Histopathology in the Diagnosis and Classification of Acute Myeloid Leukemia, Myelodysplastic Syndromes, and Myelodysplastic/Myeloproliferative Diseases

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Abstract

In spite of the impressive advances in the area of molecular pathology, bone marrow morphology remains the diagnosis cornerstone to identify the various subtypes of myeloid neoplasms. Morphological examination of the bone marrow requires both bone marrow aspirate and bone marrow trephine biopsy. Immunohistochemistry of bone marrow biopsy with markers reactive in paraffin-embedded tissues represents a powerful diagnostic tool; its results can be easily correlated with those obtained by other techniques such as flow cytometry and genetic analysis, and above all, the clinical findings. The role of the bone marrow biopsy will be particularly stressed in this review article. Particular emphasis is being given to the correct identification of cases of myeloid neoplasms associated with myelofibrosis and for which the bone marrow biopsy represents the only available diagnostic mean. Moreover, the often low cellular yield of the bone marrow aspirate in these cases may also be insufficient to obtain adequate cytogenetic information. Such cases include two subtypes of acute myeloid leukemia which typically cause diagnostic difficulties: acute megakaryoblastic leukemia and acute panmyelosis with myelofibrosis (acute myelosclerosis). Acute myeloid leukemia with multilineage

dysplasia, therapy-related myelodysplastic syndrome/therapy-related acute myeloid leukemia and de novo myelodysplastic syndromes (MDS) will also be discussed. The value of bone marrow biopsy in this group of disorders is generally well established. In MDS, in particular, bone marrow biopsy may help in confirming a suspected diagnosis by excluding reactive conditions in which dyshematopoietic changes may also be observed. It can increase the diagnostic accuracy and helps in refining the IPPS risk evaluation system. Among the alterations detected by bone marrow biopsy, a prognostically important finding is the presence of aggregates or clusters of immature myeloid precursor cells (myeloblasts and promyelocytes). These can also be identified by immunohistochemistry with CD34, an antigen expressed in progenitor and early precursor marrow cells, which can be used to demonstrate pathological accumulations of blasts in aggressive subtypes of myeloid neoplasms. Immunohistologic analysis is especially helpful in cases of MDS with fibrosis and cases with hypocellular marrows (hypoplastic MDS). In both of these variants, the presence of reticulin fibrosis or fatty changes in the bone marrow can make accurate disease characterization very difficult or impossible using bone marrow aspirates. Finally, the important group of the myelodysplastic/myeloproliferative disorders can only be accurately categorized by a careful multiparametric approach in which the bone marrow biopsy exerts a pivotal role.

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Table 1. Bone marrow aspirate and biopsy

<i>Bone marrow aspirate</i>
Blast cell count
M:E ratio and detailed myelogram
Cytochemistry
Dysplasia
Flow cytometry
Cytogenetics/molecular genetic techniques
<i>Bone marrow biopsy</i>
Cellularity, lineage prevalence, architectural disturbances
Fibrosis and stromal changes
Immunohistochemistry
Dysmegakaryopoiesis
True blast cell count
Microdissection-based approaches

Table 2. AML classification according to WHO 2001

AML with specific cytogenetic/genetic abnormalities [t(8;21), t(15;17), inv or t(16), 11q23]
AML-NOS [7 FAB types (M0–M7) plus basophilic and APMF]
AML with multilineage dysplasia ($\pm$ prior MDS)
t-AML/MDS (alkylating, topoisomerase)

In the universally adopted WHO 2001 classification system [1], disease entities are defined by a combination of morphology, immunophenotype, genetics, and clinical features. The relative importance of each of these features varies among diseases – no gold standard ‘one size fits all’ is established. Neoplasms are stratified primarily according to their lineage (e.g. myeloid, lymphoid, mast cell, histiocytic). The diagnosis follows a stepwise approach: first, in establishing a hematopoietic neoplasm versus other non-hematopoietic neoplastic diseases and reactive conditions; second, in identifying the main type of hematopoietic neoplasm, e.g. acute myeloid leukemia (AML) versus acute lymphoblastic leukemia (ALL), AML versus myelodysplastic syndromes (MDSs), and chronic myeloproliferative disorders (CMPDs) versus myelodysplastic/myeloproliferative (MDS/MPD) diseases; third, in identifying the specific WHO subtypes of a given hematopoietic neoplasm, e.g. AML, monoblastic versus megakaryoblastic, MDS, refractory anemia (RA) versus refractory anemia with excess of blasts (RAEB), and MDS/MPD, chronic myelomonocytic leukemia (CMML) versus atypical chronic myeloid leukemia (aCML).

Table 3. Immunohistological reagents useful in separating AML from ALL in paraffin sections

AML ¹
CD34, CD103, MPO, HEM, CD61, CD42b, CD68R (PG-M1), CD163, lysozyme, CD56
ALL
CD34, TdT, CD10, CD79a, PAX5, CD20, CD3, CD7, CD2, CD5, CD7, CD4, CD8, CD1a

AML = Acute myeloid leukemia; ALL= acute lymphoblastic leukemia; HEM = hemoglobin; MPO = myeloperoxidase.

¹ AML with specific cytogenetic abnormalities PAX5, CD79a [t(8;21)]; NPM cyto+ (normal karyotype).

Table 4. Paraffin immunophenotype: limited correlation with FAB subtypes

M0	CD34+; MPO+/-; CD68 (KP1)+/-; CD68 (PG-M1)-
M1/M2	CD34+/-; MPO+; CD68 (KP1)+; CD68 (PG-M1)-
M3	MPX++; HLA-DR-; CD34-; CD68 (PG-M1)-
M4	MPX+; CD68 (PG-M1)+/-; CD34-
M5	CD68 (PG-M1)+; MPO+/-; CD34+/-
M6	HB, Glyco A+ cells >50%; MPO+/-; CD34+/-
M7	CD61 >50% blasts; MPO-; CD34+/-

The separation of the various entities belonging to the group of myeloid neoplasms is best achieved by a multi-parametric approach. However, the bone marrow morphology retains its importance in reaching a correct diagnosis. Additionally, it might be the only approach available in many countries and the one upon which the practicing hematologist has to rely. Morphology supplemented by immunohistochemistry with markers reactive in routinely processed tissues represents a powerful diagnostic tool on its own. Its results can be usually easily correlated to those obtained with other non-morphologic techniques such as flow cytometry and genetic analysis, and above all the clinical findings.

