CD1a, moderate TDT, cytoplasmic CD3, and also with subset expressing surface CD3. The mature T cells or medullary thymocytes (*blue*) have the highest CD45 expression, mostly either CD4 or CD8+, only partial CD1a+, surface CD3+, and no TDT

however, in some cases, the tumor cells may be CD4 negative or CD56 negative. They can express TDT and T-cell antigens including CD2, CD7, and CD5. However, BPDCN do not express cytoplasmic or surface CD3. In addition to bright CD123, BDCA2 (CD303) or TCL1 will confirm a diagnosis of BPDCN.

Finally, an indolent T-lymphoblastic proliferation has been described [177]. These lesions typically occur in the upper aerodigestive tract and characterized by multiple local recurrences without systemic dissemination. It has been observed in patients with Castleman disease [178, 179]. They are morphologically less atypical than TALL/LBL and, immunophenotypically, similar to cortical thymocytes, CD4+CD8+TDT+, and lack TCR clonal rearrangements. It is debatable if this should be called cortical thymocyte proliferation rather than indolent T-lymphoblastic proliferation [180, 181].

References

1. Cohen JI. Epstein–Barr virus infection. N Engl J Med. 2000;343(7):481–92.
2. Cohen JI, Corey GR. Cytomegalovirus infection in the normal host. Medicine (Baltimore). 1985;64(2):100–14.
3. Karandikar NJ, Hotchkiss EC, McKenna RW, Kroft SH. Transient stress lymphocytosis: an immunophenotypic characterization of the most common cause of newly identified adult lymphocytosis in a tertiary hospital. Am J Clin Pathol. 2002;117(5):819–25.
4. Teggatz JR, Parkin J, Peterson L. Transient atypical lymphocytosis in patients with emergency medical conditions. Arch Pathol Lab Med. 1987;111(8):712–4.
5. Chabot-Richards DS, George TI. Leukocytosis. Int J Lab Hematol. 2014;36(3):279–88.
6. George TI. Malignant or benign leukocytosis. Hematology Am Soc Hematol Educ Program. 2012;2012:475–84.
7. Sun P, Kowalski EM, Cheng CK, Shawwa A, Liwski RS, Juskevicius R. Predictive significance of absolute lymphocyte count and morphology in adults with a new onset peripheral blood lymphocytosis. J Clin Pathol. 2014;67(12):1062–6.

8. Tollerud DJ, Clark JW, Brown LM, Neuland CY, Pankiw-Trost LK, Blattner WA, Hoover RN. The influence of age, race, and gender on peripheral blood mononuclear-cell subsets in healthy nonsmokers. J Clin Immunol. 1989;9(3):214–22.
9. Valiathan R, Deeb K, Diamante M, Ashman M, Sachdeva N, Asthana D. Reference ranges of lymphocyte subsets in healthy adults and adolescents with special mention of T cell maturation subsets in adults of South Florida. Immunobiology. 2014;219(7):487–96.
10. Marcos A, Nova E, Montero A. Changes in the immune system are conditioned by nutrition. Eur J Clin Nutr. 2003;57(Suppl 1):S66–9.
11. Rubin RH, Carney WP, Schooley RT, Colvin RB, Burton RC, Hoffman RA, Hansen WP, Cosimi AB, Russell PS, Hirsch MS. The effect of infection on T lymphocyte subpopulations: a preliminary report. Int J Immunopharmacol. 1981;3(3):307–12.
12. Laurence J. T-cell subsets in health, infectious disease, and idiopathic CD4+ T lymphocytopenia. Ann Intern Med. 1993;119(1):55–62.
13. Heldrup J, Kalm O, Prellner K. Blood T and B lymphocyte subpopulations in healthy infants and children. Acta Paediatr. 1992;81(2):125–32.
14. Ginaldi L, Farahat N, Matutes E, De Martinis M, Morilla R, Catovsky D. Differential expression of T cell antigens in normal peripheral blood lymphocytes: a quantitative analysis by flow cytometry. J Clin Pathol. 1996;49(7):539–44.
15. Reinhold U, Abken H, Kukel S, Moll M, Muller R, Oltermann I, Kreysel HW. CD7- T cells represent a subset of normal human blood lymphocytes. J Immunol. 1993;150(5):2081–9.
16. Flammiger A, Bacher U, Christopeit M, Horn C, Ruhlmann E, Kluge K, Vettorazzi E, Bokemeyer C, Binder M. Multiparameter flow cytometry in the differential diagnosis of aberrant T-cell clones of unclear significance. Leuk Lymphoma. 2015;56(3):639–44.
17. Wang W, Gao L, Gong M, Tang Y, Li Y, Zhang WT, Huang FZ, Zhang CX, Chen YR, Gao YY, et al. Non-malignant T-cells lacking multiple pan-T markers can be found in lymph nodes. Leuk Lymphoma. 2015:1–7.
18. Roden AC, Morice WG, Hanson CA. Immunophenotypic attributes of benign peripheral blood gamma delta T cells and conditions associated with their increase. Arch Pathol Lab Med. 2008;132(11):1774–80.
19. Shimura Y, Horiike S, Tsutsumi Y, Hatsuse M, Okano A, Fuchida S, Kobayashi T, Matsumoto Y, Kuroda J, Kawata-Iida E, et al. The longitudinal analysis of large granular lymphocytosis in patients with Philadelphia chromosome-positive leukemia treated with dasatinib. Int J Hematol. 2015;102(4):426–33.
20. Ito Y, Miyamoto T, Kamimura T, Aoki K, Henzan H, Aoki T, Shiratsuchi M, Kato K, Nagafuji K, Ogawa R, et al. Characteristics of patients with development of large granular lymphocyte expansion among dasatinib-treated patients with relapsed philadelphia chromosome-positive acute lymphoblastic leukemia after allogeneic stem cell transplantation. Cl Lymph Myelom Leuk. 2015;15(3):e47–54.
21. Mohty M, Faucher C, Vey N, Chabannon C, Sainty D, Arnoulet C, Gaugler B, Gastaut JA, Maraninchi D, Olive D, et al. Features of large granular lymphocytes (LGL) expansion following allogeneic stem cell transplantation: a long-term analysis. Leukemia. 2002;16(10):2129–33.
22. Wolniak KL, Goolsby CL, Chen YH, Chenn A, Singhal S, JayeshMehta, Peterson LA. Expansion of a clonal CD8+CD57+ large granular lymphocyte population after autologous stem cell transplant in multiple myeloma. Am J Clin Pathol. 2013;139(2):231–41.
23. Munoz-Ballester J, Chen-Liang TH, Hurtado AM, Heras I, de Arriba F, Garcia-Malo MD, Iniesta P, Lozano ML, Nieto JB, Ortuno FJ, et al. Persistent cytotoxic T lymphocyte expansions after allogeneic haematopoietic stem cell transplantation: kinetics, clinical impact and absence of STAT3 mutations. Br J Haematol. 2016;172(6):937–46.
24. Sabnani I, Tsang P. Are clonal T-cell large granular lymphocytes to blame for unexplained haematological abnormalities? Br J Haematol. 2007;136(1):30–7.
25. Dhodapkar MV, Li CY, Lust JA, Tefferi A, Phyliky RL. Clinical spectrum of clonal proliferations of T-large granular lymphocytes: a T-cell clonopathy of undetermined significance? Blood. 1994;84(5):1620–7.
26. Feng B, Jorgensen JL, Hu Y, Medeiros LJ, Wang SA. TCR-Vbeta flow cytometric analysis of peripheral blood for assessing clonality and disease burden in patients with T cell large granular lymphocyte leukaemia. J Clin Pathol. 2010;63(2):141–6.
27. Singleton TP, Yin B, Teferra A, Mao JZ. Spectrum of clonal large granular lymphocytes (LGLs) of alphabeta T cells: T-cell clones of undetermined significance, T-cell LGL leukemias, and T-cell immunoclones. Am J Clin Pathol. 2015;144(1):137–44.
28. Carding SR, Egan PJ. Gammadelta T cells: functional plasticity and heterogeneity. Nat Rev Immunol. 2002;2(5):336–45.
29. Vroom TM, Scholte G, Ossendorp F, Borst J. Tissue distribution of human gamma delta T cells: no evidence for general epithelial tropism. J Clin Pathol. 1991;44(12):1012–7.
30. Holtmeier W. Compartmentalization gamma/delta T cells and their putative role in mucosal immunity. Crit Rev Immunol. 2003;23(5-6):473–88.
31. Fahrer AM, Konigshofer Y, Kerr EM, Ghandour G, Mack DH, Davis MM, Chien YH. Attributes of gammadelta intraepithelial lymphocytes as suggested by their transcriptional profile. Proc Natl Acad Sci U S A. 2001;98(18):10261–6.
32. Ueta C, Tsuyuguchi I, Kawasumi H, Takashima T, Toba H, Kishimoto S. Increase of gamma/delta T cells in hospital workers who are in close contact with tuberculosis patients. Infect Immun. 1994;62(12):5434–41.
33. Martins EB, Graham AK, Chapman RW, Fleming KA. Elevation of gamma delta T lymphocytes in

peripheral blood and livers of patients with primary sclerosing cholangitis and other autoimmune liver diseases. Hepatology. 1996;23(5):988–93.
34. McClanahan J, Fukushima PI, Stetler-Stevenson M. Increased peripheral blood gamma delta T-cells in patients with lymphoid neoplasia: a diagnostic dilemma in flow cytometry. Cytometry. 1999;38(6): 280–5.
35. Ishiyama KI, Kitawaki T, Sugimoto N, Sozu T, Anzai N, Okada M, Nohgawa M, Hatanaka K, Arima N, Ishikawa T, et al. Principal component analysis uncovers cytomegalovirus-associated NK cell activation in Ph+ leukemia patients treated with dasatinib. Leukemia. 2017;31(1):268.
36. Kim DH, Kamel-Reid S, Chang H, Sutherland R, Jung CW, Kim HJ, Lee JJ, Lipton JH. Natural killer or natural killer/T cell lineage large granular lymphocytosis associated with dasatinib therapy for Philadelphia chromosome positive leukemia. Haematologica. 2009;94(1):135–9.
37. Schiffer CA, Cortes JE, Hochhaus A, Saglio G, le Coutre P, Porkka K, Mustjoki S, Mohamed H, Shah NP. Lymphocytosis after treatment with dasatinib in chronic myeloid leukemia: effects on response and toxicity. Cancer. 2016;122(9):1398–407.
38. Bosch M, Khan FM, Storek J. Immune reconstitution after hematopoietic cell transplantation. Curr Opin Hematol. 2012;19(4):324–35.
39. Marcenaro E, Carlomagno S, Pesce S, Moretta A, Sivori S. Bridging innate NK cell functions with adaptive immunity. Adv Exp Med Biol. 2011;780:45–55.
40. Cooper MA, Fehniger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001;22(11):633–40.
41. Epling-Burnette PK, Liu JH, Catlett-Falcone R, Turkson J, Oshiro M, Kothapalli R, Li Y, Wang JM, Yang-Yen HF, Karras J, et al. Inhibition of STAT3 signaling leads to apoptosis of leukemic large granular lymphocytes and decreased Mcl-1 expression. J Clin Invest. 2001;107(3):351–62.
42. Zhang R, Shah MV, Yang J, Nyland SB, Liu X, Yun JK, Albert R, Loughran TP Jr. Network model of survival signaling in large granular lymphocyte leukemia. Proc Natl Acad Sci U S A. 2008;105(42):16308–13.
43. Jerez A, Clemente MJ, Makishima H, Koskela H, Leblanc F, Peng Ng K, Olson T, Przychodzen B, Afable M, Gomez-Segui I, et al. STAT3 mutations unify the pathogenesis of chronic lymphoproliferative disorders of NK cells and T-cell large granular lymphocyte leukemia. Blood. 2012;120(15):3048–57.
44. Rajala HL, Porkka K, Maciejewski JP, Loughran TP Jr, Mustjoki S. Uncovering the pathogenesis of large granular lymphocytic leukemia-novel STAT3 and STAT5b mutations. Ann Med. 2014;46(3):114–22.
45. Koskela HL, Eldfors S, Ellonen P, van Adrichem AJ, Kuusanmaki H, Andersson EI, Lagstrom S, Clemente MJ, Olson T, Jalkanen SE, et al. Somatic STAT3 mutations in large granular lymphocytic leukemia. N Engl J Med. 2012;366(20):1905–13.
46. Swerdlow SH, Campo E, Pileri SA, Harris NL, Stein H, Siebert R, Advani R, Ghielmini M, Salles GA, Zelenetz AD, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. Blood. 2016;127(20):2375–90.
47. Loughran TP Jr. Clonal diseases of large granular lymphocytes. Blood. 1993;82(1):1–14.
48. Steinway SN, LeBlanc F, Loughran TP Jr. The pathogenesis and treatment of large granular lymphocyte leukemia. Blood Rev. 2014;28(3):87–94.
49. Kwong YL, Wong KF. Association of pure red cell aplasia with T large granular lymphocyte leukaemia. J Clin Pathol. 1998;51(9):672–5.
50. Bareau B, Rey J, Hamidou M, Donadieu J, Morcet J, Reman O, Schleinitz N, Tournilhac O, Roussel M, Fest T, et al. Analysis of a French cohort of patients with large granular lymphocyte leukemia: a report on 229 cases. Haematologica. 2010;95(9):1534–41.
51. Lamy T, Loughran TP Jr. How I treat LGL leukemia. Blood. 2011;117(10):2764–74.
52. Garrido P, Ruiz-Cabello F, Barcena P, Sandberg Y, Canton J, Lima M, Balanzategui A, Gonzalez M, Lopez-Nevot MA, Langerak AW, et al. Monoclonal TCR-Vbeta13.1+/CD4+/NKa+/CD8-/+dim T-LGL lymphocytosis: evidence for an antigen-driven chronic T-cell stimulation origin. Blood. 2007;109(11):4890–8.
53. Lima M, Almeida J, Dos Anjos TM, Alguero Md Mdel C, Santos AH, Balanzategui A, Queiros ML, Barcena P, Izarra A, Fonseca S, et al. TCRalphabeta+/CD4+ large granular lymphocytosis: a new clonal T-cell lymphoproliferative disorder. Am J Pathol. 2003;163(2):763–71.
54. Richards SJ, Sivakumaran M, Parapia LA, Balfour I, Norfolk DR, Kaeda J, Scott CS. A distinct large granular lymphocyte (LGL)/NK-associated (NKa) abnormality characterized by membrane CD4 and CD8 coexpression. The Yorkshire Leukaemia Group. Br J Haematol. 1992;82(3):494–501.
55. Au WY, Lam CC, Lie AK, Pang A, Kwong YL. T-cell large granular lymphocyte leukemia of donor origin after allogeneic bone marrow transplantation. Am J Clin Pathol. 2003;120(4):626–30.
56. Gill H, Ip AH, Leung R, So JC, Pang AW, Tse E, Leung AY, Lie AK, Kwong YL. Indolent T-cell large granular lymphocyte leukaemia after haematopoietic SCT: a clinicopathologic and molecular analysis. Bone Marrow Transplant. 2012;47(7):952–6.
57. Lopez JE, Yabe M, Carballo-Zarate AA, Wang SA, Jorgensen JL, Ahmed S, Lee J, Li S, Schlette E, McDonnell T, et al. Donor-derived T-cell large granular lymphocytic leukemia in a patient with peripheral T-cell lymphoma. J Natl Compr Cancer Netw. 2016;14(8):939–44.
58. Howard MT, Bejanyan N, Maciejewski JP, Hsi ED. T/NK large granular lymphocyte leukemia and coexisting monoclonal B-cell lymphocytosis-like proliferations. An unrecognized and frequent association. Am J Clin Pathol. 2010;133(6):936–41.
59. Viny AD, Lichtin A, Pohlman B, Loughran T, Maciejewski J. Chronic B-cell dyscrasias are an

important clinical feature of T-LGL leukemia. Leuk Lymphoma. 2008;49(5):932–8.
60. Hanada T, Ishida T, Kojima H, Tsuchiya T. Granular lymphocyte leukaemia in association with multiple myeloma. Br J Haematol. 1992;80(1):127–9.
61. Huh YO, Medeiros LJ, Ravandi F, Konoplev S, Jorgensen JL, Miranda RN. T-cell large granular lymphocyte leukemia associated with myelodysplastic syndrome: a clinicopathologic study of nine cases. Am J Clin Pathol. 2009;131(3):347–56.
62. Sauntharajah Y, Molldrem JL, Rivera M, Williams A, Stetler-Stevenson M, Sorbara L, Young NS, Barrett JA. Coincident myelodysplastic syndrome and T-cell large granular lymphocytic disease: clinical and pathophysiological features. Br J Haematol. 2001;112(1):195–200.
63. Chan WC, Foucar K, Morice WG. T-cell large granular lymphocytic leukemia. The world health organization classification of hematolymphoid neoplasms Lyon, France: IARC; 2008.
64. Neff JL, Howard MT, Morice WG. Distinguishing T-cell large granular lymphocytic leukemia from reactive conditions: laboratory tools and challenges in their use. Surg Pathol Clin. 2013;6(4):631–9.
65. Ohgami RS, Ohgami JK, Pereira IT, Gitana G, Zehnder JL, Arber DA. Refining the diagnosis of T-cell large granular lymphocytic leukemia by combining distinct patterns of antigen expression with T-cell clonality studies. Leukemia. 2011;25(9):1439–43.
66. Alekshun TJ, Tao J, Sokol L. Aggressive T-cell large granular lymphocyte leukemia: a case report and review of the literature. Am J Hematol. 2007;82(6):481–5.
67. Yabe M, Medeiros LJ, Wang SA, Konoplev S, Ok CY, Loghavi S, Lu G, Flores L, Khoury JD, Cason RC, et al. Clinicopathologic, immunophenotypic, cytogenetic, and molecular features of gammadelta T-cell large granular lymphocytic leukemia: an analysis of 14 patients suggests biologic differences with alpha-beta t-cell large granular lymphocytic leukemia. [corrected]. Am J Clin Pathol. 2015;144(4):607–19.
68. Yabe M, Medeiros LJ, Wang SA, Tang G, Buesso-Ramos CE, Jorgensen JL, Bhagat G, Chen W, Li S, Young KH, et al. Distinguishing between hepatosplenic T-cell lymphoma and gammadelta T-cell large granular lymphocytic leukemia: a clinicopathologic, immunophenotypic, and molecular analysis. Am J Surg Pathol. 2017;41(1):82–93.
69. Morice WG, Kurtin PJ, Tefferi A, Hanson CA. Distinct bone marrow findings in T-cell granular lymphocytic leukemia revealed by paraffin section immunoperoxidase stains for CD8, TIA-1, and granzyme B. Blood. 2002;99(1):268–74.
70. Wong KF, Chan JC, Liu HS, Man C, Kwong YL. Chromosomal abnormalities in T-cell large granular lymphocyte leukaemia: report of two cases and review of the literature. Br J Haematol. 2002;116(3):598–600.
71. Clemente MJ, Wlodarski MW, Makishima H, Viny AD, Bretschneider I, Shaik M, Bejanyan N, Lichtin AE, Hsi ED, Paquette RL, et al. Clonal drift demonstrates unexpected dynamics of the T-cell repertoire in T-large granular lymphocyte leukemia. Blood. 2011;118(16):4384–93.
72. Rajala HL, Olson T, Clemente MJ, Lagstrom S, Ellonen P, Lundan T, Hamm DE, Zaman SA, Lopez Marti JM, Andersson EI, et al. The analysis of clonal diversity and therapy responses using STAT3 mutations as a molecular marker in large granular lymphocytic leukemia. Haematologica. 2015;100(1):91–9.
73. Rajala HL, Eldfors S, Kuusanmaki H, van Adrichem AJ, Olson T, Lagstrom S, Andersson EI, Jerez A, Clemente MJ, Yan Y, et al. Discovery of somatic STAT5b mutations in large granular lymphocytic leukemia. Blood. 2013;121(22):4541–50.
74. Epling-Burnette PK, Bai F, Wei S, Chaurasia P, Painter JS, Olashaw N, Hamilton A, Sebti S, Djeu JY, Loughran TP. ERK couples chronic survival of NK cells to constitutively activated Ras in lymphoproliferative disease of granular lymphocytes (LDGL). Oncogene. 2004;23(57):9220–9.
75. Rabbani GR, Phyliky RL, Tefferi A. A long-term study of patients with chronic natural killer cell lymphocytosis. Br J Haematol. 1999;106(4):960–6.
76. Oshimi K. Progress in understanding and managing natural killer-cell malignancies. Br J Haematol. 2007;139(4):532–44.
77. Barcena P, Jara-Acevedo M, Tabernero MD, Lopez A, Sanchez ML, Garcia-Montero AC, Munoz-Garcia N, Vidriales MB, Paiva A, Lecrevisse Q, et al. Phenotypic profile of expanded NK cells in chronic lymphoproliferative disorders: a surrogate marker for NK-cell clonality. Oncotarget. 2015;6(40):42938–51.
78. Morice WG, Kurtin PJ, Leibson PJ, Tefferi A, Hanson CA. Demonstration of aberrant T-cell and natural killer-cell antigen expression in all cases of granular lymphocytic leukaemia. Br J Haematol. 2003;120(6):1026–36.
79. Tien HF, Su IJ, Tang JL, Liu MC, Lee FY, Chen YC, Chuang SM. Clonal chromosomal abnormalities as direct evidence for clonality in nasal T/natural killer cell lymphomas. Br J Haematol. 1997;97(3):621–5.
80. Zambello R, Falco M, Della Chiesa M, Trentin L, Carollo D, Castriconi R, Cannas G, Carlomagno S, Cabrelle A, Lamy T, et al. Expression and function of KIR and natural cytotoxicity receptors in NK-type lymphoproliferative diseases of granular lymphocytes. Blood. 2003;102(5):1797–805.
81. Landay A, Gebel H, Levin S, Prasthofer E, Pistoia V, Downing J, Grossi C. CD16+ NK lymphoproliferative disorders: cellular and molecular characterization. Nat Immun Cell Growth Regul. 1987;6(3):141–9.
82. Allen RC, Zoghbi HY, Moseley AB, Rosenblatt HM, Belmont JW. Methylation of HpaII and HhaI sites near the polymorphic CAG repeat in the human androgen-receptor gene correlates with X chromosome inactivation. Am J Hum Genet. 1992;51(6):1229–39.
83. Epling-Burnette PK, Painter JS, Chaurasia P, Bai F, Wei S, Djeu JY, Loughran TP Jr. Dysregulated NK receptor expression in patients with lymphopro-

liferative disease of granular lymphocytes. Blood. 2004;103(9):3431–9.
84. Suzuki R, Suzumiya J, Nakamura S, Aoki S, Notoya A, Ozaki S, Gondo H, Hino N, Mori H, Sugimori H, et al. Aggressive natural killer-cell leukemia revisited: large granular lymphocyte leukemia of cytotoxic NK cells. Leukemia. 2004;18(4):763–70.
85. Ruskova A, Thula R, Chan G. Aggressive natural killer-cell leukemia: report of five cases and review of the literature. Leuk Lymphoma. 2004;45(12):2427–38.
86. Li Y, Wei J, Mao X, Gao Q, Liu L, Cheng P, Liu L, Zhang X, Zhang K, Wang J, et al. Flow cytometric immunophenotyping is sensitive for the early diagnosis of de novo aggressive natural killer cell leukemia (ankl): a multicenter retrospective analysis. PLoS One. 2016;11(8):e0158827.
87. Nicolae A, Ganapathi KA, Pham TH, Xi L, Torres-Cabala CA, Nanaji NM, Zha HD, Fan Z, Irwin S, Pittaluga S, et al. EBV-negative aggressive NK-cell leukemia/lymphoma: clinical, pathologic, and genetic features. Am J Surg Pathol. 2017;41(1):67–74.
88. Gao LM, Zhao S, Liu WP, Zhang WY, Li GD, Kucuk C, Hu XZ, Chan WC, Tang Y, Ding WS, et al. Clinicopathologic characterization of aggressive natural killer cell leukemia involving different tissue sites. Am J Surg Pathol. 2016;40(6):836–46.
89. Shi Y, Wang E. Hepatosplenic T-cell lymphoma: a clinicopathologic review with an emphasis on diagnostic differentiation from other T-cell/natural killer-cell neoplasms. Arch Pathol Lab Med. 2015;139(9):1173–80.
90. Tripodo C, Iannitto E, Florena AM, Pucillo CE, Piccaluga PP, Franco V, Pileri SA. Gamma-delta T-cell lymphomas. Nat Rev Clin Oncol. 2009;6(12):707–17.
91. Belhadj K, Reyes F, Farcet JP, Tilly H, Bastard C, Angonin R, Deconinck E, Charlotte F, Leblond V, Labouyrie E, et al. Hepatosplenic gammadelta T-cell lymphoma is a rare clinicopathologic entity with poor outcome: report on a series of 21 patients. Blood. 2003;102(13):4261–9.
92. Parakkal D, Sifuentes H, Semer R, Ehrenpreis ED. Hepatosplenic T-cell lymphoma in patients receiving TNF-alpha inhibitor therapy: expanding the groups at risk. Eur J Gastroenterol Hepatol. 2011;23(12):1150–6.
93. Vose J, Armitage J, Weisenburger D, International TCLP. International peripheral T-cell and natural killer/T-cell lymphoma study: pathology findings and clinical outcomes. J Clin Oncol. 2008;26(25):4124–30.
94. Macon WR, Levy NB, Kurtin PJ, Salhany KE, Elkhalifa MY, Casey TT, Craig FE, Vnencak-Jones CL, Gulley ML, Park JP, et al. Hepatosplenic alpha-beta T-cell lymphomas: a report of 14 cases and comparison with hepatosplenic gammadelta T-cell lymphomas. Am J Surg Pathol. 2001;25(3):285–96.
95. Yabe M, Medeiros LJ, Tang G, Wang SA, Patel KP, Routbort M, Bhagat G, Bueso-Ramos CE, Jorgensen JL, Luthra R, et al. Dyspoietic changes associated with hepatosplenic T-cell lymphoma are not a manifestation of a myelodysplastic syndrome: analysis of 25 patients. Hum Pathol. 2016;50:109–17.
96. Yabe M, Medeiros LJ, Tang G, Wang SA, Ahmed S, Nieto Y, Hu S, Bhagat G, Oki Y, Patel KP, et al. Prognostic factors of hepatosplenic T-cell lymphoma: clinicopathologic study of 28 cases. Am J Surg Pathol. 2016;40(5):676–88.
97. Ohshima K, Haraoka S, Harada N, Kamimura T, Suzumiya J, Kanda M, Kawasaki C, Sugihara M, Kikuchi M. Hepatosplenic gammadelta T-cell lymphoma: relation to Epstein–Barr virus and activated cytotoxic molecules. Histopathology. 2000;36(2):127–35.
98. Jonveaux P, Daniel MT, Martel V, Maarek O, Berger R. Isochromosome 7q and trisomy 8 are consistent primary, non-random chromosomal abnormalities associated with hepatosplenic T gamma/delta lymphoma. Leukemia. 1996;10(9):1453–5.
99. Wang CC, Tien HF, Lin MT, Su IJ, Wang CH, Chuang SM, Shen MC, Liu CH. Consistent presence of isochromosome 7q in hepatosplenic T gamma/delta lymphoma: a new cytogenetic-clinicopathologic entity. Genes Chromosom Cancer. 1995;12(3):161–4.
100. McKinney M, Moffitt AB, Gaulard P, Travert M, De Leval L, Nicolae A, Raffeld M, Jaffe ES, Pittaluga S, Xi L, et al. The genetic basis of hepatosplenic T-cell lymphoma. Cancer Discov. 2017;7(4):369–79. https://doi.org/10.1158/2159-8290.CD-16-0330. Epub 2017 Jan 25.
101. Willemze R, Jaffe ES, Burg G, Cerroni L, Berti E, Swerdlow SH, Ralfkiaer E, Chimenti S, Diaz-Perez JL, Duncan LM, et al. WHO-EORTC classification for cutaneous lymphomas. Blood. 2005;105(10):3768–85.
102. Olsen E, Vonderheid E, Pimpinelli N, Willemze R, Kim Y, Knobler R, Zackheim H, Duvic M, Estrach T, Lamberg S, et al. Revisions to the staging and classification of mycosis fungoides and Sezary syndrome: a proposal of the International Society for Cutaneous Lymphomas (ISCL) and the cutaneous lymphoma task force of the European Organization of Research and Treatment of Cancer (EORTC). Blood. 2007;110(6):1713–22.
103. Olsen EA, Whittaker S, Kim YH, Duvic M, Prince HM, Lessin SR, Wood GS, Willemze R, Demierre MF, Pimpinelli N, et al. Clinical end points and response criteria in mycosis fungoides and Sezary syndrome: a consensus statement of the International Society for Cutaneous Lymphomas, the United States Cutaneous Lymphoma Consortium, and the Cutaneous Lymphoma Task Force of the European Organisation for Research and Treatment of Cancer. J Clin Oncol. 2011;29(18):2598–607.
104. Huang KP, Weinstock MA, Clarke CA, McMillan A, Hoppe RT, Kim YH. Second lymphomas and other malignant neoplasms in patients with mycosis fungoides and Sezary syndrome: evidence from population-based and clinical cohorts. Arch Dermatol. 2007;143(1):45–50.
105. Introcaso CE, Hess SD, Kamoun M, Ubriani R, Gelfand JM, Rook AH. Association of change in clinical status and change in the percentage of the CD4+CD26− lym-

phocyte population in patients with Sezary syndrome. J Am Acad Dermatol. 2005;53(3):428–34.
106. Boonk SE, Zoutman WH, Marie-Cardine A, van der Fits L, Out-Luiting JJ, Mitchell TJ, Tosi I, Morris SL, Moriarty B, Booken N, et al. Evaluation of immunophenotypic and molecular biomarkers for Sezary syndrome using standard operating procedures: a multicenter study of 59 patients. J Invest Dermatol. 2016;136(7):1364–72.
107. Jones D, Dang NH, Duvic M, Washington LT, Huh YO. Absence of CD26 expression is a useful marker for diagnosis of T-cell lymphoma in peripheral blood. Am J Clin Pathol. 2001;115(6):885–92.
108. Meyerson HJ, Awadallah A, Pavlidakey P, Cooper K, Honda K, Miedler J. Follicular center helper T-cell (TFH) marker positive mycosis fungoides/Sezary syndrome. Mod Pathol. 2013;26(1):32–43.
109. Bosisio FM, Cerroni L. Expression of T-follicular helper markers in sequential biopsies of progressive mycosis fungoides and other primary cutaneous T-cell lymphomas. Am J Dermatopathol. 2015;37(2):115–21.
110. McGirt LY, Jia P, Baerenwald DA, Duszynski RJ, Dahlman KB, Zic JA, Zwerner JP, Hucks D, Dave U, Zhao Z, et al. Whole-genome sequencing reveals oncogenic mutations in mycosis fungoides. Blood. 2015;126(4):508–19.
111. Choi J, Goh G, Walradt T, Hong BS, Bunick CG, Chen K, Bjornson RD, Maman Y, Wang T, Tordoff J, et al. Genomic landscape of cutaneous T cell lymphoma. Nat Genet. 2015;47(9):1011–9.
112. Woollard WJ, Pullabhatla V, Lorenc A, Patel VM, Butler RM, Bayega A, Begum N, Bakr F, Dedhia K, Fisher J, et al. Candidate driver genes involved in genome maintenance and DNA repair in Sezary syndrome. Blood. 2016;127(26):3387–97.
113. da Silva Almeida AC, Abate F, Khiabanian H, Martinez-Escala E, Guitart J, Tensen CP, Vermeer MH, Rabadan R, Ferrando A, Palomero T. The mutational landscape of cutaneous T cell lymphoma and Sezary syndrome. Nat Genet. 2015;47(12):1465–70.
114. Kiel MJ, Sahasrabuddhe AA, Rolland DC, Velusamy T, Chung F, Schaller M, Bailey NG, Betz BL, Miranda RN, Porcu P, et al. Genomic analyses reveal recurrent mutations in epigenetic modifiers and the JAK-STAT pathway in Sezary syndrome. Nat Commun. 2015;6:8470.
115. Klemke CD, Booken N, Weiss C, Nicolay JP, Goerdt S, Felcht M, Geraud C, Kempf W, Assaf C, Ortonne N, et al. Histopathological and immunophenotypical criteria for the diagnosis of Sezary syndrome in differentiation from other erythrodermic skin diseases: a European Organisation for Research and Treatment of Cancer (EORTC) Cutaneous Lymphoma Task Force Study of 97 cases. Br J Dermatol. 2015;173(1):93–105.
116. Baseggio L, Berger F, Morel D, Delfau-Larue MH, Goedert G, Salles G, Magaud JP, Felman P. Identification of circulating CD10 positive T cells in angioimmunoblastic T-cell lymphoma. Leukemia. 2006;20(2):296–303.
117. Loghavi S, Wang SA, Jeffrey Medeiros L, Jorgensen JL, Li X, Xu-Monette ZY, Miranda RN, Young KH. Immunophenotypic and diagnostic characterization of angioimmunoblastic T-cell lymphoma by advanced flow cytometric technology. Leuk Lymphoma. 2016;57(12):2804–12.
118. Matutes E, Brito-Babapulle V, Swansbury J, Ellis J, Morilla R, Dearden C, Sempere A, Catovsky D. Clinical and laboratory features of 78 cases of T-prolymphocytic leukemia. Blood. 1991;78(12):3269–74.
119. Brito-Babapulle V, Catovsky D. Inversions and tandem translocations involving chromosome 14q11 and 14q32 in T-prolymphocytic leukemia and T-cell leukemias in patients with ataxia telangiectasia. Cancer Genet Cytogenet. 1991;55(1):1–9.
120. Taylor AM, Metcalfe JA, Thick J, Mak YF. Leukemia and lymphoma in ataxia telangiectasia. Blood. 1996;87(2):423–38.
121. Chen X, Cherian S. Immunophenotypic characterization of T-cell prolymphocytic leukemia. Am J Clin Pathol. 2013;140(5):727–35.
122. Garand R, Goasguen J, Brizard A, Buisine J, Charpentier A, Claisse JF, Duchayne E, Lagrange M, Segonds C, Troussard X, et al. Indolent course as a relatively frequent presentation in T-prolymphocytic leukaemia. Groupe Francais d'Hematologie Cellulaire. Br J Haematol. 1998;103(2):488–94.
123. Krishnan B, Matutes E, Dearden C. Prolymphocytic leukemias. Semin Oncol. 2006;33(2):257–63.
124. Yuille MR, Condie A, Stone EM, Wilsher J, Bradshaw PS, Brooks L, Catovsky D. TCL1 is activated by chromosomal rearrangement or by hypomethylation. Genes Chromosom Cancer. 2001;30(4):336–41.
125. Pekarsky Y, Hallas C, Croce CM. Molecular basis of mature T-cell leukemia. JAMA. 2001;286(18):2308–14.
126. Pekarsky Y, Hallas C, Croce CM. The role of TCL1 in human T-cell leukemia. Oncogene. 2001;20(40):5638–43.
127. Herling M, Patel KA, Teitell MA, Konopleva M, Ravandi F, Kobayashi R, Jones D. High TCL1 expression and intact T-cell receptor signaling define a hyperproliferative subset of T-cell prolymphocytic leukemia. Blood. 2008;111(1):328–37.
128. Feldman AL, Law M, Grogg KL, Thorland EC, Fink S, Kurtin PJ, Macon WR, Remstein ED, Dogan A. Incidence of TCR and TCL1 gene translocations and isochromosome 7q in peripheral T-cell lymphomas using fluorescence in situ hybridization. Am J Clin Pathol. 2008;130(2):178–85.
129. Gritti C, Dastot H, Soulier J, Janin A, Daniel MT, Madani A, Grimber G, Briand P, Sigaux F, Stern MH. Transgenic mice for MTCP1 develop T-cell prolymphocytic leukemia. Blood. 1998;92(2):368–73.
130. Maljaei SH, Brito-Babapulle V, Hiorns LR, Catovsky D. Abnormalities of chromosomes 8, 11, 14, and X in T-prolymphocytic leukemia studied by fluorescence in situ hybridization. Cancer Genet Cytogenet. 1998;103(2):110–6.

131. Stilgenbauer S, Schaffner C, Litterst A, Liebisch P, Gilad S, Bar-Shira A, James MR, Lichter P, Dohner H. Biallelic mutations in the ATM gene in T-prolymphocytic leukemia. Nat Med. 1997;3(10):1155–9.
132. Vorechovsky I, Luo L, Dyer MJ, Catovsky D, Amlot PL, Yaxley JC, Foroni L, Hammarstrom L, Webster AD, Yuille MA. Clustering of missense mutations in the ataxia-telangiectasia gene in a sporadic T-cell leukaemia. Nat Genet. 1997;17(1):96–9.
133. Bellanger D, Jacquemin V, Chopin M, Pierron G, Bernard OA, Ghysdael J, Stern MH. Recurrent JAK1 and JAK3 somatic mutations in T-cell prolymphocytic leukemia. Leukemia. 2014;28(2):417–9.
134. Bergmann AK, Schneppenheim S, Seifert M, Betts MJ, Haake A, Lopez C, Maria Murga Penas E, Vater I, Jayne S, Dyer MJ, et al. Recurrent mutation of JAK3 in T-cell prolymphocytic leukemia. Genes Chromosom Cancer. 2014;53(4):309–16.
135. Lopez C, Bergmann AK, Paul U, Murga Penas EM, Nagel I, Betts MJ, Johansson P, Ritgen M, Baumann T, Aymerich M, et al. Genes encoding members of the JAK-STAT pathway or epigenetic regulators are recurrently mutated in T-cell prolymphocytic leukaemia. Br J Haematol. 2016;173(2):265–73.
136. Phillips AA, Shapira I, Willim RD, Sanmugarajah J, Solomon WB, Horwitz SM, Savage DG, Bhagat G, Soff G, Zain JM, et al. A critical analysis of prognostic factors in North American patients with human T-cell lymphotropic virus type-1-associated adult T-cell leukemia/lymphoma: a multicenter clinicopathologic experience and new prognostic score. Cancer. 2010;116(14):3438–46.
137. Matutes E. Adult T-cell leukaemia/lymphoma. J Clin Pathol. 2007;60(12):1373–7.
138. Murphy EL, Hanchard B, Figueroa JP, Gibbs WN, Lofters WS, Campbell M, Goedert JJ, Blattner WA. Modelling the risk of adult T-cell leukemia/lymphoma in persons infected with human T-lymphotropic virus type I. Int J Cancer. 1989;43(2):250–3.
139. Matsuoka M. Human T-cell leukemia virus type I (HTLV-I) infection and the onset of adult T-cell leukemia (ATL). Retrovirology. 2005;2:27.
140. Bunn PA Jr, Schechter GP, Jaffe E, Blayney D, Young RC, Matthews MJ, Blattner W, Broder S, Robert-Guroff M, Gallo RC. Clinical course of retrovirus-associated adult T-cell lymphoma in the United States. N Engl J Med. 1983;309(5):257–64.
141. Katsuya H, Ishitsuka K, Utsunomiya A, Hanada S, Eto T, Moriuchi Y, Saburi Y, Miyahara M, Sueoka E, Uike N, et al. Treatment and survival among 1594 patients with ATL. Blood. 2015;126(24):2570–7.
142. Sawada Y, Hino R, Hama K, Ohmori S, Fueki H, Yamada S, Fukamachi S, Tajiri M, Kubo R, Yoshioka M, et al. Type of skin eruption is an independent prognostic indicator for adult T-cell leukemia/lymphoma. Blood. 2011;117(15):3961–7.
143. Takasaki Y, Iwanaga M, Imaizumi Y, Tawara M, Joh T, Kohno T, Yamada Y, Kamihira S, Ikeda S, Miyazaki Y, et al. Long-term study of indolent adult T-cell leukemia-lymphoma. Blood. 2010;115(22):4337–43.
144. Jaffe ES, Blattner WA, Blayney DW, Bunn PA Jr, Cossman J, Robert-Guroff M, Gallo RC. The pathologic spectrum of adult T-cell leukemia/lymphoma in the United States. Human T-cell leukemia/lymphoma virus-associated lymphoid malignancies. Am J Surg Pathol. 1984;8(4):263–75.
145. Duggan DB, Ehrlich GD, Davey FP, Kwok S, Sninsky J, Goldberg J, Baltrucki L, Poiesz BJ. HTLV-I-induced lymphoma mimicking Hodgkin's disease. Diagnosis by polymerase chain reaction amplification of specific HTLV-I sequences in tumor DNA. Blood. 1988;71(4):1027–32.
146. Kagdi HH, Demontis MA, Fields PA, Ramos JC, Bangham CR, Taylor GP. Risk stratification of adult T-cell leukemia/lymphoma using immunophenotyping. Cancer Med. 2017;6(1):298–309.
147. Itoyama T, Chaganti RS, Yamada Y, Tsukasaki K, Atogami S, Nakamura H, Tomonaga M, Ohshima K, Kikuchi M, Sadamori N. Cytogenetic analysis and clinical significance in adult T-cell leukemia/lymphoma: a study of 50 cases from the human T-cell leukemia virus type-1 endemic area, Nagasaki. Blood. 2001;97(11):3612–20.
148. Kamada N, Sakurai M, Miyamoto K, Sanada I, Sadamori N, Fukuhara S, Abe S, Shiraishi Y, Abe T, Kaneko Y, et al. Chromosome abnormalities in adult T-cell leukemia/lymphoma: a karyotype review committee report. Cancer Res. 1992;52(6):1481–93.
149. Watanabe T. Adult T-cell leukemia (ATL): molecular basis for clonal expansion and transformation of HTLV-1-infected T cells. Blood. 2017;129(9):1071–81.
150. Onciu M, Behm FG, Raimondi SC, Moore S, Harwood EL, Pui CH, Sandlund JT. ALK-positive anaplastic large cell lymphoma with leukemic peripheral blood involvement is a clinicopathologic entity with an unfavorable prognosis. Report of three cases and review of the literature. Am J Clin Pathol. 2003;120(4):617–25.
151. van den Berg H, Noorduyn A, van Kuilenburg AB, Kroes W, de Jong D. Leukaemic expression of anaplastic large cell lymphoma with 46,XX,ins(2;5)(p23;q15q35) in a child with dihydropyrimidine dehydrogenase deficiency. Leukemia. 2000;14(4):769–70.
152. Bayle C, Charpentier A, Duchayne E, Manel AM, Pages MP, Robert A, Lamant L, Dastugue N, Bertrand Y, Dijoud F, et al. Leukaemic presentation of small cell variant anaplastic large cell lymphoma: report of four cases. Br J Haematol. 1999;104(4):680–8.
153. Ok CY, Wang SA, Amin HM. Leukemic phase of ALK(+) anaplastic large-cell lymphoma, small-cell variant: clinicopathologic pitfalls of a rare entity. Clin Lymphoma Myeloma Leuk. 2014;14(4):e123–6.
154. Awaya N, Mori S, Takeuchi H, Mori S, Sugano Y, Kamata T, Takeuchi T, Abe T. CD30 and the NPM-ALK fusion protein (p80) are differentially expressed between peripheral blood and bone marrow in primary small cell variant of anaplastic large cell lymphoma. Am J Hematol. 2002;69(3):200–4.

