Aplastic anemia (AA)

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Continued research and targeted testing of blood and bone marrow are resulting in several diagnostic recommendations for patients with aplastic anemia (AA).

Diagnostic recommendation

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

Definition and characteristics

Acquired aplastic anemias (AA) are characterized by bi- or tricytopenias resulting from hypo- or aplasia in the bone marrow. The annual incidence of the disease in Central Europe is 2-3:1,000,000. Aplastic anemias can occur at any age, with a first peak in patients between 10 and 25 years of age and a second peak in patients over 60 years of age (Marsh et al. 2003, Marsh et al. 2009, Killick et al. 2016).

Patients with aplastic anemia typically initially experience benign oligoclonal hematopoiesis due to a reduction in the stem cell pool as a result of immune-mediated pathogenesis, in which autoreactive T cells appear to play a major role (Marsh et al. 2009, Ogawa 2016). During the pathogenesis of aplastic anemia, the majority of bone marrow cells are replaced by adipocytes (Young 2018). In contrast, MDS are hematopoietic stem cell disorders with monoclonal hematopoiesis displacing normal polyclonal hematopoiesis (Afable et al. 2011).

To diagnose aplastic anemia, exclusion of other causes is essential. These include acquired cytopenias, for example, viral or drug-induced, as well as congenital syndromes with bone marrow insufficiency or hypoplastic **myelodysplastic syndrome (MDS)** (Marsh et al. 2003, Marsh et al. 2009, Killick et al. 2016). Diagnostics should allow for such exclusion and therefore include cytomorphology, histology, and cytogenetics from bone marrow in all cases.

Classification

Aplastic anemia can be classified into different severity levels, which are listed in Table 1:

Table 1: Classification of aplastic anemia (two of three criteria must be met) (Onkopedia Leitlinie AA 2018).

	Non-severe AA	**Severe AA**	**Very severe AA**
Neutrophil count	< 1,0 G/L	< 0,5 G/L	< 0,2 G/L
Platelets	< 50 G/L	< 20 G/L	< 20 G/L
Reticulocyte count	< 20 G/L	< 20 G/L	< 20 G/L

To diagnose very severe AA, < 0.2 G/L neutrophil granulocytes and hypocellular bone marrow must be present (cellularity < 25% or 25-50% with < 30% hematopoietic cells in the bone marrow), whereas evidence of hypocellular bone marrow is sufficient to diagnose non-severe AA (Onkopedia guideline AA 2018).

Diagnostics

Cytomorphology

Cytomorphology and histology are mandatory for reliable diagnosis and differentiation from acute leukemias and MDS. In principle, a bone marrow biopsy (1.5 - 2 cm biopsy cylinder) and histological examinations must be performed for every bone marrow aspiration in which insufficient material has been obtained for a definite diagnosis or in the case of *punctio sicca*. This generally applies to aplastic anemias.

Immunophenotyping

Immunophenotyping is used to clarify the question of a PNH subclone (paroxysmal nocturnal hemoglobinuria). When cytomorphology and especially histology are inconclusive in aplastic anemia, immunophenotyping may have significance for differential diagnosis.

Chromosome analysis

About half of the cases with aplastic anemia show genetic abnormalities
Genetic alterations detected by sequencing and SNP array analyses are present in approximately 50% of patients with aplastic anemia (Yoshizato et al. 2015, Ogawa 2016). Cytogenetic abnormalities have been described in 5-15% (Gupta et al. 2006, Kim et al. 2010) of adult patients with severe aplastic anemia (SAA), and these patients were generally younger (median age 32 years) than patients with normal karyotype (median age 67 years) (Kim et al. 2010).

Common cytogenetic abnormalities in aplastic anemia
Typical cytogenetic abnormalities in aplastic anemia include:
- Trisomy 8
- 7q deletion
- monosomy 7
- trisomy 6
- 13q deletion

Trisomy 8 and changes in chromosome 7 (7q deletions or monosomy 7) have been detected most frequently (Maciejewski et al. 2002, Maciejewski & Selleri 2004, Kim et al. 2010, Kulasekararaj et al. 2014). Furthermore, trisomy 6 represents a frequent abnormalities, whereby this is usually detected at initial diagnosis and does not only occur during the course (Keung et al. 2001, Maciejewski & Selleri 2004). Deletions in the long arm of chromosome 13 (13q deletion) have also been described many times (Solé et al. 2000, Maciejewski et al. 2002, Maciejewski & Selleri 2004, Kulasekararaj et al. 2014).

