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Diagnostics of myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML)

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Abstract: Myelodysplastic syndromes (MDS) are clonal malignant diseases of the hematopoietic stem cell. They are diagnosed mainly in elderly cytopenic patients and are characterized by a heterogeneous course of the disease. The diagnosis is based on blood and marrow cytology, chromosomal examination, and histology of the marrow, taking into account the degree of dysplasia, peripheral and medullary blast count, and cytogenetic findings according to the proposals of the World Health Organization (WHO) classification. The assessment of cytologic findings is important because the different types of MDS differ in terms of prognosis and therapeutic considerations. Acute myeloid leukemias are discriminated from MDS according to the blast counts in the bone marrow or peripheral blood (≥20%). Diagnosis is based on morphology and cytogenetics, and histology is also important. In addition, immunophenotyping and molecular investigations are needed. Information derived from cytogenetics and molecular markers play an important role for prognostication. Minimal residual disease can be best investigated using immunophenotyping and measurement of molecular markers.

Keywords: acute myeloid leukemia; diagnostic work up; differential diagnosis; myelodysplastic syndromes.

Introduction

Myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML) are malignant diseases of the hematopoietic stem cell, characterized by differentiation and maturation disorders of hematopoiesis, cytopenia and clonal expansion of varying degrees. Various pathophysiological phenomena are observed in this context, such as chromosomal, molecular and epigenetic changes in the hematopoietic stem cell, as well as alterations to the bone marrow stroma.

Both groups of diseases are closely related pathophysiologically, and transitions from MDS to AML are frequent. The differentiation of MDS from AML is based, on the one hand, on cytogenetic or molecular-genetic markers that define AML and, on the other hand, on the assessment of the medullary and peripheral proportion of blasts. According to World Health Organization (WHO) recommendations, detecting ≥20% of blasts in the blood and/or bone marrow is a basic prerequisite for diagnosing AML. This number is arbitrary and cannot be taken dogmatically due to the imprecision of how the percentage of blasts is estimated. It is, instead, more of a pragmatic instruction. The detection of certain cytogenetic and molecular-cytogenetic markers defines some AML entities also independently of the blasts.

The following will explain in detail the diagnostic steps taken in connection with MDS and AML, and the WHO recommendations on the classification of MDS and AML will be explained and described in a tabular format.

MDS diagnostics

The diagnosis of an MDS is typically taken into account when trying to clarify a diagnosis of anemia and bi- or pancytopenia. Most patients have an isolated anemia, and only about 20% have pancytopenia. Initially, it is necessary to rule out, through suitable methods, a varied range of hematological and non-hematological differential diagnoses (Table 1). The most common MDS differential diagnosis is an iron level disorder in terms of “tumor – inflammatory anemia”. If the cause of the cytopenia cannot be identified, it is essential to do a thorough cytomorphological test of the blood and bone marrow to diagnose MDS. In around half of the cases of patients with MDS, the differential

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Table 1: Differential diagnosis of myelodysplastic syndromes.

Differential diagnosis	Diagnostic method
Aplastic anemia, pure-red-cell-aplasia (PRCA)	Histology, cytology, virology
Toxic BM damage (alcohol, lead, NSAR, etc.)	Medical history
Copper deficiency	Laboratory
Reactive BM changes (sepsis, HIV, chronic infections, tuberculosis, autoimmune diseases, etc.)	Cytology, medical history, laboratory
Monocytosis of other origin	Medical history, laboratory, molecular genetics
Paroxysmal nocturnal hemoglobinuria (PNH)	Immunophenotyping
Immune thrombocytopenia	Medical history, course of the disease
Megaloblastic anemias	Vitamin B_{12}/folic acid levels
Hypersplenic syndrome	History/clinic (splenomegaly)
Acute leukemias (especially erythroleukemia, FAB-M6)	Cytology, genetics and molecular genetics
Myeloproliferative neoplasms (especially aCML, PMF)	Cytology, histology, cytogenetics, molecular genetics
Hairy cell leukemia, LGL	Cytology, histology, immunophenotyping
Congenital dyserythropoietic anemia (rare)	Cytology, molecular genetics

Table 2: Dysplasia in the blood and bone marrow in connection with MDS.

