6 Clinical Manifestation and Diagnostic Workup

Agnieszka Wierzbowska
and Magdalena Czemerska

6.1 Clinical Manifestation of AML

Patients with acute myeloid leukemia (AML) usually present with signs and symptoms resulting from bone marrow (BM) failure, circulation of blasts in peripheral blood, and organ infiltration with leukemic cells. The time course of leukemia symptoms is variable. In some patients, particularly younger ones, clinical symptoms develop rapidly, over a few days to 1–2 weeks. Others have a longer course, with fatigue or other symptoms preceding the proper diagnosis by 1 or 2 months. A longer course is more frequently observed in elderly patients and may suggest an antecedent hematologic disorder, such as myelodysplastic syndrome (MDS). The initial signs and symptoms of AML are usually nonspecific and may mimic those of the common chronic diseases or of casual infections like cold or flu. Patients may present with lethargy and fatigue, loss of appetite and weight, fever, or night sweats (Metzeler 2016).

6.1.1 Symptoms Related to Bone Marrow Failure

The clonal proliferation of leukemic blasts ultimately leads to the failure of normal hematopoiesis. The symptoms of bone marrow failure are related to anemia, neutropenia, and thrombocytopenia, and may vary based on the type of blood cell affected (Metzeler 2016).

The most common symptom of anemia is fatigue. Patients usually complain of a decreased energy level and deterioration of exercise tolerance over previous weeks. Underproduction of red blood cells may lead to weakness, headache, or dizziness. Anemia also causes shortness of breath, dyspnea, heart palpitation, or chest pain. Severe cardiac complications may be observed as the first symptoms of AML, especially in patients with a history of cardiovascular disease or in elderly patients.

AML patients frequently demonstrate decreased neutrophil level, regardless of white blood cell (WBC) count. The level of neutropenia correlates with the risk of infections. Patients often present with fever, which may occur with or without specific documentation of an infection. Medical history usually reveals the occurrence of frequent infections of varied clinical localization and presentation that have not improved despite treatment with oral antibiotics.

Patients with thrombocytopenia often complain of easily bruised skin, ecchymoses, and

A. Wierzbowska (✉) · M. Czemerska
Department of Hematology, Medical University of Lodz, Copernicus Memorial Hospital, Lodz, Poland

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unusual bleeding, such as frequent nosebleeds and bleeding from the gums. In women, menorrhagia is commonly observed. In some cases, especially those with co-existing coagulopathy due to disseminated intravascular coagulation (DIC), life-threatening hemorrhagic complications such as gastrointestinal, respiratory, intracranial, or subdural bleeding may occur. DIC is most commonly observed in acute promyelocytic leukemia (APL).

6.1.2 Symptoms Related to Circulation of Blasts in Peripheral Blood

Leukemic blasts may be present in the peripheral blood, resulting in an increased WBC count. About 10% of AML patients demonstrate hyperleukocytosis, defined as WBC $>100 \times 10^9$/L. Such patients, with markedly elevated WBC counts, can present with symptoms of leukostasis such as dizziness, blurred vision, headache, confusion, somnolence, and dyspnea, resulting in both respiratory and neurological distress. Leukostasis is a medical emergency that requires an urgent intervention (Metzeler 2016).

Patients with high leukemic cell burden often complain of bone pain related to increased pressure in the bone marrow.

6.1.3 Symptoms Related to Organ Infiltration with Leukemic Cells

Extramedullary infiltration is usually diagnosed simultaneously with overt AML. In rare cases, it precedes the bone marrow involvement (myeloid sarcoma). It may occur at any site and varied clinical presentation is possible. Extramedullary disease is most frequently observed in AML of monocytic origin. The most common sites of infiltration include the spleen, liver, gums, and skin. Cutaneous manifestations of AML are usually described as multiple papules. Gingival infiltration results in hypertrophy and swollen gums. In the case of spleen or liver involvement, patients may report abdominal discomfort or feelings of fullness and early satiety. In rare cases, AML can spread to the lymph nodes, leading to their enlargement (Metzeler 2016).