Morphological examination of the bone marrow requires both a properly prepared bone marrow aspirate smear and bone marrow trephine biopsy. The latter should be of adequate length and carefully processed. The two approaches effectively complement each other, as can be seen in table 1. The role of the bone marrow biopsy will be particularly stressed in this presentation.

Table 5. AML with fibrosis

Acute megakaryoblastic
Acute panmyelosis with myelofibrosis
Other AML-NOS (rare)
Acute leukemia with multilineage dysplasia de novo or secondary to MDS, MDS/MPD or CMPD
Therapy-related (alkylating agents)

Table 6. Acute megakaryoblastic leukemia (AML-M7)

5% ANLL, all ages
Associations: Down's syndrome, MNSGCTs ¹
Secondary to CMPD or MDS
Hepatosplenomegaly, lytic lesions
PB: Pancytopenia, blasts, no dacrocytosis
BM: Blasts ≥20%; ≥50% of blasts megakaryocytic lineage, fibrosis (+3)
Cytogenetics: variable, inv 3, t(1;22), i(12p)
GATA1 somatic mutations (expression of GATA1s)

¹ Mediastinal non-seminomatous germ cell tumors [Orazi et al., Cancer 1994].

Acute Myeloid Leukemia

The diagnosis and classification of acute leukemia currently requires a combination of morphology, cytochemistry, immunophenotyping and cytogenetics (or molecular genetics) for complete diagnosis of many of the disease types. The main AML subtypes including the three subtypes identified on the basis of a specific cytogenetic abnormality are listed in table 2.

Although the gold standard in diagnosing AML is a combination of bone marrow aspirate smear morphology, cytochemistry, flow cytometry, and cytogenetics, the bone marrow biopsy yields useful information which can be crucially important in several diagnostic settings and is therefore a mandatory step. What is the role of the bone marrow biopsy in the initial disease assessment? First, it is impossible to be sure 'upfront' of the adequacy of the bone marrow aspirate. In fibrotic or fatty marrows only the biopsy can provide for a correct assessment of the blast count. Additionally, given the importance of follow-up (e.g. post-chemotherapy and/or bone marrow transplant) bone marrows, it is necessary to create a baseline 'picture' against which to compare them. This can greatly help in excluding the presence of a residual minimal leukemic disease.

Table 7. Antibodies which are known to recognize abnormal MK forms and MK blasts

	MKs/atypical MKs	MK blasts
vWF	good	inadequate
CD42b	good	good
CD61 (processing-dependent)	good	good
CD31 (nonspecific)	good	good

Table 8. Acute panmyelosis with myelofibrosis (acute onset, no splenomegaly, rapidly fatal)

PB	Pancytopenia, no teardrops, non-leukemic
BM	Proliferative disorder of all three cell lines: Small-size MKs with disperse chromatin, non- or hypolobulated nuclei Blasts variable increased (10–25%) No or rare MK blasts Degree of fibrosis is variable, but usually 3+

In all cases, the diagnostic value of the bone marrow biopsy can be greatly enhanced by adding immunohistochemistry. The latter technique employs antibodies similar or comparable to those traditionally used by flow cytometry and immunofluorescence. Some of the antibodies reactive with paraffin-embedded biopsic tissue which can be useful in distinguishing AML from ALL are found listed in table 3. In the large group of the AML, the AML not otherwise specified (AML-NOS includes the 6 FAB classification-derived subtypes (M0-M6) plus acute megakaryoblastic leukemia, acute basophilic leukemia, and acute panmyelosis with myelofibrosis) [2] (table 2), immunohistochemistry may be helpful in the distinction between the various AML subtypes (table 4).

Cases of AML associated with marrow fibrosis represent a diagnostic challenge (fig. 1). The fibrotic variants of AML are summarized in table 5. Among the fibrotic AML, two subtypes which typically cause the greatest diagnostic difficulties are acute megakaryoblastic leukemia (AMKL) and acute panmyelosis with myelofibrosis (APMF; acute myelosclerosis).

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Fig. 1. AML-NOS with fibrosis. Dry tap aspirate. The myeloid nature of the blasts in this case can only be confirmed by immunostaining.

Fig. 2. AMKL. **a** AMKL, poorly differentiated example. Note the large cell lymphoma-like morphology of the blasts. **b** CD42 confirms their megakaryoblastic nature.

Fig. 3. APMF. **a** Panmyelotic proliferation with many atypical megakaryocytes. **b** CD34 confirms the presence of an increased number of blasts. **c** CD42 staining highlights the dysplastic megakaryocytes. Note the absence of megakaryoblasts.

Acute Megakaryoblastic Leukemia

De novo AMKL is an uncommon disease representing 1% or less of AMLs [1]. The pediatric forms include cases associated with Down's syndrome, often following resolution of a transient MPD, occurring at around 2 years of age, and cases associated with t(1;22)(p13;q13), which usually occurs as a sole abnormality in the first 6 months of life [3, 4]. In adult males, an association between AMKL and mediastinal non-seminomatous germ cell tumors has been reported [5]. The main features of AMKL are summarized in table 6. AMKL is characterized by a relatively uniform blast cell proliferation, often 'lymphoid'-looking cells with varying amounts of basophilic cytoplasm, and without Auer rods (fig. 2a). Cytoplasmic blebs may be present, but are not specific enough for use as an absolute diagnostic criterion. The blast cells are myeloperoxidase and Sudan black B negative, but may be positive for the non-specific esterase such as α -naphthyl acetate esterase (the blasts are sodium-fluoride sensitive though). By flow cytometry, the blasts may express myeloid-associated antigens, but should also express at least two megakaryocyte-associated antigens, such as CD41, CD42, and/or CD61. In AMKL, however, bone marrow aspiration is frequently unsuccessful due to marrow fibrosis. In these cases, cytochemistry and flow cytometry can be performed using peripheral blood samples, since circulating blasts are present in the majority of the cases. In most cases, however, bone marrow biopsy supplemented by immunohistology (table 7) may be required for appropriate characterization [6]. CD42 and CD61 antibodies are used to confirm the megakaryoblastic nature of the cells in tissue sections. Von Willebrand factor (vWF; factor VIII) can also be used but is less frequently positive. The prognosis for AMKL is poor.

