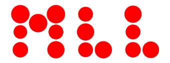


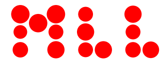
Primary myelofibrosis (PMF)

Status: July 2020

Here you can find information about the WHO classification for primary myelofibrosis and criteria for the diagnosis and prognosis of PMF.

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 of primary myelofibrosis

Primary myelofibrosis (PMF) is a myeloproliferative, *BCR-ABL1*-negative neoplasm. It is characterized by a dominant proliferation of megakaryocytes and granulocytes in the bone marrow and shows increasing reticulin and/or collagen fibrosis in advanced stages. The incidence of primary myelofibrosis is 0.5-1.5 / 100,000 per year and occurs predominantly at the age of 60-70 years.

Classification of primary myelofibrosis

According to the WHO classification 2017, primary myelofibrosis is classified as myeloproliferative neoplasm (MPN) and is divided into a prefibrotic/early stage and a fibrotic stage.

Primary myelofibrosis - WHO Classification 2017

Myeloproliferative Neoplasm (MPN)
Primary myelofibrosis (PMF):
- Prefibrotic/early stage
- Fibrotic stage

Diagnostic criteria for primary myelofibrosis
According to the WHO classification, all 3 major criteria and at least 1 minor criterion of the diagnostic criteria must be met in order to make a diagnosis of primary myelofibrosis (overt fibrotic stage).

Major criteria for primary myelofibrosis
- Megakaryocyte proliferation and atypia, accompanied by reticulin and/or collagen fibrosis grades 2 or 3 according to WHO
- WHO criteria for ET, PV, *BCR-ABL1*-positive CML, MDS or other myeloid neoplasm are not met
- *JAK2*, *CALR* or *MPL* mutation, or in the absence of these mutations, detection of another clonal marker*, or absence of minor reactive bone marrow reticulin fibrosis**

Minor criteria for primary myelofibrosis
At least 1 of the following criteria are met in 2 consecutive determinations
- anaemia not attributed to a comorbid condition
- Leukocytosis ≥ 11 x 10^9 /l
- Palpable splenomegaly
- LDH level above the upper limit of normal (ULN) of the institutional reference range
- Leukoerythroblastosis

* e.g. *ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*
** bone marrow fibrosis due to secondary infection, autoimmune disease or other chronic inflammatory diseases, hairy cell leukemia or other lymphoid neoplasm, metastatic tumor disease or toxic (chronic) myelopathy

Facts

Approx.
60%

of patients with PMF have a *JAK2* V617F mutation
(Onkopedia Guideline PMF)

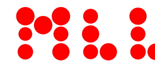
Diagnostics of primary myelofibrosis

Cytomorphology

The cytomorphological assessment in MPN involves cellularity in the total as well as in the individual hematopoietic lines. It is also important to determine the proportion of blasts. For special questions (in case of suspected refractory anaemia with ring sideroblasts and thrombocytosis; MDS/MPN-RS-T) iron staining is also relevant.

If there is fibrosis of the bone marrow, e.g. in primary myelofibrosis (PMF), the cytomorphological assessability of the preparations is often limited ("punctio sicca").

In all cases, however, the histology is decisive for the diagnosis as well as for the assessment of the degree of fibrosis and the bone marrow architecture, which must always be carried out if MPN is suspected or confirmed.



Chromosome analysis

Chromosomal abnormalities occur in 35-40% of patients with primary myelofibrosis and are predominantly unbalanced events. Thus, gains of material of the long arm of chromosome 1 (+1q), deletions in the long arm of chromosome 20 (del(20q)), trisomy 9 (+9), deletions in the long arm of chromosome 13 (del(13q)), trisomy 8 (+8), abnormality of chromosome 7 (-7, del(7q)) as well as of chromosome 5 (-5, del(5q)), deletions in the short arm of chromosome 12 (del(12p)) and rather rarely an isochromosome of the long arm of chromosome 17 (i(17q)) are observed. None of these abnormalities are specific for primary myelofibrosis, they also occur in other MPN and also in MDS (Swerdlow et al. 2017).

FISH

In the case of punctio sicca, the cytogenetic changes in the blood smear typical of primary myelofibrosis can be detected by FISH analysis.