Central nervous system (CNS) involvement at presentation is rare in adult AML patients. The typical symptoms of overt CNS infiltration consist of headache, cranial nerve palsies, visual changes, and balance problems.

6.1.4 Physical Examination

Patients usually present with pallor of the skin, conjunctiva, and oral mucosa. Examination of the mouth and pharynx may reveal mucositis, neutropenic ulcers, gum hypertrophy, gingivitis, or bleeding gums. Petechiae, a small, punctate hemorrhagic rash that is not palpable, can be detected, especially on the lower limbs. Moreover, bruises or hematoma can be seen on the skin, particularly in patients with concomitant coagulopathy. The symptoms of infection can be detected by respiratory tract auscultation, and cardiac flow murmur, heart rhythm disorder, or extrasystole may be revealed by heart examination. Moreover, in cases with extramedullary involvement, lymphadenopathy, splenomegaly, hepatomegaly or substantial reddish or purple red, firm papules, plaques, or nodules in the skin may be observed (Metzeler 2016).

6.2 Diagnostic Workup of Patients with Suspected AML

The diagnostic evaluation of patients with suspected acute leukemia has two objectives: firstly, to confirm a diagnosis, and secondly, to characterize disease-specific and patient-specific factors to provide important information for risk stratification and treatment decisions. The schema of initial diagnostic workup of acute leukemia is shown in Fig. 6.1.

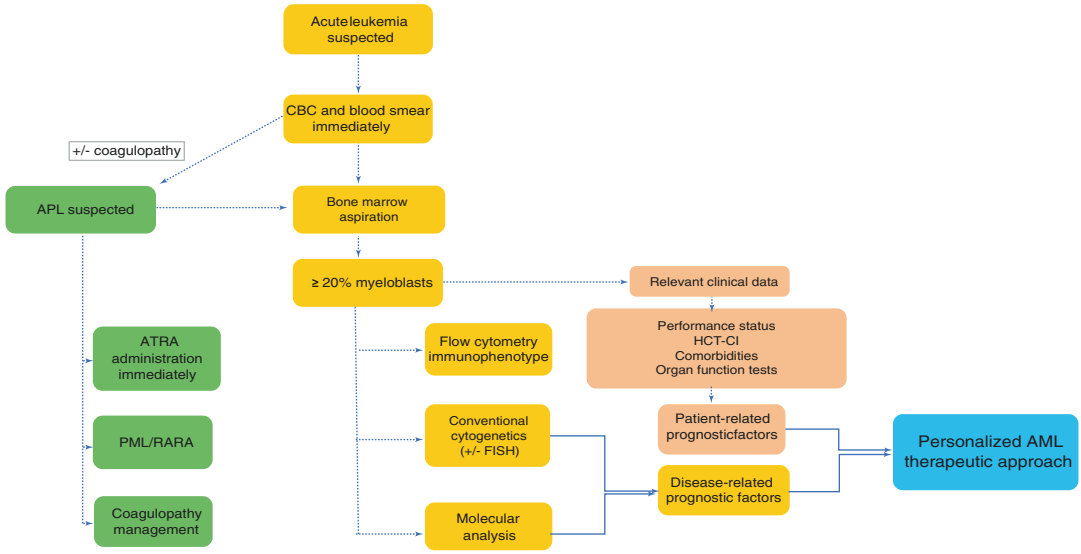

Fig. 6.1 The initial diagnostic workup of acute leukemia. *APL* acute promyelocytic leukemia, *AML* acute myeloid leukemia, *ATRA* all-trans retinoid acid, *CBC* complete blood count, *FISH* fluorenscence in situ hybridization, *HCT-CI* hematopoietic cell transplantation-comorbidity index

The diagnosis of AML requires a comprehensive medical history and physical examination as well as detailed morphologic, cytochemical, immunophenotypic, cytogenetic, and molecular evaluation of bone marrow samples; this demands close collaboration between the treating physician and the pathologist (Döhner et al. 2017).