Acute Panmyelosis with Myelofibrosis

APMF is a rare subtype of AMLs and corresponds also to <1% of cases [1]. The WHO criteria for the diagnosis of APMF include: panmyelosis, significant marrow fibrosis, pancytopenia, normal erythrocyte morphology, lack of splenomegaly, and a rapidly fatal course (table 8). Bone marrow aspiration is usually unsuccessful and the bone marrow biopsy supplemented with immunohistology is required for establishing this diagnosis [6, 7]. The bone marrow biopsy (fig. 3a) shows a hypercellular, diffusely fibrotic bone marrow with an increased number of multilineage immature hematopoietic elements, and

conspicuously dysplastic megakaryocytes predominately of small size showing variable degrees of atypia, including the presence of hypolobulated or non-lobulated nuclei with dispersed chromatin. Foci of blasts are found scattered throughout the marrow [6, 7]. The overall frequency of blasts in APMF marrows is uncertain. In a recent study based on bone marrow biopsy, we found a blast frequency comprised between 10 and 25% with a median value of 22.5% [6], however its precise determination is not considered a diagnostic requirement, according to the WHO system. Most of the blasts express CD34 (fig. 3b) and are negative for the megakaryocyte-associated markers such as CD42 (fig. 3c) and CD61 [6]. The course is rapidly fatal, often terminating with an overtly leukemic phase. In the terminal stage, splenomegaly may be observed, which usually results from leukemic infiltration of the red pulp. The disease usually occurs in adults, but rare cases have been described in children. The main differential diagnosis is MDS with fibrosis (MDS-f) associated with an excess of blasts and cases of AML with multilineage dysplasia with a low blast frequency, and AMKL with a 'dry tap' and no circulating blasts. Distinction, in some of these cases, may however be arbitrary and clinically irrelevant.

Acute Myeloid Leukemia with Multilineage Dysplasia and Therapy-Related Acute Myeloid Leukemia

AML with multilineage dysplasia (AML-MLD) may occur de novo, may follow a known MDS, or be therapy-related (TR-AML) [1, 8, 9]. This group of diseases shares an increased frequency of complex cytogenetic abnormalities, deletions or chromosomes 5 or 7, trisomies, or abnormalities of chromosome band 3q21, and a poor prognosis. AML-MLD and TR-AML are diseases that usually show dysplastic changes in the non-blast marrow and peripheral blood elements. The WHO classification defines AML-MLD as 20% or more bone marrow or peripheral blood blast cells with 50% or more dysplastic cells in at least two cell lines (erythroid, granulocytic and/or megakaryocytic) [9] (table 9). Dysplastic changes may be seen in both peripheral blood and bone marrow samples. Red blood cell changes include anisopoikilocytosis of peripheral blood red cells, including hypochromic teardrop-shaped cells and macrocytes, dimorphic red cell populations of the blood, nuclear-cytoplasmic asynchrony of red cell precursors, megaloblastic changes and irregularities of red cell precursor nuclei. Multinu-

Table 9. Acute leukemia with multilineage dysplasia

Neoplastic process predominately composed of one cell type (myeloblast)
Dysplasia in $\geq 50\%$ cells in ≥ 2 cell lines
Absence/rarity of megakaryoblasts
Lesser degrees of fibrosis

Table 10. AML – therapy-related (alkylating)

Off chemotherapy for at least 6 months
Dysplasia in ≥ 2 lines, one line is the megakaryopoietic
Biopsy role
Cellularity (hypo- and/or hypercellular)
Fibrosis
Dysmegakaryopoiesis
Increased number of CD34-positive cells
p53 overexpression

cleation, nuclear blebs and irregular nuclear contours are commonly seen in dysplastic erythroid precursors. Granulocyte dysplasia includes uneven cytoplasmic granulation or completely agranular mature neutrophils and nuclear changes include clumping of nuclear chromatin usually associated with monolobated or bilobate nuclei ('pseudo-Pelger-Huët' cells). Megakaryocytes may show great variation in size with detached, hyperlobated nuclei or hypo- or monolobated forms with hyperchromatic nuclei.

The therapy-related subtype of AML-MLD is typically characterized by the presence of fibrotic and fatty marrows with an uneven distribution of the marrow cellularity [10]. Although less frequent, the same stromal changes can also be seen both in the de novo cases and (particularly) in the cases of AML-MLD following an MDS. As previously mentioned, AML-MLD is generally associated with a poor prognosis and separation of de novo cases and those arising from myelodysplasia does not appear to have clinical relevance [11]. TR-AML is further subdivided in the WHO classification into alkylating agent-related and topoisomerase II inhibitor-related types. Alkylating agent-related disease (table 10) usually occurs 5–7 years after therapy, and shows morphologic and cytogenetic changes similar to AML-MLD with deletions of chromosome 5 and 7 common [12]. Topoisomerase II inhibitor-related disease generally has a shorter latency period of 2–3 years, tends to have monocytic fea-

tures and may or may not have associated multilineage dysplasia. Abnormalities of the *MLL* gene of 11q23 and *AML1* gene of 21q22 are commonly present in these cases, but a variety of other abnormalities including t(8;21), t(3;21), inv(16), t(8;16), t(15;17) or t(9;22) may also be seen [13]. TR-AML is also generally associated with a poor prognosis.

Biopsy Facilitates the Distinction between AML and MDS

The bone marrow biopsy can be used to provide correct blasts enumeration (see next section on MDS) and to avoid 'undercounts' in the aspirate due to spotty cellularity, fatty marrows, or fibrosis. Discrepancy between bone marrow aspirate and bone marrow biopsy: the highest blast count result should be used. Blasts are more often in clusters (3–5 cells) in aggressive MDS subtypes, RAEB in particular (fig. 4). Conversely, in AML the blasts usually are arranged in aggregates (>5 cells) or in confluent sheets. In hypoplastic AML (fig. 5a), the blasts are often interstitially located with an Indian file appearance. Their visibility can be enhanced by immunohistology (fig. 5b).

Immunohistology can also be helpful in drawing the line between RAEB and AML by facilitating the detection of the blasts and the assessment of their distribution. For cases rich in megaloblastoid erythroblasts, immunohistology for glycophorin or antihemoglobin may be helpful in distinguishing those cells from the myeloblasts (e.g. in cases of RAEB or AML-M6).

