Acute myeloid leukemia (AML)

Status: May 2020

Diagnostic recommendation
Continuous research and targeted examinations of blood and bone marrow result in various diagnostic recommendations for patients with acute myeloid leukemia (AML).

Method	Anticoagulant	Recommendation
Cytomorphology	EDTA	mandatory
Immunophenotyping	EDTA or Heparin	mandatory
Chromosome analysis	Heparin	mandatory
FISH	EDTA or Heparin	optional
Molecular genetics	EDTA or Heparin	mandatory

Definition and charateristics of AML

Acute myeloid leukemias (AML) are a heterogeneous group of diseases. AML can either develop de novo, after previous cytotoxic and/or radiotherapy (t-AML), or secondarily from a pre-existing myeloproliferative disease or MDS (s-AML). The incidence of AML is 2.5 - 3.0 / 100,000 inhabitants per year. The median age is 65 years. In children under the age of 15, AML accounts for only about 15-20% of acute leukemias.

Classification of AML

The new AML WHO Classification 2017 divides AML into specific AML subgroups, first according to the patient's medical history (de novo, t-AML, s-AML) and then taking into account a large number of recurrent, balanced cytogenetic abnormalities (see Table 1). Overall, this means that 80% - 90% of patients with AML can now be classified by cytogenetic and/or molecular genetic markers.

AML WHO Classification 2017
(Arber DA et al. 2016, Swerdlow et al. 2017)

Acute myeloid leukemia (AML) and related neoplasms
AML with recurrent genetic abnormalities
- AML with t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
- Acute promyelocytic leukaemia (APL) with *PML-RARA*
- AML with t(9;11)(p21.3;q23.3); *KMT2A-MLLT3*
- AML with t(6;9)(p23;q34.1); *DEK-NUP214*
- AML with inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM*
- AML (megakaryoblastic) with t(1;22)(p13.3;q13.3); *RBM15-MKL1*
- AML with *BCR-ABL1* (provisional entity)
- AML with mutated *NPM1*
- AML with biallelic *mutation of CEBPA*
- AML with mutated *RUNX1* (provisional entity)

AML with myelodysplasia-related changes

Therapy-related myeloid neoplasm

AML, not otherwise classified (NOS)

- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic and monocyte leukemia
- Pure erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

Myeloid sarcoma

Myeloid proliferation associated with Down syndrome

- Transient abnormal myelopoiesis associated with Down syndrome
- Myeloid leukemia associated with Down syndrome

According to the WHO classification, a blast percentage of at least 20% in peripheral blood or bone marrow is required for the diagnosis of AML. In AML with t(8;21)(q22;q22.1), *RUNX1-RUNX1T1* or inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* and acute promyelocytic leukemia (APL) with *PML-RARA*, the disease is classified as acute leukemia even if the blast count is below 20%.

Facts

≥20%

Blasts in blood or bone marrow define AML
(Oncopedia Guideline AML)

AML - Diagnostics

Cytomorphology

The cytomorphology together with the cytochemistry (myeloperoxidase and esterase) belong to the standard diagnostics. It serves to secure the diagnosis and is also required for classification according to WHO. Cytomorphologic remission assessment is still a gold standard for follow-up

examinations under therapy.

Immunophenotyping

Acute myeloid leukemia (AML) is often first diagnosed on the basis of cytomorphology and cytochemistry. The only exceptions are AML with minimal differentiation and acute megakaryoblast leukemia, which can only be diagnosed reliably by immunophenotyping. This is one of the reasons why immunophenotyping is always part of the routine diagnosis of AML. In addition, typical immunophenotypes are available for some genetic abnormalities occurring in AML. However, these correlations are not complete and therefore only have a guiding role in diagnostics.

In acute promyelocytic leukemia (APL), a characteristic finding in the scatter plot is shown in association with infiltration by atypical promyelocytes. In addition, HLA-DR and CD34 are usually not expressed. However, these findings can also be found in some other AML cases (e.g. AML with *NPM1* mutation and normal karyotype). AML with maturation and t(8;21)(q22;q22)/*RUNX1-RUNX1T1* is typically associated with aberrant coexpression of CD19 and CD56. This constellation is not specific to a particular AML subtype too. The same is true for the immunophenotype of myelo-monocytic AML with abnormal eosinophils, where one can specifically find a positivity for CD2 as well as an asynchronous coexpression of CD15 and CD34.

