BCR-ABL1-negative myeloproliferative neoplasms (MPN) – Overview

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In this review article, you will learn important information about the differential diagnosis of *BCR-ABL1*-negative myeloproliferative neoplasms (MPN), as well as their classification, diagnostics, and prognosis.

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

Myeloproliferative neoplasms (MPNs) are rare, clonal diseases of the hematopoietic stem cell that share many common features, which is why they are often difficult to distinguish from one another, especially in the early stages, and can also merge in individual cases. The annual incidence of all MPN subtypes combined is approximately 6/100,000 (Swerdlow et al. 2017). MPNs usually affect people of older age with a median of 60-65 years (Barbui 2012). Characteristic of MPN is hypercellularity of the bone marrow, specifically the myeloid cell series, and increased numbers of erythrocytes, granulocytes, and/or platelets in the peripheral blood, depending on the entity (Haferlach et al. 2008, Swerdlow et al. 2017).

MPNs are basically divided into **chronic myeloid leukemia (CML)** as *BCR-ABL1*-positive MPNs and *BCR-ABL1*-negative myeloproliferative neoplasms (Haferlach et al. 2008, Swerdlow et al. 2017).

Classification

The classification into *BCR-ABL1*-positive CML and *BCR-ABL1*-negative myeloproliferative neoplasms is according to the WHO classification (see Table 1) (Swerdlow et al. 2017).

Table 1: MPN WHO classification 2017. (Swerdlow et al., 2017)

Myeloproliferative Neoplasms (MPN)	
Chronic myeloid leukemia (CML)	*BCR-ABL1*-positive
Chronic neutrophilic leukemia (CNL)	*BCR-ABL1*-negative
Polyzythemia vera (PV)	
Primary myelofibrosis (PMF) • prefibrotic stage • Overt fibrotic stage	
Essential thrombocythemia (ET)	
Chronic eosinophilic leukemia, not otherwise specified (CEL, NOS)	
MPN, unclassifiable (MPN-U)	

The three entities **polycythemia vera (PV)**, essential thrombocythemia (ET) and **primary myelofibrosis (PMF)** are often also grouped under the term "classic" *BCR-ABL1*-negative MPN. Primary myelofibrosis was also previously referred to as osteomyelofibrosis (OMF) or chronic idiopathic myelofibrosis (CIMF).

In contrast to **CML**, which is clearly defined by the presence of a *BCR-ABL1* rearrangement or a Philadelphia chromosome, *BCR-ABL1*-negative myeloproliferative disorders are a very heterogeneous group of diseases from a cytogenetic and molecular genetic perspective. This is mainly reflected in the frequency and nature of clonal chromosomal abnormalities as well as molecular genetic abnormalities.

Diagnostics

Cytomorphology

Cytomorphologic assessment in MPNs involves cellularity in the whole as well as in individual hematopoietic series. It is also important to determine the proportion of blasts in the blood and bone marrow. If fibrosis of the bone marrow exists, e.g. in primary myelofibrosis (PMF), the cytomorphological assessability of the preparations is often limited (punctio sicca).

In all cases, the histology is decisive for the assessment of the degree of fibrosis and the bone marrow architecture, which should always be performed in the case of a suspected or confirmed MPN.

Chromosome analysis

Chromosomal abnormalities occurring in MPN present very heterogeneously. Chromosomal abnormalities are most frequently observed in PMF (40%), followed by PV (35%), whereas abnormalities in ET are rarely found (~ 3%). Over 80% of chromosomal abnormalities in MPN are unbalanced alterations (Bacher et al. 2005).

Across MPNs, trisomy 8, trisomy 9, and trisomy 9p are found. Deletion in the long arm of a chromosome 20 (del(20q)) also represents a typical abnormalities. Other cytogenetic abnormalities in MPN include loss of the Y chromosome (-Y) and gain of a chromosome 19 (+19) (Bacher et al. 2005, Gangat et al. 2008, Haferlach et al. 2008, Sever et al. 2009, Swerdlow et al. 2017, Tefferi et al. 2018).

Trisomy 8 (+8) represents the most common clonal change (~20% of PV cases, 10% of PMF cases) (Haferlach et al. 2008). However, the presence of trisomy 8 allows only limited conclusions about the type of disease, as it also occurs in MDS and AML. Other abnormalities in MPN affect chromosome 7 (-7, -7q) and often occur in combination with *JAK2* mutations (Dunlap et al. 2011).