– Differential blood count with 200 nucleated cells, including blast count
– Pseudo-Pelger cells, hypo/degranulated white precursors
– Giant platelets, platelet anisometry
– Anisocytosis, poikilocytosis, dimorphic erythrocytes, polychromasia, megalocytes, basophilic stippling, teardrop shapes, fragmentocytes
Dyserythropoiesis in the bone marrow
Proportion of erythropoiesis (%), megaloblastic cells, multinucleation, nuclear anomalies (nuclear bridges, nuclear fragmentation, nuclear unrounding) atypical mitosis, sideroblastosis, ringed sideroblasts, PAS positive cells of erythropoiesis, cytoplasmic vacuolization of proerythroblasts
Dysmegakaryopoiesis in the bone marrow
Micromegakaryocytes, mononuclear megakaryocytes, hypersegmented megakaryocytes, multinucleated megakaryocytes
Dysgranulopoiesis in the bone marrow
Hyperplasia of granulopoiesis, blast content normal or increased, specified in %, by definition <20%, Auer rods, Auer bodies (POX staining), hypo/degranulated white precursors, pseudo-Pelger cells, round nucleated granulocytes, hypersegmentation of granulocytes, peroxidase deficiency, monocyte content (esterase staining)

blood count shows signs of dysplasia, particularly of the granulocytes, and also, but less frequent, platelet anisometry [1]. In the bone marrow cytology, a number of dysplasia signs are mostly observed that, by definition, make up more than 10% of the nucleated cells of one, two or all three cell lines. None of the dysplasia signs is pathognomonic of MDS, because myeloproliferative neoplasms and AML, as well as other hematological and non-hematological conditions can show signs of dysplasia as well.

In addition to standard staining, iron staining and, ideally, a peroxidase reaction (POX) must be prepared in all cases. Ringed sideroblasts will become visible only with iron staining. They represent in many cases a clear criterion of dysplasia, and are required to diagnose ringed sideroblastic anemia. A classic indicator of dysplasia of granulopoiesis is a partial POX defect. PAS-positive erythroblasts are less common. The detection of a monocyte population is often difficult, which is why an esterase reaction is recommended, particularly in cases of suspected chronic myelomonocytic leukemia (CMML). The individual dysplasia criteria are listed in Table 2.

The main criteria for differentiating between various MDS types are the expressions of dysplasia signs (unilineage vs. multilineage), the medullary and peripheral proportions of blasts (<>5% and <>10%), as well as the detection of chromosomal changes, particularly the deletion of part of the long arm in chromosome 5 [2].

One distinguishes RCUD and RARS – entities for which the dysplasia signs are limited to one lineage, mostly erythropoiesis – from RCMD where two or mostly all three cell lines exhibit dysplastic characteristics. These patients have a median probability of survival of approximately three years, a significantly worse prognosis and a higher risk of leukemia than RCUD and RARS patients (median probability of survival of approx. 6 years). All of the aforementioned MDS types have medullary blasts of <5%. In the case of isolated del(5q) without blast proliferation, a diagnosis of MDS with del(5q) will be made. This entity comes with a good prognosis, but is also characterized by transitions to acute leukemia [3]. It is interesting to note that typical morphological characteristics are usually observed that lead attentive cytologists to think of a deletion of the long arm in chromosome 5. These characteristics are a) mononuclear megakaryocytes, b) a reduced, but usually only slightly dysplastic erythropoiesis, c) mild dysplasia signs of granulopoiesis, and d) increased lymphocytes in the bone marrow smear.

All cases of MDS require an analysis of the medullary blast proportion that must be as exact as possible, because a patient's prognosis depends greatly on the proportion of blasts according to the revised version of the International Prognostic Scoring System [4]. The classification of blast

categories (0–2%, 3–4%, 5–9%, 10–19%) demands precise morphology.