Molecular genetics

JAK2 V617F mutations are common in primary myelofibrosis
Molecular genetic evidence of a *JAK2* V617F mutation is found in about 60% of patients with primary myelofibrosis. The prognostic relevance of this mutation is controversially discussed (Campbell et al. 2006, Guglielmelli et al. 2009). In 10% of all patients with primary myelofibrosis without *JAK2* V617F mutation a mutation in codon W515 of the MPL gene was detected. A mutation in the calreticulin gene (CALR) is found in about 70% of patients with myelofibrosis in whom no *JAK2* or *MPL* mutation has been detected (Klampfl et al. 2013, Nangalia et al. 2013). Patients without one of these three mutations ("triple negative") have a higher risk of developing AML than patients with a *JAK2*, *MPL* or *CALR* mutation (Rumi et al. 2014, Tefferi et al. (1) 2014). Mutations in the *EZH2* gene are found in 5-7% of patients with PMF and are associated with an unfavourable course of disease according to the data available to date (e.g. Guglielmelli et al. 2011). A mutation of the *ASXL1* gene is detected in 20-30% of patients with primary myelofibrosis (Vannucchi et al. 2013).

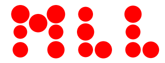
Table 1: Frequency of different mutations in primary myelofibrosis (Langabeer et al. 2015, Tefferi 2018)

Gene mutation	Frequency (%)
JAK2 V617F (Exon 14)	55-65
JAK2 Exon 12	rare
MPL	5-10
CALR	25-35
TET2	10-20
IDH1/2	4-5
DNMT3A	5-10
ASXL1	13-30
EZH2	5-10
CBL	5-10
SF3B1	5-10
SRSF2	10-17
U2AF1	5-16
TP53	4

Prognosis

Among the *BCR-ABL1*-negative myeloproliferative neoplasms, primary myelofibrosis has the most unfavourable course. There is both a risk of progression of the disease into the fibrotic stage and a risk of leukemic transformation. While the median overall survival is about 6 years (Tefferi et al. (4) 2014), the individual clinical courses are very heterogeneous. The prognosis is influenced by a variety of factors, which can be divided into clinical, cytogenetic and molecular genetic factors. Especially for genetic changes, the description of prognostically relevant factors is still a subject of research. With increasing knowledge, various prognostic scoring systems (~PSS) have been successively established or further developed. Table 2 gives an overview of the published risk stratification systems and the prognostic factors considered therein.

Table 2: Prognostic scoring systems for primary myelofibrosis and its risk factors



Score	IPSS	DIPSS	DIPSS Plus	GPSS	MIPSS	GIPSS	MIPSS70	MIPSS70+
	International Prognostic Scoring System	Dynamic International Prognostic Scoring System	Dynamic International Prognostic Scoring System Plus	Genetics-Based Prognostic Scoring System	Mutation-Enhanced International Prognostic Scoring System	Genetically Inspired Prognostic Scoring System	Mutation - Enhanced International Prognostic Scoring System for Transplantation-age Patients	Karyotype Enhanced MIPSS70
Publication	Cervantes et al. 2009	Passamonti et al. 2010	Gangat et al. 2011	Tefferi et al. (5) 2014	Vannucchi et al. 2014	Tefferi et al. (3) 2018	Guglielmelli et al. 2018	Guglielmel et al. 2018
Considered prognositc factors	• Clinic	• Clinic	• Clinic		• Clinic		• Clinic	• Clinic
			• Karyotype	• Karyotype		• Karyotype		• Karyoty
				• Mutations	• Mutations	• Mutations	• Mutations	• Mutatic

Clinical prognostic factors in primary myelofibrosis

Various studies have demonstrated an association between reduced overall survival and the following clinical parameters (Cervantes et al. 2009, Passamonti et al. 2010, Caramazza et al. 2011, Gangat et al. 2011, Guglielmelli et al. 2018):
- Age
- Anemia
- Thrombocytopenia
- Leucocytosis
- Circulating Bubbles
- Bone marrow fibrosis
- Constitutional symptomatology
- Transfusion dependence

An association with leukemia-free survival was observed for the clinical parameters of platelet count <100 x 10^9/l and circulating blasts ≥2% (Caramazza et al. 2011, Gangat et al. 2011, Tefferi et al. Leukemia (3) 2018).