In addition, various other cytogenetic alterations such as deletions in the long arm of chromosome 1, an isochromosome 17q, and gains (such as trisomy 15) or losses (such as monosomy 21) of whole chromosomes are also found (Kim et al. 2010, Kulasekararaj et al. 2014).

FISH

Often, the cytogenetic abnormalities are found only in subclones and can only be detected by FISH on interphase nuclei. Genomic array analyses can detect further chromosomal abnormalities not detectable by chromosome band analysis and FISH analysis, such as cytogenetically cryptic microdeletions and microduplications, as well as regions with LOH without copy number alteration ("copy number neutral loss of heterozygosity", CN-LOH). In particular, cytogenetically cryptic deletions as well as CN-LOH of the HLA-A locus in chromosome bands 6p22.1-6p21.33 could be detected in several patients, which might be related to the immune-mediated pathogenesis of aplastic anemia (Afable et al. 2011, Betensky et al. 2016).

Molecular genetics

In aplastic anemia, mutations occur that are also observed in MDS, but with different frequencies. Most commonly, mutations are found in the *BCOR*, *BCORL1*, *DNMT3A*, *PIGA*, and *ASXL1* genes (Yoshizato et al. 2015). In addition, an association between the presence of a mutation in the *ASXL1* or *DNMT3A* genes and an increased risk of transformation to MDS or AML, respectively, has been demonstrated (Kulasekararaj et al. 2014).

Prognosis

Good prognosis in trisomy 8 and 13q deletions
Patients with trisomy 8 show a better response to immunosuppressive therapy, a lower leukemic transformation rate, and better overall survival than patients with abnormalities on chromosome 7. This alteration is associated with a poor response to immunosuppressive therapy, persistent pancytopenia, and an adverse prognosis. Deletions in the long arm of a chromosome 13 (13q deletion), like trisomy 8, are associated with more stable blood counts and a good response to immunosuppressive therapy (Ishiyama et al. 2002, Maciejewski et al. 2002, Maciejewski & Selleri 2004, Kim et al. 2010).

Genetic alterations influence transformation rate and response to therapy
The risk of leukemic transformation is significantly increased when cytogenetic alterations are detected in chromosome banding analysis (see Fig. 1).