Deletions in the long arm of a chromosome 13 (del(13q)) and gain of material of the long arm of chromosome 1 (+1q) also occur in PV and PMF (Bacher et al. 2005). In PMF, this change affects 6 and 10% of cases, respectively (Bacher et al. 2005). Deletions in the short arm of chromosome 12 (del(12p)) are also observed in PV and PMF.

Trisomy 9 or trisomy 9p is one of the most common cytogenetic abnormalities in PV and is observed in approximately 10% of cases (Haferlach et al. 2008). It is virtually always associated with a homozygous V617F mutation in the *JAK2* gene.

Table 2: Overview of chromosomal abnormalities in classical *BCR-ABL1*-negative MPN (Bacher et al. 2005, Gangat et al. 2008, Haferlach et al. 2008, Sever et al. 2009, Swerdlow et al. 2017, Tefferi et al. 2018)

Abnormalities	PMF	PV	ET
+8	+	+	+
del(20q)	+	+	+
-Y	+	+	+
+9	+	+	+
del(5q)	+	+	+
del(13q)	+	+	
+1q	+	+	
del(12p)	+	+	
del(9p)		+	+
del(9q)			+
+19	+		
-7/-7q	+		
partial +1q	+		

FISH

Im Rahmen der Diagnostik der MPN ist die FISH-Analyse überwiegend als ergänzende Methode zur klassischen Chromosomenanalyse zu sehen. Sie wird gezielt zur Beantwortung bestimmter Fragestellungen eingesetzt. Zum Ausschluss eines *BCR-ABL1*-Rearrangements als sichere Abgrenzung gegenüber der CML sollte bei Verdacht auf MPN immer eine FISH-Analyse und/oder eine PCR durchgeführt werden, da auch beim Fehlen einer Philadelphia-Translokation in der Chromosomenanalyse ein kryptisches *BCR-ABL1*-Rearrangement vorliegen kann (sog. Philadelphia-negative *BCR-ABL1*-positive CML).

Mittels eines FISH-"Screenings" an Interphase-Kernen würde nur ein Bruchteil der bei der MPN möglichen Aberrationen erfasst werden. Daher kann eine FISH die klassische Chromosomenanalyse nicht ersetzen.

Molecular genetics

Today, molecular genetics is the most important tool in MPN diagnostics. It is used to exclude a *BCR-ABL1* rearrangement and is also used to differentiate an MPN from a reactive alteration and to diagnose progression. For this purpose, a large number of mutations (especially *JAK2 V617F*, *MPL*, *CALR*) have been identified in the last ten years (see Table 3).

Table 3: Frequency of the different mutations in PV, ET and PMF (Tefferi 2018).

Gene	Frequency (%)		
	PV	ET	PMF
JAK2	96	55	65
JAK2 Exon 12	3	N/A	N/A
CALR	0	20	25
MPL	N/A	3	10
TET2	16	5	17
ASXL1	N/A	3	13
IDH1/2	2	1	4
EZH2	3	N/A	7
DNMT3A	7	N/A	7
CBL	rare	rare	6
TP53	N/A	N/A	4
SF3B1	N/A	N/A	7
SRSF2	N/A	N/A	17
U2AF1	N/A	N/A	16

In familial MPN, mutations in the *VHL*, *EPOR*, *EPAS1* and *EGLN1* genes can occasionally be found. These mutations are very rare and should be investigated when all other markers are negative, the patient is very young, and there is a positive family history (Jones & Cross 2013, Rumi & Cazzola 2017, McMullin 2019).

JAK2 V617F mutation plays central role in MPN diagnostics

The V617F mutation in exon 14 of the *JAK2* gene (Janus kinase 2) has assumed a central role in the diagnosis of MPN. This involves an exchange of guanidine for thymidine at nucleotide position 1849, resulting in a substitution of the amino acid valine at position p.617 for phenylalanine (Baxter et al. 2005, James et al. 2005, Kralovics et al. 2005, Levine et al. 2005, Bench et al. 2013, Vainchenker & Kralovics 2017). This mutation, resulting in increased kinase activity, is found in approximately 96% of all PV patients. V617F mutations are also found in approximately 50% of all ET and PMF. In general, advanced disease appears to be associated with a higher mutation burden (i.e., mutation/wild-type ratio), with the highest mutation burdens found in PV and the lowest in ET. Homozygous V617F mutations arising from uniparental disomy are more common in PV than in ET (Stein et al. 2015).