Myelodysplastic Syndromes

The diagnostic work-up of MDS requires morphologic evaluations of peripheral blood, marrow aspirate, and bone marrow biopsy, in the light of complete blood count results and adequate clinical information. Correlation with marrow cytogenetics is always recommended. It needs to be stressed, however, that the presence of a 'negative' karyotype does by no means exclude a diagnosis of MDS. To increase the yield of cytogenetic or genetic anomalies, FISH analysis as well as other less frequently available techniques (e.g. loss of heterozygosity for tumor suppressor genes, spectral karyotyping) can also be successful in demonstrating clonal hematopoiesis in cases of MDS.

Fig. 4. MDS-RAEB. CD34 demonstrates the presence of clusters of blasts, a characteristic finding in aggressive subtypes of MDS.

Fig. 5. a Hypoplastic AML. It is difficult to appreciate the high number of blasts located interstitially among the fat lobules. **b** CD34 facilitates their identification (note that not all AML express CD34).

Fig. 6. Chronic parvovirus infection. Note the abnormal looking appearance of erythroblasts which can be mistaken for evidence of severe dyserythropoiesis.

Fig. 7. MDS-RAEB. a ALIP (arrows) as seen in an HE-stained section of bone marrow biopsy. **b** CD34 staining confirms the presence of an increased number of blasts.

Table 11. Dyshematopoietic changes MDS-like (non-clonal)

Dyserythropoiesis: non-MDS	Dysgranulopoiesis: non-MDS	Dysmegakaryopoiesis: non-MDS
B ₁₂ /folate deficiencies	Chemotherapy/bone marrow regeneration	Infections (HIV)
Methotrexate and other chemotherapy	B ₁₂ /folate deficiency	Chemotherapy
Alcohol and other toxic compounds	CSFs treatment	Paraneoplastic myelofibrosis
Autoimmune conditions	Infections (HIV, B ₁₉)	Autoimmune myelofibrosis
Aplastic anemia/PNH	Malignancy-associated (paraneoplastic)	Status post-transplant
Transplant recipients	Congenital (Fanconi anemia)	TMPD (Down syndrome)
Infections (parvovirus, HIV)	Drug (purine analog)	
Hemophagocytic lymphohistiocytosis		
Congenital dyserythropoietic anemias		

Table 12. Pseudo-ALIPs: immunohistology

Erythroblasts (megaloblastic anemia)	HB (glycophorin A)+
Promyelocytes (after G-CSF)	MPO+/CD34-/CD117-
Monocytes	CD68 (PG-M1)+
Large-sized lymphoid cells	Lymphoid markers+

Table 13. MDS with fibrosis (≥2 reticulin fibrosis) [see 37]

10–15% p-MDS
RAEB >> other types
No organomegaly
Increased MKs and dysmegakaryopoiesis
Clusters of CD34
No or rare MK-blasts

The previously used FAB classification [2] was based entirely on findings identifiable by cytological analysis of stained smears of peripheral blood and marrow aspirate. The main criteria for subdividing MDS were the percentage of blasts in the peripheral blood and in bone marrow aspirates, and the identification of dysplastic changes in at least one of the three main marrow cell lines. The new and more comprehensive approach used by the WHO system stresses the importance of integrating other techniques such as bone marrow biopsy histology, cytogenetics, and molecular genetics, which may provide clinically relevant information. However, not all of these techniques are generally available and/or can provide useful information at the time that initial treatment decisions need to be made.

The value of bone marrow biopsy in this group of disorders is generally well established [1]. Bone marrow biopsy may help in confirming a suspected MDS by excluding reactive conditions in which dyshematopoietic changes may also be observed. Reactive conditions which may simulate MDS are listed in table 11. Among the most frequent causes of 'non-clonal' dyspoiesis encountered in clinical practice, it is worthwhile mentioning HIV-related myelodysplasia, autoimmune myelofibrosis, paraneoplastic myelodysplasia, and chronic parvovirus infection

(fig. 6). All of these conditions are easily recognizable in the bone marrow biopsy (where most of them were initially described), but are not so easy to distinguish from MDS in the bone marrow aspirate smear. However, the need for clinical correlation to minimize the chances of 'overinterpretation' of the marrow findings cannot be overemphasized.

In MDS, bone marrow biopsy supplemented by immunohistochemistry can be used to increase the diagnostic accuracy and to refine the IPPS risk evaluation system [14, 15].

Bone marrow biopsy in MDS allows an accurate assessment of: marrow cellularity, predominant cell line(s), presence of fibrosis and/or other stromal changes, dysmegakaryopoiesis (which is more easily detected in histology preparations than in smears), and, finally, architectural disorganization. In normal marrow, granulopoietic precursors are mainly found in the paratrabecular region, while erythroid and megakaryocytes are more or less confined to the central marrow cavities. In MDS, topographical organization is lost, with precursors of different cell lines found in all marrow regions. Among the alterations detected by bone marrow biopsy, a prognostically important finding is the presence of aggregates or clusters of 'abnormally localized' immature myeloid pre-

cursor cells (ALIP), i.e. myeloblasts and promyelocytes, in an abnormal central marrow cavity location (fig. 7a) [16, 17]. Cases are classified as 'ALIP-positive' if at least three aggregates (>5 myeloid precursors) or clusters (3–5 myeloid precursors) are identified in each section [17]. ALIP is mainly present in the aggressive MDS subtypes and is associated with a poor prognosis and an increased incidence of progression to acute leukemia. Presence of ALIP, however, is not unique to MDS and has been reported in reactive hematologic conditions (e.g. status post-bone marrow transplantation and post-induction chemotherapy) (table 12). In addition, the identification of the presence of ALIP may be compromised by using paraffin sections of excessive thickness or otherwise suboptimal morphology. CD34, an antigen expressed in progenitor and early precursor marrow cells, can be used as a 'surrogate marker' for the presence of ALIP (fig. 7b) [18, 19]. Both an increase in the percentage of CD34-positive cells and a tendency of positive cells to form aggregates have been shown to be reliable predictors of acute leukemic transformation and of poor survival in MDS cases, irrespective of their subtype [13, 19, 20]. This approach can be used to identify patients with MDS undergoing transition to AML, who are therefore candidates for early aggressive therapy. Most of the CD34-positive cells found in MDS morphologically resemble blasts. However, a proportion of the positive cells show promyelocyte-like cytologic features. These should be counted as blasts rather than promyelocytes for the purpose of blast cell evaluation.