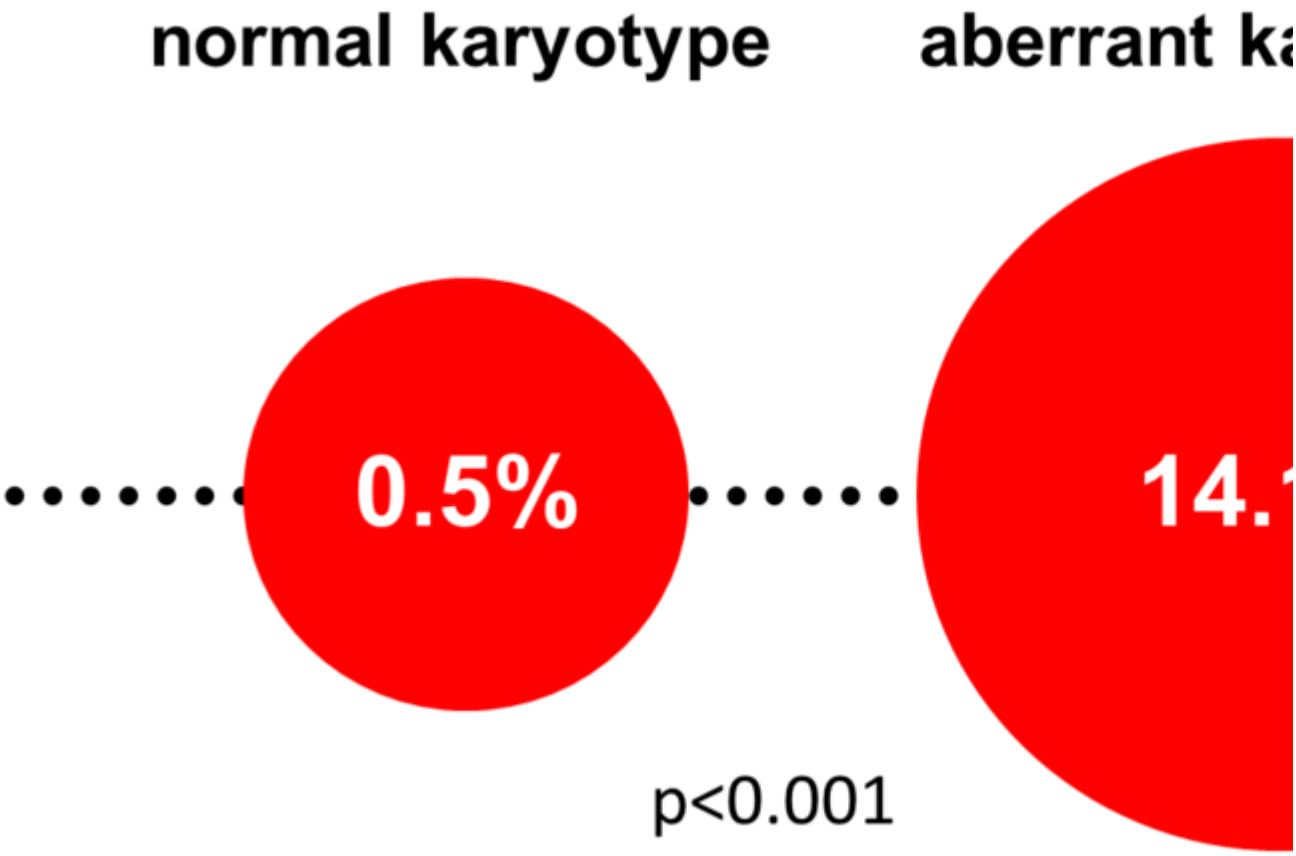

Figure 1: Cumulative probability of leukemic transformation within 5 years in AA with normal versus aberrant karyotype (created after Kim et al. 2010).
Response to immunosuppressive therapy may be worse (-7, complex karyotype, 5q syndrome) or better (+8, -13q) depending on the type of cytogenetic alteration (Maciejewski & Selleri 2004, Kim et al. 2010).

Clonal evolution to MDS occurs in up to 25% of patients with aplastic anemia within a 10-year period. Whether clonal evolution is a late complication in the pathogenesis of aplastic anemia or whether occurring myelodysplastic syndromes are related to immunosuppressive therapy remains unclear to date (Maciejewski & Selleri 2004, Afable et al. 2011).

In addition to cytogenetic alterations, molecular aberrations such as mutations of the *ASXL1* and *DNMT3A* genes may also increase the risk of developing MDS or AML (see Molecular genetics) (Kulasekararaj et al. 2014).

Therapy

With the help of immunosuppressive therapies (IST), approximately 50% of patients with aplastic anemia achieve remission, with significantly higher response in patients with age ≤ 67 years (p=0.002), coexistence of a PNH clone (p=0.017), and normal karyotype (p=0.024) (Maciejewski & Selleri 2004, Kim et al. 2010). Patients with mutations of the *PIGA*, *BCOR* and *BCORL1* genes also respond significantly better to IST (Yoshizato et al. 2015, Ogawa 2016).

Recommendation

Differentiation from MDS important for prognosis
Differentiation of aplastic anemia from hypoplastic MDS is important for prognosis assessment as well as for the choice of therapeutic strategies despite the partially overlapping symptoms (see also Fig. 2), but morphologic analyses are challenging because of the cell-poor bone marrow.

