An update on *BCR-ABL1*-negative myeloproliferative neoplasms

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Abstract

BCR-ABL 1 negative myeloproliferative neoplasms (MPN) are clonal haemopoietic stem cell disorders, characterized by proliferation of one or more of the myeloid cell lineages. Classification is based on the genetic abnormalities involved in the pathogenesis and on correlation of specific histological features with clinical findings. The diagnostic approach must take into account clinical, haematological, morphological and genetic features. A clinico-pathologically assigned diagnosis therefore forms the basis of the current consensus based classification.

Myeloproliferative neoplasms may have an insidious onset and variable progression, comprising the full spectrum of evolution from a prodromal or initial phase, through a stable phase, to a terminal phase. The latter results in bone marrow failure, myelodysplastic changes and blast crisis. Prodromal stages pose a number of diagnostic problems because they may not present with the classic clinico-pathological criteria for diagnosis. Follow-up biopsies are important to monitor progression and features of regression, stability, progression and the effects of therapy must be noted. In the MPN group particularly, quantitation of fibrosis is very important and it must be graded accurately throughout the course of the disease. The effect of therapies must be taken into account in initial and follow-up biopsies.

The last decade has seen tremendous advances in molecular biology, diagnosis and treatment of *BCR-ABL1-neg* MPN. These advances have led to re-examination of diagnostic criteria and revision of the 2008 WHO classification is required urgently.

Keywords chronic eosinophilic leukaemia; chronic neutrophilic leukaemia; essential thrombocythaemia; myeloproliferative neoplasm; polycythaemia vera; primary myelofibrosis

Introduction

In the fourth edition of the WHO classification,[1] the term 'chronic myeloproliferative disease' was replaced by 'myeloproliferative neoplasm' (MPN). Inclusion of systemic mastocytosis (SM) has attracted controversy and, together with the myeloid and lymphoid neoplasms with eosinophilia and abnormalities of *PDGFRA, PDGFRB or FGFR1*, will not be considered here as the diagnostic process is different.

Myeloproliferative neoplasms are clonal haemopoietic stem cell disorders, characterized by proliferation of one or more of the myeloid cell lineages and lack of significant dysplasia. Classification continues to be influenced by recent discoveries of genetic abnormalities involved in the pathogenesis of *BCR-ABL1*-negative MPNs, correlated with specific histological features and clinical findings.

Approach to diagnosis

The diagnostic approach must take into account clinical, haematological, morphological and genetic features (Box 1).

A clinico-pathologically assigned diagnosis forms the basis of the current consensus classification (Box 2). This classification is not without controversy and alternative views have been expressed.[2] Reproducibility of the histopathology of certain aspects of the classification also remains controversial.

Myeloproliferative neoplasms may have an insidious onset and variable progression, comprising the full spectrum of evolution from a prodromal or initial phase, through a stable phase, to a terminal phase. The latter results in bone marrow failure, myelodysplastic changes and blast crisis (Table 1).

Prodromal stages pose a number of diagnostic problems because they may not present with the classic clinico-pathological criteria for diagnosis.[3,4] They may be recognisable by bone marrow examinations but may also result in an "unclassifiable" diagnosis. Rebiopsy may eventually clarify the situation but these cases may initially have to be placed in the MPN, U or MDS/MPN, U category awaiting follow-up investigations and eventual reclassification. Likewise, the onset of 'acceleration' and acute transformation is sometimes difficult to define and there is a lack of consensus on diagnostic criteria. The WHO criteria of $\geq$20% blasts in blast crisis and 10–19% blasts in accelerated phase may be difficult to apply in practice in cases of 'dry tap' marrow aspiration and discordant bone marrow and peripheral blood findings. It is in any case arbitrary. A blast crisis may be the result of clonal evolution that is obvious from an increasingly complex karyotype. During the phase of progression, transition to an MDS/MPN phenotype preceding transformation is often parallelled by development of a complex karyotype typical for secondary AML.[5] It should also be noted that extramedullary blast proliferation indicates blast phase and that the marrow findings may be discordant.

Follow-up biopsies are important to monitor progression and features of regression, stability, progression and the effects of therapy must be noted (Table 2).

In the MPN group particularly, quantitation of fibrosis is very important and it must be accurately graded (Box 3).

The effects of therapies such as hydroxycarbamide must be taken into account.

The differential diagnosis in MPN includes other members of this category, the related refractory anaemia with ring sideroblasts with thrombocytosis (RARS-T)[6] and also entities such as MPN/MDS and MDS with isolated del(5q), which may present with thrombocytosis.[7] A variety of reactive disorders must be actively excluded before a diagnosis of MPN or MDS/MPN can be established. Of particular interest are the hereditary

Abbreviations: MPN, myeloproliferative neoplasm; MPN, U, myeloproliferative neoplasm, unclassifiable; MDS, myelodysplastic syndromes; MPN/MDS, myeloproliferative neoplasm/myelodysplastic syndrome; PV, polycythaemia vera; PMF, primary myelofibrosis; ET, essential thrombocythaemia; CML, chronic myelogenous leukaemia; CNL, chronic neutrophilic leukaemia; CEL, chronic eosinophilic leukaemia; AML, acute myeloid leukaemia; CMML, chronic myelomonocytic leukaemia; SM, systemic mastocytosis.

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Guidelines for using the revised WHO classification of myeloid neoplasms[26]

Specimen requirements

- PB and BM specimens collected prior to any definitive therapy.
- PB and cellular BM aspirate smears and/or touch preparations stained with Wright-Giemsa or similar stain.
- BM biopsy specimen, at least 1.5 cm in length and at right angles to the cortical bone, is recommended for all cases if feasible.
- BM specimens for complete cytogenetic analysis and, when indicated, for flow cytometry, with an additional specimen cryopreserved for molecular genetic studies. The latter studies should be performed based on initial karyotypic, clinical, morphologic, and immunophenotypic findings.