155. Juco J, Holden JT, Mann KP, Kelley LG, Li S. Immunophenotypic analysis of anaplastic large cell lymphoma by flow cytometry. Am J Clin Pathol. 2003;119(2):205–12.
156. Bernt KM, Armstrong SA. Leukemia stem cells and human acute lymphoblastic leukemia. Semin Hematol. 2009;46(1):33–8.
157. Yamazaki H, Nishida H, Iwata S, Dang NH, Morimoto C. CD90 and CD110 correlate with cancer stem cell potentials in human T-acute lymphoblastic leukemia cells. Biochem Biophys Res Commun. 2009;383(2):172–7.
158. Goldberg JM, Silverman LB, Levy DE, Dalton VK, Gelber RD, Lehmann L, Cohen HJ, Sallan SE, Asselin BL. Childhood T-cell acute lymphoblastic leukemia: the Dana-Farber Cancer Institute acute lymphoblastic leukemia consortium experience. J Clin Oncol. 2003;21(19):3616–22.
159. Inhorn RC, Aster JC, Roach SA, Slapak CA, Soiffer R, Tantravahi R, Stone RM. A syndrome of lymphoblastic lymphoma, eosinophilia, and myeloid hyperplasia/malignancy associated with t(8;13)(p11;q11): description of a distinctive clinicopathologic entity. Blood. 1995;85(7):1881–7.
160. Zhou Y, Fan X, Routbort M, Cameron Yin C, Singh R, Bueso-Ramos C, Thomas DA, Milton DR, Medeiros LJ, Lin P. Absence of terminal deoxynucleotidyl transferase expression identifies a subset of high-risk adult T-lymphoblastic leukemia/lymphoma. Mod Pathol. 2013;26(10):1338–45.
161. Hann IM, Richards SM, Eden OB, Hill FG. Analysis of the immunophenotype of children treated on the Medical Research Council United Kingdom Acute Lymphoblastic Leukaemia Trial XI (MRC UKALLXI). Medical Research Council Childhood Leukaemia Working Party. Leukemia. 1998;12(8):1 249–55.
162. Paietta E, Ferrando AA, Neuberg D, Bennett JM, Racevskis J, Lazarus H, Dewald G, Rowe JM, Wiernik PH, Tallman MS, et al. Activating FLT3 mutations in CD117/KIT(+) T-cell acute lymphoblastic leukemias. Blood. 2004;104(2):558–60.
163. Pilozzi E, Pulford K, Jones M, Muller-Hermelink HK, Falini B, Ralfkiaer E, Pileri S, Pezzella F, De Wolf-Peeters C, Arber D, et al. Co-expression of CD79a (JCB117) and CD3 by lymphoblastic lymphoma. J Pathol. 1998;186(2):140–3.
164. Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, van't Veer MB. Proposals for the immunological classification of acute leukemias. European Group for the Immunological Characterization of Leukemias (EGIL). Leukemia. 1995;9(10):1783–6.
165. Coustan-Smith E, Mullighan CG, Onciu M, Behm FG, Raimondi SC, Pei D, Cheng C, Su X, Rubnitz JE, Basso G, et al. Early T-cell precursor leukaemia: a subtype of very high-risk acute lymphoblastic leukaemia. Lancet Oncol. 2009;10(2):147–56.
166. Jain N, Lamb AV, O'Brien S, Ravandi F, Konopleva M, Jabbour E, Zuo Z, Jorgensen J, Lin P, Pierce S, et al. Early T-cell precursor acute lymphoblastic leukemia/lymphoma (ETP-ALL/LBL) in adolescents and adults: a high-risk subtype. Blood. 2016;127(15):1863–9.
167. Guo RJ, Bahmanyar M, Minden MD, Chang H. CD33, not early precursor T-cell phenotype, is associated with adverse outcome in adult T-cell acute lymphoblastic leukaemia. Br J Haematol. 2016;172(5):823–5.
168. Patrick K, Wade R, Goulden N, Mitchell C, Moorman AV, Rowntree C, Jenkinson S, Hough R, Vora A. Outcome for children and young people with early T-cell precursor acute lymphoblastic leukaemia treated on a contemporary protocol, UKALL 2003. Br J Haematol. 2014;166(3):421–4.
169. Graux C, Cools J, Michaux L, Vandenberghe P, Hagemeijer A. Cytogenetics and molecular genetics of T-cell acute lymphoblastic leukemia: from thymocyte to lymphoblast. Leukemia. 2006;20(9):1496–510.
170. Han X, Bueso-Ramos CE. Precursor T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma and acute biphenotypic leukemias. Am J Clin Pathol. 2007;127(4):528–44.
171. Szczepanski T, Pongers-Willemse MJ, Langerak AW, Harts WA, Wijkhuijs AJ, van Wering ER, van Dongen JJ. Ig heavy chain gene rearrangements in T-cell acute lymphoblastic leukemia exhibit predominant DH6-19 and DH7-27 gene usage, can result in complete V-D-J rearrangements, and are rare in T-cell receptor alpha beta lineage. Blood. 1999;93(12):4079–85.
172. Weng AP, Ferrando AA, Lee W, JPt M, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT, Aster JC. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. Science. 2004;306(5694):269–71.
173. Malyukova A, Dohda T, von der Lehr N, Akhoondi S, Corcoran M, Heyman M, Spruck C, Grander D, Lendahl U, Sangfelt O. The tumor suppressor gene hCDC4 is frequently mutated in human T-cell acute lymphoblastic leukemia with functional consequences for Notch signaling. Cancer Res. 2007;67(12):5611–6.
174. Zhang J, Ding L, Holmfeldt L, Wu G, Heatley SL, Payne-Turner D, Easton J, Chen X, Wang J, Rusch M, et al. The genetic basis of early T-cell precursor acute lymphoblastic leukaemia. Nature. 2012;481(7380): 157–63.
175. Neumann M, Heesch S, Schlee C, Schwartz S, Gokbuget N, Hoelzer D, Konstandin NP, Ksienzyk B, Vosberg S, Graf A, et al. Whole-exome sequencing in adult ETP-ALL reveals a high rate of DNMT3A mutations. Blood. 2013;121(23):4749–52.
176. Brunetti L, Di Battista V, Venanzi A, Schiavoni G, Martelli MP, Ascani S, Mecucci C, Tiacci E, Falini B. Blastic plasmacytoid dendritic cell neoplasm and chronic myelomonocytic leukemia: a shared clonal origin. Leukemia. 2017;31(5):1238–40.
177. Ohgami RS, Arber DA, Zehnder JL, Natkunam Y, Warnke RA. Indolent T-lymphoblastic proliferation (iT-LBP): a review of clinical and pathologic features

and distinction from malignant T-lymphoblastic lymphoma. Adv Anat Pathol. 2013;20(3):137–40.
178. Ohgami RS, Zhao S, Ohgami JK, Leavitt MO, Zehnder JL, West RB, Arber DA, Natkunam Y, Warnke RA. TdT+ T-lymphoblastic populations are increased in Castleman disease, in Castleman disease in association with follicular dendritic cell tumors, and in angioimmunoblastic T-cell lymphoma. Am J Surg Pathol. 2012;36(11):1619–28.
179. Kansal R, Nathwani BN, Yiakoumis X, Moschogiannis M, Sachanas S, Stefanaki K, Pangalis GA. Exuberant cortical thymocyte proliferation mimicking T-lymphoblastic lymphoma within recurrent large inguinal lymph node masses of localized Castleman disease. Hum Pathol. 2015;46(7):1057–61.
180. Nathwani BN, Kansal R, Yiakoumis X, Pangalis GA. Indolent T-lymphoblastic proliferation: a name with specific meaning--reply. Hum Pathol. 2015;46(11):1786–7.
181. Ohgami RS, Natkunam Y, Warnke RA. Indolent T-lymphoblastic proliferation: a name with specific meaning. Hum Pathol. 2015;46(11):1785–6.

B-Cell Lymphocytosis

12

Robert P. Hasserjian

Overview

Evaluation of the peripheral blood and/or bone marrow is often the initial testing used to make a diagnosis of many B-cell lymphomas. This chapter presents the features and differential diagnoses of B-cell lymphomas that are primarily diagnosed by evaluating peripheral blood (PB) and bone marrow (BM) samples, as well as reactive B-cell proliferations in the bone marrow and blood. The chapter will not discuss bone marrow staging of B-cell and Hodgkin lymphomas that have already been diagnosed in extramedullary tissues.

B-cell lymphomas that primarily involve the marrow and blood present in three general clinical scenarios:

1. B-cell lymphocytosis (an absolute increase in lymphocytes in the peripheral blood, with or without lymphadenopathy or splenomegaly)
2. Cytopenias(s) with neoplastic B cells circulating in the peripheral blood
3. Cytopenias(s) due to marrow infiltration by B-cell lymphoma without identifiable neoplastic cells circulating in the peripheral blood

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The above clinical scenarios can influence the approach to diagnosis, ancillary tests ordered, and type of information conveyed in the pathology report.

In patients presenting with lymphocytosis, it is important to review the peripheral smear to observe the lymphocyte morphology, which is described for each of the lymphomas discussed below. Although there is a considerable overlap in the morphologic spectrum of B-lymphoid leukemias, the cytology of the circulating lymphocytes provides helpful information for final diagnosis and classification. Flow cytometry is a critical test in distinguishing among the various lymphomas that present with lymphocytosis. In many cases (particularly chronic lymphocytic leukemia, the most common lymphoma manifesting as lymphocytosis), a definitive diagnosis can be rendered on the blood, by incorporating peripheral smear morphology, immunophenotype, and genetic studies. In other cases, it may be necessary to perform a bone marrow biopsy to clarify the specific lymphoma subtype causing the lymphocytosis.

Hairy cell leukemia is the most common B-cell leukemia that presents with cytopenias and typically lacks a significant lymphocytosis. This can pose a diagnostic challenge: although circulating neoplastic B-cells are always present, they may be overlooked on morphologic review of the smear, and other causes of cytopenia besides lymphoma may be entertained. Flow cytometry can readily identify the neoplastic circulating hairy cell population, even if it is small,

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since its phenotype is characteristic. In other cases where a B-cell clone is identified in the blood of a cytopenic patient, a definitive diagnosis may be more challenging. Monoclonal B-cell lymphocytosis is relatively common in older individuals and it may be unclear if a clonal B-cell population in the blood reflects a lymphoma or is merely an incidental finding in a patient with cytopenia due to other reasons. Bone marrow examination can be very helpful in this scenario.

Finally, in rare cases, a bone marrow sample may disclose a B-cell lymphoma in a cytopenic patient, even in the absence of any morphologically identifiable circulating neoplastic cells in the peripheral blood. Diffuse large B-cell lymphoma and intravascular large B-cell lymphoma can present in this fashion. Thus, the pathologist should always consider a lymphoma when approaching a bone marrow sample performed to evaluate unexplained cytopenia, even if peripheral blood flow cytometry is negative for lymphoma.

The sections below review the clinical, morphologic, immunophenotypic, and genetic features of B-cell lymphomas primarily involving the bone marrow and blood as well as Blymphoblastic leukemia. The first part of this chapter reviews features of normal B cells and reactive B-cell lymphocytosis; more general aspects of lymphocytosis are discussed separately in Chap. 11. An algorithm for approaching the differential diagnosis of small mature clonal B-cell populations in the blood is shown at the end of this chapter (Fig. 12.31).

Normal B-Cell Immunophenotypes

Approximately 5–20% of the peripheral blood lymphocytes are B-cells; in general, the total number of B cells decreases with age (see Table 11.2, Chap. 11) [1, 2]. Approximately two-thirds of circulating B cells are CD27-CD20+CD19+CD38- naïve B lymphocytes, and one-third are CD27+CD20+CD19+CD38- memory B cells (Table 12.1). In some individuals, especially children, some CD10+ B cells can be detected in peripheral blood. These are early-stage mature B cells (also called "transitional B cells" or "stage 3 hematogones"), which express dim CD10 and bright CD38, CD24, and IgM and lack surface CD27 expression [3]. Early-stage mature B cells can be distinguished from circulating B lymphoblasts by the absence of CD34 and TdT, dim expression of polyclonal surface immunoglobulin, and bright expression of CD20, CD45, and CD38 (Fig. 12.1). Very low numbers of plasma cells (approximately 2/μL) are found in the peripheral blood of healthy individuals, but this number can significantly increase post immunization [4]. These circulating plasma cells are CD38+ and CD27+ with variable CD138 expression and may lack surface light chain expression [5].

Many of the markers mentioned above that define various subsets of lymphocytes are not routinely used for clinical diagnosis. Using a typical antibody panel applied for the diagnosis of B-cell lymphomas, normal mature B cells are CD19+, CD22+, CD20+, CD79a+, CD79b+, CD38 partial/moderate+, FMC7+, CD23partial+, CD200partial+, CD43-, and polytypic by kappa and lambda surface and cytoplasmic light chain assessment; a subset expresses CD11c. At birth, most B cells in cord blood dimly express CD5, and by the time of adulthood, 5–25% of PB B cells and 5–20% of BM B cells are CD5+ [6, 7] (Fig. 12.2). CD5+ B cells can be increased in patients with autoimmune diseases.

Table 12.1 Normal B-cell subsets in the peripheral blood

B-cell subset	Proportion (%)	Immunophenotype	Surface light chain expression pattern
Naïve B cells	60–70	CD27-CD20+CD19+CD38-	Polytypic
Memory B cells	20–40	CD27+CD20+CD19+CD38-	Polytypic
Transitional/immature B cells	2–4	CD27-CD20+CD19+CD38 bright+ CD10+	Weak/dim polytypic
Plasma cells/plasmablasts	0–2	CD27+CD20-CD19+CD38 bright+ CD138 variable	No surface light chain

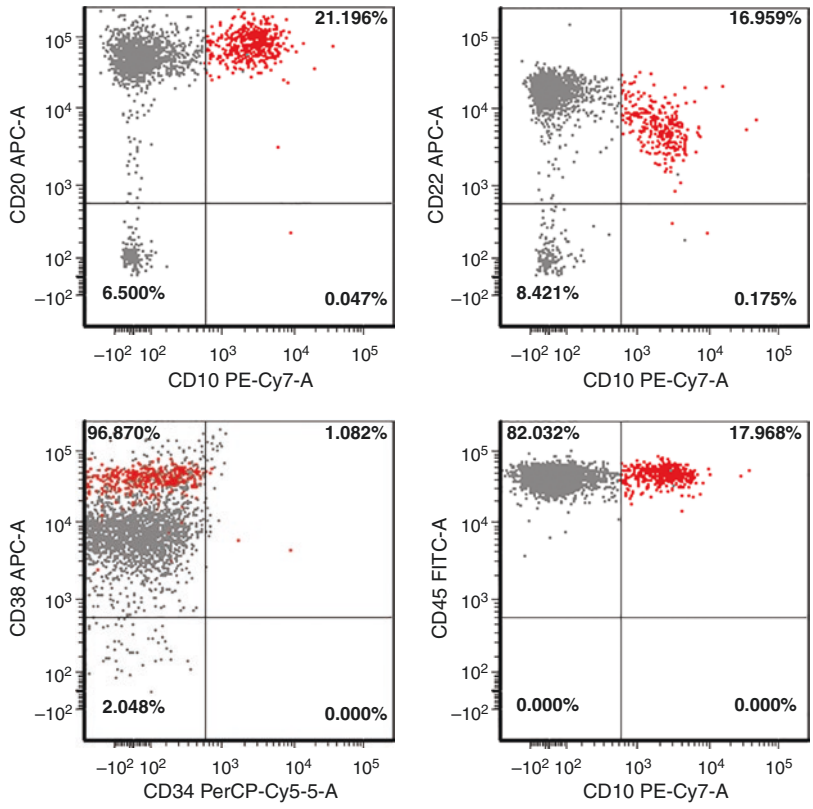

Fig. 12.1 Early-stage mature B cells (*red dots*), also called "transitional B cells," "recent bone marrow emigrant B cells," or "stage 3 hematogones," can be seen in blood, especially in children. These cells are CD10 dim+, CD22 dimmer than mature B cells (*gray dots*), negative for CD34, and brightly positive for CD38, CD45, and CD20

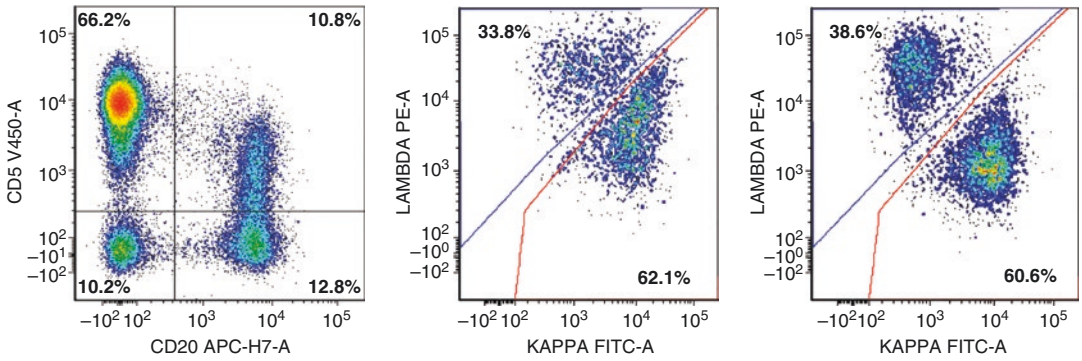

Fig. 12.2 CD5+ normal B cells (*left panel*, comprising 45.8% of the total B cells in this pediatric patient) are polytypic (*middle panel*), as are the CD5- B cells (*right panel*)

In the normal bone marrow, in addition to mature B cells, variable numbers of normal immature B cells (hematogones) are present, which can significantly increase in reactive conditions, especially in children and young adults. Hematogones have bright CD38 expression, are positive for CD43 and CD200, and are dimmer for CD45 than mature lymphocytes. They are divided into three

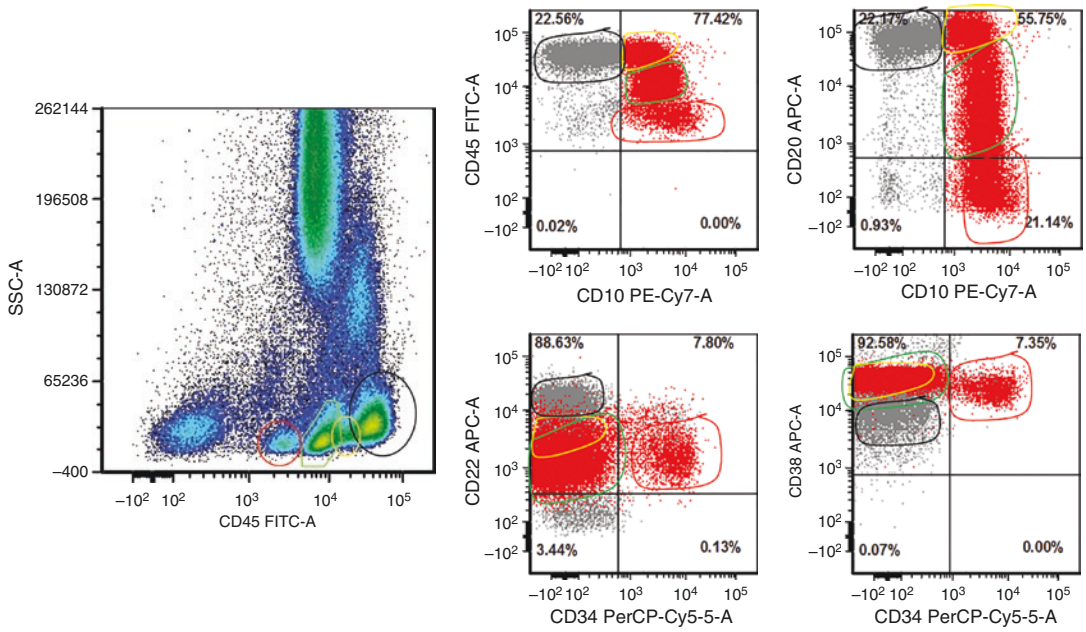

Fig. 12.3 Normal immature (hematogones) and mature B cells in bone marrow. Cell populations are circled in different colors: stage 1 hematogones (*red*), stage 2 hematogones (*green*), stage 3 hematogones (*yellow*), and mature B cells (*black*, admixed with T/NK cells on CD45/SSC). Stage 1 hematogones are CD34+ and are also TDT+ (not shown), have the brightest CD10 and dimmest CD45 expression and are CD20 negative. Stage 2 hematogones have moderate CD45 and CD10 expression and are negative for CD34, with variable CD20 expression. Stage 3 hematogones have dim CD10, bright CD45 expression and are CD20 positive. All three stages of hematogones have dimmer CD22 and brighter CD38 expression as compared to mature B cells

stages (Fig. 12.3). Stage 1 and 2 hematogones are negative for surface light chain, whereas stage 3 hematogones express dim surface light chain. Of note, skewed light chain expression or light chain expression bias by hematogones can be observed in reactive conditions (Fig. 12.4), and it is important not to misinterpret this finding as a clonal CD10+ B-cell population.

Reactive/Polytypic B-Cell Lymphocytosis

An absolute increase in peripheral blood (PB) B cells is very uncommon. Persistent polyclonal B-cell lymphocytosis is a rare disorder and is defined as a PB B-cell count above 4×10^9/L that persists for at least 6 months and is unchanged over time. Clinical symptoms are nonspecific except for mild fatigue in most individuals [8]. Patients are usually female cigarette smokers, some presenting with elevated polyclonal serum IgM [9]. Persistent polyclonal B-cell lymphocytosis has been linked to HLA-DR7 [10], and familial cases have been reported. The etiology remains unknown, and any possible role of smoking, genetic predisposition, or viral factors remains to be determined. Morphologically, the blood smear is characterized by the presence of atypical lymphocytes with abundant cytoplasm and mature nuclei (Fig. 12.5). Binucleated lymphocytes can be

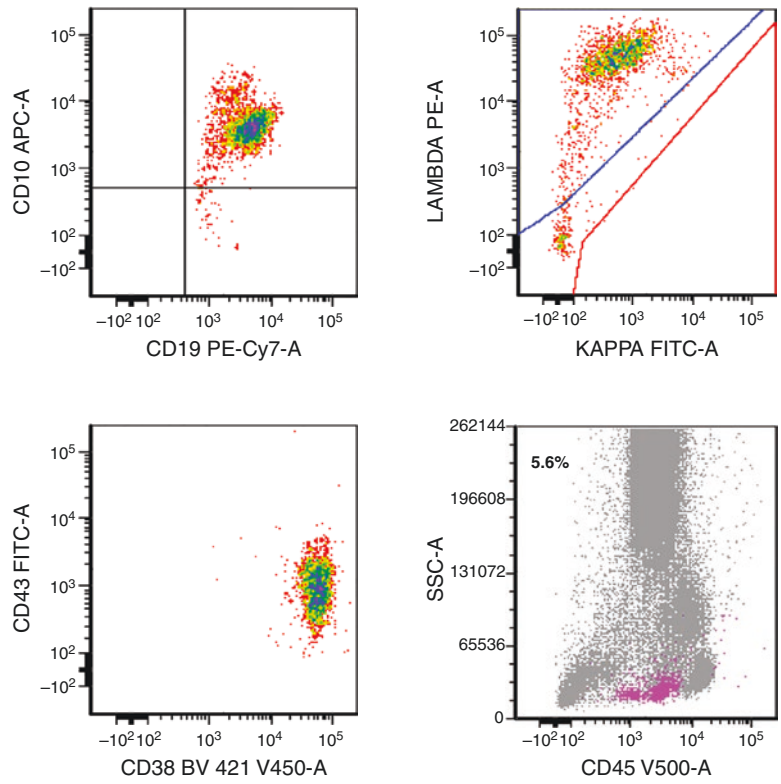

Fig. 12.4 Normal immature B cells (hematogones) can show skewed light chain expression. The hematogones in this case are CD19+, CD10+, and lambda+ but kappa-. These cells show characteristic bright CD38 expression, are CD43+ (lower left plot), and CD45 dim on the CD45/SSC plot (lower right plot)

observed, sometimes comprising up to 10% of the total lymphocytes [11]. By flow cytometry, there is an expansion of CD27+IgM+IgD+ memory B cells with a normal kappa/lambda ratio [12, 13]. A recent case study reported that the B cells are positive for CD19, CD20, CD22, CD79b, and CD200 and negative for CD5, CD10, and CD11c; FMC7 and CD23 were expressed on only a minority of cells [14]. Curiously, although the lymphocytes are polyclonal, recurrent cytogenetic abnormalities have been described, such as i(3q), trisomy 3, and dup(3)(q26q29) [11, 15, 16].

In contrast to absolute B-cell lymphocytosis, a relative increase in blood B cells can be seen in a number of conditions. Autoimmune diseases often show an increased proportion of B cells as a result of immune activation. A relative increase in B cells can also occur in some patients with plasma cell myeloma and has been reported to be a favorable prognostic feature in this setting [17]. DiGeorge syndrome is an inborn defect resulting from chromosome 22q11.2 deletion. Affected children often present with congenital heart malformation, hypoparathyroidism, thymus aplasia, and other phenotypic features [18]. Patients with DiGeorge syndrome display a marked relative (but not absolute) increase in PB B cells and increased numbers of naïve B cells, but decreased memory B cells [19].

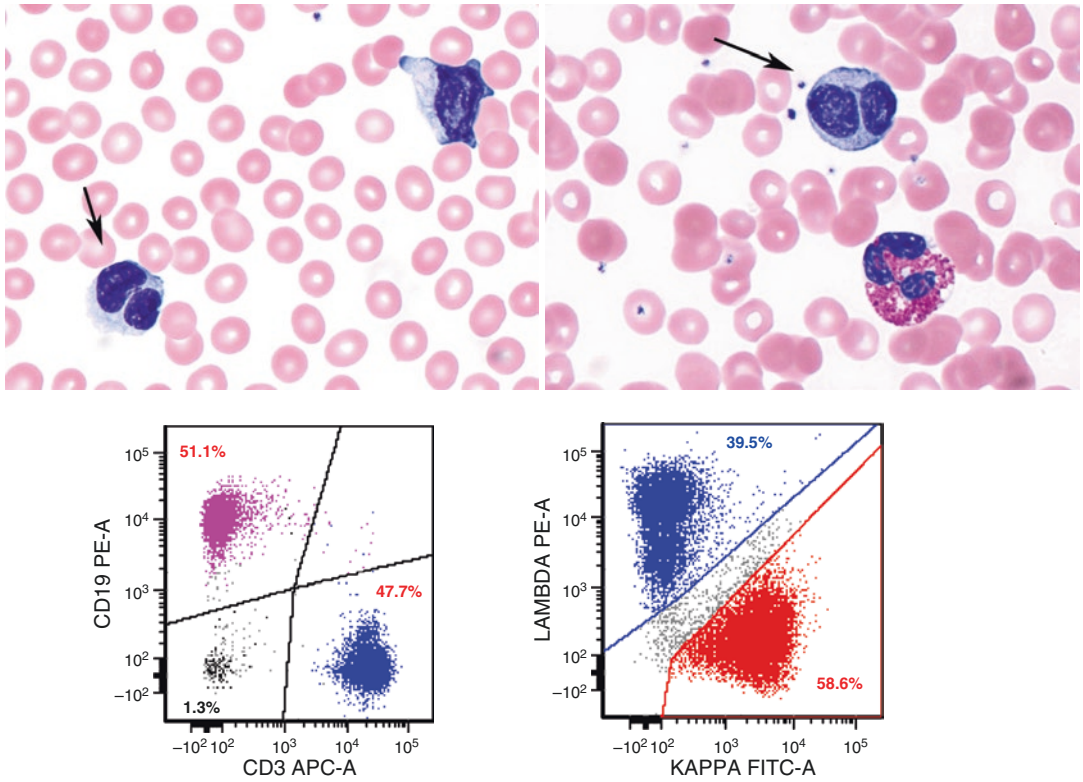

Fig. 12.5 Polyclonal B-cell lymphocytosis. (*Top panel*) The peripheral blood smear shows lymphocytes with bilobed nuclei (*arrows*). (*Bottom panel*) By flow cytometry, CD19+ B cells comprise 51.1% of lymphocytes. Despite this marked increase in numbers, the B cells are polytypic as assessed by kappa and lambda light chain expression

Mature B-cell lymphomas/leukemias

Chronic Lymphocytic Leukemia, Small Lymphocytic Lymphoma, and Monoclonal B-Cell Lymphocytosis

Chronic lymphocytic leukemia (CLL) is the most common B-cell leukemia of adults. By definition, absolute lymphocytosis is present, and by flow cytometry, the absolute level of clonal CLL cells must be at least 5×10^9/L in the peripheral blood. A diagnosis of small lymphocytic lymphoma (SLL) may be made in patients who have biopsy-confirmed SLL involving a lymph node but fewer than 5×10^9/L CLL-type B cells in the blood. In CLL-type monoclonal B-cell lymphocytosis (MBL), circulating clonal B cells with a similar immunophenotype to CLL are present but at a level of $<5 \times 10^9$/L. In the 2016 WHO Classification, MBL has been divided into cases with high count (monoclonal B cells $\geq 0.5 \times 10^9$/L but $<5 \times 10^9$/L) and low count (monoclonal B cells $<0.5 \times 10^9$/L).

Clinical Features

The median age at diagnosis of CLL is 60–70 years, with a male predominance. Patients often are asymptomatic and are diagnosed when lymphocytosis is found on routine blood testing. The range of lymphocyte counts at diagnosis varies, and the count may exceed 100×10^9/L in some patients. Symptomatic patients usually present with fatigue, symptoms related to splenomegaly, and/or peripheral lymphadenopathy. Autoimmune thrombocytopenia, hemolytic anemia, or both (Evans syndrome) may be present at diagnosis or may develop later during the course of the disease. CLL is an indolent disease, and patients often die of unrelated causes.

Morphology

The diagnosis of CLL usually can be established by identifying a clonal B-cell population in the peripheral blood (absolute count at least 5×10^9/L) with the characteristic morphology and flow cytometry immunophenotype of CLL; therefore, a bone marrow sample is usually not required for a primary diagnosis. However, bone marrow biopsy is recommended before therapy is started in order to establish the baseline level of disease and to evaluate the success of subsequent therapeutic interventions.

On PB smears, CLL cells resemble small lymphocytes. They have regularly condensed "chunky" chromatin, absent or small nucleoli, and scant, pale cytoplasm. Some cells may have slightly more abundant pale, basophilic cytoplasm, nuclear irregularities, and/or small nucleoli (Fig. 12.6a–b). The regular "checkerboard" pattern of chromatin condensation in CLL cells is subtly different from the more irregularly condensed chromatin of normal circulating lymphocytes, but this may be difficult to appreciate on many smear preparations. Smudge cells (ruptured cell nuclei devoid of cytoplasm) frequently are seen in PB smears from patients with CLL and may comprise most of the white cells in the smear. Preparing the smear by hand rather than by an automated method, or adding albumin to the blood, abrogates the tendency of the CLL cells to smudge, allowing a more accurate peripheral blood white cell differential count. Smudge cells are by no means specific for CLL and frequently can be seen in association with other circulating lymphoma cells, blasts, or even reactive atypical lymphocytes.

Prolymphocytes represent the proliferating cell component in CLL and usually are present at low levels in the peripheral blood. Prolymphocytes are 1.5–2 times the diameter of small lymphocytes and have a round nucleus, a somewhat dispersed nuclear chromatin (less condensed than that of small lymphocytes but less finely dispersed than that of blasts), and a prominent central nucleolus; the cytoplasm is moderately abundant and usually pale basophilic (Fig. 12.6c–d). Although prolymphocytes are rare in the blood in most CLL cases, they should be enumerated, because increased numbers of prolymphocytes are associated with a more aggressive disease. If prolymphocytes comprise more than 55% of the circulating lymphocytes at presentation, a diagnosis of B-cell prolymphocytic leukemia is made.

Some cases of CLL show cells intermediate in size between small lymphocytes and prolymphocytes, with variably prominent nucleoli (Fig. 12.6b); a strict definition of prolymphocytes should be used, and such cells should not be counted among the prolymphocytes. Although the CLL lymphocytes usually have round nuclear contours, a subset of cases may show cells with irregular or even clefted nuclei and/or distinct nucleoli (Fig. 12.6e, f). These cases, which have been called "atypical CLL" by some authors, may have a more aggressive course [20]. Other cases may have unusually abundant, pale cytoplasm. Despite the wide cytomorphologic spectrum manifested by CLL, atypical morphologic features do not appear to have an independent impact on the prognosis. The WHO Classification system does not recognize morphologic subtypes of CLL and a previous category of "CLL/prolymphocytic leukemia" (CLL with 10–55% prolymphocytes) has been eliminated.

In the bone marrow biopsy sample, CLL cells appear as small, round lymphocytes that percolate among the hematopoietic elements singly or as small clusters in an interstitial pattern and/or form non-paratrabecular aggregates (Fig. 12.7a–c). Paratrabecular aggregates, in which the lymphocytes are closely opposed to the bone surface, are exceedingly rare in CLL and should suggest an alternate diagnosis if present [21]. The aggregates in CLL are usually rounded and consist of monotonous, small lymphocytes with occasional admixed, larger nucleolated cells. Unlike in many types of lymphomatous infiltrates, reticulin staining usually is not significantly increased or only mildly increased in the lymphoid aggregates in CLL (Fig. 12.7d) [22]. A diffuse pattern of involvement (Fig. 12.7e) is seen in about 20% of patients biopsied and has been associated with an adverse prognosis, but this finding is not clearly independent of other prognostic risk factors, such as the genetic characteristics [23]. Proliferation centers are collections of enlarged CLL cells (medium-sized prolymphocytes and

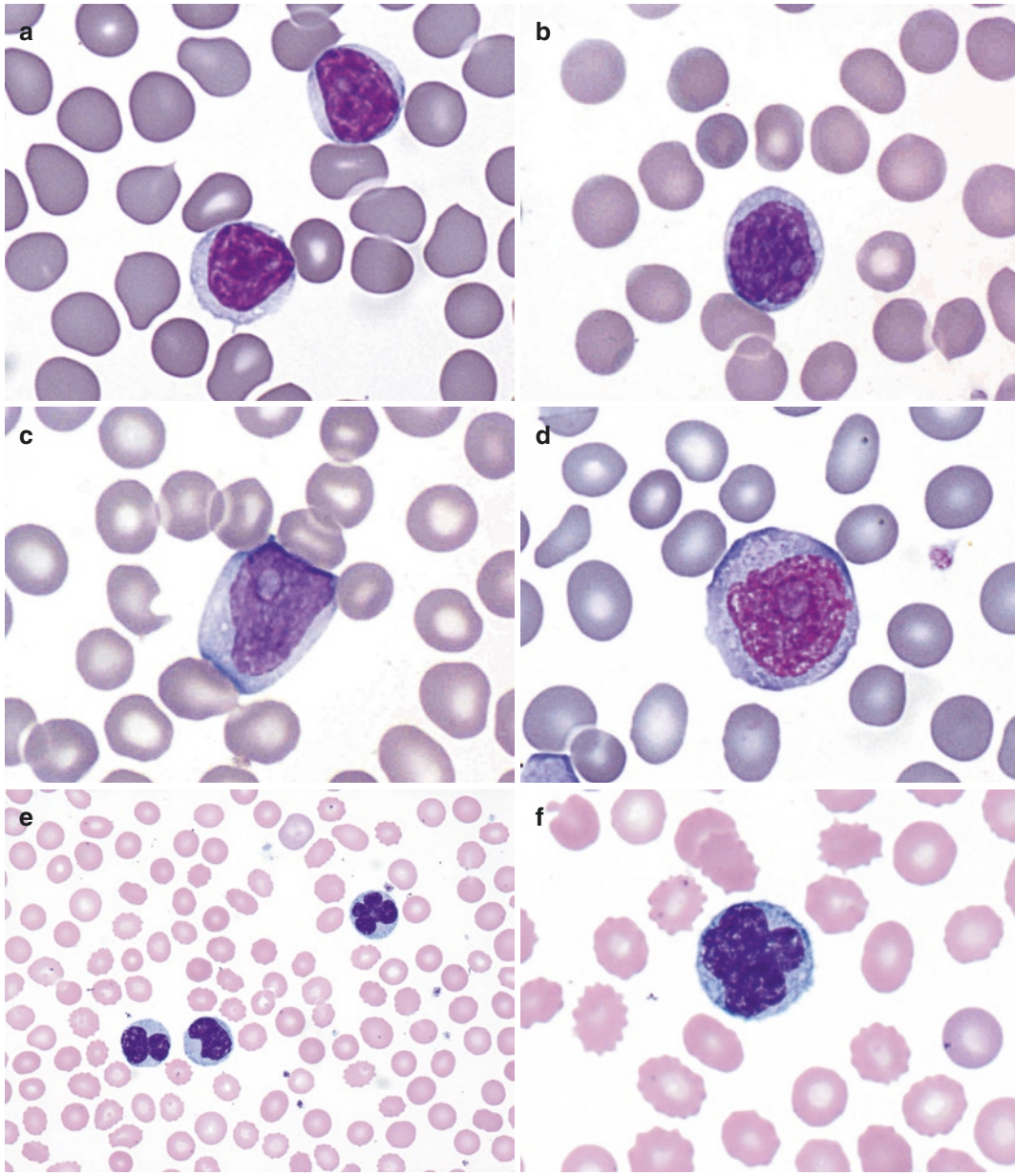

Fig. 12.6 Chronic lymphocytic leukemia (CLL) cells in the blood. (**a**, **b**) Small lymphocytes predominate. They have round to slightly irregular nuclei, condensed chromatin, and scant, pale cytoplasm. Small nucleoli are not uncommon. (**c**–**d**) Prolymphocytes vary in numbers in CLL but are usually <10% of all lymphocytes. They are at least 1.5× larger than the small lymphocytes and have more abundant cytoplasm, more dispersed chromatin, and prominent central nucleoli. Atypical variant morphology in CLL includes cells with distinct nucleoli and/or significant nuclear irregularities (**e**, **f**); these cells do not qualify for prolymphocytes

large paraimmunoblasts with vesicular nuclei) that appear pale on low-power examination among the small, dark blue CLL lymphocytes. Proliferation centers commonly are seen in extramedullary tissues involved by CLL. In the bone marrow, they may be seen in the diffuse pattern of involvement and to a lesser degree within nodular aggregates [24].

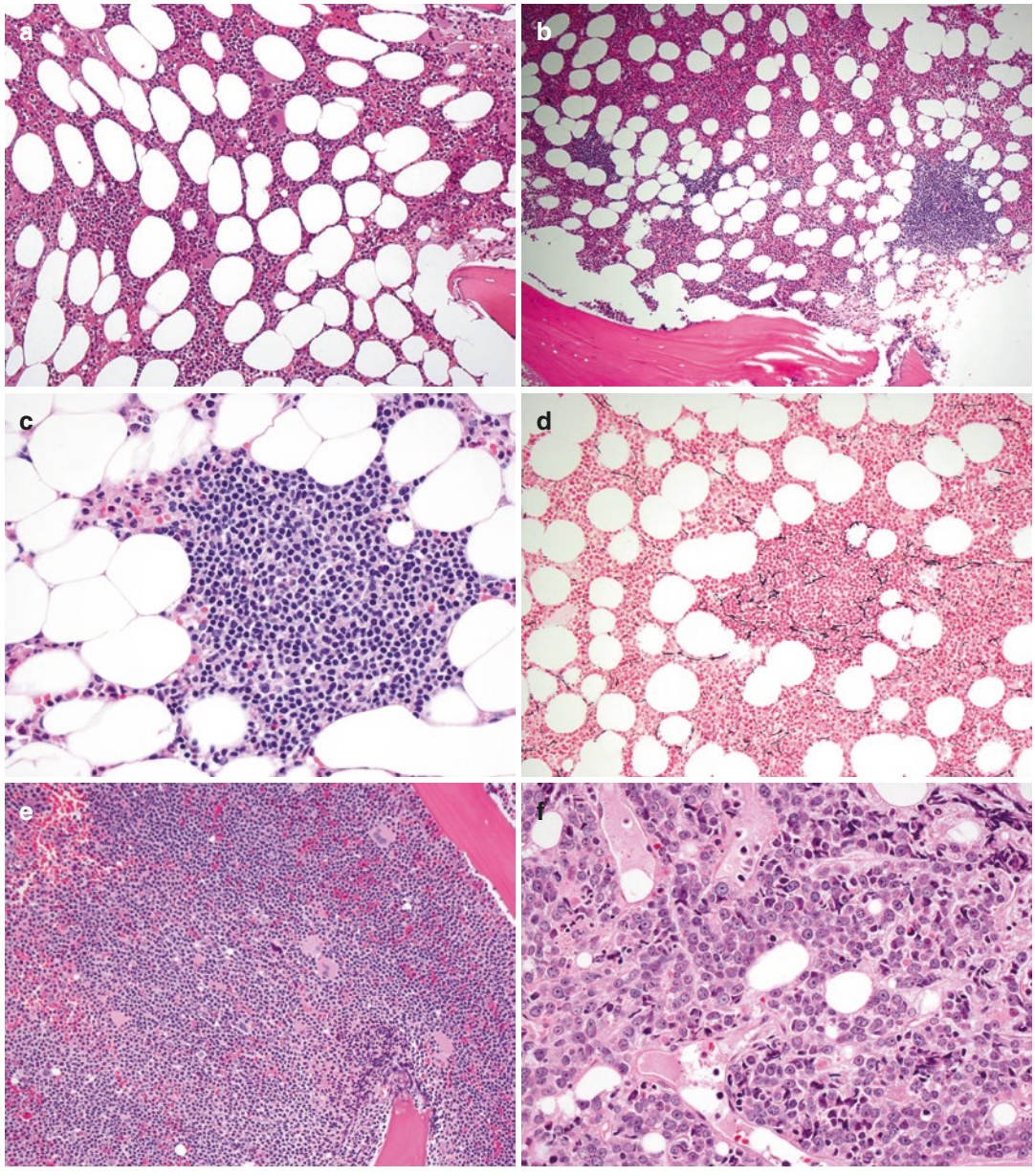

Fig. 12.7 Bone marrow biopsy morphology in chronic lymphocytic leukemia (CLL). Cases most commonly have an interstitial (**a**) or non-paratrabecular nodular (**b**) infiltration pattern. (**c**) The nodules in CLL are composed of small lymphocytes with round nuclei that often percolate into the surrounding marrow; large nucleolated cells are rare. (**d**) The nodules are usually associated with only small amounts of reticulin fibrosis, and hence the CLL cells are usually well represented in the aspirate smears. (**e**) In an advanced disease, the pattern is diffuse, with near complete effacement of the normal marrow elements. (**f**) CLL in Richter's transformation. This patient presented with rapid onset pancytopenia following treatment of CLL. Sheets of large cells replace the bone marrow. In this case, the large cell lymphoma cells retained CD5 and LEF1 expression (not shown)

Occasionally, a bone marrow sample may be taken from patient with CLL that has undergone progression to diffuse large B-cell lymphoma (Richter's syndrome), usually accompanied by rapidly increasing lymphadenopathy. These transformed CLL cases manifest as sheets of large lymphoid cells and may show areas of geographic necrosis (Fig. 12.7f).

Immunophenotype

Immunophenotypic analysis of blood and/or bone marrow by flow cytometry is a cornerstone of CLL diagnosis. CLL has a characteristic immunophenotype, typically showing expression of CD19; dim expression of CD20, CD22, and monotypic surface immunoglobulin light chain; and co-expression of CD5 (usually at a somewhat dimmer level than that of benign T cells), CD23, CD43, and uniform bright CD200. Rare CLL cases (0.5–3%) may co-express the T-cell marker CD8 [25]. FMC7, which recognizes an epitope of CD20 and is associated with bright expression of this protein, usually is dim or negative, as is CD79b. Surface immunoglobulin expression may be so low as to be undetectable by flow cytometry. However, investigation of permeabilized cells by flow cytometry usually discloses monotypic expression of cytoplasmic immunoglobulin in such cases. The hairy cell leukemia markers CD103 and CD25 are negative, although CD11c may be partially expressed in a subset of cases. Because CLL is a disease with various underlying molecular genetic alterations, the existence of some immunophenotypic variability is not surprising. Atypical immunophenotypes that occur in otherwise typical CLL cases include partial/dim CD5, partial/negative CD23, and strong CD20 and/or surface immunoglobulin expression. CD200 is uniformly positive in CLL cases with atypical immunophenotypes (Fig. 12.8) [26].

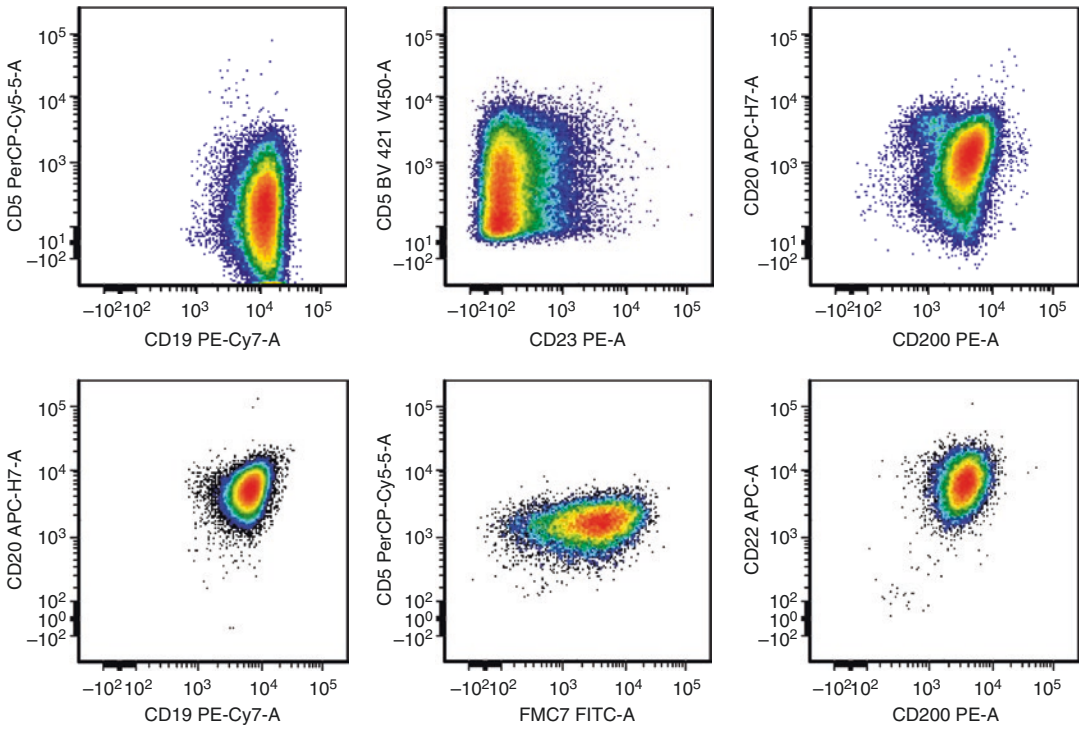

Fig. 12.8 CD200 is uniformly expressed in chronic lymphocytic leukemia (CLL), including atypical CLL cases. Upper panel shows a case of CLL with del(13q). Atypical features include dim/partial expression of CD5 and negativity for CD23. Lower panel shows a case of CLL with trisomy 12. Atypical features include bright CD20 and CD22 expression and expression of FMC7. Both cases show uniform CD200 expression

Immunohistochemistry is usually not required if full flow cytometric immunophenotyping has been performed, although applying B-cell markers such as PAX5, CD19, or CD79a can be helpful in quantifying the extent of CLL cell infiltration of the bone marrow biopsy. Performing CyclinD1 immunostaining is prudent if mantle cell lymphoma has not been excluded by cytogenetics or FISH for a t(11;14) translocation. Some cases of mantle cell lymphoma may express CD23 or demonstrate other immunophenotypic features resembling CLL [27]. If confirmatory peripheral blood or bone marrow flow cytometry is not available, immunohistochemistry can be used to evaluate non-paratrabecular lymphoid aggregates to assess for the possibility of CLL. However, there is morphologic overlap between the lymphoid aggregates of CLL and reactive lymphoid aggregates that are not infrequent in the bone marrow of older individuals. Small T cells are usually admixed with the neoplastic aggregates of CLL. Close inspection of the CD5 stain relative to the stains for B cells as well as application of other T-cell markers (such as CD3) are important in disclosing the aberrant CD5+ B-cell population in CLL, which usually shows dimmer staining for CD5 than the admixed normal T cells [28]. LEF1 immunostain is helpful in confirming a diagnosis of CLL, as it is expressed in almost all cases and is negative in normal B cells and in other B-cell lymphomas [29]. LEF1 does stain T cells, thus this stain always should be reviewed together with a CD3 immunostain (Fig. 12.9) [30].