AML with minimal differentiation shows no myeloperoxidase (MPO) positivity (<3%) in cytochemistry, therefore this diagnosis cannot be made morphologically alone. Immunophenotyping differentiates AML from ALL and detects the expression of CD13, CD33 and CD117 and other myeloid antigens. At the same time lymphatic antigens are not expressed or the lymphatic score is not sufficient for the diagnosis of a biphenotypic acute leukemia. In about half of the cases, despite the cytochemical negativity for myeloperoxidase, an expression of MPO can be detected by flow cytometry and thus the diagnosis of AML can be made.

Acute megakaryoblast leukemia is also cytochemically negative for MPO and esterase. Due to the frequently existing myelofibrosis, a cytological evaluation is difficult. In immunophenotyping, the expression of CD41 or CD61 can be detected.

Immunophenotyping plays an important and increasing role in the determination of MRD (Schuurhuis et al. 2018). Together with molecular genetics (see below), it provides the decisive parameters for therapy stratification. Depending on the expression of certain markers, the sensitivity is 10^{-4} to 10^{-5}.

Chromosome analysis

The classical chromosome analysis to determine the karyotype of leukemia cells is now part of the standard diagnostic procedure for every AML patient. The karyotype and the numerous characteristic chromosomal abnormalities are used for classification according to WHO, but also represent the most important independent prognostic parameters. For some genetically defined subtypes of AML, therapeutic consequences can be drawn from the chromosomal analysis findings. About 50-75% of adult patients and 75-85% of children with AML show clonal chromosomal alterations. While the incidence of different chromosomal abnormalities depends on age, the prognostic significance of a cytogenetic alteration is largely independent of the patient's age.

Fluoreszenz in situ Hybridisierung (FISH)

FISH is a fast method for the detection of specific abnormalities

The FISH analysis serves as an important addition to the classical chromosome analysis and answers specific questions, e.g. the detection of the translocation t(15;17)(q24;q21) (with a rearrangement of the genes *PML* and *RARA*) in suspected cases of acute promyelocyte leukemia. It is a fast and reliable method, with which a *PML-RARA* rearrangement can be detected after only 3 hours. Other genetic abnormalities frequently occurring in AML, such as t(8;21)(q22;q22), inv(16)(p13q22) and rearrangements of the *KMT2A* gene, are relevant for the classification of AML within the WHO classification. They can also be detected by FISH. Frequent unbalanced changes, such as deletions of the long arm of chromosome 5 or 7 or monosomes (-7) or trisomies (+8, +11, +13, +21) can also be detected by FISH. With the help of a manageable set of FISH probes, even a large proportion of AML cases with complex aberrant karyotype can be correctly classified.

24-color FISH for complex karyotypes

The so-called "chromosome painting" with 1-3 or 24 colours (24-colour-FISH) on metaphase chromosomes is performed in addition to the classical chromosome analysis, if the karyotype cannot be clearly clarified by chromosome analysis using the classical banding technique. This is often the case with complex aberrant karyotypes.

Detection of minimal residual disease with FISH

The FISH method can also be used in the course of AML after therapy to detect residual disease. It is more sensitive and specific than cytomorphology, but less sensitive than PCR and immunophenotyping.

Molecular genetics

For the detection of molecular mutations a variety of detection techniques are available. For example, conventional reverse transcription (RT) PCR (fusion genes), gene scan analysis (*NPM1*, *FLT3*-ITD), melting curve analysis (*FLT3*-TKD, *NPM1*, *RAS*, *KIT* D816) and real-time PCR (*KMT2A*-PTD, fusion genes, *EVI1* expression). If a mutation is detected using these techniques, sequencing can be used for precise characterization. In the following, these molecular markers open up new target structures for the PCR-based detection of minimal residual disease (MRD). Today, so-called Next-Generation-Sequencing (NGS) is often used to sequence a large number of genes (e.g. 75) in one gene panel and in one step in order to keep examination times short and to obtain a comprehensive picture of the molecular landscape of the respective AML with diagnostic, prognostic and increasingly also therapeutic significance.