Aberrant expression of CD34 by megakaryocytes in MDSs has also been reported (fig. 8) [21]. In normal conditions, the CD34+ phenotype is only found on a small subset of megakaryocyte precursors morphologically identifiable as immature blasts. This suggests that the CD34-positive megakaryocytes seen in MDS represent poorly functional neoplastic megakaryocytes showing, in addition to morphologic atypia, anomalous phenotypic differentiation. However, CD34-positive megakaryocytes can also be identified in other types of neoplastic myeloid disorders, as well as in reactive conditions (e.g. megaloblastic anemia).

Immunohistology using CD34, CD42b, or CD61 may be useful in distinguishing MDS-f [22] (blasts CD34 positive and negative with CD42b, or CD61) from AMKL, a condition associated with an increased number of megakaryoblasts (positive with CD42 or CD61, and only variably positive with CD34) [6]. CD117 (c-Kit) has been recently proposed as an additional marker to identify myeloid precursor cells in marrow biopsies of patients

with MDS. However, in our experience, this antibody is a less reliable marker than CD34, due to its weak and variable expression in MDS myeloblasts. Other antibodies which can be occasionally useful include: antihemoglobin [23] or glycophorin A to identify megaloblastic erythroblasts which can occasionally be confused with myeloid precursors (fig. 9); myeloperoxidase [24], which can be useful in differentiating background maturing myeloid precursors which are strongly myeloperoxidase positive from the leukemic myeloblasts which are characteristically weakly positive or negative in MDS cases, and CD68 and CD163 which can be used to identify background monocytes and in ruling out CMML [25, 26]. However, CMML marrows often show a predominance of granulocytes with only rare monocytes, with a characteristic discrepancy with the peripheral blood findings.

Immunohistologic analysis is especially helpful in three subsets of patients with MDS that may not have suitable marrow aspirate material for analysis: MDS-f [22, 27, 28], therapy-related MDS (TR-MDS) [10, 29, 30], and MDS with hypocellular marrow [31, 32]. The presence of reticulin fibrosis and/or fatty changes in the bone marrow of these MDS patients, by causing hemodilution and poorly cellular smears, can make accurate disease characterization very difficult or impossible. The often low cellular yield of the bone marrow aspirate may also be insufficient to obtain adequate cytogenetic information, the importance of which has been discussed previously. Besides the presence of fibrosis, other common findings include the presence of areas of edema, increased number of microvessels which can be demonstrated also by immunostaining with vWF or CD34, frequent plasmacytosis, increased mast cells, macrophages with increased cellular debris and hemosiderin, lymphocytosis and lymphoid follicles. Loss of p53 function has a major role in the transformation process in hematologic malignancy. p53 alterations are also frequent in aggressive MDS, particularly in therapy-related cases, and in secondary AML [30, 33]. A proportion of these cases have abnormalities of chromosome band 17p, site of the TP53 gene. The p53 gene product can be easily demonstrated in routinely processed bone marrow specimens (fig. 10). In our experience, p53 overexpression is almost always associated with the presence of a complex karyotypes and poor prognosis [30]. Therefore, p53 analysis of paraffin sections in MPD and MDS cases can be proposed as a 'surrogate marker' for cytogenetics when the latter approach is not available.

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Fig. 8. MDS. The presence of aberrant expression of CD34 in megakaryocytes is a characteristic although non-specific finding in cases of MDS.

Fig. 9. Antihemoglobin immunostain is helpful in confirming the erythroblastic nature of clustered megaloblastic and/or dysplastic erythroblasts.

Fig. 10. Therapy-related MDS/AML. Overexpression of p53 protein is frequently seen in cases of therapy-related (alkylating) MDS or AML.

Fig. 11. Hypoplastic MDS. The main difficulty is to separate cases such as this one from cases of aplastic anemia. Immunohistology may be helpful (see fig. 5b).

It is now well established that angiogenesis is involved in the pathogenesis of MDS. By immunostaining bone marrow biopsies with CD34 or other endothelium-reactive markers, such as CD105, CD31, vWF microvessel density has also been found to be increased in various leukemic disorders [34, 35]. Results obtained in cases of MDSs suggest a correlation between increased angiogenesis, aggressive MDS subtypes, and rate of progression to acute leukemia [36].

Myelodysplastic Syndromes with Fibrosis

MDS-f is a MDS variant which is characterized by a marked increase in bone marrow reticulin fibers and presents with pancytopenia and minimal or absent organomegaly [22, 27, 28] (table 13). The marrow shows tri-lineage dysplasia with prominent dysmegakaryopoiesis. In most cases an increased number of blasts are seen. The blasts are more easily documented in the marrow biopsy than in the aspirate, the latter being frequently suboptimal due to the presence of myelofibrosis. To qualify a case as MDS-f, a silver-stained reticulin fibrosis score of ≥ 2

on the basis of the system proposed by Manoharan et al. [37] should be obtained.

MDS-f accounts for 10–15% of primary MDS cases and >50% of TR-MDS. Within the subgroup of MDS-f, the classification according to FAB criteria reveals a majority of patients with RAEB. Only rare cases of RA with fibrosis have been reported. These rare cases seem to share the poor prognosis associated with the RAEB with fibrosis subtypes.

In MDS-f, the presence of increased CD34 expression in the marrow is often observed and the marker can be used to assess the blast count. The use of antibodies reactive with megakaryocytes has shown a higher number of these cells in MDS-f cases than either normal subjects or patients affected by MDS without fibrosis [22]. The megakaryocytes can show a particularly marked degree of pleomorphism with both small dwarf forms and large abnormal cells. The differential diagnosis of MDS-f includes several myelofibrotic myeloid neoplasms outlined in table 14. APMF is distinct from MDS-f only by its abrupt onset with fever and bone pain. In APMF, the histology of the marrow shows marked fibrosis (≥ 3 according to the Manoharan grading system [37]) associated with numerous dwarf megakaryocytes, an increased number of blasts and severe trilineage dysplasia. Cases of RAEB-2 with fibrosis, except for the usually less acute clinical presentation, may be totally indistinguishable from APMF and probably represent the same disorder [6, 7]. The differential diagnosis includes also acute megakaryocytic leukemia which can morphologically overlap with APMF and AML-MLD. Chronic idiopathic myelofibrosis is usually easily distinguished by its characteristic morphologic features (e.g. intravascular hematopoiesis, giant megakaryocytes) and the presence of significant splenomegaly.