Cytogenetics and Molecular Genetics

Routine cytogenetic analysis of CLL often is uninformative because of the poor growth of

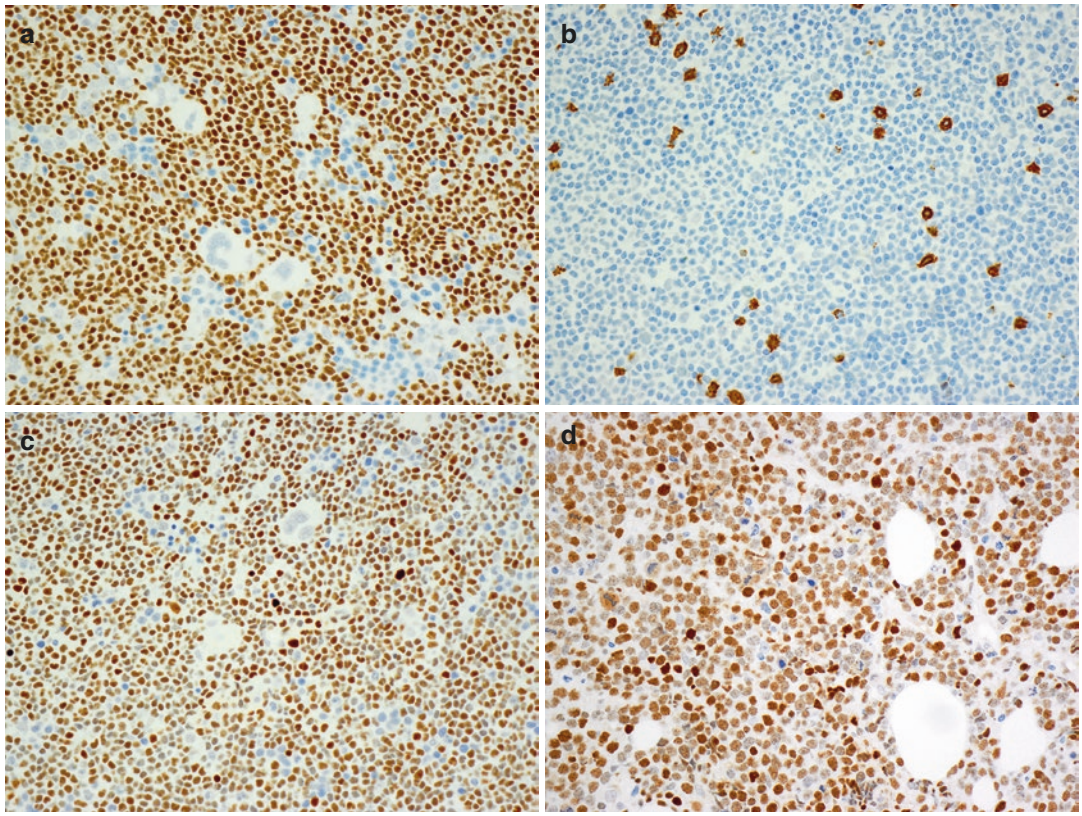

Fig. 12.9 LEF1 staining in CLL diffusely involving the bone marrow. Nearly all the cells in the bone marrow biopsy are PAX5+ B cells (**a**), with few CD3+ T cells (**b**). LEF1 immunostain (**c**) is positive in cells corresponding to the PAX5+ CLL cells. (**d**) Another case showing a Richter transformation of CLL in the bone marrow, which is also LEF1 positive

CLL cells in culture. However, interphase FISH study on the neoplastic lymphocytes from the blood or bone marrow can identify several recurring abnormalities associated with CLL that have important prognostic implications: 80% of CLL cases exhibit at least one such abnormality [31]. The most common abnormality is del(13q), which is associated with a favorable prognosis provided it is the only detected abnormality. The less frequent abnormalities del(11q), del(17p), and del(6q) are associated with an inferior prognosis, and trisomy 12 is associated with an intermediate prognosis. In particular, del(17p) that results in the loss of the *TP53* gene predicts a relatively aggressive clinical course. Mutations occur in *NOTCH1, SF3B1, ATM*, and other genes in a minority of cases [32, 33].

An important molecular genetic prognostic marker is the mutational status of the *IGH* gene variable region. About half of CLL cases show high levels of somatic hypermutation (implying origin from a lymphocyte at the germinal center or post-germinal center stage of differentiation), and these cases have a better prognosis than cases that lack somatic hypermutation (implying origin from a naïve, pre-germinal center lymphocyte). However, assessment of the somatic mutational status is a specialized test that is not available in most laboratories. Immunophenotypic surrogates that correlate with unmutated CLL (and a poorer prognosis) include expression of ZAP-70 on more than 20% of the tumor cells [34] and CD38 expression on more than 30% of the tumor cells [35]. Although these markers do not correlate perfectly with the mutational status, they do provide useful prognostic information.

Differential Diagnosis

The differential diagnosis of CLL includes other small B-cell lymphomas and some nonmalignant conditions. Among the lymphomas, mantle cell lymphoma (MCL) can bear a close morphologic resemblance to CLL and can present as a leukemia. The proliferation centers that are a clue to CLL diagnosis in lymph nodes are usually absent in CLL involving the bone marrow. Immunophenotypic analysis is critical for showing bright (as opposed to dim) expression of CD20 and surface immunoglobulin in MCL and, typically, lack of CD23. Additionally, CD200 is negative in >95% of MCL cases [26, 36] (Fig. 12.10). The gold standard of MCL diagnosis is demonstration of cyclinD1 expression by immunohistochemistry and/or *CCND1* rearrangement by cytogenetics, FISH, or polymerase chain reaction (PCR) on a bone marrow or blood sample. Of note, some cyclinD1 expression may be detected within the proliferation centers of CLL, but it is not strong and uniform as the expression in MCL [37]. Marginal zone lymphomas do not infrequently express CD5, potentially mimicking CLL. Conversely, some CLL cases have abundant cytoplasm, mimicking the villous lymphocytes of splenic marginal zone lymphoma, and splenomegaly also is relatively common in CLL. The lymphocyte count in CLL usually is higher than in splenic marginal zone lymphoma, and on well-prepared smears, the characteristic regularly condensed chromatin of CLL is a helpful clue. In difficult cases, FISH analysis can be very helpful, because splenic marginal zone lymphomas do not usually show the common cytogenetic abnormalities of CLL and may show other abnormalities, such as interstitial deletion of 7q. Cases of CLL with abundant cytoplasm on smear preparations may raise the differential diagnosis of hairy cell leukemia. However, CLL cases in bone marrow sections do not show the abundant, pale cytoplasm that characterizes almost all hairy cell leukemia cases and also lack expression of CD103 and CD25. Tables 12.2 and 12.3 present the various pathologic features that aid in the differential diagnosis of CLL with other B-cell leukemias and lymphomas that involve the bone marrow.

The main nonmalignant disorder that may be confused with CLL is monoclonal B lymphocytosis (MBL). This is a clonal proliferation of B cells that usually have an immunophenotype similar to that of CLL but are present in insufficient numbers (fewer than 5×10^9/L in the blood, without extramedullary tissue involvement) to warrant a diagnosis of CLL. MBL is relatively common and is analogous to monoclonal gammopathy of undetermined significance (MGUS). The incidence of

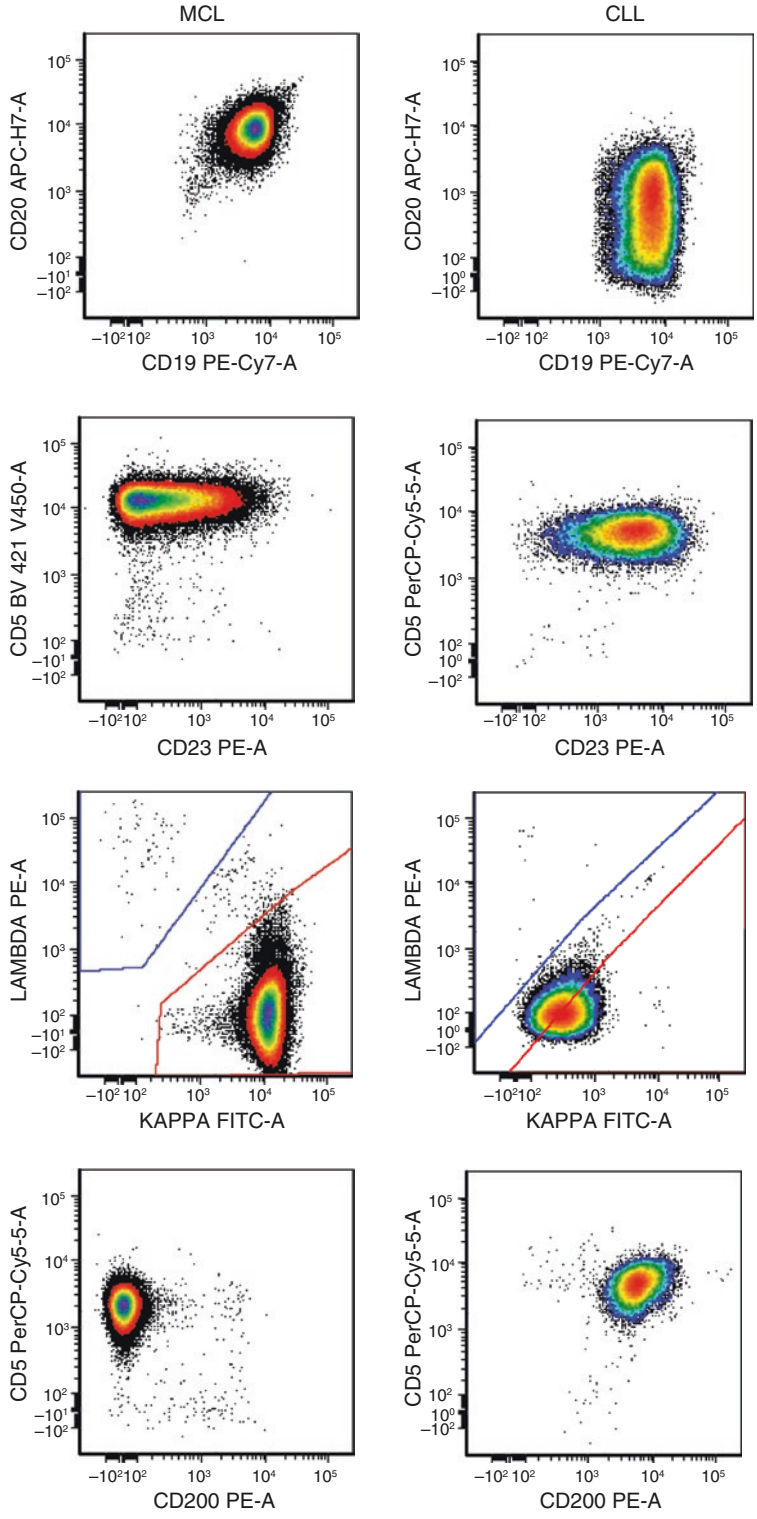

Fig. 12.10 Immunophenotypic comparison of mantle cell lymphoma (MCL, *left panel*) and chronic lymphocytic leukemia (CLL, *right panel*). MCL is typically CD20 bright+, CD5+, CD23- (or only partially positive, as in this case), surface light chain bright+, and CD200-. In contrast, CLL is typically CD20 dim or partial+, CD23+, surface light chain dim+, and CD200 uniformly+

Table 12.2 Morphologic and immunophenotypic features aiding in the differential diagnosis of B-cell lymphomas with predominantly a leukemic presentation

Feature	HCL	SMZL	HCL-v	CLL	B-PLL
White blood cell count	Usually low	Low, normal, or high	High	High	Very high
Nuclei	Medium sized, oval, or bean shaped	Small to medium sized, round	Medium sized, round to oval	Small, round	Large, round
Chromatin	Fine	Condensed	Variable	Condensed	Moderately dispersed
Nucleoli	Absent	Small to absent	Present	Small to absent	Prominent, central
Cytoplasm	Abundant, pale blue	Moderate, pale blue	Abundant, pale blue	Scant, pale	Abundant
Cell surface	Circumferential projections	Polar projections	Present, variable	Smooth	Smooth
Marrow infiltration pattern	Diffuse and interstitial	Nodular, interstitial, intrasinusoidal	Diffuse and interstitial	Nodular, interstitial, diffuse	Nodular, interstitial, diffuse
Immunophenotype	CD20 br+ FMC7+	CD20 br+ FMC7+	CD20 br+ FMC7+	CD20 dim+ FMC7-	CD20 br+ FMC7-/+
	CD5-, CD10- CD23- CD200 br+ sIg br+	CD5-, CD10- CD23-/+ CD200 dim+ sIg+	CD5-, CD10- CD23- CD200 dim+ or - sIg br+	CD5+, CD10-CD23+ CD200 br+ sIg dim+ or -	CD5-/+, CD10- CD23- CD200 dim+ or - sIg+
	CD103+ CD25+ CD11c+	CD103-,CD25-CD11c+/-	CD103+,CD25-CD11c-	CD103-, CD25- CD11c-/+	CD103-, CD25- CD11c-/+
	CD123+ LEF1- AnnexinA1+ CyclinD1 dim+ BRAF V600E+	CD123- LEF1- AnnexinA1- CyclinD1- BRAF V600E-	CD123- LEF1- AnnexinA1- CyclinD1- BRAF V600E-	CD123-/+ LEF1+ AnnexinA1- CyclinD1- BRAF V600E-	CD123- LEF1 unknown AnnexinA1- CyclinD1- BRAF V600E-

HCL hairy cell leukemia, *SMZL* splenic marginal zone lymphoma, *HCL-v* hairy cell leukemia variant, *CLL* chronic lymphocytic leukemia, *B-PLL* B-cell prolymphocytic leukemia, *br* bright, + positive in vast majority of cases, +/- positive in most cases, -/+ positive in a minority of cases, - negative in vast majority of cases, *sIg* surface immunoglobulin (kappa or lambda)

Table 12.3 Features aiding in the differential diagnosis of chronic lymphocytic leukemia versus other small B-cell lymphomas involving the bone marrow

Feature	CLL	LPL	MCL	FL
Percent of patients with marrow involvement	>90%	>90%	>80%	40–70%
Marrow infiltration pattern	Nodular (non-paratrabecular), interstitial, diffuse	Any pattern	Diffuse, nodular (often paratrabecular)	Nodular, paratrabecular
Cell morphology	Small, round to irregular nuclei, condensed chromatin	Spectrum of small round lymphocytes to mature plasma cells	Small to medium, irregular nuclei, moderately dispersed chromatin	Small, angulated nuclei, condensed chromatin; variable number of large cells
Immunophenotype	CD20 dim+	CD20+	CD20 br+	CD20+
	CD5+, CD10-CD23+ LEF1+ CD200+	CD5-/+, CD10- CD23-/+ LEF1- CD200-	CD5+, CD10-CD23/+ LEF1- CD200-	CD5-, CD10+/- CD23-/+ LEF1- CD200-
	CyclinD1- SOX11-	CyclinD1- SOX11-	CyclinD1+ SOX11+/-	CyclinD1- SOX11-
	BCL6-	BCL6-	BCL6-	BCL6+/- (in marrow)
	Usually no clonal plasma cells	IgM+ clonal plasma cells	Usually no clonal plasma cells	Usually no clonal plasma cells
Paraprotein	Usually low or absent	High; almost always IgM	Usually absent	Usually absent
Genetic findings	del(13q), del(11q), del(17q), del(6q), +12	*MYD88* mutation	t(11;14) with *CCND1* rearrangement	t(14;18) with *BCL2* rearrangement

CLL chronic lymphocytic leukemia, *LPL* lymphoplasmacytic lymphoma, *MCL* mantle cell lymphoma, *FL* follicular lymphoma, *br* bright, + positive in vast majority of cases, +/- positive in most cases, -/+ positive in a minority of cases, - negative in vast majority of cases

MBL increases with age, and 3.5% of adults over the age 40 harbor a detectable B-cell clone in the blood [38]. These circulating B-cell clones often are discovered incidentally when peripheral blood is subjected to flow cytometry for other reasons. About two-thirds of cases of MBL have a typical CLL immunophenotype; these cases are the best studied, have a relatively predictable likelihood of progressing to CLL based on the monoclonal B-cell count (see below), and are termed "CLL-type" MBL. The remaining cases encompass MBL with an atypical CLL immunophenotype (due to bright CD20 expression, bright surface light chain expression, and/or negativity for CD23) and those with a CD5- immunophenotype. Cases of MBL that do not have a typical CLL immunophenotype warrant further investigation for lymphadenopathy or other possible sites of disease, as they may represent cases of MCL or other B-cell lymphomas that have not yet come to clinical attention (Fig. 12.11) [39].

CLL-type MBL represents a precursor of CLL, and a small but significant percentage of patients (about 1% per year) progress to CLL; the incidence is somewhat higher for patients with high-count MBL (monoclonal B-cell count $0.5–5 \times 10^9$/L) [38]. The bone marrow findings for patients with MBL have not been extensively studied. In one study, almost all cases had detectable clonal B cells in the bone marrow identified by flow cytometry, while 80% had abnormal lymphoid infiltrates in the bone marrow biopsy, with variable infiltration patterns and extent of involvement [40]. It is uncertain whether patients with fewer than 5×10^9/L CLL cells in the blood

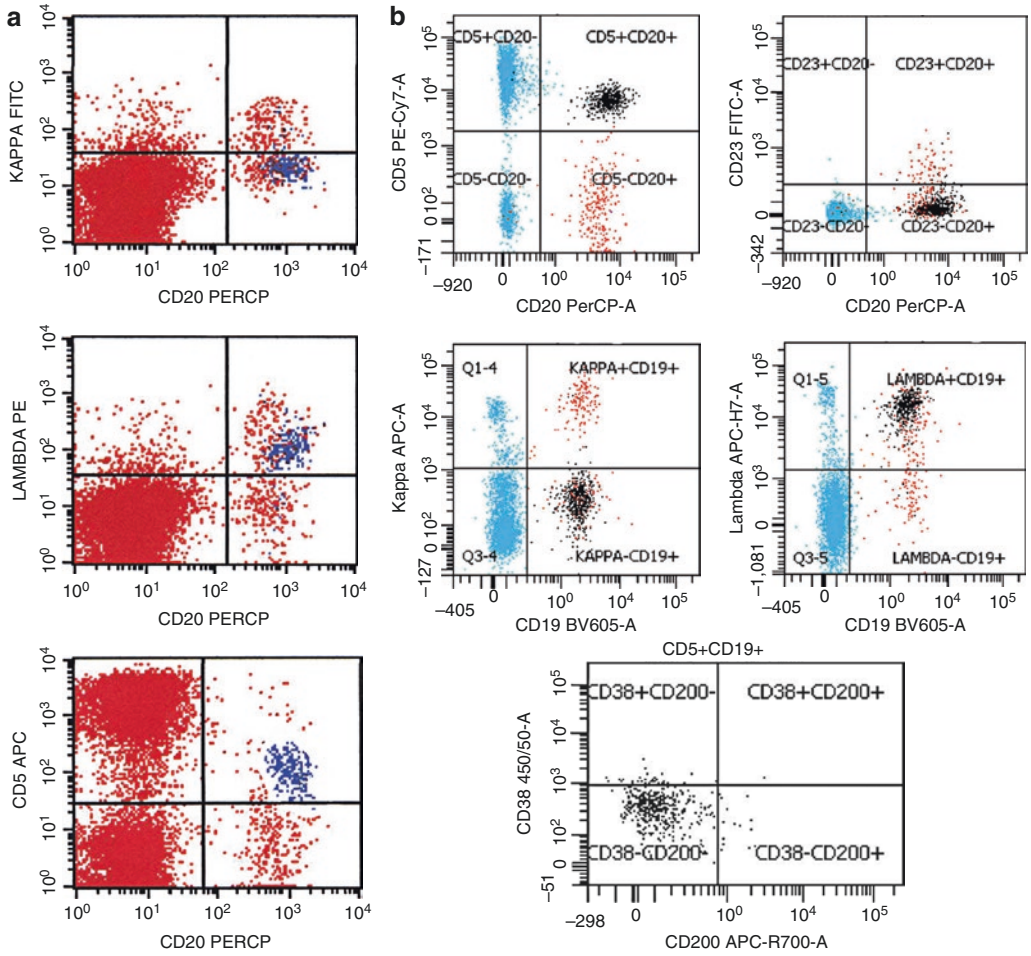

Fig. 12.11 Monoclonal B-cell lymphocytosis (MBL). (**a**) The MBL cells (*blue*) in this case have a typical CLL immunophenotype (CD5+, CD23+ [not shown], and surface lambda dim+) with the exception of somewhat brighter CD20 than the normal B cells (*red*). (**b**) In this different case, the MBL cells (*black*) have an atypical CLL immunophenotype (CD5+, but CD23-, CD20 bright, surface lambda bright+, and CD200-); normal polyclonal B cells (*red*) are also present

but with bone marrow infiltrates resembling CLL should be classified as CLL or as MBL. The International Workshop on Chronic Lymphocytic Leukemia (IWCLL) recommends that the marrow should contain at least 30% lymphoid cells to qualify for CLL, but this is an arbitrary figure and is not included in the criteria for diagnosing CLL in the 2016 WHO Classification.

B-Cell Prolymphocytic Leukemia

As defined by the current 2016 WHO Classification system, B-cell prolymphocytic leukemia (B-PLL) is a de novo leukemia in which prolymphocytes comprise at least 55% of the peripheral blood lymphoid cells. Defined as such, B-PLL is a rare disease.

Clinical Features

B-PLL affects older adults. The median age is 60–70 years, and a slight male preponderance is seen. Patients usually present with systemic symptoms and splenomegaly but, in contrast to most cases of leukemic MCL, without significant lymphadenopathy. A marked leukocytosis (typically >50 × 10^9/L) with a rapid lymphocyte doubling time is characteristic. B-PLL has a much poorer prognosis than CLL [41].

Morphology

In the blood, the vast majority of the lymphoid cells (by definition at least 55%) are prolymphocytes, resembling those seen in much smaller numbers in CLL (Figs. 12.6c–d and 12.12). Although the prolymphocyte nucleus usually is round, some cases may show prominent nuclear irregularities or lobulations, but the main uniform feature of B-PLL is a prominent "punched out" central nucleolus. The cytoplasm of prolymphocytes is typically abundant and pale basophilic. The bone marrow is involved in all cases and can show interstitial, nodular, or diffuse patterns of involvement.

Immunophenotype

Immunophenotypically, B-PLL shows expression of CD19 and strongly expresses CD20, CD22, CD79a, and CD79b. Unlike in CLL, FMC7 is strongly expressed. B-PLL also differs from CLL in its relatively infrequent expression of CD5 and CD23, seen in only a minority of cases [42].

Cytogenetics and Molecular Genetics

Cytogenetic and FISH analysis shows some genetic relationship with CLL: del(17p) is present in half the cases, *TP53* mutations are frequent, and del(13q) also is common. However, usage of the *IGH* variable region family in B-PLL appears distinct from that seen in CLL cases with increased prolymphocytes, which supports the separation of B-PLL from CLL advocated in the WHO Classification system [42].

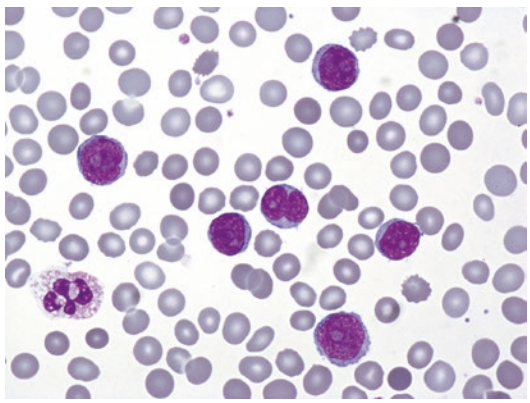

Fig. 12.12 B-cell prolymphocytic leukemia. Most of the circulating lymphocytes are large prolymphocytes with prominent central nucleoli

Differential Diagnosis

The main differential diagnosis in B-PLL is CLL with increased prolymphocytes. There is a spectrum of small lymphocytes and prolymphocytes in both B-PLL and CLL, but the latter predominate in B-PLL. The prolymphocytes in CLL show CD5 expression much more often than in B-PLL, although CD23 may be lost when CLL cases develop an increase in prolymphocytes. Documenting a previous history of CLL is critical for excluding a diagnosis of de novo B-PLL in such cases.

Assessment for t(11;14) by FISH in a blood sample and/or for cyclin D1 expression in tissue sections is mandatory for excluding a leukemic presentation of MCL. Like B-PLL, hairy cell leukemia variant (considered a subcategory of unclassifiable splenic B-cell lymphoma/leukemia in the 2016 WHO Classification system) shows prominent splenomegaly and leukocytosis at presentation. The neoplastic cells in the peripheral blood usually are somewhat enlarged compared to small lymphocytes, but they are smaller than B-PLL cells and have less prominent nucleoli. Hairy cell leukemia variant lacks many characteristic markers of hairy cell leukemia, such as CD25 and annexin A1, but unlike B-PLL, it usually is CD103+ [43]. Distinguishing between B-PLL and hairy cell leukemia variant morphologically may be difficult in some cases; an intrasinusoidal infiltrate in the bone marrow may be a helpful clue that suggests the hairy cell leukemia variant [44]. Features that aid in the differential diagnosis of B-PLL with other B-cell leukemias are listed in Table 12.2.

Mantle Cell Lymphoma

Mantle cell lymphoma is a systemic B-cell lymphoma that usually presents with diffuse lymphadenopathy. It involves the bone marrow in nearly all cases at diagnosis, and circulating lymphoma cells can be detected in the peripheral smear and by flow cytometry immunophenotyping in most patients.

Clinical Features

About 25% of patients have peripheral lymphocytosis (>5 × 10^9/L), and 5–10% of patients

manifest a frankly leukemic presentation with marked leukocytosis that may mimic an acute leukemia [45]. Such patients usually present with symptoms related to splenomegaly and/or cytopenias. Unlike in CLL, incidental discovery of leukemic MCL on a routine blood count evaluation is rare [46]. The median lymphocyte count in leukemic cases is 58×10^9/L, and one-third of patients present with a lymphocyte count greater than 100×10^9/L. Leukemic non-nodal MCL, recognized as a specific disease variant in the 2016 WHO Classification, refers to patients presenting with blood and bone marrow involvement, but without significant lymphadenopathy. Patients with this variant of disease appear to have a better prognosis compared to patients with classic MCL, often manifesting a stable disease for many years [47, 48].

Morphology

The appearance in aspirate and peripheral smears is heterogeneous. Although the neoplastic cells often vary in size in smear preparations and may or may not have prominent nucleoli, the chromatin tends to be more dispersed than that of the cells of CLL, and the nuclei usually have irregular or cleaved nuclear borders (Fig.12.13a, b). A common feature that can be a helpful clue to the diagnosis is the presence of a small subset of binucleated or trinucleated cells [45]. In cases of the blastoid variant, cells usually have finely dispersed chromatin and inconspicuous nucleoli resembling lymphoblasts; in other cases, prominent nucleoli resembling myeloblasts or prolymphocytes may be seen (Fig. 12.13c, d) [49]. Cells of the leukemic non-nodal variant are often small and can resemble the cells of CLL.

In bone marrow biopsy sections, most cases show a nodular pattern of involvement that includes paratrabecular infiltrates in almost half of the cases. Interstitial and diffuse patterns also are common (Fig. 12.13e). In biopsy sections, neoplastic cells are small to medium sized with irregular nuclei. Rare leukemic cases may show a prominent intrasinusoidal growth pattern [50]. The blastoid variant has somewhat larger cells with more dispersed chromatin and a brisk mitotic rate.

Immunophenotype

By flow cytometry, a CD20 bright+, CD10-, CD5+, CD23-, FMC7+, and CD200- phenotype is characteristic; a small subset of cases may be CD5- or CD23+ (Fig. 12.10) [46]. CD200+ MCL, comprising <5% of cases, appears to have an indolent clinical course [47]. Identification of a t(11;14) involving the *CCND1* locus by cytogenetics or FISH analysis (see below) and/or demonstration of cyclinD1 expression by immunohistochemistry (Fig. 12.13f) is critical, particularly in differentiating MCL from CLL and other small B-cell lymphomas. SOX11 is positive in the vast majority of cases, including the CD5- and CyclinD1-negative variants [51, 52]; however, SOX11 is usually negative in the leukemic non-nodal variant [47]. Demonstrating the presence of surface immunoglobulin expression and lack of TdT is helpful for distinguishing the blastoid variant of MCL from B-lymphoblastic lymphoma/leukemia.

Cytogenetics and Molecular Genetics

The genetic hallmark of MCL is the t(11;14)(q13;q32) translocation resulting in *IGH/CCND1* rearrangement, which drives overexpression of the CyclinD1 protein detected by immunohistochemistry. This is present in almost all MCL cases. The rare cases lacking *IGH/CCND1* rearrangement often have *CCND1* rearrangement with another immunoglobulin locus or have *CCND2* rearrangements [53]. Similar to CLL, deletions of 17p13 and 11q22–23 (*ATM*) loci or trisomy 12 may be observed.

Differential Diagnosis

CyclinD1 and SOX11 immunostaining have simplified the differential diagnosis of MCL with other small B-cell lymphomas. Other features that help distinguish MCL from other small B-cell lymphomas involving the bone marrow are listed in Table 12.3. The most difficult differential is with CLL, which is the most common lymphoid leukemia and can show significant immunophenotypic and morphologic overlap with mantle cell leukemia, particularly the non-nodal leukemic variant. CyclinD1 should always

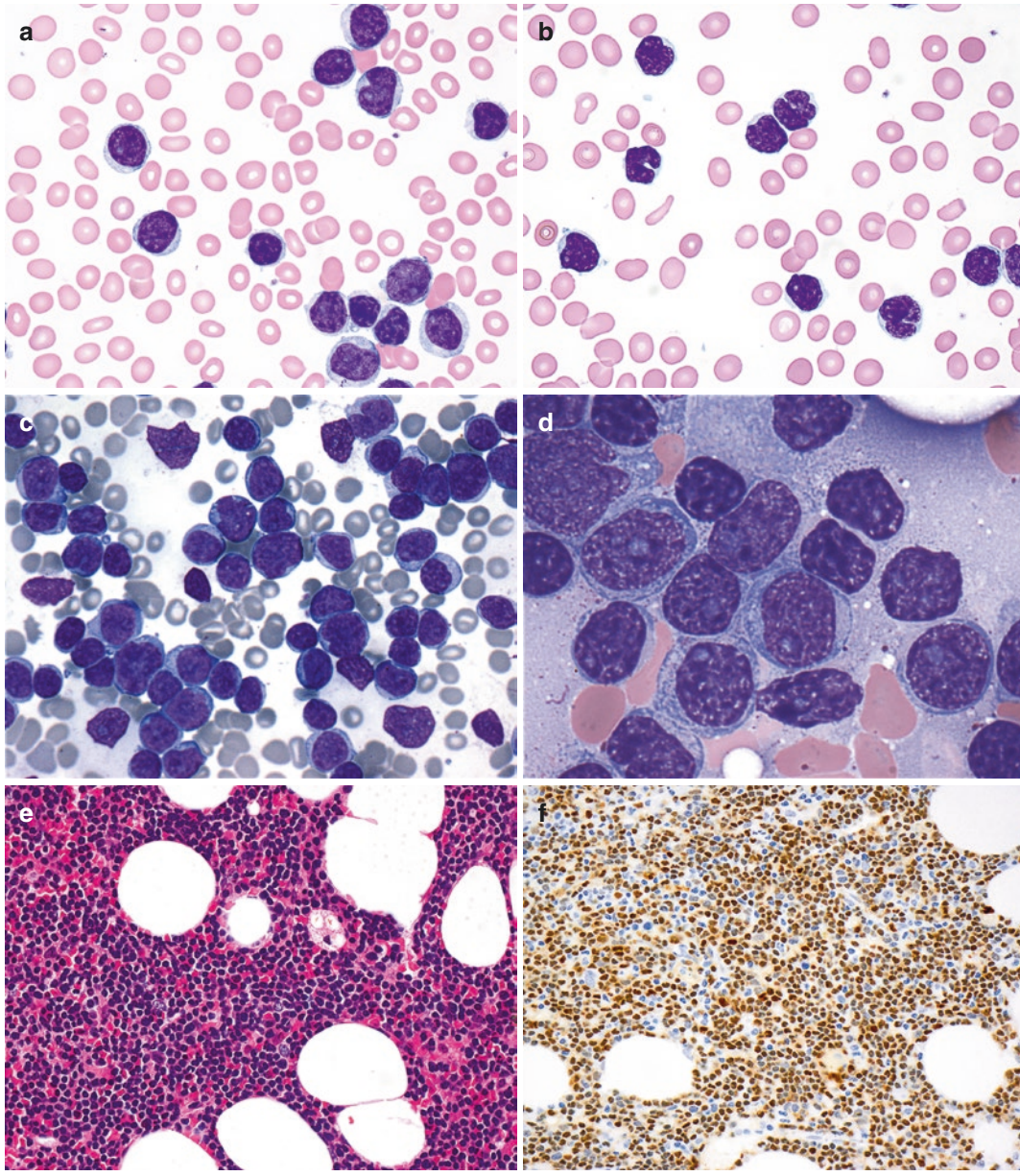

Fig. 12.13 Mantle cell lymphoma. Leukemic disease can present with a wide range of peripheral blood cytologies and degrees of leukocytosis. (**a**) In this case, there is modest lymphocytosis composed of slightly enlarged lymphoid cells with distinct nucleoli and often clefted nuclei. (**b**) In this case, the cells are small and nucleoli are inconspicuous, but there are pronounced nuclear membrane irregularities. (**c**) This patient presented with profound leukocytosis (white blood cell count of 643 × 10^9/L) and medium to large cells, representing the blastoid variant of mantle cell leukemia. (**d**) Blastoid mantle cell leukemia cells seen in the bone marrow aspirate smear with prominent nucleoli and dispersed chromatin, admixed with small, mature lymphocytes. (**e**) Mantle cell leukemia (classical type) diffusely involving the bone marrow as predominantly small, irregular lymphocytes that are shown to express CyclinD1 by immunohistochemistry (**f**)

be assessed by immunohistochemistry or by evaluating for a *CCND1* rearrangement prior to rendering a definitive diagnosis of CLL, even if the immunophenotype and morphology appear to suggest this diagnosis. Of note, hairy cell leukemia and plasma cell myeloma frequently express CyclinD1, but the characteristic morphologic and other immunophenotypic features of these neoplasms allow their distinction from MCL.

When presenting as a leukemia or as the blastoid variant, MCL often shows prominent nucleoli and dispersed chromatin and may mimic an acute myeloid or lymphoid leukemia morphologically (Fig. 12.13c, d). However, the cells in the bone marrow biopsy often lack the prominent nucleoli present in the blood smears. Immunophenotyping can readily distinguish MCL from acute leukemias. The frequently prominent nucleoli, marked leukocytosis, and splenomegaly that often characterize MCL with a leukemic presentation have led to previous classification of some cases with B-PLL. However, currently mature B-cell leukemias with *CCND1* rearrangement now are considered to represent MCL, irrespective of any morphologic resemblance to B-PLL.

Hairy Cell Leukemia

Hairy cell leukemia (HCL) is a mature B-cell lymphoma that primarily involves the blood and bone marrow, as well as the splenic red pulp. Patients usually present with one or more cytopenias and a relatively low level of circulating neoplastic hairy cells. The neoplastic B cells have characteristic surface "hairy" projections, best visualized on the peripheral blood smear. As the therapeutic approach to HCL is distinct from other lymphomas, it is essential to accurately diagnose the disease and to differentiate it from other B-cell lymphomas so that patients receive the right therapy.

Clinical Features

HCL is uncommon, representing only 2% of all leukemias [2]. The median age at diagnosis is 50 years, with a marked male predominance (male/female ratio 4:1) [54]. Patients with HCL present most often with symptoms related to one or more cytopenias, such as infections, weakness, or fatigue. Sometimes the disease is diagnosed when a complete blood count (CBC) discloses cytopenias in an asymptomatic patient [55]. Unlike most other B-cell lymphomas discussed in this chapter, HCL is characterized by a leukopenic rather than a leukemic presentation [56]. The presence of marked leukocytosis with numerous circulating neoplastic cells tends to suggest hairy cell variant (see below) or another lymphoma subtype. Monocytopenia is present in almost all cases at diagnosis and can be a helpful clue in suggesting a diagnosis of HCL in a cytopenic patient; conversely, a diagnosis of HCL would be very unusual in a patient with a normal monocyte count. Palpable splenomegaly is present in most patients at diagnosis, but up to 25% of patients lack splenomegaly at diagnosis [54, 55, 57]. Peripheral lymphadenopathy is uncommon, in contrast to many other mature B-cell leukemias.

Morphology

A diagnosis of HCL can usually be established on peripheral blood findings (morphology and immunophenotype) in the context of the typical clinical features described above. However, bone marrow biopsy is recommended in all newly diagnosed cases to determine the degree of marrow disease burden prior to treatment. Obtaining a good bone marrow core biopsy sample is essential, because the bone marrow aspirate is often poor or even unobtainable due the presence of bone marrow fibrosis [58].

Hairy cell morphology is best demonstrated on the peripheral blood smear: hairy cells are larger than small, mature lymphocytes and have oval or "bean-shaped" nuclei with dispersed chromatin and lack prominent nucleoli. In thin areas of the blood smear, hairy cell cytoplasm is relatively abundant and has a pale blue appearance. The cell surface is typically ruffled in appearance, and in many (but not all) cells, slender "hairy" projections can be visualized and are distributed around the

cell surface (Fig. 12.14a) [59]. Due to their abundant cytoplasm and dispersed chromatin, hairy cells may be erroneously counted as monocytes in the WBC differential; for this reason, the peripheral smear should always be carefully reviewed for monocytes versus hairy cells if HCL is in the differential diagnosis. The classic hairy cell morphology is often less evident in bone marrow aspirate smears or touch preparations, but can be seen in extensively involved samples with well-prepared smears (Fig. 12.14b) [60].

In the BM biopsy, the HCL infiltrate is interstitial or diffuse and does not form nodules, unlikely many B-cell lymphomas involving BM (Fig. 12.14c–e). The hairy cells appear monotonous with oval- or bean-shaped nuclei and clear cytoplasm that imparts a "fried egg" appearance to BM heavily involved by HCL. However, in more subtle examples of HCL, the BM is normocellular or even hypocellular, and the hairy cells may be obscured as they percolate among the normal hematopoietic elements [61]. Hematopoietic elements may manifest morphologic dysplasia, potentially mimicking a myelodysplastic syndrome [62]. Significant reticulin fibrosis is found in most cases (Fig. 12.14f) but may be lacking in some cases with a smaller degree of involvement [63].

Immunophenotype

Even if a BM aspirate sample is unobtainable, the diagnostic HCL immunophenotype can almost always be demonstrated by performing flow cytometry on the peripheral blood. The neoplastic cells of HCL express bright CD45, bright CD20, and bright CD19, FMC-7, CD22, and CD79a. CD5, CD10, and CD23 are usually negative, but CD5 has been reported to be positive in about 5% of cases and CD10 in up to 26% [64, 65]. Bright expression of CD11c, CD25, and CD103 is characteristic of HCL, and these markers should be added to the PB and BM flow cytometry panels in all cases where HCL is a diagnostic consideration. Light chain assessment can be difficult due to the "stickiness" of the cell surfaces that may bind both kappa and lambda nonspecifically. CD123 and CD200 are expressed in almost all cases [66]. Beyond the immunophenotypic features, the characteristic high forward and side scatter qualities of hairy cells on flow cytometry can be a clue to the diagnosis. Care must be taken not to exclude these cells from the lymphocyte gate when performing the flow cytometry analysis (Fig. 12.15).

If the characteristic HCL immunophenotype (including positivity for CD103, CD25, and CD11c) can be demonstrated by flow cytometry, paraffin section immunohistochemistry on the biopsy sample usually is unnecessary, except to help quantify involvement. Routine B-cell markers such as CD20 and CD79a often reveal far more hairy cells than are expected based on the H&E stain. HCL cells express CD25, CD123, CD11c, and Annexin A1 by immunohistochemistry, but some of these antigens also are expressed in other marrow cells (such as Annexin A1 positivity in neutrophils), which can render interpretation difficult if the degree of involvement is modest. DBA.44 stains hairy cells in bone marrow trephine sections, but it may not stain all the neoplastic cells, and it is expressed in other lymphomas, such as splenic marginal zone and splenic diffuse red pulp B-cell lymphoma. Cyclin D1 is weakly expressed in most cases [67] despite the lack of any associated *CCND1* rearrangement in HCL [68]. The neoplastic cells can also be disclosed by using an antibody against mutated BRAF protein, which is applicable to paraffin-embedded decalcified bone marrow sections and correlates with the presence of *BRAF* mutation in nearly all HCL cases (see below) [69, 70] (Fig. 12.16).