Common reciprocal chromosome rearrangements (fusion genes)

Reciprocal chromosomal rearrangements are present in about 25% of all AML. The molecular correlates or fusion genes of most reciprocal rearrangements, which can also be detected cytogenetically, are known. The most frequent reciprocal translocations t(15;17)(q24;q21), t(8;21)(q22;q22), and inv(16)(p13q22)/t(16;16)(p13;q22) lead to the formation of the fusion genes *PML-RARA*, *RUNX1-RUNX1T1* and *CBFB-MYH11* at the molecular level. Rearrangements of the *KMT2A* gene are found in about 5% of all AML, with more than 50 different translocation partners described. In addition, numerous other rare rearrangements can be detected, which often affect less than 1% of all cases, but in individual cases represent useful target structures for diagnosis. For fusion genes, quantification at the time of diagnosis is useful, since this measurement value serves as a starting point for future follow-ups (MRD).

Fusion genes detectable by RT-PCR:

- *RUNX1-RUNX1T1*
- *CBFB-MYH11*
- *PML-RARA*
- *KMT2A mergers*
- *CALM-AF10*
- *CHIC2-ETV6*
- *NUP98-HOXA9*
- *DEK-NUP214*
- *MYST3-CREBBP*
- *BCR-ABL1*

Table 1: Rearrangements with the associated fusion genes in AML

Cytogenetics	Fusion gene	Frequency	
		Children	Adults
t(8;21)(q22;q22)	*RUNX1-RUNX1T1*	10-15%	8-12%
inv(16)(p13q22)	*CBFB-MYH11*	6-12%	8-12%
t(15;17)(q24;q21)	*PML-RARA*	8-15%	8-10%
t(6;11)(q27;q23)	*KMT2A-MLLT4 (KMT2A-AF6)*	2-5%	< 1%
t(9;11)(p21;q23)	*KMT2A-MLLT3 (KMT2A-AF9)*	8-10%	1-2%
t(10;11)(p12;q23)	*KMT2A-MLLT10 (KMT2A-AF10)*	< 1%	1-2%
t(11;19)(q23;p13)	*KMT2A-MLLT1 (KMT2A-ENL)*	< 1%	< 1%
t(11;19)(q23;p13)	*KMT2A-ELL*	< 1%	< 1%
t(3;21)(q26;q22)	*RUNX1-EVI1*	1%	< 1%
t(6;9)(p23;q34)	*DEK-NUP214*	1-2%	< 1%
t(8;16)(p11;p13)	*KAT6A-CREBBP*	< 1%	< 1%
t(1;22)(p13;q13)	*RBM15-MKL1*	2%	-
t(7;11)(p15;p15)	*NUP98-HOXA9*	-	< 1%
t(10;11)(p13;q14)	*CALM-AF10*	-	< 1%
t(16;21)(p11;q22)	*FUS-ERG*	-	< 1%

Molecular mutations common in *NPM1* and *FLT3-ITD* and *RUNX1*
Mutations in the nucleophosmin *(NPM1)* gene are found in approximately 35% of all AML cases and 55% of cases with a normal karyotype. The transcription factor *RUNX1* is mutated in 15-30% of AML cases. Mutations in the transcription factor *CEBPA* have been described in 7-15% of AML cases with normal karyotype. In the WHO classification of 2017, both the *NPM1* and the biallelic *CEBPA* mutation were introduced as a separate AML entity. Internal tandem duplications (ITDs) of the FMS-like tyrosine kinase 3 gene (*FLT3*-ITD) occur in 25% of younger adults with cytogenetically normal AML. Studies have shown that the ratio of mutation load to wild type plays a prognostic role. Patients with a ratio ≥0.5 show a less favourable prognosis as well as a higher recurrence rate and are also more likely to benefit from a stem cell transplant (Schlenk et al. 2014, Döhner et al. 2017).