A diagnosis of RAEB I or II requires a relevant, malignant cell population with a blast proportion of 5–9% and/or 10–19% in the bone marrow. These patients have a high risk of developing a subsequent AML, and their median probability of survival is approximately 20 months in RAEB I and about one year in RAEB II. The current WHO classification of MDS [3] is shown in Table 3.

CMML, previously grouped with MDS, now makes up an entity within the group of mixed myelodysplastic-myeloproliferative neoplasms together with ringed sideroblastic anemia with thrombocytosis (RARS-T) [5]. CMML I and II as well as RARS-T also exhibit characteristics of myeloproliferative neoplasms that manifest themselves in an increase of monocytes in the blood (>1000/μL absolute) for CMML (I and II) and thrombocytes of >450,000/μL for RARS-T, as well as in the common detection of *JAK2* and *SF3B1* mutations. There is also a further phenotype-genotype correlation for RARS-T, because it is mostly *SF3B1* mutations that are found [6]. The classification of CMML into the types CMML I and II is analogous to the classification of RAEB I and II, that is, in accordance with the peripheral and medullary content of blasts, while promonocytes with CMML are classified as blasts.

The group of refractory anemia with excess blasts in transformation (RAEB-T), which was initiated with the first MDS classification, is now allocated to AML with multilineage dysplasia, because the new limit of medullary blasts to differentiate between MDS and AML is now at 20%.

Table 3: WHO classification of myelodysplastic syndromes and myelodysplastic/myeloproliferative neoplasms [3].

MDS subtype	Blood	Bone marrow
Refractory cytopenia with unilineage dysplasia (RCUD) RA: refractory anemia RN: refractory neutropenia RT: refractory thrombocytopenia	<1% blasts Uni- or bicytopenia	<5% blasts Dysplasia in ≥10% of the cells in one line
Refractory anemia with ringed sideroblasts (RARS)	Anemia, no blasts	<5% blasts, ≥15% ringed sideroblasts in erythropoiesis, exclusively dyserythropoiesis
Refractory cytopenia with multilineage dysplasia (RCMD) with or without ringed sideroblasts	<1% blasts Cytopenia(s) <1000/μL monocytes	<5% blasts, dysplasia signs ≥10% of cells from 2–3 cell lines
MDS with isolated del(5q)	≤1% blasts Anemia, platelets often elevated	Mostly typical mononuclear megakaryocytes, <5% blasts, isolated del(5q) abnormality
Refractory anemia with excess blasts I (RAEB I)	Cytopenia(s) <5% blasts, <1000/μL monocytes	Uni- or multilineage dysplasia, 5–9% blasts, no Auer rods
Refractory anemia with excess blasts II (RAEB II)	Cytopenia(s) <20% blasts, <1000/μL monocytes	Uni- or multilineage dysplasia, 10–19% blasts, Auer rods possible
Unclassified MDS (MDS-U) a) RCUD with pancytopenia b) RCMD/RCUD with 1% blasts in the blood c) MDS-typical chromosomal aberration with no clear signs of dysplasia	≤1% blasts, <1000/μL monocytes	<5% blasts
Chronic myelomonocytic leukemia I (CMML I)	<5% blasts Uni- or bicytopenia >1000/μL monocytes/μL no Auer rods	<10% blasts, dysplasia in >10% of cells in 1–3 lines, no Auer rods
Chronic myelomonocytic leukemia II (CMML II)	<20% blasts Uni- or bicytopenia >1000/μL monocytes/μL Auer rods possible	<20% blasts, dysplasia in >10% of cells in 1–3 lines, Auer rods possible
Refractory anemia with ringed sideroblasts and thrombocytosis (RARS-T)	Cytopenia(s), platelets >450,000/μL <1% blasts	<5% blasts, >15% ringed sideroblasts in erythropoiesis, dysplasia in >10% of cells in 1–3 lines, no Auer bodies, often *JAK2* and *SF3B1* mutations

It may also be useful diagnostically to do a histomorphological assessment of the bone marrow, which allows for a far better estimation of cellularity [7], which in turn may be especially relevant in differentiating from an aplastic syndrome, but may also detect bone marrow fibrosis [8], which can have a negative impact on the course of the disease. Ringed sideroblasts can be represented only in the smear, but not in a section slide. This is why the pathologist should be given some bone marrow smears as well.