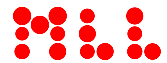
Risk assessment based on clinical risk factors: IPSS and DIPSS score

In order to be able to make a statement about the course of primary myelofibrosis, the IPSS and DIPSS scoring systems were established on the basis of clinical risk factors. In both scoring systems the risk factors age, B symptoms, haemoglobin value below 10 g/dl, leukocytes above 25 x 10^9/l and over 1% blasts in peripheral blood are considered (Cervantes et al. 2009, Passamonti et al. 2010). Both scoring systems can be used for the selection of the therapy algorithm according to German guidelines (Onkopedia guideline PMF 2018). While IPSS can only be applied at the initial diagnosis, risk classification according to DIPSS is possible throughout the course of the disease due to a different weighting of the risk factors.

Table 3: Prognosis and risk assessment of primary myelofibrosis after IPSS and DIPSS

Risk factors	Number of risk faktors		Prognosis (Risk)	Median survival risk factors (years)	
	IPSS	DPSS		IPSS	DPSS
- Age >65 years - Constitutional symptoms (Fever, weight loss, night sweat) - Hb <10g/dl* - Leukocytes>25 x 10^9 /l - Blasts in peripheral blood ≥ 1%	0	0	low	11,2	15,4
	1	1-2	intermediate 1	7,9	6,5
	2	3-4	intermediate 2	4,0	2,9
	≥ 3	≥ 5	high	2,3	1,3

*double weighted at DIPSS



Cytogenetic prognostic factors in primary myelofibrosis

Cytogenetic abnormalities are frequently observed in primary myelofibrosis, although the abnormalities present are heterogeneous and not specific to PMF (see Diagnosis of Primary Myelofibrosis, Chromosomal Analysis). Cytogenetic alterations can influence the prognosis both favourably and unfavourably. Accordingly, stratification based on the prognostic significance of the individual cytogenetic abnormality was included in various scores.

DIPSS Plus Score

For the first time the karyotype was included in the DIPSS Plus score. In addition to the clinical risk factors listed in Table 3, this also integrated the parameters of transfusion need and platelet count below 100 x 10^9/l as well as cytogenetic abnormalities (Gangat et al. 2011). Cytogenetic abnormalities were divided into two prognostic groups. A complex karyotype (≥ 3 abnormalities) and one or two abnormalities with a trisomy 8, a monosomy 7 or 7q deletion, an abnormalities of chromosome 5, an isochromosome of the long arm of chromosome 17, a 12p deletion, an inversion of chromosome 3 or an 11q23 rearrangement were evaluated as prognostically unfavorable cytogenetic abnormalities.

Refined 3-step cytogenetic risk model

As studies increasingly pointed to cytogenetic heterogeneity in the prognostically unfavourable group, this two-step model was re-examined in a study of 1,002 patients with primary myelofibrosis by Tefferi et al. and the dependence of overall survival on cytogenetic abnormalities was newly validated (Tefferi et al. (1) 2018). This led to a refined 3-step model that favours risk stratification into three prognostic groups: favourable, unfavourable and very unfavourable (= very high risk) abnormalities with regard to overall survival (see Table 4). This 3-step risk model was incorporated into the risk classifications published in 2018 in MIPSS70+ Version 2.0 (Tefferi et al. (2) 2018) and GIPSS (Tefferi et al. (3) 2018).

Table 4: Revised cytogenetic risk stratification according Tefferi et al. (1) 2018

Risk category	Specific abnormalities	Median survival
Favorable	**Normal karyotype** **Sole** 20q-, sole 13q-, sole +9, sole chromosome 1 translocationen/duplikationen, sole sex chromosome-abnormality including -Y	**4.4 years**
Unfavorable	**Sole abnormalities:** Sole +8, 7q-, sole translocation not involving chromosome 1 **Two abnormalities:** without VHR-abnormality **Single/multiple abnormalities:** 5q- abnormalities Complex karyotype without VHR-abnormality, Monosomal karyotype without VHR-abnormality, sole abnormalities not otherwise classified	**2.9 years**
Very high risk (VHR)	**Single/multiple abnormalities:** Monosomy 7, inv(3)/3q21, i(17q), 12p-/12p11.2, 11q-/11q23, autosomal trisomies other than +8 or +9 (z.B. +21, +19)	**1.2 years**

Further Prognostic Assessments of Cytogenetic Abnormalities
Other publications have discussed further prognostic assessments of cytogenetic abnormalities (see Table 5).