Primary MDS-f patients show an unfavorable prognosis mainly attributable to complications deriving from pancytopenia and continuous transfusions, with a life expectancy of 9.6 months, compared with 17.4 months in MDS without fibrosis [27].

Therapy-Related Myelodysplastic Syndrome

TR-MDS cases are usually clinically very aggressive diseases which should be considered as a separate type of MDS [38–40]. Two main types of TR-MDSs have been described. The first classical type typically occurs late, usually ≥ 7 years after use of alkylating agents and presents as MDS with -7/del 7q and/or -5/del5q [10, 29, 40].

Table 14. MDS-F and its separation from other myelofibrotic myeloid neoplasms

MDS
MDS with fibrosis (MDS-F)
AML
Acute panmyelosis with myelofibrosis (APMF)
Acute megakaryoblastic leukemia (AMKL)
AML with multilineage dysplasia (AML-MLD)
CMPDS
Chronic idiopathic myelofibrosis (CIMF)
Post polycythemic myeloid metaplasia (PPMM)
Chronic myeloid leukemia (CML) in acc/blast phases
Essential thrombocythemia (ET) – late stages (?)
Others (e.g. CEL, CMML, mastocytosis)

Table 15. MDS, hypoplastic

Cellularity <30% (adjusted for age if >65)
5–15% of MDS
F > M; same age as other MDS
Most are RA
Cytopenia more severe
Role for immunosuppressive therapy

By the FAB system most cases fall within the RAEB category [10]. The second form occurs relatively early, in 2–3 years after the use of agents targeted at topoisomerase II (epipodophyllotoxins, e.g. etoposide, teniposide, doxorubicin), and presents with chromosomal translocations involving bands 11q23 and 21q22, or less frequently, other translocation which are more typical of the de novo leukemias [41]. Many of these patients progress directly to AML without a previously documented dysplastic phase. TR-MDSs with 17p deletions have morphologic features similar to those described before for the de novo cases with the same cytogenetic abnormality and are similarly characterized by the presence of p53 mutations [42, 43].

The clinical features of TR-MDS are similar to those seen in aggressive primary MDS cases except for the more pronounced pancytopenia and anisopoikilocytosis which are usually associated with the former group. Occasional nucleated red blood cells are seen in the peripheral blood. The bone marrow is, on average, less cellular than in the primary cases, and significant reticulin fibrosis ($\geq 2+$) is also more common. In spite of the similar degrees of fibrosis in both primary MDS-f and TR-MDS, the latter

Table 16. Atypical CML

Atypical CML is a Ph ⁿ -negative, <i>BCR/ABL</i> -negative myeloid disorder
Its incidence is <2 cases for 100 cases of t(9;22), <i>BCR/ABL</i> -positive CML
Patients have some features of CML, e.g. splenomegaly, neutrophilia and anemia
No basophilia
Major characteristics to separate aCML from CML: dysgranulopoiesis which is often severe
Moreover, atypical CML may have an initial presentation more typical of MDS with anemia and normal to decreased PLT counts

Table 17. Chronic myelomonocytic leukemia

Peripheral blood	Bone marrow
Persistent monocytosis of >1 × 10 ⁹ /l with a percentage of monocytes >10% of WBC	Hypercellular Most cases show a 'myeloproliferative look' High M:E ratio due to myeloproliferation Blast count is usually <5% Variable fibrosis

group differs in terms of the number of megakaryoblasts and megakaryocytes which are significantly higher in the primary forms [22]. CD34 expression is almost always increased in this aggressive MDS subtype [10, 22]. In addition, p53 protein overexpression can be frequently observed, particularly in cases associated with prior alkylating agent chemotherapy (fig. 10) [30]. p53 expression was found to be associated with increased apoptosis in marrow hematopoietic cells and severe ineffective hematopoiesis [33].

Hypoplastic Myelodysplastic Syndrome

Hypoplastic MDS (h-MDS) accounts for about 15% of the MDS cases, is more frequent in women, and occurs with an age-related frequency which is similar to that seen in primary MDS [32] (table 15). Previous genotoxic exposure or therapy needs to be excluded since hypocellular marrows can be seen in cases of TR-MDS. h-MDS is generally associated with pronounced cytope-

nia, a finding which may suggest a diagnosis of acquired aplastic anemia. Bone marrow biopsy is necessary to diagnose this variant. Most investigators consider a case of MDS as hypocellular when the marrow cellularity is <30%. However, correction for age is recommended as a value of 20% may be still within normal range for patients >60 years of age [44]. Within the subgroup of MDS with hypoplastic bone marrow, classification according to FAB criteria reveals a majority of patients with RA (66.7% in one series). The presence of ≥20% myeloblasts in a hypocellular marrow rules out MDS in favor of hypocellular AML. Dysplastic features in hematopoietic cells occur less frequently and are of lower grade in comparison to normo- to hypocellular MDS. In the author's experience, most h-MDS patients with RA have only mild dyserythropoiesis. Since these patients do not have an increased proportion of blasts in the aspirate or ALIP in the bone marrow biopsy, the separation from aplastic anemia may be problematic (fig. 11). This is also compounded by the high proportion of cases showing a mesenchymal reaction, especially an increase of mast cells, and reactive lymphoid follicles, features similar to those observed in bone marrow biopsies obtained from patients with aplastic anemia. The presence of easily identifiable megakaryocytes within an architecturally disorganized marrow and the presence of reticulin fibrosis favor MDS over aplastic anemia. Immunohistochemistry can help in distinguishing h-MDS from acquired aplastic anemia, the former disorder being characterized by higher CD34 expression as compared to aplastic anemia [31, 45]. Finally, bone marrow cellularity does not appear to be an important prognostic factor in MDS, because patients with hypocellular MDS have a similar prognosis to those cases of MDS with normo/hypercellular marrows [46]. The distinction from aplastic anemia, however, is significant because the risk of progression to acute leukemia is much greater in h-MDS [47].