Cytogenetics and Molecular Genetics

Routine cytogenetic analysis of HCL is generally not indicated, and no prognostic cytogenetic markers have been identified [71]. Despite CyclinD1 expression in most HCL cases [67], translocations involving the *CCND1* locus do not occur in HCL [68, 72]. A point mutation in the *BRAF* gene (V600E), an oncogene located at chromosome 7q24, is present in the vast majority

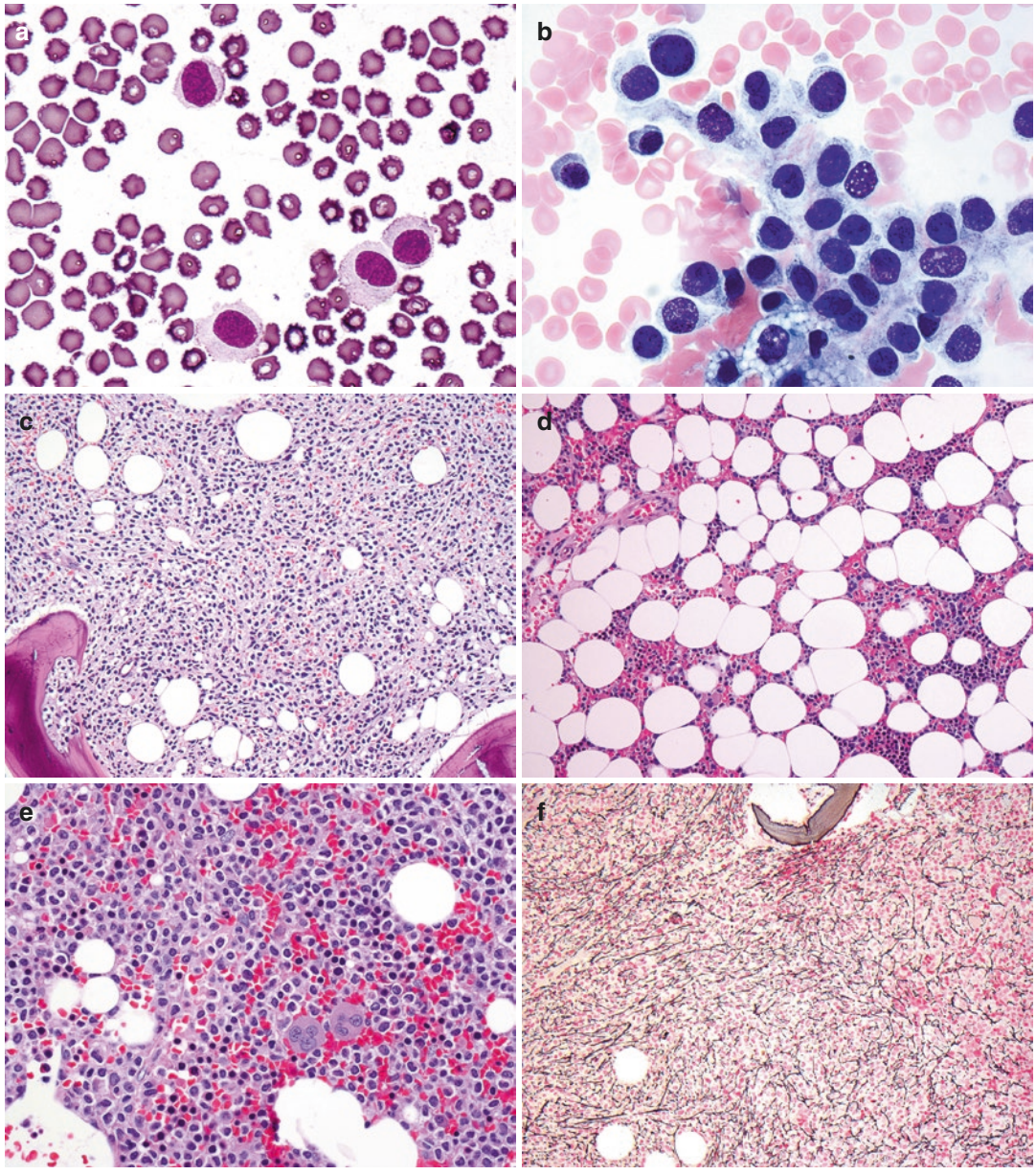

Fig. 12.14 Hairy cell leukemia (HCL) peripheral blood and bone marrow morphology. (**a**) HCL cells in the peripheral blood are usually sparse; in this rare example with leukocytosis, several neoplastic cells are seen, which have oval or indented nuclei, relatively dispersed chromatin, absent nucleoli, and abundant, flocculent cytoplasm with numerous circumferential delicate hairy projections. (**b**) In this bone marrow specimen that was heavily involved with HCL, numerous hairy cells are evident, with flocculent pale basophilic cytoplasm; hairy projections are generally not as well preserved in aspirate smears compared to peripheral blood smears. (**c**) In bone marrow heavily involved by HCL, the cells may appear markedly elongated or spindled. (**d**) Some early cases of HCL show subtle interstitial involvement that can be missed on casual inspection. In this case, the HCL cells are visible as ovoid nuclei infiltrating among marrow elements. Immunostains typically reveal a much higher number of HCL cells than suspected on the histology (CD20 immunostain for this case shown in Fig. 12.16**a**). (**e**) HCL cells on high magnification show a "fried egg" appearance in tissue sections. (**f**) Reticulin is increased in most bone marrows involved by HCL

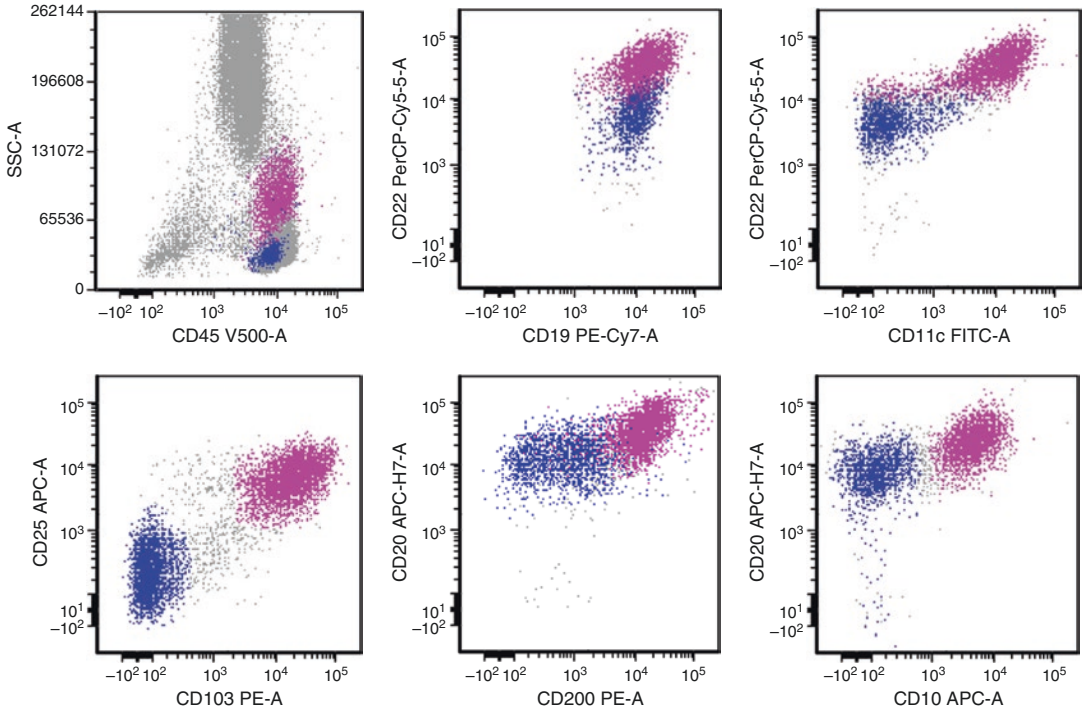

Fig. 12.15 Hairy cell leukemia flow cytometry findings. Hairy cell leukemia cells (*pink*) and background normal B cells (*blue*). Hairy cell leukemia cells have high SSC, falling into the monocyte region (CD45/SSC). Hairy cell leukemia cells are brightly positive for CD22, CD20, CD11c, CD25, and CD103. They are also uniformly bright CD200+. About 20% of hairy cell leukemias are CD10+, as illustrated in this case

of HCL and is absent from other small B-cell lymphomas [73]. The *BRAF* V600E mutation can be detected in bone marrow aspirates or peripheral blood by allele-specific PCR or next-generation sequencing technologies. Rarely, alternative *BRAF* mutations may occur in exon 11 [74]. The *BRAF* V600E mutation causes constitutive activation of the MAP kinase signaling pathway, an important pathway underlying the pathogenesis of HCL [75].

Differential Diagnosis

Hairy cell leukemia usually can be diagnosed accurately with the proper workup, as long as it is considered in the differential diagnosis. However, if the neoplastic infiltrate is subtle, erroneous diagnoses of MDS or even aplastic anemia may be rendered [61]. Immunostaining of the bone marrow biopsy specimen with a B-cell marker (such as CD20 and/or PAX5) is recommended in cases in which MDS is being considered, but HCL remains a possibility. Splenic marginal zone lymphoma is the most common differential diagnostic consideration. Although both splenic marginal zone lymphoma and HCL may show an intrasinusoidal pattern of marrow infiltration, the former usually also shows lymphoid nodules, which are characteristically absent in HCL. Circulating splenic marginal zone lymphoma cells have less prominent and blunter hairy projections, which are "polarized" to one section of the cell surface, unlike the encircling hairy projections seen in hairy cells [76]. Although splenic marginal zone lymphoma is also negative for CD5 and CD10, it does not typically manifest the CD103+, CD25+, and CD11c+ phenotype characteristic of HCL; it also is CD123- and negative for annexin A1 and cyclinD1. Morphologic and clinical features that help in the differential diagnosis of HCL with other B-cell leukemias are

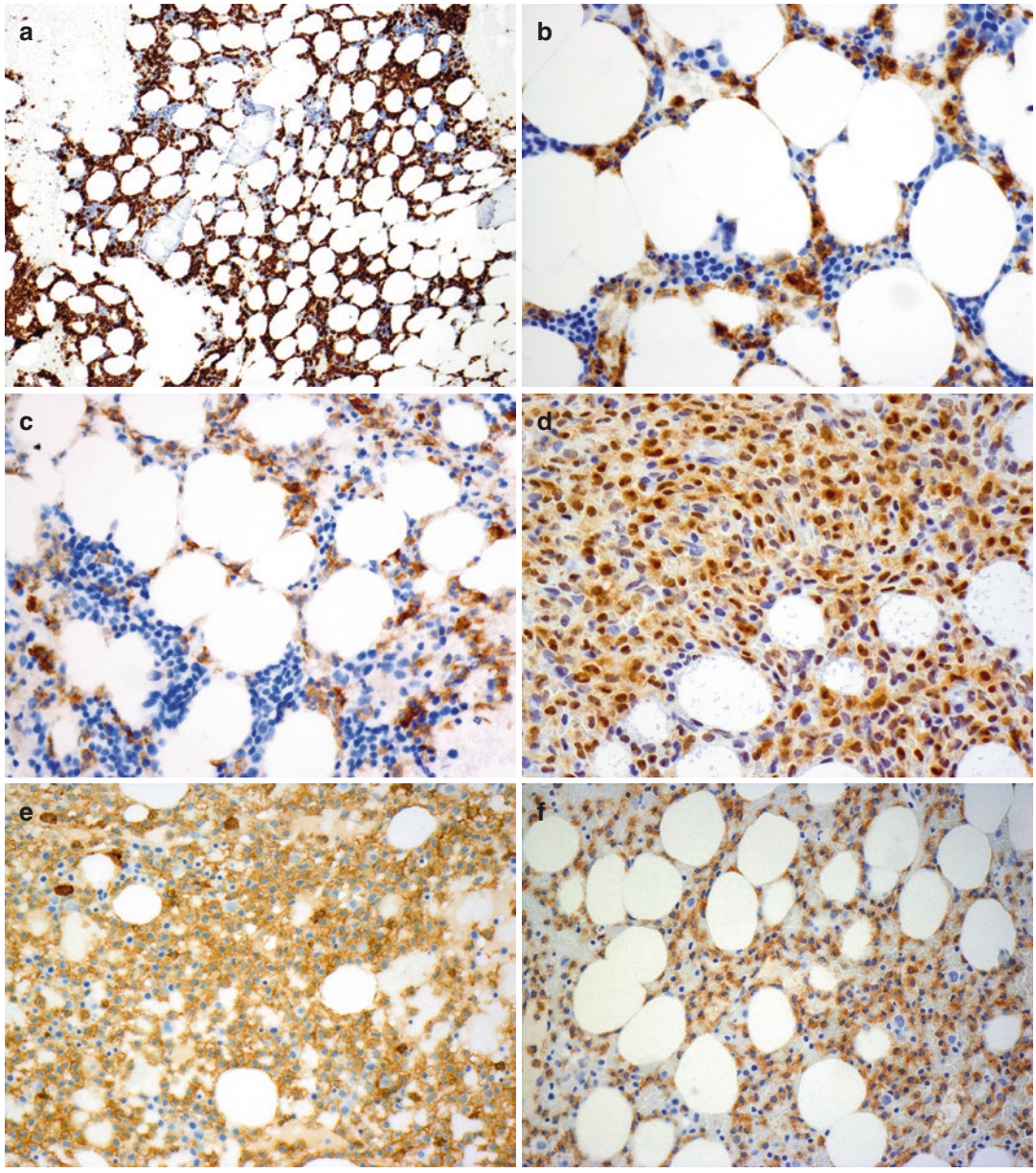

Fig. 12.16 Hairy cell leukemia (HCL) immunohistochemistry. (**a**) CD20 often reveals far more hairy cells than suspected on the histologic sections; unlike most other B-cell lymphomas, HCL does not form nodules in the bone marrow. The neoplastic HCL cells are positive for CD25 (**b**), CD123 (**c**), CyclinD1 (**d**), and AnnexinA1 (**e**). Immunostain for mutated BRAF protein (**f**) is positive in almost all HCL, although staining may be weak

listed in Table 12.2. Splenic diffuse red pulp small B-cell lymphoma remains a provisional entity within the category of "splenic B-cell lymphoma/leukemia, unclassifiable" in the 2016 WHO Classification system. It has a splenic infiltration pattern similar to that of HCL but also bone marrow intrasinusoidal involvement similar to that seen in splenic marginal zone lymphoma. The immunophenotype is more akin to splenic marginal zone lymphoma, although some cases can express CD123 or CD103, and DBA.44 frequently is positive [77, 78].

Hairy Cell Variant

Hairy cell variant (HCL-v) is a distinct B-cell leukemia, classified separately from HCL in the 2016 revised WHO Classification within the category of "splenic B-cell lymphoma/leukemia, unclassifiable." It is only about 10% as frequent as HCL (which itself is a rare disease). HCL-v differs morphologically, immunophenotypically, and clinically from HCL and also shows distinct genetic aberrations [79]. HCL-v patients tend to be older than HCL patients, with a median age of 71 years. In contrast to HCL, there is usually marked leukocytosis with a white blood count over 30 x 10^9/L and with numerous circulating neoplastic cells [80, 81]. Patients often have massive splenomegaly. The leukemic cells resemble hairy cells in terms of having abundant cytoplasm and surface projections, but also have prominent central nucleoli that are not typically seen in HCL, resembling prolymphocytes (Fig. 12.17) [80]. Monocytopenia is absent.

The pattern of bone marrow infiltration is similar to that in HCL, but HCL-v typically lacks bone marrow fibrosis [80]. An intrasinusoidal infiltration pattern may be observed [44]. Immunophenotypically, HCL-v shares expression of DBA.44 and CD103 with HCL and is often also CD11c positive, but it is negative for CD25, CD123, CyclinD1, and AnnexinA1 [81–83]. CD200 is either negative or dimly expressed (Fig. 12.18).

HCL-v lacks *BRAF* mutations, but up to half of HCL-v cases bear activating mutations in the *MAP2K1* gene [84, 85]. A high proportion of HCL-v cases show *TP53* gene deletion [86] and/or *TP53* mutations [87]. The clinical course of HCL-v is more aggressive than classical hairy cell leukemia, and patients usually do not respond to therapies administered to treat HCL; thus, it is important to correctly distinguish between HCL and HCL-v [80].

Splenic Marginal Zone Lymphoma

Splenic marginal zone lymphoma (SMZL) involves peripheral blood and the bone marrow in the vast majority of cases, and the diagnosis is often made based on the peripheral blood and bone marrow features rather than on a splenectomy specimen [88].

Clinical Features

Patients usually present with splenomegaly and lymphocytosis, often with other cytopenias due to hypersplenism and/or bone marrow infiltration. Up to 25% have lymphadenopathy, but this usually is localized to the abdomen. Peripheral lymphadenopathy at presentation is rare [89]. Most patients have absolute lymphocytosis, and rare patients may present with lymphocytosis before the development of splenomegaly [90]. Even in patients

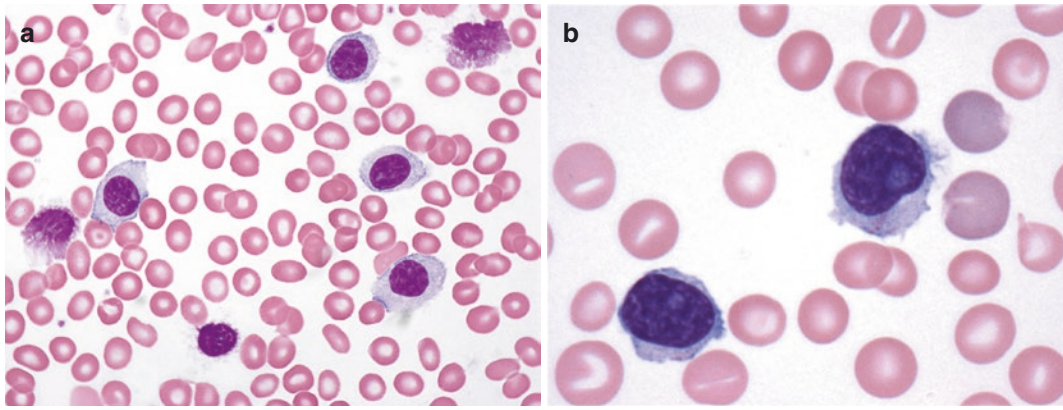

Fig. 12.17 Hairy cell variant morphology in peripheral blood. (**a**) Hairy cell variant cells resemble HCL cells, but have distinct nucleoli and are somewhat larger. (**b**) Some cases may have prominent nucleoli resembling prolymphocytes; these cells demonstrate typical surface hairy projections

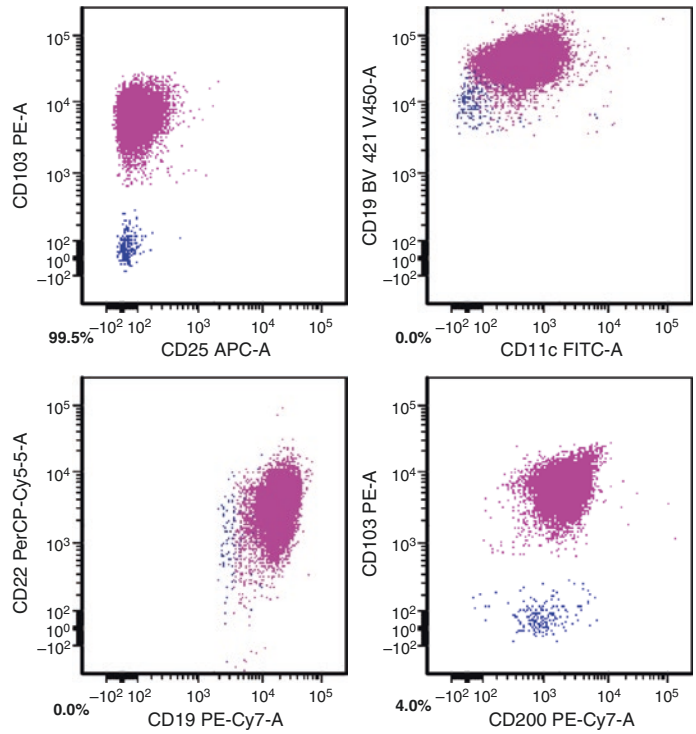

Fig. 12.18 Hairy cell leukemia variant flow cytometry findings, showing hairy cell leukemia variant cells (*pink*) and background normal B cells (*blue*). Hairy cell leukemia variant cells are CD103+ and CD25-. CD11c expression in this case is rather dim, and CD22 shows a similar level to normal B cells, contrasting typical bright CD22 expression in hairy cell leukemia. CD200 is uniformly positive but at a dimmer level than hairy cell leukemia

lacking lymphocytosis at presentation, neoplastic cells usually can be identified on the peripheral smear and/or detected by flow cytometry.

Morphology

Circulating neoplastic cells are medium sized to occasionally large in size and have oval nuclei, inconspicuous nucleoli, and a moderate amount of pale, basophilic cytoplasm; some may appear plasmacytoid. Cytoplasmic villous projections are reported to be located at one aspect of the cell surface (polarized) and are relatively short compared to the longer villi present all around the cell surface in HCL (Fig. 12.19). However, these findings may not be demonstrable in many cases [91]. In the bone marrow aspirate, the neoplastic cells are more heterogeneous and show a range of cell sizes and nuclear shapes. Monocytoid cells with moderately abundant, clear cytoplasm, small plasmacytoid cells, and occasional large cells that resemble centroblasts may be observed [92]. As with HCL, the villous projections of SMZL usually are not as well displayed in the bone marrow aspirate as in the peripheral blood smears [90].

Bone marrow involvement usually manifests as non-paratrabecular nodules, sometimes surrounding reactive germinal centers, and as intrasinusoidal infiltration. Paratrabecular and interstitial infiltrates can also occur [88, 93]. Intrasinusoidal lymphoma involvement is characterized by small chains or clusters of neoplastic lymphoid cells within vascular sinuses, usually clearly identifiable only through immunohistochemistry (see below) (Fig. 12.19). Although commonly observed in SMZL, intrasinusoidal involvement is not present in all cases and can be seen in other types of lymphoma [88, 94]. An exclusively intrasinusoidal pattern, which may be overlooked on routine histology, is present in about 10% of cases of SMZL [95].

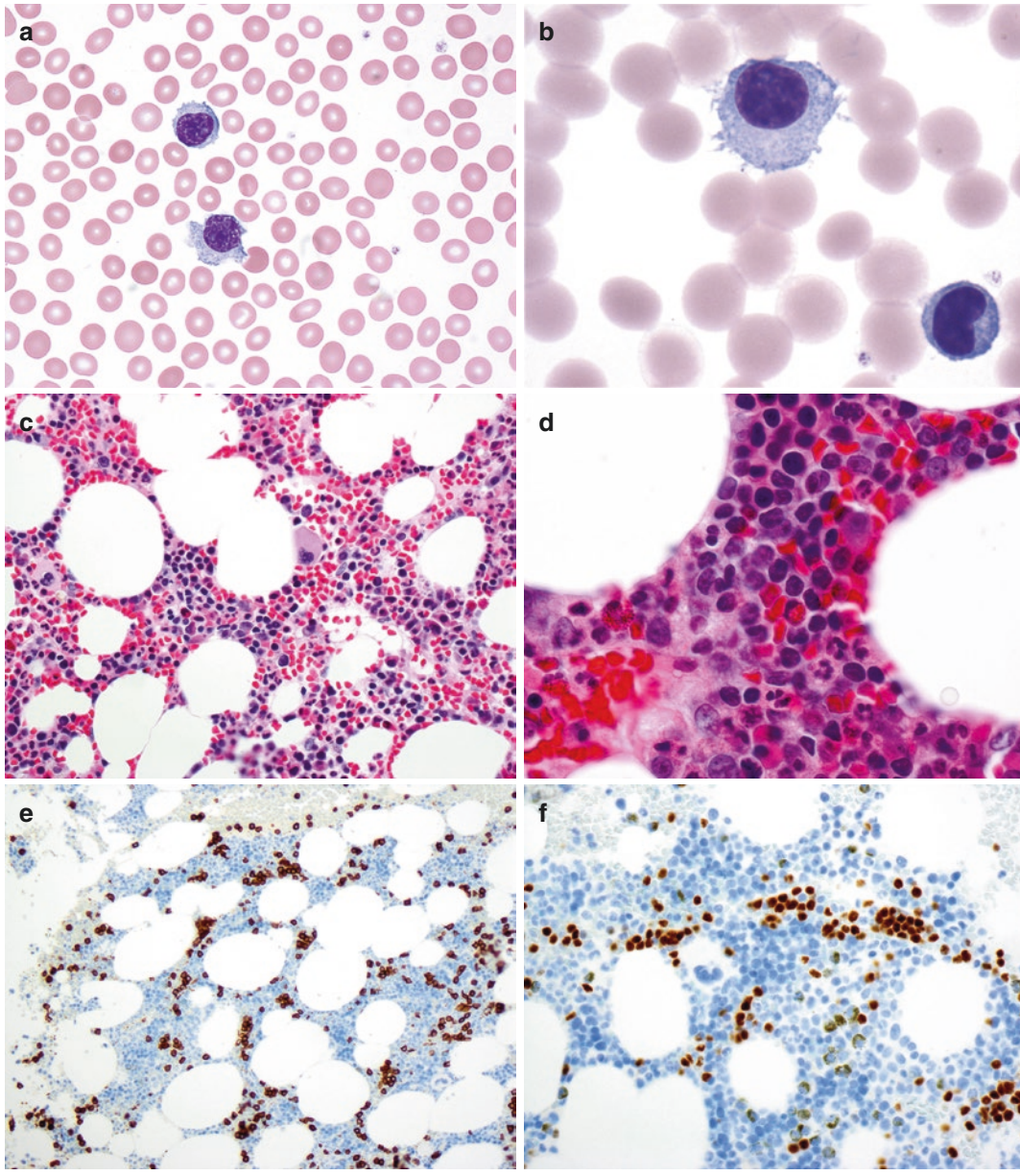

Fig. 12.19 Splenic marginal zone lymphoma (SMZL) morphology. (**a**, **b**) In the peripheral blood, SMZL cells generally resemble hairy cell leukemia cells but tend to have more basophilic cytoplasm, rounder nuclei, more condensed chromatin, and projections that are polar rather than circumferential (seen in the top cell in **a**). (**c**) The bone marrow infiltrate is often subtle. (**d**) On high power, a collection of small, mature lymphoid cells is seen in a pattern suggesting intrasinusoidal involvement. Intrasinusoidal infiltration, characteristic of SMZL, is best demonstrated with B-cell immunostains such as CD20 (**e**) or PAX5 (**f**), which show groups and linear arrays of cells within the bone marrow sinusoids

Immunophenotype

The immunophenotype of SMZL is CD19+, CD20 bright+, and CD10- and usually CD5-, CD103-, and CD25-. CD11c and CD23 can be positive or negative. CD200 is often partially expressed, in contrast to its uniform expression in HCL and CLL and its absence of expression in MCL and follicular lymphoma. DBA.44 can be

positive by immunohistochemistry, although the staining is less uniform and is weaker than in splenic diffuse red pulp small B-cell lymphoma [78]. Expression of CD5 at variable levels can be seen in 10–15% of cases, and this finding alone does not exclude the diagnosis, provided CLL and MCL can be ruled out; CD5 expression may be more frequent in the bone marrow than in the spleen [96]. Applying immunohistochemistry for B-cell markers such as CD20 or PAX5 is very helpful at disclosing the intrasinusoidal infiltration pattern that is characteristic of SMZL (Fig. 12.19e, f). Monotypic plasma cells are found in some cases [93]. Immunohistochemistry for follicular dendritic cell markers such as CD21 and CD23 may disclose colonized germinal center remnants within the lymphomatous nodules.

Cytogenetics and Molecular Genetics

There are no specific cytogenetic findings in SMZL and there are no *BCL2* or *CCND1* rearrangements. Of note, about one-third of cases show deletion of 7q, which is not frequently found in other lymphoma subtypes [97, 98], while a smaller number have a t(2;7)(p12;q21) translocation [99]. *NOTCH2* mutations are found in 10–25% of cases and are relatively infrequent in other B-cell lymphomas, while *KLF2* mutations are seen in a similar proportion of SMZL but are less specific [100–102].

Differential Diagnosis

The combination of a nodular marrow infiltrate (often with intrasinusoidal infiltration, disclosed by CD20 staining), characteristic morphology of the circulating neoplastic cells, and an immunophenotype not specific for any other subtype of B-cell lymphoma often allows a diagnosis of SMZL in a patient with splenomegaly without the need for splenectomy [90]. The main differential diagnosis in such a context usually is lymphoplasmacytic lymphoma (LPL). Although about one-third of patients with SMZL have a serum paraprotein, the level usually is relatively low (less than 2 g/dL), and the paraprotein may be IgM or some other heavy chain type [103]. In contrast, the paraprotein in LPL is usually IgM (with some cases being IgG), and the level usually is higher. The majority of LPL cases do not have PB lymphocytosis. Also, LPL involvement of the BM usually is interstitial, nodular, or diffuse, and intrasinusoidal involvement is uncommon [103]. Plasma cells are invariably present in LPL, mixed with lymphocytes, and are almost always clonal. Demonstration of a *MYD88* mutation ultimately favors a diagnosis of LPL. Cases with heavy BM involvement and a prominent intrasinusoidal pattern may raise the differential of splenic diffuse red pulp small B-cell lymphoma, an entity that can be difficult or impossible to distinguish from SMZL on bone marrow histology alone; in ambiguous cases, splenectomy may be required for a definitive diagnosis. After splenectomy and/or chemotherapy, the extent of BM involvement by SMZL may either increase or decrease. Nodular and interstitial involvement tends to predominate over intrasinusoidal infiltrates later in the course of the disease [104].

Lymphoplasmacytic Lymphoma

LPL is an uncommon B-cell neoplasm that arises from a post-germinal center B cell, shows plasmacytic differentiation, and in most cases secretes an IgM paraprotein [105]. The IgM paraprotein may result in hyperviscosity and/or cryoglobulinemia, a clinical syndrome called Waldenström macroglobulinemia.

Clinical Features

LPL affects older adults, and a male predominance is seen. Most patients present with symptoms related to BM infiltration, such as anemia or infections, or hyperviscosity [106]. There is almost always a serum M protein, usually IgM and rarely IgG or other immunoglobulin subtypes. Patients who present with the hyperviscosity picture of Waldenström macroglobulinemia have a particularly high serum IgM level: the median level in these patients is 3.8–5.1 g/dL, whereas the reported median IgM level in lymphoplasmacytic lymphoma overall is 1.5–2.2 g/dL [105–107]. Some cases may be associated with hepatitis C

infection, commonly with concomitant type II cryoglobulinemia [108]. Up to 30% of patients have lymphadenopathy and/or splenomegaly [109].

Morphology

Plasmacytoid lymphocyte cells can circulate in the peripheral blood but are usually infrequent in LPL [110], and flow cytometry on the blood may fail to disclose any clonal B-cell population; overt lymphocytosis is rare. When LPL cells are present on the peripheral smear, they show similar features to those in the BM aspirate smear (see below). Prominent RBC clumping may be seen due to red cell agglutination.

The BM is involved in almost all cases at presentation, and the diagnosis is usually made on BM biopsy. The infiltration patterns vary: interstitial and nodular non-paratrabecular patterns are most commonly seen, while paratrabecular infiltrates have been reported to occur in a minority of cases [111]. Diffuse infiltration patterns can also occur when the level of involvement is extensive. The pattern or degree of BM involvement does not appear to correlate with the level of IgM paraprotein [107]. The neoplastic infiltrates include small lymphocytes, plasma cells, and plasmacytoid lymphocytes, the latter cells having hybrid features between lymphocytes and plasma cells (Fig. 12.20). In most cases, lymphocytes outnumber the plasma cell component in the bone marrow, but plasma cells predominate in a minority of cases. Plasmacytoid lymphocytes are seen in a spectrum of forms, frequently including cells with condensed chromatin that resembles plasma cells but with the scant, pale cytoplasm of a lymphocyte. Also seen are cells with nuclear chromatin resembling lymphocytes, but with basophilic, eccentric, plasmacytoid cytoplasm. Intranuclear eosinophilic immunoglobulin pseudoinclusions (Dutcher bodies) are present in about half of the cases, while cytoplasmic immunoglobulin inclusions (Russell bodies) are rare. Large cells usually are infrequent, but some cases may have admixed large cells imparting a polymorphous appearance. Mast cells frequently are present within the lymphoma and have been postulated to play a role in tumor pathogenesis [112]. However, mast cells also can be frequent in other BM lymphomas. Thus, although their presence is expected and supports the diagnosis, they are not specific for LPL.

Immunophenotype

A clonal B-cell component is always detectable at diagnosis by flow cytometry and is characteristically CD20+, CD5-, CD10-, and CD23- (Fig. 12.21). Up to 20% of cases may express CD5, and expression of CD23 is even more frequent [113]. Unlike in CLL, these markers are usually weakly expressed in LPL. CD10 expression is very uncommon [27]. The B lymphocytes express monotypic surface IgM, and the plasma cells and plasmacytoid cells express cytoplasmic IgM. Less than 10% of cases express IgG or other heavy chain types.

Cytogenetics and Molecular Genetics

About 50% of cases bear a 6q deletion, a finding that is not specific for LPL but has been associated with an adverse prognosis [114]. A recently identified genetic hallmark of LPL is the presence of an *MYD88* L265P mutation, which characterizes over 90% of cases [115, 116]. Other reported gene mutations include *CXCR4* (about 30% of cases), *ARID1A*, as well as *TP53, CD79B, KMT2D*, and *MYBBP1A* [117].

Differential Diagnosis

It is important to note that not all lymphomas presenting with an IgM paraprotein represent LPL: CLL/SLL, marginal zone lymphomas, follicular lymphoma, diffuse large B-cell lymphoma, and some T-cell lymphomas also may be associated with an IgM paraproprotein. The characteristic features of LPL versus other small B-cell lymphomas involving the BM are shown in Table 12.3. Although rare patients with other types of lymphoma may have high IgM levels and hyperviscosity, the serum IgM level in these other lymphomas usually is much lower than in LPL [106, 118]. While a high IgM paraprotein level (over 3 g/dL) nearly always indicates LPL, only about one-third of LPL patients have a markedly elevated IgM level at diagnosis [106].

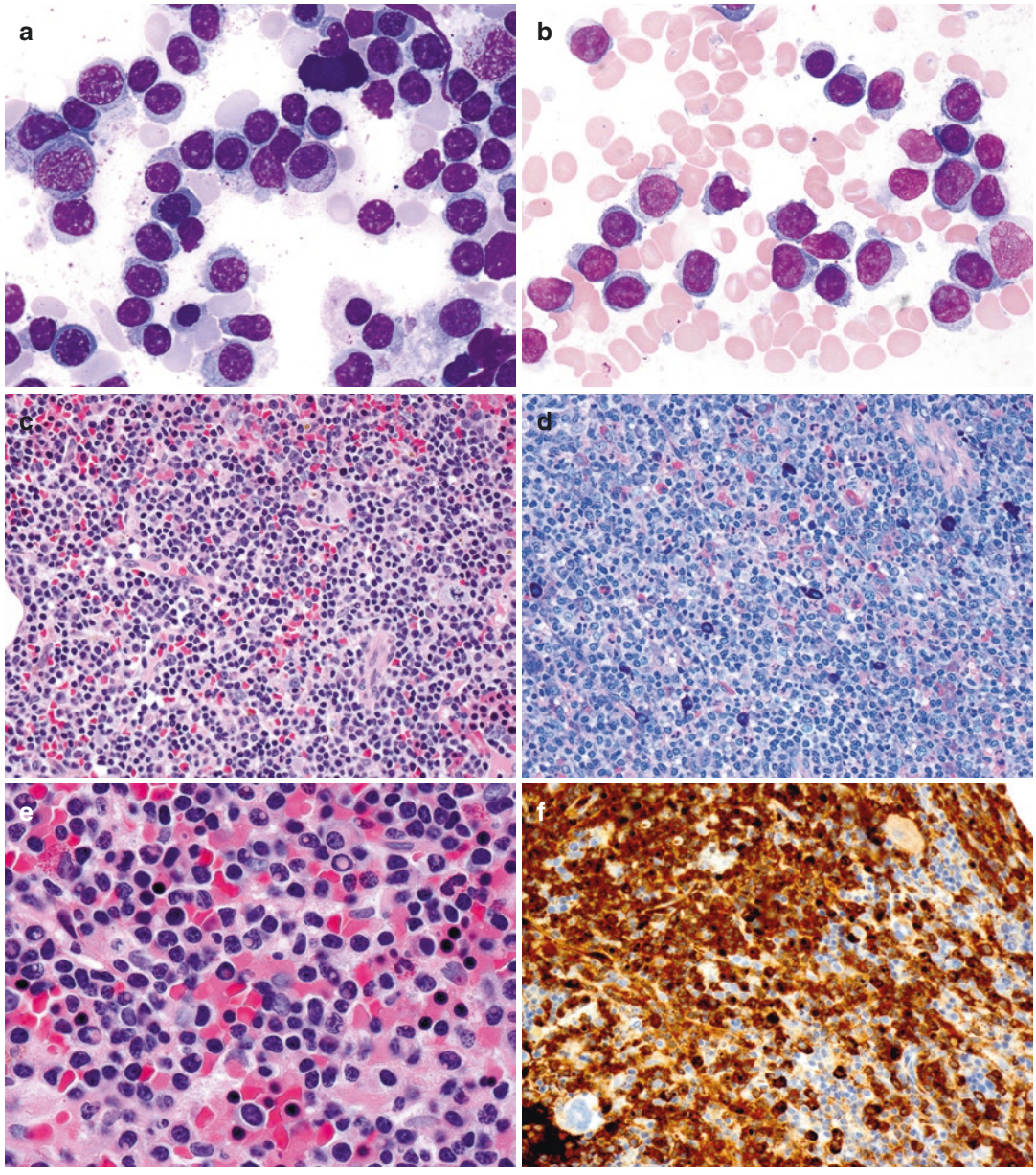

Fig. 12.20 Lymphoplasmacytic lymphoma (LPL). (**a**) In the bone marrow aspirate, there is usually a spectrum of cells ranging from small lymphocytes to plasma cells, with many intermediate forms. (**b**) Some cases have a more uniform population of lymphoplasmacytoid cells. (**c**) The range of lymphocytes, lymphoplasmacytoid forms, and plasma cells is also evident in this diffusely involved bone marrow biopsy. (**d**) Giemsa stain reveals increased mast cells, which is a typical but nonspecific finding in LPL. (**e**) On a higher magnification, the range of nuclear morphologies and Dutcher bodies can be appreciated. (**f**) An immunostain for IgM is positive in the neoplastic cells and highlights the Dutcher bodies, which contain monoclonal IgM

Conversely, MGUS may be an IgM type, and IgM MGUS is noted as a separate entity in the 2016 WHO Classification, defined as having <10% BM lymphoplasmacytic cells and <3 g/dL of serum IgM paraprotein; usually, the paraprotein level in IgM MGUS is much lower. In the absence of a significant (at least 10%) neoplastic infiltrate of clonal small lymphocytes

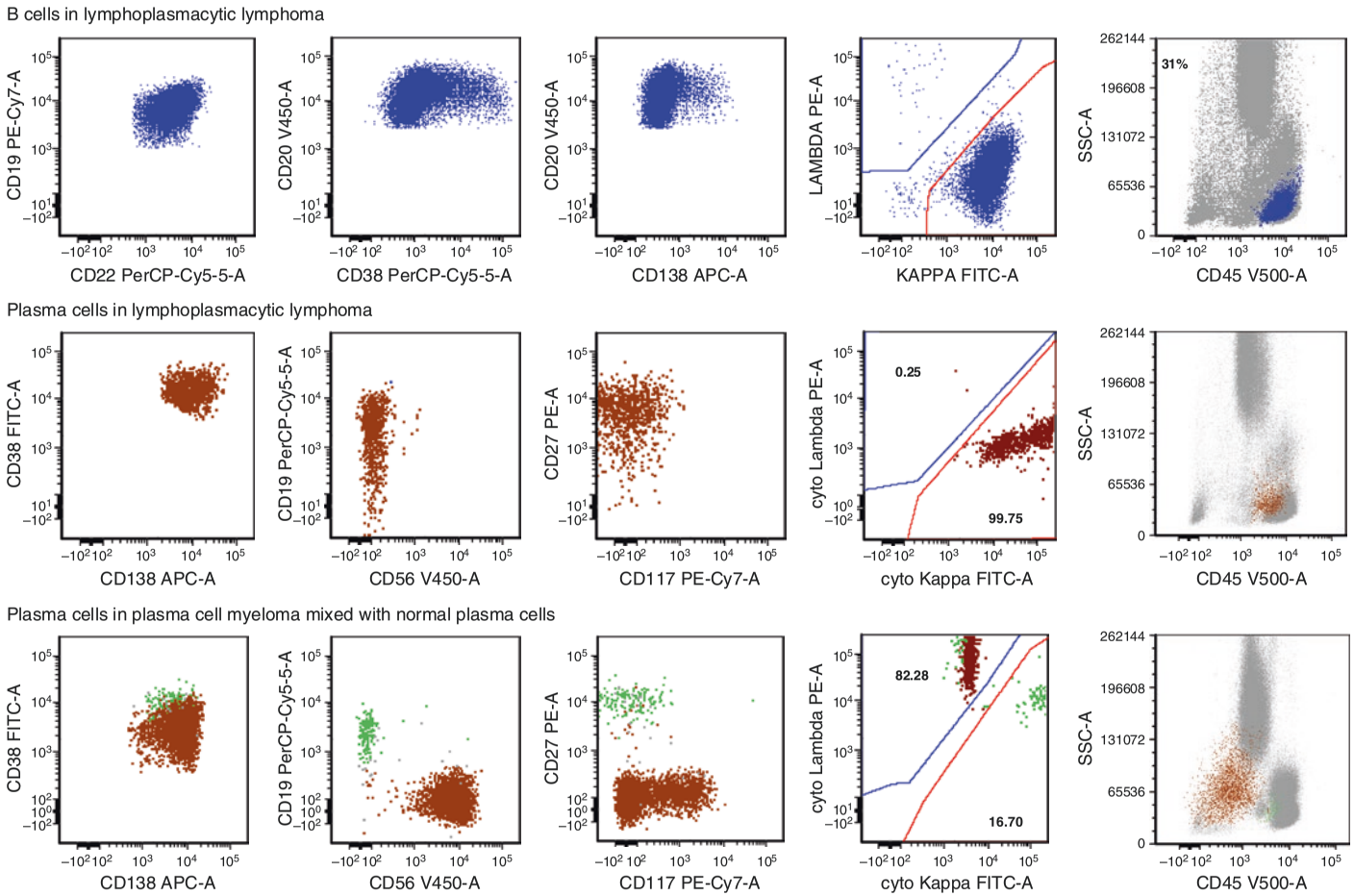

Fig. 12.21 Plasma cells in lymphoplasmacytic lymphoma (LPL) versus plasma cells in plasma cell myeloma. *Top panel* shows clonal B cells in LPL (*blue*) that are CD19+CD22+CD20+ and kappa restricted with very little CD38 or CD138 expression. *Middle panel* shows a population of plasma cells that are CD38+CD138+ and cytoplasmic kappa restricted with a normal immunophenotype (CD19+CD27+CD56-CD117-). *Lower panel* shows a case of plasma cell myeloma with clonal plasma cells (*brown*) and a few residual normal plasma cells (*green*). The neoplastic plasma cells have an aberrant immunophenotype (CD19-CD27-CD56+CD117partial+) and are cytoplasmic lambda restricted; the B cells were polytypic (not shown). The normal plasma cells are CD19+CD27+CD56-CD117-, similar to the plasma cells in LPL, but they are polytypic

and lymphoplasmacytoid cells, a diagnosis of LPL should not be made in the setting of an IgM paraprotein, even if a BM monotypic plasma cell component is present. Lower levels of BM infiltration represent IgM MGUS even in the presence of symptoms related to the paraprotein, such as cryoglobulinemia or neuropathy [119, 120].

The main pathologic differential diagnosis of LPL is with plasma cell myeloma and with other lymphomas that may show plasmacytic differentiation. Some cases of plasma cell myeloma express CD20 and have a small cell appearance that mimics LPL. However, unlike LPL, plasma cell myeloma lacks a surface immunoglobulin-positive clonal B-cell component. The plasma cell component of LPL almost always expresses IgM heavy chain and shows a normal plasma cell immunophenotype (CD19+CD45+CD56-CD117-) but with cytoplasmic light chain restriction, whereas plasma cells in myeloma are CD19- and CD45-, often with aberrant CD56, CD117, CD27, and/or CD28 expression and almost never of the IgM subtype [121] (Fig. 12.21). CD20+ plasma cell myelomas often express cyclin D1 and have a translocation of *CCND1*, findings that exclude a diagnosis of LPL. Myelomas, including CD20+ cases, also should co-express CD138 but lack PAX5 expression.

Distinction from other lymphomas with plasmacytic differentiation may be problematic, particularly when an IgM paraprotein is present. Although CD5 may be expressed in LPL, it usually lacks the uniform moderate to bright expression level characteristic of CLL and MCL and has brighter CD20 and surface immunoglobulin expression than CLL. CD200 is partially or dimly expressed in LPL, but is uniformly positive in CLL. LEF1 is positive in CLL and cyclinD1 in MCL; both of these markers are negative in LPL. Although considerable morphologic and immunophenotypic overlap is seen between marginal zone lymphomas (which often show extensive plasmacytic differentiation) and LPL, correlation with clinical and radiographic features (e.g., the presence or absence of splenomegaly or an extranodal mass) and the paraprotein level and type allow classification in most cases. Additionally, LPL does not tend to show the prominent intrasinusoidal BM infiltration pattern that characterizes splenic marginal zone lymphoma [122]. Finally, angioimmunoblastic T-cell lymphoma may show a marked reactive BM plasmacytic infiltrate, as well as an IgM paraprotein.

In ambiguous cases, demonstrating an *MYD88* mutation is very helpful, since it is present in nearly all LPL cases (including non-IgM subtypes) and is uncommon in other B-cell lymphomas. However, *MYD88* mutation does occur in a small subset of CLL and also in some cases of diffuse large B-cell lymphoma [123, 124]. Conversely, a diagnosis of LPL can be made in the absence of *MYD88* mutation if other features support the diagnosis.

Follicular lymphoma

Clinical Features

Rare cases of follicular lymphoma may present as leukemia with a high WBC count, clinically mimicking chronic lymphocytic leukemia. These patients almost always have concurrent splenomegaly and generalized lymphadenopathy.

Morphology

The circulating cells in leukemic follicular lymphoma are small and have characteristic deep nuclear clefts or grooves, as well as markedly irregular nuclear contours [125] (Fig. 12.22a, b).