A comprehensive clinical assessment should include the age, sex, and ethnicity of the patient; history of any hematologic disorder; any prior malignancy; smoking status; exposure to cytotoxic therapy, immunotherapy, radiotherapy or other possibly toxic substances, such as benzene or pesticides. Any past medical history regarding known predisposing conditions or syndromes should be carefully reviewed to avoid overlooking any myeloid neoplasms with germline predisposition (Döhner et al. 2017).

Clinical symptoms related to anemia, thrombocytopenia, and neutropenia, as well as the presence of tumor masses; other tissue lesions; the presence of organomegaly and findings from neurologic examination should be elaborately collected. Immediate evaluation of symptoms related to potentially life-threatening leukostasis, coagulopathy, and tumor lysis syndrome is of particular importance.

6.3 Blood and Bone Marrow Morphology

6.3.1 Complete Blood Count

A complete blood count (CBC) with differential demonstrates anemia, neutropenia, and thrombocytopenia of varying degrees. Anemia is usually normocytic but macrocytosis is also a common finding in AML with myelodysplasia related changes (AML-MRC). Patients with AML often display high, normal, or low WBC counts. A review of a peripheral blood smear can confirm the any findings from a CBC count and usually also the presence of circulating blasts. According to European LeukemiaNet (ELN) recommendations, at least 200 leukocytes on blood smears should be counted (Döhner et al. 2010, 2017). Dysplastic changes can be found in granulocytes and erythrocytes. Schistocytes are occasionally seen in patients with DIC.

6.3.2 Bone Marrow Morphology

Bone marrow aspiration is obligatory to establish AML diagnosis. According to WHO 2016

classification, AML is defined based on the presence of a myeloid blast count of ≥20% out of 500 nucleated cells on spiculated marrow smears following morphological BM evaluation (Vardiman et al. 2009). Counting fewer BM cells may be sufficient in patients with a high blast count (Abdulrahman et al. 2018). Myeloblasts, monoblasts, and megakaryoblasts are included in the blast count. In AML with monocytic or myelomonocytic differentiation, promonocytes are also considered as blast equivalents (Arber et al. 2016; Döhner et al. 2017). Sometimes, linear groupings of primary granules (Auer rods) may be observed in myeloblasts. Bone marrow evaluation should always include the level of dysplasia (≥ 50% or <50% of cells) in erythroid, granulocytic, and megakaryocytic line, which is required for diagnosis of AML-MRC.

The bone marrow biopsy is always mandatory in patients with a dry tap, but it can be also used to provide correct blast enumeration and to avoid "undercounts" in the aspirate due to spotty cellularity, fatty marrows, or fibrosis (Döhner et al. 2017). Bone marrow biopsy yields useful information for differential diagnosis of AML associated with marrow fibrosis (e.g., acute megakaryoblastic leukemia and acute panmyelosis with myelofibrosis) or in several other diagnostic settings.

6.4 Cytochemistry and Immunophenotyping

Although multiparameter flow cytometry (MFC) is the recommended method for determining the lineage involvement in patients with suspected AML, cytochemical staining with myeloperoxidase (MPO), Sudan Black B (SBB), or nonspecific esterase (NSE) may also be useful in the early period of diagnosis, when the MFC results are not available or when bone marrow aspirate or peripheral blood material is not available for flow cytometry study. Immunophenotyping by MFC of bone marrow or peripheral blood samples can be used to distinguish AML from acute lymphoblastic leukemia (ALL); it can also be used to further classify the subtype of AML, and also to evaluate a panel of leukemia-associated immunophenotypes (LAIP) as a background for further monitoring of measurable residual disease (MRD). At least 3-colour MFC is recommended by ELN (Béné et al. 2011; Döhner et al. 2017) for routine diagnostic workup in AML; however, a minimum of six colors is required for the diagnosis of specific diagnostic entities, such as mixed phenotype acute leukemia (MPAL), AML not otherwise specified with minimal differentiation, acute megakaryoblastic leukemia, or blastic plasmacytoid dendritic cell neoplasm (BPDCN) (Johansson et al. 2014; van Dongen et al. 2012). A minimal panel of antigens recommended for AML diagnosis is presented in Table 6.1. Because MRD is an important post-diagnosis prognostic factor in AML, included as a new response criterion (i.e., complete response (CR) with/without MRD), MRD monitoring should be considered as a part of the standard of care of AML patients (Döhner et al. 2017; Schuurhuis et al. 2018). ELN experts recommend MFC with at least eight colors to be used at diagnosis and further MRD monitoring in AML patients (Schuurhuis et al. 2018).