Table 2: Molecular mutations in AML

Mutation	Most common subtypes	Total frequency	Prognosis
NPM1	normale-karyotype (55%)	30%	favourable, if *FLT3*-ITD is not mutated
FLT3-ITD	normale-karyotype (40%)	25%	adverse
FLT3-TKD	all AML	6-7%	depending on additional abnormality
KMT2A-PTD	normale-karyotype (11%) trisomy 11 (20-50%)	6,5%	adverse
CEBPA	normale-karyotype (7%)	4%	favourable, only if biallelic
*KIT*D816	t(8;21) (12%)	1,5%	adverse
*KIT*exon8	inv(16) (10%)	< 1%	adverse, may TKI possible
NRAS	inv(16) (45%) inv(3)/t(3;3) (40%)	10%	intermediate
KRAS	inv(16); t(8;21) (5-20%) (in children)	< 1%	prognostic significance unclear
RUNX1	AML minimally differentiated (22%) trisomy 21 (30%)	15%	adverse
IDH1/2	normale-karyotype (30%)	15-20%	depending on the type of mutation, specifically treatable
TET2	-	15-25%	not yet clearly defined
DNMT3A	normale-karyotype (35%)	20%	adverse

Next-Generation-Sequencing (NGS) identified new molecular markers

The introduction of new sequencing techniques ("next-generation-sequencing", NGS) has facilitated the expansion of the spectrum of molecular markers. Mutations in the *TET2* gene ("*TET* oncogene family member 2") on chromosome 4q24 were detected in 15-25% of all AML cases. They are also detectable in numerous other myeloid entities such as MDS. Whether or not this has an influence on the prognosis is the subject of current studies. Mutations of the isocitrate dehydrogenase 1 (*IDH1*) and *IDH2* genes have been detected in up to 20% of all AML patients. Specific drugs are available for these patients. In addition, de novo AML mutations in the *ASXL1* (additional sex-comb like 1) gene, which is located on chromosome section 20q11, have been identified in about 10% of all patients. Very rare are mutations in the "Casitas B-cell lymphoma" (CBL) gene located on chromosome 11q23 (< 2% of all AML cases).

AML: MRD (Measurable Residual Disease)

The determination of Measurable Residual Disease (formerly known as minimal residual disease) opens up new possibilities for certain AML subgroups in terms of prognosis and classification with regard to response to therapy. The search for suitable parameters is often difficult. The diversity of clones can be high, and preleukemic clones and subclones exist side by side. Over time, the composition of the clones may change, especially under the selection pressure of the therapy. Nevertheless, it has been shown very well that certain markers are suitable for prognostic assessment.

The determination of the mutation load of the marker *NPM1* by means of quantitative polymerase chain reaction (qPCR) can, for example, be used very well to assess the risk of recurrence and give an indication as to which patient should be given an allogeneic stem cell transplantation or not due to MRD negativity. Patients with wild-type *NPM1* and intermediate risk benefit from an MRD determination after induction therapy by flow cytometry, which also allows a prognosis of the risk of relapse. The determination of the transcription level of fusion proteins such as *RUNX1-RUNX1T1* at specific points in time during therapy also allows an assessment of the risk of relapse. MRD negativity is a very favourable parameter for survival and a low risk of relapse (Freeman et al. 2019, Rücker et al. 2019, Schuurhuis et al. 2018).

Prognosis of AML

Karyotype and molecular genetic changes most important prognostic parameters
In addition to age, leukocyte count and general condition, the karyotype and molecular genetic changes are important prognostic parameters and have a major influence on the therapy strategy. Several studies have shown that especially patients with normal karyotype benefit from additional molecular genetic information regarding therapy choice and prognosis assessment (Döhner et al. 2010, 2015, 2017). Modern genetic prognosis systems combine molecular mutations and cytogenetics (Grimwade et al. 2016, Döhner et al. 2017).