In the BM, at least some paratrabecular aggregates are observed, although non-paratrabecular nodules also are frequently seen [111, 126]. A high level of BM lymphomatous involvement (10% or more of the marrow space) and a diffuse infiltration pattern are associated with poorer survival [127]. On close inspection, the lymphoid aggregates in follicular lymphoma contain cells with nuclear irregularity and angulation. However, these characteristic nuclear irregularities may be less marked in the bone marrow than in nodal follicular lymphoma. The neoplastic cells are arranged in linear arrays immediately adjacent to the bone trabeculae and extending along the trabecular surface

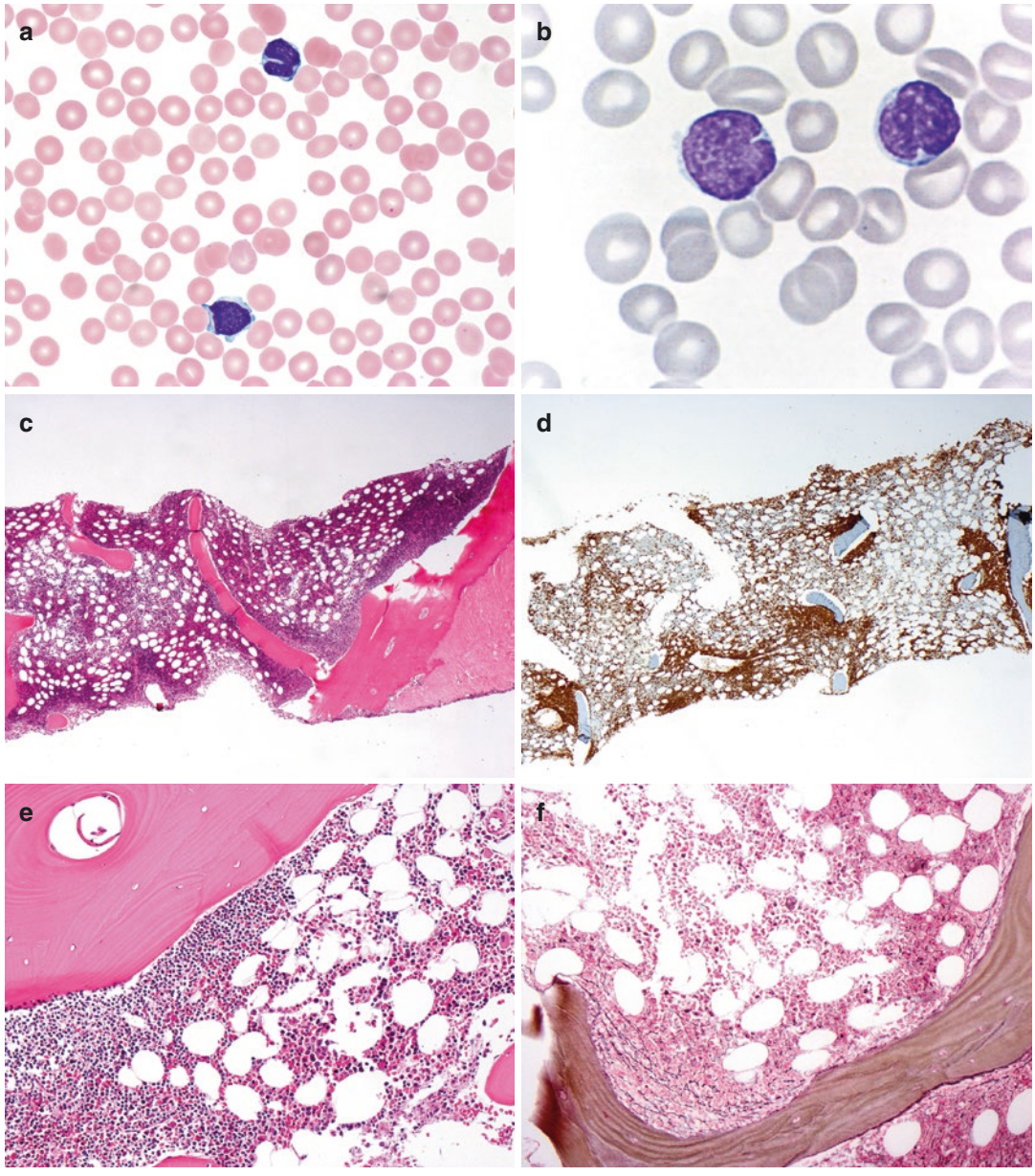

Fig. 12.22 Follicular lymphoma. (**a**, **b**) Follicular lymphoma cells in blood typically have deeply indented or clefted nuclei. (**c**) The bone marrow biopsy shows paratrabecular lymphoid infiltrates, which are highlighted by CD20 immunostaining (**d**). (**e**) Paratrabecular aggregates are closely opposed to the bone surface and have a linear or fusiform rather than the rounded shape of non-paratrabecular lymphomatous nodules. (**f**) Reticulin fibers are increased in the paratrabecular lymphomatous aggregates

(Fig. 12.22c–e). The paratrabecular aggregates in follicular lymphoma are associated with increased reticulin fibrosis (Fig. 12.22f), causing underrepresentation of the tumor cells in the aspirated marrow and occasionally false-negative flow cytometry results.

Neoplastic follicles resembling those present in involved lymph nodes are uncommon in grade 1–2 follicular lymphoma involving the BM, but they are seen more frequently in grade 3 follicular lymphoma. Unlike germinal centers, which may occur in the BM in reactive conditions and in other lym-

phomas (e.g., marginal zone lymphomas), the neoplastic follicles in grade 3 follicular lymphoma characteristically lack mantle zones and usually are bcl2+. However, some grade 3 cases may be bcl2-, and cases have been reported that show a prominent non-paratrabecular pattern with intact mantle zones [128].

Immunophenotype

Flow cytometry of the BM aspirate detects a clonal B-cell population in most cases with BM involvement, but false-negative results can be seen in up to half of the cases, most likely as a result of reticulin fibrosis associated with the paratrabecular lymphomatous aggregates [129]. Even in the absence of a confirmatory lymph node biopsy specimen, the combination of the characteristic immunophenotype (CD20 bright+, CD19 dimmer+, CD10+, CD5-, cyclin D1-) with a predominantly or exclusively paratrabecular marrow involvement pattern favors a diagnosis of follicular lymphoma; in one study, 90% of BM lymphoma cases with an exclusively paratrabecular pattern were follicular lymphoma [111]. Demonstrating co-expression of Bcl2 with Bcl6 and/or CD10 by immunohistochemistry in the BM lymphocytes can help confirm a diagnosis of follicular lymphoma, but Bcl2 and Bcl6 immunohistochemistry may be weak or falsely negative in decalcified sections [130]. Of note, absent surface light chain expression can be seen in about 10% of follicular lymphomas [131], potentially mimicking stage 1 and 2 hematogones. This can be particularly challenging in patients post-rituximab treatment in which the neoplastic B cells lack CD20 expression. Unlike hematogones, follicular lymphoma cells are usually negative for CD43 and have brighter CD45 expression and dimmer CD38 expression. Figure 12.23 illustrates a case of a BM staging sample that contains both follicular lymphoma with no surface light chain expression as well as hematogones. Recognition of the immunophenotype of hematogones is essential to reliably differentiate them from B lymphoblasts or clonal CD10+ follicular lymphoma cells.

Cytogenetics and Molecular Genetics

A t(14;18) rearrangement involving the *BCL2* gene is characteristic of follicular lymphoma and demonstrating this finding may be helpful in its

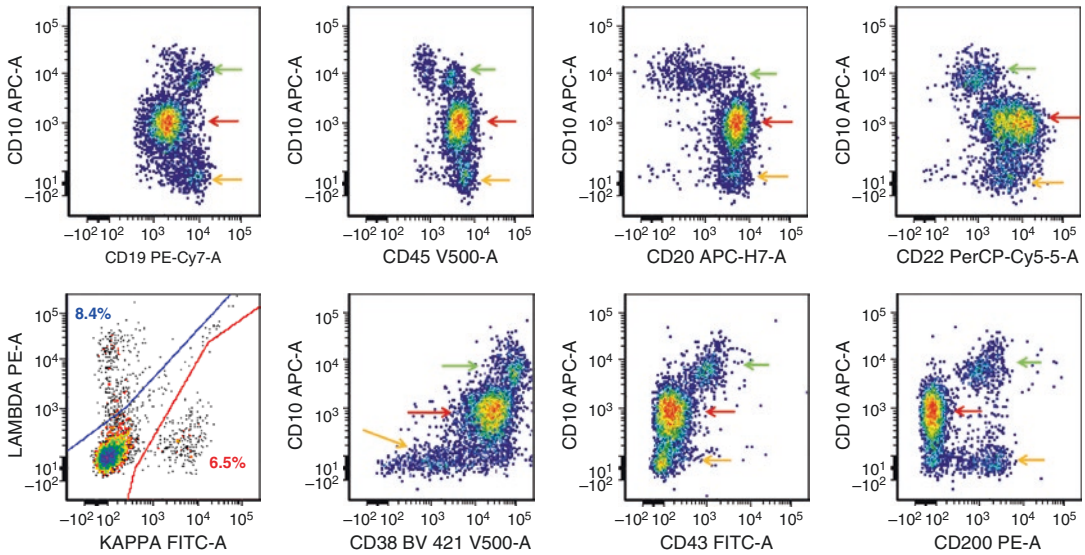

Fig. 12.23 Staging bone marrow sample from a patient with follicular lymphoma. Among the CD19+ B cells, there are several populations of CD10+ B cells, all of which have no surface light chain expression. *Green arrows* show stage 1 and stage 2 hematogones that have bright CD10 and CD38, dimmer CD45 and CD22, and variable CD20 and dim CD200 expression. *Red arrows* show follicular lymphoma cells that have bright CD45, CD22, and CD20, dimmer CD10, CD19, and CD38, and negative CD43 and CD200 expression. *Yellow arrows* indicate normal mature B cells that are CD19+,CD10-,CD20+, CD22+,CD38variable+,CD43-, CD200variable+, and are polytypic

differential diagnosis with other lymphomas that involve the BM. When the blood is involved, a PB sample can be sent for karyotype and/or FISH to document a *BCL2* rearrangement.

Differential Diagnosis

The CD10 expression, clefted cell morphology, and nearly ubiquitous presence of concurrent lymphadenopathy in follicular lymphoma involving the blood and BM usually make the diagnosis apparent. The characteristic features of follicular lymphoma involving the blood and BM relative to other B-cell lymphomas are shown in Table 12.3.

Diffuse Large B-Cell Lymphoma

In rare cases, a primary diagnosis of diffuse large B-cell lymphoma may be made from a BM sample in a patient not known to have lymphoma. Intravascular large B-cell lymphoma (IVLBL) is a rare, highly aggressive lymphoma of adults in which the tumor cells almost exclusively exist within small vascular lumina of various organs, including the BM vascular sinusoids. A BM biopsy is often the procedure that establishes a diagnosis of IVLBL.

Clinical Features

Patients with primary BM diffuse large B-cell lymphoma tend to be elderly or immunosuppressed and this type of lymphomatous presentation appears to be more common in Asian patients. Patients usually present with fever and/or unexplained cytopenias. IVLBL presents as one of the three clinical subtypes: the *classical*, or *Western form*, in which patients present most often with fever of unknown origin, skin rash, and/or neurologic symptoms; an *isolated cutaneous form*, in which the tumor is limited to skin blood vessels and has a more indolent behavior; and an *Asian variant*, characterized by hepatosplenomegaly, cytopenias, and an often rapid clinical decline because of an associated hemophagocytic syndrome. Despite their intravascular growth pattern, tumor cells are rare in PB smears (particularly in the classical form). The neoplastic cells may extravasate into tissues adjacent to the involved small vessels.

The BM is involved in about 75% of cases of the Asian variant of IVLBL, and the diagnosis often is made from a BM biopsy specimen. In contrast, only about one-third of cases of the classical form of IVLBL have marrow involvement [132]. IVLBL often is unsuspected clinically, with BM examination performed to investigate fever of unknown origin in Western cases and cytopenias and hepatosplenomegaly in Asian variant cases.

Morphology

Diffuse large B-cell lymphoma may involve the BM in any pattern. Diffuse and interstitial patterns are most common, but nodular nonparatrabecular and occasionally paratrabecular involvement also may be seen [111]. T-cell/histiocyte-rich diffuse large B-cell lymphoma is not uncommonly seen involving the BM and shows features that are similar to extramedullary tissue involvement. Large lymphoma cells may be abundant or rare in the aspirate smear and may be overlooked, as they often resemble early erythroid elements and may be mistaken for blasts (Fig. 12.24). In IVLBL, large tumor cells with variably abundant cytoplasm, vesicular nuclei, and prominent nucleoli fill dilated BM sinuses but usually are absent from extravascular marrow tissue. In subtly involved cases, the cells may be difficult to visualize on routine histology but are readily revealed by applying B-cell immunostains (Fig. 12.25). Because of their characteristic intrasinusoidal location, neoplastic cells may be sparse or absent in the aspirate. In the Asian variant, an associated hemophagocytic syndrome is present in a large proportion of the cases, and histiocytes containing engulfed erythrocytes and nucleated hematopoietic cells are identified in the extravascular marrow spaces of the biopsy specimen and in the aspirate smear.

Immunophenotype

Flow cytometry may be falsely negative in primary BM DLBCL [133]. Thus, it is reasonable to apply immunohistochemistry for CD20 and/or other B-cell markers to any BM sample from a patient with unexplained cytopenia or fever

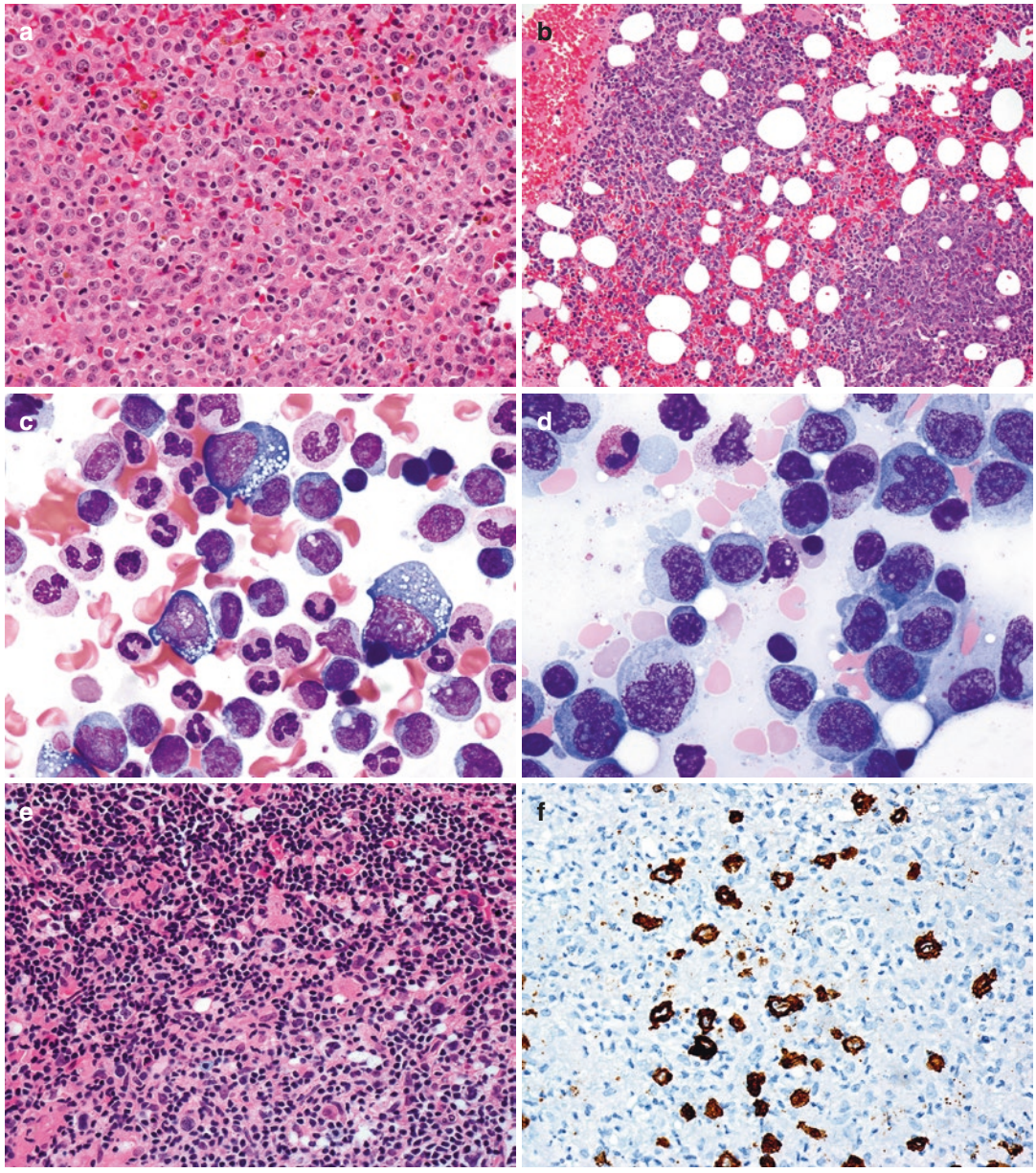

Fig. 12.24 Diffuse large B-cell lymphoma (DLBCL) involving the bone marrow. The involvement is often diffuse (**a**) but can also be nodular (**b**). DLBCL cells have variable appearance in aspirate preparations, frequently showing vacuolated, basophilic cytoplasm (**c**, **d**), potentially mimicking erythroblasts or myeloblasts. (**e**, **f**) T-cell/histiocyte-rich DLBCL not uncommonly involves the bone marrow; the scattered large neoplastic B cells occur in a prominent background of small, reactive mature lymphocytes (**e**) and are highlighted by CD20 immunostain (**f**)

of unknown origin if there are large cells of uncertain linage, either interstitial or intravascular, even if flow cytometry fails to detect a B-cell clone.

The cells of IVLBL express CD45 as well as pan-B-cell antigens such as CD79a and CD20. They frequently express MUM1 and are usually CD10 negative and are negative for EBV. About one-third of the cases express CD5, and these CD5+ cases are more likely to present with BM involvement [132].

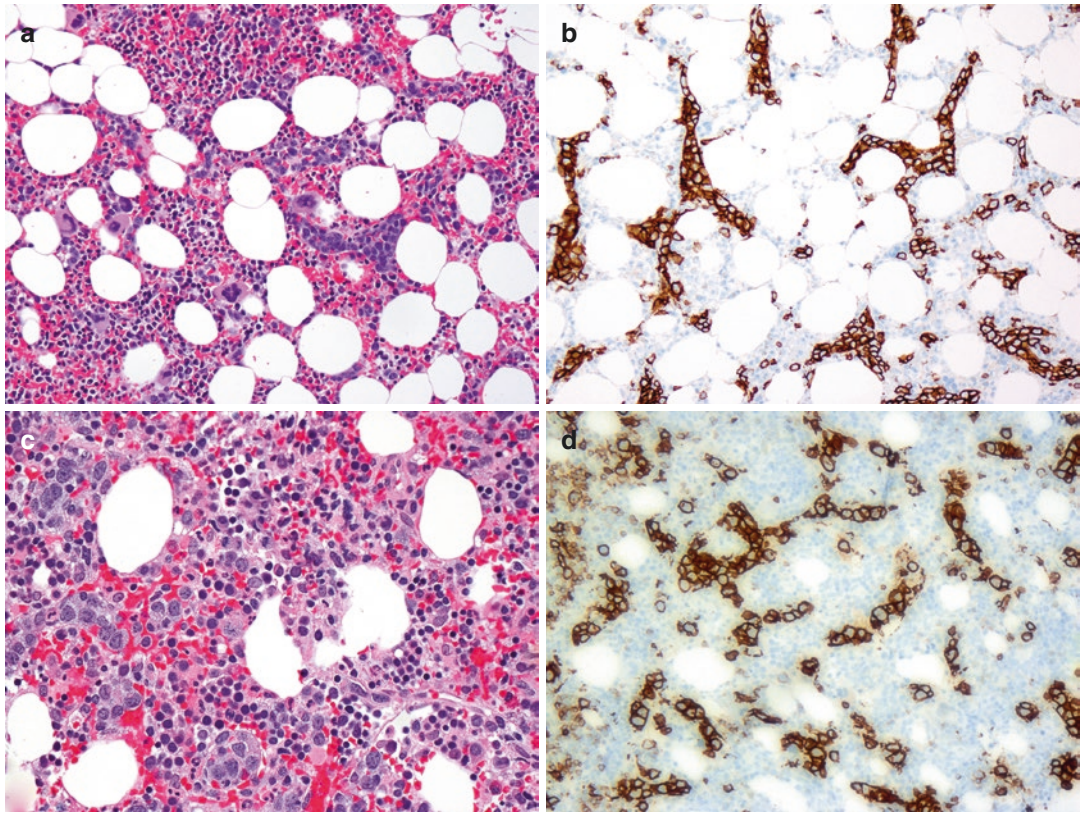

Fig. 12.25 Intravascular large B-cell lymphoma. (**a**) Often the intravascular large cell collections are obvious and conform to the sinusoidal space in the bone marrow biopsy. They are highlighted by CD20 immunostain (**b**) or with other B-cell markers. (**c**) In some cases, the intravascular pattern may not be as evident, and the large cells may resemble left-shifted hematopoietic elements. (**d**) The CD20 immunostain in this case reveals the prominently intrasinusoidal malignant large B-cell collections

Cytogenetics and Molecular Genetics

There is very little data on the cytogenetics or molecular genetic features of IVLBL, and these studies do not impact the diagnosis.

Differential Diagnosis

The differential diagnosis of IVLBL in the BM includes other lymphomas that may have an intrasinusoidal growth pattern, such as SMZL, large granular lymphocytic leukemia, and other peripheral T-cell lymphomas. The large cell size, vesicular nuclei, and frequent dilatation of the vascular sinusoids that characterize IVLBL help distinguish it from SMZL [134]. The expression of B-cell markers distinguishes this entity from T-cell lymphomas with an intrasinusoidal growth pattern. Finally, the rare disorder of polyclonal B-cell lymphocytosis may exhibit intravascular collections of B cells. The characteristic clinical features of polyclonal B-cell lymphocytosis (young females, smokers, lack of systemic symptoms) and small cell size (Fig. 12.5) help distinguish this benign disorder from IVLBL.

Burkitt Lymphoma/Leukemia

Burkitt lymphoma is a highly aggressive B-cell lymphoma that presents in children, young adults, and occasionally older adults, usually with lymphadenopathy and/or an extranodal mass. Three main epidemiological subtypes are recognized: *endemic* Burkitt lymphoma, which occurs in Africa and Papua New Guinea and affects mainly children; *sporadic* Burkitt lymphoma, which affects mainly

children and young adults worldwide; and *immunodeficiency-associated* Burkitt lymphoma, which occurs in all age groups in individuals who are immunosuppressed, especially due to HIV infection. Burkitt lymphoma is associated with EBV infection in nearly all endemic cases and in about one-third of sporadic and immunodeficiency-associated cases. About 15% of Burkitt lymphoma cases present as a leukemia [135].

Clinical Features

When presenting as a leukemia, Burkitt lymphoma is usually associated with concomitant lymphadenopathy. An exclusively leukemic presentation without lymphadenopathy is rare [135]. Leukemic presentation is most often seen in sporadic and immunodeficiency-associated Burkitt lymphoma and is rare in endemic Burkitt lymphoma. Patients often have a very high WBC count and manifest tumor lysis syndrome, clinically mimicking an acute leukemia; involvement of the CNS in such cases is common.

Morphology

Burkitt lymphoma cells in the BM aspirate and PB smears are medium sized with round nuclei, moderately dispersed chromatin, often multiple nucleoli, and deeply basophilic cytoplasm that characteristically contains numerous small vacuoles, which contain lipid (Fig. 12.26a, b). When involved, the BM usually is extensively infiltrated (more than 70% of the marrow space) and shows a diffuse pattern, frequently with areas of geographic necrosis [111]. Scattered phagocytic histiocytes impart a "starry-sky" appearance similar to that seen in Burkitt lymphoma involving extramedullary involvement, including a "starry-sky" appearance. (**d**) Giemsa stain on tissue sections highlights the basophilic cytoplasm and tendency for the tumor cells to mold to one another

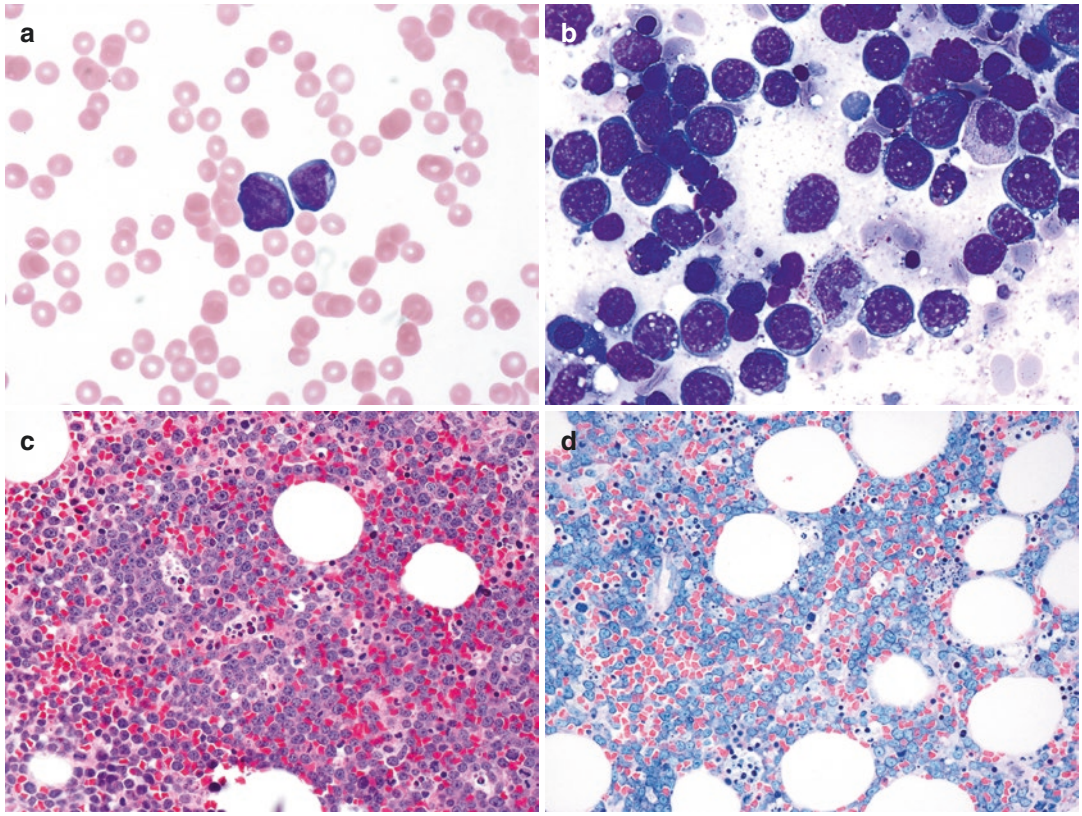

Fig. 12.26 Burkitt lymphoma. In the blood (**a**) and in bone marrow aspirate smears (**b**), Burkitt lymphoma cells are uniformly medium sized with deeply basophilic, vacuolated cytoplasm. In the bone marrow biopsy sections (**c**), Burkitt lymphoma has a similar appearance to extramedullary involvement, including a "starry-sky" appearance. (**d**) Giemsa stain on tissue sections highlights the basophilic cytoplasm and tendency for the tumor cells to mold to one another

medullary tissues. The cell borders appear polygonal, with discernible cytoplasm that stains basophilic with Giemsa stain (Fig. 12.26c, d).

Immunophenotype

Neoplastic cells express pan-B-cell markers CD19, CD20, and PAX5 and are Bcl6+, brightly CD10+, TdT-, and Bcl2-, with expression of monotypic surface immunoglobulin. Weak expression of Bcl2 may be seen in a subset of cases and does not in isolation exclude the diagnosis provided a *BCL2* rearrangement is excluded (see below). The lymphoma cells are usually bright CD38+ and CD44- (Fig. 12.27). In EBV+ Burkitt lymphoma, CD20 and CD45 expression may be slightly decreased. MUM1 is often positive, typically only in a subset of cells. The Ki67 proliferation index is nearly 100%.

Cytogenetics and Molecular Genetics

Rearrangement of the *MYC* gene, usually resulting from a t(8;14) translocation involving the *IGH* locus (or less commonly with one of the immunoglobulin light chain loci), is a hallmark of Burkitt lymphoma and is present in almost all cases. Assessment for a *MYC* rearrangement by conventional karyotype and/or FISH should be performed in all cases of possible Burkitt lymphoma. A diagnosis of Burkitt lymphoma is allowed in the absence of *MYC* rearrangement provided other features fit with the diagnosis, as these cases have a gene expression profile and biologic behavior that are similar to *MYC*-rearranged classic Burkitt lymphoma [136, 137].

A subset of otherwise typical cases have a genetic abnormality involving chromosome 11q and are classified separately, in a provisional 2016 WHO Classification entity Burkitt-like lymphoma with 11q aberration [138]. Importantly, Burkitt lymphoma must always lack *BCL2* or *BCL6* rearrangement. Cases resembling Burkitt lymphoma that have rearrangements of these

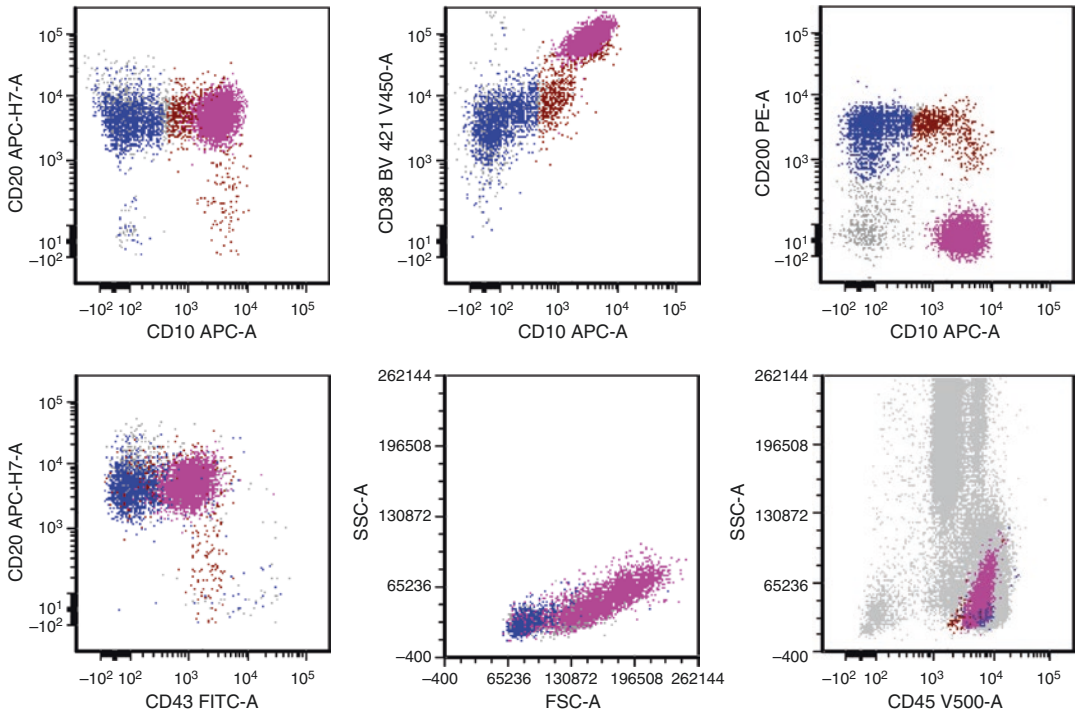

Fig. 12.27 Burkitt lymphoma cells (*pink*) involving the bone marrow admixed with normal B cells (*blue*) and hematogones (*brown*). The CD45/SSC (lower right panel) shows the locations of the three populations of B cells. The Burkitt lymphoma cells are CD20+, CD10+, CD38 bright+, CD43+, and CD200 negative. In contrast, hematogones, while CD10+ and CD43+, show variable expression of CD20 and CD38 and are CD200+. Mature B cells are CD43-,CD10-CD200+,CD38moderate+. The Burkitt lymphoma cells have higher forward and side scatter than both normal B cells and hematogones

genes in addition to a *MYC* rearrangement are classified separately, as high-grade "double-hit" lymphoma in the 2016 WHO Classification.

Recurrent mutations in the *TCF3* and *ID3* genes, which regulate B-cell receptor signaling, have been found in a high proportion of sporadic Burkitt lymphoma cases and occur at a lower frequency in endemic cases [139]. These mutations can be tested in next-generation sequencing panels but do not impact the diagnosis or classification and have no known prognostic significance.

Differential Diagnosis

Burkitt lymphoma presenting as a leukemia may mimic an acute leukemia if the lymphoma cells are mistakenly counted as blasts. Some acute myeloid leukemia cases have blasts with basophilic and vacuolated cytoplasm, resembling the cells of Burkitt lymphoma. Flow cytometry and/or immunohistochemical demonstration of myeloid rather than B-cell marker expression is helpful in this distinction. Differentiating Burkitt lymphoma from B-lymphoblastic leukemia (B-ALL) can be more difficult, because considerable morphologic overlap may be seen. Similar to Burkitt lymphoma, B-ALL can have a "starry-sky" appearance and a high proliferation index, is composed of medium-sized cells, and expresses CD19 and usually CD10. However, unlike Burkitt lymphoma, B-ALL is negative for surface immunoglobulin, is TdT+, and often expresses CD34. Rare leukemic Burkitt lymphoma cases occurring in pediatric patients exhibit a precursor B-cell immunophenotype, with expression of TdT and/or absent surface immunoglobulin expression, similar to B-ALL [140]. A subset of childhood B-ALL cases with t(1;19) translocation involving *E2A-PBX1* rearrangement can be CD34 negative, but these cases are TdT+ [141].

Cases with more cellular pleomorphism raise the differential diagnosis of DLBCL or high-grade B-cell lymphoma (with or without a "double hit" including *MYC* with a *BCL2* and/or *BCL6* rearrangement). Burkitt lymphoma is defined strictly and should not deviate significantly from the expected cytomorphology and characteristic phenotype (Bcl6+, CD10+, Bcl2-, with a Ki67 proliferation index of >95%). Cases lacking *MYC* rearrangement should only be diagnosed as Burkitt lymphoma if the morphology and immunophenotype are completely classical. Minor deviations from classical morphology and immunophenotype are acceptable for *MYC*-rearranged Burkitt lymphoma, especially if the karyotype is relatively simple with no or few other alterations aside from the *MYC* rearrangement. The presence of a *BCL2* or *BCL6* rearrangement excludes a diagnosis of Burkitt lymphoma, and such cases otherwise resembling Burkitt lymphoma are classified as high-grade B-cell lymphoma with "double hit" (Fig. 12.28).

B-Lymphoblastic Leukemia/ Lymphoma

Precursor lymphoid neoplasms encompass malignant proliferations of B or T lymphocytes that may present as leukemia, with predominant involvement of PB, or as lymphoma, with predominant involvement of lymph nodes or extranodal tissues. The distinction of leukemia versus lymphoma does not appear to have biologic relevance and is somewhat arbitrarily based on the clinical presentation. Patients with extramedullary disease at presentation (lymphadenopathy or an extranodal mass lesion), and in whom lymphoblasts make up fewer than 25% of the BM cells and are absent or infrequent in the blood, are classified as B-lymphoblastic lymphoma; all other patients are classified as having B-lymphoblastic leukemia (B-ALL), regardless of whether extramedullary disease is present.

Clinical Features

The peak incidence of B-ALL occurs between 2 and 5 years of age, and a slight male predominance is seen. The incidence is increased in children with Down syndrome. Patients usually present with symptoms related to BM replacement by lymphoblasts, such as fever, fatigue, bone pain, and bleeding. The leukocyte count at presentation is highly variable, as is the proportion of circulating lymphoblasts. Some patients are aleukemic, with pancytopenia and few or no identifiable circulating blasts; conversely, a small subset have hyperleukocytosis, with a WBC above 100×10^9/L. Due to the small size of B lymphoblasts, symptoms related to leukostasis

are rare even when the WBC is markedly elevated with numerous circulating blasts.

Morphology

The leukemic blasts in the blood and BM aspirate smears usually are small to intermediate in size and have scant cytoplasm. Often the cytoplasm is so scant that it appears as a small tag applied to the nucleus (so-called "hand mirror cell"). The nucleus may be round or irregular and has delicate, dispersed chromatin and usually indistinct nucleoli. Some cases have atypical morphology, with larger blasts and more prominent nucleoli, basophilic vacuolated cytoplasm, or deceptively mature-appearing clumped chromatin (Fig. 12.29a–c). These morphologic differences do not appear to have prognostic significance, and no morphologic subtypes of B-ALL are recognized in the 2016 WHO Classification. A subset of leukemic cases may show prominent PB and BM eosinophilia and relatively low-level blast infiltration. This presentation usually is associated with a t(5;14)(q31.1;q32.1) translocation that juxtaposes the *IL3* and *IGH* genes, resulting in a paraneoplastic eosinophilia, and is considered as a specific B-ALL subtype in the WHO Classification [142] (Fig. 12.29d).

The BM biopsy is usually diffusely infiltrated by blasts. The neoplastic cells appear as medium-sized cells with very scant cytoplasm and fine chromatin (Fig. 12.29e). Mitotic figures usually are frequent, and interspersed phagocytic histiocytes may impart a "starry-sky" appearance. Rare cases can demonstrate significant pleomorphism in the blasts, with bizarre giant forms (Fig. 12.29f).

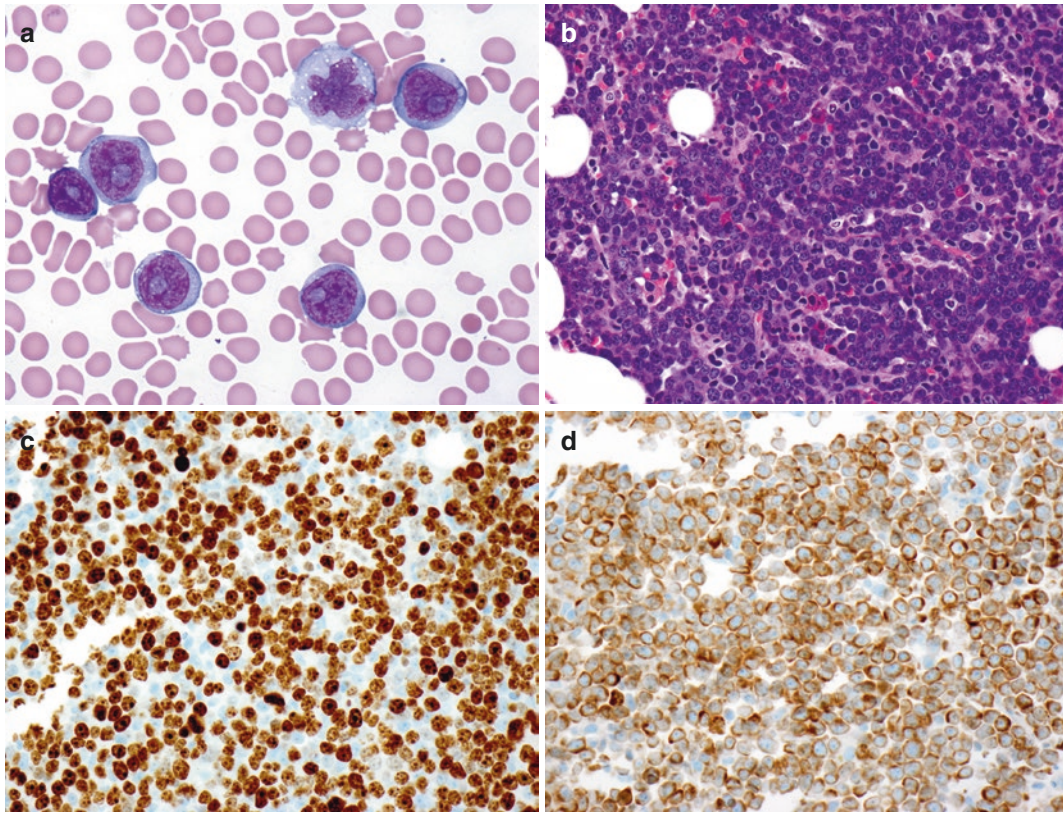

Fig. 12.28 High-grade B-cell lymphoma with *MYC* and *BCL2* rearrangements presenting as a leukemia. (**a**) The cells in the peripheral blood have irregular nuclei, pale cytoplasm, and very prominent central nucleoli, features that would be unusual for Burkitt lymphoma. (**b**) The marrow is diffusely infiltrated by monotonous-appearing tumor cells with a high mitotic rate. (**c**) The Ki67 proliferation index is high (90%) but lower than expected for typical Burkitt lymphoma, which should have almost no Ki67-negative tumor cells. (**d**) The tumor cells show uniform expression of Bcl2; an *IGH-BCL2* as well as *MYC* rearrangement were subsequently confirmed by FISH

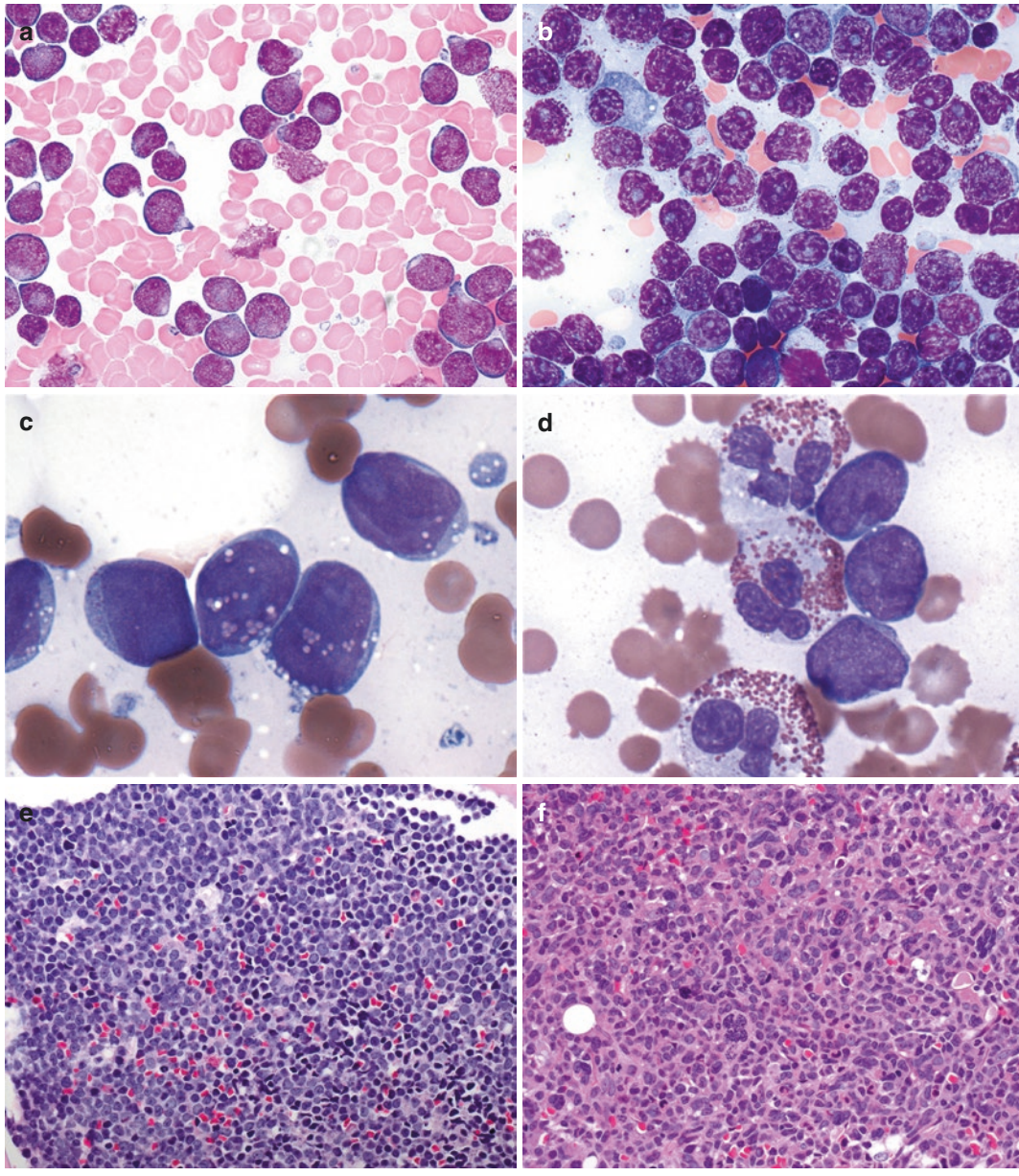

Fig. 12.29 B-lymphoblastic leukemia (B-ALL) in the bone marrow. (**a**) In most cases, the blasts are relatively small with indistinct nucleoli and scant, pale cytoplasm. (**b**) In some cases, the chromatin appears condensed, mimicking a mature B-cell lymphoma. The illustrated case had a classic B-ALL immunophenotype, with expression of TdT and CD34. (**c**) Some cases have larger blasts with cytoplasmic basophilia, vacuoles, and/or fine granules; the presence of granules in blasts does not necessarily indicate myeloid lineage. (**d**) B-ALL with t(5;14)(q31.1;q32.1);*IGH/IL3* involving the bone marrow aspirate smear, with scattered small lymphoblasts and numerous eosinophils. (**e**) B-ALL in the bone marrow biopsy, showing sheets of small blasts with fine chromatin and scant cytoplasm. (**f**) B-ALL with marked cellular pleomorphism in the bone marrow biopsy; although markedly pleomorphic, the neoplastic cells have fine chromatin typical of blasts

Immunophenotype

Although the morphology of the BM and PB may strongly suggest a primitive lymphoid neoplasm, immunophenotyping is required to distinguish B-ALL from other acute leukemias and from mature lymphoid neoplasms. B-ALL blasts express CD19, CD79a, and PAX5 and are variably positive for CD20 and CD34. CD45 usually is dim in intensity compared to normal lymphocytes and may be completely negative. TdT is almost invariably positive and most cases express CD10, although cases with *KMT2A (MLL)* rearrangement are usually CD10 negative. Surface immunoglobulin as assessed by flow cytometry is generally negative; rare cases may express monotypic surface immunoglobulin, but these cases should have morphologic and other immunophenotypic features of B-ALL, and mature B-cell lymphomas (e.g., Burkitt lymphoma and DLBCL) must be excluded [143]. The myeloid antigens CD13 and/or CD33 may be expressed and in the absence of the lineage-specific myeloid marker myeloperoxidase (MPO) or monocytic markers (lysozyme, nonspecific esterase [NSE], CD14, CD64, and/or CD11c), this finding does not mandate classification as a mixed-lineage leukemia.