6.5 Genetic Analysis

6.5.1 Cytogenetic and FISH

Cytogenetic testing is a mandatory part of any diagnostic workup of patients with AML that provides important information for prognosis and is needed for the WHO classification of "AML with recurrent genetic abnormalities" as well as "AML with myelodysplasia-related changes." In the presence of t(15;17), t(8;21) and inv(16) or t(16;16), a diagnosis of AML can be made even with less than 20% of blasts in BM (Arber et al. 2016). Cytogenetic abnormalities can also guide further treatment in selected cases (i.e., t(15;17); t(9;22); MDS-like cytogenetic abnormalities). In conventional cytogenetic analysis using karyotyping of G-banded metaphase chromosomes, at least 20 metaphases should be evaluated (Döhner et al. 2017). An abnormal clone can be reported if at least two of 20 cells carry the same karyotypic

Table 6.1 Procedures recommended for AML diagnosis and classification according to WHO 2016 classification, ELN 2017 recommendations and ELN-MRD 2019 recommendations

Diagnostic workup	Recommended
Cytological assessment	Peripheral blood smear (at least 200 leukocytes to be assessed) Bone marrow aspiration (at least 500 nucleated cells + dysplastic changes to be assessed) Bone marrow biopsy (if dry tap in bone marrow aspiration)
Flow cytometry (FC) immunophenotype	At least 3-colour FC, optimal at least 8-colour FC *Markers recommended for diagnosis of AML* – Precursors markers: CD34, CD117, CD33, CD13, HLA-DR – Granulocytic markers: CD65, cytoplasmic MPO – Monocytic markers: CD14, CD36, CD64 – Megakaryocytic markers: CD41 (glycoprotein IIb/IIIa), CD61 (glycoprotein IIIa) – Erythroid markers: CD235a (glycophorin A), CD36 Assessment of LAIPs for further MRD evaluation LSC assessment
	Markers recommended for diagnosis of MPAL – Myeloid lineage: MPO or at least 2 monocytic markers (NE, CD11c, CD14, CD64, lysozyme) – T-lineage: strong cytoplasmatic or surface CD3 B-lineage: strong CD19 + at least 1 of: cytoplasmatic CD79a, CD22, CD10 or weak CD19 and at least 2 of: CD79a, CD22, CD10
Cytogenetics	Conventional cytogenetics with GTG banding technique (at least 20 metaphases to be assessed) FISH (if conventional cytogenetics fails) – to detect: *RUNX1-RUNX1T1*, *CBFB-MYH11, KMT2A* (*MLL*), *MECOM* (*EVI1*), loss of chromosome 5q, 7q or 17p
Molecular genetic	*Gene mutations:* *NPM1, FLT3-ITD* with allelic ratio data, *FLT3-TKD* (mutations at codons D835 and I836), *CEBPA, TP53, ASXL1, IDH1, IDH2, RUNX1, cKIT* in CBF-AML *Gene rearrangements:* *PML-RARA, RUNX1-RUNX1T1, CBFB-MYH11, BCR/ABL1*

MPAL mixed phenotype acute leukemia, *LSC* leukemic stem cells, *LAIPs* leukemia associated immunophenotypes, *FISH* fluorescence in situ hybridization

abnormality. Fluorescence in situ hybridization (FISH) assays are helpful to define chromosomal aberrations in patients with complex karyotype, or partner fusion chromosomes in 11q23 translocations. FISH is always mandatory when conventional cytogenetic fails. An example of initial workup in patient with AML is presented in Fig. 6.2.