Myelodysplastic/Myeloproliferative Disorders

The category of MDS/MPD disorders includes malignant hematopoietic proliferations which, at the time of their initial presentation, display features of both MDSs and MPDs [48–50]. Cytopenias and dysplastic changes of any cell line may be seen, similar to the MDSs. Elevated white blood cell (WBC) counts, hypercellular marrows with fibrosis, and organomegaly, features more commonly associated with MPDs, may also be present. The pres-

ence of fibrosis alone in cases that are otherwise typical of myelodysplasia should not justify placement in this category. The three best-defined mixed myeloproliferative and myelodysplastic syndromes are: aCML, CMML, and juvenile myelomonocytic leukemia.

Atypical Chronic Myeloid Leukemia

aCML [48] is a Philadelphia chromosome-negative and *BCR/ABL*-negative proliferative disorder that affects elderly patients with an apparent male predominance. Its incidence is <2 cases for every 100 cases of t(9;22), *BCR/ABL*-positive CML [50] (table 16). Patients have some features of CML including: splenomegaly, an elevated WBC count of predominantly granulocytic cells, and moderate anemia. The major characteristic which distinguishes atypical CML is dysgranulopoiesis, which is often severe. Occasionally, atypical CML may have an initial presentation more typical of myelodysplasia with a low WBC count and normal to decreased platelet counts [49]. The WBCs are shifted to the left with immature granulocytes, including blast cells, promyelocytes, and myelocytes, representing 10–20% of peripheral blood white cells. Dysplastic neutrophils are typically seen. Granulocytes may show typical pseudo-Pelger-Huët changes, ‘mononuclear’ cell-like morphology due to nuclear condensation, and cytoplasmic hypogranularity. Monocytes are usually <10% of peripheral blood cells. In contrast to CML, basophilia is not prominent, usually accounting <2% of peripheral blood white cells.

The bone marrow is hypercellular, with an elevated myeloid-to-erythroid ratio, and marrow fibrosis may be present (fig. 12a). The bone marrow biopsy demonstrates granulocytic hyperplasia with an increased proportion of blasts (<20%). These cells can be highlighted by immunostaining with CD34. Dysmegakaryopoiesis is easily identified. A predominance of small megakaryocytic forms, which can be similar in appearance to those seen in MDS, may be observed. The myeloid-to-erythroid ratio is usually less than 10:1, and there is no evidence of the Philadelphia chromosome by either routine karyotype or molecular studies for the *BCR/ABL* fusion product. Although some abnormalities of granulocyte nuclear lobation may be seen in CML, particularly in the accelerated phase, atypical CML commonly has more typical dysplastic changes that may involve all cell lines (trilineage dysplasia). In addition to dysgranulopoiesis, dyserythropoiesis and megakaryocyte dysplasia (fig. 12b) are common, and megakaryocytes may be reduced in number with associated thrombocytopenia. Atypical CML is a more aggressive disease than CML, and pro-

gression usually occurs within 2 years [49, 51]. Patients may develop acute leukemia or may have bone marrow failure secondary to marked fibrosis.

Cytogenetic and molecular genetic studies are essential in the diagnosis of atypical CML to exclude t(9;22) or the *BCR/ABL* fusion product of usual-type CML. There is no known defining cytogenetic abnormality for atypical CML, but del(20q11) and trisomy 8 have been reported [50]. Other ancillary studies, particularly immunophenotyping studies, do not usually help unless blast cell numbers are elevated.

Chronic Myelomonocytic Leukemia

CMML has features both of a myeloproliferative and a myelodysplastic syndrome. The disease has been divided into myelodysplastic and myeloproliferative subtypes on the basis of a WBC count of $13 \times 10^9/l$ or higher for the myeloproliferative type and less than that number for the myelodysplastic type. Patients with leukocytosis of $>13 \times 10^9/l$ have a higher incidence of splenomegaly [48]. However, both subtypes show a similar degree of dysplastic changes. Since the percentage of bone marrow (or peripheral blood) blasts is the most important determinant of survival in CMML patients, the use of a WBC cutoff to separate the subtypes is probably arbitrary and of controversial prognostic value [52].

The diagnosis (table 17) requires a persistent peripheral blood monocytosis of $>1 \times 10^9/l$, with a percentage of monocytes >10% of the WBCs. The monocytes may be abnormal in appearance with bizarre nuclei and even cytoplasmic granules. Promonocytes, with more immature nuclear chromatin, may be present in the blood, but monoblasts are usually not present or represent <2% of peripheral blood cells. If blasts and promonocytes account for $\geq 20\%$ of the WBCs, the diagnosis should be AML rather than CMML. The peripheral blood may demonstrate dysplastic changes typical of the MDSs, or dysplastic changes may be minimal. Other changes in the peripheral blood are variable, however mild anemia is usually seen. Although an elevated peripheral blood monocyte count is necessary for the diagnosis of CMML, such a diagnosis should never be made without examination of the bone marrow. Some AMLs with monocytic blasts may show peripheral blood changes similar to those of CMML because of cytologic maturation of the blast cell population in the peripheral blood. The bone marrow of CMML is usually hypercellular and may demonstrate monocytic or granulocytic hyperplasia (fig. 13a). When granulocytic hyperplasia is prominent, it may be difficult to distinguish the abnormal monocyte population from myelocytes. Non-

12

13

Fig. 12. MDS/MPD. **a** Atypical CML. Note the predominant granulocytic proliferation (MPD-like) associated with an increased number of blasts. **b** CD42 may help in identified dysmegakaryopoietic forms frequently found in cases of MDS/MPD.

Fig. 13. CMML. **a** The striking myeloproliferative appearance makes it difficult to distinguish this case of CMML from CML or aCML. **b** CD68R immunostaining identifies a few monocytes but is not as sensitive as NBE (as shown in fig. 14).

Fig. 14. CMML. Naphthyl butyrate esterase facilitates the identification of monocytes and promonocytes in marrow aspirate of cases of CMML.