JAK2 Exon 12-Mutationen bei V617F-negativer PV

Bei etwa 80% aller V617F-negativen PV-Fälle finden sich Mutationen im Exon 12 des *JAK2*-Gens, die im Gegensatz zur V617F-Mutation heterogen sein können (Langabeer et al. 2015, Stein et al. 2015, Geyer & Orazi 2016). Diese Mutationen sind meistens Insertionen oder Deletionen im Bereich der Aminosäurereste 533-547 (Langabeer et al. 2015), wobei seltener auch Punktmutationen (Missensemutationen) oder größere Insertionen vorliegen können (Butcher et al. 2008, Pietra et al. 2008, Langabeer et al. 2015, Vainchenker & Kralovics 2017). Über die Hälfte aller Patienten mit *JAK2* Exon 12-Mutationen weisen nicht den klassischen PV-Phänotyp auf, sondern imponieren als isolierte Erythrozytosen (Pardanani et al. 2007, Scott et al. 2007, Williams et al. 2007, Pietra et al. 2008, Passamonti et al. 2011). Die klinische Symptomatik erscheint weniger ausgeprägt, jedoch liegen aufgrund der insgesamt geringen Häufigkeit bisher keine sicheren Daten zur Prognose vor.

MPL and _CALR_ mutations in JAK2 V617F-negative PMF or ET

The *MPL* gene encodes the thrombopoietin receptor and is a frequently mutated gene in *JAK2* V617F-negative patients, although the relative mutation frequency varies widely between different studies. Up to 16% have been described in PMF patients and 0-15% in ET patients (Levine & Wernig 2006, Pikman et al. 2006, Beer et al. 2008, Pietra et al. 2011). Mutations in the *MPL* gene are almost always located at position W515, resulting in an exchange of the tryptophan for leucine (W515L), lysine (W515K) or more rarely arginine (W515R), serine (W515S) or alanine (W515A). Rarely, S505N mutations are also found (Pardanani et al. 2006, Pikman et al. 2006, Tefferi 2008, Langabeer et al. 2015). These mutations also lead to constitutive activation of the JAK-STAT signaling pathway.

Overall survival of *JAK2* V617F and *MPL* W515 mutated patients does not differ (Klampfl et al. 2013). Patients with *MPL* mutation tend to show lower hemoglobin levels. ET patients with *MPL* mutation also show higher platelet levels, isolated megakaryocyte proliferation, and higher serum erythropoietin levels compared to patients with *JAK2* V617F mutation (Langabeer et al. 2015). Analogous to the *JAK2* V617F mutation, *MPL* mutations generally have a higher mutational burden in PMF than in ET and may increase with disease progression (Rumi et al. 2013).

Mutations in the calreticulin gene (*CALR*) are found in up to 70% of patients with essential thrombocythemia and in 56-88% with myelofibrosis in

TET2 mutations occur in MPN independent of _JAK2_ mutation status

Mutations in the *TET2* gene, a member of the *TET* oncogene family, have also been described in 10-20% of each PV and PMF and approximately 5% of ET, which can occur independently of *JAK2* mutation status (Langabeer et al. 2015). *TET2* mutations are found upstream or downstream of *JAK2* mutations in the developmental sequence and represent early or even late events in transformation in MPN. Because *TET2* mutations are

found in all MPN types, they do not allow discrimination between entities. However, they can be helpful in differentiating clonal disease from reactive transformation (Stein et al. 2015).

Prognosis

In addition to increased age, leukocytosis, and thrombosis (Gangat et al. 2011, Barbui et al. 2018), in general, the detection of chromosomal abnormalities at diagnosis of an MPN seems to be associated with a less favorable prognosis. The presence of complex karyotypes during the course of an MPN increases the likelihood of transition to blast crisis (Haferlach et al. 2008). SNP array analyses showed clustered deletions of the genes *ETV6* (Chr. 12), *TP53* (Chr. 17), or *RUNX1* (Chr. 21) in blast crisis (Thoennissen et al. 2010).

Gene mutations influence the risk profile of "classic" *BCR-ABL1*-negative MPN
The *JAK2* V617F mutation is the most common mutation in MPN. PV is almost always associated with this mutation (97%). In addition to the *JAK2* V617F mutation, mutations in the *CALR* (20-25%) and *MPL* (3-8%) genes often occur in ET and PMF. These mutations are among the driver mutations in MPN, but do not allow a specific diagnosis due to their cross-entity occurrence.