6.5.2 Molecular Genetic Testing (PCR, NGS)

The field of genomics in AML and related implications are evolving rapidly (Lindsley et al. 2015; Papaemmanuil et al. 2016). In AML, a variety of gene mutations (*NPM1*, biallelic *CEBPA*, *FLT3-ITD*, *RUNX1, TP53,* and *ALXL1*) are associated with specific prognoses and may guide the intensity of post-remission treatment (see Chap. 9) (Döhner et al. 2017). Molecular genetic testing for *NPM1*, biallelic *CEBPA* and *RUNX1* mutations is also required for WHO classification of "AML with recurrent genetic abnormalities." Other mutations, such as *FLT3-ITD, FLT3-TKD*, *IDH1/2* may have therapeutic implications. The panel of molecular genetic tests recommended in the diagnostic workup of newly-diagnosed AML patients is presented in Table 6.1. Screening for single

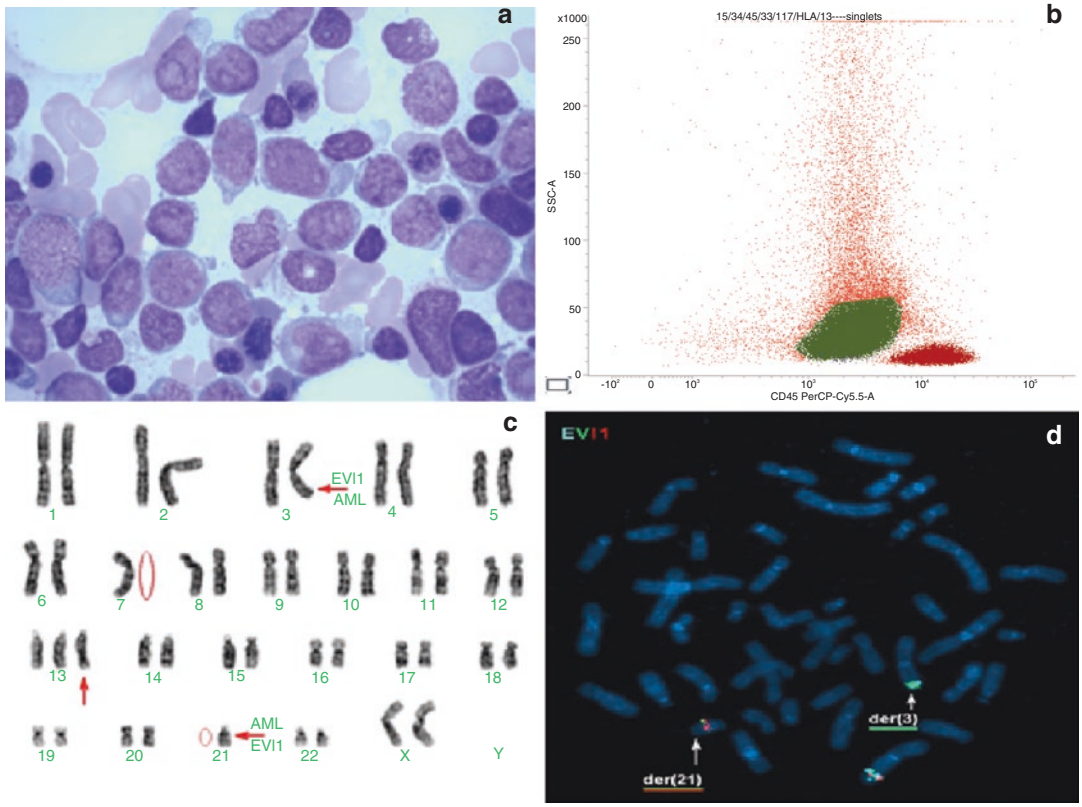

Fig. 6.2 The initial acute leukemia workup which contains cytological assessment of AML blasts (**a**), flow cytometry immunophenotyping (**b**), conventional cytogenetics that revealed complex katyotype (**c**) with MECOM (EVI1) abnormality confirmed by FISH (**d**). (Courtesy of Ewa Wawrzyniak and Agata Majchrzak)