A chromosome analysis of bone marrow cells is absolute essential, both for classification purposes and for a prognosis estimation [9]. At the time of diagnosis, around 60% of all MDS patients exhibit cytogenetic aberrations. Clonality testing may be diagnostically indicative in cases of mild dysplasia. The paramount significance of chromosomal findings in connection with MDS was most recently reflected in the revised version of the International Prognostic Scoring System (IPSS-R) [4], which is greatly dominated by cytogenetic findings. The key preparatory work with respect to cytogenetics was done under the leadership of the German-Austrian MDS Group [9].

Table 4 shows the frequency of different chromosomal findings and their allocation to risk groups. Ideally, 20–25 metaphases would be analyzed using banding cytogenetics so as to detect also smaller clones [10]. In a few cases of dry tap aspiration, the cytogenetic analysis may also be performed on cells of peripheral blood, although the analytical quality will be somewhat reduced. Fluorescence in situ hybridization (FISH) in the blood, however, is particularly successful with CD34+ selected cells. This method can also be used for monitoring treatment [11]. Immunophenotyping of bone marrow cells taken from MDS patients can generally trace and identify dysplasia as well as provide an estimation of the medullary blast percentage. But this requires an extended marker panel (ELN) in the hands of experienced personnel. This is why this method is not part of the standard repertoire of MDS diagnostics.

Testing for genetic mutations has already become part of clinical routine with respect to the pathogenesis and treatment decisions for AML. Recent years have also seen more and more molecular aberrations in MDS patients as well. Molecular analysis can already be used for diagnostic purposes where there are no cytogenetic aberrations or sufficient cytological criteria.

Several studies [12–14] have shown that molecular aberrations can be relevant to the prognosis, particularly mutations of the genes *ASXL1, TP53, RUNX1* and EZH2. Molecular markers will soon be integrated with prognostic scoring systems, and likely become therapy-relevant and predictive in nature. More recent analytical methods, such as proteomics, are of great scientific interest, but not yet part of routine diagnostics [15–17].

Table 4: Chromosomal findings and their allocation to risk groups and typical MDS-associated cytogenetic abnormalities.

Cytogenetic risk groups	
Very good:	del(11q), –Y
Good:	Normal, del(20q), del(5q), single and double, del(12p)
Intermediate:	+8, del(7q), i(17q), +19, +21, any other single or double anomaly, independent clones
Poor:	–7, inv(3)/t(3q)/del(3q), double anomaly including –7/del(7q), complex with 3 abnormalities
Very poor:	Complex >3 abnormalities
Typical MDS-associated cytogenetic abnormalities (9)	
Anomaly	Frequency
–5 or del(5q)	10–15%
–7 or del(7q)	10%
i(17q) or t(17p)	2–3%
del(12p) or t(12p)	1–2%
del(11q)	1–2%
–13 or del(13q)	1–2%
del(9q)	1%
idic(X)(q13)	1%
inv(3)(q21q26.2)	1%
t(6;9)(p23;q34)	1%
t(3;21)(q26.2;q22.1)	<1%
t(1;3)(p36.3;q21.2)	<1%
t(11;16)(q23;p13.3)	<1%
t(2;11)(p21;q23)	<1%

Table 5 summarizes standard MDS diagnostics [18, 19].

The MDS Registry, based in Düsseldorf, captures diagnostic, clinical, prognostic and therapeutic data collected by many participating centers in order to promote joint scientific projects. A central biobank has also been established, which is part of the joint MDS-Cancer Aid project. It also offers bone marrow reference cytology. An interactive, web-based learning program with lectures and demonstrations of diagnostic steps and virtual microscopy is available at www.mdsdiagnosis.com free of charge.