14

specific esterase cytochemical studies may be helpful in this setting, by highlighting the abnormal monocytes (fig. 14) [26]. Flow cytometry with CD14, CD11c, CD64, and immunohistochemistry with CD68 (KP-1), CD163, and CD68R (PG-M1) are less useful in confirming the presence of monocytic differentiation than cytochemistry (fig. 13b) [26]. Erythroid precursors and megakaryocytes may demonstrate prominent dysplastic changes, but these cell types are often normal in appearance. Ringed sideroblasts are present in increased numbers in some cases. Blast cell and promonocyte counts may be elevated up to 20%, and an elevation in blast cell numbers is associated with a poorer prognosis [53, 54]. The WHO classification scheme subdivides CMML into two subcategories, depending on the number of blasts found in the peripheral blood and bone marrow. These include: CMML-1 with blasts <5% in the blood or <10% in the bone marrow, and CMML-2 which is characterized by blasts 5–19% in the blood or 10–19% in the bone marrow, or when Auer rods are present and the blast count is <20% in blood or marrow. The finding of $\geq 20\%$ blasts in the blood or the bone marrow indicate AML rather than CMML.

Cytogenetic and molecular genetic studies, particularly the absence of the Philadelphia chromosome and *BCR/ABL*, help in excluding CML. An additional subset, CMML with eosinophilia, may be diagnosed when the criteria for CMML are present, but in addition, the eosinophil count in the peripheral blood is $>1.5 \times 10^9/l$. Patients in this category may have symptoms related to the degranulation of the eosinophils comparable to those seen in hypereosinophilic syndrome. This subset should be designated as CMML-1 or CMML-2 with eosinophilia according to the above guidelines. Mutations of *RAS* are detected in approximately one third of CMML cases. Although detection of *RAS* mutations is not generally useful for diagnosis, abnormalities involving the *RAS* pathway are thought to be an important mechanism for this and other CMPDs, including CML and cases associated with t (5;12) chromosome abnormality [55].

The differential diagnosis between atypical CML and CMML may be difficult, but is critical because of the worse prognosis of patients with atypical CML compared with CMML. CMML may be distinguished from atypical CML by peripheral blood features [26, 48], but some overlap with atypical CML may occur. Although monocyte counts may be slightly elevated in atypical CML, these cells do not usually exceed 10%, whereas monocyte counts in CMML are usually $>10\%$. The degree of granulocyte dysplasia in CMML is also not as pronounced as is usually seen in atypical CML. Atypical CML demon-

strates an increase in immature granulocytic cells, including blast cells, promyelocytes, and myelocytes, of up to 20% in the peripheral blood; these cell types are almost always $<10\%$ in the blood of patients with CMML.

Juvenile myelomonocytic leukemia, a member of the WHO MDS/MPD diseases group, is currently included within the group of pediatric MDSs [56] and is therefore not included in this review.

Other Myelodysplastic/Myeloproliferative Syndromes

Some cases demonstrate features of both myelodysplasia and myeloproliferative syndromes and do not fit well into any of the previously mentioned categories. Many of these cases have typical features of myelodysplasia as well as an atypical finding more suggestive of a MPD, such as marked marrow fibrosis, leukocytosis, or organomegaly. Such cases may be termed mixed myeloproliferative/myelodysplastic syndromes, not further classifiable, with a comment describing the atypical findings. One such syndrome has features of RA with ringed sideroblasts and thrombocythemia (acquired sideroblastic anemia associated with thrombocytosis) [57–58]. These cases have no sex predilection or specific cytogenetic abnormality and must be differentiated from the 5q- syndrome myelodysplasias. A mixed MDS/MPD disorder associated with isochromosome 17q has been described; it occurs with a male predominance in adults and is associated with severe hyposegmentation of neutrophil nuclei, monocytosis, and a high rate of transformation to AML. Poorly characterized MDS/MPD neoplasms may also occur in association with mast cell disease where they represents one of the types of clonal non-mast cell lineage hematological diseases associated with systemic mastocytosis [59].

This review article stresses the need for integration between morphology, immunophenotype, genetic features, and clinical manifestations in diagnosing myeloid neoplasms. In this article, although much emphasis has been placed on the histologic interpretation of the bone marrow biopsy, the need for a comprehensive evaluation of marrow samples cannot be overemphasized. This multiparametric approach forms the basis for the WHO classification of tumors of hematopoietic and lymphoid tissues. Only by following this principle can the hematopathologist reliably and reproducibly identify rare variants of myeloid neoplasms such as chronic myelomonocytic leukemia, atypical chronic myeloid leukemia, fibrotic and/or hypoplastic subtypes of myelodysplastic syn-

dromes and of acute myeloid leukemia. The latter two disease groups include the important subsets of patients with therapy-related or secondary (to previous MDS, MDS/MPD, or MPD) myeloid neoplasms. Since all these entities have, usually, some degree of associated myelofibrosis, bone marrow biopsy often represents the only game in town. When supplemented by immunohistology it is an essential tool for assessing the frequency of marrow blasts and their lineage derivation. Novel cytogenetics and molecular genetic approaches are likely to revolutionize the way we classify these diseases in the near future. However, it is highly unlikely that these new techniques will be capable, on their own, of adequately stratifying patients for purpose of treatment.

The bone marrow biopsy has a bright future ahead. It represents the only *in situ* approach that allows the identification of specific cell subsets within their unique spatial relationships. This is of course particularly valuable in analyzing neoplastic populations present within highly polymorphic cellular backgrounds, such as those normally seen in bone marrow. New approaches will further enhance its value. Several recent studies have demon-

strated the usefulness of microdissection techniques in the demonstration of important biologic characteristics of selected malignant cells present within a mixed bone marrow cellularity. Examples of these applications have included the detection of additional genetic events in microdissected megakaryocytes, the demonstration of clonality in low-grade myelodysplastic syndromes, and the objective confirmation of lymphomatous involvement in bone marrows containing lymphoid aggregates in patients with lymphoproliferative disorders. The possibility of analyzing, by genetic techniques, clusters of blasts microdissected from biopsies will allow a better demonstration of disease progression and of clonal evolution, in cases in which the blast count on the bone marrow aspirate is still 'falsely' low. This will open the possibility of better assessing post-transplant or post-chemotherapy marrow samples by accurately distinguishing between early regeneration, cytokine effects, and residual leukemic disorder. Even with the most sophisticated technology at one's disposal, an adequate characterization of bone marrow morphology will represent the best 'reality check' available to us for many years to come.

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