A subset of cases of *BCR-ABL1*-like B-ALL (see below) is characterized by a strong expression of CRLF2 by flow cytometry, which correlates with *CRLF2* rearrangements, *JAK2* gene mutation, and generally more aggressive clinical behavior [144] (Fig. 12.30). However, only about half of all *BCR-ABL1*-like B-ALL cases have demonstrable expression of CRLF2.

Cytogenetics and Molecular Genetics

Cytogenetic analysis is critical for risk stratification of B-ALL, and several cytogenetic subgroups define distinct entities in the 2016 WHO Classification system as listed in Table 12.4. A new provisional category of *BCR-ABL1*-like B-ALL has been included, which encompasses cases that lack *BCR-ABL1* rearrangement, but

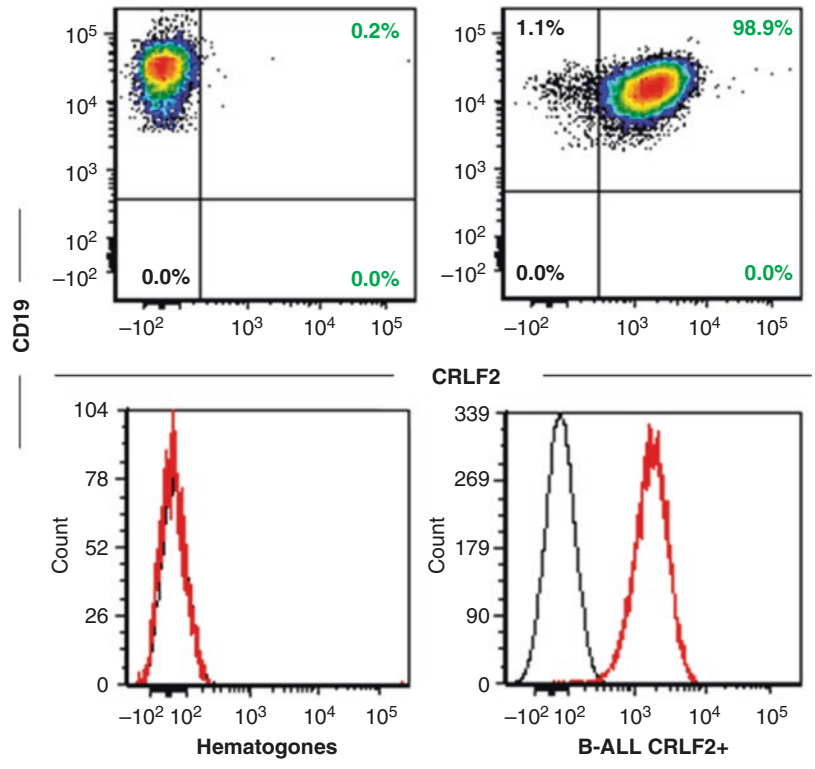

Fig. 12.30 CRLF2 expression detected by flow cytometry in *BCR-ABL1*-like B-lymphoblastic leukemia (B-ALL). CRLF2 expression is completely absent in normal precursor B cells (hematogones) (*left*) and positive in about 20–30% adult B-ALL (*right*). When positive, the expression is uniform. A positive flow cytometry predicts a *CRLF2* rearrangement essentially in almost all cases

Table 12.4 Revised 2016 WHO Classification of B-lymphoblastic leukemia (B-ALL) [152]

WHO B-ALL subtype	Morphologic and immunophenotypic features	Defining genetic features	Other features
B-ALL with recurrent genetic abnormalities			
B-ALL with t(9;22)(q34.1;q11.2); *BCR-ABL1*	No specific morphology, usually CD10+, often CD25+	t(9;22)(q34.1;q11.2) resulting in *BCR-ABL1* rearrangement	Incidence increases with age; uncommon in children. Adverse prognosis
B-ALL with t(v;11q23.3), *KMT2A* rearranged	No specific morphology, usually CD10 negative	Rearrangement of *KMT2A* (*MLL*) gene at 11q23.3 with a variety of partners	Most common in infants <1 year of age, also seen in older adults. Adverse prognosis
B-ALL with t(12;21)(p13.2;q22.1), *ETV6-RUNX1*	No specific morphology, usually CD34+ and CD20 negative	t(12;21)(p13.2;q22.1) resulting in *ETV6-RUNX1* rearrangement	Most common in children >1 year of age, rare in adults. Favorable prognosis
B-ALL with hyperdiploidy	No specific morphology, usually CD34+ and often CD45 negative	Hyperdiploidy with >50 chromosomes; extra copies of 4, 14, 21, and X are most common	Most common in children, uncommon in adults. Favorable prognosis
B-ALL with hypodiploidy	No specific morphology or immunophenotype	Hypodiploidy, with <46 chromosomes. Subdivided based on the number of chromosomes into near diploid (44–45), high hypodiploid (40–43), low hypodiploid (33–39), and near haploid (23–29) subgroups	Affects both children and adults. Adverse prognosis; near haploid subgroup has worst prognosis
B-ALL with t(5;14)(q31.1;q32.1); *IGH/IL3*	Marked increase in nonneoplastic bone marrow and blood eosinophils. Blasts are CD10+	t(5;14)(q31.1;q32.1) resulting in *IGH/IL3* rearrangement	Affects both children and adults. Rare, prognosis is uncertain
B-ALL with t(1;19)(q23;p13.3), *TCF3-PBX1*	No specific morphology, usually CD34 negative with cytoplasmic mu heavy chain expression	t(1;19)(q23;p13.3) resulting in *TCF3-PBX1* rearrangement	More common in children than adults. Prognosis relative to other B-ALL uncertain
B-ALL, *BCR-ABL1*-like (provisional entity)	No specific morphology or immunophenotype	Gene expression profile similar to B-ALL with *BCR-ABL1. CRLF2* rearrangement or rearrangement or mutation of various kinases	Incidence increases with age. Adverse prognosis, but some cases are sensitive to tyrosine kinase inhibitors
B-ALL with iAMP21	No specific morphology or immunophenotype	Amplification (multiple extra copies) of *RUNX1* locus on chromosome 21 detected by FISH	More common in children than adults. Adverse prognosis may be ameliorated by more intensive therapy
B-ALL, not otherwise specified	No specific morphology or immunophenotype	Lacks of any of the above genetic abnormalities	More common in children than adults, favorable prognosis in children

have a gene expression signature similar to B-ALL with t(9;22)(q34;q11.2);*BCR-ABL1*. This newly recognized B-ALL subtype is more common in adults than children and has an overall poor prognosis [145]. Diagnosis is challenging because this entity was originally defined by gene expression profiling, which is not available in most centers. The gene expression profile is associated with several specific genetic aberrations: *CRLF2* rearrangement (about half of the cases) or rearrangements of several tyrosine kinase genes, including *ABL1* (with genes other than *BCR*), *ABL2, PDGFRB, NTRK3, TYK2, CSF1R,* and *JAK2* with diverse partners. Some labs have been using PCR or next-generation sequencing technology to identify these recurrent rearrangements, which include gene aberrations that may be amenable to targeted therapy with tyrosine kinase inhibitors [146]. Flow cytometry detection of CRLF2 expression on B-ALL blasts can identify the subset of cases with *CRLF2* rearrangement [144].

Gene rearrangement studies are rarely needed to establish the diagnosis of B-ALL. Almost all cases show clonal *IGH* rearrangement, despite their failure to express immunoglobulin protein on the cell surface, and up to 70% may show concomitant aberrant clonal rearrangement of *TCR* genes [147].

Differential Diagnosis

Because of the broad morphologic spectrum of B-ALL, there is a broad differential diagnosis. AML and mixed-lineage leukemias (discussed in Chap. 5) should be excluded by demonstrating lack of MPO expression and lack of monocytic markers such as CD11c, CD64, and CD14. The presence of Auer rods normally would exclude B-ALL and establish a finding of malignant myeloid proliferation. However, B-ALL blasts sometimes contain fine cytoplasmic granules or even crystalline structures that superficially resemble Auer rods. An MPO cytochemical stain can be helpful in such cases, because Auer rods are MPO+, whereas lymphoblasts should show no or only very faint MPO staining. It is important to note that CD19, PAX5, and TdT are expressed in some cases of AML, and CD13 and CD33 can be expressed in B-ALL [148]. Therefore, a broad panel of immunophenotypic markers often must be used to distinguish between B-ALL, mixed-lineage leukemias, and AML. Gene rearrangement studies should not be used to establish lymphoid versus myeloid lineage or T versus B lineage, because "promiscuous" rearrangements of *TCR* may occur in a high proportion of B-ALL cases [147]. *IGH* and *TCR* rearrangements also may occur in AML.

Burkitt lymphoma often has a blastic appearance and was previously classified as a subtype of B-ALL (L3 type) in the French-American-British classification of leukemias. Although Burkitt lymphoma shares CD20 and CD10 expression with B-ALL, it is negative for CD34 and TdT, and almost all cases express surface immunoglobulin, unlike B-ALL. Rare cases of leukemias resembling B-ALL but with a t(8;14) translocation involving the *MYC* gene have been reported, as well as blastic leukemias that bear concurrent translocations of *MYC, BCL2*, and/or *BCL6* genes. These cases often show mixed immunophenotypic features of B-ALL and a mature B-cell lymphoma, including surface immunoglobulin light chain expression [149]. The classification of these cases is controversial, but according to the 2016 WHO Classification, the cases with t(8;14) are considered within the Burkitt lymphoma category. Blastoid MCL (which may present as a leukemia, see Fig. 12.13c, d) is often morphologically indistinguishable from B-ALL, but it is easily identified by its distinctive immunophenotype (CyclinD1+, CD5+, TdT-). Other small, round, blue cell tumors of childhood, such as Ewing sarcoma, may enter into the morphologic differential diagnosis; caution should be used in relying only on CD45 to exclude lymphoid neoplasms in the workup of such tumors, because B-ALL may be CD45 weak or completely negative [150].

Finally, it is important to recognize that benign B lymphoblasts (hematogones) have a morphology and immunophenotype similar to B-ALL cells. Hematogones normally comprise fewer than 5% of all BM cells, but their numbers may

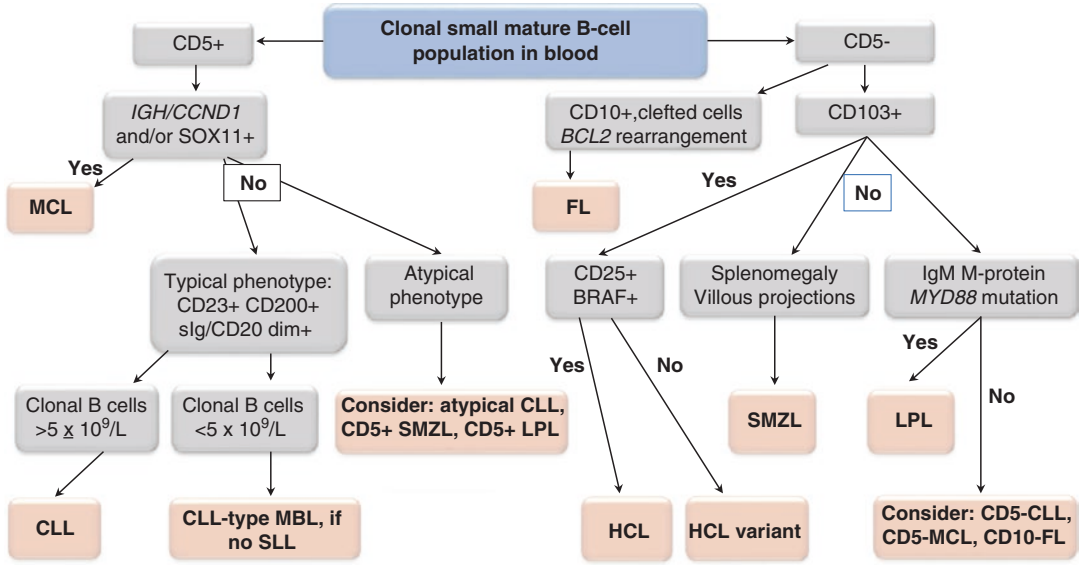

Fig. 12.31 A suggested algorithm for approaching clonal populations of small, mature B cells in the peripheral blood, combining morphology, clinical and laboratory features, and immunophenotype. Abbreviations: *CLL* chronic lymphocytic leukemia, *MCL* mantle cell lymphoma, *SLL* small lymphocytic lymphoma, *MBL* monoclonal B-cell lymphocytosis, *SMZL* splenic marginal zone lymphoma, *LPL* lymphoplasmacytic lymphoma, *HCL* hairy cell leukemia, *FL* follicular lymphoma

increase in response to marrow stress (e.g., chemotherapy recovery), particularly in pediatric patients [151]. This may present diagnostic difficulties, mainly in the posttreatment setting in patients with a history of B-ALL. Hematogones appear as small- to medium-sized cells in the aspirate smear, often with a long cleft in the nucleus, with cytologic features intermediate between small lymphocytes and blasts. Flow cytometry is critical for distinguishing benign hematogone populations from malignant B lymphoblasts. Hematogones usually show a heterogeneous pattern of CD34, TdT, CD10, and CD20 expression, as they comprise various stages of maturation, although this pattern may be distorted after chemotherapy or the use of anti-CD20 antibody therapy. Hematogones are bright CD38+ and dim CD58+ (Fig. 12.3). In contrast, B-ALL lymphoblasts are CD38 dimmer+ and CD58 bright+ and also usually show other immunophenotypic aberrancies, such as inappropriate antigen combinations and/or expression of CD13 or CD33 that can be helpful in distinguishing them from hematogones.

An algorithm for approaching the differential diagnosis of small mature clonal B-cell populations in the blood is shown in Fig. 12.31.

References

1. Morbach H, Eichhorn EM, Liese JG, Girschick HJ. Reference values for B cell subpopulations from infancy to adulthood. Clin Exp Immunol. 2010;162(2):271–9.
2. Bernstein L, Newton P, Ross RK. Epidemiology of hairy cell leukemia in Los Angeles County. Cancer Res. 1990;50(12):3605–9.
3. Sims GP, Ettinger R, Shirota Y, Yarboro CH, Illei GG, Lipsky PE. Identification and characterization of circulating human transitional B cells. Blood. 2005;105(11):4390–8.
4. Medina F, Segundo C, Campos-Caro A, Gonzalez-Garcia I, Brieva JA. The heterogeneity shown by human plasma cells from tonsil, blood, and bone marrow reveals graded stages of increasing maturity, but local profiles of adhesion molecule expression. Blood. 2002;99(6):2154–61.
5. Caraux A, Klein B, Paiva B, Bret C, Schmitz A, Fuhler GM, Bos NA, Johnsen HE, Orfao A, Perez-Andres M, et al. Circulating human B and plasma cells. Age-associated changes in counts

and detailed characterization of circulating normal CD138- and CD138+ plasma cells. Haematologica. 2010;95(6):1016–20.
6. Kipps TJ. The CD5 B cell. Adv Immunol. 1989;47:117–85.
7. Gupta R, Jain P, Deo SV, Sharma A. Flow cytometric analysis of CD5+ B cells: a frame of reference for minimal residual disease analysis in chronic lymphocytic leukemia. Am J Clin Pathol. 2004;121(3):368–72.
8. Gordon DS, Jones BM, Browning SW, Spira TJ, Lawrence DN. Persistent polyclonal lymphocytosis of B lymphocytes. N Engl J Med. 1982;307(4):232–6.
9. Carstairs KC, Francombe WH, Scott JG, Gelfand EW. Persistent polyclonal lymphocytosis of B lymphocytes, induced by cigarette smoking? Lancet. 1985;1(8437):1094.
10. Agrawal S, Matutes E, Voke J, Dyer MJ, Khokhar T, Catovsky D. Persistent polyclonal B-cell lymphocytosis. Leuk Res. 1994;18(10):791–5.
11. Troussard X, Mossafa H, Valensi F, Maynadie M, Schillinger F, Bulliard G, Malaure H, Flandrin G. Polyclonal lymphocytosis with binucleated lymphocytes. Morphological, immunological, cytogenetic and molecular analysis in 15 cases. Presse Med. 1997;26(19):895–9.
12. Loembe MM, Neron S, Delage R, Darveau A. Analysis of expressed V(H) genes in persistent polyclonal B cell lymphocytosis reveals absence of selection in CD27+IgM+IgD+ memory B cells. Eur J Immunol. 2002;32(12):3678–88.
13. Dugas-Bourdages E, Neron S, Roy A, Darveau A, Delage R. Persistent polyclonal B cell lymphocytosis B cells can be activated through CD40-CD154 interaction. Adv Hematol. 2014;2014:854124.
14. Deplano S, Nadal-Melsio E, Bain BJ. Persistent polyclonal B lymphocytosis. Am J Hematol. 2014;89(2):224.
15. Troussard X, Cornet E, Lesesve JF, Kourel C, Mossafa H. Polyclonal B-cell lymphocytosis with binucleated lymphocytes (PPBL). Onco Targets Ther. 2008;1:59–66.
16. Callet-Bauchu E, Renard N, Gazzo S, Poncet C, Morel D, Pages J, Salles G, Coeur P, Felman P. Distribution of the cytogenetic abnormality +i(3)(q10) in persistent polyclonal B-cell lymphocytosis: a FICTION study in three cases. Br J Haematol. 1997;99(3):531–6.
17. Kay NE, Leong T, Kyle RA, Greipp P, Billadeau D, Van Ness B, Bone N, Oken MM. Circulating blood B cells in multiple myeloma: analysis and relationship to circulating clonal cells and clinical parameters in a cohort of patients entered on the Eastern Cooperative Oncology Group phase III E9486 clinical trial. Blood. 1997;90(1):340–5.
18. Kirkpatrick JA Jr, DiGeorge AM. Congenital absence of the thymus. Am J Roentgenol Radium Ther Nucl Med. 1968;103(1):32–7.
19. Klocperk A, Mejstrikova E, Kayserova J, Kalina T, Sediva A. Low marginal zone-like B lymphocytes and natural antibodies characterize skewed B-lymphocyte subpopulations in del22q11 DiGeorge patients. Clin Immunol. 2015;161(2):144–9.
20. Frater JL, McCarron KF, Hammel JP, Shapiro JL, Miller ML, Tubbs RR, Pettay J, Hsi ED. Typical and atypical chronic lymphocytic leukemia differ clinically and immunophenotypically. Am J Clin Pathol. 2001;116(5):655–64.
21. Montserrat E, Villamor N, Reverter JC, Brugues RM, Tassies D, Bosch F, Aguilar JL, Vives-Corrons JL, Rozman M, Rozma C. Bone marrow assessment in B-cell chronic lymphocytic leukaemia: aspirate or biopsy? A comparative study in 258 patients. Br J Haematol. 1996;93(1):111–6.
22. Thiele J, Zirbes TK, Kvasnicka HM, Fischer R. Focal lymphoid aggregates (nodules) in bone marrow biopsies: differentiation between benign hyperplasia and malignant lymphoma: a practical guideline. J Clin Pathol. 1999;52(4):294–300.
23. Bergmann MA, Eichhorst BF, Busch R, Adorf D, Stilgenbauer S, Eckart MJ, Wendtner C-M, Hallek M. Prospective evaluation of prognostic parameters in early stage chronic lymphocytic leukemia (CLL): results of the CLL1-protocol of the German CLL Study Group (GCLLSG). Blood. 2007;110(11):165.
24. Henrique R, Achten R, Maes B, Verhoef G, De Wolf-Peeters C. Guidelines for subtyping small B-cell lymphomas in bone marrow biopsies. Virchows Arch. 1999;435(6):549–58.
25. Carulli G, Stacchini A, Marini A, Ciriello MM, Zucca A, Cannizzo E, Aliberti S, Demurtas A, Novero D, Calcagno L, et al. Aberrant expression of CD8 in B-cell non-Hodgkin lymphoma: a multicenter study of 951 bone marrow samples with lymphomatous infiltration. Am J Clin Pathol. 2009;132(2):186–90. quiz 306
26. Sandes AF, de Lourdes CM, Oliveira CR, Maekawa Y, Tamashiro N, Takao TT, Ritter EC, Rizzatti EG. CD200 has an important role in the differential diagnosis of mature B-cell neoplasms by multiparameter flow cytometry. Cytometry B Clin Cytom. 2014;86(2):98–105.
27. Morice WG, Kurtin PJ, Hodnefield JM, Shanafelt TD, Hoyer JD, Remstein ED, Hanson CA. Predictive value of blood and bone marrow flow cytometry in B-cell lymphoma classification: comparative analysis of flow cytometry and tissue biopsy in 252 patients. Mayo Clin Proc. 2008;83(7):776–85.
28. Kremer M, Quintanilla-Martinez L, Nahrig J, von Schilling C, Fend F. Immunohistochemistry in bone marrow pathology: a useful adjunct for morphologic diagnosis. Virchows Arch. 2005;447(6):920–37.
29. Tandon B, Peterson L, Gao J, Nelson B, Ma S, Rosen S, Chen YH. Nuclear overexpression of lymphoid-enhancer-binding factor 1 identifies chronic lymphocytic leukemia/small lymphocytic lymphoma in small B-cell lymphomas. Mod Pathol. 2011;24(11):1433–43.
30. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, Dohner H, Hillmen P, Keating MJ, Montserrat E, Rai KR, et al. Guidelines for the

diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood. 2008;111(12):5446–56.
31. Dohner H, Stilgenbauer S, Benner A, Leupolt E, Krober A, Bullinger L, Dohner K, Bentz M, Lichter P. Genomic aberrations and survival in chronic lymphocytic leukemia. N Engl J Med. 2000;343(26): 1910–6.
32. Landau DA, Tausch E, Taylor-Weiner AN, Stewart C, Reiter JG, Bahlo J, Kluth S, Bozic I, Lawrence M, Bottcher S, et al. Mutations driving CLL and their evolution in progression and relapse. Nature. 2015;526(7574):525–30.
33. Wang L, Lawrence MS, Wan Y, Stojanov P, Sougnez C, Stevenson K, Werner L, Sivachenko A, DeLuca DS, Zhang L, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. N Engl J Med. 2011;365(26):2497–506.
34. Rassenti LZ, Huynh L, Toy TL, Chen L, Keating MJ, Gribben JG, Neuberg DS, Flinn IW, Rai KR, Byrd JC, et al. ZAP-70 compared with immunoglobulin heavy-chain gene mutation status as a predictor of disease progression in chronic lymphocytic leukemia. N Engl J Med. 2004;351(9):893–901.
35. Hamblin TJ, Orchard JA, Ibbotson RE, Davis Z, Thomas PW, Stevenson FK, Oscier DG. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. Blood. 2002;99(3):1023–9.
36. Challagundla P, Medeiros LJ, Kanagal-Shamanna R, Miranda RN, Jorgensen JL. Differential expression of CD200 in B-cell neoplasms by flow cytometry can assist in diagnosis, subclassification, and bone marrow staging. Am J Clin Pathol. 2014;142(6):837–44.
37. O'Malley DP, Vance GH, Orazi A. Chronic lymphocytic leukemia/small lymphocytic lymphoma with trisomy 12 and focal cyclin d1 expression: a potential diagnostic pitfall. Arch Pathol Lab Med. 2005;129(1):92–5.
38. Rawstron AC, Bennett FL, O'Connor SJ, Kwok M, Fenton JA, Plummer M, de Tute R, Owen RG, Richards SJ, Jack AS, et al. Monoclonal B-cell lymphocytosis and chronic lymphocytic leukemia. N Engl J Med. 2008;359(6):575–83.
39. Karube K, Scarfo L, Campo E, Ghia P. Monoclonal B cell lymphocytosis and “in situ” lymphoma. Semin Cancer Biol. 2014;24:3–14.
40. Randen U, Tierens AM, Tjonnfjord GE, Delabie J. Bone marrow histology in monoclonal B-cell lymphocytosis shows various B-cell infiltration patterns. Am J Clin Pathol. 2013;139(3):390–5.
41. Krishnan B, Matutes E, Dearden C. Prolymphocytic leukemias. Semin Oncol. 2006;33(2):257–63.
42. Del Giudice I, Davis Z, Matutes E, Osuji N, Parry-Jones N, Morilla A, Brito-Babapulle V, Oscier D, Catovsky D. IgVH genes mutation and usage, ZAP-70 and CD38 expression provide new insights on B-cell prolymphocytic leukemia (B-PLL). Leukemia. 2006;20(7):1231–7.
43. Matutes E. Immunophenotyping and differential diagnosis of hairy cell leukemia. Hematol Oncol Clin North Am. 2006;20(5):1051–63.
44. Ya-In C, Brandwein J, Pantalony D, Chang H. Hairy cell leukemia variant with features of intrasinusoidal bone marrow involvement. Arch Pathol Lab Med. 2005;129(3):395–8.
45. Cohen PL, Kurtin PJ, Donovan KA, Hanson CA. Bone marrow and peripheral blood involvement in mantle cell lymphoma. Br J Haematol. 1998;101(2):302–10.
46. Matutes E, Parry-Jones N, Brito-Babapulle V, Wotherspoon A, Morilla R, Atkinson S, Elnenaei MO, Jain P, Giustolisi GM, A'Hern RP, et al. The leukemic presentation of mantle-cell lymphoma: disease features and prognostic factors in 58 patients. Leuk Lymphoma. 2004;45(10):2007–15.
47. Espinet B, Ferrer A, Bellosillo B, Nonell L, Salar A, Fernandez-Rodriguez C, Puigdecanet E, Gimeno J, Garcia-Garcia M, Vela MC, et al. Distinction between asymptomatic monoclonal B-cell lymphocytosis with cyclin D1 overexpression and mantle cell lymphoma: from molecular profiling to flow cytometry. Clin Cancer Res. 2014;20(4):1007–19.
48. Fernandez V, Salamero O, Espinet B, Sole F, Royo C, Navarro A, Camacho F, Bea S, Hartmann E, Amador V, et al. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res. 2010;70(4):1408–18.
49. Bernard M, Gressin R, Lefrere F, Drenou B, Branger B, Caulet-Maugendre S, Tass P, Brousse N, Valensi F, Milpied N, et al. Blastic variant of mantle cell lymphoma: a rare but highly aggressive subtype. Leukemia. 2001;15(11):1785–91.
50. Schenka AA, Gascoyne RD, Duchayne E, Delsol G, Brousset P. Prominent intrasinusoidal infiltration of the bone marrow by mantle cell lymphoma. Hum Pathol. 2003;34(8):789–91.
51. Mozos A, Royo C, Hartmann E, De Jong D, Baro C, Valera A, Fu K, Weisenburger DD, Delabie J, Chuang SS, et al. SOX11 expression is highly specific for mantle cell lymphoma and identifies the cyclin D1-negative subtype. Haematologica. 2009;94(11):1555–62.
52. Nakashima MO, Durkin L, Bodo J, Lin J, Quintanilla-Martinez L, Fu K, Hsi ED. Utility and diagnostic pitfalls of SOX11 monoclonal antibodies in mantle cell lymphoma and other lymphoproliferative disorders. Appl Immunohistochem Mol Morphol. 2014;22(10):720–7.
53. Salaverria I, Royo C, Carvajal-Cuenca A, Clot G, Navarro A, Valera A, Song JY, Woroniecka R, Rymkiewicz G, Klapper W, et al. CCND2 rearrangements are the most frequent genetic events in cyclin D1(−) mantle cell lymphoma. Blood. 2013;121(8):1394–402.

54. Frassoldati A, Lamparelli T, Federico M, Annino L, Capnist G, Pagnucco G, Dini E, Resegotti L, Damasio EE, Silingardi V. Hairy cell leukemia: a clinical review based on 725 cases of the Italian Cooperative Group (ICGHCL). Italian Cooperative Group for Hairy Cell Leukemia. Leuk Lymphoma. 1994;13(3–4):307–16.
55. Flandrin G, Sigaux F, Sebahoun G, Bouffette P. Hairy cell leukemia: clinical presentation and follow-up of 211 patients. Semin Oncol. 1984;11(4 Suppl 2):458–71.
56. Dasanu CA, Ichim TE, Alexandrescu DT. Inherent and iatrogenic immune defects in hairy cell leukemia: revisited. Expert Opin Drug Saf. 2010;9(1):55–64.
57. Golomb HM. Hairy cell leukemia: an unusual lymphoproliferative disease: a study of 24 patients. Cancer. 1978;42(2 Suppl):946–56.
58. Humphries JE. Dry tap bone marrow aspiration: clinical significance. Am J Hematol. 1990;35(4):247–50.
59. Bouroncle BA. Thirty-five years in the progress of hairy cell leukemia. Leuk Lymphoma. 1994;14(Suppl 1):1–12.
60. Sharpe RW, Bethel KJ. Hairy cell leukemia: diagnostic pathology. Hematol Oncol Clin North Am. 2006;20(5):1023–49.
61. Lee WM, Beckstead JH. Hairy cell leukemia with bone marrow hypoplasia. Cancer. 1982;50(10):2207–10.
62. Pittaluga S, Verhoef G, Maes A, Boogaerts MA, De Wolf-Peeters C. Bone marrow trephines. Findings in patients with hairy cell leukaemia before and after treatment. Histopathology. 1994;25(2):129–35.
63. Burthem J, Cawley JC. The bone marrow fibrosis of hairy-cell leukemia is caused by the synthesis and assembly of a fibronectin matrix by the hairy cells. Blood. 1994;83(2):497–504.
64. Chen YH, Tallman MS, Goolsby C, Peterson L. Immunophenotypic variations in hairy cell leukemia. Am J Clin Pathol. 2006;125(2):251–9.
65. Jasionowski TM, Hartung L, Greenwood JH, Perkins SL, Bahler DW. Analysis of CD10+ hairy cell leukemia. Am J Clin Pathol. 2003;120(2):228–35.
66. Del Giudice I, Matutes E, Morilla R, Morilla A, Owusu-Ankomah K, Rafiq F, A'Hern R, Delgado J, Bazerbashi MB, Catovsky D. The diagnostic value of CD123 in B-cell disorders with hairy or villous lymphocytes. Haematologica. 2004;89(3):303–8.
67. Miranda RN, Briggs RC, Kinney MC, Veno PA, Hammer RD, Cousar JB. Immunohistochemical detection of cyclin D1 using optimized conditions is highly specific for mantle cell lymphoma and hairy cell leukemia. Mod Pathol. 2000;13(12):1308–14.
68. Brito-Babapulle V, Matutes E, Oscier D, Mould S, Catovsky D. Chromosome abnormalities in hairy cell leukaemia variant. Genes Chromosomes Cancer. 1994;10(3):197–202.
69. Andrulis M, Penzel R, Weichert W, von Deimling A, Capper D. Application of a BRAF V600E mutation-specific antibody for the diagnosis of hairy cell leukemia. Am J Surg Pathol. 2012;36(12):1796–800.
70. Brown NA, Betz BL, Weigelin HC, Elenitoba-Johnson KS, Lim MS, Bailey NG. Evaluation of allele-specific PCR and immunohistochemistry for the detection of BRAF V600E mutations in hairy cell leukemia. Am J Clin Pathol. 2015;143(1):89–99.
71. Kluin-Nelemans HC, Beverstock GC, Mollevanger P, Wessels HW, Hoogendoorn E, Willemze R, Falkenburg JH. Proliferation and cytogenetic analysis of hairy cell leukemia upon stimulation via the CD40 antigen. Blood. 1994;84(9):3134–41.
72. Brito-Babapulle V, Ellis J, Matutes E, Oscier D, Khokhar T, MacLennan K, Catovsky D. Translocation t(11;14)(q13;q32) in chronic lymphoid disorders. Genes Chromosomes Cancer. 1992;5(2):158–65.
73. Tiacci E, Trifonov V, Schiavoni G, Holmes A, Kern W, Martelli MP, Pucciarini A, Bigerna B, Pacini R, Wells VA, et al. BRAF mutations in hairy-cell leukemia. N Engl J Med. 2011;364(24):2305–15.
74. Tschernitz S, Flossbach L, Bonengel M, Roth S, Rosenwald A, Geissinger E. Alternative BRAF mutations in BRAF V600E-negative hairy cell leukaemias. Br J Haematol. 2014;165(4):529–33.
75. Tiacci E, Schiavoni G, Martelli MP, Boveri E, Pacini R, Tabarrini A, Zibellini S, Santi A, Pettirossi V, Fortini E, et al. Constant activation of the RAF-MEK-ERK pathway as a diagnostic and therapeutic target in hairy cell leukemia. Haematologica. 2013;98(4):635–9.
76. Melo JV, Hegde U, Parreira A, Thompson I, Lampert IA, Catovsky D. Splenic B cell lymphoma with circulating villous lymphocytes: differential diagnosis of B cell leukaemias with large spleens. J Clin Pathol. 1987;40(6):642–51.
77. Traverse-Glehen A, Baseggio L, Bauchu EC, Morel D, Gazzo S, Ffrench M, Verney A, Rolland D, Thieblemont C, Magaud JP, et al. Splenic red pulp lymphoma with numerous basophilic villous lymphocytes: a distinct clinicopathologic and molecular entity? Blood. 2008;111(4):2253–60.
78. Kanellis G, Mollejo M, Montes-Moreno S, Rodriguez-Pinilla SM, Cigudosa JC, Algara P, Montalban C, Matutes E, Wotherspoon A, Piris MA. Splenic diffuse red pulp small B-cell lymphoma: revision of a series of cases reveals characteristic clinico-pathological features. Haematologica. 2010;95(7):1122–9.
79. Hockley SL, Morgan GJ, Leone PE, Walker BA, Morilla A, Else M, Wotherspoon A, Dearden C, Catovsky D, Gonzalez D, et al. High-resolution genomic profiling in hairy cell leukemia-variant compared with typical hairy cell leukemia. Leukemia. 2011;25(7):1189–92.
80. Matutes E, Wotherspoon A, Catovsky D. The variant form of hairy-cell leukaemia. Best Pract Res Clin Haematol. 2003;16(1):41–56.
81. Sainati L, Matutes E, Mulligan S, de Oliveira MP, Rani S, Lampert IA, Catovsky D. A variant form of hairy cell leukemia resistant to alpha-interferon:

clinical and phenotypic characteristics of 17 patients. Blood. 1990;76(1):157–62.
82. Shao H, Calvo KR, Gronborg M, Tembhare PR, Kreitman RJ, Stetler-Stevenson M, Yuan CM. Distinguishing hairy cell leukemia variant from hairy cell leukemia: development and validation of diagnostic criteria. Leuk Res. 2013;37(4):401–9.
83. Zhang QY, Chabot-Richards D, Evans M, Spengel K, Andrews J, Kang H, Foucar K. A retrospective study to assess the relative value of peripheral blood, bone marrow aspirate and biopsy morphology, immunohistochemical stains, and flow cytometric analysis in the diagnosis of chronic B cell lymphoproliferative neoplasms. Int J Lab Hematol. 2015;37(3):390–402.
84. Waterfall JJ, Arons E, Walker RL, Pineda M, Roth L, Killian JK, Abaan OD, Davis SR, Kreitman RJ, Meltzer PS. High prevalence of MAP2K1 mutations in variant and IGHV4-34-expressing hairy-cell leukemias. Nat Genet. 2014;46(1):8–10.
85. Xi L, Arons E, Navarro W, Calvo KR, Stetler-Stevenson M, Raffeld M, Kreitman RJ. Both variant and IGHV4-34-expressing hairy cell leukemia lack the BRAF V600E mutation. Blood. 2012;119(14):3330–2.
86. Vallianatou K, Brito-Babapulle V, Matutes E, Atkinson S, Catovsky D. p53 gene deletion and trisomy 12 in hairy cell leukemia and its variant. Leuk Res. 1999;23(11):1041–5.
87. Hockley SL, Else M, Morilla A, Wotherspoon A, Dearden C, Catovsky D, Gonzalez D, Matutes E. The prognostic impact of clinical and molecular features in hairy cell leukaemia variant and splenic marginal zone lymphoma. Br J Haematol. 2012;158(3):347–54.
88. Kent SA, Variakojis D, Peterson LC. Comparative study of marginal zone lymphoma involving bone marrow. Am J Clin Pathol. 2002;117(5):698–708.
89. Kurtin PJ. Indolent lymphomas of mature B lymphocytes. Hematol Oncol Clin North Am. 2009;23(4):769–90.
90. Matutes E, Oscier D, Montalban C, Berger F, Callet-Bauchu E, Dogan A, Felman P, Franco V, Iannitto E, Mollejo M, et al. Splenic marginal zone lymphoma proposals for a revision of diagnostic, staging and therapeutic criteria. Leukemia. 2008;22(3):487–95.
91. Mollejo M, Camacho FI, Algara P, Ruiz-Ballesteros E, Garcia JF, Piris MA. Nodal and splenic marginal zone B cell lymphomas. Hematol Oncol. 2005;23(3–4):108–18.
92. Berger F, Felman P, Thieblemont C, Pradier T, Baseggio L, Bryon PA, Salles G, Callet-Bauchu E, Coiffier B. Non-MALT marginal zone B-cell lymphomas: a description of clinical presentation and outcome in 124 patients. Blood. 2000;95(6):1950–6.
93. Audouin J, Le Tourneau A, Molina T, Camilleri-Broet S, Adida C, Comperat E, Benattar L, Delmer A, Devidas A, Rio B, et al. Patterns of bone marrow involvement in 58 patients presenting primary splenic marginal zone lymphoma with or without circulating villous lymphocytes. Br J Haematol. 2003;122(3):404–12.
94. Franco V, Florena AM, Campesi G. Intrasinusoidal bone marrow infiltration: a possible hallmark of splenic lymphoma. Histopathology. 1996;29(6):571–5.
95. Anagnostou D. Pitfalls in the pattern of bone marrow infiltration in lymphoproliferative disorders. Current Diagnostic Pathology. 2005;11(3):170–9.
96. Giannouli S, Paterakis G, Ziakas PD, Anagnostou D, Voulgarelis M. Splenic marginal zone lymphomas with peripheral CD5 expression. Haematologica. 2004;89(1):113–4.
97. Rinaldi A, Mian M, Chigrinova E, Arcaini L, Bhagat G, Novak U, Rancoita PM, De Campos CP, Forconi F, Gascoyne RD, et al. Genome-wide DNA profiling of marginal zone lymphomas identifies subtype-specific lesions with an impact on the clinical outcome. Blood. 2011;117(5):1595–604.
98. Salido M, Baro C, Oscier D, Stamatopoulos K, Dierlamm J, Matutes E, Traverse-Glehen A, Berger F, Felman P, Thieblemont C, et al. Cytogenetic aberrations and their prognostic value in a series of 330 splenic marginal zone B-cell lymphomas: a multicenter study of the Splenic B-Cell Lymphoma Group. Blood. 2010;116(9):1479–88.
99. Corcoran MM, Mould SJ, Orchard JA, Ibbotson RE, Chapman RM, Boright AP, Platt C, Tsui LC, Scherer SW, Oscier DG. Dysregulation of cyclin dependent kinase 6 expression in splenic marginal zone lymphoma through chromosome 7q translocations. Oncogene. 1999;18(46):6271–7.
100. Kiel MJ, Velusamy T, Betz BL, Zhao L, Weigelin HG, Chiang MY, Huebner-Chan DR, Bailey NG, Yang DT, Bhagat G, et al. Whole-genome sequencing identifies recurrent somatic NOTCH2 mutations in splenic marginal zone lymphoma. J Exp Med. 2012;209(9):1553–65.
101. Rossi D, Trifonov V, Fangazio M, Bruscaggin A, Rasi S, Spina V, Monti S, Vaisitti T, Arruga F, Fama R, et al. The coding genome of splenic marginal zone lymphoma: activation of NOTCH2 and other pathways regulating marginal zone development. J Exp Med. 2012;209(9):1537–51.
102. Piva R, Deaglio S, Fama R, Buonincontri R, Scarfo I, Bruscaggin A, Mereu E, Serra S, Spina V, Brusa D, et al. The Kruppel-like factor 2 transcription factor gene is recurrently mutated in splenic marginal zone lymphoma. Leukemia. 2015;29(2):503–7.
103. Catovsky D, Matutes E. Splenic lymphoma with circulating villous lymphocytes/splenic marginal-zone lymphoma. Semin Hematol. 1999;36(2):148–54.
104. Franco V, Florena AM, Stella M, Rizzo A, Iannitto E, Quintini G, Campesi G. Splenectomy influences bone marrow infiltration in patients with splenic marginal zone cell lymphoma with or without villous lymphocytes. Cancer. 2001;91(2):294–301.
105. Morice WG, Chen D, Kurtin PJ, Hanson CA, McPhail ED. Novel immunophenotypic features of marrow lymphoplasmacytic lymphoma and correla-

tion with Waldenstrom's macroglobulinemia. Mod Pathol. 2009;22(6):807–16.
106. Lin P, Hao S, Handy BC, Bueso-Ramos CE, Medeiros LJ. Lymphoid neoplasms associated with IgM paraprotein: a study of 382 patients. Am J Clin Pathol. 2005;123(2):200–5.
107. Owen RG, Barrans SL, Richards SJ, O'Connor SJ, Child JA, Parapia LA, Morgan GJ, Jack AS. Waldenstrom macroglobulinemia. Development of diagnostic criteria and identification of prognostic factors. Am J Clin Pathol. 2001;116(3):420–8.
108. Vitolo U, Ferreri AJ, Montoto S. Lymphoplasmacytic lymphoma-Waldenstrom's macroglobulinemia. Crit Rev Oncol Hematol. 2008;67(2):172–85.
109. Dimopoulos MA, Kyle RA, Anagnostopoulos A, Treon SP. Diagnosis and management of Waldenstrom's macroglobulinemia. J Clin Oncol. 2005;23(7):1564–77.
110. Swerdlow SH, Berger F, Pileri SA, Harris NL, Jaffe ES, Stein H. Lymphoplasmacytic lymphoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri S, Stein H, Thiele J, Vardiman JW, editors. WHO classification of tumours of haematopoietic and lymphoid tissues. Lyon: International Agency for Research on Cancer (IARC); 2008. p. 194–5.
111. Arber DA, George TI. Bone marrow biopsy involvement by non-Hodgkin's lymphoma: frequency of lymphoma types, patterns, blood involvement, and discordance with other sites in 450 specimens. Am J Surg Pathol. 2005;29(12):1549–57.
112. Tournilhac O, Santos DD, Xu L, Kutok J, Tai YT, Le Gouill S, Catley L, Hunter Z, Branagan AR, Boyce JA, et al. Mast cells in Waldenstrom's macroglobulinemia support lymphoplasmacytic cell growth through CD154/CD40 signaling. Ann Oncol. 2006;17(8):1275–82.
113. Konoplev S, Medeiros LJ, Bueso-Ramos CE, Jorgensen JL, Lin P. Immunophenotypic profile of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia. Am J Clin Pathol. 2005;124(3):414–20.
114. Ocio EM, Schop RF, Gonzalez B, Van Wier SA, Hernandez-Rivas JM, Gutierrez NC, Garcia-Sanz R, Moro MJ, Aguilera C, Hernandez J, et al. 6q deletion in Waldenstrom macroglobulinemia is associated with features of adverse prognosis. Br J Haematol. 2007;136(1):80–6.
115. Treon SP, Cao Y, Xu L, Yang G, Liu X, Hunter ZR. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. Blood. 2014;123(18):2791–6.
116. Treon SP, Xu L, Yang G, Zhou Y, Liu X, Cao Y, Sheehy P, Manning RJ, Patterson CJ, Tripsas C, et al. MYD88 L265P somatic mutation in Waldenstrom's macroglobulinemia. N Engl J Med. 2012;367(9):826–33.
117. Hunter ZR, Xu L, Yang G, Zhou Y, Liu X, Cao Y, Manning RJ, Tripsas C, Patterson CJ, Sheehy P, et al. The genomic landscape of Waldenstrom macroglobulinemia is characterized by highly recurring MYD88 and WHIM-like CXCR4 mutations, and small somatic deletions associated with B-cell lymphomagenesis. Blood. 2014;123(11):1637–46.
118. Owen RG, Parapia LA, Higginson J, Misbah SA, Child JA, Morgan GJ, Jack AS. Clinicopathological correlates of IgM paraproteinemias. Clin Lymphoma. 2000;1(1):39–43. discussion 44-35
119. Baldini L, Goldaniga M, Guffanti A, Broglia C, Cortelazzo S, Rossi A, Morra E, Colombi M, Callea V, Pogliani E, et al. Immunoglobulin M monoclonal gammopathies of undetermined significance and indolent Waldenstrom's macroglobulinemia recognize the same determinants of evolution into symptomatic lymphoid disorders: proposal for a common prognostic scoring system. J Clin Oncol. 2005;23(21):4662–8.
120. Owen RG, Treon SP, Al-Katib A, Fonseca R, Greipp PR, McMaster ML, Morra E, Pangalis GA, San Miguel JF, Branagan AR, et al. Clinicopathological definition of Waldenstrom's macroglobulinemia: consensus panel recommendations from the Second International Workshop on Waldenstrom's Macroglobulinemia. Semin Oncol. 2003;30(2):110–5.
121. Morice WG, Hanson CA, Kumar S, Frederick LA, Lesnick CE, Greipp PR. Novel multi-parameter flow cytometry sensitively detects phenotypically distinct plasma cell subsets in plasma cell proliferative disorders. Leukemia. 2007;21(9):2043–6.
122. Grogg KL, Morice WG, Macon WR. Spectrum of bone marrow findings in patients with angioimmunoblastic T-cell lymphoma. Br J Haematol. 2007;137(5):416–22.
123. Swerdlow SH, Kuzu I, Dogan A, Dirnhofer S, Chan JK, Sander B, Ott G, Xerri L, Quintanilla-Martinez L, Campo E. The many faces of small B cell lymphomas with plasmacytic differentiation and the contribution of MYD88 testing. Virchows Arch. 2016;468(3):259–75.
124. Insuasti-Beltran G, Gale JM, Wilson CS, Foucar K, Czuchlewski DR. Significance of MYD88 L265P mutation status in the subclassification of low-grade B-cell lymphoma/leukemia. Arch Pathol Lab Med. 2015;139(8):1035–41.
125. Hsieh YC, Lee LP, Chuang SS. Follicular lymphoma with many circulating buttock cells: a leukemic presentation mimicking mantle cell leukemia. Am J Hematol. 2006;81(4):294–5.
126. Iancu D, Hao S, Lin P, Anderson SK, Jorgensen JL, McLaughlin P, Medeiros LJ. Follicular lymphoma in staging bone marrow specimens: correlation of histologic findings with the results of flow cytometry immunophenotypic analysis. Arch Pathol Lab Med. 2007;131(2):282–7.
127. Canioni D, Brice P, Lepage E, Chababi M, Meignin V, Salles B, Xerri L, Peaud PY, Rousselot P, Peuchmaur M, et al. Bone marrow histological patterns can predict survival of patients with