Diagnosis of acute myeloid leukemia

Both the diagnosis and classification of AML are subject to the current 2008 WHO classification, like MDS (Table 6) [20].

Table 5: Diagnostic of myelodysplastic syndromes.

Peripheral blood	Bone marrow
Blood count	Cytology with iron staining, POX and esterase
Differential blood count	Cytogenetics, possibly with FISH
Reticulocytes	Histology
LDH	Immunophenotyping if necessary
Ferritin	
Erythropoietin	
Folic acid	
Vitamin B12	
HLA typing if necessary	
Molecular markers	
JAK2, *BCR-ABL1*, *PDGFR-a/b* if necessary	
ASXL1, *TET2*, *RUNX1*, *TP53*, etc. if necessary	

Table 6: Current classification of AML according to WHO, 2008 [20].

AML with recurrent cytogenetic aberrations
– with t(8;21)(q22;q22); *RUNX1-RUNX1T1*
– acute promyelocytic leukemia (AML M3 with t(15;17)(q22;q12); *PML-RARA)*
– with abnormal eosinophils and inv(16)(p13q22) or t(16;16)(p13;q22); *CBFB-MYH11*
– with 11q23 (MLL) anomalies
Other rare ones
AML with myelodysplasia-associated changes
– with prior myelodysplastic syndrome (MDS)
– with MDS-associated cytogenetic aberrations
– with multilineage dysplasia
Therapy-induced AML and MDS
– by alkylating agents
– by epipodophyllotoxin
– by radiotherapy
– by other triggers
AML without other classification option
– AML minimally differentiated
– AML without maturation
– AML with maturation
– Acute myelo-monocytic leukemia
– Acute monocytic leukemia
– Acute erythroleukemia
– Acute megakaryoblastic leukemia
– Acute basophilic leukemia
– Acute panmyelosis with myelofibrosis
– Myelosarcoma/chloroma

Cytomorphology

Like with MDS, smears of bone marrow and peripheral blood are stained using the Pappenheim or May-Grunwald-Giemsa method. In cytochemistry, myeloperoxidase (MPO) allows for the detection of myeloid cells, and esterase ("non-specific esterase", NSE) for the detection of monocytic cells. In borderline cases with MDS, ringed sideroblasts can be identified via iron and/or Prussian blue staining [21].

AML can be proved in almost all cases by means of a cytomorphological assessment of the bone marrow and blood smears. A positive test for myeloperoxidase in at least 3% of blasts continues to be conclusive evidence of AML. The boundary between AML and MDS is drawn at a blast content of ≥20% in the bone marrow or blood. However, there are specific AML subtypes, such as inversion inv(16) and/or translocation t(16;16) with the molecular correlate *CBFB-MYH11*, translocation t(8;21)/*RUNX1-RUNX1T1*, and acute promyelocytic anemia with t(15;17)/*PML-RARA*, in which cyto- or molecular-genetic rearrangement, independent of the blast content, is deemed evidence of an AML diagnosis [22].

Immunophenotyping

Findings in cytomorphology and cytochemistry provide essential clues about the composition of the antibody panel. For AML, this includes, for example, antibodies against the antigens myeloperoxidase, CD13, CD33 or CD117. Acute leukemias with mixed phenotype express antigens of both myeloid and lymphoid lines. Acute leukemias with mixed phenotype have an unfavorable prognosis [23]. Furthermore, using immunophenotyping, it is possible to identify the AML subtypes FAB M0 and M7 as myeloid beyond doubt, which is impossible with morphology and/or cytochemistry. More than 90% of AML cases have a "leukemia-associated immunophenotype" (LAIP), which can be used for monitoring "minimal residual disease" (MRD). For example, in some AML cases, the antigens CD7, CD19 or CD56, which are typically expressed on lymphatic cells, can be detected. Other possibilities include the lack of markers normally expressed on blasts (e.g. HLA-DR), the over-expression of certain antigens (e.g. CD34), or the asynchronous expression of antigens across the boundaries of different cell maturation stages. Compared to molecular genetics, the sensitivity of immunophenotyping is somewhat lower for follow-up testing (approx. 1:10,000). But its advantage lies in the fact that it can be used across different molecular-genetic subgroups.

