Clonal cytopenia of undetermined significance (CCUS)

Status: May 2020

Diagnostic recommendation

Method	Anticoagulant	Recommendation
Cytomorphology	EDTA	mandatory
Immunophenotyping	-	no
Chromosome analysis	Heparin	mandatory
FISH	EDTA or Heparin	no
Molecular genetics	EDTA or Heparin	mandatory

Definition and characteristics

If an acquired mutation or chromosomal change is detected in patients with unexplained cytopenia and the absence of cytomorphological features of a myeloid disease, clonal cytopenia of undetermined significance (CCUS) is present (Steensma et al. 2015, Valent et al. 2017). Depending on the cytogenetic change observed, MDS of the category "MDS, unclassifiable" may also be present (**CCUS, diagnostics, chromosome analysis**).

Classification

Characteristics of CCUS
(Steensma et al. 2015, Bejar Leukemia et al. 2017, Greenberg et al. 2020)

- Evidence of clonal haematopoiesis*
- Absence of dysplasia of hematopoiesis in bone marrow
- Persistent cytopenia
- No proliferation of blasts in bone marrow/blood

*Detection of somatic mutations in genes associated with myeloid neoplasm with an allele frequency ≥ 2% and/or clonal chromosomal abnormality

Delineation from CHIP and MDS
Clonal cytopenia of indeterminate significance must be distinguished from **clonal hematopoiesis of indeterminate potential (CHIP)**, in which acquired mutations or acquired chromosomal changes are also detectable but cytopenia is not present.

Furthermore, according to the WHO classification 2017, a distinction must be made between **MDS** and MDS of the category "MDS, unclassifiable" (MDS-U). This is diagnosed in the presence of persistent cytopenia, certain % limits of blasts in the peripheral blood/bone marrow and certain cytogenetic alterations (**see CCUS, Diagnostics, Chromosome analysis**) even in the absence of morphological features necessary for the diagnosis of MDS. Patients diagnosed with MDS that cannot be further classified should be carefully monitored for disease progression into a more specific MDS subtype.

Diagnostics

Cytomorphology

Cytomorphologically, the criteria for the diagnosis of MDS in CCUS are not met, as dysplasias are absent or minimal (< 10%). The proportion of blasts in the bone marrow is less than 5%.

Chromosome analysis

In chromosome analysis, clonal chromosomal abnormalities are rarely found. However, if one of the following chromosomal abnormalities is observed in the presence of persistent cytopenia (see Table 1), a diagnosis of MDS of the category "MDS, unclassifiable, due to certain cytogenetic abnormality" would be made, even in the absence of MDS-defining dysplasias.

Table 1: Chromosomal abnormality that define an entity for "MDS, unclassifiable (MDS-U), due to certain cytogenetic abnormalities" even in the absence of MDS-defining dysplasias (Swerdlow et al. 2017)

Chromsomal abnormality	
unbalanced	**balanced**
Gain of chromosome 8[a]	t(11;16)(q23.3;p13.3)
Loss of chromosome 7 or del (7q)	t(3;21)(q26.2;q22.1)
del(5q)	t(1;3)(p36.3;q21.2)
del(20q)[a]	t(2;11)(p21;q23.3)
Loss of Y-chromosome[a]	inv(3)(q21.3q26.2) oder t(3;3)(q21.3;q26.2)
Isochromosome 17q or t(17p)	t(6;9)(p23;q34.1)
Loss of chromosome 13 or del(13q)	
del(11q)	
del(12p) or t(12p)	
del(9q)	
idic(X)(q13)	

[a]As a sole cytogenetic abnormality in the absence of morphological criteria, gain of chromosome 8, del(20q) and loss of Y chromosome are not considered definitive evidence of MDS; in the setting of persistent cytopenia of undeterminded origin, the other abnormalities shown in this table are considered presumptive evidence of MDS, even in the absence of definitive morphological features. However, initial studies show that against

the background of cytopenia, trisomy 8 or 20q deletion is associated with an increased risk of developing myeloid neoplasm (Jawad et al. 2016, Petrova-Drus et al. 2017, Bewersdorf et al. 2019).

FISH

If it is not possible to carry out a chromosome analysis, clonality can also be detected by means of FISH. Here too, it should be noted that the simultaneous presence of cytopenia and certain cytogenetic changes (see Table 1) can define an "MDS, unclassifiable, due to certain cytogenetic abnormalities".

Molecular genetics

The distinction between CHIP, CCUS and MDS cannot be made on the basis of molecular genetic examinations, but is currently based on differences in the presence of cytopenia and, for MDS, diagnostic morphological or cytogenetic criteria (see also Table 1).

A smooth transition between CHIP, CCUS and MDS is assumed to be likely (Bejar Leukemia 2017). Thereby, the genetic complexity with regard to the mutation load and the number of mutations increases (Cargo et al. 2015, Bejar Curr Opin Hematol. 2017, Malcovati et al. 2017, Bewersdorf et al. 2019). The genes mutated in CCUS correspond to those also affected in CHIP and MDS. However, the mutation landscapes differ with regard to the frequently occurring mutations (see Table 2) (Bejar Curr Opin Hematol. 2017).