81% of PMF patients, 53% of PV patients, and 53% of ET patients show additional mutations (non-driver mutations) in addition to the driver mutations, including the genes *SRSF2*, *ASXL1*, *IDH2*, *EZH2*, *TP53*, *U2AF1*, *CBL*, *SF3B1* and *SH2B3* (see also Fig. 1). *IDH2* mutations are classified as a risk factor in all three classic *BCR-ABL1*-negative MPNs (Tefferi et al. 2016, Tefferi & Vannucchi 2017). Furthermore, each of the three entities has an entity-specific risk profile, an overview is given in Figure 1.

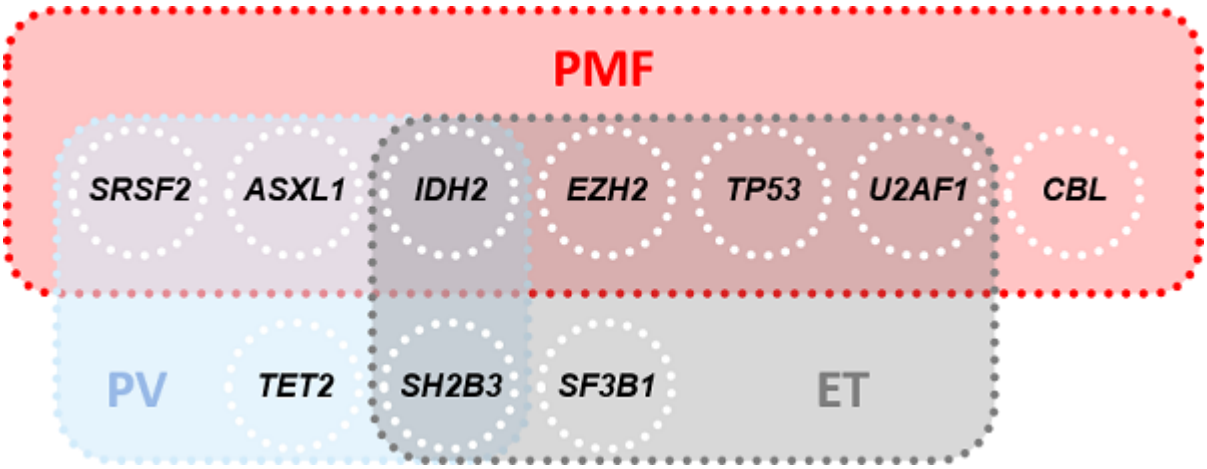

Figure 1: Recurrent non-driver gene mutations in PMF, PV and ET with prognostic impact (adapted from Tefferi et al. 2016, Tefferi & Vannucchi 2017).
Recurrent mutations in PMF patients frequently affect the *SRSF2*, *ASXL1*, *IDH2*, *EZH2*, *TP53*, *U2AF1*, and *CBL* genes. These gene mutations are prognostically significant and the presence of two or more mutations worsens prognosis (Guglielmelli et al. 2011, Vannucchi et al. 2013, Guglielmelli et al. 2014, Tefferi et al. 2014).

In PV patients, recurrent mutations include *SRSF2*, *ASXL1* and *IDH2*, each of which is associated with decreased overall survival and myelofibrosis/leukemia-free survival (Tefferi et al. 2016, Tefferi & Vannucchi 2017).

Approximately 15% of ET patients have a mutation in at least one of the *IDH2*, *EZH2*, *TP53*, *U2AF1*, *SF2B1* or *SH2B3* genes. These mutations are considered risk factors and are associated with reduced overall survival or myelofibrosis- and leukemia-free survival (Tefferi & Vannucchi 2017).

For a detailed insight into the individual MPNs and their genetic risk profiles, please refer to the infotexts of the respective entity:
- Essential thrombocythemia (ET)
- Polycythemia vera (PV)
- Primary myelofibrosis (PMF)
- Chronic eosinophil leukemia, not further specified (CEL, NOS)

References

You can find the corresponding references here:
https://www.mll.com/en/diagnostic-offer/myelodysplastic-syndrome-mds/myeloproliferative-neoplasm-mpn/bcr-abl1-negative-myeloproliferative-neoplasms.html#referenzen