Cytogenetics

The karyotype is an essential part of risk stratification at the time of diagnosis or in the event of an AML relapse.

Table 7: Classification of AML by cytogenetic risk groups, pursuant to the criteria set by the Medical Research Council (MRC) [24].

Prognostic classification	Cytogenetic subgroup
Favorable karyotype	– t(15;17)(q22;q12) – t(8;21)(q22;q22) – inv(16)(p13q22)/t(16;16)(p13;q22)
Intermediate karyotype	All karyotypes that have neither a favorable nor an unfavorable prognostic significance
Unfavorable karyotype	– Abnormalities of chromosome 3q – inv(3)(q21q26)/t(3;3)(q21;q26) – Abnormalities of chromosomes 5 or 5q – Abnormalities of chromosomes 7 or 7q – t(6;11)(q27;q23) – t(10;11)(p11~13;q23) – t(11q23) [Exceptions: t(9;11)(p21~22;q23); t(11;19)(q23;p13)] – t(9;22)(q34;q11) – –17; 17p-abnormalities – Complex changes (≥4 clonal abnormalities)

One may refer, by way of example, to the "Medical Research Council" (MRC) criteria, which categorize cases as prognostically favorable, intermediate and unfavorable [24] (Table 7). The reciprocal rearrangements t(15;17)(q22;q12)/*PML-RARA* (for acute promyelocytic anemia), t(8;21)(q22;q22)/*RUNX1-RUNX1T1*, and inv(16)(p13q22)/t(16;16)(p13;q22)/*CBFB-MYH11* are prognostically favorable. As a rule, standard chemotherapy is sufficient for an initial manifestation. In contrast to this, AML patients with complex cytogenetic changes or changes in chromosome 5 or 7 have an unfavorable prognosis. This means that allogeneic stem cell transplantation should be implemented already at the first remission of the disease in order to fully utilize the immune effect of the donor hematopoiesis as an addition to chemotherapy. Rearrangements of the *MLL*-gene (on the chromosome section 11q23) are prognostically unfavorable; one exception is translocation t(9;11)(p22;q23)/*MLL-MLLT3* (*MLL-AF9*), which has been categorized as intermediate. Some rarer reciprocal rearrangements, such as t(6;9)(p23;q34)/*DEK-NUP214* (=*DEK-CAN*), are also prognostically unfavorable. A normal karyotype is prognostically intermediate, but can be broken down further via the mutation profiles of different genes in molecular genetics (see below).

Fluorescence in situ hybridization

To detect submicroscopic cytogenetic changes, one needs to use fluorescence in situ hybridization (FISH) or Array-CGH (comparative genomic hybridization). But the probes used in the FISH analysis only capture the spectrum of cytogenetic changes for which they were selected. Interphase FISH also works with avital cell material, that is, also when the chromosome analysis could not be performed, or only with suboptimal results, due to limited metaphase quality. Furthermore, FISH can detect cryptic changes that are not visible through an optical microscope. Another benefit is the speed with which the interphase FISH method can be performed. For example, the test for suspected acute promyelocytic anemia with t(15;17)(q22;q12)/*PML-RARA* takes around 3 h.

In addition to the probes used on interphase nuclei, so-called "chromosome painting" probes can be used on metaphase spreads that mark entire chromosomes. Individual chromosomes can be selected with this method, or all 24 different chromosomes can be represented using different colors (24-color FISH). This method allows to fully clarify the structural chromosomal changes for a complex karyotype (involving ≥3 chromosomes).