Table 2: Comparison of genetic characteristics between CHIP, CCUS and MDS, according to Bejar Curr Opin Hematol. 2017

	CHIP (unselected population)	CCUS (at diagnosis)	MDS (all risk groups)
Commonly Mutated Genes	*DNMT3A*, *TET2*, *ASXL1*, *PPM1D*, *JAK2*, *TP53*	*TET2*, *DNMT3A*, *ASXL1*, *SRSF2*, *TP53*	*SF3B1*, *TET2*, *ASXL1*, *SRSF2*, *DNMT3A*
Mean # of Mutations	~1	~1,6	~2,6
Typical VAF	9-12%	30-40%	30-50%

Compared to CCUS, MDS is more complex in molecular genetic terms: there are usually two or more mutations and the mutation load is usually more than 10% (Haferlach et al. 2014, Malcovati et al. 2017, Sperling et al. 2017). Since mutations accumulate during progression, it is recommended that investigations be carried out during the course of the progression in question cases (e.g. Steensma et al. 2015).

Clinical benefit of molecular diagnostics in unexplained cytopenia

Molecular genetic diagnostics using directed panel testing is gaining in importance for the clarification of unclear cytopenia (Malcovati et al. 2017, Baer et al. 2018, Shanmugam et al. 2019, Zheng et al. 2019). Even the detection or exclusion of clonality has prognostic relevance (see also Prognosis) and benefit for differential diagnosis.

These studies show a high negative predictive value (Malcovati et al. 2017, Shanmugam et al. 2019, Zheng et al. 2019): If no mutation can be detected by directed sequencing, the probability that myeloid neoplasm will be diagnosed at the time of examination or in follow-up is low (Malcovati et al. 2017, Shanmugam et al. 2019). In the future, a molecular genetic examination of the peripheral blood could thus potentially contribute to the decision for or against a more invasive bone marrow biopsy (Shanmugam et al. 2019).

Mutation detection is associated with an increased probability that haematological neoplasm exists or occurs later. The positive predictive value for the presence of myeloid neoplasm can be maximised if a higher allele frequency (≥ 10% or ≥ 20%) and/or the presence of ≥ 2 gene mutations are used as criteria (Malcovati et al. 2017, Shanmugam et al. 2019, Zheng et al. 2019). Certain mutations are also predictive of MDS - these include in particular mutations in spliceosome factors (Malcovati et al. 2017, Shanmugam et al. 2019) or *RUNX1* and *JAK2* (Malcovati et al. 2017). If *DNMT3A*, *TET2* or *ASXL1* mutations are present in combination with at least one other mutation, this mutation pattern is also predictive for an MDS (Malcovati et al. 2017).

While currently it is also being evaluated how many genes need to be examined for the most accurate prediction of myeloid neoplasm (Malcovati et al. 2017, Shanmugam et al. 2019, Zheng et al. 2019), the National Comprehensive Cancer Network (NCCN) has so far recommended that mutation analysis be performed for the following MDS-associated genes in suspected cases of MDS or in the presence of unexplained cytopenia: *TET2*, *DNMT3A*, *ASXL1*, *EZH2*, *SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*, *RUNX1*, *TP53*, *STAG2*, *NRAS*, *CBL*, *NF1* (Greenberg et al. 2020).

Prognosis

The study by Malcovati et al. shows that there is a highly predictive mutation pattern for myeloid neoplasm among patients with cytopenia (Blood 2017). These include, as mentioned above, mutations in spliceosome genes (*SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*), *RUNX1* and *JAK2* as well as mutations in *ASXL1*, *DNMT3A* and *TET2*, each in combination with at least one other mutation. The risk of progression of CCUS to myeloid disease was also investigated in this study: a risk of progression of approximately 20% per year was present in patients with cytopenia and the above-mentioned patterns. The detection of other mutations or constellations of mutations in persistent cytopenia also speaks for the presence of CCUS, but with a lower risk of progression into myeloid neoplasm (risk of progression of about 10% per year).

Figure 1: Differential diagnosis of unexplained cytopenia. If both myeloid neoplasms and reactive/secondary forms can be excluded as the cause of cytopenia, the diagnosis is cytopenia of uncertain significance (CUS). The detection of mutations is of prognostic relevance in this context. The risk of progression in patients with clonal cytopenia of indeterminate significance (CCUS) depends on the mutations or mutation patterns present.
Our own data from our laboratory (Baer et al. 2018) also confirm that mutations occur in 21% of patients without morphological abnormalities. This means that mutation diagnostics can provide valuable information both in the context of cytomorphologically/cytogenetically confirmed MDS and in the context of unexplained cytopenia.

Mutation detection therefore has either prognostic significance or an influence on the probability of developing myeloid neoplasm. If no mutation can be detected, the risk of developing a haematological disease is considered low.

References

You can find the corresponding references here:
https://www.mll.com/en/diagnostic-offer/others/clonal-cytopenia-of-undetermined-significance-ccus.html#references