genes may be replaced by multiplex gene panels and next-generation sequencing (NGS) analysis for a comprehensive prognostic assessment. Molecular testing by reverse transcriptase–polymerase chain reaction (RT-PCR) for recurring rearrangements can also be helpful if rapid information is needed for recommendation of suitable therapy (i.e., *PML-RARA*) or if chromosome morphology is of poor quality (Döhner et al. 2017). As midostaurin, the FLT3 inhibitor, is currently approved in AML treatment, molecular results confirming the presence of *FLT3* gene mutations should be available rapidly (optimally within 72 h from the diagnosis) in order to allow timely initiation of midostaurin treatment by day 8 (Stone et al. 2017). Procedures recommended for diagnosis and classification of AML are presented in Table 6.1.

6.6 Additional Procedures Recommended at Diagnosis of AML

As coagulopathy is common at presentation of AML, an evaluation of prothrombin time, activated partial thromboplastin time and fibrinogen activity is a part of the routine initial evaluation and is advisable before performing any invasive procedures.

Other laboratory tests frequently performed during the diagnostic workup in AML include a comprehensive metabolic panel, serum uric acid and lactate dehydrogenase, liver function tests, tumor lysis syndrome (TLS) panel, uric acid measurement, urine analysis and viral screening (i.e., evaluation of HBV, HCV, HIV, and CMV antibodies). In women of childbearing potential, a pregnancy test should be performed.

For patients with neurologic signs or symptoms at diagnosis, cranial magnetic resonance imaging (MRI) or computed tomography (CT) should be performed to detect meningeal disease or CNS hemorrhage. Lumbar puncture (LP) should be performed if no mass lesion is detected on the imaging study and a coagulopathy is excluded (Tallman et al. 2019). In APL with suspected CNS involvement, due to high hemorrhagic risk, LP should be postponed to the end of induction (Sanz et al. 2019).

If extramedullary disease is suspected, positron emission tomography (PET/CT) or CT of the relevant organ should be performed with a biopsy in rare cases of myeloid sarcoma without bone marrow involvement. Imaging technics (CT, X-ray) are also useful to diagnose and monitor concomitant pulmonary infections.

An ECG, echocardiogram or MUGA (multigated acquisition) scan evaluation is of particular importance in AML patients with a history or symptoms of cardiac disease or prior/planned exposure to cardiotoxic drugs or radiation to the thorax (Tallman et al. 2019). In case of pulmonary comorbidity, the function tests of respiratory track should be performed.

Human leukocyte antigen (HLA) typing and an early search for family or an alternative donor is recommended in all patients with newly-diagnosed AML for whom an allogenic hematopoietic cell transplantation (alloHCT) is being considered.

Sperm cryopreservation before starting chemotherapy (ChT), should be proposed to younger patients, particularly if they are planned for alloHCT. Cryopreservation of ovarian tissue is rarely feasible at diagnosis because of the urgent need for ChT and the possibility that ovarian fragments may be contaminated with leukemic cells (Shapira et al. 2014).

Because of the relationship between level of fitness and the treatment outcomes, an evaluation should be performed of the patient's performance status according to ECOG/WHO score as well as a careful assessment of their pre-existing comorbidities (i.e., based on hematopoietic cell transplantation-comorbidity index [HCT-CI] score) (Sorror et al. 2005; Sorror et al. 2017). In elderly patients (>65 years), a comprehensive geriatric assessment may provide additional information to determine eligibility to conventional chemotherapy (Klepin et al. 2020; Pettit and Odenike 2015).

6.7 Differential Diagnosis

It is crucial to quickly and accurately distinguish AML from less urgent hematological or other diseases, the most common being the following: infectious causes, such as mononucleosis, Plaut-Vincent angina, and severe infections such as sepsis or tuberculosis; other hematological disorders such as acute lymphoblastic leukemia, aplastic anemia, paroxysmal nocturnal hemoglobinuria, and MDS; other miscellaneous causes such as solid tumors metastases and bone marrow failure due to drug toxicity.

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