Molecular genetics

AML patients with a normal karyotype can be characterized further and divided into subtypes by way of molecular tests: Mutations in the nucleophosmin gene (*NPM1* mutations) are prognostically favorable in the event of an isolated occurrence [25]. Internal tandem duplications in the *FLT3* gene (*FLT3*-ITD) have an unfavorable risk profile [26] – also when coinciding with *NPM1* mutations. An unfavorable prognosis attaches also to partial tandem duplications within the *MLL* gene (*MLL*-PTD) [27]. *CEPBα*-mutations are prognostically favorable in connection with a biallelic AML with a normal karyotype [28–30]. This molecular subtyping and prognostic classification allows for improved risk assessment of AML with a normal karyotype, and increasingly also for a more specific choice of treatment.

The European Leukemia Net (ELN) has proposed that the diagnostic cytogenetic and molecular-genetic findings be combined (Table 8) [31].

At this point, high-throughput sequencing is being integrated with the screening for molecular mutations in connection with hematological neoplasms [32]. This allows for the sequencing process to occur in parallel with the generation of thousands, and up to millions, of sequences in an analysis. Even now, the new sequencing methods already make it possible to detect a large number of new mutations in connection with AML [33, 34]. High-throughput sequencing is also crucial to MRD

Table 8: AML prognostic groups, combination of cytogenetics and molecular genetics ELN proposal [31].

Risk group	Cytogenetic aberration	Comment
Favorable	– t(8;21)(q22;q22); *RUNX1-RUNX1T1*	
	– inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*	
	– *NPM1* mutated and no *FLT3* -ITD	Within the normal karyotype
	– *CEBPA* mutated	
Intermediate-I	– *NPM1* mutated and *FLT3*-ITD	
	– No *NPM1* but *FLT3*-ITD	
	– No *NPM1* and no *FLT3*-ITD	
Intermediate-II	– t(9;11)(p22;q23); *MLLT3-MLL*	
	– All cases not classified as favorable or unfavorable	
Unfavorable	– inv(3)(q21q26.2) or t(3;3)(q21;q26.2); *RPN1-EVI1*	
	– t(6;9)(p23;q34); *DEK-NUP214*	
	– t(v;11)(v;q23); *MLL* rearranged	
	– –5 or del(5q);	
	– –7	
	– abn(17p)	
	– Complex karyotypes	

diagnostics, because it can capture subclones up to a sensitivity of under 1%. Moreover, the new sequencing techniques create a better understanding of how relapses occur and/or of the clonal evolution in the course of acute leukemia [35], as well as of the molecular characterization of secondary AML [36].

Histopathology

Another test for suspected AML is the bone marrow biopsy with the results of round plugs. In some cases of dry tap aspiration, it may be helpful to create impression smears from a bone marrow cylinder. Histological analysis is particularly important for patients with unclear cytopenia.

A link to a myeloid line may be suspected, but a definitive conclusion is mostly impossible in histology on a purely conventional-morphological basis. For some entities, one may suspect a specific subtype of leukemia (acute monocytic leukemia, acute panmyelosis with myelofibrosis, AML with myelodysplasia-related changes, etc.) based on the morphology of the blasts or the characteristics of the concomitant infiltrate in the bone marrow biopsy. But for AML, this should always be followed by immunohistology: Approximately 80% of AML cases are CD34-positive. In case of acute leukemia, which has developed against a background of myelodysplasia, the CD34 staining, however, is an essential criterion. As for AML with an *NPM1* mutation, immunohistochemical proof of an aberrant localization of the nucleophosmin protein in the cytoplasm of myeloid blasts can be delivered in addition to or in lieu of a molecular-genetic analysis [22, 37]. Cases of AML FAB M7 (in terms of megakaryocytic AML) are often associated with dry tap aspiration, which renders a cytomorphological assessment difficult. Here, great importance must be attached to bone marrow biopsy and/or immunohistochemistry coupled with proof of expression of CD41 and CD61. Furthermore, pathology in combination with immunohistochemistry is essential to diagnosing a myeloid sarcoma (as defined by WHO) and/or a chloroma in terms of extramedullary AML manifestations [24].

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