The cytogenetic abnormalities and molecular genetic changes with prognostic relevance defined so far are listed in Tables 3 and 4

Table 3: Risk distribution with independent prognostic relevance in younger AML patients (16 - 60 years) (according to Grimwade et al. 2016)

Risk category	Genetic abnormality
Favourable	t(15;17)(q24;q21) / *PML-RARA* t(8;21)(q22;q22) / *RUNX1-RUNX1T1* inv(16)(p13q22) / t(16;16)(p13;q22) / *CBFB-MYH11* Mutated *NPM1* (no *FLT3*-ITD and no mutated *DNMT3A*) Biallelic mutated *CEBPA*
Intermediate	aberrations, which are not classified as favourable or adverse
Adverse*	abn(3q) except t(3;5)(q21~25;q31~35)/ *NPM1-MLF1*) inv(3)(q21q26) / t(3;3)(q21;q26) / *GATA2/EVI1* add(5q) / del(5q), -5- t(5;11)(q35;p15.1) / *NUP98-NSD1* t(6;9)(p23;q34) / *DEK-NUP214* add(7q) / del(7q), -7 t(11q23) except t(9;11)(p21~22;q23) and t(11;19)(q23;p13) t(9;22)(q34;q11) / *BCR-ABL1* -17 / abn(17p) / mutated *TP53* complex karyotype (≥ 4 independent abnormalities) mutated *ASXL1*, mutated *DNMT3A*, mutated *RUNX1* *FLT3*-ITD, *KMT2A*-PTD

*only if none of the cyto- and molecular genetic abnormalities classified as favourable are present

Table 4: Risk classification according to ELN recommendation (Döhner et al. 2017)

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22); *RUNX1-RUNX1T1* inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11* mutated *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^{low} Biallelic mutated *CEBPA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^{high} Wild-type *NPM1* without *FLT3*-ITD bzw. *FLT3*-ITD^{low} (without adverse-risk genetic lesions) t(9;11)(p21.3;q23.3); *MLLT3-KMT2A* cytogenetic abnormalities not classified as favorable or adverse
Poor*	t(6;9)(p23;q34.1); *DEK-NUP214* t(v;11q23.3); *KMT2A* rearranged t(9;22)(q34.1;q11.2); *BCR-ABL1* inv(3)(q21.3q26.2) oder t(3;3)(q21.3;q26.2); *GATA2*-MECOM (*EVI1*) -5 or del(5q); -7; -17/abn(17p) Complex karyotype (≥ 3 unrelated abnormalities)* Monosomal karyotype (one single monosomy (excluding loss of X or Y) in association with at least 1 additional monosomy or structural chromosome abnormality)# Wild-type *NPM1* and *FLT3*-ITD^{high} Mutated *RUNX1* Mutated *ASXL1* Mutated *TP53*

* three or more unrelated chromosome abnormalities in the absence of 1 of the WHO-designated recurring
# excluding core-binding-factor (CBF) AML

AML - Therapy

For a long time, all AML subtypes were treated with the same standard therapy 7+3 or similar protocols, which were specifically designed for fit patients with a biological age below 75 years. A fast start and intensive treatment was considered important, which consisted of induction therapy with the aim of complete remission (CR) and post-remission therapy to maintain CR. Allogeneic transplantation played a major role in this process. The response to therapy was very much determined by the genetic background of AML. For the first time, it was possible to introduce a specific therapy for acute promyelocytic leukemia with all-trans-retinoic acid (ATRA) (later also in combination with arsenic), which was specifically oriented on the genetics of AML. In recent years, the spectrum of targeted therapies has expanded for several other AML subgroups, relapsed patients and especially patients who did not seem suitable for intensive therapy due to comorbidities. The most accurate genetic characterization of AML is essential for the use of targeted therapies.
For clinically stable patients at the time of the initial diagnosis delay in the start of therapy due to the genetic analyses, which can take several days, does not lead to a worse response but rather to an improved long-term prognosis through the use of adapted therapies.

Today, according to the Onkopedia guidelines (status: 10/2019), patients with

- CD33-positive Core Binding Factor AML (CBF-AML) and with CD33-positive *NPM1* mutation in *FLT3*wt
- *FLT3* mutation

- AML-MRC and patients with therapy-associated AML (t-AML) at *FLT3wt*
- CD33-positive intermediary risk-AML in *FLT3wt*

can be treated with an alternative induction scheme.