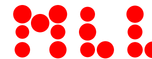
Table 5: Prognostic assessment of cytogenetic changes

	Favorable	Intermediate	Unfavorable	Very high risk
Tam et al. 2009	+9 (also with additional abnormality), 13q-, 20q-	NK	Abnormalities including chromosome 5 or 7, complex	Abnormality of Chromosom 17
Hussein et al. 2010	+9, 13q-, 20q-	NK	Other abnormalities	+8, complex
Caramazza et al. 2011	NK and abnormalities, which are not unfavorable		Complex, +8, -7/7q-, i(17q), -5/5q-, 12p-, inv(3), 11q23- rearrangements	
Brecqueville et al. 2014	Assoziation between 12p-, 17q-, 20q- and transformation into AML			
Tefferi et al. (5) 2014	NK, +9, 13q-	20q-, 1q+, -Y	Complex-not-monosomal, 5q-, +8, sole or two other abnormalities	monosomal KT, inv(3), i(17q), -7/7q-, 11q or 12p abnormality

KT: karyotype, NK: normal karyotype, complex ≥ 3 abnormalities

Molecular genetic prognostic factors in primary myelofibrosis

Influence of driver mutations



The driver mutations influence the overall survival in different ways. With a median overall survival of 15.9 years, patients with a *CALR* mutation have the best prognosis (Tefferi et al. (4) 2014), as long as there is no concomitant *ASXL1* mutation (Tefferi et al. (2) 2014). Patients with *MPL* mutation (9.9 years) and *JAK2* mutation (5.9 years) follow. With a median overall survival of 2.3 years, triple negative cases have the worst prognosis (Tefferi et al. (4) 2014).

For patients with a *CALR* mutation the subtype is of prognostic relevance. A distinction is mainly made between type 1 and type 2 mutations. Both involve exon 9 of the *CALR* gene. With ~69-80%, the type 1 mutation represents the majority of the *CALR* mutations, it leads to a deletion in exon 9. Type 2 mutations are detected in 11-21% of the cases, this mutation results in an insertion (Klampfl et al. 2013, Tefferi et al. (3) 2014). The apparent prognostically favorable course for *CALR*-mutated PMF is due to the high number of *CALR* type 1 mutations and possibly limited to these. PMF patients with *CALR* type 2 mutations show a similar clinical course as patients with *JAK2* mutation (Tefferi et al. (3) 2014).

"High molecular risk" mutations affect overall and leukemia-free survival
Cooperative non-driver mutations can greatly affect the prognosis in primary myelofibrosis. For this purpose, the category of HMR mutations ("high molecular risk") was introduced (Guglielmelli et al. 2014), which is now considered in various scoring systems. The established HMR mutations include *ASXL1*, *EZH2*, *SRSF2*, *IDH1* and *IDH2*. Independent of IPSS and DIPSS Plus, they are associated with a shorter survival and higher risk of transformation into acute leukemia (Vannucchi et al. 2013). The occurrence of two or more mutations of these genes is 20-051505

less favourable compared to one or no mutation (Guglielmelli et al. 2014). Mutations of the splicing factor *U2AF1* affecting the amino acid glutamine at position 157 (Q157) have also been identified as a risk factor. They are independently associated with reduced survival in DIPSS, but the transformation rate was not affected (Tefferi et al. (4) 2018). Based on this study, *U2AF1* Q157 mutations are now also counted as HMR category (Tefferi et al. (2) 2018, Tefferi et al. (3) 2018).

A further study indicates that *NRAS/KRAS* mutations also strongly impair the prognosis. Although they occur rarely in primary myelofibrosis (about 6% of patients), they are associated with reduced overall and leukemia-free survival (Santos et al. 2020).