grade 1 or 2 follicular lymphoma: a study from the Groupe d'Etude des Lymphomes Folliculaires. Br J Haematol. 2004;126(3):364–71.
128. Torlakovic E, Torlakovic G, Brunning RD. Follicular pattern of bone marrow involvement by follicular lymphoma. Am J Clin Pathol. 2002;118(5):780–6.
129. Schmidt B, Kremer M, Gotze K, John K, Peschel C, Hofler H, Fend F. Bone marrow involvement in follicular lymphoma: comparison of histology and flow cytometry as staging procedures. Leuk Lymphoma. 2006;47(9):1857–62.
130. Zhang QY, Foucar K. Bone marrow involvement by hodgkin and non-hodgkin lymphomas. Hematol Oncol Clin North Am. 2009;23(4):873–902.
131. de Martini RM, Turner RR, Boone DC, Lukes RJ, Parker JW. Lymphocyte immunophenotyping of B-cell lymphomas: a flow cytometric analysis of neoplastic and nonneoplastic cells in 271 cases. Clin Immunol Immunopathol. 1988;49(3):365–79.
132. Murase T, Yamaguchi M, Suzuki R, Okamoto M, Sato Y, Tamaru J, Kojima M, Miura I, Mori N, Yoshino T, et al. Intravascular large B-cell lymphoma (IVLBCL): a clinicopathologic study of 96 cases with special reference to the immunophenotypic heterogeneity of CD5. Blood. 2007;109(2):478–85.
133. Kremer M, Spitzer M, Mandl-Weber S, Stecker K, Schmidt B, Hofler H, Quintanilla-Martinez L, Fend F. Discordant bone marrow involvement in diffuse large B-cell lymphoma: comparative molecular analysis reveals a heterogeneous group of disorders. Lab Invest. 2003;83(1):107–14.
134. Costes V, Duchayne E, Taib J, Delfour C, Rousset T, Baldet P, Delsol G, Brousset P. Intrasinusoidal bone marrow infiltration: a common growth pattern for different lymphoma subtypes. Br J Haematol. 2002;119(4):916–22.
135. Soussain C, Patte C, Ostronoff M, Delmer A, Rigal-Huguet F, Cambier N, Leprise PY, Francois S, Cony-Makhoul P, Harousseau JL, et al. Small noncleaved cell lymphoma and leukemia in adults. A retrospective study of 65 adults treated with the LMB pediatric protocols. Blood. 1995;85(3):664–74.
136. Dave SS, Fu K, Wright GW, Lam LT, Kluin P, Boerma EJ, Greiner TC, Weisenburger DD, Rosenwald A, Ott G, et al. Molecular diagnosis of Burkitt's lymphoma. N Engl J Med. 2006;354(23):2431–42.
137. Hummel M, Bentink S, Berger H, Klapper W, Wessendorf S, Barth TF, Bernd HW, Cogliatti SB, Dierlamm J, Feller AC, et al. A biologic definition of Burkitt's lymphoma from transcriptional and genomic profiling. N Engl J Med. 2006;354(23):2419–30.
138. Salaverria I, Martin-Guerrero I, Wagener R, Kreuz M, Kohler CW, Richter J, Pienkowska-Grela B, Adam P, Burkhardt B, Claviez A, et al. A recurrent 11q aberration pattern characterizes a subset of MYC-negative high-grade B-cell lymphomas resembling Burkitt lymphoma. Blood. 2014;123(8):1187–98.
139. Schmitz R, Young RM, Ceribelli M, Jhavar S, Xiao W, Zhang M, Wright G, Shaffer AL, Hodson DJ, Buras E, et al. Burkitt lymphoma pathogenesis and therapeutic targets from structural and functional genomics. Nature. 2012;490(7418):116–20.
140. Navid F, Mosijczuk AD, Head DR, Borowitz MJ, Carroll AJ, Brandt JM, Link MP, Rozans MK, Thomas GA, Schwenn MR, et al. Acute lymphoblastic leukemia with the (8;14)(q24;q32) translocation and FAB L3 morphology associated with a B-precursor immunophenotype: the Pediatric Oncology Group experience. Leukemia. 1999;13(1):135–41.
141. Borowitz MJ, Hunger SP, Carroll AJ, Shuster JJ, Pullen DJ, Steuber CP, Cleary ML. Predictability of the t(1;19)(q23;p13) from surface antigen phenotype: implications for screening cases of childhood acute lymphoblastic leukemia for molecular analysis: a Pediatric Oncology Group study. Blood. 1993;82(4):1086–91.
142. Grimaldi JC, Meeker TC. The t(5;14) chromosomal translocation in a case of acute lymphocytic leukemia joins the interleukin-3 gene to the immunoglobulin heavy chain gene. Blood. 1989;73(8):2081–5.
143. Nelson BP, Treaba D, Goolsby C, Williams S, Dewald G, Gordon L, Peterson LC. Surface immunoglobulin positive lymphoblastic leukemia in adults; a genetic spectrum. Leuk Lymphoma. 2006;47(7):1352–9.
144. Konoplev S, Lu X, Konopleva M, Jain N, Ouyang J, Goswami M, Roberts KG, Valentine M, Mullighan CG, Bueso-Ramos C, et al. CRLF2-positive B-cell acute lymphoblastic leukemia in adult patients: a single-institution experience. *Am J Clin Pathol*. 2017;147(4):357–63.
145. Boer JM, Koenders JE, van der Holt B, Exalto C, Sanders MA, Cornelissen JJ, Valk PJ, den Boer ML, Rijneveld AW. Expression profiling of adult acute lymphoblastic leukemia identifies a BCR-ABL1-like subgroup characterized by high non-response and relapse rates. Haematologica. 2015;100(7):e261–4.
146. Weston BW, Hayden MA, Roberts KG, Bowyer S, Hsu J, Fedoriw G, Rao KW, Mullighan CG. Tyrosine kinase inhibitor therapy induces remission in a patient with refractory EBF1-PDGFRB-positive acute lymphoblastic leukemia. J Clin Oncol. 2013;31(25):e413–6.
147. van der Velden VH, Bruggemann M, Hoogeveen PG, de Bie M, Hart PG, Raff T, Pfeifer H, Luschen S, Szczepanski T, van Wering ER, et al. TCRB gene rearrangements in childhood and adult precursor-B-ALL: frequency, applicability as MRD-PCR target, and stability between diagnosis and relapse. Leukemia. 2004;18(12):1971–80.
148. Tiacci E, Pileri S, Orleth A, Pacini R, Tabarrini A, Frenguelli F, Liso A, Diverio D, Lo-Coco F, Falini B. PAX5 expression in acute leukemias: higher B-lineage specificity than CD79a and selective association with t(8;21)-acute myelogenous leukemia. Cancer Res. 2004;64(20):7399–404.

149. Komrokji R, Lancet J, Felgar R, Wang N, Bennett JM. Burkitt's leukemia with precursor B-cell immunophenotype and atypical morphology (atypical Burkitt's leukemia/lymphoma): case report and review of literature. Leuk Res. 2003;27(6):561–6.
150. Ozdemirli M, Fanburg-Smith JC, Hartmann DP, Azumi N, Miettinen M. Differentiating lymphoblastic lymphoma and Ewing's sarcoma: lymphocyte markers and gene rearrangement. Mod Pathol. 2001;14(11):1175–82.
151. McKenna RW, Washington LT, Aquino DB, Picker LJ, Kroft SH. Immunophenotypic analysis of hematogones (B-lymphocyte precursors) in 662 consecutive bone marrow specimens by 4-color flow cytometry. Blood. 2001;98(8):2498–507.
152. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–405.

Multiple Myeloma and Other Plasma Cell Neoplasms

13

Pei Lin

Overview

Plasma cell neoplasms (PCN) encompass a range of diseases ranging from indolent to aggressive. They include monoclonal gammopathy of undetermined significance (MGUS) (67%), multiple myeloma (14%), solitary plasmacytoma of the bone or extraosseous tissue (3%), primary amyloidosis (9%), immunoglobulin (Ig) chain deposition diseases (<1%), POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, M component, and skin changes), and more recently recognized TEMPI syndrome (telangiectasias, elevated erythropoietin level and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting).

Multiple myeloma is the prototype of plasma cell neoplasm that generally evolves from MGUS as a result of accumulated genetic hits and altered microenvironment. The monoclonal proliferation of plasma cells is characterized by intra-clonal heterogeneity and clonal evolution over time. Markedly improved patient outcome owning to novel therapy over the past decade is redefining the disease paradigm. This chapter focuses mainly on myeloma and briefly describes other related PCN. Figure 13.1 shows an algorithm that assists in the diagnosis and differential diagnosis of plasmacytosis.

Multiple Myeloma

Multiple myeloma represents 1% of all malignancy and 10% of hematologic tumors. It is characterized by multifocal involvement of the bone marrow by a monoclonal expansion of plasma cells associated with lytic bone lesions and M protein in the serum and/or urine. The diagnosis of myeloma requires ≥ 10% monoclonal plasma cells in the bone marrow or a biopsy-proven plasmacytoma, though patients who meet the criteria may be symptomatic or asymptomatic. In symptomatic patients, unexplained anemia coupled with M protein and other clinical symptoms such as bone pain usually lead to bone marrow examination and other ancillary studies that confirm the diagnosis. Asymptomatic patients are usually diagnosed incidentally during routine laboratory workup. Multiple myeloma is also known as plasma cell myeloma or Kahler's disease.

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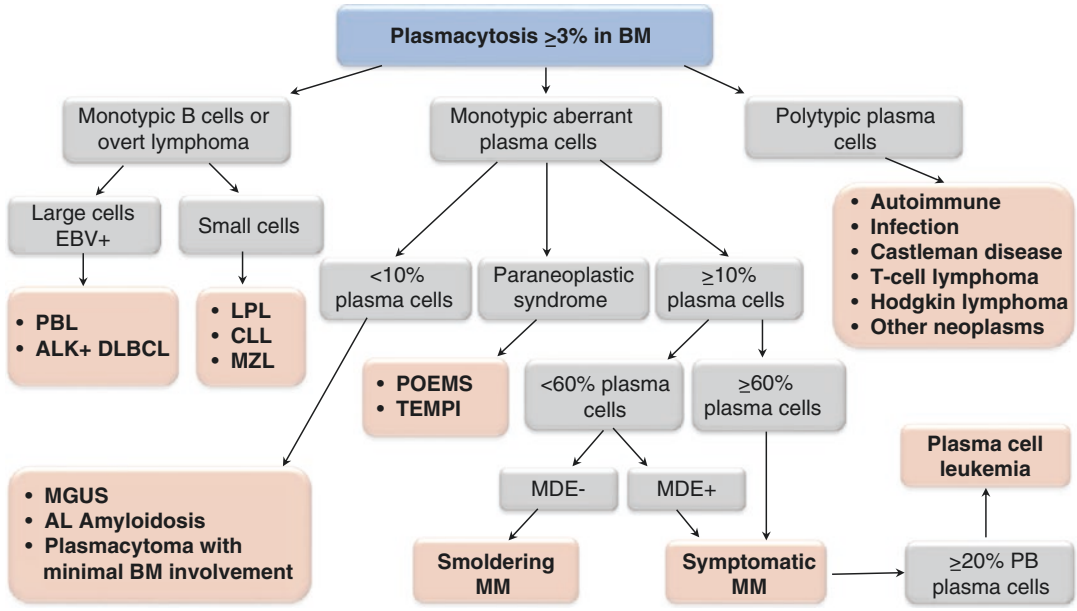

Fig. 13.1 Diagnosis and differential diagnosis of patients with plasmacytosis with or without M proteins. *PBL* plasmablastic lymphoma, *DLBCL* diffuse large B cell lymphoma, *LPL* lymphoplasmacytic lymphoma, *CLL* chronic lymphocytic leukemia, *MZL* marginal zone lymphoma, *MGUS* monoclonal gammopathy of undetermined significance, *MDE* myeloma-defining disease, *POEMS* polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes, *TEMPI* telangiectasias, elevated erythropoietin level and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting, *MM* multiple myeloma

Clinical Features

Multiple myeloma is a disease of older people with a median age of 69 years. Each year over 20,000 new patients are diagnosed with the disease. Blacks are twice more likely affected than whites, and Asians are least susceptible. Most patients present with bone pain due to tumor infiltration and pathologic fractures. Fatigue and weakness secondary to anemia are common, and some may present with renal failure, weight loss, or recurrent infection. Less frequent symptoms include hyperviscosity syndrome, hypercalcemia, and spinal cord compression as a result of vertebral body fracture or epidural tumor mass.

According to the recently proposed revised International Myeloma Working Group (IMWG) criteria [1, 2], the diagnosis of symptomatic myeloma requires the presence of one or more myeloma-defining events (MDEs) such as CRAB features (hypercalcemia >11 mg/dL, renal dysfunction, anemia, and destructive bone lesions). The new criteria also recognize patients who have not yet developed CRAB but are on the verge of becoming symptomatic due to the presence of ultrahigh risk features (Table 13.1). These include (a) clonal plasma cells of ≥60%, (b) involved to uninvolved serum light chain ratio of ≥100, or (c) more than one focal lesion of 5 mm on imaging studies. Patients who meet the criteria of myeloma but are asymptomatic are considered to have smoldering (asymptomatic or indolent) multiple myeloma (SMM). SMM represents about 8–20% of all cases of myeloma, including those with up to 60% of monoclonal plasma cells. It is distinguished from MGUS by >10% plasma cells in the bone marrow and/or 30g/L of M protein in the serum. SMM patients have a much higher risk of progression to symptomatic myeloma than MGUS patients. The rate of progression of MGUS is at 1% per year, but the rate of progression of SMM is 10% per year for the first 5 years, 3% per year for the next 5 years, and 1% per year for the following 10 years [3]. Identifying patients at high risk for early intervention to prevent transformation has

Table 13.1 Revised International Myeloma Working Group Diagnostic Criteria for MM, SMM, and MGUS (2014)

MGUS	Serum M protein <3 g/dL and/or abnormal FLC ratio (<0.26 or >1.65) with increased level of appropriate involved light chain Clonal BM plasma cells <10% Absence of MDEs or amyloidosis
SMM	Serum M protein >3 g/dL Clonal BM plasma cells 10–59% Urinary monoclonal protein >500 mg per 24 h Absence of MDEs or amyloidosis
MM	Clonal BM plasma cells ≥10% or biopsy-proven plasmacytoma *and* any one or more of the following myeloma-defining events (MDEs) • Evidence of end-organ damage (CRAB criteria) that can be attributed to the underlying plasma cell proliferative disorder, specifically: – Hypercalcemia: serum Ca^{2+} > 0.25 mmol/L (>1 mg/dL) above the upper limit of normal or >2.75 mmol/L (>11 mg/dL) – Renal insufficiency: serum creatinine >177 μmol/L (>2 mg/dL) – Anemia: HGB value of >2 g/dL below the lower limit of normal or a HGB <10 g/dL – Bone lesions: one or more lytic lesion(s) on skeletal radiography, CT, and PET-CT • Or more than one of the following three biomarkers – Clonal BM plasma cells ≥60% – Involved/uninvolved serum FLC ratio ≥100 (involved FLC level must be ≥ 100 mg/L) – >1 focal lesion on MRI study (at least 5 mm)

MGUS Monoclonal gammopathy of undetermined significance, *SMM* smoldering multiple myeloma, *MM* multiple myeloma, *MDEs* myeloma-defining events, *FLC* free light chain, *BM* bone marrow

become the focus of more recent studies [4]. Features associated with a high risk to progression include 50–60% BM plasma cells, adverse cytogenetic findings (see below), >95% aberrant plasma cells (of the total plasma cells) detected by flow cytometry, IgA-type paraprotein, and decreased levels of uninvolved immunoglobulin [5].

Solitary plasmacytoma of the bone is a single localized neoplasm usually involving axial skeleton. The diagnosis requires extensive radiologic imaging, especially magnetic resonance imaging (MRI) and positron emission tomography (PET) scan, to exclude occult systemic disease. A low level of serum or urine M protein is detectable in majority of patients, and there is a higher risk for systemic disease. Patients with bone marrow clonal plasma cells <10% are classified as solitary plasmacytoma with minimal bone marrow involvement.

Solitary extramedullary plasmacytoma most commonly occurs in the head and neck regions but may involve any organs including the gastrointestinal tract, central nervous system, and skin. Most cases produce no detectable serum protein and rarely evolve to myeloma.

Plasma cell leukemia (PCL) is an aggressive disease defined as an absolute plasma cell count of more than 2×10^9/L or plasma cells constituting more than 20% of the total leukocyte count in the peripheral blood. Primary PCL comprises 5% of the newly diagnosed cases of myeloma but 60% of all leukemic cases. Secondary PCL results from excessive tumor growth and represents 1% of all myeloma cases. Primary PCL is frequently associated with hepatosplenomegaly, extramedullary dissemination, and a poor outcome.

Osteosclerotic myeloma is a rare form of myeloma usually seen in POEMS (polyneuropathy, organomegaly, endocrinopathy, M component, and skin changes) syndrome. The bone changes are characterized by osteosclerosis rather than lytic lesions. Lymph nodes may show Castleman disease. The serum vascular endothelial growth factor receptor (VGEF) level is elevated.

Laboratory evaluations are essential for diagnosis, follow-up, and prognosis. Serum protein electrophoresis (SPEP), urine protein electrophoresis (UPEP) of an aliquot from 24 h urine collection, and immunofixation electrophoresis (IFE) are used to detect and quantify M protein (Fig. 13.2). Most patients have M protein and suppression of uninvolved immunoglobulin. IgG is most common (55%), followed by IgA (20%),

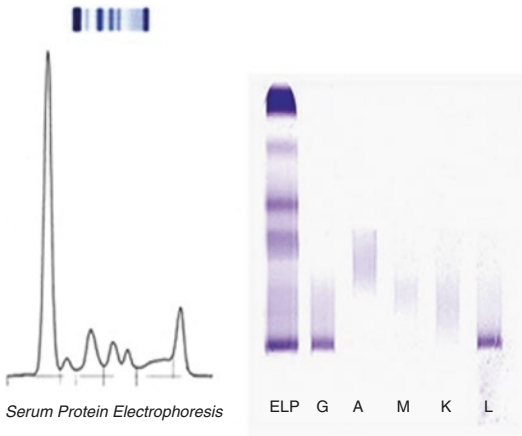

Fig. 13.2 Serum protein electrophoresis (*left*) shows a spike in the gamma region and immunofixation electrophoresis show monoclonal protein of IgGL type (*right*)

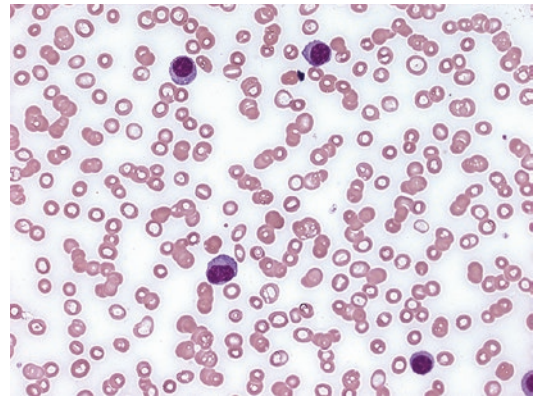

Fig. 13.3 Peripheral blood smear of a patient with plasma cell leukemia. The circulating plasma cells resemble plasmacytoid lymphocytes. The background red blood cells show rouleaux formation due to a high level of M protein

light chain only (20%), IgD (1.5%), IgM (0.5%), and IgE (0. 5 %). Nonsecretory myelomas account for 2% cases. Rarely biclonal disease is observed. In light chain only myeloma, hypogammaglobulinemia may be the only finding in the serum, but Bence-Jones protein can be identified in the urine. Serum free light chains (FLC) ratio is most useful in cases with minimal serum M protein and nonsecretory or light chain only myeloma. It is also useful in diagnosis of MGUS, primary amyloidosis, and light chain deposition disease (LCDD) when M component is low.

Morphology

The most common finding in the peripheral blood is normochromic normocytic anemia. The peripheral blood smear may have a blue hue and show rouleaux formation due to a high level of immunoglobulin. Leukopenia and thrombocytopenia are usually features of advanced disease. A varying number of circulating plasma cells may be identified. Primary PCL is often associated with small cell morphology resembling low-grade B-cell lymphoma or leukemia with plasmacytoid differentiation (Fig. 13.3). Secondary PCL usually shows more immature or atypical plasma cells.

Plasma cells in normal bone marrow are mature forms with abundant basophilic cytoplasm, perinuclear hof, and a "spoke-wheel" nuclear pattern, comprising fewer than <4% of the total marrow elements. The neoplastic plasma cells are marked by a variable degree of immaturity or nuclear atypia. Asynchronous maturation of the nuclear and cytoplasm, dispersed chromatin, a high nuclear and cytoplasmic ratio, and prominent nucleoli are features of immaturity. Atypia usually manifests as bizarre nuclear forms and shapes or marked variation in nuclear size. Accumulation of cytoplasmic immunoglobulin forms cytoplasmic inclusions. Russell bodies are the most common form. Cells containing inclusions are described as grape cells, flaming cells, Mott cells, or Gaucher-like cells. Dutcher bodies are nuclear inclusions. In rare cases, crystalline material can be found also in histiocytes causing crystal-storing histiocytosis (Fig. 13.4).

Historically, two grading systems have been proposed to describe the spectrum of plasma cell morphology in myeloma: the Greipp system and the Bartl system. The Greipp system has four subgroups: mature, intermediate, immature, and plasmablastic. Bartl system contains seven subtypes: small cell, Marschalko, asynchronous, polymorphic, cleaved, blastic, and sarcomatoid (Fig. 13.5a–f) [6].

As plasma cell enumeration is essential for classification and prognostication, core biopsy is usually obtained to better estimate the extent of

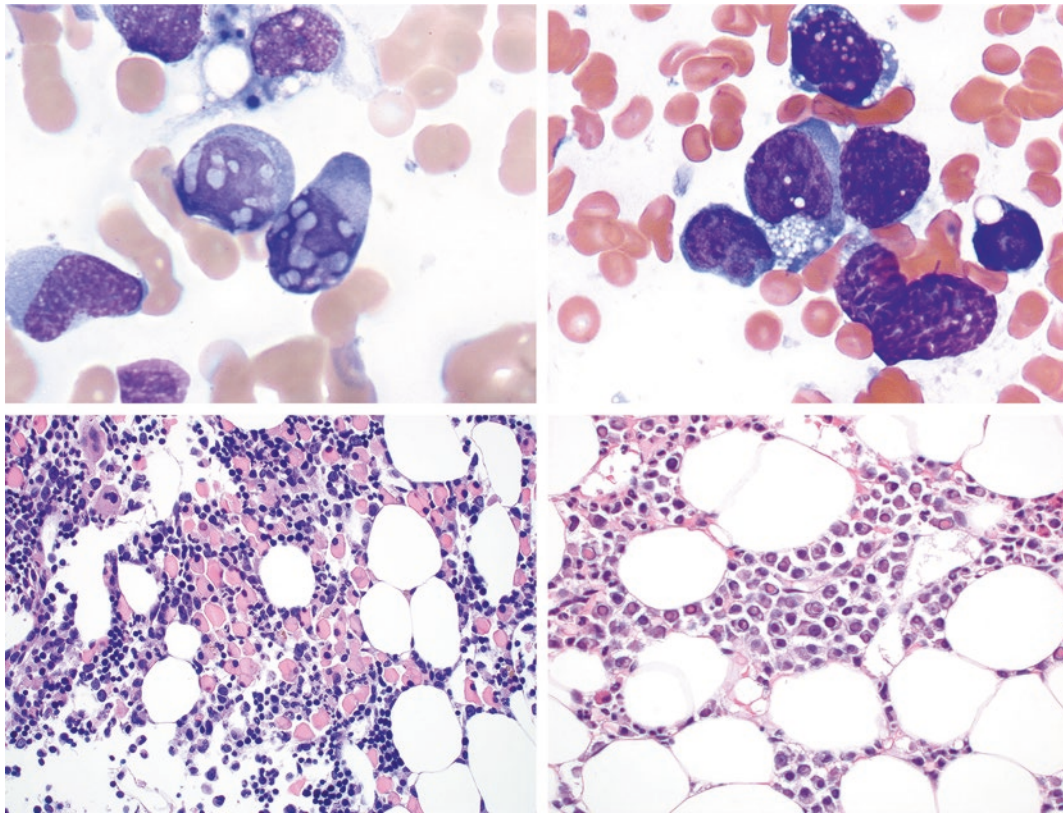

Fig. 13.4 Various types of cytoplasmic and nuclear inclusions in myeloma cells, including crystal-like inclusions (*upper left*), cytoplasmic vacuoles resembling Burkitt lymphoma (*upper right*), numerous Russell body containing plasma cells in the core biopsy (*lower left*), and Dutcher bodies seen in many plasma cells (*lower right*)

disease as aspirate may underestimate the tumor burden. In addition, evidence of myeloma-associated bone changes, amyloid deposition, or fibrosis can be assessed (Fig. 13.6). Symptomatic myeloma usually has a higher tumor burden with plasma cells forming large clusters or sheets, whereas low-risk SMM and MGUS have more dispersed or small clusters of plasma cells. Increased bone resorption and remodeling are usually features of symptomatic myeloma.

Immunophenotype

Flow cytometry immunophenotyping is useful for diagnosis, risk stratification, and guiding therapy. In morphologically challenging tumors or nonsecretory myeloma, identification of monotypic or phenotypically aberrant plasma cells facilitates the diagnosis. Multiparameter flow cytometry of eight or more color allows sensitive detection of minimal residual disease (MRD) which correlates with clinical outcome and can serve as a surrogate for drug efficacy in clinical trials [7].

CD138 is the best marker for identification of benign and malignant plasma cells. CD38 is less specific than CD138 as it is also brightly expressed in T cells, NK cells, normal precursor B cells, and some mature B cells. The new anti-CD38 antibody daratumumab (Darzalex) therapy also renders the routinely used antibody nonreactive and may also interfere with direct antiglobulin test (DAT). Plasma cell gating is best achieved by using a combination of CD138, CD38, and CD45 and light scatter patterns. Additional markers such as CD19, CD27, CD28, CD56, CD81, CD117, and cytoplasmic light chain further dis-

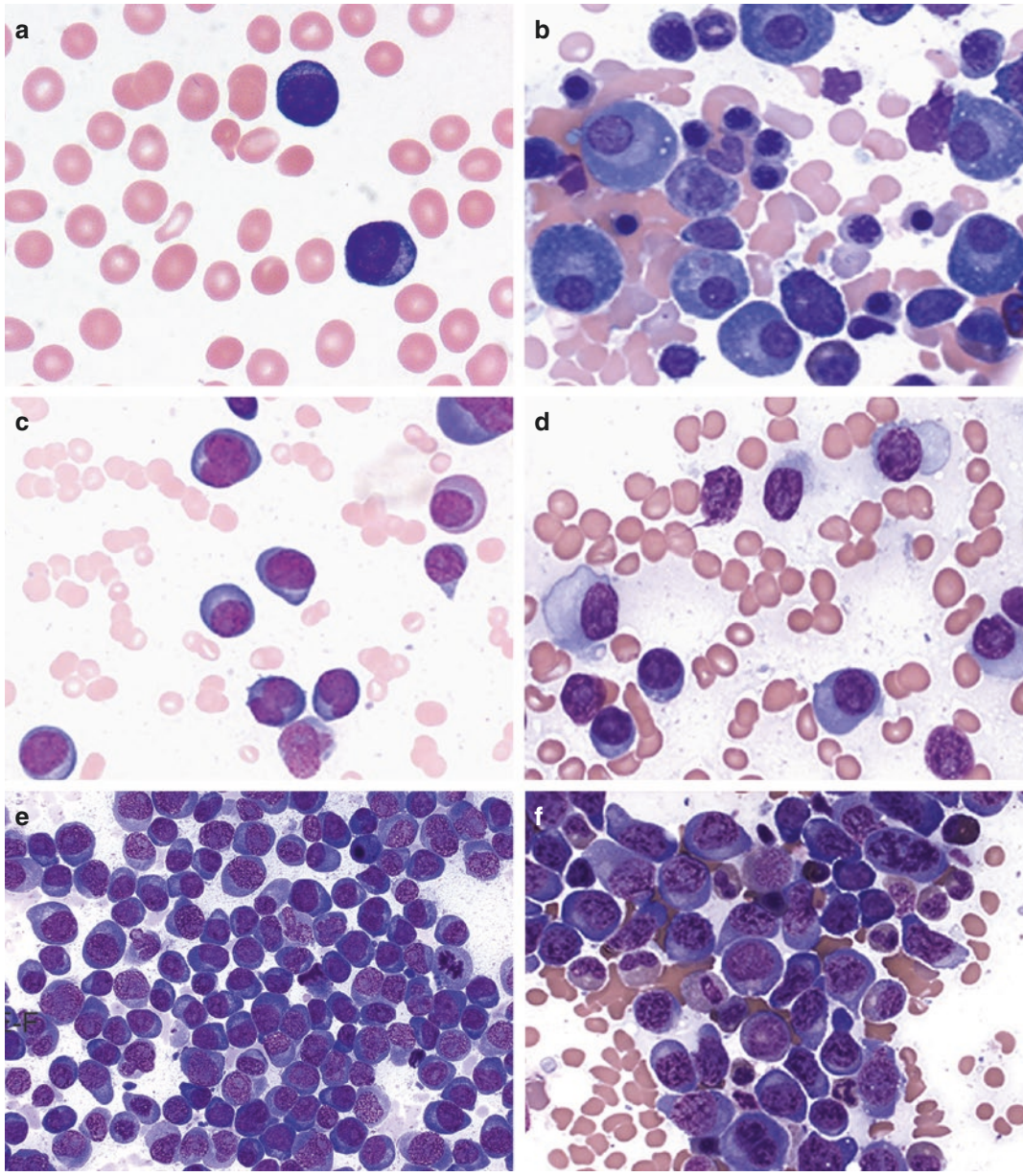

Fig. 13.5 Myeloma cytological grade. Myeloma cells of low-grade cytology including small cell variant (**a**) and Marschalko type (**b**). Myeloma cells of intermediate-grade cytology show polymorphic size and nuclear shape (**c**) or prominent nucleoli and asynchronous cytoplasmic-nuclear maturation (**d**). Myeloma of high-grade cytology includes plasmablastic variant (**e**) and anaplastic variant (**f**)

criminate benign from aberrant PCs. CD38 is bright in benign plasma cells but can be dimmer in malignant plasma cells. Benign plasma cells are typically positive for CD19, CD27, and CD45 and negative for CD20, CD28, CD117, and CD56. Malignant plasma cells usually show decreased or absent CD19, CD27, or CD45 but increased CD28, CD56, and CD117. However, CD45 and CD20 can be expressed at moderate or higher intensity in 10% and 20% of cases, respectively. Rarely, the myeloma cells may express CD19. CD56 is positive in 70–75% of cases. CD117 is

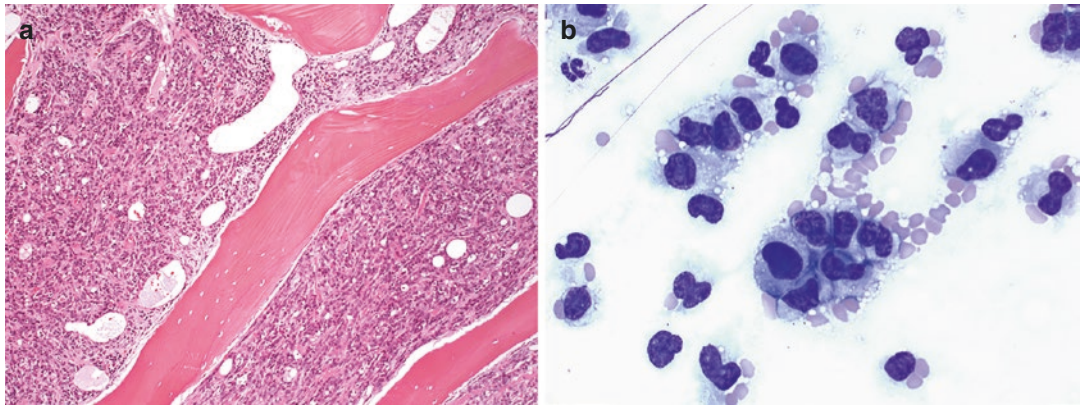

Fig. 13.6 (**a**) Bone marrow core biopsy shows marked fibrosis associated with an extensive infiltrate of abnormal plasma cells. (**b**) The aspirate smear shows marked nuclear irregularity of plasma cells

aberrantly expressed in 30% of cases and is a useful marker for malignancy. Myeloma cells are also positive for CD200 in 70% of cases. Myeloid markers CD13 and CD33 or other markers such as CD10 can also be identified in a small subset of cases. Primary plasma cell leukemia (PCL) tends to be positive for CD20 (50%) but negative for CD117 and CD56 compared to myeloma. The plasma cells usually express cytoplasmic but not surface Ig light chain with rare exception. They also display sideward (SSC) and forward (FSC) light scatter properties different from non-plasma cells in the bone marrow (Fig. 13.7a–g).

Assays designed to detect MRD by flow cytometry are usually based on the differential expression patterns of surface markers between benign and malignant plasma cells [8]. The international consensus panel proposed for MRD assay includes CD138, CD38, CD45, CD19, CD27, CD56, CD81, and CD117. As normal plasma cells also have $CD45^{-/low}$ or $CD19^{-/low}$ subsets that overlap with that of neoplastic plasma cells, and even CD56 can be observed in up to 15% of normal plasma cells, MRD should be determined on the basis of multiple aberrancies and overall pattern. Due to loss of plasma cells during processing and various other factors, the number of plasma cells quantified by flow cytometry is consistently lower than aspirate smears. However, the detection sensitivity reaches 1 in 10^5 or higher when a minimum of 2×10^6 events are analyzed. Assessment of cytoplasmic light chain is not routinely required but may enhance sensitivity in cases with less surface aberrancy. Flow cytometry-based assay is widely applicable and has sensitivity comparable to allele-specific oligonucleotide polymerase chain reaction (ASO-PCR) but is about 1 log less sensitive than next-generation sequencing-based assay.

Immunostaining for plasma cell markers should be performed on the biopsy specimen, at least in the initial evaluation to assess the extent and pattern of involvement. CD138 (syndecan-1) is the most useful antibody in paraffin-embedded tissue. CD138 also stains neoplastic cells in immunoblastic/plasmablastic large B-cell lymphoma and plasmablastic lymphoma, as well as epithelial neoplasm. In rare cases negative for CD138, a panel of antibodies can be used in combination with the Ig κ and λ light chain to confirm plasmacytic/plasmablastic differentiation. BLIMP-1 encoded by *PRDM1* gene and MUM1 are usually expressed in myeloma. CD79a is expressed in about 75% of cases. Cyclin D1 is expressed in 40–45% of cases, typically in a nuclear and cytoplasmic pattern (Fig. 13.8a, b). PAX5 is usually negative or weakly positive with occasional exceptions. The combination of positive CD79a and negative PAX5 suggests plasmacytic differentiation. Ki-67 can be used to assess proliferation rate and (Fig. 13.9a, b) is best used with CD138 as hematopoietic components are also positive for Ki-67. Myeloma cells can be positive for cytokeratin in a dot-like pattern (Fig. 13.10a, b).

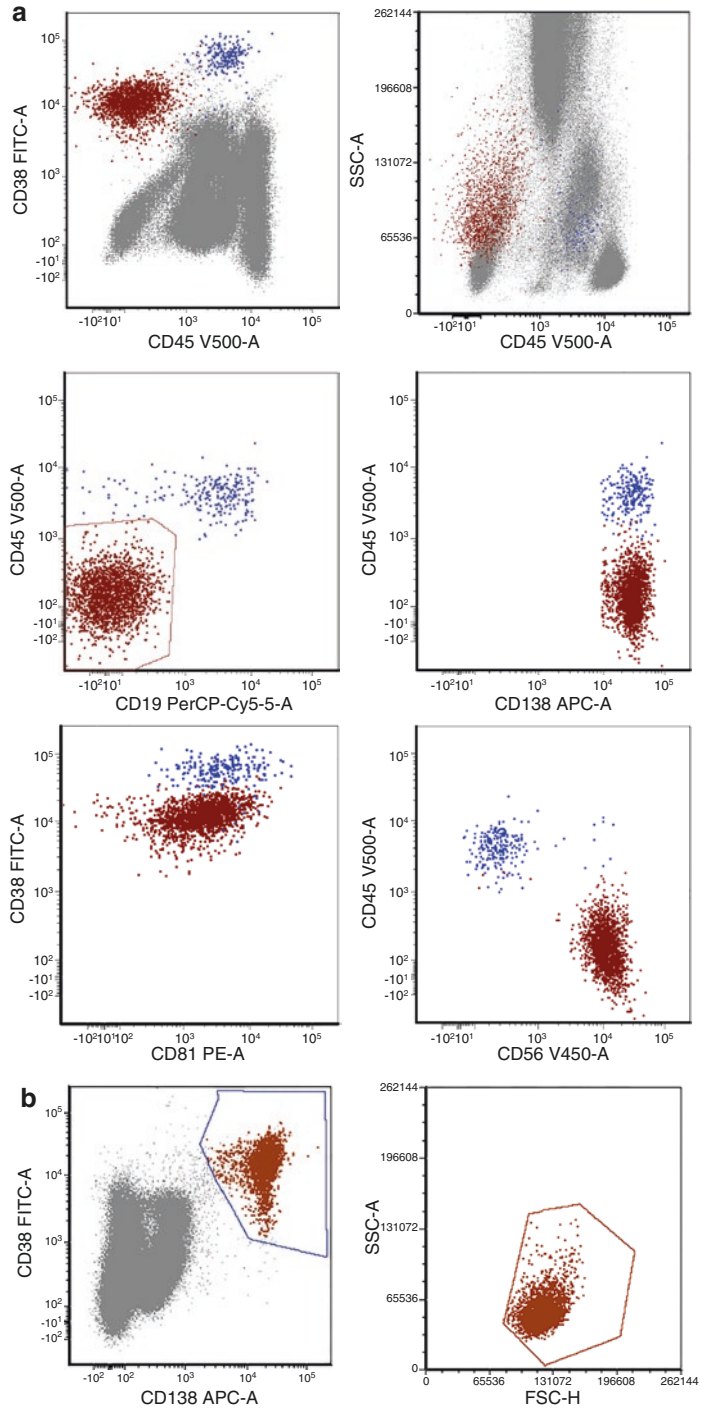

Fig. 13.7 (**a**) Flow cytometry immunophenotyping of normal and aberrant plasma cells. Comparison of neoplastic (*brown*) versus normal plasma cells (*blue*). Normal plasma cells are CD19+, CD45+, and CD81+, and the aberrant plasma cells show decreased intensity of CD38, CD45−, CD19−, CD56+ and decreased intensity of CD81 Fig. 13.7 (continued) (**b**) Immunophenotypic heterogeneity of plasma cells in a case of myeloma. Three different subsets of myeloma cells with variable CD56 intensity ranging from negative to intermediate to bright. These cells are all CD19−, CD45−, CD38+, CD138+, and cytoplasmic lambda restricted

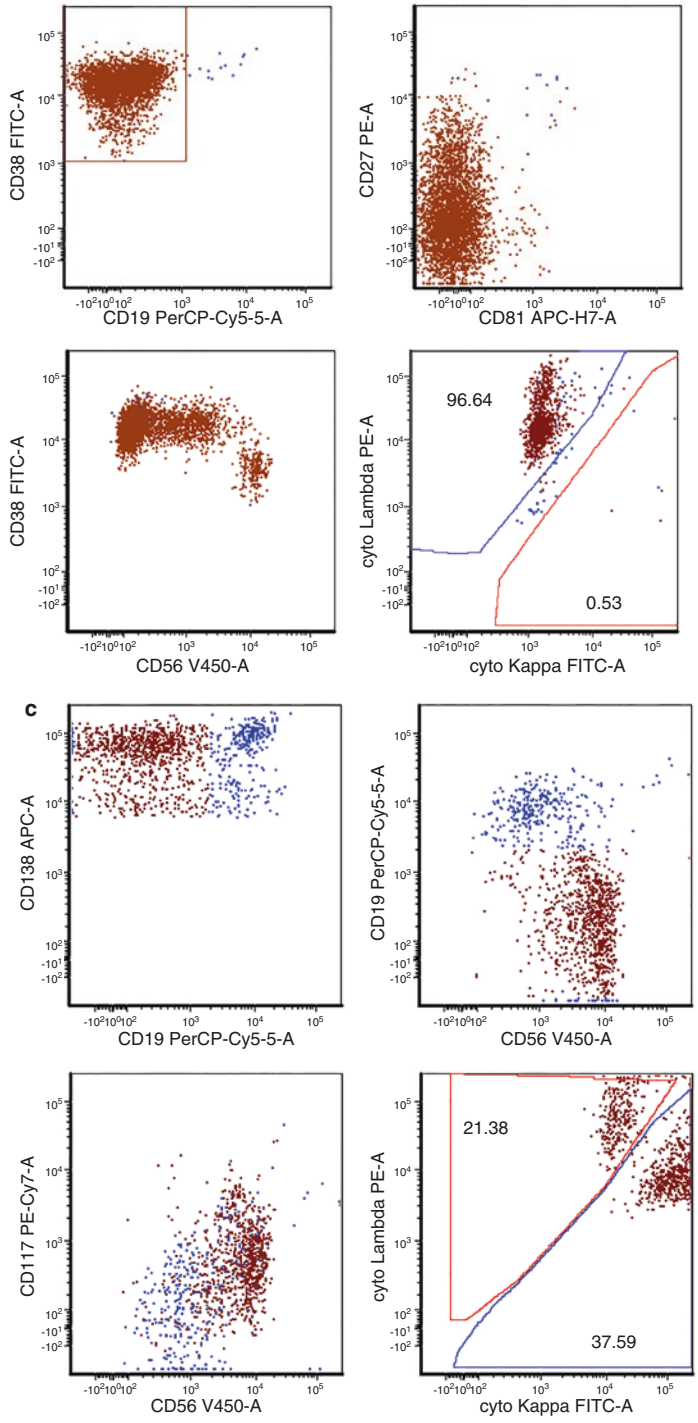

Fig. 13.7 (continued) (**c**) Biclonal myeloma cells. Neoplastic myeloma cells are highlighted in brown and normal plasma cells highlighted in blue. The neoplastic cells appear to have a normal kappa/lambda ratio. However, both populations show aberrant CD19−, CD56+, and CD117dim+. One monotypic cytoplasmic kappa and the other monotypic cytoplasmic lambda