The B-cell lymphoma (*BCL-2*) inhibitor Venetoclax, in combination with hypomethylating agent (HMA) therapy, showed very good success in older patients with *NPM1* mutant AML, who otherwise have a comparatively adverse prognosis compared to younger patients. Studies with a small cohort of older de novo AML patients with an intermediate or adverse risk profile after ELN also showed that venetoclax in combination with decitabine or azacidine is a very good alternative to standard therapy. CR rates of between 54% and 67% were shown, with a response being achieved as early as 1 to 2 cycles, mortality was only 3 to 6% and survival was significantly prolonged (DiNardo et al. 2019).

FLT3 inhibitors represent a new therapeutic option for patients with mutated *FLT3*. *FLT3* inhibitors of class I (midostaurine) or II (gilteritinib, quizartinib) are used as first-line therapy as well as in recurrence. The *FLT3* inhibitor midostaurine, together with conventional chemotherapy, has a positive effect on all risk groups grouped by ELN (Döhner et al. 2020). Studies have shown that second-generation *FLT3* inhibitors in combination with the 7+3 regimen in refractory patients lead to improved CR rates (48% vs. 27%), longer survival (6.2 months vs. 4.7 months) and a higher probability of receiving a stem cell transplant. So far it is questionable whether these inhibitors also have a positive effect in maintenance therapy, although initial study results suggest that they do (DiNardo et al. 2020).

Older patients who do not respond to HMA in first-line therapy have had an adverse prognosis. This prognosis has been improved by the introduction of *IDH* antagonists (*IDH1*-ivosidenib and *IDH2*-enasidinib) for AML patients with *IDH* mutation. Response rates of 29% to 34% and a median survival of 9 months demonstrate the potency of these drugs. Of the patients who responded to an *IDH* antagonist, 50% were still alive after 18 months. In addition, 21% of the patients achieved deep remission of *IDH1* (DiNardo et al. 2020).

Gemtuzumab ozogamicin (GO) is currently used together with the 7+3 regimen as first-line therapy for low and intermediate risk groups classified as ELN. The positive effect occurs mainly with mutations that affect cell signalling (*FLT3*-ITD, *FLT3*-TKD, *NRAS*) and correlate with high CD33 expression (Fournier et al. 2020).

For patients with secondary AML, therapy-associated AML or AML associated with MDS, Vyxeos (CPX-351) represents a new alternative. It consists of a fixed combination of daunorubicin and cytarabine in an optimal 5:1 ratio for the treatment and a liposomal formulation that leads to improved biological accessibility in the body. A group of patients in the intermediate and adverse risk group benefited in several studies in terms of response rate (58% in CR and 55% MRD<10^{3}), overall survival (5.95 vs. 9.56 months) and survival rate after two years (31% vs. 12%).

AML: Recommendation

AML is defined by ≥20% blasts in peripheral blood and/or bone marrow. This differentiates AML from myelodysplastic syndromes. To confirm the diagnosis the following tests are recommended according to the current oncopedia guidelines for AML.

Diagnostic confirmation in case of suspected AML (Oncopedia-LL AML; 10/2019)
Investigations
Medical history and physical examination

Blood count and differential blood count

Bone marrow cytology and cytochemistry

Bone marrow biopsy (absolutely necessary in case of punctio sicca)

Immunophenotyping

Cytogenetics

FISH

(if the cytogenetic analysis is not successful: detection of translocations such as *RUNX1-RUNX1T1*, *CBFB-MYH11*, *KMT2A* and *EVI1*; or loss of chromosome 5q, 7q or 17p)

Molecular genetics (mutations)
- *NPM1*
- *CEBPA*
- *RUNX1*
- *FLT3* (internal tandem duplications (ITD), mutant wild type ratio)
- *FLT3*-TKD (Codon D853 and I836)
- *TP53*
- *ASXL1*

Molecular genetics (gene fusions)
- *PML-RARA*
- *CBFB-MYH11*
- *RUNX1-RUNX1T1*
- *BCR-ABL1*

References

You can find the corresponding references here:
https://www.mll.com/en/diagnostic-offer/acute-myeloid-leukemias/acute-myeloid-leukemia-aml.html#references