Epigenetic and splicing factors with potential association to rapid progression
In a cohort of 77 PMF patients with disease progression an association to the presence of mutations of certain splicing factors (*SRSF2*, *U2AF1*, *SF3B1*) or epigenetic factors (*IDH2*, *EZH2*) could be observed. For 27 patients with stable clinical course, which were used as control group, no mutation in any of the mentioned genes could be detected in any case. For the 19 patients with mutation in *SRSF2*, *U2AF1*, *SF3B1*, *IDH2* or *EZH2* a rapid progression was observed, in the median after 2 years. In comparison, the median progression-free survival for the 58 patients with a different genetic background was 7.25 years (Bartels et al. 2020).

Integration of molecular genetic factors in prognostic risk models
In order to take account of the prognostic influence of mutations, these were increasingly integrated into risk stratification models. Table 6 provides an overview.

Table 6: Overview of risk scores that take molecular genetic factors into account. For CALR driver mutations a distinction is made between type 1 and type 2 mutations. CALR type 1 mutations have the most favourable prognosis among driver mutations and therefore do not represent a risk factor. In contrast, CALR type 2 mutations appear to be prognostically equivalent to JAK2 mutations (Tefferi et al. (3) 2014).

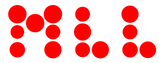
	MIPSS	**GPSS**	**GIPSS**	**MIPSS70 & MIPSS70+**	**MIPSS70+ Version 2.0**
Publication	Vannucchi et al. 2014	Tefferi et al. (5) 2014	Tefferi et al. (3) 2018	Guglielmelli et al. 2018	Tefferi et al. (2) 2018
Risc factors taken into account with regard to Driver mutations / mutation status	• *JAK2* or *MPL* • triple negative	• *JAK2* • *MPL* • *CALR* type 2 mutation • triple negative	• any driver-mutation or -mutation-status, except *CALR* type 1 mutation	• any driver mutation or -mutation status, except *CALR* type 1 mutation	• any driver mutation or -mutation status, except *CALR* type 1 mutation
Risk factors considered in relation to HMR mutations	• *ASXL1* • *SRSF2*	• *ASXL1* • *SRSF2*	• *ASXL1* • *SRSF2* • *U2AF1* Q157	• 1 HMR-mutation* • ≥2 HMR-mutations* **ASXL1*, *SRSF2*, *EZH2*, *IDH1/2*	• 1 HMR-mutation* • ≥2 HMR-mutations* **ASXL1*, *SRSF2*, *EZH2*, *IDH1/2*, *U2AF1* Q157

Calculation for primary myelofibrosis (PMF)
The calculation of the clinical scoring systems IPSS or DIPSS is recommended for the selection of the therapy algorithm according to the DGHO guideline (Onkopedia guideline PMF 2018). Here you can access the forecast calculation of the **DIPSS score**.

Consideration of cytogenetic and molecular genetic factors would lead to re-classification into a higher risk category for an estimated 25% of patients assigned to the low or intermediate group according to IPSS/DIPSS classification (Onkopedia Guideline PMF 2018). The determination of genetic risk factors can therefore be useful for weighing up for or against a stem cell transplantation (Kröger et al. 2015, Tefferi 2018).

Here you can access the calculation of the **DIPSS plus score**, which takes a two-step cytogenetic risk model into account. A refined three-level cytogenetic risk model as well as molecular genetic risk factors are integrated in the scoring systems of **GIPSS** and **MIPSS70+ Version 2.0**.

Recommendation



In addition to the collection of clinical and laboratory chemical parameters, histological and cytomorphological examination of the bone marrow and blood, cytogenetic analysis and molecular genetic examinations (*JAK2* V617F mutation, if negative, *CALR*, if negative *MPL*) are recommended. According to the WHO 2017 classification, additional molecular genetic analyses should be added for primary myelofibrosis (*ASXL1*, *EZH2*, *TET2*, *IDH1/IDH2*, *SRSF2*, *SF3B1*).

References

Here you can find the corresponding references:
https://www.mll.com/en/diagnostic-offer/myelodysplastic-syndrome-mds/myeloproliferative-neoplasm-mpn/primary-myelofibrosis-pmf.html#references