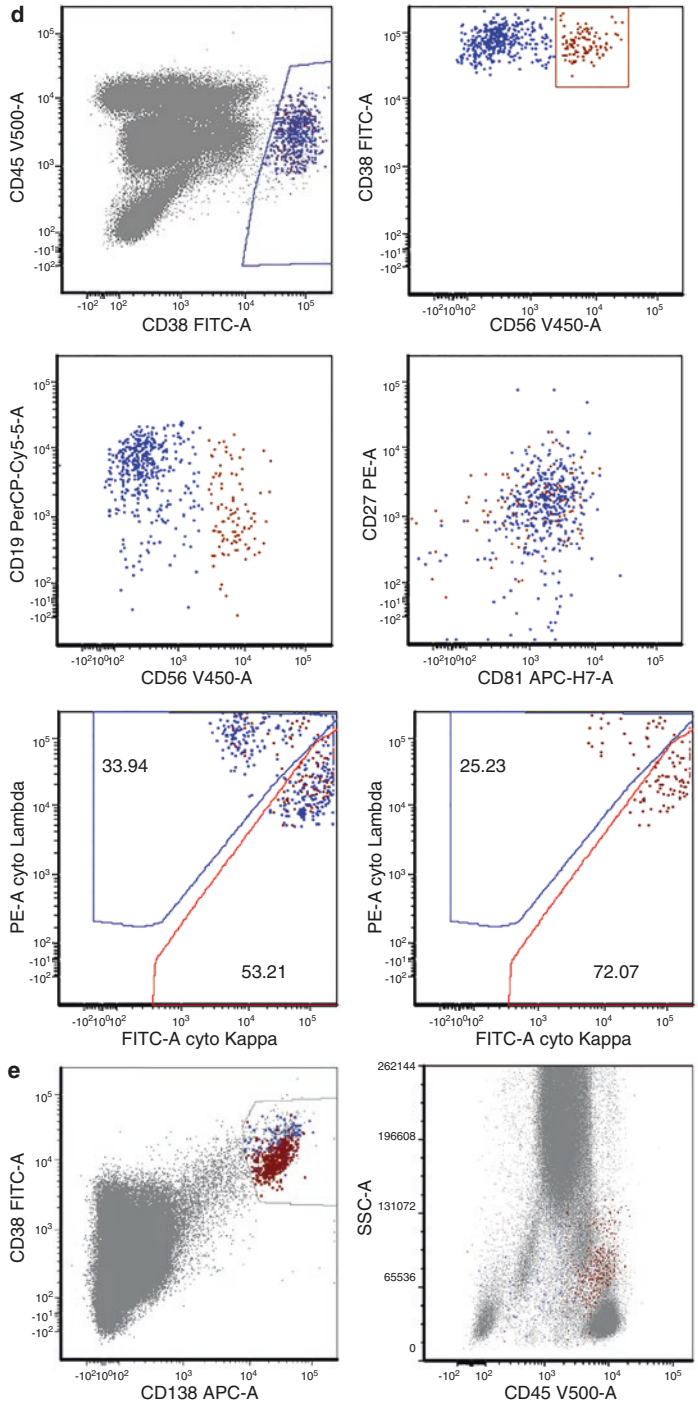

Fig. 13.7 (continued) (**d**) Normal plasma cells with reactive atypia. A subset of normal plasma cells (*brown*) shows CD56 expression with slightly decreased CD19 but polytypic for cytoplasmic light chain. Note also that they show normal CD45, CD27, and CD81 expression levels. (**e**) The myeloma cells (*brown*) in this case show increased CD45 expression compared to the normal plasma cells (highlighted in *blue*). The neoplastic plasma cells are also CD56+, CD117+, and cytoplasmic lambda light chain restricted

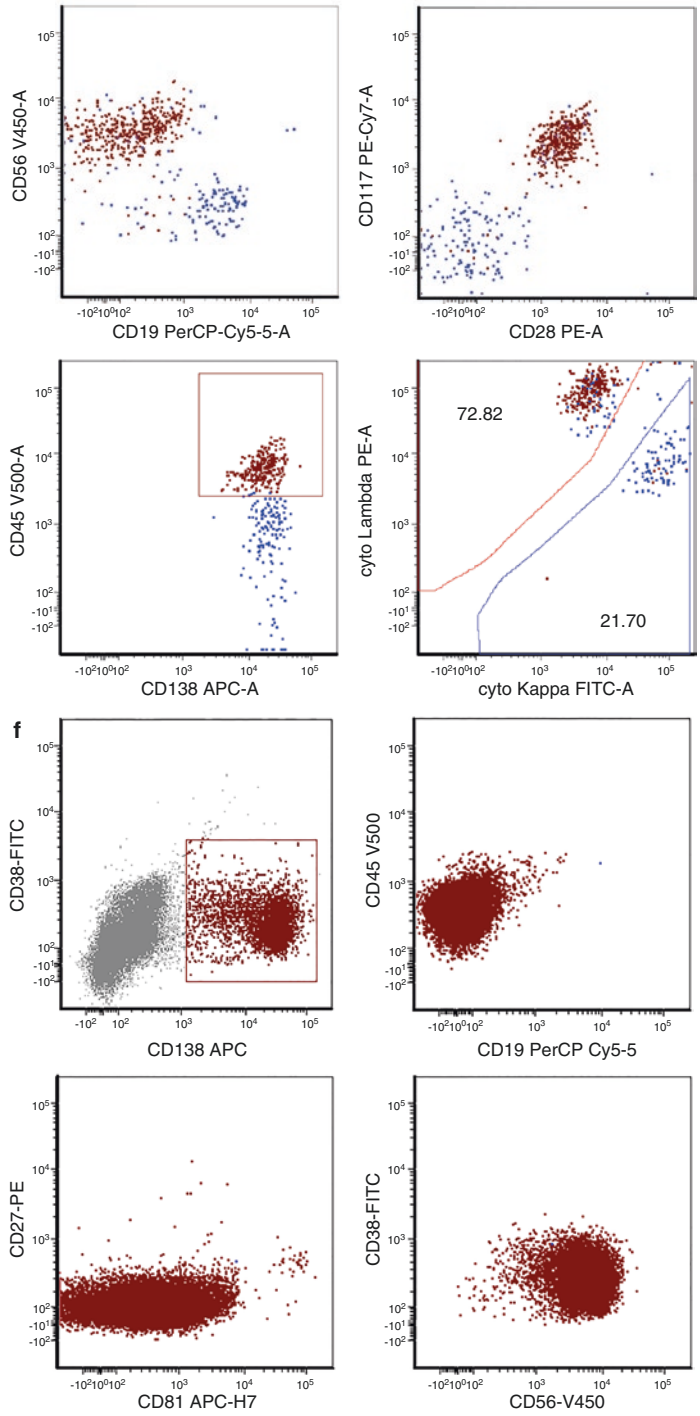

Fig. 13.7 (continued) (**f**) Myeloma cells in a patient treated with daratumumab (anti-CD38 antibody) showing no detectable CD38 expression when tested with CD38 FITC (BD Bioscience). The neoplastic cells are CD138+, aberrantly positive for CD56, decreased for CD81, and negative for CD27, CD19, and CD45

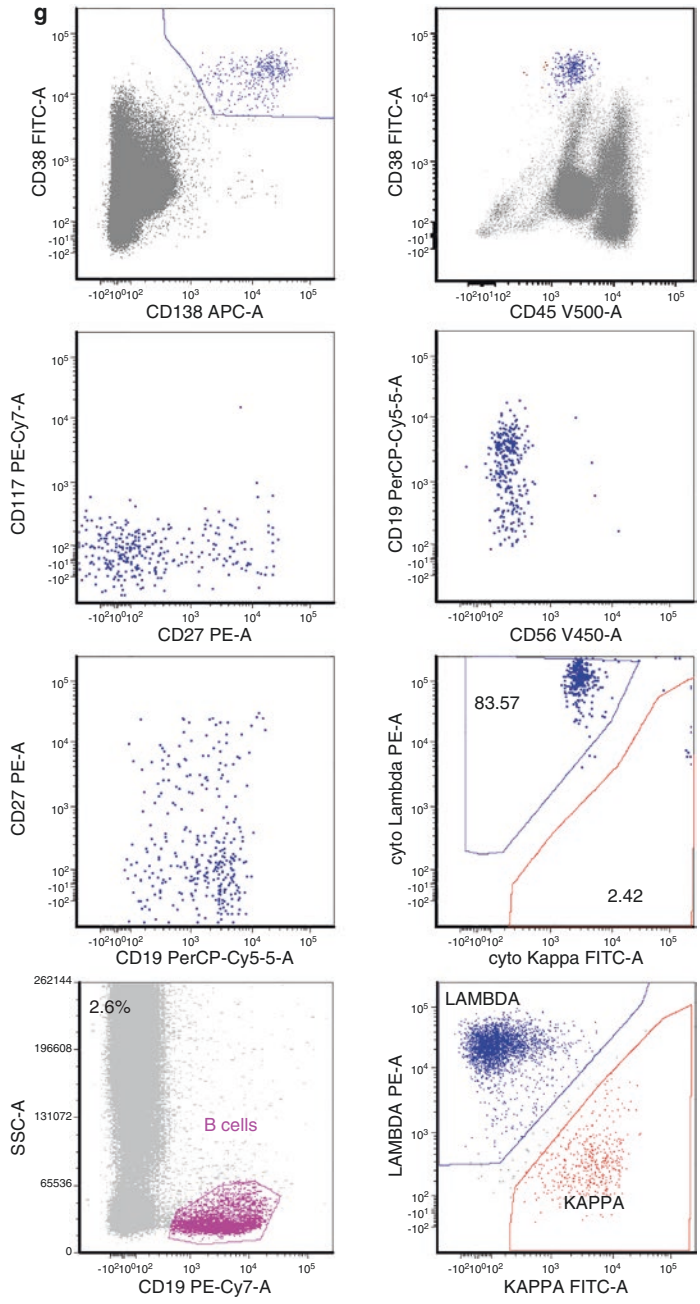

Fig. 13.7 (continued) (**g**) A case of lymphoplasmacytic lymphoma/Waldenstrom macroglobulinemia. The plasma cells are CD138+, CD38+, CD45 slightly dim, CD19+, CD27 decreased, CD56−, CD117−, and cytoplasmic lambda light chain restricted. The B cells are CD19+ with surface lambda light chain restriction

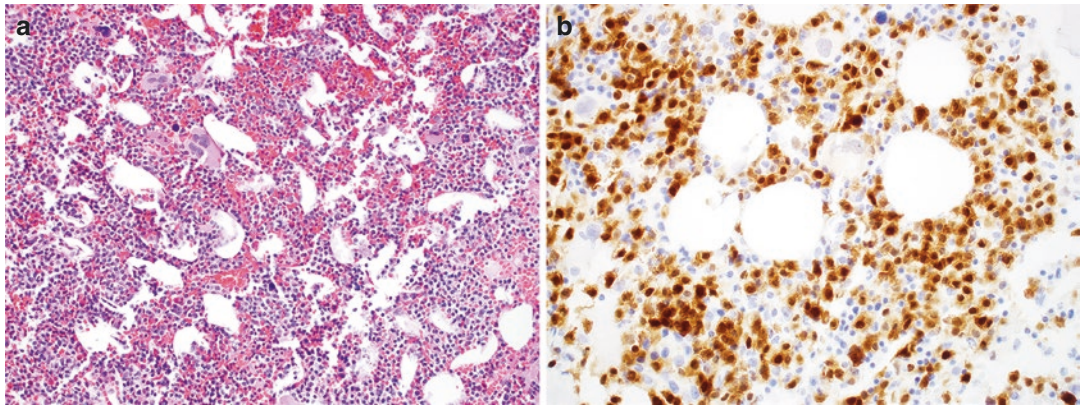

Fig. 13.8 The neoplastic plasma cells in this multiple myeloma case are positive for cyclin D1 (nuclear and cytoplasmic staining). (**a**) H&E and (**b**) cyclin D1 immunohistochemistry

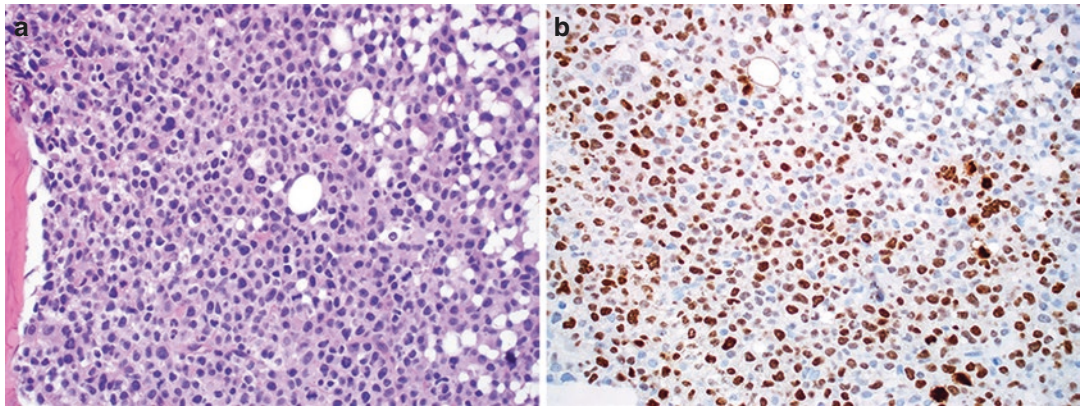

Fig. 13.9 Aggressive myeloma with a high ki-67 proliferation (**a**) H&E 200×, (**b**) Ki-67 immunohistochemistry

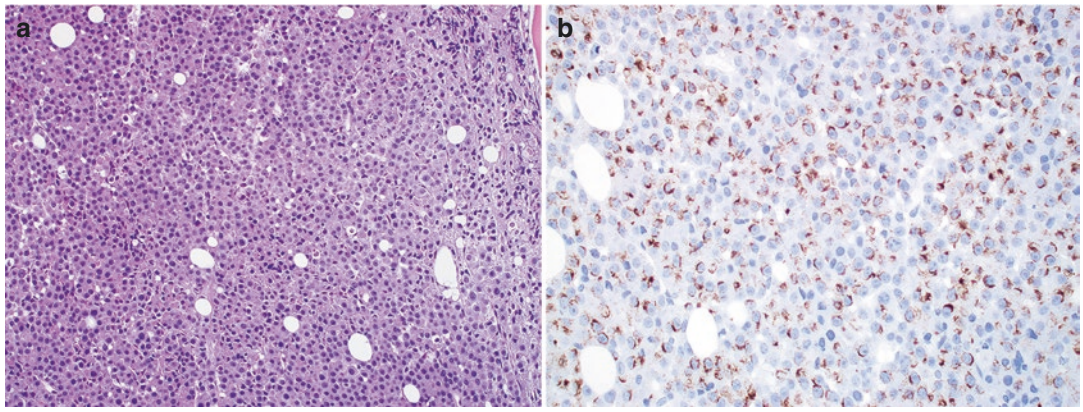

Fig. 13.10 Plasma cells in this multiple myeloma case are positive for keratin in a dot-like pattern (**a**) H&E and (**b**) cytokeratin immunohistochemistry

Cytogenetics and Molecular Genetics

Conventional cytogenetic analysis identifies an abnormal karyotype, usually complex, in only 30% of cases at diagnosis. However, genetic aberrations can be demonstrated in nearly all myelomas by interphase FISH analysis. Plasma cell enrichment by CD138 magnetic beads may enhance sensitivity of FISH.

Translocations involving Ig heavy chain locus on chromosome 14q32 are the most common recurrent cytogenetic abnormalities, occurring in about 50 % of cases. The five most common partners are located at 11q, 4p, 16q, 6p, and 20q, involving genes regulating cyclin D family protein and cell cycle (Table 13.2). These five translocations are also detectable in MGUS and therefore considered primary events.

The breakpoint in t(11;14) involves part of *CCND1* gene that is located more centromeric than that in mantle cell lymphoma. The t(11;14) is more common in primary PCL (71%) compared with secondary PCL (23%). The t(4;14) interrupts both fibroblast growth factor (FGF) receptor 3 gene (*FGFR3*) and multiple myeloma SET domain (*MMSET*) genes (Fig. 13.11). The t(14;16) and t(14;20) result in overexpression of MAF/MAFB, regulating CCND2, and therefore overexpression of cyclin D2 (Fig. 13.11a–d).

Apart from structural aberrations, numerical aberrations are also observed. Trisomy most frequently involves odd chromosomes 3, 5, 7, 9, 11, 15, 19, and 21, whereas monosomy and partial deletions affect mainly chromosomes 6, 13, 16, and 22. Deletion of 1p can be found in 30% of cases affecting putative genes *CDKN2C, FAM46C*. Monosomy 13 or interstitial deletion of 13 (13q14) is detectable in 15–20% of newly diagnosed myeloma by conventional cytogenetics and 50% of cases by interphase FISH. Del(13q) can also be found in MGUS.

Table 13.2 The five most common partner genes involved in translocations of *IGH* gene

Chromosome	Gene	Percentage (%)
11q13	*CCND1*	15
4p16.3	*FGFR3* and *MMSET*	15
16q23	*C-MAF*	5
6p21	*CCND3*	3
20q11	*MAFB*	2

Based on the number of chromosomes, myeloma cases can be broadly divided into two groups: the hyperdiploid (HRD; 48–75 chromosomes) and non-hyperdiploid myeloma (NHRD; <48 or >75 chromosomes). HRD represents about half of cases and frequently carries trisomies. The five recurrent translocations described above and del(13q) are more common in NHRD than HRD, suggesting that different molecular genetic pathways are involved in pathogenesis of the two subgroups.

Other genetic aberrations described in myeloma include *MYC* rearrangement, occurring in up to 50% of cases, often cryptic and involving non-*IGH* partners, and amplification of 1q21 (resulting in putative cyclin-dependent kinase subunit 1 (*CKS1*B) gene amplification) in 40%. Deletion of *TP53* is identified in 10% of newly diagnosed myeloma but at a much higher rate in refractory/relapsed myeloma or PCL, in approximately 50% of the primary PCL and 70% of the secondary PCL.

The advent of new technology allows global investigation of genes altered in myeloma. Molecular classification is based on the predominant activation pattern of cyclin D protein and the pattern of *IGH* translocations identified by DNA microarray. Gene expression profiling (GEP) has been increasingly utilized in clinical trials to stratify patients beyond conventional techniques. Other technologies such as complimentary genomic hybridization (CGH)-based mapping array also allow dissection of complex chromosomal gain and loss. Single nucleotide polymorphism (SNP) array has found independent prognostic markers including del(12p13.31) as an adverse marker and amplification of (5q31.1) as a favorable marker.

Next-generation sequencing has not only confirmed known recurrent mutations but also discovered new mutations in myeloma. Mutations of *NRAS (20–30%), KRAS (15–20%), and NF-κB* (6–17%) pathway components such as *TRAF3*, *cIAP1/2*, *CYLD*, and *NIK* are most common, followed by, *TP53* (7%), *BRAF* (7%), *CCND1* (3%), *ATM (3%)*,

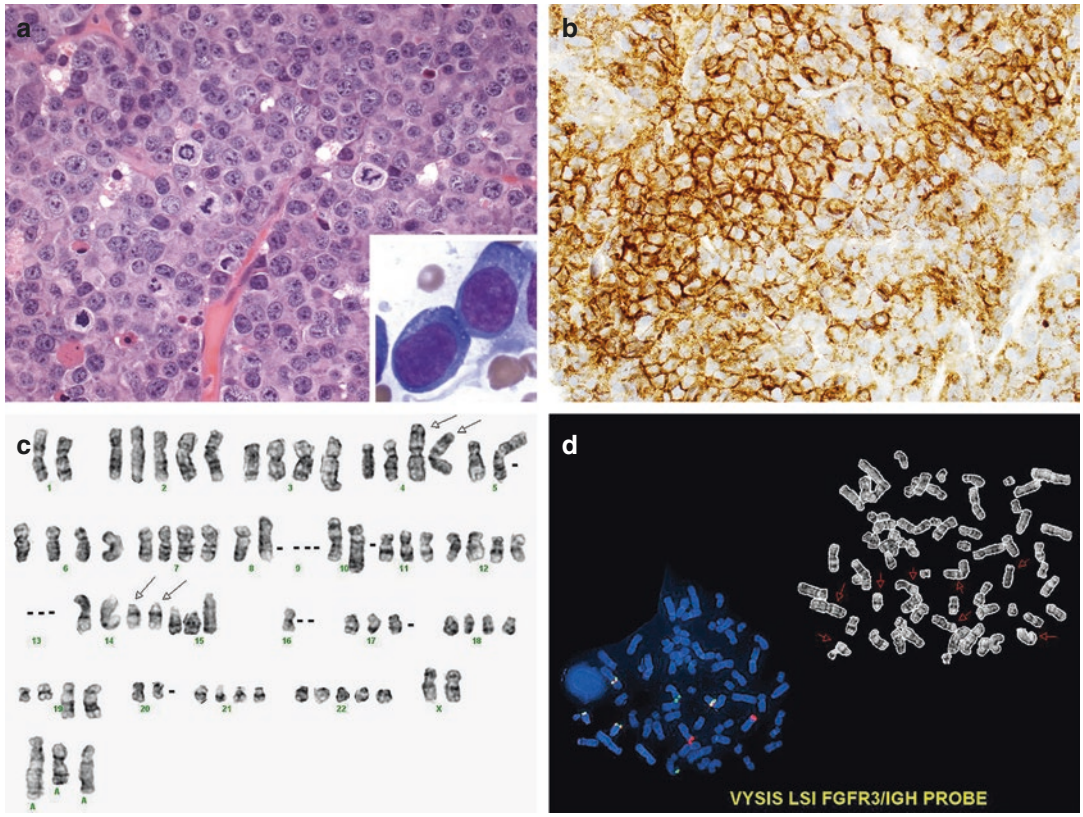

Fig. 13.11 (**a**) Bone marrow biopsy of plasmablastic myeloma with increased mitotic figures. (*Inset*) Smear shows markedly immature nuclear features (*upper left*); the plasma cells are positive for CD138 (**b**). A complex karyotype with t(4;14) (**c**). Fluorescence in situ hybridization confirms *FGFR3/IGH* gene rearrangement (**d**)

ATR (1.5%), or MYC (1%). Mutations in *CCND1* and DNA repair pathway alterations (*TP53*, *ATM*, *ATR*, and *ZNFHX4* mutations) are negatively associated with survival. In contrast, mutations in *IRF4* and *EGR1* are positively associated with survival. The combination of various types of genetic hits ultimately affects multiple signaling pathways including the MYC, NF-κB, and MAPK pathways with downstream effect on plasma cell differentiation, cell cycle regulation, or DNA damage repair, driving disease progression [9, 10].

Genomic and single-cell sequencing have identified marked intra-clonal heterogeneity and clonal evolution upon therapeutic intervention. Focal lesions from different parts of the patients may harbor divergent genetic hits competing in a Darwinian fashion [11]. The so-called clonal tide has profound therapeutic implication as drug-resistant clones, such as the ones with deleted or mutated *TP53* detected as a minor clone at diagnosis, may be "selected" to expand. To eradicate the myeloma stem cells will inevitably need to overcome the challenge of clonal heterogeneity and evolution.

Molecular genetics and cytogenetics are markers of tumor biology and the basis of risk stratification. An abnormal karyotype identified by conventional cytogenetics is indicative of highly proliferative tumors and an inferior patient survival. Deletion of chromosome 13 or 13q14 by conventional karyotyping, hypodiploidy, and monosomy 17 is associated with a poor prognosis. Detection of t(4;14), t(14;16), or t(14;20) and deletion of the *TP53* locus by FISH predict an inferior survival though the adverse effect of t(4;14) could be partially ameliorated by protea-

some inhibitor Velcade (bortezomib). Gain of chromosome 1q (1q+) and loss of chromosome 1p are also unfavorable.

Differential Diagnosis

A variety of reactive conditions are associated with plasmacytosis and sometimes up to 30% of marrow cells. Autoimmune disorders, hepatitis C, human immunodeficiency virus infections, Hodgkin lymphoma, and angioimmunoblastic T-cell lymphoma are a few examples. Nuclear immaturity and marked atypia are not features of reactive plasmacytosis and are strong indications of malignancy although binucleated or occasionally trinucleated forms may be observed in reactive conditions. Reactive plasmacytosis is also frequently associated with increased eosinophils, mast cells, and megakaryocytes. Benign plasma cells usually present in small clusters of five or six cells in perivascular areas, while the myeloma cells proliferate in sheets and replace normal hematopoietic elements with increased bone resorption. Evidence of immunoglobulin light chain restriction supports a neoplastic process. Identification of aberrant marker expression such as CD56 and CD117 also provides further evidence of malignancy.

M protein can be detected in a variety of non-Hodgkin lymphomas, especially lymphoplasmacytic lymphoma (LPL) or chronic lymphocytic leukemia . A small amount of monotypic B cells can also be detected in myeloma patients causing confusion. Myeloma cases with t(11;14) translocation often have lymphoid features (Figs. 13.5a and 13.8). Post-therapy samples from patients with LPL often have persistent plasma cells but devoid of lymphoid cells, mimicking myeloma. Immunophenotyping and molecular studies can help make the distinction between myeloma versus lymphoma. Pax5/BSAP is usually absent or weak and variable in myeloma, but CD20 can be positive in both myeloma and LPL. Myeloma is more often positive for immunoglobulin heavy chain IgG or IgA type, whereas LPL is more likely to be IgM type. By flow cytometry, plasma cells in myeloma are usually $CD19^{-}CD45^{-/low}$ with a higher side scatter property, whereas plasma cells in LPL are usually CD19+ CD45+/slightly dim with a lower side scatter. The plasma cells in LPL usually lack CD56 and CD117. Identification of cyclin D1 overexpression and t(11;14)(*CCND1/IGH*) supports a diagnosis of myeloma (Fig. 13.8). *MYD88* L265P mutations or *CXCR4* mutations support a diagnosis of lymphoma.

Epstein-Barr virus-encoded RNA in situ hybridization is often positive in plasmablastic lymphoma and negative in myeloma except in immune compromised individuals. Correlation with other clinical features such as serum M protein and radiographic imaging may be helpful in difficult cases.

TEMPI syndrome (telangiectasias, elevated erythropoietin level and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting) is a rare, recently recognized disease considered to be a paraneoplastic syndrome resulting from monoclonal gammopathy. The bone marrow may show erythrocytosis mimicking polycythemia vera [12] . Patients with TEMPI syndrome may show nonspecific erythroid hyperplasia and mild nonspecific megakaryocytic hyperplasia. There is however usually no clustering of megakaryocytes or fibrosis. The syndrome should be suspected when erythrocytosis coexists with plasma cell dyscrasia. Elevated erythropoietin and a normal VGEF level allow the diagnosis and distinction from POEMS syndrome.

Monoclonal Gammopathy of Unknown Significance

MGUS is an asymptomatic disorder in which a monoclonal immunoglobulin is secreted by low proliferating plasma cells in the bone marrow. The diagnosis of MGUS requires serum M protein <30 g/L, bone marrow plasma cells <10%, and absence of myeloma-defining events such as CRAB.

Clinical Features

The incidence of MGUS increases with age, occurring in 3% of the population older than age 50 years, 5% older than age 70 years, and 10% older than age 80 years. MGUS is more prevalent in men (4.0%) than in women (2.7%). Like myeloma,

blacks are more affected than white (14.8% versus 7.8%), and asians have the lowest rate.

Currently, three major types of MGUS are recognized: non-immunoglobulin M (IgM) MGUS, IgM-MGUS, and light chain MGUS. Overall, IgG type is most common (65–81%) followed by IgA (5–19%) or IgM (2–17%), biclonal (2–17%), and light chain only type (3%). The IgM-MGUS is related to lymphoproliferative disorders rather than myeloma and may evolve to LPL/WM, other non-Hodgkin lymphomas, amyloidosis, or CLL. The light chain type, also known as Bence-Jones proteinuria of undetermined significance, is defined by an abnormal FLC ratio of <0.26 or >1.65 without evidence of Ig heavy chain expression by IFE. It may evolve to light chain only myeloma.

The risk of progression from MGUS to myeloma is 1% each year. Cumulative incidence of progression is about 10% in 5 years, 15% in 10 years, and 20% in 15 years. The interval between recognitions of MGUS to the onset of myeloma is widely variable with a median of 10.4 years. The risk for progression is measured by biomarkers. In the model proposed by the Mayo Clinic group, patients with low-risk MGUS have <15 g/L M-protein, IgG type, and a normal FLC ratio. Patients with intermediate- or high-risk MGUS have a >15 g/L M-protein, IgA type, and an abnormal FLC ratio: <0.26 or > 1.65. In the model proposed by the Spanish group, a ratio of aberrant plasma cells to normal plasma cell with ≥ 95%, immune paresis and DNA aneuploidy are high-risk features.

Morphology

There is usually no anemia. Bone marrow biopsy and aspirate show mildly increased plasma cells (median, 3%) that are scattered throughout the interstitium. Large aggregates of plasma cells are not seen, and bone trabeculae are unremarkable. Lack of marked cytological atypia and an infiltrative pattern help distinguish MGUS from myeloma.

Immunophenotype

Immunoglobulin κ to λ ratio may fall within the normal range when analyzed by immunohistochemistry due to the presence of admixed polytypic plasma cells. Flow cytometry identifies both aberrant and normal plasma cells. It is helpful to document the percentage of abnormal plasma cells out of the total plasma cells, since the ratio has prognostic implication. Gating on the subset of aberrant plasma cells (CD45−/low CD19− or CD56+ CD117+ plasma cells) may help more sensitive discrimination of monotypic plasma cells from background polytypic plasma cells.

Cytogenetics and Molecular Genetics

By FISH analysis, aneuploidy is a consistent finding in MGUS. Monosomy 13 and 14q32 translocations are detectable in 21–45% and 50%, respectively, with the former being usually detected in a subset of clonal population.

Differential Diagnosis

There are no known molecular or cytogenetic markers that reliably distinguish MGUS from myeloma. Microarray analysis reveals significant overlap between gene expression patterns of MGUS and myeloma. However, *RAS* mutations are usually not observed in MGUS, and *MYC* rearrangement is rare.

The IgM type of MGUS is distinguished from the so-called IgM-related disorders by the absence of symptoms attributable to the M protein. In IgM-related disorders, the patients may present with minimal serum M protein, but there is no evidence of lymphoma. The patients, however, suffer from more debilitating complications such as autoimmune hemolytic anemia, cryoglobulinemia, and peripheral neuropathy due to IgM binding to the myelin-associated glycoprotein and cryoglobulinemia.

Primary Amyloidosis

Amyloidosis is caused by accumulation of insoluble proteins with a β-pleated sheet configuration. Primary amyloidosis, amyloid light chain (AL) type, is associated with plasma cell neo-

plasm, and the amyloid contains a part of the variable region of the light chain, most often λ (75%) and sometimes κ (25%). The diagnosis of amyloidosis AL type requires direct evidence of amyloid-induced organ damage as a result of a monoclonal plasma cell proliferation [13]. All of the following criteria need to be fulfilled: the presence of amyloid-related systemic syndrome (such as renal, liver, heart, gastrointestinal tract, or peripheral nerve involvement); positive amyloid staining by Congo red or EM in any tissue, confirming that the amyloid is composed of Ig chain by immunostaining or mass spectrometry, and detection of monoclonal plasma cell proliferation such as serum or urine M protein, abnormal free light chain FLC ratio, or monotypic plasma cells in the bone marrow.

Clinical Features

The incidence of AL is eight per million person-years with a median age of 63 years at onset. There is a male predominance. The symptoms are vague and nonspecific in the early stage. The most common complaints are fatigue and malaise. Amyloid infiltrates cause organ damage and organ dysfunction. These include nephrotic syndrome or renal failure, restrictive cardiomyopathy or arrhythmia, gastrointestinal symptoms, peripheral neuropathy, and coagulopathy due to binding of the amyloid fibers with factor X. Congestive heart failure and fatal arrhythmia are the major causes of death.

The median survival of patients with AL is 1–2 years. The patients usually succumb to progressive organ failure. The prognosis largely depends on the dominant organ involvement. Cardiac involvement predicts a poor prognosis.

In one-third of patients with the AL, no distinct spike is visible by SPEP. IFE is more sensitive. Serum FLC assays can determine the type of the M protein and confirm the diagnosis. They are also useful for monitoring response to therapy.

Morphology

Grossly, the involved organs appear to be waxy and stiff. On hematoxylin and eosin sections, the amyloid fibers have an amorphous, eosinophilic appearance often associated with cracking artifact and multinucleated giant cell reactions. The amyloid fibers appear apple-green birefringence when stained with Congo red and viewed under polarized light. By electronic microscopy, amyloid fibers appear as a mass of nonbranching linear fibrils 7–10 nm in diameter and of variable length. The deposit can be seen in vasculature and periosteum or medullary cavity (Fig. 13.12). Abdominal subcutaneous fat pad aspiration is often obtained to identify amyloid in addition to bone marrow biopsy. The numbers of neoplastic plasma cells in AL are usually in the range of MGUS in the bone marrow, and the cytological atypia is usually mild.

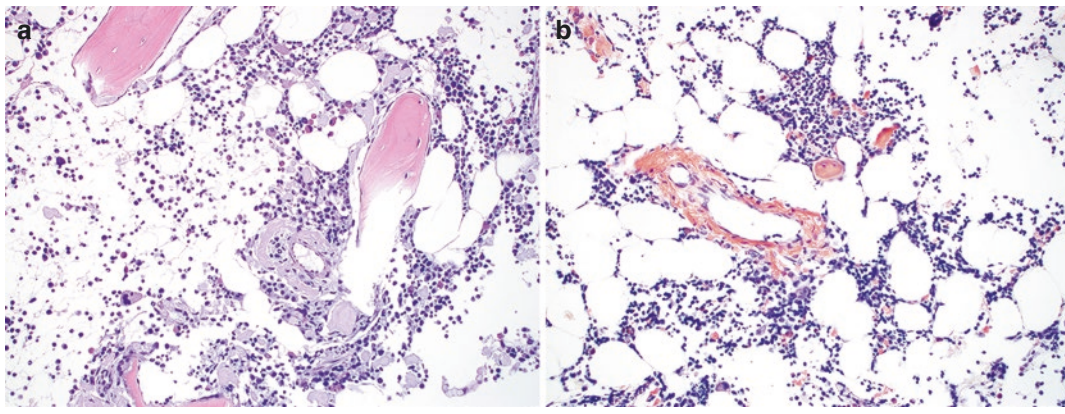

Fig. 13.12 Amyloid deposits in the bone marrow vascular wall. (*Left*) H&E (200×) and (*right*) Congo red stain (200×)

Immunophenotype

The clonal bone marrow plasma cells can be revealed by immunohistochemistry or flow cytometry using a panel of antibodies including CD138, in conjunction with immunoglobulin light chains. Flow cytometry aberrations in the clonal plasma cells of AL amyloidosis are similar to those observed in multiple myeloma, with frequent aberrant expression of CD56, CD117, CD28, and CD27 and frequent negativity for CD45 and CD19. Lambda light chain is more frequently expressed than kappa light chain. In some cases, the neoplastic plasma cell clone is small and may be obscured in a background of polyclonal reactive plasma cells [14]. Systemic AL amyloidosis patients with polyclonal BM plasma cells by light chain immunohistochemistry appear to have better prognosis than patients with a detectable BM plasma cell clone; however, unlike in patients with plasma cell myeloma, the quantity of BM clonal plasma cells does not appear to impact the prognosis [15]. The AL amyloid can be detected using antibody specific for AL or amyloid P component.

Cytogenetics and Molecular Genetics

The genetic abnormalities in the plasma cells of systemic AL amyloidosis patients are similar to those of MGUS and include del(13q) and gain at 1q21 [16]. However, t(11;14) *CCND1-IGH* is more common in amyloidosis compared to MGUS, being present in almost 50–60% of cases [17].

Differential Diagnosis

Amyloidosis AL type should be distinguished from other types of amyloidosis, mainly AA (amyloid A), AF (amyloid familial), and Aβ2M. Secondary amyloidosis (AA) is associated with chronic inflammation. AF is found in familial amyloidosis such as ATTRwt (senile systemic amyloidosis) and ATTR mutated (transthyretin V122I variant), whereas Aβ2m is related to hemodialysis. Molecular studies with polymerase chain reaction amplification and DNA sequence analysis will detect patients with mutant transthyretin or mutated fibrinogen A α-chain. Of note, given the common occurrence of MGUS in elderly patients, the presence of amyloid in the setting of a serum paraprotein does not necessarily indicate that the amyloid is of AL origin. Mass spectrometry allows specific typing of amyloid fibers and is considered the gold standard to confirm AL amyloidosis [18].

Localized amyloid deposition can occur as part of lymphoma or plasmacytoma, sometimes with mass formation, the so-called amyloidoma. They are not associated with systemic disease as AL.

The monoclonal immunoglobulin in AL can be IgM or non-IgM as well as light chain only type similar to those seen in MGUS. As such, the organ dysfunction caused by amyloid deposition should not be taken as evidence of end-organ damage meeting the criteria for active multiple myeloma. The distinction between AL and light or heavy chain deposition diseases is discussed below.

Monoclonal Light and Heavy Chain Deposition Diseases

Monoclonal LCDD and HCDD are caused by deposition of abnormal heavy or light chains secreted by neoplastic plasma cells. In HCDD, the CH1 and VH region abnormalities of heavy chain lead to their early secretion from the plasma cells prior to conjugation with light chain. In LCDD, HCDD, or combined LCDD and HCDD, the abnormal proteins do not have a sheet structure or contain P component but are deposited in the tissue, as in amyloidosis, causing organ damage and dysfunctions. LCDD and HCDD are frequently underdiagnosed. Adult patients with unexplained nephrotic syndrome should be worked up for possible amyloidosis or LCDD.

Free light chains filtered through the glomeruli tend to deposit in the kidney when the amount exceeds the degradation capacity of the renal tubular epithelia. Free heavy chains usually do not circulate in blood [19]. For LCDD, serum M protein is also minimal. However, even without an abnormal finding with serum and/or urine electrophoresis with immunofixation, an abnormal serum free light chain ratio is found in about 25% patients.

Clinical Features

Combined LCDD and HCDD and isolated HCDD are rare. The patients with LCDD are usually younger (average age of 55–60 years old) and predominantly female compared with patients with AL. The patients usually present with acute renal failure with profound proteinuria or hematuria. Less commonly, the patients present with cardiomyopathy or hepatic or pulmonary insufficiency.

The overall prognosis is poor with an overall survival of 1–2 years, regardless of plasma cell tumor load.

Morphology

Similar to amyloidosis AL type, most cases (50–60%) of LCDD have minimal plasmacytosis in the bone marrow. Immunohistochemistry is usually required to demonstrate monoclonality of the plasma cells. The bone marrow is involved with overt myeloma only in 10% of patients.

Monoclonal immunoglobulin deposits in the tissue as amorphous eosinophilic material resembling amyloid but does not stain for Congo red. The diagnosis of LCDD is usually established by renal biopsy with appropriate ancillary studies. In the kidney, LCDD is characterized by nodular glomerulosclerosis, resembling diabetic nephropathy. Deposition in the renal vasculature results in proliferative vasculopathy. The renal tubules show interstitial disease. By immunofluorescence, abnormal immunoglobulin appears along the renal glomerular and tubular basement membrane as linear ribbonlike deposition. By electron microscopy, the abnormal immunoglobulin typically appears as discrete, electron-dense, punctate deposits. These deposits are usually demonstrated in the kidney biopsy specimen. Similar changes can also be observed in the dermal-epidermal junction of the skin biopsy specimens.

Immunophenotype

Unlike AL amyloidosis, the tissue deposits in LCDD are almost always composed of kappa light chains; they are granular, not fibrillar, and do not bind Congo red. Immunofluorescence microscopy is typically strongly positive for monoclonal light chain. In HCDD, immunofluorescence staining with anti-heavy chain antibodies will highlight the deposition. Gamma heavy chain deposition is the most common.

Similar to AL amyloidosis, flow cytometry immunophenotyping can help to identify abnormal plasma cells in the bone marrow, which often fulfill diagnostic features of plasma cell myeloma [20]. Rarely, LCDD and HCDD may be associated with B-cell lymphomas, particularly lymphoplasmacytic lymphoma, chronic lymphocytic leukemia, or marginal zone lymphoma [21].

Differential Diagnosis

The main differential diagnosis is amyloidosis. The abnormal immunoglobulin in LCDD or HCDD is Congo red negative.

References

1. Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol. 2014;15(12):e538–48.
2. Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, et al. Revised international staging system for multiple myeloma: a report from International Myeloma Working Group. J Clin Oncol. 2015;33(26):2863–9.
3. Kyle RA, San-Miguel JF, Mateos MV, Rajkumar SV. Monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. Hematol Oncol Clin North Am. 2014;28(5):775–90.
4. Rajkumar SV, Landgren O, Mateos MV. Smoldering multiple myeloma. Blood. 2015;125(20):3069–75.
5. Neben K, Jauch A, Hielscher T, Hillengass J, Lehners N, Seckinger A, et al. Progression in smoldering myeloma is independently determined by the chromosomal abnormalities del(17p), t(4;14), gain 1q, hyperdiploidy, and tumor load. J Clin Oncol. 2013;31(34):4325–32.
6. Bartl R, Frisch B. Clinical significance of bone marrow biopsy and plasma cell morphology in MM and MGUS. Pathol Biol. 1999;47(2):158–68.
7. Paiva B, Merino J, San Miguel JF. Utility of flow cytometry studies in the management of patients with multiple myeloma. Curr Opin Oncol. 2016;28(6):511–7.
8. Flores-Montero J, de Tute R, Paiva B, Perez JJ, Bottcher S, Wind H, et al. Immunophenotype of normal vs. myeloma plasma cells: toward antibody panel specifications for MRD detection in multiple myeloma. Cytometry B Clin Cytom. 2016;90(1):61–72.

9. Morgan GJ, Walker BA, Davies FE. The genetic architecture of multiple myeloma. Nat Rev Cancer. 2012;12(5):335–48.
10. Walker BA, Boyle EM, Wardell CP, Murison A, Begum DB, Dahir NM, et al. Mutational spectrum, copy number changes, and outcome: results of a sequencing study of patients with newly diagnosed myeloma. J Clin Oncol. 2015;33(33):3911–20.
11. Keats JJ, Chesi M, Egan JB, Garbitt VM, Palmer SE, Braggio E, et al. Clonal competition with alternating dominance in multiple myeloma. Blood. 2012;120(5):1067–76.
12. Rosado FG, Oliveira JL, Sohani AR, Schroyens W, Sykes DB, Kenderian SS, et al. Bone marrow findings of the newly described TEMPI syndrome: when erythrocytosis and plasma cell dyscrasia coexist. Modern Pathol. 2015;28(3):367–72.
13. Gertz MA. Immunoglobulin light chain amyloidosis: 2016 update on diagnosis, prognosis, and treatment. Am J Hematol. 2016;91(9):947–56.
14. Wolf BC, Kumar A, Vera JC, Neiman RS. Bone marrow morphology and immunology in systemic amyloidosis. Am J Clin Pathol. 1986;86(1):84–8.
15. Hasserjian RP, Goodman HJ, Lachmann HJ, Muzikansky A, Hawkins PN. Bone marrow findings correlate with clinical outcome in systemic AL amyloidosis patients. Histopathology. 2007;50(5):567–73.
16. Bochtler T, Hegenbart U, Cremer FW, Heiss C, Benner A, Hose D, et al. Evaluation of the cytogenetic aberration pattern in amyloid light chain amyloidosis as compared with monoclonal gammopathy of undetermined significance reveals common pathways of karyotypic instability. Blood. 2008;111(9):4700–5.
17. Bryce AH, Ketterling RP, Gertz MA, Lacy M, Knudson RA, Zeldenrust S, et al. Translocation t(11;14) and survival of patients with light chain (AL) amyloidosis. Haematologica. 2009;94(3):380–6.
18. Vrana JA, Gamez JD, Madden BJ, Theis JD, Bergen HR 3rd, Dogan A. Classification of amyloidosis by laser microdissection and mass spectrometry-based proteomic analysis in clinical biopsy specimens. Blood. 2009;114(24):4957–9.
19. Sethi S, Fervenza FC, Rajkumar SV. Spectrum of manifestations of monoclonal gammopathy-associated renal lesions. Curr Opin Nephrol Hypertens. 2016;25(2):127–37.
20. Pozzi C, D'Amico M, Fogazzi GB, Curioni S, Ferrario F, Pasquali S, et al. Light chain deposition disease with renal involvement: clinical characteristics and prognostic factors. Am J Kidney Dis. 2003;42(6):1154–63.
21. Went P, Ascani S, Strom E, Brorson SH, Musso M, Zinzani PL, et al. Nodal marginal-zone lymphoma associated with monoclonal light-chain and heavy-chain deposition disease. Lancet Oncol. 2004;5(6):381–3.

Index

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