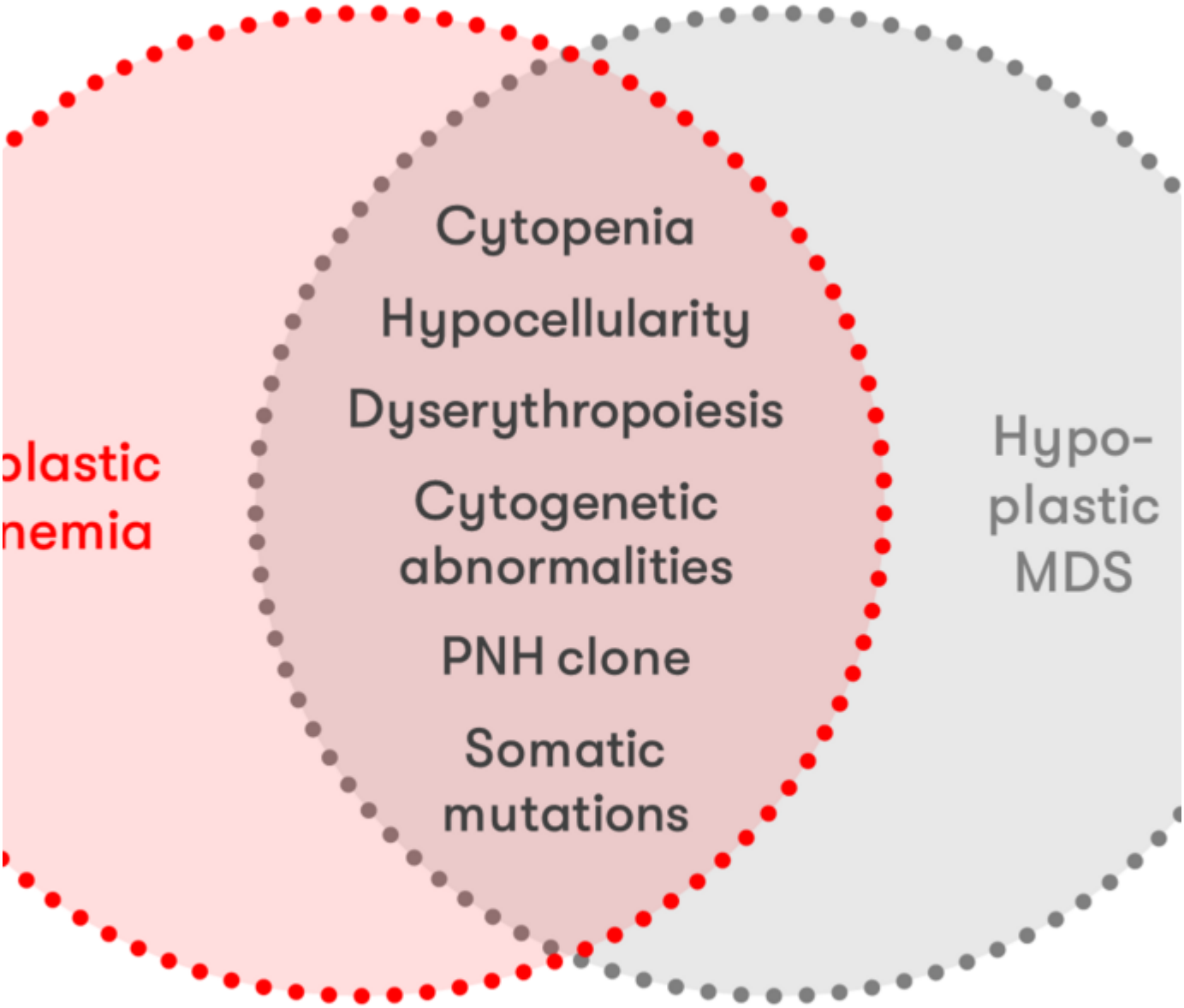

Fig. 2: Overview of common symptoms in AA and hypoplastic MDS (modeled after Mufti et al. 2018).
Therefore, histological examination on a sufficiently large bone marrow cylinder (at least 15 mm biopsy length) should definitely be performed to establish the diagnosis. Indications of clonal evolution in the course of aplastic anemia are an increased blast percentage, hypercellular bone marrow in the presence of recurrent or persistent cytopenia, and the appearance of new cytogenetic or molecular genetic abnormalities (Maciejewski & Selleri 2004, Afable et al. 2011).

Other differential diagnoses include **clonal cytopenia of undetermined significance (CCUS)** and paroxysmal nocturnal hemoglobinuria (PNH). In CCUS, the bone marrow is hypo- to hypercellular, and somatic mutations are more frequent than in AA, particularly affecting the genes *TET2*, *DNMT3A*, *ASXL1*, *TP53*, and splicing factors, among others; therefore, molecular genetic analyses could also aid diagnosis in this case. AA and PNH can each develop into each other (AA/PNH syndrome). Major clues for the presence of PNH or a PNH clone are CD59-negative cells in immunophenotyping and mutations of the *PIGA* gene (Ogawa 2016, Bejar 2020).

Because patients with somatic mutations are at higher risk of developing MDS, early molecular genetic diagnosis may be informative. This is also supported by the improved response to IST in mutations of the *PIGA*, *BCOR*, and *BCORL1* genes, which may contribute to treatment decisions (Kulasekararaj et al. 2014, Yoshizato et al. 2015).

References

You can find the corresponding references here:
https://www.mll.com/en/diagnostic-offer/others/aplastic-anemia-aa.html#referenzen