Assessment of blasts

- Blast percentage in PB and BM is determined by visual inspection.
- Myeloblasts, monoblasts, promonocytes, megakaryoblasts (but not dysplastic megakaryocytes) are counted as blasts when summing blast percentage for diagnosis of AML or blast transformation; count abnormal promyelocytes as "blast equivalents" in APL.
- Proerythroblasts are not counted as blasts except in rare instances of "pure" acute erythroleukaemia.
- Flow cytometric assessment of CD34+ cells is not recommended as a substitute for visual inspection; not all blasts express CD34, and artifacts introduced by specimen processing may result in erroneous estimates.
- If the aspirate is poor and/or marrow fibrosis is present, immunohistochemistry (IHC) on biopsy sections for CD34 may be informative if blasts are CD34+.

Assessment of blast lineage

- Multiparameter flow cytometry (at least three colours) is recommended; the panel should be sufficient to determine lineage as well as aberrant antigen profile of a neoplastic population.
- Cytochemistry, such as myeloperoxidase or nonspecific esterase, may be helpful, particularly in AML, NOS, but it is not essential in all cases.
- IHC on biopsy sections may be helpful; many antibodies are now available for recognition of myeloid and lymphoid antigens.

Assessment of genetic features

- Complete cytogenetic analysis from BM at initial diagnosis when possible.
- Additional studies, such as FISH, RT-PCR, mutational status, should be guided by clinical, laboratory, and morphologic information.
- Mutational studies for mutated *NPM1*, *CEBPA*, and *FLT3* are recommended in all cytogenetically normal AML; mutated *JAK2* should be sought in *BCR-ABL1*—negative MPN, and mutational analysis for *MPL*, *CALR*, *KIT*, *NRAS*, *PTNP11*, etc, should be performed as clinically indicated.

Correlation/reporting of data

- All data should be assimilated into one report that states the WHO diagnosis.

Box 1

myeloproliferative disorders,[8] hereditary thombocytosis[9] and congenital erythrocytosis.[10,11] Knowledge of the family history and appreciation of variant morphology are crucial for the diagnosis. With the decline in biopsy rates for PV in the post *JAK2*^{V617F} era, a higher proportion of cases of congenital or acquired erythrocytosis are likely to encountered. Reactive conditions such as infection with HIV[12] may cause thrombocytosis. The use of thrombopoietin receptor agonists in thrombocytopenic states[13] may mimic ET or PV, with increased reticulin and MPN-like morphology.

Advances in the molecular pathology of MPN

Acquired somatic mutations of *JAK2*^{V617F} and *JAK2* exon 12 have been shown to play a crucial role in the pathogenesis of many cases of *BCR-ABL1*-negative MPN and these discoveries opened a new era in the understanding of the biology and therapy of MPN.[14,15] *JAK2*^{V617F} mutation is detected in about 95% of patients with PV as a secondary genetic event that is preceded by an as yet undefined molecular abnormality. As a consequence of this mutation, transformation and proliferation of haematopoietic progenitor cells are promoted by downstream signal transduction pathways. In ET and PMF, this mutation is found in only about 50% of patients and in *JAK2*^{V617F} negative PV, an activating *JAK2* exon 12 mutation is detectable.[16] In a small number of patients with ET and PMF, an activating mutation of *MPL*$^{W515L/K}$ is present.[17] It must be noted that *JAK 2*^{V617F} is not specific for MPN and its absence does not exclude the diagnosis.

In 2013, two groups independently reported novel calreticulin gene (*CALR*) mutations (exon 9 deletions and insertions) in *JAK2* or *MPL* unmutated PMF and ET.[18,19] In one of the studies, among 1107 patient samples with MPN, *CALR* mutations were not seen in PV but were seen in ET (25%) and PMF (35%).[19] *CALR* mutations were mutually exclusive of *JAK2* or *MPL* mutations. When *JAK2/MPL*-unmutated cases were analysed, *CALR* mutational were found to be 67% in ET and 88% in PMF. *CALR* mutations were not detected in AML, MDS, CMML or CML. Three patients (13%) with RARS-T displayed *CALR* mutations and all three were JAK2/MPL-negative. Subsequent studies of CML cases have shown concurrent *CALR* and *BCR-ABL1*; these have been described[20,21] as 'ET evolving to CML' a*nd* 'A *CALR* Mutation Preceding *BCR-ABL1* in an Atypical Myeloproliferative Neoplasm'. These complex cases require synthesis of haematological, morphological and molecular findings to reach a diagnosis.

Other mutations, including *TET2*, *IDH*, *ASXL1* or *DNMT3A*, are occasionally seen in PV and ET.[22] The presence or absence of the various mutations has been found to have prognostic implications. In a recent study of PMF, *CALR* mutations were mutually exclusive of *JAK2* or *MPL* mutations and were associated with younger age and higher platelet counts, with patients being less likely to be anaemic, require transfusions or display leucocytosis. *CALR* mutations had a favourable impact on survival while '*CALR*$^{-}$*ASXL1*$^{+}$' and 'triple-negative' cases were identified as high-risk molecular signatures in PMF.[23] In another study, ET patients with *CALR* mutation had lower leucocyte counts and ages compared with *JAK2*-mutated ET patients.[24] Thus, evaluation of JAK2, *MPL*, and *CALR* mutation status has become mandatory not only for diagnosis but also for prognostication.[25]